GENETIC MAPPING OF FRUIT QUALITY TRAITS IN APPLE (*MALUS* X *DOMESTICA* BORKH.).

MOGAMAT KHASHIEF SOEKER

A thesis submitted in partial fulfillment of the requirements for the degree of *philosophiae doctorae* in the Faculty of Science, University of the Western Cape.

Supervisor: Dr. D. Jasper G. Rees.

October 2011

ABSTRACT ..... **I**

DECLARATION ..... **IV**

ACKNOWLEDGEMENTS ..... **V**

LIST OF TABLES ..... **VI**

LIST OF FIGURES ..... **IX**

APPENDICES ..... **XIII**

ABBREVIATIONS ..... **XIV**

CHAPTER 1: LITERATURE REVIEW **1**

1.1 INTRODUCTION **1**
1.2 CONSUMER PERSPECTIVE OF FRUIT QUALITY **6**
1.3 FACTORS AFFECTING FRUIT QUALITY **6**
1.3.1 SIZE AND SHAPE 7
1.3.2 COLOUR AND RUSSETTING 10
1.3.3 FIRMNESS AND TEXTURE 18
1.3.4 FLAVOUR 29
1.4 APPLE GENETICS **42**
1.4.1 TRADITIONAL BREEDING 44
1.4.2 ESTIMATION OF HERITABILITY 45
1.4.3 MODERN / ADVANCED BREEDING TECHNIQUES 46
1.4.4 MARKERS AND GENETIC MAPPING 49
1.4.5 MARKER-ASSISTED SELECTION 53
1.5 QUANTITATIVE TRAIT LOCI (QTLS) **54**
1.5.1 DEFINITION OF A QTL 55
1.5.2 CHARACTERISATION OF POLYGENIC TRAITS 55
1.5.3 DETECTION AND MAPPING OF QTLS 58
1.5.4 QTL ANALYSIS 59
1.5.5 STATISTICAL APPROACH FOR DETERMINATION OF LINKAGE 63
1.5.6 QTL AND GENE MAPPING IN APPLE 63
1.5.7 APPLE GENOME SEQUENCING 69
1.6 OBJECTIVES OF THIS STUDY **70**

CHAPTER 2: MATERIALS AND METHODS **72**

2.1 GENERAL CHEMICALS AND ENZYMES **72**
2.2 GENERAL STOCK SOLUTIONS AND BUFFERS **74**
2.3 PHENOTYPIC DATA **76**
2.3.1 MAPPING POPULATIONS 76
2.3.2 ASSESSMENT OF PHENOTYPIC TRAITS 77
2.3.3 DATA ANALYSIS 81
2.3.4 VARIANCE STRUCTURE 81
2.4 GENOTYPIC DATA **83**
2.4.1 EXTRACTION OF GENOMIC DNA FROM APPLE LEAVES 83
2.4.2 1% AGAROSE GEL PREPARATION AND ELECTROPHORESIS 84
2.4.3 SSR DETECTION, PRIMER DESIGN AND PRIMER SYNTHESIS 84
2.4.4 PCR AMPLIFICATION 87
2.4.5 MEGAPLEX PCR 88

2.4.6 AMPLIFICATION OF ACS-1, ACO-1 AND EXPANSIN-7 USING PCR 88
2.4.7 AUTOMATED FRAGMENT ANALYSIS 90
2.4.8 AMPLIFIED PRODUCT ANALYSIS 91
2.4.9 SEGREGATION ANALYSIS OF MAPPING POPULATIONS 92
2.4.10 DART ANALYSIS 92
2.4.11 GENETIC LINKAGE MAP CONSTRUCTION 92
2.4.12 QTL MAPPING 93

CHAPTER 3: PHENOTYPIC RESULTS 95

3.1 PHENOTYPIC ANALYSIS 95
3.1.1 SUBJECTIVE ANALYSIS 95
3.1.2 INSTRUMENTAL ANALYSIS 111
3.2 HERITABILITY 112
3.3 CORRELATION ANALYSIS OF PRE-STORAGE AND POST-STORAGE EVALUATIONS 115
3.4 YEAR BY YEAR CORRELATIONS 120
3.5 SUMMARY 122

CHAPTER 4: GENOTYPIC RESULTS 123

4.1 ISOLATION OF GENOMIC DNA 123
4.2 PRIMER DESIGN AND SYNTHESIS 125
4.3 OPTIMISATION OF SSR MARKERS 125
4.4 SCORING ACS1, ACO1 AND EXPANSIN-7 MARKERS ON MAPPING POPULATIONS 127
4.5 SEGREGATION ANALYSIS OF MAPPING POPULATIONS 132
4.6 GENETIC LINKAGE MAP CONSTRUCTION 136
4.7 QTL IDENTIFICATION 151
4.8 COMPARING DIFFERENT MAPQTL® 5.0 MAPPING FUNCTIONS 183
4.9 STABILITY OF QTLS 187

CHAPTER 5: DISCUSSION 190

5.1 PHENOTYPIC RESULTS 190
5.1.1 ANALYSIS OF VARIANCE 190
5.1.2 HERITABILITY 192
5.1.3 CORRELATION ANALYSIS 194
5.2 GENOTYPIC RESULTS 201
5.2.1 NEWLY DESIGNED SSRS (SAMS) 201
5.2.2 SEGREGATION ANALYSIS 202
5.2.3 GENETIC MAPPING 205
5.2.4 QTL MAPPING 209

CHAPTER 6: CONCLUSION 227

REFERENCES 233

ABSTRACT

Genetic mapping of fruit quality traits in apple (Malus x Domestica Borkh.)

PhD thesis, Department of Biotechnology, Faculty of Science, University of the Western Cape.

Mogamat Khashief Soeker

Apple fruit quality is of utmost importance to apple farmers and breeders in the selection and commercialization of new cultivars. Fruit size, colour, texture, firmness and taste are all traits that affect the quality of fruit. In this study the genetic contribution of these traits, and others were evaluated in order to generate the genetic markers required for the application of marker assisted selection in fruit quality breeding.

Three mapping populations, 'Prima' x 'Anna', 'Golden Delicious' x 'Priscilla' and 'Golden Delicious' x 'Anna', consisting of 87, 87 and 141 respectively, were used in the study. Fruit samples were analysed, using a range of visual, physical and sensory measurements, over a period of three years, and the data was then correlated using statistical analysis. Traits analysed included stripe-ness, fruit colour, fruit size, fruit form, ground colour, russet, texture, fruit firmness,

juiciness, sugar content, acidity, taste, skin toughness, %TSS, fruit mass and diameter.

ANOVA detected significant levels of variation between the three families for all traits except taste and russet; while highly significant 'within family' variation was also observed for all traits in pre- and post-storage analyses, except for sugar content (sweetness) and fruit form. Within family variation also contributed the largest percentage towards the variance components of all traits. Heritability estimates found stripe-ness to be the most heritable trait, from subjective analyses, while heritability values ranged from 0.41 to 0.84 for instrumentally measured traits.

The genetic maps for the three populations were generated using both published microsatellites and new EST-SSR and DART markers, using JoinMap 4.0™. The integrated genetic linkage maps of 'Prima' x 'Anna', 'Golden Delicious' x 'Priscilla', 'Golden Delicious' x 'Anna' consisted of 398 (133 SSR and 265 DArT), 353 (80 SSR and 273 DArT) and 213 (87 SSR and 126 DArT) markers respectively. The maps were 1021.6cM, 1079cM and 1302.7cM in length, respectively. Location of quantitative trait loci (QTL) for 14 fruit quality traits was detected using MapQTL 5.0™ and a total of 79 pre-storage and 60 post-storage QTLs were identified on the three mapping populations.

Comparative genome analysis and the role of various genes on the outcome of fruit quality can now be investigated. Using the integrated genetic maps, and the

QTLs identified, candidate markers associated with these QTL can be used for marker-assisted selection, to increase the speed and efficiency of the apple-breeding program.

DECLARATION

I declare that, **Genetic mapping of fruit quality traits in apple (*Malus x domestica* Borkh.)**, is my own work that has not been submitted for any degree or examination in any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Mogamat Khashief Soeker October 2011

Signed:…….

ACKNOWLEDGEMENTS

I would like to especially thank the following people and institutions:

- The Almighty, Allah, for the health, patience, perseverance and strength to fulfill my dreams.
- My parents, Mogamat Sediek and Kalthoema Soeker, my wife Zaida, my brothers Mogamat Fatiegh and Mogamat Ameen and my sister-in-law, Hameedah Soeker for your love, support, inspiration and motivation to do what was required to finish this degree.
- My supervisor, Jasper Rees, for the opportunity to become part of the plant genetics research group at the University of the Western Cape and for all the guidance during my PhD studies. Thank you for all the opportunities to present my work nationally as well as internationally and all the associated opportunities to travel and attend workshops related to my field of interest. Thank you for the financial support that made it possible for me to further my academic career.
- Dr Iwan Labuschagnè, for all your help and advice during the phenotypic data collection phase of the project.
- All the members of the Fruit tree genetics group, formerly at the University of the Western Cape, for your friendship, support and helpful discussions. A special thank you to Dr Daleen van Dyk for all the guidance, as my honours supervisor, and a constant source of help and advice.
- Support staff in the Department of Biotechnology at the University of the Western Cape and Agricultural Research Council at Infruitec.
- Funding agencies, the National Research Foundation, Deciduous Fruit Producer's Trust and Department of Trade and Industry-THRIP.

LIST OF TABLES

Table 1. Lexicon of sensory texture attributes and their associated reference standards……...…..21

Table 2. Selected aroma volatiles found in apples……..…..34

Table 3. Comparison of molecular marker systems……..…48

Table 4. Summary of linkage groups (LG's) on which QTL's have been identified for a variety of phenotypic traits……..…..66

Table 5. Summary of linkage groups (LG's) on which candidate genes have been mapped for a variety of phenotypic traits……..67

Table 6. Number of seedlings form each mapping population……..…76

Table 7. The different structures that ANOVA is broken down into……....82

Table 8. Classes of segregation types encountered when working with a full-sib family…….……91

Table 9. Variation within families recorded for sensory traits during pre-storage evaluation of apple fruit…….……96

Table 10. Variation within families recorded for sensory traits during post-storage evaluation of apple fruit…….……97

Table 11. Yearly variation recorded for sensory traits during pre-storage evaluation of apple fruit…….……98

Table 12. Yearly variation recorded for sensory traits during post-storage evaluation of apple fruit……....99

Table 13. Within family variation recorded for traits during pre-storage evaluation of apple fruit…….…..100

Table 14. Within family variation recorded for traits during post-storage evaluation of apple fruit…….……..100

Table 15. Yearly variation recorded for traits during pre-storage evaluation of apple fruit…….....101

Table 16. Yearly variation recorded for traits during post-storage evaluation of apple fruit…….…101

Table 17. Analysis of variance for traits involved in sensory development of apple fruit…….…104

Table 18. Summary of the variance components for sensory traits……....107

Table 19. Analysis of variance for characteristics associated with apple colour development…….……109

Table 20. Analysis of variance for mass, diameter, firmness and total soluble solids in apple fruit……..…….110

Table 21. Summary of the variance components for maturity traits estimated from pre-storage evaluation measurements……..……114

Table 22. Summary of the variance components for maturity traits estimated from post-storage evaluation measurements……114

Table 23. Pre-storage correlations and post-storage correlations among 13 apple fruit characteristics of 'Prima' x 'Anna'……..…..…117

Table 24. Pre-storage correlations and post-storage correlations among 13 apple fruit characteristics of 'GD' x 'Anna'…….……..…118

Table 25. Pre-storage correlations and post-storage correlations among 13 apple fruit characteristics of 'GD' x 'Priscilla'……..…119

Table 26. Yearly correlations for subjective and instrumentally measured fruit traits over a three-year period…….….120

Table 27. Newly designed SSR markers and the mapping populations they are located on…….....145

Table 28. Overview of fruit quality trait QTLs detected, pre-storage…….....176

Table 29. Overview of fruit quality trait QTLs detected, post-storage……...180

LIST OF FIGURES

Figure 1. Anthocyanin distribution in cross-sections of apple skin……..12

Figure 2. The putative anthocyanin biosynthetic pathway in apple skin……..…15

Figure 3. Anaerobic biosynthetic pathway for the formation of acetaldehyde, ethanol, and esters…….…..……37

Figure 4. Partial metabolic pathway of sorbitol synthesis and degradation in Rosaceae……..……...40

Figure 5. Colorimeter and penetrometer used to capture readings for colour components and firmness……...……79

Figure 6. Sensory evaluation form……...…...….80

Figure 7. Outputs of the tandem repeats finder database……..……...….86

Figure 8. Agarose gel electrophoresis of genomic DNA on a 1% agarose gel...124

Figure 9. Representation of a 6% PAGE showing polymorphic nature of primer A222……....126

Figure 10a. Agarose gel electrophoresis showing segregation of ACS-1 in a representative sample of the 'Prima' x 'Anna' mapping population…….…..127

Figure 10b. 2% agarose gel electrophoresis showing segregation of ACO-1 in a representative sample of ‘Prima’ x ‘Anna’ mapping population……..129

Figure 10c. Electropherogram showing the segregation of EXPANSIN7 in ‘Golden Delicious’ and ‘Anna’.....131

Figure 11. Electropherogram obtained after implementation of Megaplex 26 on ‘Prima’ parent cultivar…….……134

Figure 12. Electropherograms obtained after implementation of the marker CH04a12 on the cultivars 'Prima' and 'Anna', as well as the four different classes (ac, ad, bc, bd) observed in the F1 population……135

Figure 13. Genetic linkage map constructed using 87 individuals of a F1 population derived from a cross between 'Prima' (female) and 'Anna' (male parent)…….….139

Figure 14. Genetic linkage map constructed using 87 individuals of a F1 population derived from a cross between 'Golden Delicious' (female parent) and 'Priscilla' (male parent)……..…...142

Figure 15. Genetic linkage map constructed using 141 individuals of a F1 population derived from a cross between 'Golden Delicious' (female parent) and 'Anna' (male parent)…….……144

Figure 16. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'Prima' x 'Anna' mapping population, pre-storage, using rMQM mapping...……..…158

Figure 17. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'GD' x 'Anna' mapping population pre-storage, using rMQM mapping…....……..….161

Figure 18. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'GD' x 'Priscilla' mapping population pre-storage, using rMQM……..……..….....165

Figure 19. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'Prima' x 'Anna' mapping population, post-storage, using rMQM mapping……..……..169

Figure 20. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'GD' x 'Anna' mapping population, post-storage, using rMQM mapping……...…….172

Figure 21. Graphical representation of the genetic positions (in cM) of QTL's, of fruit quality traits, identified in the 'GD' x 'Anna' mapping population, post-storage, using rMQM mapping……....175

Figure 22. Graphical representation of the KW output……..…..….184

Figure 23. A graphical representation of LG15b of the 'Prima' x 'Anna' mapping population found using the interval mapping function of MapQTL 5.0……..……...185

Figure 24. Graphical representation of the rMQM output file LG15b of 'Prima' x 'Anna'……...……...……186

Figure 25. The stability of the stripe-ness QTL localized around the SSR marker CH01h021, in the 'Prima' x 'Anna' progeny…….….187

Figure 26. The stability of the colour QTL in the 'Prima' x 'Anna' progeny, localizing around SSR markers SAmsDR998909 and SAmsCO540769 ..……...189

APPENDICES

Appendix A. The 444 SSR markers used in this study…..……...…..….276

Appendix B. Megaplexes designed for 451 SSR markers.……..……...295

Appendix C. Simple statisics and histograms of fruit quality trait scores collected pre- and post-storage, in the ‘Prima’ x ‘Anna’ population……...…CD

Appendix D. Simple statisics and histograms of fruit quality trait scores collected pre- and post-storage, in the ‘Golden Delicious’ x ‘Anna’ population……CD

Appendix E. Simple statisics and histograms of fruit quality trait scores collected pre- and post-storage, in the ‘Golden Delcious’ x ‘Priscilla’ population……CD

Appendix F. Segregation analysis and Joinmap codes of DArT markers in the ‘Prima’ x ‘Anna’ population……CD

Appendix G. Segregation analysis and Joinmap codes of DArT markers in the ‘Golden Delicious’ x ‘Anna’ population…….……CD

Appendix H. Segregation analysis and Joinmap codes of DArT markers in the ‘Golden Delicious’ x ‘Priscilla’ population……CD

Appendix I. Basic Local alignment Search Tool (BLAST) of all apple SSRs against the apple genome……CD

Appendix J. MapQTL® 5.0 Kruskal Wallis test output file……..CD

ABBREVIATIONS

1-MCP	1-Methylcyclopropene
AAT	Alcohol acyl CoA transferase
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ACS	1-aminocyclopropane-1-carboxylic acid synthase
ADH	Alcohol dehydrogenase
AFLP	Amplified fragment length polymorphism
ANS	Anthocyanidin synthase
AVG	Aminoethoxyvinylglycine
BLAST	Basic local alignment search tool
CA	Controlled atmosphere
CHI	Chalcone isomerase
CHS	Chalcone synthase
cM	Centi-Morgen
CO_2	Carbon dioxide
CoA	Acyl co-enzyme A
DAFB	Days after full bloom
DArT	Diversity array technology markers
DFR	Dihydroflavonol 4-reductase
DH	Double haploid
EST	Expressed sequence tag
F3H	Favanone 3-hydroxylase

G6P	Glucose 6-phosphate
IM	Interval mapping
KW	Kruskal Wallis
LD	Linkage disequilibrium
LOD	Log of odds
LOX	Lipxogenase
MdH1	Malus domestica histoe
MdExp3	Malus domestica Expansin
MQM	Multiple QTL mapping
NAD	Nicotinamide adenine dinucleotide
NAD-SDH	Nicotinamide adenine dinucleotide-sorbitol dehydrogenase
PAL	Phenylalanine ammonia-lyase
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
PDH	Pyruvate dehydrogenase
QTL	Quantitative trait loci
RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
S6PDH	Sorbitol-6-phosphate dehydrogenase
SADH	Succinic acid 2,2-dimethyl hydrazide
SCAR	Sequence characterised amplified region
SI	Self incompatibility
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat

TSS	Total soluble solid
UFGalT	UDP galactose: flavonoid 3-*O*-galactosyltransferase
UFGluT	UDP glucose: flavonoid 3-*O*-glucosyltransferase
w/v	weight per volume
v/v	volume per volume

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The major temperate fruit tree crops, apple (*Malus* x *domestica*), peach (*Prunus persica*), cherry (*Prunus avium* and *Prunus cerasus*), plum (*Prunus domestica* and *Prunus salicina*), apricot (*Prunus armeniaca*), almond (*Prunus dulcis*), pear (*Pyrus comunis*) and loquat (*Eriobotrya japonica*) all belong to the Rosaceae family. These woody perennials have long intergenerational periods due to their juvenile phase and large size. They are therefore poorly suited organisms for classical genetic analysis. The breeding methods used for these species have not changed much over the last fifty years and the incorporation of alleles of interest from wild or exotic materials into elite breeding lines has rarely produced new commercial cultivars (Dirlewanger *et al.,* 2004).

Apple growing and breeding can be dated back thousands of years and has maintained its popularity due to its fleshy nature, nutritional value and desirable taste. The exact origin of the cultivated, or domesticated, apple is not exactly known but is believed to originate in the Tien Shan Mountains (Juniper *et al*., 2001), which is located in eastern China, Kazakhstan and Krygyzstan.

The occurrence of the cultivated apple can be explained by the hybridization of "*M. sieversii*" with "*M. prunifolia*", "*M. baccata*" and "*M. sieboldii*" in the east, and in the west with the hybridization of "*M. sieversii*" with "*M. turkmenorum*"

and "*M. sylvestris*" (Juniper *et al*., 1999). Authors suggest that this as the most likely scenario, as man moved from western China to the Black Sea in the late Neolithic or early Bronze age, on the so-called Old Silk Road. As the Romans had practised excellent grafting and hybridisation techniques, there was a further progression in the cultivated apple, and the resultant introduction of it into Western Europe. More recently, studies undertaken using nuclear DNA and chloroplast DNA sequences, have shown that the domesticated apple is most closely related to the *Malus sp*. (Harris *et al*., 2002). Another important finding from Harris *et al*., (2002) is that the Central Asian wild apple, "*M. sieversii*", is also most closely related to the domesticated apple. The most accurate nomenclature for the domesticated apple is disputed between "*Malus* x *domestica* Borkh." and "*Malus* x *pumila* Mill." but the former is more commonly used (Korban and Skirvin, 1984). The Maloideae are believed to be allopolyploids, which is not a rare phenomenon in the plant kingdom and usually results in larger and more vigorous plants. The Rosaceae family has four sub-families each with a specific basic chromosome number, the Rosoideae has a base chromosome number of x=7, the Prunoideae x=8, the Spiraeoideae x=9 and the Maloideae (including *Malus* and *Pyrus*) x=17. It is hypothesized that the latter have originated through an ancient hybridization event between the Prunoideae and the Spiroideae. At present the binominal *Malus X domestica* has been generally accepted as the appropriate scientific name for the cultivated apple (Gardiner *et al*., 2007).

Today, the demand for new apple cultivars and the resultant industry is extremely

competitive. “Pink Lady”, “Royal Gala” and “Fuji”, to name a few, have been cultivars produced from successful breeding programs. As agriculture forms an integral part of the economy of many countries, methods in improving crop production through research has increase steadily in recent years. Janick *et al*., (1996) mentions that increased marketability is the principal breeding objective in apples. They also highlight that there are many markets viz. fresh, stored or processed; local market, commercial market or export.

The consumer plays an integral part in this market, as their interests and demands needs to be met. China is the world’s leading apple producer with millions of metric tonnes being produced annually. Gardiner *et al*., (2007) summarises that fruit quality (viz. colour, texture, size, shape, texture and taste) are the main criteria used by consumers. The most attractive feature that would result in the purchasing of apples would be its skin colour. Once this criterion has been met, the other qualities are “evaluated” by the consumer until the ultimate purchasing of it.

The export industry is a quality-driven market, and this requires fruit breeders to increase breeding efficiency and use more modern fruit breeding techniques in combination with traditional techniques, to improve fruit quality. Quality can be defined as all those characteristics of a food (not just sensory characteristics) that lead a consumer to be satisfied with the product (Cardello, 1995). Apples are recognized, worldwide, for their flavour, health and nutritional attributes (Harker *et al.,* 2003). Because of this, apple fruit quality is of utmost importance to apple

farmers and breeders. This is reflected in major international markets, which are experiencing a period of intense competition (Harker *et al.,* 2002), where failure to meet specifications can result in shipment rejections, reduced returns to growers and a damaged reputation as a supplier of top quality fruit. The modern apple industry relies on a narrow array of cultivars that meet basic levels of size, firmness, eye appeal and other standards necessary for successful marketing (Bassi and Selli, 1990). It is very difficult to get a reliable measure of apple fruit quality, since cultivars ripen at different times (Redalen, 1988), and even though size, external colour and firmness have been steadily improved through selection, the maintenance and improvement of flavour is more difficult to achieve as this is composed of a complex of different quality components (Redalen, 1988).

In South Africa, the deciduous fruit industry is a multi-million rand industry, yielding 1 653 556 tons in the 2008/2009 seasons alone. The 2008 season was a very good one for pome fruits, because producer profitability was high due to the availability of large export quantities and weaker exchange rates. This number is forecast to increase by 4.3% in the 2009/2010 seasons. Apple production showed the largest percentage increase of 7.1% compared to previous year's production, there was a 12.5 % decrease in the amount of catrons passed for export in the 2010 season. This decrease was due to a heat wave that hit the apple production region, leading to sunburnt fruits which were not passed for export (Ntombela, 2010). Most of the fruit are produced in the Western Cape, with its favourable Mediterranean climate. Majority of fruit produced in other parts of the country is sold domestically, but fruit of the Western Cape makes up 50% of total amount

exported to foreign markets. Fruits, such as apples, can fetch up to twice as much, per ton sold, on foreign markets than on South African markets and by the end of 2009, apples made up the majority of deciduous fruit produced i.e. 56% of the total deciduous fruit yield, with the rest being made up by pears, grapes, peaches, apricots, plums and nectarines.

Breeding new apple cultivars is a long and tedious process requiring more than 20 years, including periods of cross-pollination, seedling selection and field trials. Selection processes is complicated by the slow growth, the long juvenile phase, the high level of heterozygosity and the strong self-incompatibility present in this species. These factors has lead to the release of two South African bred apple cultivars, 'African Carmine', in 1999, and more recently, 'Elegant', in 2007, athough the breeding program at the Agricultural Research Council (ARC) that has been established for almost 35 years. The time constraints often make conventional breeding, or then conventional selection methods, impractical and this has stimulated an interest in the apple genome and molecular marker techniques in order to apply Marker Assisted Breeding (MAB). Conventional breeding will be complemeted by these techniques, in order to produce cultivars with desired traits after a shorter period of time and with less cost involved in maintaining trees that will only show their 'undesirable' characteristics after years of costly field maintenance.

1.2 CONSUMER PERSPECTIVE OF FRUIT QUALITY

Plant breeders have been criticized for their concentration on yield and appearance to the detriment of colour, taste and nutrient value (Francis, 1970). Consumers are becoming more and more vocal about the characteristics they expect fruit to have. Consumer responses to fruit drive (i) the need of the industry to improve its competitiveness (Ricks *et al.,* 2000) and (ii) the need to improve consumption of fruit for health reasons (Krebs-Smith *et al.,* 1996; Harker *et al.,* 2003). Consumers are no longer focusing only on size and eye-appeal to decide; they also focus on flavour and texture as major determinants. Firmer fruit are favoured as compared to softer fruit (Lui and King, 1978; Prange *et al.,* 1993) and crispness accounted for most of the variation seen in "texture liking", among consumers (Hampson *et al.,* 2000). Despite some research being done to determine consumer preferences, consumer tests are impossible for routine screening of breeding selections, due to the limited availability of fruit, and other resources required for the large number of evaluations (between 75 and 200 consumers are required**)**. To overcome this, fruit are evaluated by a panel of trained judges who judge fruit according to consumer ideals for size, colour, firmness and percentage total soluble solids (% TSS) (Hampson *et al.,* 2000).

1.3 FACTORS AFFECTING FRUIT QUALITY

The concept of fruit quality is derived from a variety of factors, all of which play a very important role in the marketing of that particular fruit. Fruit size and shape,

colour and firmness are among the most important of these traits. More recently, factors such as texture and percentage total soluble solids (TSS) have become essential in the determination of top quality fruit. These traits are strongly influenced by genetics and are triggered by various environmental factors, some of which will be discussed later.

1.3.1 Size and Shape

Good fruit size and shape are undoubtedly two of the important traits required for a better quality fruit, with premiums paid for a larger sized fruit. Fruit size is influenced by both environmental and genetic conditions (Harada *et al.,* 2005). Of the genetic factors affecting fruit size, the cultivar plays the dominant role. It is well known that some cultivars have larger fruit than others. The other major genetic factor affecting fruit size is the rootstock genotype. The type of rootstock used can affect fruit size. Genotypes such as M.27 and OAR.1 tend to produce smaller fruit than others (Ferree, 2000). Another important factor that can determine fruit size is the size and presence of the spurs, since not all cultivars are spur bearers. The spur leaves are the only leaves supplying the carbohydrates during the critical cell division stage. If early defoliation occurs the reserves in the spur would not be sufficient for fruit to set, thus resulting in a smaller fruit being produced (Ferree, 2000). The presence of lateral fruit also leads to the development of smaller fruit (Ferree, 2000). According to Janick and Moore (1975), optimal fruit size for good quality fruit varies between 65mm and 75mm.

Environmental factors affecting fruit size include light, temperature and moisture. According to a study in Europe's more northerly latitudes, cooler temperatures tend to result in smaller fruit (Ferree, 2000). Good cultural practices also lead to the development of good-sized fruit. Since light is the most important environmental factor affecting fruit size, cultural practices need to address good light interception to encourage improved light penetration through the canopy e.g. by thinning and pruning. Enhanced fruit size can also be obtained by removing competitive grasses from the orchard floor, thus enabling better nutrient availability (Ferree, 2000).

McKenzie (1971) observed that fruit growing in mild, moist regions of Northern New Zealand were more flattened than those developing in cooler, drier areas. It was also shown that apples grown on hills higher above sea level (500m-800m) tend to be more elongated with smoother skins than those found in valleys 250m above sea level (Eccher, 1986; Noè *et al.,* 1994). Air and soil temperature, day and night temperatures and relative humidity can also affect apple fruit shape (Sullivan, 1965; Greenhalgh and Goodley, 1976 and Tromp, 1990).

1.3.1.1 Cell Number and Cell Size

In 1951, Bain and Robertson showed that that difference in size of apple fruit between varieties is the result of differences in cell number and/or cell size (Harada *et al.,* 2005). Cell number is usually determined in the first thirty-five to fifty days after full bloom (DAFB) (Denne, 1960; Harada *et al.,* 2005). Once this

cell division stage is completed, the enlargement of cells determines the size of fruit. Wakasa *et al*. (2003) showed that the cell proliferation stage and cell enlargement stage are characterized by high expression of the histone (MdH1) and expansin (MdExp3) genes, respectively. Species such as *Malus floribunda* and *Malus coronaria,* with small crabapple-like fruit show low levels of expression of these genes, whereas, larger domesticated species such as 'Fuji', 'Mutsu' and 'Sekaiichi' show high expression of these genes (Harada *et al.,* 2005).

It has also been shown that the difference in size of many cultivars, as well as their final cell size, is linked to the ploidy level of the cultivar (Taas *et al.,* 1998). The *Malus domestica* cultivar, 'Mutsu', a triploid cultivar, has larger fruit than a diploid cultivar viz. 'Fuji' (Janick *et al.,* 1996; Harada *et al.,* 2005). According to protoplast data, the cells from 'Mutsu' have been shown to be 1.1 times the size of cells from 'Fuji' (Harada *et al.,* 2005).

Genetic mapping of fruit size was performed by Liebhard *et al*., (2003), who detected a QTL on LG 8 and 17 on the 'Fiesta' x 'Discovery' mapping population. These results expanded on those found by Seglias and Gessler (1997), who reported a QTL on LG 5 of 'A679-2'. Conner et al., (1997, 1998) mapped this trait to LG 7 on 'Wijcik McIntosh' and LG 1 on 'NY75441-58'.

1.3.2 Colour And Russetting

1.3.2.1 Colour

The colour of apples is determined by two factors viz. (i) the ground colour of the skin and (ii) the anthocyanin pigmentation (over colour). The red colour of anthocyanin is superimposed on the ground colour. Most fruit, when immature, start off green in colour. The green colour then lightens and fades until the fruit appears from pale cream to deep yellow. Ground colours in the greenish-yellow, to yellowish green range appear when the green colour seen in the immature fruit does not fade completely. Finally, if the green colour does not fade at all, a mature green fruit results (Janick and Moore, 1975).

Anthocyanin is either present or absent from the fruit, and the colour can tbe distributed in several ways on the fruit. Fruits can either have small red flecks to thick, red stripes or a faint blush to solid red. The presence of anthocyanin is dominant over the lack of it, with heterozygous seedlings, all showing some sort of colouration (Crane, 1953; Janick and Moore, 1975). The shade of red that develops depends on the ground colour of the fruit, with the most brilliant red forming when the ground colour is almost white. The area of the fruit covered with anthocyanin is inherited quantitatively (Janick and Moore, 1975).

Striped fruit colour was reported as early as the 1930's, where Crane and Lawrence (1933) reported the dominant gene, R*f*. Cultivars that are homozygous

dominant for this gene include ‘Worcester Pearmain’ and ‘Delicious’, with ‘Golden Delicious’ representing a homozygous recessive cultivar (Browne, 1992).

Anthocyanin biosynthesis

The red colour in the skin of fruit is due to the presence of anthocyanin pigments that belong to a class of flavonoids (Honda *et al.,* 2002). The accumulation of anthocyanins is influenced by environmental stimuli, such as light, temperature and nutrition, as well as by genetic factors. Anthocyanin pigments are also responsible for the red color in the leaves, flowers and fruits of some apples. Saito *et al*. (2002) reported that anthocyanins are the main pigments in flowers and fruits and they serve as visual signals that attract insects and animals for pollination and seed dispersal. Anthocyanins also play a role in photoprotection in autumn foliage and in the rapidly developing shoots of tropical trees (Saito *et al.,* 2002)**.**

The anthocyanin pigments accumulate in the epidermal cell vacuoles (Figure 1); their intensity and color depends on external conditions, as well as on the microenvironment conditions in the vacuole (Harborne and Grayer, 1988). Unlike pigmentation in flowers and fruit, anthocyanin accumulation in leaves is normally due to environmental stress. Since the pigments absorb green/blue UV light, their accumulation possibly serves as an adaptive mechanism to protect plants from strong sunlight (Batschauer *et al.,* 1996). Curry, (1997) reported that low temperatures induced red color development in many fruit crops, e.g., apples,

while Christie *et al.,* (1994), also showed that low temperatures induced anthocyanin synthesis in vegetative tissues. The main point of control of anthocyanin production varies according to plant species. Red and blushed apples acquire their red colour from anthocyanins present in their peel (Francis, 1970).

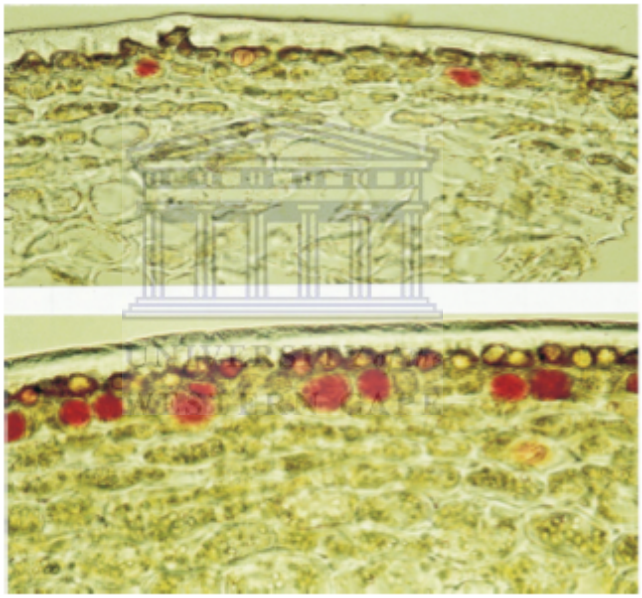


Figure 1. Anthocyanin distribution in cross-sections of apple skin. (Top) 'Jonagold' and (bottom) its mutant, 'Red Jonaprince'. Magnification 350x (Awad *et al.,* 2000)

The environment in the vacuole may also affect anthocyanin concentration. Mazza and Miniati (1993) reported that tin, copper, and aluminium ions form stable complexes with anthocyanins. Stable ternary complexes containing

anthocyanin, an unidentified colorless compound and magnesium have been described (Takeda *et al.,* 1990, 1994; Kondo *et al.,* 1992). The metals can also change the hue of flower colour as well. Shaked-Sachray *et al*. (2002) reported that magnesium treatment increased concentrations of anthocyanin in aster 'Sungal' flowers without stimulating synthesis, suggesting that the ion increased the stability of the pigment**.**

Nissim-Levi *et al*. (2003) discovered that the accumulation of magnesium in plant tissues inhibits anthocyanin degradation. It was also hypothesized, that magnesium forms a stable complex with the anthocyanin, delaying its degradation (Takeda *et al.,* 1990, 1994; Kondo *et al.,* 1992).

Genes involved in Anthocyanin Biosynthesis

Biosynthesis of anthocyanins is well established, with the exception of a few enzymatic steps (Macheix *et al.,* 1990). The enzymes and genes involved in anthocyanin biosynthesis are most investigated in petunia, snapdragon and maize as model plant species, resulting in the accumulation of knowledge regarding elucidation of the anthocyanin biosynthetic pathway (Heller and Forkmann, 1988; Forkmann, 1993; Holton and Cornish, 1995). According to Yamazaki and Saito (2002), most genes for the biosynthetic enzymes have been isolated, and the biochemical reactions catalyzed by those enzymes from those model plants have been characterized. In addition, the regulatory proteins and their genes were also isolated through analysis of genetic mutants, which exhibit altered flower colour.

In most cases, these regulatory genes encoded transcriptional factors, to control the expression of the genes for biosynthetic enzymes (Mol *et al.,* 1996; Mol *et al.,* 1998; Winkel-Shirley, 2001).

Anthocyanin biosynthesis has been well characterized in other species e. g. flowers petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), and in the kernels of maize (*Zea mays*) (Kim *et al.,* 2003). Anthocyanin biosynthetic genes from grapes (*Vitis vinifera*) were studied and UDP glucose: flavonoid 3-O-glucosyltranseferase (UFGluT) was found to be a major enzyme controlling the red colour in grape skin. Anthocyanin pigments are produced from phenylalanine via the flavonoid biosynthesis pathways involving at least seven key enzymes in apple, these include: (i) Phenylalanine ammonia lyase (PAL); (ii) Chalcone synthase (CHS); (iii) Chalcone isomerase (CHI); (iv) Flavanone 3-hydroxylase (F3H); (v) Dihydroflavonol 4-reductase (DFR); (vi) Anthocyanidin synthase (ANS) and (vii) UDP-galactose:flavonoid 3-O-galactosyltransferase (UFGalT) (Figure 2). UDP-galactose:flavonoid 3-O-galactosyltransferase (UFGalT) is involoved in this final step since cyanidin 3-galactoside is the major pigment in the red skin of apple.

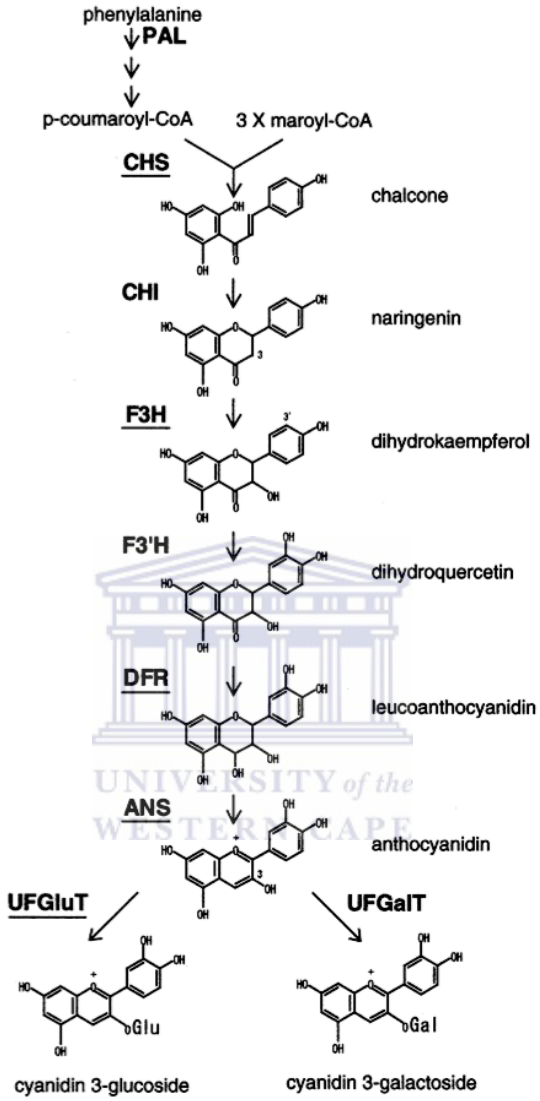


Figure 2. The putative anthocyanin biosynthetic pathway in apple skin. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavanoid 3'-hydroxylase; DFR, dihydroflavanol 4-reductase; ANS, anthocyanin synthase; UFGluT, UDP glucose:flavanoid 3-*O*-glucosyltransferase; UFGalT, UDP galactose:flavanoid 3-*O*-galactosyltransferase; glu, glucose; gal, galactose (Honda *et al.,* 2002).

Anthocyanin degradation

Role of low, high temperature and of sunlight

Marais *et al*. (2001a) reported that irradiation, with light for 144h at 37°C, to simulate temperatures experienced in summer, reduced the anthocyanin content of detached 'Cripps' Pink' apples by more than half, resulting in red colour loss. Steyn *et al*. (2004) discovered in their study that high temperature (30°C) accelerated the degradation of anthocyanin and the fading of red colour in detached fruit in 'Forelle' pears and in 'Royal Gala' apples. In their findings, light was not a prerequisite for anthocyanin degradation, though it increased the rate of anthocyanin degradation and color loss in 'Royal Gala' apples. Little is known about the mechanism of anthocyanin degradation in fruit (Lancaster, 1992).

Francis (1989) reported that anthocyanins were degraded in food products in response to heat and light. Degradation was reported to be nonenzymatic, but may also be mediated by common enzyme groups, i.e., the glycosidases, polyphenoloxidases and peroxidases (Francis, 1989; Piffaut *et al.,* 1994). Piffaut *et al*. (1994) found that anthocyanin degradation was mediated by B-glycosidases or induced by high temperature and proceeded via the same pathway.

A study undertaken by Plant and Food Research (New Zealand), in collaboration with Spanish research groups, using a cross between 'Scigold' and 'T22' (Envy™), positioned a SNP marker less than 1Mb away from the MdMYB10

(Chagné *et al*., 2007) locus on LG 9. MdMYB10 was highly expressed in red-fleshed fruit, as well as red foliage.

Carotenoids

Carotenoids play a very important role in light harvesting, stabilization of the thylakoid membrane and energy distribution and dissipation in pigment-protein complexes, in plant photosynthetic apparatus (Biswall, 1995; Demmig-Adams *et al.,* 1996; Young and Frank, 1996; Edge *et al.,* 1997; Havaux, 1998; Merzylak and Solovchenko, 2002). However, little is known on the physiological significance of carotenoid retention and accumulation that occurs in senescing leaves and ripening fruit (Gitelson *et al.,* 2002; Merzylak and Solovchenko, 2002). In both senescing leaves and ripening fruit, the pool of carotenoids is comprised of xanthophylls and carotenol fatty acid esters (Biswall, 1995; Merzylak and Solovchenko, 2002). Carotenoids do, however, undergo rapid destruction when exposed to visible light, in the presence of chlorophyll (Merzylak *et al.,* 1996; Tregub *et al.,* 1996).

Thus far, no QTLs for carotenoid pigmentation have been mapped in apple, but it has been located in other species e. g. Asiatic hybrid lily (*Lilium sp*) (Nakano *et al*., 2004).

1.3.2.2 Russett

Russet on apple, (*Malus* x*domestica* Borkh) and other fruits results in lowered fruit quality and substantial economic losses to growers (Cummins *et al*., 1977). Russetting on fruit varies from one cultivar to the next and is caused by the yeasts, *Aureobasidium pullulan*s and *Rhodotorula glutinis.* It can found on different areas of the fruit, but some older varieties are completely russetted e.g. 'D'Arcy Spice'. On some fruits, russetting occurs in the stalk cavity, while on others it is confined to areas surrounding the calyx. Some cultivars such as 'Golden Delicious' are preferred if no russet is present, but others such as 'Cox's Orange Pippin' are tolerated if russet is present, with some people associating russet with favourable flavour (Janick and Moore, 1975). Alston and Watkins (1975) reported that Cox' Orange Pippin carried a dominant gene for russetting as well as a few minor genes with modifying effects (Brown, 1992).

1.3.3 Firmness And Texture

Fruit firmness is one of the most important characteristics of apple quality and obtaining and maintaining apple fruit firmness, from the orchard through to the consumer, is important to the industry (DeEll *et al.,* 2001) in more recent years. Firmness, or hardness, of a fruit can be defined as the force required to "compress" the sample with the back teeth (Harker *et al.,* 1997) (Table 1).

Bourne (1980) indicated that it is difficult to give an accurate description of this property, but many horticulturalists use fruit firmness to measure the mechanical properties of a fruit. It is usually determined as the maximum force required to "push" an 11 mm diameter probe of specific shape into the flesh (DeEll *et al.,* 2001). The instrument most widely used to perform this operation is the penetrometer. The Magness-Taylor tester, the Effegi firmness tester or the Electronic Pressure tester is used worldwide (DeEll *et al.,* 2001; Abbott, 1994; Abbott *et al.,* 1976; Lehmann-Salada, 1996). Despite there being a few non-destructive methods of determining firmness in sorting machines, none of these are commercially used (Abbott *et al.,* 1997).

Fruit firmness, as a quality trait, is not only influenced by climate, but by many other factors, most notably, pre-harvest and post-harvest factors. Pre-harvest factors include the genetic background of the fruit, cultural practices and the application of various fertilizers and growth regulators, whereas post-harvest factors include maturity at harvest, cooling, post-harvest dips and storage conditions (DeEll *et al.,* 2001). At the cellular level, firmness depends on the structure of the cells themselves, their size, shape, cell wall firmness and thickness, turgor pressure and the manner in which these cells bind to each other (Harker *et al.,* 1997).

The term texture covers a wide range of attributes that determine the feel of food in the mouth, and the way these characteristics can be measured using sensory and

instrumental methods. Food scientists have suggested a number of definitions, and some of these are summarized in Table 1.

Table 1. Lexicon of sensory texture attributes and their associated reference standards as developed and used with fruit by trained sensory panels at The Horticulture and Food Research Institute of New Zealand (Harker *et al.,* 1997: *Horticultural Reviews*.)

Attribute	Description	Reference standard (Absent/Low)	Reference standard (Extreme/High)
Crispness	The amount and pitch of sound generated when the sample is first bitten with the front teeth.	Ripe banana	Fresh potato crisp
Crunchiness	The amount of sound of noise generated when the sample is chewed at a fast rate with the back teeth.	Ripe banana	Raw celery
Ease of breakdown	The amount of chewing required to break down the sample so that it can be swallowed.	Apple puree	Raw swede
Fibrousness	The amount of readily separated filaments present.	Ripe banana	Celery
Flouriness	The amount of dry, fine, powdery particles that can coat the mouth during chewing.	Raw carrot	Overcooked garbanzo Beans (chick peas)
Graininess	The presence of small firm particles detected during chewing	Cream (liquid)	Semolina
Grittiness	The presence of small hard sharp particles detected during chewing	Cream (liquid)	White sugar crystals
Hardness	The force required to compress the sample with the back teeth.	Ripe banana	Raw carrot
Juiciness	The amount of free fluid released from the sample during chewing.	Ripe banana	Watermelon
Mealiness	The amount of small, lumpy particles that become apparent during chewing.	Canned mango slices	Porridge (made with rolled oats)
Melting	The degree to which the sample disintegrates evenly in the mouth, often without chewing	Raw swede	Canned mango
Pastiness	The amount of soft, smooth mass that doesn't release moisture during chewing.	Watermelon	Peanut butter
Pulpiness	The amount of wet, weblike material that develops during chewing	Raw carrot	Watermelon
Starchiness	The amount of fine particles that coat the mouth during chewing	Raw carrot	Raw potato

Physiologically the loss of firmnessin apple is related to ethylene (Costa *et al.*, 2005). Ethylene's biosynthetic pathway is controlled by two large gene families coding for 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO). As apple is a climacteric fruit, it experiences a burst of ethylene accompanied by a an increase in respiration. The first enzyme (ACS) represents the rate limiting step in the pathway and is responsible for the conversion of Sadenosyl-L-methionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC). The second enzyme, viz. ACO, or ethylene forming enzyme, is responsible for the conversion of ACC to ethylene. These genes have been mapped to LG 15 (ACS) (Harada *et al*., 2000) and LG 10 (ACO) on 'Prima' x 'Fiesta' and 'Fuji' x 'Mondial Gala' (Costa *et al*., 2005).

In 2008, Costa *et al*. mapped a functional marker, based on a simple sequence repeat (SSR) motif, MdEXP-7 to LG1 in apple. This class of proteins, known as expansins are believed to play a role in cell wall remodeling, by disrupting non-covalent bonds between the hemicellulose matrix and the cellulose microfibril (Cosgrove, 1997), thus exposing the structural polymer, of the cell wall, to the action of other cell-wall enzymes. It it believed to act in conjuction with the polygalacturonases that play a role in regulating fruit softening.

1.3.3.1 Pre-harvest Factors Affecting Firmness

Cultivar (genotype)

Most post-harvest quality characteristics, including firmness are genetically influenced and may vary with cultivar (Beverley *et al.,* 1993). This is seen in the case of 'Granny Smith' apples, which tend to be firmer than most other cultivars, whereas 'McIntosh' apples tend to be among the softest. Fruit firmness can also be influenced by the strain within a specific cultivar (DeEll and Prange, 1994).

The type of rootstock used may also have an effect on firmness, but this also tends to vary with cultivars and/or strains. Certain rootstocks, such as M.26, produce softer fruit than trees grown on rootstocks such as Seedling, M.1, MM.106, M.7 and OAR.1 (Fallahi *et al.,* 1985). The main effect of rootstock on apple storability is related to maturity and calcium levels, with high calcium content and low nitrogen to calcium ratio producing a firmer fruit (Drake *et al.,* 1993).

Nutrient Management

Various nutrients have been shown to have an effect on fruit firmness, whether directly or indirectly.

Calcium (Ca)

Although there are conflicting reports discussing the effect of calcium on fruit firmness, it was shown, by Webster (1978) that fair or poor quality 'McIntosh' apples contained a consistently lower concentration of Ca^{2+} than that seen in good-keeping apples. Results of using pre-harvest sprays with $CaCl_2$ have been inconsistent, with not much effect seen on the calcium concentration in 'Golden Delicious' and 'Anna' cultivars (Peryea, 1991; El-Ansary *et al.,* 1992). The use of Ca sprays on individual apples has shown a positive correlation between calcium concentration and fruit firmness, before and after storage (Riley *et al.,* 1976). Ca was also shown to delay softening during storage, as it delayed the degradation of polysaccharides in the cell wall (Sams and Conway, 1984) as well as maintaining cell-to-cell adhesion (Porritt and Lidster, 1978). Apples with high calcium also tend to respire more slowly and therefore have a longer shelf life (Shear and Faust, 1975).

Despite the conflicting data, it is clear that calcium levels in the fruit have to be maintained or increased in order to reduce the onset of disorders such as bitterpit and cork spot and thus also sometimes enhance firmness (DeEll *et al.,* 2001).

Nitrogen (N)

The application of nitrogen, to apple trees, was shown to have no direct effect on fruit firmness, but it does play a role indirectly. Bramlage *et al*. (1980)

demonstrated that fruits with a high N-content were larger, softer and more prone to pre-harvest drop.

Phosphorus (P)

The application of phosphorus, in sprays, was shown to increase fruit firmness to the same extent that post-harvest Ca dips do (Mason, 1976; Mason *et al.,* 1974; Mason *et al.,* 1975 and Webster *et al.,* 1986).

Fruit size

Generally, larger fruit tend to be softer than smaller fruit. However, Johnson (1992) showed that early thinning during cell division resulted in larger, firmer fruit, since fruit size relates to both cell number and cell size.

Bioregulators

Bioregulators are sprayed on apple trees to control vegetative growth, hasten or delay ripening, delay apple abscission or simply to increase fruit quality characteristics (DeEll *et al.,* 2001). The use of sprays containing cytokinins, succinic acid 2,2-dimethyl hydrazide (SADH) and aminoethoxyvinylglycine (AVG), which effectively block initiation of autocatalytic ethylene production and

ripening of harvested apples, have been shown to increase fruit firmness (Bufler, 1984; Greene, 1993; Bartram *et al.,* 1971; Greene, 1996).

Other cultural practices such as planting, crop density, root and tree pruning, flower and fruit thinning and trunk scoring have also been shown to have an effect on fruit firmness. Most if not all of these practices increase fruit firmness but results vary among cultivars (DeEll *et al.,* 2001).

1.3.3.2 Post-harvest Factors Affecting Fruit Firmness

Maturity at harvest

Maturity at harvest can affect the post harvest quality of apples (DeEll *et al.,* 2001). Fruit mature at different times of the season, and therefore requires more than one harvest (Harker *et al.,* 1997). Fruit firmness was greatest in fruits that are harvested earlier, with loss of firmness occurring later in the season, but this varies from cultivar to cultivar. Some apple cultivars such as 'Granny Smith' are not affected by harvest date (Sfakiotakis *et al.,* 1993b; Testoni *et al.,* 1989). The rate at which apples soften during storage is also affected by maturity at harvest, with early harvested 'Cox's Orange Pippin' showing greater firmness retention than late harvested apples, stored at 0°C (Tu *et al.,* 1997).

Pre-storage Treatments

A variety of pre-storage treatments can be used on apples with the aim of maintaining or increasing fruit firmness. These include Ca applications, heat application and positive results seen when using the Ca sprays and dips. Permeability of cultivars to Ca^{2+} varies; most if not all tend to show an increased firmness prior to storage (DeEll *et al.,* 2001).

The application of heat also works well in some cultivars, and is aimed at reducing losses caused by post-harvest pathogens (Burchill, 1964 and Sharples, 1967), thus maintaining fruit firmness in storage. Hot air is the preferred method of heat application rather than hot water, since hot water resulted in an increase in tissue breakdown (Porritt and Lidster, 1978). The application of heat is not as reliable as Ca, since not all cultivars respond positively to it (Chiu, 1984).

The latest pre-storage treatment used was the novel gaseous compound 1-methylcyclopropene (MCP). This compound inhibits the action of ethylene, by blocking ethylene receptors, and has been shown to improve firmness retention (DeEll *et al.,* 2001). Since ethylene is the plant growth regulator involved in fruit ripening, preventing its action by the use of 1-MCP has the potential to extend the storage life of apple fruit (Pre-Aymard *et al.,* 2003).

Storage Conditions

Temperature

The single most important factor governing the maintenance of post-harvest quality is temperature (DeEll *et al.,* 2001). Low temperature is very important in the retention of fruit firmness. A rapid decrease in temperature, from room temperature to refrigeration temperature, slows the rate of respiration thus resulting in a longer storage life.

Controlled Atmosphere (CA) Storage

Controlled atmosphere (CA) storage is technique in which oxygen, carbon dioxide and nitrogen concentrations as well as temperature and humidity are regulated. CA has reduced the loss in firmness in many cultivars viz., 'Prima', 'Priscilla', 'Moira', 'Nova' and 'Novaspy' (DeEll and Prange, 1992b). This technique involves the removal or addition of certain gases e.g. oxygen and carbon dioxide (CO_2) from the storage chambers. The downside to this is that mealiness is hastened in certain cultivars due to the levels of CO_2 (Fisher, 1939).

Low Ethylene

The presence or absence of ethylene in storage rooms affects apple fruit firmness differently depending on cultivar and storage conditions. Removal of this ethylene

from the storage chambers can result in an increase in fruit firmness in certain cultivars such as 'McIntosh' (Blanpied *et al.,* 1975; Forsyth *et al.,* 1969; Granger and Rousselle, 1984). Ethylene is removed from the chambers by the addition of alumina/potassium permanganate (Granger and Rousselle, 1984). Since low ethylene CA only works for some cultivars, its commercial use is cultivar dependent.

1.3.3.3 Summary

Given the genetic limitations of fruit firmness imposed by the cultivar, the post-harvest factors, especially the rapid imposition of low temperatures, have the greatest effect on apple firmness. Since many of these factors (pre-harvest and post-harvest) interact with one another, to influence fruit firmness, there is no one simple solution to the problem of consistently producing and maintaining superior fruit firmness.

1.3.4 Flavour

Most market research indicates that sensory characteristics (texture, odour and flavour) are the primary reason consumers purchase a particular type of fruit (Harker, 2002; Wismer *et al.,* 2005). Consumers today are becoming more and more aware of flavour in the fruit they eat. Hewett *et al*. (1999) suggested that

growers producing fruit having an intense and characteristic flavour are more likely to have a marketing advantage over those who do not.

The flavours of various fruits result from complex interactions of physical and chemical attributes. It combines the four basic tastes of sweet, sour, salty and bitter, with aroma and mouth feel. The flavours of different foods are perceived with taste receptors in different parts of the mouth, back of the throat and in the retro-nasal cavity in the nose, while chewing (Hewett *et al.,* 1999). Total soluble solids and titratable acidity play an important role in taste indication, with high solids resulting in high sugar levels and therefore a sweet taste whereas high acid generally means a more sour taste.

1.3.4.1 Apple volatiles

Fruit aroma is due to a complex mixture of a large number of volatile compounds that contribute to the overall sensory quality of fruit specific to species and cultivar (Sanz *et al.,* 1997). Most of the aromatic character of apples comes from volatile compounds known as esters (80-90%), with some alcohols (10-20%), ketones and ethers making minor contributions (Table 2) (Dimick and Hoskin, 1983; Hewett *et al.,* 1999). Free fatty acids, or those liberated by lipase activity and further metabolized by β-oxidative enzymes and/or lipoxygenase (Sanz *et al.,* 1997) are generally regarded as being the main precursors of ester-, alcohol-, and aldehyde volatiles produced by apple fruit during development and maturation (Fellman *et al.,* 2000).

Apple aroma in different cultivars

Apple volatile production has been categorised according to: type and quantity of esters or alcohols (Dirinck & Schamp, 1989; Paillard, 1990; Dixon and Hewett, 2000), aroma production pattern (Dirinck & Schamp 1989), skin colour (Paillard, 1990), or C6 aldehydes (Paillard 1990). Yellow-skinned cultivars have been reported to produce mainly acetic acid esters and red-skinned cultivars mostly butyric acid esters (Paillard, 1990). High concentrations of hexyl acetate and butyl acetate were considered to characterise 'Cox's Orange Pippin' 'Elstar', 'Golden Delicious', 'Jonagold' and 'Jublie Delbar', with 'Granny Smith', 'Nico', 'Paulared', and 'Summerred' being characterised by high concentrations of ethyl butanoate and hexan-1-ol (Dixon and Hewett, 2000).

1.3.4.2 Biogenesis of volatiles

There are several ways in which volatiles can be synthesized, since they are comprised of five chemical classes. Volatiles important for aroma, and flavour are synthesized from amino acids, membrane lipids and carbohydrates (Sanz *et al*., 1997), and these pathways appear to be common for different fruits.

Fatty acids

Fatty acids are the major precursors of aroma volatiles in most fruit (Sanz *et al*., 1997). Aroma volatiles in intact fruit are formed via the β-oxidation biosynthetic pathway, whereas, when fruit tissue is disrupted, volatiles form via the

lipoxygenase pathway (Schreier, 1984; Sanz *et al.,* 1997; Dixon and Hewett, 2000). The proportion of linolenic acid in lipids of post-climacteric apples is lower than in pre-climacteric apples. This low level of linolenic acid is associated with plastid structure, and results from the decreased concentrations of monogalactosyl diglyceride, digalactosyl diglyceride and phosphatidal glycerol, and not from a change to the fatty acid distribution of individual lipids (Galliard, 1968; Dixon and Hewett, 2000). When apples ripen, chloroplasts break down and therefore provide a major source of linoleic and linolenic fatty acids for volatile biosynthesis. This also explains why a decrease in chlorophyll concentration is observed with the decrease in lipids (Dixon and Hewett, 2000).

β-oxidation biosynthetic pathway

β-oxidation of fatty acids is the primary biosynthetic process providing alcohols and acyl co-enzyme A (CoA) for ester formation (Sanz *et al*., 1997). Rowan *et al*. (1997) showed that saturated ester volatiles arise by β-oxidation of fatty acid precursors, rather than by peroxidation of these precursors. Rowan *et al*. (1997) also showed that an α-oxidation pathway existed and that it resulted in a range of labelled volatiles, including ethyl butanoate and pentyl acetate (Table 2).

Lipoxygenase biosynthetic pathway (LOX)

In intact fruit, enzymes in the lipoxygenase (LOX) biosynthetic pathway and their substrates have different sub-cellular locations, preventing formation of volatile

compounds. However, during ripening, cell walls and membranes become more permeable, allowing the LOX pathway to become active without tissue disruption (Sanz *et al.,* 1997). This pathway also has the potential to provide substrates for ester production, and if it were active during ripening, it would act as an alternative to β-oxidation of fatty acids (Dixon and Hewett, 2000).

Table 2. Selected aroma volatiles found in apples, their sensory description and human detection threshold (Hewett *et al.,* 1999)

Compound	sensory description	Detection threshold ($\mu L.L^{-1}$)	Cultivar
Aldehydes			
hexanal	Green apple, grass-like, earthy	0.005	Golden Delicious, Delicious
trans-2-hexanal	Green/sharp, fruity, grass-like, harmonious	0.001 – 0.017	Golden Delicious, Delicious, McIntosh, others
Alcohols			
butanol	Sweet aroma, overall flavour	0.5	Royal Gala, Golden Delicious
hexanol	Earthy, unpleasant	0.15 - 0.5	Golden Delicious
Esters			
ethyl butanoate	Fruity, banana, pineapple, sweet, ester-like	0.001 – 0.007	
ethyl hexanoate	Fruity, fresh, winey, sweet, ester-like	0.001 – 0.003	
butyl acetate	Red apple, Cox-like, nail polish	0.066	Royal Gala, Cox, Gala
hexyl acetate	Red apple aroma, sweet, ripe, fruity, pear-like	0.002 – 0.12	Royal Gala, Cox, Golden Delicious
2 – methyl butyl acetate	Typical apple, banana-like	0.005 – 0.11	Royal Gala, Gala, Cox
ethyl-2-methyl butanoate	Fruity, apple-like, sweet strawberry, pungent	0.000006 – 0.0001	Golden Delicious, Delicious, Gala

Amino acids

Sanz *et al*. (1997) and Heath and Reineccius (1986) showed that branched chain alcohols, carbonyls and esters are produced when the amino acids valine, leucine, iso-leucine, alanine and aspartic acid are metabolised. Varying concentrations of free amino acids are responsible for different concentrations of these branched chain volatiles (Dixon and Hewett, 2000). In apples, iso-leucine is considered to be the biosynthetic precursor of 2-methyl butanoic acid and its esters (Paillard, 1990). It has also been shown that different ratios of amino acid conversion to volatiles occur in 'Braeburn', 'Granny Smith', 'Fuji', 'Red Delicious' and 'Royal Gala' apples (Rowan *et al.,* 1997). Little is known about the concentration and availability of amino acids during ripening and senescence of apples, and it is therefore unclear if amino acid concentrations determine the type of volatile compounds produced by apples.

Esters

The ester biosynthetic pathway (Figure 3) is not fully understood, but it is well documented that esters form the largest group of volatile compounds produced by fruit (Paillard, 1990; Song and Bangerth, 1994; Sanz *et al.,* 1997; Dixon and Hewett, 2000). Ester production in fruit tissue is a result of esterification of alcohols, carboxylic acids and acyl CoA, an oxygen dependent reaction, and is considered to be most active in the epidermis (Berger *et al.,* 1992). The enzyme responsible for the synthesis of esters is known as alcohol acyl CoA transferase (AAT) (Bartley *et al.,* 1985). It has been shown that similarities exist between substrate specificity of AAT enzymes from different fruits. Sulfydryl goups are

essential for activity (Dixon and Hewett, 2000). The mixture of esters produced in different fruits depends on activity and substrate specificity of AAT. Rowan *et al*., 1996 showed that esterification of straight-chain alcohols is preferred over branched-chain alcohols. Such differences in preference for acyl CoA's and alcohols may determine the concentration of different esters in fruit aroma profiles. In addition to AAT, the enzyme esterase, which converts esters to alcohols and carboxylic acids, may have some synthetic capacity. Therefore, ester synthesis in apple tissue may be a result of ester formation by AAT, and ester hydrolysis by esterase (Knee and Hatfield, 1981; Sanz *et al.,* 1997).

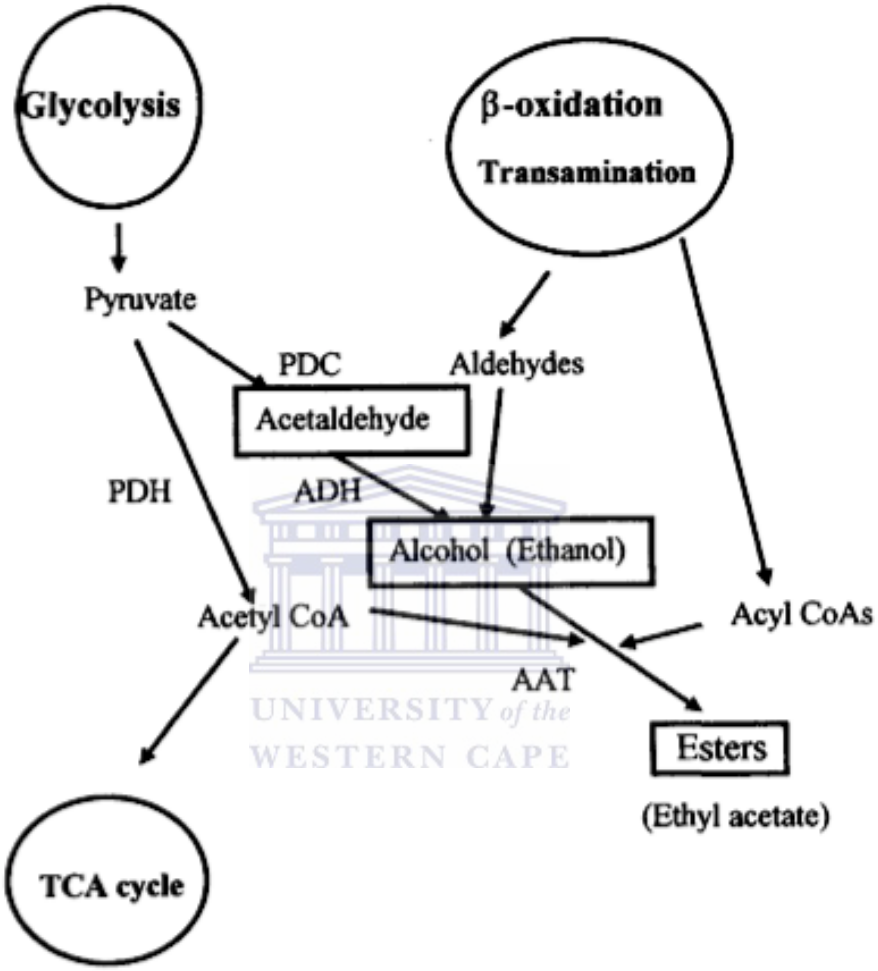


Figure 3. Anaerobic biosynthetic pathway for the formation of acetaldehyde, ethanol, and esters (adapted from Mathews & van Hold 1996). Highlighted text represents compounds that accumulate under hypoxic conditions. (PDH = pyruvate dehydrogenase, PDC = pyruvate decarboxylase, ADH = alcohol dehydrogenase, AAT = alcohol acyl CoA transferase, TCA = tri-carboxylic acid.) (Dixon and Hewett, 2000)

1.3.4.3 Effect of temperature

Volatile concentrations increase as temperature increases, but production is reduced above 32°C (Dixon and Hewett, 2000). Apples transferred to 20°C after a period at low temperature, produce higher concentrations of volatiles and reach maximum production quicker than freshly harvested apples. This trait is cultivar specific and results from an accumulation of volatile precursors in the fruit at low temperatures (Dixon and Hewett, 2000). Storage at low temperatures for more than three months reduced production and concentration of volatiles in apples (Ampun, 1997).

1.3.4.4 Other Flavour Determinants

Sugars and organic acids, along with cellulose and pectic substances, make up the edible portion of an apple. These substances vary among different cultivars, and depend on the local climate as well as the location of the particular tree in the orchard (Ackermann *et al.,* 1992).

Sugars

Sugar content influences the sensory quality of most, if not all fruit (Ackermann *et al.,* 1992). The most important sugars present include fructose, sucrose and glucose. The metabolism of these sugars, during development, is influenced by sorbitol concentration, present to a larger extent in the leaves than in the fruit

itself (Ackermann *et al.,* 1992; Wang *et al.,* 1999; Park *et al.,* 2002; Zhou *et al.,* 2003). Sorbitol accounts for approximately 80%of the total carbohydrates in apple leaves (Park *et al.,* 2002) and also plays a role in the metabolism of sugar accumulation during development (Ackermann *et al.,* 1992). Sucrose, fructose and glucose make up the other 20%.

Role of Sorbitol

Sorbitol is a sugar alcohol that is distributed among the woody members of Rosaceae, which represent some of the important fruit and nut crops, such as *Malus*, *Prunus*, *Pyrus*, *Eriobotrya* and *Rubus* (Touster and Shaw, 1962; Bieleski, 1982; Gao *et al.,* 2001). The true function of sorbitol is still unknown, but it is known to serve as a major photosynthetic product translocated from mature leaves to growing tissues such as fruits and young leaves in the woody Rosaceae (Zimmermann and Zeigler, 1975; Loescher, 1987; Park *et al.,* 2002).

Biosyntheisis and Breakdown of Sorbitol

The enzyme responsible for the biosynthesis of sorbitol in apple leaves is NADP-dependent sorbitol-6-phosphate dehydrogenase (S6PDH) (Park *et al.,* 2002). The activity of this enzyme increases gradually according to the transition from sink to source (Loescher *et al.,* 1982; Park *et al.,* 2002).

The enzyme involved in sorbitol breakdown is NAD-dependent sorbitol dehydrogenase (NAD-SDH) (Figure 4). This enzyme gradually decreases as the leaf develops (Papageorigiou and Murata, 1995) and it is therefore believed that these two enzymes are associated with sink to source transition. NAD-SDH also plays a role in the development of fruits. This enzyme increases in activity from early development to the late maturity stage (Touster and Shaw, 1962; Hirai. 1981; Tarczynski *et al.,* 1993; Gao *et al.,* 2001). This explains why NAD-SDH is more active than any other sorbitol metabolizing enzyme viz. sorbitol oxidase, S6PDH and NADP-dependent sorbitol dehydrogenase (Touster and Shaw, 1962).

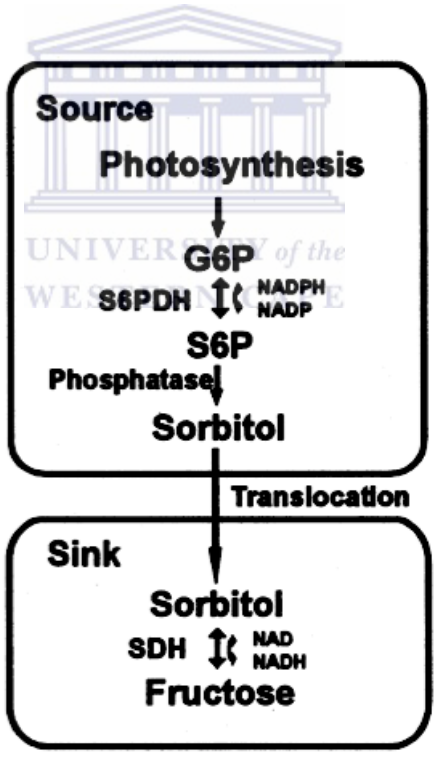


Figure 4. Partial metabolic pathway of sorbitol synthesis and degradation in Rosaceae. G6P, glucose-6-phosphate; S6PDH, sorbitol-6-phosphate dehydrogenase; S6P, sorbitol-6-phosphate; and SDH, sorbitol dehydrogenase (Gao *et al.,* 2001).

Acids

In apple, the predominant factor of variation inflavour is the balance between sugars and acids. Of all the acids present in apple fruits, Malic acid, constitutes the greatest percentage, approximately 90 percent (Ackermann *et al.,* 1992). Citric and succinic acids make up the rest. Malic acid constitutes most of the acid present in apple fruits, and contributes to a sour, acidic taste, especially if very little sucrose is detected (Wismer *et al.,* 2005). In 1998, Maliepaard *et al*. reported the position of the major gene for malic acid, *Ma*, on LG16 of 'Prima' x 'Fiesta' mapping population. The amount of acid present in a particular fruit varies among different cultivars, but it also depends on whether fruit are harvested too early (Harker *et al.,* 2003). To some extent, the taste of the apple depends on the absolute level of acids present, and not just the relative proportions of the different acids.

1.3.5 Ethylene-related genes

One of the important role players in fruit flavour composition is the hormone ethylene that has been shown to influence the physiology and biochemistry of tomato via the expression of specific genes involved in ripening (Theologis, 1994; Fluhr and Mattoo, 1996; Ciardi and Klee, 2001; Giovannoni, 2001). In apple, the exponential increase in ethylene production coincides with a rise in respiration and correlates with the development of fruit flavour composition (Yang and Hoffmann, 1984; Knee, 1993; Dandekar *et al.,* 2004).

Dandekar *et al*., (2004) showed successful silencing of the genes involved in ethylene biosynthesis viz. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). During ripening, the expression of ACO and ACS genes and the activity of their encoded enzymes govern the rate of ethylene production. The fruits produced from these transgenic trees were shown to have a significant increase in firmness and an extended shelf life and thus improving fruit quality.

1.4 APPLE GENETICS

For many years farmers have relied on the more traditional and conventional methods of breeding. Kumar, (1999) summarises it as crossing the genomes and allowing the population to express its phenotypic traits and then selecting the superior or desired recombinants from the several segregation products. Several crosses and several generations need to be produced for a successful selection and this is extremely tedious, time consuming and costly. In addition, there might be a tight linkage of the desirable loci with the undesirable loci; therefore producing the desirable outcome is difficult.

In the case of apples, which have a very long juvenile period (3-10 years), this problem is further worsened, as certain assessments can only be done after this period (Janick *et al*., 1996). In addition to the long juvenile phase, apples also pose the problem of being self-incompatible due to the arrested development of

the pollen tubes controlled by S-alleles. Today breeders have adopted modern-biotechnological driven methods to facilitate this process. There are two main streams available i.e. the transgenic method and the marker-assisted selection/breeding (MAS/MAB) method. Due to consumer demand, breeders tend to rely on the "safer" marker-assisted selection. In contrast to transgenics, MAS utilises the already present genetic characteristics of a plant to produce their desirable phenotypic characteristics.

In the late 20th century, the development of various molecular marker techniques led to an increase in molecular marker research. The aim of this research was often to construct a genetic linkage map. Ideally, a linkage map should include molecular markers linked to traits important to breeders. Characteristics of choice include disease resistance, fruit quality, low temperature tolerance and early budbreak.

Genetic studies concentrate on a specific locus that affects a trait of interest. Once the genetic map is constructed, fine mapping usually follows with the identification of markers closely linked to the target locus. Scab resistance controlled by the V*f* gene, derived from *Malus floribunda* 821, is one such trait (Xu and Korban, 2002; Xu and Korban 2004; Silfverberg-Dilworth *et al.,* 2005)

The main reason for breeding is to continue to develop and improve superior breeding families to enable genetic advancement through successive generations (Labuschagné *et al*., 2003) i.e. to develop better apples. Breeding also allows

breeders to develop and choose crops displaying favourable phenotypic traits over those that do not.

Another important consideration when breeding crops is to eliminate the effects of pathogens and diseases that destroy the production of top quality crops. In apple, there are many problematic diseases which threaten production in most parts of the world viz. apple scab (*Venturia inaequalis*) and apple powdery mildew *(Podosphaera leucotrichia*), fireblight and invasion by pests such as Wooly Apple Aphid and Codling moth. Producers are currently compelled to use chemicals against these pathogens. This use of chemicals causes many problems in the commercial food market, since consumers tend to favour unprocessed fruits of high quality and free of chemical residues (White, 2000).

1.4.1 Traditional Breeding

Traditional breeding involves the choosing of parents on the basis of the desirable traits they contain and the knowledge of which traits are likely to be passed on to their progeny. Emphasis is placed on obtaining a hybrid, containing a combination of desirable traits from the parents, but also minimizing any undesirable traits they might inherit.

When two parents are crossed, the two genomes combine to form a new combination, containing 50% of each parents' characteristics. Seedlings/ hybrids

are then observed to identify those with the desired combination of characteristics. This can take several years, depending on the characteristic/ trait of interest.

This form of breeding, in apples, is hindered by its long generation time, which is about 3 to 10 years. There have been many attempts to shorten the juvenility period by inducing seedlings to flower early (Janick *et al.,* 1996). However, many attempts to interfere with seedling growth have led to an increase juvenility time. To an extent, this has been overcome by grafting onto M9 rootstocks, which showed a reduction in seedling growth as well as a shorter juvenile period.

1.4.2 Estimation Of Heritability

The extent and the nature of the genotypic versus the non-genotypic variation in traits determines whether or not there is progress in the breeding programme. It is therefore important to understand the pattern of inheritance of the traits in question to devise effective breeding strategies (Hauagge and Cummins, 1991b). The relationships among genetic traits affecting physiological processes can therefore be investigated among families, within families or within individuals propagated vegetatively as clones (Kester *et al.,* 1977). Heritability of traits is therefore dependent on the separation of the variance among the breeding stock phenotypes, σ^2_P, into genetic (σ^2_G) and environmental (σ^2_E) variance/components i.e. σ^2_G can be written as the sum of σ^2_G and σ^2_E. Therefore, $\sigma^2_P = \sigma^2_G + \sigma^2_E$ (Wright, 1921).

These variance components can be easily determined in fruit breeding programmes. σ^2_P is estimated as the phenotypic variance among individuals, σ^2_E, the variance between clones of a common genotype and σ^2_G, as the variance components between clones or by subtraction of σ^2_G from σ^2_P. The importance of genetic and environmental causes of variation can also be estimated by calculation of the ratio of genetic and environmental variances, i.e. σ^2_G/σ^2_P. This is also known as the "heritability of broad sense" or the maximum value of heritability. This definition of heritability states that the additive and non-additive components of genetic variance are inseparable. To calculate this additive component, σ^2_A, one requires an experimental design that allows for estimation of co-variance between half-sibs or parents of offspring (Falconer and McKay, 1996) leading to "heritability in the narrow sense", σ^2_A/σ^2_P, but high heritability estimates indicate that selection should be effective. The most important function of heritability is its role in selection and expressing the reliability of the phenotypic value as a breeding value.

1.4.3 Modern / Advanced Breeding Techniques

Traditional breeding techniques are being revolutionized by advanced biotechnology techniques that complement conventional breeding approaches. DNA markers are unique sequences found distributed throughout the plant, animal and human genome. These markers are used to identify and locate linked DNA polymorphisms on the genome. Molecular marker techniques that generate genomic DNA fingerprints were developed in the last two decades of the 20th

century. Breeders using markers proven to be linked to the genes of interest, can now select seedlings with specific genes. Marker-assisted breeding would potentially save time and money on seedlings that would usually be planted out in the field and discarded at a later stage (Gardiner *et al.,* 1998). Previously, Randomly Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length polymorphism (AFLPs) and Restriction Fragment Length Polymorphisms (RFLPs) were used to produce more dense genetic maps, while more recently microsatellites or simple sequence repeats (SSRs) (Maliepaard *et al.,* 1998; King *et al.,* 2001; Liebhard *et al*., 2002; 2003; Silverberg-Dilworth *et al*., 2006; Kenis *et al*., 2008, van Dyk *et al*., 2010) and Diversity Array Technology (DArT) (Schouten *et al*. -in press) markers have been used to generate these maps. These technologies utilise the polymerase chain reaction (PCR) (Mullis, 1990) technique. Since these technologies are not influenced by the environment and are detectable at all stages in the plant's growth, they are extremely reliable (Mohan *et al*., 1997).

The advantages and disadvantages of the different markers have been compared, and are summarized in Table 3.

Table 3. Comparison of molecular marker systems (Breyne *et al.,* 1997).

	RAPD	RFLP	SSR	AFLP
Principle	Random PCR amplification of genomic region	PCR amplification restriction digestion	PCR amplification of microsatellites	Restriction digestion, adaptor annealing, selective PCR
Nature of Polymorphism	Base changes, insertions deletions	Base changes, insertions deletions	Variation in repeat length	Base changes, insertions, deletions
Level of Polymorphism	Medium	Medium	Very high	Medium
Abundance	Very high	High	Medium	Very high
Dominance	Dominant	Co-dominant	Co-dominant	Mixed
Multiplex ratio	5 – 20	1	1	50 – 100
Sequence information required	No	Yes	Yes	No
Costs	Low	High	High	Medium

1.4.4 Markers and Genetic Mapping

The advent of molecular marker techniques triggered research on apple towards the genetic-mapping. Genetic linkage maps are useful in many areas of genetics, e.g. quantitative trait loci (QTL) analysis, marker-assisted selection (Jansen *et al.,* 2001) and map-based cloning of genes (Liebhard *et al.,* 2002). The main goal of the genetic mapping projects worldwide was to construct linkage maps comprising of molecular markers as well as genes governing characters of importance to breeders. These characters include resistance to scab, powdery mildew and woolly apple aphid, and more complex traits such as low temperature tolerance, early budbreak, fruit quality and also rootstock influences such as dwarfing. To obtain these genetic maps, increasing numbers of molecular genetic markers e.g. Simple sequence repeats and Amplified fragment length polymorphisms are genotyped on various apple cultivars and mapping populations (Table 3).

To map QTLs successfully, a saturated reference genetic map from which regularly spaced markers can be selected is essential. Large gaps between markers on the linkage group or missing (unmapped) chromosome segments can lead to inaccurate analyses (Liebhard *et al.,* 2002). Such maps are becoming increasingly available for woody perennials like *Malus* (Hemmat *et al.,* 1994; Conner *et al.,* 1997; Seglias and Gessler, 1997; Maliepaard *et al.,* 1998; King *et al.,* 2001; Liebhard *et al.,* 2002; Liebhard *et al.,* 2003; Silfverberg-Dilworth *et al.,* 2006, Celton *et al*., 2008; van Dyk *et al*., 2010).

1.4.3.1 Molecular markers

Since the structure of DNA was deciphered (Watson and Crick, 1953), the study of DNA variation emerged as a field of scientific endeavour only in the last 25 years. Throughout this time two groups of technologies were developing in parallel: DNA sequencing (Maxam and Gilbert, 1977; Sanger *et al*., 1977) and molecular markers. Both these techniques enabled the field of genomics (Wenzl *et al*., 2004).

Molecular marker techniques developed rapidly and progressed from techniques like Southern blotting from which Botsein *et al*. (1980) developed the RFLP technique as a method for generating genetic maps. The development of PCR techniques then gave rise to techniques such as AFLPs (Vos *et al*., 1995) and simple sequence repeats (SSRs) (Weber and May, 1989). The merging of DNA sequencing and molecular markers then gave rise to the analysis of single nucleotide polyphorphisms (SNP), as more and more sequence data started producing information on sequence variation among different accessions. These SNPs were quickly identified as the most abundant marker type, promising an unlimited number of markers. Variations on this SNP array technology then followed, as it laid the foundation for technologies such as DNA chips, MALDI-TOF and self-assembling arrays, that allows for high throughput typing of these markers.

1.4.3.2 Simple sequence repeats (SSR)

Simple sequence repeats (SSRs) are short stretches of DNA, consisting of

tandemly repeated nucleotide units, which are 1-6 nucleotides in length. They are the preferred markers, used worldwide in mapping studies, due to them being highly polymorphic, co-dominant (making them highly informative) and present in most eukaryote genomes. SSRs were first used in 1989 (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989) and these are also PCR based. Each SSR locus has a unique set of primers designed from the conserved, flanking regions of the microsatellite, making them easily reproducible.

SSRs are easily transferred to other apple progenies and can also be transferred across genera. In other words, apple SSRs are not only used between different apple cultivars, they can be successfully used in pear (*Pyrus*) cultivars as well (Yamamoto *et al*., 2001). Yamamoto *et al*., (2002a) successfully mapped apple SSRs on a pear cross and more recently, Celton *et al*. (2008) and van Dyk *et al*. (2010) reported successful mapping of pear SSRs on various apple crosses. Not only were apple SSRs mapped, but peach and cherry (*Prunus*) SSRs as well. Pear and apple, however, belong to same subfamily of *Maloideae,* but the *Prunus* genus belongs to a different subfamily viz. *Prunoideae*. Therefore, due to this difference, Yamamoto *et al*., (2002a) suggest this transfer of SSRs is less common and more difficult.

1.4.3.3 Diversity Array Technolgy (DArT)

Diversity array technology (DArT) (Wenzl *et al*., 2004) is one such technology that enables whole genome profiling of species without the need for sequence

information. DArT is based on microarray hybridizations that detect the presence versus absence of individual fragments in genomic representations as described by Jaccoud *et al*., (2001).

DArT loci are therefore scored as binary characters and must be treated as dominant markers and this limits the genetic information provided by a given locus. It does, however, generate the highest throughput genotyping available, scoring hundreds of polymorphic markers across the genome in a single assay (Jaccoud *et al.,* 2001; Wenzl *et al.,* 2004). In 2004, Wenzl *et al*. generated a DArT map in barley for a cross between 'Steptoe' and 'Morex' cultivars. This map comprised 385 DArT markers and spanned 1137cM. More recently van Dyk *et al.* (personal communication), generated a genetic map consisting of ≈ 240 SSR and ≈ 550 DArT markers for a cross between 'Golden Delicious' and 'Anna' apple cultivars. These two maps show that DArT markers can be used, not only to generate medium density genetic maps, but also to saturate maps consisting of other DNA markers (van Dyk *et al.* (personal communication)).

1.4.3.3 Current apple genetic linkage maps

In the last decade of the twentieth century, genetic maps in apple have been constructed by Weeden *et al.* (1994); Conner *et al.* (1997); Seglias and Gessler (1997); Maliepaard *et al.* (1998); Liebhard *et al.* (2002); Liebhard *et al*. (2003); Fernandez-Fernandez *et al*., (2008); Celton *et al*., (2008); and Van Dyk *et al*., (2010).

In 2002 Liebhard *et al.* constructed an apple genetic linkage map on the basis of a segregating population of the cross between the cultivars 'Fiesta' and 'Discovery'. Using a total of 214 RAPDs, 115 SSRs, 1 SCAR and 475 AFLPs. They then proceeded to saturate the genetic map consisting of 840 markers comprised of 235 RAPDs, 129 SSRs, 1 SCAR and 475 AFLPs (Liebhard *et al.,* 2003). Silfverberg–Dilworth *et al*. (2006) then developed a new set of 148 microsatellite markers and mapped these on the existing 'Fiesta' x 'Discovery' reference linkage map. It is the most advanced linkage map, in apple, with regard to genome coverage and marker density. The genetic map represents an ideal starting point for future mapping projects in *Malus* since the stable and transferable SSR frame of the map can be saturated quickly with dominant AFLP and DArT markers.

1.4.5 Marker-assisted selection

Marker-assisted selection allows an accurate screening of seedlings many years before the traits can be evaluated in the field. This procedure not only allows for the possible accumulation of different resistance factors in a genotype of interest, but also shortens the number of generations needed to recover the genotype of the cultivated species after a cross with an exotic genotype or wild species (Dirlewanger *et al.,* 2004). This technique results in saving of time and space, two factors important to woody perennials such as *Malus* sp.

1.5 QUANTITATIVE TRAIT LOCI (QTLs)

Most variation occurring within populations or between lines or breeds is quantitative in nature. The variation that occurs between individuals does not fall into discrete classes in Mendelian proportions but is continuous, showing a gradation from one extreme to the other (Haley and Andersson, 1997). This lack of discrete phenotypic segregation has all but prevented the use of classical Mendelian techniques for studying polygenetic traits. Earlier this century a subspecialty of genetics viz. quantitative genetics emerged, to deal with quantitative traits. This approach relied upon statistics to study the characteristics of continuous phenotypic distribution, and allowed several things to be estimated. Of these, the approximation of the number of loci affecting the character in a particular mating, the average gene action and the degree to which the various polygenes interact with each other and the environment in determining the phenotype are the most important.

In the early 20th century, the linkage of a single gene controlling one character with one or more of the polygenes controlling another character was reported. Since then, the analysis of quantitative trait loci using molecular markers has become routine in genetic studies of plant and animal species (Maliepaard *et al.,* 2001; Tanksley, 1993; Haley, 1995; Doerge *et al.,* 1997; Hoechele *et al.,* 1997; Kearsey and Farquhar, 1998).

1.5.1 Definition Of A QTL

Macromutations found in genetic stocks occur very seldom in natural populations. If they do, they are weeded out by natural selection. Even though the occurrence of these mutations is relatively low, there is no lack of phenotypic or genetic variation within the population. However, the phenotypic variation is usually continuous, instead of discrete and conditioned by allelic variation at several genetic loci, each with a relatively small effect (Tanksley, 1993).

A quantitative trait locus or polygene can therefore be regarded as a polymorphic locus that contains alleles that differentially affect the expression of a continuously distributed phenotypic effect. Usually, it is a marker described by statistical association to quantitative variation in the particular phenotypic trait that is controlled by the cumulative action of alleles at multiple loci.

1.5.2 Characterisation Of Polygenic Traits

Polygenic traits can be characterised by estimating the number of QTLs present and by determining the magnitude of the effects exerted on a certain character.

1.5.2.1 Number of polygenes

It is quite straightforward to estimate the number of polygenes, using molecular marker approaches, but even this is not without limitations (Sasaki and Yano, 1997; Serono *et al.,* 1998). In this approach, the number of QTLs detected in a particular study is added up to give a value, which is an estimate of the number of segregating polygenes affecting a particular character in a population. Probably the most important limitation of the molecular marker approach is that underestimation of the number of polygenes can occur. This occurs when only the genes with a large enough phenotypic effect to be detected statistically, are counted. Genes that do not affect the phenotype as much, fall below the threshold of detection depending on the size of the segregating population. These "lesser" genes are more likely to be detected statistically if a large segregating population is studied. A certain LOD score is the score that describes the statistical likelihood of the individual QTL it relates to. LOD scores above the threshold value, usually 3, are indicated as significant, whereas those less than 3 are considered non-significant. This approach is biased towards detection of larger phenotypic effects (Lynch and Walsh, 1998). Underestimation of the number of genes also occurs when two or more polygenes closer than 20 centi-Morgans (cM) appear as a single QTL. They therefore cannot be easily distinguished as separate genes (Nelson *et al.,* 1995; Yunbi, 1995).

1.5.2.2 Effect of QTLs

The magnitude of the effects exerted on a character by different polygenes is usually different for each gene. QTLs with major effects have been identified for most characters studied, but most QTLs reported are those of small effects. It is therefore unlikely that one will ever detect and characterize all polygenes affecting a segregating population, due to the bias towards detecting QTLs with larger effects. The smallest effect a QTL can have and still be detected by the molecular marker depends on a number of factors, viz.

i) Map distance from the nearest marker to the QTL.

The closer it is to a marker, the more likely a QTL with a smaller effect will still be detected statistically. This is because the effects of the QTL closer to the marker will not be interfered with by recombination events occurring between the marker and the QTL.

ii) The size of the segregating population.

The larger the population size, the more likely the effects of lesser QTLs will reach statistical significance.

iii) Heritability of the trait.

The larger the environmental effects on a particular character, the less likely are QTLs affecting the trait to be detected, since heritability is lowered.

iv) Probability criteria used to declare a QTL effect significant.

If probability criteria (LOD scores) are set too high, this will reduce the chances of the QTL being reported.

From a plant breeder's perspective, the fact that only QTLs contributing most toward the phenotype are detected is not a major problem, since it is these QTLs that are of greatest interest to them. They are therefore not concerned if QTLs contributing lesser effects are not detected.

1.5.3 Detection And Mapping Of Qtls

Detection and mapping of QTLs is important for many reasons. It allows insight into actions and interactions of individual genes, at a molecular level. This in turn allows a more realistic modeling of phenotypic variation, responses to selection and evolutionary processes. These models not only augment our understanding of trait variation in humans and our ability to predict breeding values, but also allow us to implement selection on plant and livestock species (Haley and Andersson, 1997).

Mapping of a QTL opens the door to positional cloning of genes. This will then allow for the study of molecular causes of existing variation. It may also allow improved alleles to be produced by direct molecular intervention, for use in plant or animal breeding programs (Haley and Andersson, 1997).

Very fine mapping of major genes can be performed, using a random population sample, when the amount of disequilibrium between tightly linked markers is generated by random drift, in small populations. This approach is known as linkage disequilibrium (LD) (Lynch and Walsh, 1998). LD works relatively well if individuals displaying the trait are traced to a single allele at a single locus. If a trait is influenced by multiple loci, marker associations will be obscured. So, given its extreme sensitivity to allelic heterogeneity, it is unlikely that LD mapping can be applied to QTLs of small to moderate effects.

1.5.4 QTL Analysis

1.5.4.1 Single Point Analysis

Analysing data using one marker at a time does not require a complete molecular linkage map. This is the simplest approach for detecting QTLs and is known as single point analysis. There are advantages and disadvantages of this approach. The first disadvantage is that the further away a QTL is from a marker gene, the less likely it is to be detected statistically due to crossing over events between the marker and the QTL. This results in an inaccurate classification. Secondly, the magnitude of the effect of a QTL will almost certainly be underestimated. This is also due to recombination between the marker and the QTL.

Increasing the number of the segregating molecular markers used, to cover the entire genome, minimizes these disadvantages. Marker intervals should be less than 15 cM. This distance allows any potential QTLs to be linked to at least one marker (Jansen, 1994).

1.5.4.2 Interval analysis (IM)

Since the advent of molecular linkage maps, covering the entire genome, it has become possible to overcome the problems associated with single point analysis. Interval analysis is preferred, because sets of markers can now be used and analysed to determine their effect on quantitative traits, rather than a single marker at a time. Any recombination occurring between the markers and the QTLs is compensated for, when using interval analysis. An unbiased estimate of the effect of the QTL on the character is therefore provided. This increases the chance of a QTL being statistically detected. As opposed to point analysis, where markers cannot be spaced more than 15cM apart, interval analysis allows markers to be spaced more than 20cM apart. If markers are spaced more than 35cM apart, even interval analysis will be inefficient in detecting QTLs between the marker loci (Jansen, 1994).

1.5.4.3 Molecular marker-QTL linkage

This method of analysis involves testing DNA markers, throughout the genome, for the likelihood they are associated with a QTL (Beer *et al.,* 1997). This approach has taken off, since the explosion of DNA marker techniques in the late 20th century. Since then more and more DNA markers have been mapped throughout many genomes using computer software programs such as QTL-Mapmaker (Castiglioli *et al.,* 1998) and MapQTL (Van Ooijen and Maliepaard 1996).

Detecting QTLs using molecular markers normally requires a large segregating population (> 100 individuals), but because not all species produce offspring in such large numbers, alternative approaches have to be used. One such approach is the Half-sib analysis approach, which is used in livestock. Half-sibs arise when a single individual is mated to random individuals of a population. If the original individual in the mating is heterozygous for both markers and QTL, the linkage can be detected by analyzing a Half-sib population (Lynch and Walsh, 1998).

1.5.4.4 Nonparametric Mapping (Kruskal Wallis Analysis)

The Kruskal-Wallis (KW) test is regarded as the nonparametric equivalent of the one-way analysis of variance (Van Ooijen *et al*., 1993). The test gives all individuals a ranking according to the quantitative trait, while it classifies them according to their marker genotype. A segregating QTL (with a large effect) that

is closely linked to the tested marker will result in large differences in average rank of the marker genotype classes. A test statistic based on the ranks in the genotype classes is calculated. This test is generally performed on both linked and unlinked loci, and for this reason, it is important that a stringent significance (P-value) be used for the individual tests. Van Ooijen, (2004), recommends a significance level of 0.005, to obtain the overall significance of about 0.05.

1.5.4.5 Multiple QTL Model Mapping (MQM Mapping)

The MQM mapping method, developed by Jansen (1993, 1994) and Jansen and Stam (1994), can be used to locate markers and the multiple QTLs associated around these markers. Currently, MapQTL® versions 5.0 and 6.0 (Van Ooijen, 2004, 2008) only allows for markers to be used as co-factors to approximate the multiple-QTL model with additive and dominant gene actions only. To use MQM mapping effectively to detect and map QTLs requires a multidimensional search over the linkage groups, which cannot be performed without the necessary computational power (Van Ooijen, 2004).

With this MQM mapping a one-dimensional search over the genome is done by testing for a single segregating QTL as in interval mapping, while simultaneously fitting the selected cofactors and these cofactors will reduce the residual variance (Van Ooijen, 2004). If a QTL explains a large proportion of the total variance, then the use of a linked marker as cofactor in subsequent MQM mapping will importantly enhance the power in the search for other segregating QTLs.

1.5.5 Statistical Approach For Determination Of Linkage

Most statistical procedures for determining linkage between a polygene and a marker follow the same basic approach.

(i) Partitioning of the segregating population into different genotypic classes based on genotypes at a marker locus.

(ii) Using correlative statistics to determine whether individuals in the different genotypic classes differ from each other with respect to the trait being measured. If phenotypic means between the different genotypic classes are significantly different, it means that the particular trait is linked to the molecular marker locus used to subdivide the population.

(iii) Repeat the procedures for additional marker loci, to detect as many QTLs as possible.

Usually, it is not possible to determine whether the effect detected is due to one or more linked genes affecting the trait (Young, 1996).

1.5.6 QTL and gene mapping in apple

Various research groups worldwide are actively involoved in the mapping of QTLs and the identification of specific genes responsible for economically important fruit traits. These include groups forming part of the European projects

ISAFRUIT, 'Durable Apple Resistance in Europe' (DARE), 'High Quality Disease Resistant Apples for a Sustainable Agriculture' (HIDRAS) (Gianfranceschi and Soglio, 2004), Plant and Food Research (PFR) from New Zealand and the Fruit Tree Genetics group from South Africa. QTLs have been detected for various apple traits e. g. resistance to various apple diseases viz. powdery mildew (Kellerhals *et al*. 2000; Calenge and Durel, 2006**),** apple scab (Durel *et al*., 2003; Liebhard *et al*., 2003; Calenge *et al*., 2004) and fire blight (Calenge *et al*., 2005; Khan *et al*., 2006); tree growth and development (Lawson *et al*., 1995; Conner *et al*., 1998; Liebhard *et al*., 2003); time of budbreak (van Dyk *et al*., 2010) and fruit quality (King *et al*., 2000, King *et al*., 2001; Liebhard *et al*., 2003; Kenis *et al*., 2008) (Table 4).

Although a number of QTLs and candidate genes (Table 5) have been identified, the larger number of QTLs suggests that, for most of the traits for which candidate genes have been mapped, there are more genes playing a role in the determination of the expression of the trait in the seedlings. Candidate genes have been identified for many fruit quality traits including genes for malic acid (Ma) (Liebhard *et al*., 2003), fruit softening (Md-EXP7) (Costa *et al*., 2008) and ethylene production (Md-ACO1 and Md-ACS1) (Table 5). QTLs have also been identified for many important traits for which genes have yet to be mapped. The identification of new QTLs, in additional to known and mapped candidate genes, are the first step towards unraveling complex traits into all the contributing genetic factors. The next step will be the identification of markers that can be

linked to these QTLs and that can be used in MAS in breeding programs, where the ultimate goal is the pyramiding of favourable genes.

Table 4. Summary of linkage groups (LG's) on which QTLs have been identified for a variety of phenotypic traits in apple. (This table was constructed using information gathered from various sources(Conner *et al*., 1998; Dunemann *et al*., 1999; Kellerhals *et al*., 2000; King et al., 2000; King *et al*., 2001; Durel *et al*., 2003; Liebhard *et al*., 2003a; Liebhard *et al*., 2003b; Calenge *et al*., 2004; Calenge *et al*., 2005a; Calenge *et al*., 2005b; Stankiewicz-Kosyl *et al*., 2005; Calenge and Durel, 2006; Durel *et al*., 2006; Khan *et al*., 2007; Peil *et al*., 2007; Kenis *et al*., 2008; Van Dyk *et al*., 2010)

TRAIT	LINKAGE GROUPS																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
QTLS IDENTIFIED																	
Scab resistance	X	X	X		X					X	X	X			X		X
Fire blight resistance			X		X		X					X	X				
Powdery mildew resist.		X						X		X		X	X				X
Fruit harvest date			X														
Fruit flesh firmness*	X					X		X		X	X	X		X			
Fruit weight*	X		X	X		X		X		X		X			X	X	X
Fruit acidity*		X						X		X			X		X	X	
Sugar content*			X			X		X	X					X			
Number of fruit					X										X	X	
Fruit sensory descriptors*	X		X	X		X	X	X				X	X		X	X	
Blooming time							X			X							X
Leaf size									X								X
Height increment			X		X			X			X		X				X
Juvenile phase length			X												X		
Number of bunches								X							X		
Stem diameter	X	X	X					X			X		X	X	X		X
Time of budbreak									X								
Fruit diameter*										X							X
Rate of Browning*			X														X
Fruit height			X						X	X						X	

* QTLs relating to fruit quality

Table 5. Summary of linkage groups (LG's) on which genes have been mapped for a variety of phenotypic traits in apple. This table was constructed using information gathered from various sources (Weeden *et al*., 1994; Seglias and Gessler, 1997; Maliepaard *et al*., 1998; Cevik and King, 2002; Hemmat *et al*., 2002; Liebhard *et al*., 2003; Bus *et al*., 2004; Gygax *et al*., 2004; James *et al*., 2004; James and Evans, 2004; Patocchi *et al*., 2004; Tartarini *et al*., 2004; Vinatzer *et al*., 2004; Bus *et al*., 2005a; Bus *et al*., 2005b; Costa *et al*., 2005; Gao *et al*., 2005; Patocchi *et al*., 2005; Celton *et al*., 2006; Durel *et al*., 2006; Freslon *et al*., 2006; Lesemann and Dunemann, 2006; Peil *et al*., 2007; Chagné *et al*., 2007; Bus *et al*.,, 2008; Costa *et al*., 2008; Yao *et al*., 2008)

TRAIT	LINKAGE GROUP																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
CANDIDATE GENES																	
Major Scab resistance	Vf Va	Vr2 Vh2 Vh4 Vh8 Vbj Vt5				Vdr1		Vfh		Vd		Vg Vb					Vm
Powdery Mildew resistance								Plw			Pl2	Pld Pl1					
Wooly apple aphid resistance								Er1 Er3									
Rosy leaf curly aphid resistance							Sd1 Sd2										
Rosy apple aphid resistance								Dpfl									
Malic acid- fruit acidity																Ma	
Fruit skin colour						MdF3’ HII			Rf					MdF3’ HI			
Self incompatibility																	SI

Non-specific lipid transfer protein		Mal d4	Mal d3		Mal d1			Mal d4	Mal d2 Mal d4			Mal d3	Mal d1			Mal d1	
Ethylene production										Md-ACO1					Md ACS1		
Rootstock formation																	Rs
Columnar growth										Co							
Dwarfing					Dw												
Fruit softening	Md Exp7																
Red flesh and foliage									Md-MyB10								

1.5.7 Apple Genome Sequencing

With one of the largest genomes in the Rosaceae family, apple (*Malus* spp), with a genome size of 750Mb per haploid complement (Shulaev *et al*., 2008), was at the centre of two independent sequencing strategies. The Istituto Agrario San Michele all'Adige, Trento (IASMA) chose the economically important cultivar, 'Golden Delicious', on which to carry out it's sequencing initiative (Velasco, 2009). The project was carried out by IASMA Myriad Genetics Inc., Amplicon and 454 Life Science, and was based on the integration of 4x coverage with Sanger sequencing and 12x coverage with 454 pyrosequencing. This is essentially made possible by the greater depth of sequencing, which is guaranteed by pyrosequencing, even though shorter read sequences are produced, then by the Sanger method (Ronaghi, 2001). Pyrosequencing also creates the possibility of applying pair-end sequencing approaches to short and long libraries, thus allowing partial substitution of fosmid and BAC clones. Using these sequencing technologies allowed for a 16.9x coverage of the genome, of which 26% was provided by Sanger dye primer sequencing of paired reads, and the remaining 74% was from 454 sequencing by synthesis of paired and unpaired reads. The assembly of the genome produced 122,146 contigs, 103,076 of which were assembled into 1,629 metacontigs. The total contig length (603.9 Mb) covers about 81.3% of the apple genome (Velasco *et al*., 2010). Velasco *et al*. (2010) performed pairwise comparisons of the chromosomes and reported regions of collinearity between regions of chromosomes 3 and 11, 5 and 10, 9 and 17, and 13 and 16, as well as between shorter fragments of chromosomes 1 and 7, 2 and 7, 2 and 15, 4 and 12, 12 and

14, 6 and 14 and 8 and 15. These regions, as well as remnants of older duplication events were also reported.

IASMA, together with INRA Angers (France), and HortResearch (New Zealand), supplied five apple progeny populations, allowing for the anchoring of the genomic scaffolds to the 17 linkage groups. This will ultimately produce a dense, reliable integrated molecular map, based on internationally shared microsatellites, as well as single nucleotide polymorphisms (SNPs), developed in 'Golden Delicious' (Velasco, 2009).

Complementary to this project, is the public initiative to sequence a double haploid (DH) selection derived from a 'Golden Delicious' variety provided by INRA Angers. This initiative is currently underway at Washington State University (WSU). The DH material is expected to simplify downstream genome assembly, due to its relatively simple genetic organization. Both these sequencing initiatives have joined into an International Program for Apple Sequencing, that also includes the INRA research institute as well as the University of the Western Cape (South Africa), who is currently generating sequences from DH material, using Illumina's Solexa technology.

1.6 OBJECTIVES OF THIS STUDY

The ojectives of this study is to, firstly, generate genetic linkage maps for 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x Priscilla progenies, which form part of the ARC apple breeding program, using

published and newly developed SSR markers. Secondly, highly polymorphic DArT markers will be implemented onto these maps in order to saturate them. The DArT markers, together with SSR markers will provide insight into the coverage of DArT markers in the apple genome. These maps will then be used to identify regions of the genome that contain putative QTLs for fruit quality. Phenotypic data recorded over a three-year period (2005, 2006 and 2007) during this study, for all fruit quality traits, was used during QTL analysis. Once QTLs are identified, the larger aim of linking SSR markers to the traits of interest, as well as the efficiency of these markers for use in MAS, will be determined. This will allow for MAS for components of good fruit quality, as a whole, to be applied to future progenies and thus improving the apple breeding program.

CHAPTER 2: MATERIALS AND METHODS

2.1 GENERAL CHEMICALS AND ENZYMES

Agarose D1 LE	Promega
APS (Ammonium persulphate)	Merck
Boric acid	Merck
Bromophenol blue	Sigma
CTAB (N-cetyl-NNN-trimethyl ammonium bromide)	Saarchem
Chloroform	BDH
dNTPs (Deoxyribonucleotide triphosphate)	ABgene
DTT (1,4 – Dithiothreitol)	Roche
Ethanol	Merck
EDTA (Ethylene diamine tetra-acetic acid)	Merck
Ethidium bromide	Sigma
Formamide	Merck
Gelatin	Merck
GeneScan® 500 LIZ™ standard	Applied Biosystems
Hydrochloric acid	BDH
Isoamyl alcohol	Merck
Iso-propyl alcohol	BDH
Magnesium chloride	Riedel-de Haën
Megaplex Kit	Qiagen
Oligonucleotides	Applied Biosystems
Polyvinyl-pyrolidone (PVP-40)	Sigma

POP 7	Applied Biosystems
Potassium chloride	Saarchem
Proteinase K solution	Applied Biosystems
RNase A	Roche
Sodium acetate	Riedel-de Haën
Sodium borohydride	Saarchem
Sodium chloride	Merck
Sodium hydroxide	BDH
Excel T*aq* polymerase®	Southern Cross
Tris (hydroxymethyl) aminomethane	Merck
Urea	Merck
Xylene cyanol	BDH

2.2 GENERAL STOCK SOLUTIONS AND BUFFERS

Agarose loading buffer	0.25 % (w/v) bromophenol blue, 0.25 % (w/v) xylene cyanol in 30% (v/v) glycerol in deionised water.
CIA (Chloroform-isoamyl alcohol)	24:1 (v/v) chloroform and isoamyl alcohol.
DTT	10 % (w/v) in deionised water.
PCR reagents	10x buffer: 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01 % gelatin, pH 8.3, in deionised water. $MgCl_2$: 50 mM in deionised water. dNTPs: 5 mM in deionised water.
Polyacrylamide loading buffer	80 % (v/v) formamide, 10 mM NaOH, 1 mM EDTA, 0.1 % (w/v) xylene cyanol, 0.1 % (w/v) bromophenol blue in deionised water.
RNase A buffer	0.1 M sodium acetate, 0.3 mM EDTA, pH 4.8.
RNase A (DNase free)	20 mg/ml RNase in RNase A buffer (see above).
Sodium Acetate	3 M NaOAc with 1 mM EDTA, pH 5.2.
2x CTAB	2 % (w/v) CTAB, 1% (w/v) PVP-40, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM

	EDTA, 1 mM DTT, pH 8.0
10x TBE	0.9 M Tris, 0.89 M boric acid, 0.032 M EDTA.
10x TE	100 mM Tris-HCl, 10 mM EDTA, pH 7.5.
1 % agarose	1 % (w/v) agarose in 1x TBE.
2 % agarose	2 % (w/v) agarose in 1x TBE

2.3 PHENOTYPIC DATA

2.3.1 Mapping Populations

Three mapping populations, situated at ARC's Drostersnes experimental farm in the Vyeboom area (34° 4' 15" S 19° 4' 47" E), viz. 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla' were used in the study. All three populations, grafted onto M793 rootstocks, were resistant to apple scab, as they were pre-screened in the greenhouse, before being planted in the orchard. Fruit from each seedling of the three progenies were harvested at weekly intervals for three harvests (2005-2007). These three seasons of data were sufficient for the study, and even though data from more seasons would be beneficial to the analyses, it was not possible due to the removal of trees in orchard planning. Fruit were considered mature and at an appropriate stage for harvest at 70% to 90% starch breakdown and eating ripeness. Seedling trees were labelled as row number, and position in the row (i.e. seedling 3-124, refers to row 3, and tree number 124) and were planted one metre apart.

Table 6. Number of seedlings form each apple mapping population used in this study, for construction of genetic linkage maps

Mapping Population	No. of seedlings
'Prima' x 'Anna'	87
'Golden Delicious' x 'Anna'	141
'Golden Delicious' x 'Priscilla'	94

2.3.2 Assessment of phenotypic traits

Visual, sensory and instrumental analyses were performed on a sample of five fruits from each seedling tree. Apples were considered ready to be picked when they came off the tree when gently twisted and when the seeds had a dark brown colour. Fruit were also tasted to estimate starch-sugar conversion. The first instrumental evaluation was performed on the same day as harvest and the second after twelve weeks at cold storage (-0.5°C) and seven days at room temperature, using both non-destructive (fruit mass, diameter, colour) and destructive (firmness, % total soluble solids) procedures (Kenis *et al*., 2008).

Fruit mass was measured using a scale, while diameter was measured using electronic calipers. Colour changes were documented over the duration of the experiment. L* values indicate lightness (black [L* = 0] and white [L* = 100]), a* values indicate redness-greenness (red [a* = 100] and green [a*= {-100}]), b* values indicate yellow-ness-blueness (yellow [b* = 100] and blue [b* ={100}]). Chroma (C) (C = [(a*)2 + (b*)2]0.5) measures colour saturation or intensity and the hue angle (h = arc tan b*/a*) determines the red, yellow, green, blue, purple, or intermediate colors between adjacent pairs of these basic colours (Ayala-Silva *et al*., 2005). Colour measurements were determined using a colorimeter (Minolta Chroma Meter CR 400, Osaka, Japan) (Figure 5).

Fruit firmness was determined, as the maximum force required pushing a 7 mm-diameter probe with a convex tip into the flesh after peeling an equatorial site on a sample of five fruits per tree. Firmness measurements were performed using a

motorized penetrometer (Figure 5) (Gus Instruments, Bien Donne, Paarl, South Africa). % Total soluble solids were measured when a drop of juice, squeezed from the fruit, was analysed using a digital refractometer.

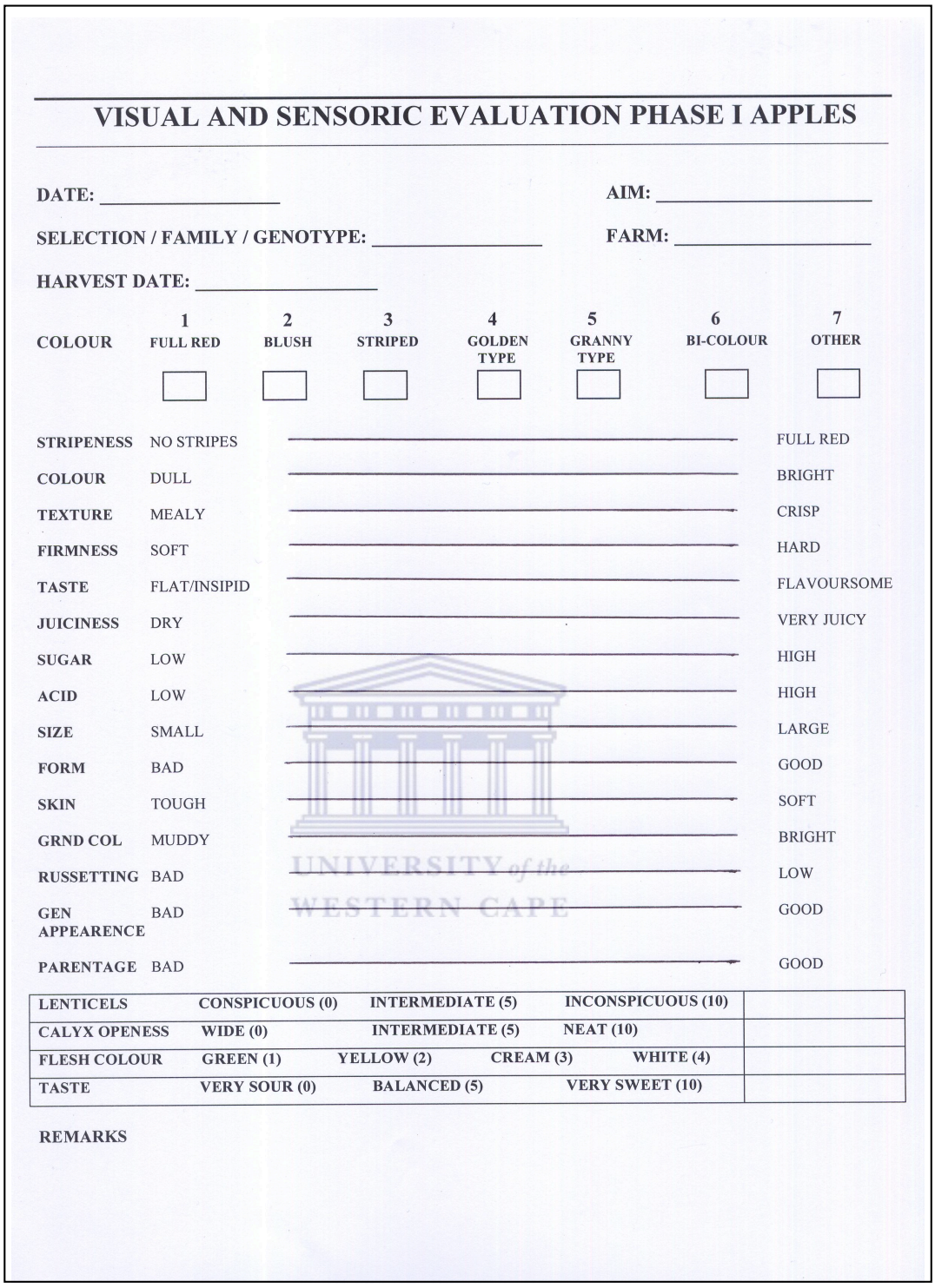
Fruit traits were also analysed subjectively, and these include stripe-ness, colour, texture, firmness, taste, juiciness, sugar, acidity, size, skin toughness, ground colour and russetting. Fruits were evaluated using a 10cm line scale from low to high as described by Heintz and Kader (1983) (Figure 6). General appearance, parentage, lenticels, calyx openness, flesh colour and taste (lower end of form) were not evaluated in this study. All traits were given a rating of 0 to 100%. Fruits scored for stripe-ness were ranged from those having no stripes to those completely covered in stripes. Fruit colour was visually measured from 0, for dull fruits, to 100%, for very brightly-coloured fruit. When scoring fruit size and form, small and irregular shaped fruit were given low scores and large and round, regular shaped fruit were allocated higher scores. Ground colour was measured by allocating lower scores to muddy fruit and higher scores to fruit with brighter ground colours. Fruit with a high russet coverage were scored considerably lower than those with very little or no russet.

The sensory traits measured, viz. texture, firmness, fruit taste/flavour, juiciness, skin toughness, sweetness (sugar content) and acidity were all measured by tasting pieces of fruit from each seedling. Texture was given a rating from 0, for mealy fruit, to 100% for crispy fruit. Sweetness and acidity were given a scale from low to high, describing ‘how sweet’ or ‘how sour’ the fruit is. Fruit firmness was rated form soft to hard, and juiciness, although given a rating from

dry to very juicy, was considered dry when fruits were mealy and juicy when fruits were crispy. Fruit taste/flavour were rated 0 for flat/insipid (fruit lacking acidity) to 100% for flavoursome fruit. The last trait measured was skin toughness, and this proved one of the hardest traits to measure, subjectively, as hard fruit tends to have a softer skin than a soft fruit.

The evaluation form (Figure 6) used in this study was one used by the apple breeder for phase 1 fruit evaluation, and therefore not all traits were measured

Figure 5. Colorimeter and penetrometer used to capture readings for colour components and firmness.



VISUAL AND SENSORIC EVALUATION PHASE I APPLES

DATE: _____ AIM: _____

SELECTION / FAMILY / GENOTYPE: _____ FARM: _____

HARVEST DATE: _____

	1	2	3	4	5	6	7
COLOUR	FULL RED	BLUSH	STRIPED	GOLDEN TYPE	GRANNY TYPE	BI-COLOUR	OTHER
	☐	☐	☐	☐	☐	☐	☐

STRIPENESS	NO STRIPES	_____	FULL RED
COLOUR	DULL	_____	BRIGHT
TEXTURE	MEALY	_____	CRISP
FIRMNESS	SOFT	_____	HARD
TASTE	FLAT/INSIPID	_____	FLAVOURSOME
JUICINESS	DRY	_____	VERY JUICY
SUGAR	LOW	_____	HIGH
ACID	LOW	_____	HIGH
SIZE	SMALL	_____	LARGE
FORM	BAD	_____	GOOD
SKIN	TOUGH	_____	SOFT
GRND COL	MUDDY	_____	BRIGHT
RUSSETTING	BAD	_____	LOW
GEN APPEARENCE	BAD	_____	GOOD
PARENTAGE	BAD	_____	GOOD

LENTICELS	CONSPICUOUS (0)	INTERMEDIATE (5)	INCONSPICUOUS (10)	
CALYX OPENESS	WIDE (0)	INTERMEDIATE (5)	NEAT (10)	
FLESH COLOUR	GREEN (1)	YELLOW (2)	CREAM (3)	WHITE (4)
TASTE	VERY SOUR (0)	BALANCED (5)	VERY SWEET (10)	

REMARKS

Figure 6. Sensory evaluation form, described by Heintz and Kader (1983), used in Phase I evaluation.

2.3.3 Data analysis

ANOVA (analysis of variance) was performed on all measurements for each of the populations. Separate analyses were performed for each year and a joint analysis for the 3 years in order to test for year x family interaction effects. The mean square for seedlings within families was used for the comparison between families. Where a significant year x family interaction was found in the joint analysis, the mean square for year x family was used as error. Intraclass correlation coefficients and variance component analyses was performed using SAS Variance Component Estimation Procedure (SAS Institute, Inc., 1996) at Infruitec-Nietvoorbij, Stellenbosch, South Africa (Chapter 3).

2.3.4 Variance structure

Standard quantitative genetic principles (Falconer and Mackay, 1996) were applied so as to estimate the underlying causal components of variance from all observations recorded. This is the primary interest of variance structure in seedling populations, and was broken down as follows:

i) variance of seedlings trees within families of the same cross

$$\sigma_w^2 = \sigma_g^2 + \sigma_e^2$$

where σ_g^2 = a genetic component (generated by crossing in this case), and

σ_e^2 = a component ascribable to environmental variable within the trial orchard

ii) variance between families

$$\sigma_b^2 = \sigma_G^2 + \sigma_W^2$$

where σ_G^2 = the genetic variance between families for a given common parent

The intraclass correlation coefficient relevant to selection between families is

$$t = \sigma_g^2 / \sigma_g^2 + \sigma_e^2$$

In these experiments, the repetition was performed on the same tree in different seasons, involving possible genotype-environment interactions at two levels, viz.,

i) year x family interactions, σ_{GE}^2 and

ii) year x seedling interaction within families, σ_{gE}^2.

Conceptionally, ANOVA and expected mean squares (EMS) can be performed in two parts (Kempthorne, 1957), assuming *y* years of measurement and *N* trees per family (table 7).

Table 7. The different structures that ANOVA is broken down into.

1	Years (Y)	not relevant
	Families (F)	$\sigma^2 + N\sigma_{GE}^2 + Ny\sigma_G^2$
	Y x F interaction	$\sigma^2 + N\sigma_{GE}^2$
	Residual	σ^2
2	Seedlings within families	$(\sigma_e^2 + N\sigma_{gE}^2) + y\sigma_g^2$
	Y x trees within families	$(\sigma_e^2 + \sigma_{gE}^2)$

Since only one observation was made on each tree each year, environmental variance (within the orchard) and genotype x environment interaction could not be estimated separately.

2.4 GENOTYPIC DATA

2.4.1 Extraction of genomic DNA from apple leaves

Leaves were collected from each of the three mapping populations and stored at –20ºC until ready to use. DNA was extracted from the leaf material using the 2x CTAB (Cetyltrimetylammoniumbromide) method. One leaf was put in a sterile mortar and liquid nitrogen was added. The leaf was gently ground using a pestle. The powder was transferred into 2ml tubes and 1ml of pre-warmed (60ºC) 2x CTAB was added. The samples were incubated at 62ºC for 30 minutes to homogenize. An aliquot of 10µl of Proteinase K at 20mg/ml was added to the homogenates and incubated at 37ºC for 30 minutes. An equal volume of Chloroform:Isoamylalcohol (CIA) was added. The samples were then vortexed briefly and inverted for 10 minutes. The tubes were centrifuged at 16.1g for 10 minutes. The supernatant was collected and transferred into new 2ml tubes. An aliquot of 2.5µl RNase at 10mg/ml was added and the samples were then incubated at 37 ºC for 30 minutes. Equal volumes of CIA were added and the samples were briefly vortexed, followed by five minutes of tube inversion. The tubes were centrifuged for 10 minutes at 16.1g, the supernatant transferred into new 1.5ml tubes and 2/3 of ice-cold isopropanol was added. The tubes were then inverted several times and incubated at –20ºC for 20 minutes. After 20 minutes the tubes were centrifuged for 10 minutes at 16.1g and the supernatant was carefully discarded. The pellet was washed twice with 70% ethanol and after each wash, centrifuged for five minutes. The pellets were air dried and resuspended in 50µl of 1 x TE.

2.4.2 1% Agarose gel preparation and electrophoresis

1g of agarose was weighed and added to 100ml of 1x TBE and dissolved by boiling. Once cool, 3µl of Ethidium Bromide (EtBr) was added to the dissolved agarose. The liquid was poured into a gel-caster and allowed to solidify. Prior to loading, 5µl of DNA loading buffer was added to 5µl of each DNA sample. The samples were then electrophoresed at 10V/cm on a 1% agarose gel.

2.4.3 SSR detection, primer design and primer synthesis

Tandem repeats finder (http//:tandem.bu.edu) (Benson, 1995) was used to search publicly available EST's for *Malus*, for simple sequence repeats (SSRs). These search through sequence data for SSRs, according to specified criteria (Figure 7). SSRs were selected based on pattern size, copy number, % matches and position of the first base of the repeat (first index). A pattern size, viz. di- tri- or tetra-nucleotide, was set at more than two. The percentage matches were set at greater than or equal to 90%, thus eliminating sequences showing insertions, deletions and substitutions within the repetitive region. A first index of greater than or equal to 20 allows for a forward primer to be designed before the first base of the repeat sequence, and a score of greater than 40 was used as a cut-off, with sequences having lower scores showing higher percentage of mismatches within the repetitive regions (Figure 7).

Primer pairs flanking the SSR were designed by visual inspection of the conserved sequences flanking repeats. Primers were chosen in such a way that the resulting amplicons vary in size, ranging from 75 bp to 500 bp. Primers had a GC-content of between 40 and 60% and an ideal melting temperature (T_m) of 60°C.

All primer pairs used during this study were synthesized at Applied Biosystems (Foster City CA, USA) and the primer closest to the repeat was labelled with one of four fluorescent dye colours viz. 6-carboxy fluorescein (6-FAM), VIC, NED and PET) (the chemical names are proprietary to Applied Biosystems).

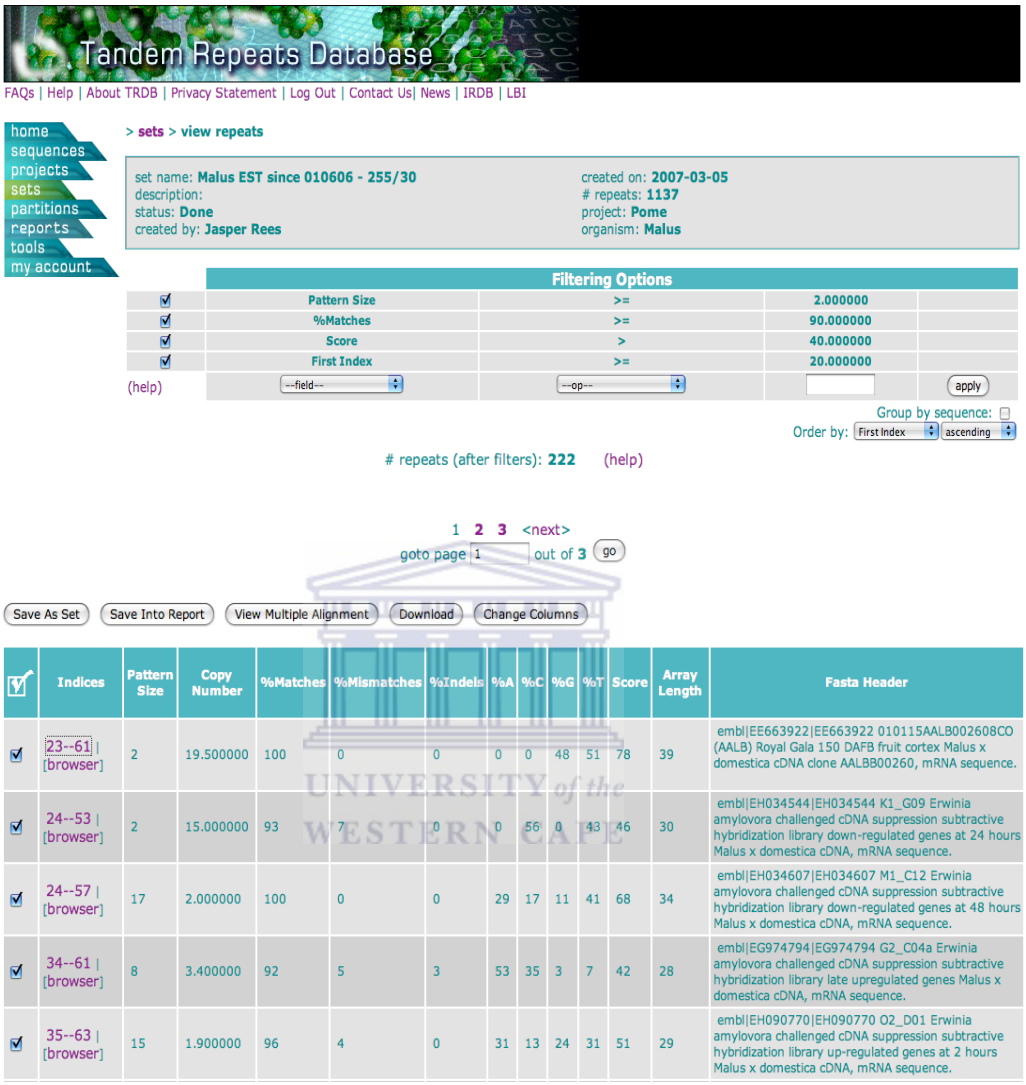


Figure 7. Outputs of the tandem repeats finder database, showing the initial number of repeats, and the filtering options used to eliminate unwanted sequences.

2.4.4 PCR amplification

Microsatellite markers were screened to test the ability of specific primer pairs to amplify target genomic DNA and generate amplification products or fragments.

Simplex amplifications were performed in volumes of 20 µl with 1 unit T*aq* polymerase (Excel), 0.2 µM Tris-HCl (pH8.3), 1 µM KCl, 0.07 µM $MgCl_2$, 50 µM each dNTP's, 0.016 µM each primer and 1 µl DNA template. PCR reactions were optimized, in order to obtain the correct annealing temperature for a specific primer pair, using a 'touch down' approach on an Eppendorf Mastercycler® gradient PCR machine (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). The thermal cycling conditions were as follows: (1) 96°C for 5 min, (2) 10 cycles: 94°C for 40 sec, (65°C - 55°C) touch down to (60°C - 45°C) for 40 sec, 72°C for 2 min, (3) 30 cycles: 94°C for 40 sec, (55°C - 45°C) for 40 sec, 72°C for 2 min, (4) 72°C for 45 min and (5) 4°C hold. Amplification was performed on a 2720-Thermal Cycler (Applied Biosystems, Foster City CA, USA). Amplicons were electrophoresed on 6% polyacrylamide gel at 120 V/cm. Primer pairs generating such products were then assessed on cultivars used as parents, viz. 'Anna', 'Golden Delicious', 'Priscilla' and 'Prima' as well as 'Braeburn', 'Cox's Orange Pippin', 'Mildew resistant', 'Austin' and 'Sharpe's Early' (SE) in order to determine polymorphic information content and heterozygosity. Thermal conditions were as mentioned above with the exception that no gradient was used for annealing temperature.

On the basis of the above-mentioned criteria, microsatellites were then selected for megaplexing, and used to screen the three mapping populations.

2.4.5 Megaplex PCR

Twelve to sixteen primer pairs, labelled with the same fluorescent dye, but amplifying differently sized fragments, were selected, pooled and amplified in the same PCR reaction. 5ng of genomic DNA template, as well as 0.2μM of each primer, was added to the Qiagen multiplex kit as per the manufacturer's instructions. The thermal cycling conditions were as follows: (1) 15min at 95°C, (2) 40 cycles: 30s at 94°C, 90s at 60°C, 60s at 72°C, (3) 30min at 60°C and (4) 4°C hold, and amplification was performed in a 9700-Thermal Cycler (Applied Biosystems, Foster City CA, USA).

2.4.6 Amplification of ACS-1, ACO-1 and EXPANSIN-7 using PCR

For the non-fluorescently-labelled primer pairs, ACS-1 and ACO-1, reaction conditions were as follows: $MgCl_2$ was at a final concentration of 3 mM; dNTPs were 100 μM; the primers were at 1.0 μM.

The PCR temperature profile for ASC-1 was performed as follows: 2min at 94˚C; 45s at 94˚C, 45s at 58˚C, 20s at 72 ˚C, repeated for 35 cycles; followed by 7min at 72˚C. PCR products were then electrophoresed on 1% agarose gel.

The PCR temperature profile for ACO-1 was performed as follows: 2min at 94˚C; 45s at 94˚C, 45s at 65˚C, 2min at 72 ˚C, repeated for 35 cycles; followed by 10min at 72˚C. PCR products were then electrophoresed on 2% agarose gel.

The general PCR temperature profile for EXPANSIN-7 was performed as follows: 15min at 95°C, 30s at 94°C, 90s at 60°C, 60s at 72°C, repeated for 40 cycles; followed by 30min at 60°C. PCR fragments were then separated and analysed on the ABI 3130xl (16-capillary array system) Genetic Analyzer (Applied Biosystems, Foster City CA, USA).

2.4.7 Automated fragment analysis

Since actual fragment size determination and differentiation between larger fragments and 2bp repeats are difficult to accomplish with the use of gel electrophoresis, the ABI 3130xl (16-capillary array system) Genetic Analyzer (Applied Biosystems, Foster City CA, USA) was used. Size determination of 6-FAM, VIC, NED and PET labelled primers were done with size standards labelled with LIZ (Applied Biosystems) (GeneScan^{TM} 500 LIZ^{TM}) fluorescent dyes. POP-7 sieving polymer matrix, 1x Genetic analyzer buffer with EDTA and 16 x 36 cm x 50 μm uncoated capillaries were used.

Samples were prepared by adding 3 μl of a 1:10 diluted PCR product to 10 μl Hi-Di formamide (Applied Biosystems) containing 0.2 μl size standard. In cases where PCR products were pooled to maximize throughput, 1:10 PCR product dilutions were pooled in the ratio 6-FAM:VIC:NED:PET = 1:1:3:2. The samples were heat denatured at 96°C for 5 min and then snap cooled on ice prior to loading them into the autosampler tray. Samples were injected for 15s at 15,000 V and separated at 15,000 V for 24 min with a run temperature of 60°C. The resulting data can be displayed as an electropherogram using GeneMapper 4.0® software (Applied Biosystems, Foster City, CA).

2.4.8 Amplified product analysis

SSR markers were allocated to megaplexes and these were used to screen each of the three mapping populations, 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla'. Allele sizes were automatically detected using the ABI 31030xl Genetic Analyser (Applied Biosystems, Foster City CA, USA) and output files were analysed using GeneMapper 4.0® software (Applied Biosystems, Foster City, CA). Each seedling was genotyped for a specific locus, using the JoinMap® 4.0 (Van Ooijen, 2006) coding system, according to preset criteria regarding fragment size and intensity (Table 8). Reliability of a subset of the data was tested before a complete analysis was performed. All SSRs genotyped on the 'Golden Delicious' x 'Anna' progeny were compared to results obtained from a previous study (van Dyk *et al*., 2010). SSRs found to show a different segregation profile were rescored, with adjustments to expected product sizes.

Table 8. Classes of segregation types encountered when working with a full-sib family, derived from an outbreeding species, as described by JoinMap® 4.0 codes.

Class	Segregation type	Number of alleles	Segregating alleles		F1	
			Parent 1	Parent 2	Genotypic codes	Expected ratio
1	ab x cd	4	Yes	Yes	ac; ad; bc; bd	1:1:1:1
	ef x eg	4	Yes	Yes	ee; ef; eg; fg	1:1:1:1
2	hk x hk	2	Yes	Yes	hh; hk; kk	1:2:1
3	nn x np	2 or 3	No	Yes	nn; np	1:1
	lm x ll	2 or 3	Yes	No	lm; ll	1:1

2.4.9 Segregation analysis of mapping populations

Segregation analyses were performed on the three mapping populations, as well as the parents of these populations. 'Golden Delicious' was used as the female parent in crosses with 'Anna' and 'Priscilla', as male parent, for two of the populations. The third mapping population had 'Prima' as the female parent, with 'Anna' being the male parent in this cross. A relevant JoinMap code was allocated to each seedling based on the segregation type (Table 9) identified from the parents of each population.

2.4.10 DArT analysis

An aliquot of 20µl at a concentration of 50ng/µl of each genomic DNA sample, of the progenies of the three mapping populations, were sent to Diversity Array Technology Pty Limited (Yarralumla, Australia) for DArT analysis. These dominant markers were then converted to JoinMap codes as instructed by the supplier, added to SSR data and used in the construction of the genetic maps for each family.

2.4.11 Genetic Linkage Map Construction

Integrated genetic linkage maps were constructed for the F1 populations generated from each of the three mapping populations used, viz. 'Prima' x 'Anna', 'Golden Delicious' x 'Priscilla' and 'Golden Delicious' x 'Anna', using

JoinMap® 4.0 (Van Ooijen, 2006). The logarithm of odds (LOD score) of 4 was used to define linkage groups (LGs) and genetic distances between markers were calculated using the Kosambi mapping function. The numbering of LGs is in accordance with Maliepaard *et al*. (1998). Alignment with the reference markers proposed by Silfverberg-Dilworth *et al*. (2006) allowed for the generation of extra segments that belong to specific linkage groups.

2.4.12 QTL Mapping

2.4.12.1 Phenotypic trait data

Phenotypic trait data were used to identify QTLs for each of the quality traits mentioned earlier. Datasets for each of the three years of harvest, as well as a dataset representing the mean values for each trait were analyzed independently so as to compare and contrast any similarities or differences between each year of harvest. Yearly harvests were also treated to determine which of the QTLs identified remained consistent over the three-year period.

2.4.12.2 Mapping of QTLs

QTL analysis was performed using the MapQTL 5.0® (Van Ooijen, 2004) software package, for each of the three mapping populations 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla', using the average phenotypic trait assessment performed, for three years (2005, 2006 and

2007). The integrated genetic linkage maps for the three populations were used together with this phenotypic data, to identify prospective QTLs. Interval mapping was the performed for each year of phenotypic assessment, for the mean dataset of the three years, and this was also performed for the first evaluation i.e. pre-storage and also for the second evaluation i.e. post-storage. A QTL was declared significant if it had a LOD threshold of 3.8 and the maximum LOD score attained, as well as the percentage of the population variation explained by that QTL. Prospective QTLs found here were then analyzed with the Kruskal-Wallis nonparametric mapping option of MapQTL 5.0® to identify the SSR markers that were associated with these QTLs and that might be good candidates for marker assisted selection.

2.4.12.3 Multiple QTL Mapping (MQM)

Multiple QTL mapping was performed, using MapQTL 5.0®, on prospective QTLs to identify if there were any other QTLs, which might be present in the population, for a specific trait. A genome-wide (GW) LOD threshold of 3.8 was chosen as the cut-off for presence or absence of QTLs. In MQM mapping, markers found to be associated with LOD scores greater than the threshold value were used as co-factors, to identify any other QTLs that may be present. SSR markers associated with these QTLs were then identified and tabulated. QTLs were declared significant if the maximum LOD obtained after multiple rounds of MQM mapping exceeded the genome wide LOD threshold (calculated with an error rate of 0.05 over 1000 permutations).

CHAPTER 3: PHENOTYPIC RESULTS

3.1 Phenotypic analysis

The level of genetic diversity available, together with the methods used for its use ultimately determines whether a crop improvement programme is a success or not (de Souza and Byrne, 1998). The knowledge of genetic parameters, such as heritability, variances and correlations, help to make predictions of genetic progress among the offspring (Falconer, 1989). These phenotypic data, or analyses, form an integral component of identifying QTLs, for specific traits of interest. In this study, traits involved in fruit quality were analysed. Analysis of fruit quality traits was measured using both subjective and instrumental techniques, independently, or simultaneously. Statistical analyses performed on all datasets include ANOVA, heritability and variance coefficient analyses, as well as correlational analyses.

3.1.1 Subjective analysis

Thirteen fruit quality traits were successfully measured and analysed during the subjective analysis component of this study. These include stripeness, colour, texture, firmness, taste, juiciness, sweetness (sugar content), acidity, size, form, skin toughness, ground colour and russetting. All histograms of raw data for each trait, in each population are found in Appendix B, C and D and simple statistics in Table 9, 10, 11 and 12.

Table 9. Variation within three apple families recorded for sensory traits during pre-storage evaluation of apple fruit. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Family	**Stripeness (%)**				**Colour (%)**			
Prima x Anna	2	99	72.33a	17.49	11	98	62.96b	18.51
GD x Anna	3	99	59.33b	23.04	12	97	61.80b	17.57
GD x Priscilla	1	96	40.79c	32.72	20	97	67.26a	18.61
	Texture (%)				**Firmness (%)**			
Prima x Anna	16	91	63.24a	16.96	15	89	55.50a	17.03
GD x Anna	23	93	62.61a	15.85	20	86	53.25ab	15.70
GD x Priscilla	7	87	54.88b	23.24	7	95	48.20c	17.75
	Taste (%)				**Juiciness (%)**			
Prima x Anna	5	87	53.00ab	16.51	10	87	54.89b	14.58
GD x Anna	14	92	54.60a	14.83	15	84	58.70a	13.46
GD x Priscilla	4	86	53.08ba	17.90	6	87	47.39c	18.94
	Sugar (%)				**Acid (%)**			
Prima x Anna	7	92	43.66b	14.11	6	89	48.00a	16.09
GD x Anna	16	83	47.28a	13.70	9	75	45.41ab	13.86
GD x Priscilla	9	78	47.12a	16.10	9	79	44.41b	13.79
	Size (%)				**Form (%)**			
Prima x Anna	17	98	45.11ab	11.58	18	92	45.93b	13.58
GD x Anna	14	77	47.43a	10.69	15	78	46.00b	12.54
GD x Priscilla	3	72	37.73c	12.36	16	74	51.56a	12.2
	Skin toughness (%)				**Ground colour (%)**			
Prima x Anna	13	86	52.75c	15.89	6	98	64.96c	22.65
GD x Anna	15	89	55.84b	15.33	17	97	70.42b	20.87
GD x Priscilla	23	90	60.43a	16.46	11	96	77.95a	18.12
	Russet (%)							
Prima x Anna	6	97	64.40a	20.60				
GD x Anna	12	99	66.35a	19.84				
GD x Priscilla	15	96	66.68a	20.55				

Table 10.Variation within families recorded for sensory traits during post-storage of apple fruit, after 12 weeks of cold storage. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Family	**Stripeness (%)**				**Colour (%)**			
Prima x Anna	1	97	65.92a	18.95	30	97	68.82ab	16.28
GD x Anna	2	98	61.97a	20.18	14	93	62.07c	17.27
GD x Priscilla	1	95	42.41b	31.43	10	99	71.23a	15.62
	Texture (%)				**Firmness (%)**			
Prima x Anna	5	93	50.35b	20.18	6	79	40c	17.24
GD x Anna	11	86	57.88a	17.44	12	87	44.40b	16.28
GD x Priscilla	6	84	48.8b	24.2	6	86	48.66a	20.42
	Taste (%)				**Juiciness (%)**			
Prima x Anna	7	84	46.85c	13.90	6	89	41.99b	17.19
GD x Anna	22	89	56.75a	15.54	16	86	52.83a	14.87
GD x Priscilla	8	95	51.77b	18.95	7	86	44.32b	18.64
	Sugar (%)				**Acid (%)**			
Prima x Anna	5	79	41.38c	14.94	3	91	45.23ab	16.12
GD x Anna	14	89	52.71a	14.35	11	90	46.96a	14.44
GD x Priscilla	4	86	47.55b	18.97	5	96	41c	22.08
	Size (%)				**Form (%)**			
Prima x Anna	19	73	43.98b	10.70	17	80	45.98b	11.27
GD x Anna	15	82	47.60a	11.37	15	96	48.84a	12.44
GD x Priscilla	9	85	39.70c	13.48	20	88	54.61a	13.17
	Skin toughness (%)				**Ground colour (%)**			
Prima x Anna	20	93	65.43a	17.16	3	98	80.25a	16.73
GD x Anna	14	89	61.46b	14.73	17	96	76.77a	17.69
GD x Priscilla	7	97	59.13b	19.26	9	97	77.14a	19.81
	Russet (%)							
Prima x Anna	15	98	73.72a	17.78				
GD x Anna	9	97	69.12b	19.48				
GD x Priscilla	7	98	66.95b	22.80				

Table 11. Yearly variation recorded for sensory traits during pre-storage evaluation of apple fruit. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Year	**Stripeness (%)**				**Colour (%)**			
2005	1	99	57.21ab	33.20	4	99	56.99c	22.87
2006	1	99	55.57b	33.34	12	98	71.65a	16.19
2007	6	93	58.88a	26.40	25	89	65.64b	13.17
	Texture (%)				**Firmness (%)**			
2005	7	93	58.14b	17.91	8	83	49.72b	14.68
2006	7	92	62.80a	22.12	7	95	56.00a	20.18
2007	12	91	62.52a	18.79	15	87	50.20b	16.70
	Taste (%)				**Juiciness (%)**			
2005	10	92	52.49b	18.04	10	90	58.63a	15.55
2006	4	92	55.58a	17.95	6	91	54.38b	19.07
2007	5	84	51.27b	12.99	13	86	49.56c	14.41
	Sugar (%)				**Acid (%)**			
2005	8	78	44.05b	16.03	7	79	40.69b	14.82
2006	7	92	45.47b	15.88	6	89	46.85a	16.49
2007	17	78	48.12a	13.40	18	87	45.48a	11.80
	Size (%)				**Form (%)**			
2005	3	76	44.53a	13.47	15	78	46.91b	13.12
2006	14	98	42.75b	12.03	16	92	49.77a	13.91
2007	13	87	43.67ab	10.22	18	74	47.25b	9.70
	Skin toughness (%)				**Ground colour (%)**			
2005	9	85	50.00b	16.44	11	95	58.03b	22.99
2006	13	90	58.76a	18.89	6	98	81.86a	15.70
2007	23	85	60.12a	13.22	30	94	80.53a	10.64
	Russet (%)							
2005	11	97	56.50b	22.02				
2006	6	99	69.10a	19.31				
2007	10	93	70.55a	14.26				

Table 12. Yearly variation recorded for sensory traits during post-storage evaluation of apple fruit, after 12 weeks in cold storage. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Year	**Stripeness (%)**				**Colour (%)**			
2005	1	99	54.09b	32.02	10	99	67.96a	18.68
2006	2	98	53.13b	29.36	4	97	67.84a	19.65
2007	7	93	61.88a	23.89	14	90	63.28b	13.96
	Texture (%)				**Firmness (%)**			
2005	4	93	51.85b	21.13	6	92	49.00a	17.85
2006	5	92	58.51a	22.28	6	89	46.20a	20.76
2007	12	82	57.97a	19.73	12	84	42.45b	16.83
	Taste (%)				**Juiciness (%)**			
2005	7	95	56.11a	18.34	7	91	54.34a	19.80
2006	8	88	57.90a	17.06	6	80	47.58b	16.47
2007	16	78	48.39b	11.70	12	76	46.54b	12.93
	Sugar (%)				**Acid (%)**			
2005	4	89	50.88a	17.91	2	96	45.17a	19.63
2006	10	92	47.65b	18.77	8	94	43.25a	18.50
2007	13	79	48.68ab	12.75	13	73	43.70a	10.12
	Size (%)				**Form (%)**			
2005	1	85	43.69b	14.41	17	96	50.57a	13.96
2006	14	72	43.83ab	11.44	15	80	48.65a	12.38
2007	16	75	45.54a	9.35	23	76	50.25a	9.55
	Skin toughness (%)				**Ground colour (%)**			
2005	7	97	55.04b	18.88	3	97	72.99c	21.00
2006	5	92	64.62a	17.46	7	98	77.76b	24.21
2007	25	93	62.40a	14.06	43	92	82.35a	8.53
	Russet (%)							
2005	7	98	61.81b	22.87				
2006	9	99	72.11a	19.10				
2007	38	93	74.27a	11.84				

Table 13. Within family variation recorded for traits during pre-storage evaluation of apple fruit. Values are averaged over three years for all traits. Letters indicate significant differences between means a $P \leq 0.05$.

Family	Min	Max	Mean	SD	Min	Max	Mean	SD
	Mass (g)				**Diameter (cm)**			
Prima x Anna	38.00	313.00	113.83b	36.84	49.10	202.00	94.85a	50.06
GD x Anna	34.00	257.00	131.10a	39.51	20.91	192.00	79.62b	35.90
GD x Priscilla	21.00	207.00	90.89c	30.03	39.33	82.65	60.75c	7.29
	Firmness (kg/cm)				**% Total soluble solids**			
Prima x Anna	1.23	18.74	8.81a	2.47	10.70	23.20	15.07c	1.74
GD x Anna	1.17	14.05	7.67b	2.09	11.20	25.80	15.76b	1.91
GD x Priscilla	1.09	14.07	7.47b	2.33	9.40	25.90	18.03a	2.14
	Overcolour L				**Background colour L**			
Prima x Anna	20.17	73.13	36.28c	7.78	28.03	85.62	66.33b	12.2
GD x Anna	25.70	81.38	45.93b	9.41	28.25	89.87	72.06a	10.14
GD x Priscilla	26.48	91.85	53.15a	15.84	20.43	87.05	72.16a	10.85
	Overcolour C				**Background colour C**			
Prima x Anna	7.76	77.89	34.47c	7.59	2.80	65.76	41.42c	7.96
GD x Anna	8.54	62.31	40.86a	6.09	1.99	109.51	41.15c	9.18
GD x Priscilla	16.67	70.81	41.74a	9.39	23.15	79.82	47.01a	7.60
	Overcolour H				**Background colour H**			
Prima x Anna	4.25	103.19	24.2d	12.34	12.86	113.26	76.46b	27.01
GD x Anna	9.53	100.90	32.90c	15.88	11.70	112.57	83.91a	22.38
GD x Priscilla	10.33	102.83	53.54a	27.63	18.04	106.86	85.36a	16.10

Table 14. Within family variation recorded for traits during post-storage evaluation of apple fruit. Values are averaged over three years for all traits. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Family	**Firmness**				**% Total soluble solids**			
Prima x Anna	0.90	13.35	5.15c	2.07	6.80	22.80	15.26d	1.94
GD x Anna	0.03	13.26	4.85c	1.25	10.5	25.10	16.26c	1.94
GD x Priscilla	0.99	13.68	6.63a	2.01	9.80	26.10	17.65b	2.08

Table 15. Yearly variation recorded for traits during pre-storage evaluation of apple fruit. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Year	**Mass**				**Diameter**			
2005	27.33	213.67	114.1b	34.57	41.54	82.18	64.46b	47.27
2006	51.50	268.00	123.43a	39.01	49.53	90.30	66.38b	47.11
2007	52.33	210.75	109.51c	31.90	50.20	194.00	86.35a	46.38
	Firmness				**% Total soluble solids**			
2005	2.09	13.97	7.76a	2.24	6.80	24.26	15.95b	2.06
2006	1.69	13.40	7.61a	2.01	12.76	24.90	17.22a	2.13
2007	1.75	13.60	7.79a	2.21	12.40	28.37	17.18a	2.14
	Overcolour L				**Background colour L**			
2005	25.98	76.73	45.09b	13.12	35.54	83.81	67.63b	11.17
2006	27.38	74.00	45.95a	12.25	36.20	82.95	69.79a	10.82
2007	27.71	81.03	46.48a	13.32	34.23	83.78	66.13c	11.17
	Overcolour C				**Background colour C**			
2005	15.21	65.59	37.18c	8.33	4.08	65.83	41.66c	9.19
2006	15.49	62.81	38.07b	7.60	28.68	65.39	45.50a	6.53
2007	23.77	65.38	40.24a	6.90	24.92	59.58	43.08b	6.21
	Overcolour H				**Background colour H**			
2005	7.92	94.12	37.62b	24.4	17.90	106.43	73.27c	24.67
2006	13.72	96.19	39.82a	22.6	24.09	109.40	82.22a	19.07
2007	13.65	97.45	38.63ab	22.4	23.56	107.92	78.40b	20.71

Table 16. Yearly variation recorded for traits during post-storage evaluation of apple fruit. Letters indicate significant differences between means a $P \leq 0.05$.

	Min	Max	Mean	SD	Min	Max	Mean	SD
Year	**Firmness**				**% Total soluble solids**			
2005	2.63	11.35	5.68b	1.79	6.80	24.46	16.12c	2.24
2006	1.69	11.68	5.88a	1.67	13.6	24.90	17.69a	2.24
2007	1.75	12.55	5.47c	1.77	12.4	28.37	17.36b	2.11

3.1.1.1 Within family variation

It is very clear that variation within family was highly significant in both pre- and post-storage subjective evaluations (Table 17). We also saw that genotypic variation within the family was much higher than between families (Table 18). Stripe-ness, for example, makes up 60% and 56% of the variance components, for pre-storage and post-storage evaluations, as opposed to 15% and 9.5% for between family variance components.

3.1.1.2 Between family variation

ANOVA detected significant levels of variation for stripe-ness, colour, sugar content, acid content, firmness, texture, juiciness, size, form, skin-toughness and ground-colour, with only taste and russet showing no significant variation between the three families, in the pre-storage evaluation. $P < 0.05$ indicated significant differences, with taste and russet yielding *P*-values of 0.086 and 0.085 respectively (Table 17). All traits showed significant variation between the families in post-storage evaluation, except for ground-colour, which has a *P*-value of 0.16.

3.1.1.3 Year x Family interaction (Y x F interaction)

Significant Y x F interaction was apparent in pre-storage measurements for all traits except colour, juiciness and ground colour (Table 17). Expressed as a

percentage, Y x F interaction was small compared to other components contributing to variance (Table 14). In the post-storage analysis, firmness, taste, juiciness, sugar and acid content, size, form and skin toughness were all found to show significant levels of variation in the Y x F interactions (Table 17).

3.1.1.4 Year to year performance

The ANOVA indicates significant differences between the years in all measurements (Table 17), and this might have been predicted from the fluctuation in weather patterns in the region (van Rooyen, 2008), during the three-year period.

Table 17. Analysis of variance for traits involved in subjective assessment of apple fruit. Data were recorded on fruit from seedling trees of three progenies over three years (2005, 2006 and 2007) for stripeness, colour, texture, firmness, taste, juiciness, sugar, acid, size, form, skin toughness, ground colour and russet.

Stripeness	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	37021.7	146.5	<.0001	3	15709.4	56.3	<.0001
Year	2	1245.9	4.9	0.0076	2	2385.5	8.5	0.0002
Seedling (w Family)	485	1479.1	5.9	<.0001	416	1110.7	3.9	<.0001
Y x F interaction	6	921.9	3.7	0.0015	6	309.7	1.1	0.3569
Residual	511	252.7			290	279.2		
Corrected Total	1007				717			

Colour	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	1460.5	6.5	0.0003	3	2650.4	12.7	<.0001
Year	2	12669.2	56.2	<.0001	2	306.7	1.5	0.2322
Seedling (w Family)	485	437.8	1.9	<.0001	416	353.9	1.7	<.0001
Y x F interaction	6	93.1	0.4	0.8707	6	414.4	1.9	0.0680
Residual	511	225.6			290	208.9		
Corrected Total	1007				717			

Texture	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	3972.2	16.6	<.0001	3	6272.3	18.8	<.0001
Year	2	1465.8	6.1	0.0024	2	2593.3	7.8	0.0005
Seedling (w Family)	485	509.3	2.1	<.0001	416	474.6	1.4	0.0007
Y x F interaction	6	676.9	2.8	0.0104	6	258.4	0.8	0.5908
Residual	511	239.9			290	333.8		
Corrected Total	1007				717			

Firmness	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	2237.7	9.4	<.0001	3	3477.2	13.8	<.0001
Year	2	3471.3	14.6	<.0001	2	2766.2	11.0	<.0001
Seedling (w Family)	485	329.9	1.4	0.0001	416	359.7	1.4	0.0005
Y x F interaction	6	1390.3	5.9	<.0001	6	887.2	3.5	0.0022
Residual	511	237.8			290	251.0		
Corrected Total	1007				717			

Taste	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	470.3	2.2	0.0866	3	4029.4	20.6	<.0001
Year	2	1025.5	4.8	0.0085	2	3178.2	16.2	<.0001
Seedling (w Family)	485	308.1	1.4	<.0001	416	270.3	1.4	0.0017
Y x F interaction	6	1822.6	8.6	<.0001	6	780.1	3.9	0.0008
Residual	511	213.3			290	195.8		
Corrected Total	1007				717			

Juiciness	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	5483.3	29.7	<.0001	3	7835.0	45.8	<.0001
Year	2	4691.3	25.4	<.0001	2	4213.3	24.6	<.0001
Seedling (w Family)	485	329.9	1.8	<.0001	416	290.7	1.7	<.0001
Y x F interaction	6	203.8	1.1	0.3582	6	1230.5	7.2	<.0001
Residual	511	184.5			290	171.1		
Corrected Total	1007				717			

Sugar	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	647.0	3.4	0.0171	3	5140.2	22.8	<.0001
Year	2	1287.9	6.8	0.0012	2	414.4	1.8	0.1608
Seedling (w Family)	485	258.5	1.4	0.0002	416	258.6	1.2	0.1038
Y x F interaction	6	912.9	4.8	<.0001	6	876.8	3.9	0.0009
Residual	511	188.9			290	225.3		
Corrected Total	1007				717			

Acid	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	2376.2	14.5	<.0001	3	1148.9	6.30	0.0004
Year	2	2555.9	15.6	<.0001	2	9.4	0.05	0.9497
Seedling (w Family)	485	232.3	1.4	<.0001	416	316.2	1.7	<.0001
Y x F interaction	6	919.4	5.6	<.0001	6	1028.6	5.6	<.0001
Residual	511	163.8			290	182.3		
Corrected Total	1007				717			

Size	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	4163.8	54.3	<.0001	3	1636.2	18.2	<.0001
Year	2	631.4	8.2	0.0003	2	374.5	4.2	0.0165
Seedling (w Family)	485	183.3	2.4	<.0001	416	171.3	1.9	<.0001
Y x F interaction	6	396.2	5.2	<.0001	6	200.1	2.2	0.0409
Residual	511	76.7			290	89.9		
Corrected Total	1007				717			

Form	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	1627.3	15.0	<.0001	3	1958.2	15.9	<.0001
Year	2	457.7	4.2	0.0152	2	429.9	3.5	0.0313
Seedling (w Family)	485	182.7	1.7	<.0001	416	146.7	1.2	0.0505
Y x F interaction	6	484.7	4.5	0.0002	6	344.4	2.8	0.0114
Residual	511	108.4			290	122.6		
Corrected Total	1007				717			

Skin toughness	Pre-storage				Post-storage			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	2255.5	11.1	<.0001	3	3195.2	15.3	<.0001
Year	2	11657.4	57.1	<.0001	2	6099.6	29.3	<.0001
Seedling (w Family)	485	289.6	1.4	<.0001	416	308.2	1.5	0.0002
Y x F interaction	6	1460.9	7.2	<.0001	6	998.4	4.8	0.0001
Residual	511	204.1			290	208.4		
Corrected Total	1007				717			

Gound colour	Pre-storgae				Post-storgae			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	8359.5	34.8	<.0001	3	491.5	1.7	0.1655
Year	2	44751.4	186.1	<.0001	2	2380.7	8.3	0.0003
Seedling (w Family)	485	372.2	1.6	<.0001	416	390.1	1.4	0.0028
Y x F interaction	6	394.4	1.6	0.1338	6	569.0	1.9	0.0688
Residual	511	240.4			290	287.8		
Corrected Total	1007				717			

Russet	Evaluation 1				Evaluation 2			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	534.9	2.2	0.0847	3	2036.9	8.5	<.0001
Year	2	11925.4	49.5	<.0001	2	6622.4	27.8	<.0001
Seedling (w Family)	485	495.8	2.1	<.0001	416	429.2	1.8	<.0001
Y x F interaction	6	844.3	3.5	0.0021	6	424.6	1.7	0.1029
Residual	511	240.8			290	238.5		
Corrected Total	1007				717			

Table 18. Summary of the variance components for subjective traits estimated from pre- and post-storage evaluation measurements. Heritability is shown by t2, and calculated by t2=sdl(fam)/[sdl(fam)+err]. Variance as a % of the total is shown in brackets.

	Pre-storage evaluation								
Trait	**Stripeness**	**Colour**	**Texture**	**Firmness**	**Taste**	**Juiciness**	**Sugar**	**Acid**	**Size**
Fam	150.88 (15)	5.18 (1)	11.98 (3)	3.32 (1)	0.00 (0)	19.71 (6.8)	0.00 (0)	4.75 (2.2)	15.81 (10.6)
Sdl (Fam)	625.71 (60)	90.40 (24)	128.92 (33)	46.20 (5)	42.05 (15.3)	65.77 (22.8)	29.91 (12.9)	26.08 (12)	51.95 (34.9)
Yr	0.00 (0)	51.88 (14)	4.57 (1)	6.91 (2.2)	0.65 (0.2)	17.64 (6.1)	1.86 (0.8)	7.89 (3.6)	0.00 (0)
Yr * Fam	9.96 (1)	0.00 (0)	6.41 (2)	16.52 (5.4)	15.59 (5.7)	1.82 (0.6)	7.96 (3.4)	10.46 (4.8)	3.88 (2.6)
Error (Residual)	257.29 (25)	228.17 (61)	240.52 (61.3)	234.42(76.3)	215.98 (78.7)	183.00 (63.6)	192.62 (82.5)	168.82 (77.4)	77.34 (51.9)
TOTAL	1043.84	375.63	392.41	307.38	274.28	287.94	232.35	217.99	148.99
t1	0.14	0.013	0.031	0.011	0	0.07	0	0.021	0.11
t2	0.71	0.28	0.35	0.16	0.16	0.26	0.13	0.13	0.40

Trait	**Form**	**Skin**	**Ground Colour**	**Russet**
Fam	2.73 (1.8)	4.15 (1.4)	36.46 (7.5)	0.00 (0)
Sdl (Fam)	35.58 (23)	37.10 (12.6)	32.14 (6.6)	107.57(26.7)
Yr	0.49 (0.3)	24.71 (8.4)	178.69 (36.5)	50.70 (12.6)
Yr * Fam	7.23 (4.7)	20.10 (6.8)	4.13 (0.8)	5.27 (1.3)
Error (Residual)	107.17 (70)	208.24 (70.8)	237.58 (48.6)	239.86 (59)
TOTAL	153.20	294.31	488.99	403.41
t1	0.02	0.01	0.07	0
t2	0.25	0.15	0.12	0.31

Post-storage evaluation									
Trait	**Stripeness**	**Colour**	**Texture**	**Firmness**	**Taste**	**Juiciness**	**Sugar**	**Acid**	**Size**
Fam	84.33 (9.5)	12.21 (3.9)	27.84 (6.1)	14.50 (4.2)	19.98 (7.2)	31.25 (10.1)	27.01 (9.6)	0.00 (0)	5.65 (3.8)
Sdl (Fam)	499.63(56.2)	67.23 (21.6)	71.29 (15.6)	57.28 (16.4)	18.26 (6.5)	56.64 (18.4)	3.31 (1.2)	49.67 (18.1)	42.20 (28.7)
Yr	14.78 (1.7)	3.04 (1)	9.24 (2)	5.93 (1.7)	18.39 (6.5)	9.71 (3.1)	0.00 (0)	0.00 (0)	0.00 (0)
Yr * Fam	6.01 (0.7)	4.27 (1.4)	4.51 (1)	13.01 (3.7)	7.94 (2.8)	35.35 (11.5)	11.49 (4.1)	15.43 (5.6)	6.44 (4.4)
Error (Residual)	284.47 (32)	224.67 (72)	343.09 (75.2)	257.82 (74)	214.81(76.9)	175.36 (56.9)	240.62 (85.2)	209.99 (76.3)	92.59 (63)
TOTAL	889.22	311.43	455.96	348.54	279.39	308.31	282.44	275.09	146.88
t1	0.09	0.04	0.06	0.04	0.07	0.10	0.10	0	0.04
t2	0.64	0.23	0.17	0.18	0.08	0.24	0.01	0.19	0.31

Trait	**Form**	**Skin**	**Ground Colour**	**Russet**
Fam	12.67 (8.3)	11.27 (3.4)	0.00 (0)	5.71 (1.5)
Sdl (Fam)	10.48 (6.8)	51.06 (15.8)	20.98 (5.8)	91.20 (23.3)
Yr	0.23 (0)	22.39 (6.9)	18.77 (5.2)	43.22 (11.1)
Yr * Fam	0.00 (0)	25.01 (7.8)	8.11 (2.2)	5.15 (1.3)
Error (Residual)	128.84 (84.6)	212.91 (66)	314.52 (86.8)	245.35(62.8)
TOTAL	152.21	322.65	362.39	390.63
t1	0.08	0.03	0	0.01
t2	0.08	0.19	0.06	0.27

Table 19. Analysis of variance for characteristics associated with apple colour development. Data were recorded, pre-storage, on apple fruit from seedling trees over three years for LCH values (2005, 2006 and 2007). P< 0.05 was regarded as being significant.

Over colour	*L*				*C*				*H*			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	10082.3	374.4	<.0001	3	2949.9	179.2	<.0001	3	33601.3	450.4	<.0001
Year	2	632.8	23.5	<.0001	2	1070.5	65.0	<.0001	2	1332.2	17.9	<.0001
Seedling (w Family)	486	252.5	9.4	<.0001	486	83.7	5.1	<.0001	486	814.2	10.9	<.0001
Y x F interaction	6	108.2	4.02	0.0006	6	30.1	1.83	0.0911				
Residual	509	26.9			509	16.5			506	74.6		
Corrected Total	1006				1006				1003			
Background colour	*L*				*C*				*H*			
Family	3	6888.0	169.5	<.0001	3	2058.4	76.02	<.0001	3	16124.4	89.5	<.0001
Year	2	539.6	13.3	<.0001	2	1112.6	41.09	<.0001	2	5904.9	32.8	<.0001
Seedling (w Family)	486	168.6	4.2	<.0001	482	72.0	2.7	<.0001	485	661.8	3.8	<.0001
Y x F interaction	6	148.2	3.7	0.0015	6	82.2	3.03	0.0064	6	1918.4	10.7	<.0001
Residual	509	40.6			499	27.1			509	180.2		
Corrected Total	1006				992				1005			

Table 20. Analysis of variance for mass, diameter, firmness and total soluble solids in apple fruit. Data were collected from adult seedling trees over three years (2005, 2006 and 2007) after 12 weeks in cold storage.

Mass	Pre-storage evaluation			
Source of variation	*df*	*MS*	*F*	*P*
Family	3	73377.2	155.5	<0.001
Year	2	9524.4	20.2	<0.001
Seedling (w Family)	486	1594.4	3.4	<0.001
Y x F interaction	6	1622.2	3.4	0.0025
Residual	509	472.0		
Corrected Total	1006			

Diameter	Pre-storage evaluation			
	df	*MS*	*F*	*P*
Family	3	43021.6	78.1	<.0001
Year	2	49265.7	89.5	<.0001
Seedling (w Family)	483	560.9	1.02	0.4173
Y x F interaction	6	25211.2	45.8	<.0001
Residual	471	550.2		
Corrected Total	965			

Firmness	Pre-storage evaluation				Post-storage evaluation			
	df	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	76.7	34.1	<.0001	3	104.6	117.3	<.0001
Year	2	0.4	0.2	0.8352	2	1.7	1.9	0.1553
Seedling (w Family)	439	5.1	2.3	<.0001	420	4.0	4.5	<.0001
Y x F interaction	6	14.2	6.3	<.0001	6	2.4	2.6	0.0164
Residual	334	2.3			312	0.9		
Corrected Total	784				743			
% TSS	Pre-storage evaluation				Post-storage evaluation			
	df	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Family	3	447.0	324.1	<.0001	3	294.7	152.2	<.0001
Year	2	121.5	88.1	<.0001	2	82.3	42.5	<.0001
Seedling (w Family)	484	4.5	3.2	<.0001	405	5.0	2.6	<.0001
Y x F interaction	6	16.7	12.1	<.0001	6	5.2	2.7	0.0142
Residual	495	1.3			288	1.9		
Corrected Total	990				704			

3.1.2 Instrumental Analysis

Data was successfully recorded for five instrumentally measured traits. These traits include fruit mass, diameter, firmness, %TSS and colour. Tables 13 and 14 compares the simple statistics between the three progenies and tables 15 and 16, the differences between the data in each of the three years (2005, 2006 and 2007). Only firmness and %TSS were evaluated both pre- and post-storage.

3.1.2.1 Within family variation

There was significant variation within the families for all traits, except the pre-storage evaluation of fruit diameter. A *P*-value of 0.42 was estimated, with all other traits showing values of less than 0.0001 (Table 20).

3.1.2.2 Between family variation

ANOVA revealed that significant variation is present between each of the three families, for all traits evaluated (Table 20).

3.1.2.3 Year x Family interaction (Y x F interaction)

As with between family variations, Y x F interaction was significantly different for all traits, except chromatic over-colour (1st C colour). This produced a *P*-value of 0.0911 (Table 19)

3.1.2.4 Year to year performance

All traits showed significant changes from one year to the next, except for firmness, which showed no significant variation in both the pre-storage and post-storage evaluations (Table 20). P-values of 0.84 and 0.16 for pre-storage and post-storage respectively are seen. Year to year performance also accounts for 36% and 53.4% of the variance components of these evaluations (Table 21 and 22).

3.2 Heritability

Heritability estimates are useful when studying genetics in a breeding population that is undergoing selection (Falconer, 1989). Estimates calculated in the sensory component of this project ranged from very low (0.06 for fruit flesh colour), to high (0.71 for fruit stripe-ness) (Table 18). The stripe-ness trait showed high heritability in both pre-storage evaluation and post-storage evaluation, with high values of 0.71 and 0.64 respectively. Heritability of most, if not all, of the traits measured instrumentally were found to be higher than that of the subjectively analysed traits. Intermediate heritability values of 0.4 and 0.35 and 0.31 were observed for size, texture and russet, respectively, with other subjectively analysed traits showing very weak heritability values of less than 0.3 (Table 18). Table 22, however, shows a heritability value of 0.53 for fruit mass, which is significantly higher than the size estimate observed in subjective evaluation. It was also shown that fruit size had a relatively low standard deviation (Table 9 and 10), when compared to fruit mass (Table 13), with values of 36.8g, 39.5g and 30.0g estimated for ‘Prima’ x ‘Anna’, ‘Golden Delicious’ x ‘Anna’ and ‘Golden Delicious’ x

‘Priscilla’, respectively. It would therefore be easier to select for fruit mass rather than fruit size. Even though low heritability values were encountered for both sugar and acid content (Table 18), it found that the heritability of % TSS to be 0.51 (pre-storage) and 0.45 (post-storage), as seen in table 21 and 22, respectively.

Table 21. Summary of the variance components for instrumentally measured traits estimated from pre-storage measurements. Heritability is shown by t2 is calculated by t2=sdl(fam)/[sdl(fam)+err]. Variance as a % of the total is shown in brackets. Over refers to overcolour and back to background colour.

Trait	Mass	Over L	C	H	Back L	C	H	Diameter	Firmness	% TSS
Fam	287.54 (21.4)	41.81 (3.1)	12.82 (19.5)	129.90 (21.6)	22.62 (17.5)	8.84 (14)	43.14 (8.5)	33.15 (3.2)	0.27 (6.3)	1.86 (35.2)
Sdl (Fam)	535.91 (39.8)	114.49 (61.4)	32.83 (49.9)	390.57 (64.9)	62.56 (48.3)	21.43 (33.9)	233.11 (46.1)	0.00 (0)	1.54 (36)	1.43 (27)
Yr	36.15 (2.7)	1.42 (0.8)	3.35 (5)	3.21 (0.5)	1.54 (1.2)	4.01 (6.3)	15.29 (3)	111.21 (10.6)	0.00 (0)	0.41 (7.7)
Yr * Fam	12.37 (0.9)	1.41 (0.8)	0.36 (0.5)	2.47 (0.4)	1.78 (1.4)	1.05 (1.7)	29.56 (5.8)	397.64 (37.9)	0.22 (5.1)	0.19 (19.5)
Error (Residual)	473.01 (35.2)	27.13 (14.6)	16.49 (25)	75.38 (12.5)	40.97 (31.6)	27.92 (44)	184.97 (36.6)	506.77 (48.3)	2.25 (52.6)	1.40 (26.5)
TOTAL	1344.99	186.25	65.84	601.54	129.47	63.24	506.07	1048.78	4.28	5.29
t1	0.21	0.22	0.19	0.22	0.17	0.14	0.09	0.03	0.06	0.35
Heritability	0.53	0.81	0.67	0.84	0.60	0.43	0.56	0.00	0.41	0.51

Table 22. Summary of the variance components for instrumentally measured traits estimated from post-storage measurements. Heritability is shown by t2 and is calculated by t2=sdl(fam)/[sdl(fam)+err]. Variance as a % of the total is shown in brackets.

Trait	Firmness	%TSS
Fam	0.56 (17.4)	1.77 (29.8)
Sdl (Fam)	1.72 (53.4)	1.55 (26.1)
Yr	0.02 (0.6)	0.61 (10.3)
Yr * Fam	0.04 (1.2)	0.08 (1.3)
Error (Residual)	0.88 (27.3)	1.94 (32.7)
TOTAL	3.22	5.94
t1	0.17	0.30
Heritability	0.66	0.45

3.3 Correlation analysis of Pre-storage and Post-storage evaluations

Correlational analysis was performed between pre- and post-storage evaluations. Tables 23, 24 and 25 compares these correlations for the 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla' progenies, respectively. The traits being correlated are those measured subjectively in section 3.1.1.

About 22% of pre-storage and 15% of post-storage correlations were greater than 0.30, in the 'Prima' x 'Anna' population. Stripe-ness showed a negative correlation with texture, firmness, taste, juiciness, acid content and size, before and after cold storage. Moderately positive correlations were seen between sugar and acid content, with an r-value of 0.50 being estimated (Table 23). Flavour and texture traits, viz texture, firmness, juiciness, acidity, sugar content and taste all showed moderately strong positive correlations, with acid, in particular, showing good correlations to texture, taste, juiciness and sugar content, both before and after cold storage.

The 'Golden Delicious' x 'Anna' population, showed positive correlations in flavour and texture traits, with acid (Table 24), as well, but unlike 'Prima' x 'Anna', the correlations were less strong. The highest correlation value was observed between taste and percentage sugar content, in the pre-storage evaluation. This moderately strong r-value was 0.55 and 0.45 in pre- and post-storage evaluations, respectively. There was a lower percentage of negative correlations in both pre- and post-storage evaluations of the 'Golden Delicious' x

‘Anna’ population when compared to the ‘Prima’ x ‘Anna’ progeny (Tables 23 and 24).

A strong positive correlation of 0.76 was observed between juiciness and texture in the pre-storage evaluation of ‘Golden Delicious’ x ‘Priscilla’, but following the trend of the other mapping populations, this value decreased in post-storage evaluation. Juiciness also showed moderately positive correlations to firmness, taste and percentage sugar content. It did not, on the other hand, correlate too well with acid content, with a low estimate of 0.20 observed. Skin toughness is negatively correlated to fruit firmness in all three populations, both before and after cold-storage, with values of -0.37, -0.26 and -0.41 observed in pre-storage evaluation of ‘Prima’ x ‘Anna’, ‘Golden Delicious’ x ‘Anna’ and ‘Golden Delicious’ x ‘Priscilla’, respectively (Table 25).

Table 23. Pre-storage correlations (above diagonal) and post-storage correlations (below diagonal) among 13 apple fruit characteristics evaluated subjectively for 3 years, on the 'Prima' x 'Anna' mapping population. Correlation values r ≥ 0.65; 0.64 ≥ r or r ≥ 0.50; 0.49 ≥ r or r ≥ 0.30 and r < 0.30 were considered strong or very strong, moderately strong, moderately weak and weak or very weak, respectively. Correlation values ≥ 0.30 are highlighted in red, and negative correlations are in blue.

Trait	Stripeness	Colour	Texture	Firmness	Taste	Juiciness	Sugar	Acid	Size	Form	Skin	Ground colour	Russet
Stripeness	--	-0.04	-0.24	-0.28	-0.05	-0.14	-0.09	-0.13	-0.15	-0.13	0.08	0.13	0.12
Colour	0.17	--	0.05	0.11	0.17	0.03	0.20	0.13	0.16	0.39	0.15	0.39	0.39
Texture	-0.18	-0.07	--	0.47	0.39	0.50	0.24	0.37	0.18	0.10	-0.21	-0.06	-0.08
Firmness	-0.06	-0.07	0.05	--	0.28	0.39	0.22	0.34	0.09	0.15	-0.37	-0.05	-0.03
Taste	-0.04	0.05	0.48	0.16	--	0.46	0.49	0.50	0.26	0.33	0.14	0.19	0.14
Juiciness	-0.08	0.13	0.57	0.28	0.60	--	0.27	0.32	0.17	0.30	-0.10	-0.07	0.07
Sugar	0.07	-0.09	0.37	0.15	0.50	0.54	--	0.50	0.25	0.32	0.09	0.24	0.08
Acid	-0.16	-0.09	0.37	0.23	0.45	0.52	0.50	--	0.11	0.22	0.06	0.26	0.07
Size	-0.06	-0.01	-0.03	-0.09	-0.03	0.08	0.17	0.03	--	0.37	0.07	0.11	0.08
Form	0.00	0.14	0.08	0.08	0.05	0.13	-0.05	0.04	0.33	--	0.07	0.17	0.27
Skin Toughness	0.06	0.20	-0.02	-0.31	-0.05	-0.25	-0.16	-0.18	-0.06	0.33	--	0.26	0.16
Ground Colour	0.18	0.30	-0.11	-0.16	-0.01	-0.21	-0.05	-0.33	-0.03	-0.06	0.24	--	0.20
Russet	-0.04	0.29	-0.02	-0.19	-0.05	-0.13	-0.02	-0.07	0.02	-0.03	0.13	0.29	--

Table 24. Pre-storage correlations (above diagonal) and post-storage correlations (below diagonal) among 14 apple fruit characteristics evaluated for 3 years, on the 'Golden Delicious' x 'Anna' mapping population. Correlation values r ≥ 0.65; 0.64 ≥ r or r ≥ 0.50; 0.49 ≥ r or r ≥ 0.30 and r < 0.30 were considered strong or very strong, moderately strong, moderately weak and weak or very weak, respectively. Correlation values ≥ 0.30 are highlighted in red, and negative correlations are in blue.

Trait	Stripeness	Colour	Texture	Firmness	Taste	Juiciness	Sugar	Acid	Size	Form	Skin	Ground colour	Russet
Stripeness	--	0.03	0.05	-0.19	0.08	-0.05	0.03	-0.08	0.04	0.17	-0.02	0.11	0.12
Colour	0.00	--	0.24	0.16	0.20	0.03	0.05	0.21	0.12	0.24	-0.02	0.36	0.23
Texture	-0.06	0.10	--	0.05	0.32	0.46	0.12	0.25	0.14	0.15	-0.05	0.15	0.16
Firmness	-0.10	-0.07	-0.26	--	0.03	0.11	-0.11	0.06	-0.04	-0.01	-0.26	0.03	0.05
Taste	-0.05	0.14	0.25	0.08	--	0.36	0.55	0.47	0.17	0.25	0.21	0.36	0.13
Juiciness	0.15	0.09	0.45	0.00	0.45	--	0.29	0.23	0.32	0.15	0.00	-0.05	0.02
Sugar	0.15	0.01	0.07	-0.02	0.45	0.28	--	0.54	0.21	0.17	0.23	0.30	0.06
Acid	-0.19	0.17	0.11	0.04	0.28	0.19	0.12	--	0.18	0.09	0.21	0.26	-0.02
Size	0.01	0.10	0.22	-0.18	0.18	0.28	0.25	0.17	--	0.23	0.13	0.02	0.04
Form	0.14	0.22	0.07	-0.01	0.11	0.11	0.10	0.03	0.29	--	0.09	0.21	0.31
Skin Toughness	0.15	0.07	-0.13	-0.31	0.03	-0.13	0.04	-0.05	-0.08	-0.18	--	0.23	-0.03
Ground Colour	0.26	0.15	0.15	-0.18	-0.01	0.00	0.05	-0.09	-0.12	-0.16	0.27	--	0.32
Russetting	0.10	0.18	-0.06	-0.07	-0.07	-0.04	-0.03	0.03	0.03	0.12	0.17	0.29	--

Table 25. Pre-storage correlations (above diagonal) and post-storage correlations (below diagonal) among 14 apple fruit characteristics evaluated for 3 years, on the 'Golden Delicious' x 'Priscilla' mapping population. Correlation values r ≥ 0.65; 0.64 ≥ r or r ≥ 0.50; 0.49 ≥ r or r ≥ 0.30 and r < 0.30 were considered strong or very strong, moderately strong, moderately weak and weak or very weak, respectively. Correlation values ≥ 0.30 are highlighted in red, and negative correlations are in blue.

Trait	Stripeness	Colour	Texture	Firmness	Taste	Juiciness	Sugar	Acid	Size	Form	Skin	Ground colour	Russet
Stripeness	--	-0.14	0.22	0.04	0.22	0.19	0.32	0.01	0.23	0.18	-0.14	-0.23	0.12
Colour	-0.07	--	0.09	-0.08	0.03	0.02	-0.03	0.21	-0.16	-0.02	0.25	0.47	0.33
Texture	0.16	0.01	--	0.40	0.57	0.76	0.47	0.24	0.22	0.16	-0.16	-0.02	0.20
Firmness	0.05	0.00	0.21	--	0.29	0.43	0.24	0.20	-0.01	0.09	-0.41	-0.11	0.13
Taste	0.23	0.21	0.48	0.40	--	0.66	0.66	0.48	0.20	0.22	-0.21	-0.24	0.07
Juiciness	0.26	0.00	0.71	0.30	0.53	--	0.54	0.34	0.22	0.21	-0.23	-0.17	0.13
Sugar	0.12	0.09	0.42	0.39	0.65	0.45	--	0.52	0.20	0.22	-0.08	-0.14	0.06
Acid	0.13	0.15	0.24	0.20	0.37	0.23	0.24	--	-0.03	0.15	0.05	0.04	-0.09
Size	0.05	0.20	0.06	0.00	0.18	0.13	0.14	0.01	--	0.26	-0.10	-0.23	-0.03
Form	0.01	0.29	0.00	0.23	0.22	0.05	0.24	-0.12	0.40	--	-0.06	-0.08	-0.08
Skin Toughness	-0.06	0.05	-0.01	-0.18	0.06	-0.03	0.00	-0.04	0.19	0.12	--	0.33	0.08
Ground Colour	-0.13	0.17	0.14	0.10	0.04	0.14	0.13	-0.03	0.12	0.10	0.14	--	0.35
Russetting	0.13	0.02	0.23	0.05	0.11	0.34	0.11	0.02	-0.10	-0.24	-0.01	0.16	--

3.4 Year by year correlations

Correlation analysis was performed for the three years of data collection viz. 2005, 2006 and 2007. Table 26 compares the correlations of 2005 with 2006, 2005 with 2007 and 2006 with 2007. The traits correlated were those measured subjectively and instrumentally in section 3.1.1 and 3.1.2, respectively.

Table 26. Correlation between years for subjectively and instrumentally measured fruit traits, over a three-year period. Correlation values r ≥ 0.65; 0.64 ≥ r or r ≥ 0.50; 0.49 ≥ r or r ≥ 0.30 and r < 0.30 were considered strong or very strong, moderately strong, moderately weak and weak or very weak, respectively. Correlation values ≥ 0.30 are highlighted in red.

	2005/06	2005/07	2006/07
Instrumentally measured traits			
Diameter	0.55	0.05	0.04
Mass	0.62	0.64	0.63
Firmness	0.62	0.71	0.66
%TSS	0.61	0.59	0.62
Subjectively measured traits			
Stripe-ness	0.69	0.72	0.67
Colour	0.17	0.09	0.31
Ground colour	0.12	0.14	0.17
Russet	0.28	0.37	0.31
Size	0.38	0.44	0.43
Texture	0.34	0.29	0.30
Firmness	0.05	0.22	0.26
Taste	0.11	0.09	0.17
Juiciness	0.27	0.33	0.39
Sugar content	0.09	0.09	0.16
Acidity	0.11	0.14	0.13
Form	0.24	0.24	0.17
Skin toughness	0.17	0.09	0.32

Positive correlations were observed for all subjectively measured traits, although these varied from strong to very weak. Stripe-ness showed the strongest correlation between the years, with values of 0.69, 0.72 and 0.67 seen for 2005/2006, 2005/2007 and 2006/2007, respectively. Fruit size had moderately weak correlations in 2005/2006, 2005/2007 and 2006/2007 with values of 0.38, 0.44 and 0.43 respectively. Colour, ground colour, firmness, taste, sugar content, acidity and fruit form all showed very weak correlation between 2005/2006, 2005/2007 and 2006/2007, with values well below 0.3.

Texture showed a weak positive correlation of 0.34 between 2005/2006 and this value decreased to 0.29 for 2005/2007 with not much change seen for 2006/2007. Other moderately weak correlations were seen for russet, with 2005/2006 giving a value of 0.37 and 2006/2007 having a value of 0.31. Juiciness showed similar results with correlations of 0.33 and 0.39 being seen for 2005/2006 and 2006/2007, respectively.

Instrumentally measured traits on the other hand showed strong positive correlation for all years of analysis, compared to those measured subjectively. Mass, firmness and %TSS showed a certain amount of consistency, with very strong correlations for 2005/2006, 2005/2007 and 2006/2007, with all r-values being greater than 0.59.

3.5 Summary

The different ways in which data was viewed in this section allowed us to easily identify which traits varied significantly form year to year, between the three mapping populations, as well as within the different mapping populations. It also confirmed that traits measured instrumentally gave more consistent, stronger heritability and correlation coefficient values as opposed to those same traits measured subjectively.

CHAPTER 4: GENOTYPIC RESULTS

4.1 Isolation of Genomic DNA

Total genomic DNA was successfully isolated from the three mapping populations, 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla', using the 2 x CTAB method. Figure 8 shows a 1% agarose gel with genomic DNA isolated in lanes 3 to 16. The four parental plants ('Anna', 'Golden Delicious', 'Prima' and 'Priscilla'), of the mapping population, were shown in lane 1, 2, 3 and 4, of Figure 8, respectively. Figure 8 also shows that the isolated DNA was larger than 10 kb with no RNA contamination.

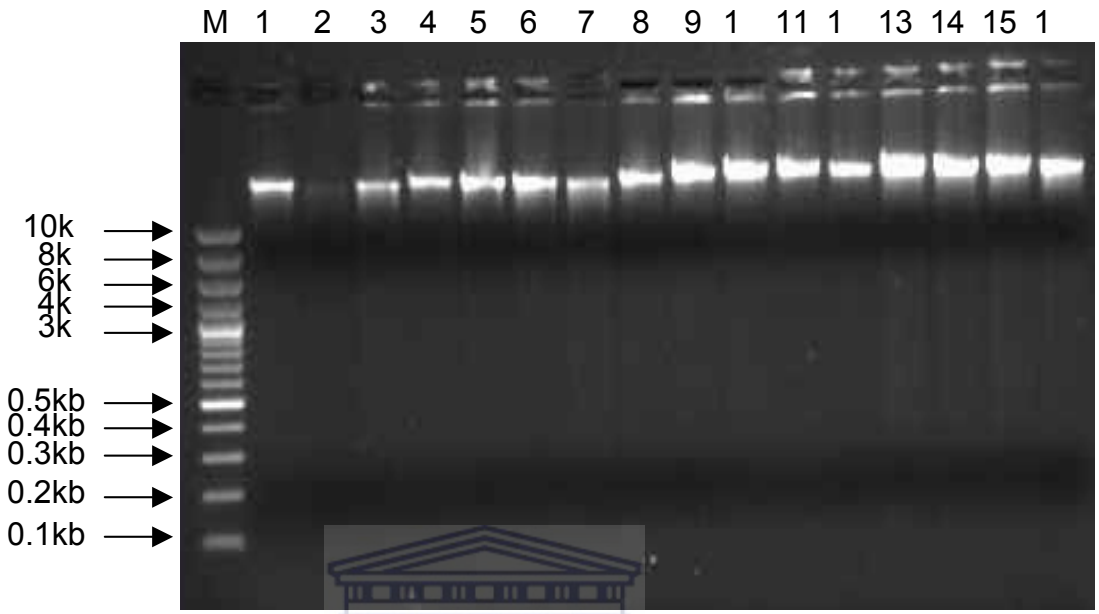


Figure 8. Agarose gel electrophoresis of genomic DNA on a 1% agarose gel. Lane M: GeneRuler™ DNA ladder. Lane 1: 'Golden Delicious', Lane 2: 'Priscilla', Lane 3: 'Anna', Lane 4: 'Prima', Lane 5-8: 'Golden Delicious' x 'Anna' seedlings (6-71, 6-86, 7-123, 7-124), Lane 9-12: 'Prima' x 'Anna' seedlings (3-129, 3-130, 4-78, 4-79), Lane 13-16: (8-110, 8-112, 8-114, 8-116). Seedlings were labelled as row number, and position in the row i.e. seedling 6-71, refers to row 6, and tree number 71.

4.2 Primer design and synthesis

540 new SSR primer pairs were designed and synthesized. Of these, 382 amplified the targeted DNA sequence. These were classified as operational. Of these primers, 297 revealed polymorphism among the nine parental cultivars used previously. These, together with 293 published microsatellites (Liebhard *et al*., 2003; Silfverberg-Dilworth *et al*., 2006) were then allocated into megaplexes and used to screen the three mapping populations. 42 of these "new" primer sets were previously unmapped in other mapping projects.

4.3 Optimisation of SSR markers

Working primers, both published (Liebhard *et al*., 2003; Silfverberg-Dilworth *et al*., 2006) and those designed in the project from EST sequences were optimized in the gradient PCR and optimal annealing temperatures for each primer pair was determined. All primers were then screened across nine parent cultivars to determine whether they were polymorphic or not (Figure 9). Table 28 shows 27 megaplexes generated from 451 (Appendix A) of the 590 markers, i.e. 293 published markers and 297 newly designed, polymorphic primer sets, which were used to screen the three mapping populations.

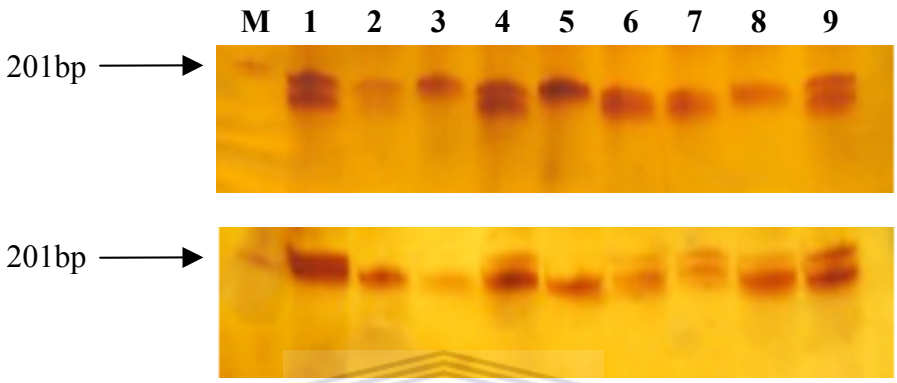


Figure 9. 6% PAGE gel showing polymorphism revealed by (top) primer SAmsCN581649 and (bottom) primer SAmsCN490740 in nine apple parents. (top) Lane M: pTz/Hinf*I* molecular weight marker, Lane 1: **'**Austin', Lane 2: 'Anna', Lane 3: 'Golden Delicious', Lane 4: 'Priscilla', Lane 5: 'Sharpe's Early', Lane 6: 'Braeburn', Lane 7: 'Cox's Orange Pippin', Lane 8: 'Mildew resistant', and Lane 9: 'Prima'.

4.4 Scoring ACS1, ACO1 and EXPANSIN-7 markers on mapping populations

ACS-1 was scored on each of the three progenies viz. 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla. In the 'Prima' x 'Anna' population, both parents, viz. 'Prima' and 'Anna' were heterozygous for ACS1, producing the ACS1-1 and ACS1-2 products, which had been reported to be 489bp and 655bp respectively (Zhu and Barritt, 2008). About 28% of the population were homozygous for ASC-1/1, while 38% were heterozygous and amplified ACS1-1/2 and 28% was homozygous for ACS1-2/2. Figure 10a shows the segregation of ACS1 in the 'Prima' x 'Anna' mapping population.

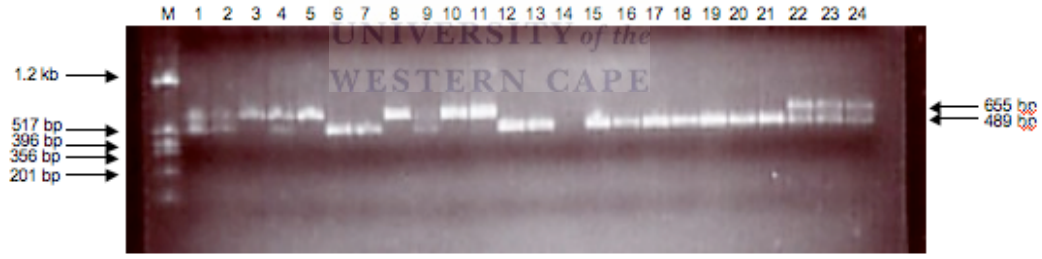


Figure 10a. Agarose gel electrophoresis showing segregation of ACS-1 in a representative sample of the 'Prima' x 'Anna' apple mapping population. Lane M- pTz/Hinf*I* molecular weight marker, Lane 1- 'Prima', Lane 2-'Anna', Lane 3-'3-124', Lane 4-'3-125', Lane 5-'3-126', Lane 6-'3-127', Lane 7-'3-128', Lane 8-'3-129', Lane 9-'3-130', Lane 10-'3-131', Lane 11-'3-132', Lane 12-'3-133', Lane 13-'3-134', Lane 14-'3-135', Lane 15-'3-136', Lane 16-'3-137', Lane 17-'3-138', Lane 18-'3-139', Lane 19-'3-140', Lane 20-'3-141', Lane 21-'3-142', Lane 22-'4-41', Lane 23-'4-44' and Lane 24-'4-45'.

Both ‘Golden Delicious’ x’ Anna’ and the ‘Golden Delicious’ x ‘Priscilla’ populations showed similar segregation patterns as ‘Prima’ x ‘Anna’, with both parents, viz. ‘Golden Delicious’, ‘Priscilla’ and ‘Anna’ being heterozygous and amplifying the ACS1-1 and ACS1-2 products.

ACO1 was scored on each of the three progenies viz. ‘Prima’ x ‘Anna’, ‘Golden Delicious’ x ‘Anna’ and ‘Golden Delicious’ x ‘Priscilla. ‘Prima’, ‘Priscilla’ and ‘Golden Delicious’ were homozygous for ACO1/2, with all amplifying the 587bp top fragment, while ‘Anna’ was heterozygous, amplifying both ACO1/1 and ACO1/2, the 525bp and 587bp fragments, respectively (Costa *et al*., 2005) (Figure 10b). About 40% of the ‘Prima’ x ‘Anna’ population was heterozygous, amplifying ACO1/1 as well as ACO1/2, 45% was homozygous, amplifying only ACO1/2.

Similar results were seen for ‘Golden Delicious’ x ‘Anna’ as ‘Golden Delicious’ was homozygous for ACO1/2, while ‘Anna’ was heterozygous amplifying both ACO1/1 and ACO1/2. Similar segregation ratios were seen in this population, as with ‘Prima’ x ‘Anna’. The third population, ‘Golden Delicious’ x ‘Priscilla’ however resulted in ACO1/2 homozygous seedlings, as both parents were homozygous for ACO1/2.

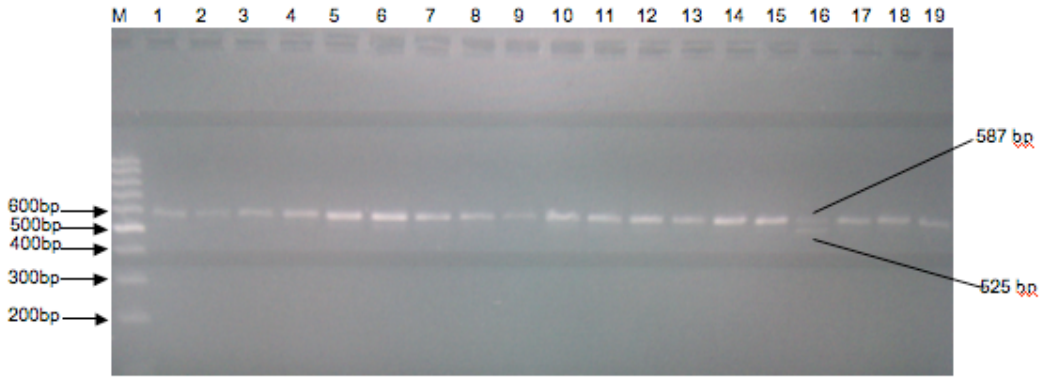


Figure 10b. 2% agarose gel electrophoresis showing segregation of ACO-1 in a representative sample of the 'Prima' x 'Anna' apple mapping population. Lane M-100bp DNA ladder, Lane 1-'4-95', Lane 2- '4-96', Lane 3- '4-97', Lane 4- '4-98', Lane 5- '4-99' Lane 6- '4-100', Lane 7- '4-101' Lane 8- '4-102', Lane 9- '4-103' Lane 10- '4-104', Lane 11- '4-105' Lane 12- '4-106', Lane 13- '4-107', Lane 14- '4-108', Lane 15- '4-109', Lane 16- 'Anna', Lane 17-'Prima', Lane 18-' Golden Delicious' and Lane 19-'Priscilla'

Expansin7 was scored on each of the three progenies viz. ‘Prima’ x ‘Anna’, ‘Golden Delicious’ x ‘Anna’ and ‘Golden Delicious’ x ‘Priscilla. ‘Priscilla’ and ‘Golden Delicious’ were homozygous for Expansin7 (Figure 10c), amplifying a 200bp fragment, while ‘Anna’ and ‘Prima’ were heterozygous, with ‘Anna’ amplifying 200bp and 202bp fragments (Figure 10c), while ‘Prima’ amplified 200bp and 214bp fragments. The sizes for ‘Golden Delicious’, ‘Priscilla’ and ‘Prima’ differed from published data, where a 198bp fragment, instead of 200bp, was reported (Costa *et al*., 2008).

The ‘Golden Delicious’ x ‘Priscilla’ population showed no segregation, with all seedlings amplifying the 200bp fragment. This was expected, as both parents were homozygous for this allele. The ‘Golden Delicious’ x ‘Anna’ population exhibited a 45% to 55% segregation ratio, as ‘Golden Delicious’ was homozygous (Figure 10c) and ‘Anna’ was heterozygous for this marker (Figure 10c). The third mapping population, viz. ‘Prima’ x ‘Anna’ exhibited approximately a 1:1:1:1 segregation ratio, with 24% segregating for 200/200bp, 26% segregating for 200/202bp, 25% segregating for 200/214bp and 21% segregating for 202/214bp.

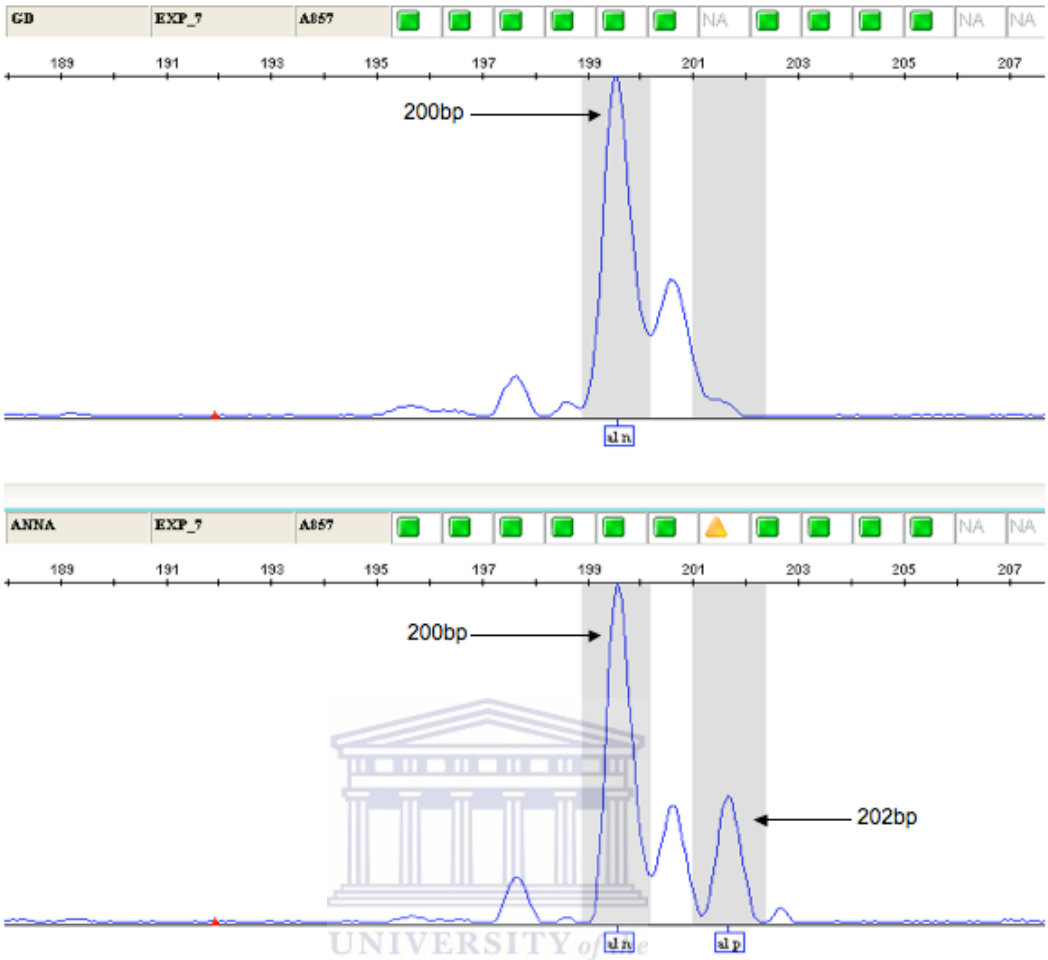


Figure 10c. Electropherogram showing the segregation of EXPANSIN7 in 'Golden Delicious' and 'Anna'.

4.5 Segregation analysis of mapping populations

The 449 of the 590 SSR markers used for segregation analysis in this study were allocated into 27 megaplexes/multiplexes. 241 of these are previously unpublished markers, generated inhouse. The other 208 SSRs are all previously published SSRs, most of which are positioned on the reference linkage map (Silfverberg-Dilworth *et al*., 2006; Liebhard *et al*., 2002, 2003). Allele sizes for each of the four parents were determined (Appendix B) so as to allocate a JoinMap code to each genotype of the three progenies. 312 SSR markers were successfully scored on the 'Prima' x 'Anna' mapping population, of which 36 were homozygous in the parents. 271 markers were successfully scored on the 'Golden Delicious' x 'Anna' progeny, of which 41 were found to be homozygous for both parents. 261 SSRs were successfully scored on the 'Golden Delicious' x 'Priscilla' mapping population, 42 of which were homozygous in the parents.

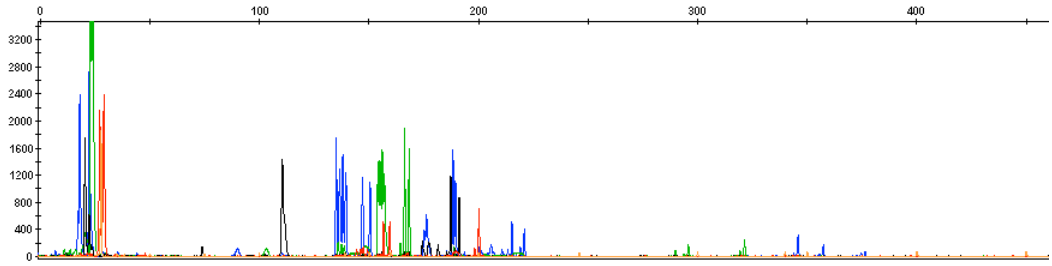
Segregation of alleles from all classes of loci was easily studied through the interpretation of electropherograms obtained from automated genetic analyzers (Figure 11). Not all markers successfully yielded alleles for both parents of the population, but JoinMap codes were easily assigned, when studying the segregation of these alleles within that specific population. An example of this is seen in Figure 12, where both parents were heterozygous for SSR marker CH04a12, and segregated within the mapping population. The presence of null alleles, which are encountered when multiplexing many primers together, could also be determined. An example of this was seen with SAmsCO068842, where 457/-bp alleles and 436/449bp alleles were scored on the 'Anna' and 'Prima'

cultivars, respectively. The presence of the null allele therefore allowed for the correct segregation ratio to be determined.

There were very few multilocus markers scored, with Hi04g11, AG11, CH03g12 identified in 'Golden Delicious', SAmsCO903298 and NZmsDR033893 identified in 'Priscilla' and SAmsDR990381 in 'Anna'. No multilocus markers were identified in 'Prima'.

DaRT Markers

A total of 492, 432 and 556 DArT markers were scored on the 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla' mapping population, respectively (Appendix F, G and H).



Sample File	Sample Name	Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Size 1	Size 2	Size 3	Size 4	Size 5	OS	BIN	AN	GQ
Run_UWC_2008-1	PRIMA	A564	m	l				146.93	150.47							
Run_UWC_2008-1	PRIMA	A256	h	k				188.31	189.48							
Run_UWC_2008-1	PRIMA	A582												NA		
Run_UWC_2008-1	PRIMA	A383	l	m				345.76	357.28							
Run_UWC_2008-1	PRIMA	A86	L1	m				166.25	168.32							
Run_UWC_2008-1	PRIMA	A577	?					219.74								
Run_UWC_2008-1	PRIMA	A761	m	l				289.8	295.64							
Run_UWC_2008-1	PRIMA	A107	n					110.39								
Run_UWC_2008-1	PRIMA	A572	e	f				187.25	191.07							
Run_UWC_2008-1	PRIMA	A586												NA		
Run_UWC_2008-1	PRIMA	A566	h	k				156.54	159.45							
Run_UWC_2008-1	PRIMA	A588	a					200.21								

Figure 11. Electropherogram obtained after amplification of 'Prima' DNA with Megaplex 26 (Table 27) on 'Prima' parent cultivar. Data are represented both graphically and in tabular form, with the table listing the SSR markers used in the megaplex, the JoinMap code allocated and the allele size scored. Red and green blocks, on the right of the figure, represent failed and passed scores respectively.

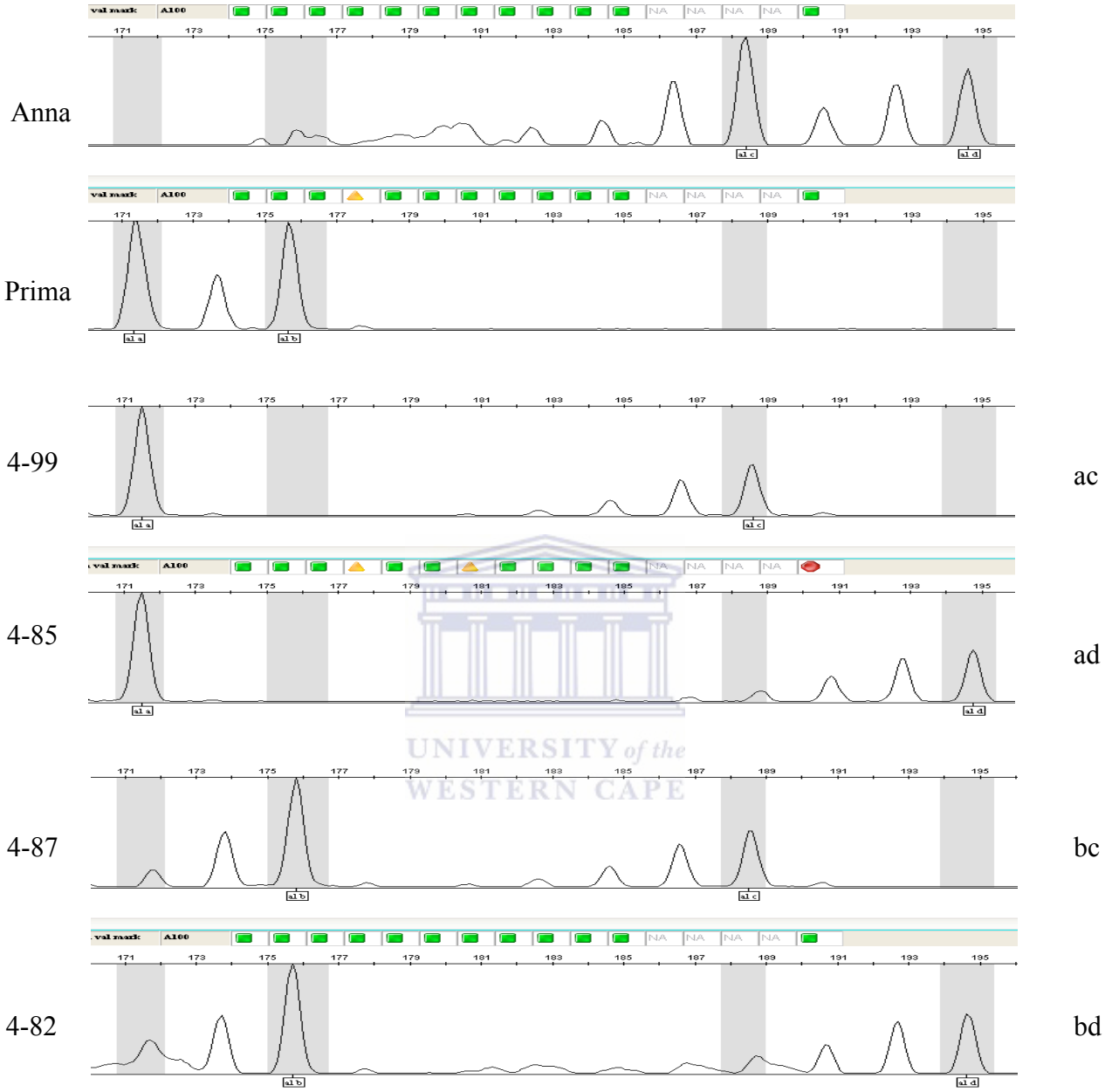
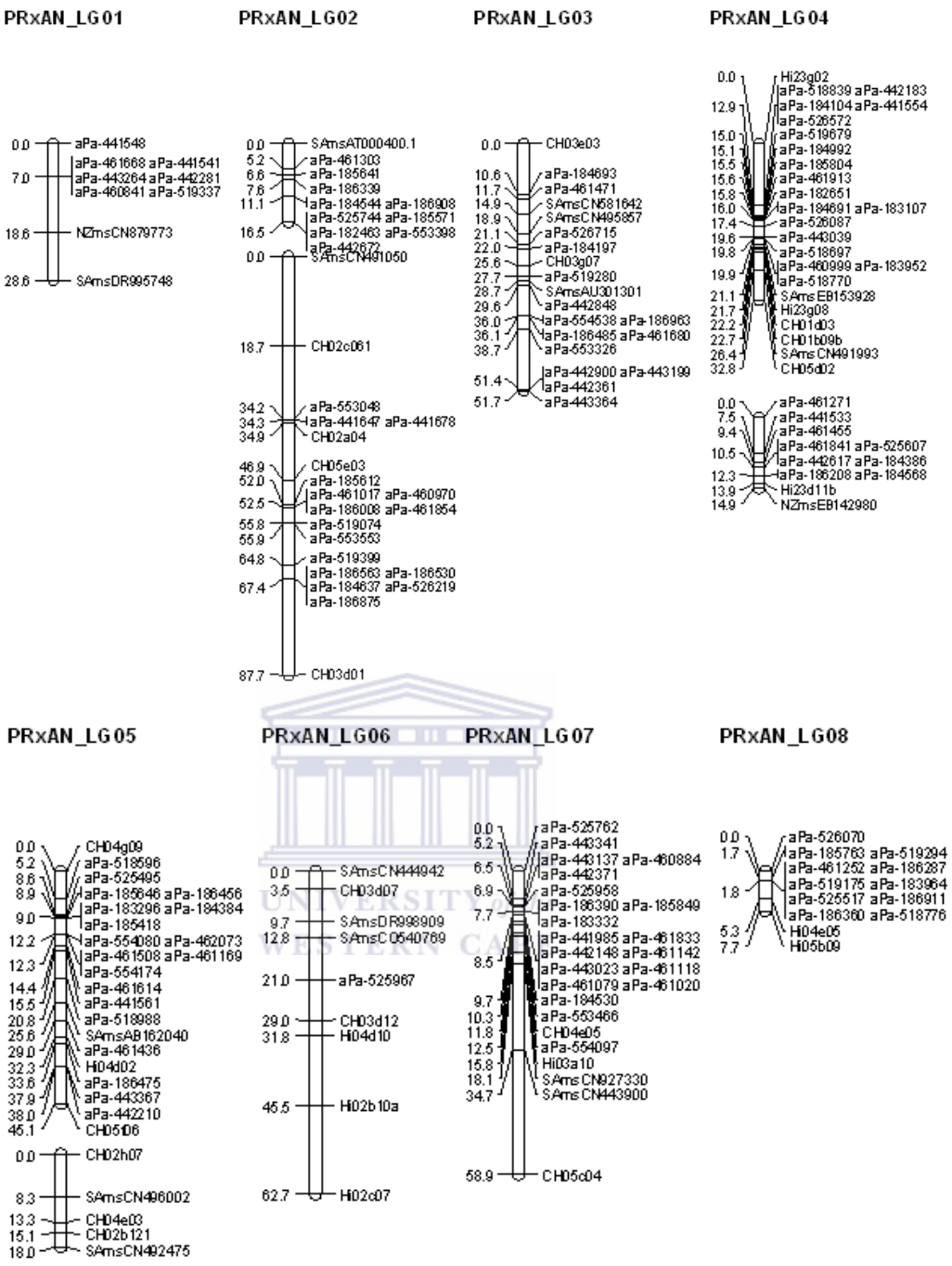


Figure 12. Electropherograms obtained after amplification of 'Prima' and 'Anna' with CH04a12 as well as the four different classes (ac, ad, bc, bd) observed in the F1 progeny (4-99, 4-85, 4-87, 4-82) derived from a cross between these two cultivars.

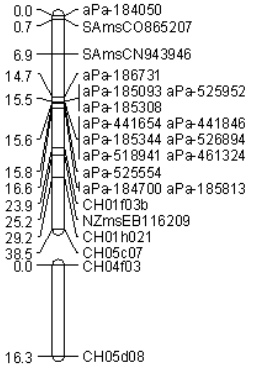
4.6 Genetic linkage map construction

Genetic linkage maps were constructed for the F1 populations derived from each of the three different mapping populations used during this study, viz 'Prima' x 'Anna' (Figure 13), 'Golden Delicious' x 'Priscilla' (Figure 14) and 'Golden Delicious' x 'Anna' (Figure 15), using JoinMap® 4. The numbering of linkage groups was in accordance with Maliepaard *et al*. (1998) and different segments belonging to the same linkage group were identified through the alignment with reference markers proposed by Silfverberg-Dilworth *et al*. (2006).

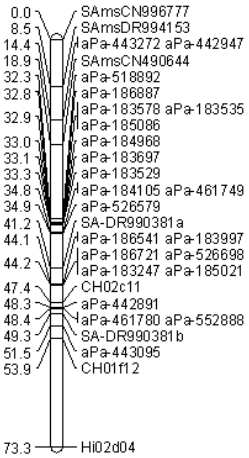
All 17 linkage groups were generated, for each genetic linkage map of the three mapping populations. 'Prima' x 'Anna' (Figure 13) was calculated to be 1021.6cM in length and consisted of 135 SSR and 265 DArT markers on 17 linkage groups. The 'Golden Delicious' x 'Priscilla' integrated map (Figure 14) consists of 353 markers in total, 80 of which are SSR markers, and 273 DArT markers on 17 linkage groups or segments thereof. The map covers a distance of 1079cM. The 'Golden Delicious' x 'Anna' integrated map (Figure 15) consists of 213 markers, in total, on 17 linkage groups, or segments thereof. In total 87 SSRs and 126 DArT markers were positioned on the genetic map that covers a distance of 1302.7cM.



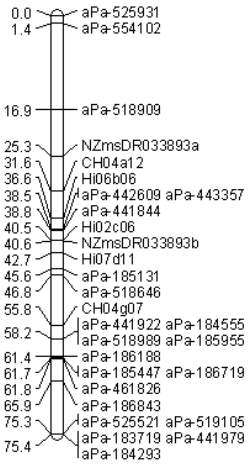
PRxAN_LG09



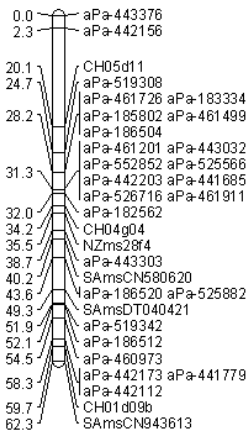
PRxAN_LG10



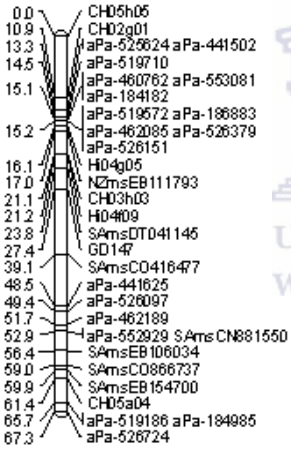
PRxAN_LG11



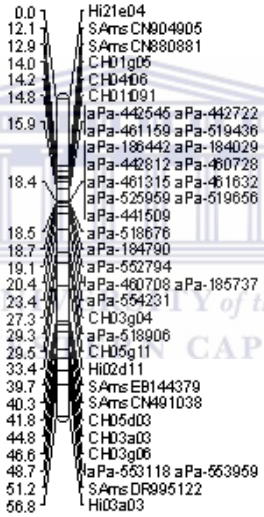
PRxAN_LG12



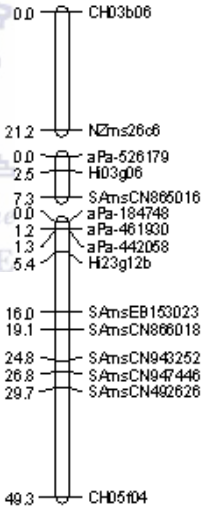
PRxAN_LG13



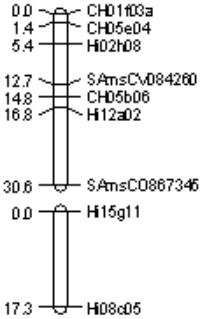
PRxAN_LG14



PRxAN_LG15



PRxAN_LG16



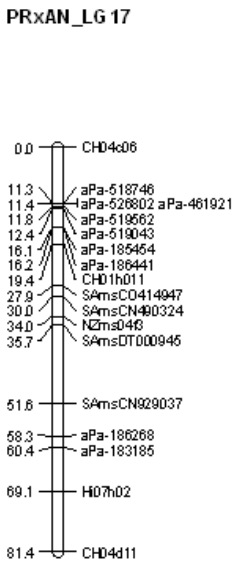
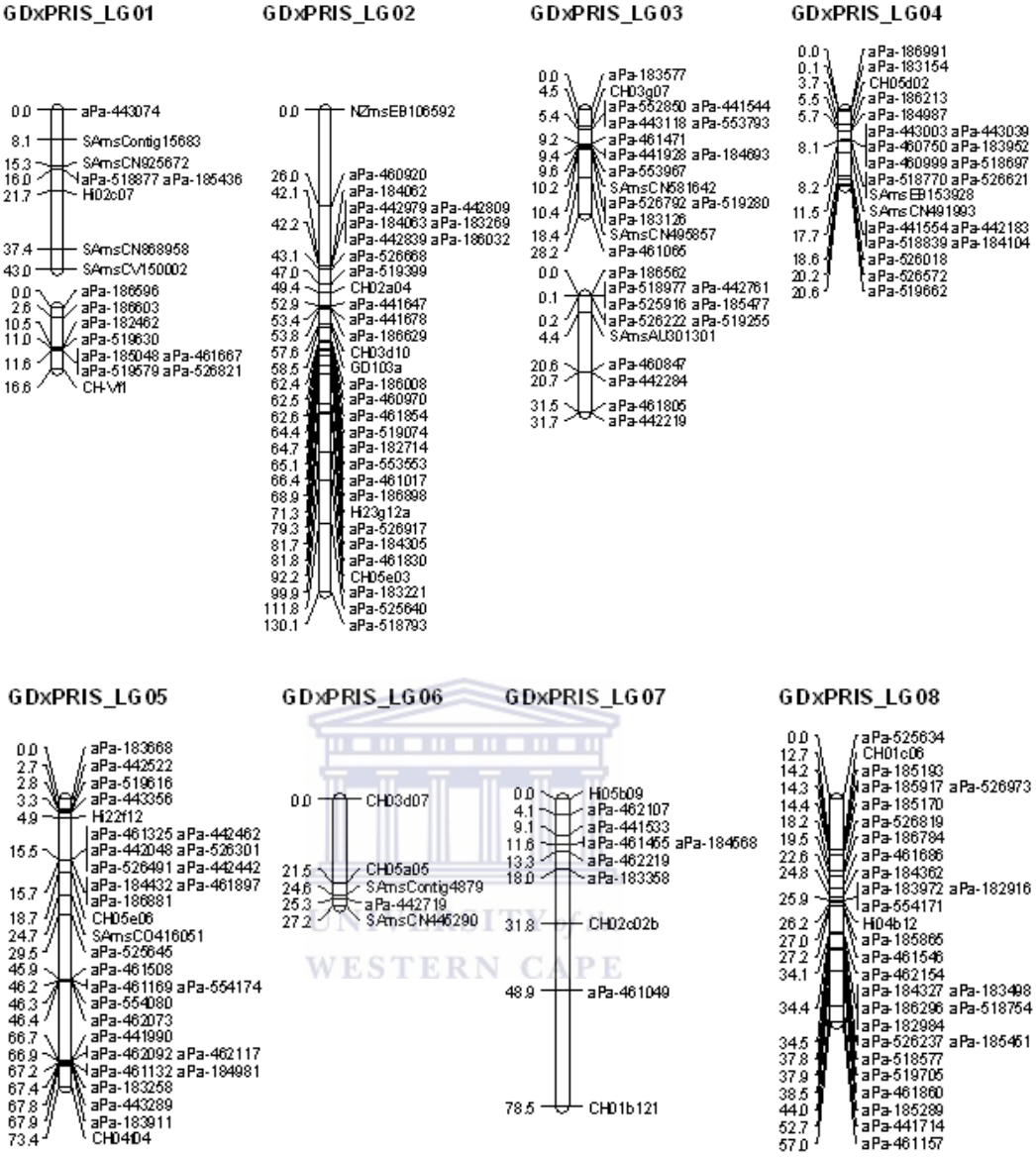


Figure 13. Genetic linkage map constructed using 87 individuals of the F1 progeny derived from a cross between 'Prima' (female parent) and 'Anna' (male parent). The 17 linkage groups obtained, are numbered in accordance with Maliepaard *et al*. (1998). Newly developed and mapped SSR markers are labelled with the prefix 'SAms'. Published markers are labelled with the prefixes 'CH', 'Hi', 'NZms' and 'MS'



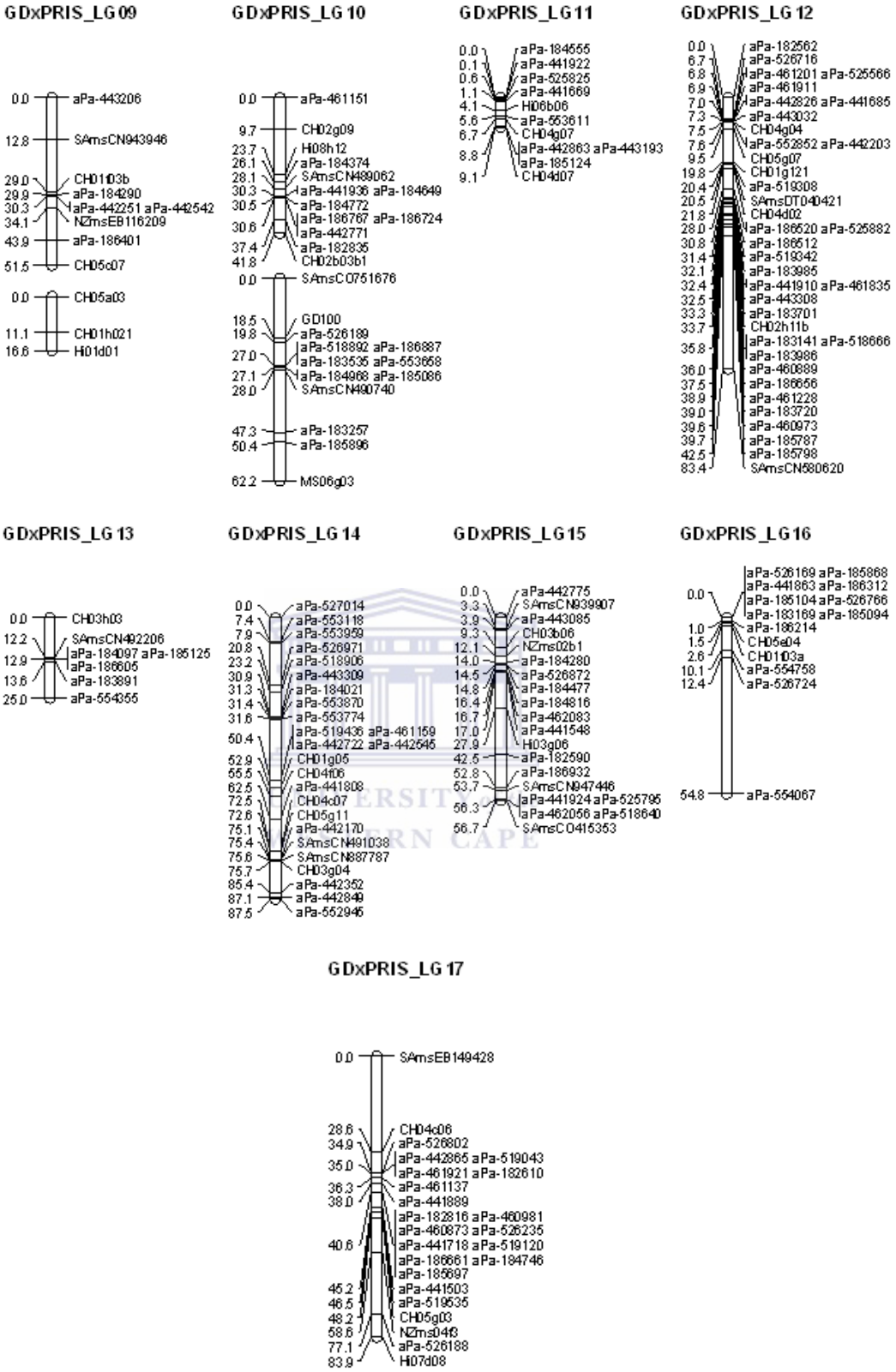
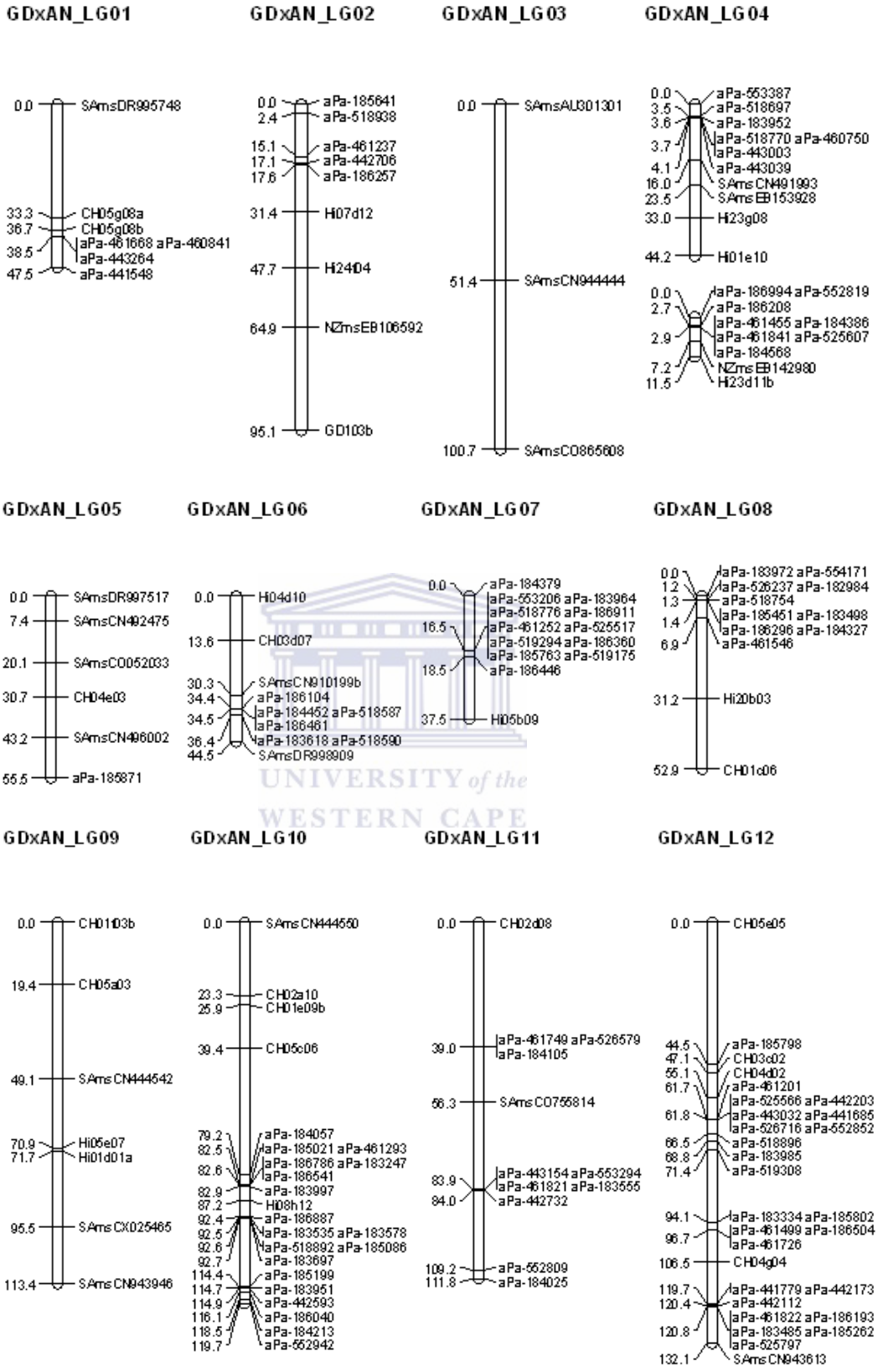

GDxPRIS_LG 09
0.0 aPa-443206
12.8 SAmsCN943946
29.0 CH01i03b
29.9 aPa-184290
30.3 aPa-442251 aPa-442542
34.1 NZmsEB116209
43.9 aPa-186401
51.5 CH05c07
0.0 CH05a03
11.1 CH01h021
16.6 Hi01d01
GDxPRIS_LG 10
0.0 aPa-461151
9.7 CH02g09
23.7 Hi08h12
26.1 aPa-184374
28.1 SAmsCN489062
30.3 aPa-441936 aPa-184649
30.5 aPa-184772
30.6 aPa-186767 aPa-186724
aPa-442771
37.4 aPa-182835
41.8 CH02b03b1
0.0 SAmsCO751676
18.5 GD100
19.8 aPa-526189
27.0 aPa-518892 aPa-186887
aPa-183535 aPa-553658
27.1 aPa-184968 aPa-185086
28.0 SAmsCN490740
47.3 aPa-183257
50.4 aPa-185896
62.2 MS06g03
GDxPRIS_LG 11
0.0 aPa-184555
0.1 aPa-441922
0.6 aPa-525825
1.1 aPa-441669
4.1 Hi06b06
5.6 aPa-553611
6.7 CH04g07
aPa-442863 aPa-443193
8.8 aPa-185124
9.1 CH04d07
GDxPRIS_LG 12
0.0 aPa-182562
6.7 aPa-526716
6.8 aPa-461201 aPa-525566
6.9 aPa-461911
7.0 aPa-442826 aPa-441685
7.3 aPa-443032
7.5 CH04g04
7.6 aPa-552852 aPa-442203
9.5 CH05g07
19.8 CH01g121
20.4 aPa-519308
20.5 SAmsDT040421
21.8 CH04d02
28.0 aPa-186520 aPa-525882
30.8 aPa-186512
31.4 aPa-519342
32.1 aPa-183985
32.4 aPa-441910 aPa-461835
32.5 aPa-443308
33.3 aPa-183701
33.7 CH02h11b
35.8 aPa-183141 aPa-518666
aPa-183986
36.0 aPa-460889
37.5 aPa-186656
38.9 aPa-461228
39.0 aPa-183720
39.6 aPa-460973
39.7 aPa-185787
42.5 aPa-185798
83.4 SAmsCN580620
GDxPRIS_LG 13
0.0 CH03h03
12.2 SAmsCN492206
12.9 aPa-184097 aPa-185125
aPa-186605
13.6 aPa-183891
25.0 aPa-554355
GDxPRIS_LG 14
0.0 aPa-527014
7.4 aPa-553118
7.9 aPa-553959
20.8 aPa-526971
23.2 aPa-518906
30.9 aPa-443309
31.3 aPa-184021
31.4 aPa-553870
31.6 aPa-553774
aPa-519436 aPa-461159
50.4 aPa-442722 aPa-442545
52.9 CH01g05
55.5 CH04f06
62.5 aPa-441808
72.5 CH04c07
72.6 CH05g11
75.1 aPa-442170
75.4 SAmsCN491038
75.6 SAmsCN887787
75.7 CH03g04
85.4 aPa-442352
87.1 aPa-442849
87.5 aPa-552946
GDxPRIS_LG 15
0.0 aPa-442775
3.3 SAmsCN939907
3.9 aPa-443085
9.3 CH03b06
12.1 NZms02b1
14.0 aPa-184280
14.5 aPa-526872
14.8 aPa-184477
16.4 aPa-184816
16.7 aPa-462083
17.0 aPa-441548
27.9 Hi03g06
42.5 aPa-182590
52.8 aPa-186932
53.7 SAmsCN947446
aPa-441924 aPa-525795
56.3 aPa-462056 aPa-518640
56.7 SAmsCO415353
GDxPRIS_LG 16
0.0 aPa-526169 aPa-185868
aPa-441863 aPa-186312
aPa-185104 aPa-526766
aPa-183169 aPa-185094
1.0 aPa-186214
1.5 CH05e04
2.6 CH01i03a
10.1 aPa-554758
12.4 aPa-526724
54.8 aPa-554067
GDxPRIS_LG 17
0.0 SAmsEB149428
28.6 CH04c06
34.9 aPa-526802
35.0 aPa-442865 aPa-519043
aPa-461921 aPa-182610
36.3 aPa-461137
38.0 aPa-441889
aPa-182816 aPa-460981
40.6 aPa-460873 aPa-526235
aPa-441718 aPa-519120
aPa-186661 aPa-184746
aPa-185697
45.2 aPa-441503
46.5 aPa-519535
48.2 CH05g03
58.6 NZms04f3
77.1 aPa-526188
83.9 Hi07d08

Figure 14. Genetic linkage map constructed using 87 individuals of the F1 progeny derived from a cross between ‘Golden Delicious’ (female parent) and 'Priscilla' (male parent). The 17 linkage groups obtained, are numbered in accordance with Maliepaard *et al*. (1998). Newly developed and mapped SSR markers are labelled with the prefix ‘SAms’. Published markers are labelled with the prefixes ‘CH’, ‘Hi’, ‘NZms’ and ‘MS’. DArT markers are labeled with the prefix ‘aPa’.



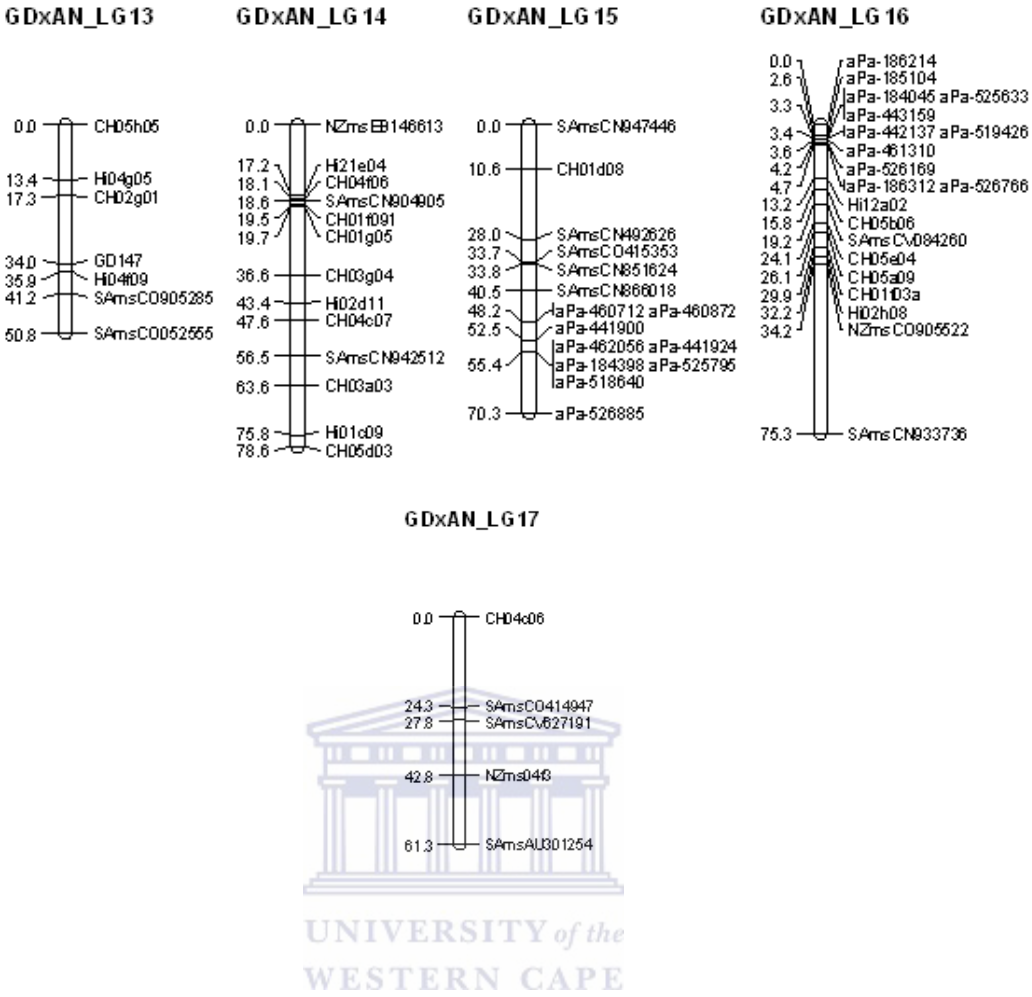


Figure 15. Genetic linkage map constructed using 141 individuals of the F1 progeny derived from a cross between ‘Golden Delicious’ (female parent) and 'Anna' (male parent). The 17 linkage groups obtained, are numbered in accordance with Maliepaard *et al*. (1998). Newly developed and mapped SSR markers are labelled with the prefix ‘SAms’. Published markers are labelled with the prefixes ‘CH’, ‘Hi’, ‘NZms’ and ‘MS’

Table 27. Newly designed SSR markers (SAms), the mapping populations in which they were mapped, as well as their position on the linkage group.

SSR Marker	Prima x Anna	Golden Delicious x Anna	GD x Priscilla	Position (LG)	Position (cM)
SAmsCN868958			X	1	37.36
SAmsCN925672			X	1	15.26
SAmsContig15683			X	1	8.09
SAmsCV150002			X	1	42.98
*SAmsDR995748	X	X		1	28.61
SAmsAT000400.1	X			2	0.00
SAmsCN491050	X			2	0.00
*SAmsAU301301	X	X	X	3	
SAmsCN495857	X		X	3	
SAmsCN581642	X		X	3	
*SAmsCN944444		X		3	49.27
*SAmsCO865608		X		3	0.00
*SAmsEB153928	X	X	X	3	
SAmsCN491993	X	X	X	4	
*SAmsAB162040	X			5	25.56
SAmsCN492475	X	X		5	
SAmsCN496002	X	X		5	
*SAmsCO052033		X		5	20.10
*SAmsCO416051			X	5	24.71
*SAmsDR997517		X		5	0.00
SAmsCN444942	X			6	0.00
SAmsCN445290			X	6	21.15
SAmsCN910199		X		6	30.26
SAmsCO540769	X			6	12.76
SAmsContig4879			X	6	24.59
SAmsDR998909	X	X		6	
SAmsCN443900	X			7	34.72
*SAmsCN927330	X			7	18.06
SAmsCN444542		X		9	49.09
SAmsCN943946	X	X	X	9	
*SAmsCO865207	X			9	0.71
*SAmsCX025465		X		9	95.53
*SAmsCN444550		X		10	119.72
SAmsCN490644	X			10	18.91
SAmsCN490740			X	10	27.99
*SAmsCN996777	X			10	0.00
*SAmsCO751676			X	10	
*SAmsDR990381	X			10	41.23
*SAmsDR994153	X			10	8.50
*SAmsCO755814		X		11	56.27
*SAmsCN580620	X		X	12	
*SAmsCN943613	X	X		12	
SAmsDT040421	X		X	12	

*SAmsCN492206			X	13	12.22
*SAmsCO052555		X		13	50.85
*SAmsCO416477	X			13	39.14
SAmsCO866737	X			13	59.02
SAmsCO905285		X		13	41.17
SAmsDT041145	X			13	23.85
*SAmsEB106034	X			13	56.41
*SAmsEB154700	X			13	59.85
*SAmsCN491038	X			14	40.29
*SAmsCN880881	X			14	12.86
SAmsCN887787			X	14	75.59
SAmsCN904905	X	X		14	
SAmsCN942512		X		14	56.45
SAmsDR995122	X			14	51.20
*SAmsEB144379	X			14	39.71
SAmsCN492626	X	X		15	
SAmsCN851624		X		15	36.56
*SAmsCN865016	X			15	
SAmsCN866018	X	X		15	
SAmsCN939907			X	15	3.33
SAmsCN492626	X	X		15	
*SAmsCN943252	X			15	
*SAmsCN947446	X	X	X	15	
*SAmsCO415353		X	X	15	
SAmsEB153023	X			15	
SAmsCN933736		X		16	0.00
SAmsCO867345	X			16	30.59
*SAmsCV084260	X	X		16	
*SAmsAU301254		X		17	61.31
*SAmsCN490324	X			17	29.97
*SAmsCN929037	X			17	51.57
*SAmsCO414947	X	X		17	
SAmsCV627191		X		17	27.76
SAmsDT000945	X			17	35.70
SAmsEB149428			X	17	0.00

* represents SSR markers published by Van Dyk *et al*. (2010)

Twenty-three new SSR markers (Table 27)(Figure 13) were positioned on the integrated genetic linkage map for 'Prima' x 'Anna', with nine previously unmapped, published markers (Liebhard *et al*., 2002, 2003) also positioned. These were CH01b09b, CH01d03 (LG4), CH02h07 (LG5), CH04f03 (LG9), CH02h11b (LG12), CH03a03 (LG13), CH03g06 (LG14), CH01f03a (LG16) and CH04d11 (LG17). Differences in marker position were observed for 12 markers in this population. Liebhard *et al*. (2002, 2003) reported that the locus amplified by CH04g09 was to be found on LG10, but it was positioned on LG5 in this study. After BLASTing the SSRs in this study against the apple genome contigs, released by Velasco *et al*. (2010), it was found that LG 5 was a more accurate position for the marker CH04g09 (Appendix I). Two other markers, viz. Hi02b10 and Hi02c07 were mapped to LG6, after reportedly amplifying loci on LGs 16 and 1, respectively (Silfverberg-Dilworth *et al*., 2006). The BLAST results for Hi02b10, did not confirm our result, but rather that of Silfverberg-Dilworth *et al*. (2006), as contig MDC012438.222 was one of the 10 top matches, with a 92% identity to this chromosome. Hi02c07 also mapped to LG 6, even though it shared 98% identity with chromosome 1. Marker CH05c04 was reportedly found on LG13 but it was positioned on LG7 in this study. This position differs from the top ten best matches, for this marker, on the apple genome. Hi05b09 was positioned on LG8 in this study after Silfverberg-Dilworth *et al*. (2006) placed it on LG7. This new position was not one of the top ten best matches for the marker, as the highest identity was found on LG 7. Markers CH05e05 and CH05a04 were positioned on LG12 and 13 in this study after being reportedly being placed on LG14 and 16, respectively. Another two markers, CH01f09 and Hi03a03 were

placed on LG14, whereas previously they were placed on LG8 and 6, respectively. LG 14 was the best match for Hi03a03 when it was BLASTed against the apple genome contigs and showing a 98% identity to the chromosome. CH01f09 however, was shown to a position on LG 8, as reported by Liebhard *et al*. (2002). The last two markers, CH05f04 and Hi08c05 were placed on LG15 and 16, but were reportedly placed on LG8 and 14, respectively. BLAST results confirmed these outputs, as CH05f04 had a 98% identity to LG 15, while the best match of Hi08c05 was previously unanchored to the apple genome. There was one multi-locus marker in this population, viz. Hi23g12, with the second locus (Hi23g12b) mapping to LG15 in this study, confirming the results after using the BLAST algorithm. ACS1 segregated on this population, but failed to map to the correct LG, viz. LG 15.

Fifteen new SSR markers (Table 27)(Figure 14) were positioned on the 'Golden Delicious' x 'Priscilla' genetic map, five of which were also found on the 'Prima' x 'Anna' map. These include SAmsCN491993, SAmsCN495857, SAmsCN581642, SAmsCN943946 and SAmsDT040421. There were five discrepancies on this map viz. CH02c02b and CH01b121 that were reported to be found on LG4 was now placed on LG7. Although both of these markers were now positioned on LG 7, BLAST searches identified LG 4 as the correct LG for CH02c02b, with 98% identity to LG 4. The best identity (100%), however, was found to be LG 8. CH01b121, however, was best positioned to LG 12, according to BLAST analyses. CH02g09 was placed on LG10 after reportedly being found on LG8. BLAST analyses confirmed the map position reported by Liebhard *et al*.

(2002). Hi07d08 was placed on LG17 after it was reportedly placed on LG1. The most accurate marker position was LG 9, which matched the contig with 99% identity. Finally, the multi-locus Hi23g12 placed on LG8 of the 'Fiesta' x 'Discovery' map (Liebhard *et al*., 2003) was now placed on LG 2. Six previously unmapped published SSRs were located on this maps, viz. GD100 (LG10), CH04f04 (LG5), CH05a03 (LG09), 02b1 (LG15), GD103 (LG02) and CH02h11b (LG12). Van Dyk *et al*. (2010) recently mapped the locus amplified by CH02h11b to LG12 on 'Golden Delicious' x 'Anna' and 'Sharpe's Early' x 'Anna'.

Sixteen new SSR markers (Table 27)(Figure 15) were located on the 'Golden Delicious' x 'Anna' genetic map, nine of which were also found on the 'Prima' x 'Anna' map (Table 28). These were SAmsCN491993, SAmsCN492425, SAmsCN492626, SAmsCN496002, SAmsCN866018, SAmsCN904905, SAmsCN943946, SAmsDR998909 and SAmsEB153928. Seven previously unmapped, published markers were also positioned, viz. GD103 (LG2), Ch05a03 (LG9), Ch01e09b (LG10), CH03a03 (LG14), CH01f03a, CH05a09 (LG16) and 04f3 (LG17) Again, there were some discrepancies on this map, with markers CH05e05, Ch05c06 and CH01f091 all amplifying loci on linkage groups other than those published for the 'Fiesta' x 'Discovery' reference map (Liebhard *et al*., 2002, 2003). CH05e05 and was reportedly mapped to LG14, now amplified loci on LG12. The BLAST analysis performed against the apple genome contigs, however, revealed that neither of these linkage groups are the best position, as LG 1 had the highest identity with this marker. CH05c06 is now mapped to LG10, when reportedly it amplified a locus on LG16. This was an inaccurate position for

the marker, as LG 16 was identified as the chromosome with the highest identity. The final discrepancy occurred on LG14, where CH01f091 was mapped, after reportedly being mapped to LG8. The marker, CH01f091, was correctly mapped to LG 8 by Liebhard *et al*. (2002). However, the mean chi-squared contribution for this marker within the LG was low (0.543), and suggested that CH01f091 fitted well with other markers on LG 14.

For the 'Prima' x 'Anna' mapping population only one individual was excluded based on missing data, while no loci was excluded when generating this map. For the 'Golden Delicious' x 'Anna', 54 seedlings were excluded, while 46 loci were excluded when generating the map. Although this number of seedlings seems high, they were excluded, as no DArT data was available for these seedlings, giving them a high ratio of missing data points. For the 'Golden Delicious' x 'Priscilla' mapping population, six seedlings had more than 25% missing data points and four loci contained more than 40% data was missing. These were removed from the analysis when generating the 'Golden Delicious' x 'Priscilla' integrated map.

Markers already positioned on the 'Golden Delicious' x 'Anna' genetic map (van Dyk *et al*., 2010), found in any of these three mapping populations were not considered 'new' markers. Table 27 shows 36 'new' previously unpublished markers located on the mapping populations in this study.

4.7 QTL Identification

Maximum likelihood interval mapping, Kruskal-Walllis mapping and restricted multiple QTL mapping (rMQM) were used to identify regions on the map of 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla', for QTLs responsible for subjective and instrumentally measured traits involved in fruit quality. QTLs were identified, over three years, viz. 2005, 2006, 2007 and a mean of these years, for the following traits, colour, stripe-ness, size, form, ground colour, russet, texture, firmness, taste, juiciness, sweetness (sugar content), acidity, skin toughness, mass, diameter and % total soluble solids (% TSS).

rMQM mapping in the 'Prima' x 'Anna' yielded 30 pre-storage QTLs, for the mean of the years, one each for stripe-ness, size, form, ground colour, russet, sugar content, acidity, juiciness, taste and skin toughness; two for colour, texture and %TSS; three for russet and mass, and five for diameter(Table 28, 29) (Figure 16). A genome-wide LOD threshold of 3.8 was used for all traits, with only taste and skin toughness showing LOD values lower than the threshold (Table 28). All QTLs were detected on the integrated map for this population (Figure 16).

The 'Golden Delicious' x 'Anna' mapping population was also subjected to rMQM analyses, and QTLs were detected for all the traits mentioned above. 27 pre-storage QTLs were detected for the mean of the years (Figure 17), one for stripe-ness (3.7), colour (3.1), size (4.2), firmness (4.4), sweetness (4.3), skin

toughness (3.2) and %TSS (3.7); two for texture (3.8), juiciness (4.4), acidity (5.3), form (3.27), taste (6.1) and mass (7.1); three for form (3.9) and seven for diameter (26.4) (Table 28)(Figure 17). No QTLs were detected for ground colour and russet in this population. The highest LOD score for each trait is shown in brackets and percentage of the population variance explained by the QTL is shown in Table 28.

31 pre-storage QTLs were detected on the map of the 'Golden Delicious' x 'Priscilla' mapping population (Figure 18). One QTL was detected for firmness (4.3), acidity (3.6), taste (5.6), %TSS (5.2). Two QTLs were detected for mass (8.6), ground colour (7.4), colour (4.7), size (6.3), form (7.5), russet (10.7), juiciness (7.1) sweetness (7.7), skin toughness (5.1) and diameter (7.4). Three QTLs were detected for stripe-ness (28.2) and four were detected for texture (6.7). The highest LOD score for each trait is shown in brackets and percentage of the population explained is shown in Table 28.

All three mapping populations were also evaluated for post-storage QTLs. However, QTLs detected from post-storage analysis differed from those in pre-storage, with the exception of a few. In the 'Prima' x 'Anna' population, 24 post-storage QTLs were detected (Figure 19) and of this only stripe-ness, fruit form, fruit firmness and %TSS produced common QTLs in both pre- and post-storage. The QTL for stripe-ness was found on LG 9 while the QTL for firmness was found on LG 15, in both analyses. A QTL for fruit form was found on LG 2, while %TSS had two QTLs on LG 2 and 15. Single QTLs were identified for

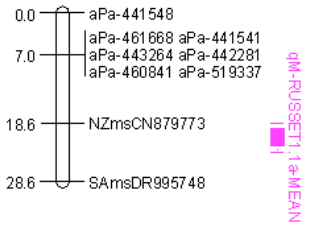
stripe-ness, colour, texture, firmness, acidity, taste and skin toughness with russet, size, juiciness and sugar content having two QTLs each. Three QTLs were identified for form, ground colour and %TSS. The lowest population variance explained was 13.7%, on LG 16 for taste and the highest variance (56.3%) was seen for colour on LG 3. The highest LOD score for any trait was 7.1, observed for size on LG 14 and sugar content on LG 15.

Seventeen post-storage QTLs were detected in the 'Golden Delicious' x 'Anna' (Figure 20). Of these, 5 are common between pre- and post-storage analyses. These QTLs include texture (LG 2), firmness (LG 10), juiciness (LG 10), taste (LG 11) and %TSS (LG 2). Two QTLs were only detected for colour (4.8) and form (5.1), whereas all other traits were detected as single QTLs. The highest LOD score for these two traits are shown in brackets. The QTL with the highest population variance was texture, yielding a variance of 74.6%, with a corresponding LOD score of 6.6. This QTL was found on LG 2 of the 'Golden Delicious' x 'Anna' population. Juiciness, acidity and skin toughness were traits displaying LOD scores below the threshold of 3.8, with values of 3.7, 3.4 and 3.2 respectively. Table 29 shows the LOD scores and variances obtained for all traits on each of the three mapping populations. The highest LOD score for each trait is shown in brackets and percentage of the population variance explained by the QTL is shown in Table 29.

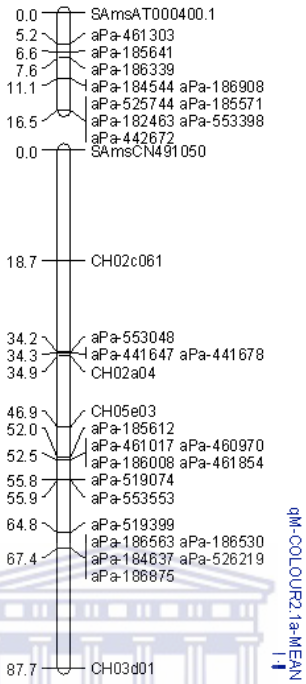
The 'Golden Delicious' x 'Priscilla' mapping population yielded 21 post-storage QTLs (Figure 21), with a LOD score of 12.2 and 83.7% of the variance explained

by the QTL, for ground colour. The QTL for stripe-ness has a LOD score of 13.7 and explained 84% of the population variance. Two QTLs were detected for colour (4.8), size (3.6), russet (7.5), taste (6.0) and skin toughness (7.2), with all other traits only detecting a single QTL (Table 29). Only stripe-ness, russet and texture produced QTLs common to both pre- and post-storage. Stripe-ness was found on LG 9, russet on LG 2 and texture on LG 14. Size, form and firmness were the only traits to produce LOD scores below the threshold of 3.8, with values of 3.6, 3.5 (size), 3.2 and 3.5 for form and firmness respectively. The highest LOD score for those traits detecting more than one QTL is shown in brackets.

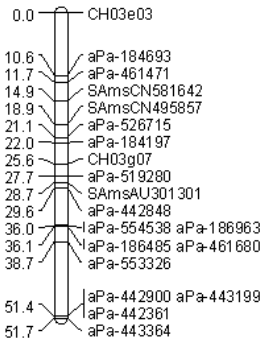
PRxAN_LG01



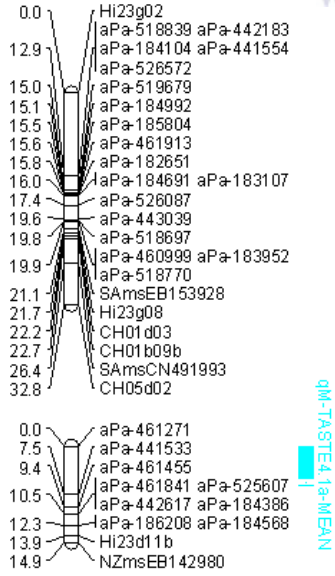
PRxAN_LG02



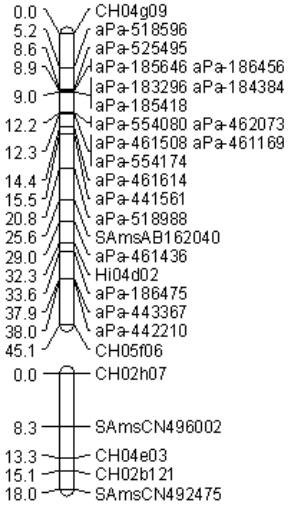
PRxAN_LG03



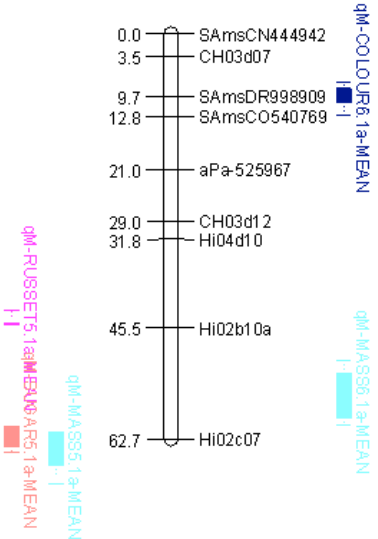
PRxAN_LG04



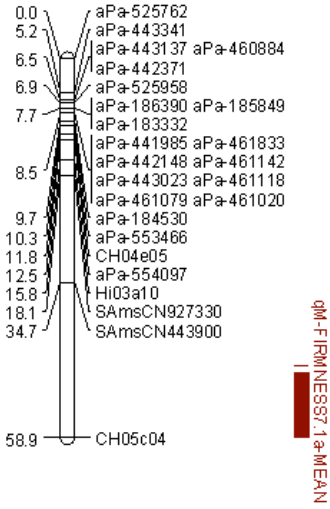
PRxAN_LG05



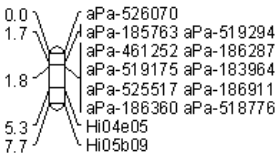
PRxAN_LG06



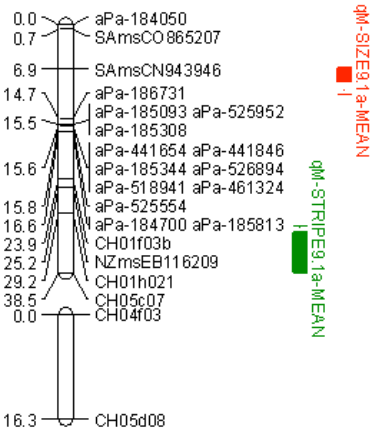
PRxAN_LG07



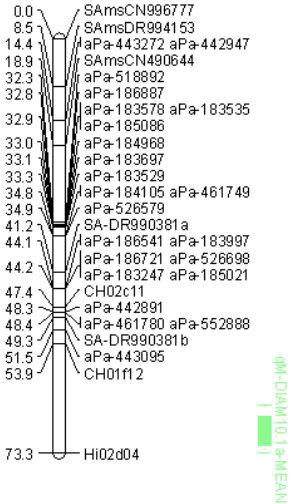
PRxAN_LG08



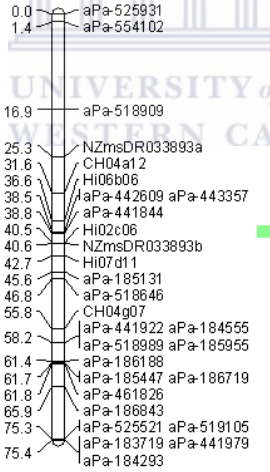
PRxAN_LG09



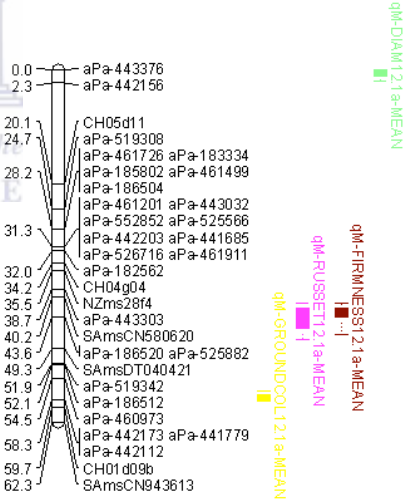
PRxAN_LG10



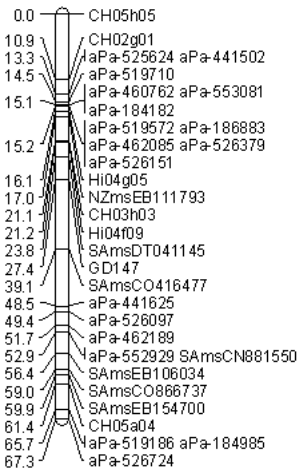
PRxAN_LG11



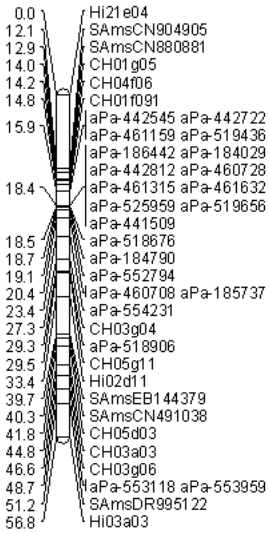
PRxAN_LG12



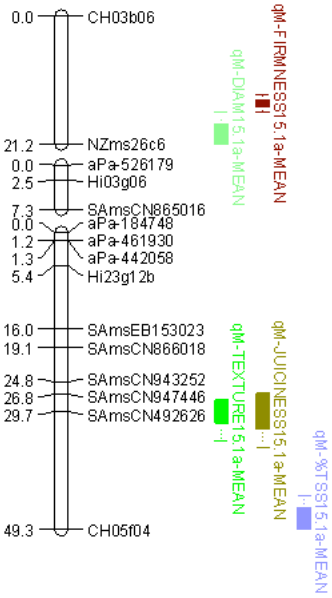
PRxAN_LG13



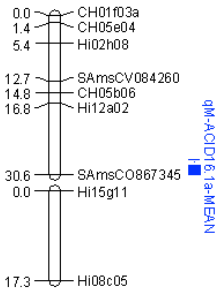
PRxAN_LG14



PRxAN_LG15



PRxAN_LG16



PRxAN_LG17

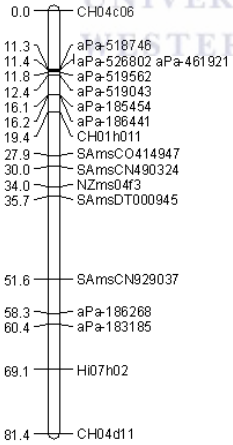
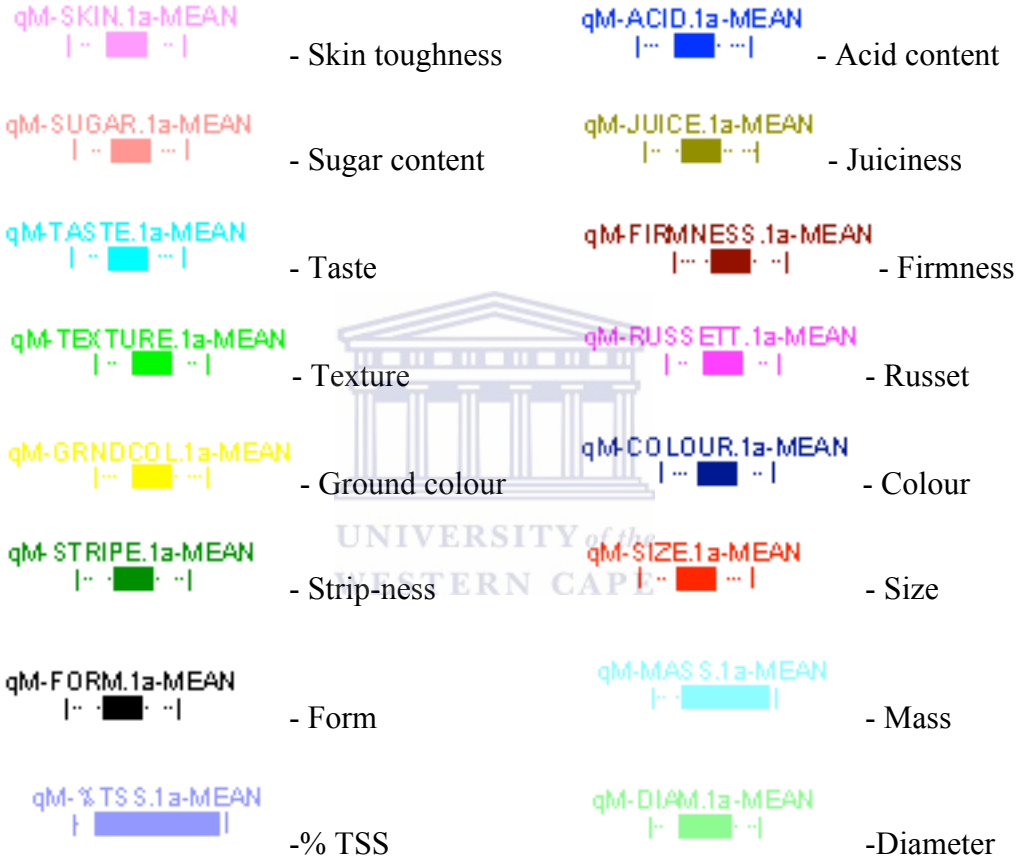
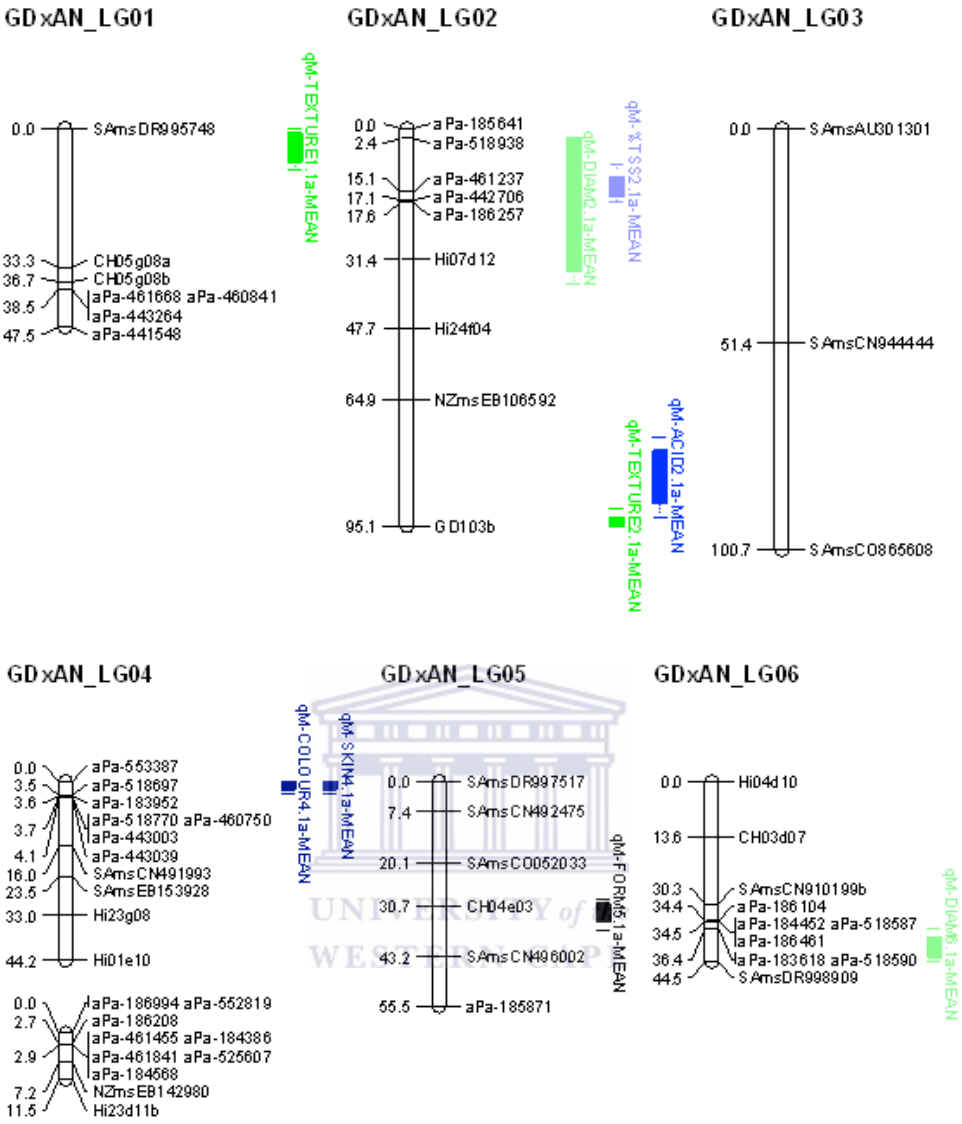
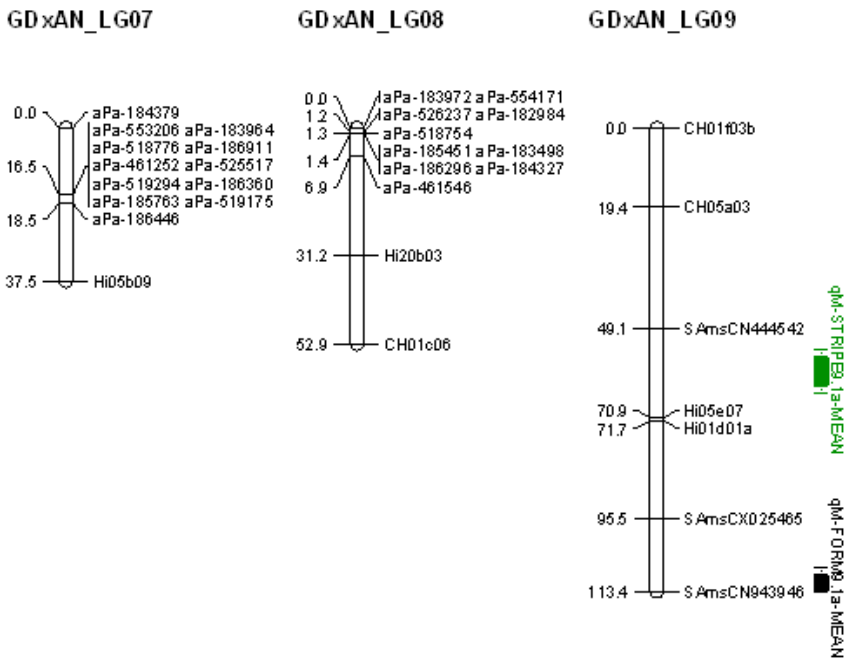


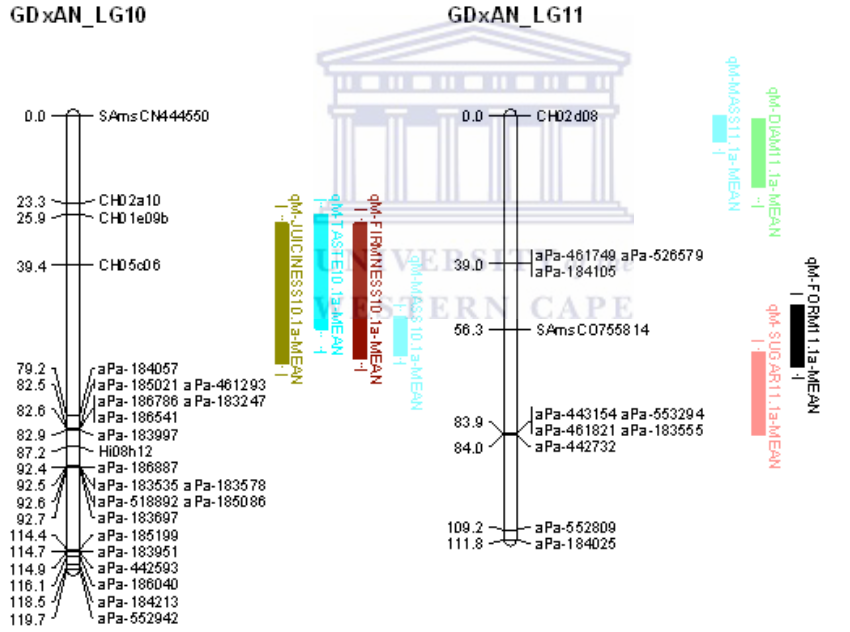
Figure 16. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Prima' x 'Anna' mapping population, pre-storage, using rMQM mapping. The 17 linkage groups obtained are numbered according Maliepaard *et al*. (1998). QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

QTL Colour Legend:









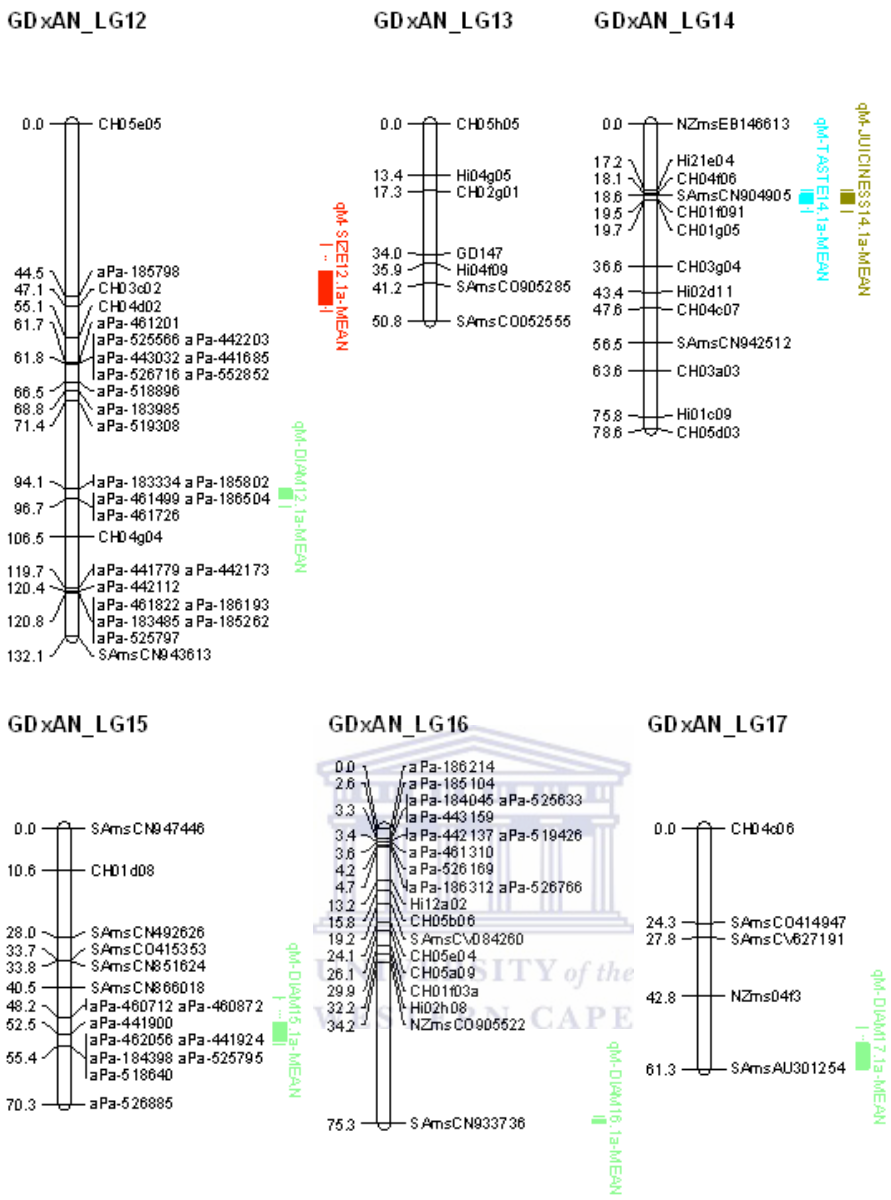


Figure 17. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Golden Delicious' x 'Anna' mapping population, pre-storage, using rMQM mapping. The 17 linkage groups obtained, are numbered according Maliepaard *et al*. (1998). All QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

QTL Colour Legend:

- Skin toughness

- Acid content

- Sugar content

- Juiciness

- Taste

- Firmness

- Texture

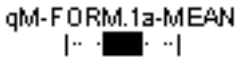
- Russet

- Ground colour

- Colour

- Strip-ness

- Size

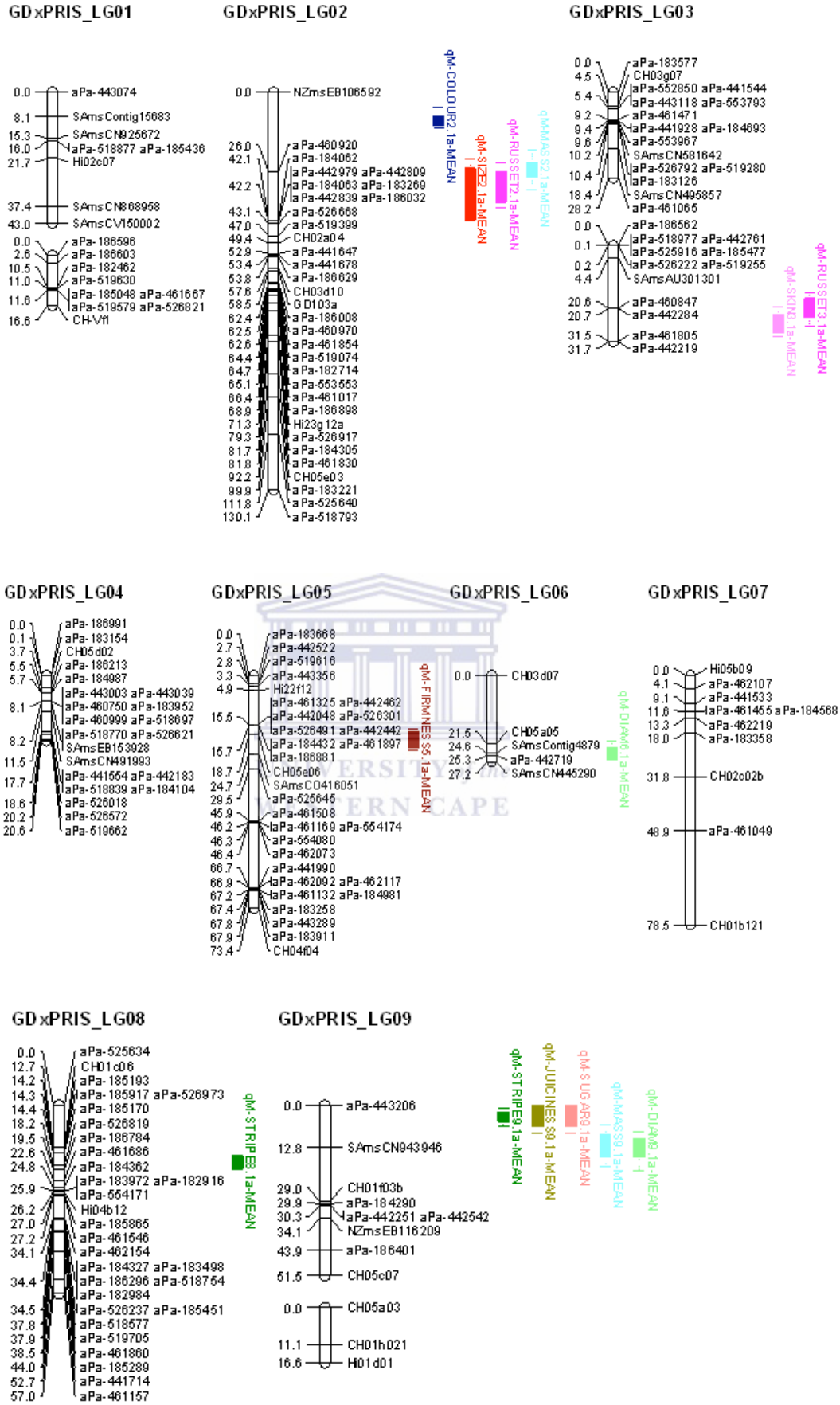


- Form

- Mass

-% TSS

-Diameter


GDxPRIS_LG01
0.0 aPa-443074
8.1 SAmsContig15683
15.3 SAmsCN925672
16.0 aPa-518877 aPa-185436
21.7 Hi02c07
37.4 SAmsCN868958
43.0 SAmsCV150002
0.0 aPa-186596
2.6 aPa-186603
10.5 aPa-182462
11.0 aPa-519630
11.6 aPa-185048 aPa-461667
11.6 aPa-519579 aPa-526821
16.6 CH-Vfl
GDxPRIS_LG02
0.0 NZmsEB106592
26.0 aPa-460920
42.1 aPa-184062
aPa-442979 aPa-442809
42.2 aPa-184063 aPa-183269
aPa-442839 aPa-186032
43.1 aPa-526668
47.0 aPa-519399
49.4 CH02a04
52.9 aPa-441647
53.4 aPa-441678
53.8 aPa-186629
57.6 CH03d10
58.5 GD103a
62.4 aPa-186008
62.5 aPa-460970
62.6 aPa-461854
64.4 aPa-519074
64.7 aPa-182714
65.1 aPa-553553
66.4 aPa-461017
68.9 aPa-186898
71.3 Hi23g12a
79.3 aPa-526917
81.7 aPa-184305
81.8 aPa-461830
92.2 CH05e03
99.9 aPa-183221
111.8 aPa-525640
130.1 aPa-518793
qM-COLOUR2.1a-MEAN
qM-SIZE2.1a-MEAN
qM-RUSSET2.1a-MEAN
qM-MAS2.1a-MEAN
GDxPRIS_LG03
0.0 aPa-183577
4.5 CH03g07
5.4 aPa-552850 aPa-441544
aPa-443118 aPa-553793
9.2 aPa-461471
9.4 aPa-441928 aPa-184693
9.6 aPa-553967
10.2 SAmsCN581642
10.4 aPa-526792 aPa-519280
aPa-183126
18.4 SAmsCN495857
28.2 aPa-461065
0.0 aPa-186562
0.1 aPa-518977 aPa-442761
aPa-525916 aPa-185477
0.2 aPa-526222 aPa-519255
4.4 SAmsAU301301
20.6 aPa-460847
20.7 aPa-442284
31.5 aPa-461805
31.7 aPa-442219
qM-SKIN2.1a-MEAN
qM-RUSSET3.1a-MEAN
GDxPRIS_LG04
0.0 aPa-186991
0.1 aPa-183154
3.7 CH05d02
5.5 aPa-186213
5.7 aPa-184987
aPa-443003 aPa-443039
8.1 aPa-460750 aPa-183952
aPa-460999 aPa-518697
8.2 aPa-518770 aPa-526621
11.5 SAmsEB153928
SAmsCN491993
17.7 aPa-441554 aPa-442183
aPa-518839 aPa-184104
18.6 aPa-526018
20.2 aPa-526572
20.6 aPa-519662
GDxPRIS_LG05
0.0 aPa-183668
2.7 aPa-442522
2.8 aPa-519816
3.3 aPa-443356
4.9 Hi22f12
aPa-461325 aPa-442462
15.5 aPa-442048 aPa-526301
aPa-526491 aPa-442442
15.7 aPa-184432 aPa-461897
aPa-186881
18.7 CH05e06
24.7 SAmsCO416051
29.5 aPa-526645
45.9 aPa-461508
46.2 aPa-461169 aPa-554174
46.3 aPa-554080
46.4 aPa-462073
66.7 aPa-441990
66.9 aPa-462092 aPa-462117
67.2 aPa-461132 aPa-184981
67.4 aPa-183258
67.8 aPa-443289
67.9 aPa-183911
73.4 CH04f04
qM-FIRMNESS6.1a-MEAN
GDxPRIS_LG06
0.0 CH03d07
21.5 CH05a05
24.6 SAmsContig4879
25.3 aPa-442719
27.2 SAmsCN445290
qM-DIAM6.1a-MEAN
GDxPRIS_LG07
0.0 Hi05b09
4.1 aPa-462107
9.1 aPa-441533
11.6 aPa-461455 aPa-184568
13.3 aPa-462219
18.0 aPa-183358
31.8 CH02c02b
48.9 aPa-461049
78.5 CH01b121
GDxPRIS_LG08
0.0 aPa-525634
12.7 CH01c06
14.2 aPa-185193
14.3 aPa-185917 aPa-526973
14.4 aPa-185170
18.2 aPa-526819
19.5 aPa-186784
22.6 aPa-461686
24.8 aPa-184362
aPa-183972 aPa-182916
25.9 aPa-554171
26.2 Hi04b12
27.0 aPa-185865
27.2 aPa-461546
34.1 aPa-462154
aPa-184327 aPa-183498
34.4 aPa-186296 aPa-518754
aPa-182984
34.5 aPa-526237 aPa-185451
37.8 aPa-518577
37.9 aPa-519705
38.5 aPa-461860
44.0 aPa-185289
52.7 aPa-441714
57.0 aPa-461157
qM-STRIPE8.1a-MEAN
GDxPRIS_LG09
0.0 aPa-443206
12.8 SAmsCN943946
29.0 CH01f03b
29.9 aPa-184290
30.3 aPa-442251 aPa-442542
34.1 NZmsEB116209
43.9 aPa-186401
51.5 CH05c07
0.0 CH05a03
11.1 CH01h021
16.6 Hi01d01
qM-STRIPE9.1a-MEAN
qM-JUICINESS9.1a-MEAN
qM-SUGAR9.1a-MEAN
qM-MAS9.1a-MEAN
qM-DIAM9.1a-MEAN

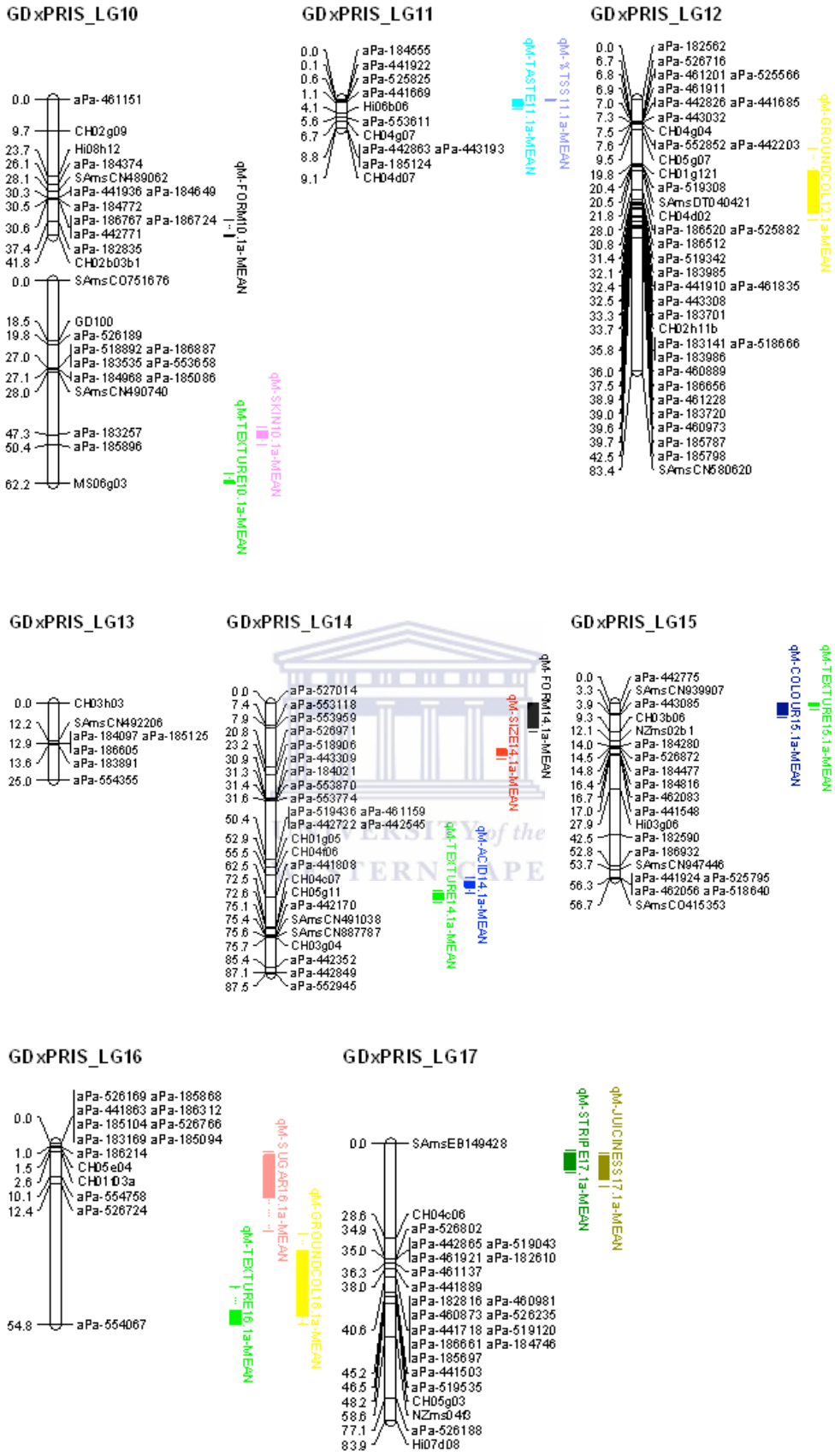
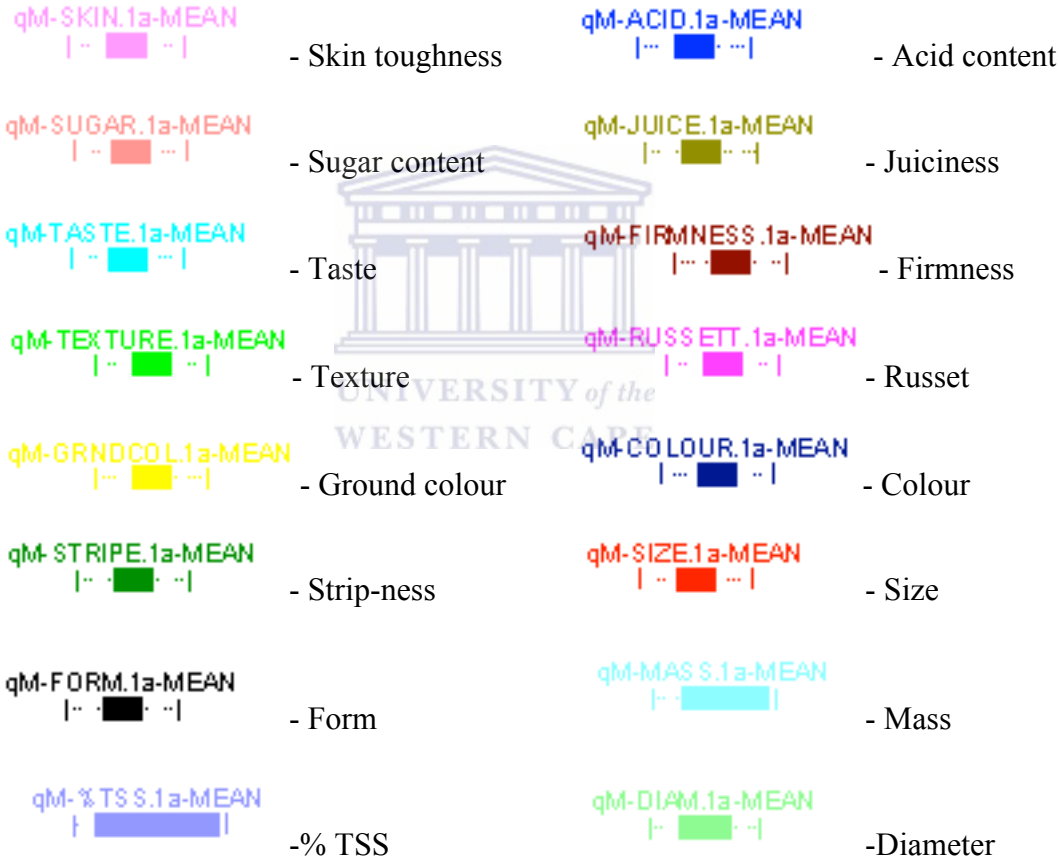
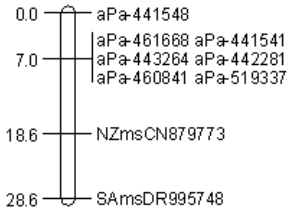

GDxPRIS_LG10
0.0 aPa-461151
9.7 CH02g09
23.7 Hi08h12
26.1 aPa-184374
28.1 SAmsCN489062
30.3 aPa-441936 aPa-184649
30.5 aPa-184772
30.6 aPa-186767 aPa-186724
37.4 aPa-442771
41.8 aPa-182835
CH02b03b1
0.0 SAmsCO751676
18.5 GD100
19.8 aPa-526189
27.0 aPa-518892 aPa-186887
27.1 aPa-183535 aPa-553658
28.0 aPa-184968 aPa-185086
SAmsCN490740
47.3 aPa-183257
50.4 aPa-185896
62.2 MS06g03
qM-FORM10.1a-MEAN
qM-SKIN10.1a-MEAN
qM-TEXTURE10.1a-MEAN
GDxPRIS_LG11
0.0 aPa-184555
0.1 aPa-441922
0.6 aPa-525825
1.1 aPa-441669
4.1 Hi06b06
5.6 aPa-553611
6.7 CH04g07
8.8 aPa-442863 aPa-443193
9.1 aPa-185124
CH04d07
qM-TASTE11.1a-MEAN
qM-%TSS11.1a-MEAN
GDxPRIS_LG12
0.0 aPa-182562
6.7 aPa-526716
6.8 aPa-461201 aPa-525566
6.9 aPa-461911
7.0 aPa-442826 aPa-441685
7.3 aPa-443032
7.5 CH04g04
7.6 aPa-552852 aPa-442203
9.5 CH05g07
19.8 CH01g121
20.4 aPa-519308
20.5 SAmsDT040421
21.8 CH04d02
28.0 aPa-186520 aPa-525882
30.8 aPa-186512
31.4 aPa-519342
32.1 aPa-183985
32.4 aPa-441910 aPa-461835
32.5 aPa-443308
33.3 aPa-183701
33.7 CH02h11b
35.8 aPa-183141 aPa-518666
aPa-183986
36.0 aPa-460889
37.5 aPa-186656
38.9 aPa-461228
39.0 aPa-183720
39.6 aPa-460973
39.7 aPa-185787
42.5 aPa-185798
83.4 SAmsCN580620
qM-GROUNDCOL12.1a-MEAN
GDxPRIS_LG13
0.0 CH03h03
12.2 SAmsCN492206
12.9 aPa-184097 aPa-185125
aPa-186605
13.6 aPa-183891
25.0 aPa-554355
GDxPRIS_LG14
0.0 aPa-527014
7.4 aPa-553118
7.9 aPa-553959
20.8 aPa-526971
23.2 aPa-518906
30.9 aPa-443309
31.3 aPa-184021
31.4 aPa-553870
31.6 aPa-553774
50.4 aPa-519436 aPa-461159
aPa-442722 aPa-442545
52.9 CH01g05
55.5 CH04f06
62.5 aPa-441808
72.5 CH04c07
72.6 CH05g11
75.1 aPa-442170
75.4 SAmsCN491038
75.6 SAmsCN887787
75.7 CH03g04
85.4 aPa-442352
87.1 aPa-442849
87.5 aPa-552945
qM-FORM14.1a-MEAN
qM-SIZE14.1a-MEAN
qM-ACID14.1a-MEAN
qM-TEXTURE14.1a-MEAN
GDxPRIS_LG15
0.0 aPa-442775
3.3 SAmsCN939907
3.9 aPa-443085
9.3 CH03b06
12.1 NZms02b1
14.0 aPa-184280
14.5 aPa-526872
14.8 aPa-184477
16.4 aPa-184816
16.7 aPa-462083
17.0 aPa-441548
27.9 Hi03g06
42.5 aPa-182590
52.8 aPa-186932
53.7 SAmsCN947446
aPa-441924 aPa-525795
56.3 aPa-462056 aPa-518640
56.7 SAmsCO415353
qM-COLOUR15.1a-MEAN
qM-TEXTURE15.1a-MEAN
GDxPRIS_LG16
0.0 aPa-526169 aPa-185868
aPa-441863 aPa-186312
aPa-185104 aPa-526766
aPa-183169 aPa-185094
1.0 aPa-186214
1.5 CH05e04
2.6 CH01d03a
10.1 aPa-554758
12.4 aPa-526724
54.8 aPa-554067
qM-SUGAR16.1a-MEAN
qM-TEXTURE16.1a-MEAN
qM-GROUNDCOL16.1a-MEAN
GDxPRIS_LG17
0.0 SAmsEB149428
28.6 CH04c06
34.9 aPa-526802
35.0 aPa-442865 aPa-519043
aPa-461921 aPa-182610
36.3 aPa-461137
38.0 aPa-441889
aPa-182816 aPa-460981
aPa-460873 aPa-526235
40.6 aPa-441718 aPa-519120
aPa-186661 aPa-184746
aPa-185697
45.2 aPa-441503
46.5 aPa-519535
48.2 CH05g03
58.6 NZms048
77.1 aPa-526188
83.9 Hi07d08
qM-STRIPE17.1a-MEAN
qM-JUICINESS17.1a-MEAN

Figure 18. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Golden Delicious' x 'Priscilla' mapping population, pre-storage, using rMQM mapping. The 17 linkage groups obtained, are numbered according to Maliepaard *et al*. (1998). All QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

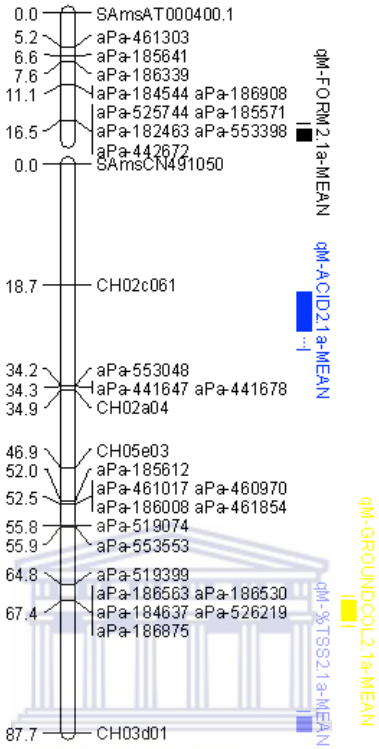
QTL Colour Legend:



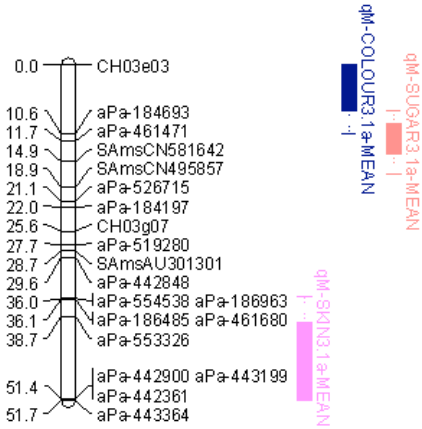
PRxAN_LG01



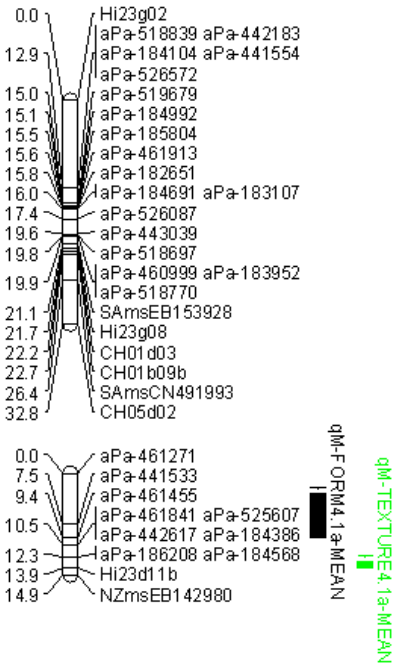
PRxAN_LG02



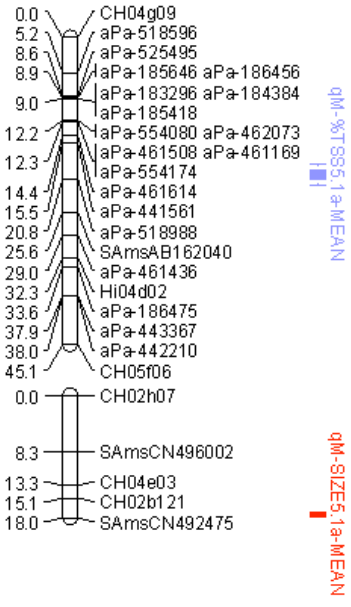
PRxAN_LG03



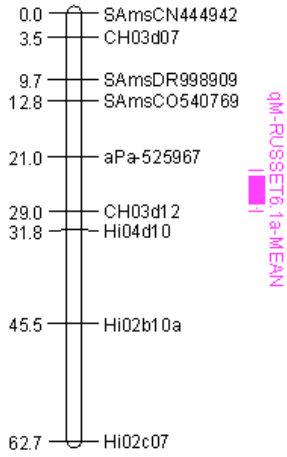
PRxAN_LG04



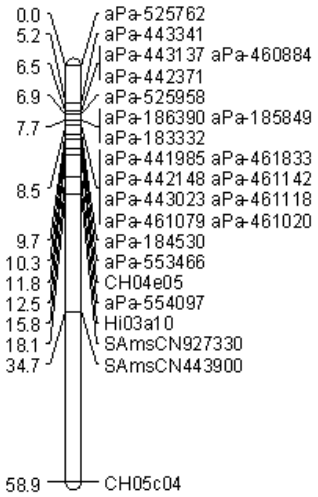
PRxAN_LG05



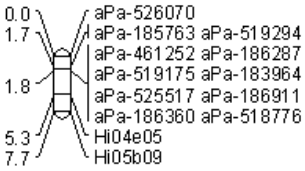
PRxAN_LG06



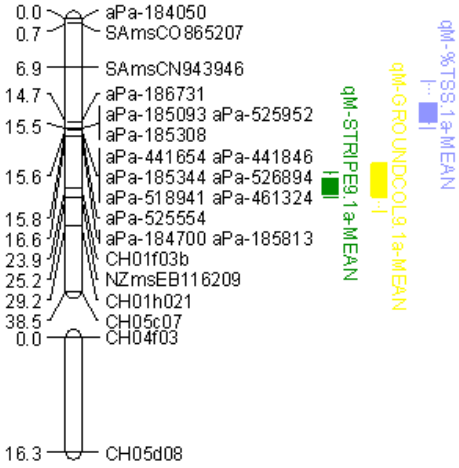
PRxAN_LG07



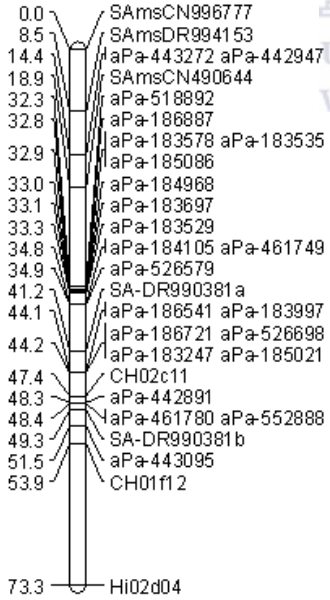
PRxAN_LG08



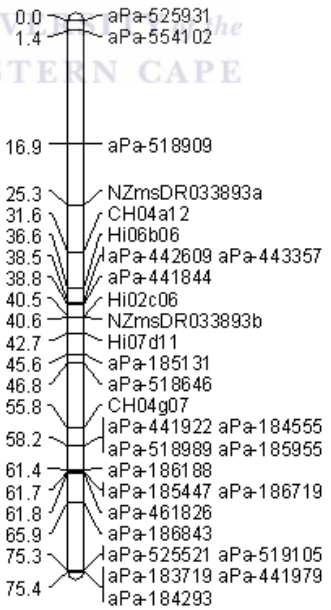
PRxAN_LG09



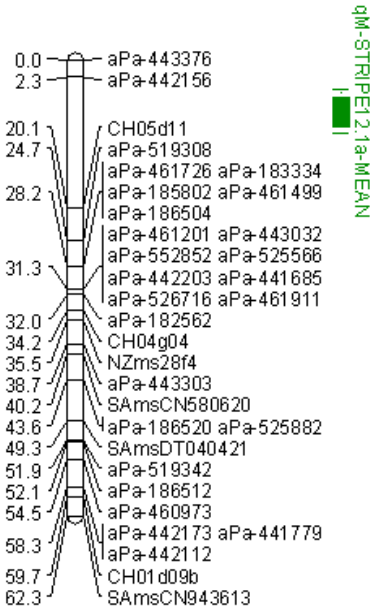
PRxAN_LG10



PRxAN_LG11



PRxAN_LG12



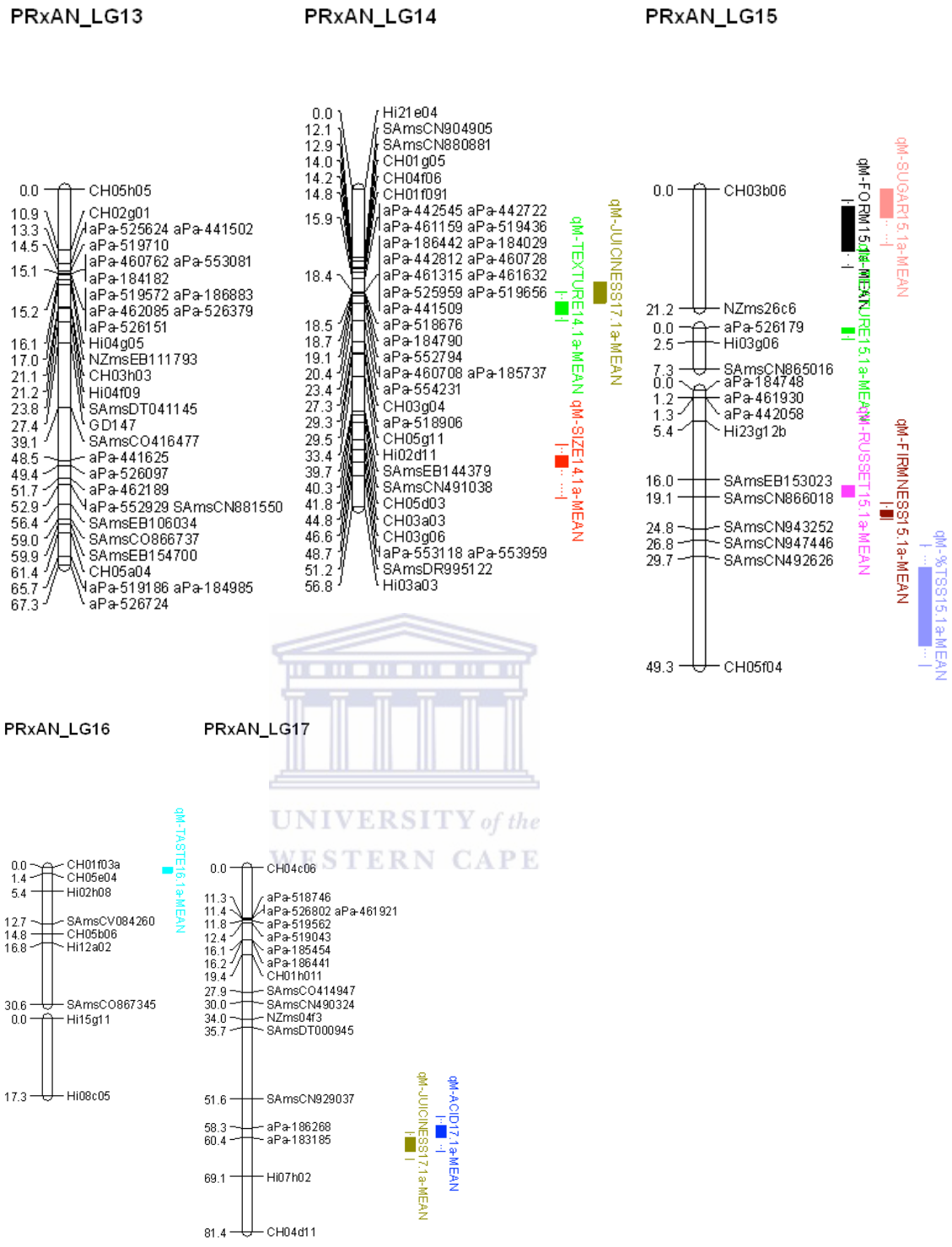
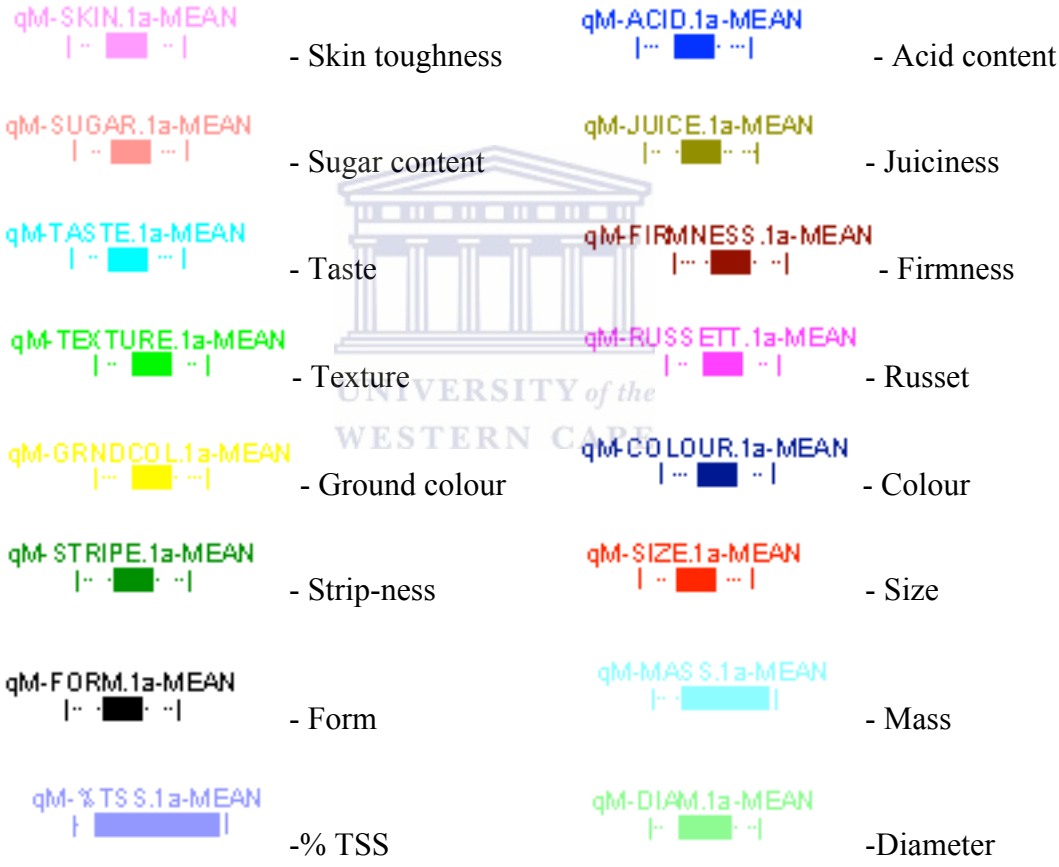
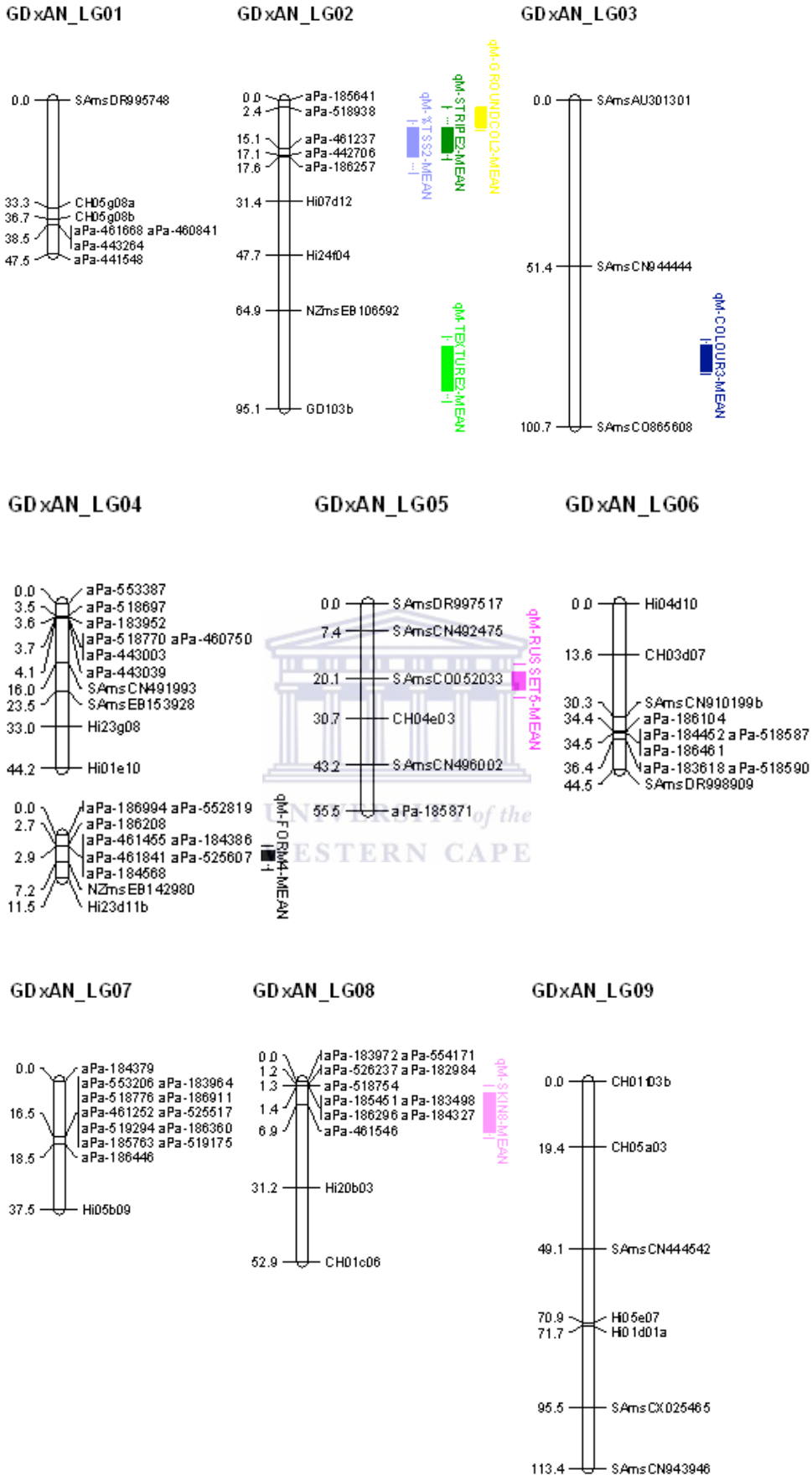


Figure 19. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Prima' x 'Anna' mapping population, post-storage, using rMQM mapping. The 17 linkage groups obtained, are numbered according to Maliepaard *et al*. (1998). All QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

QTL Colour Legend:





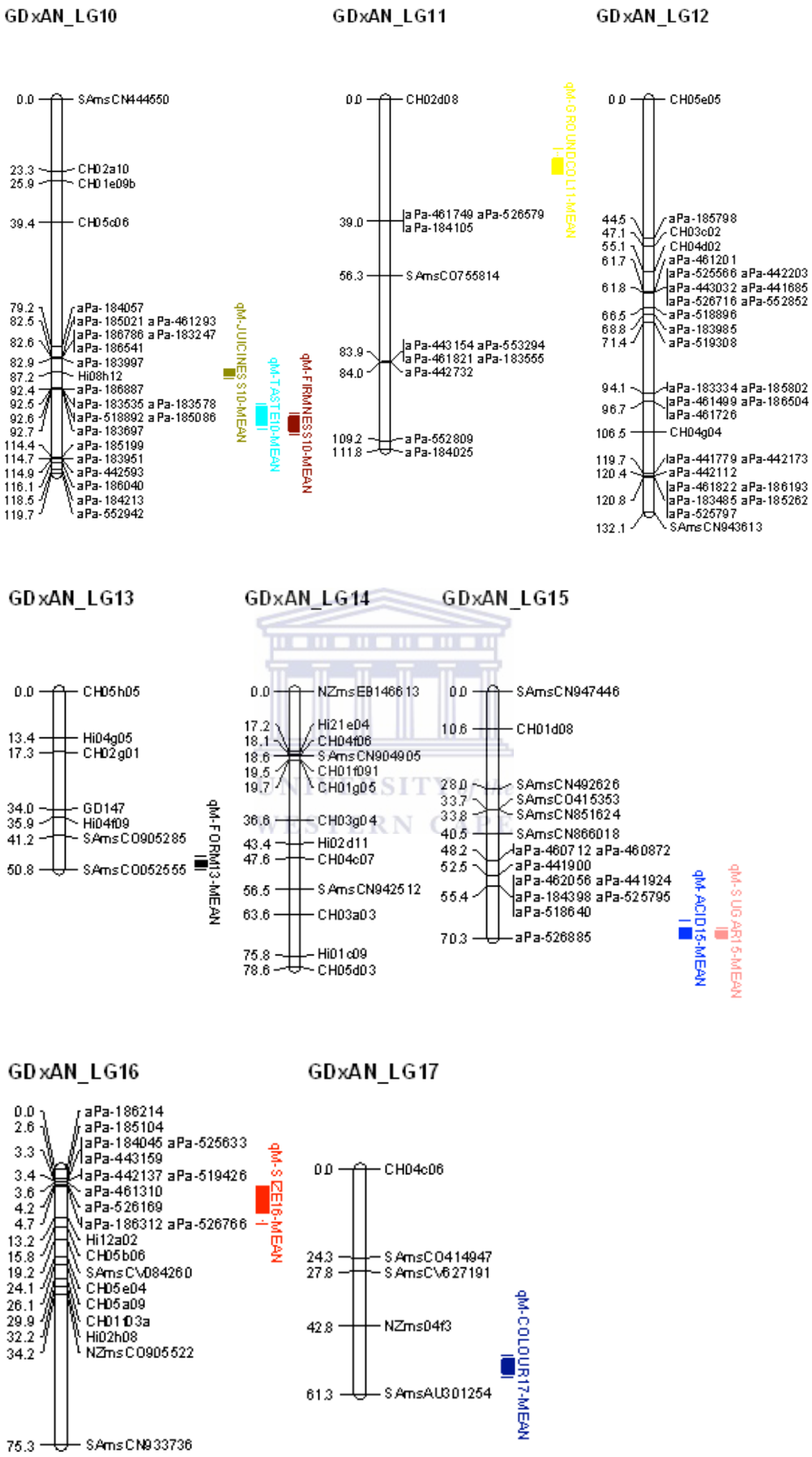
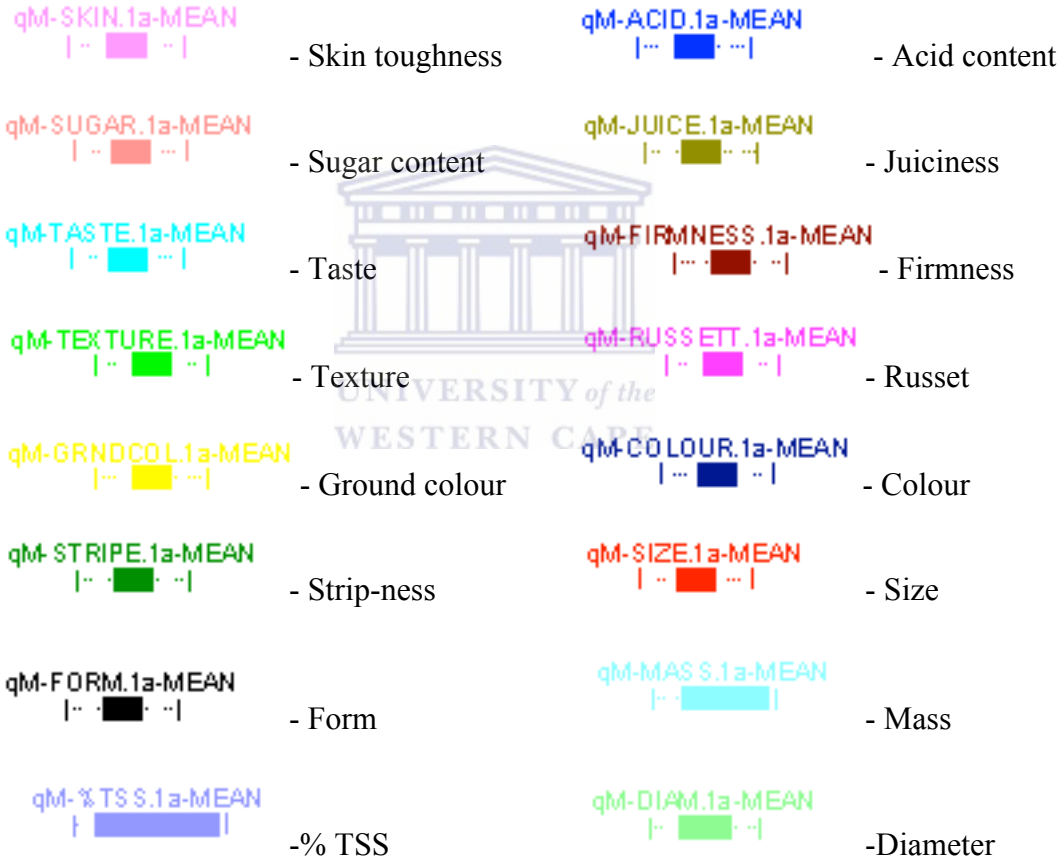
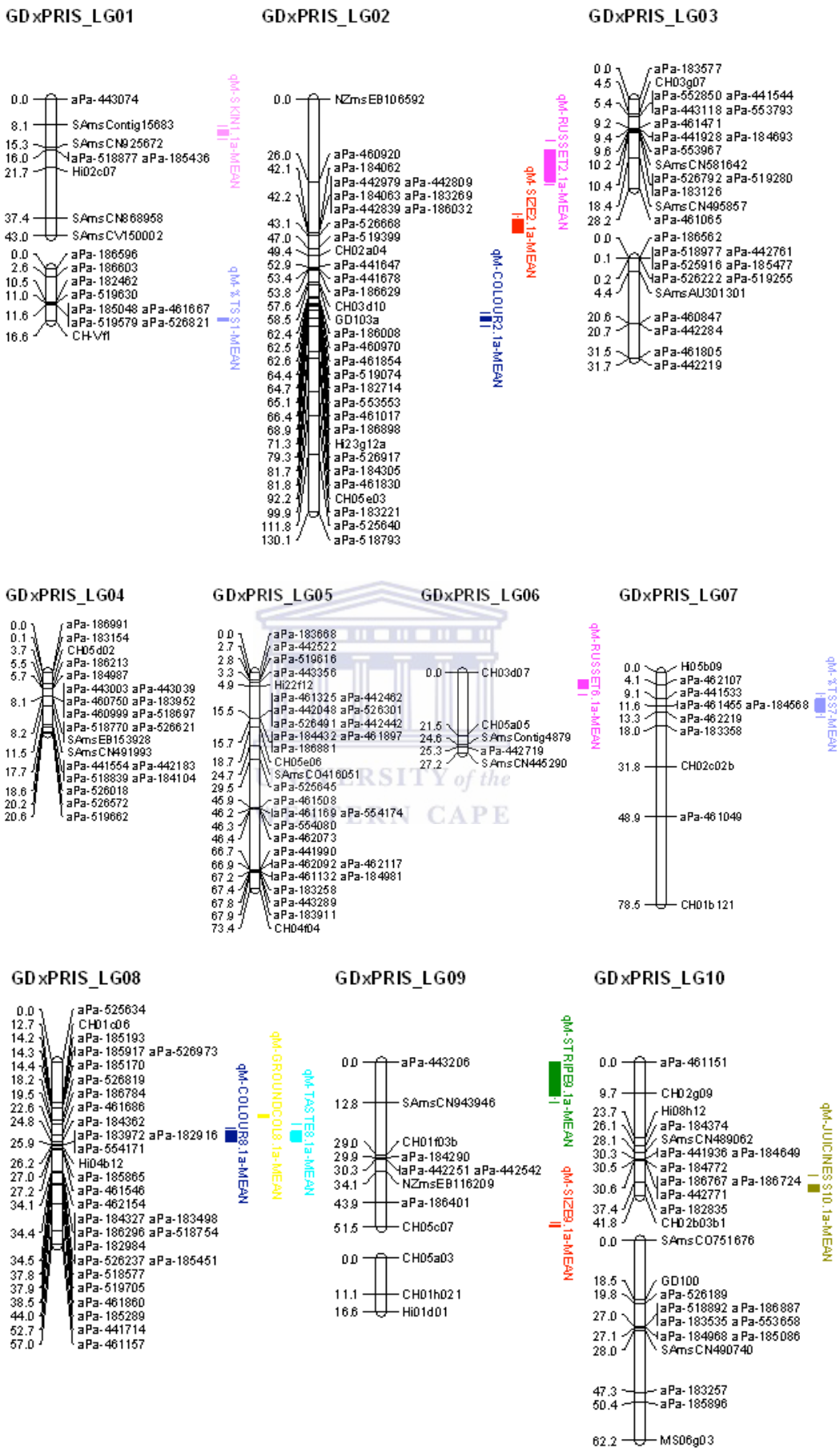

GDxAN_LG10
0.0 SAmsCN444650
23.3 CH02a10
25.9 CH01e09b
39.4 CH05c06
79.2 aPa-184057
82.5 aPa-185021 aPa-461293
82.6 aPa-186786 aPa-183247
aPa-186541
82.9 aPa-183997
87.2 Hi08h12
92.4 aPa-186887
92.5 aPa-183535 aPa-183578
92.6 aPa-518892 aPa-185086
92.7 aPa-183697
114.4 aPa-185199
114.7 aPa-183951
114.9 aPa-442593
116.1 aPa-186040
118.5 aPa-184213
119.7 aPa-552942
qM-JUICINESS10-MEAN
qM-TASTE10-MEAN
qM-FIRMNESS10-MEAN
GDxAN_LG11
0.0 CH02d08
39.0 aPa-461749 aPa-526579
aPa-184105
56.3 SAmsCO755814
83.9 aPa-443154 aPa-553294
84.0 aPa-461821 aPa-183555
aPa-442732
109.2 aPa-552809
111.8 aPa-184025
qM-GROUNDCOL11-MEAN
GDxAN_LG12
0.0 CH05e05
44.5 aPa-185798
47.1 CH03c02
55.1 CH04d02
61.7 aPa-461201
aPa-525566 aPa-442203
61.8 aPa-443032 aPa-441685
aPa-526716 aPa-552852
66.5 aPa-518896
68.8 aPa-183985
71.4 aPa-519308
94.1 aPa-183334 aPa-185802
96.7 aPa-461499 aPa-186504
aPa-461726
106.5 CH04g04
119.7 aPa-441779 aPa-442173
120.4 aPa-442112
aPa-461822 aPa-186193
120.8 aPa-183485 aPa-185262
aPa-525797
132.1 SAmsCN943613
GDxAN_LG13
0.0 CH05h05
13.4 Hi04g05
17.3 CH02g01
34.0 GD147
35.9 Hi04f09
41.2 SAmsCO905285
50.8 SAmsCO052555
qM-FORM13-MEAN
GDxAN_LG14
0.0 NZmsEB146613
17.2 Hi21e04
18.1 CH04f06
18.6 SAmsCN904905
19.5 CH01f091
19.7 CH01g05
36.6 CH03g04
43.4 Hi02d11
47.6 CH04c07
56.5 SAmsCN942512
63.6 CH03a03
75.8 Hi01c09
78.6 CH05d03
GDxAN_LG15
0.0 SAmsCN947446
10.6 CH01d08
28.0 SAmsCN492626
33.7 SAmsCO415353
33.8 SAmsCN851624
40.5 SAmsCN866018
48.2 aPa-460712 aPa-460872
52.5 aPa-441900
aPa-462056 aPa-441924
55.4 aPa-184398 aPa-525795
aPa-518640
70.3 aPa-526885
qM-ACID15-MEAN
qM-SUGAR15-MEAN
GDxAN_LG16
0.0 aPa-186214
2.6 aPa-185104
3.3 aPa-184045 aPa-525633
aPa-443159
3.4 aPa-442137 aPa-519426
3.6 aPa-461310
4.2 aPa-526169
4.7 aPa-186312 aPa-526766
13.2 Hi12a02
15.8 CH05b06
19.2 SAmsCV084260
24.1 CH05e04
26.1 CH05a09
29.9 CH01f03a
32.2 Hi02h08
34.2 NZmsCO905522
75.3 SAmsCN933736
qM-SIZE16-MEAN
GDxAN_LG17
0.0 CH04c06
24.3 SAmsCO414947
27.8 SAmsCV627191
42.8 NZms04f3
61.3 SAmsAU301254
qM-COLOUR17-MEAN

Figure 20. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Golden Delicious' x 'Anna' mapping population, post-storage, using rMQM mapping. The 17 linkage groups obtained, are numbered according to Maliepaard *et al*. (1998). QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

QTL Colour Legend:





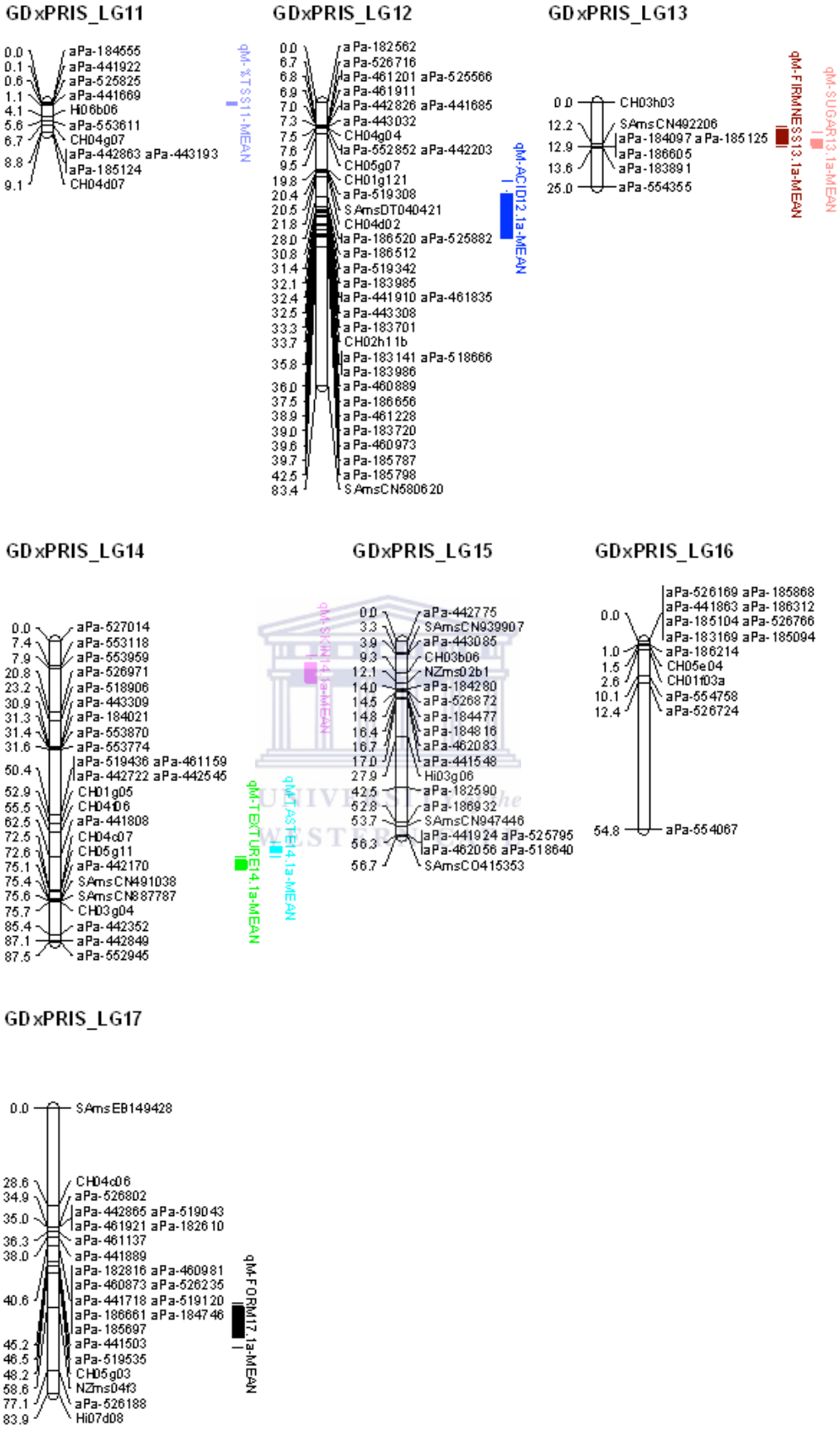

GDxPRIS_LG11
0.0 aPa-184655
0.1 aPa-441922
0.6 aPa-525825
1.1 aPa-441669
4.1 Hi06b06
5.6 aPa-553611
6.7 CH04g07
8.8 aPa-442863 aPa-443193
9.1 aPa-185124
CH04d07
qM-%TSS11-MEAN
GDxPRIS_LG12
0.0 aPa-182562
6.7 aPa-526716
6.8 aPa-461201 aPa-525566
6.9 aPa-461911
7.0 aPa-442826 aPa-441685
7.3 aPa-443032
7.5 CH04g04
7.6 aPa-552852 aPa-442203
9.5 CH05g07
19.8 CH01g121
20.4 aPa-519308
20.5 SAmsDT040421
21.8 CH04d02
28.0 aPa-186520 aPa-525882
30.8 aPa-186512
31.4 aPa-519342
32.1 aPa-183985
32.4 aPa-441910 aPa-461835
32.5 aPa-443308
33.3 aPa-183701
33.7 CH02h11b
35.8 aPa-183141 aPa-518666
aPa-183986
36.0 aPa-460889
37.5 aPa-186656
38.9 aPa-461228
39.0 aPa-183720
39.6 aPa-460973
39.7 aPa-185787
42.5 aPa-185798
83.4 SAmsCN580620
qM-ACID12.1a-MEAN
GDxPRIS_LG13
0.0 CH03h03
12.2 SAmsCN492206
12.9 aPa-184097 aPa-185125
aPa-186605
13.6 aPa-183891
25.0 aPa-554355
qM-FIRMNESS13.1a-MEAN
qM-SUGAR13.1a-MEAN
GDxPRIS_LG14
0.0 aPa-527014
7.4 aPa-553118
7.9 aPa-553959
20.8 aPa-526971
23.2 aPa-518906
30.9 aPa-443309
31.3 aPa-184021
31.4 aPa-553870
31.6 aPa-553774
50.4 aPa-519436 aPa-461159
aPa-442722 aPa-442545
52.9 CH01g05
55.5 CH04d06
62.5 aPa-441808
72.5 CH04c07
72.6 CH05g11
75.1 aPa-442170
75.4 SAmsCN491038
75.6 SAmsCN887787
75.7 CH03g04
85.4 aPa-442352
87.1 aPa-442849
87.5 aPa-552946
qM-TEXTURE14.1a-MEAN
qM-TASTE14.1a-MEAN
GDxPRIS_LG15
0.0 aPa-442775
3.3 SAmsCN939907
3.9 aPa-443085
9.3 CH03b06
12.1 NZms02b1
14.0 aPa-184280
14.5 aPa-526872
14.8 aPa-184477
16.4 aPa-184816
16.7 aPa-462083
17.0 aPa-441548
27.9 Hi03g06
42.5 aPa-182590
52.8 aPa-186932
53.7 SAmsCN947446
aPa-441924 aPa-525795
56.3 aPa-462056 aPa-518640
56.7 SAmsCO415353
GDxPRIS_LG16
aPa-526169 aPa-185868
aPa-441863 aPa-186312
aPa-185104 aPa-526766
aPa-183169 aPa-185094
0.0
1.0 aPa-186214
1.5 CH05e04
2.6 CH01f03a
10.1 aPa-554758
12.4 aPa-526724
54.8 aPa-554067
GDxPRIS_LG17
0.0 SAmsEB149428
28.6 CH04c06
34.9 aPa-526802
35.0 aPa-442865 aPa-519043
aPa-461921 aPa-182610
36.3 aPa-461137
38.0 aPa-441889
aPa-182816 aPa-460981
40.6 aPa-460873 aPa-526235
aPa-441718 aPa-519120
aPa-186661 aPa-184746
aPa-185697
45.2 aPa-441503
46.5 aPa-519535
48.2 CH05g03
58.6 NZms04f3
77.1 aPa-526188
83.9 Hi07d08
qM-FORM17.1a-MEAN

Figure 21. Graphical representation of the genetic positions (in cM) of QTLs for fruit quality traits identified in the 'Golden Delicious' x 'Anna' mapping population, post-storage, using rMQM mapping. The 17 linkage groups obtained, are numbered according Maliepaard *et al*. (1998). All QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

QTL Colour Legend:

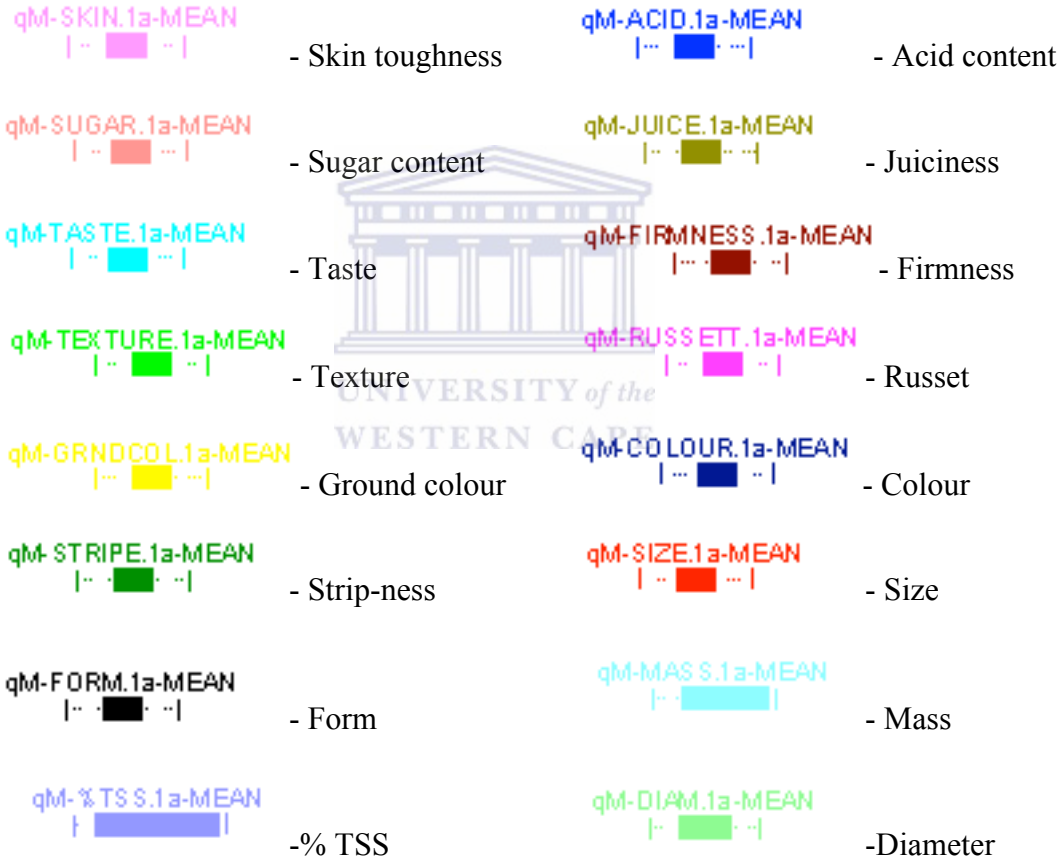


Table 28. Overview of fruit quality trait QTLs detected, pre-storage, in segregating progeny of the 'Prima' x 'Anna' (PxA), 'Golden Delicious' x 'Priscilla' (GxP) and 'Golden Delicious' x Anna' (GxA), for the mean of the years, with results listed per LG. For each QTL, significance (LOD score) is presented first followed by the value for the % population variance explained by that QTL. LOD scores below the threshold are indicated in bold.

TRAIT	MAP	LG01	LG02	LG03	LG04	LG05	LG06	LG07	LG08	LG09	LG10	LG11	LG12	LG13	LG14	LG15	LG16	LG17
Stripy-ness	PxA									8.3 / 58								
	GxP								14.6 /28.2	28.2 /62.2								7.3 /8.9
	GxA									3.7 /27.8								
Colour	PxA		4.5 /17.4				6.5 /36.4											
	GxP		4.3 /53.7													4.7 /30.5		
	GxA				**3.1 /33.3**													
Size	PxA									5.6 / 28.4								
	GxP			6.3 /47.1											5.5 /12.6			
	GxA												4.2 /20.7					
Form	PxA		5.8 /29.6															
	GxP										7.5 /57.4				4.2 /20.7			

	GxA					**3.5 /27**				3.8 /19.5		3.9 /50.7						
Ground Colour	PxA												3.8 / 45.6					
	GxP												6.5 /35				7.4 /63.8	
	GxA																	
Russet	PxA	5.1 / 35.3				7.0 / 21.9							4.9 / 15.5					
	GxP		6.1 /33.5	10.7 /33.9														
	GxA																	
Texture	PxA		4.9 / 42													7.7 /56.7		
	GxP										6.7 /14.8				5.8 /15.3	5.4 /13.5	4.5 / 11	
	GxA	**3.4 /39.1**	3.8 /44.3															
Firmness	PxA							4.5 / 53.7					5.5 / 25.9	4.4 / 7.9		7.3 / 13.3		
	GxP					4.3 /25.7												
	GxA										4.4 /70.9							
Juiciness	PxA															4.5 /22.1		
	GxP									7.1 /35.6								5.0 /25.2
	GxA										4.4				3.8			

											/71.1				/4.9			
Sweetness (sugar content)	PxA					4.5 /28.7												
	GxP									7.7 /29.8							5.9 /42.1	
	GxA											4.3 /30.4						
Acidity	PxA																4.3 / 23.5	
	GxP														**3.6 / 68**			
	GxA		5.3 /63.6									4.0 /14.7						
Taste	PxA				**3.6 /24.4**													
	GxP											5.6 /50.3						
	GxA										6.1 /66.3				3.8 /4.9			
Skin toughness	PxA													**3.7 /19.7**				
	GxP			5.0 /35.3							5.1 /34.7							
	GxA				**3.2 /45.7**													
% TSS	PxA		4.2 /14.7													7.0 /52		
	GxP											5.2 /33.1						
	GxA		**3.7 / 40**															

Mass	PxA	9.1 / 34.3		6.3 / 25.4	5.7 / 19.6									
	GxP	4.3 /16.9				8.6 /46.2								
	GxA						7.1 /21.4	5.4 / 58						
Diameter	PxA	6.0 / 4.2					7.2 / 4.1	4.9 / 34.5	5.4 / 3.7		12.2 / 54.2			
	GxP				5.0 /26.5	7.4 /41.9								
	GxA		13.4 /6.2		16.3 /30.8			26.4 /27.9	17 / 6.3		14.6 /6.6	12.4 /6.4	14.8 /5.7	

Table 29. Overview of fruit quality trait QTLs detected, post-storage, in segregating progeny of the 'Prima' x 'Anna' (PxA), 'Golden Delicious' x 'Priscilla' (GxP) and 'Golden Delicious' x Anna' (GxA), for the mean of the years, with results listed per LG. For each QTL, significance (LOD score) is presented first followed by the value for the % population variance' explained by that QTL.

TRAIT	MAP	LG01	LG02	LG03	LG04	LG05	LG06	LG07	LG08	LG09	LG10	LG11	LG12	LG13	LG14	LG15	LG16	LG17
Stripyness	PxA									**3.4 /23.4**								
	GxP									13.7 /84								
	GxA		3.9 /37.3															
Colour	PxA			5.2 / 56.3														
	GxP		4.6 /31.3						4.8 /31.2									
	GxA			4.8 /16.2														**3.7 /13.5**
Size	PxA					5.9 /25.1									7.1 /30			
	GxP		**3.6 /37.5**							**3.5 /39.2**								
	GxA																4.6 /31.7	
Form	PxA		4.6 / 22.6		4.3 / 24.9	4.2 / 31.1												
	GxP																	**3.2 /49.5**
	GxA				4.5 /35.6									5.1 /38.8				

Ground Colour	PxA	6.9 / 35.7							9.2 / 47.2	4.7 /17.3							
	GxP							12.2 /83.7									
	GxA	5.8 /45.9									6.4 /36.4						
Russet	PxA					4.3 /17									5.0 /58.2		
	GxP	5.7 /30.5				7.5 /64.1											
	GxA				5.5 /55.2												
Texture	PxA			4.8 / 34.3													
	GxP													4.6 /74.8			
	GxA	6.6 /74.6															
Firmness	PxA														5.8 / 55.3		
	GxP												**3.5 /33.9**				
	GxA									4.0 /58.9							
Juiciness	PxA													4.1 / 26			5.0 /29.3
	GxP									4.4 /49.2							
	GxA									**3.7 /18.5**							
Sweetness	PxA		7.0 /												7.1 /		

(sugar content)	PxA			39.4								52.4		
	GxP										5.1 /42.1			
	GxA											5.8 /77		
Acidity	PxA													5.4 / 25.1
	GxP									4.4 /59				
	GxA											**3.4 /48.1**		
Taste	PxA												4.7 / 13.7	
	GxP					4.6 /44.4					6.0 /54.8			
	GxA							4.3 /56.5						
Skin toughness	PxA			5.1 /42										
	GxP	7.2 /68.8										6.1 /21.4		
	GxA					**3.2 /36.4**								
% TSS	PxA		6.5 / 29.1				5.8 / 21.5					4.9 / 29.2		
	GxP	6.7 /26.8			6.9 /34.7				8.7 /29					
	GxA		3.8 /33.9											

4.8 Comparing different MapQTL® 5.0 mapping functions

Nonparametric, interval and multiple QTL were the different mapping techniques used to locate QTLs on the linkage map. The three techniques were compared for all the traits evaluated and results of the texture QTL on LG15 of 'Prima' x 'Anna', was discussed below.

4.8.1 Kruskal-Wallis (KW) nonparametric mapping function

The KW test gives all individuals a ranking according to the quantitative trait, while it classifies them according to their marker genotype. The KW mapping function identified LG15 as the highest ranked location for the position of the texture QTL on 'Prima' x 'Anna'. It also identified SSR marker SAmsCN492626, or more specifically, alleles at this locus, at position 29.65cM, as a candidate marker for marker-assisted selection (Appendix J and Figure 22).

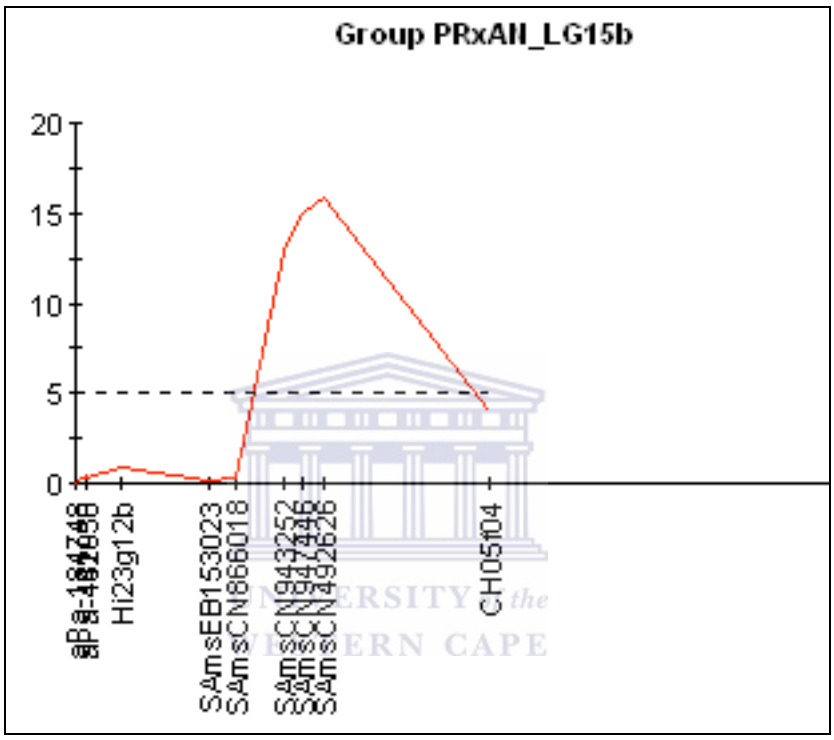


Figure 22. Graphical representation of the KW output for texture on LG 15b. The red line shows the Kruskal-Wallis coefficient, peaking at 15.8, around SAmsCN492626 of LG15b of the 'Prima' x 'Anna' mapping population. The dashed line indicates a threshold of 5. The red line indicates the Kruskal-Wallis (K*) test statistic.

4.8.2 Simple interval mapping

The interval mapping function allowed for the detection of segregating QTLs, using the maximum likelihood approach (van Ooijen, 2004). The QTL for fruit texture was detected at 29.65cM, on LG15b in the 'Prima' x 'Anna' mapping population, with a LOD score of 4.78 (Figure 23).

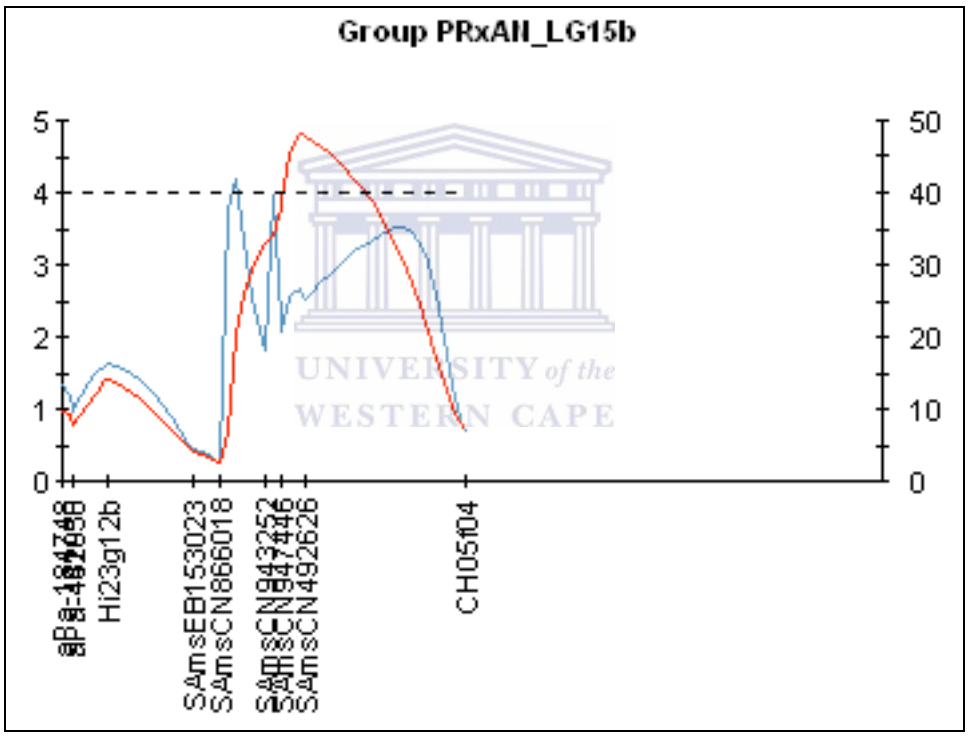


Figure 23. A graphical representation of LG15b of the 'Prima' x 'Anna' mapping population, showing a QTL, with LOD of 4.78, found using the interval mapping function of MapQTL 5.0. The red line shows the change in the LOD score over the entire linkage group, and the dashed line, the LOD threshold of 4.

4.8.3 Restricted Multiple QTL mapping (rMQM)

An extension of the simple interval mapping function (van Ooijen, 2004), the MQM function allows for co-factors to be selected (Figure 24) close to the QTL of interest, and this in turn assumes the role of the QTL itself. The QTL, identified in simple interval mapping (Figure 23), is thus enhanced, after selecting SAmsCN492626 as the marker (co-factor) closest to the QTL. The QTL has a LOD score of 7.43, and explains 53.6% of the population variance.

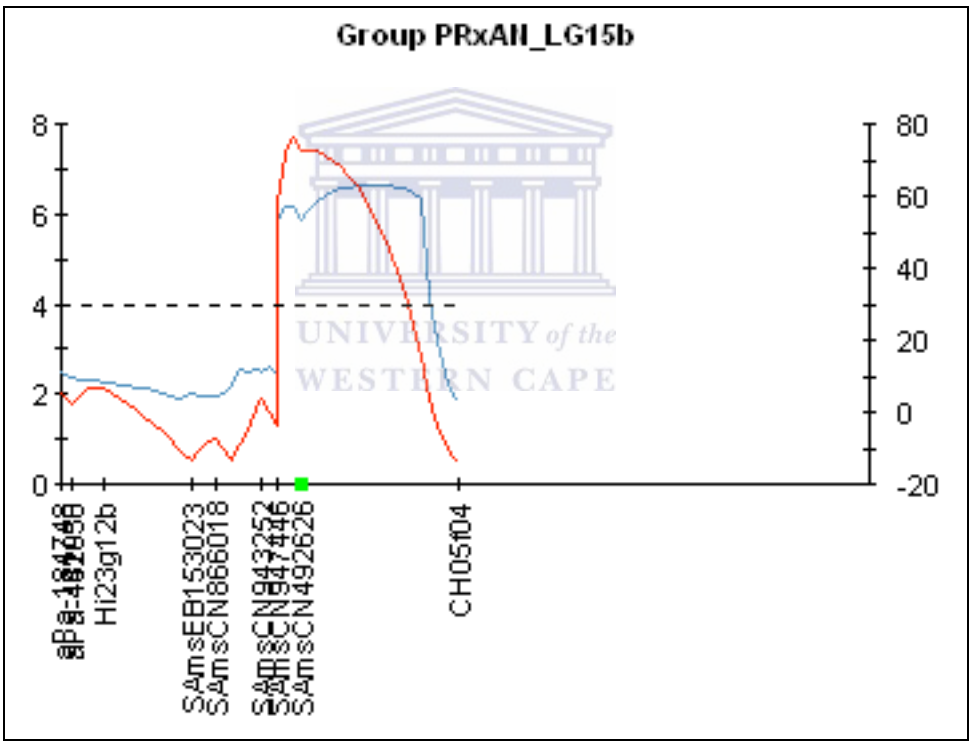


Figure 24. Graphical representation of the rMQM output file for texture on LG15b of 'Prima' x 'Anna'. The QTL is positioned around the co-factor (SAmsCN492626), and now has a LOD score of 7.43. The QTL for texture explains 53.6% of the population variance.

4.9 Stability of QTLs

QTLs were considered to be stable if they were detected in successive years in homologous regions of the same LG. Often these year-stable QTLs also had the same marker as the most significant (highest LOD score) marker. Such markers are currently the best candidates for further development into breeding tools. An example of this is marker NZmsEB116209 (Celton *et al*., 2008) found on LG9, around which a QTL for fruit stripe-ness is located (Figure 26).

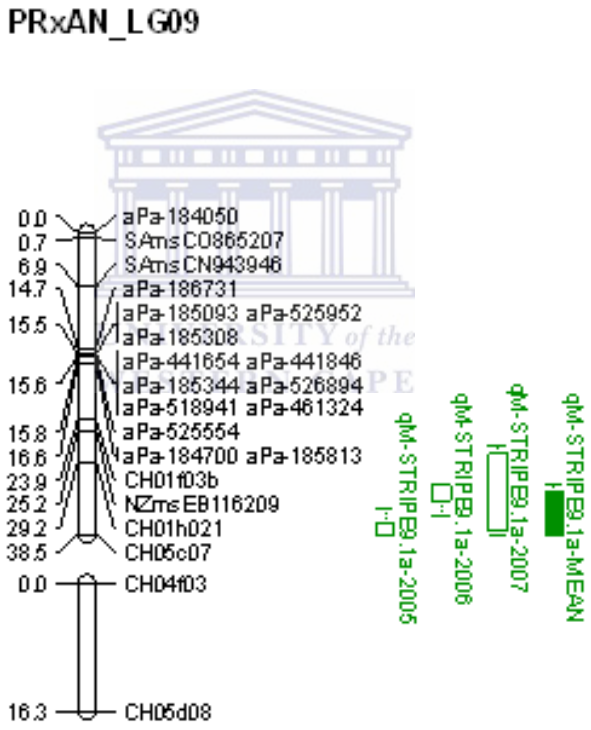


Figure 25. The stability of the stripe-ness QTL localized around the SSR marker NZmsEB116209, in the pre-storage 'Prima' x 'Anna' progeny. The solid part of the bars of the QTL symbols indicates the most likely position of the QTLs, while the thin lines represent the confidence interval at the 95% level. The QTL symbols for the harvest of 2005, 2006 and 2007 are open whereas the mean of the years are filled green.

Colour, texture, acidity and taste are some of the other traits found to be stable in two or more years of harvest. A colour QTL was identified on LG 6 in 2005, 2006 and 2007. In 2005 the QTL produced a LOD score of 8.6 and explained 61.2% of the population variance, in 2006 the same QTL had a LOD score of 4.5 and explained 26.9% of the variance, while in 2007 this QTL had a LOD score of 4.2 and explained 27.1% of the population variance. This QTL for colour localized around the SSR marker, SAmsDR998909 (Figure 26).

The QTL for texture is quite stable, being found on LG15, for two seasons of harvest viz. 2006 and 2007, as well as the mean of the years, in the 'Prima' x 'Anna' mapping population with the LOD scores of 9.1, 4.2 and 7.4 being identified for 2006, 2007 and the mean respectively, explaining 81.6%, 79.6% and 53.6% of the variation. However, these QTLs did not localize around a particular marker, but instead were found on different sections of LG 15.

Taste was another trait that produced year-stable QTLs in 2005 and 2006 seasons of harvest. These QTLs were detected on LG 4 and had LOD produced LOD scores of 3.3 and 6.1, respectively, while explaining 24.5% and 25.4% of the population variance in the two years mentioned earlier. In 2007 this QTL was found on LG 14, which is homologous to LG 4 (Velasco *et al*., 2010). In 2005 QTL was found near the SSR marker CH05d02 and in 2006 near the DArT marker aPa-416271, both in the middle of LG 4.

Similar analyses were undertaken on the 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla' mapping populations but results were not

displayed.

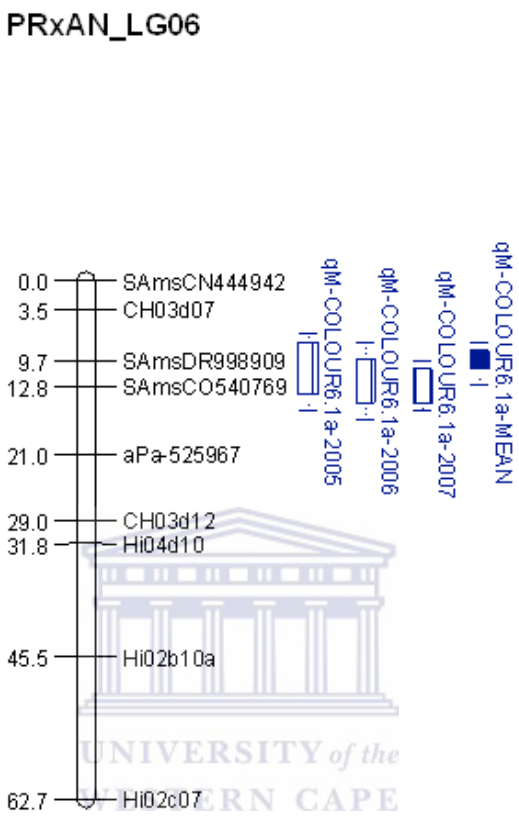


Figure 26. The stability of the colour QTL in the 'Prima' x 'Anna' mapping population, localizing around SSR markers SAmsDR998909 and SAmsCO540769. The solid part of the bars of the QTL symbols indicates the most likely position of the QTLs, while the thin lines represent the confidence interval at the 95% level. The QTL symbols for the harvest of 2005, 2006 and 2007 are open boxes, while filled boxes represent the mean of the years.

CHAPTER 5: DISCUSSION

5.1 PHENOTYPIC RESULTS

Genetic analyses of fruit traits at the ARC have usually been performed retrospectively from the breeding program data and were often deficient in appropriate experimental design, thus impeding estimation of variance components and heritability (Labuschagné *et al*., 2002). In these trials seedlings were grafted onto the same rootstock thus giving the trees a similar physiological status. For this study, three seasons of data collected from 2005 to 2007 was sufficient, although further data collection would have been beneficial, this was not posibble as the trees from all populations were removed due to orchard planning. From the results of analysis of variance (ANOVA), the total variance (σ_T) of each fruit trait could be separated into the variance components associated with genotype (σ^2_g) (Family), season (σ^2_s) (Year), and genotype by season interaction (σ^2_{gs}) (Seedling within Family), and trees within genotype interaction (σ^2_t) (Year by Family interaction), shown in Table 17, 19 and 20.

5.1.1 Analysis of variance

ANOVA showed that the effect of family interaction was significant for stripe-ness, colour, texture, firmness, juiciness, % sugar content, % acid content, size, form, skin toughness, ground colour, mass, diameter and % total soluble solids, but not for taste and russetting (Table 17 and 20), of the pre-storage evaluation, indicating that there are genetic differences between the genotypes. These results

are confirmed by the simple statistics in Table 9, where the Student's t-test showed no significant differences between the means of the three mapping populations. These traits showed considerable genetic variation suggesting that genetic gain through breeding were practical assuming additive genetic variance contributed significantly to phenotypic variance. In comparison, post harvest analyses showed only ground colour not to be significantly different between the three families (Table 10 and 17), with all other traits exhibiting a *P*-value of less than 0.0001 in post-storage analyses of all fruit traits.

All apple fruit traits, in pre-storage analysis, except firmness (P=0.84) (Table 17) were significantly influenced by the envirnoment (Table 10). This results echoes that of Rathore (1976) who studied the effect of season on the growth and chemical composition of guava (*Psidium guajava L.*) fruits. After cold storage, Table 18, however, does show that the season did not influence sugar and acid content significantly, with *P*-values of 0.16 and 0.95, respectively. The season also did not affect instrumentally measured firmness (Table 20), with value a P-value of 0.16 being seen

The year x family interaction (Y x F), (σ^2_{gs}) (Table 17 and 20), was significant in pre-storage analysis, for stripe-ness, texture, firmness, taste, sugar and acid content, form, skin toughness and russetting, but not for colour, juiciness and ground colour. The significant genotype by season interaction for stripe-ness, texture, form and russetting. The family by season interaction variance component was small for most traits, except fruit diameter (37.9%) and skin toughness (8.6%) in pre-storage analysis, and juiciness (11.47%) in post-storage analysis. Since interaction of juiciness was mainly due to a change in magnitude rather than a ranking change among genotypes, statistically only one season of evaluation would be sufficient for selection. Diameter and skin toughness, on the other hand, would vary between seasons and would therefore need a few seasons

of evaluation for selection. This is indicated by the large variance component for both σ^2_{gs} as well as σ^2_s (Table 18 and 21), for these traits (diameter and skin toughness).

The effect of seedling within family (Table 10 and 17) was significant for all traits, pre-storage, but sugar content and fruit form were not significant in post-storage. Within-family seedling variance was generally higher than that for between families for all measurements (Table 18). It is common knowledge that the apple, cultivars are highly heterozygous and this is reflected in the variation in seedling families. (Brown, 1960). Except for stripe-ness, mass and texture, all traits showed relatively low variance components, σ^2_t (Table 18 and 21), which implied that effective field evaluation of the fruit traits, could be based on a single tree rather than several trees (Thaipong and Boonprakob, 2005).

Although fruits within tree variance was not analysed in this study it could serve as a better measure for minimizing environmental variance than increasing the number of seedlings within a genotype, for genetic evaluation.

5.1.2 Heritability

Knowledge of the genetic systems controlling the inheritance of desirable traits such as good texture and flavour, and of the genetic and environmental factors that influence their expression, is essential for a successful breeding programme. Heritability estimates can not only be used to make predictions of genetic progress in the offspring, when the parents are selected on the basis of their own performance and for choosing among selection strategies to improve breeding

efficiency, but also explain the major changes in the amount and nature of genetic variability (Hansche, 1986).

The high value of heritability across families for stripe-ness, both before and after cold storage, indicate that this trait may be controlled primarily by one or a set of major genes. Table 18 shows that heritability values for all other traits, measured subjectively, are quite low, with only firmness, acidity and size showing an increase from pre- to post-storage. This variation in heritability is not unexpected as it is a function of the population's variability and the environment in which it is grown (Falconer, 1989). This is also because different sets of five fruits were used when performing pre- and post-storage analysis. Imprecise measurement of these traits could be a reason why heritability values were so low for many of the other traits in Table 18. Intermediate heritability estimates were seen for fruit size and mass. Values of 0.4 and 0.53, respectively, were similar to values in previous reports on the inheritance of fruit size and mass in other fruit species, such as peach and nectarine (Hansche and Beres, 1980). Hansche and Beres reported a value of 0.26, and Hansche (1986) reported a value of between 0.5 and 0.6.

Another moderately heritable trait is % TSS (h=0.51 and 0.45) (table 22 and 23). Even though the phenotypic standard deviations for this trait are very low for each family (Table 17), the heritabilities are sufficiently high to allow genetic advance. These heritability values were significantly higher than those estimated in the subjective analysis of sugar content content and acidity, before and after cold storage (Table 18) and also suggests that when instrumental measurements

are captured, there is no need to do a subjective analysis as well. The low heritability for acidity was, however, shown to be similar to that found in grape (Firoozabadi and Olmo, 1987).

The heritability of striped fruit colour was consistently high at 0.71 and 0.64 during pre- and post-storage analysis, respectively, during subjective evaluation of the fruit. Skin colour variables (L, C, h-values) were measured separately for over- and ground colour of fruit. The L-value represents the light-dark scale; the C-value colour saturation or intensity of the fruit, and h-value the hue angle. These traits showed particularly high heritability values for all three, colour dimensions of over-colour, as well as intermediate heritability values for back or ground colour (Table 21). These high heritability values show that the genotype had a large influence on the lightness/darkness and the hue angle. This shows that the colour traits with intermediate heritabilities are largely affected by the environment (Couranjou, 1995).

The heritability of fruit firmness is almost 2.5 times higher, when measured instrumentally, than subjectively, in pre-storage and almost four times higher in post-storage. This again shows that data collected instrumentally is more useful than that collected subjectively, as this can be very imprecise.

5.1.3 Correlation analysis

Both positive and negative correlations were observed for each of the three mapping populations. Generally, positive correlations are desirable from a

breeding perpective when two desirable traits are associated one another, but undesirable when the character of interest is associated with an undesirable character (de Souza and Byrne, 1998). A negative correlation on the other hand is not wanted when it involves two desirable traits, such as total soluble solids and fruit blush, for example. Positive correlation between traits, of all three mapping populations, ranged from 0 (post-storage) to 0.71 (pre-storage) (Table 23, 24 and 25). The strongest positive correlation occurred between texture and juiciness, of the 'Golden Delicious' x 'Priscilla' progeny.

In the 'Prima' x 'Anna' progeny, pre-storage analyses showed positive correlations between colour and all other traits, although, these were very weak, with r-values of less than 0.3 for all traits, except form, ground colour and russet. Overall fruit colour, although affected by ground colour and russet during measurement, is not produced as a combination of these traits. This is observed for post-storage analysis as well. Only ground colour shows a weak positive correlation with fruit colour. Moderately strong positive correlations were observed between percentage acidity and overall fruit taste and sugar content, with weaker correlations between acid and texture, firmness and juiciness. The same result was observed in post-storage acid content, although there was a stronger correlation between acid and juiciness, post-storage, as compared to pre-storage, and a much weaker correlation between acid and firmness, at the same stage i.e. post-storage. Juiciness was also a good indicator of texture and firmness, both pre- and post-storage, showing moderately strong correlations for texture in both evaluations. Traits showing very low positive correlations or low

negative correlations, with most, if not all traits, include size, skin toughness, russet and ground colour.

Negative correlations, in the ‘Prima’ x ‘Anna’ progeny were seen between colour parameters such as ground colour with texture, firmness and juiciness. Likewise stripe-ness with texture, firmness, taste, juiciness, sugar and acid content, size and form. This could be because the striped-colour trait in apple skin is genetically controlled, by the R*f* gene (Crane and Lawrence, 1933), whereas pathways responsible for fruit texture, aroma and sugar content and acidity are dependent on the environment. Firmness was moderately correlated to texture in pre-storage, but showed almost no correlation to texture in post-storage analysis.

The ‘Golden Delicious’ x ‘Anna’ mapping population (Table 26) showed much lower correlations for all the trait compared to the ‘Prima’ x ‘Anna’ family. As with the ‘Prima’ x ‘Anna’ population, stripe-ness showed very low or negative correlations with all other traits, in both pre- and post-storage analysis.

Negative correlations were also seen in the ‘Golden Delicious’ x ‘Anna’ progeny between firmness and sugar, size, form and skin toughness. Moderately strong correlations were seen between taste and sugar content and fruit acidity, both before and after cold storage. This was expected, as overall taste is directly determined by the perception of sweetness and sourness, which are not independent of one another (Stevens *et al*., 1977). As with the ‘Prima’ x ‘Anna’ population, this population also showed low, or negative correlations between colour parameters and traits related to sensory measurements (Table 24). These

traits include stripe-ness, with firmness, juiciness, acidity and skin toughness. Overall fruit colour was weakly correlated to ground colour, in pre-storage analysis (r=0.36), but showed no significant correlation after cold storage (r=0.15). Some 83.3% of all correlations between traits of this population were positive, pre-storage, but only 59% of these were considered significant. The correlation of firmness with sugar content, acid content, size and form, did, however, differ significantly from the same correlations in the 'Prima' x 'Anna' population, in pre-storage analysis (Table 23 and 24).

It is interesting to note, that a good negative correlation exists between skin toughness and firmness in all three populations, both before and after cold storage. As skin toughness is not related to fruit firmness (Bourne, 2002), the skin needs to be removed when performing this measurement for accurate readings to be recorded and is a reason why a negative correlation is seen in all three populations (Table 23, 24 and 25). As skin toughness was measured subjectively, it is possible that this trait was measured in relation to the firmness of the fruit i.e. a softer fruit would tend to have tougher skin than a firmer fruit. A positive correlation exists between these two traits in other species of the Rosaceae family, e.g. strawberry, when the skin does not need to be removed during firmness measurements, as it increases the firmness reading negligibly (Ourecky and Bourne, 1968).

In the 'Golden Delicious' x 'Priscilla' population there were significant, positive correlations between all sensory traits viz. taste, firmness, texture, sugar content and acidity, in pre-storage analysis, although some were stronger than others

(Table 25). Very strong correlations were observed between juiciness and texture (pre- and post-storage), and juiciness and taste (only pre-storage), with r-values of more than 0.65 being observed. Therefore, improvement of fruit juiciness through selection for fruit texture should be effective. The other traits showing a very strong correlation were sugar content and taste (pre- and post-storage). Skin toughness, again, was negatively correlated to all sensory traits, with a strong negative correlation to firmness. As was explained above, this could be because skin toughness was measured subjectively in relation to firmness, and not independently thereof. This trait showed very low positive correlation to colour, in both pre- and post-storage analysis. Sugar content and acidity were moderately correlated in this population, and showed similar results observed for these traits in the 'Prima' x 'Anna' and 'Golden Delicious' x 'Anna' populations (Tables 23, 24 and 25).

Although fruit size and form showed positive correlations with most traits, these were less than 0.3 and were considered extremely weak. It therefore is not possible to improve either of these traits, by selecting for the other. In post-storage, however, there was a moderately weak correlation between these two traits (r=0.4). These results were similar to those observed for the 'Golden Delicious' x 'Anna' progeny, although stronger correlations were observed between form and the fruit texture traits in the 'Prima' x 'Anna' progeny.

It is clear throughout the correlation analyses of the three progenies, that moderately strong to strong correlations exist between the fruit texture traits, whereas very low positive or even negative correlations exist between visual

traits viz. fruit form, colour, russet, on the one hand and the texture traits, on the other.

Year by year correlations on the other hand varied over the three-year period for all traits evaluated. Correlations for subjectively measured traits were generally very weak, except for fruit stripe-ness. 39% of all correlations measured here were significant, with the rest all well below the r=0.3. Stripe-ness showed a strong positive correlation, with r-values above 0.67. Improvement of the striped colour in fruit, through selection should be effective based on these strong year-by-year correlations. It shows that there is a strong genetic component to this trait as it is not affected by the change in season.

Another trait showing moderately weak correlations from year to year was fruit size. Although, not as high as stripe-ness, this trait will also be improved by selection, based on these correlations. Ground colour, taste, sugar content and acidity all produced consistently low, positive correlations over all three years of evaluation. As the correlation values are consistent from season to season, it is safe to say that the environment has little effect on the expression of these traits.

Some other traits, viz. fruit colour, russet, texture, juiciness and skin toughness showed varying correlations, with at least one year showing a significant correlation. Based on these varying correlations between the years it is safe to say that these traits are dependent, even if only partly, on the environment as well as the climate of the region. The firmness trait also varied over the three years but none of these values were significant.

Correlation coefficients of instrumentally measured traits were very strong, with 83% being significant over the three years. Mass, firmness and %TSS therefore do not need to be measured instrumentally and subjectively, as instrumental data suggests that good selections can be made based purely on instrumental data. This could be due to the low error rate when measuring traits instrumentally.

Since all other correlations seen for instrumentally measured traits were significant over the three years if was quite odd to see very weak values for diameter in 2005/2007 and 2006/2007. This result occurred due to a discrepancy in the measurement of one of the samples in 2007.

5.2 GENOTYPIC RESULTS

5.2.1 Newly designed SSRs (SAms)

Published (Guilford *et al*., 1997; Liebhard *et al*., 2002, 2003; Silfverberg-Dilworth *et al*., 2006; Celton *et al*., 2008 and van Dyk *et al*., 2010) as well as newly designed SSR markers were implemented on all three mapping populations used in this study. A set of 540 new SSR markers were designed, 525 of these from EST sequences, using the tandem repeats finder algorithm (Benson, 1999), and extensively optimized on parental cultivars, to determine whether markers revealed polymorphism or not. The optimization process was completed using 6% polyacrylamide gels and silver staining.

This study yielded 42 previously unpublished SSR markers, with the prefix 'SAms', on the three mapping populations. These markers will allow for greater saturation of genetic maps and might fill in any gaps, or stretches on the chromosomes where no markers are located. As all the SAms are SSR markers, they are very informative and can be transferred to other mapping populations.

Newly developed SSR markers were combined with published markers to generate the 27 megaplexes used. Multi-/megaplexing can be an efficient way to reduce the cost of using SSR markers for the construction of genetic linkage maps. The success of multiplexing depends on the principle that primers should have comparable annealing temperatures and that the primer sequences should not contain excessive regions of complementarity (Butler *et al*., 2001), which

could lead to primers binding to each other rather than to template DNA (also known as the formation of primer-dimers). In total, 449 SSR markers (241 newly designed and 208 published markers) were implemented on the three mapping populations, and fragment analysis was performed on all seedlings' DNA. The success rate of the megaplexes were variable (Appendix B), with megaplex 7 having the highest success rate (100%) and megaplex 15b, producing a 58% success rate, being the lowest. Success rate was determined as the number of markers producing a scorable allele, from any of the three mapping populations.

SSRs were chosen as the marker of choice for this project because of their many advantages over other DNA markers. The SSR markers are known to be very transferable and informative, and can be used to genotype different mapping populations, as seen by the use of published SSR markers (Liebhard *et al*., 2002; Liebhard *et al*., 2003; Silfverberg-Dilworth *et al*., 2006; Celton *et al*., 2009; Van Dyk *et al*., 2010) Even though SNP markers are the markers of choice for many mapping projects, currently, when the project started little to no sequence data was available to genotype with SNP markers. These SNP markers will however be used to saturate the three genetic maps generated in this project, using the Illumina 9K SNP BeadChip array.

5.2.2 Segregation analysis

Three hundred and twelve SSR markers were successfully scored on the 'Prima' x 'Anna' mapping population, of which 36 was homozygous within the population. Two hundred and seventy-one markers were successfully scored on

the ‘Golden Delicious’ x ‘Anna’ mapping population, of which 41 was found to be homozygous for both parents. Two hundred and sixty-one SSRs were successfully scored on the ‘Golden Delicious’ x ‘Priscilla’ mapping population, 42 of which were homozygous in the parents. Segregation of alleles from all classes of loci was easily studied through the interpretation of electropherograms obtained from automated genetic analyzers. All markers that failed to amplify in under megaplex conditions, whether heterozygous or homozygous, left until all markers were analysed, before they were re-analysed. However, due to time constraints, the three mapping populations were not rescreened with any of the markers that failed previously. The genetic mapping process therefore started with a total of 746 markers in the ‘Prima’ x ‘Anna’ population, consisting of 254 SSRs and 492 DArT markers. 677 markers were used for mapping the ‘Golden Delicious’ x ‘Anna’ population and 831 markers were used to map ‘Golden Delicious’ x ‘Priscilla’. The success rate of primer amplification was 63%, 61.4% and 53% for ‘Prima’ x’ Anna’, ‘Golden Delicious’ x ‘Anna’ and ‘Golden Delicious’ x ‘Priscilla’, respectively. These ratios lie at the bottom end of the amplification success rate reported by Varshney *et al*., (2005). The failure of PCR amplification of SSR-containing regions could be due to poor quality sequence data in the primer designing step (SAms), poor genomic DNA quality, or even the number of primer sets used when performing multiplex or megaplex PCR. The use of megaplexes worked better when they contained between eight and twelve primers, as more primers affected the chemical balance of the PCR reaction, which caused failure in amplification of products. The new primers amplifying trinucleotide repeats also produced PCR products bigger than expected in some cases. Expansions within these repeats have been shown, in

other species viz. *Arabidopsis thaliana* (Sureshkumar *et al*., 2009), to be the cause for these increases in product sizes.

Non-specificity of primers to the DNA could be another reason for sizes differing to those expected, being produced. Primer pairs amplifying more than two fragments per individual were also encountered and are probably multi-locus markers where the primers anneal to more than one site. The complexity of these multi-locus markers depends largely on the number of loci amplified (two or more) as well as the difference in product sizes obtained from the two (or more) different loci. The ease with which these markers could be used only became clear during segregation analysis of alleles in mapping populations in preparation for genetic linkage map construction. Not all markers used yielded products for both parental cultivars, but studying the allele segregation in the progeny, allowed the correct allele products to be identified, and a JoinMap code to be allocated.

It is very important to ensure that observed segregation ratios resemble expected ratios and this is indicative of whether the fragments observed in parental cultivars are correct. Due to sampling error in mapping populations of limited size, it is necessary to bear in mind that segregation distortion is a phenomenon that does occur in nature. This could be due to gametic selection, zygotic selection or both. Xu and Hu (2009) successfully mapped markers with distorted ratios in order to use all resources at their disposable. They did this using an algorithm that estimates QTL and segregation distortion loci.

When observed ratios clearly indicate the presence or absence of a segregation type in the seedlings, caution should be taken and the possibility of the presence of a null allele should be explored.

5.2.3 Genetic mapping

Each mapping population varied with the number of seedlings containing more than 25% missing data points, and these seedlings were omitted when constructing the genetic linkage maps (both integrated and parental). Although markers with more than 40% missing data points were excluded during the determination of genetic linkage groups, many of these markers were successfully assigned to groups, based on strong cross linked (SCL) values (van Ooijen, 2006), particularly in the 'Prima' x 'Anna' mapping population. For the 'Prima' x 'Anna' mapping population a only one individual was excluded based on missing data, while no loci was excluded when generating this map. For the 'Golden Delicious' x 'Anna', 54 seedlings were excluded, while 46 loci were excluded when generating the map. Although this number of seedlings seems high, they were excluded, as no DArT data was available for these seedlings, giving them a high ratio of missing data points. For the 'Golden Delicious' x 'Priscilla' mapping population, six seedlings had more than 25% missing data points and four loci contained more than 40% data was missing. These were removed from the analysis when generating the 'Golden Delicious' x 'Priscilla' integrated map.

A number of markers that did not show sufficient linkage with the linkage groups (LG) obtained using selected grouping criteria were also successfully added to linkage groups using SCL values. Markers that remained excluded after assignment of markers to SCL groups, in F1 populations were not mapped on account of the large amounts of missing data making the placement of these markers very difficult. Markers having enough data to be included in initial linkage group determination steps but that did not show small enough recombination frequencies with any other markers to enable their assignment to linkage groups, might be situated so far apart from any other marker/s that the recombination frequency observed between them is similar to that of markers residing on other linkage groups.

The fragmentation of linkage groups could also be due to regions showing a higher rate of recombination. It is, however, possible to overcome these breakages by the targeted implementation of more markers in order to generate genetic linkage maps that are more saturated. The absence of linkage groups could also be due to published markers, with no known map position, and newly developed markers being linked together, and there is no way of knowing where they are to be positioned on the map, unless a published SSR of known position proves to be linked when more SSRs are added. Many of these markers were among those that did not link and it could well be that they are to be found at the edge of the chromosomes, too far away from a marker of known position.

Previously unknown markers, with no known map position, were included in the study as these showed polymorphism between the parents used. Examples of

these include CH01b09b (LG4) and CH01f03a (LG16) positioned on the 'Prima' x 'Anna' genetic map.

The genetic linkage maps constructed are composed of SSR and DArT markers. The lengths of published maps varied between different research groups, with the 'Fiesta' x 'Discovery' genetic map (Liebhard *et al*., 2003) with a length of 1371cM being the most complete. The 'Prima' x 'Anna' integrated map consists of 400 loci (135 SSRs and 265 DArT loci) and spans a distance of 1021.6cM. The 'Golden Delicious' x 'Anna' map consists of 213 loci (87 SSRs and 126 DArT loci) and spans a distance of 1302.7cM and the 'Golden Delicious' x 'Priscilla' map consists of 353 loci (80 SSRs and 273 DArT loci) and spans a distance of 1079cM. The mapping of SSR markers makes it possible to align these maps with other published maps (Guilford *et al*., 1997; Liebhard *et al*., 2002, 2003; Kenis and Keulemans, 2005; Fernandez-Fernandez *et al*., 2008; Celton *et al*., 2008 and van Dyk *et al*., 2010). Each map does however need to be more saturated, i.e no gaps larger than 10cM, with more SSR, DArT, and SNP markers, as there are large areas of various chromosomes on which no markers are located. Despite having the most SSRs of the three maps, the 'Prima' x 'Anna' map can still be saturated, with particular attention given to linkage groups 1, 8 and 16, with LG 1 being the smallest of the three groups, at 28.6cM long. As all three mapping populations were screened for apple scab, in the green house, the V*f* locus could not be fully mapped in any of the three maps, as shown by the fragmented linkage groups for all maps. This also applies to Expansin-7, which is found in close proximity to the V*f* locus on LG 1(Costa *et al*., 2008).

The mapping of a set of SSRs to two different linkage groups (LG8 and LG15) may indicate homoeology between chromosomes 8 and 15 in the apple genome (Velasco *et al*., 2010). This has been reported previously when Maliepaard *et al*. (1998), found that markers mapping to LG 5 amplified a second locus on LG10.

The 'Golden Delicious' x 'Priscilla' map contains the fewest SSRs of the three. Linkage groups 6, 11 and 13 require more SSRs to be mapped so as to gain the true length of the linkage group. Targeted screening of this population with more SSR markers, will allow more of the unlinked SSRs and DArT markers to link, thus filling out the ends of certain chromosomes, as well as positioning more markers on the map itself.

The third integrated map ('Golden Delicious' x 'Anna') is the largest map constructed, and consists of both SSR and DArT markers. All 17 linkage groups were identified, with LG12 being the largest at 132.1cM and LG7, the smallest, spanning 37.5cM.

In order to fill the gaps in all three genetic maps, SSR markers that were found to be homozygous in each population, but reported to map in other populations (Liebhard *et al*., 2002; Liebhard *et al*., 2003; Silfverberg-Dilworth *et al*., 2006, Celton *et al*., 2009; Van Dyk *et al*., 2010), where gaps are observed could be sequenced to identify SNPs in the alleles that would allow them to be mapped at the desired location.

Despite mapping of SSR markers being an expensive and time consuming exercise, the genetic maps of the three mapping populations generated were successfully used for the identification of putative QTLs for different fruit quality traits, the main objective of this study.

5.2.4 QTL mapping

QTL analyses were carried out using fruit from the each of the three mapping populations mentioned earlier and QTLs were identified using the interval mapping method in combination with restricted multiple QTL mapping as recommended by Van Ooijen *et al*. (2002). The fruit was assessed using a range of sensory and mechanical parameters. Using MapQTL® 5.0, QTLs accounting for stripe-ness, colour, size, form, ground colour, russet, texture, firmness, juiciness, sweetness, acidity, taste, skin toughness, %TSS, mass and diameter were identified. The results confirm the quantitative nature of all the traits analysed because generally one or more QTLs were detected per trait. Because of the small size of the populations, only the most significant QTLs were reported. QTL results also depend on the percentage of population phenotypic variance explained or accounted for by that particular QTL. Kenis *et al*. (2008) distinguished between major and minor QTLs as those explaining greater than 20% of the variance, and those explaining less than 20% of the variance, respectively. QTL clustering similar to those found in other species (Kenis *et al*., 2008; Quilot *et al*., 2004; Cuasse *et al*., 2002) was detected on LG 2, 9, 10, 11, 14, 15 and 16 in all three populations studied.

In this study, QTLs were located from the mean of the three years in which phenotypic data was collected.

Evaluation of identified QTLs based on individual linkage groups

Evaluation of identified QTLs can be analysed based on individual linkage groups. In pre-storage analysis, no QTLs were located on LG 7 and could be as a result of to few makers present in unlinked groups.

On LG 1, the *Md-expansin 7* gene was mapped in apple ('Prima' x 'Fiesta') and pear ('Passe Crassane' x 'Harrow Sweet') genomes in a region where one major apple QTL for fruit firmness had been previously identified (Costa *et al*., 2008). In this study, however, no QTL for texture was identified on this LG in the 'Prima' x 'Anna' population. We did however identify a QTL for texture on LG 1 for 'Golden Delicious' x 'Anna' as well, but the LOD score for this was lower than the genome-wide LOD threshold of 3.8. The QTL however, explains 39.1% of the population variance for the texture trait. LG 1 also contained one of three QTLs for russet, in the 'Prima' x 'Anna' population. This was a major QTL with a LOD score of 5.1 and explained 35.3 of the population variance. LG 1 was also home to one of the four QTLs identified for diameter. This however was a minor QTL that explained only 4.2% of the population variance, even though it had a LOD score of 6.

LG 2 yielded pre-storage QTLs for colour, form, russet, texture, %TSS, acidity, mass and diameter, as well as stripe-ness, colour, size, form, ground colour,

russet, texture and %TSS in post-storage analysis. Table 30 and 31 distinguishes between the QTLs in each mapping population. In 2008, Kenis *et al*. conducted a comprehensive study on fruit physiological traits that located minor QTLs for fruit weight, diameter, acidity and Brix content on LG 2 on a cross between 'Telemon' and 'Braeburn'. These QTLs compare favourably to those identified in this study. The highest LOD score from pre-storage analysis, on this LG was found for diameter (13.4), in the 'Golden Delicious' x 'Anna' population and explained 6.2% of the population variance of the trait, while the lowest was identified for %TSS (3.7), explaining 40% of the population variance of the trait. In post-storage analysis, the QTL for acidity produce the lowest LOD score (3.2), explaining 27.5 % of the population variance of the trait, while the highest LOD score was identified for %TSS (8.3), and this explained 58.5% of the 'Prima' x 'Anna' population variance for this trait.

Pre-storage QTLs for size, russet and skin toughness on LG 3 were all identified on the 'Golden Delicious' x 'Priscilla' mapping population. These all appear to be major QTLs with LOD scores of 6.3, 10.7 and 5, explaining 47.1%, 33.9% and 35.3% of the population variance, respectively. As LG 3 proved the most troublesome to obtain during linkage, it did not yield as many QTLs. We did however locate a QTL for sugar content during post-storage analysis on the 'Prima' x 'Anna' population. This corresponds to a similar QTL identified by Liebhard *et al*., (2003), also found on LG 3, on the 'Fiesta' x 'Discovery' population. Other QTLs identified on 'Prima' x 'Anna' include a major, post-storage colour QTL, with a LOD score of 5.21, explaining 56.3% of the variance, as well as a single QTL for skin toughness. This QTL explained 42% of the

variance of the population and had a LOD score of 5.1.

Pre-storage QTLs were detected on LG4 of the 'Golden Delicious' x 'Anna' population for colour and skin toughness, whereas a QTL for taste was detected on the same LG in the 'Prima' x 'Anna' population. Post-storage analyses yielded a single QTL for fruit form on LG4 of the 'Golden Delicious' x 'Anna' population. In previous studies, King *et al*. (2001) detected a QTL for fruit weight on LG4 of the 'Prima' x 'Fiesta' mapping population. However, since LG 4 and LG 12 were found to be homologous in apple (Velasco *et al*., 2010), similar QTLs on LG 12 on the 'Fiesta' x 'Discovery' population were detected by Liebhard *et al*. (2003). The 'Prima' x 'Anna' population yielded two QTLs on LG 4 from post-storage phenotypic data. These include one for fruit form, with a LOD score of 4.3 and 24.9% of the variance explained by the trait; as well as one for texture, with a LOD score of 4.8 and explaining 34.3% of the variance within the population.

With regards to LG 5, five pre-storage and three post-storage QTLs were detected. A pre-storage QTL was detected for fruit form on the 'Golden Delicious' x 'Anna' population, for firmness on 'Golden Delicious' x 'Priscilla' and sweetness, russet and mass on 'Prima' x 'Anna'. The QTL for fruit form had a LOD score of 3.5, which was lower than the threshold, and a population variance explaining the trait of 27%. It is not surprising to find a QTL for fruit firmness on LG 5 as this LG and LG 10 are known to be homologs of each other, with the Md-ACO gene located on LG 10 (Liebhard *et al*., 2003). The QTL for sweetness differs from those identified earlier, by Liebhard *et al*. (2003), who

detected QTLs on LGs 3, 6, 8, 9 and 14. From post-storage analyses, QTLs for size and form were detected in the 'Prima' x 'Anna' population, as well as a russet QTL in the 'Golden Delicious' x 'Anna'.

The QTLs for diameter on 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla', as well as colour and mass on 'Prima' x 'Anna', were detected on LG 6, from pre-storage analyses. These all appear to be major QTLs as the population variation explaining the trait were 26.5, 30.8, 36.4% and 20% respectively. There were also QTLs for russet on this LG, in post-storage analyses, on the 'Prima' x 'Anna' and 'Golden Delicious' x 'Priscilla' mapping populations.

Very few QTLs for fruit components were detected on this LG 7, in previous studies, with only King *et al*. (2001) detecting QTLs for wedge measure and wedge fracture tests. A similar QTL was detected for fruit firmness on the 'Prima' x 'Anna' population on LG 7, with a LOD score of 4.5 and explaining 53.7% of the population variance. A single post-storage QTL for %TSS was identified on LG 7 on the 'Golden Delicious' x 'Priscilla'. This QTL explained 34.7% of the population variance of the trait, and was detected at a LOD score of 6.9.

A pre-storage QTL for stripe-ness was detected on LG 8, in the 'Golden Delicious' x 'Priscilla' population. This QTL explained 28.2% of the population variance observed for the trait, and had a LOD score of 14.6. Although, no QTLs, for stripe-ness, were detected from post-storage analyses, on this LG, there were

QTLs for both colour and ground colour, in the 'Golden Delicious' x 'Priscilla' population. The QTL for both ground colour and colour are major QTLs, explaining 83.7 and 31.2% of population variance observed for the traits, respectively. Other QTLs observed include, a single QTL for taste ('Golden Delicious' x 'Priscilla') and a major QTL for skin toughness on 'Golden Delicious' x 'Anna', explaining 36.4% of the population variance of the trait.

Pre-storage QTL analysis yielded QTLs for seven traits on LG 9. These include a major QTL for stripe-ness in all three mapping populations. A single QTL for size (5.6; 28.4%) in 'Prima' x 'Anna' and form (3.8; 19.5%) in 'Golden Delicious' x'Anna', as well as QTLs for juiciness (7.1; 35.6%), sugar (7.7; 29.8%), mass (8.6; 46.2%) and diameter (7.4; 41.9%), in the 'Golden Delicious' x 'Priscilla' mapping population. Kenis *et al*. (2008) also detected QTLs for diameter and weight on LG 9 on the 'Telamon' x 'Braeburn' mapping population. Post-storage analyses of LG 9, yielded QTLs for stripe-ness for both 'Prima' x 'Anna' as well as 'Golden Delicious' x 'Priscilla', with the QTL on 'Golden Delicious' x 'Priscilla' explaining 84% of the population variance observed for the trait. Other QTLs include those for size on 'Golden Delicious' x 'Priscilla' and ground colour on 'Prima' x 'Anna'.

Pre-storage QTLs were detected on LG 10 for eight traits viz. fruit form, texture, firmness, juiciness, taste, skin toughness, mass and diameter. QTLs for form, texture and skin toughness were detected on 'Golden Delicious' x 'Priscilla', firmness, juiciness, taste and %TSS were dectected on 'Golden Delicious' x 'Anna' and a diameter QTL was located on LG 10 of 'Prima' x 'Anna'. King *et*

al. (2000) previously identified QTLs for firmness and crispness on LG 10 as well, and Kenis *et al*. (2008) found QTLs for Brix content on this LG as well. It is well known that the Md-ACO1 locus resides on LG 10, and that ACO, also known as ethylene forming enzyme (EFE), is involved in the conversion of ACC to ethylene (Costa *et al*., 2005). Kenis *et al*. (2008) also detected a minor QTL for fruit weight on LG 10, and even though the QTL detected on 'Golden Delicious' x 'Anna' explained more than 20% of the population variance, it was not considered 'major' as the QTL, for mass, on LG 11 explained more than 50% of the population variance observed for the trait. A minor QTL for diameter was detected on 'Prima' x 'Anna' that had a LOD score of 7.2 and explained only 4.1% of the population variance. Firmness, juiciness and taste QTLs were also detected on 'Golden Delicious' x 'Anna' in post-storage analyses, although these were not detected on the other two mapping populations. There was also a minor, post-storage QTL for ground colour on LG 10 in the 'Prima' x 'Anna' population that explained only 17.3% of the variance in the population.

As with LG 10, LG 11 contained QTLs for fruit form, taste, %TSS and mass, as well as QTLs for sweetness, acidity and diameter. The QTLs for fruit form, sweetness, acidity, mass and diameter were detected on 'Golden Delicious' x 'Anna', those for %TSS and taste on 'Golden Delicious' x 'Priscilla', with only a single QTL for diameter detected on LG 11 for 'Prima' x 'Anna'. A single post-storage QTL for ground colour was detected on the 'Golden Delicious' x 'Anna' population and explained 36.4% of the population variance. A post-storage QTL for %TSS was also detected on 'Golden Delicious' x 'Priscilla'. Liebhard *et al*. (2003) detected a minor QTL for fruit firmness, which explained 8% of the

population variance, on LG 11 on the 'Fiesta' x 'Discovery' mapping population. This study, however, did not produce any firmness QTLs on LG 11 in any of the three mapping populations used.

The QTLs detected, using the mean of the phenotypic data, on LG 12 include those for size and diameter on 'Golden Delicious' x 'Anna' from pre-storage phenotypic data. Liebhard *et al*. (2003) also reported a minor QTL for fruit weight on 'Fiesta' x 'Discovery' that explained 6% of the population variance, and a LOD score of 2.7, which could be the same QTL as the ones found in our study. QTLs for ground colour on 'Golden Delicious' x 'Priscilla' and 'Prima' x 'Anna' were also detected, as well as a minor QTL for russet and major QTL for fruit firmness on 'Prima' x 'Anna', from analysis of pre-storage data. This firmness QTL had a LOD score of 5.5 and explained 25.9% of the variance within the population. Liebhard *et al*., (2003) also detected a similar QTL for fruit firmness on LG 12 of the 'Fiesta' x 'Discovery' population, however, King *et al*., (2000) did not identify LG 12, as one that contains a QTL for firmness, on the 'Prima' x 'Fiesta' map. There was a major post-storage QTL for acidity on 'Golden Delicious' x 'Priscilla', with a LOD score of 4.4 and explained 59% of the population variance, observed for the trait. This result was unexpected as it is well known that the major gene for malic acid is located on LG 16.

Only two QTLs were identified from pre-storage analysis on LG 13. This was a minor QTL for skin toughness on LG 13 of 'Prima' x 'Anna' and explained 19.7% of the population variance observed for the trait, while the second was a minor QTL for firmness, which explained only 7.9% of the variance within the

'Prima' x 'Anna' mapping population. There were four post-storage QTLs identified, with three of these on 'Golden Delicious' x 'Priscilla'. These include firmness, sweetness and taste and were all major QTLs with population variances of 33.9, 42.1 and 54.8% respectively. A major QTL for fruit form was detected on 'Golden Delicious' x 'Anna', with a LOD score of 5.1 and explaining 38.8% of the population variance observed for the trait.

Six QTLs were detected on LG 14, with those for size, form, texture and acidity found from pre-storage analysis on 'Golden Delicious' x 'Priscilla'. Two QTLs, viz. juiciness and taste were detected on 'Golden Delicious' x 'Anna'. These QTLs all had a minor effect on the population genotype with % of population variance explained ranging from 4.9% to 20.7%. Fruit size and juiciness QTLs were detected from post-storage phenotypic data on 'Prima' x 'Anna' while a texture QTL was also found on LG 14 of 'Golden Delicious' x 'Priscilla'. This result confirms those reported by Liebhard *et al*. (2003), who detected a QTL for firmness on LG 14 of 'Fiesta' x 'Discovery'. This firmness QTL on 'Golden Delicious' x 'Priscilla' did have a major effect on the population genotype as it explained 74.8% of the population variance, with a LOD score of 5.8, as opposed to 6% and a LOD score of 3.6 in 'Fiesta' x 'Discovery'.

Analysis of pre-storage phenotypic data detected QTLs for texture, firmness, juiciness, %TSS and diameter on LG 15 of 'Prima' x 'Anna'. These QTLs conferred a major effect on the population genotype, with population variance ranging from 22.1% to 56.7%. Costa *et al*. (2005) reportedly mapped the marker *Md*-ACS1 to LG 15, and the QTLs for firmness and texture found here confirm

that the gene for ethylene production is located on LG 15. It was shown (Costa *et al*., 2005) that descendants homozygous for *Md-ACS1-2* have the lowest ethylene production as well as superior shelf-life. A texture QTL was also detected on LG 15 for the 'Golden Delicious' x 'Priscilla' population, but this was not a major QTL, with a LOD score and population variance of 5.4 and 13.5% respectively. A minor QTL for diameter also detected on the 'Golden Delicious' x 'Anna' population, and was one of seven QTLs detected for this trait on the 'Golden Delicious' x 'Anna' population. QTLs from post-storage analysis were detected on 'Prima' x 'Anna' for russet, firmness, sugar content and %TSS. QTLs for sweetness and acidity were detected on 'Golden Delicious' x 'Anna' on LG 15 and these both had a major effect on the population genotype, with population variances explaining 77% and 48.1% observed for the traits, respectively. A minor QTL for skin toughness was detected on 'Golden Delicious' x 'Priscilla', explaining 21.4% of the population variance.

A major, pre-storage QTL for acidity was detected on LG 16 of the 'Prima' x 'Anna' population. This corresponds to the findings of Maliepaard *et al*. (1998) who reported the position of the major gene for malic acid, *Ma*, on LG16 of 'Prima' x 'Fiesta' mapping population. The QTL explains 23.5% of the population variance observed for the trait, and had a LOD score of 4.3. More QTLs for ground colour, texture and sweetness were identified on LG 16 on the 'Golden Delicious' x' Priscilla' mapping population, with sweetness conferring a major effect on the population genotype after explaining 42.1% of the population variance. A minor QTL for diameter was detected on LG 16 of 'Golden Delicious' x 'Anna' and explained only 16.4% of the population variance. A

post-storage QTL for taste was also detected on LG 16 of 'Prima' x 'Anna', that explained 13.7% of the population variance observed for the trait. Since titratable acidity plays an important role in taste indication, it would make sense that the QTL for taste also be found on the same linkage group, as for acidity. A single post-storage QTL was detected on 'Golden Delicious' x 'Priscilla', for fruit size. This QTL explained 31.7% of the population variance observed for the trait.

The final linkage group, LG 17 contained three QTLs form pre-storage analysis as well as three from post-storage analysis. QTLs for stripe-ness, juiciness were detected for 'Golden Delicious' x 'Priscilla' and a QTL was also detected on 'Golden Delicious' x 'Anna', for diameter. Kenis *et al*. (2008) also reported a QTL for diameter on LG 17 of 'Telamon' x 'Braeburn', and like the one reported in this study also only conferred a minor effect on the population genotype. The four QTLs detected from post-storage analysis were found separately on each of the three mapping populations. A colour QTL was detected on 'Golden Delicious' x 'Anna', explaining only 13.5% of the population variance. A second QTL was detected on 'Golden Delicious' x 'Priscilla', for fruit form, and was a major QTL, explaining 49.5% of the population variation observed for the trait. Two QTLs were detected on 'Prima' x 'Anna', the first, for juiciness, that explained 29.5% of the population variance observed for the trait, conferring a major effect on the population genotype; and the second, for acidity which had a LOD score of 5.4 and explained 25.1% of the population variance observed for the trait.

The results of this study can be compared with previous studies in which QTLs

were identified because the genetic linkage maps used were constructed in part using co-dominant and transferable SSR markers (King *et al.,* 2000; Liebhard *et al*., 2003; Kenis *et al*., 2008).

'Prima' x 'Anna' population

QTLs were detected for all traits analysed with only skin toughness showing a LOD score lower than the threshold. The number of QTLs detected for each trait, varied from one (stripe-ness, size, form, ground colour, sweetness, juiciness, sugar content, acidity, taste and skin toughness) to four (firmness), but up to 12 QTLs per trait has been reported by other groups (e.g. fruit flesh firmness; Liebhard *et al*., 2003). QTLs were uniformly spread throughout the genome, but, interestingly, the clustering of five pre-storage QTLs were found localized to LG 2 and LG15, with a grouping of four QTLs detected on LG 12, and three QTLs on LG 5. Similar clusters of fruit quality QTLs have been reported in other apple varieties (Liebhard *et al*., 2003), as well as other fruit species viz. peach (Quilot *et al*., 2004), and tomato (Causse *et al*., 2002). QTL analyses of the same population ('Prima' x 'Anna') after a three-month post-harvest storage with seven-day room temperature storage revealed a cluster of five QTLs on LG15, as in pre-storage analysis. But, in this analysis, only the QTLs for firmness and %TSS were common before and after storage. Four significant QTLs, for fruit firmness were detected, in pre-storage analysis on LG 7, 12, 13 and 15 of which LG15 has been reported to contain Md-ACS1 gene (Costa *et al*., 2005), known to have a strong effect on internal ethylene concentration and thus affecting fruit softening and texture (Zhu and Barritt, 2008). LG 15 also contained a significant

QTLs for texture and juiciness; two traits that correlated fairly well, with a moderately strong r-value, identified in pre-storage analyses of this population.

The 'Prima' x 'Anna' population also showed the presence of five pre-storage QTLs for fruit diameter. LG 1, 10, 11, 12 and 15 were identified for this trait. Kenis *et al*., (2008) did report the presence of a QTL for diameter on LG 10, 'Telamon' x 'Braeburn' which could be similar to the one reported in this study. The QTL on 'Prima' x 'Anna', however, was not a major QTL, as it only explained 4.1% of the population variance. The major QTL for this trait was identified on LG 15 on this population.

Two significant QTLs were also detected for %TSS in the harvested fruit. These were detected on LG2, and 15 in pre-storage analysis with the % variation ranging from 14.7 to 52 and some level of stability with LG 7 and 15 containing QTLs from post-storage analysis.

The QTL for fruit acidity was identified on LG 16 on this population. Maliepaard *et al*., (1998) previously reported LG 16 as containing the *Ma* locus. Although no other QTLs were identified, on this population, for any other sensory traits, there were QTLs for texture and sweetness (sugar content) on LG 16 of 'Golden Delicious' x 'Priscilla'. King *et al*., (2001), however, determined that any association of the *Ma* gene with the regions contributing to sensory traits was unlikely to be a result of the 'perceptual interactions' with the *Ma* locus.

A major QTL for fruit form was detected on LG2 of 'Prima' x 'Anna', in pre-

storage and LG 2, 4 and 5 in post-storage, explaining 22.6, 24.9 and 31.1% percent of the population variation observed for the trait. We therefore see that the QTL for fruit form is stable, on LG 2, from pre-storage to post-storage analyses. LG2 also contains a QTL for mass, and Kenis *et al*. (2008) showed that minor QTLs for fruit weight and diameter also localized to this LG, in one or more years of their study, on 'Telamon' x 'Braeburn'. This clustering together of traits related to fruit development led us to believe that this linkage group could also contain genes, which might control fruit size as a whole. Clustering of QTLs have been reported by Kenis *et al*. (2008), Etienne *et al*. (2002) and Causse *et al*. (2002), in other species such as peach and tomato. However, the QTL specifically for fruit size was not found on LG2, but on LG9, explaining 29.1% of the population variation. This trait was subjectively measured and could be a reason why it was not detected on LG2.

A few very good candidate markers for marker-assisted selection were identified. The first was found on LG9, for stripe-ness. The QTL localized around the New Zealand marker NZmsEB116209 (Celton *et al*., 2008) and was identified in all years of analyses. This QTL for stripe-ness confirms reports of the presence of the major gene, Rf, for stripe-ness, on LG 9 (Crane and Lawrence, 1933; Maliepaard *et al*., 1998). The second QTL was found on LG 06, for colour. This QTL localized around one of the newly designed markers in the study (SAms), viz. SAmsDR998909. This QTL was stable over all three years of evaluation. These QTLs were regarded as being reproducible as they were detected in all years of the study.

‘Golden Delicious’ x ‘Priscilla’ population

Thirty pre-storage and 21 post-storage QTLs were identified in the ‘Golden Delicious’ x ‘Priscilla’ population. Some QTLs identified on this population were very significant, and were identified as ‘major’ QTLs (Kenis *et al*., 2008). Twenty-three of these QTLs were ‘major’ QTLs, accounting for over 20% of the observed population variance of the trait. Clusters of QTLs were identified on LG 3, 9, 14 and 16 in pre-storage analyses and similar clusters were detected from post-storage analyses on LG2 and 13.

A QTL for fruit texture was identified on LG14 in both pre- and post-storage analysis of this population, with LOD scores of 5.8 and 4.6 being identified, respectively and accounting for 13.5 and 74.8% of the observed population variance of the trait. Liebhard *et al*. (2003) reported a QTL for flesh firmness on LG14, as well, encouraging the argument that this chromosome does play a role during expression of genes relating to texture and firmness of fruit. Pre-storage analysis also allowed for the detection of texture QTLs on LG 10, 15 and 16 all of which are reported to contain QTLs relating to fruit texture and firmness (Liebhard *et al*., 2003; King *et al*., 2000 and King *et al*., 2001).

The QTL detected for stripe-ness, on LG 9, was stable in both pre- and post-storage analysis and localized around DArT marker aPa-443206 in both evaluations. LOD scores of 28.2 and 13.7 were observed and accounted for 62.2 and 84% of the population variance, respectively. This result was expected as the major gene for stripe-ness, R*f,* was reportedly found on LG 9 in previous studies

(Crane and Lawrence, 1933; Maliepaard *et al*., 1998).

Liebhard *et al*. (2003) reportedly identified a QTL for sugar content on LG 9 on the 'Fiesta' x 'Discovery' genetic map and comparably, in this study, the same QTL was identified for sweetness. A second QTL for sweetness was also identified on LG 16 of this population. This second QTL also appears to be major, 42.1% of the population variance is explained by this trait. LG 16 also contained the significant QTLs for texture, as well as sugar content (sweetness), on this population. The QTL for texture confirmed results reported by King *et al*., (2001), who also identified LG 16 as the location of a 'texture' QTL, from wedge fracture tests.

There are also traits that do not meet the LOD threshold defining a QTL in this study. These QTLs include the size, form and firmness from post-storage analysis. The QTLs do, however, explain a large percentage of the population variance, of each trait, in this population.

'Golden Delicious' x 'Anna' population

Even though the 'Golden Delicious' x 'Anna' map was largest of the three genetic maps, it contains the least SSR markers, and this accounts for the low LOD scores calculated for possible QTLs. There was, however, a cluster of 4 QTLs on LG10 of this population. These traits include firmness, juiciness, taste and %TSS from pre-storage analyses and firmness, juiciness and taste in post-storage analyses. The QTL identified for firmness was located at the lower-end

of LG10, in post-storage analysis and confirms the results of previous work identifying LG10 as the chromosome containing the QTL and candidate gene, for firmness on 'Prima' x 'Fiesta' and 'Fuji' x 'Mondial Gala' (Costa *et al*., 2010; Zhu and Barritt, 2008; Costa *et al*., 2005). However, the QTL for firmness identified from pre-storage analysis was located on a different region of LG10 as opposed to post-storage analysis. Kenis and Keulemans (2008) reported QTLs for a number of architechtural characteristics viz. height increment, growth rate, internode length, number and length of branches and growth increment, on LG 10. This region of the genome is believed to control aspects of tree growth and therefore have an impact on fruit quality traits (Kenis and Keulemans, 2008) due to its pleiotropic effects.

Another region where clustering of QTLs was detected was on LG 11. Five QTLs were located on this chromosome, for form, sweetness, acidity, mass and diameter. Correlation analyses from pre-storage data showed moderately strong correlations between acidity and sugar content, in this population. Post-storage analyses showed poor correlation between these traits on this population. As LG 11 is known to be homologous to LG 16 (Velasco *et al*., 2010), it is understandable that a QTL for acidity is also found on this chromosome. In 1998, Maliepaard *et al*. reported the position of the major gene for malic acid, *Ma*, on LG16 of the 'Prima' x 'Fiesta' mapping population.

QTLs for fruit size and diameter were located on LG12 and explained 20.7 and 6.3% of the population variance, respectively with LOD scores of 4.2 and 17, respectively. The QTL for size appears to be a major QTL, as it explains greater

than 20% of the population variance.

There are also traits that do not meet the LOD threshold defining a QTL in this study on the ‘Golden Delicious’ population. These include QTLs for colour, skin toughness, %TSS and to a lesser extent, fruit texture and form. The QTLs do, however, explain a large percentage of the population variance, of each trait, in this population.

CHAPTER 6: CONCLUSION

The results of a comprehensive study of various fruit physiological traits, conducted over three years are presented in this thesis. The results obtained here were compared and found to be consistent with previously published studies.

The organoleptic quality of fresh market apples can be described by a set of parameters, including fruit appearance, taste, flavour and texture. The phenotypic analyses performed showed that variance components of seedlings within the population were quite low and it was therefore safe to say that effective field-evaluation of the fruit traits could be based on a single tree, within a population rather than several trees. There were also many relationships shown among sensory and instrumental traits, with some traits being more difficult to score due to imprecision of measurements, and also because of the interactions between the traits. Correlation analyses showed that biochemical pathways that control sensory traits are dependent on the environment, whereas, visual traits such as stripe-ness are genetically controlled. The results also showed that traits that can be measured instrumentally are more useful than those measured subjectively, with correlations from year to year as well as between traits being higher and more reliable than those traits measured subjectively. This was mainly due to the consistency in measurement, as opposed to subjective measurements that leave considerable room for error. This is also because single data points are collected each year i. e. fruit harvested from one tree still only count as one data point, whereas this data set would be more robust if clonal replicates of each tree were present, in order to do data collection. These relicated experiments are therefore very important if consistent subjective analyses are to be performed. Fruit traits such as texture/firmness, size, colour and %TSS are all traits that do not need

subjective measurements, as shown previously. This will, in turn, make detection of year-stable QTLs easier and more accurate. Reliability of data collected from harvested fruit could be affected by various issues. Issues associated with edge effects, weak grafts onto rootstocks, poor health of the individuals and shading are some of the issues encountered when trees, of the progeny, are located in the same field/orchard.

Heritability estimates varied among the different traits, with some, such as stripeness being highly heritable, and probably being controlled by a single or single set of genes, viz. the R*f* gene, mentioned previously. The highest heritability were calculated for those traits that were instrumentally measured, as opposed to subjectively evaluated traits that had much lower heritability, most likely caused by imprecise measurements.

An approach focusing on a single mapping population rather than multiple populations would also be more viable, and save one time when harvesting fruit, as the broad time scale for harvesting more than one population will be avoided. Although using a single mapping population would limit phenotypic analyses strictly to, within family and yearly variation, it would be less complicated than trying to compare more than one mapping population at a time. It would also allow a shorter time period for harvesting of fruit.

The use of SSR markers, and the development of megaplex PCRs, greatly increased the efficiency and reduced the cost involved in the implementation of this type of molecular characterization studies. The mapping of SSR markers in common makes it possible to align the individual and integrated maps with published maps (Maliepaard *et al*., 1998; Liebhard *et al*., 2002; Silfverber-Dilworth *et al*., 2006; Celton *et al*., 2008; van Dyk *et al*., 2010). This efficiency

is primarily based on the high number of markers amplified in a single reaction. The allocation of SSR markers into megaplexes, also increased efficiency and reduced the cost of generating integrated genetic linkage maps in *Malus* spp. This study produced genetic linkage maps using SSR, as well as DArT markers, for the three mapping populations reported, viz. 'Prima' x 'Anna', 'Golden Delicious' x 'Anna' and 'Golden Delicious' x 'Priscilla'. The general coverage of the 17 linkage groups obtained for the three maps in this study, is comparable with the reference maps of 'Fiesta' x 'Discovery' (Maliepaard *et al*., 1998) and 'Malling 9' x 'Robusta 5' (Celton *et al*., 2008). In all three maps very few markers mapped to LG 1, because some markers like CH-Vf1, disease resistance associated marker (Bus *et al*., 2008; Celton *et al*., 2008) could not link. Cultivars in this study may not be used for studies related to scab resistance, as the mapping populations were previously screened, in the greenhouse, for *Venturia inaqualis.*

The use of the DArT technique to saturate apple linkage maps has also been reported before (van Dyk-personal communication) and was applied here to saturate the linkage maps, and allowed for all 17 linkage groups to be identified and saturated in these populations. Even though the DArT technique allows for the analysis of many loci per experiment, without requiring sequence information, and low-cost data production, there are a few disadvantages. The main disadvantage being that they are dominant markers and scoring is performed based on the presence or absence of products at a particular locus.

For effective screening of progeny, using megaplexes, it would be advisable to use a single mapping population. Megaplexes can then be generated and optimized using only the parents of the particular population. This would eliminate the chance of parents being homozygous for a particular primer, at a particular locus and would immediately remove any uninformative markers from the scoring process. These markers can, however, be used in megaplexes to be used on other mapping populations, or rather, populations with parents, polymorphic for that marker. The number of markers added to these megaplexes would also affect the success rate of PCR amplification, as more markers would change the chemical composition of the PCR reaction thus inhibiting the amplification of products. Thus increasing the chance of any scoring errors that could occur.

Due to the presence of co-dominant microsatellite markers, full alignment and comparison of the identified QTLs in this study with previous studies of 'Prima' x 'Fiesta' (Maliepaard *et al*., 1998; King *et al*., 2000, 2001), 'Fiesta' x 'Discovery' (Liebhard *et al*., 2003), 'Telamon' x 'Braeburn' (Kenis and Keulemans, 2006; Kenis *et al*., 2008) and 'Ralls Janet' x 'Delicious' (Igarashi *et al.*, 2008) was feasible. These comparisons are of great importance and value for breeding purposes since they provide information about alleles associated with the QTL, reveal more QTLs affecting the same trait and allow an estimation of the effectiveness of the QTL associated alleles in other genetic environments. In total, 79 and 60 QTLs were detected in pre- and post storage analyses, respectively. As the populations used were relatively small, the effect of mainly major QTLs was detected. The QTL results produced in this study, will, however, need to be reproduced in other mapping populations, so as to confirm

the stability of these QTLs, at different locations and over several years. This would have to be performed before the co-segregating molecular markers can be considered for progeny screening in the apple-breeding programme. Nonetheless, these results have identified population- and year-stable QTLs, in one or more of the mapping populations used, which hold some promise for further development. Further linkage map saturation, with SSR and SNP molecular markers, of the three mapping populations in this study, will allow integration and alignment of these with existing maps, and thus provide a more comprehensive interpretation and analysis of the QTL results obtained. This has been made easier with the release of the apple genome in August 2010 (Velasco *et al*., 2010), as SNP approaches were largely dependent on the availability of sequence information. This is also the reason why SNPs were not chosen as the marker of choice for this project. Only more recently, with the development of the Illumina 9K SNP BeadChip, has it become a viable option to generate genetic maps consisting entirely of SNP markers.

Several QTL clusters were detected and using multivariate analyses could help in describing these clusters, but a better solution would be to use fine mapping experiments, to dissect these clusters. The analyses from this study form the basis of QTL characterization, following either a candidate gene approach or through positional cloning. Some linkage groups that were detected from previous studies could not be associated with QTLs in this thesis. This can be explained firstly by, lack of enough markers on some linkage groups e.g. LG 1, therefore no exact positioning of QTLs was feasible, secondly, high heterozygosity among

cultivars, thus influencing the presence or absence of a locus and also its position on genome and thirdly different environmental influence upon cultivars.

It is also important to note that some previous studies (Liebhard *et al*., 2003; Kenis *et al*., 2008) focused on fruit traits at harvest, whereas this study followed up with post-storage QTL analysis as well, to show how the position of QTLs as well as correlations, between traits, change from pre- to post-storage.

The few candidate markers identified as being stable in all years of the study, viz. NZmsEB116209 and SAmsDR998909, on LG 9 and 6, in the 'Prima' x 'Anna' mapping population, respectively can now be looked into in more detail to determine whether or not they can, firstly, be validated in other mapping populations, and secondly, be used in marker-assisetd selection.

In conclusion, this study forms the basis for further comparative genome analysis. Using this, the role of various genes on the outcome of fruit quality can now be investigated. Using the integrated genetic maps, and the QTLs identified, candidate markers associated with these QTLs can be used for marker-assisted selection, to increase the speed and efficiency of the apple-breeding program.

REFERENCES

Abbott, J. A., Watada, A. E., Massie, D. R., 1976. Effegi, Magness-Taylor, and Instron fruit pressure testing devices for apples, peaches, and nectarines. *J Amer Soc Hort Sci*. **101**, 698-700

Abbott, J. A., 1994. Firmness measurement of freshly harvested "Delicious" apples by sensory methods, sonic transmission, Magness-Taylor, and compression. *J Amer Soc Hort Sci*. **119**, 510-515

Abbott, J. A., Lu, R., Upchurch, B. L., Stroshine, R. L., 1997.Technologies for nondestructive quality evaluation of fruits and vegetables. *Hortic Rev*. **20**, 1-12

Ackermann, J., Fischer, M., Amado, R., 1992. Changes in Sugars, Acids, and Amino Acids during Ripening and Storage of Apples (Cv. Glockenapfel). *J Agric Food Chem*. **40***,* 1131-1134

Alexander, L., Grierson, D., 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *J Exp Bot*. **53**, 2039-2055

Alston, F. H., Watkins, R., 1975. Apple breeding at East Malling Proceedings EUCARPIA Symposium on Tree Fruit Breeding 1973, Canterbury, pp. 14-29.

American Chemical Society. 2000. Genetically modified trees sow seeds of growing conflict. *Environ News*. **34**, 69-70

Ampun, W., 1997. Enhancement of aroma volatile compounds in apples. PhD thesis, Department of Plant Science, Massey University, Palmerston North, New Zealand. 500 pp

Awad, M. A., Jager, A., van Westing, L. M., 2000. Flavanoid and chlorogenic acid levels in apple fruit: Characterisation of variation. *Sci Hort*. **83**, 249-263

Ayala-Silva, T., Schnell, R. J., Meerow, A. W., Winterstein, M., Cervantes, C., Brown, J. S., 2005. Determination Of Color And Fruit Traits Of Half-Sib Families Of Mango (*Mangifera Indica* L.). *Proc Fla State Hort. Soc.* **118**, 253-257

Bain, J. M., Robertson, R. N., 1951. The physiology of growth in apple fruits. 1. Cell size, cell number, and fruit development. *Aust J Sci Res*. **4,** 75-91

Bartley, I. M., Stoker, P. G., Martin, A. D. E., Hatfield, S. G. S., Knee, M., 1985. Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. *J Sci Food Agric.* **36***,* 567-574

Bartram, R. D., Olsen, K. L., Williams, M. W., 1971. Influence of summer application of succinic acid-2, 2 dimethylhydrazide on firmness of 'Delicious' apples in storage. *HortSci*. **6**, 240-241

Bassi, D., Selli, R., 1990. Evaluation of Fruit Quality in Peach and Apricot. *Adv Hort Sci*. **4**, 107-112

Batschauer, A., Rocholl, M., Kaiser, T., Nagatani, A., Furuya, M., Schäfer, E., 1996**.** B**l**ue and UV-a light regulated CHS expression in *Arabidopsis* independent of phytochrome A and phytochrome B. *Plant Jour.* **9**, 63-69

Beer, S.C., Matthews, D., O' Donouyhue, L. S., Sirpoowiwat, W., Sorrells, M. E., Souza E., 1997. Associations between Molecular Markers and Quantitative Trait in an oat Germplasm pool: can we infer linkages? *J QTL.* **3**, 1-12

Benson, G., 1999. Tandem repeats finder: a program to analyze DNA sequences. *Nucl Acid Res.* **27**, 573-580

Berger, R. G., Dettweiler, G. R., Krempler, M. R., Drawert, F., 1992. Precursor atmosphere technology. Efficient aroma enrichment in fruit cells. *In:* Teranishi, R., Takeoka, G. R., Guntert, M., *ed.* Flavour precursors. Thermal and enzymatic conversions. *ACS Symposium Series.* **490**, 59-71

Beverley, R. B., Latimer, J. G. Smitle, D. A., 1993. Preharvest physiological and cultural effects on postharvest quality, In Shewfelt, R. L., Prussia, S. E. (Eds), Postharvest Handling: a system approach. pp 73-98. Academic Press, Inc., London

Bieleski, R. L., 1982, Sugar alcohols, in: (Loewus, F., Tanner, W., Eds.), Encyclopedia of Plant Physiology New Series, vol. 13A, pp 158-192. Springer, Berlin

Biswall, B., 1995. Carotenoid catabolism during leaf senescence and its control by light. *J Photochem Photobiol.* **30**, 3-14

Blanpied, G. D., 1975. The effect of cooling rate on the market quality of "McIntosh" apples. *Proc Amer Soc Hort Sci*. **70**, 58-70

Botstein, D., White, R. L., Skolnick, M., Davis, R. W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer J Hum Genet*. **32**, 314 –331

Bourne, M. C., 1980. Texture evaluation of horticultural crops. *Hort Sci*. **15***,* 51-56

Bourne, M. C., 2002. Food texture and viscosity: Concept and measurement. pp 187-223. Academic press, San Diego, CA, USA

Bramlage, W. J., Drake, M., Lord, W. J., 1980. The influence of mineral nutrition on the quality and storage performance of pome fruits grown in North America. *Acta Hort*. **92**, 29-40

Breyne, P., Boerjan, W., Gerats, T., Van-Montagu, M., Van-Gysel, A., 1997. Application of AFLP in plant breeding, molecular biology and genetics. *Belg Jour Bot*. **129**, 107-117

Broothaerts, W., Keulemans, J., van Nerum, I., 2004. Self-fertile apple resulting from S-RNase gene silencing. *Plant Cell Rep*. **22**, 497-501

Brown, A.G., 1960. The inheritance of shape, size and season of ripening in families of the cultivated apple. *Euphytica.* **9**, 327-337.

Brown, A. G., 1975. Apples. In: Advances in fruit breeding (Janick and Moore Ed.). Purdue University Press. West Lafayette, Indiana

Browne, S. K., 1992. Genetics of apple. *Amer Soc Hort Sci.* **9**, 333-358

Bufler, G., 1984. Ethylene-Enhanced 1-aminocyclpropane-1-1carboxylic Acid Synthase activity in ripening apples. *Plant physiol*. **75**, 192-195

Burchill, R. T., 1964. Hot water as a possible post-harvest control of Gloesporium rots of stored apples. *Plant Pathol*. **13**, 106-107

Bus, V. G. M., Van de Weg, W. E., Durel, C. E., Gessler, C., Calenge, F., Parisi, L., Rikkerink, E. H. A., Gardiner, S. E., Patocchi, A., Meulenbroek, M., Schouten, H., Laurens, F. N. D., 2004. Delineation of a scab resistance gene cluster on linkage group 2 of apple. *Acta Hort*. **663**, 57-62

Bus, V. G. M., Laurens, F. N. D., Van de Weg, W. E., Rusholme, R. L., Rikkerink, E. H. A., Gardiner, S. E., Bassett, H. C. M., Kodde, L. P., Plummer, K. M., 2005. The *Vh8* locus of a new gene-for-gene interaction between *Venturia*

inaequalis and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *Malus pumila* R12740-7A. *New Phytol.* **166**, 1035-1049.

Bus, V. G. M., Rikkerink, E. H. A., Van de Weg, W. E., Rusholme, R. L., Gardiner, S. E., Bassett, H. C. M., Kodde, L. P., Parisi, L., Laurens, F. N. D., Meulenbroek, E. J., Plummer, K. M., 2005. The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. *Molecul Breed*. **15**, 103-116

Calenge, F., Faure, A., Goerre, M., Gebhardt, C., Van de Weg, W. E., Parisi, L., Durel, C-E., 2004. Quantitative trait loci (QTL) analysis reveals both broad-spectrum and isolate specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. *Phytopathol*. **94**, 370-379

Calenge, F., Drouet, D., Denance, C., Van de Weg, W. E., Brisset, M-N., Paulin, J-P., Durel, C-E., 2005. Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theor Appl Genet*. **111**, 128-135

Calenge, F., Durel, C-E., 2006. Both stable and unstable QTLs for resistance to powdery mildew are detected in apple after four years of field assessments. *Mol Breed*. **17**, 329-339

Cardello, A. V., 1995. Food Quality: Relativity, Context and Consumer Expectations. *Food Qual Pref*. **6**, 163-170

Castiglioli, P., Heun, M., Muller, K. J., Pose, C., Rohde, W., Salamini, F., Terzi, V., 1998. An AFLP-Based Procedure for the Efficient Mapping of Mutations and DNA Probes in Barley. *Genetics*. **149**, 2039-2056

Causse, M., Saliba-Colombani, V., Lecomte, L., Duffe, P., Rousselle, P., Buret, M., 2002. QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. *J Exp Bot.* **53**, 2089-2098

Celton, J., Tustin, D. S., Chagné, D., Gardiner, S. E., 2008. Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. *Tree Genetics and Genomes.* **5**, 93-107

Cevik, V., King G. J., 2002. High-resolution genetic analysis of the *Sd-1* aphid resistance locus in *Malus* spp. *Theor Appl Genet.* **105**, 346-354

Chagné, D., Carlisle, C. M., Blond, C., Volz, R. K., Whitworth, C. J., Oraguzie, N. C., Crowhurst, R. N., Allan, A. C., Espley, R. V., Hellens, R. P., Gardiner, S. E., 2007. Mapping a candidate gene (MdMYB10) for red flesh and foliage colour in apple. *BMC Genomics*. **8**, 212

Chiu, H. J., 1984. Effects of pre-storage and storage treatments of brown core development in “McIntosh” apples. *Acta Hort.* **157**, 135-142

Christie, P. J., Alfenito, M. R., Walbot, V., 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta*. **194**, 541-549

Ciardi, J., Klee, H., 2001. Regulation of ethylene-mediated responses at the level of the receptor. *Ann Bot*. **88**, 813-822

Conner, J. P., Brown, S. K., Weeden, N. F., 1997. Randomly Amplified Polymorphic DNA-based genetic linkage maps of three apple cultivars. *J Am Soc Hort Sci.* **122**, 350-359

Conner, J. P., Brown, S. K., Weeden, N. F., 1998. Molecular-marker analysis of quantative traits for growth and development in juvenile apple trees. *Theor Apple Genet*. **96**, 1027-1035

Cosgrove, D. J., 1997. Assembly and enlargement of the primary cell wall in plants. *Annu Rev Cell Dev Biol*. **13**, 171-201

Costa, F., Sara, S., Van de Weg, W. E., Guerra, W., Cecchinel, M., Dallivina, J., Koller, B., Sansivini, S., 2005. Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (*Malus domestica Borkh*). *Euphytica*. **141**, 181-190

Costa, F., Van de Weg, W. E., Stella, S., Dondini, L., Pratesi, D., Musacchi, S., Sansavini, S., 2008. Map position and functional allelic diversity of Md-Exp7, a new putative expansin gene associated with fruit softening in apple (*Malus x domestica* Borkh.) and pear (*Pyrus communis*). *Tree Genetics & Genomes.* **4**, 575-586

Couranjou J., 1995. Genetic studies of 11 quantitative characters in apricot. *Hort Sci*. **61**, 61-75

Crane, M. B., Lawrence, W. J. C. 1933. Genetic studies in cultivated apple. *J Genet.* ***28***, 285-290

Crane, M. B., 1953. The genetics and breeding of tree fruits. Rpt. 13th Int Hor Cong. London, pp 687-695

Cummins, J. N., Forsline, P. L., Way, R. D., 1977. A comparison of russeting among 'Golden Delicious' subclones. *Hort Sci.* **12**, 241-242

Curry, E. A., 1997. Temperatures for optimal anthocyanin accumulation in apple tissue. *J Hort Sci*. **72**, 723-729

Dandekar, A. D., Teo, G., Defilippi, B. G., Uratsu, S. L., Passey, A. J., Kader, A. A., Stow, J. R., Colgan, R. J., James, C. J., 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. *Transgen Res*. **13**, 373-384

DeEll, J. R., Prange, R. K., 1992. Post-harvest quality and storage of scab-resistant apple cultivars. *HortTechnol.* **2**, 352-358

DeEll, J. R., Prange, R. K., 1994. Maturity and storage requirements of "McIntosh" and "Cortland" strains, pp 86-87. 131st Annual Report of the Nova Scotia Fruit Growers' Association.

DeEll, J. R., Khanizadeh, S., Saad, F., Ferree, D. C., 2001. Factors affecting apple fruit firmness-A Review. *J Am Pomol Soc*. **55**, 8-27

Demmig-Adams, B., Gilmore, A. M., Adams, W. W., 1996. In vivo functions of carotenoids in higher plants. *FASEB J*. **10**, 403

Denne, M. P., 1960. The growth of apple fruitlets, and the effect of early thinning on fruit development. *Annu Bot*. **24,** 397-406

De Souza, V. A. B., Byrne, D. H., 1998. Heritability, Genetic and Phenotypic Correlations, and Predicted selection response of Quantitative traits in Peach: II. An analysis of several fruit traits. *J Amer Soc Hort Sci*. **123,** 604-611

Dimick, P. S., Hoskin, J. C., 1983. Review of apple flavor - state of the art. *CRC Crit Rev Food Sci Nutr.* **18**, 387-409

Dirinck, P., Schamp, N., 1989. Instrumental aroma analysis for objective evaluation of the parameters influencing aroma formation in apples and for

prediction of the optimum picking date. *Ada Hort.* **258***,* 421-428

Dirlewanger, E., Graziano, E., Joobeur, T., Garriga-Calere, F., Cosson, P., Howad, W., Arus, P., 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. *Proc Natl Acad Sci.* **101**, 9891-9896

Dixon, J., Hewett, E. W., 2000. Factors affecting apple aroma/flavour volatile concentration: a review. *NZ J Crop and Hort Sci*. **28**, 155-173

Doerge, R. W., Zeng, Z., Weir, B., 1997. Maximum Likelihood from incomplete data via the EM algorithm. *J R Stat Soc Ser B*. **39**, 1-38

Doyle, J. J., Doyle, J. L., 1990. Isolation of plant DNA from fresh tissue. *Focus.* **12**, 13-15.

Drake, S. R., Larsen, F. E., Higgins, S. S., 1993. Greenspur on selected rootstocks- a better green. *Good Fruit Grower*. **44**, 75-77

Dunemann, F., Bräcker, G., Markussen, T., Roche, P., 1999. Identification of molecular markers for the major mildew resistance gene *Pl2* in apple. *Acta Hort*. **484**, 411-416

Durel, C-E., Parisi, L., Laurens, F., Van de Weg, W. E., Liebhard, R., Jourjon, M. F., 2003. Genetic dissection of partial resistance to race 6 *Venturia inaequalis* in apple. *Genome*. **46**, 224-234

Eccher, T., 1986. Russetting and shape of Golden Delicious apples as related to Endogenous GA content of Fruitlets. *Acta Hort*. **179**, 767-770

Edge, R., McGarvey, D. J., Truscott, T. G., 1997. The carotenoids as anti-oxidants, *J Photochem Photobio*l. **41**, 189-200

El-Ansary, M. M., Ayaad, H. M., El-Morshed, F., 1992. The effect of spraying and post-harvest treatment with calcium chloride on the fruit quality changes of "Anna" apples during cold storageat 4°C. *Acta Agronomica Hungarica*. **41**, 209-915

Falconer, D. S., 1989. Introduction to quantitative genetics. 3rd ed. Longman Science and Technology. London

Falconer, D. S., Mackay, T. F. C., 1996. Introduction to quantitative genetics., 4th ed. Pearson, England.

Fallahi, E. T., Richardson, D. G., Westwood, M. N., 1985. Quality of apple fruit from high density orchard as influenced by rootstocks, fertilizer, maturity and storage. *J Amer Soc Hort Sci.* **119**, 71-74

Fellman, J. K., Miller, T. W., Mattinson, D. S., Mattheis, J. P., 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. *Hort Sci*. **35**, 1026-1033

Fernández-Fernández, F., Evans. K. M., Clarke, J. B., Govan, C. L., James, C. M., Marić, S., Tobutt, K. R., 2008. Development of an STS map of an interspecific progeny of *Malus*. *Tree Genetics & Genomes.* **4**, 587

Ferree, D. C., 2000. What determines fruit size? *Fruit Grower*. pp 33-34

Firoozabady, E., Olmo, H. P., 1987. Heritability and correlation studies in certain quantitative traits in table grapes, *Vitis* spp. *Vitis*. **26**, 132-146

Fisher, D. V., 1939. Storage of "Delicious" apples in artificial atmospheres. *Proc Amer Soc Hort Sci*. **37**, 459-462

Fluhr, R., Mattoo, A. K., 1996. Ethylene: biosynthesis and perception. *Critical Rev Plant Sci*. **15**, 479-523

Forkmann, G., 1993. Genetics of Flavonoids. In: (harborne, J.B., Ed*.)*. The flavonoids: advance in research since 1986. London, UK: Chapman and Hall, 537-564

Forsyth, F. R., Eaves, C. A., Lightfoot, H. J., 1969. Storage quality of "McIntosh apples as affected by removal of ethylene from the storage atmosphere. *Can J Plant Sci*. **49**, 567-572

Francis, F. J., 1970. Color Measurement in Plant Breeding. *Hort Sci*. **5**, 102-106

Francis, F. J., 1970. Anthocyanins in pears. *Hort Sci*. **5**, 42

Francis, F. J., 1989. Food colorants: Anthocyanins. *Crit. Rev. food Sci. Nutr*. **28**, 273-314

Freslon, V., Bus, V., Gianfranceschi, L., Patocchi, A., Durel, C. E., 2006. Presented at the 3rd International Rosaceae genomics conference, Napier, New zealand, 19-22 March 2006

Galliard, T., 1968. Aspects of lipid metabolism in higher plants-II. The identification and quantitative analysis of lipids from the pulp of pre- and post-climacteric apples. *Phytochemistry*. **7**, 1915-1922

Gao, M., Tao, R., Miura, K., Dandekar, A. M., Sugiura, A., 2001. Transformation of Japanese persimmon (*Diospyros kaki Thunb.)* with apple cDNA encoding NADP dependent sorbitol-6-phosphate dehydrogenase. *Plant Sci*. **160**, 837-845

Gao, Z. S., Van de Weg, W. E., Schaart, J. G., Schouten, H. J., Tran, D. H., Kodde, L. P., Van der Meer, I. M., Van der Geest, A. H. M., Kodde, J., Breiteneder, H., Hoffmann-Sommergruber, K., Bosch, D., 2005. Genomic cloning and linkage mapping of the *Mal d 1* (PR-10) gene family in apple (*Malus domestica*). *Theor Appl Genet*. **111**, 171-183.

Gao, Z. S., Van de Weg, W. E., Schaart, J. G., Van der Meer, I. M., Kodde, L., Laimer, M., Breiteneder, H., Hoffmann-Sommergruber, K., Gilissen, L. J. W. J., 2005. Linkage map positions and allelic diversity of two *Mal d 3* (non-specific lipid transfer protein) genes in the cultivated apple (*Malus domestica*). *Theor Appl Genet*. **10**, 479-491

Gardiner, S., Bus, V., 1998. Pest and Resistance: III Marker-assisted breeding. *The Orchardist.* **8**, 59-61

Gardiner, S., Bus, V. G. M., Rusholme, R. L., Chagné, D., Rikkerink, E. H. A., 2007. Apple, p. 1-62. *In* C. Kole (ed.), Genome mapping and molecular breeding in plants. Fruits and Nuts., vol. 4. Springer-Verlag, Berlin Heidelberg.

Gianfranceschi, L., Soglio, V., 2004. The European project HIDRAS: Innovative Multidisciplinary approaches to breeding high quality disease resistant apples, Eucarpia Symposium on fruit breeding and genetics. *Acta Hort.* **663**, 327-330,

Giovannoni, J., 2001. Molecular biology of fruit maturation and ripening. *Ann Rev Plant Physiol Plant Mol Biol.* **52**, 725-749

Gitelson, A. A., Zur, Y., Chivkunova, O. B., Merzlyak, M. N., 2002. Assessing carotenoid content in plant leaves with reflectance spectroscopy. *Photochem Photobiol*. **75**, 272-281

Granger, R. L., Rouselle, G. L., 1984. Effect of ethylene removal by

alumina/potassium permanganate on “McIntosh” apples in regular and controlled atmosphere storages. *Acta Hort*. **157**, 57-64

Greene, D. W., 1993. A comparisons of the effects of several cytokinins on apple fruit set and fruit quality. *Acta Hort*. **329**, 144-146

Greene, D. W., 1996. AVG: a new pre-harvest drop control compound for apples. *Proc 101st Annual Meeting of the Massachusetts Fruit Growers' Association*. **102**, 79-84

Greenhalgh, W. T., Goodley, G. L., 1976. Studies of fruit shape in apples:a survey of factors influencing the development of “typiness” of the cultivar “Red delicious”. *Aust J Exp Agric Anim Husb*. **16**, 592-595

Grierson, D., Fray, R. G., 1994. Control of ripening in transgenic tomatoes. *Euphytica*. **79**, 251-263

Grierson, D., Schuch, W., 1993. Control of ripening. *Philos Trans Roy Soc London*. **342**, 241-250

Guilford, P., Prakash, S., Zhu, J. M., Rikkerink, E., Gardiner, S., Bassett, H., Forster, R., 1997. Microsatellites in *Malus x domestica* (apple): abundance, polymorphism and cultivar identification. *Theor Appl Genet.* **94**, 249-254

Gygax, M., Gianfranceschi, L., Liebhard, R., Kellerhals, M., Gessler, C., Patocchi, A., 2004. Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata jackii*. Theor Appl Genet **109,** 1702-1709

Haley, C. S., 1995. Livestock QTLs – bringing home the bacon? *Trends Genet*. **11**, 463-524

Haley, C. S., Andersson, C., 1997. Linkage mapping or Quantitative Trait Loci in Plants and Animals IN Dear P. H., ed. Genome Mapping: A Practical Approach. Oxford University Press. Ch 3 pp 49-70

Hamilton, A. J., Lycett, G. W., Grierson, D., 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature.* **346**, 284-287

Hampson, C. R., Quamme, H. A., Hall, J. W., MacDonald, R. A., King, M. C., Cliff, M. A., 2000. Sensory Evaluation as a Selection Tool in Apple Breeding. *Euphytica*. **111**, 79-90

Hansche, P. E., Beres, V., 1980. Genetic remodeling of fruit and nut trees to facilitate cultivar development. *Hort Sci*. **15**, 710-715

Hansche, P. E., 1986. Heriatbility of fruit quality traits in peach and nectarine breeding stocks dwarfed by the *dw* gene. *Hort Sci*. **25,** 1193-1195

Harada, T., Sunako, T., Wakasa, Y., Soejima, J., Satoh, T., Niizeki, M., 2000. An allele of 1-aminocyclopropane-1-carboxylate synthase gene (Md-ACS1) accounts for the low ethylene production in climacteric fruits of some apple cultivars. *Theor Appl Genet*, **101**, 742-746

Harada, T., Kurahashi, W., Yanai, M., Wakasa, Y., Satoh, T., 2005. Involvement of cell proliferation and cell enlargement in increasing the fruit size of Malus species. *Sci Hort*. **105,** 447-456

Harborne, J. B., Grayer, R. J., 1988. The anthocyanins. In: (Harborne, J.B., Ed.). The flavonoids: Advances in research since 1980. Chapman and Hall Ltd. London, 526-537

Harker, F. R., Redgwell, R. J., Hallett, I. C., Murray, S. H., 1997. Texture of fresh fruit. In Horticutural Reviews. Vol. **20**. Wiley and Sons, pp121-224

Harker, F. R., Marsh, K. B., Young, H., Murray, S. H., Gunson, F. A., Walker, S. B., 2002. Sensory Interpretation of Instrumental Measurements 2: sweet and acid taste of apple fruit. *Postharvest Biol Technol.* **24**, 2441-250

Harker, F. R., Gunson, F. A., Jaeger, S. R., 2003. The Case for Fruit Quality: an interpretive review of consumer attitudes, and preference for apples. *Postharvest Biol Technol*. **28**, 333-347

Harris, S. A., Robinson, J. P., Juniper, B. E., 2002. Genetic clues to the origin of the apple. *Trends Genet*. **18**, 426-429

Hauagge, R., Cummins, J. N., 1991. Genetics of length of dormancy period in *Malus* vegetative buds. Journal of the American Society for Horticultural *Science*. **116,** 121-126.

Havaux, M., 1998. Carotenoids as stabilisers in chloroplasts. *Trends Plant Sci*. **3,** 147-151

Heath, H. B., Reineccius, G., 1986. Flavour chemistry and technology. Westport, Connecticut, AVI Publishing, pp 442

Heintz, C. M., Kader, A. A.,1983. Procedures for Sensory Evaluation of Horticultural Crops. *HortSci*. **18**, 18-22

Heller, W., Forkmann, G., 1988. Biosynthesis. In: (Harborne, J.B., Ed.). The flavonoids. Chapman and Hall, pp399-425, London

Hemmat, M., Weeden, N. K., Mangenaris, A. G., Lawson, D. M., 1994. Molecular Marker Linkage Map for Apple. *J Hered.* **85**, 4-11

Hemmat, M., Brown, S. K., Weeden, N. F., 2002. Tagging and mapping scab-resistance genes from R12740-7A apple. *Jour Amer Soc Hort Sci*. **127,** 365-370

Hewett, E. W., Kim, H. O., Lallu, N., 1999. Postharvest physiology of kiwifruit: the challenges ahead. *Acta Hort.* **498**, 203-216

Hirai, M., 1981. Purification and characteristics of sorbitol-6-phosphate dehydrogenase from loquat leaves. *Plant Physiol*. **67**, 221-224

Hoeschele, I., Uimari, P., Grignola, F. E., Zhang, Q., Gage, K. M., 1997. Advances in Statistical Methods to Map Quantitative Trait Loci in Outbred Populations. *Genetics*. **147**, 1445-1457

Holton, T. A., Cornish, E. C., 1995. Genetics and biochemistry of anthocyanin biosynthesis. *The Plant Cell*. **7**, 1071-1083

Honda, C., Kotoda, N., Wada, M., Kondo, S., Kobayashi, S., Soejima, J., Zhang, Z., Tsuda, T., Moriguchi, T., 2002. Anthocyanin biosynthetic genes are coordinately expressed during red coloraration in apple skin. *Plant Physiol Biochem*. **40**, 955-962

Jaccoud, D., Peng, K., Feinstein, D., Kilian, A., 2001. Diversity Arrays: a solid-state technology for sequence information independent genotyping. *Nucl Acid Res*. **29**, e25

James, C. M., Clarke, J. B., Evans, K. M., 2004. Identification of molecular markers linked to the mildew resistance gene *Pl-d* in apple. *Theor Appl Genet*. **110,** 175-181.

James, C. M., Evans, K. M. 2004. Identification of molecular markers linked to the powdery mildew resistance genes *Pl-d* and *Pl-w* in apple. *Acta Hort*. **663,** 123-127

Janick, J., Cummins, J. N., Brown, S. K., Hemmat, M., 1996. Apples. In: (Janick, J., Moore, J. N. Eds.), In Fruit Breeding, vol 1. pp 25. Wiley

Janick, J., Cummins, J. N., Brown, S. K., Hemmet, M., 1996. Tree and tropical Fruits. *Fruit Breed*. **1**, 1-76

Janick, J. and Moore, J. N., 1975. Advances in fruit breeding. Pardue University Press. W. Lafayette, Ind.

Jansen, R.C., 1993. Interval mapping of multiple quantitative trait loci. *Genetics.* **135**, 205-211.

Jansen, R.C., 1994. Controlling the type I and type II errors in mapping quantitative trait loci. *Genetics.* **138**, 871-881

Jansen, R.C., Stam, P., 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics.* **136**, 14471455

Jansen, J., de Jong, A. G., van Ooijen, J. W., 2001. Constructing dense genetic linkage maps. *Theor Apple Genet*. **102**, 1113-1122

Jansen, R. C., 2003. Quantitative trait loci in Inbred Lines. In: (Balding, D. J., Bishop, M., Cannings, C., Ed.). *Handbook of Statistical Genetics*, *2nd Edition.* 445-476. John Wiley and Sons Ltd. West Sussex

Johnson, D. S., 1992. The effect of flower and fruit thinning on the firmness of "Cox's Orange Pippin" apples at harvest and after storage. *J Hort Sci*. **67**, 95-101

Joobeur, T., Viruel, M. A., de Vicente, M. C., Jauregui, B., Ballester, J., Dettori, M. T., Verde, I., Truco, M. J., Messeguer, R., Batlle, I., Quarta, R., Dirlewanger, E., Arus, P., 1998. Construction of a saturated linkage map of *Prunus* using an almond. *Theor Appl Genet.* **97**, 1034-1041

Juniper, B. E., Watkins, R., Harris, S. A., 1999. The origin of the apple. *Acta Hort*. **484**, 27-33

Juniper, B. E., Robinson, J, Harris, S. A., Watkins, R., 2001. Origin of the apple (*Malus x domestica* Borkh.). In: Reeve ECR (ed) Encyclopedia of Genetics. London: FitzroyDearborn, pp. 674-677

Kanamaru, N., Ito, Y., Komori, S., Saito, M., Kato, H., Takahashi, S., Omura, M., Soejima, J., Shiratake, K., Yamada, K., Yamaki, S., 2004. Transgenic apple transformed by sorbitol-6-phosphate dehydrogenase cDNA: Switch between

sorbitol and sucrose supply due to its gene expression. *Plant Sci*. **167**, 55-61

Kearsey, J. J., Farquhar, G. L., 1998. Short review: QTL Analysis in Plants; where are we now? *Heredity.* **80**, 137-142

Kellerhals, M., Gianfranceschi, L., Seglias, N., Gessler, C., 2000. Marker-assisted selection in apple breeding. *Acta Hort*. **521**, 255-265

Kempthorne, O., 1957. An Introduction to Genetic Statistics. Wiley & Sons. New York.

Kenis, K., Keulemans, J., Davey, M. W., 2008. Identification and stability of QTLs for fruit quality tarits in apple. *Tree Genetics and Genomes*. **4**, 647-661

Kester, D. E., Hansche, P. E., Beres, W., Asay, R. N., 1977. Variance components and heritability of nut and kernel traits in almond. *J Amer Soc Hort Sci.* **102**, 264-266

Khan, M. A., Duffy, B., Gessler, C., Patocchi, A., 2006. QTL mapping of fire blight resistance in apple. *Mol Breed*. **7**, 299-306

Kim, S., Lee, J., Hong, S., Yoo, Y., An, G., Kim, S., 2003. Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. *Plant Sci*. **165**, 403-413

King, G. J., Maliepaard, C., Lynn, J. R., Alston, F. H., Durel, C-E., Evans, K. M., Griffon, B., Laurens, F., Manganaris, A. G., Schrevens, E., Tartarini, S., Verhaegh, J., 2000. Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill). *Theor Appl Genet*. **100**, 1074-1084

King, G. J., Lynn, J. R., Dover, C. J., Evans, K. M., Seymour, G. B., 2001. Resolution of quantitative trait loci for mechanical measures accounting for variation in fruit texture of apple (*Malus pumila* Mill.). *Theor Appl Genet*. **102**, 1227-1235

Knee, M., Hatfield, S. G. S., 1981. The metabolism of alcohols by apple fruit tissue. *Jour Sci Food and Agric*. **32**, 593-600

Knee, M., 1993. Pome fruits. In: (Seymour, G. B., Taylor, J. E., Tucker, G. A., Eds), *Biochemistry of Fruit Ripening*. pp. 325-346. Chapman and Hall, London

Kobayashi, S., Ishimaru, M., Ding, C. K., Yakushiji, H., Goto, N., 2001. Comparison of UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin, *Plant Sci.* **160,** 543-550

Kondo, S., 1992. Effect of environmental conditions on the contents of sugar and ascorbic acid in Shensu apple fruit. *Jour Jap Soc Food Sci Technol*. **39**, 1112-1118

Korban, S. S., Skirvin, R.M., 1994. Nomenclature of the cultivated apple. *HortSci*. **19,** 177-180

Krebs-Smith, S., Cook, D. A., Subar, A. F., Cleveland, L., Friday, J., Kahle, L. L., 1996. Fruit and Vegetable Intake of Children and adolescents in the United States. *Arch Pediatr Adolesc Med*. **150**, 81-86

Kumar, L. S., 1999. DNA markers in plant improvement-Chromosome organization and evolution in a disomic polyploid. *Biotechnol Adv*. **17**, 143-182

Labuschagné, I., 2003. Fruit Breeding: Basic Principles. *SA Fruit Journal*. **16,** 10-12

Lancaster, J. E., 1992. Regulation of skin color in apples. *Crit Rev Plant Sci*. **10**, 487-502

Lander, E. S., Botstein, D., 1988. Mapping Mendelian Factors underlying Quantitative Traits using RFLP Linkage Maps. *Genetics*. **121**, 183-199

Lawson, D. M., Hemmet, M., Weeden, N., 1995. The use os molecular markers to analyze the inheritance of morphological and developmental traits in apple. *J Am Soc Hort Sci*. **120**, 532-537

Lehman-Salada, L., 1996. Instrument and operator effects on apple firmness readings. *Hort Sci*. **31**, 994-997

Lesemann, S., Dunemann, F., 2006. Presented at the The 3rd International Rosaceae Genomics Conference, Napier, New Zealand, March 19-22

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C. D., Tarchini, R., Van de Weg, E., Gessler, C., 2002. Development and characterization of 140 new microsatellites in apple (*Malus x domestica* Borhk.). *Mol Breeding*. **10**, 217-241

Liebhard, R., Kellerhals, M., Plammatter, W., Jertmni, M., Gessler, C., 2003. Mapping quantitative physiological traits in apple (*Malus* X *domestica* Borkh.). *Plant Mol Biol.* **52,** 511-526

Liebhard, R., Gianfranceschi, L., Koller, B., Gessler C., 2003. Creating a Saturated Reference Map for Apple (*Malus* x *domestica borkh*.) Genome. *Theor Appl Genet.* **106**, 1497-1508

Loescher, W. H., Marlow, G. C., Kennedy, R. A., 1982. Sorbitol metabolism and sink-source interconversion of developing apple leaves. *Plant Physiol*. **70**, 335-339

Loescher, W. H., 1987. Physiology and metabolism of sugar alcohols in higher plants. *Physiol. Plant.* **70**, 553-557

Lui, F. W., King, M. M., 1978. Common Evaluation of "McIntosh" apple firmness. *HortSci*. **13**, 162-163

Lynch, M., Walsh, B., 1998. Genetics and Analysis of Quantitative Traits. Sunderland, Sinauer Associates, Inc

Macheix, J-J., Fleuriet, A., Billot, J., 1990. Fruit phenolics, pp149-237. CRC Press Inc., Boca Raton, Fla.

Maliepaard, C., Alston, F.H., Van Arkel, G., Brown, L. M., Chevreau, E., Dunemann, F., Evans, K. M., Gardiner, S., Guilford, P., van Heusden, A. W., Jansen, J., Laurens, F., Lynn, J. R., Manganaris, A. G., Den Nijs, A. P. M., Periam, N., Rikkerink, E., Roche, P., Ryder, C., Sansavini, S., Schmidt, H., Tartarini, S., Verhaegh, J. J., Vrielink-Van Ginkel, M., King, G. J., 1998. Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. *Theor Appl Genet*. **97**, 60-73

Maliepaard, C., Sillanpää, M. J., van Ooijen, J. W., Jansen, R. C., Arjas, E., 2001. Bayesian versus frequentist Analysis of Multiple Quantitative Trait Loci with an Application to an Outbred apple cross. *Theor Appl Genet.* **103**, 1243-1253

Marais, E., Jacobs, G., Holcroft, D. M., 2001. Color response of 'Cripps'Pink' apples to postharvest irradiation is influenced by maturity and temperature. *Sci Hort*. **90**, 31-41

Mason, J. L., Drought, B. G., McDougald, J. M., 1974. Effect of a calcium dip on senescent breakdown, firmness and calcium concentration in 'Spartan' apple. *Hort Sci*. **9**, 596

Mason, J. L., Jasmin, J. J., Granger, R. L., 1975. Softening of McIntosh apples reduced by a post-harvest dip in calcium chloride solution plus thickener. *Hort Sci*. **10**, 524-525

Mason, J. L., 1976. Calcium concentration and firmness of stored 'McIntosh' apples increased by calcium chloride solution plus thickener. *Hort Sci*. **11**, 504-505

Maxam, A. M., Gilbert, W., 1977. A new method for sequencing DNA. *Proc Natl Acad Sci*. **74**, 560-564

Mazza, G., Miniati, E., 1993. Color stabilization and intensification. In: Anthocyanins in fruits, vegetables and grains. (Mazza, G. and Miniati, E., Eds). pp1-10. CRC Press, Boca Raton, Florida, USA

McKenzie, D. W., 1971. A survey of shape variations in New Zealand apples. *NZ J Agric Res*. **14**, 491-498

Merzylak, M. N., Chivkunova, O. B., Lekhimena, L., Belevich, N. P., 1996. Some limitations and potentialities of the spectrophotometric assay of pigments extracted from leaves of higher plants. *Russ J Plant Physiol*. **43**, 800-809

Merzylak, M. N., Solovchenko, A. E., 2002. Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. *Plant Sci*. **163**, 881-888

Michelmore, R. W., Paran, I., Kesseli, R. V., 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci*. **88**, 9828-9832

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C. R., Sasaki, T., 1997. Genome mapping, moleculsr markers and marker assisted selection in crop plants. *Molec breed*. **3**, 87-103

Mol, J., Jenkins, G., Schafer, E., Weiss, D., 1996. Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. *CRC Crit Rev Plant Sci.* **15**, 525-557

Mol, J., Grotewold, E., Koes, R., 1998. How genes paint flowers and seeds. *TRENDS Plant Sci.* **3**, 212-217

Mullis, K. B., 1990. The unusual origin of the polymerase chain reaction. *Sci Amer*, 36-43

Nakano, M., Nakatsuka, A., Nakayama, M., Koshiok, M., Yamagishi, M., 2004. Mapping of quantitative trait loci for carotenoid pigmentation in flower tepals of Asiatic hybrid lily. *SciHort*. **104**, 57-64

Nelson, J. C., Sorrells, M. E., Van Deynze, A. E., Lu, Y. H., Atkinson, M., Bernard, M., Leroy P., Faris J. D., Anderson J. A., 1995. Molecular Mapping of Wheat Major Genes and Rearrangements in Homologous Groups 4, 5, and 7. *Genetics*. **141**, 721-731

Nissim-Levi, A., Kagan, S., Ovadia, R., Oren-Shamir, M., 2003. Effects of temperature, UV-light and magnesium on anthocyanin pigmentation in cocoplum leaves. *J Hort Sci Biotechnol*. **78**, 61-64

Noe, N., Eccher, T., 1996. "Golden Delicious" Apple Fruit Shape and Russetting are affected by light conditions. *Sci Hort*. **65**, 209-213

Ntombela, S., 2010. "South African Fruit Trade Flow". National Agriculture Marketing Council. **1**, 8-9

Ourecky, D.K., Bourne, M. C., 1968. Measurements of strawberry texture with an I nstron machine. *Proc Amer Soc Hort. Soc*. **93**, 317- 325

Paillard, N. M. M., 1990. The flavour of apples, pears and quinces. *In:* Morton, 1. D.; MacLeod, A. J. *ed.* Food flavours, Part C. The flavour of fruits. pp1-41. Amsterdam, The Netherlands, Elsevier Science Publishing Company Inc.

Papageorigiou, G. C., Murata, N., 1995. The unusually strong stabilizing effects

of glycinebetaine on the structure and function of the oxygen-evolving photosystem II complex. *Photosynth. Res*. **44**, 243-252

Park, S. W., Song, K. J., Kim M. Y., Hwang, J-H., Shin, Y. U., Kim W-C., Chung, W-I., 2002. Molecular cloning and characterization of four cDNAs encoding the isoforms of NAD-dependent sorbitol dehydrogenase from the Fuji apple. *Plant Sci*. **162**, 513-519

Patocchi, A., Bigler, B., Koller, B., Kellerhals, M., Gessler, C., 2004. *Vr(2)*: a new apple scab resistance gene. *Theor Appl Genet*. **109**, 1087-1092.

Patocchi, A., Walser, M., Tartarini, S., Broggini, G. A. L., Gennari, F., Sansavini, S., Gessler, C., 2005. Identification by genome scanning approach (GSA) of a microsatellite tightly associated to the apple scab resistance gene *Vm*. *Genome*. **48**, 630-636.

Pechous, S. W., Whitaker, B. D., 2004. Cloning and functional expression of an (E, E)-α-farnesene synthase cDNA from peel tissue of apple fruit. *Planta*, **219**, 84-94

Peil, A., Dunemann, F., Garcia, T., Richter, K., Trognitz, B., Hanke, V., Flachowsky, H., 2006. Presented at the The 3rd International Rosaceae Genomics Conference, March 19-22

Peryea, F. J., 1991. Preharvest calcium sprays and apple firmness. *Good Fruit*

Growers. **42,** 12-15

Piffaut, B., Kader, F., Girardin, M., Metche, M., 1994**.** Comparative degradation pathways of malvidin 3,5-diglucoside after enzymatic and thermal treatments. *Food Chem*. **50**, 115-120

Porritt, S. W., Lidster, P. D., 1978. The effect of pre-storage heating on ripening and senescence of apples during cold storage. *J Amer Soc Hort Sci*. **109**, 53-57

Prange, R. K., Meheriuk, M., Lougheed, E. C., Lidster, P. D., 1993. Harvest and Storage. In: Producing apples in eastern and central Canada. Agriculture Canada. pp 64-69

Pre-Aymard, C., Weksler, A., Lurie, S., 2003. Responses of 'Anna', a rapidly ripening summer apple, to 1-methylcyclopropene. *Postharv Biol Technol*. **27**, 163-170

Quilot, B., Wu, B. H., Kervella, J., Genard, M., Foulongne, M., Moreau, K., 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. *Theor Appl Genet.* **109**, 884–897

Redalen, G., 1988. Quality Assessment of Apple Cultivars and Selections. *Acta Hort*. **224**, 441-445

Ricks, D., Woods, T., Sterns, J., 2000. Chain Management and Marketing Performance in Fruit Quality. *Acta Hort*. **536**, 661-668

Riley, R. G., Kolattukudy, P. E., 1976. Effect of tretmant with calcium ion-containing formulations on the firmness of "Golden Delicious" apples. *Hort Sci.* **11**, 249-251

Risterucci, A. M.,, Grivet, L., N'Goran, J. A. K., Pieretti, I., Flament, M. H., Lanaud, C., 2000. A high-density linkage map of *Theobroma cacao* L. *Theor Appl Genet*. 101, 948-955

Ronaghi, M., 2001. Pyrosequencing Sheds Light on DNA Sequencing. *Genome Res,* **11**, 3-11

Rathore, D.S., 1976. Effect of season on the growth and chemical composition of guava (Psidium guajava L.) fruits. *J Hort Sci*. **51**, 41-47.

Rowan, D. D., Lane, H. P., Allen, J. M., Fielder, S., Hunt, M. B., 1996. Biosynthesis of 2-methylbutyl, 2- methyl-2-butenyl, and 2-methylbutanoate esters in "Red Delicious" and "Granny Smith" apples using deuterium-labeled substrates. *J Agric Food Chem.* **44***,* 3276-3285

Rowan, D. D., Allen, J. M., Fielder, S., Hunt, M. B., 1997. Deuterium labelling to study aroma biosynthesis in stored apples. *In:* Mitcham, E. J. *ed.* CA '97 Proceedings Volume 2: apples and pears. pp. 227-233. University of California, California, United States.

Saito, K., Yamasaki, M., 2002. Biochemistry of molecular biology of the late-stage of biosynthesis of anthocyanin: lessons from *Perilla frutescens* as a model plant. *New Phytologist*. **155**, 9-23

Sams, C. E., Conway, W. S., 1984. Effect of calcium infiltration on ethylene production, respiration rate, soluble polyuronide content, and quality of "Golden Delicious" apple fruit. *J Amer Soc Hort Sci*. **109**, 53-57

Sanger, F., Nicklen, S., Coulsen, A. R., 1977. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci*. **74,** 5463-5467

Sankar, A. A., Moore, G. A., 2001. Evaluation of inter-simple sequence repeat analysis for mapping in *Citrus* and extension of the genetic linkage map. *Theor Appl Genet*. **102**, 206-214

Sanz , C., Olias, J. M., Perez, A. G., 1997. Aroma biochemistry of fruits and Vegetables. In Tomas-Barberan, F.A., Robins, R. J. (ed) Phytochemistry of fruit and vegetables. pp125-155, New York, Oxford University Press Inc.

Sasaki, T., Yano, M., 1997. Genetic and Molecular dissection of Quantitative Traits in Rice. *Plant Mol Biol*. **35**, 145-153

Schreier, P., 1984. Chromatographic studies of biogenesis of plant volatiles. Alfred Hiithig Verlag GmbH. Heidelberg, Basel, New York

Seglias, N. P., Gessler, C., 1997, Genetics of apple powdery mildew resistance from Malus zumi (Pl2). Integrated control of pome fruit diseases. *IOBC/WPRS Bulletin*. **20**, 195-208

Serono, J., Hayes, P. M., Hoffman, D., Kleinhofs, A., Kuiper, M., Kudrna, D., Kilien, D., Saghai-maroof, M., Sato, K., Witsenhoer, H., Zabeau, M., 1998 Characterisation and Exploiting Genetic Diversity and Quantitative Traits in Barley. *Plant Breeding Series*. **830**, 1-10

Sfakiotakis, E., Naonos, G., Stavroulakis, G., 1993. Effect of growing location, harvest maturity and ventilation during storage on ripening and superficial scald of "Starking Delicious" apples. *Acta Hort*. **326**, 231-235

Shaked-Sachray, L., Weiss, D., Reuveni, M., Nissim-Levi, A., Oren-Shamir, M., 2002. Increased anthocyanin accumulation in aster flowers at elevated temperatures due to magnesium treatment. *Physiol Planta*. **144**, 559-65

Sharples, R. O., 1967. The effect of post-harvest heat treatment on the storage behavior of "Cox's Orange Pippin" apple fruits. *Ann Appl Biol*. **59**, 401-406

Shear, C. B., Faust, M., 1975. Preharvest nutrition and postharvest physiology of apples, pp35-42. In: Haard, N. F., Salunkhe, D. K. (eds) Postharvest Biology and Handling of Fruit. AVI Publication Inc., West Port, Conneticut

Shulaev, V., Korban, S. S., Sosinski, B., Abbott, A. G., Aldwinckle, H. S., Folta, K. M., Iezzoni, A., Main, D., Arús, P., Dandekar, A. M., Lewers, K., Brown, S. K., Davis, T. M., Gardiner, S. E., Potter, D., Veilleux, R. E., 2008. Multiple Models for Rosaceae Genomics. *Amer Soc Plant Bio*. **147**, 984-1003

Silfverberg-Dilworth, E., Besse, S., Paris, R., Belfanti, E., Tartarini, S., Sansavini, S., Patocchi, A., Gessler, C., 2005. Identification of functional apple scab reesistance gene promoters. *Theor Appl Genet.* **110**, 1119-1126

Silfverberg-Dilworth, E., Matasci, C. L., Van de Weg, W. E., Van Kaauwen, M. P. W., Walser, M., Kodde, L. P., Soglio, V., Gianfranceschi, L., Durel, C. E., Costa, F., Yamamoto, T., Koller, B., Gessler, C., Patocchi, A., 2006. Microsatellite markers spanning the apple (*Malus* x *domestica* Borkh.) genome. *Tree Genet and Genomes*. **2**, 202-204

Song, J., Bangerth, F., 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. *Ada Hort.* **368***,* 150-159

Stankiewicz-Kosyl, M., Pitera, E., Gawronski, S. W., 2005. Mapping QTL involved in powdery mildew resistance of the apple clone U 211. *Plant Breed*. **124,** 63-66

Stevens, M. A., Kader, A. A., Albright-Halton, M., Algazi, M., 1977. Genotypic variation for flavour and composition in fresh market tomatoes. *J Amer Soc Hort Sci.* **102**, 680-689

Steyn, W. J., Holcroft, D. M., Wand, S. J. E., Jacobs, G., 2004. Anthocyanin degradation in detached pome fruit with reference to pre-harvest red color loss and pigmentation patterns of blushed and fully red pears. *J Amer Soc Hort Sci*. **129,** 13-19

Sullivan, D. T., 1965. The effect of time of bloom of individual flowers on the size, shape and maturity of apple fruits. *Proc Am Soc Hortic Sci*. **87**, 41-46

Sureshkumar, S., Todesco, M., Schneeberger, K., Harilal, R., Balasubramanian, S., Weigel, D., 2009. A genetic defect caused by a triplet repeat expansion in *Arabidopsis thaliana*. *Science*, **323,** 1060-1063.

Taas, J., Hulskamp, M., Gendreau, E., Hofte, H., 1998. Endoreduplication and development: rule without dividing? *Curr Opt Plant Biol*. **1**, 498-503

Takeda, K., Yamashita, T., Takahashi, A., Timberlake, C.F., 1990. Stable blue complexes of anthocyanin-aluminium-3-p-coumarol-or 3-caffeol-quinic acid involved in the blueing of *Hydrangea* flower. *Phytochem.* **29**, 1089-91

Takeda, K., Yanagisawa, M., Kifune, T., Kinoshita, T., Timberlake, C. F., 1994. A blue pigment complex in flowers of *Salvia patens*. *Phytochem*. **35**, 1167-1169

Tanksley, S. D., 1993. Mapping of Polygenes. *Ann Rev Genet*. **2**, 205-233

Tarczynski, M. C., Jensen, R. G., Bohnert, H. J., 1993. Stress protection of

transgenic tobacco by production of the osmolyte mannitol. *Sci*. **259**, 508-510

Tartarini, S., Gennari, F., Pratesi, D., Palazzetti, C., Sansavini, S., Parisi, L., Fouillet, A., Fouillet, V., Durel, C. E., 2004. Characterisation and genetic mapping of a major scab resistance gene from the old Italian apple cultivar 'Durello di Forli'. *Acta Hort*. **663**, 129-133

Testoni, A., Zerbini, P. E., 1989. Picking time and quality in apple storage. *Acta Hort*. **258**, 445-454

Theologis, T., 1994. Control of ripening. *Curr Opin Biotechnol*. **5**, 152-157

Touster, O., Shaw, D. R. D., 1962. Biochemistry of acyclic polyols, *Physiol Rev.* **42**, 181-225

Tregub, I., Schoch, S., Erazo, S. G., Scheer, H., 1996. Red-light-induced photoreactions of chlorophyll a mixtures with all-trans- or 9-cis-β-carotene. *J Photochem Photobiol*. **98**, 51-58

Tromp, J., 1990. Fruit shape in apple under various controlled environment conditions. *Sci Hort*. **43**, 109-115

Tu, K., Waldron, K., Ingham, L., de Barsy, T., de Baerdemaker, J., 1997. Effect of picking time and storage conditions on 'Cox's Orange Pippin' apple texture in relation to cell wall changes. *J Hort Sci*. **72**, 971-980

Van Dyk, M. M., Soeker, M. K., Labuschagné, I. F., Rees, D. J. G., 2010. Identification of a major QTL for time of initial vegetative budbreak in apple (Malus x domestica Borkh.). *Tree Genetics and Genomes.* **6**, 489-502

Van Ooijen, J., Sandbrink, H., Purimahua, C., Vrielink, R., Verkerk, R., Zabel, P., Lindhout, P., 1993. Mapping quantitative genes involved in a trait assessed on an ordinal scale: A case study with bacterial canker in Lycopersicon peruvianum. In: J. I. Yoder (Ed.) Molecular Biology of Tomato, Ch 7, pp59-74. Technomic Publishing Co. Inc., Lancaster PA

van Ooijen, J. W., Maliepaard, C., 1996. MapQTL version 3.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Van Ooijen, J. W., 2004. MapQTL® 5.0: Software for the mapping of quantitative trait loci in experimental populations. Wageningen, Netherlands

Van Ooijen, J. W., 2006. JoinMap® 4.0: Software for the calculation of genetic linkage maps in experimental populations. Wageningen, Netherlands

Velasco, R., 2009. *The 'Golden Delicious' Apple Genome: An International Whole Genome Sequencing Initiative.* Paper presented at the Plant & Animal Genomes XVII Conference, San Diego, CA, Jan 10-14

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S. K., Troggio, M., Pruss, D., Salvi, S., Pindo, M.,

Baldi, P., Castelletti, S., Cavaiuolo, M., Coppola, G., Costa, F., Cova, V., Dal Ri, A., Goremykin, V., Komjanc, M., Longhi, S., Magnago, P., Malacarne, G., Malnoy, M., Micheletti, D., Moretto, M., Perazzolli, M., Si-Ammour, A., Vezzulli, S., Zini, E., Eldredge, G., Fitzgerald, L. M., Gutin, N., Lanchbury, J., Macalma, T., Mitchell, J. T., Reid, J., Wardell, B., Kodira, C., Chen, Z., Desany, B., Niazi, F., Palmer, M., Koepke, T., Jiwan, D., Schaeffer, S., Krishnan, V., Wu, C., Chu, V. T., King, S. T., Vick, J., Tao, Q., Mraz, A., Stormo, A., Stormo, K., Bogden, R., Ederle, D., Stella, A., Vecchietti, A., Kater, M. M., Masiero, S., Lasserre, P., Lespinasse, Y., Allan, A. C., Bus, V., Chagné, D., Crowhurst, R. N., Gleave, A. P., Lavezzo, E., Fawcett, J. A., Proost, S., Rouzé, P., Sterck, L., Toppo, S., Lazzari, B., Hellens, R. P., Durel, C-E., Gutin, A., Bumgarner, R. E., Gardiner, S. E., Skolnick, M., Egholm, M., Van de Peer, Y., Salamini, F., Viola, R., 2010. The genome of the domesticated apple (*Malus* × *domestica* Borkh.). *Nature Genet*. **42(10)**, 833-83

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucl Acid Res*. **23**, 4407-4414

Wakasa, Y., Hatsuyama, Y., Takahashi, A., Sato, T., Niizeki, M., Harada, T., 2003. Divergent expression of six expansin genes during apple fruit ontogeny. *Eur J Hort Sci*. **68**, 253-259

Wang, Z., Pan, Q., Quebedeaux, B., 1999. Carbon partitioning into sorbitol, sucrose, and starch in source and sink apple leaves as affected by elevated CO_2.

Environ Exp Bot. **41**, 39-46

Watson, J. D. and Crick, F. H., 1953. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. G. D. Nature 248, 737–738. *Nature.* **170**, 737.

Weber, J., May, P. E., 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction *Amer J Hum Genet*. **44**, 388-396

Webster, D. H., 1978. Soft McIntosh and calcium content of apple fruit tissue. The Canadian Horticulture Council Report, Ottawa.

Webster, D. H., Lidster, P. D., 1986. Effects of phosphate sprays on "McIntosh" apple fruit and leaf composition, flesh firmness and susceptibility to low-temperature disorders. *Can J Plant Sci*. **66**, 617-626

Weeden, N. F., Hemmat, M., Lawson, D. M., Lodhi, M., Bell, R. L., Manganaris, A. G., Reisch, B. I., Brown, S. K., Ye, G. N., 1994. Development and application of molecular marker linkage maps in woody fruit crops. *Euphytica*. **77**, 71-75

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., and Kilian, A., 2004. Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proc Natl Acad Sci.* **101**, 9915-9920

White, A., 2000. Breeding Apple Varieties for the Worldmarket. *Hort Res.* 43rd February, 116-118

Winkel-Shirley, B., 2001 Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. *Plant Physiol*. **126**, 485-493

Wismer, W. V., Harker, F. R., Gunson, F. A., Rossiter, K. L., Lau, K., Seal, A. G., Lowe, R. G., Beatson, R., 2005. Identifying flavour targets for fruit breeding: A kiwifruit example. *Euphytica,* **141**, 93-104

Wright, S., 1921. Systems of mating. I. The biometric relations between parent and offspring. *Genetics*. **6**, 111-123

Xu, M., Korban, S. S., 2002. Positional cloning of the Apple scab resistance gene V*f*. *Genome*. **44**, 1104-1113

Xu, M., Korban, S. S., 2004. Somatic variation plays a key role in the evolution of the V*f* gene family residing in the V*f* locus that confers resistance to apple scab disease. *Mol Phylogenet Evol*. **32**, 57-65

Xu, S., Hu, Z., 2009. Mapping Quantitative Trait Loci Using Distorted Markers. *Int J Plant Genom.* **2009**, 1-11

Yang, S. F., Hoffman, N. E., 1984. Ethylene biosynthesis and its regulation in

higher plant. *Ann Rev Plant Physiol*. **35**, 126-138

Young, A. J., Frank, H. A., 1996. Energy transfer reactions involving carotenoids: quenching of chlorophyll fluorescence, *J. Photochem. Photobiol. B.* **36**, 3-15

Young, N. D., 1996. QTL Mapping and Disease Resistance in Plants. *Ann Rev Phytopath.* **34**, 479-501

Yunbi, X., 1995. Quantitative Trait Loci: separating, pyramiding and cloning. *Plant Breed Rev.* **15**, 85-129

Zhou, R., Cheng, L., Wayne, R., 2003. Purification and characterization of sorbitol-6-phosphate phosphatase from apple leaves. *Plant Sci*. **165**, 227-232

Zhu, Y., Barritt, H., 2008. Md-ACS1 and Md-ACO1 genotyping of apple (Malus x domestica Borkh.) breeding parents and suitability for marker-assisted selection. *Tree Genetics & Genomes*. **4**, 555-562

Zimmermann, M. H., Zeigler, H., 1975. List of sugars and sugar alcohols in sieve-tube exudates, In: (Zimmermann, M. H., Milburn, J. A., Eds.) Encyclopedia of Plant physiology, New series, vol. 1, pp480-503, Springer, Berlin

Appendix A. The 444 SSR markers used in this study, together with its name, dye colour, expected amplicon range, repeat type and forward and reverse sequences. The four fluorecent labels are indicated by P, F, N and V corresponding to Pet (red), 6-Fam (blue), Ned (yellow) and Vic (green). Size range was identified from allele sizes from the nine parents mentioned previously (section 2.4.6). Repeat refers to whether a marker is di-, tri-, tetra-, penta-, or hexanucleotide.

SSR Marker	Marker name	Colour	Size range (bp)	Repeat	Forward sequence	Reverse sequence
4	GD 100	P	223-238	2	aca gca agg tgt tgg gta aga agg t	tgc gga caa agg aaa aaa aaa agt g
5	GD 103	F	78-130	2	cgg cga gaa aaa aaa aca atg	gga taa ccg tcc ccc tct tc
7	GD 147	N	129-152	2	tcc cgc cat ttc tct gc	aaa ccg ctg ctg ctg aac
9	01a6	F	87-155	2	agg att gct gga aaa gga gg	tta gac gac gct act tgt cct
10	02b1	N	188-288	2	ccg tga tga caa agt gca tga	atg agt ttg atg ccc ttg ga
11	04h11	V	175-275	2	ctt cca tcg aga ttg cat cat a	cga att gag agg tcg tcg tt
12	05g8	F	71-171	2	cgg cca tcg att atc tta ctc tt	gga tca atg cac tga aat aaa cg
13	22c6	V	63-142	2	gac ctt tcc ctc tcc tga	ctg gat atg att att gca ga
14	23g4	F	70-130	2	ttt ctc tct ctt tcc caa ctc	agc cgc ctt gca tta aat ac
15	28f4	N	90-110	2	tgc ctc cct tat ata gct ac	tga gga cgg tga gat ttg
29	SAmsAT000141	V	56-100	4	gaa ata aac acc gag taa aca g	tgc tat ctg gtt ttc ttt tag c
30	SAmsAT000400.1	N	175-181	3	cgt atc gaa gta gaa cga cg	cag ggt tgt acg gat tca cg
32	CH05g08	F	161-179	2	cca aga cca agg caa cat tt	ccc ttc acc tca ttc tca cc
34	CH01c06	N	146-188	2	ttc ccc atc atc gat ctc tc	aaa ctg aag cca tga ggg c
35	CH01f021	V	174-206	2	acc aca tta gag cag ttg agg	ctg gtt tgt ttt cct cca gc
36	CH02g09	V	98-138	2	tca gac aga aga gga act gta ttt g	caa aca aac cag tac cgc aa
37	CH02c061	V	216-254	2	tga cga aat cca cta cta atg ca	gat tgc gcg ctt ttt aac at

38	CH05e03	V	158-190	2	cga ata ttt tca ctc tga ctg gg	caa gtt gtt gta ctg ctc cga c
39	CH03g07	V	115-181	2	aat aag cat tca aag caa tcc g	ttt ttc caa atc gag ttt cgt t
40	MS14h03	V	114-140	2	cgc tca cct cgt aga cgt	atg caa tgg cta agc ata
41	CH02c02b	V	78-126	2	tgc atg cat gga aac gac	tgg aaa aag tca cac tgc tcc
42	CH05d02	N	203-225	2	aaa ctc cct cac ctc aca tca c	aat agt cca atg gtg tgg atg g
43	CH04e03	F	179-222	2	ttg aag atg ttt ggc tgt gc	tgc atg tct gtc tcc tcc at
44	CH05e06	F	125-222	2	aca cgc aca gag aca gag aca t	gtt gaa tag cat ccc aaa tgg t
45	CH03d07	N	186-226	2	caa atc aat gca aaa ctg tca	ggc ttc tgg cca tga ttt ta
46	CH05a05	F	198-230	2	tgt atc agt ggt ttg cat gaa c	gca act ccc aac tct tct ttc t
47	CH04e05	V	174-227	2	agg cta aca gaa atg tgg ttt g	atg gct cct att gcc atc at
48	CH01h021	F	236-256	2	aga gct tcg agc ttc gtt tg	atc ttt tgg tgc tcc cac ac
49	CH05c07	N	111-149	2	tga tgc att agg gct tgt act t	ggg atg cat tgc taa ata gga t
50	CH01f07a	F	174-206	2	ccc tac aca gtt tct caa ccc	cgt ttt tgg agc gta gga ac
52	CH02d08	F	210-254	2	tcc aaa atg gcg tac ctc tc	gca gac act cac tca cta tct ctc
53	CH04g07	V	149-211	2	ccc taa cct caa tcc cca at	atg agg cag gtg aag aag ga
54	CH05d04	V	154-214	2	act tgt gag ccg tga gag gt	tcc gaa ggt atg ctt cga tt
56	CH05f04	V	160-172	2	gat gat ggt gct ctc ggt tat t	tta tgt tgg gta atg tct tcc g
57	CH01g05	V	140-188	2	cat cag tct ctt gca ctg gaa a	gac aga gta agc tag ggc tag gg
59	CH03d08	F	129-161	2	cat cag tct ctt gca ctg gaa a	tag ggc tag gga gag atg atg a
60	CH03g04	V	122-144	2	atg tcc aat gta gac acg caa c	ttg aag atg gcc taa cct tgt t
61	CH04c07	N	98-135	2	ggc ctt cca tgt ctc aga ag	cct cat gcc ctc cac taa ca
62	CH04f06	N	159-179	2	ggc tca gag tac ttg cag agg	atc ctt aag cgc tct cca ca
63	CH05d03	F	152-187	2	tac ctg aaa gag gaa gcc ct	tca ttc ctt ctc aca tcc act
64	CH05e05	N	138-160	2	tcc tag cga tag ctt gtg aga g	gaa acc acc aaa ccg tta caa t

65	CH05g11	F	201-255	2	gca aac caa cct ctg gtg at	aaa ctg ttc caa cga cgc ta
66	MS01a05	V	158-176	2	gga agg aac atg cag act	tga tgt ttc atc ttt aca
67	CH02c09	N	233-257	2	tta tgt acc aac ttt gct aac ctc	aga agc agc aga gga gga tg
70	CH05c06	F	104-149	2	att gga act ctc cgt att gtg c	atc aac agt agt ggt agc cgg t
71	CH01h011	N	114-134	2	gaa aga ctt gca gtg gga gc	gga gtg ggt ttg aga agg tt
72	CH05g03	N	135-192	2	gct ttg aat gga tac agg aac c	cct gtc tca tgg cat tgt tg
73	CH01f12	F	145-162	2	ctc ctc caa gct tca acc ac	gca aaa acc aca ggc ata ac
74	CH02a10	N	143-177	2	atg cca atg cat gag aca aa	aca cgc agc tga aac act tg
75	CH02b03b1	F	77-109	2	ata agg ata caa aaa ccc tac aca g	gac atg ttt ggt tga aaa ctt g
76	CH02c11	N	219-239	2	tga agg caa tca ctc tgt gc	ttc cga gaa tcc tct tcg ac
78	Cola	F	220-240	2	agg aga aag gcg ttt acc tg	gac tca ttc ttc gtc gtc act g
79	MS01a03	V	235-249	2	agc agt ata ggt ctt cag	tgc gta gat aac act cga t
80	MS02a01	N	170-194	2	ctc cta cat tga cat tgc at	tag aca ttt gat gag act g
81	MS06g03	V	154-190	2	cgg agg gtg tgc tgc cga ag	gcc cag ccc ata tct gct
82	CH02b101	N	121-159	2	caa gga aat cat caa aga ttc aag	caa gtg gct tcg gat agt tg
84	CH02f061	V	135-158	2	ccc tct tca gac ctg cat atg	act gtt tcc aag cga tca gg
85	CH03d01	F	95-115	2	cgc acc aca aat cca act c	aga gtc aga agc aca gcc tc
86	CH03d10	V	152-182	2	ctc cct tac caa aaa cac caa a	gtg att aag aga gtg atc ggg g
87	CH03e03	F	106-216	2	gca cat tct gcc tta tct tgg	aaa acc cac aaa tag cgc c
88	CH02h11a	V	104-132	2	cgt ggc atg cct atc att tg	ctg ttt gaa ccg ctt cct tc
89	CH04e02	F	143-163	2	ggc gat gac tac cag gaa aa	atg tag cca agc cag cgt at
90	CH02b121	V	101-143	2	ggc agg ctt tac gat tat gc	ccc act aaa agt tca cag gc
91	CH03a04	V	92-124	2	gac gca taa ctt ctc ttc cac c	tca agg tgt gct aga caa gga g
92	CH03a09	V	125-143	2	gcc agg tgt gac tcc ttc tc	ctg cag ctg ctg aaa ctg g

93	CH05f06	V	166-184	2	tta gat ccg gtc act ctc cac t	tgg agg aag acg aag aag aaa g
94	CH03d12	V	108-154	2	gcc cag aag caa taa gta aac c	att gct cca tgc ata aag gg
95	CH01f091	F	125-160	2	atg tac atc aaa gtg tgg att g	aat tcc aat ttc aga aca gg
96	CH01h101	N	94-114	2	tgc aaa gat agg tag ata tat gcc a	agg agg gat tgt ttg tgc ac
97	CH01f03b	V	139-183	2	gag aag caa atg caa aac cc	ctc ccc ggc tcc tat tct ac
98	CH02d121	F	177-199	2	aac cag att tgc ttg cca tc	gct ggt ggt aaa cgt ggt g
99	CH03d02	F	201-223	2	aaa ctt tca ctt tca ccc acg	act aca ttt tta gat ttg tgc gtc
100	CH04a12	V	158-196	2	cag cct gca act gca ctt at	atc cat ggt ccc ata aac ca
101	CH04d07	F	119-142	2	tgt cct cca atc tta acc cg	cac aca gac gac aca ttc acc
104	CH01d09	V	131-172	2	gcc atc tga aca gaa tgt gc	ccc ttc att cac att tcc ag
105	CH01g121	F	112-186	2	ccc acc aat caa aaa tca cc	tga agt atg gtg gtg cgt tc
106	CH03c02	F	116-136	2	tca cta ttt acg gga tca agc a	gtg cag agt ctt tga caa ggc
107	CH04d02	N	118-146	2	cgt acg ctg ctt ctt ttg ct	cta tcc acc acc cgt caa ct
108	CH04g04	F	170-186	2	agt ggc tga tga gga tga gg	gct agt tgc acc aag ttc aca
109	CH05d11	N	171-211	2	cac aac ctg ata tcc ggg ac	gag aag gtc gta cat tcc tca a
110	MS14b04	V	230-292	2	cct taa gaa tca tgt gat	act aat ggc aca aag att gt
111	CH03h03	F	72-120	2	aag aaa tcg gat cca aaa caa c	tcc ctc aaa gat tgc tcc tg
112	CH05c04	V	186-258	2	cct tcg tta tct tcc ttg cat t	gag ctt aag aat aag aga agg gg
113	CH01d08	N	238-290	2	ctc cgc cgc tat aac act tc	tac tct gga ggg tat gtc aaa g
114	CH03b06	F	111-131	2	gca tcc ttg aat gag gtt cac t	cca atc acc aaa tca atg tca c
115	CH03b10	N	99-121	2	ccc tcc aaa ata tct cct cct c	cgt tgt cct gct cat cat act c
116	CH04g10	N	127-168	2	caa aga tgt ggt gtg aag agg a	gga ggc aaa aag agt gaa cct
117	CH02a03	N	122-170	2	aga agt ttt cac ggg tgc c	tgg aga cat gca gaa tgg ag
118	CH02d10a	V	215-229	2	tga ttt cct ttt tcg caa gg	ttc atc gtt ccc tct cca ac

119	CH05a04	F	159-189	2	gaa gcg aat ttt gca cga at	gct ttt gtt tca ttg aat ccc c
120	CH05e04	F	153-234	2	aag gag aag acc gtg tga aat c	cat gga taa ggc ata gtc agg a
121	CH02g04	F	132-197	2	ttt tac ctt ttt acg tac ttg agc g	agg caa aac tct gca agt cc
122	CH04c06	V	155-186	2	gct gct gct gct tct agg tt	gct tgg aaa agg tca ctt gc
125	CH02g01	P	91-121	2	ccg cga gat gac aag tcc	atc ttg caa tct tct tgc ata gg
126	CH01b09b	P	172-182	2	tta tag cag caa cag gag cg	tat tcg gga ggc atg gta tg
128	CH01b121	P	125-178	2	cgc atg ctg aca tgt tga at	cgg tga gcc ctc tta tgt ga
130	CH01c09	P	92-108	2	tca tct ttc tcg cct gcc	tcc atc aaa acc aag ttt tcg
131	CH01c11	P	109-155	2	aaa tcc taa aac aca agc aaa acc	tga acc aag tcc tcc act cc
133	CH01d03	P	136-160	2	cca ctt ggc aat gac tcc tc	acc tta ccg cca atg tga ag
135	CH01e09b	P	118-140	2	cca tcc aac tac tgc ctt tcc	ttt gat gaa ccc ctt ctt cc
136	CH01e121	P	246-278	2	aaa ctg aag cca tga ggg c	ttc caa ttc aca tga ggc tg
137	CH01f03a	P	210-224	2	cac cta aaa agt ttc tcc cct tc	aat ggg tta gag atg ggt gc
139	CH02a04	P	66-112	2	gaa aca ggc gcc att att tg	aaa gga gac gtt gca agt gg
140	CH02a08	P	128-177	2	gag gag ctg aag cag cag ag	atg cca aca aaa gca tag cc
145	CH02g01	P	198-238	2	gat gac gtc ggc agg taa ag	caa cca aca gct ctg caa tc
146	CH02h07	P	214-236	2	tga gct gac aag tgt aaa atg c	gcc gaa caa tgt aaa gct cg
147	CH02h11b	P	214-240	2	ggg acg taa aca ggt att ctc tc	atg gtt agg cca agc aca tc
148	CH03a03	P	154-182	2	gtg gtg gta atg acg aga acc t	aag caa agt agc caa act gca t
151	CH03g06	P	137-171	2	atc cca cag ctt ctg ttt ttg	tca cag aga atc aca agg tgg a
152	CH03g12	P	150-200	2	gcg ctg aaa aag gtc agt tt	caa gga tgc gca tgt att tg
158	CH04d08	P	116-142	2	aat tcc aca ttc acg cat ct	ttg aaa gac gga aac gat ca
159	CH04d11	P	85-152	2	att agg caa tac aca gca c	gct gct ttg ctt ctc act cc
161	CH04f03	P	175-191	2	ctt gcc cta gct tca aat gc	tcg atc cgg tta ggt ttc tg

162	CH04f04	P	144-166	2	gtc ggt aca aac tca gga cc	cga cgt tcg atc ttc ctc tc
163	CH04f07	P	82-113	2	cag atc atg aat gat tga aa	gaa aat cac acc ctc aaa cca t
165	CH04g09	P	141-177	2	ttg tcg cac aag cca gtt ta	gaa gac tca tgg gtg cca tt
166	CH05a09	P	141-186	2	cac cga tgg tgt caa ctt gt	caa caa aat gtg atc gcc ac
167	CH05a02	P	111-135	2	gtt gca aga gtt gca tgt tag c	ttt tga ccc cat aaa acc cac
168	CH05a03	P	182-220	2	cgg ctg agc atg gtt act tc	tga tcg ttg tga aag ctc ca
169	CH05a09	P	152-200	2	tga ttt aga cgt cca ctt cac ct	tga ttg gat cat ggt gac tag g
170	CH05b06	P	185-215	2	aca agc aaa cct aat acc acc g	gag act gga aga gtt gca gag g
171	CH05c02	P	168-200	2	tta aac tgt cac caa atc cac a	gcg aag ctt tag aga gac atc c
172	CH05d08	P	91-143	2	tca tgg atg gga aaa aga gg	tga ttg cca cat gtc agt gtt
173	CH05g01	P	236-276	2	ttt cat tca act tca cct ctc	ctc ctt tcc gat tct tct att tca
174	CH05g02	P	133-155	2	agt gca gct ttc agc tca gat t	agt cag aca cac caa aat ccc t
176	CH05g07	P	149-197	2	ccc aag caa tat agt gaa tct caa	ttc atc tcc tgc tgc aaa taa c
177	CH05h05	P	168-184	2	aca tgt cac tcc tac gcg g	gtg cag tga tta gca ttg ctg t
178	CH05h12	P	164-192	2	ttg cgg agt agg ttt gct tt	tca atc ctc atc tgt gcc aa
179	MS06c09	P	102-118	2	act att gga gta agt cga	aat ata aga gcc aga ggc
180	SAmsCN444111	N	409	3	tga ggc cac cta aat atc ac	cag gat gag agt tct tga gc
181	SAmsCN444846	N	150-152	3	cta gtt tcc tcc gtg gtt tct	cgg aaa gtt tgt agt ggt gg
182	SAmsCN445253	F	265-365	3	tgc aag aat cat cca ctt cc	ttg gac ctg tga gga ctc c
186	SAmsCN90349	N	207	3	gta cta tca gca gaa act gg	gat ttg agc aca aca tac gg
187	SAmsCN490566	V	286-386	3	agc gca atg gcg ttc tag g	agc tgc gct atc ttc tca gc
188	SAmsCN490740	F	213	3	agg atc ctt cct cga ttt gc	ggc att gag gtt ctt gat cc
189	SAmsCN490897	F	458-462	2	gcg gag ata agg atg ctt cg	cct cag tac caa act agg ct
192	SAmsCN491993	F	245-284	3	aag cag tcg cag cag gtg	aac aac cgt tcg gat tct cg

193	SAmsCN492206	F	329-429	3	aca tac tgg agt ctg cga gc	caa tac gct agt gaa gac gc
195	SAmsCN492475	N	175-185	3	act cac ccc ctt cct ttc c	gaa gaa agg tag ggg tca gc
196	SAmsCN492626	N	260-360	3	tgc agg ttg aga tgg ttt gg	gac cca aga aca aca aaa cc
200	SAmsCN493925	N	366-466	3	tct cct tca ctt ccc att cc	tgg tga tgg cat aca cat cc
201	SAmsCN493973	F	252-329	3	tac tct ctg atc ttc tga ttg c	cag tgc acc acc aag ttg c
202	SAmsCN494248	V	266-366	3	acc tct ctt cat tct tct cc	gaa gag cat aga aga aca cc
204	SAmsCN494928	V	209-229	3	aat tat atc cgt ccg act cca	tta gag tag tca cga taa tgg
206	SAmsCN495278	N	214-240	3	ccc aga atc att cag aga cc	gca ggc tcc atg cag ttc g
207	SAmsCN495433	V	213-313	3	aca aga gca gca gca ttt cg	gta gcg tgt ttc agg cag tc
208	SAmsCN495651	V	348-448	3	ctt ctc cca gaa ctg act gc	tct aca acc gca aac acg ag
209	SAmsCN495857	F	145-155	3	tca aaa ccc acc tca tat tgc	tag gaa gga gat gag att tgg
212	SAmsCN496144	V	303-349	3	ctc aga ctc ctg ctg cac c	tac tgc ctg gtg ttt ctt cc
213	SAmsCN496756	N	423-523	3	tcg gtg gaa gac caa gca g	cat gat cat gtg gcg ccg t
214	SAmsCN496821	F	358-410	3	aat gcc act gaa atg act gc	agc ttc gtc tat gga gtg c
215	SAmsCN496844	V	243-343	3	gga tca aca gca aca gca gc	ctt gga ccg gag cat gtc c
217	SAmsCN579502	F	230-330	3	tcg tga agt gcc aag tat cg	tgg cgg act gct caa ttg c
218	SAmsCN580519	F	120-135	3	tcc cca cac ca ttg att tgc	acc ttg gaa gct ccc ttc c
219	SAmsCN580620	F	333-433	3	tgc ggt caa cga tgt ctt cg	aag gta caa gcc cgc aaa gg
220	SAmsCN580732	F	300-400	3	atg ggg cca gtt aca gga g	ctg aag aaa tcg cag gtt cc
221	SAmsCN580954	V	106-118	3	tct ctt gtc aag gat gga cc	gaa tcc gaa gca acg gaa gc
222	SAmsCN581649	N	332-432	3	agc cct gat ctt cct cta gc	acg aac tac cac ctc aaa cc
226	SAmsCN444745	V	455-480	4	agg aaa taa aca ccg agt aaa c	cac aag cat ctc gag cac c
227	SAmsCN493171	N	295-395	4	tct tac ttc gtc ggt gga cc	tgt gtg gct att acc tga gg
228	SAmsCN496055	N	360-364	4	cca cac aga aac gag tcc tc	att ttg gtc ctc ctt gct gg

229	SAmsCN496966	N	167-171	4	gga gga gaa tat gtg att ttg ag	gat tgc gac agc att tat gg
231	SAmsCN580271	V	156-256	4	tct ggc tct cat cgg ttt gc	tcg atg ccc ttg taa cgc c
234	SAmsCN938125	N	303-403	3	gcc ttc atc ccc cct tga	ggt gta tag gaa tct tgg ag
235	SAmsCN881550	P	305-405	3	atc caa aca acc cca ttg cg	agt cga tgt tga acg ctc ca
236	SAmsCN910036	P	192-292	3	gag aaa ccg ttt gat tac agc	ctc cat ccc caa tca cac c
238	SAmsCN865016	F	294-394	3	ttc ttc aca ccc ttc aat cc	aaa gcg cct gcg att gcg
241	SAmsCN887787	N	254-257	3	cac ttt agc tta gta cac agc	tga ggt agt aag agt aga agg
243	SAmsCN907588	N	304-307	3	ccg aag aca att ctg tct gg	ggt act tgt tgg tga tct cg
244	SamsCN947446	V	136-236	3	ccg tta cag cta tcc aaa cc	ata atg gcc att ctg ttc agc
245	SAmsCN943613	F	165-174	3	tag cag aaa cca gca gat gg	tga ggc ctc gaa gaa gtg c
253	SAmsCO540769	N	213-313	3	tcc tag ggt cgg aga gca g	ctc aag aat cac caa caa tgc
254	SAmsCN933736	F	291-334	3	tgg cag ctc cac cac aat c	gcc aga ttc aca cga aag c
256	SAmsCN868958	F	181-202	3	caa ccc tca ccg act ttg c	cag aac cat tga tgg tca cc
259	SAmsCN904905	P	114-138	3	gtt caa tga ctt gaa caa gag g	ttc tga tga atg aaa gca cct
260	SAmsCN935817	V	189-289	2	gcc ttc caa gcg tct tgg	tta tca aca agc gcc gtt cc
261	SAmsCO541090	P	403-407	3	cct cgg cat cca caa atc g	gag aag aca aac aga cac ca
262	SAmsCO865955	F	200-214	3	tac tca tgg cgg caa ctc c	gcg gac ggt gat ttc ttg g
265	SAmsCO723438	P	182-202	3	tcc gat tct cta tca gat cca t	tgg atc ggg aca tgg aag g
266	SAmsCN851624	N	359-459	4	aac tgt aga aaa aac act ccc	ggt cct cct ttc aca aat gc
272	SAmsCN942512	P	389-397	4	atc cat cat cgg aaa cct gc	aaa gaa act gga gga ccg c
274	SAmsCN925672	V	214-314	4	aca cgg taa aca cta cca cc	gcg aac ttc acc ttc gca aa
277	SAmsCN866018	P	273-373	2	ttc ctc tca tct atc ctt tcg	gag gtg aca gac aaa ttc gg
279	SAmsCN887525	N	167-267	4	tag tag cta cac act ctt tcc	gca ttg cct tga gct cca g
281	SAmsCN870040	V	260-360	4	cct cag cat cat caa ccc c	gga aat gcg att tcg aac cc

283	SAmsCN921216	F	329-429	4	cgc aca ccc cca aat gcg	aga gct tgt cgc cct cgg
284	SAmsCO752155	F	189-192	3	tgc cta aga atc cat ctg gc	tct cga act tac taa cta ggc
288	SAmsCN909118	F	218-318	3	ctg agg act ctt cta ccc c	cag cag cca cag aat cag c
290	SAmsCN864595	P	358-394	3	ctc tgc aaa cta cca ccg c	tcc tcc tca aca gcg ggg
293	SAmsCN944444	N	333-433	3	tag tgc aag tac tgg ggc c	cat cga tag aat agg acg gc
294	SAmsCN946851	V	311-411	3	aat gac tca agc gat cag gg	ccg atc caa gta gtt aac gg
296	SAmsCN880881	F	406-430	3	ata gct cat acc gct tct cc	gtg acg aaa acc aag aac cc
298	SAmsCN943252	V	148-248	3	tcc cac tga cac tat cac c	tgc agg aaa tga gaa tgc gc
300	SAmsCN939907	N	257-357	3	atc cgc aga act gaa ggc g	act ggt cgg tta tcg acg g
301	Z71981/MDKN1GN	P	331-345	3	ctt gca cta gtg tgc ttt gg	ctt gtt ggg att aaa tcc ggc
302	SAmsCN581539	F	450->500	2	aca aca gct gac gac caa gc	gtc tcc atg act ttt ctg tcc
304	SAmsAJ291492	F	344-418	2	gcg aac tcc agg tga gtg g	taa gca cta aac cac ggt gc
305	SAmsCN491050	V	177-269	2	aat caa tgg aga aac gtc tgc	aaa gga aac cga ctt cac cc
307	SAmsCN445290	N	298-398	2	tca ctt tct cag ttg ctc tgg	atg gaa gct tac tct ttt ccg
308	SAmsCN444942	N	260-273	2	gct ctc aaa gtc tct cca gc	tac gga ctc tct ttg ggg c
310	SAmsAU301301	N	182-282	2	ggc ata gca atg ctt gaa gg	gaa tag cac aaa gga ggt tgc
311	SAmsAU301254	F	232-244	2	tcc cgg aaa ttt ttc aac gc	aac gct agg gat tgg tcg c
312	SAmsCN493139	V	378-478	2	caa acc tat gca ttg tga cag g	cag tct taa gat ccc tgt gg
316	SAmsCN496913	P	240-340	2	gaa agg atg gta cac tct tcg	tta gat gcc tta aat act tcc g
318	SAmsCN580227	N	196-296	2	gac gta aaa tcc cta att ccc	tca tcc cag tcg tct tcc c
319	SAmsAF527800	V	290-390	2	ttg gtc aga cat aca ctg gg	ttg gtc aga cat aca ctg gg
320	SAmsCN580637	F	163-263	2	aca aca gct gac gaa caa gc	cta ctc gtc gaa gta cgc c
322	SAmsAJoo1681	P	349-423	2	atc agg att gga acc tga gg	ctc ttc agc tcc act ctt cc
323	SAmsCN490058	P	196-296	2	cat tgc tca aat cac cct cc	gtc gca gga caa gta gag g

327	SAmsCN490324	V	180-280	2	ata gag agg tag agg act gg	ttc gcc cag tgt aac att gg
328	SAmsCN489396	N	448-540	2	tgg gtc tgc tga gta att agg	ttg ggc ttg gtc gaa aca cc
329	SAmsCN496002	N	177-277	2	agc agc agc tag gct aga gc	aaa ttg cct tgc cag att agc
331	SAmsAB162040	V	244-344	2	gga gtg cta tta gct cct cc	tcc ttg aat ctc aac tct agg
334	SAmsCN444542	F	190-223	2	aag cca ggc cac caa atc c	gag agc tgc att att tgg tcc
335	SAmsCO052033	N	142-242	2	ttg cca atc cgc att cgc c	tga ggt tcc cgc cct tgc
336	SAmsCO168310	F	386-474	2	gtc gac ttc gcc cga agc	acg acc agg ttc atg aac tg
339	SAmsCO066563	V	420-438	2	aca aag gaa cag tga aga ctc	tac ttg ctc tgc ata gtt tgg
340	SAmsCO416051	N	267-367	2	cct cac taa acg cat tgc ac	cgg tac gat gag gat cat cc
341	SAmsCO723148	P	81-181	2	cgg tgg tga cta gta tca gc	tat gga gga aga aac tga ggc
343	SAmsCV084260	F	265	2	caa agc aaa aca gag gat ttg	gga gcg cat gaa att act gc
344	SAmsCO905375	F	407-435	2	agt ctc tgt ttt tgc tcg ttc	gaa cgc cgg gtc cct gc
345	SAmsCO755814	F	211-311	2	aac atc aag aca gag aag agc	cgt ctt ctt cac aaa ctc cg
346	SAmsCO753022	P	421-468	2	ctg agt ctt tgt ttt tgc tcg	gct ccg cct ctc tgt acc
352	SAmsCO866862	P	124-224	2	cat acg cag ctc cca cac g	agg aac ttc tcc agt gag g
355	SAmsCO903877	N	222-232	2	aac agg cgc cat tat ttg cc	cct cgc cat tcg act ttc c
359	SAmsCO756752	V	293-345	2	ctc tct gct ttc ttt cca gc	ggt ggc tcc gct ttc tcc
361	SAmsCO903775	F	239-251	2	cat cga tcc ttc atg aaa ggc	ggt ggt ctg ata tga ttg gcg
365	SAmsCO903680	P	200-300	2	cag cag ttg caa caa gtc c	gtg gaa atg gct aag caa gc
368	SAmsCO723511	V	356-434	2	ctg tcg gga ttc att gtt gc	ccg agt aga agg ctg aag
369	SAmsCO865608	P	109-209	2	caa caa gtg tgc ctc tgt gg	agc aag caa cag atc aag cc
370	SAmsCO052793	F	171-186	2	cca tcc ctt cct cct aca tc	tgg gcc tct tgt tca tta gg
372	SAmsCO052555	N	238	2	gaa gtt ctc atc aag tct tgc	gct tct gca caa tgg ctg g
376	SAmsCO867345	N	318-418	2	tac atc cac cat gga aag atc	ctg gtc gga cag gtt aac g

377	SAmsCO068842	N	283-283	2	tgg ttg gag atg ttc cat gg	acc agc tag att atc ttc tgc
378	SAmsCO753033	V	273-296	2	aca cag tca ttg ctt cct cc	acc cag cat gtg gtc gaa g
379	SAmsCO865207	N	120-138	2	tgc acc aaa taa gcc gat cc	caa gaa gtg caa cca gtc ga
380	SAmsCO866737	F	192-292	2	agc agc ttc cgt ttc cct g	aaa caa ccc acg ctc gga g
381	SAmsCO751676	V	210-260	2	tgt ggc tct gga tgg ttc c	tac cag tcc atc cgt ata gc
382	SAmsCO067152	V	218-233	2	atc atg gcc aac aat atc tcc	gtt gga tta cgc tca cat gg
383	SAmsCO 903298	F	342-356	2	ttg aga agc aat gct gcc tc	tgc cac agt tgg aag gtg g
385	SAmsCO865258	P	170-190	2	ctc ctg tga atc tgc cac c	aga agc agc tct ggc agg
386	SAmsCO901343	P	208-233	2	cac ctc ttc cct cat cag tc	cga caa agg aga ctg aga gg
390	SAmsCN544851	P	250-350	2	ttg tcg gat ttg taa ccc tag	ttc cat atc agt ttg gac acc
395	SAmsCN495393	N	200-219	2	tcc caa gct ccc aac aaa cc	cta tct ggg tcg gcc agg
397	SAmsCN491038	N	498-510	2	gct ctg tct cgt tga tcg g	agc tgc ttc acc ctc ttg c
398	SAmsCN490644	N	214-314	2	atc tca cac ctc agc agt ga	ctt ctg ccc aat tca aga cc
400	SAmsCN578608	N	192-196	2	ctt cgc ctc agt ttc aaa cc	gaa gcc aga gtc tgt tgc c
401	SAmsCN544835	V	137-237	2	agg aga gct ttc tgc att cc	agc gct atc ccc agc tgc
402	SAmsAT000420	N	162-174	2	gtt gga cca att atc tct gc	ata tac tgg gga ggt tga gg
403	SAmsCN494091	P	253-289	2	ctt caa ctt ctc aaa tcg acg	ctt ctg gaa ctc agc ctc c
411	SAmsCN581642	V	162-170	2	caa gaa tac gtt ggg cat gg	aca acg aca taa caa aca cg
412	SAmsCN492999	P	165-265	3	atg aga gag agc tac ctc ac	gta caa gtt cag cag tga cc
413	SAmsCN492417	N	116-145	2	tac cat gtt tta gca cca tgg	ggc caa gtt agg tca aga cg
414	SAmsCN489062	V	284-306	2	aca act tgg tta cgc gac ac	gaa cag att agg gtc gct gg
416	SAmsCO168103	N	141-241	2	ctc aaa aca aga aca atg agc c	ccc aaa agg ttt tcc aca cg
417	SAmsCV128959	P	179-270	2	aaa tag tgt gga aga cgc gg	caat ata cta atg agt cct tcg
418	SAmsCV150384	F	235-250	2	aca aac cac cac caa ttc cc	cct gag aga gcc aat tga gc

419	SAmsCO755991	V	150-154	2	aat ctc tcg tct gca aac cc	gta tga gta tcc agc acc cg
420	SAmsCO903145	N	261-263	2	ggg cac tga acg gtt cgc	ctt tat gca gag aca tgg tcc
421	SAmsCO865954	P	452-455	2	aac acc gtc cag gaa tgc g	aca cac agg tct tcg cag g
422	SAmsCV627191	F	250-385	2	ctt aat cac cca tca ttc ccc	ctc tgt cgg cta act aac cc
424	SAmsCO415353	N	330	2	atg aac agt cac aga cta tgc	aac gaa gca aag gaa gac gg
425	SAmsCO756781	P	281-381	2	ata agt tta ggc tca tct gcc	aaa ccc atc cca ctt aag gc
428	SAmsCO902639	V	293-393	2	ctc ctt tat ctc ttt cct ccc	ttg tcg tcc caa atc aag cc
429	SAmsCO905285	P	344-382	2	gtt gat tct tat ggc acc gg	acc caa atg gcg caa tgc c
435	SAmsCO867454	V	377-392	2	acc gct aaa tgc tgt tca gg	ctt cac tgt gtt agc att ggg
440	SAmsCO416477	N	218-224	2	cca cac aac aca aac caa cc	tgt ggt cat ttg gtg agt cc
443	SAmsCO903797	V	399-413	2	att gat atc aca gct aag cc	cca aaa tct cag aaa cgg gg
444	SAmsCO752447	N	439-453	2	aac ccg caa aca aaa atc cag	tcg gtg atc cgt ttc gcc
445	SAmsCO068219	P	433-437	2	att gct tgc acc gca acg c	gga ctg atc aat gac act cg
448	SAmsCV150002	N	426-456	2	agt tcg atc ttt aat gcc cc	gaa aga gca aga gag act gg
451	SAmsAF429983	F	174-219	2	tac aca gac cag tac tct gc	gga gtc cca ttt caa tgt gg
452	SAmsCO900827	N	394-494	2	acc ttg gtg gcc aag tag c	ctt gcg tat caa agc tgc cg
458	04f3	F	93-143	2	caa aac cac cct cat cct cga a	ccc caa gca gac ctg aag aaa
459	17e6	V	60-158	2	aac acg cca tca cac atc	ctg ttt gct aga aga gaa gtc
460	26c6	N	102-165	2	gac gaa gaa ctc gcc gga gc	cga gga cca acc cac aca caa
461	SAmsDT000945	F	370-421	2	agt tga cta cct cct ccg c	gta agc gat gaa act gat gc
462	SAmsDR994153	V	462-474	2	cac gag gtc tgc atc tac c	tcc aag tcg gtc tga gac g
466	SAmsDT040421	N	325-350	2	ggc aga gca gat gca gat aa	tat aag atg gaa gcc aat gcc
472	SAmsDR995122	P	296-328	2	cga ggc ctt ttt tta ctc gg	att gct ctc ctg tgg tgc c
473	SAmsDR996674	N	424-428	2	caa gca gag tag caa ctg c	gag gcc tct tgc aat tgc g

484	SAmsDT041144	V	335-396	2	aaa tgc tgc agt gag gcc c	gaa ttc cat cta aac gag agc
485	SAmsDR993043	P	279-315	2	cac gag ggt aag ctc ccc	ttg ggg tta ttg ctc tga cg
490	SAmsDR995748	V	315-338	2	tac acc agc gcc aca ccg	tgg cga gca cga tga gcg
491	SAmsDT041234	F	158-176	2	gca act gca agt gag agg g	aga aga agc cat ggc cac c
496	SAmsDT003221	P	319-330	2	ccc aat tac aga gcg agg g	ata cct gaa gaa gca gct cc
498	SAmsDR992457	V	356-375	3	tct cca agt gga cga atc ag	tcc tca gtg aag aca aac cc
502	SAmsDR990381	N	264-300	3	aaa cac tac tgt gct ggt gg	agt cca ctt act act cct cc
505	SAmsDR995002	F	324-334	3	atc tga tgg tgc atc ggt ag	tta ggg tct tct tgt cac gc
506	SAmsDR997517	P	287-324	3	tct aca cca ccc cgc ctc	cga att cgt cat tgg aga gg
507	SAmsDR998909	P	216-221	3	ggg gct gca aca ccc ttc	cat cca tgt ctt cct ttg cc
508	SAmsDT041145	F	63-131	3	tgg ctg tga tgt cat gat gg	tct aga gtt cat cac aaa gaa g
510	SAmsCN881550	V	241-253	3	tcg cgg gaa gtt ccg cag	ggc ctc aag gac cca tcg
512	SAmsCN944528	F	205-214	3	gac gac gga aag gaa gac g	att acg ctg ttg cag aga gc
514	SAmsCX025465	V	227-235	3	tgc tag agc tgc gtt ctc c	tcg cag act gct cgc tgc
515	SAmsCV657225	V	173-200	3	tcc ctg tca tcg aat gat gc	gca aac cca atc aga agg ac
516	SAmsCO900034	P	353-367	3	aaa gtc cgt ttt ggg ctg ag	gct ctc tgc tgc cat ttc c
525	SAmsCV186968	N	389-397	4	acg tac atg cat gcc ttt gg	agt caa gag gca cta tga gc
529	SAmsCN443900	P	418-498	4	agc aat ttt gcc taa aac cga a	gct cat gag gtg cga ttg g
531	SAmsCN943946	N	327-341	4	cac ttg cag cct tgc aca g	tca ctg tct tca tag cct cc
533	SAmsDR993168	P	249-253	4	act tcc ctg ccg cag agg	cac ttg aag cag acc gag g
534	SAmsDR997824	N	319-330	4	gac tgg tga gat aga gag g	atg agc atc gga tag ctgg
535	SAmsDR997862	P	275-283	4	cac aat cat att ccc gca cg	ttc ttc tcc gat gag caa gc
536	Hi02c07	V	108-149	2	aga gct acg ggg atc caa at	gtt taa gca tcc cga ttg aaa gg
538	CH-V*f*1	V	137-169	2	atc acc acc agc agc aaa g	cat aca aat caa agc aca acc c

540	Hi16d02	V	141-160	3	aac cca act gcc tcc ttt tc	gtt tcg aca tga tct gcc ttg
542	Hi03g06	P	172-210	2	tgc caa tac tcc ctc att tac c	gtt taa aca gaa ctg cac cac atc c
543	Hi04g05	V	190-158	2	ctg aaa cag gaa acc aat gc	gtt tcg tag aag cat cgt tgc ag
544	Hi07d11	V	200-232	2	cct tag ggc ctt tgt ggt aag	gtt tga gcc gat tag ggt tta ggg
545	Hi07f01	P	207-215	2	gga ggg ctt tag ttg gga ac	gtt tga gct cca ctt cca act cc
546	Hi22f12	N	207-212	3	ggc ctc acc cag tct aca tt	gtt tgg tgt gat ggg gta ctt tgc
547	Hi03a10	V	206-292	2	gga cct gct tcc cct tat tc	gtt tca ggg aac ttg ttt gat gg
548	Hi04a08	F	211-250	2	ttg aag gag ttt ccg gtt tg	gtt tca ctc tgt gct gga tta tgc
549	Hi05e07	P	194-228	2	ccc aag tcc cta tcc ctc tc	gtt tat ggt gat ggt gtg aac gtg
550	Hi04e04	V	224-242	2	gac cac gaa gcg ctg tta ag	gtt tcg gta att cct tcc atc ttg
551	Hi23g02	F	229-250	3	ttt tcc agg ata tac tac cct tcc	gtt tct tcg agg tca ggg ttt g
552	CN444794-ssr	V	230-306	2	cat ggc agg tgc taa act tg	gtt tgc aac tca cac aat gca ac
553	Hi07h02	F	242-276	2	caa att ggc aac tgg gtc tg	gtt tag gtg gag gtg aag gga tg
554	Hi03c05	N	179-221	2	gaa gag aga ggc cat gat ac	gtt taa ctg aaa ctt caa tct agg
555	Hi02d04	P	217-239	2	tgc tga gtt ggc tag aag agc	gtt taa gtt cgc caa cat cgt ctc
556	Hi23g12	N	223-241	3	ccc ttc cct acc aaa tgg ac	gtt taa agg ggc cca caa agt g
558	Hi01e10	F	198-220	2	tgg gct tgt tta gtg tgt cag	gtt tgg cta gtg atg gtg gag gtg
559	Hi03e04	P	132-160	2	ctt cac acc gtt tgg acc tc	gtt tca tat ccc acc acc aca gaa g
561	Hi05b09	V	123-140	2	aaa ccc aac cca aag agt gg	gtt tct aac gtg cgc cta acg tg
563	Hi04b12	P	138-154	2	ccc aaa ctc cca aca aag c	gtt tga gca gag gtt gct gtt gc
564	Hi24f04	F	144-153	3	ccg acg gct caa aga caa c	tga aaa gtg aag gga atg gaa g
565	Hi08h12	N	101-202	3	gaa gga aat cat cat caa gac g	gtt tca aga cca tgg aac aac ttg g
566	Hi21g05	P	155-164	3	gac gag ctc aag aag cga ac	gtt tgc tct tgc cat ttt ctt tcg
567	Hi03a03	F	205-223	2	aca ctt ccg gat ttc tgc tc	gtt tgt tgc tgt tgg att atg cc

572	Hi01d01	N	191-221	2	ctg aaa tgg aag gct tgg ag	gtt tac caa tta gga ctt aaa gct g
574	Hi02b07	N	204-216	2	tca ctg tct tca tag cct cc	tgg cag tca tct aac ctc cc
576	Hi05g12	P	208-288	2	tct cta gca tcc att gct tct g	gtt tgt gtg ttc tct cat cgg att c
577	Hi05d10	V	212	2	aat ggg tgg ttt ggg ctt a	gtt tct ttg gct att agg cct gc
579	Hi07b06	F	216-222	2	agc tgc agg tag agt tcc aag	gtt tca tta cca tta cac gta cag c
580	Hi20b03	N	215-238	3	aaa ctg caa tcc aca act gc	gtt tag ttg cta atg gcg tgt cg
582	Hi07d08	F	222-232	2	tga cat gct ttt aga ggt gga c	gtt tga ggg gtg tcc gta caa g
583	Hi04f09	V	222-258	2	act ggg tgg ctt gat ttg ag	gtt tca act cac acc ctc tac atg c
584	Hi06b06	P	236-262	2	ggt ggg att gtg gtt act gg	gtt tca tcg tcg gca aga act aga g
586	CH-V*f*2	N	87-115	2	ttt gtt ttt cga gca gga gc	ttt cac att cgg agc atg ag
588	Aj320188-ssr	P	191-245	2	aac gat gct tga gga aga aca	gct taa cag aaa cat cgc tga
592	SAmsEB149750	V	246-265	2	atc aag gtg tga gtg tgt gc	aag ctt gca tct cta ggt cc
593	SAmsEB138715	F	315-338	2	gcg cga tgc cat ctc tgc	ggg atc gca gct cac tcc
594	SAmsEB151342	F	359-376	2	gct gaa aga tgt cac cta cc	cgt gga tcc agc ctt agg g
595	SAmsEB148060	F	374-441	2	act ctc att tct cca cct cc	ctc ctc tgt ctt cct ctg g
597	SAmsEB109450	V	527-539	4	gtt gat atc ggt acg cta gc	gag gca tct ctg ttg gtg
598	SAmsEB138859	V	162-169	4	tac gct agt gct aca gaa gc	aaa ctc cat agc agt agt tcg
601	SAmsEB154700	N	229-236	2	ttt gtt ggg att gtg ggt cg	gtt gct gag agt gat gat gg
602	SAmsEB144676	F	161-197	2	cat cag cca tct tct tct cc	ccg atg gaa atg cag aag c
603	SAmsEB114458	P	119-219	2	tat gat cca tca ccc gaa gg	agt cat aca gct tca cat tcg
610	SAmsEB133782	P	508-543	2	ctc cca gct cac ttt ctcc	cag agg atg cac cac ttg g
612	SAmsEB1155894	F	258-287	2	ttt gcg aca cgt ctc cac c	ttg cac cga gct cct agt c
614	SAmsEB155789	N	323-358	2	ccc cgt tcc ctt gaa ttg ta	cca gtg gaa cga tga ctg c
615	SAmsEB153928	N	348-358	2	ctc aaa tcc cag aag att atc c	gtc ctc gga atc gtc ctc c

617	SAmsEB114260	P	274-290	2	tca tcc tca tcg ttt cct cg	tgt agt tgc ctg cga cac c
623	SAmsEB149589	V	401-404	2	tct tta cct tct tct cca tcc	cgg tac gct gtg gac tcg
626	SAmsEB135470	F	291-301	2	cat ctt tat atg agc cac ttc c	gtt gat gct att ggt agt agg
629	SAmsEB149808	N	269-286	4	tta aag ctc gag ccg agc c	tcc aac cca cta aga tta tcc
630	SAmsDY255319	V	181-211	4	atc gaa ttc cgt tgc tgt cg	atc aat cag cag gct ctt cc
635	SAmsEB149433	N	285-309	3	ctg caa cgt ata ctc taa tcc	gaa agt aac aaa gta cca ggc
636	SAmsEB121159	V	175-194	3	gga tca gag agc tct cag c	tgt gta gag cag tca tgt gg
638	SAmsEB147667	P	411-420	3	agg tct cag gac tct cag g	att gtt aat gtc ggc gaa tcg
639	SAmsEB149851	N	187-202	3	gaa cag agg gaa gca gac g	aga agt ggc aac cat gtt gc
645	SAmsEB156254	V	329-358	2	tat tga ttg tgt gtg tgt gcg	taa gag aag acg aca ttg tcg
647	SAmsEB146894	N	422-438	2	aag gaa gga gcc atg gag g	ata tgg aat cta caa gcc acc
656	SAmsEB139609	F	311-351	2	acc ata tac atc tct ctc tgc	ttc aga agc tgt tgt tgt tgg
661	SAmsEB126773	P	442-470	3	gtt tgt gtt tga aca acg acc	gtg gtt gtt gag gtc gtg g
662	SAmsEB138222	P	264-266	2	tgg aag att gtg aag gca gc	ttg tgg gtg gtt ctt cat cc
664	SAmsEB153442	P	365-373	2	ggt tca caa ggc caa ctt tg	atg gtt cga tcg gtt taa tgc
665	SAmsEB132264	F	119-148	2	ctc att gct act cac taa tcc	gtt cag aaa aga gag aga gag
671	SAmsEB149428	N	255-281	2	gtt aat tcc gct ccc ctc c	atg ctt ctg ggc tcg aac c
673	SAmsEB153023	V	476-494	2	atg tct gca ttc ttg ggt cc	aaa cgc aac att aca agg acg
676	SAmsEB106537	F	178-188	3	gta cag atc tcg ttt cat cac	tga ttg aag ggc agt ctt gg
678	SAmsEB128431	N	322-342	3	acg tag tga tac cgg att cg	aga gct agc tag aga tat tcc
680	SAmsEB106034	N	189-196	3	aga aga agc cca tcc cag c	ttc acc ttc gtc ggc atg g
686	SAmsEB106592	P	234-237	3	ctt gga agc cca acg aac c	aga gga gct tgt tgt tga gg
687	SAmsEB132187	F	220-275	3	tct ccc tca ctc gac gtt g	gtt gca gga agg agt gtc g
688	SAmsEB142061	P	339-341	3	tcg acc agc cag aca aag c	aag agt tgc agg tgg gtc g

701	SAmsEG631386	V	389	2	aca acc tct tct tcc tca gc	gat atc aga agg tac act gaa g
712	SAmsEB 112897	P	330-390	3	caa atc cag ttc gaa gtt tgg	gtc tcc gcg tcc tta aac g
714	SAmsCO417701	V	325-395	2	gtc gat gat ctc tgc gag g	agc aag caa agc atc aga ttg
715	SAmsCN444550	V	320-380	5	agc atc aag cca atc ttt aag c	gta tgc tct tct tct tca tgg
716	SAmsCO051709	F	190-221	6	ctg tgc cgt cat cta tat gc	aac caa aga ggg aag aga cg
717	SAmsContig4879	P	351-361	6	agt tac aag gcg cat tga gg	ttt cga gta gct aaa gag tcg
718	SAmsCN927330	F	400-470	3	tta aac tgc caa att gca cgg	gtt ggg tat ttg cat ggt gg
720	SAmsCN900718	V	259-296	3	agc atc tga act acc aat acc	acc gat ata gtg ctg ttg c
722	SAmsContig21019	F	240-320	5	aac tcg ttt gtc agc aga gg	gtg gaa tat gaa caa atc acg
724	SAmsContig14444	V	282-288	6	ctc ttc atc tga gaa tac acc	aga ctc gag tca tcc ata cc
725	SAmsContig6533	N	228-353	2	tgg tgg ttc tca gtc cag g	cca ata gtg ata agc agt tc
726	SAmsCN877882	F	485-505	5	aac ttg ctg aga gag taa tgg	caa cca aag ggc ctg aag c
728	SAmsCN868149	P	210-285	2	ttg ctg ctg tct gtg ttt gc	gtc tcg tcg aaa tct taa agg
732	SAmsGO566418	V	269-309	2	tat cgt aga gca ggt tgc tg	tat cag tat gca tca cct ac
735	SAmsContig5280	V	284-295	3	tat cag att cgt gcc aca gc	ctt tga cat aga ccc tgt cc
736	SAmsCO414947	V	325-380	2	ttt gat tgg acc tgc agt gg	tta gca gct gct tca gtg tg
738	SAmsCV883434	F	332-351	2	cga aac tgg tcg aag aac ct	aaa cta cac aga gca aga tgg
740	SAmsContig22587	N	305-325	3	ttc acc caa ttc cac aac cg	tca ctg tcg tcc aaa tca gg
742	SAmsCN996777	F	266-275	5	tga caa cta tga tcg aag tgg	ttt cat atc aca tga cgt ggc
744	SAmsCN850743	N	260-20	3	tct acc aat cgt tca aag tcc	tta tca gct ttc cga acc ttc
753	SAmsGO522086	V	249-261	3	tctttgctttgcccttgtgg	agt cca att ctt cct ctt cac
754	SAmsEB144379	P	380-510	6	agc tga tgg cca gaa ctg c	gag ggt cca agt tac aaa gg
756	SAmsCN942929	V	480-550	4	acg cta gga gag agg aac g	gag cat tcc gta tta aat ccg
759	SAmsCN929037	P	187-239	2	agt tga cta cct cct ccg c	gtg gtt ctc acg gta cac g

760	SAmsContig15066	P	274-301	6	gtc ttt gga agc ttg gtt gg	aag tta ctc ttt gtt gct c
761	SAmsCN910199	V	285-301	2	agg aga ata tca gag aaa ggg	gaa tgg tga aat gct cct gg
763	SAmsContig11936	N	344-355	6	cac cga acc aat ccg tag c	aga gag tat gaa agg tgt tcc
766	Ag11	Y	195-220	2	cag aca acc tcc tca cct ca	agt gcc ctg aaa tct gga tg
768	Hi04g11	F	108-150	2	cag agg att atc aat tgg acg c	aaa cta tct cca gtt atc ctg ctt c
769	Hi22d06	V	115-140	3	ccc cga gct cta cct caa a	cat tat gtt tcc ggt ttt tgg
771	Hi21e04	P	110-160	3	tgg aaa cct gtt gtg gga tt	tgc aga gcg gat gta agt tg
772	Hi02a09	F	110-195	2	atc tct aag ggc agg cag ac	ctg act ctt tgg gaa ggg c
773	Hi23b12	V	125-175	3	tga gcg caa tga cgt ttt ag	gtt tca ggc ttt ccc ttc agt gtc
774	Hi04e05	N	116-179	2	aag ggt gtt tgc gga gtt ag	ggt gcg ctg tct tcc ata aa
775	Hi08e06	P	120-164	3	gca atg gcg ttc tag gat tc	ggt ggt gaa ccc tta att gg
776	Hi23d02	F	100-155	3	ccg gca tat caa agt ctt cc	gtt tga tgg tct gag gca atg gag
777	Hi23d06	V	140-175	3	ttg aaa ccc gta cat tca act c	gtt tca aga acc gtg cga aat g
778	Hi15g11	N	80-192	3	tga cat gca tag ggt tac atg c	gtt tgg gtt cgt aat cgt tct tgt g
779	Hi04d10	R	140-200	2	aaa ttc cca ctc ctc cct gt	gtt tga gac gga ttg gg gta g
780	Hi08f05	F	142-170	3	gtg tgg gcg att cta act gc	gtt tcc ttt att cta aac atg cca cgt c
781	Hi02a07	V	170-200	2	gcc act cat acc cat cgt att g	gtt tgg ctg gga ata tat gat cag gtg
783	Hi23d11b	P	165-205	3	gac agc cag aag aac cca ac	gtt tat tgg tcc att tcc cag gag
784	Hi08d09	F	171-220	3	aac ggc ttc ttg tca aca cc	gtt tac tgc atc cct tac cac cac
785	Hi09a01	V	174-199	3	gaa gca acc acc aga aga gc	gtt tcc cat tcg ctg gta ctt gag
786	Hi07d12	N	184-250	2	gga atg agg gag aag gaa gtg	gtt tcc tct tca cgt ggg atg tac c
788	Hi04a05	F	180-220	2	ggc agc agg gat gta ttc tg	gtt tca tgt caa atc cga tca tca c
789	Hi02b10	V	177-270	2	tgt ctc aag aac aca gct atc acc	gtt tct tgg agg cag tag tgc ag
791	Hi02c06	P	180-270	3	agc aag cgg ttg gag aga	gtt tgc aac agg tgg act tgc tct

792	Hi01d05	F	210->330	2	ggt atc ctc ttc atc gcc tg	tta gat tga cgt tcc gac cc
793	Hi23g08	V	200-230	3	agc cgt ttc cct ccg ttt	gtt tgt gga tga gaa gca cag tca
794	Hi01c09	N	193-250	2	aaa ggc gag gga taa gaa gc	gtt tgc aca ttt gag ctg tca agc
796	Hi08c05	F	180-260	3	tca tat agc cga ccc cac tta g	gtt tca cac tcc aag att gca tac g
797	Hi02d11	V	176-285	2	gca atg ttg tgg gtg aca ag	gtt tgc aga atc aaa acc aag caa g
800	Hi12a02	F	223-280	3	gca agt cgt agg gtg aag ctc	gtt tag tat gtt ccc tcg gtg acg
801	Hi02a07	V	210-320	2	ttg aag cta gca ttt gcc tgt	tag att gcc caa aga ctg gg
802	NzmsCN879773	N	125-195	2	ccc tct gtt act ttg act ctt ctc	tgg ttt ggg ttg aaa atg gt
804	NzmsEB106592	F	240-243	3	ctc cca cta cta gcc aaa cg	ttg gga ttt gaa gga cag g
806	NzmsEB107305	Y	110-190	2	aac ttc caa acc cca tct cc	aga gca acc tca cca tct tca
810	NzmsEB142980	N	80-140	4	cca gtt ggt tat aca aat cgc aaa g	cct gat cct caa aat tac agc a
813	NZmsCO754252	V	195-197	2	ctg ccc tca agg aga atg tc	aca ggt gca gca aag gct at
820	NzmsEB116209	F	100-140	3	aaa atc cca att cca aaa cc	ttg gag cag tga aag att gg
822	NZmsDR033893	N	194-225	3	cac tta ggg tgt atg ggt gtg a	tca ttt tgg gca ggc act
824	NzmsEB153947	F	166-180	3	ggg aga gtt agg gga aaa gg	act gag gcc tgc aac ata cc
826	NZmsEB111793	N	275-281	2	ttg agg gct gct ttc cag	gga gac ata caa gat ttc caa tga g
827	NzmsEB146613	P	140-210	4	aga gtt ccg ttc ccc tct ct	gtg gat tcg gaa atg cac tc
828	NZmsCN914822	F	190-193	3	gac gat gat cag gcc att ct	tgt tca tgt cgg tgc tca at
829	NzmsCO905522	V	155-172	2	cag ggc act gac aaa gac ag	aat tgg aga ttt gcg gtg tc
833	NZmsEB137525	V	172-192	2	tct ttc gct ggt gtc ctc tt	gtg ctg ctt gct gtt gtt gt

Appendix B. Megaplexes designed for 451 SSR markers, the dye colour of each marker, the observed size range, across the nine parents used previously, for each marker and the alleles sizes produced for each of the four parents used in this study, after PCR with the Qiagen megaplex PCR kit. JoinMap codes for each of the three mapping populations are also given.

Megaplex 1	Accession number	Dye	Contig no. / Chromosome (Velasco et al., 2010)	Size range (bp)	Anna Alleles	GD Alleles	Priscilla alleles	Prima Alleles	'Prima' x 'Anna JoinMap code	'Golden Delicious' x 'Anna' JoinMap code	'Golden Delicious' x 'Priscilla' JoinMap code
93	CH05f06	V	MDC011855.327/ CHR05	166-184	180/184	176/184	176/184	184	nn x np	ef x eg	hk x hk
107	CH04d02	N	MDC019740.197/ CHR12	118-146	-	-	-	-	--	--	--
114	CH03b06	F	MDC022202.499/ CHR15	105-131	106/115	115	118/125	106/115	hk x hk	nn x np	nn x np
120	CH05e04	F	MDC004808.272/ CHR16	140-234	147/159	159/167	167	161/167	ab x cd	ef x eg	lm x ll
166	CH05a09	P	MDC006875.277/ CHR16	141-186	-	-	-	-	--	--	--
227	SAmsCN493171	N	MDC010527.333/ CHR06	295-395	347	347	347	347	--	--	--
236	CH01e09b	P	MDC016291.91/ CHR15	192-292	233/241	233/236	233/241	236/241	ef x eg	ef x eg	ef x eg
281	SAmsCN870040	V	MDC005271.182/ CHR16	260-360	305	301/304	301	301	lm x ll	lm x ll	lm x ll
288	SAmsCN909118	F	MDC022525.56/ CHR05	218-318	248/251	248	251	248/251	hk x hk	nn x np	--
294	SAmsCN946851	V	MDC019585.198/ CHR13	190-250	242	243	228/243	158	--	--	nn x np
318	SAmsCN580227	N	MDC015010.269/	196-296	276	276	276	271	--	--	--

			CHR06								
320	SAmsCN580637	F	MDC018988.253/ CHR15	415-425	420	408	408/420	420	--	--	nn x np
329	SAmsCN496002	N	MDC008539.361/ CHR05	177-277	209/214	209/226	209/212	209/214	hk x hk	ef x eg	ef x eg
335	SAmsCO052033	N	MDC003753.230/ CHR05	142-242	196	190/196	196/199	190/196	lm x ll	lm x ll	ef x eg
341	SAmsCO723148	P	MDC001241.304/ CHR11	81-181	149/153	149/153	153	144/153	ef x eg	hk x hk	lm x ll
425	SAmsCO756781	P	MDC002994.270/ CHR10	281-381	-	333/361	333/358	-	--	--	ef x eg
Megaplex 2											
15	28f4	N	LG 12	90-110	102/110	95/110	110	95/110	ef x eg	ef x eg	lm x ll
43	CH04e03	F	MDC041220.7/ CHR05	179-222	204/206	194	177/197	185/204	ab x cd	nn x np	nn x np
56	CH05f04	V	MDC006621.180/ CHR04	160-172	163/169	163	163/171	163/171	ef x eg	nn x np	nn x np
59	CH03d08	F	MDC020317.340/ CHR14	129-161	133/135	126/133	138	136	ef x eg	ef x eg	lm x ll
60	CH03g04	V	MDC004274.213/ CHR14	122-144	127/135	132	127/132	135/142	ef x eg	nn x np	nn x np
80	MS02a01	N	MDC011588.208 / CHR10	170-194	192/194	180/199	199	185	nn x np	ab x cd	lm x ll
128	CH01b121	P	MDC019519.278 / CHR12	125-178	127/133	124/127	126	126/134	hk x hk	ef x eg	lm x ll
139	CH02a04	P	MDC022150.298/ CHR 02	66-112	68/107	92/103	92/99	99/103	ab x cd	ab x cd	ef x eg
219	SAmsCN580620	F	MDC008517.277/ CHR12	333-433	378	377	334/380	378/383	lm x ll	--	nn x np
244	SAmsCN947446	V	MDC019062.252/ unanchored	136-236	181/184	184/187	181/190	180/190	ab x cd	ef x eg	ab x cd

279	SAmsCN887525	N	MDC004101.200/ CHR05	167-267	-	209/216	212	-	--	--	lm x ll
293	SAmsCN944444	N	MDC001204.808/ CHR03	365-433	374/376	374/378	374/378	374/380	ef x eg	ef x eg	hk x hk
327	SAmsCN490324	V	MDC013709.214/ CHR17	180-280	229/231	229/232	229/231	231/235	ef x eg	hk x hk	hk x hk
346	SAmsCO753022	P	MDC012584.88/ CHR15	305-480	-	438	438/457	-	--	--	nn x np
398	SAmsCN490644	N	MDC003800.283/ CHR10	214-314	263/267	264/356	358	282/356	ab x cd	ef x eg	lm x ll
417	SAmsCV128959	P	MDC003450.371/ CHR06	179-270	240	232/242	-	-	--	lm x ll	--
Megaplex 3											
66	MS01a05	V	LG 14	158-176	170/174	145/172	-	145/175	ef x eg	ab x cd	--
71	CH01h011	N	MDC013304.239 / CHR17	100-134	105/121	117	117/119	115/119	ab x cd	nn x np	nn x np
74	CH02a10	N	MDC016803.330/ CHR03	143-177	147/154	128/146	154/152	147/154	hk x hk	ab x cd	ab x cd
112	CH05c04	V	MDC008148.499/ CHR13	186-258	220/224	185/198	-	220	nn x np	ab x cd	--
113	CH01d08	N	MDC021953.346/ CHR15	238-290	252/260	253/273	253	253/270	ef x eg	ef x eg	lm x ll
119	CH05a04	F	MDC010246.376/ CHR16	159-189	170/175	165/174	166/189	165/184	ab x cd	ef x eg	ef x eg
125	CH02g01	P	MDC008787.433/ CHR05	91-121	-	-	-	-	--	--	--
182	SAmsCN445253	F	MDC019975.203/ CHR12	410-430	417/420	-	-	420	nn x np	--	--
187	SAmsCN490566	V	MDC015511.204/ CHR06	286-386	-	336	-	-	--	--	--
207	SAmsCN495433	V	MDC031287.8/ CHR05	213-313	-	-	-	-	--	--	--

234	SAmsCN938125	N	MDC012545.302/ CHR17	303-403	339/347	342/354	347	342/354	ab x cd	ab x cd	nn x np
235	CH01b09b	P	MDC004291.249/ CHR17	305-405	345/348	-	-	-	--	--	--
345	SAmsCO755814	F	MDC003399.279/ CHR10	211-311	261/341	-	-	341	nn x np	--	--
352	SAmsCO866862	P	MDC012661.305/ CHR03	124-224	-	-	-	-	--	--	--
355	SAmsCO903877	N	MDC022150.298/ CHR02	222-232	-	224/228	224/228	-	--	--	hk x hk
369	SAmsCO865608	P	MDC006289.408/ CHR01	109-209	161/163	160/164	-	163	nn x np	ab x cd	--
452	SAmsCO900827	N	MDC010624.539/ CHR02	394-494	-	443	-	-	--	--	--
Megaplex 4											
29	SAmsAT000141	V	MDC015190.83/ CHR09	56-100	88/89	94	94	88/89	--	--	--
63	CH05d03	F	MDC018782.299/ CHR06	152-187	167/175	154/164	166	152/181	ab x cd	ab x cd	lm x ll
64	CH05e05	N	MDC009350.182/ CHR02	138-160	162	157/160	157	157/160	lm x ll	lm x ll	nn x np
67	CH02c09	N	MDC011137.202/ CHR15	233-257	243/249	240/255	232/243	241	nn x np	ab x cd	ab x cd
99	CH03d02	F	MDC005828.284/ CHR11	201-223	-	211	-	-	--	--	--
106	CH03c02	F	MDC018186.206/ CHR12	116-136	125	125/127	125/127	116/125	lm x ll	lm x ll	hk x hk
109	CH05d11	N	MDC018277.209/ CHR12	171-211	183	169/173	169/173	169/173	lm x ll	lm x ll	hk x hk
122	CH04c06	V	MDC017603.123/ CHR17	155-186	171/177	175/179	171/179	171/175	ef x eg	ab x cd	ef x eg
137	CH01f03a	P	MDC015290.99/	210-224	212/224	213/224	224	212	nn x np	hk x hk	lm x ll

			CHR16								
148	CH03a03	P	MDC015486.182/ CHR14	154-182	156/158	160/170	158/160	156/167	ef x eg	ab x cd	ef x eg
158	CH04d08	P	MDC019260.152/ CHR11	116-142	-	117	117/135	-	--	--	nn x np
200	SAmsCN493925	N	MDC015011.163/ CHR02	366-410	-	405	405	-	--	--	--
220	SAmsCN580732	F	MDC015169.163/ CHR02	300-400	-	-	-	-	--	--	--
231	SAmsCN580271	V	MDC003949.200/ CHR01	156-256	-	217/271	240	-	--	nn x np	lm x ll
377	SAmsCO068842	N	MDC018268.352/ CHR13	399-466	457/-	401/448	401/457	436/449	ab x cd	ab x cd	ef x eg
380	SAmsCO866737	F	MDC011713.137/ CHR16	192-292	240	240	240	240/254	lm x ll	--	--
390	SAmsCN544851	P	MDC006391.297/ CHR04	250-350	-	242	228/242	-	--	--	nn x np
Megaplex 5											
10	02b1	N	LG 15	188-288	218	218/229	230/238	218/229	lm x ll	lm x ll	ab x cd
14	23g4	F	LG 06	70-130	91	80/84	91	91	--	lm x ll	lm x ll
49	CH05c07	N	MDC005293.195/ CHR09	111-149	139	137/149	111/137	139/149	lm x ll	lm x ll	ef x eg
62	CH04f06	N	MDC011094.321/ CHR14	159-179	176/180	160/179	176/179	176/179	ef x eg	ef x eg	ef x eg
73	CH01f12	F	MDC019380.166/ CHR10	145-162	149/151	146/162	151/162	162	nn x np	ab x cd	ef x eg
87	CH03e03	F	MDC005190.587/ CHR03	106-216	184/190	197	199/203	185	nn x np	ab x cd	nn x np
94	CH03d12	V	MDC007389.248/ CHR06	108-154	113/121	121	113/121	113/121	hk x hk	nn x np	nn x np
171	CH05c02	P	MDC004471.532/	168-200	-	172/178	160/171	-	--	--	ef x eg

			CHR11								
172	CH05d08	P	MDC013234.266/ CHR 17	91-143	116/123	122/125	123	123	nn x np	ef x eg	lm x ll
173	CH05g01	P	MDC017682.301/ CHR11	236-276	-	-	252/254	-	--	--	--
217	SAmsCN579502	F	MDC012292.266/ CHR07	230-330	280/288	281/289	281/289	280/288	hk x hk	hk x hk	hk x hk
238	SAmsCN865016	F	MDC009136.399/ CHR15	294-394	341/347	-	-	341	nn x np	--	--
253	SAmsCO540769	N	MDC017032.162/ CHR06	213-313	264/266	262/266	250/266	250	nn x np	ef x eg	ef x eg
260	SAmsCN935817	V	MDC005588.270/ unanchored	189-289	223/239	238	227	-	--	nn x np	--
331	SAmsAB162040	V	MDC020034.222/ CHR12	244-344	303/305	280/288	280/288	303/305	hk x hk	ab x cd	hk x hk
376	SAmsCO867345	N	MDC000910.324/ CHR16	318-418	366/439	366/440	366/440	366/439	hk x hk	hk x hk	hk x hk
401	SAmsCN544835	V	MDC009798.251/ CHR05	137-237	-	-	161/174	-	--	--	--
412	SAmsCN492999	P	MDC002480.238/ CHR16	165-265	-	215	-	-	--	--	--
Megaplex 6											
44	CH05e06	F	MDC002834.158/ CHR05	125-222	136/150	130/145	146	202/220	ab x cd	ab x cd	lm x ll
48	CH01h021	F	MDC003767.335/ CHR09	236-256	236	-	246	235	--	--	--
57	CH01g05	V	MDC020317.340/ CHR14	140-188	138/155	137/144	151	136	nn x np	ef x eg	lm x ll
72	CH05g03	N	MDC010787.146/ CHR17	135-192	175/183	132/164	162	162/183	ef x eg	ab x cd	lm x ll
76	CH02c11	N	MDC001758.144/ CHR10	219-239	228/237	194/220	194/234	229/233	ef x eg	ab x cd	ef x eg

85	CH03d01	F	MDC022695.138/ CHR02	95-115	98/110	101/111	110	100/108	ab x cd	ab x cd	lm x ll
115	CH03b10	N	MDC012303.704/ CHR15	99-121	111/116	102/118	111	101/111	ef x eg	ab x cd	lm x ll
165	CH04g09	P	MDC012537.142/ CHR 05	141-177	145/147	149/174	157/174	154/174	ab x cd	ab x cd	ef x eg
167	CH05a02	P	MDC021095.21/ CHR 15	111-135	132/137	116/136	116/136	130	nn x np	ef x eg	hk x hk
168	CH05a03	P	MDC018744.266/ CHR09	182-220	183/193	191/193	190/192	196	nn x np	ef x eg	hk x hk
193	SAmsCN492206	F	MDC021083.97/ CHR13	329-429	397	398/471	379/471	397	--	lm x ll	ef x eg
196	SAmsCN492626	N	MDC020254.241/ CHR15	260-360	308/314	309/314	309/314	308/314	hk x hk	hk x hk	hk x hk
202	SAmsCN494248	V	MDC011588.205/ CHR05	266-366	313	314	314	314	--	--	--
213	SAmsCN496756	N	MDC042546.8/ CHR14	423-523	468	469	469	468	--	--	--
222	SAmsCN581649	N	MDC000908.450/ CHR14	140-200	175/183	184	175/183	174/182	hk x hk	nn x np	nn x np
323	SAmsCN490058	P	MDC022454.244/ CHR15	196-296	224/227	-	227/229	224/227	hk x hk	--	--
Megaplex 7											
12	05g8	F		71-171	127	118	118/124	123	--	--	nn x np
36	CH02g09	V	MDC002525.346/ CHR 08	98-138	120/144	144/156	144/156	-	--	ef x eg	hk x hk
38	CH05e03	V	MDC008217.277/ CHR 02	158-190	169/172	178/184	184/189	178/184	ab x cd	ab x cd	ef x eg
78	COLa	F	MDC001085.297/ CHR 10	220-240	226/229	219/231	220/231	223/-	ab x cd	ab x cd	hk x hk
81	MS06g03	V	MDC016163.84 / CHR 10	154-190	156/177	142/156	156	160/165	ab x cd	ef x eg	lm x ll

89	CH04e02	F	MDC019231.92/ CHR 09	143-163	152/163	157	148/150	154/156	ab x cd	nn x np	nn x np
90	CH02b121	V	MDC022137.130/ CHR 05	101-143	130/136	139	130/145	119/128	ab x cd	nn x np	nn x np
98	CH02d121	F	MDC018548.59/ CHR 11	177-199	179/198	177/198	177	185/191	ab x cd	ef x eg	lm x ll
118	CH02d10a	V	MDC006455.384/ CHR 16	215-245	213/242	218	216/221	211/217	ab x cd	nn x np	nn x np
133	CH01d03	P	MDC014207.192/ CHR 04	136-160	139/144	129/139	138/160	139/144	hk x hk	ef x eg	ef x eg
136	CH01e121	P	MDC012891.303/ CHR 08	246-278	252	248/254	252	248/252	lm x ll	lm x ll	lm x ll
147	CH02h11b	P	MDC007676.537/ CHR 04	214-240	220/222	216/222	220	222	nn x np	ef x eg	lm x ll
177	CH05h05	P	MDC022738.132/ CHR 13	168-184	-	159/169	181/184	-	--	--	ab x cd
215	SAmsCN496844	V	MDC021142.191/ CHR 15	192-210	194/208	207	194/208	208	nn x np	nn x np	nn x np
274	SAmsCN925672	V	MDC011928.397/ CHR 04	214-314	305	298/303	304	305/309	lm x ll	lm x ll	lm x ll
283	SAmsCN921216	F	MDC013463.226/ CHR 09	329-429	368	385	366/374	368	--	--	nn x np
Megaplex 8											
34	CH01c06	N	MDC012891.303/ CHR 08	146-188	160/162	156/162	156/159	156/160	ef x eg	ef x eg	ef x eg
35	CH01f021	V	MDC022471.103/ CHR 12	174-206	170/184	180	173/183	180	nn x np	nn x np	nn x np
42	CH05d02	N	MDC005153.453/ CHR 04	203-225	213/217	196/223	218/223	213/223	ef x eg	ab x cd	ef x eg
61	CH04c07	N	MDC022423.57/ CHR 14	98-135	97/134	94/112	94/133	107	nn x np	ab x cd	ef x eg
65	CH05g11	F	MDC001583.305/	201-255	214/249	238/249	213/252	245	nn x np	ef x eg	ab x cd

			CHR 14								
75	CH02b03b1	F	MDC009271.511/ CHR 10	77-109	94/96	74/86	83/89	90/94	ef x eg	ab x cd	ab x cd
84	CH02f061	V	MDC000307.248/ CHR 02	135-158	-	158	145/149	-	--	--	nn x np
91	CH03a04	V	MDC000528.538/ CHR 05	92-124	97/100	96/119	93	104/107	ab x cd	ef x eg	lm x ll
95	CH01f091	F	MDC002525.346/ CHR 08	125-160	-	120/136	120	-	--	--	lm x ll
108	CH04g04	F	MDC004400.583/ CHR 12	170-186	173	172/180	172	173/181	lm x ll	lm x ll	lm x ll
126	CH01b09b	P	MDC001010.290/ CHR 04	172-182	177/181	173/183	183	174/181	ef x eg	ab x cd	lm x ll
145	CH02g01	P	MDC007396.58/ CHR 13	198-238	200/220	227	228	228	nn x np	nn x np	--
162	CH04f04	P	MDC017371.119/ CHR 05	144-166	151/159	151/169	169	151/157	ef x eg	ef x eg	lm x ll
365	SAmsCO903680	P	MDC009439.435/ CHR 11	200-300	250	242/244	-	246/250	lm x ll	lm x ll	--
381	SAmsCO751676	V	MDC010150.221/ CHR 10	210-260	221/235	219/234	219/234	219/235	ef x eg	ef x eg	hk x hk
428	SAmsCO902639	V	MDC000636.613/ CHR 15	293-393	-	343	-	-	--	--	--
Megaplex 9											
37	CH02c061	V	MDC026455.33/ CHR 02	216-254	-	237/241	215/238	-	--	--	ef x eg
41	CH02c02b	V	MDC007362.400/ CHR 04	78-126	103/111	114/121	109/113	111/115	ef x eg	ab x cd	ef x eg
45	CH03d07	N	MDC018191.399/ CHR 06	186-226	-	-	185	-	--	--	--
52	CH02d08	F	MDC005828.284/ CHR 11	210-254	225	224/226	211/217	225	--	lm x ll	ab x cd

96	CH01h101	N	MDC005828.284/ CHR 08	94-114	91/98	88/99	89/120	91/98	hk x hk	ef x eg	ef x eg
97	CH01f03b	V	MDC020937.110/ CHR 09	139-183	148/160	138/171	160/179	148/155	ef x eg	ab x cd	ab x cd
111	CH03h03	F	MDC004556.326/ CHR 10	72-120	76/82	75/117	75	76/91	ab x cd	ef x eg	lm x ll
116	CH04g10	N	MDC012425.163/ CHR 15	127-168	133/148	132	120/140	129/155	ab x cd	nn x np	lm x ll
121	CH02g04	F	MDC013381.253/ CHR 17	132-197	148	179/194	189/192	149	--	lm x ll	ab x cd
130	CH01c09	P	MDC004126.509/ CHR 13	92-108	-	87/94	-	-	--	--	--
135	CH01e09b	P	MDC016291.91/ CHR 15	118-140	126/136	122/136	120/138	138/-	ab x cd	ef x eg	ab x cd
146	CH02h07	P	MDC010531.484/ CHR 09	214-236	218/-	227	226/236	221/236	ab x cd	nn x np	nn x np
169	CH05a09	P	MDC006875.277/ CHR 16	152-200	157/176	178/184	178/184	155/184	ab x cd	ab x cd	ef x eg
266	SAmsCN851624	N	MDC001342.390/CH R 16	359-459	-	248	253	-	--	--	--
319	SAmsAF527800	V	MDC021880.118/ CHR 17	290-390	330	330	330	330	--	--	--
422	SAmsCV627191	F	MDC020007.246/ CHR 17	250-350	310/312	310/312	296/312	310/312	hk x hk	hk x hk	ef x eg
Megaplex 10											
46	CH05a05	F	MDC017021.252/ CHR 06	198-230	-	217/220	217	-	--	--	lm x ll
53	CH04g07	V	MDC012022.139/ CHR 11	149-211	171/181	177/205	148/150	150/171	ef x eg	ab x cd	ab x cd
88	CH02h11a	V	MDC007676.537/ CHR 04	104-132	-	126	120/126	-	--	--	nn x np
101	CH04d07	F	MDC005248.149/	119-142	128	115/128	114	128/138	lm x ll	lm x ll	lm x ll

			CHR 11								
208	SAmsCN495651	V	MDC021781.288/ CHR 06	348-448	-	-	-	-	--	--	--
277	SAmsCN866018	P	MDC029130.40/ CHR 15	220-235	222	195/223	221	222/226	lm x ll	lm x ll	lm x ll
300	SAmsCN939907	N	MDC007320.447/ CHR 15	257-357	302	302/308	302/308	302/309	lm x ll	lm x ll	hk x hk
307	SamsCN445290	N	MDC002235.539/ CHR 06	298-398	340	340/352	352	340	--	lm x ll	lm x ll
310	SAmsAU301301	N	MDC018350.223/ CHR 03	182-282	230/244	223/238	219/242	242/252	ab x cd	lm x ll	ab x cd
316	SAmsCN496913	P	MDC017405.92/ CHR 13	240-340	-	-	302/308	-	--	--	--
346	SAmsCO753022	P	MDC012584.88/ CHR 15	350-460	-	436	437/440	-	--	--	nn x np
416	SAmsCO168103	N	MDC019586.334/ CHR 15	141-241	194	194	194	194	--	--	--
603	SAmsEB114458	P	MDC019757.125/ CHR 06	119-215	-	-	-	-	--	--	--
Megaplex 11											
105	CH01g121	F	MDC002525.336/ CHR 12	112-186	107/130	102/143	105/127	107/151	ef x eg	ab x cd	ab x cd
110	MS14b04	V	MDC008313.329/ CHR 12	230-292	-	-	-	-	--	--	--
161	CH04f03	P	MDC022821.76/ CHR 10	175-191	177/189	185	176/186	187	nn x np	nn x np	lm x ll
179	MS06c09	P	MDC019138.228/ CHR 08	102-118	-	-	113	-	--	--	--
180	SAmsCN444111	N	MDC011837.83/ CHR 09	409	406	405	406	406	--	--	--
186	SAmsCN90349	N	MDC018282.133/ CHR 15	207	-	196/206	206	-	--	--	lm x ll

188	SAmsCN490740	F	MDC013217.295/ CHR 10	213	195/207	190	190/212	192/213	ab x cd	nn x np	nn x np
340	SAmsCO416051	N	MDC000020.209/ CHR 05	317	-	121/130	119/121	-	--	--	ef x eg
343	SAmsCV084260	F	MDC005861.294/ unanchored	265	228/257	262	238/267	228/263	ef x efg	nn x np	nn x np
372	SAmsCO052555	N	MDC007544.497/ CHR 13	238	234/236	237	234/237	234/237	hk x hk	nn x np	nn x np
424	SAmsCO415353	N	MDC017127.194/ CHR 15	330	-	331/335	331	-	--	--	lm x ll
536	Hi02c07	V	MDC014016.450/ CHR 01	108-149	112/148	107/113	108/114	108/116	ab x cd	ef x eg	hk x hk
559	Hi03e04	P	MDC041875.12/ CHR 13	132-160	141/144	131/151	141/159	141/144	hk x hk	ab x cd	ab x cd
584	Hi06b06	P	MDC017030.295/ CHR 03	236-262	259/261	258/261	261	259/261	hk x hk	hk x hk	lm x ll
781	Hi02a07	V	MDC010932.713/ CHR 16	170-200	-	185	179/185	-	--	--	nn x np
Megaplex 12											
50	CH01f07a	F	MDC009271.511/ CHR 10	174-206	192/194	176	191/204	189/192	ef x eg	nn x np	nn x np
100	CH04a12	V	MDC010999.445/ CHR 11	158-196	190/203	176/182	174	188/192	ab x cd	ab x cd	lm x ll
221	SAmsCN580954	V	MDC012238.252/ CHR 03	106-118	-	-	-	-	--	--	--
259	SAmsCN904905	P	MDC008622.281/ CHR 14	114-138	116/122	116	116/122	116/122	hk x hk	nn x np	nn x np
311	SAmsAU301254	F	MDC005145.116/ CHR 17	232-244	233/246	234	242/246	233/246	hk x hk	nn x np	nn x np
385	SAmsCO865258	P	MDC011523.287/ CHR 12	170-190	-	-	-	-	--	--	--
395	SAmsCN495393	N	MDC001276.321/	200-219	203/214	219	-	204/216	ab x cd	nn x np	--

			CHR 10								
413	SAmsCN492417	N	MDC015871.265/ CHR 02	116-145	-	-	-	-	--	--	--
540	Hi16d02	V	MDC020977.553 /CHR 05	141-160	143	143	143	141	--	--	--
550	Hi04e04	V	MDC025815.15/ CHR 16	224-242	225/237	244	225	225/244	ef x eg	nn x np	--
555	Hi02d04	P	MDC016662.359/ CHR 15	217-239	219/235	219/235	241	235/241	ef x eg	hk x hk	lm x ll
579	Hi07b06	F	MDC009002.127/ CHR 06	216-222	221	219	217/219	219/223	lm x ll	--	nn x np
662	SAmsEB138222	P	MDC008411.143/ CHR 09	264-266	-	-	-	-	--	--	--
725	Contig6533	N	MDC010551.377/ CHR 05	228-353	228/329	333	229/333	228/329	hk x hk	nn x np	nn x np
813	NZmsCO754252	V	MDC017371.127/ CHR 06	195-197	195/197	-	-	195/197	hk x hk	--	--
Megaplex 13a											
189	SAmsCN490897	F	MDC020416.37/ CHR 12	458-462	463	463	463	463	--	--	--
284	SAmsCO752155	F	MDC010250.69/ CHR 12	189-192	192/195	195	195/200	192/195	hk x hk	nn x np	nn x np
344	SAmsCO905375	F	MDC012584.88/ CHR 15	407-435	407	408/428	408	427/435	lm x ll	lm x ll	lm x ll
491	SAmsDT041234	F	MDC020535.246/ CHR 12	158-176	165/167	165	165	165	nn x np	nn x np	--
512	SAmsCN944528	F	MDC003532.156/ CHR 02	205-214	205	215	205/215	205/214	lm x ll	--	hk x hk
551	Hi23g02	F	MDC009192.441/ CHR 15	229-250	236/251	245/251	251	236	nn x np	ef x eg	lm x ll
593	SAmsEB138715	F	MDC022702.107/ CHR 02	315-338	-	-	-	-	--	--	--

594	SAmsEB151342	F	MDC013761.438/ unanchored	359-376	-	-	-	-	--	--	--
626	SAmsEB135470	F	MDC019582.266/ CHR 06	291-301	-	-	-	-	--	--	--
665	SAmsEB132264	F	MDC022516.234/ CHR 07	119-148	126/141	-	-	-	--	--	--
742	SAmsCN996777	F	MDC002085.537/ CHR 15	266-275	270/274	274	264/274	274	nn x np	nn x np	lm x ll
Megaplex 13b											
181	SAmsCN444846	N	MDC005133.90/ CHR 13	150-152	-	460	-	-	--	--	--
229	SAmsCN496966	N	MDC003594.382/ CHR 15	167-171	-	167	-	-	--	--	--
241	SAmsCN887787	N	MDC010065.349/ unanchored	254-257	255/257	258	255/258	255/257	hk x hk	nn x np	nn x np
243	SAmsCN907588	N	MDC011946.321/ CHR 11	304-307	306	305	306	306	--	--	--
379	SAmsCO865207	N	MDC014214.260/ CHR 13	120-138	134/138	120	-	134/138	hk x hk	nn x np	--
397	SAmsCN491038	N	MDC020705.116/ CHR 14	498-510	500/513	510	500/513	513	nn x np	nn x np	nn x np
440	SAmsCO416477	N	MDC013556.555/ CHR 07	218-224	221	218/224	220/226	221/226	lm x ll	lm x ll	hk x hk
525	SAmsCV186968	N	MDC019148.87/ CHR 08	389-397	396	-	396	396	--	--	--
534	SAmsDR997824	N	MDC007440.255/ CHR 10	319-330	325	-	325	325	--	--	--
629	SAmsEB149808	N	MDC012989.567/ CHR 02	269-286	283	-	283	286	--	--	--
639	SAmsEB149851	N	MDC016474.226/ CHR 10	187-202	-	-	-	-	--	--	--
647	SAmsEB146894	N	MDC017945.196/	422-438	423/426	424/438	426	423/426	hk x hk	ef x eg	lm x ll

			CHR 08								
763	SAmsContig11936	N	MDC013753.167/ CHR 02	344-355	347	344	346	347	--	--	--
Megaplex 13c											
163	CH04f07	P	MDC021718.251/ CHR 09	82-113	-	94/98	-	-	--	--	--
174	CH05g02	P	MDC021144.114/ CHR 12	133-155	-	141/146	-	-	--	--	--
178	CH05h12	P	MDC005658.277/ unanchored	164-192	-	-	-	-	--	--	--
272	SAmsCN942512	P	MDC012121.557/ CHR 14	389-397	390/392	390/392	390/393	388/392	ef x eg	hk x hk	hk x hk
472	SAmsDR995122	P	MDC014092.189/ CHR 14	296-328	311/318	318/322	318	307/325	ab x cd	ef x eg	lm x ll
516	SAmsCO900034	P	MDC007820.597/ CHR 15	353-367	361/367	354/367	353/367	354/367	ef x eg	ef x eg	hk x hk
533	SAmsDR993168	P	MDC007844.642/ CHR 15	249-253	249/253	249/251	249/251	249/251	ef x eg	ef x eg	hk x hk
545	Hi07f01	P	MDC003391.187/ CHR 12	207-215	209/213	207/209	-	207	nn x np	ef x eg	--
610	SAmsEB133782	P	MDC015102.351/ CHR 04	508-543	-	-	-	-	--	--	--
638	SAmsEB147667	P	MDC007467.200/ CHR 11	411-420	411/420	411/420	-	411/420	hk x hk	hk x hk	--
661	SAmsEB126773	P	MDC034420.7/ CHR 15	442-470	-	-	-	-	--	--	--
686	SAmsEB106592	P	MDC011198.306/ CHR 02	234-237	236	234/237	235/237	234/237	lm x ll	lm x ll	hk x hk
688	SAmsEB142061	P	MDC006613.339/ CHR 14	339-341	339	339	-	339/341	lm x ll	--	--
Megaplex 13d											

104	CH01d09	V	MDC010076.456/ CHR 12	131-172	145/147	132/141	136/147	145/147	hk x hk	ab x cd	ab x cd
226	SAmsCN444745	V	MDC015190.83/ CHR 09	455-480	-	-	-	-	--	--	--
443	SAmsCO903797	V	MDC021940.79/ CHR 16	399-413	411	401/409	406/-	407/409	lm x ll	lm x ll	ab x cd
490	SAmsDR995748	V	MDC012914.254/ CHR 14	315-338	316/333	336	336	333/336	ef x eg	nn x np	--
498	SAmsDR992457	V	MDC013008.333/ CHR 09	356-375	356/362	359/370	359/376	362/365	ef x eg	ab x cd	ef x eg
514	SAmsCX025465	V	MDC015326.172/ CHR 09	227-235	231/236	230	230/236	231/236	hk x hk	nn x np	nn x np
592	SAmsEB149750	V	MDC013258.236/ CHR 13	246-265	258/264	256	-	262	nn x np	nn x np	--
597	SAmsEB109450	V	MDC011235.284/ CHR 13	527-539	532/544	-	-	532/544	hk x hk	--	--
630	SAmsDY255319	V	MDC022862.53/ CHR 05	181-211	-	181	181	-	--	--	--
724	SAmsCN996777	V	MDC009274.258/ CHR 10	282-288	270/275	-	-	275	nn x np	--	--
Megaplex 14a											
30	SAmsAT000400.1	N	MDC002235.548/ CHR 02	175-181	176/183	175/179	176/183	183	nn x np	ef x eg	ef x eg
220	SAmsCN580732	F	MDC015169.163/ CHR 02	340-375	370	369	341/369	347	--	--	nn x np
254	SAmsCN933736	F	MDC019787.50/ CHR 16	291-334	311/335	310/318	310	335	nn x np	ab x cd	lm x ll
262	SAmsCO865955	F	MDC015520.222/ CHR 01	200-214	202/216	202	202/208	202/216	hk x hk	nn x np	nn x np
400	SAmsCN578608	N	MDC010461.160/ CHR 12	192-196	197	194	194/197	197	--	--	nn x np
418	SAmsCV150384	F	MDC017449.236/	235-250	231/248	235/-	-	248	nn x np	ab x cd	--

			CHR 17								
448	SAmsCV150002	N	MDC026285.8/ unanchored	426-456	-	-	-	-	--	--	--
460	26c6	N		102-165	140	-	-	129/140	lm x ll	--	--
461	SAmsDT000945	F	MDC017026.232/ CHR 17	390-425	369/400	396/420	396/400	369	nn x np	ab x cd	ef x eg
502	SAmsDR990381	N	MDC007681.179/ CHR 10	264-300	265/289/ 301	264/300	-	290/301	--	--	--
508	SAmsDT041145	F	MDC017144.293/ CHR 13	63-131	78/87	-	-	87	nn x np	--	--
531	SAmsCN943946	N	MDC016731.254/ CHR 09	327-341	329/339	329/343	329/347	329/344	ef x eg	ef x eg	ef x eg
574	Hi02b07	N	MDC009491.388/ CHR 12	204-216	204/216	204/216	-	207	nn x np	hk x hk	--
601	SAmsEB154700	N	MDC006620.372/ CHR 16	229-236	228/234	229/236	-	228	nn x np	ef x eg	--
602	SAmsEB144676	F	MDC008781.274/ CHR 08	161-197	164/188	189/197	189	164/188	hk x hk	ef x eg	lm x ll
615	SAmsEB153928	N	MDC013377.330/ CHR 15	348-358	350/353	353/359	353	353	nn x np	ef x eg	lm x ll
Megaplex 14b											
4	GD 100	P	LG 10	223-238	229	227	235/237	226/228	lm x ll	--	nn x np
13	22c6	V		63-142	-	-	-	-	--	--	--
159	CH04d11	P	MDC010450.930/ CHR 03	85-152	129/138	89	129/138	129/138	hk x hk	nn x np	nn x np
265	SAmsCO723438	P	MDC001167.326/ CHR 02	182-202	202	199/205	205	204	--	lm x ll	lm x ll
414	SAmsCN489062	V	MDC021085.739/ CHR 10	284-306	297/301	284/298	298	297/401	ef x eg	ef x eg	lm x ll
419	SAmsCO755991	V	MDC020003.312/ unanchored	150-154	-	150/154	-	-	--	--	--

448	SAmsCV150002	N	MDC026285.8/ unanchored	426-465	428/460	428/430	-	428/460	hk x hk	ef x eg	--
484	SAmsDT041144	V	MDC017371.127/ CHR 06	335-396	350/352	350	350	350/352	hk x hk	lm x ll	--
496	SAmsDT003221	P	MDC012972.308/ CHR 15	319-330	-	-	-	-	--	--	--
507	SAmsDR998909	P	MDC002325.395/ CHR 06	216-221	216/219	216/224	219	219	nn x np	ef x eg	lm x ll
583	Hi04f09	V	MDC005047.173/CH R 13	222-258	241/253	252	238/253	243/259	ab x cd	nn x np	nn x np
598	SAmsEB138859	V	MDC014091.117/ CHR 09	162-169	-	-	-	-	--	--	--
617	SAmsEB114260	P	MDC008416.202/ CHR 10	274-290	-	-	-	-	--	--	--
833	NZmsEB137525	V	MDC014091.117/ CHR 09	172-192	174/188	184/190	174/188	184/194	ab x cd	ab x cd	ab x cd
Megaplex 15a											
5	GD 103	F	LG 5/10	78-130	78/105	78/104	78/86/93/105	84/93	ab x cd	hk x hk	nn x np
82	CH02b101	N	MDC022150.298/ CHR 02	121-159	-	-	148/155	-	--	--	--
195	SAmsCN492475	N	MDC010740.412/ unanchored	175-185	177/183	195	174/185	177/186	ef x eg	nn x np	nn x np
201	SAmsCN493973	F	MDC001897.482/ CHR 02	252-329	314	284	325	-	--	--	--
214	SAmsCN496821	F	MDC015102.349/ CHR 04	358-410	410	410	383/411	410	--	--	nn x np
245	SAmsCN943613	F	MDC005388.315/CH R 15	165-174	166/175	174	175	175	nn x np	nn x np	--
466	SAmsDT040421	N	MDC015817.303/ CHR 12	325-350	348	348/354	339/348	389/346	lm x ll	lm x ll	ef x eg
567	Hi03a03	F	MDC016112.100/ CHR 14	205-223	214/222	222	222/226	225	nn x np	nn x np	nn x np

676	SAmsEB106537	F	MDC017002.101/ CHR 08	178-188	183	183	184	-	--	--	--
738	SAmsCV883434	F	MDC016637.26/ CHR 06	332-351	334	351/357	346	334	--	lm x ll	lm x ll
822	NZmsDR033893	N	MDC017604.504/ CHR 11	194-225	202/214	202/216	196/202/216/222	202	nn x np	ef x eg	nn x np
826	NZmsEB111793	N	MDC021681.173/ CHR 13	275-281	275	275	275/279	275/280	lm x ll	--	nn x np
Megaplex 15b											
40	MS14h03	V	MDC015605.102/ CHR 03	114-140	115	111	-	-	--	--	--
131	CH01c11	P	MDC004106.267/ CHR 11	109-155	112/144	-	-	112	nn x np	--	--
339	SAmsCO066563	V	MDC015340.304/ CHR 13	420-438	-	-	-	-	--	--	--
359	SAmsCO756752	V	MDC002458.1854/ CHR 03	293-345	-	-	-	-	--	--	--
382	SAmsCO067152	V	MDC016102.192/ CHR 10	218-233	-	218/233	-	-	--	--	--
444	SAmsCO752447	N	MDC021843.193/ unanchored	439-453	-	-	-	-	--	--	--
506	SAmsDR997517	P	MDC033581.12/ CHR 12	287-324	306/309	293	293	287	nn x np	nn x np	--
510	SAmsCN881550	V	MDC004291.249/ CHR 17	241-253	251/254	-	-	242/248	ab x cd	--	--
515	SAmsCV657225	V	MDC016649.157/ CHR 06	173-200	182/194	194/200	194	194	nn x np	ef x eg	lm x ll
529	SAmsCN443900	P	MDC020042.326/ CHR 14	418-498	441/456	-	-	443	nn x np	--	--
664	SAmsEB153442	P	MDC018604.406/ CHR 10	365-373	367/373	373	-	-	--	nn x np	--
759	SAmsCN929037	P	MDC017026.232/	187-239	219/225	214	215/219	231/239	ab x cd	nn xnp	nn x np

			CHR 17								
Megaplex 16a											
9	01a6	F	LG 04	87-155	123	133/143	132/143	132/143	lm x ll	lm x ll	hk x hk
328	SAmsCN489396	N	MDC015986.169/ CHR 02	448-540	-	495	-	-	--	--	--
336	SAmsCO168310	F	MDC020043.176/ CHR 12	386-474	-	-	397/428	-	--	--	--
361	SAmsCO903775	F	MDC010201.199/ CHR 05	239-251	-	239	-	-	--	--	--
370	SAmsCO052793	F	MDC015381.190/ CHR 04	171-186	181	-	182	181	--	--	--
473	SAmsDR996674	N	MDC015516.245/ CHR 06	424-428	428	428	428	428	--	--	--
558	Hi01e10	F	MDC002171.593/ CHR 09	198-220	213	220	207/222	201/210	lm x ll	--	nn x np
565	Hi08h12	N	MDC010803.260/ CHR 10	101-202	102/151	150/171	151/171	167/204	ab x cd	ef x eg	hk x hk
580	Hi20b03	N	MDC014200.253/ CHR 08	215-238	218/226	215/238	218/226	226	nn x np	ab x cd	ab x cd
656	SAmsEB139609	F	MDC007147.92/ CHR 08	311-351	-	-	-	-	--	--	--
671	SAmsEB149428	N	MDC021125.349/ CHR 04	255-281	256/258	255/277	255/277	256	nn x np	ef x eg	hk x hk
678	SAmsEB128431	N	MDC004449.266/ CHR 13	322-342	-	342	333	-	--	--	--
828	NZmsCN914822	F	MDC010773.182/ CHR 14	190-193	-	190/193	-	-	--	--	--
Megaplex 16b											
140	CH02a08	P	MDC019763.88/ CHR 10	128-177	-	136/152	140/154	-	--	--	ab x cd

204	SAmsCN494928	V	MDC016112.100/ CHR 14	209-229	211/228	209/219	211	211	nn x np	ab x cd	lm x ll
368	SAmsCO723511	V	MDC006682.168/ CHR17	356-434	-	356/434	-	-	--	--	--
386	SAmsCO901343	P	MDC003451.570/ CHR 04	208-233	-	208/233	209	-	--	--	--
411	SAmsCN581642	V	MDC005839.240/ CHR 13	162-170	167/171	166/170	167	165/171	--	hk x hk	lm x ll
429	SAmsCO905285	P	MDC020851.240/ CHR 13	344-382	345/381	345	345/382	345/369	ef x eg	nn x np	nn x np
445	SAmsCO068219	P	MDC018327.114/ CHR 01	433-437	-	-	-	-	--	--	--
636	SAmsEB121159	V	MDC011090.394/ CHR 15	175-194	-	181	181/184	-	--	--	nn x np
673	SAmsEB153023	V	MDC009294.148/ CHR 05	476-494	477	491/494	477	477/496	lm x ll	lm x ll	lm x ll
732	SAmsGO566418	V	MDC007950.564/ CHR 16	269-309	-	300/-	-	-	--	--	--
753	SAmsGO522086	V	MDC008749.41/ CHR 05	249-261	256	258/261	-	247/256	lm x ll	lm x ll	--
760	SAmsContig15066	P	MDC010751.331/ CHR 04	274-301	-	-	-	-	--	--	--
Megaplex 17											
47	CH04e05	V	MDC011989.191/ CHR 07	174-227	202/214	174	202/204	175/219	ab x cd	nn x np	nn x np
170	CH05b06	P	MDC018507.307/ CHR 10	185-215	188/193	198/218	218/221	199	nn x np	ab x cd	ef x eg
192	SAmsCN491993	F	MDC004698.235/ CHR 05	245-284	282	252/283	282	282/284	lm x ll	lm x ll	lm x ll
228	SAmsCN496055	N	MDC005479.52/ CHR 14	360-364	-	363	-	-	--	--	--
308	SAmsCN444942	N	MDC015532.141/	260-273	265/275	273	259/275	275	nn x np	nn x np	nn x np

			CHR 06								
378	SAmsCO753033	V	MDC021781.288/ CHR 06	273-296	274	273	275	274	--	--	--
403	SAmsCN494091	P	MDC008371.455/ CHR 04	253-289	-	-	-	-	--	--	--
421	SAmsCO865954	P	MDC022559.265/ unanchored	452-455	-	454	454/458	-	--	--	nn x np
451	SAmsAF429983	F	MDC017091.105/ CHR 04	356-371	-	356/367	-	-	--	--	--
458	04f3	F	LG 09	93-143	108/114	120	118/120	120	nn x np	nn x np	nn x np
505	SAmsDR995002	F	MDC000262.256/ CHR 12	324-334	331/334	333	329/332	331/334	hk x hk	nn x np	nn x np
546	Hi22f12	N	MDC021414.198/ CHR 05	207-212	209	200/207	215	209	--	lm x ll	lm x ll
561	Hi05b09	V	MDC006588.64/ CHR 07	123-140	138/140	135	133/138	138/142	ef x eg	nn x np	nn x np
563	Hi04b12	P	MDC016797.262/ CHR 08	138-160	141/148	147/154	135/139	157	nn x np	ef x eg	ab x cd
595	SAmsEB148060	F	MDC008726.377/ CHR 04	374-441	-	-	-	-	--	--	--
623	SAmsEB149589	V	MDC001040.257/ CHR 02	401-404	-	401	-	-	--	--	--
717	SAmsContig4879	P	MDC007228.344/ CHR 06	351-361	355/360	355/361	350/360	351/355	ef x eg	hk x hk	ef x eg
740	SAmsContig22587	N	MDC006300.120/ CHR 12	305-325	317	315	317	317	--	--	--
774	Hi04e05	N	MDC024246.13/ CHR 08	116-179	138/140	138/142	133/138	138/142	ef x eg	ef x eg	ab x cd
Megaplex 18a											
7	GD 147	N	LG 13	129-152	135/152	134	147	129/148	ab x cd	nn x np	--
206	SAmsCN495278	N	MDC011995.314/	214-240	-	-	-	-	--	--	--

			CHR 15								
209	SAmsCN495857	F	MDC020525.273/ CHR 03	145-155	149/152	145/148	152	152	nn x np	ef x eg	lm x ll
218	SAmsCN580519	F	MDC011588.205/ CHR 05	120-135	123/128	120/135	-	123	nn x np	ab x cd	--
296	SAmsCN880881	F	MDC008622.281/ CHR 14	406-430	430/433	406	430	411/430	ef x eg	nn x np	--
402	SAmsAT000420	N	MDC002412.304/ CHR 04	162-174	-	170/172	-	-	--	--	--
420	SAmsCO903145	N	MDC003918.382/ CHR 02	261-263	-	261	-	-	--	--	--
422	SAmsCV627191	F	MDC020007.246/ CHR 17	296-385	311/313	313	296/312	311/313	hk x hk	nn x np	lm x ll
612	SAmsEB1155894	F	MDC009328.385/ CHR 16	258-287	-	-	277/285	-	--	--	--
680	SAmsEB106034	N	MDC004713.230/ unanchored	189-196	193/197	169	178/191	193	nn x np	nn x np	nn x np
716	SAmsCO051709	F	MDC005414.494/ CHR 15	190-221	195	195/221	195/221	195/221	lm x ll	lm x ll	hk x hk
804	NZmsEB106592	F	MDC011198.306/ CHR 02	240-243	243	240/243	240/242	240/243	lm x ll	lm x ll	hk x hk
824	NZmsEB153947	F	MDC022425.139/ CHR 11	166-180	167/171	167/170	167/171	165/171	ef x eg	hk x hk	hk x hk
Megaplex 18b											
151	CH03g06	P	MDC015735.303/ CHR 11	137-171	139/167	139/164	139/167	150/167	ef x eg	ef x eg	--
212	SAmsCN496144	V	MDC019010.307/ CHR 06	303-349	-	338	338	-	--	--	--
261	SAmsCO541090	P	MDC011995.314/ CHR 15	403-407	405	405	405	405	--	--	--
290	SAmsCN864595	P	MDC007691.315/ CHR 15	358-394	362	358	362	362	--	--	--

301	Z71981/MDKN1GN	P	MDC016467.170/ CHR 15	331-345	338/348	337	334/340	338	nn x np	nn x np	nn x np
305	SAmsCN491050	V	MDC013938.271/ CHR 03	177-269	189	173/189	173/189	189/231	lm x ll	lm x ll	hk x hk
419	SAmsCO755991	N	MDC020003.312/ unanchored	148-156	154	150/154	-	-	--	lm x ll	--
435	SAmsCO867454	V	MDC004223.800/ unanchored	377-392	-	396	391/396	-	--		lm x ll
462	SAmsDR994153	V	MDC017740.298/ CHR 10	462-474	465/471	-	466/472	463/471	ef x eg	--	--
485	SAmsDR993043	P	MDC022656.93/ CHR 11	279-315	298/315	-	281/284	279/298	ef x eg	--	--
538	CH-Vf1	V	LG 01	137-169	163	137/169	159	139/159	lm x ll	lm x ll	lm x ll
549	Hi05e07	P	MDC003262.348/ CHR 09	194-228	215	213/228	214/229	-	--	lm x ll	hk x hk
614	SAmsEB155789	N	MDC012906.325/ CHR 14	323-358	326	323	324/333	324	--	--	lm x ll
635	SAmsEB149433	N	MDC013012.212/ CHR 11	285-309	305/310	309	285/288	286	nn x np	nn x np	nn x np
735	SAmsContig5280	V	MDC020462.181/ CHR 05	284-295	284/287	287/295	287/290	284/296	ef x eg	ef x eg	ef x eg
Megaplex 19											
718	SAmsCN927330	F	MDC016235.85/ CHR 07	400-470	439/443	432/440	-	429/431	ef x eg	ef x eg	--
722	SAmsContig21019	F	MDC011822.222/ CHR 12	240-320	-	278/284	-	-	--	--	--
724	SAmsContig14444	V	MDC009274.258/ CHR 10	240-315	-	-	-	-	--	--	--
736	SAmsCO414947	V	MDC008623.473/ CHR 17	325-380	348/351	343/356	-	342/349	ab x cd	ab x cd	--
768	Hi04g11	F	MDC006738.419/ CHR 11	108-150	116	116/123/126/ 140/147	-	116	--	--	--

769	Hi22d06	V	MDC001013.218/ CHR 02	115-140	127/133	124/127	-	124/127	ef x eg	ef x eg	--
785	Hi09a01	V	MDC017714.167/ CHR 11	174-199	192	187	-	184/187	lm x ll	--	--
788	Hi04a05	F	MDC010937.194/ CHR 01	180-220	186/192	186	-	186/188	ef x eg	nn x np	--
793	Hi23g08	V	MDC001907.204/ CHR 09	200-230	210/219	219	-	213/219	ef x eg	nn x np	--
802	NZmsCN879773	N	MDC018532.138/ CHR 01	125-195	140/187	140/147	-	142	nn x np	ef x eg	--
827	NZmsEB146613	P	MDC002255.84/ CHR 14	140-210	171/176	160/180	-	176	nn x np	ab x cd	--
829	NZmsCO905522	V	MDC017428.71/ CHR 16	155-172	165	165/170	-	165	--	lm x ll	--
Megaplex 20											
687	SAmsEB132187	F	MDC001593.313/ CHR 01	220-275	239	253	234	-	--	--	--
712	SAmsEB 112897	P	MDC001100.222/ CHR 12	330-390	381	380	381	381	--	--	--
714	SAmsCO417701	V	MDC022324.112/ CHR 09	325-395	-	354/360	349/354	-	--	--	ef x eg
726	SAmsCN877882	F	MDC019147.47/ CHR 02	460-510	485	495/502	502/507	502/507	lm x ll	lm x ll	ef x eg
728	SAmsCN868149	P	MDC008453.906/ CHR 13	210-285	-	252	248/250	-	--	--	nn x np
772	Hi02a09	F	MDC012004.220/ CHR 11	110-195	145/157	127/135	135	126/135	ab x cd	ab x cd	lm x ll
773	Hi23b12	V	MDC005900.178/ CHR 14	125-175	-	-	154	-	--	--	--
775	Hi08e06	P	MDC011043.394/ CHR 05	120-164	-	156	-	-	--	--	--
783	Hi23d11b	P	LG 04	165-205	180/186	180/186	183/185	186	nn x np	hk x hk	ef x eg

797	Hi02d11	V	MDC011578.52/ CHR 14	176-285	197/257	233/246	254/257	243/253	ab x cd	ab x cd	ab x cd
810	NZmsEB142980	N	MDC018496.52/ CHR 04	80-140	112/123	108/120	123	123	nn x np	ab x cd	lm x ll
Megaplex 21											
715	SAmsCN444550	V	MDC015575.172/ CHR 10	320-380	343/352	343	-	348/352	hk x hk	nn x np	--
744	SAmsCN850743	N	MDC021608.178/ CHR 01	260-290	279/282	279/282	-	273/279	ab x cd	hk x hk	--
754	SAmsEB144379	P	MDC016553.87/ CHR 14	380-510	417	411/417	-	411/423	lm x ll	lm x ll	--
771	Hi21e04	P	MDC000164.370/ CHR 14	110-160	136/151	134/138	-	133/151	ab x cd	ab x cd	--
776	Hi23d02	F	MDC000442.224/ CHR 11	100-155	125/146	134/146	-	146	nn x np	ef x eg	--
777	Hi23d06	V	MDC007040.105/ CHR 09	140-175	154/160	160/169	-	160	nn x np	ef x eg	--
778	Hi15g11	N	MDC006465.421/ CHR 16	80-192	98/159	-	-	98	nn x np	--	--
789	Hi02b10	V	MDC012697.251/ CHR 02	177-270	200/202	202/218	-	200/202	hk x hk	ef x eg	--
791	Hi02c06	P	LG 11	180-270	224	224/243	-	228/244	lm x ll	lm x ll	--
794	Hi01c09	N	MDC020259.182/ CHR 14	193-250	205/219	216/218	-	203/217	ab x cd	ef x eg	--
796	Hi08c05	F	MDC021778.347/ unanchored	180-260	219/232	233/236	-	233	nn x np	ef x eg	--
Megaplex 22											
756	SAmsCN942929	V	MDC004462.498/ CHR 03	480-550	523/526	530	530	531	--	nn x np	--
766	AG11	Y	LG 01	195-220	203/206	199/203/205	-	203/206	hk x hk	--	--

779	Hi04d10	R	LG 06	140-200	176/182	166	166/184	184	nn x np	nn x np	nn x np
780	Hi08f05	F	MDC005649.355/ CHR 02	142-170	162/164	-	-	162/164	hk x hk	--	--
781	Hi02h08	V	MDC010932.713/ CHR 16	140-185	166/170	173/184	161/173	172	nn x np	ab x cd	ef x eg
784	Hi08d09	F	MDC002262.69/ CHR 16	171-220	182	182	182/185	182	--	--	nn x np
800	Hi12a02	F	MDC009686.144/ CHR 10	223-280	252/255	-	-	252	nn x np	--	--
801	Hi02a07	V	MDC000017.398/ CHR 02	210-320	264/281	281	279/281	264/281	hk x hk	nn x np	nn x np
806	NZmsEB107305	N	MDC022702.107/ CHR 02	110-190	167	149/161	-	152/162	lm x ll	lm x ll	--
820	NZmsEB116209	F	MDC000625.521/ CHR 09	100-140	132	115/129	129/132	115/132	lm x ll	lm x ll	ef x eg
Megaplex 23											
46	CH05a05	F	MDC017021.252/ CHR 06	198-260	207/220	217/256	214	199/220	ef x eg	ab x cd	lm x ll
54	CH05d04	V	MDC004971.319/ CHR 12	154-214	-	155/187	210/212	-	--	--	ab x cd
70	CH05c06	F	MDC017428.71/ CHR 16	104-149	106/112	106	106/114	110	nn x np	nn x np	nn x np
92	CH03a09	V	MDC002901.281/ CHR 05	122-151	124	126/130	128	124/128	lm x ll	lm x ll	lm x ll
117	CH02a03	N	MDC021909.329/ CHR 16	122-170	124/134	134	-	150	nn x np	nn x np	--
543	Hi04g05	V	MDC011851.278/ CHR 13	190-258	252/256	227	230	229	nnx np	nn x np	--
549	Hi05e07	P	MDC003262.348/ CHR 09	194-228	214	213/228	214/229	215	--	lm x ll	hk x hk
554	Hi03c05	N	MDC018788.94/ CHR 17	179-221	-	205/217	-	-	--	--	

Megaplex 24											
57	CH01g05	V	MDC020317.340/ CHR 14	134-188	157/159	137/144	151/157	139/157	ef x eg	ef x eg	ab x cd
95	CH01f091	F	MDC002525.346/ CHR 08	114-160	157/159	120/136	120	139/159	ef x eg	ab x cd	lm x ll
163	CH04f07	P	MDC021718.251/ CHR 09	82-113	-	94/98	-	-	--	--	--
334	SAmsCN444542	F	MDC013323.310/ CHR 09	190-223	203/217	190/209	203/217	203/217	hk x hk	ab x cd	ab x cd
542	Hi03g06	P	MDC016820.135/ CHR 15	172-210	175/206	184/206	196/198	206	nn x np	ef x eg	ab x cd
544	Hi07d11	V	MDC009608.253/ CHR 13	200-232	208/216	217	217/219	216	nn x np	nnx np	nnx np
553	Hi07h02	F	MDC019711.264/ CHR 17	242-276	246/254	244/252	246/254	246/254	hk x hk	ab x cd	ab x cd
556a	Hi23g12a	N	MDC016662.359/ CHR 15	223-241	221	221/224	224	221/224	lm x ll	lm x ll	lm x ll
556b	Hi23g12b	N	MDC016662.359/ CHR 15	223-241	233	231	233/236	233/238	lm x ll	--	nn x np
Megaplex 25											
39	CH03g07	V	MDC010653.386/ CHR 03	115-181	125/127	115/125	118/122	127/166	ef x eg	ef x eg	ab x cd
45	CH03d07	N	MDC018191.399/ CHR 06	163-226	187/205	188/205	188/205	205/217	ef x eg	hk x hk	hk x hk
107	CH04d02	N	MDC019740.197/ CHR 12	106-164	-	111/114	111	111	--	--	lm x ll
152	CH03g12	P	MDC000180.101/ CHR 01	150-200	-	160/170/181/ 197	-	-	--	--	--
547	Hi03a10	V	MDC009304.358/ CHR 07	206-292	290	254/290	215/290	215/241	lm x ll	lm x ll	ef x eg
548	Hi04a08	F	MDC007686.156/	211-250	210/213	211	213	209/213	hk x hk	nn x np	--

			CHR 05								
Megaplex 26											
86	CH03d10	V	MDC021153.205/ CHR 02	152-182	172	166/172	168/172	166/168	lm x ll	lm x ll	ef x eg
107	CH04d02	N	MDC019740.197/ CHR 12	106-164	111	111/114	111	110	--	lm x ll	lm x ll
256	SAmsCN868958	F	MDC021905.407/ CHR 02	181-202	188/190	180/-	183/189	188/190	hk x hk	ab x cd	ab x cd
383	SAmsCO903298	F	MDC007382.112/ CHR 17	342-356	346	343/357	342/351/353/357	346/357	lm x ll	lm x ll	nn x np
564	Hi24f04	F	MDC006022.710/ CHR 14	144-153	150	144/147	150	147/150	lm x ll	lm x ll	lm x ll
566	Hi21g05	P	MDC020046.235/ CHR 04	155-164	157/159	159	159	157/159	hk x hk	nn x np	--
572	Hi01d01	N	MDC015015.42/ CHR 09	191-221	187/199	194/219	194/220	187/191	ef x eg	ab x cd	hk x hk
577	Hi05d10	V	MDC024409.26/ CHR 10	212	-	-	-	-	--	--	--
582	Hi07d08	F	MDC003205.158/ CHR 01	222-232	230	230	221	-	--	--	--
586	CH-Vf2	N		87-115	-	-	-	-	--	--	--
588	AJ320188-SSR	P	MDC022377.146/ CHR 09	191-245	200	200	195/208	200	--	--	nn x np
761	SAmsCN910199	V	MDC017817.285/ unanchored	285-301	296	296/300	296/302	296	--	lm x ll	ef x eg
Megaplex 27											
32	CH05g08	F	MDC012059.23/ CHR 01	161-179	175/177	175/177	161/176	165/177	ef x eg	hk x hk	ab x cd
176	CH05g07	P	MDC015780.141/ CHR 14	149-197	149/157	156/164	156	157	nn x np	ef x eg	--

312	SAmsCN493139	V	MDC020235.543/ CHR 02	378-478	-	-	-	-	--	--	--
552	CN444794-SSR	V	MDC022754.106/ CHR 07	230-306	259	251/253	255	256/274	lm x ll	lm x ll	lm x ll
576	Hi05g12	P	MDC013149.564/ CHR 01	208-288	246	270/279	246	270/279	lm x ll	lm x ll	lm x ll
786	Hi07d12	N	MDC002532.193/ CHR 02	184-250	-	191/244	192	-	--	--	lm x ll
792	Hi01d05	F	MDC011710.245/ CHR 06	210-360	208	296/326	349/354	208/326	lm x ll	lm x ll	ab x cd

‘ - ‘ represents failure to amplify a product

‘--‘ represents the lack of a JoinMap code due to homozygous nature of the marker, or lack of information from the population

UNIVERSITY of the
WESTERN CAPE