SSR-based genetic mapping of QTLs determining chilling requirements for time of initial vegetative budbreak in domesticated apple (*Malus* x *domestica* Borkh.) cultivar 'Anna' x 'Austin'.

by

Paidashe Hove

A thesis submitted in partial fulfilment of the requirements for the degree of
MAGISTER SCIENTIAE (M.Sc.)

Department of Biotechnology
University of the Western Cape
Bellville

Supervisor: Dr. D. J. G. Rees
Co-supervisor: Prof. B. Ndimba

June 2012

ABSTRACT

The Rosaceae family contains major temperate crops such as the domesticated apple (*Malus x domestica* Borkh.), peach (*Prunus persica* L. Batsch) and European pear (*Pyrus communis* L.). However, despite its evident economic importance, it is generally poorly studied in genomic terms, relative to the other major crop groups. Microsatellite and Diversity Array Technology (DArT) genetic markers have been exploited in this work and are essential tools in genetic map construction and marker-assisted selection (MAS) of high quality apples and other rosaceous crops. Microsatellites are advantageous in that they are co-dominant, highly polymorphic, abundant, transferable and reliably reproducible; hence their use in this study. In order for budbreak to take place in a timely and homogenous fashion, apple trees need a period of exposure to low temperatures. Within orchards the application of chemicals that induce budbreak in unsuitable environments is required to produce apples from cultivars that require high chilling levels. However, this and other practices using chemicals in orchards tend to pollute the environment. One of the solutions to this problem is to breed low chill apples such as 'Anna' cultivar, which was used as one of the parents in this study.

This work was aimed at understanding the underlying genetic factors that determine chilling requirements for the time of initial vegetative budbreak trait in the apple cross 'Anna' x 'Austin'. This was achieved through linkage map construction using SSR and DArT molecular markers followed by QTL analysis. This thesis has therefore exploited the large number of Expressed Sequence Tags (ESTs) and genome sequence data for the apple, using Tandem Repeats Finder, to design a total of 98 new SSR primers pairs. The

UNIVERSITY of the WESTERN CAPE

other 369 SSR markers used in this work were from published work. JoinMap® 4.1 software was used to create an integrated genetic map with 17 linkage groups, for the domesticated apple cultivar, ‘Austin’ x ‘Anna’ mapping population with 80 individuals. The result of this process was a genetic map 1 212cM in length, and a total of 429 markers (314 DArT and 115 SSR), at an average density of a marker every 4 cM. This map was used identify the Quantitative Trait Loci (QTLs) determining chilling requirements for time of vegetative budbreak (IVB). In this process, putative IVB QTLs were identified in the ‘Anna’ x ‘Austin’ mapping population using the rMQM analysis function of MapQTL® 6.0, for both adult and seedling data collected over 3 growing seasons from 1996 to 1998. These QTLs were detected on linkage groups 2, 9 and 14, and explained 0.3 to 12.8 % of the observed phenotypic variation for the adult population, and 5.3 - 21 % for the seedling population. Seedling (LG 14) and adult (LGs 5, 7, 10) specific QTLs were also detected for the ‘Anna’ x ‘Austin’ cross. These QTLs will provide the basis for marker validation on related mapping populations in the apple breeding programme, and for the future identification of candidate genes controlling the process of budbreak.

UNIVERSITY of the
WESTERN CAPE

LIST OF ACRONYMS AND ABBREVIATIONS

ABI	Applied Biosystems
AFLP	Amplified Fragment Length Polymorphism
$AgNO_3$	Silver nitrate
An	Anna
APS	Ammonium PeroxidiSulphate
ARC	Agricultural Research Council
Au	Austin
BLAST	Basic Local Alignment Search Tool
bp	base pair
CIA	Chloroform Isoamyl Alcohol
cm	centimetre
cM	centiMorgan
CR	Chilling Requirement
CTAB	N-acetyl-N-N-N trimethyl ammonium bromide
CU	Chilling Units/ Cold Units
DArT	Diversity Array Technology
DFPT	Deciduous Fruit Producers Trust
dNTPs	DeoxyriboNucleic-5′-TriPhosphate
DNA	DeoxyriboNucleic Acid
DNOC	DiNitro Ortho Cresol mineral oil
°C	degrees Celcius

UNIVERSITY of the
WESTERN CAPE

EtOH	ethanol
EDTA	Ethylene Diamine Tetraacetic Acid (disodium salt)
EST	Expressed Sequence Tag
FAOSTAT	Food and Agricultural Organization Statistical Database (United Nations)
F_1	First filial generation
g	gram
GD	'Golden Delicious'
gDNA	genomic DNA
x g	centrifugal force
ha	hectare
IRB	time of Initial Vegetative Budbreak
IVB	time of Initial Reproductive Budbreak
kb	kilo basepairs
kV	kilo Volt
LG	Linkage Group
l	litre
LOD	Logarithm (base 10) of ODds
M	Molar
MAB	Marker Assisted Breeding
MAS	Marker Assisted Selection
Mbp	Mega basepairs/ Million basepairs
ml	milli litre

UNIVERSITY of the
WESTERN CAPE

min	minute
mM	milli Molar
MT	Metric Tons
$NaBH_4$	Sodium Borohydride
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nano gram
NH_4Ac	Ammonium Acetate
PAGE	PolyAcrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PDS	Prolonged Dormancy Symptoms
PIC	Polymorphism Information Content
PPECB	Perishable Product Export Control Board
QTL	Quantitative Trait Locus
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	RiboNucleic Acid
s	second
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA
TE	Tris EDTA
TEMED	N. N. N′. N′-Tetra Ethyl Methyl-Ethylene Diamine

UNIVERSITY of the
WESTERN CAPE

T_m	melting temperature
Tris (base)	Tris hydroxymethyl amino methane
μg	microgram
μl	microlitre
UV	Ultra Violet
V	Volts
v/v	volume per volume
w/v	weight per volume
x g	centrifugal force

UNIVERSITY of the
WESTERN CAPE

ACKNOWLEDGEMENTS

Firstly, I would like to express my most sincere gratitude to my supervisor Dr. Jasper Rees and co-supervisor Prof. Bongani Ndimba for their guidance and mentorship throughout the duration of this research. Special thanks also goes to Dr. Iwan Labuschagné and Mr. Trevor Koopman for the work they did at the ARC in generating and maintaining the plant material used in this study. I would also like to thank Dr. Daleen van Dyk and Dr. Khashief Soeker; many thanks are in order for your insight and help with linkage and QTL mapping. Members of the ARC Biotechnology Platform and the Department of Biotechnology at the University of the Western Cape deserve special mention for their camaraderie and support. Financial support for this work was supplied by THRIP and DFPT and was most appreciated. To my parents, family and friends, thank you for your continued great patience and encouragement thus far. Above all, I would like to thank God for affording me the life and good health to be able to apply myself in this academic endeavour.

UNIVERSITY of the
WESTERN CAPE

DECLARATION

I herewith declare that the work presented in this thesis is my own work and 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.

Paidashe Hove June 2012

Signed………………………

UNIVERSITY of the
WESTERN CAPE

LIST OF FIGURES

Figure 1: A diagrammatic representation of the signals and the typical seasons corresponding to the three different types of dormancy. **17**

Figure 2: The annual cycle of a *Populus* tree growing at Umea University, Umea Sweden.. **19**

Figure 3: A simplified view of the four pathways that control flowering time in Arabidopsis, showing the major genes involved. **30**

Figure 4: 'Anna' x 'Austin' adult tree IVB frequency distribution data for the years 1996 to 1998. **74**

Figure 5: 'Anna' x 'Austin' seedling IVB frequency distribution data for the years 1996 to 1998. **75**

Figure 6: A graphical representation of 'Anna' x 'Austin' adult tree year-to-year IVB data compared as a data trend, over the years 1996, 1997 and 1998. **76**

Figure 7: A graphical representation of 'Anna' x 'Austin' seedling year-to-year IVB data compared as a trend, over the years 1996, 1997 and 1998. **76**

Figure 8: A 1 % Agarose gel run of the 'Austin' x 'Anna' genomic DNA. **78**

Figure 9: The user interface of the Tandem Repeats Database **80**

Figure 10: A graphical display of a typical sequence shown in the tandem repeats database. **81**

Figure 11: A nucleotide level or fasta format view of the dinucleotide repeat sequence array. **82**

Figure 12: A 6 % silver stained polyacrylamide gel of PCR amplicons derived form parental cultivar DNA and marker CH04e03. **83**

UNIVERSITY of the
WESTERN CAPE

Figure 13: A 6 % silver stained PAGE of a four-primer PCR multiplex run for DNA from four apple cultivars.. 84

Figure 14: Electropherograms obtained after amplification of the 'Anna' parental DNA using megaplex 17 85

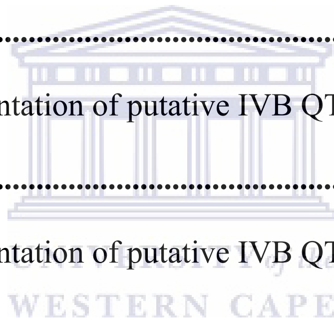
Figure 15: Electropherograms showing four PCR samples run on the ABI 3130*xl* Genetic Analyzer. 86

Figure 16: A snapshot of the raw DArT results before conversion to JoinMap® 4.1 codes. 87

Figure 17: An integrated genetic linkage map developed for a F_1 generation 'Anna' x 'Austin' mapping population 107

Figure 18: A graphical representation of putative IVB QTLs detected by rMQM analysis, for adult trees. 116

Figure 19: A graphical representation of putative IVB QTLs detected by rMQM analysis, for seedling trees. 118


WESTERN CAPE

LIST OF TABLES

Table 1: Global ranking of estimated apple production volumes by leading producer countries for 2009.. 9

Table 2: Costs of running a high throughput SSR MAS experiment in US$ for various combinations of sample size and number of markers analysed. 48

Table 3: Conversion codes for DArT markers to JoinMap codes according to the segregation ratios of the offspring. 68

Table 4: The JoinMap® 4.1 codes for segregation types observed when working with a full-sib family, derived from an outbreeding species. 70

Table 5: Pearson's correlation coefficients (R) showing phenotypic association (P<0.0001) between different years for IVB 77

Table 6: A list of the respective quantities of genomic DNA quantitated spectrophotometrically. 79

Table 7: Putative IVB QTLs identified by Interval mapping consistently from 1996 to 1998 for adult trees. 109

Table 8: Putative IVB QTLs identified by Interval mapping consistently from 1996 to 1998 for seedling apple trees. 111

Table 9: Putative IVB QTLs localized by rMQM mapping at consistently from 1996 to 1998 for adult apple trees of the 114

Table 10: Putative IVB QTLs found by rMQM mapping at consistently from 1996 to 1998 for seedling apple trees of the. 117

UNIVERSITY of the
WESTERN CAPE

Table of Contents

ABSTRACT i

LIST OF ACRONYMS AND ABBREVIATIONS iii

ACKNOWLEDGEMENTS vii

DECLARATION viii

LIST OF FIGURES ix

LIST OF TABLES xi

1.0 LITERATURE REVIEW 1

1.1 Introduction to the Apple 1

1.1.1 Classification and nomenclature 4

1.1.2 The apple genome sequencing initiative 6

1.1.3 Commercial Production 7

1.1.4 Breeding history 10

1.1.5 Current breeding objectives 12

1.1.6 Apple cultivars 14

1.2 Dormancy and other dormancy related traits 15

1.2.1 Defining dormancy 15

1.2.2 The seasonal plant cycle and dormancy 18

1.2.3 Chilling requirement and dormancy 20

1.2.4 Characteristics employed in dormancy studies 24

1.2.5 Flowering time and dormancy-related genes 28

1.3 Molecular markers and mapping polygenes 31

1.3.1 A brief introduction to markers 31

1.3.2 Markers systems available 33

1.4 Qualitative and Quantitative traits 36

1.5 Quantitative trait loci and their analysis 37

1.5.1 QTL analysis in apple 41

1.6 Linkage mapping 44

1.6.1 Genetic linkage maps in apple 45

1.7 Marker Assisted Selection (MAS) and Marker Assisted Breeding (MAB) 46

1.8 Aims of the study 51

2.0 MATERIALS AND METHODS 52

2.1 General chemicals 52

2.2 General stock solutions and buffers 54

2.3 Phenotypic trait assessment 56

2.4 Genomic DNA extraction 57

2.5 Agarose gel electrophoresis 59

2.6 Genomic DNA quantification 59

2.7 SSR primer design 60

2.8 Simplex PCR 63

2.9 Multiplex/Megaplex PCR 64

2.10 PAGE based PCR product detection 65

2.11 Capillary electrophoresis PCR product sizing 66

UNIVERSITY of the WESTERN CAPE

2.12 DArT marker analysis **67**
2.13 Linkage mapping **69**
2.14 QTL mapping **71**
2.14.1 Interval mapping 72
2.14.2 rMQM analysis 72

3.0 RESULTS **74**
3.1 Phenotypic trait assessment data **74**
3.1.1 Time of IVB frequency distributions 74
3.1.2 Time of IVB data trend graphs 75
3.2 Genomic DNA extraction **78**
3.2.1 Agarose gel electrophoresis 78
3.2.2 Spectrophotometric genomic DNA quantification 78
3.4 SSR Primer design **80**
3.5 Simplex PCR primer testing **83**
3.6 Multiplex and Megaplex PCR development **84**
3.6.1 Polyacrylamide gel electrophoresis based detection 84
3.6.2 Megaplex PCR 85
3.7 DArT markers **87**
3.8 Genetic linkage map construction **88**
3.9 QTL Mapping **108**
3.9.1 Interval mapping 108
3.9.2 Restricted MQM (rMQM) analysis 113
3.10 TECHNICAL DISCUSSION AND CONCLUSIONS **119**
3.10.2 Genomic DNA quantification 120
3.10.3 Simple Sequence Repeat Primer design and Simplex PCR testing 120
3.10.4 Megaplex PCR development and testing 121
3.10.5 Capillary electrophoresis and PAGE amplicon based detection 123
3.10.6 DArT marker analysis 125

4.0 DISCUSSION AND CONCLUSIONS **127**
4.1 Introduction **127**
4.2 Phenotypic trait assessment data **127**
4.2.1 Time of IVB frequency distributions 127
4.1.2 Time of IVB data trend graphs 129
4.2 Genetic linkage map construction **130**
4.3 QTL Mapping **135**
4.3.1 Interval mapping 137
4.3.2 rMQM analysis 139
4.9 CONCLUSIONS AND RECOMMENDATIONS **142**

5.0 REFERENCES **144**

APPENDIX 1 ..... **168**

UNIVERSITY of the
WESTERN CAPE

1.0 LITERATURE REVIEW

1.1 Introduction to the Apple

The domesticated or 'sweet' apple (Janick, 2005; Juniper and Mabberley, 2006) (*Malus x domestica* Borkh.) is a member of the Rosaceae or rose family, which is a vast and diverse assembly of deciduous and evergreen trees, shrubs and herbs, comprising from about 100 genera and more than 2 000 species (Arus *et al.,* 2006). It is the most important temperate fruit crop, grown for its large fruit, a consequence of many cycles of breeding. Other species of apple such as the crab apple are collectively grown for their ornamental value as they produce an attractive array of fruit, flowers and foliage. The apple is believed to be an interspecific hybrid (Janick *et al.,* 1996). Insect mediated cross-pollination, promoted by self-incompatibility in most apple plants and the conspicuous nature of apple flowers, results in fruit set. This pistil pollination leads to the formation of fruit typically containing 7 to 10 seeds (Ibanez and Dandekar, 2007). The fruit therefore develops into a fruit referred to as a pome, hence the placement of apples (and their close relative the pear) into the subfamily Pomoideae (Janick *et al.,* 1996; Forsline *et al.,* 2003) or Maloideae (Luby, 2003).

UNIVERSITY of the
WESTERN CAPE

Due to its vast genetic variability, the apple can be grown in a variety of environments, from the cold regions of the world, such as Siberia and northern China to the warmer and much higher altitude locations of the globe like Colombia and Indonesia (Janick *et al.,* 1996). Apple trees can be growing and fruiting found on all continents except Antarctica (Luby, 2003). The apple is known to have a complex origin (Luby, 2003), though most

experts agree that the apple originates in central Asia or areas around southern China, in particular, the Kazakhstan area (Almaty or Alma Ata also known as 'Father of Apples'), as this is where the greatest diversity of wild apples can be found today (Janick, 2005; Janick *et al.,* 1996; Juniper and Mabberley, 2006; Luby, 2003).

The family Rosaceae enjoys a global cultivation and distribution with members grown for their fruits, nuts, timber or ornamental value. This plant family is the third most economically important in the temperate regions (Dirlewanger *et al.,* 2002; Folta and Dhingra, 2006; Potter *et al.,* 2007) and includes popular fruit crops such as pears, peaches, plums, nectarines, cherries, apricots, strawberry and raspberry, to name a few. Members of the Rosaceae family are generally characterised by a frequently large and conspicuous insect-pollinated flower with radial symmetry, five sepals and petals together with numerous stamens, whose number varies within each respective subfamily, Furthermore, the number of carpels and the ovary position varies, giving rise to different fruit types, that is achenes, drupes, pomes or follicles. These are important features employed in placing members of the grouping into respective subfamilies (Arus *et al.,* 2006; Shulaev *et al.,* 2008). A majority of the species in the Rosaceae have a gametophytic incompatibility system that prevents self-pollination and makes the presence of two compatible genotypes, a pre-requisite for fertilisation and ultimate fruit production (Arus *et al.,* 2006).

The Rosaceae family has traditionally been classified using morphological characteristics into four subfamilies namely the: Spiraeoideae, Maloideae, Prunoideae, and Rosoideae

UNIVERSITY of the
WESTERN CAPE

with the major species cultivated being in the three latter subfamilies (Arus *et al.,* 2006). However, more recent phylogenetic analyses employing nuclear and chloroplast nucleotide sequence data in various combinations, coupled with parsimony and likelihood-based Bayesian approaches, classify the family into 3 subfamilies; the Rosoideae containing genera such as *Rubus* (raspberry) and *Rosa* (rose) and three tribes; the Dryadoideae consisting of actinorhizal genera; and the Spiraeoideae and seven tribes. This classification system recognises all genera previously assigned to the Amygdaloideae and Maloideae as members of the Spiraeoideae (Potter *et al.,* 2007). However, this thesis will employ the older nomenclature that recognises four subfamilies in the family Rosaceae. Accordingly, the latter classification system places several species in the Rosaceae family as close relatives of the apple, and these are the common fruit and ornamental genera namely, *Eryobotrya, Pyrus, Cydonia, Amelanchier, Aronia, Chaenomeles, Cotoneaster, Crategus, Pyracantha and Sorbus*. These genera all belong to the subfamily Maloideae (Gardiner *et al.,* 2007).

UNIVERSITY of the
WESTERN CAPE

For most, if not all commercially produced rosaceous fruit (and nuts), years of selection and breeding have lead to their significantly larger flesh, in comparison with their wild relatives, which are much more reduced in size (Shulaev *et al.,* 2008). Ornamentals have also been bred so that they exhibit characteristics favoured by breeders such as larger inflorescences and reduced plant size, Rosaceous fruits are therefore consumed in a variety of forms including fresh, dried, processed into juices, sauces, and various confectionaries. Fruit juices such as that of apple, may be consumed fresh, fermented into ciders, wines, brandy, or transformed into vinegar (Janick *et al.,* 1996). As such they

provide a healthy dietary source of for example, chemicals with known anticancer/ anti-oxidant activity such as anthocyanins, *L*-Ascorbic, gallic and ellagic acids, among a vast range of dietary phytochemicals (Shulaev *et al.,* 2008).

1.1.1 Classification and nomenclature

Consisting of approximately 1 000 species in 30 genera, this subfamily is characterised by a unique pome fruit (Arus *et al.,* 2006), hence the name Pomoideae sometimes afforded to this grouping (Janick *et al.,* 1996). Lespinasse *et al.* (1999) describe the subfamily Maloideae, which contains the relatively well-known and important fruit genera *Malus* (apple), *Pyrus* (pear), *Eryobotrya* (loquat) *and Cydonia* (quince), is an allopolyploid grouping believed to have evolved from a hybridisation between a Spiraeoidae (x = 9) and a Prunoidae (x = 8) ancestor, resulting in the basic x = 17 haploid number for the Pomoidae. The genus *Malus* is thought to consist of about 20 to 30 species, including the domesticated apple, whose widely accepted names are *Malus x domestica* Borkh. (Korban and Skirvin, 1994) and *Malus x domestica* or *Malus domestica* Borkh. (Phipps *et al.,* 1991). Other species included in the subfamily are the wild crab apple species *Malus sieversii,* and *M. orientalis, M. sylvestris* (the European crab apple), *M. baccata* (the Siberian crab apple), *M. mandshurica* (the Manchurian crab apple) and *M. prunifolia* (the larger Chinese crab apple) among others (Janick, 2005; Janick *et al.,* 1996).

Morgan *et al.* (2003), like other experts in the field (Arus *et al.,* 2006; Forsline *et al.,* 2003; Harris *et al.,* 2002; Ibanez and Dandekar, 2007; Kellerhals, 2009) regard the main

UNIVERSITY of the WESTERN CAPE

ancestor of apple (*M. x domestica*) to be a wild species *Malus sieversii* Lebed., which is native to central Asia. Even though in very recent times arguments that do not agree with the current name for the domesticated apple have still arisen (Juniper and Mabberley, 2006; Kartesz and Gandhi, 1992), the general consensus accepts the name *Malus domestica* (Borkh.) over *M. pumila* (Mill) (Gardiner *et al.,* 2007; Harris *et al.,* 2002; Ibanez and Dandekar, 2007; Korban and Skirvin, 1994; Luby, 2003). Despite the adoption of the name *M. domestica*, scholars still argue for and employ names such as *M. sylvestris* (Kartesz and Gandhi, 1992; Labuschagné *et al.,* 2002a, b) and *M. pumila* Mill. (Juniper and Mabberley, 2006; Mabberley *et al.,* 2001). It is also worth noting that to this day taxonomists have failed to reach an agreement as to the exact number of species in the genus *Malus,* thus the numbers ranging from 8 to 122 (Arus *et al.,* 2006; Harris *et al.,* 2002; Janick *et al.,* 1996; Robinson *et al.,* 2001). Therefore, taxonomic hierarchy of an apple cultivar named 'Bramley's Seedling', in accordance with the classification employed by Harris *et al.* (2002) would be:

UNIVERSITY of the
WESTERN CAPE

Family - Rosaceae

Subfamily - Maloideae

Genus - *Malus*

Section - *Malus*

Series - *Malus*

Species - *domestica*

Variety/ Cultivar - 'Bramley's Seedling'

1.1.2 The apple genome sequencing initiative

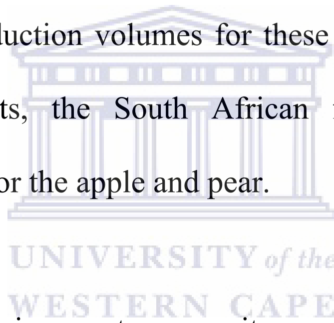
In a massive international effort centred at IASMA Italy, Velasco *et al.* (2010) produced an estimated 742.3 Mega base pair, high quality, first draft of the apple genome sequence, For this purpose, the diploid 'Golden Delicious' cultivar was sequenced at a 16.9-fold genome coverage, using Sanger and Roche 454 sequencing by synthesis of paired and unpaired reads. This produced a total of 122 146 contigs, 103 076 of which, were assembled into 1 629 metacontigs and grouped into 17 chromosomes, after anchoring onto a high-density integrated genetic map with 1 643 markers.

The paper highlighted the high level of genome-wide duplication and co-linearity (homology) after a pair-wise comparison of the 17 chromosomes, that is large segment similarity between 3 and 11, 5 and 10, 9 and 17, and 13 with 16. To a smaller degree co-linearity was shown between 1 and 17, 2 and 7, 2 and 15, 4 and 12, 12 and 14, 6 and 14, and 8 and 15. Velasco *et al.* (2010) also point out the evolutionary significance of this genome-wide duplication that occurred about 50 million years ago, in support of the theory of monophyletic origin of the Pyreae, from the nine chromosome ancestor to the seventeen chromosome extant species. In an effort to clarify the origins of apple, Velasco *et al.* (2010) posit that *Malus sieversii* is the progenitor of modern apples, a view shared by numerous experts in the field as highlighted earlier in this review. The generation of a high quality draft of the apple genome sequence has opened doors for advanced *in silico* work that will enable among many useful analyses; gene and marker prediction at loci of interest, the elucidation of complex gene networks and experiments that clarify interspecific synteny and positional cloning of genes.

UNIVERSITY of the
WESTERN CAPE

1.1.3 Commercial Production

Approximately 64.3 million metric tons (MT) of apple fruit are produced globally, along with 20 million MT of pears. Apples and pears are by far the two most common fruits in the Maloideae, if not the whole of the rosaceous family. These two fruit outputs represent 12.9 % and 4 % of total world fruit production, respectively. In addition, relative to production levels in the recent past, worldwide apple production levels have witnessed an increase of about 10 % in the last 4 to 5 years. South Africa produced 650 000 and 325 000 MT of apples and pears, respectively in 2006 (FAOSTAT data 2007; Janick, 2005; Juniper and Mabberley, 2006). These figures correspondingly represent almost 35 % and 53 % of the total African production volumes for these fruits. However, relative to the global output for these fruits, the South African figures significantly lower to approximately 1 % and 1.6 % for the apple and pear.


UNIVERSITY of the
WESTERN CAPE

Global production of apples has in recent years, witnessed significant increases, primarily due to the introduction of new cultivars/ breeds into southern hemisphere countries. These countries capitalised on the contrast between theirs and their northern hemisphere apple growing counterpart's growing seasons. This has resulted in the development of apple industries that have begun to contribute extensively to global output (Morgan *et al.,* 2003). In 2007 about 64.3 million metric tonnes (MT) of apples were produced worldwide on trees in an area of 4.9 million hectares (ha). This therefore, placed apples in third place in terms of relative production, after bananas (81.3 million MT) and grapes (66.3 million MT) (FAOSTAT data for 2007; (Janick, 2005; Juniper and Mabberley, 2006)). Apple production in the world has recently, marginally surpassed that of oranges

(63.9 million MT), which were third in global production terms in the year 2004 (Gardiner *et al.,* 2007). China, whose annual output of apples was 31.7 million MT, is the leading producer of apples and produces about a third of the world's apples. The USA (4.4 million MT), Turkey 2.8 (million MT) and Iran (2.6 million MT) are far behind in second, third and fourth place respectively (**Table 1:** FAOSTAT data 2009. http://faostat.fao.org). Being the leading global producer, China is the major supplier of apples to Asia and Russia (and to a much lesser extent, to other global regions) with figures of 68.2 % and 54.1 % of all apple supplies in south-east and south Asia respectively, coming from there (Kellerhals, 2009).

UNIVERSITY of the
WESTERN CAPE

As noted by Gardiner *et al.* (2007), the largest production for apples is for the well-developed local markets and to a far less extent, for export. Consequently, only about 10 % of the world apple crop is for export. Luby (2003) however, states that most southern hemisphere apple producing countries, do so for the primary purpose of export to northern hemisphere countries during their summer and spring.

In terms of its total commercial production, South Africa produces six main cultivars, which are 'Granny Smith' (25 %), 'Golden Delicious' (22 %), 'Royal Gala' (12 %), 'Pink Lady' (7 %), 'Starking' (6 %) and 'Topred' (6 %). Recent production figures, on an approximately 22 000 hectare area, have been estimated at between 815 000 MT (**Table 1**) and 822 000 MT in the 2005 - 2006 season (Deciduous Fruit Producers' Trust (DFPT): http://www.dfpt.co.za). South Africa exports about 40 - 45 % of its apples mainly to the

UK, which constitutes 44 % of South Africa's exports (Perishable Product Export Control Board (PPECB): http://www.ppecb.com).

Table 1: Global ranking of estimated apple production volumes by leading producer countries for 2009 (metric tonnes) and total value of production (x $1 000). All values have been rounded off to 3 significant figures.

Ranking	Country	Production (MT)	Production (x $1 000)
1	China	31 700 000	13 400 000
2	USA	4 400 000	1 910 000
3	Turkey	2 780 000	1 180 000
4	Poland	2 630 000	1 110 000
5	Iran	2 000 000	1 030 000
6	Italy	2 330 000	978 000
7	France	1 730 000	826 000
8	India	1 800 000	759 000
9	Russian Federation	1 440 000	675 000
10	Brazil	1 220 000	517 000
11	Chile	1 090 000	461 000
12	Germany	1 070 000	453 000
13	Argentina	1 030 000	434 000
14	Japan	846 000	358 000
15	Ukraine	853 000	343 000
16	North Korea	720 000	304 000
17	South Africa	816 000	297 000
18	Uzbekistan	635 000	258 000
19	Spain	595 000	247 000
20	Hungary	575 000	243 000

Source: FAOSTAT data for 2009: (http://faostat.fao.org).

UNIVERSITY of the
WESTERN CAPE

1.1.4 Breeding history

Apples were at the beginning of the apple breeding practice, improved by picking seedlings with the most favourable phenotype from open pollinated seeds (Gardiner *et al.,* 2007). This was the breeding practice employed from at least as far back as 2 000 years ago when the Greeks and Romans travelled and conquered extensively. They are credited for the spread of the apple to Europe and Asia (Ibanez and Dandekar, 2007; Janick *et al.,* 1996; Luby, 2003). Even though archaeological evidence of apple remains dated back to 6 500 BC were found in Anatolia (the area around modern day Turkey), these finds do not give concrete evidence pointing to cultivation of the apple at this time period. However, evidence suggests that cultivation of the apple was probably started at around the second millennium BC in northern Mesopotamia (the area corresponding to a greater part of modern day Iraq) and Anatolia (Luby, 2003). The dominant breeding practice, which entailed selecting superior naturally pollinated phenotypes for breeding, was however replaced about 200 years ago by controlled cross-pollination. Of mention in this regard is the first recognised apple breeder, Thomas A. Knight (1759 - 1838), in the early 19th century, who bred the first cultivars with known parentage (Gardiner *et al.,* 2007; Janick *et al.,* 1996; Luby, 2003). Janick *et al.* (1996) state that despite the adoption by virtually all apple breeders today, of the breeding methods developed by Knight, the process until recently, has gained the notoriety of being unsuccessful with apples compared with other fruit. This is chiefly attributed to poor selection of parents.

Luby (2003) attributes the spread of the apple to the Americas by European colony settlers in the 16th and the 17th centuries, who supposedly set up orchards in eastern North

America. Alternative hypotheses, which attempt to explain the westward passage of apples from Central Asia to areas afar off as North America, have been given by Janick (2005). The first, postulates the introduction of apple seed carried via the saddlebags of caravans along trade routes, with the seed germinating in horse droppings. Secondly, the propagation of root suckers is also a possibility. This plausible explanation can be tied to the fact that Harris *et al.* (2002) state in their paper, that grafting technology can be dated as far back as 3 800 years ago. Persia served as an intermediary stop for apples via Greece and the Roman empire, which is thought to have not only naturalised the apple across Europe (in what was part of the vast expanse of the Roman empire), but also to have perfected orchard economies (Harris *et al.,* 2002; Janick, 2005). Between the 18th and 19th century fruit growers introduced apples to the United States of America via importation of seed from European cider mills. This (probably coupled with intensive apple breeding programs) resulted in this area being a secondary source of apple diversity, as evidenced by the fact that most new grown cultivars worldwide are of American and Canadian origin (Janick, 2005).

UNIVERSITY of the
WESTERN CAPE

Spanish and Portuguese priests and settlers, who grew apples at their missions and settlements respectively, have been credited with introducing apples to the suitable temperate zones of the Americas in locations such as Chile and California. The first known apple orchards near Cape town, South Africa were established to supply settlers and the Dutch East India company ships, With the aim of replacing a failing wine industry, commercial production of apples in the Western cape region was only commenced by Cecil John Rhodes and associates, between the late 19th and early 20th

century. Australian apple introductions began in Tasmania and Sydney in 1788. Orchards were then established by settlers in Tasmania and New South Wales in the early 1800s. New Zealand apples introduced by English missionaries in 1814 from Australia were developed into two significant growing regions by the penultimate years of the 19th and early 20th centuries (Luby, 2003).

1.1.5 Current breeding objectives

Today, apples are usually grown from a tree, which is constituted of a rootstock and fruiting scion. The fruit tree may though uncommonly, be comprised of a tree of three distinct sections, that is, a genetically distinct trunk or interstem in addition to the rootstock and fruiting scion. The modern breeding strategy is focused on genetic improvement of existing cultivar rootstocks and scions so as to increase fruit and tree (in the case of ornamentals) marketability and to introduce traits that reduce production costs (Janick *et al.,* 1996).

UNIVERSITY of the
WESTERN CAPE

Janick *et al.* (1996) also highlight the fact that the apple industry mainly aims to breed apples of quality that satisfies the customer, which are those apples that are marketable according to the specifications of the customer. As a result, the apple industry strives to produce apples that can be stored for prolonged time periods and still be marketed well; and with favourable appearance (skin colour, pattern and overall surface covered with colour combined with size and shape of the fruit) and particular eating quality characteristics (flesh texture and flavour). Due to the fact that rosaceous crop breeding and ultimately production are driven by quality and not yield, a vast amount of effort has

been directed towards breeding and producing profitable, quality produce (Peace and Norelli, 2009). Additionally, the US White Paper on Rosaceae Genomics, Genetics and Breeding Initiatives (2006) outlines three fundamental recurring themes of highest priority that form the foundation of the needs and aims of the US industry. Other Rosaceae breeding groups have taken up essentially similar themes in their programmes. These are: i. Improved quality - this includes production of new cultivars for better customer satisfaction. ii. Reduced chemical usage and better stress tolerance for environmental sustainability and. iii. Lowered labour, energy and crop production crops.

A review by Laurens (1999) gives a summary of the breeding objectives shared by modern breeders. The first is to combine in new cultivars, high quality fruit with resistance from the major fungal diseases apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotrica*); and the bacterial disease fire blight caused by *Erwinia amylovora*. Another important aim is the addition of tree habits that allow for high productivity and regular fruit bearing. Adaptation to climatic conditions (or adaptedness) is a measure of how plants can survive and reproduce in specific environments according to (Hill *et al.,* 1998). It is a complex interaction between various environmental factors and the plant. This characteristic was found to be a major objective only for countries in marginal areas (Laurens, 1999); Labuschagné *et al.* (2002a, b) and (van Dyk *et al.,* 2010) pointed out that breeding for traits related to adaptedness would be important in the South African context, so as to maintain and expand growing areas. Labuschagné *et al.* (2002b) also adds weight to this view by saying that fruit tree breeders should be paying more attention to economically important adaptedness-related traits as these can improve

UNIVERSITY of the
WESTERN CAPE

cultivar yields in their climatic regions. Janick *et al.* (1996) and Laurens (1999) also stated that storage ability and harvesting are also important breeding objectives. Despite the importance of durable pest resistance as a breeding objective in breeding programmes, fruit quality traits modern programmes are driven by customer quality requirements (Janick *et al.,* 1996; Kellerhals, 2009; Laurens, 1999). However, despite the numerous successes of modern breeding, conventional apple breeding cycles are normally as long as 20 years mainly due to the long juvenile phase of 3 to 10 years for the apple (Janick *et al.,* 1996). This represents a significant problem, to which molecular markers (in particular, DNA markers) are one solution (Agarwal *et al.,* 2008; Janick *et al.,* 1996).

UNIVERSITY of the
WESTERN CAPE

1.1.6 Apple cultivars

Luby (2003) states that a few strains of apples dominate world production. Globally, there have been several cultivars developed to suit the country-specific growing conditions and requirements of the local populations in host countries. As a result, popular cultivars discovered to be of commercial importance, among the several thousand recorded in history include: 'McIntosh' (1796, USA). 'Jonathan' (1826, USA), 'Rome Beauty' (1848, USA), 'Cox Orange' (1820, UK), 'Granny Smith' (1868, Australia), 'Red Delicious' (1880, USA), and 'Golden Delicious' (1890, USA). 'Golden Delicious' and 'Rome Beauty' remain among the important apple cultivars in the world (Kellerhals, 2009). Other varieties including the newer ones enjoying widespread commercial success as a result of breeding programmes are those such as 'Elstar' (Netherlands). 'Fuji'

(Japan); 'Braeburn' and 'Gala' (New Zealand); and 'Delicious' and 'Jonagold' (North America) (Laurens, 1999; Luby, 2003).

1.2 Dormancy and other dormancy related traits

1.2.1 Defining dormancy

Dormancy enjoys a multiplicity of definitions that have been offered and reviewed in several research articles over time; all carried out in an attempt to describe this complex phenomenon and closely related processes. It has therefore been the subject of study in a myriad of studies in *Malus* spp. (Cook and Bellstedt, 2001; Cook *et al.,* 2005; Cook *et al.,* 2001; Hauagge and Cummins, 1991a, b; Jackson and Bepete, 1995; Labuschagné *et al.,* 2002a, 2002b; Mexal *et al.,* 2009; van Dyk *et al.,* 2010), *Prunus* spp, (Campoy *et al.,* 2010; Dirlewanger, 2010; Fan *et al.,* 2010; Gariglio *et al.,* 2006; Gratacos and Cortes, 2009), raspberry, grape, mango, poplar, and spruce among several other important plant species (Arora *et al.,* 2003; Gao *et al.,* 2003; Jansson and Douglas, 2007; Mexal *et al.,* 2009). The complex nature of dormancy has led to various, vague and poorly defined terms that describe dormancy and its release (Labuschagné *et al.,* 2002a). It is known to affect a diversity of plant organs such as buds, seeds, and bulbs (Arora *et al.,* 2003).

UNIVERSITY of the
WESTERN CAPE

Lang *et al.* (1987) define dormancy as the temporary suspension of visible growth of any plant structure containing a meristem, though Okubo (2000) highlights the shortfall in this definition, as it does not refer to the commencement of temporary suspensions of growth. Several scholars in recent times however (Anderson *et al.,* 2005; Arora *et al.,*

2003; Horvath *et al.,* 2003; Labuschagné *et al.,* 2002b), still recognise the validity, completely or in part, of this definition, and division of dormancy into three sub-categories (**Fig. 1**) by Lang *et al.* (1987), with some (review by Arora *et al.,* 2003) referring to its being more physiologically descriptive nature as its greatest strength.

Okubo (2000) in his review of the subject, and in an attempt to more clearly redefine dormancy, mentions how a definition of dormancy should be clear and simple, covering all aspects of the process in almost all species of flowering plants. Okubo (2000) therefore defines dormancy as the imposed regulation on the progressing growth processes at various stages, which may or may not include morphological modification. Another important definition of a virtually inseparable feature given by Okubo (2000) is that of the induction of dormancy, which is 'the change of the primordia that cease growing for a while or that initiate special organs instead of producing shoots'.

UNIVERSITY of the
WESTERN CAPE

Cesaraccio *et al.* (2004) also offer an alternative view to dormancy and in their attempt to define it, separate dormancy two major phases: (i) a rest period: buds remain dormant due to growth arresting physiological conditions and (ii) a quiescent period where buds remain dormant due to unfavourable environmental conditions. Even more recently, Rohde and Bhalerao (2007) state two important shortfalls of the definition by Lang *et al.* (1987). First, that the meristematic growth is not readily visible due to its hidden nature within organs or highly reduced nature. Secondly and more importantly, the absence of growth being an ambiguous term as dormancy constitutes an inability to resume growth.

The authors also point out that growth consists of both cell division and elongation, which may occur as separate events it time and space.

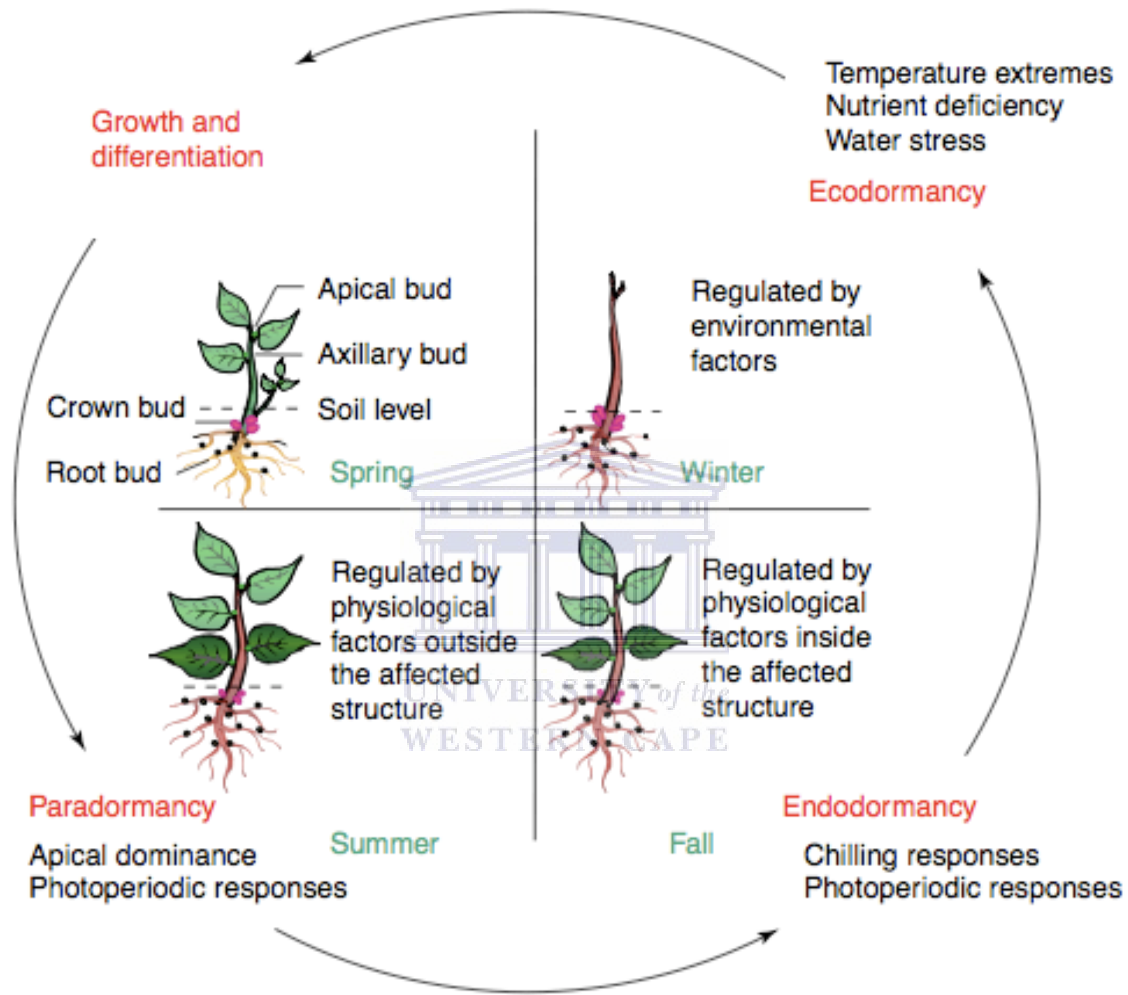


Figure 1: A diagrammatic representation of the signals and the typical seasons corresponding to the three different types of dormancy in perennial weeds, woody plants (shrubs and trees) and shoot buds of tubers (potato and yam).
Adapted from Horvath *et al.* (2003).

Rohde and Bhalerao (2007) therefore, define dormancy with the goal of unravelling the molecular components that govern the transition into and out of dormancy, in particular

at cellular level. Consequently, their dormancy definition is as follows: 'the inability to initiate growth from meristems (and other organs and cells with capacity to resume growth) under favourable conditions'. This review will however, adopt the definition of Lang *et al.* (1987), as it is ubiquitously employed by most modern researchers, who use its subcomponents to aid in defining specific objectives in their work.

1.2.2 The seasonal plant cycle and dormancy

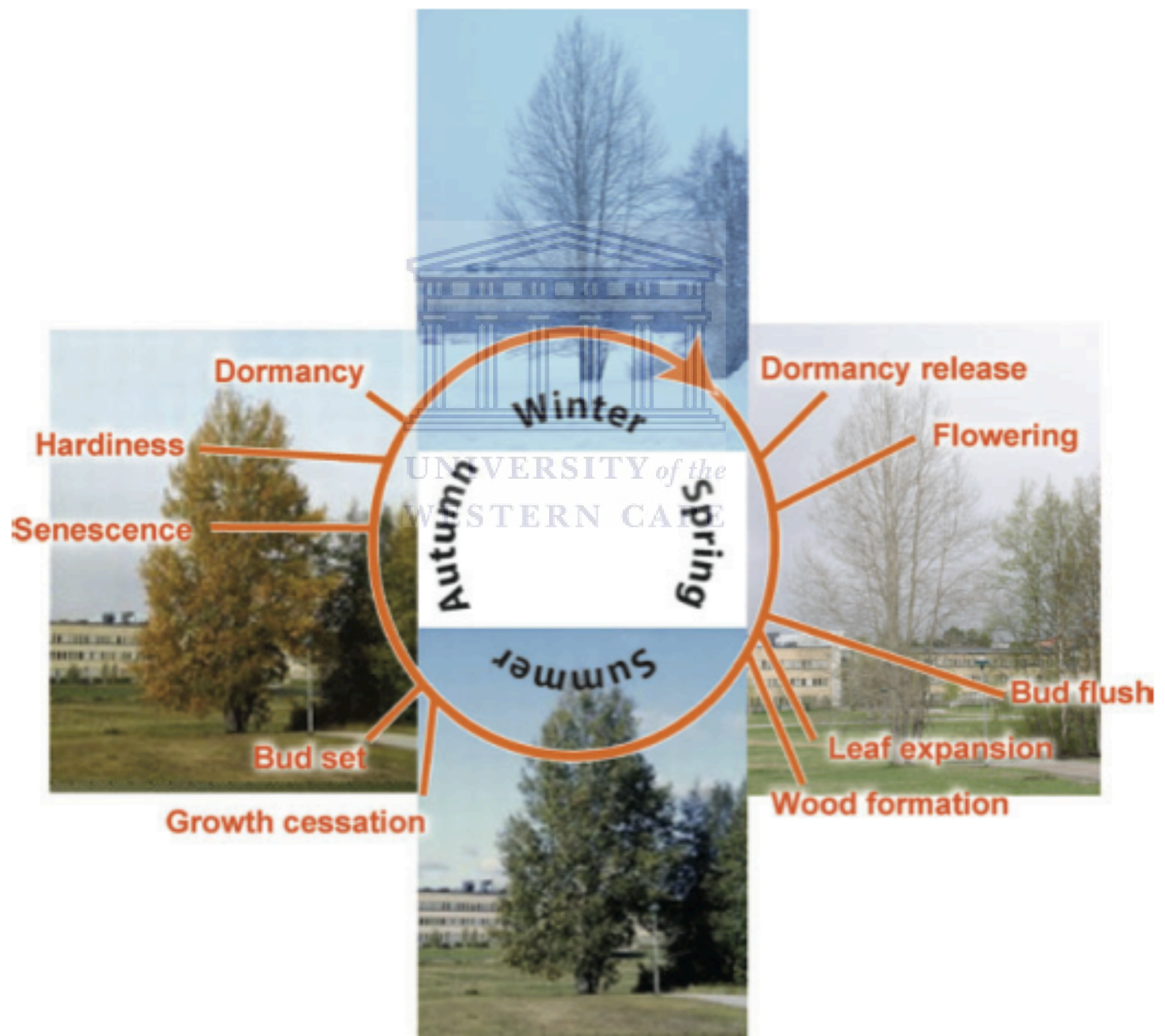
Adaptation to harsh environments such as very cold winters is an important trait and in preparation for winter, woody perennial trees cease growth and set dormant buds (Jansson and Douglas, 2007). Furthermore, in winter, orchard species as do forest species, exhibit reduced activity from the season's end until budburst in the season that follows (Arora *et al.,* 2003; Borchert *et al.,* 2004; Bradshaw and Stettler, 1995; Cesaraccio *et al.,* 2004; Jansson and Douglas, 2007). These characteristics and others like dormancy in its entirety, represent a section in the annual cycle of a typical woody perennial growing in temperate climatic regions, as can be seen in that of model tree species, poplar (*Populus trichocarpa*) (**Fig. 2**).

UNIVERSITY of the
WESTERN CAPE

Two important cues that induce the change from the paradormant (summer dormancy) to the endodormant (winter dormancy or rest) states in buds are shorter photoperiods (detected by photoreceptors and phytochromes in the plant) and progressive lowering of temperatures from warm to cold (Arora *et al.,* 2003; Jansson and Douglas, 2007).

Arora *et al.* (2003) point out that gain and loss of cold hardiness, the onset and release of

bud dormancy are separate processes though they are superimposed. Additionally, once in the winter months, plant buds are fully endodormant and then ecodormant (imposed dormancy quiescence), while all plant tissues achieve maximum hardiness. When winter ends and the warmer temperatures return in spring, there is dormancy release and fully dehardened plant tissues (Arora *et al.,* 2003).



UNIVERSITY of the
WESTERN CAPE

Figure 2: The annual cycle of a *Populus* tree growing at Umea University, Umea Sweden.

Adapted from Jansson and Douglas (2007).

Dehardened buds therefore experience increased apical meristematic tissue activity, naotably increased cell division, bud development and growth. These events are regulated by the restored cell-to-cell signalling networks via the plasmodesmata, which is thought to facilitate symplastic movement of proteins, hormones and essential signalling molecules (Arora *et al.,* 2003). Okubo (2000) also states that budbreak leads to growth of lateral primordia (branches) or buds with consequent flowering, leafing and fruiting through vegetative growth. If budset and cold acclimation do not occur before the first frost of autumn, the tree will be damaged (Chen *et al.,* 2000).

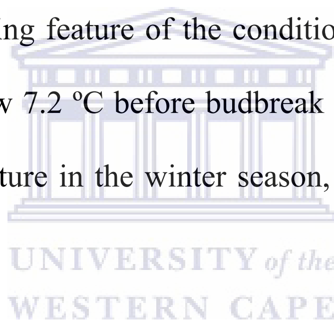
1.2.3 Chilling requirement and dormancy

Most temperate orchard crops and deciduous forest species, including the apple, must undergo a mandatory period of chilling to break endodormancy before any active shoots can grow from set buds in winter to spring transition, a phenomenon known as the chilling requirement (CR) (Sorenson, 1983; Martinez *et al.,* 1999; Howe *et al.,* 2000). Hauagge and Cummins (1991a) have found that a wide variation in CR exists between cultivated, wild and hybrid species, and this CR has been determined by the practical success of cultivars in different environments (Jackson and Bepete, 1995).

UNIVERSITY of the
WESTERN CAPE

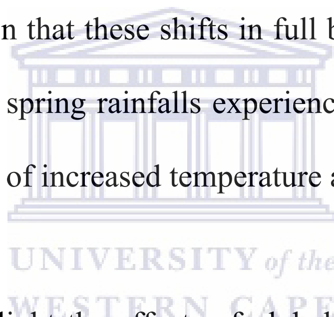
The CR has been found to be a major determinant of time of budbreak (Labuschagné *et al.,* 2002a) and when it is not met (at temperatures in the range of between 4 and 9 °C during the rest period), the consequence is an abnormal growth characteristic phenomenon known as prolonged dormancy syndrome. It is also referred to as delayed foliation or extended rest. It is also caused by a lack of favourable temperatures, which

are observable as in the growing regions, as significant chilling unit fluctuations, during the period of normal budbreak (Hauagge and Cummins, 1991a; Labuschagné *et al.,* 2002a). Martinez *et al.* (1999) notably point out the fact that prolonged dormancy reflects poor adaptation to mild winter climates. Prolonged winter symptoms were observed in conditions with a mild winter that failed to meet the CR in apple (Janick *et al.,* 1996). The symptoms of this condition, also observed by Labuschagné *et al.* (2002a) in orchard systems in the Western Cape region of South Africa, include significantly reduced vegetative and reproductive budbreak, prolonged flowering duration, lowered fruit set and size. The absence or a lengthy delay of the onset of budbreak in lateral vegetative structures is the most outstanding feature of the condition. Linsley-Noakes *et al.* (1994) uses the number of hours below 7.2 ºC before budbreak occurs, as a measure of CR and because of their cumulative nature in the winter season, expresses them as chill or cold unit (CU) accumulation.


UNIVERSITY of the
WESTERN CAPE

In the South African context Labuschagné *et al.* (2002a) highlight how apple production suffers from chilling unit fluctuations during and between winters in production areas. This undesirable temperature dynamic results in lowered fruit set and quality, which ultimately lowers saleable fruit output. Also, according to the work carried out in Germany (Chmielewski *et al.,* 2004) and the United States of America (Baldocchi and Wong, 2008) among many other examples, there has been a notable decrease of chilling hours worldwide via global warming, which are decreasing the suitability of fruit and nut growing areas. Though no major decreases in crop yield have been noted, they are predicted to be coming soon.

Recent evidence shows the effect of increased global temperatures on flowering times in three apple (*Malus domestica*) cultivars Golden Delicious, Sayaka and Granny Smith; and one pear (*Pyrus communis*) cultivar Bon Chrétien by Grab and Craparo (2011) in the Elgin-Villiersdorp-Vyeboom region of South Africa's south-western Cape. This group has shown significant early Spring (August/September) temperatures increases from 1973 to 2009 by +0.45 ºC/decade associated with a mean full bloom date increase of 1.6 days/decade. The Golden delicious cultivar was found to be most affected by this temperature change, having a +4.2 days/ºC alteration to its full blooming dates, whereas the Granny Smith trees had the least affected with a sensitivity of 2.4 days/ºC. Grab and Craparo (2011) however caution that these shifts in full bloom dates are also affected by the decreased winter and early spring rainfalls experienced at their study sites, and they hypothesize a synergistic effect of increased temperature and decreased rainfall.


UNIVERSITY of the
WESTERN CAPE

Legave *et al.* (2012) also highlight the effects of global warming on flowering dates at three French sites where Golden delicious apple trees were grown; by modelling their flowering time dates from 1976 to 2002. This study showed that the flowering time for the F1 stage of the Golden delicious apple trees had advanced by 7 to 8 days since the late 1980s. Furthermore, a 3 to 5 day increase in the duration of the chilling effect and a 10 to 13 day decrease in the duration of the heating effect in the same time period were also shown in this study.

Work by Jimenez *et al.* (2010) on a peach (*Prunus persica* (L.) Batsch] *evergrowing* (*evg*) mutant, which cannot enter dormancy under short days, gives insight into the

genetics of bud dormancy control and growth cessation in tree species. Specifically, 23 genes up-regulated in the wild type relative to the mutant were identified under short day conditions. Three general expression patterns were shown in this paper, namely: a group of genes that decreased at the time of growth cessation; another that increased immediately after the short day exposure and then remained steady, and another that increased throughout exposure to short day conditions.

Even though there may be continuing debate on the actual evidence and effects of global warming. Labuschagné *et al.* (2002a) and van Dyk *et al.* (2010) acknowledge the potentially negative influence of global warming in apple production through CU reduction, and therefore emphasize the need for the development of newer, better adapted low-chill cultivars in South Africa. Also in the southern African setting, the lack of adequate CU accumulation has led to apple producers in growing regions in South Africa. Elgin and Bokkeveld (Labuschagné *et al.,* 2002a) and Zimbabwe, Eastern highlands (Jackson and Bepete, 1995) utilizing, on a commercial scale, Dinitro-ortho-cresol oil (DNOC) and hydrogen cyanamide respectively, to induce uniform budbreak. These are among other chemicals used to bring about uniform budbreak in orchards such as azides, cyanides, mineral oil and thidiazuron (Arora *et al.,* 2003). All these chemicals have however induced heated debates on issues like the proper application time, efficacy and phytotoxicity (Arora *et al.,* 2003) combined with other environmental and health concerns (Labuschagné *et al.,* 2002a). These need to be discontinued because of these numerous setbacks.

UNIVERSITY of the
WESTERN CAPE

The best alternative according to Labuschagné *et al.* (2002a) and van Dyk *et al.* (2010) is the rapid development of new low-chill cultivars, which may decrease or otherwise eliminate the need for such chemical treatments in orchards. In a possible response to the need to develop low chill cultivars in the apple industry, Hauagge (2010) has reported two low chill cultivars developed at the Instituto Agronômico Paraná (IAPAR)'s apple breeding programme in Curitiba, Brazil. Unlike the important commercial cultivars, which need up to 1 000 CU to break bud dormancy, the two cultivars 'IPR Julieta' and 'IAPAR 75 - Eva' were developed at IAPAR and require between 100 and 500 CU to achieve budbreak. These two cultivars like the 'Anna' x 'Austin' cross used in this study, share the low chill 'Anna' male parent developed by Abba Stein in Israel in the 1950s (Hauagge, 2010) .

1.2.4 Characteristics employed in dormancy studies

Several characteristics have been used in studies of dormancy or dormancy release by researchers (Bradshaw and Stettler, 1995; Chen *et al.,* 2000; Labuschagné *et al.,* 2002a, 2002b). Time of budbreak (Initial Vegetative Budbreak (IVB) and Initial Reproductive Budbreak (IRB)), also described as budflush or budburst (Bradshaw and Stettler, 1995; Chen *et al.,* 2000; Labuschagné *et al.,* 2002b; van Dyk *et al.,* 2010), is measured from a reference date (Bradshaw and Stettler, 1995) and occurs after fulfilment of the CR. It marks the initiation of shoot elongation as an indicator of dormancy release and CR fulfilment. As is well known in plant genetics studies, environmental and genetic effects collectively influence variation in plants. Plant geneticists therefore, attempt to show just how much of the variation is due to genetic effects alone. This is normally done by using

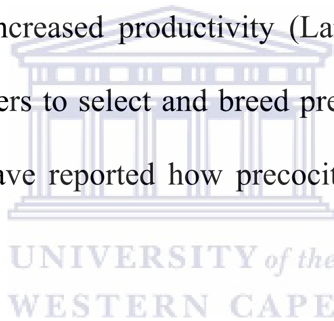
at least three clonal replicates of a mapping population over a number of growing seasons or years, which also shows consistent heritability of a trait by correctly apportioning the contribution to total observed variance of the environment on the trait under investigation. Studies by Labuschagné *et al.* (2002b) and van Dyk *et al.* (2010) also verified the quantitative nature of the IVB trait, carried the year-to-year variation and clonal similarity analysis as measures of usefulness of the mapping population. Bradshaw and Stettler (1995) and Chen *et al.* (2000) reported the usefulness of such an approach in their work on mapping Quantitative Trait Loci (QTLs) that influence growth, form and phenology traits in *Populus*. They reported figures of 98 % and 94 % for estimates of heritability for the QTLs affecting time of spring budflush. This means that 98 % and 94 % of the total observed phenotypic variation they found in *Populus* could be attributed to genetic factors and only 2 to 6 % to environmental factors. Chen *et al.* (2000) also gave an estimate of heritability for bud set at 91 % in *Populus* spp. This points to the fact that spring budflush and budset have very high heritability, making them and closely related characteristics excellent traits for genetic study. This high level of heritability is consistent with that shown by Labuschagné *et al.* (2002b) for IVB and IRB in apple of 69 % and 75 % respectively.

UNIVERSITY of the
WESTERN CAPE

Timing of bud set (at the end of the growing season) is phenotypically easy to score, is under strong genetic influence, though it does not seem to be influenced by chilling and has been used to assess dormancy (Jansson and Douglas, 2007). Both timing of bud set and budbreak are tied to climatic cycles (Howe *et al.,* 2000). Number, distribution and rating stages of budbreak have also been applied in dormancy research in various species

(Labuschagné *et al.,* 2002a).

Precocity, the earliness to flowering or fruiting in plants, or the reduced time from planting to cropping, has been studied extensively in genetics and physiology terms, in many plant species (Atkinson and Else, 2001; Hanke *et al.,* 2007). These include, but are not limited to, work reported on almond (Socias, 1998), apple (Lauri *et al.,* 2006; Hanke *et al.,* 2007), *Arabidopsis* (Roux *et al.,* 2006; Hanke *et al.,* 2007), cherry (Lang, 2000; Lang 2001), *Eucalyptus* (Chambers *et al.,* 1997; Dutkowski and Potts, 1999), loblolly pine (Schmidtling, 1981), and tobacco (Mauro *et al.,* 1996). It is generally agreed on that increased precocity leads to increased productivity (Lang, 2000; Hanke *et al.,* 2007), hence the drive by plant breeders to select and breed precocious crop cultivars. Lauri *et al.* (2006) and Lang (2000) have reported how precocity is strongly affected by scion (cultivar) choice.


UNIVERSITY of the
WESTERN CAPE

Even though Lang (2000) highlights how choice dwarfing rootstock positively influences precocity. Lauri *et al.* (2006) points out that the effect of dwarfing rootstocks in promoting precocity in fruit crops is still quite controversial. Precocity and its closely associated features such as length of juvenility (years from planting to first flowering) and flowering or fruiting time, have been found to have high broad sense heritability estimates in several important tree species such as almond (Chandrababu and Sharma, 1999), *Eucalyptus* (Chambers *et al.,* 1997; Dutkowski and Potts, 1999) loblolly pine (Schmidtling, 1981), *Prunus* spp. (apricot: Campoy *et al.,* 2010b; peach, apricot and sweet cherry: Dirlewanger, 2010), and more importantly apple (Oraguzie *et al.,* 2001;

Celton *et al.* 2011; Kumar *et al.,* 2010). The aforementioned work carried out on apple and other tree species collectively give a good basis for this genetic analysis of precocity/ early flowering in apple.

Several workers in the Rosaceae research community have focused on important phenological traits such as flowering time, IVB and IRB with outputs being linkage maps, QTL and candidate gene localization for these fruit crops (apple: Celton *et al.,* 2010; van Dyk *et al.,* 2010; Celton *et al.,* 2011); (apricot, peach and sweet cherry: Dirlewanger, 2010; Campoy *et al.,* 2010a, b; Fan *et al.,* 2010). Celton *et al.* (2011) mapped three important QTLs for the dormancy related phenological traits vegetative budbreak (VB), floral budbreak (FB) and green point (GP), where GP describes the time at which 50% of either floral or vegetative buds begin to show any green foliage.

UNIVERSITY of the
WESTERN CAPE

Recent work by Campoy *et al.* (2010b) reveal one major QTLs for flowering time on Linkage group (LG) 5 of apricot cultivar 'Z506-07', and a tight linkage between two SSR markers UDAp-423r and AMPA-105 and this trait. Still in *Prunus* spp., Fan *et al.* (2010) show one major QTL for floral bud chilling requirement and two major QTLs for bloom date for a F_2 peach population, among 18 other QTLs of additive effect. QTLs for both traits co-localized to LGs 1 and 7. Of note is the mapping of two QTLs for bloom date and floral bud chilling requirement to LG 5, the same LG by Campoy *et al.* (2010b) mapping a major flowering time QTL. Interspecific synteny (genetic sequence similarity) within the Rosaceae and the availability of co-dominant, transferable markers presents the interesting possibility of exploiting sequence homology to investigate these traits in

apple for example. For example although unpublished (J. Rees, pers. comm.) it is known that *Prunus* LG 5 and *Malus* LG 9 are homologous, which sets the stage for the above-mentioned gene synteny work.

The IVB and IRB QTLs have been shown to co-localize be at the top of LG 9 in apple, by studies by van Dyk *et al.,* (2010) and Celton *et al.* (2011) in three 'Anna' and 'X3263' x 'Belréne' mapping populations, respectively. This major QTL seems to co-localize with the markers NZmsCN943946 and GD142 in the aforementioned linkage maps. However, the study by Celton *et al.* (2011) also suggests the presence of other QTLs at the top of LG 8 for the 'Starkrimson' x 'Granny Smith' mapping population and LG 1 and LG 3 for the two mapping populations. The presence of these QTLs may be explained by the multigenic (and very possibly multilocus) nature of the genes controlling the complex dormancy trait. M. M. van Dyk (PhD thesis, 2008) also found several putative QTLs for IVB in her work. of interest being that on LG 8 of 'Sharpe's Early' x 'Anna' population, which may be the same or closely related QTLs to that found by Celton *et al.* (2011).

UNIVERSITY of the
WESTERN CAPE

1.2.5 Flowering time and dormancy-related genes

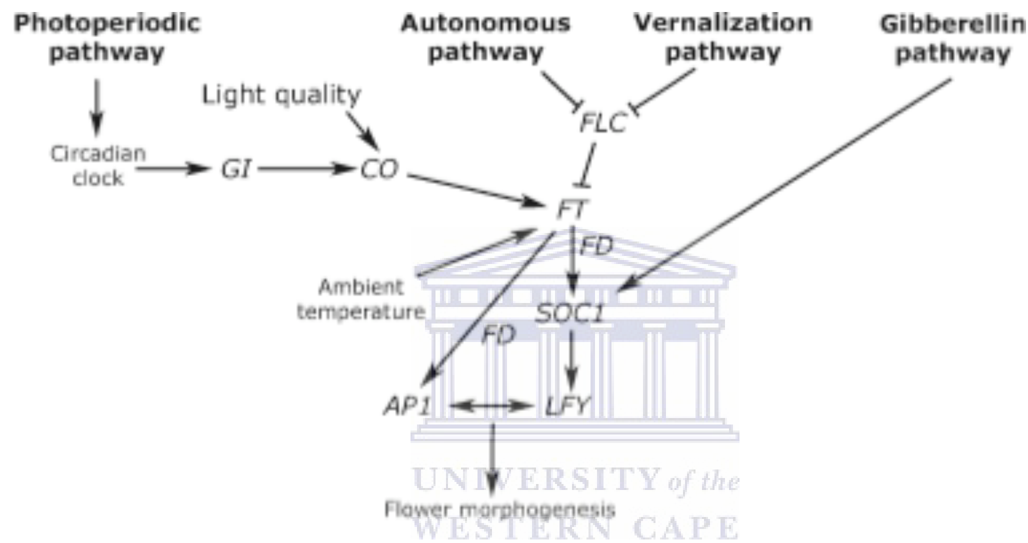
Flowering time in plant species has been shown to be a complex interaction between gene networks and environmental cues (Coupland, 1995) such as exposure to low temperature/ vernalization (Amasino, 2005) and photoperiod (Corbesier and Coupland, 2005; Jaeger *et al.,* 2006). Campoy *et al.* (2010b) show high genetic heritability and strong positive influence by chill accumulation of flowering time in apricot. It also appears to be under epigenetic control (Dennis and Peacock, 2007; Zhebentyayeva *et al.,* 2010). Though

flowering time has been well studied and characterised in herbaceous plants, with the majority of work has been carried out in *Arabidopsis thaliana,* elucidating this trait fully in woody perennials still lags behind (Bernier and Perilleux, 2005; Zhang *et al.,* 2011). This may be attributed to the fact that the *Arabidopsis* genome (The Arabidospsis Genome Initiative, 2000) was the first plant genome to be sequenced due to its inherent advantages such as short generation time, ease of propagation and ease of genetic manipulation *etc*. Despite being to a much lesser extent, efforts to study genomics and genetics of flowering time in woody perennials have been undertaken. Linkage mapping and QTL analysis, expression profiling using next generation high sequencing technology and transgenic approaches have been utilized in these studies (almond: Silva *et al.,* 2005; apricot: Yamane *et al.,* 2008. Campoy *et al.,* 2010b; apple: Kotoda *et al.,* 2010; orange: Zhang *et al.,* 2011). Gene homologues of genes in these pathways have been used as a basis to give an insight into flowering pathways in fruit trees like orange (Zhang *et al.,* 2011). It is important to note that with the present knowledge base, chilling requirement impacts on blooming and vegetative budbreak, but they are not directly linked with flower initiation, which occurs in the previous season.

UNIVERSITY of the
WESTERN CAPE

The control of flowering time can be summarised in **Fig. 3**, which shows a simplified view of the gene, hormone and environmental interaction and control in flowering (Michaels, 2009; Zhang *et al.,* 2011). In a nutshell, four main pathways control flowering time in *Arabidopsis* namely, the Gibberellin, Photoperiod (floral promoting), Autonomous and Vernalization (floral enabling) pathways (reviewed in Boss *et al.,* 2004; Corbesier and Coupland, 2006; Michaels, 2009), all of which interact via their targeting

of *Suppressor of Overexpression of CO1* (*SOC1*) (encodes a MADS-box transcription factor) and *Flowering Locus T* (*FT*) (encodes an animal RAF kinase-like protein) genes. These in turn control the expression of *APETALA 1* (*AP1*) and *LEAFY (LFY)* genes, which are shown to govern floral identity in floral morphogenesis of *Arabidopsis* (Corbesier and Coupland, 2006).



UNIVERSITY of the WESTERN CAPE

Figure 3: A simplified view of the four pathways that control flowering time in *Arabidopsis* spp., showing the major genes involved.
Adapted from Corbesier and Coupland (2006).

The Photoperiodic and Gibberellin pathways promote flowering under long and short days respectively. The transcription of *CONSTANS (CO)* and *GIGANTEA (GI)* genes, which are flowering time genes, is regulated by the circadian clock. Light quality controls CO protein abundance. Finally, the Autonomous pathway is chiefly responsible for the regulating *Flowering Locus C (FLC)*(a MADS box transcription factor suppressing

flowering) mRNA abundance, which is also repressed independent of the autonomous pathway, by the vernalization pathway (Corbesier and Coupland, 2006).

In an attempt to dissect flowering time in apple, Kotoda *et al.* (2010) isolated and characterized two *FT-like* genes in apple (*MdFT1* and *MDFT2*). These genes have been mapped to LG 12 and LG 4 respectively, which are known to have partial homology. These genes were expressed mainly in fruit-bearing shoots and in reproductive organs (flower buds and young fruit) of adult apple plants. Furthermore, they were found to induce early flowering in mutant *Arabidopsis* spp. and not in the wild type. Because of their expression in buds and fruit bearing shoots, these genes could be implicated in reproductive or vegetative bud dormancy. Interestingly too, the over expression of *MdFT1* in apple resulted in precious flowering, suggesting an important role of this gene in flowering regulation and timing in apple. This in combination with work by Zhang *et al.* (2011), could in turn help as a starting point in painting the full picture of the much needed apple florigen and election of candidate flowering genes in apple.

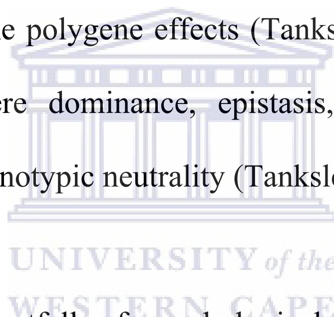
UNIVERSITY of the
WESTERN CAPE

1.3 Molecular markers and mapping polygenes

1.3.1 A brief introduction to markers

The idea of using markers to map genes, began with the employment of monogenic (as they are referred to by Tanksley, (1993)) or morphological markers, was developed about 85 years ago by Sax (1923). Jansen (1996) states that the important idea behind markers, particularly molecular markers, is that observed marker genotypes are used to infer

indirect genotypic information at target genes, but only when the marker is in close enough proximity to the gene target. In essence, the gene target and the marker should be linked. Jones *et al.* (1997) and Semagn *et al.* (2006), place genetic markers into three categories namely, visually assessable traits (morphological and agronomic traits), those based on a gene product (biochemical markers) and those reliant on a DNA assay (molecular markers). Besides the fact they are few in number and can be scored only at certain stages of plant development, these markers were limited in that they were associated with genes that have a large effect on quantitative character than did the linked polygene. This prevented polygene detection and inaccurate estimation through either the over- or under estimation of the polygene effects (Tanksley, 1993). Other limitations of this marker methodology were dominance, epistasis, inability to detect sufficient polymorphisms and lack of phenotypic neutrality (Tanksley, 1993; Jones *et al.,* 1997).


UNIVERSITY of the
WESTERN CAPE

The answer to the numerous shortfalls of morphological markers was found in molecular markers. They offered several advantages in that they offered greater polymorphism, were more abundant, co-dominant, lacked epistatic or pleiotropic effects and offered phenotypic neutrality in that alternate alleles which caused no phenotypic change could be detected at molecular loci. Most importantly, the latter advantage they offered, gave geneticists an unbiased way to estimate the phenotypic effect of each polygene or QTL without interference by the marker locus (Tanksley, 1993; Jones *et al.,* 1997). Agarwal *et al.* (2008) give the characteristics ideal molecular genetic markers should possess as the following: simple, quick and inexpensive to implement; need small amounts of tissue or DNA for the analysis and require no prior genome sequence information.

1.3.2 Markers systems available

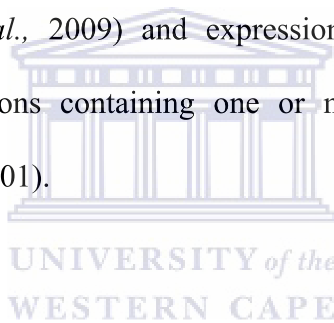
This review will focus on three marker technologies, namely Diversity Array Technology (DArT), Single Nucleotide Polymorphisms and SSR markers. For an in-depth analysis of these and other marker systems such as isozymes, Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP) *etc.*, see reviews by Kumar (1999), Semagn *et al.* (2006), Agarwal *et al.* (2008) and Gupta *et al.* (2008).

1.3.2.1 DArT and SNP markers

DArT and SNP markers are hybridization-based, high-density and high throughput platforms that assay several hundred polymorphic loci (Jaccoud *et al.,* 2001) and several thousand loci (Ganal *et al.,* 2009), respectively. DArT markers require no prior sequence information and generate polymorphic loci spread over the entire genome based on DNA Insertions, deletions and rearrangements within restriction endonuclease treated metagenomic fragments and sometimes DNA methylation patterns of the endonuclease used (Jaccoud *et al.,* 2001). SNP markers, which are the most abundant marker in the genomes of prokaryotes and eukaryotes, are also favoured for their low cost (approximately 0.01 Euro per SNP marker), low mutation rates and that they are amenable to automation (Sobrino, 2005). They are generated via several methods, that is from genomic or genic libraries. Expressed Sequence Tag (EST) data from EST databases (*in silico* to give electronic SNPs or eSNPs), array analysis, amplicon re-sequencing with or without pre-screening, next generation sequencing technologies with or without prior genomic sequence data (Rafalski, 2002; Ganal *et al.,* 2009).

UNIVERSITY of the
WESTERN CAPE

Both methods share in their need for the development of a primary hybridization platform or array, a resource-intensive stage involving the creation of genotyping toolkit which detects polymorphisms by selective binding to DNA samples being assayed (Kilian *et al.,* 2003; Ganal *et al.,* 2009). Popular SNP genotyping assays include GoldenGate® and Infinium® BeadArray® or BeadChip® genotyping by Illumina, MIP® and GeneChip® Oligonucleotide® or Tag array® on glass applications by Affymetrix, and the SNPstream® Tag array from Beckman Coulter (Ganal *et al.,* 2009). Such recent advances in SNP typing have led to new concepts in gene mapping such as Quantitative Trait Nucleotides (QTN) genotyped in genes as opposed to anonymous loci (Morgante and Salamini, 2003; Mackay *et al.,* 2009) and expression QTLs (eQTLs) - expression quantitative trait loci or regions containing one or more genes which affect gene expression (Jansen and Nap, 2001).


UNIVERSITY of the
WESTERN CAPE

1.3.2.2 SSR markers

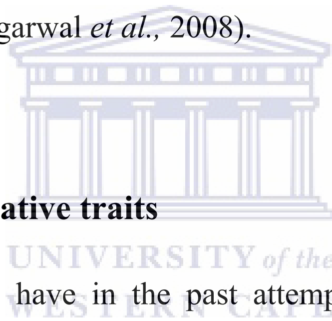
SSR or microsatellite markers, are have been referred to by several names in literature. These are Sequence Tagged Microsatellite Site (STMS). Short Tandem Repeat (STR). Simple Sequence Length Polymorphism (SSLP) and Variable Number of Tandem Repeat markers (Beckman and Soller, 1990; Gupta *et al.,* 1999; Rakoczy-Trojanowska and Bolibok, 2004). They are based on the PCR amplification of simple sequence repeats or microsatellites using primers designed to flank them. Microsatellites are defined as 2 to 6 nucleotide array of repetitive, tandem sequences that are ubiquitous in higher organisms (Chambers and MacAvoy, 2000; Holton, 2001; Rakoczy-Trojanowska and Bolibok, 2004. Jung *et al.,* 2005). These markers have been developed using *in silico* isolation

methodologies and associated tools from EST data repositories (Benson, 1999; Varshney *et al.,* 2002; Varshney *et al.,* 2005; Jung *et al.,* 2004; Lazzari *et al.,* 2005; Nagaraj *et al.,* 2006; Gelfand *et al.,* 2007; Jung *et al.,* 2008) and through screening of a clone library through hybridisation-based methods, with or without enrichment, for microsatellites (Cipriani *et al.,* 1999; Ramsay *et al.,* 2000; Cipriani *et al.,* 2008). Polymorphisms at SSR loci arise due to a change in the number of repeats caused by mutations and by strand slippage during DNA replication (Powell *et al.,* 1996; Jung *et al.,* 2005; Agarwal *et al.,* 2005).

Frequencies of SSRs vary between organisms (Varshney *et al.,* 2005), and Wang *et al.* (1994) assayed SSRs to be about one for every 64.6 kilobases (kb) in monocotyledonous and one in every 24.2 kb in dicotyledonous plants. Because of the different screening and analysis methodologies used other figures in terms of SSR abundance have been given (Varshney *et al.,* 2005) in plants. In terms of relative abundances of SSR classes, Varshney *et al.,* (2002) report that tri-nucleotide repeats (TNRs) have been found to be most common, followed by di-nucleotide repeats (DNRs) or tetra-nucleotide repeats (TTNRs). Gao *et al.* (2003) and Kantety *et al.* (2002) however, report different frequencies for these SSR classes in wheat and rice with TNRs the most common, then TTNRs and DNRs with equal abundances. For TNRs, considered to be the most common microsatellite class, Yu *et al,* (2004) has reported that the highest SSR abundance at 74 %, to be in coding regions of the genome, with 20 % and 6 % in the 5' and 3' untranslated regions respectively.

UNIVERSITY of the
WESTERN CAPE

Their high abundance and wide distribution in the genome (second only to SNPs - Mackay *et al.,* 2009), coupled with their high levels of polymorphism and ease of detection compared to other marker systems (Holton, 2001; Jung *et al.,* 2005) have led to their being a marker of choice in gene mapping experiments (Kumar, 1999; Agarwal *et al.,* 2009). These markers also exhibit co-dominant inheritance and display ease of integration into high throughput automated systems (Mitchell *et al.,* 1997; Wenz *et al.,* 1998; Holton, 2000; Jung *et al.,* 2005). SSR markers have also been shared between research groups for genetic linkage mapping and genotyping projects as they are co-dominant and highly reproducible, making them easily transferable between closely related species and cultivars (Agarwal *et al.,* 2008).



1.4 Qualitative and Quantitative traits

UNIVERSITY of the WESTERN CAPE

Geneticists and plant breeders have in the past attempted to analyse horticultural or agronomic traits that interest them with little or no success due to their quantitative or polygenic nature (Peace and Norelli. 2009). Advances in the fields of genetics and genomics, namely extensions to Mendelian genetic theory and use of genomics technologies (Peace and Norelli, 2009) and quantitative genetics (Walsh, 2002) have led to plant scientists as a whole choosing the integrated approach to dissecting important crop traits. Such an integrated approach also incorporates the disciplines of plant physiology, molecular biology and practical breeding aspects to elucidate the complex genetic architecture of for example fruit attributes such as fruit texture (firmness, softening rate, hardness, crispness, and juiciness to mention a few) in apple and peach (Peace and Norelli, 2009).

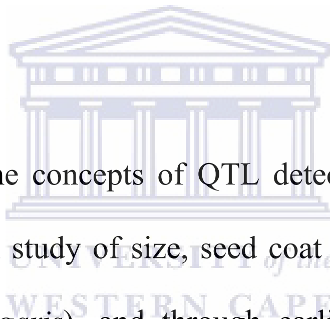
Traits of agronomic interest are classified as qualitative (also referred to as simple, Mendelian or discrete traits) or quantitative (also known as complex or continuous traits) (Peace and Norelli, 2009). Qualitative traits are typically under the control of single genes having high heritability, a term describing the degree of genetic as opposed to environmental control (Tanksley, 1993; Jones *et al.,* 1997; Collard *et al.,* 2005). They are readily dissected using genetics methodologies developed to elucidate the genetics underlying genetic architecture of useful agronomic traits. Quantitative traits on the other hand are, according to Tanksley (1993), characters in nature with polygenic inheritance and continuous variation, determined by the segregation of multiple loci. Quantitative traits, also known as poly- or multi-genic, or complex traits on the other hand (Collard *et al.,* 2005), have been found to be under the control of a few or several genes with an additive effect, and a low heritability. They are not under simple Mendelian control (Jansen, 1996; Peace and Norelli, 2009) and hence are difficult to study (Kearsey, 2002). Such traits can also be simply described as phenotypic variation of characters governed by segregation of multiple genetic loci, each with relatively small genetic effect that follow a polygenic inheritance (Tanksley, 1993).

UNIVERSITY of the
WESTERN CAPE

1.5 Quantitative trait loci and their analysis

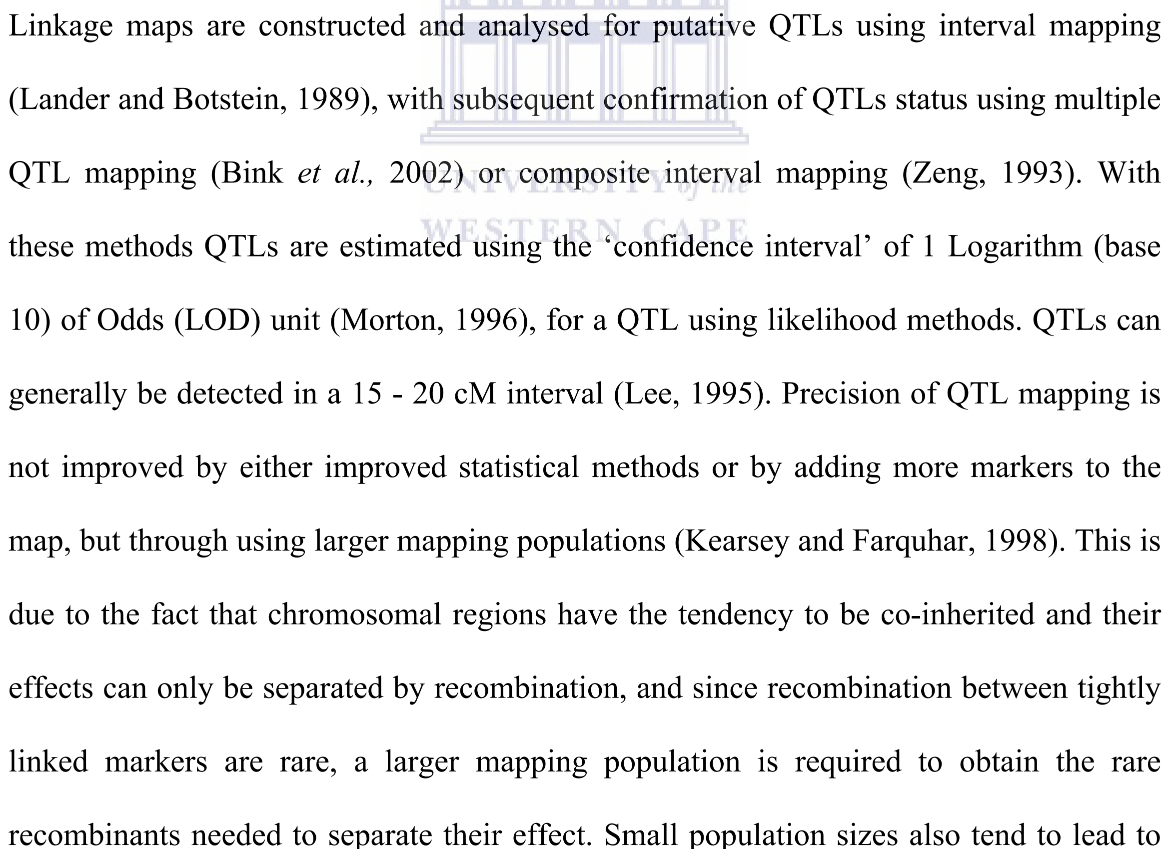
Quantitative genes are different from those having a major effect on phenotype (qualitative traits) unless they are single quantitative trait loci. Genotype can be unambiguously inferred from phenotype in what are described as macromutations (alleles with major phenotypic effect) and these are rare in nature, compared those under complex multigenic control (Tanksley, 1993). In his review of quantitatively inherited traits

(governed by poly- or multi-genes), which gives and outlines the fundamental theory of modern Quantitative Trait Locus (QTL) mapping, Tanksley (1993) states that these traits are under the influence of several minor genes expressed, mapped and characterised utilizing molecular markers. For clarity, the term QTLs will from this point be used to describe Quantitative Trait Loci. Tanksley (1993) also in this same paper, defines a QTL as the individual loci controlling a quantitative trait, which are responsible for most of the naturally occurring variation in populations. Mackay *et al.* (2009) in a more recent review of the subject, defined a QTL as a genomic region containing one to several genes that affect variation in a quantitative trait, which are localized by virtue of their linkage to polymorphic loci.


UNIVERSITY of the
WESTERN CAPE

It is generally accepted that the concepts of QTL detection were developed from Sax (1923), through his association study of size, seed coat pattern and pigmentation in the common bean (*Phaseolus vulgaris*), and through earlier work done in 1909 by W. Johannsen in which demonstrated how quantitative variation results from the combined action of segregating genes and environmental factors, and how more importantly, these factors could be analysed using statistical measures (population means, variances, co-variances between relatives and heritabilities) (Asins, 2002). With the considerable advances in DNA marker technologies and powerful biometric methods, QTL analysis has witnessed significant growth in recent years (Asins, 2002; Kearsey and Farquhar, 1997; Kearsey, 2002; Zhang and Gai, 2009).

Simply put, a joint analysis of phenotypic values and genotype marker segregation enables one to analyse QTLs through their detection and mapping (Asins, 2002), Mackay *et al.* (2009) in their review of quantitative traits, added further clarity to the matter in stating that QTL mapping is based on the concept that QTLs can be localized through their genetic linkage to visible marker loci with genotypes that can be readily classified. QTL mapping can be carried out using two fundamental bases, that is segregating populations with progeny derived from crosses between genetically divergent strains (linkage mapping), or in unrelated individuals (association mapping) (Mackay *et al.,* 2009).



Linkage maps are constructed and analysed for putative QTLs using interval mapping (Lander and Botstein, 1989), with subsequent confirmation of QTLs status using multiple QTL mapping (Bink *et al.,* 2002) or composite interval mapping (Zeng, 1993). With these methods QTLs are estimated using the 'confidence interval' of 1 Logarithm (base 10) of Odds (LOD) unit (Morton, 1996), for a QTL using likelihood methods. QTLs can generally be detected in a 15 - 20 cM interval (Lee, 1995). Precision of QTL mapping is not improved by either improved statistical methods or by adding more markers to the map, but through using larger mapping populations (Kearsey and Farquhar, 1998). This is due to the fact that chromosomal regions have the tendency to be co-inherited and their effects can only be separated by recombination, and since recombination between tightly linked markers are rare, a larger mapping population is required to obtain the rare recombinants needed to separate their effect. Small population sizes also tend to lead to

UNIVERSITY of the
WESTERN CAPE

the underestimation of QTL numbers because of the lowered power of significance tests for QTL detection (Schon *et al.,* 2004; Vales *et al.,* 2005; Collard *et al.,* 2005).

Kearsey (2002) points out another potential error source in QTL analysis that is insufficient replication in the source of phenotypic data for the mapping experiment. This together with the bias that could be introduced via using too high or too low LOD thresholds, may lead to missed QTLs or false positives in the process (Jansen, 1994). Kearsey (2002) also adds that methods in QTL analysis detect only QTLs of major effect while mostly neglecting those of small effect. Kenis and Keulemans (2004), caution against inaccurate QTL determination and localization caused by QTL instability, which results from varying tree physiology with age, environmental factors and rooting differences in plants. As a solution to this problem, van Dyk *et al.* (2010) suggest the use of several clonal replicates and grafting onto the same rootstock selection as was done by Segura *et al.,* (2007), and the gathering of phenotypic data over a number of years. Furthermore, when QTLs are in close proximity to one another on chromosomes they are difficult to detect (Zeng, 1993, 1994). To answer some of the problems mentioned above, some authors suggest alternative methods such as linkage disequilibrium and association mapping to map genes of interest (Gupta *et al.,* 2005; Mackay and Powell. 2006).

UNIVERSITY of the
WESTERN CAPE

1.5.1 QTL analysis in apple

Several QTLs have been found in several fruit and forest tree species (Bradshaw and Stettler, 1995; Chen *et al.,* 2000; Arora *et al.,* 2003; Morgante and Salamini, 2003), including apple (Maliepaard *et al.,* 1998; Vinatzer *et al.,* 2001; Gygax *et al.,* 2004; Silfverberg-Dilworth *et al.,* 2006; Segura *et al.,* 2007; Oraguzie and Bell, 2008; Celton *et al.,* 2010; van Dyk *et al.,* 2010; Celton *et al.,* 2011; among many others). More importantly, in accordance with the focus of this study, marker trait associations have been established for morphological or physiological traits (Silfverberg-Dilworth *et al.,* 2006; Celton *et al.,* 2010; van Dyk *et al.,* 2010; Celton *et al.,* 2011), Lawson *et al.,* (1995) have mapped QTLs for dormancy-associated timing of vegetative budbreak and terminal bearing to LG 6 of the 'White Angel' x 'Rome Beauty' cross, which correspond to LG 10 in the Maliepaard *et al.* (1998) reference map and (Conner *et al.,* 1998) mapped leaf break QTLs in the 'Wijcik McIntosh' x 'NY 75441-58' cross to LGs 3, 7, 9 which correspond to LGs 9, 8, and 7 in the Maliepaard *et al.* (1998) reference map. The date of budbreak QTLs have been localized in the 'Starkrimson' x 'Granny Smith' map to LGs 6 and 8 (Segura *et al.,* 2007).

UNIVERSITY of the
WESTERN CAPE

Additionally, van Dyk *et al.* (2010) mapped IVB to a major QTL in LG 9 (corresponding to LG 3 in work by Conner *et al.,* 1998) of the 'Golden' x 'Anna' and 'Sharpe's Early' x 'Anna' cross, in seedling and adult apple plants using data from a 3 to 6 year phenotypic data collection period. These explained between 40 and 46.4 % of the phenotypic variation, with heritability of 0.69 as reported previously by Labuschagné *et al.* (2002). Celton *et al.* (2010) identified QTLs controlling phenological traits namely flowering

time and budbreak, in two apple mapping populations (‘X3263’ x ‘Belrené’ and ‘Starkrimson’ x ‘Granny Smith’), and identified major and stable QTL on LGs 8 and 9 and other minor ones on LGs 1 and 3.

Using the same F_1 segregating populations they used in their 2010 budbreak study, Celton *et al,* (2011) mapped QTLs for the phonological traits VB, FB and GP. This work used phenotypic data they had gathered from 2005 - 2010 at French INRA experimental stations. Like most recent work QTLs were mapped using the extracted best linear unbiased predictor (BLUP) data and average genotypic value for each year. In brief, Robinson (1991) and Henderson (1975) describe BLUP as a mathematical method first implemented in animal breeding, which estimates genetic merits in a population through an unbiased estimation of random or environmental effects. This method allows for the making of accurate selection decisions in breeding, by selecting the best performing genotypes. As a result of this part of the study, stable QTLs were identified for the three phenotypic traits assayed in both populations, that is; VB: ‘X3263’ x ‘Belrené’ - 4 QTLs detected on LG 1, LG 9, LG 10 and LG 15, explaining 14.9%, 8.3%, 6.7% and 7.2% of phenotypic variation and ‘Starkrimson x Granny Smith’ - 4 QTLs were located on LG 2, LG 3, LG 5 and LG 6, and explained 21.6%, 16.5%, 15.9% and 9.9% of the phenotypic variation, respectively; FB: ‘X3263 x Belrené’ - 2 QTLs detected on LG 1 and LG 9, which explained 10.9% and 9.3% of phenotypic variability and ‘Starkrimson’ x ‘Granny Smith’ - 1 QTL explaining 19.5% of phenotypic variability was mapped onto LG08; and GP: ‘X3263’ x ‘Belrené’ - 3 QTLs detected on LG 1, LG 3 and LG 9 explaining 5.2%,

UNIVERSITY of the
WESTERN CAPE

10.9% and 32.1% of phenotypic variation and 'Starkrimson' x 'Granny Smith' - 1 major QTL, on the top half of LG 8 explaining 23.1% of phenotypic variation.

Celton *et al.* (2011) also reported dormancy candidate genes (CGs) for the region containing the QTL reported on LG 9, through an *in-silico* approach. In this approach, Celton *et al.* (2011) used contigs from the Velasco *et al.* (2010) apple genome data corresponding to the VB QTL in the LG 9 interval of published apple linkage maps, to predict protein sequences and perform subsequent gene ontology and annotation using BLAST2GO. Of special interest were any genes involved in the cell cycle, division and their hormonal control for putative CGs. Putative CGs were also detected at the region in and around the LOD peak for the LG 9 QTL, as this region had the statistically highest probability of containing the genes that control the variation to the trait. Single gene copies of Cyclin-A3, Cytokinin-N-glucosyltransferase 2, Myb-related protein Pp2 and Phytosulfokine receptor 1; along with two copies of Auxin signaling F-BOX 3; and fourteen copies of E3 ubiquitin-protein ligase genes were identified within 900 kb of the marker GD_SNP01189 on LG 9 as putative dormancy influencing CGs. Though not in a Rosaceaous crop, Hedley *et al.* (2010) have reported three putative genes in blackcurrant (*Ribes nigrum* L.) encoding calmodulin-binding protein, beta tubulin and acetyl CoA carboxylase respectively to co-localize with a budbreak QTL.

UNIVERSITY of the WESTERN CAPE

1.6 Linkage mapping

Jones *et al.* (1997) define linkage mapping simply, as the placement of markers in order, indicating relative genetic distance between them and assigning them to their respective LGs. based on their recombination frequencies and their pair-wise combinations, as determined by mapping functions. The above statement can be summarised as that several segregating loci are evaluated and mapped in a linkage map by virtue of their co-segregation (Jones *et al.,* 1997; M.M. van Dyk PhD thesis, 2008). In describing the fundamental basis of mapping, Maliepaard *et al.* (1997) state that linkage mapping requires analyses of polymorphic, multi-allelic loci and F_2 progeny derived from F_1 individuals obtained from homozygous parents. This helps in the separation of parental homologous LGs when integrating male and female linkage maps into one combined map. Markers such as SSRs may be employed for this process and their transferability over a wide variety of closely related crosses (Jones *et al.,* 1997; Maliepaard *et al.,* 1997). Computer software used to generate linkage maps from such markers downstream after scoring includes *MapMaker*® (Lander *et al.,* 1997) and *JoinMap*® (van Ooijen, 2006). These computer programs, like many of their contemporaries, primarily operate based on the Kosambi mapping function (Kosambi, 1944), which is the mathematical basis of the genetic theory by Morgan in 1911, that positions markers at specific loci on a genome by relating their recombination frequencies with other markers at different loci. Linkage groups generated in this map building process represent chromosomes; initially partial segments and then with wider coverage gained through adding markers to the map, entire chromosomes (Peace and Norelli, 2009).

UNIVERSITY of the
WESTERN CAPE

LGs are established by a statistical basis referred to as the Logarithm of Odds (LOD) (Morton, 1996), which is defined as the logarithm to base 10 of the likelihood ratio of linkage to independent segregation. Distances between loci in the linkage map are given in cM. Because of high levels of heterozygozity (except in peach) and widespread self-incompatibility and long generation times for the tree crops, F_1 populations are used due to the difficulties associated with developing an F_2 population. F_1 populations that mimic the backcross model for each of the heterozygous parents of the cross have been utilized for map construction and QTL analysis (Peace and Norelli, 2009).

1.6.1 Genetic linkage maps in apple

Apple research groups around the word have strived to produce saturated genetic maps so as to facilitate QTL analysis (Gardiner *et al.,* 2007; Segura *et al.,* 2007). One of the most up-to-date, highest coverage (saturated) map referred to as the reference map in apple is considered to be the one by Silfverberg-Dilworth (2006). Other maps such as those Liebhard *et al.* (2002, 2003b) are also used as reference maps when attempting to establish the correct. These three maps confirm the fact that a complete linkage map in apple has 17 LGs corresponding to the 17 chromosomes of apple.

UNIVERSITY of the
WESTERN CAPE

Previous maps, including the aforementioned maps, have consisted of most of the popular markers reviewed previously in this work, namely Isozyme, RAPD, RFLP, SCAR, AFLP and SSR markers. Other, more current maps in apple other than those mentioned above are have been generated [Khan *et al.,* 2007; Igarashi *et al.,* 2008; Celton *et al.,* 2009 (the first comprehensive apple rootstock map); Celton *et al.,* 2010; van Dyk *et al.,* 2010].

Using among other markers SSRs, Liebhard *et al.* (2003b) constructed a map using 115 new markers they developed (these were mostly from Liebhard *et al.,* 2002) and this map therefore had a total of 840 markers. Including these SSR markers, a total of 160 makers had been developed for the 'Fiesta' x 'Discovery' cross. Additions to this and other maps in apple in the other maps more current than the reference map mentioned above, brings the total of SSR markers to 300 developed for use in apple.

The Silfverberg-Dilworth (2006) reference map created using the 'Fiesta' x 'Discovery' cross has the respective parental map lengths of 1 145 and 1 417 centiMorgans (cM), the accepted standard for recombination units in linkage maps. Needless to say, this map builds on those markers and maps of its predecessors (Hemmat *et al.,* 1994; Hokanson *et al.,* 1998; Guilford *et al.,* 1997; Gianfranceschi *et al.,* 1998; Maliepaard *et al.,* 1998; Hemmat *et al.,* 2003; Vinatzer *et al.,* 2004) through the implementation of transferable, co-dominant markers, the major one of which is SSRs.

UNIVERSITY of the
WESTERN CAPE

1.7 Marker Assisted Selection (MAS) and Marker Assisted Breeding (MAB)

Marker Assisted Selection (MAS) is defined simply as the implementation of markers for selection in breeding (Collard *et al.,* 2005; Peace and Norelli, 2009). This is for both parents and seedlings though usually for seedlings. Marker Assisted Breeding (MAB) refers to the use of markers to assist with one or more aspects of breeding programmes including parent and seedling selection, family size planning, parentage verification, performance evaluation and cultivar commercialisation (Peace and Norelli, 2009). Due to the fact that a number of crossings and backcrosses required in conventional selection and

breeding programmes, about 20 years are needed to develop a new apple cultivar for example (Janick *et al.,* 1996). MAS and MAB have become essential to shorten this time (Luby and Shaw, 2001; Janick *et al.,* 1996). The use of molecular genetic markers permits genetic dissection of progeny at each generation and vastly decreases the time needed for selection compared with phenotypic trait based selection (Ribaut *et al.,* 2002).

Furthermore, genotyped cultivars and germplasm have been used in crop improvement by for example revealing heterotic crops and have well-established protocols for implementation at present. These practices have been employed to monitor and understand existing cultivar field performance and to select parental and seedling stocks in breeding programmes (parental and seedling selection). This genotyping process has a pre-requisite robust marker toolkit that needs to be developed through rigorous screening of germplasm and verification of efficiency in marker-trait associations that may reveal QTLs that are essential MAS and MAB projects (Ribaut *et al.,* 2002; Peace and Norelli, 2009).

UNIVERSITY of the
WESTERN CAPE

Luby and Shaw (2001), who analyse the cost-benefit relationship for MAS compared to conventional breeding, work with the logical assumption that it is more beneficial to for use with fruit crops, rather than annual crops. This is because apple and peach for example, have a large tree size and long juvenile phase of 3 to 10 years or more (Janick *et al.,* 1996). Ribaut *et al.* (2002) have however, performed MAS and MAB experiments on maize (*Zea mays* L.) whose results they report in this paper. They also give the costs of

running a MAS and MAB experiment, coupled with the costs and efficiency of running a hypothetical high-throughput experiment based on SSRs (**Table 2**).

Table 2: Costs of running a high throughput SSR MAS experiment in US$ for various combinations of sample size and number of markers analysed.

	Number of markers analysed (mk)					
Sample size	1 mk	10 mk	50 mk	100 mk	200 mk	500 mk
2	33.55	4.37	1.83	1.53	1.38	1.30
10	7.79	1.85	1.35	1.31	1.27	1.25
100	2.26	1.35	1.26	1.25	1.25	1.24
280	2.00	1.32	1.26	1.25	1.24	1.24
500	1.96	1.31	1.26	1.25	1.24	1.24
1000	1.94	1.31	1.26	1.25	1.24	1.24
5000	1.91	1.31	1.26	1.25	1.24	1.24

UNIVERSITY of the
WESTERN CAPE

It is important to note that the costs for the experiment whose results are summarised in **Table 2** evolve from the exact methodology used to get this data (Adapted from Ribaut *et al.,* 2002). In essence, **Table 2** follows the law of economies of scale, that is: more markers and more samples are cheapest to run in one genotyping run using SSRs. The cost of running ten or more marker combinations with 100 or more samples drops the cost of generating a data point to below US$1.35, and may vary significantly depending on the protocols used and technical available parameters of running such an experiment.
The figure of US$1.35 reported by Ribaut *et al.* (2002), corresponds well with that given per data point of US$1.33 for the Apple Research (Department of Biotechnology, University of the Western Cape, South Africa) Group's SSR-marker work for the October

2009 period (unpublished). Factors such as Megaplex PCR (Meuzelaar *et al.,* 2007 - running PCR multiplexes of up to 16 primer pairs in one reaction) and improved capillary electrophoresis systems may help to reduce costs.

Luby and Shaw (2001) like Ribaut *et al.* (2002), have found it more convenient to split MAS programmes into those targeting; i. Monogenic or simply inherited and, ii. Polygenic, quantitative or metric traits. The former provided a cost benefit analysis in grape, apple and strawberry (two of which are important Rosaceous fruit crops - Shulaev *et al.,* 2008) and the latter focused on maize (Z*ea mays* L.). For the three species, Luby and Shaw (2001) modelled and concluded that the MAS programme such as the one typified by these species could be most effective when the trait is simply inherited, expressed at the mature phase of a long-lived tree with a long juvenile phase. Furthermore, the trait(s) should require vast costs using conventional screening, and should have an inexpensive or economical marker technology, with robust marker-linked associations established, available. Finally, the MAS programme should significantly increase the probability of selecting superior individuals compared with the best available conventional breeding and evaluation methods. In concluding the matter, Luby and Shaw (2001) also raise the important question of which simply inherited trait would offer enough justification for the injection of the large initial investment required to initiate and sustain MAS, and then eventually generate sufficient external profits in the right markets.

UNIVERSITY of the
WESTERN CAPE

Ribaut *et al.* (2002) also raise the concern of the expense of running a MAS programme when they recommend solutions to problems they encountered when they implemented

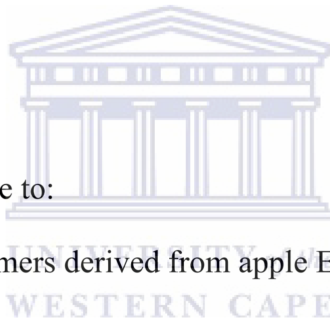
MAS on maize with partial success. The main problem they encountered was the inability to predict phenotype given the allelic constitution of an individual. They highlight the central importance of creating a consensus linkage map from many crosses of a crop, with phenotypic evaluation, so as to map markers that could be implemented in MAS for several cultivars, though the process is expensive. This was after Ribaut *et al.* (2002) estimated the cost of creating a linkage map alone to be US$25 000 with the cost multiplying with involvement of the phenotypic selection process, to US$ 98 000. Ribaut *et al.* (2002)

Caution is however given by Dudley (2002) against forgetting the relevance and importance and potential contributions of quantitative genetics in MAS and MAB, as these are founded on its principles. Ribaut *et al.* (2002) also reiterates this idea in adding that when only when quantitative genetics methods are used in unison with the identification and characterisation of genomic regions involved in expression of target traits, this gives a much fuller understanding of the genetic basis for agronomic traits of interest.

UNIVERSITY of the
WESTERN CAPE

1.8 Aims of the study

Mapping of QTLs responsible for chilling requirements in apple has come a long way and this study aims to elucidate the QTLs responsible for this dormancy-related trait, so that fruit production in horticultural systems is optimised through the development of apple cultivars better adapted to warmer climatic conditions. This could be done through the identification of markers linked to genes or QTLs that influence chilling requirement, which would then be implemented in marker assisted selection and breeding programmes. The addition of novel SSR and DArT markers at a higher density in the QTL mapping process of this work is expected to improve on the findings by van Dyk *et al.* (2010).


UNIVERSITY of the
WESTERN CAPE

The main aims of the study were to:

i. Design and test SSR primers derived from apple EST and genome sequence data.
ii. Map SSR markers for on the 'Austin' x 'Anna' mapping population.
iii. Map DArT markers on the 'Austin' x 'Anna' mapping population.
iv. Construct an integrated SSR and DArT genetic linkage map for the 'Austin' x 'Anna' mapping population.
v. Map QTLs responsible for IVB in the 'Austin' x 'Anna' mapping population.

2.0 MATERIALS AND METHODS

2.1 General chemicals

Reagents	Suppliers
Agarose D1 LE	Promega
Ammonium persulphate	Merck
β-Mercapto ethanol	Merck
Boric acid	Merck
Bromophenol blue	Sigma
Chloroform	BDH
CTAB	Saarchem
dNTPs	ABgene
EDTA	Merck
Ethidium bromide	Sigma
Ethanol (absolute)	Merck
Excel *Taq* polymerase®	Southern Cross
Formamide	Riedel-de Haën
Gelatin	Merck
Genescan® 500 (-250) LIZ ™ size standard	Applied Biosystems
Isoamyl alcohol	Saarchem

UNIVERSITY of the
WESTERN CAPE

Oligonucleotides (fluorescently labelled)	Applied Biosystems
Parafilm paper	Lasec
PCR grade DNAse free water	Qiagen
Polyvinyl pyrrolidone 40 (PVP40)	Sigma
POP-7	Applied Biosystems
Potassium chloride	Saarchem
Proteinase K	Roche
Multiplex PCR kit	Qiagen
RNase A	Roche
Silver nitrate	Merck
Sodium acetate	Merck
Sodium borohydride	Saarchem
Sodium chloride	Merck
Sodium hydroxide	BDH
Sodium sulphite	Sigma
Tris hydroxymethyl aminomethane	Merck
Urea	Merck
Xylene cyanol	BDH

UNIVERSITY of the
WESTERN CAPE

2.2 General stock solutions and buffers

1 % Agarose gel

1 % agarose (w/v) in 1x TBE.

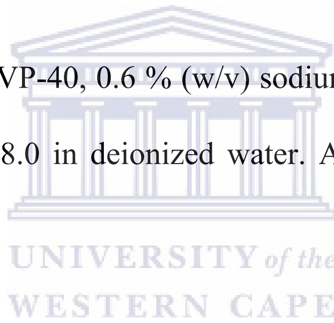
Agarose gel sample loading buffer

0.25 % (w/v) bromophenol blue, 0.25 % (w/v) xylene cyanol in 30% (v/v) glycerol in deionized water.

CIA (Chloroform-isoamyl alcohol)

24 parts chloroform: 1 part isoamyl alcohol (v/v).

2x CTAB solution


UNIVERSITY of the
WESTERN CAPE

2 % (w/v) CTAB, 2 % (w/v) PVP-40, 0.6 % (w/v) sodium sulphite, 1.4 M NaCl, 50 mM Tris-HCl, 25 mM EDTA, pH 8.0 in deionized water. Add β-Mercapto ethanol at 2 % (v/v) prior to use.

70 % Ethanol

70 % absolute ethanol (v/v) in deionized water.

PCR reagents

10x buffer: 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01 % gelatin, pH 8.3, in deionized water. $MgCl_2$: 50 mM in deionized water, dNTPs: 5 mM in deionized water.

Polyacrylamide gel loading buffer

80 % (v/v) deionized formamide, 10 mM NaOH, 1 mM EDTA, 0.1 % (w/v) xylene cyanol, 0.1 % (w/v) bromophenol blue in deionized water.

Polyacrylamide gel $AgNO_3$ staining solution

0.1 % (w/v) $AgNO_3$ in deionized water.

Polyacrylamide gel developing solution

1.5 % (w/v) NaOH, 0.15 % (v/v) formaldehyde and 0.01 % (w/v) $NaBH_4$ in deionized water.

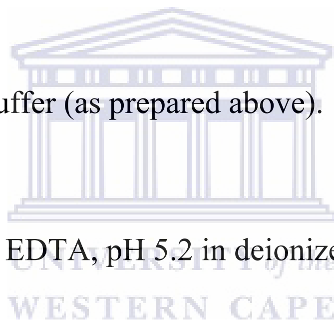
pTZ Molecular weight marker

The pTZ molecular weight marker prepared by digesting the pTZ 18 R vector with the *Hinf* I restriction enzyme. It contained the following fragments in base pairs (bp): 1 200, 517, 396, 356 and 201.

RNase A buffer

0.1 M sodium acetate, 0.3 mM EDTA, pH 4.8 in deionized water.

RNase A (DNase free)



20 mg/ml RNase in RNase A buffer (as prepared above).

Sodium Acetate

3 M sodium acetate with 1 mM EDTA, pH 5.2 in deionized water.

UNIVERSITY of the
WESTERN CAPE

10x TBE

0.9 M Tris, 0.89 M boric acid, 0.032 M EDTA, pH 8.3 in deionized water.

1x TBE

90 mM Tris, 89 mM boric acid, 3.2 mM EDTA, pH 8.3 in deionized water.

10x TE

100 mM Tris-HCl, 10 mM EDTA, pH 7.5 in deionized water.

1x TE

10mM Tris-HCl, 1mM EDTA, pH 7.5 in deionized water.

2.3 Phenotypic trait assessment

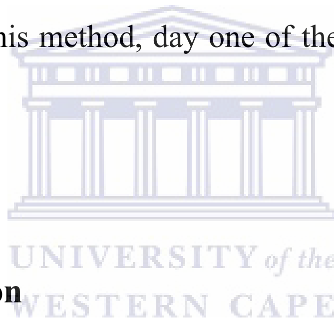
The 'Anna' x 'Austin' cultivar was developed and supplied by Dr. I. F. Labuschagné (Agricultural Research Council (ARC), Nietvoorbij, Stellenbosch, South Africa) and was also given in work by Labuschagné *et al.* (2002a). It is recognized that 'Anna' is the male parent and 'Austin' the female parent; and according to some convention used in other research, this cross should be named 'Austin' x 'Anna'. However, the name 'Anna' x 'Austin' was retained throughout this thesis in accordance to the breeder's naming and for ease of records access.

Phenotypic assessment of IVB was carried out on seedling and adult apple plants generated by Dr. I. F. Labuschagné in the years 1996, 1997 and 1998. According to his methodology, adult and seedling plants were grafted onto the M9 rootstock so as to afford the plants a similar physiological status. Sibling seedlings within the mapping population were planted in adjacent rows, with regular inspection of the area to ensure site uniformity. Seven clonal replicates on different sites were made of 'Anna' x 'Austin' seedlings so as minimize the influence of environmental differences (nutrients, water received, soil composition and human error in phenotyping) on the validity of phenotypic data. The Pearson's correlation test, which measures statistical linear interdependence between two variables or two sets of data was used in this thesis to assess the correlation of seedling and adult tree IVB data for all the yearly pairwise combinations in which data were collected. That is: 1996 and 1997; 1996 and 1998; 1997 and 1998. A confidence level of 99.9 % (p-value of $P<0.001$ or $\alpha = 0.1$ %) was used for the analysis which

UNIVERSITY of the
WESTERN CAPE

carried out using the Pearson's Linear Correlation function in StatPlus:mac LE Mac OS, Version 2009. These resulting data are presented in **section 3.1**, **Table 5.**

Furthermore, year-to-year IVB was compared graphically in terms of frequency distribution and data trend per plant (**sections 3.1.1** and **3.1.2** respectively) and these plants were a collection of 60 seedlings and 78 adult trees from the 'Anna' x 'Austin' mapping population maintained at the ARC's Drostersnes orchard (34°04′15″ S 19°04′47″ E) in the Elgin valley of the Western Cape, South Africa. The date of IVB was scored as the day of the year the first green leaves were observed to emerge from any vegetative bud. According to this method, day one of the year as 1 January and day 365 as 31 December.


UNIVERSITY of the
WESTERN CAPE

2.4 Genomic DNA extraction

Depending on the leaf size, two to three leaf samples were collected from each plant in the 'Anna' x 'Austin' mapping population. These were collected into zip lock polythene bags and placed on ice immediately after collection for transportation and then eventual storage at -20 °C until needed for extraction. The modified CTAB method by Doyle and Doyle (1990) was utilized in order to extract genomic DNA. Approximately 0.1 g of leaf material was ground to a fine powder using a pestle and mortar, in liquid nitrogen. The ground-up mass of leaf material was transferred into clean 2 ml Eppendorf tubes and mixed with a volume of 2x CTAB containing 0.2 % β-mercaptoethanol and pre-warmed

to 62 °C. This mixture was homogenized by vortexing to give consistent, free-flowing slurry and then incubated at 62 °C for a period of 30 min.

Thereafter, a 10 µl volume of 10 mg/ml Proteinase K was added, followed by a further incubation at 37 °C for 60 min. An equal volume of a 24:1 CIA was then added to the mixture, with slight vortexing and inversion to acquire a good mix. The samples were then centrifuged at 10 000x g for 10 min, with the resultant top aqueous layer carefully collected and transferred into clean 2ml Eppendorf tubes. Genomic DNA precipitation was then carried out by adding to the collected volume of aqueous layer, a 0.1 v/v 3M ammonium acetate and a 2.5 v/v cold absolute ethanol, with mixing by inverting the tube several times. The mixture was then incubated at -20 °C for 2 hours or overnight. After this, the samples were centrifuged at the 13 200x g for 10 min, with the supernatant discarded so as to retain the pellet. The pellet was the washed 2 times with as 500 µl of ice-cold 70 % ethanol, at each time centrifuging at 13 200x g for 2 - 3 min. The pellet was then air-dried until all the residual ethanol is removed. Following this, the pellet was re-suspended in a 150 µl volume of TE buffer containing RNase (at a final concentration of 0,0625 mg/ml), with a 30 - 60 min incubation at 37 °C. A final precipitation and washing of the RNase-treated gDNA was done once more with two more washing steps in 500 µl of ice-cold 70 % ethanol at each time centrifuging at 13 200x g for 2 - 3 min. The resulting pellet was then air-dried until all the residual ethanol was eliminated, in a fume cupboard, The DNA pellet was then re-suspended in a 100 µl volume 1x TE buffer.

UNIVERSITY of the
WESTERN CAPE

2.5 Agarose gel electrophoresis

The extracted DNA yield and integrity was checked on an ethidium bromide stained, 1 % agarose gel by electrophoresis. A 1 g mass of agarose powder was weighed out and placed in a conical flask. Thereafter, it was made up to a 100 ml final volume with 1x TBE and melted in a microwave by heating at medium power for about 2 to 5 min, with intermittent swirling. Ethidium bromide at a concentration of 10 mg/ml was added at 2 µl to the 100 ml volume of gel, only after the gel had been cooled for about 3 to 5 min. A 5 µl volume of DNA was mixed with an equal volume of loading dye, mixed on parafilm paper and then loaded onto the agarose gel, using a micropipette. The pTZ molecular weight marker was also loaded into the gel and a means of estimating the size and concentration of the gDNA. The gel was then run at 10 V/cm for 1 hour, in a 1x TBE buffer. After the gel was run, it was viewed under ultraviolet light using the BIO-RAD® TransIlluminator (BIORAD, South Africa). For PCR, gDNA was dilute from the stock to a 10 ng/µl working solution in volume of low salt 1x TE buffer.

UNIVERSITY of the
WESTERN CAPE

2.6 Genomic DNA quantification

The extracted gDNA was quantified by adding a 2 µl volume to the analysis lense of the NanoDrop® ND-1000 spectrophotometer (NanoDrop technologies, USA), according to the manufacturer's instructions. The OD 260/280 ratio was also measured and for the purposes of this work, used as an overall measure of DNA sample purity.

2.7 SSR primer design

Primers pairs used in this study were designed and synthesized from two major and one minor source. The first source is from publications in apple (Guilford *et al.,* 1997; Liebhard *et al.,* 2002; Liebhard *et al.,* 2003; Silfverberg-Dilworth *et al.,* 2006; van Dyk *et al.,* 2010) and pear (Yamamoto *et al.,* 2002a, b, c) mapping studies. Of note is the fact that some of these primers were pig-tailed, so as so increase their PCR efficiency. This 'pig-tailing' was characterized by the addition of a different number of nucleotides to the 5′ end of the reverse primer, so that the GTTT sequence is obtained (Brownstein *et al.* 1996). The second source was *in-silico* from publicly available apple EST (Korban *et al.,* 2005; Naik *et al.,* 2006; Newcomb *et al.,* 2006) data sets in the *Malus* assembly v3. This was mined for SSR sequences using the tandem repeats finder tool (Benson 1999) after loading the data onto the tandem repeats database (Gelfand *et al.,* 2007; http://tandem.bu.edu). The primers were designed around the regions containing a copy number of at least 10 di-, tri-, tetra-, penta- or hexa-nucleotide SSRs, to detect genetic variation within the mapping population. The minimum length of the sequence flanking the SSR repeat (first or last index) was set at 20 bp to allow enough sequence for primer design. Setting a 95 % sequence match as a minimum criterion for inclusion, minimized erroneous sequences from single base changes, via substitutions or in-dels in the SSR sequence.

A third and minor source of *in-silico* primer design was the apple genome sequence (Velasco *et al.,* 2010) that retrieved online at the Genome Database for Rosaceae (GDR; www.rosaceae.org) as a set of contigs grouped per chromosome. For the *in-silico* primer

UNIVERSITY of the
WESTERN CAPE

design work, primers were either selected around the sequences flanking SSRs by inspection, or using the software BatchPrimer3®, which is an online tool from http://probes.pw.usda.gov/batchprimer3/. This software is based in the software Primer3® (Rozen and Skaletsky, 2000). BatchPrimer3® is an online tool used to design primer pairs from a batch of sequences, based on user specifications.

As general rules, all primers were designed to give a melting temperature (T_m) of 60 °C, a 40 - 60 % GC content. Primers were designed to generate a PCR product of between 100 bp and 450 bp, because this was the efficient sizing range for the Genescan® 500 (-250) LIZ ™ size standard (Applied Biosystems, Foster City, CA, USA) in the automated product sizing process. A primer was designed using a set of rules that increased the probability of making high efficiency primers for PCR. The first was that the primer had to be between 18 and 30 bases long, as this was determined to be the optimum length for efficient primers for PCR. Secondly, the primer sequence needed to be free of long tracts of A or T bases, as these would result in poor priming in PCR as a result of the formation of weak bonds of the primer with its target sequence. Furthermore, the primer sequence had to end and begin in a GC, GG, or CC to ensure strong bonds as primer bonds its target sequence in PCR, while avoiding any internal sequence complementarity between its 3′ and 5′ ends, and any neighbouring bases. This was done so as to avoid secondary stem-loop structures within the primer that would stop it from binding and amplifying its target sequence. Sequence complementarity between the forward and reverse primers was also avoided so as to prevent primer dimers while performing PCR. A melting

UNIVERSITY of the
WESTERN CAPE

temperature (T_m) of 60 °C was aimed for all primers so as to increase their capacity for multiplexing using similar PCR conditions.

The following the formula was used to calculate T_m:

$$T_m = 2(\text{number of A + T bases}) + 4(\text{number of G + C bases})\ °C$$

For each primer pair (forward and reverse), the primer designed from sequence closest to the SSR repeat, was the one that was labelled with one of the following dyes: 6-FAM™ (blue). VIC™ (green). NED™ (yellow) and PET™ (red) (Applied Biosystems, Foster City CA, USA). This was done so that it would be easier to design process alternate unlabelled primers later. In the cases where the primers failed in the primer-optimization phase of simplex PCR, these were re-designed if sufficient sequence was available for this.

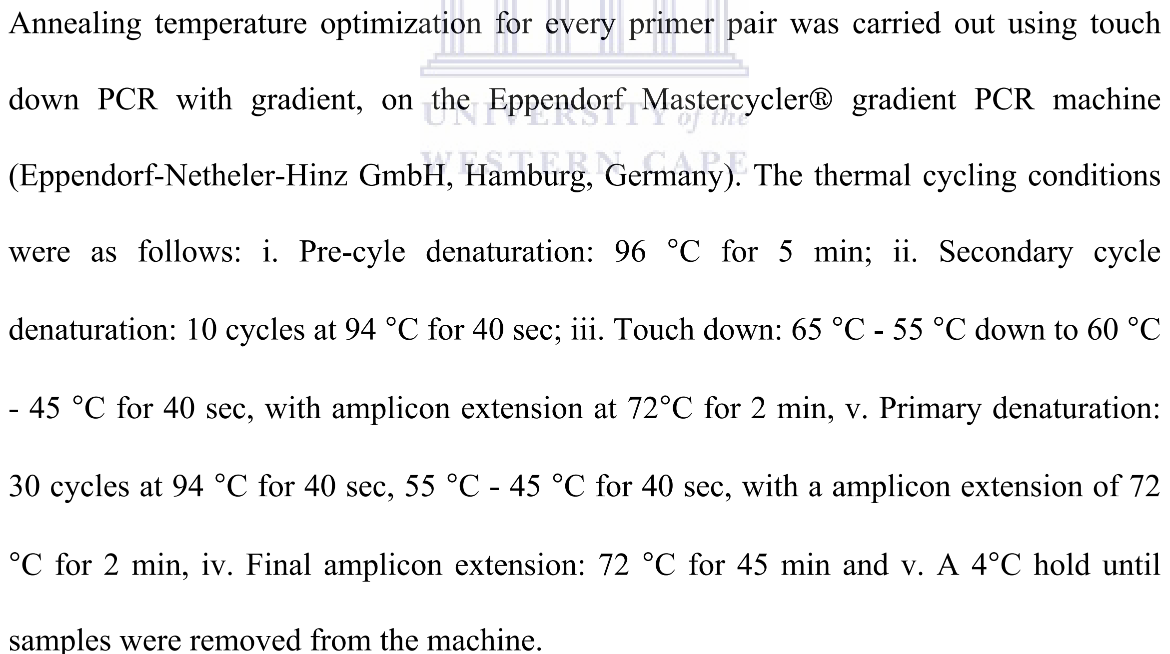
UNIVERSITY of the
WESTERN CAPE

All the primer information was then captured and indexed to create a searchable database using FileMaker Pro® 8.5v1 software. This information was organized so that information such as sequence used to design primers, individual primer sequences, expected and observed PCR amplicon sizes across the various cultivars tested in the primer optimization phase and all the megaplexes' primer constituents could be easily accessed in a tabular form. Finally, the sequences used to generate the SSR primers were used to search for a sequence homologous to them on the apple genome using the BLAST algorithm, so as to predict a most likely position the SSR marker would be mapped to. The results are shown in **Table B**, **Appendix 1** (these 98 SSR primer pairs developed in

this thesis are numbered 592 - 763 and 834, 836 - 870 and have the bold and italicized prefix "***SAms***". All other markers in **Table B, Appendix 1** are derived from published sources).

2.8 Simplex PCR

Simplex PCR reactions were carried out in volumes of 20 µl with 1 unit of Excel T*aq* polymerase, 0.2 µM Tris-HCl (pH 8.3), 1 µM KCl, 0.07 µM $MgCl_2$, 50 µM dNTPs, 0.016 µM the forward and reverse primers respectively. The DNA template was used at a 10 ng/µl final concentration.



Annealing temperature optimization for every primer pair was carried out using touch down PCR with gradient, on the Eppendorf Mastercycler® gradient PCR machine (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). The thermal cycling conditions were as follows: i. Pre-cyle denaturation: 96 °C for 5 min; ii. Secondary cycle denaturation: 10 cycles at 94 °C for 40 sec; iii. Touch down: 65 °C - 55 °C down to 60 °C - 45 °C for 40 sec, with amplicon extension at 72°C for 2 min, v. Primary denaturation: 30 cycles at 94 °C for 40 sec, 55 °C - 45 °C for 40 sec, with a amplicon extension of 72 °C for 2 min, iv. Final amplicon extension: 72 °C for 45 min and v. A 4°C hold until samples were removed from the machine.

Upon determining the optimum annealing temperature, primers were singly tested on a selection of parental cultivars *e.g.* 'Elegant', 'Priscilla', 'Dietrich', 'Jonathan', '*Malus*

floribunda', 'Liberty', 'Resista', 'Prima' and 'Lady Williams'. Similar PCR cycling conditions were used as mentioned above with the only major difference being in the annealing temperature. Instead of using a temperature gradient as above, a set annealing temperature obtained from the gradient PCR optimization was used. The Applied Biosystems® 2720 thermal Cycler (Applied Biosystems, Foster City CA, USA) was used to amplify the respective PCR products. Amplicons were then electrophoresed on 6 % polyacrylamide gel at 15 V/cm for 70 min and silver stained for viewing.

2.9 Multiplex/Megaplex PCR

A megaplex PCR strategy utilizing between ten and sixteen primer pairs in one amplification reaction was used in this study. The main advantage of this method is the higher throughput from large number of markers that can be analysed and generated in one experiment, by virtue of its amenability to automation (Meuzelaar *et al.,* 2007; Campoy *et al.,* 2010a; van Dyk *et al.,* 2010). Essential to the success of megaplex (which is a more advanced form of regular four to five primer multiplex PCR) is recognition of important factors that influence amplicon yield. These are a good prior knowledge of the PCR target and primer sequence to avoid primer dimer formation, the correctly increased $MgCl_2$ and dNTP concentrations. Furthermore, it has been suggested the increasing of weakly amplifying primer set concentrations compared to the stronger amplifying counterparts in the same reaction (Markoulatos *et al.,* 2002; Masi *et al.,* 2003).

UNIVERSITY of the
WESTERN CAPE

Fluorescently labelled primers were selected and grouped into megaplexes based on dye colour and amplicon size (results are shown in **Table C**, **Appendix 1**). These were used

to generate amplicons in the same PCR reaction. It must be noted that an expected PCR product size separation of at least 50 bp was allowed between primers of the same dye colour in a megaplex. A 2 - 5ng amount of genomic DNA template, as well as 0.2μM of each primer, was added to the Qiagen multiplex kit (Qiagen, Germany) as specified by the manufacturer. The thermal cycling conditions were as follows: i. Pre-cycle denaturation: 95 °C for 15min; ii. 40 cycles of: Primary denaturation: 94 °C for 30 sec, Primer annealing: 60°C for 90 sec, Amplicon extension: 72°C for 60 sec; iii. Final amplicon extension: 30 min at 60 °C and iii. A 4°C hold. The PCR reaction was performed using the 9700-Thermal Cycler (Applied Biosystems, Foster City CA, USA).

2.10 PAGE based PCR product detection

UNIVERSITY of the
WESTERN CAPE

In order to test the whether or not the primers designed worked, the expected amplicons were checked by polyacrylamide gel electrophoresis (PAGE) and then by automated capillary electrophoresis. For each sample, a 2.5x volume of polyacrylamide gel loading buffer was mixed with the sample with subsequent denaturation at 95 °C for 5 min, prior to loading it onto the gel. The denatured samples were then loaded on a 6 % polyacrylamide (19:1 acrylamide: bis acrylamide) gel, which was then was run in a 1x TBE solution at 15 V/cm for 70 min. Thereafter, the gels were silver stained in a modified quick-stain method for PCR product visualization. This method involved soaking gels in a 0.1 % (w/v) $AgNO_3$ solution for 10 minutes, followed by rinsing in water three times. After this, the gels were then soaked in a developing solution containing 1.5 % (w/v) NaOH, 0.15 % (v/v) formaldehyde and 0.01 % (w/v) $NaBH_4$, with a final rinse in water to stop the gel staining.

2.11 Capillary electrophoresis PCR product sizing

A 2 µl volume of PCR product was mixed with 10 µl of formamide in an Applied Biosystems® (ABI) plate that contained 0.2 µl of Genescan® 500 (-250) LIZ ™ size standard and denatured by heating at 96 °C for 5 min on a heating block. Immediately after denaturation was completed, the denatured mixture was then snap-cooled by placing the plate on ice for 3 to 5 min. Snap cooling was done to ensure that the now single-stranded PCR products did not re-anneal to revert to their double-stranded form once denatured. Following snap cooling, the ABI plates were set into the ABI 3130*xl* Genetic Analyzer machine stations (Applied Biosystems, Foster City CA, USA) and run with the appropriate 5-dye matrix [(containing the fluorescent dyes 6-FAM™, VIC™, NED™, PET™ and the LIZ™ or Genescan® 500 (-250) LIZ™ internal size standard (orange)] so as to assess fragment or amplicon size. A POP-7 polymer matrix and a 1x EDTA buffer were used with the 36 cm 16-capillary array in the Genetic Analyzer. Samples were injected for 15 sec at 15.000 V and separated at 15.000 V for 24 min at a run temperature of 60 °C. The resulting data was displayed as an electropherogram in the GeneMapper 4.0® amplicon-sizing software (Applied Biosystems, Foster City, CA), which was used to also score the SSR markers.

UNIVERSITY of the
WESTERN CAPE

2.12 DArT marker analysis

Diversity Array markers were implemented on the 'Anna' x 'Austin' DNA. After the genomic DNA was quantified spectrophotometrically and its integrity assessed via agarose gel electrophoresis, it was diluted to 50 ng/µl in PCR-grade DNAse free water (Qiagen, Germany) and then sent for DArT analysis (Diversity Arrays, Canberra, Australia) in a standard 20 µl volume.

The analysis was subsequently carried out by the Diversity Arrays (Canberra, Australia) according to their specifications and methodology. Resulting DArT markers were scored and converted into common codes in Microsoft® Excel™ according to the specifications in **Table 3** below. Once the sequences used to generate the DArT markers were obtained, their homologous sequences were searched for on the apple genome using the BLAST algorithm, so as to predict the likely position the sequence would map to. The results are shown in **Table D** in **Appendix 1**.

UNIVERSITY of the
WESTERN CAPE

Table 3: Conversion codes for DArT markers to JoinMap® codes according to the segregation ratios of the offspring.

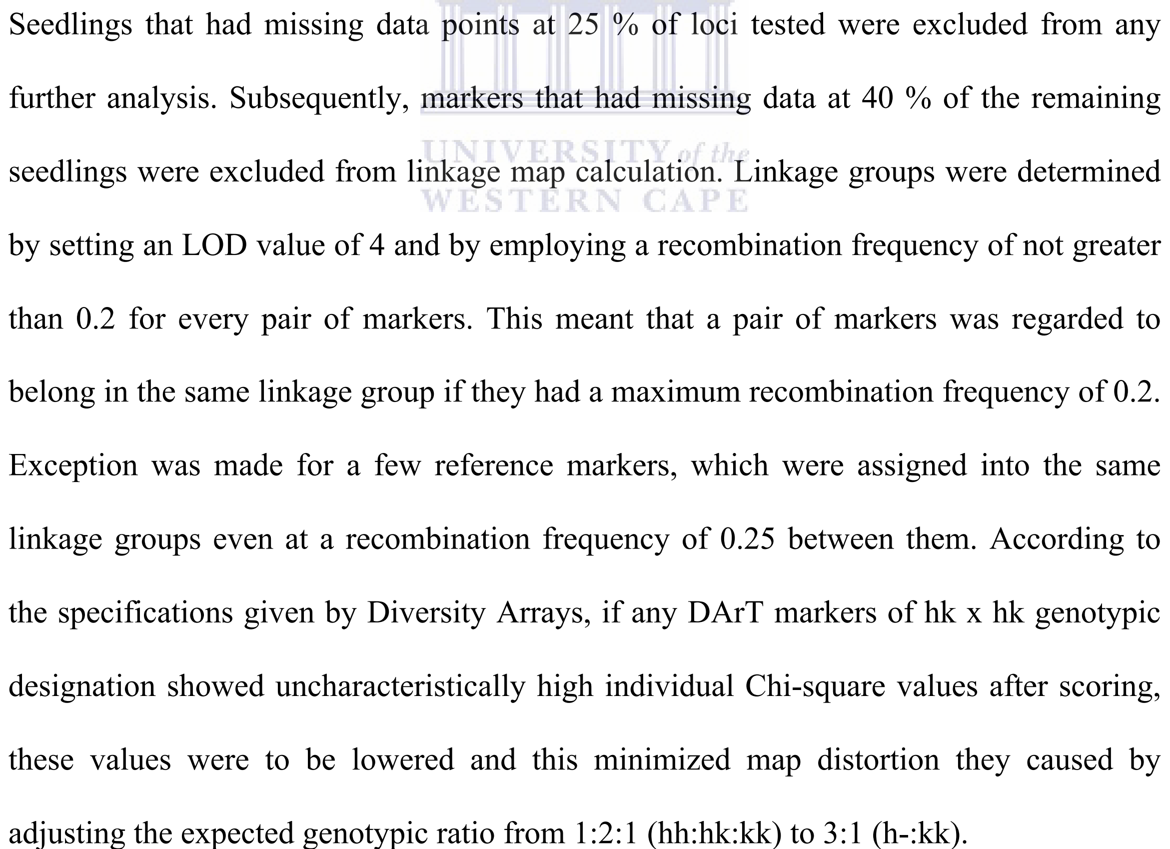
Under the parent 1 and 2 columns, results are reported as 1 or 0, indicating hybridization and non-hybridization to oligonucleotides on an array. The - indicates that the marker is either a 1 or 0 on the array, which is verified by checking the segregation ratios of individuals with that marker.

Parent 1	Parent 2	Segregation ratio guideline	Join Map code
-	**0**	1:1	lm x ll
-	**1**	1:1	lm x ll or nn x np
		3:1	hk x hk
-	**-**	1:1	lm x ll or nn x np
		3:1	hk x hk
1	**0**	-	lm x ll
1	**1**	1:1	lm x ll or nn x np
		3:1	hk x hk
0	**0**	Exclude markers	
0	**1**	-	nn x np
1	**-**	1:1	lm x ll or nn x np
		3:1	hk x hk
0	**-**	1:1	nn x np

UNIVERSITY of the WESTERN CAPE

2.13 Linkage mapping

An integrated linkage map was built using scored SSR data from GeneMapper 4.0® and DArT data scored in Microsoft® Excel™ software. These data were scored to give JoinMap® 4.1 (van Ooijen, 2006) codes nn x np, lm x ll, hk x hk, ef x eg and ab x cd depending on the segregation of the parental alleles in the 'Anna' x 'Austin' F_1 mapping population (**Table 4** below; see also **Table C, Appendix 1** for SSR markers scored in this thesis). Maps constructed in JoinMap® 4.1 were visualized graphically using MapChart® 4.0 (Voorips, 2002). Map distances and marker order were calculated according to the mapping function by Kosambi (1944).



Seedlings that had missing data points at 25 % of loci tested were excluded from any further analysis. Subsequently, markers that had missing data at 40 % of the remaining seedlings were excluded from linkage map calculation. Linkage groups were determined by setting an LOD value of 4 and by employing a recombination frequency of not greater than 0.2 for every pair of markers. This meant that a pair of markers was regarded to belong in the same linkage group if they had a maximum recombination frequency of 0.2. Exception was made for a few reference markers, which were assigned into the same linkage groups even at a recombination frequency of 0.25 between them. According to the specifications given by Diversity Arrays, if any DArT markers of hk x hk genotypic designation showed uncharacteristically high individual Chi-square values after scoring, these values were to be lowered and this minimized map distortion they caused by adjusting the expected genotypic ratio from 1:2:1 (hh:hk:kk) to 3:1 (h-:kk).

Table 4: The JoinMap® 4.1 codes for segregation types observed when working with a full-sib family, derived from an outbreeding species.

Class	Segregation type	Number of alleles	Segregating alleles		F_1 genotypes and their expected ratios	
			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	3	Yes	Yes	ee; ef; eg	2:1:1
2	hk x hk	2	Yes	Yes	hh; hk; kk h-:kk	1:2:1 3:1
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

UNIVERSITY of the WESTERN CAPE

After LG assignment of markers, markers that had been excluded as well as markers showing insufficient linkage were assigned to LGs based on Strongest Cross-Linkage Information (SCL) values. Also markers that caused suspect linkages or insufficient linkages were re-scored or excluded in any further analysis if its inclusion led a to higher mean Chi-square value for the LG. Such a case could imply a double recombination event, which is extremely rare. It is also less likely to be a true double recombination event if the markers exhibiting this behaviour are in very close proximity of a few cM from one another. This process enabled the calculation of individual LGs until all the LGs were generated.

2.14 QTL mapping

The MapQTL® 6.0 software (van Ooijen, 2004) was used to perform QTL analysis on the IVB phenotypic data collected over three years and two other essential data sets. Besides the phenotypic or quantitative data (the .qua file), each locus' information (the .loc file) and the mapped markers' data (the .map file) were required to compute putative QTLs in MapQTL® 6.0. Each year's phenotypic data was a mean value calculated from the data from seven clonal replicates. An average was also calculated for the 3 years' data. As a result there were four data sets, namely the 1996, 1997, 1998 and mean IVB data. The .loc file was generated from the markers used to create the linkage map that were exported from JoinMap® 4.1. This file among other important marker information contained each marker's phase information and segregation data. The .map file data were exported from MapChart® 2.1, showed the positions in cM. to which the linking markers mapped in their respective LGs. A putative QTL was identified as such over the LOD threshold determined by the Permutation test in MapQTL® 6.0, using 10 000 permutations as prescribed by van Ooijen (2009), but only for restricted MQM (rMQM) mapping. Co-factors for QTL analysis were chosen using the automatic co-factor selection option in MapQTL® 6.0.

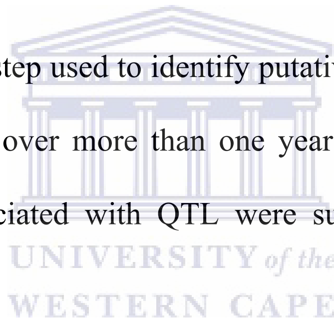
UNIVERSITY of the
WESTERN CAPE

It must be noted that in QTL mapping the mu_ac{00}, mu_ad{00} mu_bc{00}, mu_bd{00} values shown for example in **Tables 7**, **8**, **9**, and **10** represent the estimated mean of the distribution of the phenotypic value under investigation (in this thesis, time of IVB), associated with each genotypic class ac, ad, bc and bd, for the ab x cd genotype cross. This means that 'a' and 'b' alleles are inherited from the first parent ('Anna' in this

thesis) and the ‘c’ and ‘d’ alleles from the second parent (‘Austin’ in this thesis). These values can be used to assess which of the parents is contributing the most to the QTL controlling the trait. This is done by checking which of the tabulated phenotypic values mu_ac{00}, mu_ad{00} mu_bc{00}, mu_bd{00}, are closest parental means in the collected phenotypic data set. In this study however, these values (date of IVB) were only available for seedling trees and they were: 1996 (Anna - 234, Austin - 260), 1997 (Anna - 214, Austin - 250) and 1998 (Anna - 213, Austin - 253).

2.14.1 Interval mapping

Interval mapping was the first step used to identify putative QTLs. If putative QTLs were found in the same LG region over more than one year (for the years 1996, 1997 and 1998), then the markers associated with QTL were subjected to rMQM mapping to validate these QTLs.


UNIVERSITY of the
WESTERN CAPE

2.14.2 rMQM analysis

rMQM mapping is a powerful tool that can be used to localize QTLs using a limited set of carefully selected markers known as co-factors (Doerge, 2002). The rMQM mapping tool in MapQTL® 6.0 was used to check for QTLs at an error rate of 0.05 per 1000 permutations. A genome-wide LOD of 4.7 was used as a minimum threshold for putative QTLs detection, which may not have been detected for interval mapping. In this procedure, QTLs with a higher than 4.7 LOD value were used as co-factors for the discovery of other QTL in the genome, with markers associated with such QTLs noted.

Finally, QTL status was ascribed to those QTLs that were localized after multiple permutations and with a LOD value above the genome wide value of 4.7.

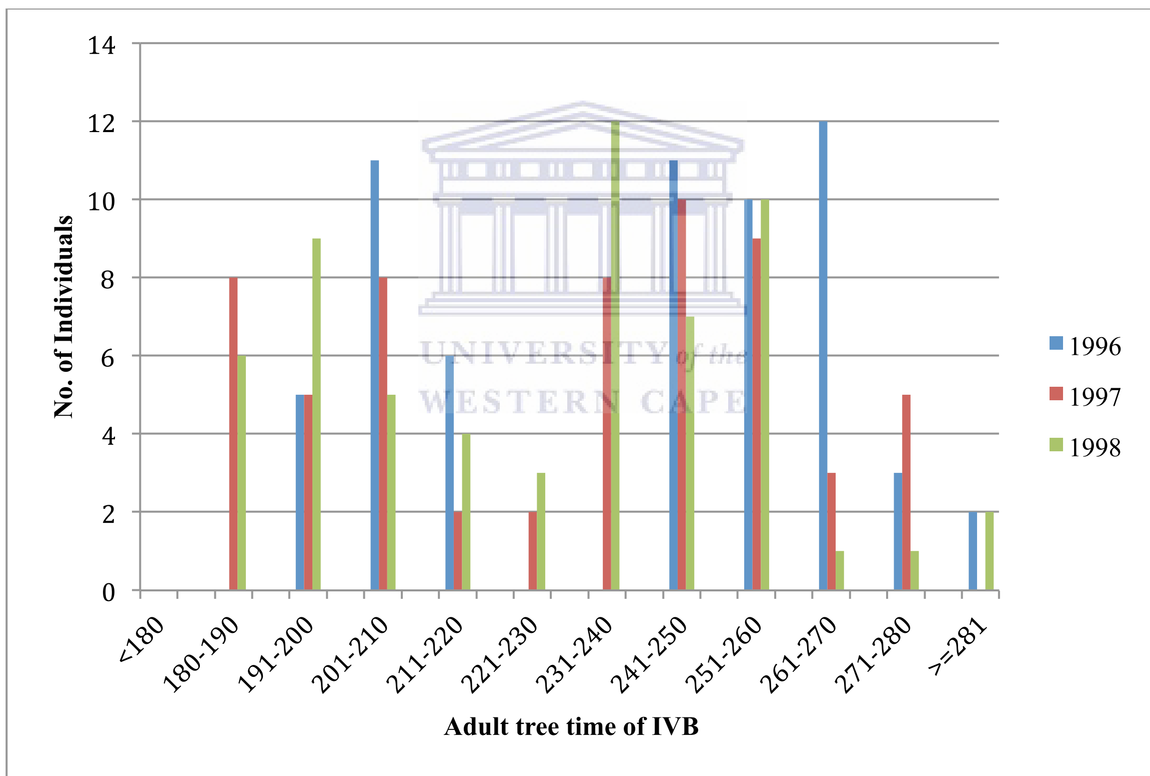
UNIVERSITY of the
WESTERN CAPE

3.0 RESULTS

3.1 Phenotypic trait assessment data

3.1.1 Time of IVB frequency distributions

Frequency distribution graphs of initial vegetative budbreak (IVB) data were generated for adult and seedling apple trees of the 'Anna' x 'Austin' mapping population, with raw data supplied by Dr. I. F. Labuschagné for the years 1996, 1997 and 1998. **Fig. 4** and **Fig. 5** below show these data summarized as frequency distribution bar graphs.



UNIVERSITY of the
WESTERN CAPE

Figure 4: 'Anna' x 'Austin' adult tree IVB frequency distribution data for the years 1996 to 1998.

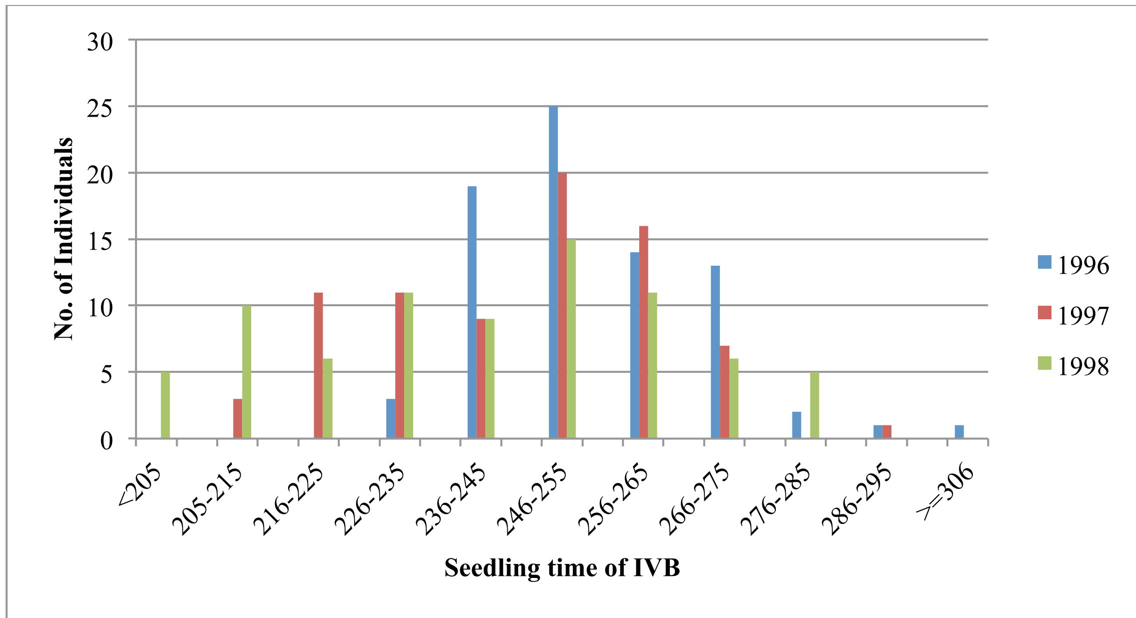


Figure 5: 'Anna' x 'Austin' seedling IVB frequency distribution data for the years 1996 to 1998.

UNIVERSITY of the
WESTERN CAPE

3.1.2 Time of IVB data trend graphs

Year-to-year data trend graphs for IVB in both adult and seedling apple trees of the 'Anna' x 'Austin' mapping population were also generated for the years 1996, 1997 and 1998 and these are shown in **Fig. 6** and **Fig. 7** below, These graphs reveal a similar data trend of pattern for all seedling and adult tree individuals over the three years 1996 to 1998, as the graphs are all virtually superimposed. These graphs give a good visual appreciation of the fact that the data followed a similar trend with few to no outliers.

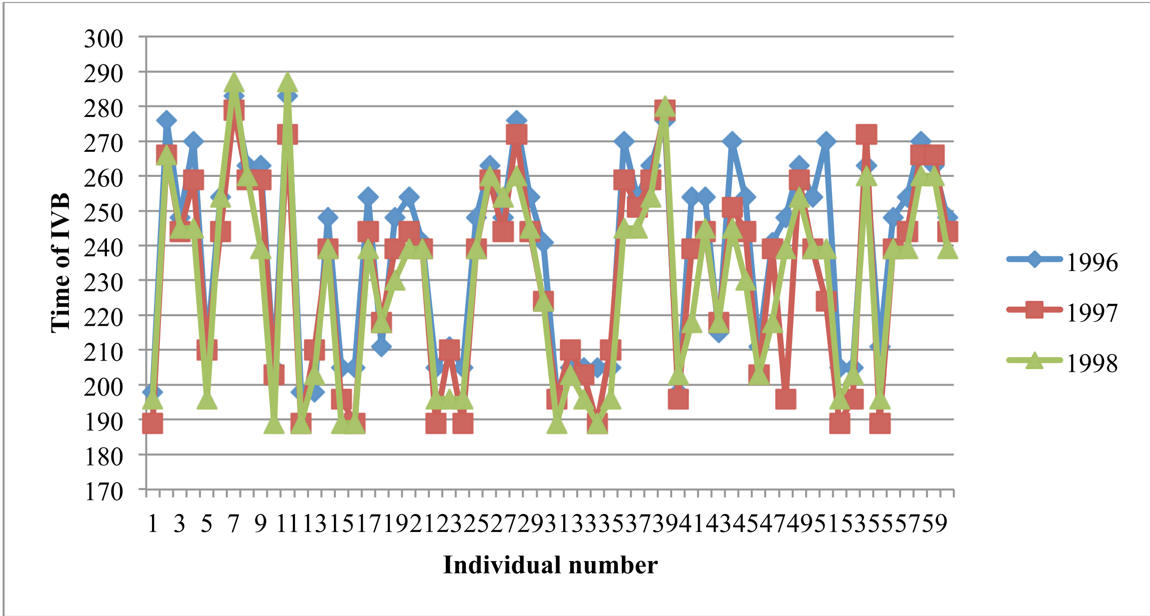


Figure 6: A graphical representation of ‘Anna’ x ‘Austin’ adult tree year-to-year IVB data compared as a data trend, over the years 1996, 1997 and 1998.

UNIVERSITY of the
WESTERN CAPE

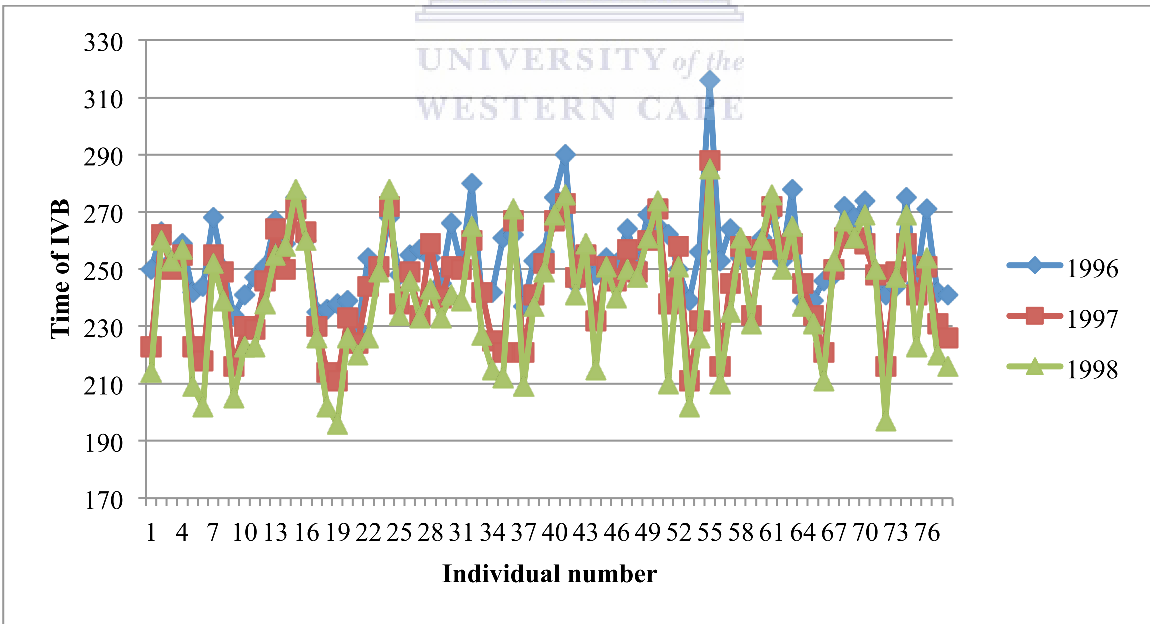


Figure 7: A graphical representation of ‘Anna’ x ‘Austin’ seedling year-to-year IVB data compared as a trend, over the years 1996, 1997 and 1998.

Table 5: Pearson's correlation coefficients (R) showing phenotypic association ($P<0.0001$) between different years for time of initial vegetative budbreak (IVB) for 'Anna' x 'Austin' adult and seedling trees.

Tree growth stage	Association between different years of phenotypic trait assessment		
	1996 + 1997	1996 + 1998	1997 + 1998
Seedling tree* R-values	0.80	0.78	0.96
Adult tree R-values	0.93	0.94	0.92

*Clonal replicates

UNIVERSITY of the
WESTERN CAPE

For both seedling and adult trees there was a high correlation or association, with the minimum and maximum R-values being 0.78 (seedling trees 1996 + 1997 comparison) and 0.96 (seedling trees 1997 + 1998 comparison) respectively, given that a correlation of 1.00 would indicate the best possible correlation. Also, seedling and adult tree R-values were on average 0.85 and 0.93 respectively over the three sets of data comparisons. The latter information is a reflection of the fact that there is a higher association of the adult tree data compared to the seedling data, as the adult trees show higher R-values over the three pair-wise data comparisons.

3.2 Genomic DNA extraction

3.2.1 Agarose gel electrophoresis

The ‘Anna’ x ‘Austin’ mapping population’s genomic DNA was extracted via the CTAB extraction protocol (**section 2.4**) was electrophoresed on a 1 % Agarose gel stained with ethidium bromide and visualized under UV light. This is shown is **Fig. 8** below.

UNIVERSITY of the
WESTERN CAPE

Figure 8: A 1 % Agarose gel run of the ‘Austin’ x ‘Anna’ population’s genomic DNA. The gel was run as follows; Lane 1: pTZ molecular weight marker; Lanes 2 to 11: offspring DNA and; Lanes 12 and 13 ‘Austin’ and ‘Anna’ parental DNA respectively.

3.2.2 Spectrophotometric genomic DNA quantification

As described in **section 2.6**, the genomic DNA extracted from the ‘Anna’ x ‘Austin’ population using the CTAB method, was quantified spectrophotometrically. **Table 6** below shows the respective quantities of genomic DNA extracted for each of the samples used in this work. Samples 182 and 159 had the lowest genomic DNA quantities of 972.3 ng/µl and 51 ng/µl, at $OD_{260/280}$ ratios of 1.78 and 1.64 respectively. The average DNA yield for the samples was 380.7 ng/µl at an average $OD_{260/280}$ ratio of 1.76.

Table 6: A list of the respective quantities of genomic DNA quantitated spectrophotometrically using the NanoDrop® ND-1000 system (NanoDrop® Technologies).

Sample name	DNA quantity (ng/µl)	OD $_{260/280}$ ratio	Sample name	DNA quantity (ng/µl)	OD $_{260/280}$ ratio
Austin	926.9	1.73	157	287.5	1.78
Anna	380.4	1.78	158	158.2	1.81
11	464.5	1.82	159	51.0	1.64
14	264.5	1.84	160	588.7	1.81
16	265.5	1.80	161	397.8	1.72
19	108.0	1.74	162	334.3	1.77
110	147.4	1.84	163	519.2	1.76
115	175.3	1.79	164	210.7	1.73
118	437.9	1.84	165	906.8	1.76
121	96.5	1.75	166	295.1	1.77
122	606.6	1.75	167	350.6	1.78
123	256.5	1.82	168	101.7	1.81
124	343.3	1.82	169	508.1	1.75
125	82.8	1.60	171	214.3	1.70
126	143.5	1.76	172	571.5	1.75
127	908.2	1.95	175	162.4	1.68
128	802.9	1.83	176	311.6	1.76
129	506.5	1.75	177	57.2	1.44
130	601.1	1.73	178	554.6	1.84
131	342.4	1.75	179	430.8	1.81
132	460.5	1.75	181	306.6	1.74
133	178.0	1.69	182	972.3	1.78
134	214.1	1.76	183	472.2	1.72
136	104.6	1.74	184	413.9	1.85
137	440.4	1.72	185	281.2	1.82
139	672.8	1.77	186	201.1	1.77
141	120.3	1.84	187	267.1	1.76
142	249.3	1.74	188	134.9	1.76
143	328.5	1.77	189	930.2	1.87
144	205.7	1.74	190	135.2	1.78
145	640.9	1.75	191	461.7	1.75
147	354.2	1.79	192	524.9	1.74
148	302.8	1.77	193	479.0	1.73
149	362.3	1.77	194	556.9	1.74
150	439.6	1.74	195	196.6	1.82
152	425.5	1.78	196	679.9	1.74
153	232.6	1.73	197	70.3	1.48
154	426.4	1.75	198	321.3	1.70
155	702.1	1.68	199	173.0	1.64
156	629.5	1.86	1100	511.5	1.81

UNIVERSITY of the
WESTERN CAPE

3.4 SSR Primer design

Primers were developed from the methodology described in **section 2.7**. **Fig. 9** below shows the user interface in the Tandem Repeats Database. One of these datasets used for primer design contained approximately 1 137 repeats in set of *Malus* Expressed Sequence Tags (ESTs). Also, as exemplified by **Fig. 9**, these repeats were filtered using the Tandem Repeats Finder program (Benson, 1999) employing a set of parameters that determined the quality of the repeats mined from the database.

Tandem Repeats Database

FAQs | Help | About TRDB | Privacy Statement | Log Out | Contact Us| News | IRDB | LBI

home
sequences
projects
sets
partitions
reports
tools
my account

> sets > view repeats

set name: **Malus EST since 010606 - 255/30**
description:
status: **Done**
created by: **Jasper Rees**
created on: **2007-03-05**
repeats: **1137**
project: **Pome**
organism: **Malus**

	Filtering Options			
☑	Pattern Size	>=	2.000000	
☑	%Matches	>=	90.000000	
☑	Score	>	40.000000	
☑	First Index	>=	20.000000	
(help)	--field--	--op--		apply

Group by sequence: ☐
Order by: First Index ascending

repeats (after filters): **222** (help)

1 **2** **3** <next>
goto page 1 out of **3** go

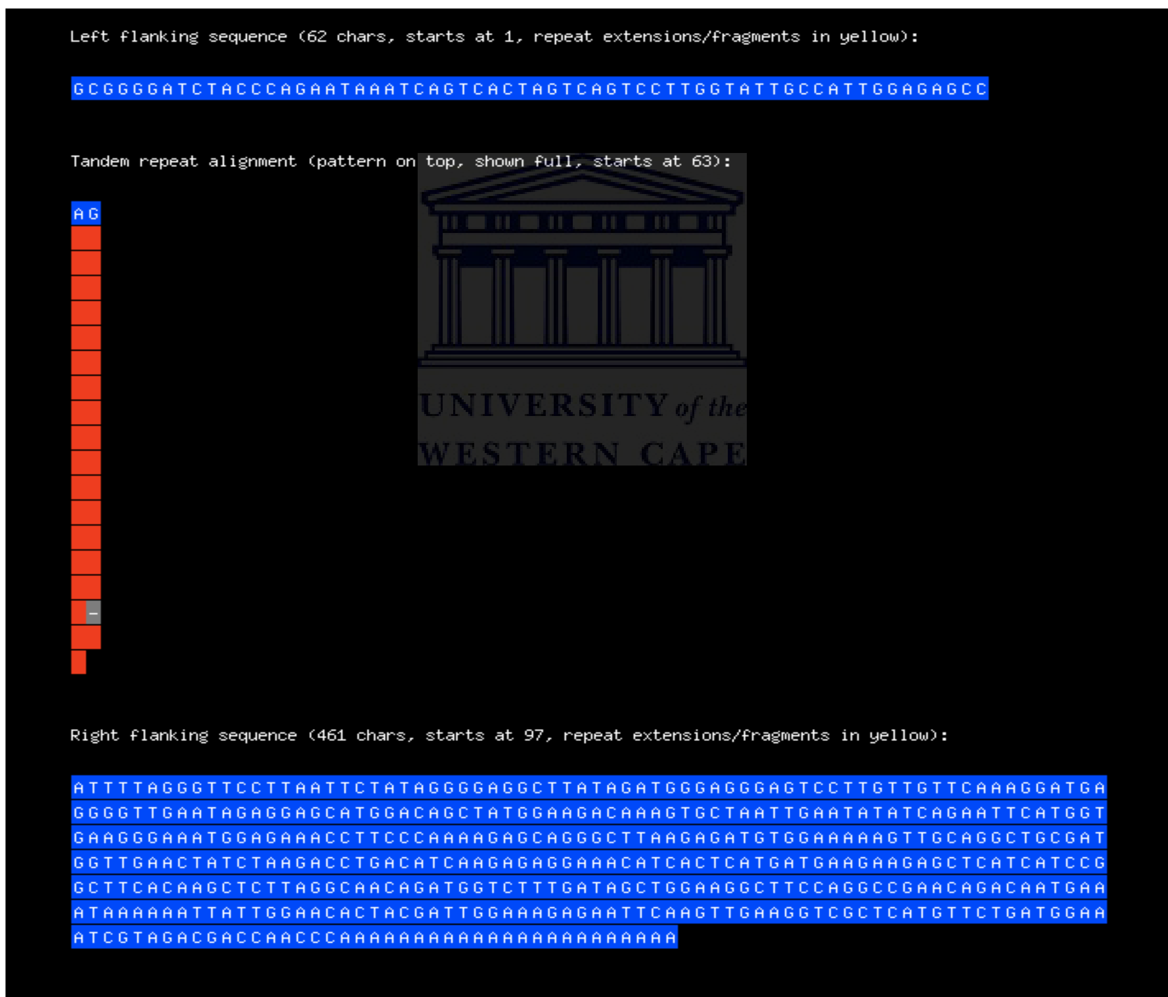
Save As Set | Save Into Report | View Multiple Alignment | Download | Change Columns

☑	Indices	Pattern Size	Copy Number	%Matches	%Mismatches	%Indels	%A	%C	%G	%T	Score	Array Length	Fasta Header
☑	23--61 | [browser]	2	19.500000	100	0	0	0	0	48	51	78	39	embl|EE663922|EE663922 010115AALB002608CO (AALB) Royal Gala 150 DAFB fruit cortex Malus x domestica cDNA clone AALBB00260, mRNA sequence.
☑	24--53 | [browser]	2	15.000000	93	7	0	0	56	0	43	46	30	embl|EH034544|EH034544 K1_G09 Erwinia amylovora challenged cDNA suppression subtractive hybridization library down-regulated genes at 24 hours Malus x domestica cDNA, mRNA sequence.
☑	24--57 | [browser]	17	2.000000	100	0	0	29	17	11	41	68	34	embl|EH034607|EH034607 M1_C12 Erwinia amylovora challenged cDNA suppression subtractive hybridization library down-regulated genes at 48 hours Malus x domestica cDNA, mRNA sequence.
☑	34--61 | [browser]	8	3.400000	92	5	3	53	35	3	7	42	28	embl|EG974794|EG974794 G2_C04a Erwinia amylovora challenged cDNA suppression subtractive hybridization library late upregulated genes Malus x domestica cDNA, mRNA sequence.
☑	35--63 | [browser]	15	1.900000	96	4	0	31	13	24	31	51	29	embl|EH090770|EH090770 O2_D01 Erwinia amylovora challenged cDNA suppression subtractive hybridization library up-regulated genes at 2 hours Malus x domestica cDNA, mRNA sequence.

UNIVERSITY of the
WESTERN CAPE

Figure 9: The user interface of the Tandem Repeats Database in which the *Malus* ESTs data set.

These parameters were a pattern size of greater than or equal to 2 (dinucleotide repeats); a percentage match of greater than or equal to 90 %; an overall sequence match score of greater that 40 and a first index (number of bases that could be used for primer design) of greater or equal to 20. In the example below, this filtering process led to the lowering of the number of repeats from 1 137 to 222. **Fig. 10** below shows a snapshot of such a sequence selected from the previous list shown in **Fig. 9.**



UNIVERSITY of the
WESTERN CAPE

Figure 10: A graphical display of a typical sequence shown in the tandem repeats database. This sequence is centred on a dinucleotide repeat array.

Furthermore, the sequence in **Fig. 10** in a simple fasta format could be viewed without the graphical display as is shown in **Fig. 11** below. This view is less detailed than any of the previously shown sequence displays and only shows the repeat array and its pattern and its left and right flanking sequences. The length of the left and right flanking sequences can be specified here, but this is limited to the length of flanking sequence available around the repeat array.

left flanking sequence:

gcggggatctacccagaataaatcagtcactagtcagtccttggtattgccattggagagcc

pattern:

AG

sequence:

agagagagagagagagagagagagagagagaaga

UNIVERSITY of the
WESTERN CAPE

right flanking sequence:

attttagggttccttaattctatagggaggcttatagatgggagggagtccttgttgttcaaaggatga
ggggttgaatagaggagcatggacagctatggaagacaaagtgctaattgaatatatcagaattcatggt
gaagggaaatggagaaaccttcccaaaagagcagggcttaagagatgtggaaaaagttgcaggctgcgat
ggttgaactatctaagacctgacatcaagagaggaaacatcactcatgatgaagaagagctcatcatccg
gcttcacaagctcttaggcaacagatggtctttgatagctggaaggcttccaggccgaacagacaatgaa
ataaaaaattattggaacactacgattggaaagagaattcaagttgaaggtcgctcatgttctgatggaa
atcgtagacgaccaacccaaaaaaaaaaaaaaaaaaaaaa

HIDE FLANKING SEQUENCES

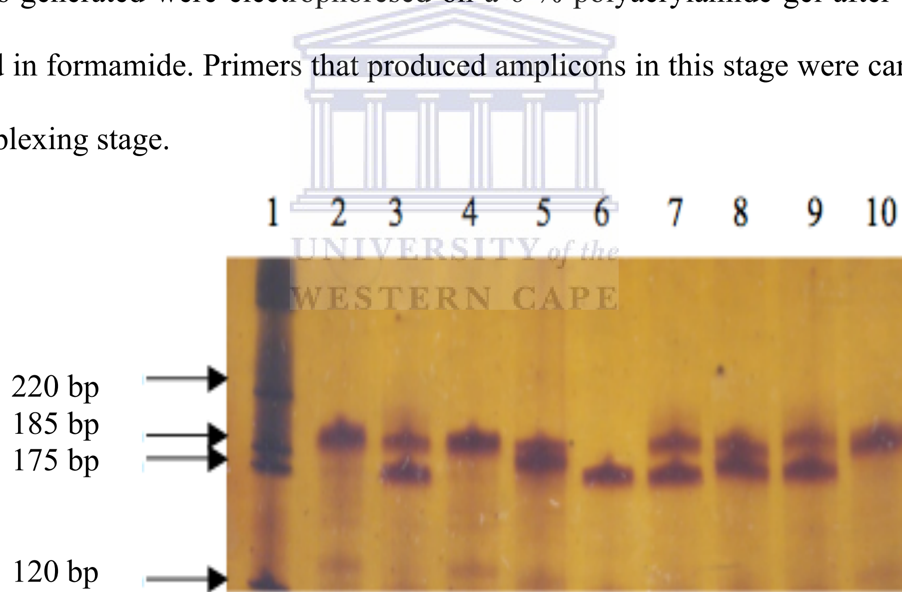
Figure 11: A nucleotide level or fasta format view of the dinucleotide repeat sequence array, the left and right flanking sequence and the description of the dinucleotide repeat pattern.

Primers were designed based on the rules specified in **section 2.7**, from the left and right flanking sequences of a repeat array, such as that shown in **Fig. 11** above. The results are

shown in **Table B**, **Appendix 1** (the 98 SSR primer pairs developed in this thesis numbered 592 - 763 and 834. 836 - 870 and have the bold and italicized prefix "***SAms***").

3.5 Simplex PCR primer testing

Once designed, primers were tested on DNA extracted from parental cultivars using touchdown PCR with gradient, using the conditions mentioned in **section 2.8**. Shown below in **Fig. 12** is the polymorphic PCR amplification products for published primer CH04e03 and genomic DNA from a selected set of parental cultivars. The PCR amplicons generated were electrophoresed on a 6 % polyacrylamide gel after they were denatured in formamide. Primers that produced amplicons in this stage were carried on to the multiplexing stage.



UNIVERSITY of the
WESTERN CAPE

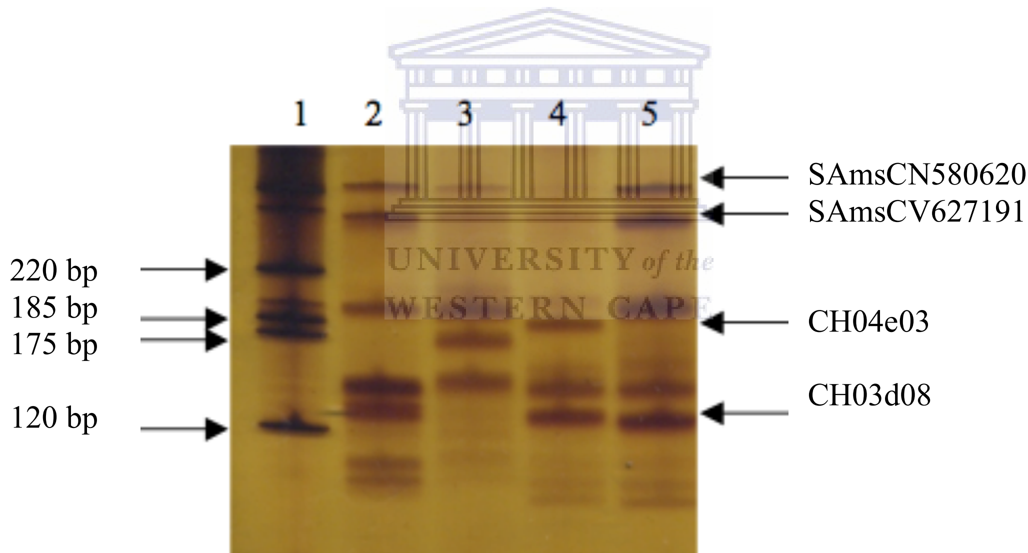
Figure 12: A 6 % silver stained polyacrylamide gel of PCR amplicons derived form parental cultivar DNA and marker CH04e03.

Lane 1: pTZ molecular weight marker, Lane 2: 'Elegant', Lane 3: 'Priscilla', Lane 4: 'Dietrich' Lane 5: 'Jonathan', Lane 6: '*Malus floribunda*', Lane 7: 'Liberty', Lane 8: 'Resista', Lane 9: 'Prima' and Lane 10: 'Lady Williams'.

3.6 Multiplex and Megaplex PCR development

3.6.1 Polyacrylamide gel electrophoresis based detection

Once primers had passed the simplex PCR testing phase they were grouped according to expected amplicon size and fluorescent dye colour as described in **section 2.9**. An example of a set of four primers that were employed in constructing a multiplex are shown in **Fig. 13** below, where DNA from four apple cultivars was run with markers SAmsCN580620, SAmsCV6277191, CH04e03 and CH03d08. They were visualized using the silver stained gel electrophoresis as specified in **section 2.10.** Primers that were tested and could be successfully multiplexed were then used in megaplex PCR.



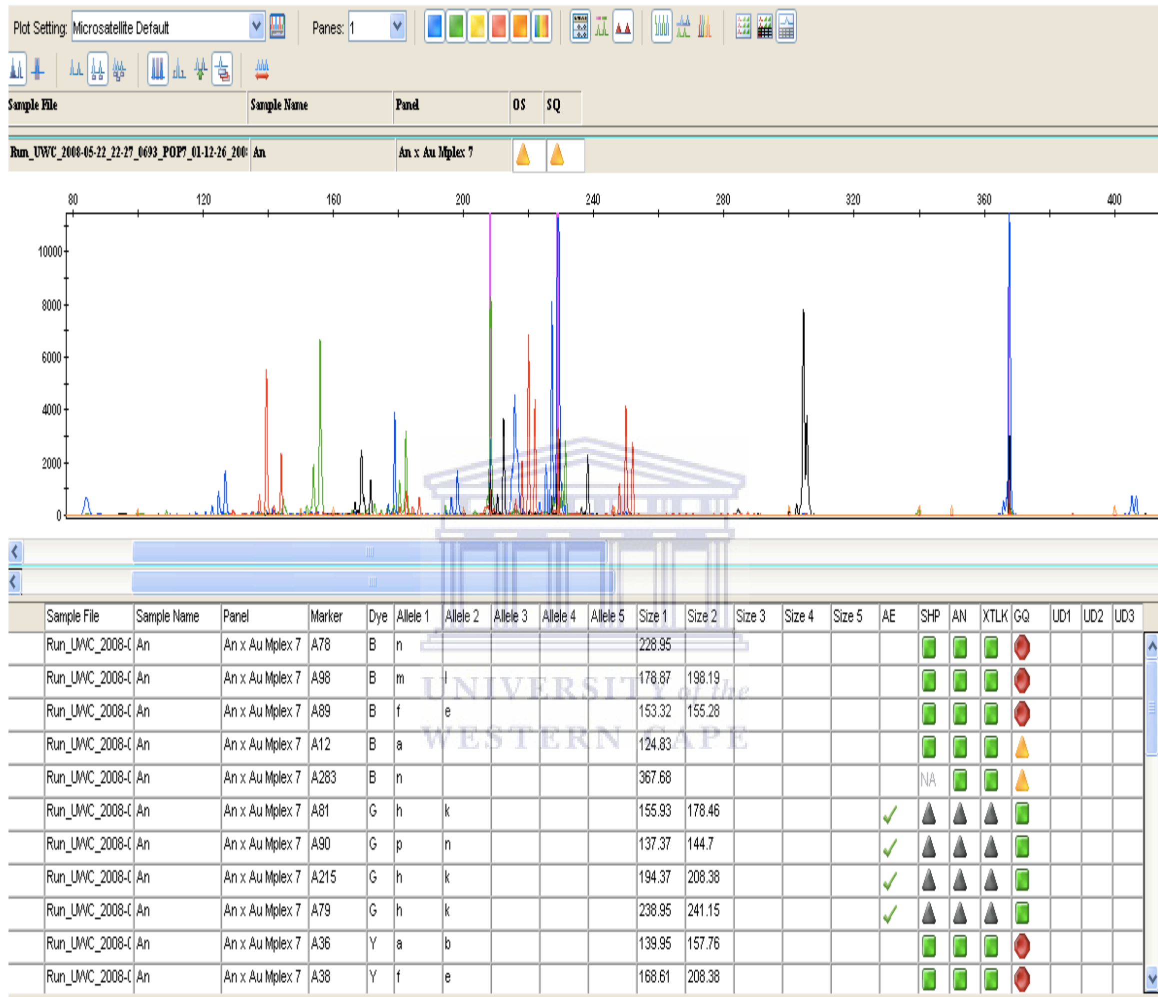
UNIVERSITY of the WESTERN CAPE

Figure 13: A 6 % silver stained PAGE of a four-primer PCR multiplex run for DNA from four apple cultivars. Lane 1: pTZ molecular weight marker, Lane 2: 'Golden Hornet', Lane 3: 'Russian Seedling'; Lane 4: 'Prima', Lane 5: 'Lady Williams'.

Because of the complex nature of the PCR amplicon visualization on a polyacrylamide gel, the megaplex PCR (employing 12 to 16 primer pairs) products were visualized using capillary electrophoresis on the ABI 3130*xl* Genetic Analyzer.

3.6.2 Megaplex PCR

Primers were grouped together into megaplexes (**Table C** in **Appendix 1)** according to expected size and fluorescent dye colour as specified in **section 2.9** and run in PCR.



Sample File	Sample Name	Panel	Marker	Dye	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Size 1	Size 2	Size 3	Size 4	Size 5	AE	SHP	AN	XTLK	GQ	UD1	UD2	UD3
Run_UWC_2008-(	An	An x Au Mplex 7	A78	B	n					228.95												
Run_UWC_2008-(	An	An x Au Mplex 7	A98	B	m	l				178.87	198.19											
Run_UWC_2008-(	An	An x Au Mplex 7	A89	B	f	e				153.32	155.28											
Run_UWC_2008-(	An	An x Au Mplex 7	A12	B	a					124.83												
Run_UWC_2008-(	An	An x Au Mplex 7	A283	B	n					367.68						NA						
Run_UWC_2008-(	An	An x Au Mplex 7	A81	G	h	k				155.93	178.46				✓							
Run_UWC_2008-(	An	An x Au Mplex 7	A90	G	p	n				137.37	144.7				✓							
Run_UWC_2008-(	An	An x Au Mplex 7	A215	G	h	k				194.37	208.38				✓							
Run_UWC_2008-(	An	An x Au Mplex 7	A79	G	h	k				238.95	241.15				✓							
Run_UWC_2008-(	An	An x Au Mplex 7	A36	Y	a	b				139.95	157.76											
Run_UWC_2008-(	An	An x Au Mplex 7	A38	Y	f	e				168.61	208.38											

UNIVERSITY of the WESTERN CAPE

Figure 14: Electropherograms obtained after amplification of the 'Anna' parental DNA using megaplex 17 (**Table C** in **Appendix 1**).

Fig. 14 above gives scoring data graphically and as a table, with the latter showing some of the SSR primers used in the megaplex and the size and alleles scored (columns 3 to 7 and 11 and 12). Red circles indicate that the marker needs checking before proceeding, and the green square shows that a marker is of god enough quality for scoring.

3.6.3 Capillary electrophoresis using ABI 3130*xl* Genetic Analyzer

Capillary electrophoresis was carried out as described in **section 2.11** on PCR fragments generated from a set of primers used to design megaplexes, the results of which were analysed and visualised as electropherograms using GeneMapper® 4.0 shown below in **Fig. 15.**

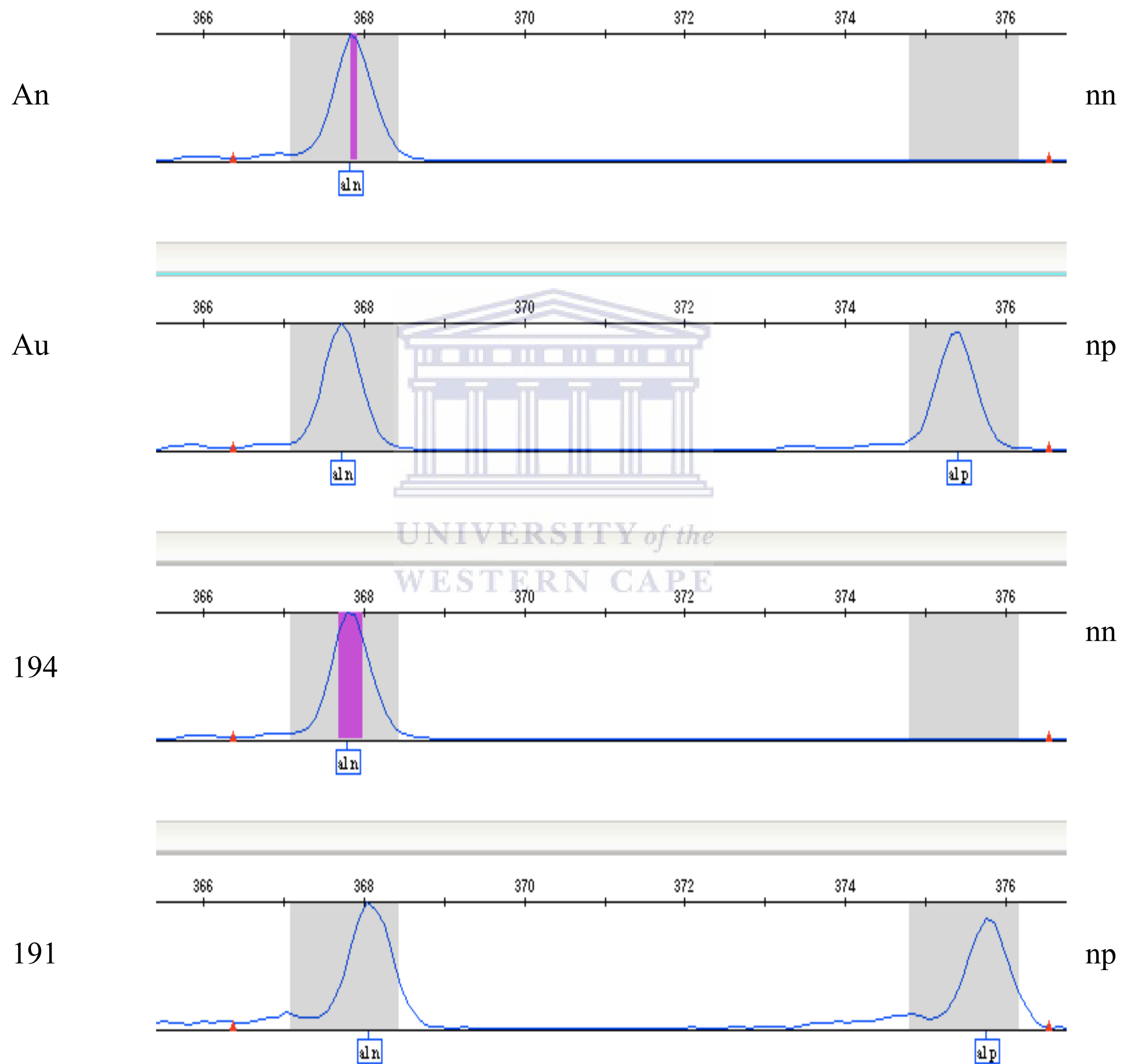


Figure 15: Electropherograms showing four PCR samples run on the ABI 3130*xl* Genetic Analyzer.

UNIVERSITY of the
WESTERN CAPE

The SAmsCN921216 marker was run on DNA from the samples ‘Anna’, ‘Austin’, 194 and 191.The two different genotype classes are shown in **Fig. 15** for the progeny, namely nn and np, meaning the alleles generated by the marker segregate for the second parent ‘Austin’, as this is the parent in which variation is detected at the locus being analysed in this example.

3.7 DArT markers

1	CloneID	MarkerName	P	Q	Call Rate	PIC	P-ANNA	P-AUSTIN	P-137	P-129	P-183	P-193	P-157	P-192	P-186	P-154	P-162	P-188	P-130
2																			
3	So	aPa-442046	96,016	95,093	98,113	0,479	1	0	0	0	0	1	1	1	0	1	0	1	0
4	183485	aPa-183485	95,911	95,006	99,057	0,496	1	0	-	0	0	0	0	1	1	1	1	1	1
5	441502	aPa-441502	95,792	94,888	99,057	0,5	1	0	0	0	0	1	1	1	1	1	0	1	1
6	185262	aPa-185262	95,584	94,682	98,113	0,497	1	0	-	0	0	0	0	1	1	1	1	1	1
7	442112	aPa-442112	95,464	94,564	99,057	0,499	1	0	0	0	0	0	0	1	1	1	1	1	1
8	442750	aPa-442750	95,48	94,553	97,17	0,481	1	0	0	0	0	1	1	1	0	1	0	1	0
9	526121	aPa-526121	95,273	94,348	97,17	0,481	1	0	0	0	0	1	1	1	0	1	0	1	0
10	525699	aPa-525699	95,191	94,266	97,17	0,481	1	0	0	0	0	1	1	1	0	1	0	1	0
11	442173	aPa-442173	95,138	94,24	98,113	0,499	1	0	0	0	0	-	0	1	1	1	1	1	1
12	442722	aPa-442722	95,028	94,096	95,283	0,495	1	0	0	0	1	0	-	1	0	1	0	1	1
13	461514	aPa-461514	94,97	94,074	99,057	0,478	1	0	0	1	0	1	1	0	1	1	1	1	0

Figure 16: A snapshot of the raw DArT results before conversion to JoinMap® 4.1 codes.

UNIVERSITY of the WESTERN CAPE

In **Fig. 16** above, marker names are shown with the ‘aPa-’ prefix and sample names are shown with a ‘P-’ prefix. DArT markers were converted to useable JoinMap® format from their raw form shown in **Fig. 16** above according to the ratio of hybridizing (1) and non-hybridizing markers (0) in the population. This was done in accordance to the methodology described in **section 2.12**. A total set of 787 markers was generated for this work by Diversity Arrays. Of this total, 285 (36.3 %) were excluded from scoring because of their skewed segregation ratios. A collection of 502 were taken forward and

scored according to the methodology described in **section 2.12** and used in JoinMap® 4.1. A final subset of 314, which represent 62.5 % of the DArT markers that were found to be useful, were mapped using JoinMap® 4.1. Useful markers that could not be mapped, clustered into small linkage groups in the map building exercise with JoinMap® 4.1 and could therefore, not be assigned to any linkage group with certainty as there was no reference map to do this with. **Table D** in **Appendix 1** shows the entire set of 502 DArT markers scored for the 'Anna' x 'Austin' population with their segregation types.

3.8 Genetic linkage map construction

An integrated genetic linkage map was constructed for the F_1 population derived from the 'Anna' x 'Austin' population using JoinMap® 4.1. The expected 17 linkage groups were named according to those published by Maliepaard *et al.* (1998). The Silfverberg-Dilworth *et al.* (2006) map was also used as a reference map, to designate markers in unknown groups to the appropriate linkage groups.

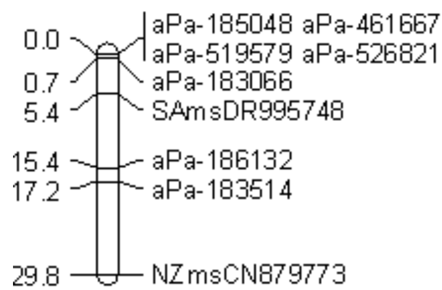
UNIVERSITY of the
WESTERN CAPE

In the 'Anna' x 'Austin' integrated genetic map shown in **Fig. 17** below, 17 linkage groups were generated and these spanned 1 212.6 cM. This map consists of a total of 429 markers: 115 SSR and 314 DArT markers. This represents roughly, an average marker density of a marker every 3 to 10 cM. The longest linkage group created was LG 17, which was a total of 152,7 cM, though it was in three segments of 54.4 cM, 53.9 cM and 44.4 cM. The shortest linkage group was LG 3, which was 30.1 cM. The average linkage group length was 71.3 cM for the map. The largest gap between markers in the map was

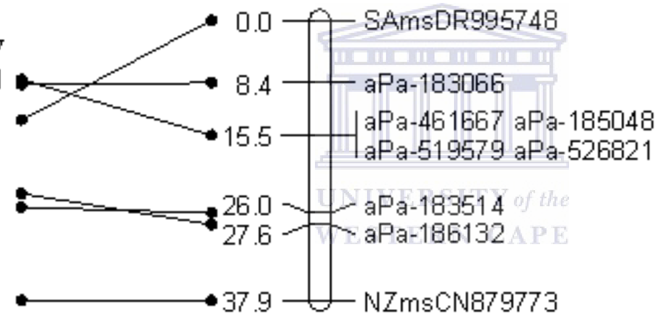
30.2 cM and 30 cM in linkage groups 11 and 2 respectively. Four LGs were composed of more than one segment and these were LGs 7, 8, 16 and 17.

UNIVERSITY of the
WESTERN CAPE

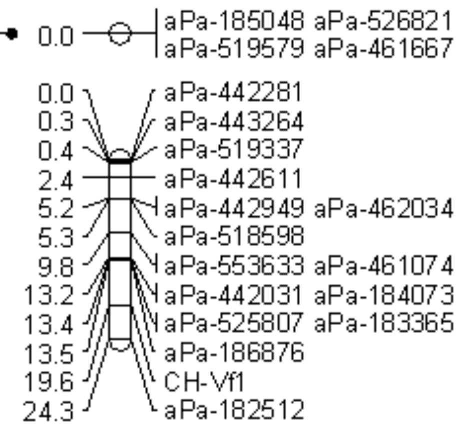
An LG1

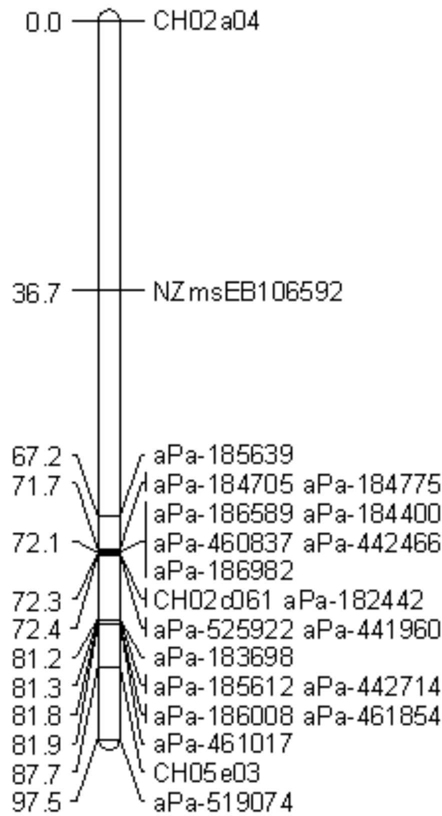


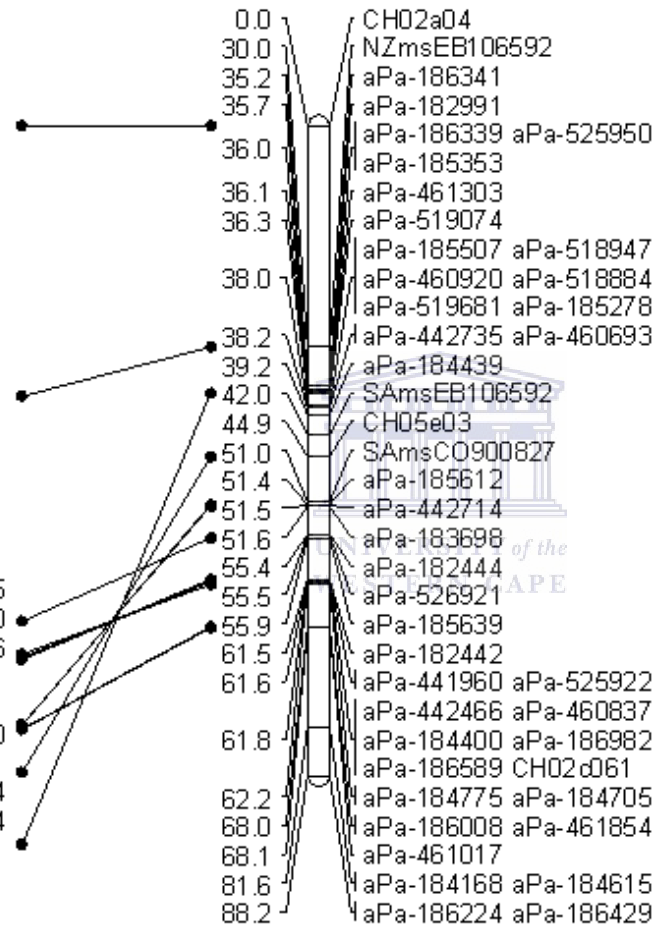
AnxAu LG1

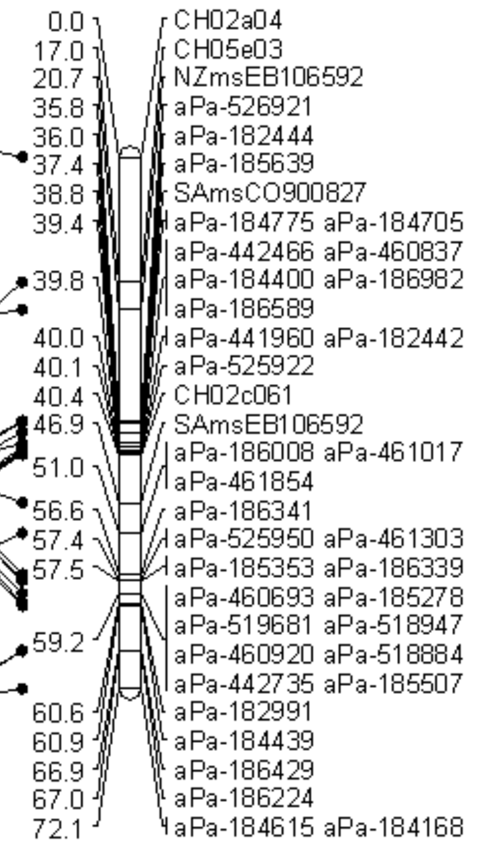


Au LG1




An LG2
0.0 CH02a04
36.7 NZmsEB106592
67.2 aPa-185639
71.7 aPa-184705 aPa-184775
aPa-186589 aPa-184400
72.1 aPa-460837 aPa-442466
aPa-186982
72.3 CH02c061 aPa-182442
72.4 aPa-525922 aPa-441960
81.2 aPa-183698
81.3 aPa-185612 aPa-442714
81.8 aPa-186008 aPa-461854
81.9 aPa-461017
87.7 CH05e03
97.5 aPa-519074


AnxAu LG2
0.0
30.0
35.2
35.7
36.0
36.1
36.3
38.0
38.2
39.2
42.0
44.9
51.0
51.4
51.5
51.6
55.4
55.5
55.9
61.5
61.6
61.8
62.2
68.0
68.1
81.6
88.2
CH02a04
NZmsEB106592
aPa-186341
aPa-182991
aPa-186339 aPa-525950
aPa-185353
aPa-461303
aPa-519074
aPa-185507 aPa-518947
aPa-460920 aPa-518884
aPa-519681 aPa-185278
aPa-442735 aPa-460693
aPa-184439
SAmsEB106592
CH05e03
SAmsCO900827
aPa-185612
aPa-442714
aPa-183698
aPa-182444
aPa-526921
aPa-185639
aPa-182442
aPa-441960 aPa-525922
aPa-442466 aPa-460837
aPa-184400 aPa-186982
aPa-186589 CH02c061
aPa-184775 aPa-184705
aPa-186008 aPa-461854
aPa-461017
aPa-184168 aPa-184615
aPa-186224 aPa-186429


Au LG2
0.0
17.0
20.7
35.8
36.0
37.4
38.8
39.4
39.8
40.0
40.1
40.4
46.9
51.0
56.6
57.4
57.5
59.2
60.6
60.9
66.9
67.0
72.1
CH02a04
CH05e03
NZmsEB106592
aPa-526921
aPa-182444
aPa-185639
SAmsCO900827
aPa-184775 aPa-184705
aPa-442466 aPa-460837
aPa-184400 aPa-186982
aPa-186589
aPa-441960 aPa-182442
aPa-525922
CH02c061
SAmsEB106592
aPa-186008 aPa-461017
aPa-461854
aPa-186341
aPa-525950 aPa-461303
aPa-185353 aPa-186339
aPa-460693 aPa-185278
aPa-519681 aPa-518947
aPa-460920 aPa-518884
aPa-442735 aPa-185507
aPa-182991
aPa-184439
aPa-186429
aPa-186224
aPa-184615 aPa-184168

An LG3

0.0 aPa-442825
0.5 aPa-461576
0.7 aPa-461291
2.2 aPa-442855
2.3 aPa-443364
30.1 SAmsCO866862

AnxAu LG3

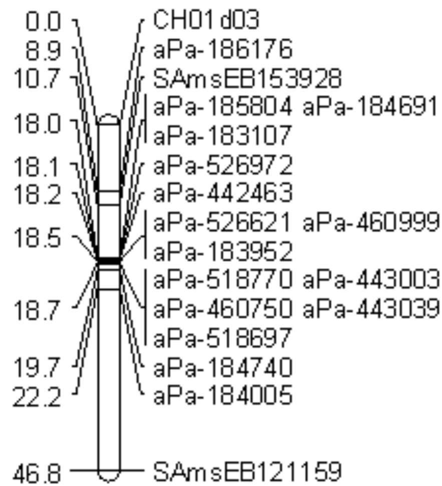
0.0 aPa-442825
0.5 aPa-461576
0.7 aPa-461291
2.2 aPa-442855
2.3 aPa-443364
30.1 SAmsCO866862

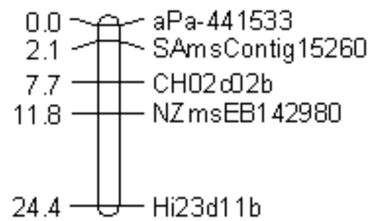
Au LG3

0.0 SAmsMDC002362.239
19.3 SAmsCN944444
35.3 SAmsCO866862

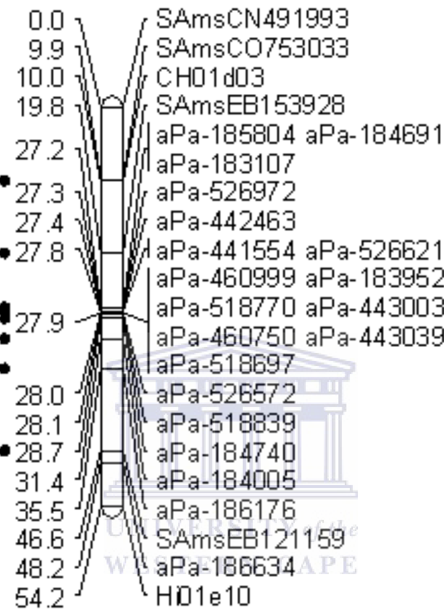
UNIVERSITY of the
WESTERN CAPE

An LG4

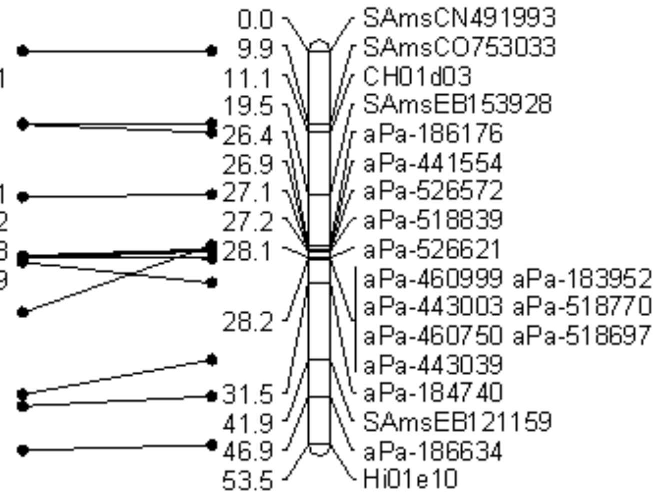


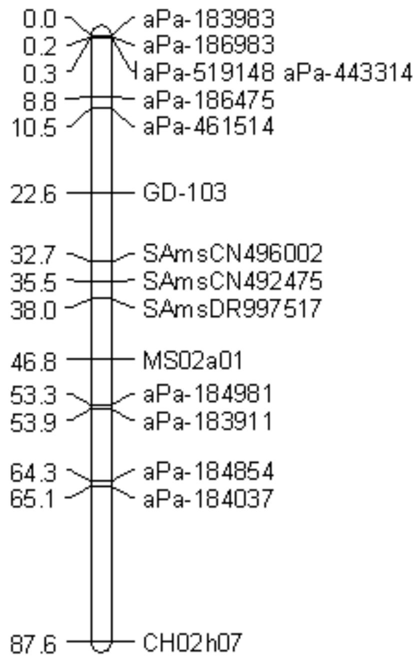


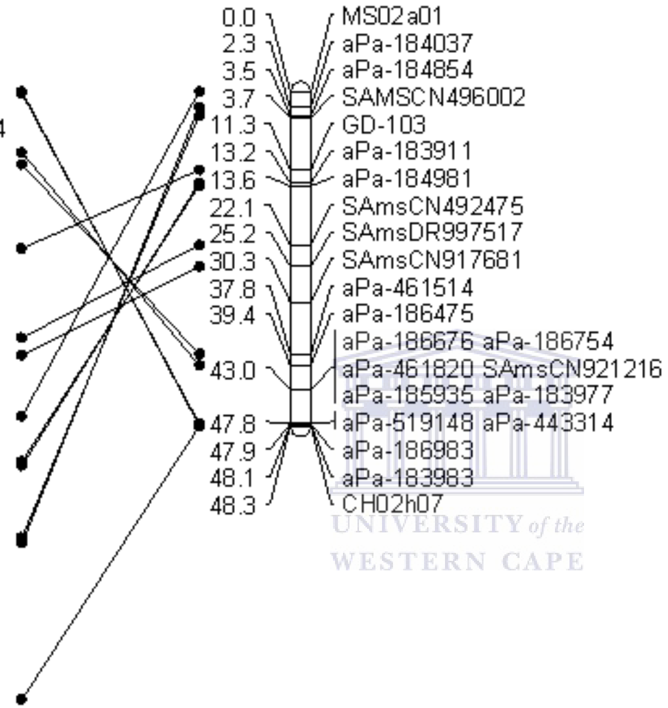
AnxAu LG4



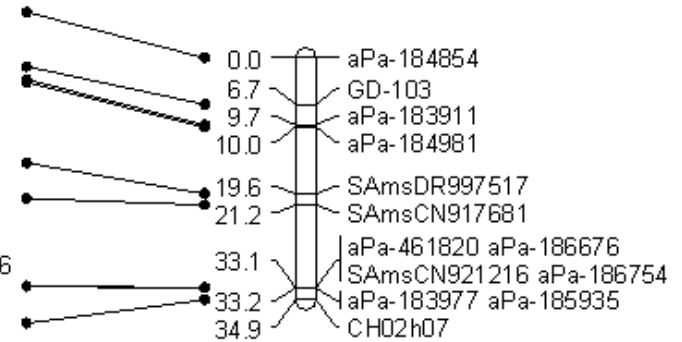
Au LG4

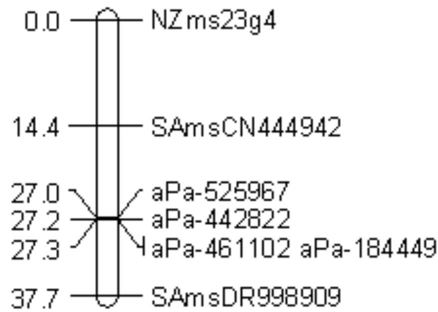


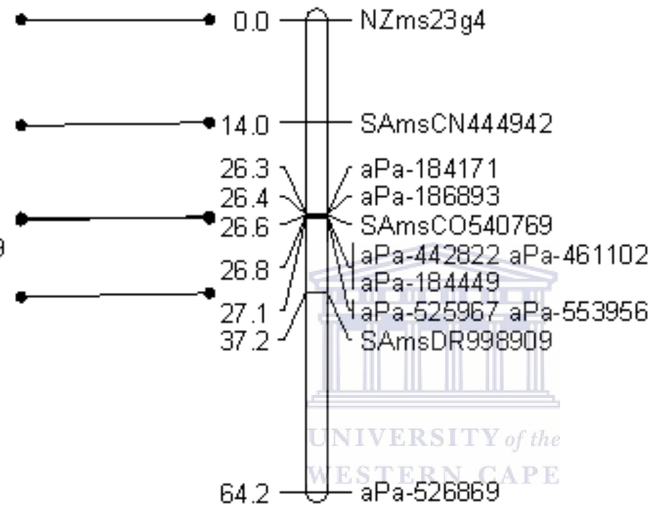


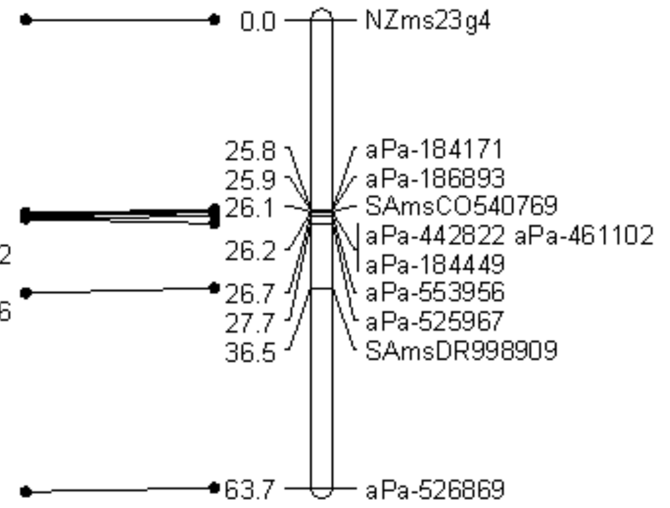


UNIVERSITY of the
WESTERN CAPE

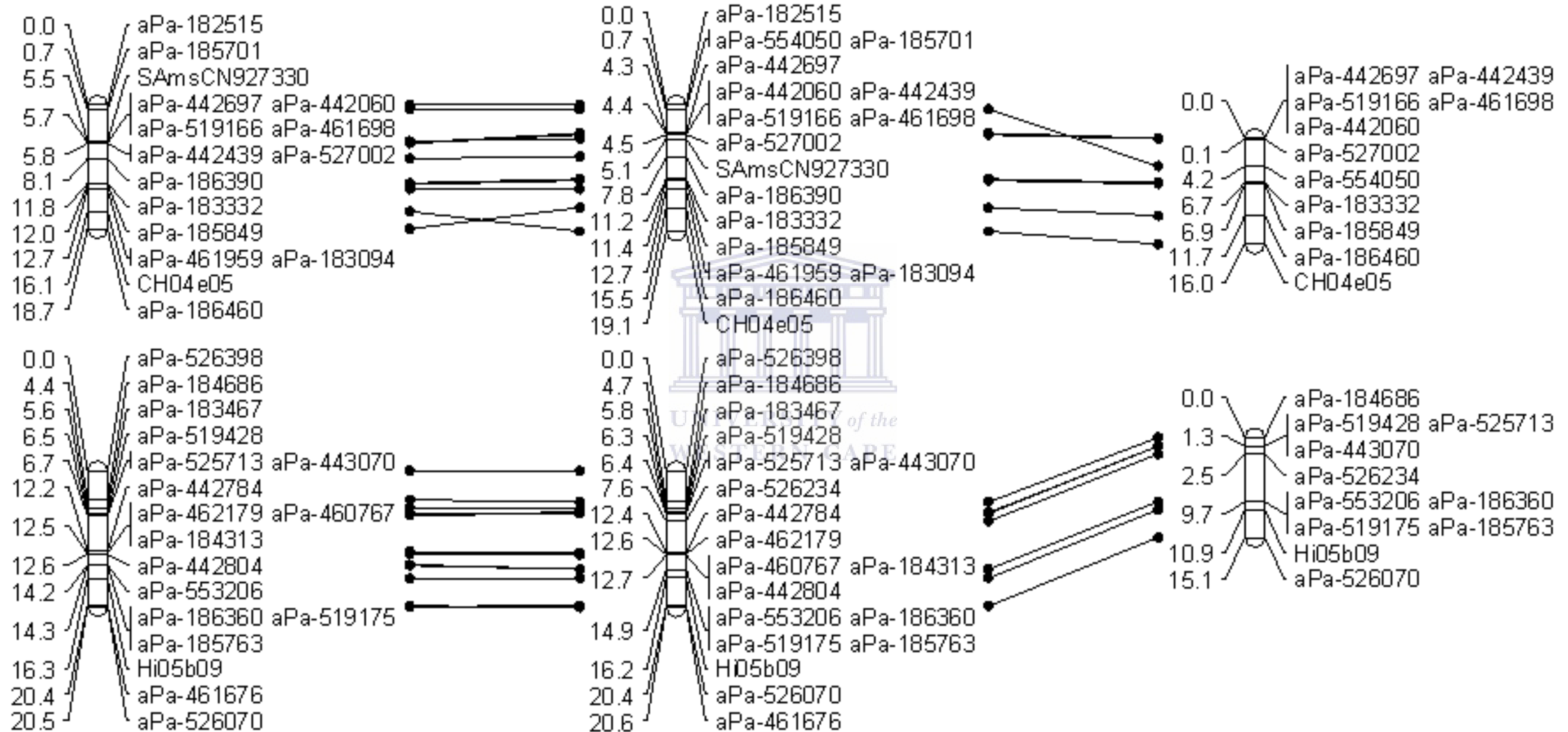






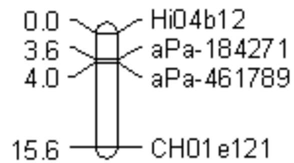


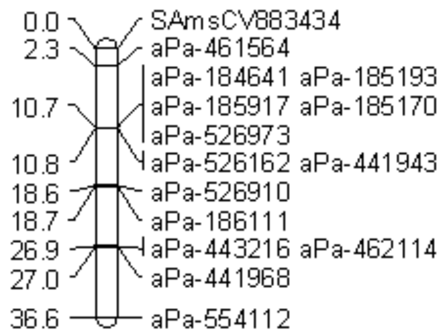
UNIVERSITY of the
WESTERN CAPE

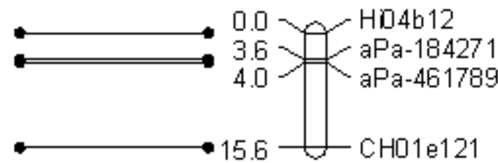


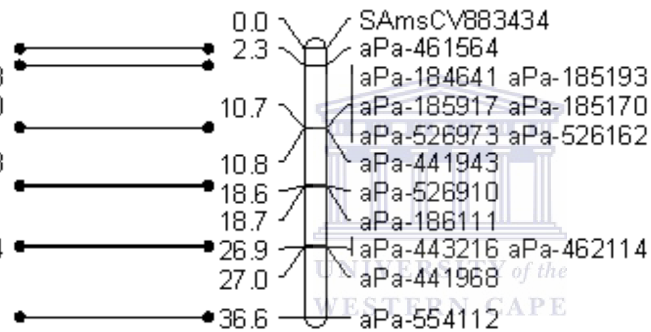
An LG7
AnxAu LG7
Au LG7
0.0 aPa-182515
0.7 aPa-185701
5.5 SAmsCN927330
5.7 aPa-442697 aPa-442060
aPa-519166 aPa-461698
5.8 aPa-442439 aPa-527002
8.1 aPa-186390
11.8 aPa-183332
12.0 aPa-185849
12.7 aPa-461959 aPa-183094
16.1 CH04e05
18.7 aPa-186460
0.0 aPa-526398
4.4 aPa-184686
5.6 aPa-183467
6.5 aPa-519428
6.7 aPa-525713 aPa-443070
12.2 aPa-442784
12.5 aPa-462179 aPa-460767
aPa-184313
12.6 aPa-442804
14.2 aPa-553206
14.3 aPa-186360 aPa-519175
aPa-185763
16.3 Hi05b09
20.4 aPa-461676
20.5 aPa-526070
0.0 aPa-182515
0.7 aPa-554050 aPa-185701
4.3 aPa-442697
4.4 aPa-442060 aPa-442439
aPa-519166 aPa-461698
4.5 aPa-527002
5.1 SAmsCN927330
7.8 aPa-186390
11.2 aPa-183332
11.4 aPa-185849
12.7 aPa-461959 aPa-183094
15.5 aPa-186460
19.1 CH04e05
0.0 aPa-526398
4.7 aPa-184686
5.8 aPa-183467
6.3 aPa-519428
6.4 aPa-525713 aPa-443070
7.6 aPa-526234
12.4 aPa-442784
12.6 aPa-462179
12.7 aPa-460767 aPa-184313
aPa-442804
14.9 aPa-553206 aPa-186360
aPa-519175 aPa-185763
16.2 Hi05b09
20.4 aPa-526070
20.6 aPa-461676
0.0 aPa-442697 aPa-442439
aPa-519166 aPa-461698
aPa-442060
0.1 aPa-527002
4.2 aPa-554050
6.7 aPa-183332
6.9 aPa-185849
11.7 aPa-186460
16.0 CH04e05
0.0 aPa-184686
1.3 aPa-519428 aPa-525713
aPa-443070
2.5 aPa-526234
9.7 aPa-553206 aPa-186360
aPa-519175 aPa-185763
10.9 Hi05b09
15.1 aPa-526070

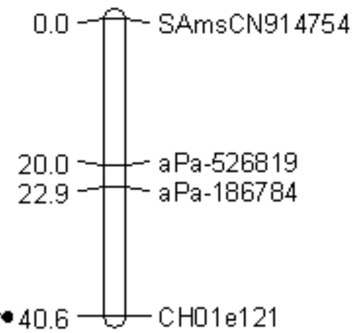
An LG 8 **AnxAu LG8** **Au LG8**

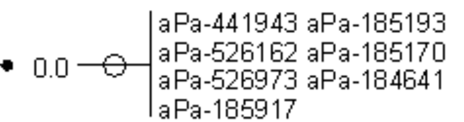




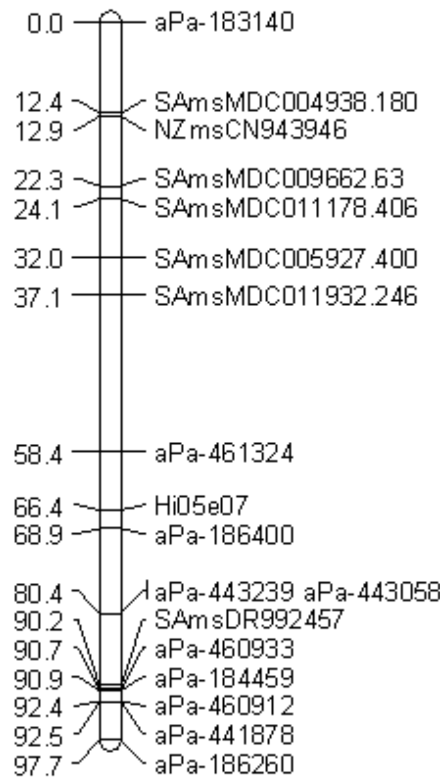


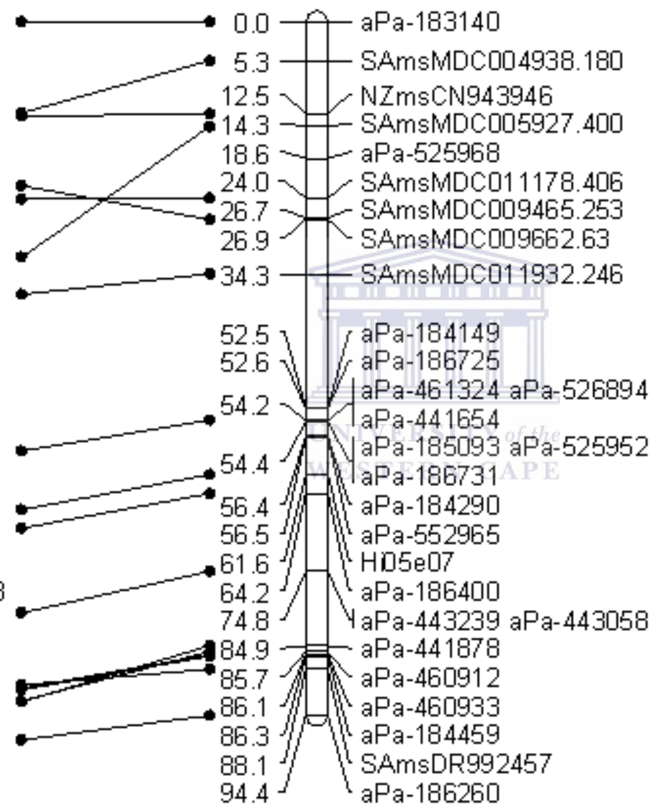


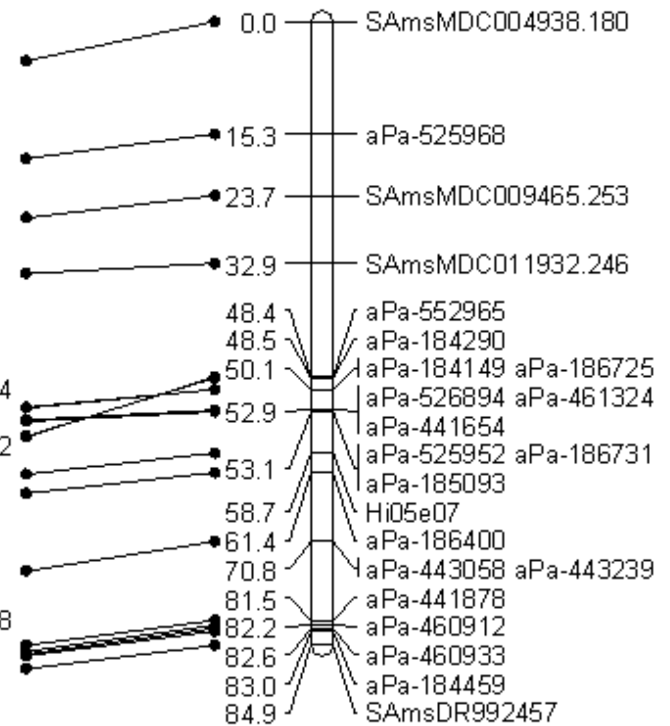




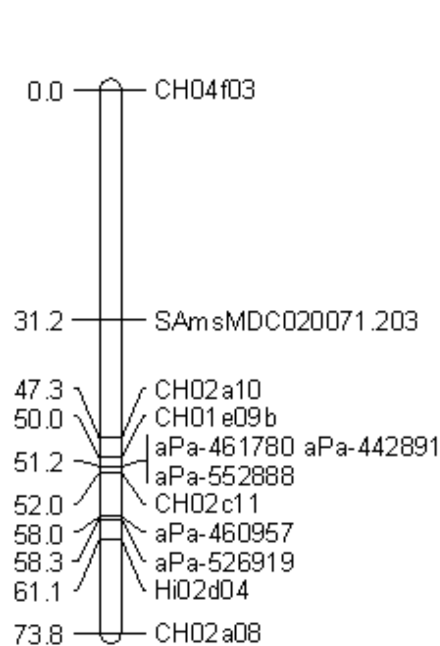
UNIVERSITY of the
WESTERN CAPE


An LG9
0.0 aPa-183140
12.4 SAmsMDC004938.180
12.9 NZmsCN943946
22.3 SAmsMDC009662.63
24.1 SAmsMDC011178.406
32.0 SAmsMDC005927.400
37.1 SAmsMDC011932.246
58.4 aPa-461324
66.4 Hi05e07
68.9 aPa-186400
80.4 aPa-443239 aPa-443058
90.2 SAmsDR992457
90.7 aPa-460933
90.9 aPa-184459
92.4 aPa-460912
92.5 aPa-441878
97.7 aPa-186260

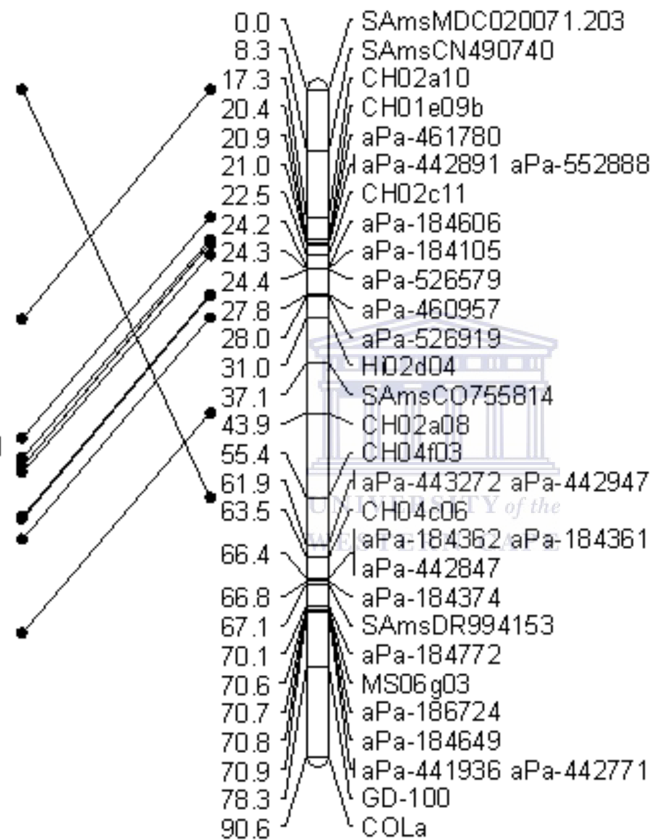

AnxAu LG9
0.0 aPa-183140
5.3 SAmsMDC004938.180
12.5 NZmsCN943946
14.3 SAmsMDC005927.400
18.6 aPa-525968
24.0 SAmsMDC011178.406
26.7 SAmsMDC009465.253
26.9 SAmsMDC009662.63
34.3 SAmsMDC011932.246
52.5 aPa-184149
52.6 aPa-186725
54.2 aPa-461324 aPa-526894
aPa-441654
aPa-185093 aPa-525952
54.4 aPa-186731
56.4 aPa-184290
56.5 aPa-552965
61.6 Hi05e07
64.2 aPa-186400
74.8 aPa-443239 aPa-443058
84.9 aPa-441878
85.7 aPa-460912
86.1 aPa-460933
86.3 aPa-184459
88.1 SAmsDR992457
94.4 aPa-186260


Au LG9
0.0 SAmsMDC004938.180
15.3 aPa-525968
23.7 SAmsMDC009465.253
32.9 SAmsMDC011932.246
48.4 aPa-552965
48.5 aPa-184290
50.1 aPa-184149 aPa-186725
52.9 aPa-526894 aPa-461324
aPa-441654
53.1 aPa-525952 aPa-186731
aPa-185093
58.7 Hi05e07
61.4 aPa-186400
70.8 aPa-443058 aPa-443239
81.5 aPa-441878
82.2 aPa-460912
82.6 aPa-460933
83.0 aPa-184459
84.9 SAmsDR992457

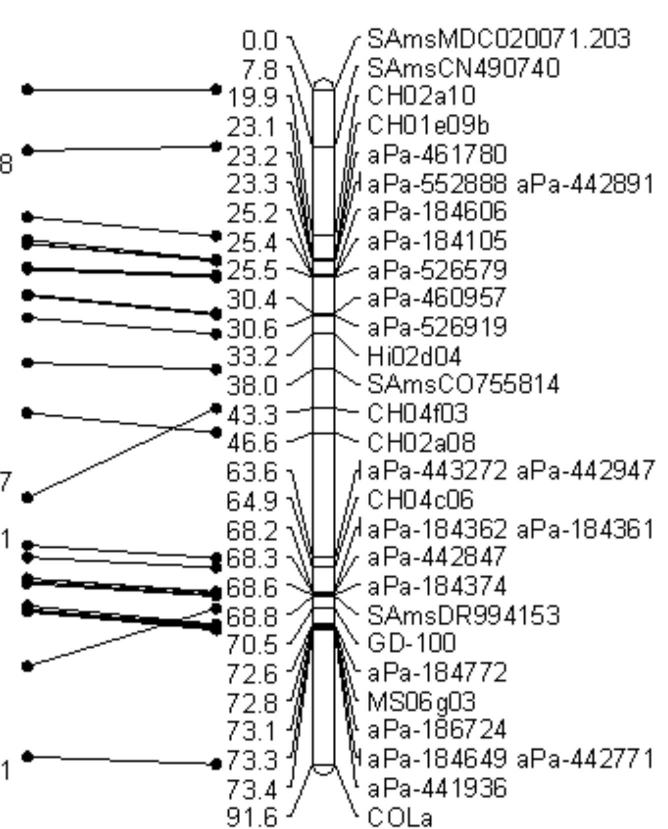
An LG10

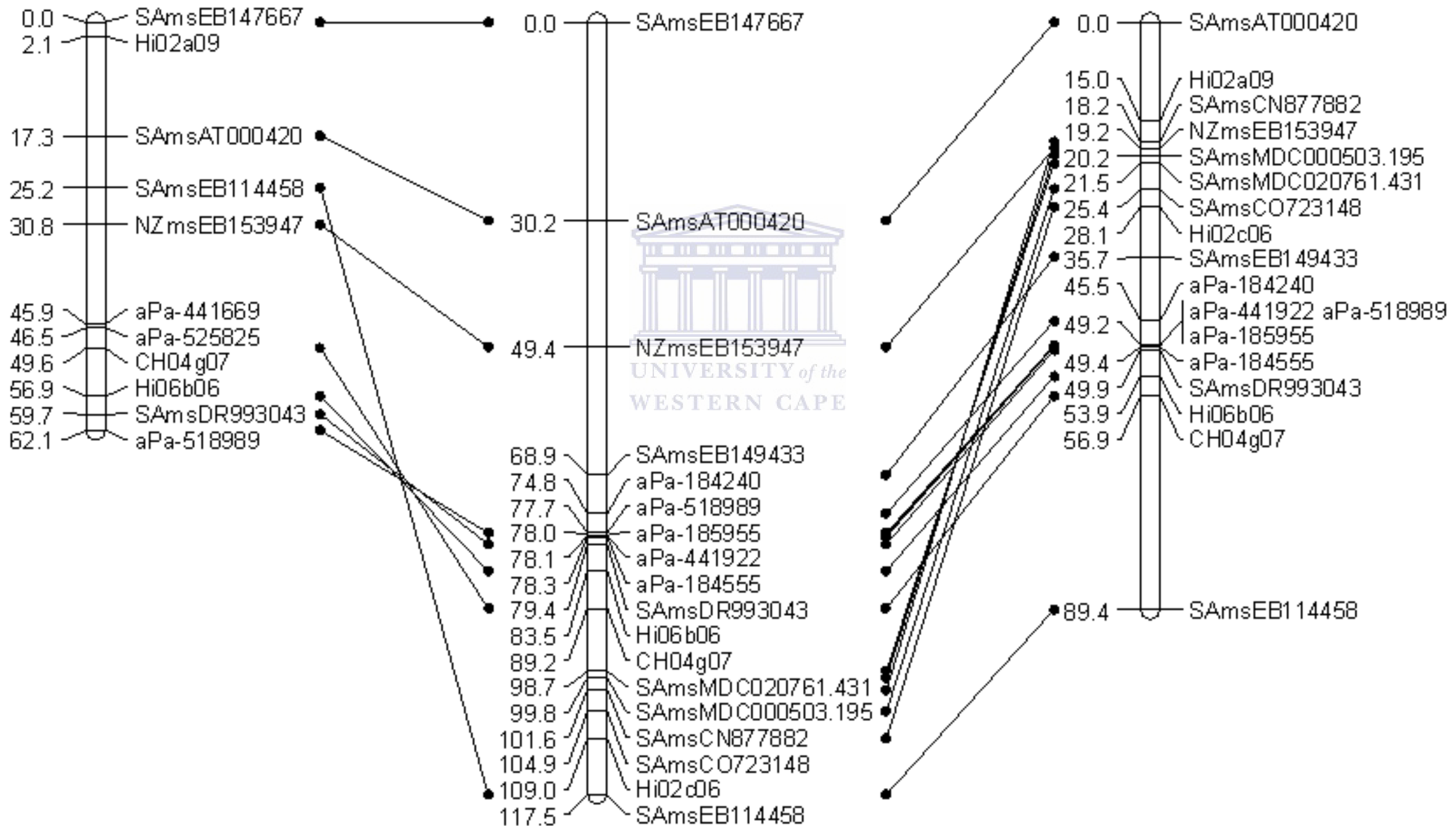


AnxAu LG10

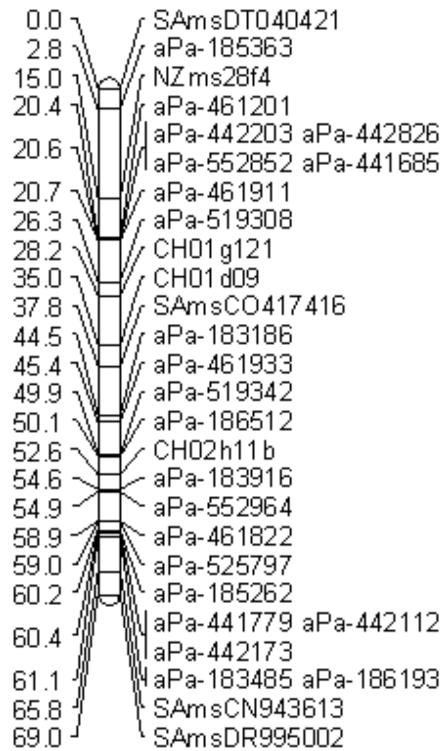


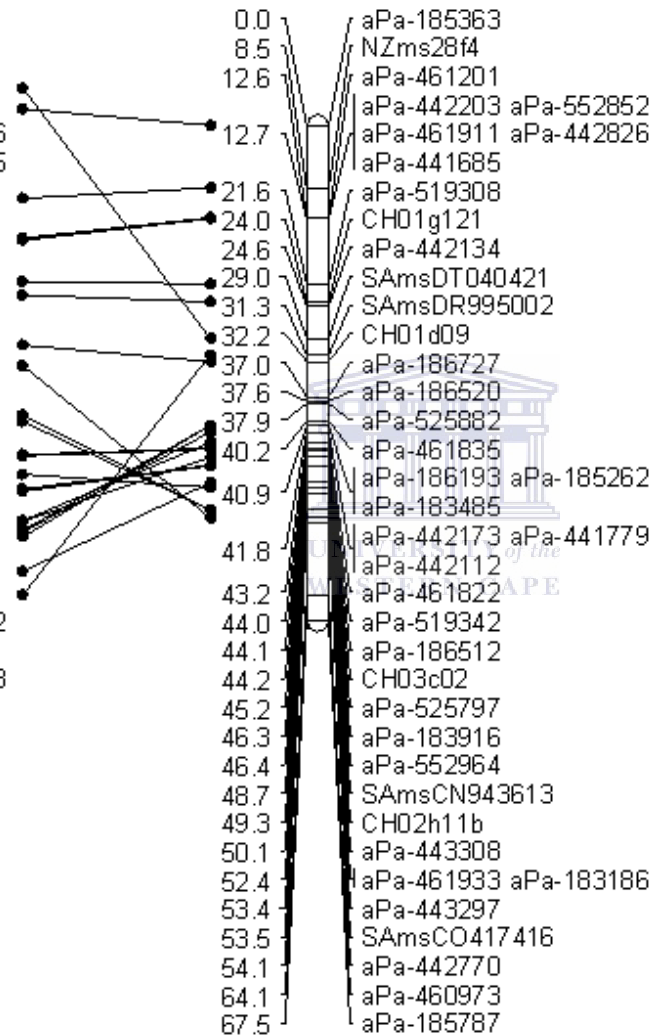
Au LG10

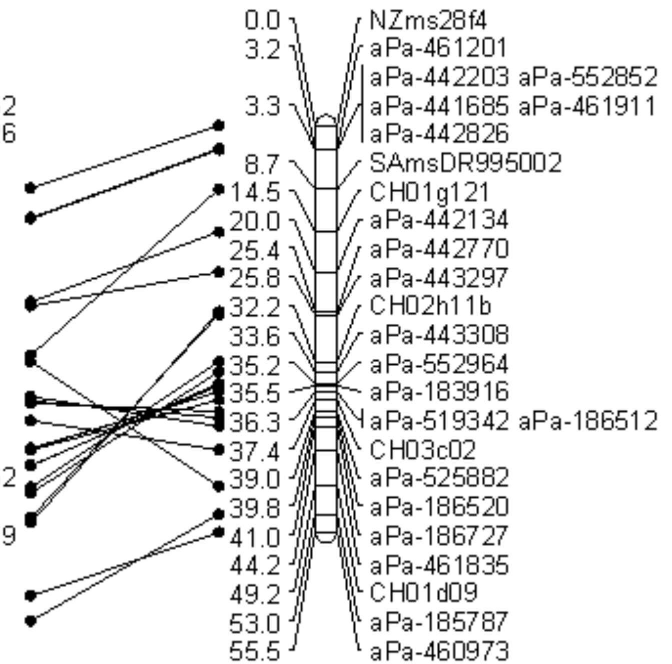




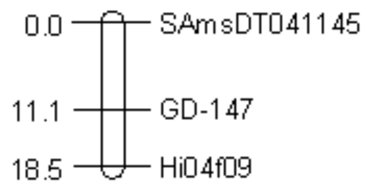
UNIVERSITY of the
WESTERN CAPE


An LG12
0.0
2.8
15.0
20.4
20.6
20.7
26.3
28.2
35.0
37.8
44.5
45.4
49.9
50.1
52.6
54.6
54.9
58.9
59.0
60.2
60.4
61.1
65.8
69.0
SAmsDT040421
aPa-185363
NZms28f4
aPa-461201
aPa-442203 aPa-442826
aPa-552852 aPa-441685
aPa-461911
aPa-519308
CH01g121
CH01d09
SAmsCO417416
aPa-183186
aPa-461933
aPa-519342
aPa-186512
CH02h11b
aPa-183916
aPa-552964
aPa-461822
aPa-525797
aPa-185262
aPa-441779 aPa-442112
aPa-442173
aPa-183485 aPa-186193
SAmsCN943613
SAmsDR995002

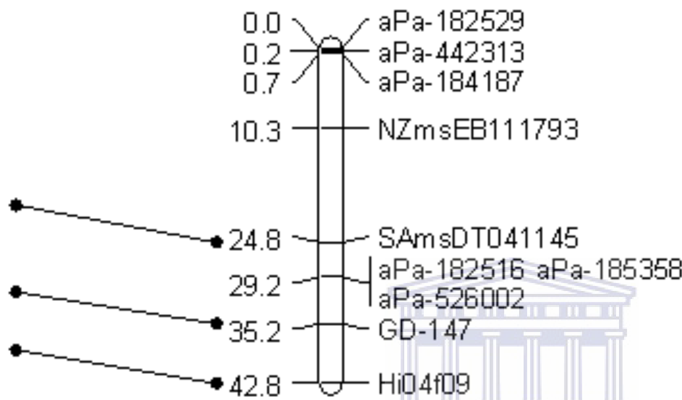

AnxAu LG 12
0.0
8.5
12.6
12.7
21.6
24.0
24.6
29.0
31.3
32.2
37.0
37.6
37.9
40.2
40.9
41.8
43.2
44.0
44.1
44.2
45.2
46.3
46.4
48.7
49.3
50.1
52.4
53.4
53.5
54.1
64.1
67.5
aPa-185363
NZms28f4
aPa-461201
aPa-442203 aPa-552852
aPa-461911 aPa-442826
aPa-441685
aPa-519308
CH01g121
aPa-442134
SAmsDT040421
SAmsDR995002
CH01d09
aPa-186727
aPa-186520
aPa-525882
aPa-461835
aPa-186193 aPa-185262
aPa-183485
aPa-442173 aPa-441779
aPa-442112
aPa-461822
aPa-519342
aPa-186512
CH03c02
aPa-525797
aPa-183916
aPa-552964
SAmsCN943613
CH02h11b
aPa-443308
aPa-461933 aPa-183186
aPa-443297
SAmsCO417416
aPa-442770
aPa-460973
aPa-185787


Au LG 12
0.0
3.2
3.3
8.7
14.5
20.0
25.4
25.8
32.2
33.6
35.2
35.5
36.3
37.4
39.0
39.8
41.0
44.2
49.2
53.0
55.5
NZms28f4
aPa-461201
aPa-442203 aPa-552852
aPa-441685 aPa-461911
aPa-442826
SAmsDR995002
CH01g121
aPa-442134
aPa-442770
aPa-443297
CH02h11b
aPa-443308
aPa-552964
aPa-183916
aPa-519342 aPa-186512
CH03c02
aPa-525882
aPa-186520
aPa-186727
aPa-461835
CH01d09
aPa-185787
aPa-460973

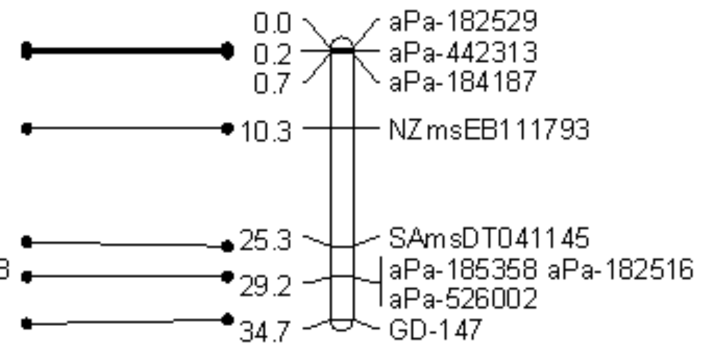
An LG13



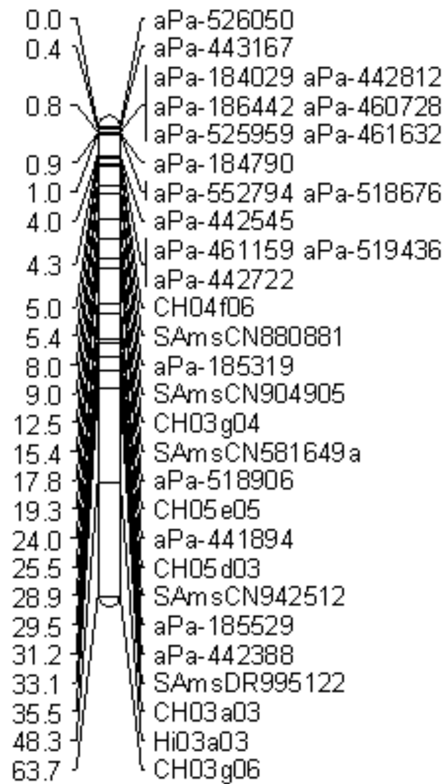
AnxAu LG13

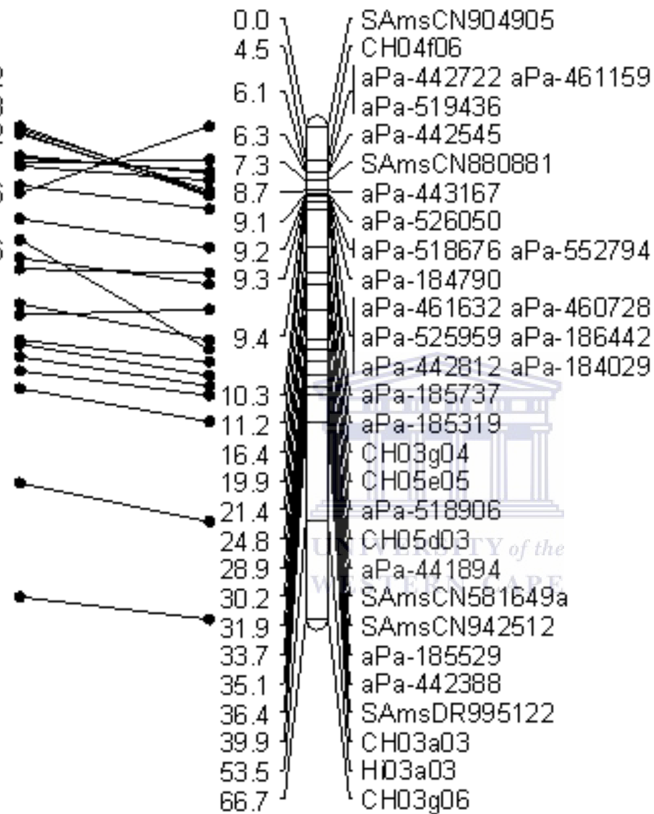


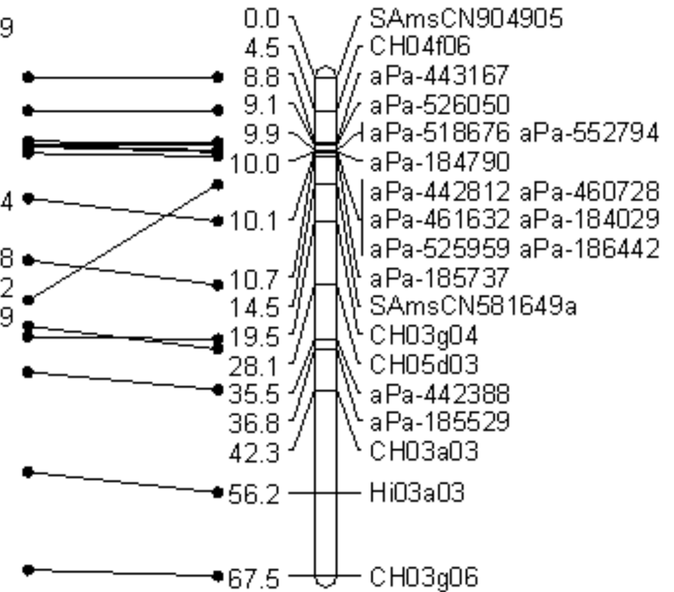
Au LG13

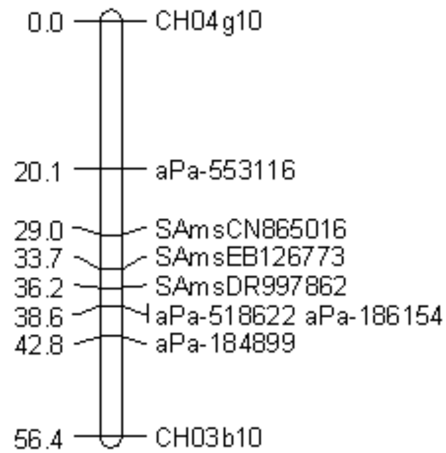


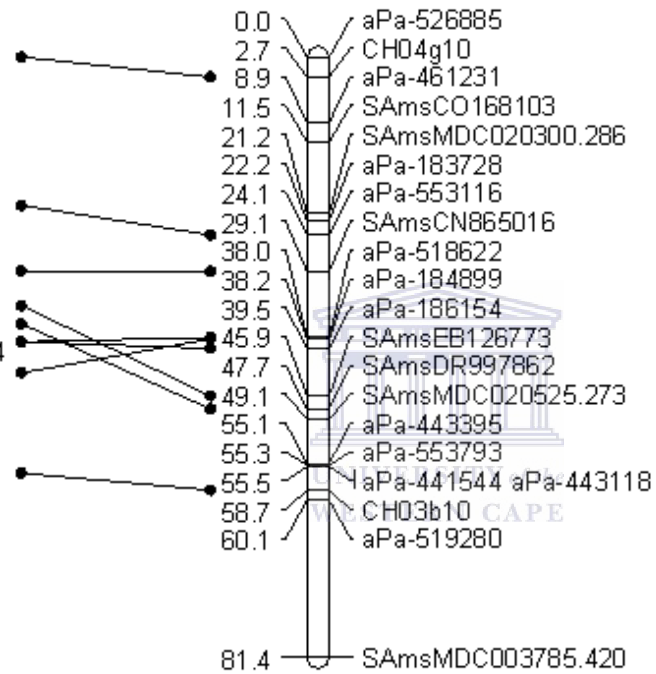
UNIVERSITY of the
WESTERN CAPE

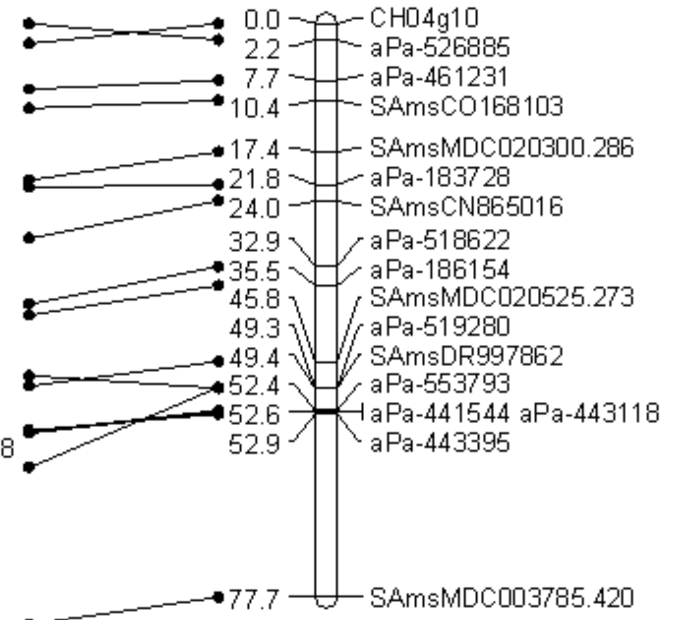

An LG14
0.0 aPa-526050
0.4 aPa-443167
aPa-184029 aPa-442812
0.8 aPa-186442 aPa-460728
aPa-525959 aPa-461632
0.9 aPa-184790
1.0 aPa-552794 aPa-518676
4.0 aPa-442545
aPa-461159 aPa-519436
4.3 aPa-442722
5.0 CH04f06
5.4 SAmsCN880881
8.0 aPa-185319
9.0 SAmsCN904905
12.5 CH03g04
15.4 SAmsCN581649a
17.8 aPa-518906
19.3 CH05e05
24.0 aPa-441894
25.5 CH05d03
28.9 SAmsCN942512
29.5 aPa-185529
31.2 aPa-442388
33.1 SAmsDR995122
35.5 CH03a03
48.3 Hi03a03
63.7 CH03g06


AnxAu LG14
0.0 SAmsCN904905
4.5 CH04f06
aPa-442722 aPa-461159
6.1 aPa-519436
6.3 aPa-442545
7.3 SAmsCN880881
8.7 aPa-443167
9.1 aPa-526050
9.2 aPa-518676 aPa-552794
9.3 aPa-184790
aPa-461632 aPa-460728
9.4 aPa-525959 aPa-186442
aPa-442812 aPa-184029
10.3 aPa-185737
11.2 aPa-185319
16.4 CH03g04
19.9 CH05e05
21.4 aPa-518906
24.8 CH05d03
28.9 aPa-441894
30.2 SAmsCN581649a
31.9 SAmsCN942512
33.7 aPa-185529
35.1 aPa-442388
36.4 SAmsDR995122
39.9 CH03a03
53.5 Hi03a03
66.7 CH03g06

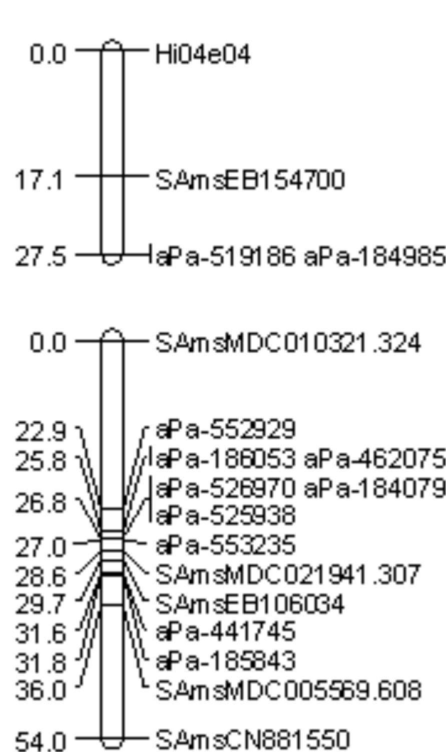

Au LG14
0.0 SAmsCN904905
4.5 CH04f06
8.8 aPa-443167
9.1 aPa-526050
9.9 aPa-518676 aPa-552794
10.0 aPa-184790
aPa-442812 aPa-460728
10.1 aPa-461632 aPa-184029
aPa-525959 aPa-186442
10.7 aPa-185737
14.5 SAmsCN581649a
19.5 CH03g04
28.1 CH05d03
35.5 aPa-442388
36.8 aPa-185529
42.3 CH03a03
56.2 Hi03a03
67.5 CH03g06

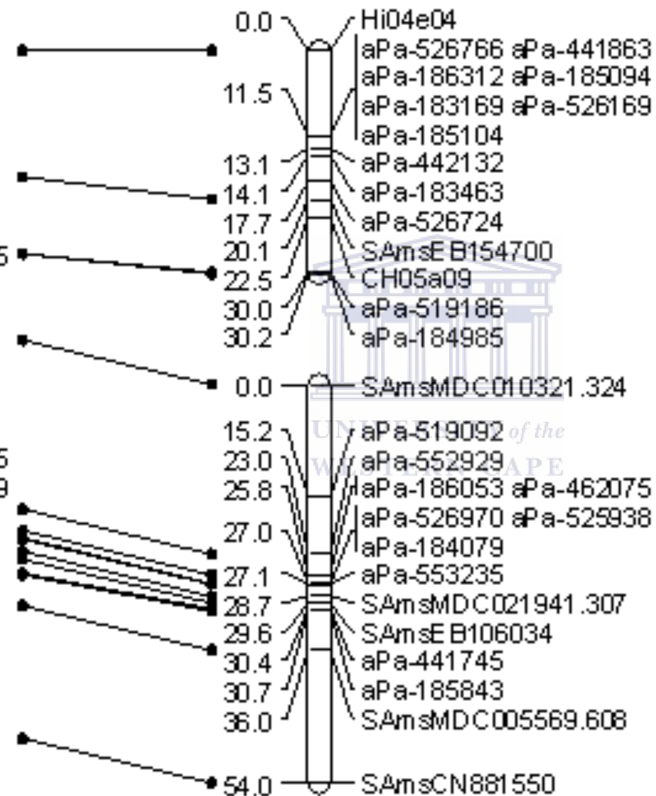


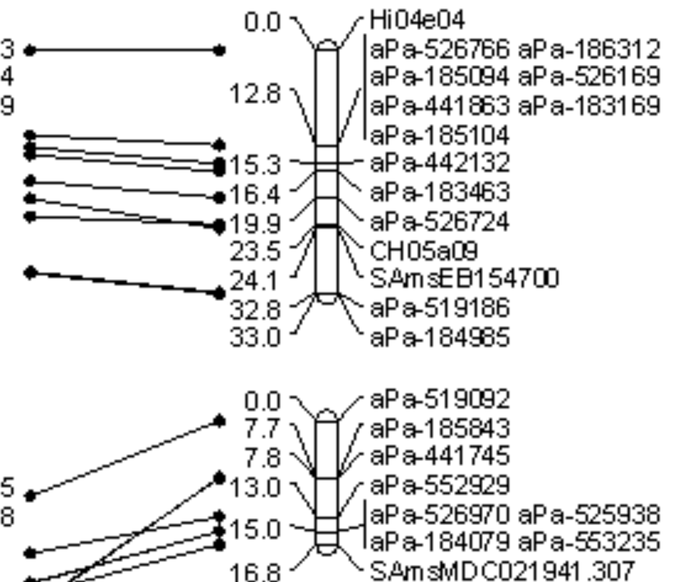


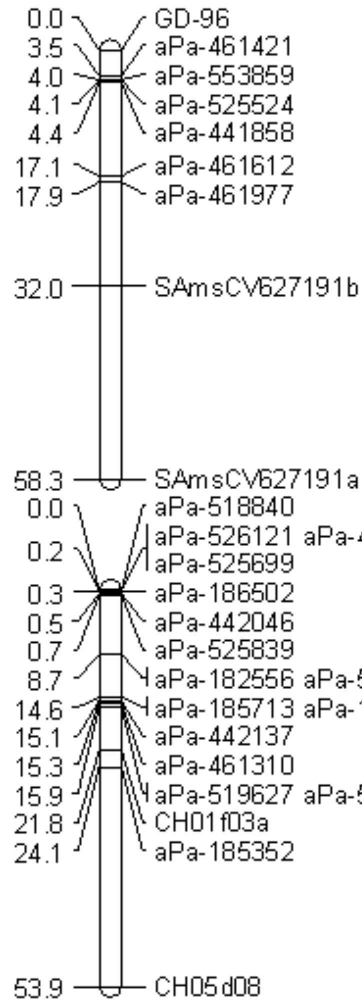


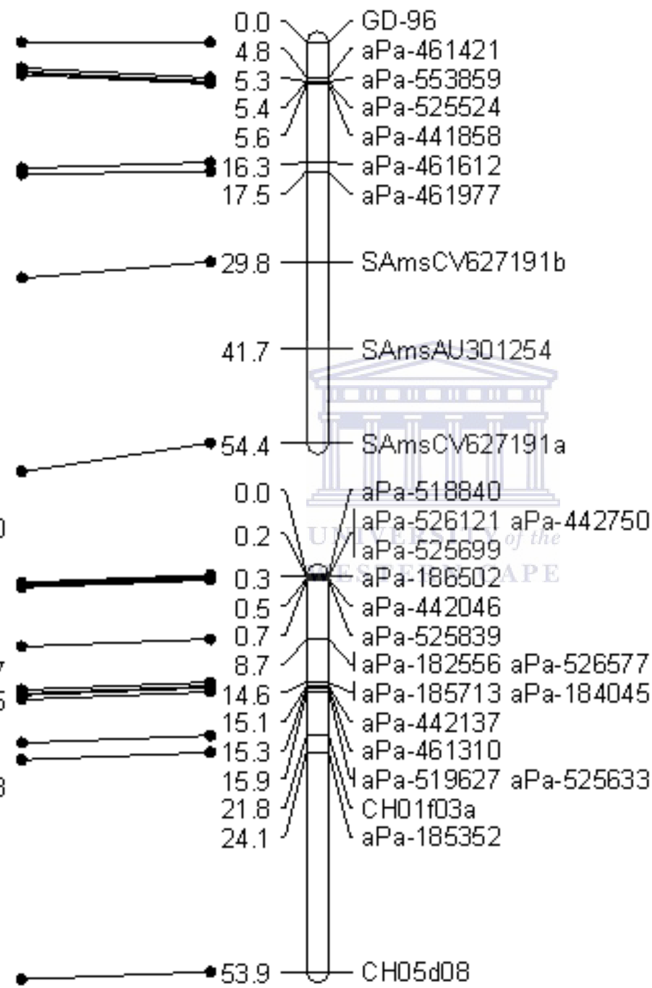
UNIVERSITY of the WESTERN CAPE

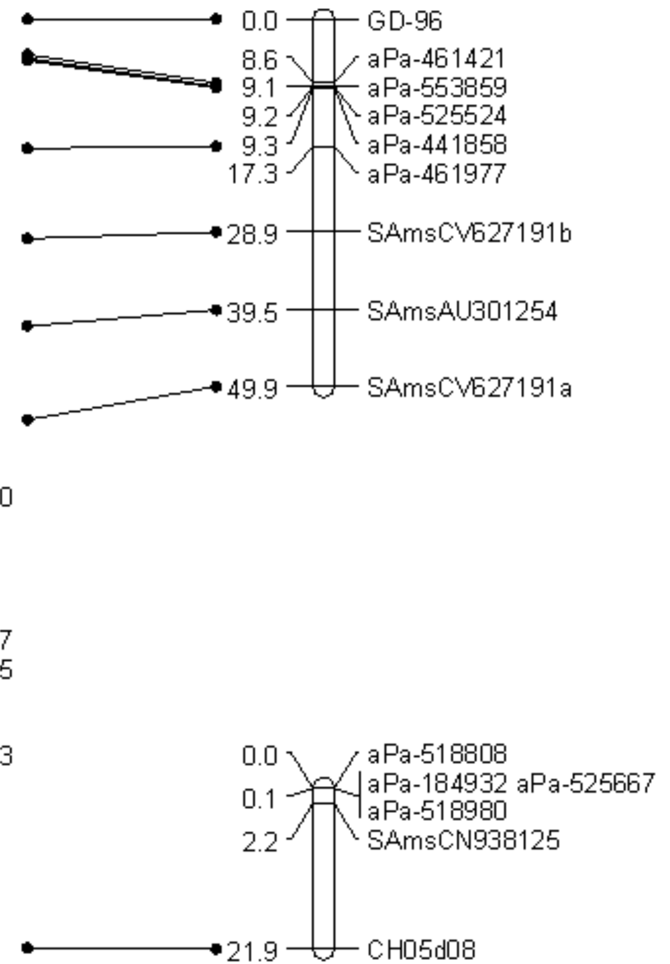

An LG16
0.0
Hi04e04
17.1
SAmsEB154700
27.5
aPa-519186 aPa-184985
0.0
SAmsMDC010321.324
22.9
25.8
26.8
27.0
28.6
29.7
31.6
31.8
36.0
aPa-552929
aPa-186053 aPa-462075
aPa-526970 aPa-184079
aPa-525938
aPa-553235
SAmsMDC021941.307
SAmsEB106034
aPa-441745
aPa-185843
SAmsMDC005569.608
54.0
SAmsCN881550


AnxAu LG16
0.0
11.5
13.1
14.1
17.7
20.1
22.5
30.0
30.2
Hi04e04
aPa-526766 aPa-441863
aPa-186312 aPa-185094
aPa-183169 aPa-526169
aPa-185104
aPa-442132
aPa-183463
aPa-526724
SAmsEB154700
CH05a09
aPa-519186
aPa-184985
0.0
SAmsMDC010321.324
15.2
23.0
25.8
27.0
27.1
28.7
29.6
30.4
30.7
36.0
aPa-519092
aPa-552929
aPa-186053 aPa-462075
aPa-526970 aPa-525938
aPa-184079
aPa-553235
SAmsMDC021941.307
SAmsEB106034
aPa-441745
aPa-185843
SAmsMDC005569.608
54.0
SAmsCN881550


Au LG16
0.0
12.8
15.3
16.4
19.9
23.5
24.1
32.8
33.0
Hi04e04
aPa-526766 aPa-186312
aPa-185094 aPa-526169
aPa-441863 aPa-183169
aPa-185104
aPa-442132
aPa-183463
aPa-526724
CH05a09
SAmsEB154700
aPa-519186
aPa-184985
0.0
7.7
7.8
13.0
15.0
16.8
aPa-519092
aPa-185843
aPa-441745
aPa-552929
aPa-526970 aPa-525938
aPa-184079 aPa-553235
SAmsMDC021941.307


An LG17
0.0 GD-96
3.5 aPa-461421
4.0 aPa-553859
4.1 aPa-525524
4.4 aPa-441858
17.1 aPa-461612
17.9 aPa-461977
32.0 SAmsCV627191b
58.3 SAmsCV627191a
0.0 aPa-518840
aPa-526121 aPa-442750
0.2 aPa-525699
0.3 aPa-186502
0.5 aPa-442046
0.7 aPa-525839
8.7 aPa-182556 aPa-526577
14.6 aPa-185713 aPa-184045
15.1 aPa-442137
15.3 aPa-461310
15.9 aPa-519627 aPa-525633
21.8 CH01f03a
24.1 aPa-185352
53.9 CH05d08


AnxAu LG17
0.0 GD-96
4.8 aPa-461421
5.3 aPa-553859
5.4 aPa-525524
5.6 aPa-441858
16.3 aPa-461612
17.5 aPa-461977
29.8 SAmsCV627191b
41.7 SAmsAU301254
54.4 SAmsCV627191a
0.0 aPa-518840
aPa-526121 aPa-442750
0.2 aPa-525699
0.3 aPa-186502
0.5 aPa-442046
0.7 aPa-525839
8.7 aPa-182556 aPa-526577
14.6 aPa-185713 aPa-184045
15.1 aPa-442137
15.3 aPa-461310
15.9 aPa-519627 aPa-525633
21.8 CH01f03a
24.1 aPa-185352
53.9 CH05d08


Au LG17
0.0 GD-96
8.6 aPa-461421
9.1 aPa-553859
9.2 aPa-525524
9.3 aPa-441858
17.3 aPa-461977
28.9 SAmsCV627191b
39.5 SAmsAU301254
49.9 SAmsCV627191a
0.0 aPa-518808
aPa-184932 aPa-525667
0.1 aPa-518980
2.2 SAmsCN938125
21.9 CH05d08

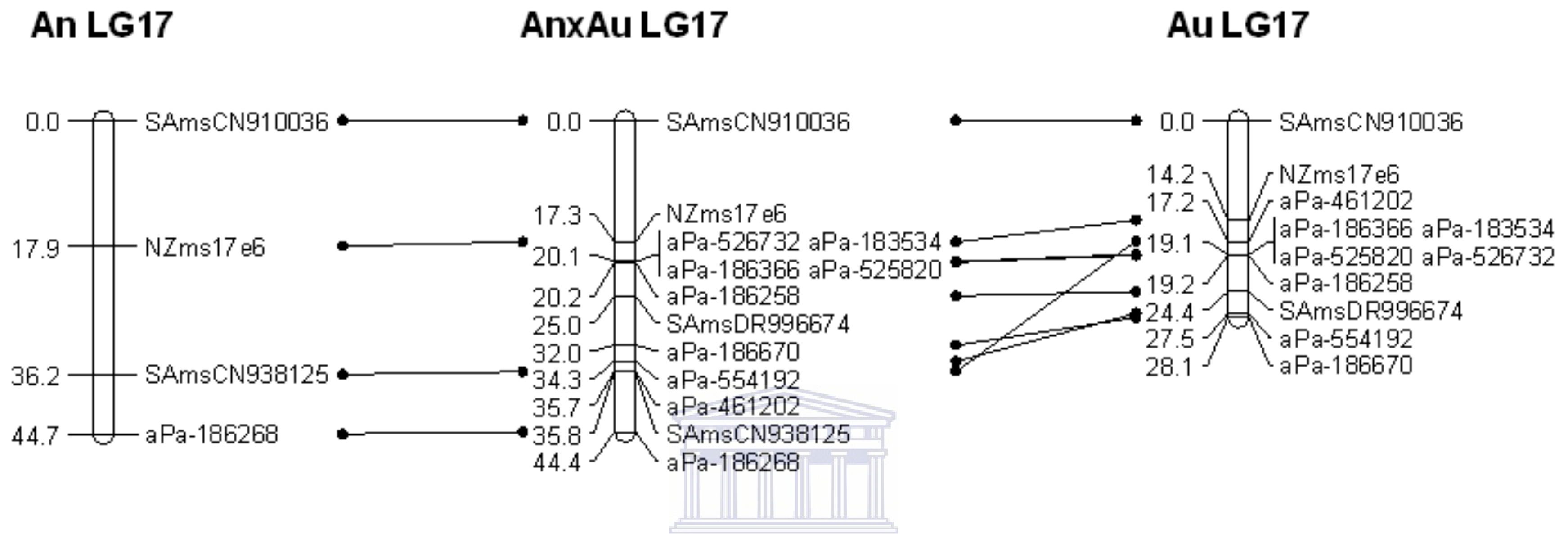


Figure 17: An integrated genetic linkage map developed for a F_1 generation 'Anna' x 'Austin' mapping population consisting of 80 individuals. Individual parental maps for 'Anna' and 'Austin' are also shown.

Newly developed and mapped SSR markers are labelled with the prefix 'SAms'. Published markers are labelled with the prefixes 'GD', 'CH', 'Hi', 'NZms' and 'MS'. DArT makers are prefixed by 'aPa-'. The Maliepaard *et al.* (1998) reference map was used to assign linkage groups.

3.9 QTL Mapping

QTL mapping was carried out using the Interval Mapping (IM) and restricted MQM (rMQM) functions of MapQTL® 6.0 according to the methodology described in **section 2.14.**

3.9.1 Interval mapping

Interval Mapping was carried out on the adult and seedling data used in MapQTL® 6.0. Results generated by Interval mapping are shown below in **Table 7** and **Table 8** for adult and seedling data respectively, for the years 1996, 1997 and 1998.

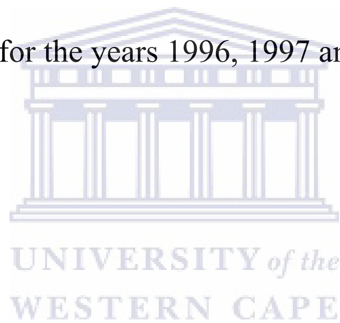

UNIVERSITY of the
WESTERN CAPE

Table 7: Putative IVB QTLs identified by Interval mapping consistently from 1996 to 1998 for adult trees of the 'Anna' x 'Austin' mapping population.

LG	Position (cM)	Locus	LOD	mu_ac{00}	mu_ad{00}	mu_bc{00}	mu_bd{00}	%Expl.	Year
1	27.6	aPa-186132	1.29	220	245	246	256	13.2	1996
			1.29	212	239	227	254	13.2	1997
			1.35	207	236	243	239	13.8	1998
2	50.2	aPa-460920	3.22	268	196	234	258	29.7	1996
			3.61	252	187	228	253	32.7	1997
			2.68	255	189	224	246	25.5	1998
3	30.1	SAmsCO866862	2.08	226	253	246	220	20.4	1996
			1.75	222	244	238	209	17.5	1997
			1.52	218	241	234	211	15.3	1998
4	48.2	aPa-186634	1.28	246	231	229	266	13.1	1996
			1.77	239	222	218	265	17.7	1997
			1.33	232	221	221	263	13.6	1998
5	43.0	aPa-183977	1.97	242	231	221	260	19.4	1996
			1.91	230	226	213	255	18.9	1997
			1.49	231	222	211	246	15.1	1998
6	37.1	aPa-553956	1.22	225	238	252	231	12.5	1996
			1.08	220	228	245	224	11.2	1997
			0.90	212	229	238	224	9.4	1998
8b	26.9	aPa-462114	1.18	232	236	263	242	12.2	1996
			1.39	224	226	258	235	14.1	1997
			0.43	221	231	245	225	4.6	1998
9	5.3	SAmsMDC004938.180	1.29	232	233	265	238	13.2	1996

UNIVERSITY of the
WESTERN CAPE

			1.77	227	219	260	234	17.7	1997
			0.57	227	222	244	228	6.1	1998
10	35.3	CH04f03	1.71	232	205	257	237	17.1	1996
			1.16	225	210	249	228	12.0	1997
			1.08	224	203	243	225	11.1	1998
11	49.4	NzmsEB153947	2.79	247	315	228	204	26.3	1996
			2.54	238	322	221	192	24.3	1997
			3.10	235	323	217	196	28.8	1998
12	21.6	aPa-519308	1.65	245	217	242	247	16.6	1996
			1.60	239	208	232	242	16.1	1997
			1.48	234	207	232	235	15.0	1998
13	10.3	NzmsEB111793	1.99	261	235	211	243	19.6	1996
			2.51	254	228	195	238	24.0	1997
			1.73	248	224	202	233	17.3	1998
14	7.3	SamsCN880881	2.07	248	249	224	225	20.3	1996
			1.92	234	246	216	219	19.0	1997
			1.84	232	241	214	217	18.2	1998
15	26.0	aPa-443118	1.37	214	249	360	225	13.9	1996
			1.20	206	240	368	216	12.3	1997
			1.09	207	237	344	214	11.3	1998
16b	38.8	aPa-519092	1.88	226	278	235	245	18.6	1996
			2.72	209	274	231	240	25.8	1997
			2.38	214	267	223	237	23.0	1998
17c	24.3	aPa-526732	1.93	226	246	256	229	19.0	1996
			1.45	217	240	245	223	14.7	1997
			1.67	218	231	246	220	16.7	1998

UNIVERSITY of the
WESTERN CAPE

Table 8: Putative IVB QTLs identified by Interval mapping consistently from 1996 to 1998 for seedling apple trees of the 'Anna' x 'Austin' mapping population.

LG	Position (cM)	Locus	LOD	mu_ac{00}	mu_ad{00}	mu_bc{00}	mu_bd{00}	%Expl.	Year
1	27.6	aPa-186132	1.43	237	246	253	250	8.1	1996
			1.19	235	237	252	244	6.8	1997
			0.65	246	262	261	254	3.8	1998
2	46.3	SAmsEB106592	0.92	257	249	255	262	5.3	1996
			1.57	246	237	246	258	8.8	1997
			1.42	241	230	241	256	8.1	1998
4	27.8	aPa-441554	1.16	259	249	258	253	6.6	1996
			0.86	249	241	247	241	5.0	1997
			0.51	244	237	244	236	2.9	1998
6	37.1	aPa-525967	1.23	249	259	258	252	7.0	1996
			1.64	238	248	251	241	9.2	1997
			1.49	232	245	248	234	8.4	1998
7	12.7	aPa-184313	2.31	253	253	251	266	12.7	1996
			2.06	239	246	241	257	11.4	1997
			2.86	232	242	234	258	15.5	1998
8	0.0	Hi04b12	1.11	249	255	260	258	6.3	1996
			0.52	232	244	245	240	3.0	1997
			0.52	232	244	245	240	3.0	1998
9	64.2	aPa-186400	0.95	254	255	252	268	5.5	1996
			1.35	242	248	241	259	7.7	1997
			1.24	236	244	235	259	7.1	1998
10	62.7	aPa-526919	1.56	253	259	259	248	8.8	1996

UNIVERSITY of the
WESTERN CAPE

			3.31	242	250	254	234	17.8	1997
			3.05	235	246	252	227	16.5	1998
11	109.0	Hi02c06	0.78	257	251	258	252	4.5	1996
			1.36	254	241	246	240	7.7	1997
			1.31	252	235	241	234	7.5	1998
12	21.6	aPa-519308	2.23	253	249	255	264	12.3	1996
			1.53	247	238	244	253	8.7	1997
			1.30	242	233	238	251	7.4	1998
13	10.3	NZmsEB111793	0.40	259	254	250	256	2.3	1996
			1.48	254	245	232	247	8.4	1997
			1.69	255	241	223	240	9.5	1998
14	4.5	CH04f06	1.23	255	254	249	261	7.0	1996
			0.94	248	244	238	248	5.4	1997
			0.69	243	239	233	246	4.0	1998
16	20.1	SAmsEB154700	0.93	252	255	251	259	5.4	1996
			1.92	237	249	239	250	10.7	1997
			1.86	232	246	231	247	10.4	1998
17	41.7	SAmsAU301254	1.88	254	258	266	250	10.5	1996
			0.84	246	251	251	240	4.9	1997
			1.09	240	250	251	233	6.2	1998

UNIVERSITY of the
WESTERN CAPE

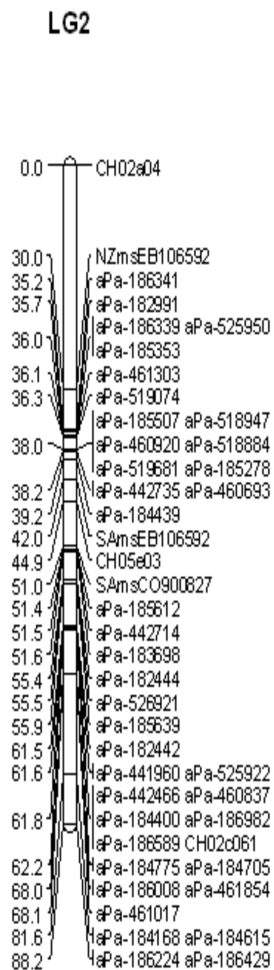
3.9.2 Restricted MQM (rMQM) analysis

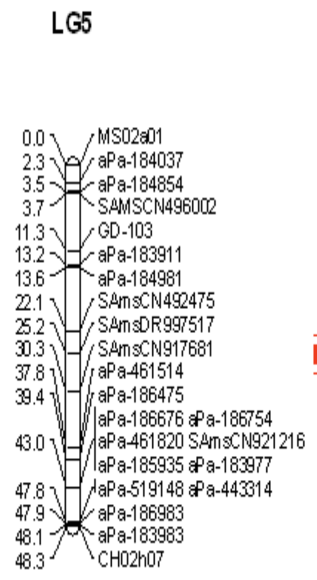
rMQM analysis was carried out on the adult and seedling data used in MapQTL® 6.0. Results generated by rMQM are shown below in **Tables 9** and **10** respectively, for those QTLs found consistently for adult and seedling data, in consecutive years of data collection (1996, 1997 and 1998).

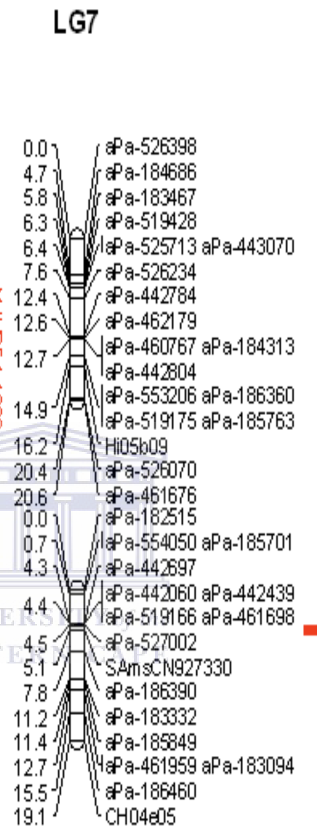
Table 9: Putative IVB QTLs localized by rMQM mapping at consistently from 1996 to 1998 for adult apple trees of the 'Anna' x 'Austin' mapping population.

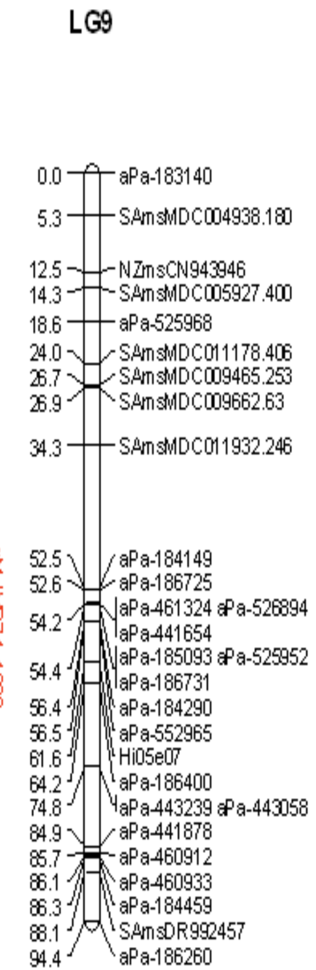
LG	Position (cM)	Locus	LOD	mu_ac{00}	mu_ad{00}	mu_bc{00}	mu_bd{00}	% Expl.	Year
2	50.2	aPa-185278	12.49	245	260	279	282	3.9	1996
			12.73	287	311	299	339	3.1	1997
			8.88	360	360	342	391	4.8	1998
5	25.2	SAmsDR997517	8.27	267	258	263	280	3.4	1996
			16.02	374	357	334	371	7.6	1997
			6.52	381	366	346	375	6.9	1998
7b	5.1	SAmsCN927330	12.18	275	262	304	290	5.7	1996
			21.35	354	332	357	404	10.7	1997
			11.63	401	368	395	445	12.8	1998
9	34.3	SAmsMDC011932.246	7.54	264	287	275	248	3.1	1996
			27.60	369	353	339	259	11.2	1997
			13.91	412	403	377	300	12.8	1998
10	27.1	CH04c06	5.58	257	271	262	259	1.5	1996
			6.90	76	181	183	178	7.2	1997
			4.95	104	208	202	205	6.8	1998
17	29.8	SAmsCV627191b	9.86	256	264	287	260	1.5	1996
			6.66	448	423	417	421	0.3	1997
			5.23	380	409	391	415	1.6	1998

UNIVERSITY of the
WESTERN CAPE


LG2


LG5


LG7


LG9

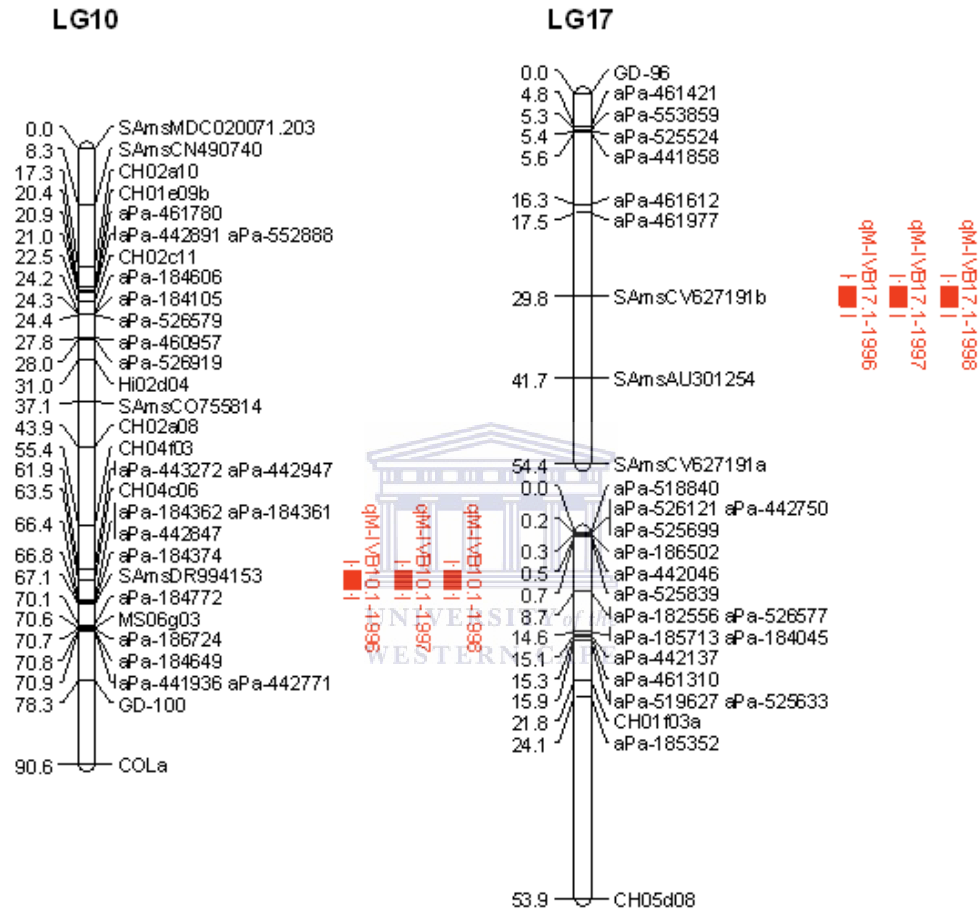


Figure 18: A graphical representation of putative IVB QTLs detected by rMQM analysis, for adult trees using the integrated 'Anna' x 'Austin' genetic map. QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

Table 10: Putative IVB QTLs found by rMQM mapping at consistently from 1996 to 1998 for seedling apple trees of the 'Anna' x 'Austin' mapping population.

LG	Position (cM)	Locus	LOD	mu_ac{00}	mu_ad{00}	mu_bc{00}	mu_bd{00}	% Expl.	Year
2	46.3	SAmsEB106592	9.33	238	220	231	246	11.2	1996
			12.46	189	176	191	223	21.0	1997
			13.62	152	134	150	196	17.8	1998
2	50.2	aPa-519681	8.84	229	209	229	225	10.7	1996
			11.78	180	168	201	201	20.2	1997
			13.54	139	124	167	166	17.8	1998
9	64.2	aPa-186400	10.67	238	236	232	272	13.2	1996
			15.13	181	205	190	223	19.5	1997
			12.11	153	182	174	213	15.1	1998
14	16.4	CH03g04	5.95	219	218	200	225	8.0	1996
			9.02	181	203	186	205	9.5	1997
			7.21	150	177	161	184	9.2	1998
14	24.8	CH05d03	6.20	226	224	219	237	8.2	1996
			4.42	208	217	209	224	5.3	1997
			6.30	177	198	193	213	8.3	1998
17	29.8	SAmsCV627191b	10.21	238	211	229	218	12.6	1996
			7.11	181	161	173	166	7.0	1997
			6.08	152	137	149	129	6.2	1998

UNIVERSITY of the
WESTERN CAPE

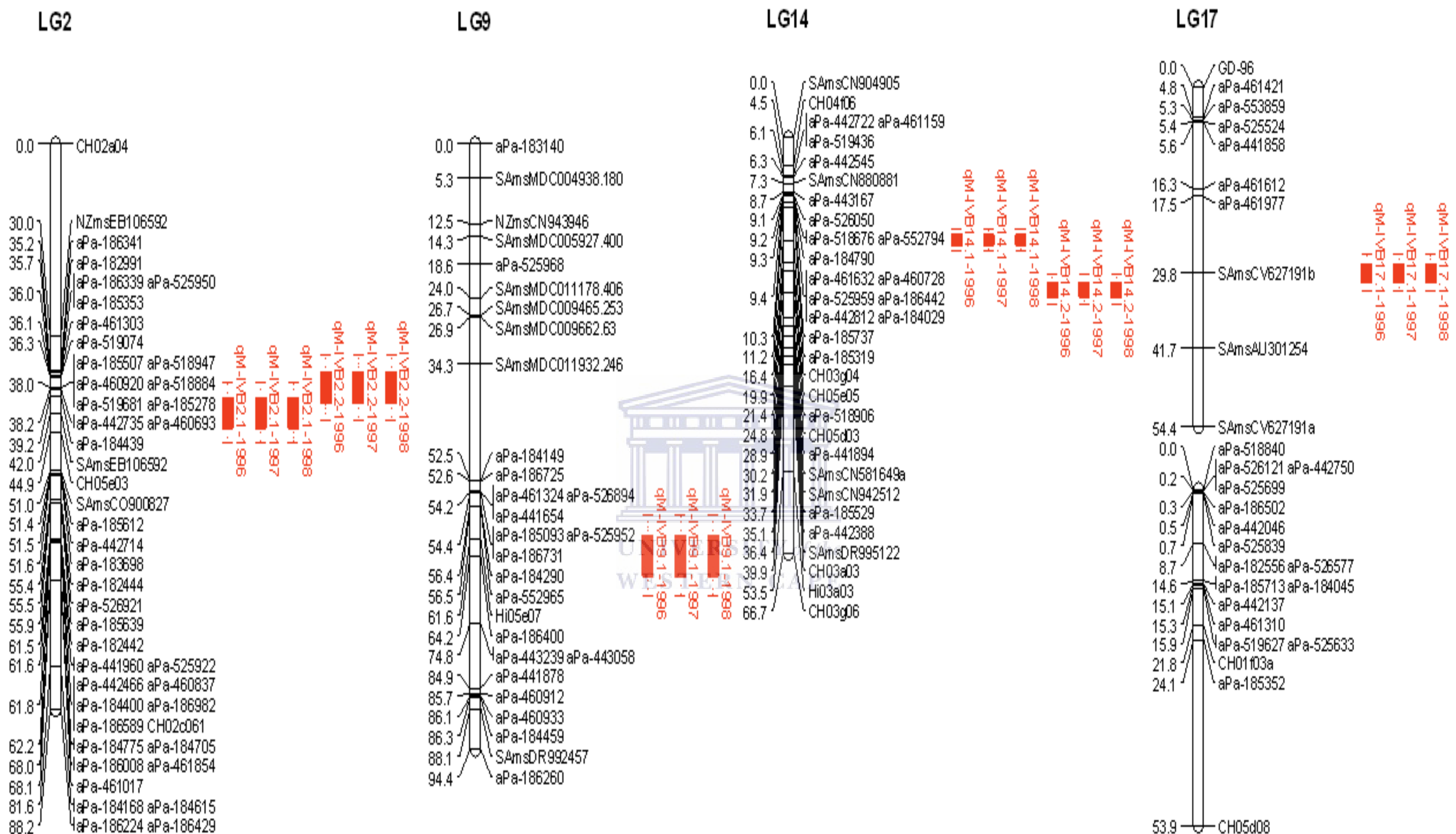


Figure 19: A graphical representation of putative IVB QTLs detected by rMQM analysis, for seedling trees using the integrated ‘Anna’ x ‘Austin’ genetic map. QTLs are represented by bars indicating 5% confidence intervals and broken lines indicating 10% confidence intervals.

3.10 TECHNICAL DISCUSSION AND CONCLUSIONS

Introduction

A technical discussion and conclusion is given in this section and it analyses the more technical methodologies in this thesis, which are genomic DNA extraction and quantitation; SSR primer testing and analysis (simplex and megaplex); PCR amplicon detection by PAGE and Capillary electrophoresis and DArT marker analysis.

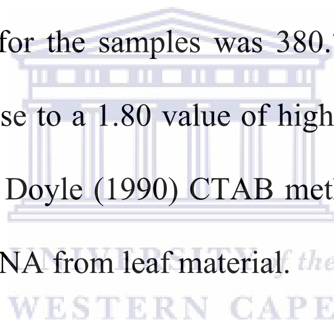
3.10.1 Genomic DNA Extraction and Agarose gel electrophoresis

Genomic DNA was successfully extracted employing the CTAB methodology as can be observed in **Fig. 8**, as single, intense bands of high molecular weight DNA. DNA samples were not observed to contain any polyphenolic compounds and polysaccharide entrapment, as this phenomenon presents as a high molecular weight smear between the gel wells and the bands of genomic DNA, after electrophoresis. Polyphenols and polysaccharides are known to inhibit downstream enzymatic reactions or simply impede DNA extraction (Bashalkhanov and Rajora, 2008). DNA samples were made up in a 1x TE solution at pH 7.5, because of the enhanced buffering and chelating capacity of the TE. The buffering capacity is attributed to Tris and the chelation of Mg^{2+} ions, which DNAases utilize to catalyse degradation genomic DNA, to the EDTA in the TE solution (Sambrook *et al.,* 1989).

UNIVERSITY of the
WESTERN CAPE

3.10.2 Genomic DNA quantification

As mentioned in **section 3.2.2**, once extracted, the genomic DNA was quantified using the NanoDrop® spectrophotometer. DNA quantities extracted ranged from as low as 51.0 ng/µl for sample 159 to 972.3 ng/µl for sample 182. This variation in genomic DNA quantity though not of any significant consequence, may most likely the result of the variable leaf quality used as starting material for the extraction. Despite efforts to acquire a uniform set of young and soft leaves from each plant in the mapping population, leaf quality between trees or seedlings varied because of age and infection with mildew or other pathogens. Therefore, lignin, cellulose and hemicellulose content also varied. On average, genomic DNA yield for the samples was 380.7 ng/µl at an average $OD_{260/280}$ ratio of 1.76, which is very close to a 1.80 value of high purity DNA. This indicates the effectiveness of the Doyle and Doyle (1990) CTAB method in rapidly extracting a high yield of highly pure genomic DNA from leaf material.

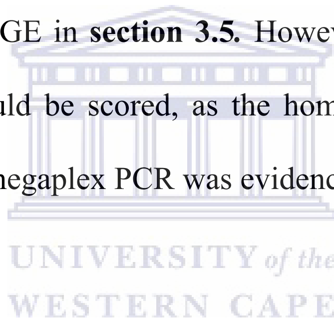

UNIVERSITY of the
WESTERN CAPE

3.10.3 Simple Sequence Repeat Primer design and Simplex PCR testing

Primer design carried out using the rules described in **section 2.8** yielded a set of 268 new primers pairs, designed by several members in the Apple genomics group (Department of Biotechnology, University of the Western Cape, South Africa) research with the 'SAms' prefix (see **Table B, Appendix 1**). Of this latter total, 98 were designed in this thesis and have the bold and italicized prefix ***'SAms'***. Using the conditions specified in **section 2.8**, these primers were tested on a 6 % polyacrylamide gel as described in **section 3.5**, successfully producing PCR bands that could be easily scored. Of these, a set of 30 primers (with the '***SAmsMDC'*** prefix) were generated from the apple genome data, with

the use of the BatchPrimer3® software (http://probes.pw.usda.gov/batchprimer3/) found on the GDR website (www.rosaceae.org). This subset of 30 primers was part of a total of 35 primers designed from the apple genome. The other 5 primers from this set of 35 are not given here, as they did not generate PCR products when tested on the parents, or did not produce consistent, bands that could be scored. The other 199 primers were acquired from published work on apple (Guilford *et al.,* 1997; Liebhard *et al.,* 2002; Liebhard *et al.,* 2003; Silfverberg-Dilworth *et al.,* 2006) and pear (Yamamoto *et al.,* 2002a, b, c).

When tested singly on the parental cultivars, the whole set of PCR primers yielded a product as was seen in the PAGE in **section 3.5.** However, this does not imply that all yielded PCR products that could be scored, as the homozygous nature of the markers these primers produced in the megaplex PCR was evidence of this.


UNIVERSITY of the
WESTERN CAPE

3.10.4 Megaplex PCR development and testing

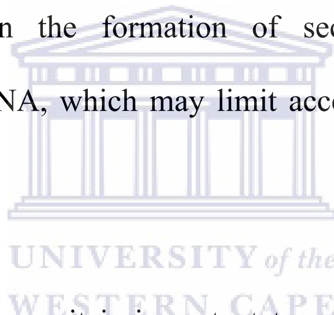
As was highlighted in the **section 2.9**, megaplex PCR was employed in this study because it utilized up to 16 primer pairs in one PCR reaction, with the use of the Qiagen® Multiplex kit. It must be noted that in the case of all the new 'SAms' SSR primers were designed with an annealing temperature as close as possible to 60 °C, so that they could be easily multiplexed. Additionally, this strategy takes advantage of the observed difference in size range of PCR amplicons for every primer pair, coupled with the use of four difference fluorescent dyes. This method was used successfully in recent published work by van Dyk *et al.* (2010) and Campoy *et al.* (2010 a, b).

The primers that were grouped for mega- or multi-plexing can be observed in **Table C**, **Appendix 1** and some of these did not produce a PCR product that could be scored. From the total of 467 primer pairs grouped into megaplexes, 77 % (359) generated a PCR product, and from this group that worked 12.4 % (58) were homozygous. This means that 65 % (301) were polymorphic and were used in the generation of the linkage map. Furthermore, 23 % (108) of the total number of primer pairs failed to produce a PCR product. So far as the success of individual megaplexes is concerned, megaplexes 1 and 3 had the lowest amplification success rate of 53 % (8/15) and megaplexes 7 (16/16), 22 (10/10), 26 (6/6) and 29 (4/4) had the highest amplification success rates of 100 %. On average, the amplification success rate for all the multiplexes was 79 % with an average of 12 markers per megaplex. These figures seem higher as compared to the Campoy *et al.* (2010b) paper, which generated an average of amplification rate of 66 %, with a lowest observed amplification rate being at about 29 %, compared to 79 % and 54 % in this thesis, respectively. The highest achieved amplification rate of 100 % was however similar in this thesis and the Campoy *et al.* (2010b) paper, M. K. Soeker (PhD thesis, 2012) and M. M. van Dyk (PhD thesis, 2008) who mapped SSR markers in three different apple mapping populations each also report similar results as those reported in this work, for the lowest and highest success rates of megaplex amplification of 60 % and 100 % respectively.

The lower end of the amplification success of PCR in the megaplexes can be explained by the fact that that other primer pairs may have complementary sequences leading to primer-dimers, or the fact there is more stereo chemical interference between primers as

UNIVERSITY of the
WESTERN CAPE

they are increased in a single PCR reaction, as they may compete for resources in the PCR. Furthermore, primers can be designed on poor and good sequence quality, leading to other primer pairs amplifying more efficiently than others to the extent that the efficient ones restrict the amplification of the others (Markoulatos *et al.,* 2002; Masi *et al.,* 2003). Another possible reason that has been suggested recently, is that there are regions on the eukaryotic genome, which do not enjoy as high a rate of PCR ability as others due to their inherent sequence characteristics, and resultantly amplify poorly in PCR (Baker, 2010). Another reason, though less plausible, is that this difference in amplification of certain genome regions may also be because of the three dimensional arrangement of the DNA in the formation of secondary helical structures by complimentary segments of DNA, which may limit access of *Taq* DNA polymerase to such areas (Baker, 2010).


UNIVERSITY of the
WESTERN CAPE

Despite its apparent shortcomings, it is important to note that megaplex PCR has the distinct advantage that when coupled with capillary electrophoresis, that it drives a high and throughput, generating reproducible markers, which is essential for genotyping and/ or the construction of genetic maps for important species. Furthermore, this entire system is amenable to semi-automation, thus reducing the possibility of human error associated with the genotyping experimental bench work, which involves large number of samples.

3.10.5 Capillary electrophoresis and PAGE amplicon based detection

Simple Sequence Repeat markers take advantage of the fact that SSR length variations in individuals result from inefficiency of the replication mechanism. This variation can be is

more frequent than can be explained by mutation alone (Powell *et al.,* 1996; Jung *et al.,* 2005). These variations can be viewed using PAGE and capillary electrophoresis. Polyacrylamide gel electrophoresis is know to be sufficient to easily distinguish between allelic variants of a marker which are at least 2 base pairs apart in size (Sambrook *et al.,* 1989). In the case of singleplex reactions as was seen in **Fig. 12**, it is easy to distinguish the two alleles in the parental PCR products run on the gel, even those that are very close in size such as those of the sample 'Jonathan' in lane 5. As a result, this is easy to score. However, for the multiplexed primers shown in **Fig. 13**, the PCR amplicons are much more difficult to score because of the complex mixture of the PCR amplicons where the contribution of each primer pair is more difficult to distinguish. And bearing in mind the fact that these are only four primers, it would be extremely difficult to score megaplexes of up to 16 primer pairs on such a platform, due to the increased complexity associated with such a high number of primer pairs in one PCR reaction. This limitation was overcome using capillary electrophoresis, on the ABI 3130*xl* Genetic Analyzer as shown in **Fig. 14**.

UNIVERSITY of the
WESTERN CAPE

As supported by a vast number of published works seen to date (Chambers and MacAvoy, 2000; Campoy *et al.,* 2010a, b; van Dyk *et al.,* 2010; Celton *et. al..* 2009 and Celton *et al.,* 2011 among many others) capillary electrophoresis is a superior method for genotyping and in this study could be used to detect a various number of alleles of SSR markers produced. This method yielded superior resolution compared to PAGE, as was mentioned in the previous paragraph. This is because; unlike PAGE which fails to clearly differentiate nucleic acid at the one two base pair level size difference, capillary

electrophoresis, provides high resolution even at such low base-pair differences. This high detection level is afforded by the semi-automated and fluorescence-based fragment detection of the system. The semi-automation leads to higher precision and that combined with the 5-dye detection system previously alluded to in this work, means even PCR fragments of overlapping size ranges can be differentiated, given that they are labelled by different dyes. Furthermore, the fragment detection software GeneMapper® 4.0 make PCR fragment analysis easier and more manageable, especially for the high numbers of markers utilized in the genotyping exercise. Chambers and MacAvoy (2000), also point to the utility of capillary electrophoresis in detection of SSR markers because of the high signal strength and purity for many analytical applications, mainly because of the precision of such systems. To emphasize this point, amplicon size-differences of 1 base pair have been reported and easily detected in grape genotyping work by Grando and Frisinghelli (1998), who also reported marked differences in amplicons size from those given by groups who had previously used agarose and polyacrylamide gel detection and scoring.

UNIVERSITY of the
WESTERN CAPE

3.10.6 DArT marker analysis

DArT markers were successfully implemented on the 'Anna' x 'Austin' population, as was the objective of this study. Though dominant markers by nature, DArT markers were useful in increasing marker density and map coverage for this apple cross under investigation. DArT markers seem to have a good complementarity to SSR markers as they have been mapped together successfully in all the LGs presented in this thesis. In

some of the LGs such as 2, 12, and 17. DArT markers were located at the distal ends of the linkage groups and up to 6 markers mapped to a single locus.

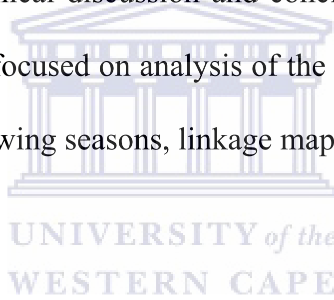
Out of a total set of 787 DArT markers generated, 502 (63.7 %) markers were used for further analysis and 285 (36.3 %) were not used due to their observed genotypic segregation ratios, which deviated significantly from the expected ratios. Such markers would distort the map if used, and were excluded from any further analysis. This unsuitability of such markers may be result from and be explained by the inefficient hybridisation or complete failure of the test DNA to hybridize onto the hybridisation array resulting in false negatives, which result in skewed segregation rations. Also excluded was a group of 202 markers from the set of markers, which could not be used for mapping. This is because these markers either did not have sufficient linkage with other linkage groups, or mapped into small clusters or groups, which did not link with any SSR marker of known position. The latter markers could therefore not be assigned into linkage groups as there was no reference map that could be used to allocate them into linkage groups. A total of 314 DArT markers, which represent 62.5 % of the useable DArT markers that were generated in this work, were mapped onto the 'Anna' x 'Austin' linkage map.

UNIVERSITY of the
WESTERN CAPE

4.0 DISCUSSION AND CONCLUSIONS

4.1 Introduction

This work aimed at the two major objectives of producing a genetic linkage map for the 'Anna' x 'Austin' cross with the use SSR and DArT markers; and to map QTLs that may be implicated in the control of dormancy-related trait, time of IVB. These two major objectives were achieved through various stages such as the generation of high quality apple genomic DNA from leaves and SSR and DArT marker implementation. These were however discussed in the technical discussion and conclusion in the preceding chapter. Therefore, this section will be focused on analysis of the phenotypic data supplied by Dr. I. F. Labuschagné for three growing seasons, linkage map construction and QTL analysis.


UNIVERSITY of the
WESTERN CAPE

4.2 Phenotypic trait assessment data

4.2.1 Time of IVB frequency distributions

Phenotypic data collected for the time of vegetative budbreak followed a normal distribution with for both the adult and seedling trees as can be observed in the frequency distribution bar graphs shown in **Fig. 4** and **Fig. 5**. These data reveal the continuous and quantitative nature of vegetative budbreak trait (and dormancy) as was highlighted by Hauagge and Cummins (1991b) and by Labuschagné *et al.* (2002b). The latter researcher was responsible breeding efforts that created the 'Anna' x 'Austin', 'Anna' x 'Golden Delicious' and 'Anna' x 'Sharpe's Early' mapping populations used to investigate dormancy. The latter populations have been employed in recent dormancy studies by van

Dyk *et al.* (2010), in which these populations also displayed a similar data trend, thereby providing further evidence in support of the hypothesis that the vegetative budbreak trait is a quantitative or continuous one in nature. This also holds true for not only the aforementioned apple mapping populations but other rosaceous crop mapping populations in which dormancy or chilling requirement QTLs are dissected such as Campoy *et al.* (2010a, b) and Fan *et al.* (2010) among countless others. The pioneer work on quantitative traits by Tanksley (1993) is the basis of much of this work.

Though seedling tree data is normally distributed, it is apparent that the adult tree frequency distribution shows a bimodal normal distribution, showing two distinct peaks of data. This is analogous to having two smaller normal distribution graphs sitting adjacent to one another, and point to the effect of at least one major QTL and a few minor QTLs in that control a quantitative trait. This hypothesis has been corroborated by results from dormancy-related QTL mapping work by van Dyk *et al.* (2010), Celton *et al.,* (2010) and Celton *et al.* (2011), which show the influence of at least one major and a few minor ones QTLs in the control of vegetative budbreak, also referred to as Green Point (GP) in the latter author's work. However, Labuschagné *et al.* (2002b) also explains this bimodal nature of the data using the fact the adult and seedling trees are not in the same physiological (therefore phenological) states.

UNIVERSITY of the
WESTERN CAPE

In terms of frequency distribution patters shown in **Fig. 4** and **Fig. 5,** both adult and seedling tree data seem to follow similar frequency distribution trends over the three years over which these data were collected. This is evident in the generally similar data

fluctuations shown in the respective, yearly coloured bar graphs represented in **Fig. 4** and **Fig. 5**, This may indicate the uniform or consistent phenotypic output or each genotype's performance in this environment, for each genotype present in the mapping population.

4.1.2 Time of IVB data trend graphs

Data trends associated with yearly recordings of the vegetative budbreak trait over three years, found in **Fig. 6** and **Fig**. **7** for adult and seedling trees respectively, show a generally uniform trend of this data over the three years it was collected. These data trends may be indicative of the near homogenous nature of the conditions present at the different sites where the adult and seedling apple trees were maintained. It is also apparent that the data over the three-year period seem to fluctuate similarly in the years 1996 to 1998 for both the adult and seedling plants, indicating a near similar response of the two population types to the varying environmental conditions in which they were grown.

UNIVERSITY of the
WESTERN CAPE

4.1.3 Correlation analysis

Correlation analysis was carried out using the Pearson's Linear Correlation test at $P<0.001$ s was described in **Table 5** (**section 3.1.2**), and revealed a higher correlation for the adult tree (R- value average of 0.93) compared to the seedling tree data correlation (R-value average of 0.85). This may be due to the fact that the adult tree datasets had a lower variation between them from year -to-year compared to the seedling trees, which seemed have higher variation. These differences may be attributed to different phenological and physiological status of the seedling and adult trees, which may likely

react differently to how they accumulate chill units. Furthermore, because the seedling trees are growing vigorously with high levels of cell division and growth, they are more likely to be physiologically different in one growing season (or year) compared to the other, therefore accumulate chilling units differently. This is, there is a high inter-season variation in chill unit accumulation because of the much higher difference in physiological and phenological changes in seedling trees compared to adults trees. However, it can be argued that the seedling and adult tree datasets show variation of 80 and 100 days respectively, between the earliest and the latest date of IVB. This view may point to the fact that the adult tree dataset is not less variable than the seedling dataset.

UNIVERSITY of the
WESTERN CAPE

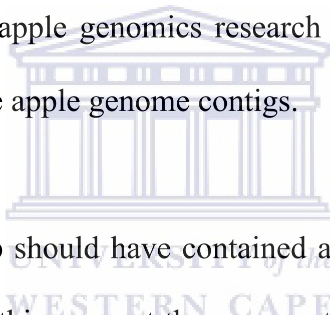
R-values in a similar range to those reported in this thesis of 0.78 to 0.96, have been reported by van Dyk *et al.* (2010) and these range from 0.68 to 0.96 for three mapping populations of adult and seedling plants. Furthermore, this aforementioned paper also reports similarly high R-value average for an adult tree population of 0.95 in the 'Sharpe's Early' x 'Anna' cross, which is similar to the 0.93 R-value average in the 'Anna' x 'Austin' adult tree mapping population.

4.2 Genetic linkage map construction

As previously mentioned in **section 3.8,** a linkage map consisting of 429 markers and spanning 1 212.6 cM was generated, with an average marker density of a marker every 3 - 10 cM. This compared favourably with the genetic distances spanned by the linkage maps reported in published work such as that of Silfverberg-Dilworth *et al.* (2006) - 1

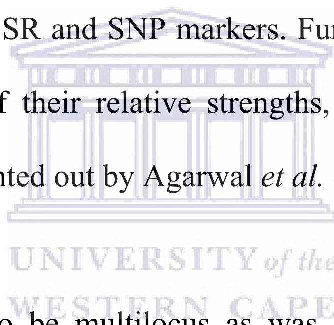
250cM, Igarashi *et al.* (2008) - 1 031 cM, Celton *et al.* (2009) - 1 100cM and van Dyk *et al.,* (2010) 1 102.3 cM (average coverage of three maps).

A total of 314 DArT markers, which represent 62.5 % of the useable DArT markers that were generated in this work, were mapped onto the 'Anna' x 'Austin' linkage map. Of the 273 total of scored SSR markers generated, 115 were mapped, which represents 42.1 % of this set of markers. In addition to this, of the 115 SSR markers mapped, 67 of these generated from primers generated collectively in the apple genomics research group at UWC and 48 from published primers. Also notable is that 15 of the 67 markers generated from primers produced in the apple genomics research group at UWC were novel and generated in this thesis from the apple genome contigs.



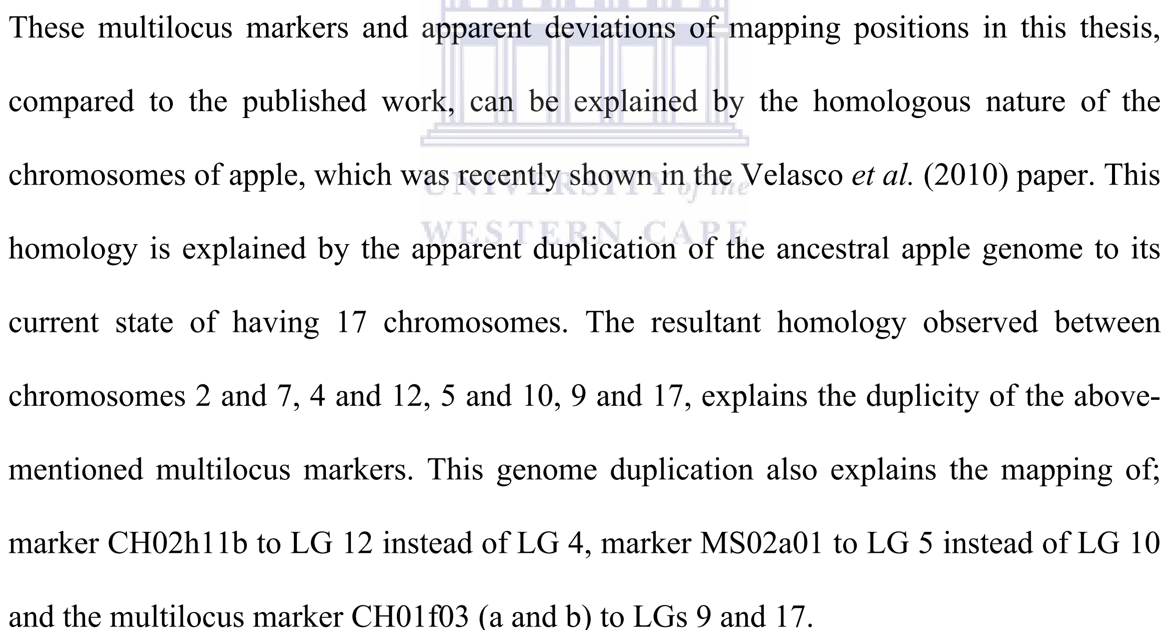
This means each linkage group should have contained approximately 18 DArT markers and 7 SSR markers. However, this was not the case, as the distribution of the DArT and SSR markers throughout the linkage groups of the apple map generated was not even. Linkage group 3, which was the shortest in length, contained 5 distally positioned and clustered DArT markers, and one SSR marker at the bottom of the linkage group. On the other hand, LG 17, which was the longest linkage group, contained 31 DArT markers and 10 SSR markers most of which seemed to be well distributed across the linkage group, with the exception of a number of clustered and distally distributed DArT markers. As the linkage groups are representative of actual chromosomes, it was not expected to find the lengths of all linkage groups be the same, as the chromosomes of apple are known to be of varying lengths (Velasco *et al.,* 2010).

Kenis and Keulemans (2008) among other workers have highlighted the importance of constructing a linkage map using co-dominant markers such as SSR markers, for the sake of producing integrated maps that are transferable and easily aligned between different apple cultivars. The map produced in this work also reiterates the importance of SSR markers in the view of producing integrated maps that are transferable between cultivars. The approach that employs various marker types such as DArT and SSR markers to build a linkage maps is a powerful strategy that has been utilized in the several modern maps such as the Silfverberg-Dilworth *et al.* (2006) reference map. This approach takes into account the way other marker types such as DArT and AFLP markers, even if dominant, complement the co-dominant SSR and SNP markers. Furthermore, this complementarity of markers takes advantage of their relative strengths, which tend to overcome their weaknesses when alone, as pointed out by Agarwal *et al.* (2008) and Gupta *et al.* (2008).


UNIVERSITY of the
WESTERN CAPE

Markers that were expected to be multilocus as was stated in research papers were mapped in this thesis. However, they have only been mapped onto one of their multilocus regions. These include, CH02a04 (LG 2 and LG 7, Liebhard *et al.,* 2002 and LG 7 in this work). CH01d03 (LG 4 and 12, Liebhard *et al.,* 2002 and LG 4 in this work) and CH05d08 (LG 9 and 17, Liebhard *et al.,* 2002 and LG 17 in this work), CH02a08 (LG 5 and LG 10, Liebhard *et al.,* 2002 and LG 10 in this work). Hi23d11b, which is presumed to be multilocus by Silfverberg-Dilworth *et al.* (2006), was mapped to LG 4 in this work as in the aforementioned work.

A few deviations in results were observed in mapping a few markers in this work, compared to published work. Examples of these include the marker CH02h11b, which is reported to be an LG 4 multilocus marker by Celton *et al.* (2009), was mapped to LG 12 in this work. It was also mapped to LG 12 by van Dyk *et al.* (2010). Furthermore, the marker CH01f03a is thought to be a multilocus marker and is mapped to LG 17 in this work. It has the CH01f03b marker mapped to LG 9 by Celton *et al.* (2009). Another marker, which had displayed a difference in the place where it mapped to relative to published work, was the marker MS02a01, which is mapped to LG 10 by Liebhard *et al.* (2002), but maps to LG 5 in this work.


UNIVERSITY of the
WESTERN CAPE

These multilocus markers and apparent deviations of mapping positions in this thesis, compared to the published work, can be explained by the homologous nature of the chromosomes of apple, which was recently shown in the Velasco *et al.* (2010) paper. This homology is explained by the apparent duplication of the ancestral apple genome to its current state of having 17 chromosomes. The resultant homology observed between chromosomes 2 and 7, 4 and 12, 5 and 10, 9 and 17, explains the duplicity of the above-mentioned multilocus markers. This genome duplication also explains the mapping of; marker CH02h11b to LG 12 instead of LG 4, marker MS02a01 to LG 5 instead of LG 10 and the multilocus marker CH01f03 (a and b) to LGs 9 and 17.

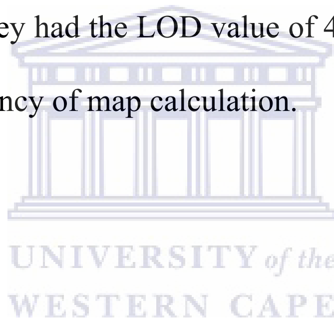
With regards to the map position, most markers were mapped in the LGs they were expected, with a few notable exceptions that could not be explained by the homology between apple chromosomes. Such examples include some of the novel, apple-genome

derived 'SAmsMDC' markers that were designed from the contigs of chromosomes 3 and 9. to target and increase marker density in LGs 3 and 9. This was in accordance with **section 2.7**. For LG 3, only one of the four markers that were mapped was placed onto the expected LG. This was the SAmsMDC002362.239 marker, mapped in the 'Austin' parental map. Unexpectedly, the three other markers, namely SAmsMDC003785.420, SAmsMDC020525.273 and SAmsMDC020300.286 mapped to LG 15 and not LG 3 (see markers in **Table A and Table B, Appendix 1**).

For LG 9, six out of a total of 12 markers mapped to positions they were not expected to. These are: SAmsMDC020071.203 (LG 10), SAmsMDC020761.431 and SAmsMDC000503.195 (LG 11), SAmsMDC010321.324, SAmsMDC021941.307 and SAmsMDC005569.608 (LG 16). However, the other six markers were successfully mapped to LG 9 as was expected and these were: SAmsMDC004938.180, SAmsMDC005927.400, SAmsMDC011178.406, SAmsMDC009465.253, SAmsMDC009662.63 and SAmsMDC011932.246. The anomalies observed here may be attributed to an inefficient pre-screening process of the sequences used to generate the primers, which may possibly have erroneously included sequences that have high homology to other sequences in other chromosomes. However, it is also plausible that some of these contigs may have been incorrectly assembled into the wrong chromosomes and that the positions they have been mapped to, may be their correct chromosomes. Furthermore, there were some major marker order inversions observed in LGs 2, 5 and 11 when individual parental maps were combined to generate the integrated map.

UNIVERSITY of the
WESTERN CAPE

The above-mentioned marker order may also be the result of erroneous mapping, which may be as a result of the inclusion of low quality markers with missing scores across the population, the incorrect scoring of a marker, or the inclusion of rogue offspring in the mapping population. These scenarios are however less likely, because of the strict adherence to rules (**section 2.13**) that would prevent such errors, though still possible because of human error. In fact, in the mapping process, offspring 185, 182, 176, 175, 147 and 131 were excluded from the analysis because they gave unexpected alleles given the parental combinations of alleles. This led to the conclusion that they were most likely rogue offspring pollinated by other parents completely. Furthermore, assigning markers to linkage groups only when they had the LOD value of 4.0, along with the rules given in **section 2.13** ensured the efficiency of map calculation.


UNIVERSITY of the
WESTERN CAPE

4.3 QTL Mapping

The two methods used for QTL analysis (Interval Mapping and Multiple QTL Mapping) yielded different results, which may be attributed to the differences in QTL detection power of the two methods. Doerge (2002) and van Ooijen (1999) give a good insight on the differences in power of the two methods, with the former reviewing the subject area in greater depth. In short, Interval Mapping is far less powerful because it assumes a single QTL model and incrementally adds single QTLs to previously detected QTLs. As a major weakness, IM does not factor in the interaction of various QTLs like MQM analysis, which is based on a multiple, interacting QTL model, which is closest to the natural model where epistasis among other gene-gene interactions are observed. IM mapping is however preferred, compared to single marker-QTL models because it utilizes

and gains strength from structured genotypic information acquired from the genetic map. IM was therefore used in this study to test its efficacy, to give insights on possible QTLs that could be investigated with MQM analysis, and to compare it to MQM analysis. The superiority of MQM analysis, though resulting in more complex statistical calculations and higher computational demands, emanates from its genome-wide, multidimensional analysis of potentially interacting QTLs. Furthermore, it is assumed that the selection of co-factors in detection of QTLs makes the process of detecting QTLs more efficient computationally by using ‘anchoring markers’ that the software can utilize for detecting other surrounding QTLs.

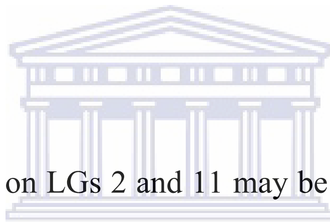
As mentioned in **section 2.14,** the mu_{00} values for the seedling rMQM analysis data were compared and 95 % of the data over the three sampling years (data not shown) show, The ‘Anna’ parent is main genetic contributor to the IVB QTLs observed in the rMQM seedling data analysis in **Table 10**. The same was reported in the van Dyk *et al.* (2010) paper, which states that ‘Anna’ parent has the higher contribution to the QTLs for time of IVB. This was as expected as the ‘Anna’ cultivar is a low chill cultivar which acts as pollinator (male parent) for many low chill cultivars as was reported by Hauagge (2010).

UNIVERSITY of the
WESTERN CAPE

4.3.1 Interval mapping

The 'Anna' x 'Austin' data was analysed for putative QTLs using IM and based on QTLs detected consistently throughout the three years 1996, 1997 and 1998 for adult and seedling trees, as shown in **section 3.9.1**, **Tables 7** and **8** respectively.

For adult trees, IM analysis localized putative QTLs on all of the 17 linkage groups, except LG 7. Though they had relatively low LOD values ranging from 0.43 to 3.61, these QTLs explained between 4.6 % and 32.7 % of the phenotypic variation. Although M. M. van Dyk (PhD thesis, 2008) used a minimum detection threshold of 2.0 for QTLs using IM analysis, at a 1.5 LOD threshold, only QTLs on LGs 2, 3, 11, 13, 14 and 16 could be detected.


UNIVERSITY of the
WESTERN CAPE

Furthermore, only two of these on LGs 2 and 11 may be declared putative QTLs at a 2.0 LOD threshold or above and these two QTLs respectively explain an average 29.3 % and 26.5 % phenotypic variation. As such, these may be major QTLs affecting the IVB trait in adult trees. Also, based on these IM results, the markers linked to the QTLs may be candidates for a marker assisted selection (MAS) or breeding (MAB) program, especially the SSR marker NZmsEB153947 on LG 11 detected, though further validation of these QTLs is necessary across different populations, environments and growth stages. The marker that seems linked to the QTL detected in LG 2 is a DArT marker, which is dominant and likely not very transferable. So for it to be more useful, an SSR marker could be designed around such a marker then if successfully mapped to the same locus, may be tested appropriately for use in MAB and/or MAS. It is noted however, that these two putative QTLs are not detected in the seedling plants too, which may reduce the

power and efficacy of the associated markers in MAS and MAB, as their use may only be limited to adult apple populations.

Seedlings on the other hand, yielded nearly similar numbers of QTLs on the 17 LGs with the exception on LGs 3, 5, 15. The LOD values were also low similarly ranging from 0.40 to 3.3 and though explaining much lower phenotypic variation of between 2.3 % and 17.8 %. Only LGs 7 and 10, however yield QTLs at an above 1.5 LOD detection threshold, with the putative QTLs detected respectively explaining 13.2 % and 14.4 % phenotypic variation on average. Both QTLs co-localize with DArT markers and as suggested before, such markers may be more useful in MAB or MAS if SSR markers may need to be designed using the sequences used for DArT marker design. However, these putative QTLs detected in the seedling plants also have the same major shortfall as those detected the adult stage. Such a weakness may greatly limit the usefulness of such markers in MAS and MAB projects.

UNIVERSITY of the
WESTERN CAPE

It was expected, according to previous work by van Dyk *et al.* (2010) that shared QTLs involved in the control of IVB would be detected in both the adult and seedling plants. However, major QTLs reported for IVB in various populations for LGs 8 and 9 by van Dyk *et al.* (2010) and Celton *et al.* (2011) were not detected as expected. Failure to detect these QTLs may strongly point to the low power of IM analysis as was suggested by Doerge (2002), especially given the low LOD thresholds at which these QTLs were detected. Such low LOD values may be described as false positives and may lead one to consider these results with caution.

4.3.2 rMQM analysis

As shown in **section 3.9.2**, **Table 9**, single putative QTLs were detected consistently and above the LOD of 4.7 thresholds on LGs 2, 5, 7, 9, 10 and 17, for the adult tree data. Putative QTLs were detected on these and LODs ranged from 4.95 to 27.60, with observed phenotypic variation explained ranging from 0.3 to 12.8 %.

The seedling rMQM results also reveal consistently localized putative QTLs on LGs 2(two QTLs), 9, 14(two QTLs) and 17. These explained between 5.3 and 21 % of the phenotypic variation observed. Also, M. M. van Dyk (PhD thesis, 2008) reports QTLs in the same LGs though in different regions of the map. However, some putative QTLs such as that detected by M. M. van Dyk (PhD thesis, 2008) in LG 2 of the 'Anna' x 'Golden Delicious' cross, overlap within a not more than 4cM genetic distance of with the LG 2 QTL found in this work. These two may represent the same QTL, which shifted by a few cM.

UNIVERSITY of the
WESTERN CAPE

The fact that QTLs are consistently located in LG 2 using either IM or rMQM analysis, using the adult and seedling data makes this LG a strong candidate to house QTLs that control budbreak. Furthermore, according to these findings markers found to associate with the QTLs in LG 2 may be strong candidates for MAS and MAB programs.

An unexpected result was found with the major QTL reported on LG 9 by van Dyk *et al.* (2010) and Celton *et al.* (2010) and Celton *et al.* (2011). It was would have been detected at the top of LG 9 for both the adult and seedling data. However, it was found only at the

top (34.3 cM) where the LOD peak was at the SSR marker SAmsMDC011932.246 in the adult tree, whereas it was found at the bottom (64.2 cM) of the LG, with the LOD peak at the DArT marker aPa-186400. This was in contrast with van Dyk *et al.* (2010) who report a consistent QTL at the top of LG 9 for both seedling (juvenile) and adult trees.

Two QTLs co-localized with specific markers for both adult and seedling trees and these were in LG 2 (aPa-519681 and aPa-185278) and LG 17 (SAmsCV627191b). The QTLs these markers co-localize with (**section 3.9.2**, **Fig. 18** and **Fig. 19**) QTLs in LGs 2 and 17. These putative QTLs explain 3.9 % (LG 2 adult tree data), 0.8 % (LG 17 adult tree data); 16.7% (LG 2 seedling data) and 8.3 % (LG 17 seedling data) of the observed phenotypic variation. These may be good candidates for a MAB or MAS program as they are detected in both adult and seedling stages. As was previously suggested, the 'aPa-' prefixed DArT markers may be used as candidates for SSR primer design, as this type of marker is co-dominant and more readily transferable, So far as the SAmsCV627191b SSR marker is concerned, testing across different mapping populations, developmental stages, and environments may help to validate this marker further.

UNIVERSITY of the
WESTERN CAPE

Other QTLs and major have been reported in apple where SSRs markers and other molecular genetic markers have been employed in construction of the dense maps. These are: apple scab resistance genes *Va, Vb, Vbj, Vb, Vf, Vg Vh2, Vh4, Vm,* and *Vr2* genes (Durel *et al.,* 2003, 2004; Erdin *et al.,* 2006; Vinatzer, 2001; Gygax *et al.,* 2004; Bus *et al.,* 2004; Liebhard *et al.,* 2003c; Xu and Korban, 2000), powdery mildew (Liebhard *et al.,* 2002; Dunemann *et al.,* 2007; Maliepaard *et al.,* 1998; Calenge and Durel, 2006), fire

blight resistance (Calenge *et al.,* 2005a, b; Khan, 2006, 2007; Durel *et al.,* 2009), Woolly apple aphid (Gardiner *et al.,* 2007; Bus *et al.,* 2008) and dormancy-related traits such as initial IRB and IVB (Labuschagné *et al.,* 2002a, 2002b; van Dyk *et al.,* 2010; Celton *et al.,* 2011). For a comprehensive list of the various QTLs. major genes and candidate genes localized in fruit crops, see Oraguzie and Bell (2008).

It needs to be noted that the major weaknesses of the QTL analysis in this work is the low population size of 80 individuals, which was too low to effect stronger QTL analysis. The strengths of this work however, are the replication of phenotypic data collection over 3 years/ seasons and the comparison between seedling and adult tree data.

UNIVERSITY of the
WESTERN CAPE

4.9 CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study has led to the successful development of novel SSR markers, which were tested in megaplexes. Furthermore, as was of the major the objectives of this work, SSR and DArT markers were successfully tested, scored and mapped on the 'Anna' x 'Austin' mapping population to create an integrated genetic linkage map. It is therefore recommended that future studies utilize SSR and DArT marker in genetic map construction as they supply good marker density and complementarity. Sequenced genomes also present a wealth of data for marker development as was demonstrated in this thesis and the next level analysis for the mapping population would be to use SNP markers to saturate the linkage map regions where QTLs have been localized. Putative QTLs responsible for the control of the time of IVB in both seedling and adult populations have also been detected over a 4.7 LOD threshold in LGs 2, 9 and 17 using the rMQM technique. Other QTLs were also found to be seedling (in LG 14) and adult (in LG 5, 7 and 10) specific at above the same LOD threshold. Furthermore, from this study, rMQM mapping is recommended above interval mapping for QTL analysis as it more powerful and gives less false negatives. Further work is required in this regard and and the regression method of QTL analysis can be used to check some of the QTLs found in this work which were found to have a high LOD and explained less than 5 % of the phenotypic variation.

UNIVERSITY of the
WESTERN CAPE

Two putative QTLs in LGs 2 and 17, along with their associated markers are potential candidates for testing in apple MAB and MAS programs for the development of low chill apples, as they seem stable and consistent over three years in both adult and seedling

populations. Furthermore, the regions in which the QTLs localize should be interrogated for candidate genes by mining the corresponding regions on the apple genome as an approach that was successfully used by Celton *et al.* (2011). Once these candidate genes have been identified they can be isolated for transformation experiments as was done by the Kotoda *et al.* (2010) group, who isolated and characterised *Malus domestica FT*-like genes *MdFT1* and *MdFT2* derived from *Arabidopsis thaliana*, on the induction of early flowering in apple and *Arabidopsis*. Genes with potential for such work also include those reported by Celton *et al.* (2011) in apple and Hadley *et al.* (2010) in blackcurrant (*Ribes nigrum*).

UNIVERSITY of the
WESTERN CAPE

5.0 REFERENCES

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports, 27*(4), 617-631.

Albert, V. A., Soltis, D. E., Carlson, J. E., Farmerie, W. G., Wall, P. K., Ilut, D. C., et al. (2005). Floral gene resources from basal angiosperms for comparative genomics research. *BMC plant biology, 5*(5).

Amasino, R. M. (1996). Control of flowering time in plants. *Current Opinion in Genetics & Development, 6*(4), 480-487.

Amasino, R. M. (2005). Vernalization and flowering time. *Current Opinion in Biotechnology*, 154-158.

AnalystSoft-Inc (2009). StatPlus:mac LE - free statistical analysis program. (Version Mac OS. Version 2009).

Anderson, J. V., Gesch, R. W., Jia, Y., Chao, W. S., & Horvath, D. P. (2005). Seasonal shifts in dormancy status, carbohydrate metabolism, and related gene expression in crown buds of leafy spurge. *Plant, Cell and Environment, 28*, 1567-1578.

Arora, R., Rowland, L. J., & Tanino, K. (2003). Induction and Release of Bud Dormancy in Woody Perennials: A Science Comes of Age. *HortScience, 38*(5), 911-921.

Arus, P., Yamamoto, T., Dirlewanger, E., & Abbot., A. G. (2006). Synteny in the Rosaceae. In J. Janick (Ed.), *Plant Breeding Reviews* (Vol. 27, pp. 175-211). New Jersey, USA: John Wiley and Sons, Inc.

Asins, M. J. (2002). Review: Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding, 121*, 281-291.

Baker, M. (2010). Clever PCR: more genotyping, smaller volumes. *Nature Methods, 7*(5), 351-

UNIVERSITY of the
WESTERN CAPE

356.

Bashalkhanov, S., & Rajora, O. (2008). Protocol: A high-throughput DNA extraction system suitable for conifers. *Plant Methods, 4*, 20.

Beckmann, J. S., & Soller, M. (1990). Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Bio/Technology, 8*, 930-932.

Benson, G. (1999). Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research, 27*(2), 573-580.

Bernier, G., & Périlleux, C. (2005). A physiological overview of the genetics of flowering time control. *Plant Biotechnology Journal, 3*, 3-16.

Bink, M. C. A. M., Uimari, P., Sillanpää, M. J., Janss, L. L. G., & Jansen, R. C. (2002). Multiple QTL mapping in related plant populations via a pedigree-analysis approach. *Theoretical and Applied Genetics, 104*, 751-762.

Borchert, D. M., Stinner, R. E., Walgenbach, J. F., & Kennedy, G. G. (2004). Oriental fruit moth (Lepidoptera: Tortricidae) phenology and management with methoxyfenozide in North Carolina apples. *Journal of Economic Entomology, 97*(4), 1353-1364.

Boss, P. K., Bastow, R. M., Mylne, J. S., & Dean, C. (2004). Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting. *The Plant Cell, 16*(Supplement), 18-31.

Bradshaw, H. D., Jr., & Stettler, R. F. (1995). Molecular genetics of growth and development in populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. *Genetics, 139*(2), 963-973.

Bus, V. G. M., Chagné, D., Bassett, H. C. M., Bowatte, D., Calenge, F., Celton, J.-M., et al. (2008). Genome mapping of three major resistance genes to wooly apple aphid (*Eriosoma lanigerum* Hausm.). *Tree Genetics and Genomes, 4*, 223-236.

UNIVERSITY of the
WESTERN CAPE

Calenge, F., Drouet, D., Denance, C., Van de Weg, W. E., Brisset, M. N., Paulin, J. P., et al. (2005b). Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theoretical and Applied Genetics, 111*(1), 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 assessment. *Molecular Breeding, 17*, 329-339.

Calenge, F., Van der Linden, C. G., Van de Weg, E., Schouten, H. J., Van Arkel, G., Denance, C., et al. (2005a). Resistance gene analogues identified through the NBS-profiling method map close to major genes and QTL for disease resistance in apple. *Theoretical and Applied Genetics, 110*(4), 660-668.

Campoy, J. A., Martínez-Gómez, P., Ruiz, D., Rees, J., & Celton, J. M. (2010a). Developing Microsatellite Multiplex and Megaplex PCR Systems for High-Throughput Characterization of Breeding Progenies and Linkage Maps Spanning the Apricot (*Prunus armeciaca* L .) Genome. *Plant Molecular Biology Reporter, 28*, 560-568.

Campoy, J. A., Ruiz, D., Egea, J., Rees, D. J. G., Celton, J. M., & Martínez-Gómez, P. (2010b). Inheritance of Flowering Time in Apricot (*Prunus armeniaca* L .) and Analysis of Linked Quantitative Trait Loci (QTLs) using Simple Sequence Repeat (SSR) Markers. *Plant Molecular Biology Reporter, 29*(2), 404-410.

Celton, J.-M., Kelner, J. J., Martinez, S., Garcia, G., & Costes, E. (2010). *QTL detection for phenological traits using two independent segregating F1 apple (Malus x domestica Borkh.) populations.* Paper presented at the Rosaceae Genome Conference 5 (RGC5), Stellenbosch, Cape town, South Africa.

UNIVERSITY of the
WESTERN CAPE

Celton, J.-M., Martinez, S., Jammes, M.-J., Bechti, A., Salvi, S., Legave, J.-M., et al. (2011). Deciphering the genetic determinism of bud phenology in apple progenies: a new insight into chilling and heat requirement effects on flowering dates and positional candidate genes. *New Phytologist*, 1-15.

Celton, J.-M., Tustin, D. S., Chagné, D., & Gardiner, S. E. (2009). Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from Malus ESTs and Pyrus genomic sequences. *Tree Genetics and Genomics, 5*(1), 93-107.

Cesaraccio, C., Spano, D., Snyder, R. L., & Duce, P. (2004). Chilling and forcing model to predict bud-burst of crop and forest species. *Agricultural and Forest Meteorology, 126*, 1-13.

Chambers, G. K., & MacAvoy, E. S. (2000). Microsatellites: consensus and controversy *Comparative Biochemistry and Physiology Part B, 126*, 455-476.

Chandrababu, R. J., & Sharma, R. K. (1999). Heritability estimates in almond [*Prunus dulcis* (Miller) D.A. Webb]. *Scientia Horticulturae 79*, 237-243.

Chen, T. H. H., Davis, J., Frewen, B. E., Howe, G. T., & Bradshaw Jr, H. D. (2000). Molecular Genetic Analysis of Bud Dormancy-related Traits in *Populus*. In J.-D. Viemont & J. Crabbe (Eds.), *Dormancy in Plants: From whole plant behaviour to cellular control* (pp. 319-329). New York, USA: CABI Publishing.

Chmielewski, F.-M., Müller, A., & Bruns, E. (2004). Climate changes and trends in phenology of fruit trees and field crops in Germany, 1961–2000. *Agricultural and Forest Meteorology, 121*, 69-79.

Choi, K., Park, C., Lee, J., Oh, M., Noh, B., & Lee, I. (2007). Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development

UNIVERSITY of the
WESTERN CAPE

Development (Cambridge, England), 134(10), 1931-1941.

Cipriani, G., Lot, G., Huang, W.-G., Marrazzo, M. T., Peterlunger, E., & Testolin, R. (1999). AC / GT and AG / CT microsatellite repeats in peach [*Prunus persica* (L) Batsch] : isolation, characterisation and cross-species amplification in *Prunus*. *Theoretical and Applied Genetics, 99*, 65-72.

Cipriani, G., Marrazzo, M. T., Di Gaspero, G., Pfeiffer, A., Morgante, M., & Testolin, R. (2008). A set of microsatellite markers with long core repeat optimized for grape (Vitis spp.) genotyping. *BMC Plant Biology, 8*, 127.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica, 142*, 169-196.

Conner, P. J., Brown, S. K., & Weeden, N. F. (1998). Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. *Theoretical and Applied Genetics, 96*, 1027-1035.

Cook, N. C., Bellen, A., Cronje, P. J. R., De Wit, I., Keulemans, W., Van den Putte, A., et al. (2005). Freezing temperature treatment induces bud dormancy in ‘Granny Smith’ apple shoots. *Scientia Horticulturae, 106*, 170–176.

Cook, N. C., & Bellstedt, D. U. (2001). Chilling response of 'Granny Smith' apple lateral buds inhibited by distal shoot tissues. *Scientia Horticulturae, 89*, 299-308.

Cook, N. C., Bellstedt, D. U., & Jacobs, G. (2001). Endogenous cytokinin distribution patterns at budburst in Granny Smith and Braeburn apple shoots in relation to bud growth. *Scientia Horticulturae, 87*, 53-63.

Cook, N. C., & Jacobs, G. (2000). Progression of apple (*Malus x domestica* Borkh.) bud

UNIVERSITY of the
WESTERN CAPE

dormancy in two mild winter climates. *Journal of Horticultural Science and Biotechnology, 75*(2), 233-236.

Corbesier, L., & Coupland, G. (2005). Photoperiodic flowering of Arabidopsis: integrating genetic and physiological approaches to characterization of the floral stimulus. *Plant, Cell and Environment, 28*, 54-66.

Corbesier, L., & Coupland, G. (2006). The quest for florigen: a review of recent progress. *Journal of Experimental Botany, 57*(13), 3395-3403.

Coupland, G. (1995). Genetic and Environmental Control of Flowering time in *Arabidopsis*. *Trends in Genetics, 11*(10), 393-397.

Dandekar, A., Iezzoni, A., Abbott, B., Sosinski, B., Peace, C., Crisoto, C., et al. (2006). The US Rosaceae Genomics, Genetics and Breeding Initiative White paper.

Deciduous Fruit Producers' Trust, D. F. P. T. (2008). Apple production figures for South Africa, 2005 - 2006 season Retrieved 10 August, 2008, from http://www.dfpt.co.za

Dennis, E. S., & Peacock, W. J. (2007). Epigenetic regulation of flowering. *Current Opinion in Plant Biology*, 520–527.

Dirlewanger, E. (2010). *QTLs detection for phenological traits within the Rosaceae family: Prunus, Fragaria, Malus.* Paper presented at the Rosaceae Genome Conference 5 (RGC5), Stellenbosch, Cape town, South Africa.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M. J., Poizat, C., Zanetto, A., et al. (2002). Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theoretical and Applied Genetics, 105*, 127-138.

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental

UNIVERSITY of the
WESTERN CAPE

populations. *Nature reviews. Genetics, 3*(1), 43-52.

Dudley, J. W. (2002). Integrating Molecular Techniques into Quantitative Genetics and Plant Breeding. In M. S. Kang (Ed.), *Quantitative Genetics, Genomics, and Plant Breeding* (pp. 69-84). New York, USA: CAB International.

Dunemann, F., Peil, A., Urbanietz, A., & Garcia-Libreros, T. (2007). Mapping of the apple powdery mildew resistance gene *Pl1* and its genetic association with an NBS-LRR candidate resistance gene *Plant Breeding, 126*, 476-481.

Durel, C. E., Calenge, F., Parisi, L., Van de Weg, W. E., Kodde, L. P., Liebhard, R., et al. (2004). Overview on position and robustness of scab resistance QTL and major genes by alignment of genetic maps in five apple progenies. *Acta Horticulturae, 663* 135-140.

Durel, C. E., Denance, C., & Brisset, M. N. (2009). Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes 'Evereste' and *Malus floribunda* clone 821. *Genome, 52*(2), 139-147.

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 of *Venturia inaequalis* in apple. . *Genome, 46*, 224-234.

Erdin, N., Tartarini, S., Broggini, G. A., Gennari, F., Sansavini, S., Gessler, C., et al. (2006). Mapping of the apple scab-resistance gene Vb. *Genome, 49*(10), 1238-1245.

Fan, S., Bielenberg, D. G., Zhebentyayeva, T. N., Reighard, G. L., Okie, W. R., Holland, D., et al. (2010). Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). *The New phytologist, 185*(4), 917-930.

Forsline, P. L., Aldwinkle, H. S., Dickson, E. E., Luby, J. J., & Hokanson, S. C. (2003).

UNIVERSITY of the
WESTERN CAPE

Collection, Maintenance, Characterization and Utilization of Wild Apples of Central Asia. In J. Janick (Ed.), *Horticultural Reviews* (Vol. 29, pp. 1-62). New York, USA: John Wiley and Sons, Inc.

Ganal, M. W., Altmann, T., & Roder, M. S. (2009). SNP identification in crop plants. *Current Opinion in Plant Biology, 12*, 211-217.

Gao, L., Tang, J., Li, H., & Jia, J. (2003). Analysis of microsatellites in major crops assessed by computational and experimental approaches. *Molecular Breeding, 12*, 245–261.

Gao, W., Clancy, J. A., Han, F., Prada, D., Kleinhofs, A., & Ullrich, S. E. (2003). Molecular dissection of a dormancy QTL region near the chromosome 7 (5H) L telomere in barley. *Theoretical and Applied Genetics, 107*(3), 552-559.

Gardiner, S. E., Bus, V. G. M., Rusholme, R. L., Chagne, D., & Rikkerink, E. H. A. (2007). Apple. In C. Kole (Ed.), *Genome Mapping and Molecular Breeding in Plants: Fruits and Nuts* (Vol. 4, pp. 1-62). Berlin Heidelberg, Germany: Springer-Verlag.

Gariglio, N., Gonza, D. E., Reig, C., Agusti, M., & Mendow, M. (2006). Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots. *Scientia Horticulturae, 108*, 371-377.

Gelfand, Y., Rodriguez, A., & Benson, G. (2007). TRDB-The Tandem Repeats Database. *Nucleic Acids Research, 35*(Database), 80-87.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M., & Gessler, C. (1998). Simple sequence repeats for the genetic analysis of apple. *Theoretical and Applied Genetics, 96*, 1069-1076.

Grab, S., & Craparo, A. (2011). Advance of apple and pear tree full bloom dates in response to climate change in the southwestern Cape, South Africa: 1973–2009. *Agricultural and*

UNIVERSITY of the
WESTERN CAPE

Forest Meteorology, 151(3), 406-413.

Grando, M. S., & Frisinghelli, C. (1998). Grape microsatellite markers: Sizing of DNA alleles and genotype analysis of some grapevine cultivars. *Vitis-Geilweilerhof, 37*(2), 79-82.

Gratacos, E., & Cortes, A. (2009). Phenology and Production of Sweet Cherry Cultivars in a Low Chilling Area of Central Chile Retrieved 03 February, 2009, from http-//www.biocerezas.cl/docs/gratacos/Turkia/Phenology_and_Production_of_Sweet_Cherry_Cultivars_in_a_Low_Chilling_Area_of_Central_Chile.pdf

Guilford, P., Prakash, S., Zhu, J. M., Rikkerink, E., Gardiner, S., Bassett, H., et al. (1997). Microsatellites in *Malus* x *domestica* (apple): abundance, polymorphism and cultivar identification. *Theoretical and Applied Genetics, 94*, 249-254.

Gupta, P. K., Rustgi, S., & Kulwal, P. L. (2005). Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Molecular Biology, 57*(4), 461-485.

Gupta, P. K., Rustgi, S., & Mir, R. R. (2008). Array-based high-throughput DNA markers for crop improvement. *Heredity, 101*(1), 5-18.

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. *Theoretical and Applied Genetics, 109*(8), 1702-1709.

Harris, S. A., Robinson, J. P., & Juniper, B. E. (2002). Genetic clues to the origin of the apple. *Trends in Genetics, 18*(8), 426-430.

Hauagge, R. (2010). ‘IPR Julieta’, a New Early Low Chill Requirement Apple Cultivar. *Acta Horticulturae, 872*, 193-196.

Hauagge, R., & Cummins, J. N. (1991a). Phenotypic variation of length of bud dormancy in

UNIVERSITY of the
WESTERN CAPE

apple cultivars and related *Malus* species. *Journal of the American Society for Horticultural Science, 116*, 100-106.

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

Hedley, P. E., Russell, J. R., Jorgensen, L., Gordon, S., Morris, J. A., Hackett, C. A., et al. (2010). Candidate genes associated with bud dormancy release in blackcurrant (*Ribes nigrum* L.). *BMC Plant Biology, 10*, 1-13.

Hemmat, M., Weeden, N. F., & Brown, S. K. (2003). Mapping and evaluation of *Malus* x *domestica* microsatellites in apple and pear. *Journal of the American Society for Horticultural Science, 128*, 515-520.

Hemmat, M., Weeden, N. F., Manganaris, A. G., & Lawson, D. M. (1994). Molecular marker linkage map for apple. *The Journal of Heredity, 85*(1), 4-11.

Henderson, C. R. (1975). Best Linear Unbiased Estimation and Prediction under a Selection Model. *Biometrics, 31*(2), 423-447.

Hill, J., Becker, H. C., & Tigerstedt, P. M. A. (1998). *Quantitative and ecological aspects of plant breeding*. Suffolk, UK: St. Edmundsbury Press.

Holton, T. A. (2001). Plant Genotyping by Analysis of Microsatellites. In R. J. Henry (Ed.), *Plant Genotyping: the DNA Fingerprinting of Plants* (pp. 15-28). New York, USA: CAB International.

Horvath, D. P., Anderson, J. V., Chao, W. S., & Foley, M. E. (2003). Knowing when to grow: signals regulating bud dormancy. *Trends in Plant Science, 8*(11), 534-540.

Howe, G. T., Sarmuul, P., Davis, J., & Chen, T. H. H. (2000). Quantitative genetics of bud phenology, frost damagem and winter, and winter survival in an F2 family of hybrid

UNIVERSITY of the
WESTERN CAPE

poplars. *Theoretical and Applied Genetics 101*, 632-642.

Ibanez, A. M., & Dandekar, A. M. (2007). Apple. In E. C. Pua, and Darvey, M.R. (Ed.), *Biotechnology in Agriculture and Forestry: Transgenic Crops V* (Vol. 60, pp. 241-282). Berlin Heidelberg, Germany: Springer-Verlag.

Igarashi, M., Abe, Y., Hatsuyama, Y., Ueda, T., Fukasawa-Akada, T., Kon, T., et al. (2008). Linkage maps of the apple (*Malus x domestica* Borkh.) cvs 'Ralls Janet' and 'Delicious' include newly developed EST markers *Molecular Breeding, 22*, 95-118.

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research, 29*(4), 1-25.

Jackson, E. W., Feng, C., Fenn, P., & Chen, P. (2009). Genetic mapping of resistance to Phomopsis seed decay in the soybean breeding line MO/PSD-0259 (PI562694) and Plant Introduction 80837. *The Journal of Heredity, 100*(6), 777-783.

Jackson, J., & Bepete, M. (1995). The effect of hydrogen cyanamide (Dormex) on flowering and cropping of different apple cultivars under tropical conditions of sub-optimal winter chilling. *Scientia horticulturae, 60*(94), 293-304.

Jaeger, K. E., Graf, A., & Wigge, P. A. (2006). The control of flowering in time and space. *Journal of Experimental Botany, 57*(13), 3415-3418.

Janick, J. (2005). The origin of fruits, fruit growing, and fruit breeding. In J. Janick (Ed.), *Plant Breeding Reviews* (Vol. 25, pp. 255-320). New Jersey, USA: John Wiley and Sons, Inc.

Janick, J., Cummins, J. N., Brown, S. K., & Hemmat, M. (1996). Apples. In J. Janick, and Moore, J. (Ed.), *Fruit Breeding: Tree and Tropical Fruits* (Vol. 1, pp. 1-77). New York, USA: John Wiley and Sons, Inc.

UNIVERSITY of the
WESTERN CAPE

Jansen (1996). Complex plant traits: time for polygenic analysis *Trends in Plant Science, 1*(3), 89-94.

Jansen, R. C. (1994). Controlling the Type I and Type II Errors in Mapping Quantitative Trait Loci *Genetics 138* 871-881.

Jansen, R. C., & Nap, J.-P. (2001). Genetical Genomics: the added value from segregation. *Trends in Genetics, 17*, 388-391.

Jansson, S., & Douglas, C. J. (2007). Populus: A Model System for Plant Biology. *Annual Review of Plant Biology, 58*, 435-458.

Jiménez, S., Li, Z., Reighard, G. L., & Bielenberg, D. G. (2010). Identification of genes associated with growth cessation and bud dormancy entrance using a dormancy-incapable tree mutant. *BMC plant biology, 10*, 25.

Jones, N., Ougham, H., & Thomas, H. (1997). Markers and mapping: we are all geneticists now. *The New Phytologist, 137*, 165-177.

Jordan, S. A., & Humphries, P. (1994). Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35 *Human molecular genetics, 3* 1915.

Jung, S., Abbott, A., Jesudurai, C., Tomkins, J., & Main, D. (2005). Frequency, type, distribution and annotation of simple sequence repeats in Rosaceae ESTs. *Functional and Integrative Genomics 5*, 136-143.

Jung, S., Jesudurai, C., Staton, M., Du, Z., Ficklin, S., Cho, I., et al. (2004). GDR (Genome Database for Rosaceae): integrated web resources for Rosaceae genomics and genetics research. *BMC Bioinformatics, 5*(130).

Jung, S., Staton, M., Lee, T., Blenda, A., Svancara, R., Abbott, A., et al. (2008). GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics

UNIVERSITY of the
WESTERN CAPE

data. *Nucleic Acids Research, 36*(Database), 1034-1040.

Juniper, B. E., & Mabberly, D. J. (2006). *The story of the apple*. Portland, Oregon: Timber Press.

Kantety, R. V., La Rota, M., Matthews, D. E., & Sorrells, M. E. (2002). Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Molecular Biology, 48*, 501–510.

Kartesz, J. T., Gandhi, K. N., & (1992). Nomenclatural notes for the North American Flora. *Phytologia, 73*, 124–136.

Kearsey, M. J. (2002). QTL Analysis: Problems and (Possible) Solutions. In M. S. Kang (Ed.), *Quantitative Genetics, Genomics, and Plant Breeding* (pp. 45-58). New York, USA: CAB International.

Kearsey, M. J., & Farquhar, G. L. (1998). QTL analysis in plants; where are we now? *Heredity, 80*, 137-142.

Kellerhals, M. (2009). Introduction to Apple (*Malus x domestica*). In K. M. Folta, and Gardiner, S. E. (Ed.), *Genetics and Genomics of Rosaceae* (Vol. 6, pp. 78-84). New York, USA: Springer Science+Business Media.

Kenis, K., & Keulemans (2004). QTL Analysis of growth characteristics in apple. *Acta Horticulturae, 663*, 369-374.

Kenis, K., & Keulemans, J. (2005). Genetic linkage maps of two apple apple cultivars (*Malus* x *domestica* Borkh.) based on AFLP and microsatellite markers. *Molecular Breeding, 15*, 205-219.

Khan, M. A., Durel, C. E., Duffy, B., Drouet, D., Kellerhals, M., Gessler, C., et al. (2007). Development of molecular markers linked to the 'Fiesta' linkage group 7 major QTL for fire blight resistance and their application for marker-assisted selection. *Genome, 50*(6),

UNIVERSITY of the
WESTERN CAPE

568-577.

Khan, M. A. B., Duffy, C., Gessler, C., & Patocchi, A. (2006). QTL mapping of fire blight resistance in apple. *Molecular Breeding, 17*, 299-306.

Kilian, A., Huttner, E., Wenzl, P., Jaccoud, D., Carling, J., Caig, V., et al. (2003). *The fast and cheap: SNP and DArT-based whole genome profiling for crop improvement.* Paper presented at the Proceedings of the International Congress " In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution", Bologna, Italy.

Korban, S. S., & Skirvin, R. M. (1994). Nomenclature of the cultivated apple *HortScience, 19*, 177-180.

Kosambi, D. D. (1944). The estimation of map distance from recombination values. *Annals of Eugenics, 12*, 172–175.

Kotoda, N., Hayashi, H., Suzuki, M., Igarashi, M., Hatsuyama, Y., Kidou, S.-i., et al. (2010). Molecular characterization of *FLOWERING LOCUS T-like* genes of apple (*Malus x domestica* Borkh.). *Plant and Cell Physiology, 51*(4), 561-575.

Kumar, L. S. (1999). DNA markers in plant improvement: An overview *Biotechnology Advances, 17*, 143–182

Labuschagne, I., Louw, B., Schmidt, K., & Sadie, A. (2002a). Genotypic Variation in Prolonged Dormancy Symptoms in Apple Progenies. *HortScience, 37*(1), 157-163.

Labuschagne, I., Louw, J. H., Schmidt, K., & Sadie, A. (2002b). Genetic Variation in Chilling Requirement in Apple Progeny. *Journal of the American Society for Horticultural Science, 127*(4), 663-672.

Labuschagne, I. F., Louw, J. H., Schmidt, K., & Sadie, A. (2003). Selection for increased budbreak in apple *Journal of the American Society for Horticultural Science, 128*, 363-

UNIVERSITY of the
WESTERN CAPE

374.

Lang, G. A., Early, J. D., Martin, G. C., & Darnell, R. L. (1987). Endo-, para-, and eco-dormancy: physiological terminology and classification for dormancy research *HortScience, 22*, 371-377.

Laurens, F. (1999). *Review of The Current Apple Breeding Programmes in the World: Objectives for Scion Cultivar Improvement.* Paper presented at the Proceedings of the Eucarpia Symposium on Fruit Breeding and Genetics.

Lawson, D. M., Hemmat, M., & Weeden, N. F. (1995). The use of molecular markers to analyze the inheritance of morphological and developmental traits in apple. *Journal of the American Society for Horticultural Science, 120*, 532-537.

Lazzari, B., Caprera, A., Vecchietti, A., Stella, A., Milanesi, L., & Pozzi, C. (2005). ESTree db: a Tool for Peach Functional Genomics. *BMC Bioinformatics, 6*(Suppl 4), S16.

Lee, M. (1995). DNA markers and plant breeding programs. *Advances in Agronomy, 55*, 265-344

Legave, J. M., Farrera, I., Almeras, T., Santamaria, P., & Calleja, M. (2008). Selecting models of apple flowering time and understanding how global warming has had an impact on this trait. *The Journal of Horticultural Science & Biotechnology, 83*(1), 76-84.

Lespinasse, Y., Bouvier, L., Djulbic, M., & Chevreau, E. (1999). Haploidy in apple and pear. *Acta Horticulturae, 484*, 49-54.

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C. D., Tarchini, R., Van De Weg, E., et al. (2002). Development and characterisation of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). *Molecular Breeding, 10* 217-241.

Liebhard, R., Kellerhals, M., Pfammatter, W., Jertmini, M., & Gessler, C. (2003a). Mapping quantitative physiological traits in apple (Malus x domestica Borkh.). *Plant Molecular*

UNIVERSITY of the WESTERN CAPE

Biology, 52(3), 511-526.

Liebhard, R., Koller, B., Gianfranceschi, L., & Gessler, C. (2003b). Creating a saturated reference map for the apple (Malus x domestica Borkh.) genome. *Theoretical and Applied Genetics, 106*(8), 1497-1508.

Liebhard, R., Koller, B., Patocchi, A., Kellerhals, M., Pfammatter, W., Jermini, M., et al. (2003c). Mapping quantitative field resistance against apple scab in a "Fiesta" × "Discovery" progeny. *Phytopathology, 93* 493–501.

Linsley-Noakes, G. C., Allan, P., & Matthee, G. (1994). Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. *Journal of the Southern African Society for Horticultural Sciences, 4*, 13-15.

Luby, J. J. (2003). Taxonomic Classification and Brief History. In D. C. Ferree, and Warrington, I.J. (Ed.), *Apples: Botany, Production and Uses* (pp. 1-14). Cambidge, Massachusetts, USA: CABI Publishing.

Luby, J. J., & Shaw, D. V. (2001). Does Marker-assisted Selection Make Dollars and Sense in a Fruit Breeding Program. *HortScience, 36*(5), 872-879.

Mabberley, D. J., Jarvis, C. E., & Juniper, B. E. (2001). The name of the apple. *Telopea, 9*(2), 421-430.

Mackay, I., & Powell, W. (2006). Methods for linkage disequilibrium mapping in crops *Trends in Plant Science, 12*(2), 57-63.

Mackay, T. F. C., Stone, E. A., & Ayroles, J. F. (2009). The genetics of quantitative traits: challenges and prospects *Nature Reviews: Genetics, 10*, 565-577.

Maliepaard, C., Alston, F. H., Van Arkel, G., Brown, L. M., Chevreau, E., Dunemann, F., et al. (1998). Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using

UNIVERSITY of the
WESTERN CAPE

multi-allelic markers *Theoretical and Applied Genetics, 97*, 60–73.

Maliepaard, C., Jansen, J., & Van Ooijen, J. W. (1997). Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. *Genetical Research, 70*, 237-250.

Markoulatos, P., Siafakas, N., & Moncany, M. (2002). Multiplex polymerase chain reaction: a practical approach. *Journal of Clinical Laboratory Analysis, 16*, 47-51.

Martinez, J. J., Gardea, A. A., Sagnelli, S., & Olivas, J. (1999). Sweet cherry adaptation to mild winters. *Fruit Varieties Journal, 53*, 181-183.

Masi, P., Spagnoletti Zeuli, P., & Donini, P. (2003). Development and analysis of multiplex microsatellite markers sets in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding, 11*, 303- 313.

Meuzelaar, L. S., Lancaster, O., Pasche, J. P., Kopal, G., & Brookes, A. (2007). MegaPlex PCR: a strategy for multiplex amplification. *Nature Methods, 4*(10), 835-837.

Mexal, J. G., Sammis, T. W., & Herrera, E. A. (2009). Cold Hardiness and Dormancy of apple trees. What's the difference? Retrieved 03 February, 2009, from http-//weather.nmsu.edu/nmcrops/Trees/Apples/coldhard_dorm/cold-hardiness.htm

Michaels, S. D. (2009). Flowering time regulation produces much fruit. *Current Opinion in Plant Biology, 12*, 75-80.

Mitchell, S. E., Kresovich, S., Jester, C. A., Hernandez, C. A., & Szewe-McFadden, A. K. (1997). Application of multiplex PCR and fluorescence based, semi-automated allele-sizing technology for genotyping plant genetic resources. *Crop Science, 37*, 617-624.

Morgan, J., Richards, A., & Dowle, E. (2003). *The New Book of Apples: The Definitive Guide to Over 2,000 Varieties*. London, UK: Ebury Press.

UNIVERSITY of the
WESTERN CAPE

Morgante, M., & Salamini, F. (2003). From plant genomics to breeding practice. *Current Opinion in Biotechnology, 14*(2), 214-219.

Morton, N. E. (1996). Logarithm of odds (lods) for linkage in complex inheritance (polygenic linkage) *Proceedings of the National Academy of Science US, Vol. 93*, 3471-3476.

Nagaraj, S. H., Gasser, R. B., & Ranganathan, S. (2006). A hitchhiker's guide to expressed sequence tag (EST) analysis. *Briefings in Bioinformatics, 8*(1), 6-21.

Okubo, H. (2000). Growth Cycle and Dormancy in Plants. In J.-D. Viemont & J. Crabbe (Eds.), *Dormancy in Plants: From whole plant behaviour to cellular control* (pp. 1-22). New York, USA: CABI Publishing.

Oraguzie, N., & Bell, R. (2008). Table of Marker-Trait associations for website Sept. 2008 Retrieved 10 October, 2009, from http://hrt2.msu.edu

Peace, C., & Norelli, J. L. (2009). Genomics Approaches to Crop Improvement in the Rosaceae. In K. M. Folta & S. E. Gardiner (Eds.), *Genetics and Genomics of Rosaceae* (Vol. 6, pp. 19-54). New York, USA: Springer Science+Business Media.

Perishable Product Export Control Board, P. P. E. C. B. (2008). Apple exports volumes for South Africa, 2005 - 2006 season Retrieved 10 August, 2008, from http://www.ppecb.com

Phipps, J. B., Robertson, K. R., Rohrer, J. R., & Smith, P. G. (1991). Origins and evolution of subfam. *Maloideae* (Rosacea). *Systematic Botany, 16*(2), 303-332.

Potter, D., Still, S. M., Grebenc, T., Ballian, D., Bozic, G., Franjiae, J., et al. (2007). Phylogenetic relationships in tribe Spiraeeae (Rosaceae) inferred from nucleotide sequence data. *Plant Systematics and Evolution, 266*, 105-118.

Powell, W., Machray, G. C., & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science, 1*(7), 214-222.

UNIVERSITY of the
WESTERN CAPE

Rafalski, A. (2002). Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology, 5*(2), 94-100.

Rakoczy-Trojanowska, M., & H., B. (2004). Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. *Cellular & Molecular Biology Letters, 9*, 221-238.

Ramsay, L., Macaulay, M., Ivanissevich, S., MacLean, K., Cardle, L., Fuller, J., et al. (2000). A simple sequence repeat-based linkage map of barley *Genetics, 156*, 1997-2005.

Ribaut, J.-M., Banziner, M., Betran, J., Jian, C., Edmeades, G. O., Dreher, K., et al. (2002). Use of Molecular Markers in Plant Breeding: Drought Tolerance Improvement in Tropical Maize. In M. S. Kang (Ed.), *Quantitative Genetics, Genomics, and Plant Breeding* (pp. 85-100). New York, USA: CAB International.

Robinson, G. K. (1991). That BLUP is a good thing: The estimation of random effects. *Statistical Science, 6*(1), 15-51.

Robinson, J. P., Harris, S. A., & Juniper, B. E. (2001). Taxonomy of the genus *Malus* Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Systematics and Evolution, 226*, 35-58.

Rohde, A., & Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends in Plant Science, 12*(5), 217-223.

Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology, 132*, 365-386.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (Vol. 1). New York: Cold Spring Harbor Laboratory Press.

Sax, K. (1923). The association of size differences with seed-coat pattern and pigmentation in

UNIVERSITY of the WESTERN CAPE

Phaseolus vulgaris. *Genetics, 8*, 552-560.

Schon, C. C., Utz, H. F., Groh, S., Truberg, B., Openshaw, S., & Melchinger, A. E. (2004). Quantitative Trait Locus Mapping Based on Resampling in a Vast Maize Testcross Experiment and Its Relevance to Quantitative Genetics for Complex Traits. *Genetics, 167* 485-498.

Segura, V., Denance, C., Durel, C. E., & Costes, E. (2007). Wide range QTL analysis for complex architectural traits in a 1-year-old apple progeny. *Genome, 50*(2), 159-171.

Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology, 5* (25), 2540-2568.

Shulaev, V., Korban, S. S., Sosinski, B., Abbott, A. G., Aldwinckle, H. S., Folta, K. M., et al. (2008). Multiple models for Rosaceae genomics. *Plant Physiology, 147*(3), 985-1003.

Silfverberg-Dilworth, E., Matasci, C. L., Van de Weg, W. E., Van Kaauwen, M. P. W., Walser, M., Kodde, L. P., et al. (2006). Microsatellite markers spanning the apple (*Malus x domestica* Borkh.) genome. *Tree Genetics & Genomes, 2*, 202–224.

Silva, C., Garcia-Mas, J., Sanchez, A. M., Arus, P., & Oliveira, M. M. (2005). Looking into flowering time in almond (*Prunus dulcis* (Mill) D. A. Webb): the candidate gene approach. *Theoretical and Applied Genetics, 110*, 959-968.

Sobrino, B., Briona, M., & Carracedoa, A. (2005). SNPs in forensic genetics: a review on SNP typing methodologies. *Forensic Science International, 154*, 181-194.

Soeker, M. K. (2012). *Genetic mapping of fruit quality traits in Apple (Malus x domestica Borkh.).* University of the Western Cape.

Sorenson, F. C. (1983). Relationship between logarithms of chilling period and germination or bud flush rate is linear for many tree species. *Forest Science, 29*, 237-240.

UNIVERSITY of the
WESTERN CAPE

Tanksley, S. D. (1993). Mapping polygenes. Retrieved 02 October 2008, from Academic OneFile. Gale: http://find.galegroup.com/ips/start.do?prodId=IPS

The_Arabidopsis_Genome_Iniative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature, 408*, 796-815.

United Nations Food and Agricultural Organisation, F. A. O. Top world apple production, 2009. Retrieved 12 February, 2012, from http://faostat.fao.org/site/339/default.aspx

United Nations Food and Agricultural Organisation, F. A. O. Top world apple production, 2007. Retrieved 15 June, 2008, from http://faostat.fao.org

Vales, M. I., Schon, C. C., Capettini, F., Chen, X. M., Corey, A. E., Mather, D. E., et al. (2005). Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. *Theoretical and Applied Genetics, Volume 111*, 1260-1270.

Van Dyk, M. M. (2008). *Identification of Quantitative Trait Loci Controlling The Requirement for Chilling in Vegetative Budbreak in Apple (Malus x domestica Borkh.).* Unpublished PhD, University of the Western Cape, Bellville.

Van Dyk, M. M., Soeker, M. K., Labuschagne, 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 & Genomes, 6*, 489-502.

Van Ooijen, J. (1999). LOD significance thresholds for QTL analysis in experimental populations of diploid species. *Heredity, 83*(May), 613-624.

Van Ooijen, J. W. (2006). JoinMap® 4 Software for the calculation of genetic linkage maps in experimental populations. Wageningen, Netherlands.

Varshney, R. K., Thiel, T., Stein, N., Landridge, P., & Graner, A. (2002). *In silico* analysis of frequency and distribution of microsatellites in ESTs of some cereal species. *Cellular and*

UNIVERSITY of the
WESTERN CAPE

Molecular Biology Letters, 7, 537-546.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., et al. (2010). The genome of the domesticated apple (*Malus* × *domestica* Borkh.). *Nature Genetics, 42*, 833-839.

Vinatzer, B. A., Patocchi, A., Gianfranceschi, L., Tartarini, S., Zhang, H.-B., Gessler, C., et al. (2001). Apple Contains Receptor-like Genes Homologous to the Cladosporium fulvum Resistance Gene Family of Tomato with a Cluster of Genes Cosegregating with *Vf* Apple Scab Resistance. *Molecular Plant-Microbe Interactions, 14*(4), 508-515.

Vinatzer, B. A., Patocchi, A., Tartarini, S., Gianfranceschi, L., Sansavini, S., & Gessler, C. (2004). Isolation of two microsatellite markers from BAC clones of the *Vf* scab resistance region and molecular characterization of scab-resistant accessions in *Malus* germplasm. *Plant Breeding, 123*, 321–326

Walsh, B. (2002). Quantitative Genetics, Genomics and the Future of Plant Breeding. In M. S. Kang (Ed.), *Quantitative Genetics, Genomics, and Plant Breeding* (pp. 23-33). New York, USA: CAB International.

Wang, Z., Weber, J. L., & Tanksley, S. D. (1994). Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics, Volume 88*(1), 1-6.

Wenz, H. M., Robertson, J. M., Menchen, S., Oaks, F., Demorest, D. M., Scheibler, D., et al. (1998). High-precision genotyping by denaturing capillary electrophoresis. *Genome Research, 3*, 69-80.

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., et al. (2004). Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Science US, 101*(26), 9915-9920.

UNIVERSITY of the
WESTERN CAPE

Xu, M. L., & Korban, S. S. (2000). Saturation mapping of the apple scab resistance gene *Vf* using AFLP markers. *Theoretical and Applied Genetics, 101*, 844–851.

Yamamoto, T., Kimura, T., Sawamura, Y., Manabe, T., Kotobuki, K., Hayashi, T., et al. (2002c). Simple sequence repeats for genetic analysis in pear. *Euphytica, 124*, 129-137.

Yamamoto, T., Kimura, T., Shoda, M., Ban, Y., Hayashi, T., & Matsuta, N. (2002b). Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). *Molecular Ecology Notes, 2*, 14-16.

Yamamoto, T., Kimura, T., Shoda, M., Imai, T., Saito, T., Sawamura, Y., et al. (2002a). Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theoretical and Applied Genetics, 106*, 9-18.

Yamane, H., Kashiwa, Y., Ooka, T., Tao, R., & Yonemori, K. (2008). Suppression Subtractive Hybridization and Differential Screening Reveals Endodormancy-associated Expression of an SVP/AGL24-type MADS-box Gene in Lateral Vegetative Buds of Japanese Apricot. *Journal of the American Society for Horticultural Science, 133*(5), 708-716.

Yu, J.-K., Dake, T. M., Singh, S., Benscher, D., Li, W., GIll, B., et al. (2004). Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome, 47*, 805–818.

Zeng, Z.-B. (1993). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Science US, 90* 10972-10976.

Zeng, Z.-B. (1994). Precision Mapping of Quantitative Trait Loci *Genetics, 136*, 1457-1468.

Zhang, J.-Z., Ai, X.-Y., Sun, L.-M., Zhang, D.-L., Guo, W.-W., Deng, X.-X., et al. (2011). Transcriptome profile analysis of flowering molecular processes of early flowering

UNIVERSITY of the
WESTERN CAPE

trifoliate orange mutant and the wild-type [Poncirus trifoliata (L .) Raf.] by massively parallel signature sequencing. *BMC Genomics, 12*(63), 1-20.

Zhang, Y. M., & Gai, J. (2009). Methodologies for segregation analysis and QTL mapping in plants. *Genetica, 136*(2), 311-318.

Zhebentyayeva, T., Fan, S., Olukolu, B., Hughes-Murphree, S., Barakat, A., Leida, C., et al. (2010). *From genetics to epigenetics in control of chilling requirement and blooming time in peach.* Paper presented at the Rosaceae Genome Conference 5 (RGC5), Stellenbosch, Cape town, South Africa.

APPENDIX 1

Table A: Markers mapped into respective LGs in the 'Anna' x 'Austin' linkage map.

Linkage group	Accession number	Reference(s) or Source
1	SAmsDR995748	van Dyk *et al.,* 2010
	aPa-183066 aPa-461667 aPa-185048 aPa-519579 aPa-526821 aPa-183514 aPa-186132	-
	NZmsCN879773	Celton *et al.,* 2009
2	aPa-186429 aPa-186224 aPa-184615 aPa-184168 aPa-461017 aPa-461854 aPa-186008 aPa-184705 aPa-184775	-
	CH02c061	Liebhard *et al.,* 2002, 2003a
	aPa-186589 aPa-186982 aPa-184400 aPa-460837 aPa-442466 aPa-525922 aPa-441960 aPa-182442 aPa-185639 aPa-526921 aPa-182444 aPa-183698 aPa-442714 aPa-185612	-
	SAmsCO900827	van Dyk *et al.,* 2010
	CH05e03	Liebhard *et al.,* 2002, 2003a
	SAmsEB106592	van Dyk *et al.,* 2010
	aPa-184439 aPa-460693	-
	aPa-442735 aPa-185278 aPa-519681 aPa-518884 aPa-460920 aPa-518947 aPa-185507 aPa-519074	-

UNIVERSITY of the WESTERN CAPE

	aPa-461303 aPa-185353 aPa-525950 aPa-186339 aPa-182991 aPa-186341	
	NZmsEB106592	Liebhard *et al.,* 2002, 2003a
	CH02a04	Celton *et al.,* 2009
3	aPa-442825 aPa-461576 aPa-461291 aPa-442855 aPa-443364	-
	SAmsCO866862	van Dyk *et al.,* 2010 (PU)
4	SAmsCN491993	van Dyk *et al.,* 2010 (PU)
	SAmsCO753033	van Dyk *et al.,* 2010 (PU)
	CH01d03	Liebhard *et al.,* 2002, 2003a
	SAmsEB153928	van Dyk *et al.,* 2010
	aPa-185804 aPa-184691 aPa-183107 aPa-526972 aPa-442463 aPa-441554 aPa-526621 aPa-460999 aPa-183952 aPa-518770 aPa-443003 aPa-460750 aPa-443039 aPa-518697 aPa-526572 aPa-518839 aPa-184740 aPa-184005 aPa-186176	-
	SAmsEB121159	van Dyk *et al.,* 2010 (PU)
	aPa-186634	-
	Hi01e10	Silfverberg-Dilworth *et al.,* 2006
5	MS02a01	Maliepaard *et al.,* 1998
	aPa-184037 aPa-184854	-
	SAmsCN496002	van Dyk *et al.,* 2010 (PU)
	GD-103	Hokanson *et al.,* 1998; Celton *et al.,* 2009
	aPa-183911 aPa-184981	-
	SAmsCN492475	van Dyk *et al.,* 2010 (PU)
	SAmsDR997517	van Dyk *et al.,* 2010 (PU)
	SAmsCN917681	van Dyk *et al.,* 2010 (PU)
	aPa-461514aPa-186475	

	aPa-186676aPa-186754 aPa-461820	-
	SAmsCN921216	van Dyk *et al.,* 2010 (PU)
6	aPa-526869	-
	SAmsDR998909	van Dyk *et al.,* 2010 (PU)
	aPa-55395611aPa-525967 aPa-184449aPa-4611023 aPa-442822	-
	SAmsCO540769	van Dyk *et al.,* 2010 (PU)
	SAmsCN444942	van Dyk *et al.,* 2010
	NZms23g4	Celton *et al.,* 2009; Guilford *et al.,* 1997
7	aPa-526398 aPa-184686 aPa-183467 aPa-519428 aPa-525713 aPa-443070 aPa-526234 aPa-442784 aPa-46217911 aPa-460767 aPa-184313 aPa-442804 aPa-553206 aPa-186360 aPa-519175 aPa-185763	-
	Hi05b09	Silfverberg-Dilworth *et al.,* 2006
	aPa-526070 aPa-461676 aPa-182515 aPa-554050 aPa-185701 aPa-442697 aPa-442060 aPa-442439 aPa-519166 aPa-461698 aPa-527002	-
	SAmsCN927330	van Dyk *et al.,* 2010
	aPa-186390 aPa-183332 aPa-185849 aPa-461959 aPa-183094 aPa-186460	-
	CH04e05	Liebhard *et al.,* 2002, 2003a
8	Hi04b12	Silfverberg-Dilworth *et al.,* 2006
	aPa-184271 aPa-461789	-
	CH01e121	Liebhard *et al.,* 2002, 2003a
	SAmsCV883434	van Dyk *et al.,* 2010

UNIVERSITY of the
WESTERN CAPE

	aPa-461564 aPa-184641 aPa-185193 aPa-185917 aPa-185170 aPa-526973 aPa-526162aPa-441943 aPa-526910 aPa-186111 aPa-443216 aPa-462114 aPa-441968 aPa-554112	-
9	aPa-183140	-
	SAmsMDC004938.180	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
	NZmsCN943946	Celton *et al.,* 2009
	SAmsMDC005927.400	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
	aPa-525968	-
	SAmsMDC011178.406 SAmsMDC009465.253 SAmsMDC009662.63 SAmsMDC011932.246	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
	aPa-184149 aPa-186725 aPa-461324 aPa-526894 aPa-441654 aPa-185093 aPa-525952 aPa-186731 aPa-184290 aPa-552965	- -
		Silfverberg-Dilworth *et al.,* 2006
	aPa-186400 aPa-443239 aPa-443058 aPa-441878 aPa-460912 aPa-460933 aPa-184459	-
	SAmsDR992457	van Dyk *et al.,* 2010
10	COLa	van Dyk *et al.,* 2010
	GD-100	Hokanson *et al.,* 1998; Celton *et al.,* 2009
	aPa-442771 aPa-441936 aPa-184649 aPa-186724	-
	MS06g03	Guilford *et al.,* 1997; van Dyk *et al.,* 2010
	aPa-184772	-

UNIVERSITY of the
WESTERN CAPE

	SAmsDR994153	van Dyk *et al.,* 2010
	aPa-184374 aPa-442847 aPa-184361 aPa-184362	-
	CH04c06	
	aPa-442947 aPa-443272	-
	CH04f03 CH02a08	Liebhard *et al.,* 2002, 2003a
	SAmsCO755814	van Dyk *et al.,* 2010
	Hi02d04	Silfverberg-Dilworth *et al.,* 2006
	aPa-526919 aPa-460957 aPa-526579 aPa-184105 aPa-184606	-
	CH02c11	Liebhard *et al.,* 2002, 2003a
	aPa-552888 aPa-442891 aPa-461780	-
	CH01e09b CH02a10	Liebhard *et al.,* 2002, 2003a
	SAmsCN490740	van Dyk *et al.,* 2010 (PU)
	SAmsMDC020071.203	**Hove 2012 (PU)**; Chromosome 3 Velasco *et al.,* 2010
	COLa	van Dyk *et al.,* 2010
11	SAmsEB147667 SAmsAT000420	van Dyk *et al.,* 2010 (PU)
	NZmsEB153947	Celton *et al.,* 2009
	SAmsEB149433	van Dyk *et al.,* 2010 (PU)
	aPa-184240 aPa-518989 aPa-185955 aPa-441922 aPa-184555	-
	SAmsDR993043	van Dyk *et al.,* 2010
	Hi06b06	Silfverberg-Dilworth *et al.,* 2006
	CH04g07	Liebhard *et al.,* 2002. 2003a
	SAmsMDC020761.431 SAmsMDC000503.195	Hove 2011 (PU); Chromosome 9 Velasco *et al.,* 2010
	SAmsCN877882 SAmsCO723148	van Dyk *et al.,* 2010
	Hi02c06	Silfverberg-Dilworth *et al.,* 2006

UNIVERSITY of the
WESTERN CAPE

	SAmsEB114458	van Dyk *et al.,* 2010 (PU)
12	aPa-185363	-
	NZms28f4	Kenis *et al.,* 2008
	aPa-461201 aPa-442203 aPa-552852 aPa-461911 aPa-442826 aPa-441685 aPa-519308	-
	CH01g121	Liebhard *et al.,* 2002, 2003a
	aPa-442134	-
	SAmsDT040421	van Dyk *et al.,* 2010 (PU)
	SAmsDR995002	van Dyk *et al.,* 2010
	CH01d09	Liebhard *et al.,* 2002. 2003a
	aPa-186727 aPa-186520 aPa-525882 aPa-461835 aPa-186193 aPa-185262 aPa-183485 aPa-442173 aPa-441779 aPa-442112 aPa-461822 aPa-519342 aPa-186512	-
	CH03c02	Liebhard *et al.,* 2002, 2003a
	aPa-525797 aPa-183916 aPa-552964	-
	SAmsCN943613	van Dyk *et al.,* 2010
	CH02h11b	Liebhard *et al.,* 2002, 2003a
	aPa-443308 aPa-461933 aPa-183186 aPa-443297 aPa-442770 aPa-460973 aPa-185787	-
	SAmsCO417416	van Dyk *et al.,* 2010 (PU)
	aPa-442770 aPa-460973 aPa-185787	-
13	aPa-182529 aPa-442313 aPa-184187	-
	NZmsEB111793	Celton *et al.,* 2009 (PU)
	SAmsDT041145	van Dyk *et al.,* 2010 (PU)
	aPa-182516 aPa-185358	-

UNIVERSITY of the
WESTERN CAPE

	aPa-526002	
	GD-147	Hokanson *et al.,* 1998; Silfverberg-Dilworth *et al.,* 2006
	Hi04f09	Silfverberg-Dilworth *et al.,* 2006
	aPa-182529 aPa-442313 aPa-184187	-
14	SAmsCN904905	van Dyk *et al.,* 2010
	CH04f06	Liebhard *et al.,* 2002, 2003a
	aPa-442722 aPa-461159 aPa-519436 aPa-442545	-
	SAmsCN880881	van Dyk *et al.,* 2010
	aPa-443167 aPa-526050 aPa-518676 aPa-552794 aPa-184790 aPa-461632 aPa-460728 aPa-525959 aPa-186442 aPa-442812 aPa-184029 aPa-185737 aPa-185319	-
	CH03g04 CH05e05	Liebhard *et al.,* 2002, 2003a
	aPa-518906	-
	CH05d03	Liebhard *et al.,* 2002, 2003a
	aPa-441894	-
	SAmsCN581649a SAmsCN942512	van Dyk *et al.,* 2010
	aPa-185529 aPa-442388	-
	SAmsDR995122	van Dyk *et al.,* 2010 (PU)
	CH03a03	Liebhard *et al.,* 2002, 2003a
	Hi03a03	Silfverberg-Dilworth *et al.,* 2006
	CH03g06	Liebhard *et al.,* 2002. 2003a
15	SAmsMDC003785.420	**Hove 2012 (PU);** Chromosome 3 Velasco *et al.,* 2010
	aPa-519280	-
	CH03b10	Liebhard *et al.,* 2002, 2003a
	aPa-443118 aPa-441544 aPa-553793 aPa-443395	-
	SAmsMDC020525.273	**Hove 2012 (PU);**

UNIVERSITY of the WESTERN CAPE

		Chromosome 3 Velasco *et al.,* 2010
	SAmsDR997862 SAmsEB126773	van Dyk *et al.,* 2010
	aPa-186154 aPa-184899 aPa-518622	-
	SAmsCN865016	van Dyk *et al.,* 2010
	aPa-553116 aPa-183728	-
	SAmsMDC020300.286	-
	SAmsCO168103	**Hove 2012 (PU)**; Chromosome 3 Velasco *et al.,* 2010
	aPa-461231 aPa-526885	-
	CH04g10	Liebhard *et al.,* 2002, 2003a
	SAmsMDC003785.420	
16	Hi04e04	Silfverberg-Dilworth *et al.,* 2006
	aPa-526766 aPa-441863 aPa-186312 aPa-185094 aPa-183169 aPa-526169 aPa-185104 aPa-442132 aPa-183463 aPa-526724	-
	SAmsEB154700	van Dyk *et al.,* 2010
	CH05a09	Liebhard *et al.,* 2002, 2003a
	aPa-519186 aPa-184985	-
	SAmsCN881550	van Dyk *et al.,* 2010
	SAmsMDC005569.608	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
	aPa-185843 aPa-441745	-
	SAmsEB106034	van Dyk *et al.,* 2010
	SAmsMDC021941.307	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
	aPa-553235 aPa-184079 aPa-525938 aPa-526970 aPa-462075 aPa-186053 aPa-552929 aPa-519092	-
	SAmsMDC010321.324	**Hove 2012 (PU)**; Chromosome 9 Velasco *et al.,* 2010
17	GD-96	Hokanson *et al.,* 1998; Celton *et al.,* 2009

UNIVERSITY of the WESTERN CAPE

	aPa-461421 aPa-553859 aPa-525524 aPa-441858 aPa-461612 aPa-461977	-
	SAmsCV627191b	van Dyk *et al.,* 2010 (PU)
	SAmsAU301254	van Dyk *et al.,* 2010
	SAmsCV627191a	van Dyk *et al.,* 2010 (PU)
	aPa-518840 aPa-526121 aPa-442750 aPa-525699 aPa-186502 aPa-442046 aPa-525839 aPa-182556 aPa-526577 aPa-185713 aPa-184045 aPa-442137 aPa-461310 aPa-519627 aPa-525633	-
	CH01f03a	Liebhard *et al.,* 2002, 2003a
	aPa-185352	-
	CH05d08	Liebhard *et al.,* 2002, 2003a
	aPa-186268	-
	SAmsCN938125	van Dyk *et al.,* 2010
	aPa-461202 aPa-554192 aPa-186670	-
	SAmsDR996674	van Dyk *et al.,* 2010 (PU)
	aPa-186258 aPa-525820 aPa-186366 aPa-183534 aPa-526732	-
	NZms17e6	Guilford *et al.,* 1997
	SAmsCN910036	van Dyk *et al.,* 2010

UNIVERSITY of the
WESTERN CAPE

Key

PU - SSR marker previously unmapped in any known publication.

- \- DArT markers previously unmapped in any known publication.

Newly developed and mapped SSR markers are labelled with the prefix 'SAms'. Published markers are labelled with the prefixes 'GD'. 'CH'. 'Hi'. 'NZms' and 'MS'.

DArT makers are prefixed by 'aPa-'. The Maliepaard *et al.* (1998) reference map was used to assign linkage groups. **Table A** above shows a total of 429 markers mapped. of which 115 of these are SSR and 314 DArT markers respectively.

UNIVERSITY of the
WESTERN CAPE

Table B: A set of 467 SSR markers used in this study, together with its accession number, dye colour, expected amplicon range. repeat type and forward and reverse sequences. The four fluorescent 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 parents as mentioned previously in **Section 2.8**.

SSR Marker	Marker name (accession number based)	Dye	Size range (bp)	SSR Repeat type	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

UNIVERSITY of the WESTERN CAPE

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	CGT TTT TGG AGC GTA GGA

UNIVERSITY of the
WESTERN CAPE

					CCC	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	GCA AAA ACC ACA GGC ATA

UNIVERSITY of the
WESTERN CAPE

					AC	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

UNIVERSITY of the WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the WESTERN CAPE

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	GTC TCC ATG ACT TTT CTG

UNIVERSITY of the WESTERN CAPE

					GC	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

UNIVERSITY of the
WESTERN CAPE

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	CTG GTC GGA CAG GTT AAC G

UNIVERSITY of the
WESTERN CAPE

					ATC	
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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

						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-Vf1	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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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-Vf2	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	AAA CTC CAT AGC AGT AGT

UNIVERSITY of the WESTERN CAPE

					GC	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

UNIVERSITY of the
WESTERN CAPE

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	***SAmsEB112897***	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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the
WESTERN CAPE

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

UNIVERSITY of the WESTERN CAPE

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

UNIVERSITY of the WESTERN CAPE

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
834	***SAmsMDC021941.303***	F	219-230	3	CTA ACG GAG AAC ATG ACA CAA AAC	GCT CTT TTG CTA CAT TGT GTT TGC
836	***SAmsMDC000503.195***	N	197-246	3	TGA AGA GAG TAG AGG AAA GGG ATG	TCC GTT AAG AGT TGA TGT GAC TGT
837	***SAmsMDC020761.431***	P	219-250	3	GGG CAG TTG GAA GTT TGA AGT TA	TGA GCC TTA TTT CTA GTC AGT CCA
838	***SAmsMDC017895.317***	F	242-257	3	ATG GTT GAT TAG GGT TCA GTG AGT	CCT CGA CTA ACG GGG TTT ATA CAT
840	***SAmsMDC009477.96***	N	219-259	3	ATA GCA CTC TTG GGA TGA ATG AGT	GAC CTA GGA CAA CTT TGG AAG AGA
841	***SAmsMDC010321.324***	P	328-341	4	TAA CTG TTC GTC TTT CCC TCT CTC	TCC ATT AAT CCA CCA ATT AGG C
842	***SAmsMDC017003.269***	F	331-369	4	CTTGATTAGTGAGCTGTTGTCA CC	TGA TCC AGC TAG CTA CAA GAA ATC
843	***SAmsMDC009858.304***	V	336-352	3	AGT GAT AGA CCC CAA TAA ACC GTA	ATC CGA GGT AAA ATA GGA ATA GCC
844	***SAmsMDC004938.180***	N	326-353	3	GAT TCT TAT TCC CCT CTT TCA AGG	CTA AGC ATA GAC GTG AAT GTC AAG A
845	***SAmsMDC009662.63***	P	334-372	4	CAG TTT CAC TTC CCC TCT CAC TAT	GCC GTA ATC AAC TAT CGA AAG ATA C
846	***SAmsMDC005569.608***	F	370-424	3	GCT CCT TGT TGA ATG TGT AAA GC	CCT CTG CAT AAG ACT TCG TTT AGC

UNIVERSITY of the
WESTERN CAPE

847	***SAmsMDC011932.246***	V	382-425	4	CAC TTT TAC GTT ACA TGC ACC ACT	AGG TTG CAC TCA TTC TTT ATA CAC C
848	***SAmsMDC015239.225***	N	370-401	3	TGT TAG TTA GGT TCA GTG GGA CAA	GGA ATG AGG ATA TCC GAG GTA AAG
849	***SAmsMDC011178.406***	P	355-429	3	GTA CAC CGT TCA ATC TAG CTT TCG	TTG GGA GCT TCT ACT ATC TTG GAC
850	***SAmsMDC005927.400***	F	391-424	3	TAT TCG CAA GTA GAG GAA GAG TGA	ACC ATC ATT CCT CTG CAA TTC T
851	***SAmsMDC010403.411***	V	390-408	3	TAG AGT AAG AGG ATG GGA GCC ATT	TAA TCT ACT ACG TGG GAC ACT TGC
852	***SAmsMDC021941.307***	N	381-411	4	CAT TCA TCA GAA TCA CCA CAC C	AGA GAG AGG TAT CAC GTG GAA TTT
853	***SAmsMDC010935.355***	P	395-410	3	GTG TTG TGA AGA TGA AAA CCA GTG	GTT TTG CTA ACC TTC AGA AGA TGC
854	***SAmsMDC009465.253***	F	397-429	3	GCA TTT CAG TCT TGT AGA GGA TCA	GGA TAT AAG GTT TGT GCC ATG TG
855	***SAmsMDC005939.185***	V	395-410	3	CCC CTC TTC CCA TAG GTA GAT AAT	GGA TCT GAT CAA ACT AGA CGA GAA
856	***SAmsMDC003421.411***	N	397-429	4	TTA CTC ATT TAC GCA GAG CTT CCT	CAT CAT TCG AAG ATC ACT CGT ACA
859	***SAmsMDC000907.297***	F	351-376	3	6-FAM-CTG CCT TAT TAA AGT GAA GAA CAG G	TGG GTA CGA CTA GGT GAC TGT ATG
860	***SAmsMDC020071.203***	V	384-408	3	GCT GGA AAG GTG TTG AGT TCT T	VIC-CAC AAG GAA CCC CGT TTT TAC TA
861	***SAmsMDC000834.114***	N	350-377	3	NED-CTG CCT TAT TAA AGT GAA GAA CAG G	TGG GTA CGA CTA GGT GAC TGT ATG
862	***SAmsMDC020525.273***	P	381-407	3	TAT GAT TCC CAC TAG GCT TAA CCA	PET-CTT TTA ACC CAG GTT TGT AGA TGG
863	***SAmsMDC002362.239***	F	396-403	3	6-FAM-ATC AGC TGG GTT TGT TTC TTT C	AAT TTG TAG GGT GTA GGG GTT AGG
864	***SAms MDC038751.8***	V	385-404	3	CCC ACT AAA ATA CCA CCA TAG ACC	VIC-CAA GTT TTG AGC TTG ATG TTC CTC
865	***SAmsMDC020300.286***	N	389-428	4	NED-GCT ACG TTC CAT CAA CAT ATC AGT	AGC ACT AAC ACC AGA GTG CAA CTA
866	***SAmsMDC007193.563***	P	359-406	3	GCC CAT TTA TTT TGG GCT TT	PET-CAT GTT TTC GAT GAT GAG TAG CAC

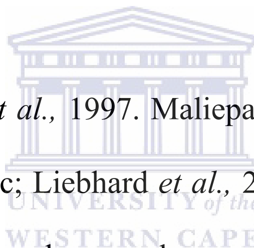
UNIVERSITY of the
WESTERN CAPE

868	***SAmsMDC003785.420***	V	405-429	3	AGG GTT TAG TCT CCA ACA ATG AAG	VIC-ACC GAT CAA TCA AAG ATC CAA C
869	***SAms MDC016904.88***	N	331-342	3	NED-CTA ATG AAA GTC GGA TAC CAG TGA	GTT GTT GCT GTT GTA TAT GAG TTG C
870	***SAmsMDC012739.316***	P	387-412	4	CTG AAG TCC AAA ATA AAC CCC ATC	PET-TTG TCC TCC ATT TTT CTG AAG C

Key:

All **SAms** markers in bold *eg.* marker **510. SAmsCN881550** were designed by Dr. MM van Dyk and Mr. K. Soeker.

All ***SAms*** markers in bold and italics *eg.* marker ***592. SAmsEB149750*** were designed by Mr. P. Hove.


UNIVERSITY of the
WESTERN CAPE

Other markers were from published sources (Guilford *et al.,* 1997. Maliepaard *et al.,* 1998; Liebhard *et al.,* 2002; Yamamoto *et al.,* 2002a; Yamamoto *et al.,* 2002b; Yamamoto *et al.,* 2002c; Liebhard *et al.,* 2003a. Newcomb *et al.,* 2006 and Silfverberg-Dilworth *et al.,* 2006). All the **SAms** markers were regarded as novel and were subsequently published in the van Dyk *et al.,* 2010 paper.

Table C: A list of 467 SSR markers grouped into megaplexes as described in **section 2.9**, showing the respective alleles and JoinMap® codes after PCR with the Qiagen® megaplex PCR kit. for the 'Austin' x 'Anna' mapping population. Markers generated were scored using GeneMapper® 4.0.

	Accession number	Best BLAST hit: Contig number and Chromosome (Velasco *et al.*, 2010)	Dye	Size range (bp)	'Anna' Alleles	'Austin' Alleles	'Anna' x 'Austin' JoinMap® code
Megaplex 1							
93	CH05f06	MDC011855.327 Chr 5	V	166-184	-	-	-
114	CH03b06	MDC022202.499 Chr 15	F	105-131	-	-	-
120	CH05e04	MDC004808.272 Chr 16	F	140-234	144/151	146/148	abxcd
169	CH05a09	MDC006875.277 Chr 16	P	141-186	-	-	-
227	SAmsCN493171	MDC029130.40 Chr 15	N	295-395	-	-	-
236	CH01e09b	MDC003427.426 Chr 17	P	192-292	236/242	233/242	efxeg
281	SAmsCN870040	MDC005271.182 Chr 16	V	260-360	305	305	--
288	SAmsCN909118	MDC022525.56 Chr 5	F	218-318	-	-	-
294	SAmsCN946851	MDC019585.198 Chr 13	V	190-250	-	-	-
318	SAmsCN580227	MDC015010.269 Chr 6	N	196-296	-	-	-
320	SAmsCN580637	MDC018988.253 Chr 15	F	415-425	418/420	418	lmxll
329	SAmsCN496002	MDC008539.361 Chr 5	N	177-277	210/214	210	lmxll

UNIVERSITY of the WESTERN CAPE

335	SAmsCO052033	MDC003753.230 Chr 5	N	142-242	196	189/196	nnxnp
341	SAmsCO723148	MDC001241.304 Chr 11	P	81-181	145	145/150	nnxnp
425	SAmsCO756781	MDC002994.270 Chr 10	P	281-381	321	321	--
Megaplex 2							
15	28f4	LG 12	N	90-110	102/110	96/110	efxeg
43	CH04e03	MDC041220.7 Chr 5	F	179-222	204/206	204	lmxll
56	CH05f04	MDC006621.180 Chr 4	V	160-172	-	-	-
59	CH03d08	MDC020317.340 Chr 14	F	129-161	141/143	143	lmxll
60	CH03g04	MDC004274.213 Chr 14	V	122-144	128/135	140/142	abxcd
80	MS02a01	MDC11588.208 Chr 10	N	170-194	204	204/206	nnxnp
128	CH01b121	MDC019519.278 Chr 12	P	125-178	135	-	-
139	CH02a04	MDC022150.298 Chr 2	P	66-112	68/107	105/108	abxcd
219	SAmsCN580620	MDC008517.277 Chr 12	F	333-433	380	380	--
244	SAmsCN947446	MDC019062.252 unanchored:22408332..22425771	V	136-236	181/184	184/190	efxeg
279	SAmsCN887525	MDC004101.200 Chr 5	N	167-267	210	-	-
293	SAmsCN944444	MDC001204.808 Chr 3	N	365-433	374	374/376	nnxnp
327	SAmsCN490324	MDC013709.214 Chr 17	V	180-280	229/231	231	llxlm
346	SAmsCO753022	MDC012584.88 Chr 15	P	305-480	-	-	-
398	SAmsCN490644	MDC003800.283	N	214-314	263/267	-	-

UNIVERSITY of the WESTERN CAPE

		Chr 10					
417	SAmsCV128959	MDC003450.371 Chr 6	P	179-270	195	-	-
Megaplex 3							
66	MS01a05	MDC022516.234 Chr 7	V	158-176	-	-	-
71	CH01h011	MDC013304.239 Chr 17	N	100-134	115/121	121/131	efxeg
74	CH02a10	MDC016803.330 Chr 3	N	143-177	147/154	150/154	efxeg
112	CH05c04	MDC008148.499 Chr 13	V	186-258	-	-	-
113	CH01d08	MDC021953.346 Chr 15	N	238-290	253	238	--
119	CH05a04	MDC010246.376 Chr 16	F	159-189	169/175	192	abxc
125	CH02g01	MDC007396.58 Chr 13	P	91-121	98/100	94/104	--
182	SAmsCN445253	MDC019975.203 Chr 12	F	410-430	417/420	-	-
187	SAmsCN490566	MDC015511.204 Chr 6	V	286-386	337	387	--
207	SAmsCN495433	MDC031287.8 Chr 5	V	213-313	264/295	306	--
234	SAmsCN938125	MDC012545.302 Chr 17	N	303-403	340/347	337/356	abxcd
235	CH01b09b	MDC001010.290 Chr 4	P	305-405	347/351	355	--
345	SAmsCO755814	MDC003399.279 Chr 10	F	211-311	261	255/269	nnxnp
352	SAmsCO866862	MDC012661.305 Chr 3	P	124-224	188/190	190/192	efxeg
369	SAmsCO865608	MDC006289.408 Chr 1	P	109-209	161/163	-	-
452	SAmsCO900827	MDC010624.539	N	394-494	444	427/444	nnxnp

UNIVERSITY of the WESTERN CAPE

		Chr 2					
Megaplex 4							
29	SAmsAT000141	MDC015190.83 Chr 9	V	56-100	89	89/97	nn x np
63	CH05d03	MDC018782.299 Chr 6	F	152-187	167/175	159/167	ef x eg
64	CH05e05	MDC009350.182 Chr 2	N	138-160	150/161	160	lm x ll
67	CH02c09	MDC011137.202 Chr 15	N	233-257	244/250	248/254	ab x cd
99	CH03d02	MDC005828.284 Chr 11	F	201-223	--	--	--
106	CH03c02	MDC018186.206 Chr 12	F	116-136	125	127	nn x np
109	CH05d11	MDC018277.209 Chr 12	N	171-211	173/183	173/181	ef x eg
122	CH04c06	MDC017603.123 Chr 17	V	155-186	171/177	158/171	ef x eg
137	CH01f03a	MDC015290.99 Chr 16	P	210-224	212/224	224	lm x ll
148	CH03a03	MDC015486.182 Chr 14	P	154-182	156/158	170/172	ab x cd
158	CH04d08	MDC019260.152 Chr 11	P	116-142	-	-	-
200	SAmsCN493925	MDC015011.163 Chr 2	N	366-410	355	355/405	nn x np
220	SAmsCN580732	MDC015169.163 Chr 2	F	300-400	-	-	-
231	SAmsCN580271	MDC003949.200 Chr 1	V	156-256	240	240/252	nn x np
377	SAmsCO068842	MDC018268.352 Chr 13	N	399-466	457	436/457	nn x np
380	SAmsCO866737	MDC011713.137 Chr 16	F	192-292	240	240/252	nn x np
390	SAmsCN544851	MDC006391.297	P	250-350	-	-	-

UNIVERSITY of the WESTERN CAPE

		Chr 4					
Megaplex 5							
10	02b1	LG 15	N	188-288	218	232	--
14	23g4	LG 6	F	70-130	90/103	82/90	efxeg
49	CH05c07	MDC005293.195 Chr 9	N	111-149	139	111/125	aaxbc
62	CH04f06	MDC011094.321 Chr 14	N	159-179	176/180	176/178	efxeg
73	CH01f12	MDC019380.166 Chr 10	F	145-162	151	151	--
87	CH03e03	MDC005190.587 Chr 3	F	106-216	201203	201/203	hkxhk
94	CH03d12	MDC007389.248 Chr 6	V	108-154	-	-	-
171	CH05c02	MDC004471.532 Chr 11	P	168-200	174/179	172/176	abxcd
172	CH05d08	MDC013234.266 Chr 17	P	91-143	102/123	123/139	efxeg
173	CH05g01	MDC017682.301 Chr 11	P	236-276	-	-	-
217	SAmsCN579502	MDC012292.266 Chr 7	F	230-330	280/288	280/288	hkxhk
238	SAmsCN865016	MDC009136.399 Chr 15	F	294-394	341/347	341/347	hkxhk
253	SAmsCO540769	MDC017032.162 Chr 6	N	213-313	265	250/265	nnxnp
260	SAmsCN935817	MDC005588.270 unanchored:76057334..76063257	V	189-289	239	226/239	nnxnp
331	SAmsAB162040	MDC020034.222 Chr 12	V	244-344	268/281	281/288	efxeg
376	SAmsCO867345	MDC000910.324 Chr 16	N	318-418	438/440	364/440	efxeg
401	SAmsCN544835	MDC009798.251 Chr 5	V	137-237	178/185	174/178	efxeg

UNIVERSITY of the WESTERN CAPE

412	SAmsCN492999	MDC002480.238 Chr 16	P	165-265	-	-	-
Megaplex 6							
44	CH05e06	MDC002834.158 Chr 5	F	125-222	137	131/137	nnxnp
48	CH01h021	MDC003767.335 Chr 9	F	236-256	-	-	-
57	CH01g05	MDC020317.340 Chr 14	V	140-188	155	155/167	nnxnp
72	CH05g03	MDC010787.146 Chr 17	N	135-192	175/184	175/190	efxeg
76	CH02c11	MDC001758.144 Chr 10	N	219-239	227/237	227	lmxll
85	CH03d01	MDC022695.138 Chr 2	F	95-115	110/112	110/112	hkxhk
115	CH03b10	MDC012303.704 Chr 15	N	99-121	118	101/118	nnxnp
165	CH04g09	MDC012537.142 Chr 5	P	141-177	149	149/152	nnxnp
167	CH05a02	MDC021095.21 Chr 15	P	111-135	131/137	131	lmxll
168	CH05a03	MDC018744.266 Chr 9	P	182-220	183/193	193	lmxll
193	SAmsCN492206	MDC021083.97 Chr 13	F	329-429	393/397	393/397	hkxhk
196	SAmsCN492626	MDC020254.241 Chr 15	N	260-360	309/314	309/314	hkxhk
202	SAmsCN494248	MDC011588.205 Chr 5	V	266-366	294/313	294/313	hkxhk
213	SAmsCN496756	MDC042546.8 Chr 14	N	423-523	438/468	437/468	efxeg
222	SAmsCN581649	MDC000908.450 Chr 14	N	140-200	175/184	175/184	hkxhk
323	SAmsCN490058	MDC022454.244 Chr 15	P	196-296	224	224/226	nnxnp

UNIVERSITY of the WESTERN CAPE

Megaplex 7							
12	05g8	LG 4	F	71-171	124	124	--
36	CH02g09	MDC002525.346 Chr 8	V	98-138	140/157	153/159	abxcd
38	CH05e03	MDC008217.277 Chr 2	V	158-190	168/208	172/208	efxeg
78	COLa	MDC001085.297 Chr 10	F	220-240	229	229/238	nnxnp
81	MS06g03	MDC016163.84 Chr 10	V	154-190	156/178	156	lmxll
89	CH04e02	MDC019231.92 Chr 9	F	143-163	153/155	155/162	efxeg
90	CH02b121	MDC022137.130 Chr 5	V	101-143	137/144	144	lmxll
98	CH02d121	MDC018548.59 Chr 11	F	177-199	178/198	198	lmxll
118	CH02d10a	MDC006455.384 Chr 16	V	215-245	216/220	216/220	hkxhk
133	CH01d03	MDC014207.192 Chr 4	P	136-160	139/144	141/146	abxcd
136	CH01e121	MDC012891.303 Chr 8	P	246-278	249/252	249/252	hkxhk
147	CH02h11b	MDC007676.537 Chr 4	P	214-240	220/222	216/220	efxeg
177	CH05h05	MDC022738.132 Chr 13	P	168-184	170/182	182	lmxll
215	SAmsCN496844	MDC021142.191 Chr 12	V	192-210	194/208	194/208	hkxhk
274	SAmsCN925672	MDC011928.397 Chr 4	V	214-314	304	304	--
283	SAmsCN921216	MDC013463.226 Chr 9	F	329-429	367	367/375	nnxnp
Megaplex 8							
34	CH01c06	MDC012891.303 Chr 8	N	146-188	176/180	176/178	efxeg

UNIVERSITY of the WESTERN CAPE

35	CH01f021	MDC022471.103 Chr 12	V	174-206	184/203	203	lmxll
42	CH05d02	MDC005153.453 Chr 4	N	203-225	217/222	217/222	hkxhk
61	CH04c07	MDC022423.57 Chr 14	N	98-135	129/133	111/125	abxcd
65	CH05g11	MDC001583.305 Chr 14	F	201-255	203	203/205	nnxnp
75	CH02b03b1	MDC009271.511 Chr 10	F	77-109	89/103	82/89	efxeg
84	CH02f061	MDC000307.248 Chr 2	V	135-158	149/151	149/151	hkxhk
91	CH03a04	MDC000528.538 Chr 5	V	92-124	100/110	100/110	hkxhk
95	CH01f091	MDC002525.346 Chr 8	F	125-160	149/151	149/151	hkxhk
108	CH04g04	MDC004400.583 Chr 12	F	170-186	184	176	--
126	CH01b09b	MDC001010.290 Chr 4	P	172-182	174/176	176	lmxll
145	CH02g01	MDC007396.58 Chr 13	P	198-238	201	176	--
162	CH04f04	MDC017371.119 Chr 5	P	144-166	-	-	-
365	SAmsCO903680	MDC009439.435 Chr 11	P	200-300	250	250	--
381	SAmsCO751676	MDC010150.221 Chr 10	V	210-260	226/233	226/233	hkxhk
428	SAmsCO902639	MDC000636.613 Chr 15	V	293-393	346	346	--
Megaplex 9							
37	CH02c061	MDC026455.33 Chr 2	V	216-254	231/251	217/249	abxcd
41	CH02c02b	MDC007362.400 Chr 4	V	78-126	111/120	111	lmxll

UNIVERSITY of the WESTERN CAPE

45	CH03d07	MDC018191.399 Chr 6	N	186-226	205/225	187/225	efxeg
52	CH02d08	MDC005828.284 Chr 11	F	210-254	225	254	--
96	CH01h101	MDC017945.181 Chr 8	N	94-114	98	98/111	nnxnp
97	CH01f03b	MDC020937.110 Chr 9	V	139-183	155/160	160/174	efxeg
111	CH03h03	MDC004556.326 Chr 10	F	72-120	89/98	89/98	hkxhk
116	CH04g10	MDC012425.163 Chr 15	N	127-168	133/166	133/155	efxeg
121	CH02g04	MDC013381.253 Chr 17	F	132-197	192/194	134/194	efxeg
130	CH01c09	MDC004126.509 Chr 13	P	92-108	106	95/106	nnxnp
135	CH01e09b	MDC016291.91 Chr 15	P	118-140	126/136	122/126	efxeg
146	CH02h07	MDC010531.484 Chr 9	P	214-236	218	218/220	nnxnp
169	CH05a09	MDC006875.277 Chr 16	P	152-200	157/176	153/178	abxcd
266	SAmsCN851624	MDC001342.390 Chr 16	N	359-459	-	-	-
319	SAmsAF527800	MDC021880.118 Chr 17	V	290-390	331	331	--
422	SAmsCV627191	MDC020007.246 Chr 17	F	250-350	310/312	314/320	abxcd
Megaplex 10							
46	CH05a05	MDC017021.252 Chr 6	F	198-230	-	-	-
53	CH04g07	MDC012022.139 Chr 11	V	149-211	171/181	150/166	abxcd
88	CH02h11a	MDC007676.537 Chr 4	V	104-132	-	-	-

UNIVERSITY of the WESTERN CAPE

101	CH04d07	MDC005248.149 Chr 11	F	119-142	129	131	nnxnp
208	SAmsCN495651	MDC021781.288 Chr 6	V	348-448	-	-	-
277	SAmsCN866018	MDC029130.40 Chr 15	P	220-235	207/222	207/222	hkxhk
300	SAmsCN939907	MDC007320.447 Chr 15	N	257-357	296/302	302	lmxll
307	SamsCN445290	MDC002235.539 Chr 6	N	298-398	340	340	--
310	SAmsAU301301	MDC018350.223 Chr 3	N	182-282	230/244	230	lmxll
316	SAmsCN496913	MDC017405.92 Chr 13	P	240-340	275/300	275/277	efxeg
32	CH05g08	MDC012059.23 Chr 1	F	161-179	170/-	166/-	abxcd
346	SAmsCO753022	MDC012584.88 Chr 15	P	350-460	437/440	437/440	hkxhk
416	SAmsCO168103	MDC019586.334 Chr 15	N	141-241	194	188/194	nnxnp
603	SAmsEB114458	MDC019757.125 Chr 6	P	119-215	-	-	-
Megaplex 11							
105	CH01g121	MDC002525.336 Chr 12	F	112-186	130/182	133/183	efxeg
110	MS14b04	MDC008313.329 Chr 12	V	230-292	-	-	-
161	CH04f03	MDC022821.76 chr10:11677427..11737802	P	175-191	177/189	177/189	hkxhk
179	MS06c09	MDC019138.228 Chr 8	P	102-118	105	113	--
180	SAmsCN444111	MDC011837.83 Chr 9	N	353-405-	380/404	380/404	hkxhk
186	SAmsCN90349	MDC018282.133 Chr 15	N	196-200	198	198	--

UNIVERSITY of the
WESTERN CAPE

188	SAmsCN490740	MDC013217.295 Chr 10	F	190-212	195/207	192/195	efxeg
340	SAmsCO416051	MDC000020.209 Chr 5	N	120-134	121/133	131/133	efxeg
343	SAmsCV084260	MDC005861.294 unanchored:26293998..26308033	F	219-264	221/228	221/228	hkxhk
372	SAmsCO052555	MDC007544.497 Chr 13	N	232-238	233/235	233	lmxll
424	SAmsCO415353	MDC017127.194 Chr 15	N	329-333	331	331	--
536	Hi02c07	MDC014016.450 Chr 1	V	108-149	112/148	112/120	efxeg
559	Hi03e04	MDC041875.12 Chr 13	P	132-160	139/148	153/159	abxcd
584	Hi06b06	MDC017030.295 Chr 3	P	236-262	259/261	255/259	efxeg
781	Hi02a07	MDC010932.713 Chr 16	V	170-200	195	192/195	nnxnp
Megaplex 12							
50	CH01f07a	MDC009271.511 Chr 10	F	174-206	193/195	193	lmxll
100	CH04a12	MDC010999.445 Chr 11	V	158-196	189/193	169/172	abxcd
221	SAmsCN580954	MDC012238.252 Chr 3	V	106-118	108/113	108/116	efxeg
259	SAmsCN904905	MDC008622.281 Chr 14	P	114-138	116/122	116/122	hkxhk
311	SAmsAU301254	MDC005145.116 Chr 17	F	232-244	233	233/239	nnxnp
385	SAmsCO865258	MDC011523.287 Chr 12	P	170-190	-	-	-
395	SAmsCN495393	MDC001276.321 Chr 10	N	200-219	204	204/218	nnxnp
413	SAmsCN492417	MDC015871.265 Chr 2	N	116-145	120/142	120	lmxll

UNIVERSITY of the
WESTERN CAPE

540	Hi16d02	MDC020977.553 Chr 5	V	141-160	143/146	143	lmxll
550	Hi04e04	MDC025815.15 Chr 16	V	224-242	225/237	225/242	efxeg
555	Hi02d04	MDC015312.249 Chr 10	P	217-239	219/235	231/239	abxcd
579	Hi07b06	MDC009002.127 Chr 6	F	216-222	221	217	--
662	SAmsEB138222	MDC008411.143 Chr 9	P	264-266	-	-	-
725	Contig6533	MDC010551.377 Chr5	N	228-353	-	-	-
813	NZmsCO754252	MDC017371.127 Chr 6	V	195-197	195/197	195-197	hkxhk
Megaplex 13a							
189	SAmsCN490897	MDC020416.37 Chr 12	F	458-462	463	-	-
284	SAmsCO752155	MDC010250.69 Chr 12	F	189-192	192	192	--
344	SAmsCO905375	MDC012584.88 Chr 1570286	F	407-435	407	407	--
491	SAmsDT041234	MDC020535.246 Chr 12	F	158-176	165/167	165	lmxll
512	SAmsCN944528	MDC003532.156 Chr 2	F	205-214	206	206/210	nnxnp
551	Hi23g02	MDC009192.441 Chr 15	F	229-250	236	230	--
593	SAmsEB138715	MDC022702.107 Chr 2	F	315-338	-	-	-
594	SAmsEB151342	MDC013761.438 unanchored:56054903..56065036	F	359-376	-	-	-
626	SAmsEB135470	MDC019582.266 Chr 6	F	291-301	-	-	-
665	SAmsEB132264	MDC022516.234 Chr 7	F	119-148	126/141	-	-

UNIVERSITY of the
WESTERN CAPE

742	SAmsCN996777	MDC002085.537 Chr 15	F	266-275	269/274	269/274	hkxhk
Megaplex 13b							
181	SAmsCN444846	MDC005133.90 Chr 13	N	150-152	-	-	-
229	SAmsCN496966	MDC003594.382 Chr 15	N	167-171	167	168	--
241	SAmsCN887787	MDC010065.349 unanchored:9403298..9415982	N	254-257	255	255	--
243	SAmsCN907588	MDC011946.321 Chr 11	N	304-307	306	306	--
379	SAmsCO865207	MDC014214.260 Chr 13	N	120-138	134/138	132/137	efxeg
397	SAmsCN491038	MDC020705.116 Chr 14	N	498-510	499	499	--
440	SAmsCO416477	MDC013556.555 Chr 7	N	218-224	220	220/222	nnxnp
525	SAmsCV186968	MDC019148.87 Chr 8	N	389-397	396	396	--
534	SAmsDR997824	MDC007440.255 Chr 10	N	319-330	325	-	-
629	SAmsEB149808	MDC012989.567 Chr 2	N	269-286	283	-	-
639	SAmsEB149851	MDC016474.226 Chr 10	N	187-202	-	-	-
647	SAmsEB146894	MDC017945.196 Chr 8	N	422-438	423/426	-	-
763	SAmsContig1193 6	MDC013753.167 Chr 2	N	344-355	347	-	-
Megaplex 13c							
163	CH04f07	MDC021718.251 Chr 9	P	82-113	-	-	-
174	CH05g02	MDC021144.114 Chr12	P	133-155	148/152	137/148	efxeg
178	CH05h12	MDC005658.277	P	164-192	165/167	165/167	hkxhk

UNIVERSITY of the WESTERN CAPE

		unanchored:33417955..33448238					
272	SAmsCN942512	MDC012121.557 Chr14	P	389-397	389/392	389	lmxll
472	SAmsDR995122	MDC014092.189 Chr14	P	296-328	312/318	312	lmxll
516	SAmsCO900034	MDC007820.597 Chr15	P	353-367	361/366	361/366	hkxhk
533	SAmsDR993168	MDC007844.642 Chr15	P	249-253	249/253	249/253	hkxhk
535	SAmsDR997862	MDC017127.194 Chr15	P	275-283	277/282	277/282	hkxhk
610	SAmsEB133782	MDC015102.351 Chr4	P	508-543	-	-	-
638	SAmsEB147667	MDC007467.200 Chr11	P	411-420	411	417	--
661	SAmsEB126773	MDC034420.7 Chr15	P	442-470	438/454	454	lmxll
686	SAmsEB106592	MDC011198.306 Chr2	P	234-237	236	233/236	nnxnp
688	SAmsEB142061	MDC006613.339 Chr14	P	339-341	339/341	339/341	hkxhk
Megaplex 13d							
104	CH01d09	MDC010076.456 Chr 12	V	131-172	155/165	149/165	efxeg
226	SAmsCN444745	MDC015190.83 Chr 9	V	455-480	-	-	-
443	SAmsCO903797	MDC021940.79 Chr 16	V	399-413	409/411	409	lmxll
490	SAmsDR995748	MDC012914.254 Chr 14	V	315-338	316/336	336	lmxll
498	SAmsDR992457	MDC013008.333 Chr 9	V	356-375	362/373	362/367	efxeg
514	SAmsCX025465	MDC015326.172 Chr 9	V	227-235	230/234	230/234	hkxhk
536	Hi02c07	MDC014016.450	V	107-119	113/117	113/117	hkxhk

UNIVERSITY of the WESTERN CAPE

		Chr 1					
592	SAmsEB149750	MDC013258.236 Chr 13	V	246-265	258/264	258/264	hkxhk
597	SAmsEB109450	MDC011235.284 Chr 13	V	527-539	532/544	-	-
630	SAmsDY255319	MDC022862.53 Chr 5	V	181-211	182/203	182/203	hkxhk
724	SAmsCN996777	MDC009274.258 Chr 10	V	282-288	282/289	289	lmxll
Megaplex 14a							
30	SAmsAT000400.1	MDC002235.548 Chr 2	N	175-181	176/181	176/181	hkxhk
220	SAmsCN580732	MDC015169.163 Chr 2	F	340-375	370	-	-
254	SAmsCN933736	MDC019787.50 Chr 16	F	291-334	301/312	301/312	hkxhk
262	SAmsCO865955	MDC015520.222 Chr 1	F	200-214	202	202	--
400	SAmsCN578608	MDC010461.160 Chr 12	N	192-196	195	195	--
418	SAmsCV150384	MDC017449.236 Chr 17	F	235-250	248	248	--
460	NZ26c6	LG 6	N	102-165	-	-	-
461	SAmsDT000945	MDC017026.232 Chr 17	F	390-425	400/406	398/394	abxcd
502	SAmsDR990381	MDC007681.179 Chr 10	N	264-300	265/289	265/278	efxeg
508	SAmsDT041145	MDC017144.293 Chr 13	F	63-131	84/94	82/88	abxcd
531	SAmsCN943946	MDC016731.254 Chr 9	N	327-341	329/339	329/339	hkxhk
574	Hi02b07	MDC009491.388 Chr 12	N	204-216	204	204/213	nnxnp
601	SAmsEB154700	MDC006620.372 Chr 16:	N	229-236	228/234	230/234	efxeg

UNIVERSITY of the WESTERN CAPE

602	SAmsEB144676	MDC008781.274 Chr 8	F	161-197	164/188	164/188	efxeg
615	SAmsEB153928	MDC013377.330 Chr 15	N	348-358	350/353	348/353	efxeg
Megaplex 14b							
4	GD 100	LG 10	P	223-238	229	229/237	nnxnp
13	22c6	-	V	63-142	76/100	76/100	hkxhk
159	CH04d11	MDC010450.930 Chr 3	P	85-152	130/143	143	lmxll
265	SAmsCO723438	MDC001167.326 Chr 2	P	182-202	202	202	--
414	SAmsCN489062	MDC021085.739 Chr 10	V	284-306	297/301	301	lmxll
419	SAmsCO755991	MDC020003.312 unanchored:33958454..33973500	V	150-154	-	-	-
448	SAmsCV150002	MDC026285.8 unanchored:19572400..19615489	N	426-465	428/430	428	lmxll
484	SAmsDT041144	MDC017371.127 Chr 6	V	335-396	350/352	350/352	hkxhk
496	SAmsDT003221	MDC012972.308 Chr 15	P	319-330	-	-	-
507	SAmsDR998909	MDC002325.395 Chr 6	P	216-221	216/-	219/-	abxcd
583	Hi04f09	MDC005047.173 Chr 13	V	222-258	241/253	241	lmxll
598	SAmsEB138859	MDC014091.117 Chr 9	V	162-169	-	-	-
617	SAmsEB114260	MDC008416.202 Chr 10	P	274-290	-	-	-
833	NZmsEB137525	MDC014091.117 Chr 9	V	172-192	174/188	174/184	efxeg
Megaplex 15a							
5	GD 103	LG 5/10	F	78-130	78/105	78/105	hkxhk
82	CH02b101	MDC022150.298	N	121-159	-	-	-

UNIVERSITY of the WESTERN CAPE

		Chr 2					
195	SAmsCN492475	MDC010740.412 unanchored:7014859..7029800	N	175-185	177/183	177	lmxll
201	SAmsCN493973	MDC001897.482 Chr 2	F	252-329	275/314	314	lmxll
214	SAmsCN496821	MDC015102.349 Chr 4	F	358-410	383/408	370/408	efxeg
245	SAmsCN943613	MDC005388.315 Chr 12	F	165-174	166/175	175	lmxll
466	SAmsDT040421	MDC015817.303 Chr 12	N	325-350	348	341/348	nnxnp
567	Hi03a03	MDC016112.100 Chr 14	F	205-223	214/222	207/214	efxeg
676	SAmsEB106537	MDC017002.101 Chr 8	F	178-188	183	178/183	nnxnp
738	SAmsCV883434	MDC016637.26 Chr 6	F	332-351	334/337	334	lmxll
822	NZmsDR033893	MDC017604.504 Chr 11	N	194-225	202/214	214	lmxll
826	NZmsEB111793	MDC021681.173 Chr 13	N	275-281	275	275/279	nnxnp
Megaplex 15b							
40	MS14h03	MDC015605.102 Chr 3	V	114-140	115	117	--
131	CH01c11	MDC004106.267 Chr 11	P	109-155	111/145	145	lmxll
339	SAmsCO066563	MDC015340.304 Chr 13	V	420-438	-	-	-
359	SAmsCO756752	MDC002458.1854 Chr 3	V	293-345	-	-	-
382	SAmsCO067152	MDC016102.192 Chr 10	V	218-233	-	-	-
444	SAmsCO752447	MDC021843.193 unanchored:114490114..1144921 63	N	439-453	-	-	-

UNIVERSITY of the WESTERN CAPE

506	SAmsDR997517	MDC033581.12 Chr 9	P	287-324	305/308	299/305	efxeg
510	SAmsCN881550	MDC004291.249 Chr 17	V	241-253	250/253	253	lmxll
515	SAmsCV657225	MDC016649.157 Chr 6	V	173-200	194	194	--
529	SAmsCN443900	MDC020042.326 Chr 14	P	418-498	441/445	-	-
664	SAmsEB153442	MDC018604.406 Chr 10	P	365-373	-	-	-
759	SAmsCN929037	MDC017026.232 Chr 17	P	187-239	219/225	219	lmxll
Megaplex 16a							
9	01a6	LG 4	F	87-155	123	149	--
328	SAmsCN489396	MDC015986.169 Chr 2	N	448-540	-	-	-
336	SAmsCO168310	MDC020043.176 Chr 12	F	386-474	428	428	--
361	SAmsCO903775	MDC010201.199 Chr 5	F	239-251	-	-	-
370	SAmsCO052793	MDC015381.190 Chr 4	F	171-186	181	-	-
473	SAmsDR996674	MDC015516.245 Chr 6	N	424-428	428	426/428	nnxnp
558	Hi01e10	MDC002171.593 Chr 9	F	198-220	211/213	213/215	efxeg
565	Hi08h12	MDC010803.260 Chr 10	N	101-202	102/151	-	-
580	Hi20b03	MDC014200.253 Chr 8	N	215-238	218/227	218	lmxll
656	SAmsEB139609	MDC007147.92 Chr 8	F	311-351	-	-	-
671	SAmsEB149428	MDC021125.349 Chr 4	N	255-281	256/278	278	lmxll
678	SAmsEB128431	MDC004449.266	N	322-342	328/332	328/336/34	efxeg

UNIVERSITY of the WESTERN CAPE

		Chr 13				1	
828	NZmsCN914822	MDC010773.182 Chr 14	F	190-193	191	-	-
Megaplex 16b							
140	CH02a08	MDC019763.88 Chr 10	P	128-177	140/154	140/149	efxeg
204	SAmsCN494928	MDC016112.100 Chr 14	V	209-229	211/220	211/224	efxeg
368	SAmsCO723511	MDC006682.168 Chr 17	V	356-434	-	-	-
386	SAmsCO901343	MDC003451.570 Chr 4	P	208-233	214/228	214/228	hkxhk
411	SAmsCN581642	MDC005839.240 Chr 13	V	162-170	167/171	167/171	hkxhk
429	SAmsCO905285	MDC020851.240 Chr 13	P	344-382	344/359	359/382	efxeg
445	SAmsCO068219	MDC018327.114 Chr 1	P	433-437	-	-	-
636	SAmsEB121159	MDC011090.394 Chr 15	V	175-194	178/181	178/181	hkxhk
673	SAmsEB153023	MDC009294.148 Chr 5	V	476-494	477	477	--
732	SAmsGO566418	MDC007950.564 Chr 16	V	269-309	269/-	300/-	abxcd
753	SAmsGO522086	MDC008749.41 Chr 5	V	249-261	247/256	256	lmxll
760	SAmsContig1506 6	MDC010751.331 Chr 4	P	274-301	276/-	292/-	abxcd
Megaplex 17							
47	CH04e05	MDC011989.191 Chr 7	V	174-227	175/202	175/202	efxeg
170	CH05b06	MDC018507.307 Chr 10	P	185-215	193/198	193/198	hkxhk
192	SAmsCN491993	MDC004698.235 Chr 5	F	245-284	278/282	278/282	hkxhk

228	SAmsCN496055	MDC005479.52 Chr 14	N	360-364	361/363	361	lmxll
308	SAmsCN444942	MDC015532.141 Chr 6	N	260-273	265	259/265	nnxnp
378	SAmsCO753033	MDC021781.288 Chr 6	V	273-296	275	275/279	nnxnp
403	SAmsCN494091	MDC008371.455 Chr 4	P	253-289	275	275/282	nnxnp
421	SAmsCO865954	MDC022559.265 unanchored:58193505..58196638	P	452-455	454	454/458	nnxnp
451	SAmsAF429983	MDC017091.105 Chr 4	F	356-371	210/213	175	--
458	04f3	LG 9	F	93-143	108/114	108/124	efxeg
505	SAmsDR995002	MDC000262.256 Chr 12	F	324-334	330/333	330/333	hkxhk
546	Hi22f12	MDC021414.198 Chr5	N	207-212	209	209	--
561	Hi05b09	MDC006588.64 Chr 7	V	123-140	138/140	125/133	abxcd
563	Hi04b12	MDC016797.262 Chr 8	P	138-160	141/148	148	lmxll
595	SAmsEB148060	MDC008726.377 Chr 4	F	374-441	390/401	394/401	efxeg
623	SAmsEB149589	MDC001040.257 Chr 2	V	401-404	402/404	401	lmxll
717	SAmsContig4879	MDC007228.344 Chr 6	P	351-361	355/360	355/360	hkxhk
740	SAmsContig22587	MDC006300.120 Chr 12	N	305-325	317/323	317/323	hkxhk
774	Hi04e05	MDC024246.13 Chr 8	N	116-179	138/142	138/142	hkxhk
Megaplex 18a							
7	GD 147	LG 13	N	129-152	135/152	129/139	abxcd
206	SAmsCN495278	MDC011995.314 Chr 15	N	214-240	-	-	-

UNIVERSITY of the WESTERN CAPE

209	SAmsCN495857	MDC020525.273 Chr 3	F	145-155	148/152	148/151	hkxhk
218	SAmsCN580519	MDC011588.205 Chr 5	F	120-135	122/137	122/137	hkxhk
296	SAmsCN880881	MDC008622.281 Chr 14	F	406-430	430/433	430	lmxll
402	SAmsAT000420	MDC002412.304 Chr 4	N	162-174	168/172	168/172	hkxhk
420	SAmsCO903145	MDC003918.382 Chr 2	N	261-263	263/265	263/265	hkxhk
422	SAmsCV627191	MDC020007.246 Chr17	F	296-385	311/313	312/321	efxeg
612	SAmsEB1155894	MDC009328.385 Chr 16	F	258-287	265	282	--
680	SAmsEB106034	MDC004713.230 unanchored:10543549..10564414	N	189-196	194/197	194	lmxll
716	SAmsCO051709	MDC005414.494 Chr 15	F	190-221	195	195/220	nnxnp
804	NZmsEB106592	MDC011198.306 Chr 2	F	240-243	239/243	239/243	hkxhk
824	NZmsEB153947	MDC022425.139 Chr 11	F	166-180	167/171	167/179	efxeg
Megaplex 18b							
151	CH03g06	MDC015735.303 Chr 11	P	137-171	139/150	139/150	hkxhk
212	SAmsCN496144	MDC019010.307 Chr 6	V	303-349	340/350	340/350	hkxhk
261	SAmsCO541090	MDC011995.314 Chr 15	P	403-407	405	405	--
290	SAmsCN864595	MDC007691.315 Chr 15	P	358-394	361/381	361/376	efxeg
301	Z71981/MDKN1 GN	MDC016467.170 Chr15	P	331-345	344/342	342	lmxll
305	SAmsCN491050	MDC013938.271 Chr3	V	177-269	189	189	--

UNIVERSITY of the
WESTERN CAPE

419	SAmsCO755991	MDC020003.312 unanchored:33958454..33973500	N	148-156	-	-	-
435	SAmsCO867454	MDC004223.800 unanchored:41825184..41838513	V	377-392	-	-	-
462	SAmsDR994153	MDC017740.298 Chr 10	V	462-474	463/471	463/471	efxeg
485	SAmsDR993043	MDC022656.93 Chr11	P	279-315	299/315	284/293	abxcd
538	CH-Vf1	LG 1	V	137-169	156	141/156	nnxnp
549	Hi05e07	MDC003262.348 Chr 9	P	194-228	196/202	196/202	hkxhk
614	SAmsEB155789	MDC012906.325 Chr 14	N	323-358	326	326	--
635	SAmsEB149433	MDC013012.212 Chr 11	N	285-309	310	286/310	nnxnp
735	SAmsContig5280	MDC020462.181 Chr 5	V	284-295	287	287/293	nnxnp
Megaplex 19a							
184	SAmsCN489175	MDC006990.204 Chr 5	V	233-243	238/241	241	lmxll
282	SAmsCO756306	MDC009766.454 Chr 4	V	101-185	-	-	-
286	SAmsCN917681	MDC002753.399 Chr 5	P	401-427	426	411/426	nnxnp
315	SAmsCN496099	MDC013184.220 Chr 10	P	210-222	211/216	211/216	hkxhk
396	SAmsCN496160	MDC020235.546 Chr 17	P	144-151	-	-	-
469	SAmsDT041836	MDC005198.371 Chr 6	V	323-331	330	328/330	nnxnp
517	SAmsCN914754	MDC007598.175 Chr 8	P	315-317	317	315/317	nnxnp
677	SAmsEB114797	MDC008031.166 Chr 10	V	217/251	-	-	-
743	SAmsContig1526	MDC008682.135	V	254-259	255/258	258	lmxll

UNIVERSITY of the WESTERN CAPE

	0	Chr 4					
Megaplex 20a							
687	SAmsEB132187	MDC001593.313 Chr 1	F	220-275	239	235	--
712	SAmsEB112897	MDC001100.222 Chr 12	P	330-390	382	-	-
714	SAmsCO417701	MDC022324.112 Chr 9	V	325-395	-	-	-
726	SAmsCN877882	MDC019147.47 Chr 2	F	460-510	485	485/501	nnxnp
728	SAmsCN868149	MDC008453.906 Chr 13	P	210-285	244/250	248/250	efxeg
772	Hi02a09	MDC012004.220 Chr 11	F	110-195	146/156	135/146	efxeg
773	Hi23b12	MDC005900.178 Chr 14	V	125-175	153	133/153	nnxnp
775	Hi08e06	MDC011043.394 Chr 5	P	120-164	156	135	--
783	Hi23d11b	LG 4	P	165-205	180/186	183	lmxll
797	Hi02d11	MDC011578.52 Chr 14	V	176-285	197/257	233/245	abxcd
810	NZmsEB142980	MDC018496.52 Chr 4	N	80-140	112/123	123	lmxll
Megaplex 21							
715	SAmsCN444550	MDC015575.172 Chr10	V	320-380	347/352	343/352	efxeg
744	SAmsCN850743	MDC021608.178 Chr1	N	260-290	278/281	278/281	hkxhk
754	SAmsEB144379	MDC016553.87 Chr14	P	380-510	417	417/423	nnxnp
771	Hi21e04	MDC000164.370 Chr14	P	110-160	133/135	133/135	hkxhk
776	Hi23d02	MDC000442.224 Chr11	F	100-155	124/147	121	lmxll

UNIVERSITY of the WESTERN CAPE

777	Hi23d06	MDC007040.105 Chr9	V	140-175	154/160	158	lmxll
778	Hi15g11	MDC006465.421 Chr16	N	80-192	159	-	-
789	Hi02b10	MDC012697.251 Chr2	V	177-270	200/202	202/221	efxeg
791	Hi02c06	LG 11	P	180-270	223	223/227	nnxnp
794	Hi01c09	MDC020259.182 Chr14	N	193-250	216/218	216/218	lmxll
796	Hi08c05	MDC021778.347 unanchored:87054581..87058720	F	180-260	232/236	232/238	efxeg
Megaplex 22							
756	SAmsCN942929	MDC004462.498 Chr3	V	480-550	522/526	526	lmxll
766	AG11	LG 1	Y	195-220	205/207	201/205	efxeg
779	Hi04d10	LG 6	R	140-200	176/182	166/182	efxeg
780	Hi08f05	MDC005649.355 Chr 2	F	142-170	163/167	163/167	hkxhk
781	Hi02h08	MDC010932.713 Chr 16	V	140-185	165/170	170/172	efxeg
784	Hi08d09	MDC002262.69 Chr 16	F	171-220	182	182	--
800	Hi12a02	MDC009686.144 Chr 10	F	223-280	252/255	252	lmxll
801	Hi02a07	MDC000017.398 Chr 2	V	210-320	281/283	281/183	hkxhk
806	NZmsEB107305	MDC022702.107 Chr 2	N	110-190	167	155	--
820	NZmsEB116209	MDC000625.521 Chr9	F	100-140	129/132	114/129	efxeg
Megaplex 23							
3	GD 96	LG 17	N	172-182	174/178	174/178	hkxhk-
205	SAmsCN495161	MDC019977.94 Chr 16	N	232-241	-	217/228	-

UNIVERSITY of the WESTERN CAPE

285	SAmsCO753983	MDC002920.156 Chr 9	F	197-203	199/201	199/201	hkxhk
342	SAmsCO067420	MDC020415.111 Chr 14	N	144-147	147/150	147/150	hkxhk
360	SAmsCO900737	MDC019824.74 Chr 7	F	249-268	264	264	--
393	SAmsCN490103	MDC007213.361 Chr 3	F	135-162	136/147	136/147	hkxhk
488	SAmsDT000773	MDC022111.144 Chr 9	F	348	-	-	-
511	SAmsCN865016	MDC009136.399 Chr 15	N	311-316	311/316	312	--
607	SAmsEB144570	MDC037296.14 Chr 10	F	437-464	408/466	439/464	abxcd
632	SAmsEB138370	MDC012797.142 Chr 6	N	262	-	-	-
812	NZmsEB132582	LG 6	F	171-174	173	171/173	nnxnp
Megaplex 24							
153	CH03h06	MDC017708.244 Chr 15	P	145-175	147/168	145/168	efxeg
303	SAmsCN491062	MDC009945.303 Chr 2	V	360-388	360/383	360/383	hkxhk
363	SAmsCO052202	MDC010937.192 Chr 17	V	208-236	218/226	218/226	hkxhk
367	SAmsCO417416	MDC011072.388 Chr 12	P	202-232	220/230	230	lmxll
503	SAmsDR990622	MDC007008.336 Chr 15	V	333-347	334/339	334/339	hkxhk
611	SAmsEB127535	MDC014229.560 Chr 9	N	326-330	-	-	-
621	SAmsEB119062	MDC002616.414 Chr 16	N	419-421	383/420	383/422	efxeg
663	SAmsEB147331	MDC000335.312 Chr 6	V	254-292	256	271/291	nnxnp
730	SAmsContig16216	MDC010186.403	P	295	-	-	-

UNIVERSITY of the WESTERN CAPE

		Chr 15					
77	CH03d11	MDC015757.243 Chr 10	V	115-181	123/127	119/127	efxeg
Megaplex 25							
718	SAmsContig16166	MDC016235.85 Chr 7	F	427-438	439/44	439	lmxll
722	SAmsContig21019	MDC011822.222 Chr 12	F	278-283	-	-	-
724	SAmsContig14444	MDC009274.258 Chr 10	V	282-288	-	-	-
736	SAmsContig12510	MDC008623.473 Chr 17	V	341-354	348/351	348/351	hkxhk
768	Hi04g11	MDC006738.419 Chr 11	F	115-166	116/126	126	lmxll
769	Hi22d06	MDC001013.218 Chr 2	V	122-131	127/133	127	lmxll
785	Hi09a01	MDC017714.167 Chr 11	V	183-192	193	187/193	nnxnp
788	Hi04a05	MDC010937.194 Chr 1	F	190-204	192	187/192	nnxnp
793	Hi23g08	MDC001907.204 Chr 9	V	208-219	210/219	210/219	hkxhk
802	NZmsCN879773	LG 14	N	137-185	140/187	187	lmxll
827	NZmsEB146613	MDC002255.84 Chr 14	P	158-178	172/176	159/176	efxeg
829	NZmsCO905522	MDC017428.71 Chr 16	V	162-168	165	170	--
Megaplex 26							
834	SAmsMDC021941.303	MDC021941.303 Chr 9	F	219-230	219/230	219/230	hkxhk
836	SAmsMDC000503.195	MDC000503.195 Chr 9	N	197-246	243	243/249	nnxnp
837	SAmsMDC020761.431	MDC020761.431 Chr 9	P	219-250	244	244/250	nnxnp

UNIVERSITY of the WESTERN CAPE

841	SAmsMDC010321.324	MDC010321.324 Chr 9	P	328-341	391/408	394/352	efxeg
843	SAmsMDC009858.304	MDC009858.304 Chr 9	V	336-352	341	341/352	nnxnp
844	SAmsMDC004938.180	MDC004938.180 Chr 9	N	326-353	341	341/353	nnxnp
846	SAmsMDC005569.608	MDC005569.608 Chr 9	F	370-424	391/407	391	lmxll
851	SAmsMDC010403.411	MDC010403.411 Chr 9	V	390-408	389/408	389/394/408	abxabc
856	SAmsMDC003421.411	MDC003421.411 Chr 9	N	397-429	420	420	--
Megaplex 27							
838	SAmsMDC017895.317	MDC017895.317 Chr 9	F	242-257	254	251/254	nnxnp
840	SAmsMDC009477.96	MDC009477.96 Chr 9	N	219-259	237/250	237	-
842	SAmsMDC017003.269	MDC017003.269 Chr 9	F	331-369	339/369	339/364/369	abxabc
845	SAmsMDC009662.63	MDC004938.180 Chr 9	P	334-372	364/368	368	lmxll
847	SAmsMDC011932.246	MDC011932.246 Chr 9	V	382-425	389/420	380/416	abxcd
848	SAmsMDC015239.225	MDC015239.225 Chr 9	N	370-401	-	-	-
853	SAmsMDC010935.355	MDC010935.355 Chr 9	P	395-410	-	-	-
854	SAmsMDC009465.253	MDC009465.253 Chr 9	F	397-429	406	406/416	nnxnp
Megaplex 28							
849	SAmsMDC011178.406	MDC011178.406 Chr 9	P	355-429	397/400	397	lmxll
850	SAmsMDC005927.400	MDC005927.400 Chr 9	F	391-424	400/408	400	lmxll
852	SAmsMDC021941.307	MDC021941.307	N	381-411	382/410	394/406	abxcd

UNIVERSITY of the
WESTERN CAPE

		Chr 9					
859	SAmsMDC000907.297	MDC000907.297 Chr 3	F	351-376	350/373	350/373/376	abxabc
860	SAmsMDC020071.203	MDC020071.203 Chr 3	V	384-408	396/405	396/405	hkxhk
869	SAmsMDC016904.88	MDC016904.88 Chr 3	N	331-342	331/342	331/342	hkxhk
Megaplex 29							
861	SAmsMDC000834.114	MDC000834.114 Chr 3	N	350-377	351/374	351/374/376	abxabc
862	SAmsMDC020525.273	MDC020525.273 Chr 3	P	381-407	401	407/407	nnxnp
863	SAmsMDC002362.239	MDC002362.239 Chr 3	F	396-403	398	395/398	nnxnp
864	SAmsMDC038751.8	MDC038751.8 Chr 3	V	385-404	400/403	400/403	hkxhk
Megaplex 30							
865	SAmsMDC020300.286	MDC020300.286 Chr 3	N	389-428	408/412	408/428	efxeg
866	SAmsMDC007193.563	MDC007193.563 Chr 3	P	359-406	-	-	-
868	SAmsMDC003785.420	MDC003785.420 Chr 3	V	405-429	406/429	406/415	efxeg
Megaplex 31							
855	SAmsMDC005939.185	MDC005939.185 Chr 9	V	395-410	-	-	-
870	SAmsMDC012739.316	MDC012739.316 Chr 3	P	387-412	405	405	--

Key

‘ - ’ represents failure to amplify a product.

‘ -- ’ represents no JoinMap code due to homozygous nature of the marker. or lack of information from the population.

UNIVERSITY of the WESTERN CAPE

Fluorescent dyes: F - 6-Fam (Blue). V - Vic (Green). N - Ned (Yellow). P - Pet (Red)

UNIVERSITY of the
WESTERN CAPE

Table D: A set of 502 DArT markers scored in this study, together with their segregation data in the 'Anna' x 'Austin' population. DArT markers are prefixed with the letters '**aPa-**'. Also shown is the best blast hit for each DarT marker on the apple genome.

DArT marker ID	Best BLAST hit on genome (Velasco *et al.,* 2010)	'Anna' x 'Austin' JoinMap® code
aPa-442046	MDC018131.78 Chr 16	lmxll
aPa-183485	MDC011101.167 Chr 12	lmxll
aPa-441502	MDC004453.322 Chr 13	lmxll
aPa-185262	MDC011101.167 Chr 12	lmxll
aPa-442112	MDC017440.326 Chr 12	lmxll
aPa-442750	MDC018131.78 Chr 16	lmxll
aPa-526121	MDC018131.78 Chr 16	lmxll
aPa-525699	MDC018131.78 Chr 16	lmxll
aPa-442173	MDC017440.326 Chr 12	lmxll
aPa-442722	MDC018505.86 unanchored:15588754..15592927	lmxll
aPa-461514	MDC013637.349 Chr 5	lmxll
aPa-186193	MDC011101.167 Chr 12	lmxll
aPa-186502	MDC018131.78 Chr 16	lmxll
aPa-441779	MDC017440.326 Chr 12	lmxll
aPa-525839	MDC018131.78 Chr 16	lmxll
aPa-185641	MDC010162.210 Chr 2	lmxll
aPa-525624	MDC004453.322 Chr 13	lmxll
aPa-443216	MDC012135.190 Chr 8	lmxll
aPa-441533	MDC017710.278 Chr 4	lmxll
aPa-442545	MDC018505.86 unanchored:15588754..15592927	lmxll
aPa-519436	MDC018505.86 unanchored:15588754..15592927	lmxll
aPa-461159	MDC018505.86 unanchored:15588754..15592927	lmxll
aPa-441968	MDC012135.190 Chr 8	lmxll
aPa-462114	MDC012135.190 Chr 8	lmxll
aPa-184271	MDC043953.5 Chr 14	lmxll
aPa-525825	MDC009148.298 Chr 11	lmxll
aPa-462075	MDC011715.382 Chr 13	lmxll
aPa-461789	MDC043953.5 Chr 14	lmxll
aPa-442706	MDC011530.503 Chr 2	lmxll
aPa-518906	MDC010525.308 Chr 14	lmxll
aPa-185612	MDC022214.483 Chr 11	lmxll

aPa-186257	MDC011530.503 Chr 2	lmxll
aPa-185317	MDC002798.552 Chr 17	lmxll
aPa-184313	MDC005050.92 Chr 4	lmxll
aPa-442804	MDC005050.92 Chr 4	lmxll
aPa-186475	MDC000091.164 unanchored:34860552..34890639	lmxll
aPa-186053	MDC011715.382 Chr 13	lmxll
aPa-183983	MDC009184.279 Chr 17	lmxll
aPa-460767	MDC005050.92 Chr 4	lmxll
aPa-443364	MDC013609.385 Chr 3	lmxll
aPa-186983	MDC009184.144 Chr 5	lmxll
aPa-442855	MDC013609.385 Chr 3	lmxll
aPa-184725	MDC002798.552 Chr 17	lmxll
aPa-183107	MDC011815.218 Chr 4	lmxll
aPa-518840	MDC018131.78 Chr 16	lmxll
aPa-184691	MDC011815.218 Chr 4	lmxll
aPa-184194	MDC016529.125 Chr 1	lmxll
aPa-183087	MDC004935.291 Chr 3	lmxll
aPa-442998	MDC002798.552 Chr 17:23857284..23887486	lmxll
aPa-182926	MDC004935.291 Chr 3	lmxll
aPa-462187	MDC002798.552 Chr 17	lmxll
aPa-185953	MDC017163.385 Chr 2	lmxll
aPa-462179	MDC005050.92 Chr 4	lmxll
aPa-461612	MDC022482.297 Chr 17:8670091..8682797	lmxll
aPa-443314	MDC009184.144 Chr 5	lmxll
aPa-185803	MDC017163.385 Chr 2	lmxll
aPa-461564	MDC002661.352 Chr 8	lmxll
aPa-185804	MDC011815.218 Chr 4	lmxll
aPa-461676	MDC015655.486 Chr 7	lmxll
aPa-553116	-	lmxll
aPa-442573	MDC017163.385 Chr 2	lmxll
aPa-442784	MDC005050.92 Chr 4	lmxll
aPa-182413	MDC004935.291 Chr 3	lmxll
aPa-186111	MDC027013.64 Chr 8	lmxll
aPa-442825	MDC007928.208 Chr 3	lmxll
aPa-185333	MDC003134.366 Chr 9	lmxll
aPa-186268	MDC021458.180 Chr 15	lmxll
aPa-184544	MDC023694.36 Chr 15	lmxll
aPa-553673	-	lmxll
aPa-183140	MDC006325.435 Chr 9	lmxll

UNIVERSITY of the
WESTERN CAPE

aPa-461800	MDC033994.4 Chr 3	lmxll
aPa-442463	MDC011815.218 Chr 4	lmxll
aPa-442714	MDC028007.44 Chr 3	lmxll
aPa-461263	MDC009345.183 Chr 15	lmxll
aPa-525797	MDC022347.26 Chr 12	lmxll
aPa-526972	-	lmxll
aPa-442609	MDC012428.223 Chr 11	lmxll
aPa-441888	MDC017163.385 Chr 2	lmxll
aPa-461291	MDC009596.266 Chr 3	lmxll
aPa-442250	MDC017163.385 Chr 2	lmxll
aPa-186390	MDC004410.713 Chr 10	lmxll
aPa-526910	MDC027013.64 Chr 8	lmxll
aPa-461822	MDC022347.26 Chr 12	lmxll
aPa-519148	MDC009184.144 Chr 5	lmxll
aPa-183698	MDC028007.44 Chr 3	lmxll
aPa-185194	MDC017163.385 Chr 2	lmxll
aPa-184495	MDC011759.259 Chr 3	lmxll
aPa-443357	MDC012428.223 Chr 11	lmxll
aPa-186813	-	lmxll
aPa-184005	MDC005553.339 Chr 4	lmxll
aPa-553658	-	lmxll
aPa-526562	MDC009630.367 Chr 13	lmxll
aPa-185713	MDC027868.28 Chr 16	lmxll
aPa-462085	MDC009630.367 Chr 13	lmxll
aPa-519308	MDC007958.145 Chr 12	lmxll
aPa-182515	MDC006893.485 Chr 7	lmxll
aPa-185701	MDC006893.485 Chr 7	lmxll
aPa-183066	MDC008970.267 Chr 14	lmxll
aPa-460762	MDC009630.367 Chr 13	lmxll
aPa-461402	MDC018274.379 Chr 11	lmxll
aPa-186132	MDC005363.525 Chr 1	lmxll
aPa-461310	MDC027868.28 Chr 16	lmxll
aPa-553081	-	lmxll
aPa-525490	MDC008217.277 Chr 2	lmxll
aPa-185352	MDC007750.139 Chr 12	lmxll
aPa-443213	MDC018586.320 Chr 2	lmxll
aPa-526879	MDC009630.367 Chr 13	lmxll
aPa-185086	MDC007972.470 Chr 5	lmxll
aPa-185349	MDC009630.367 Chr 13	lmxll

UNIVERSITY of the WESTERN CAPE

aPa-184899	MDC004236.254 Chr 15	lmxll
aPa-186069	MDC003134.366 Chr 9	lmxll
aPa-441844	MDC012428.223 Chr 11	lmxll
aPa-519627	MDC027868.28 Chr 16	lmxll
aPa-461959	MDC009148.317 unanchored:8434699..8449838	lmxll
aPa-183094	MDC009148.317 unanchored:8434699..8449838	lmxll
aPa-525633	MDC027868.28 Chr 16	lmxll
aPa-518892	MDC007972.470 Chr 5	lmxll
aPa-461933	MDC011523.287 Chr 12	lmxll
aPa-441894	MDC013599.1534 Chr 14	lmxll
aPa-183467	MDC013703.213 Chr 7	lmxll
aPa-184045	MDC027868.28 Chr 16	lmxll
aPa-183186	MDC011523.287 Chr 12	lmxll
aPa-184975	MDC024438.18 Chr 15	lmxll
aPa-186883	MDC009630.367 Chr 13	lmxll
aPa-518833	MDC017169.202 Chr 8	lmxll
aPa-183514	MDC005363.525 Chr 1	lmxll
aPa-554112	-	lmxll
aPa-519128	MDC012831.158 Chr 15	lmxll
aPa-519074	MDC006373.412 Chr 6	lmxll
aPa-553229	-	lmxll
aPa-462083	MDC009782.581 Chr 15	lmxll
aPa-184477	MDC009782.581 Chr 15	lmxll
aPa-184037	MDC016102.191 Chr 15	lmxll
aPa-442137	MDC027868.28 Chr 16	lmxll
aPa-183257	MDC017105.196 Chr 2	lmxll
aPa-526398	MDC001957.239 Chr 14	lmxll
aPa-460966	MDC007308.338 Chr 1	lmxll
aPa-443125	MDC010421.358 Chr 8	lmxll
aPa-442430	MDC007308.338 Chr 1	lmxll
aPa-443009	MDC012000.74 Chr 1	lmxll
aPa-186278	-	lmxll
aPa-184816	MDC009782.581 Chr 15	lmxll
aPa-442007	MDC010421.358 Chr 8	lmxll
aPa-186937	MDC017768.322 Chr 17	lmxll
aPa-461459	MDC011418.240 Chr 8	lmxll
aPa-460950	MDC010421.358 Chr 8	lmxll
aPa-184690	MDC006907.385 Chr 3	lmxll
aPa-443102	MDC018586.320 Chr 2	lmxll

UNIVERSITY of the
WESTERN CAPE

aPa-519033	MDC010974.393 Chr 12	lmxll
aPa-519519	MDC008705.205 Chr 2	lmxll
aPa-461576	MDC009596.266 Chr 3	lmxll
aPa-461381	MDC015452.177 Chr 5	lmxll
aPa-185363	MDC009894.238 Chr 12	lmxll
aPa-441669	MDC009148.298 Chr 11	lmxll
aPa-443108	MDC018359.70 Chr 12	lmxll
aPa-186260	MDC021201.196 Chr 9	lmxll
aPa-185428	MDC007272.508 Chr 5	nxnp
aPa-442759	MDC007272.508 Chr 5	nxnp
aPa-184280	MDC018256.380 Chr 15	nxnp
aPa-185358	MDC015890.82 Chr 13	nxnp
aPa-183977	MDC028004.28 Chr 9	nxnp
aPa-443118	MDC012097.541 Chr 3	nxnp
aPa-441922	MDC020870.303 unanchored:5069772..5091374	nxnp
aPa-442654	MDC007272.508 Chr 5	nxnp
aPa-519484	MDC017931.276 unanchored:61950560..61965395	nxnp
aPa-185955	MDC020870.303 unanchored:5069772..5091374	nxnp
aPa-526894	MDC005111.360 Chr 9	nxnp
aPa-186676	MDC028004.28 Chr 9	nxnp
aPa-185935	MDC028004.28 Chr 9	nxnp
aPa-526572	MDC004219.393 Chr 4	nxnp
aPa-526596	MDC022768.226 Chr 11	nxnp
aPa-441654	MDC005111.360 Chr 9	nxnp
aPa-526002	MDC015890.82 Chr 13	nxnp
aPa-442847	MDC015174.161 Chr 10	nxnp
aPa-441544	MDC012097.541 Chr 3	nxnp
aPa-184615	MDC019537.395 Chr 2	nxnp
aPa-182516	MDC015890.82 Chr 13	nxnp
aPa-184555	MDC020870.303 unanchored:5069772..5091374	nxnp
aPa-461820	MDC028004.28 Chr 9	nxnp
aPa-186754	MDC028004.28 Chr 9	nxnp
aPa-184168	MDC019537.395 Chr 2	nxnp
aPa-183534	MDC011601.169 unanchored:64849731..64863967	nxnp
aPa-553793	-	nxnp
aPa-442947	MDC000839.489 Chr 10	nxnp
aPa-184149	MDC001185.117 Chr 7	nxnp
aPa-442949	MDC002355.265 Chr 15	nxnp
aPa-184606	MDC003956.268 Chr 15	nxnp

UNIVERSITY of the
WESTERN CAPE

aPa-462034	MDC002355.265 Chr 15	nxnp
aPa-186634	MDC010708.304 Chr 9	nxnp
aPa-185093	MDC005111.360 Chr 9	nxnp
aPa-186366	MDC011601.169 unanchored:64849731..64863967	nxnp
aPa-184361	MDC015174.161 Chr 10	nxnp
aPa-186429	MDC019400.146 Chr 2	nxnp
aPa-443272	MDC000839.489 Chr 10:32429947..32436543	nxnp
aPa-186258	MDC011601.169 unanchored:64849731..64863967	nxnp
aPa-525952	MDC005111.360 Chr 9:10996894..11024561	nxnp
aPa-525820	MDC011601.169 unanchored:64849731..64863967	nxnp
aPa-526819	MDC006925.448 unanchored:67493805..67504998	nxnp
aPa-186224	MDC019400.146 Chr 2	nxnp
aPa-186725	MDC001185.117 Chr 7	nxnp
aPa-518598	MDC002355.265 Chr 15	nxnp
aPa-184447	MDC005242.172 Chr 13	nxnp
aPa-526732	MDC011601.169 unanchored:64849731..64863967	nxnp
aPa-441554	MDC009757.32 Chr 4	nxnp
aPa-183336	MDC005266.192 unanchored:83364288..83368862	nxnp
aPa-518980	MDC018177.205 unanchored:50842138..50866567	nxnp
aPa-186731	MDC005111.360 Chr 9	nxnp
aPa-184290	MDC007727.187 Chr 9	nxnp
aPa-525667	MDC010450.933 Chr 17	nxnp
aPa-518839	MDC009757.32 Chr 4	nxnp
aPa-554102	-	nxnp
aPa-526724	MDC001530.208 Chr 5	nxnp
aPa-443308	MDC017405.92 Chr 13	nxnp
aPa-554192	-	nxnp
aPa-525931	MDC017198.416 Chr 11	nxnp
aPa-184240	MDC003736.495 Chr 11	nxnp
aPa-552965	-	nxnp
aPa-185518	MDC005160.375 Chr 7	nxnp
aPa-186724	MDC022597.70 Chr 10	nxnp
aPa-182991	MDC019023.184 Chr 2	nxnp
aPa-185737	MDC005617.547 Chr 2	nxnp
aPa-186784	MDC011184.199 Chr 17	nxnp
aPa-182855	MDC020471.132 unanchored:99891384..99894446	nxnp
aPa-184187	MDC006704.247 Chr 13	nxnp
aPa-186893	MDC003506.327 Chr 6	nxnp
aPa-461583	MDC005160.375 Chr 7	nxnp

aPa-461231	MDC010071.356 Chr 15	nxnp
aPa-518635	MDC005160.375 Chr 7	nxnp
aPa-442611	-	nxnp
aPa-184772	MDC022597.70 Chr 10	nxnp
aPa-441938	MDC005160.375 Chr 7	nxnp
aPa-184105	MDC022220.141 Chr 10	nxnp
aPa-441714	MDC008047.500 Chr 8	nxnp
aPa-442313	MDC006704.247 Chr 13	nxnp
aPa-526869	MDC027766.33 Chr 6	nxnp
aPa-442771	MDC022597.70 Chr 10	nxnp
aPa-184171	MDC003506.327 Chr 6	nxnp
aPa-525715	MDC005160.375 Chr 7	nxnp
aPa-460973	MDC004339.376 Chr 6	nxnp
aPa-184932	MDC013755.139 unanchored:50874944..50891830	nxnp
aPa-184649	MDC022597.70 Chr 10	nxnp
aPa-526885	MDC012386.258 Chr 1	nxnp
aPa-441936	MDC022597.70 Chr 10	nxnp
aPa-442542	MDC005055.925 Chr 9	nxnp
aPa-182529	MDC006704.247 Chr 13	nxnp
aPa-186876	MDC005640.336 Chr 13	nxnp
aPa-185218	MDC011753.226 Chr 3	nxnp
aPa-462007	MDC011753.226 Chr 3	nxnp
aPa-525807	MDC005640.336 Chr 13	nxnp
aPa-186987	MDC001653.268 Chr 5	nxnp
aPa-184362	MDC011715.382 Chr 13	nxnp
aPa-526579	MDC022220.141 Chr 10	nxnp
aPa-186670	MDC000714.184 Chr 17:20027204..20034046	nxnp
aPa-554050	-	nxnp
aPa-183365	MDC005640.336 Chr 13	nxnp
aPa-184073	MDC005640.336 Chr 13	nxnp
aPa-526234	MDC002826.269 Chr 10	nxnp
aPa-441884	MDC005055.925 Chr 9	nxnp
aPa-442766	MDC033994.4 Chr 3	nxnp
aPa-525968	MDC021487.434 Chr 9	nxnp
aPa-186188	MDC008500.738 Chr 11	nxnp
aPa-461202	MDC000714.184 Chr 17	nxnp
aPa-186956	MDC011753.226 Chr 3	nxnp
aPa-553326	-	nxnp
aPa-461983	MDC011753.226 Chr 3	nxnp

UNIVERSITY of the
WESTERN CAPE

aPa-518808	MDC010450.929 Chr 17	nxnp
aPa-182444	MDC015445.345 Chr 2	nxnp
aPa-441806	MDC008895.256 Chr 10	nxnp
aPa-461826	MDC008500.738 Chr 11	nxnp
aPa-185733	MDC007380.334 Chr 14	nxnp
aPa-443297	MDC011202.332 Chr 12	nxnp
aPa-184175	MDC026708.10 Chr 5	nxnp
aPa-184828	MDC008895.256 Chr 10	nxnp
aPa-442031	MDC005640.336 Chr 13	nxnp
aPa-442132	MDC020039.190 Chr 16	nxnp
aPa-554201	-	nxnp
aPa-442770	MDC011202.332 Chr 12	nxnp
aPa-441808	MDC041528.3 Chr 14	nxnp
aPa-185787	MDC018726.176 Chr 12	nxnp
aPa-461835	MDC002016.263 Chr 12	nxnp
aPa-186727	MDC014123.202 Chr 12	nxnp
aPa-185447	MDC013648.312 Chr 13	nxnp
aPa-462025	MDC008500.738 Chr 11	nxnp
aPa-183483	MDC022173.275 Chr 4	nxnp
aPa-526921	MDC015445.345 Chr 2	nxnp
aPa-182512	MDC015166.43 Chr 1	nxnp
aPa-526698	MDC008895.256 Chr 10	nxnp
aPa-186719	MDC008500.738 Chr 11	nxnp
aPa-186721	MDC008895.256 Chr 10	nxnp
aPa-183826	MDC008895.256 Chr 10	nxnp
aPa-183463	MDC020039.190 Chr 16	nxnp
aPa-519092	MDC027078.28 Chr 16	nxnp
aPa-518790	MDC010002.120 Chr 3	nxnp
aPa-518577	MDC011277.211 Chr 8	nxnp
aPa-462115	MDC011277.211 Chr 8	nxnp
aPa-185375	MDC006182.365 Chr 5	nxnp
aPa-443303	MDC008151.427 Chr 12:3968318..3970103	nxnp
aPa-526237	MDC014115.474 Chr 8	nxnp
aPa-460890	MDC010002.120 Chr 3	nxnp
aPa-184327	-	nxnp
aPa-186296	MDC014115.474 Chr 8	nxnp
aPa-443148	MDC018806.178 Chr 11	nxnp
aPa-462150	MDC010449.276 Chr 9	nxnp
aPa-185451	MDC014115.474 Chr 8	nxnp

UNIVERSITY of the
WESTERN CAPE

aPa-182984	MDC014115.474 Chr 8	nxnp
aPa-518754	MDC014115.474 Chr 8	nxnp
aPa-183498	MDC014115.474 Chr 8	nxnp
aPa-186452	MDC019319.333 Chr 5	nxnp
aPa-185278	MDC014014.341 Chr 2	hkxhk
aPa-186157	-	hkxhk
aPa-443003	MDC010811.313 Chr 15	hkxhk
aPa-186339	MDC021900.23 Chr 2	hkxhk
aPa-553235	-	hkxhk
aPa-183952	MDC010811.313 Chr 15	hkxhk
aPa-460957	MDC021449.408 Chr 2	hkxhk
aPa-460999	MDC010811.313 Chr 15	hkxhk
aPa-186176	MDC027681.22 Chr 4	hkxhk
aPa-518770	MDC010811.313 Chr 15	hkxhk
aPa-441685	MDC022098.101 Chr 9	hkxhk
aPa-442593	MDC020558.334 Chr 10	hkxhk
aPa-518884	MDC014014.341 Chr 2	hkxhk
aPa-552888	MDC027261.9 Chr 12	hkxhk
aPa-442891	MDC027261.9 Chr 12	hkxhk
aPa-525950	MDC021900.23 Chr 2	hkxhk
aPa-184439	-	hkxhk
aPa-443039	MDC010811.313 Chr 15	hkxhk
aPa-442826	MDC022098.101 Chr 9	hkxhk
aPa-185199	MDC020558.334 Chr 10	hkxhk
aPa-460695	MDC020558.334 Chr 10	hkxhk
aPa-460933	MDC019670.396 Chr 12	hkxhk
aPa-442203	MDC022098.101 Chr 9	hkxhk
aPa-461911	MDC022098.101 Chr 9	hkxhk
aPa-552852	MDC022098.101 Chr 9	hkxhk
aPa-461780	MDC027261.9 Chr 12	hkxhk
aPa-460750	MDC010811.313 Chr 15	hkxhk
aPa-526162	MDC007459.193 Chr 8	hkxhk
aPa-441943	MDC007459.193 Chr 8	hkxhk
aPa-186589	MDC025914.43 Chr 2	hkxhk
aPa-461421	MDC003149.360 Chr 17	hkxhk
aPa-186714	MDC025340.14 Chr 12	hkxhk
aPa-443239	MDC005670.636 Chr 7	hkxhk
aPa-184374	MDC011427.201 Chr 10	hkxhk
aPa-554538	-	hkxhk

UNIVERSITY of the
WESTERN CAPE

aPa-184705	MDC013248.183 Chr 3	hkxhk
aPa-460837	MDC025914.43 Chr 2	hkxhk
aPa-525938	MDC001803.467 Chr 16	hkxhk
aPa-526970	MDC001803.467 Chr 16	hkxhk
aPa-184459	MDC019670.396 Chr 12	hkxhk
aPa-441878	MDC019670.396 Chr 12	hkxhk
aPa-461303	MDC021900.23 Chr 2	hkxhk
aPa-443058	MDC005670.636 Chr 7	hkxhk
aPa-519579	MDC013496.652 Chr 1	hkxhk
aPa-526621	MDC010811.313 Chr 15	hkxhk
aPa-442048	MDC027595.86 Chr 4	hkxhk
aPa-185048	MDC013496.652 Chr 1	hkxhk
aPa-184775	MDC025914.43 Chr 2	hkxhk
aPa-184985	MDC007837.354 Chr 8	hkxhk
aPa-461201	MDC022098.101 Chr 9	hkxhk
aPa-186982	MDC025914.43 Chr 2	hkxhk
aPa-184400	MDC025914.43 Chr 2	hkxhk
aPa-442466	MDC025914.43 Chr 2	hkxhk
aPa-183332	MDC006356.308 Chr 4	hkxhk
aPa-525922	MDC025914.43 Chr 2	hkxhk
aPa-442439	MDC021104.80 Chr 7	hkxhk
aPa-525967	MDC003136.610 Chr 6	hkxhk
aPa-186520	MDC008313.318 Chr 12	hkxhk
aPa-182442	MDC025914.43 Chr 2	hkxhk
aPa-460912	MDC019670.396 Chr 12	hkxhk
aPa-185849	MDC006356.308 Chr 4	hkxhk
aPa-185763	MDC000971.392 Chr 7	hkxhk
aPa-441960	MDC025914.43 Chr 2	hkxhk
aPa-443395	MDC009934.335 Chr 3	hkxhk
aPa-186460	MDC014055.191 Chr 7	hkxhk
aPa-518793	MDC022837.459 unanchored:49663511..49677147	hkxhk
aPa-461667	MDC013496.652 Chr 1	hkxhk
aPa-183911	MDC021912.332 Chr 10	hkxhk
aPa-461854	MDC002966.257 Chr 7	hkxhk
aPa-186360	MDC000971.392 Chr 7	hkxhk
aPa-184213	MDC010200.469 Chr 10	hkxhk
aPa-525882	MDC008313.318 Chr 12	hkxhk
aPa-519013	MDC010200.469 Chr 10	hkxhk
aPa-442388	MDC008339.624 Chr 14	hkxhk

UNIVERSITY of the
WESTERN CAPE

aPa-443167	MDC013978.597 Chr 14	hkxhk
aPa-526919	MDC021449.408 Chr 2	hkxhk
aPa-183916	MDC006692.265 Chr 12	hkxhk
aPa-526821	MDC013496.652 Chr 1	hkxhk
aPa-519175	MDC000971.392 Chr 7	hkxhk
aPa-442060	MDC002039.611 Chr 7	hkxhk
aPa-184089	MDC022173.275 Chr 4	nxnp
aPa-186400	MDC002135.739 Chr 9	nxnp
aPa-518989	MDC020870.303 unanchored:5069772..5091374	nxnp
aPa-461324	MDC005111.360 Chr 9	nxnp
aPa-518573	MDC020265.285 Chr 2	nxnp
aPa-518622	MDC006499.378 Chr 12	hkxhk
aPa-184379	MDC006459.179 Chr 7	hkxhk
aPa-442134	MDC011202.326 Chr 12	hkxhk
aPa-519166	MDC021104.80 Chr 7	hkxhk
aPa-525495	MDC018018.175 unanchored:9692396..9697877	hkxhk
aPa-184854	MDC016102.191 Chr 15	hkxhk
aPa-184079	MDC017659.305 Chr 6	hkxhk
aPa-185094	MDC020106.51 Chr 16	hkxhk
aPa-186312	MDC020106.51 Chr 16	hkxhk
aPa-186442	MDC005848.465 Chr 14	hkxhk
aPa-183169	MDC020106.51 Chr 16	hkxhk
aPa-183997	MDC018796.138 Chr 10	hkxhk
aPa-441863	MDC020106.51 Chr 16	hkxhk
aPa-526766	MDC020106.51 Chr 16	hkxhk
aPa-184029	MDC005848.465 Chr 14	hkxhk
aPa-186963	MDC008862.367 unanchored:21098768..21108804	hkxhk
aPa-525959	MDC005848.465 Chr 14	hkxhk
aPa-526169	MDC020106.51 Chr 16	hkxhk
aPa-185507	MDC014014.341 Chr 2	hkxhk
aPa-460920	MDC014014.341 Chr 2	hkxhk
aPa-184057	MDC018796.138 Chr 10	hkxhk
aPa-185104	MDC020106.51 Chr 16	hkxhk
aPa-518947	MDC014014.341 Chr 2	hkxhk
aPa-186541	MDC018796.138 Chr 10	hkxhk
aPa-460728	MDC005848.465 Chr 14	hkxhk
aPa-442735	MDC014014.341 Chr 2	hkxhk
aPa-186485	MDC008862.367 unanchored:21098768..21108804	hkxhk
aPa-460693	MDC014014.341 Chr 2	hkxhk

aPa-184197	MDC011288.353 Chr 3	hkxhk
aPa-185021	MDC018796.138 Chr 10	hkxhk
aPa-461632	MDC005848.465 Chr 14	hkxhk
aPa-185170	MDC034539.9 Chr 8	hkxhk
aPa-461224	MDC022837.459 unanchored:49663511..49677147	hkxhk
aPa-183951	MDC020558.334 Chr 10	hkxhk
aPa-183247	MDC018796.138 Chr 10	hkxhk
aPa-186512	MDC012842.348 Chr 12	hkxhk
aPa-518798	MDC022837.459 unanchored:49663511..49677147	hkxhk
aPa-185843	MDC015823.454 Chr 9	hkxhk
aPa-526973	MDC034539.9 Chr 8	hkxhk
aPa-185529	MDC008339.624 Chr 14	hkxhk
aPa-519342	MDC012842.348 Chr 12	hkxhk
aPa-442552	MDC018796.138 Chr 10	hkxhk
aPa-185193	MDC034539.9 Chr 8	hkxhk
aPa-186040	MDC020558.334 Chr 10	hkxhk
aPa-185917	MDC034539.9 Chr 8	hkxhk
aPa-186786	MDC018796.138 Chr 10	hkxhk
aPa-461293	MDC018796.138 Chr 10	hkxhk
aPa-519280	MDC016958.365 Chr 10	hkxhk
aPa-182556	MDC000948.270 Chr 16	hkxhk
aPa-526577	MDC000948.270 Chr 16	hkxhk
aPa-184790	MDC029176.7 Chr 12	hkxhk
aPa-518697	MDC010811.313 Chr 15	hkxhk
aPa-442822	MDC025782.26 Chr 6	hkxhk
aPa-519053	MDC001006.638 Chr 4	hkxhk
aPa-184449	MDC025782.26 Chr 6	hkxhk
aPa-518676	MDC029176.7 Chr 12	hkxhk
aPa-186341	MDC021900.23 Chr 2	hkxhk
aPa-552794	MDC029176.7 Chr 12	hkxhk
aPa-441745	MDC015823.454 Chr 9	hkxhk
aPa-185353	MDC021900.23 Chr 2	hkxhk
aPa-461102	-	hkxhk
aPa-184740	MDC027763.27 Chr 14	hkxhk
aPa-186154	MDC006295.205 unanchored:2027695..2031070	hkxhk
aPa-186998	MDC004599.215 Chr 1	hkxhk
aPa-526070	MDC019559.200 Chr 7	hkxhk
aPa-525497	MDC004599.215 Chr 1	hkxhk
aPa-183728	MDC005033.573 unanchored:103161777..103164608	hkxhk

aPa-185319	MDC003804.268 Chr 6	hkxhk
aPa-185639	MDC017212.105 Chr 8	hkxhk
aPa-519186	MDC007837.354 Chr 8	hkxhk
aPa-461074	MDC006675.304 Chr 1	hkxhk
aPa-552929	MDC024669.31 Chr 13	hkxhk
aPa-443070	MDC013034.68 Chr 4	hkxhk
aPa-442281	MDC013327.284 Chr 13	hkxhk
aPa-553633	-	hkxhk
aPa-553206	-	hkxhk
aPa-443264	MDC002706.286 Chr 13	hkxhk
aPa-519337	MDC005909.464 Chr 3	hkxhk
aPa-184693	MDC022184.226 Chr 3	hkxhk
aPa-519428	MDC013034.68 Chr 4	hkxhk
aPa-526050	MDC013978.597 Chr 14	hkxhk
aPa-526087	MDC021467.174 Chr 4	hkxhk
aPa-183710	MDC026241.22 Chr 3	hkxhk
aPa-184686	MDC013034.68 Chr 4	hkxhk
aPa-443171	MDC020076.247 Chr 12	hkxhk
aPa-442056	MDC007283.1041 Chr 13	hkxhk
aPa-553859	-	hkxhk
aPa-441858	MDC003149.360 Chr 17	hkxhk
aPa-525524	MDC003149.360 Chr 17	hkxhk
aPa-184122	MDC003026.402 Chr 16	hkxhk
aPa-184641	MDC007459.193 Chr 8	hkxhk
aPa-519681	MDC014014.341 Chr 2	hkxhk
aPa-186008	MDC002966.257 Chr 7	hkxhk
aPa-461017	MDC002966.257 Chr 7	hkxhk
aPa-442812	MDC005848.465 Chr 14	hkxhk
aPa-184981	MDC021912.332 Chr 10	hkxhk
aPa-442911	MDC022837.459 unanchored:49663511..49677147	hkxhk
aPa-442368	MDC017464.166 Chr 9	hkxhk
aPa-461698	MDC021104.80 Chr 7	hkxhk
aPa-442251	MDC005055.925 Chr 9	hkxhk
aPa-526320	MDC001006.638 Chr 4	hkxhk
aPa-442697	MDC002039.611 Chr 7	hkxhk
aPa-527002	MDC002039.611 Chr 7	hkxhk
aPa-552964	-	hkxhk
aPa-461977	MDC022482.297 Chr 17	hkxhk
aPa-525713	MDC013034.68 Chr 4	hkxhk

aPa-553956	-	hkxhk
aPa-555037	-	hkxhk
aPa-526019	MDC021460.100 Chr 11	hkxhk

UNIVERSITY of the
WESTERN CAPE