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Utilization Of Microsatellite Markers For A Comparative Assessment Of Norton And Cynthiana, And The Linkage Map Construction Of A 'Chambourcin' X 'Cabernet Sauvignon' Population

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UTILIZATION OF MICROSATELLITE MARKERS FOR A COMPARATIVE ASSESSMENT OF NORTON AND CYNTHIANA, AND THE LINKAGE MAP CONSTRUCTION OF A 'CHAMBOURCIN' X 'CABERNET SAUVIGNON' POPULATION

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By

Mia Mann

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ABSTRACT

The first part of this study utilized microsatellites to comparatively assess the cultivars Norton and Cynthiana. Although isozyme and simple sequence repeat (SSR) marker analyses in 1993 and 2009 provided preliminary evidence that Norton and Cynthiana grapes are genetically identical, only five banding patterns and four microsatellite loci were reported. Microsatellites (n=185) spanning 19 linkage groups were used to compare the cultivars for a genome-wide analysis. Capillary electrophoresis results revealed Norton and Cynthiana to be identical at 98.6% of alleles. In the second part of this study, an interspecific hybrid population was generated by crossing *V.* interspecific hybrid 'Chambourcin' and *V. vinifera* 'Cabernet Sauvignon'. The ultimate goal of performing this cross is to create a cultivar with the cold hardiness of 'Chambourcin' combined with the superior wine quality of *V. vinifera* 'Cabernet Sauvignon'. Cross-population (CP) maps were generated using the statistical software JoinMap 4.1 by genotyping 90 F_1 progenies using microsatellites. Map sizes ranged from 999.3 cM to 1821.9 cM and contained a maximum of 276 SSR markers.

KEYWORDS: Norton, Cynthiana, Chambourcin, Cabernet Sauvignon, microsatellite markers, hybrids, comparative assessment, linkage map, JoinMap

This abstract is approved as to form and content

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INTRODUCTION

Grapevines have become a popular staple in agriculture due to their major product, wine. There are many different uses for grape including juice making, raisins, and table grapes but wine is by far the most popular (Mullins et al. 1992). It has been estimated that grapevines originated approximately 65 million years ago (This et al. 2006). Early records show that the cultivation of grapevines did not begin until 7,000-8,000 years ago (Mullins et al. 1992; Terral et al. 2010). The first grapevines were cultivated in the South Caucasus (Myles et al. 2011) and viticulture and enology had spread across Europe by the first century (Mullins et al. 1992). Although wine is more of a luxury crop than a staple one, it has been extremely popular for thousands of years and made many appearances in literature, including both the new and old testaments of the bible (Mullins et al. 1992). Several mythological gods have also been dedicated to wine, such as Dionysus, Osiris, and Bacchus (Mullins et al. 1992; This et al. 2006).

Grapevines belong to the *Vitaceae* family, meaning they are characterized by having tendrils and flower clusters located across from leaves (Mullins et al. 1992). The majority of grape products marketed today belong to the *Vitis* genus (This et al. 2006). Within this genus, there are more than 80 species, the most popular of which is *Vitis vinifera* (This et al. 2006). Economically, grapevines are an important woody perennial. In the United States, there were over one million reported acres of vineyard production in 2014, as reported by the USDA. There were approximately 4.5 million tons of wines produced in the United States in 2014 averaging $767 per ton (USDA 2015). This totals to nearly $3.5 billion, making wine a very large economic contributor.

Even though the structure of DNA was just discovered in 1954, 60 years later researchers are under pressure to utilize the knowledge of DNA to increase crop production and tolerance to climatic factors (Neidle 2008; Tuberosa et al. 2008). This is made especially difficult by the changing environmental trends caused by climate change (Tuberosa et al. 2008).

Plant Breeding

Evidence has indicated that the cultivation of plants began approximately 10,000 years ago when humans would select mutated plants which were easier to harvest (Sleper and Poehlman 2006). This plant domestication seems to have occurred in many different places around the same time period and it is unknown whether the seeds from the mutated, higher quality crops were planted for the purpose of domestication or not (Bennett 2010). Following the initial cultivation, improvement of domesticated crops progressed slowly and crop improvement efforts did not begin until around the 18th century when the Age of Enlightenment led to a curiosity about crop improvement which humans began to act upon (Bennett 2010).

Traditional plant breeding involves the improvement of plant lines and future generations for economic improvement (Scaboo et al. 2010). Early civilization humans performed plant breeding by intentionally or unintentionally selecting seeds which were mutated, making them easier to collect. In this case, plants and seeds were selected for their ability to benefit humans, rather than the economy (Murphy 2007; Scaboo et al. 2010). This type of plant breeding has also led to a decrease in genetic diversity. The development of hybrid populations is a plant breeding method which can be used to

increase genetic diversity while still providing the same benefits (Morgante and Salamini 2003).

Plant breeding is essential because a need exists to improve crop outputs and the quality of the yields produced while using fewer inputs (Tester and Langridge 2010; Henry and Nevo 2014). It is important to increase yield and quality in optimal *and* stressed conditions because the environment is changing and crops need to change with it in order to prevent an increase in inputs needed (Tester and Langridge 2010). Breeding for disease resistance in crops is also extremely important for reducing input.

Breeding must be a constantly evolving tool for crop improvement because it has to change as agriculture and organisms evolve. Breeders also need to be able to adapt to the changes in consumer demands (Collard and Mackill 2008). Breeding for improved crops will help producers adapt to a changing environment and the ever-growing population (Collard and Mackill 2008).

Molecular Breeding

Plant breeding as a scientific measure did not truly begin until after Mendel's work became known (Scaboo et al. 2010). The speed at which molecular breeding has evolved has been very rapid, with much progress being made in the past few decades (Somerville and Somerville 1999; Wijerathna et al. 2015). For instance, in the 1990s, researchers were just beginning to sequence the genome of model organisms such as *Arabidposis* and it was believed that genome sequencing would not be widespread within a decade because of high cost (Somerville and Somerville 1999). Today, genome sequencing of new plants is extremely common and a wide variety of plant genomes have

been sequenced, including grape (Jaillon et al. 2007). Crops such as tomato (Foolad 2007) and rice (Wijerathna et al. 2015) have played an integral role in the improvement of plant and molecular breeding because they are model organisms and economic staples. It is important to identify and genetically combat disease and climate stressors in all crops. Use of molecular markers is one of the best ways to ensure that this will happen (Collard and Mackill 2008).

Marker-assisted selection (MAS) has revolutionized the breeding process. MAS is the process of mapping markers to a plant's genome and identifying which markers are linked to the trait, or quantitative trait loci (QTL), of interest using statistical software (Tester and Langridge 2010). MAS can be utilized to breed plants resistant to multiple biotic and abiotic stressors (Miklas et al. 2006). For instance, it is helpful for selecting for salt tolerant crops in areas where irrigation is needed to compensate for drought in order to prevent damage to crops from the salt left in the soil (Ashraf and Foolad 2012). In grapes, Dr. Walker of UC Davis has also successfully implemented MAS to breed grapevines resistant to nematodes and Pierce's disease (Lund 2015).

MAS can also be utilized for gene pyramiding—a technique where markers are mapped to multiple genes controlling a trait of interest and used to 'pyramid' resistance genes on top of one another to combat the disease (Tester and Langridge 2010). MAS has been useful for gene pyramiding in important cereal crops such as wheat. This technique has been employed to prevent disease resistance in the crop, specifically to control rust diseases (Randhawa et al. 2013). Not only is it time and cost efficient compared to traditional breeding methods, but it is even more effective at producing crops with ideal traits (Randhawa et al. 2013).

For MAS to work, population sizes need to be sufficiently large. Furthermore, a high quality set of markers are needed for success (Collard and Mackill 2008). Many factors go into the selection of which marker to use including the complexity of analysis, frequency of differences (or polymorphisms), and cost of implementation (Staub et al. 1996). Of these factors, simple sequence repeat (SSR) markers align very well with the specified requirements (Collard and Mackill 2008). Once the population is established and the marker type selected, there are a series of needs which need to be met for MAS to be effective. Markers closely linked to the gene need to be identified and then confirmed through plant growth and phenotyping. The process also needs to be time and cost efficient enough to be worthwhile (Randhawa et al. 2013).

Molecular Markers

Molecular markers are frequently utilized in many disciplines of research ranging from animals to plants (Dekkers and Van Der Werf 2007; Walker et al. 2010). Molecular markers have been used frequently in staple crops like soybeans and corn, but are progressively being utilized in specialty crops like grapevine (Cipriani et al. 2011). In grapes, markers can be used to distinguish within and among cultivars and to assess genetic relationships (Bautista et al. 2008).

There are a multitude of different molecular markers that have been used for genetic mapping and MAS, beginning around the 1970s (Cipriani et al. 2011). Before DNA-based markers became available, biochemical markers such as isozymes were utilized. Isozymes separate based on mutations that result in a change in the charge of an

amino acid and can be visualized through separation using gel electrophoresis (Staub et al. 1996).

DNA-based markers include restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs), and microsatellites (Schlötterer 2004). RFLPs allow for a visualization of polymorphisms since they result from single nucleotide mutations that alter the cleavage sites for restriction endonucleases. This causes polymorphisms, or differences, that result in different banding patterns and can be visualized using hybridization probes and Southern blots (Kumar 1999). RAPDs use a combination of short primer sequences of around 10 base pairs and polymerase chain reactions (PCR) to amplify DNA fragments. Gel electrophoresis can then be used to evaluate the fragment differences. However, the results gathered from RAPDs may be difficult to interpret because the short primer sequences have low specificity to DNA sequences (Walker et al. 2010). AFLPs are similar to RFLPs in that variations are seen in fragment banding patterns caused by mutations. AFLPs and RAPDs are advantageous over RFLPs because fragments are amplified using PCR—a much faster and cheaper method than the Southern blot process. Despite their efficiency, AFLPs and RAPDs are typically dominant markers, meaning it is difficult to identify heterozygous individuals (Walker et al. 2010; Mueller and Wolfenbarger 1999). SNPs are markers that display differences at a single nucleotide location (Vignal et al. 2002). They can be generated using next-generation sequencing techniques which can produce thousands of SNPs in a mapping population (Cipriani et al. 2011; Barba et al. 2013).

Microsatellites

Microsatellites, or SSRs, are very valuable in molecular breeding because of their PCR-derived, polymorphic, and co-dominant nature (Merdinoglu et al. 2005). SSR markers are often used in *V. vinifera* genetic analyses (Blondon et al. 2004) but have become increasingly used in other grapevine species due to their high interspecies transferability (Doligez et al. 2006; Li et al. 2013). They have been implemented for rootstock identification (Lin and Walker 1998), survey of germplasms (Giannetto et al. 2010), comparison of cultivars (Lefort and Roubelakis-Angelakis 2001), and breeding for resistance (Riaz et al. 2009). In addition, several SSR-based linkage maps have been developed that have allowed for the identification of quantitative trait loci (QTLs) controlling agronomic traits and can be used for MAS to improve the efficiency of grape breeding (Doucleff et al. 2004; Riaz et al 2009).

Native North American Grapevines

Very few native North American species can be seen in commercial production today but they are frequently seen as the rootstocks on many *V. vinifera* vines to protect from fungal disease outbreaks due to their high level of resistance. Breeding of interspecific hybrids has also been used to confer resistance upon more popular cultivated varieties. Of the many *Vitis* species growing throughout the world, the majority of them are native to North America (Aradhya et al. 2003). Grapevines which are native to North America include *V. arizonica, V. aestivalis, V. cinerea, V. labrusca, V. riparia, V. rupestris,* and *Muscadinia rotundifolia* (Stafne et al. 2015). *Vitis aestivalis* has become

one of few native *Vitis* species utilized in the grape industry today for its resistance characteristics (This et al. 2006; Stafne et al. 2015).

Of the many native species used for hybrid breeding, *V. aestivalis* has the most potential for warding off environmental stresses such as disease and cold temperatures (Wagner 1996). Although other native species such as *V. labrusca, V. riparia,* and *V. rotundifolia* display these qualities individually, *V. aestivalis* is the only vine which displays both characteristics (Wagner 1996).

Origin of Norton and Cynthiana

'Norton is a *V. aestivalis*-derived cultivar with ambiguous origins. Norton is believed to have been developed by Dr. Daniel Norborne Norton. Based on early records and correspondences, Dr. Norton developed the cultivar (originally known as 'Norton's Virginia Seedling') in one of his Virginian vineyards (Ambers and Ambers 2004). The cultivar is believed to be the result of a cross between Bland and a native *V. aestivalis* vine performed unintentionally by D.N. Norton (Ambers 2013). In a letter, Dr. Norton described the development of the 'Norton' cultivar through emasculation of 'Bland' and pollination with 'Pinot Meunier'. However, 'Norton' bears a very strong resemblance to *V. aestivalis* so it is believed that the 'Bland' clusters were pollinated when the flowers were not yet receptive to pollen and bags were likely not applied to protect the clusters from interfering factors. Therefore, *V. aestivalis* pollen likely traveled to the emasculated 'Bland' clusters through wind or insects and pollinated the flowers during a time when they were receptive to pollen. However, 'Bland' is no longer in existence so if it is

involved in the parentage of 'Norton', it would be nearly impossible to verify this speculation (Ambers 2013).

Similarly to Norton, Cynthiana grape cultivars are also described to be largely derived from *V. aestivalis* (Parker et al. 2005, 2009; Stover et al. 2009). Cynthiana was reportedly sent to the Prince of Flushing in New York by someone who discovered it in the woods of Arkansas (Hendrick 1908). The cultivar was then conveyed to Hermann, Missouri to be grown in the vineyards there (Hendrick 1908).

However, as previously stated, the precise origin of the two cultivars can only be hypothesized. Norton and Cynthiana vines are very popular in Missouri and Arkansas, respectively. Early records report that Norton was introduced in Missouri vineyards in the late 1840s while Cynthiana was introduced in the late 1850s (Husmann 1883; Hendrick 1908). Since this time, it has been speculated that Norton and Cynthiana are actually the same cultivar (Hendrick 1908).

Norton Characteristics

'Norton' produces a dry, red wine and displays fungal resistance and winter hardiness characteristics (Reisch et al. 1993; Ali et al. 2011). Due to its ability to withstand these environmental conditions, Norton has become increasingly popular in the Midwestern United States. Since its discovery in Virginia, 'Norton' was quickly established in vineyards west of the state and is commonly found in Midwest states such as Missouri and Arkansas (Hussmann 1883). Norton has become so popular in Missouri that Missouri is the leading producer of this cultivar (Robinson et al. 2012). Out of 500 total acres of Norton planted in the United States, the majority is constituted by vineyards

in Missouri with 300 acres planted in the state (Ambers 2013). In 2014, it was reported that Missouri had a total of 1,700 acres bearing grapes. Thus, Norton's production makes up approximately 18% of Missouri's total grape acreage (USDA 2015).

Similarities and Differences of Norton and Cynthiana

Many phenotypical similarities have been noted between Norton and Cynthiana. The two cultivars display similar cluster, berry, and peduncle sizes (Main and Morris 2004). They also display resistance to many different fungal diseases, such as powdery and downy mildew, and a variety of berry rots which can severely damage vineyards across the world (Harris 2012). Another likeness is the difficulty of rooting ability from dormant hardwood cuttings (Galet 1998; Keeley et al. 2003), and a high sensitivity to sulfur spray (unpublished data). The vines are cold hardy, withstanding a temperature range as low as -32°C, and require a long growing season (~125 days) to fully ripen (Dami et al. 2005). The two cultivars produce a dry, red wine with a high titratable acidity (8.5 to 13 g/L) which may be attributed to the high amount of malic acid present within the fruit (Main and Morris 2004).

Some phenotypical differences, however, exist between Norton and Cynthiana. For instance, differences in the ideal soil type have been noted. Though they both thrive in sandy soils, Cynthiana favors a loam soil better than Norton and Norton favors clay and gravelly soils better than Cynthiana (Hendrick 1908; Harris 2012). Differences between the fruit and wine quality of Norton and Cynthiana have also been identified (Hendrick 1908). It has been stated that, if grown beside one another in the vineyard, enough differences can be seen to discredit the cultivars being the same and that

Cynthiana is the superior cultivar (Hendrick 1908). Some researchers have accepted Norton and Cynthiana as the same cultivar (Morris and Main 2010), but many growers and wine-makers still assert that distinctions exist in their respective viticultural performance and enological quality (Hendrick 1908).

Comparison of Norton and Cynthiana

Reisch et al. (1993) provided preliminary evidence that Norton and Cynthiana are genetically indistinguishable using isozyme analysis. This study evaluated five biochemical markers across seven samples of Norton, two samples of Cynthiana, and one sample of Melody which was used as a control. The results from this study revealed similar banding patterns for Norton and Cynthiana but the use of only five banding patterns provides a low resolution view of the genome.

Similarly, in a study by Parker et al. (2009), four microsatellite loci were used to identify Norton and Cynthiana as genetically synonymous cultivars. However, this is an extremely low number of microsatellites and testing a larger number of microsatellites in order to carry out a genome-wide assessment may help to better confirm or refute conclusions made from isozyme analyses.

French-American Hybrids

French-American hybrids are developed from crosses between native American and *V. vinifera* grapes. Most of these hybrids were developed by breeders in France as a method of combating fungal diseases such as phylloxera without sacrificing the wine quality (Wagner 1996; Pollefeys and Bousquet 2003). French-American hybrid breeders

developed the hybrids using traditional methods, meaning it took many years to get a final product (Wagner 1996; Reynolds 2015). Native American rootstocks could be grafted onto *V. vinifera* to protect against phylloxera but this method did not protect against fungal diseases such as downy and powdery mildew. French growers also planted American varieties as an attempt to avoid pest and disease problems but the wine produced from these vines were far too inferior and low quality to continue production (Wagner 1996). Thus, French-American hybrids were developed to provide natural protection from these diseases without sacrificing the wine quality. (Reynolds 2015). The development of successful French-American hybrids saved growers a great deal of money spent to combat the biotic and abiotic stressors present in France (Wagner 1996).

Although developed in France and bred for European conditions, the native grape contributions to French-American hybrid grapes makes them suitable for growth in both France and North America (Wagner 1996). As a result, they have been planted more frequently in the United States. The cold hardy characteristics they carry along with the high quality wine produced makes them an overall suitable wine for the Eastern and Midwest United States (Wagner 1996; Pollefeys and Bousquet 2003). Despite their beneficial qualities, very little molecular profiling has been done with them (Pollefeys and Bousquet 2003).

Chambourcin

Chambourcin is a French-American hybrid which was developed by Johannès Sevye in France and became available on the market in 1963 (Galet 1979; Scheef 1991). According to the Vitis International Variety Catalogue (VIVC), the parents of

Chambourcin are Seyve Villard 12-417 and Chancellor (Maul and Eibach 2003). It is a hybrid with good wine qualities and is becoming very popular in Missouri vineyards (Scheef 1991). It also displays moderate cold hardiness, withstanding temperatures as low as -20° F, and has a long growing season (Dami et al. 2005; Homich et al. 2016). The cold hardiness of Chambourcin is often impacted by early frost and freezing events which fall during the vine's long growing season before acclimation can occur (Zhang and Dami 2012). However, Chambourcin is more tolerant of disease and cold temperatures than *V. vinifera* and cluster thinning can be implemented for optimal productivity and prevention of winter injuries (Zhang and Dami 2012; Reynolds 2015).

The pedigree of Chambourcin is extremely complex because many generations of crosses were often made before a final French-American hybrid was complete (Reynolds 2015). According to the VIVC, Chambourcin's pedigree goes back up to eight generations on the mother's side and seven generations on the father's side (Maul and Eibach 2003). The pedigree of Chambourcin includes contributions from *V. vinifera*, *V. rupestris*, *V. labrusca, V. riparia, V. labruscana, V. aestivalis,* and *V. cinerea* (Maul and Eibach 2003).

Vitis vinifera 'Cabernet Sauvignon'

Vitis vinifera is a popular European grape which can be utilized for eating and drinking (Riaz et al. 2004). The species has been the largest contributor to the improvement of grapevines (Olmo 1995). Although *V. vinifera* is the only grapevine originating from Europe, there are over 10,000 *V. vinifera* cultivars present today (Mullins et al. 1992; Olmo 1995; Aradhya et al. 2003). It was cultivated from the wild

European grape *V. vinifera* L. ssp. *sylvestris* (Zohary 1995). However, very few wild *vinifera* vines are still in existence, as the majority have been cultivated in some way (Olmo 1995; Zohary 1995).

Cabernet Sauvignon is a *V. vinifera* cultivar developed by crossing Cabernet Franc and Sauvignon Blanc (Myles et al. 2011). The parentage of the cultivar was identified in 1996 at UC Davis (Bowers and Meredith 1997). It is also a half-sibling to Merlot, who shares Cabernet Franc as a parent (Boursiquot et al. 2009). Cabernet Sauvignon produces an acidic, red wine which is high in tannins (Robinson et al. 2012). The vine originated in the Bordeaux region of France but has spread across the world (Kolpan et al. 1996). Out of 10,000 *V. vinifera* cultivars, Cabernet Sauvignon is one of the most popular globally (Mullins et al. 1992; Riaz et al. 2004). As of 2010, there were over 77,000 acres of Cabernet Sauvignon planted in California alone, making it the most popular red wine variety in the state (Robinson et al. 2012). Like most *V. vinifera* cultivars, Cabernet Sauvignon displays low disease resistance and is susceptible to cold temperatures (Reisch et al. 1993).

Grapevine Breeding

Grape molecular breeding is important because grapes are woody perennials and require a great deal of time and money to grow out (Lodhi et al. 1995). As a result, researchers have been working to understand the grape genome since the 1990s (Lodhi et al. 1995) The first grape linkage map was published in 1995 using isozyme, RFLP, and RAPD markers (Lodhi et al. 1995) and MAS efforts in grapevine were initiated by Dalbó et al. (2001).

The development of a hybrid grape population involves a series of steps. The first step is the emasculation of the female grape clusters. Emasculation is performed by removing the male portion of the grape flowers without harming the female portions. Paper bags are then used to cover the emasculated clusters to prevent accidental pollination from occurring (Eibach and Töpfer 2015). Pollen must then be collected from the intended male parent and dried. The dried pollen is used to pollinate the emasculated clusters. For optimal yield, pollen should be applied when the stigma is secreting fluid and the clusters recovered with bags (Eibach and Töpfer 2015). Once berries have reached veraison, seeds can be extracted. The seeds are placed into a container of water and those which float to the top are discarded because this indicates poor embryo development. A cold stratification period of approximately 2.5 months at 4°C is used to provide the seeds with a dormant period (Eibach and Töpfer 2015).

Linkage Mapping

A linkage map is essentially a "road map" of the genome which is generated, or mapped, using molecular markers (Paterson 1996). Linkage mapping is established on the basis that genes are aligned along chromosomes and crossing-over, or recombination, may occur between them (Azhaguvel et al. 2008). Linkage between genes is determined by evaluating the frequency of recombination in order to estimate their positions relative to one another on the chromosome (Sanders and Bowman 2012). The first linkage map was constructed by Alfred Sturtevant in 1911 using *Drosophila melanogaster* (Sanders and Bowman 2012). In the first linkage maps published by Sturtevant and Morgan, the map distance was equal to recombination frequency (Liu 1998). In linkage maps today,

mapping functions are utilized to convert recombination frequencies to distances for mapping (Reyes-Valdés 2003). Since the development of the first linkage map, two primary mapping functions have emerged: Haldane's and Kosambi's. Haldane's mapping function differs from Morgan's because it takes double crossovers between loci into account (Ott 1991). Kosambi's mapping function differs from both because it takes double crossovers and interference into account (Ott 1991). Interference can happen when the occurrence of a crossover event affects the probability of other crossover events occurring on the chromosome (Huehn 2011).

For genetic mapping to occur a sufficient population size must be obtained and informative markers must be available (Young 1994; Isobe and Tabata 2010). The informative markers are then screened across the population and a mapping software is used to generate a genetic map for each parent which can then be integrated into one map (Abbott 2008). Multiple mapping software programs are in existence today which can be used for map development (Kang 2003). JoinMap is a popular mapping software which was developed in order to integrate linkage maps (Stam 1993; Isobe and Tabata 2010). JoinMap has two mapping algorithms the user can choose from which are 1) the regression mapping algorithm and 2) the Monte Carlo maximum likelihood algorithm. The regression algorithm is useful to construct maps with less than 50 markers on each linkage group since it works by adding markers one at a time based on how informative they are. This can cause the program to run slowly if too many markers are being screened per linkage group (Van Ooijen 2006). JoinMap provides the option of using either Haldane's or Kosambi's mapping functions when using the regression algorithm (Van Ooijen 2006). The Monte Carlo algorithm is ideal for mapping if over 50 markers

are present on a linkage group. However, any errors or missing genotype data can cause issues if the map distance is too small (Van Ooijen 2006).

To date, many linkage maps have been constructed for grape interspecific hybrid populations (Grando et al. 2003; Doucleff et al. 2004; Lowe and Walker 2006; Moreira et al. 2011). A pseudo-test cross approach must be used for grape linkage mapping because grapes are highly heterozygous (Costantini et al. 2009). The linkage maps produced from grape populations have been useful for identifying QTLs for a variety of traits including downy mildew (Blasi et al. 2011; Moreira et al. 2011), powdery mildew (Hoffman et al. 2008; Riaz et al. 2011), seedlessness (Doligez et al. 2002; Mejía et al. 2007), and berry weight (Fischer et al. 2004; Cabezas et al. 2006).

Study Overview

The first study utilized SSR markers to compare Norton and Cynthiana at each of their 19 chromosomes to determine if they are genetically identical cultivars. Prior to this study, a genetic map was constructed of a *V. aestivalis*-derived 'Norton' and *V. vinifera* 'Cabernet Sauvignon' population by testing 600 SSR markers—359 of which were informative markers that are polymorphic for Norton in 19 chromosomes. A total of 185 markers, about 10 markers from each linkage group, were randomly selected and screened using capillary electrophoresis, and the resulting banding patterns were compared between Norton and Cynthiana.

For the second study, a *V.* interspecific hybrid 'Chambourcin' x *V. vinifera* 'Cabernet Sauvignon' population was developed in May 2013. The seeds produced from this cross were harvested fall 2013. Following germination, DNA was extracted from

seedling leaf tissue and capillary electrophoresis was used to identify true-hybrids. The crosses made typically result in some self-fertilized seedlings so true-hybrid testing is often necessary. Out of 215 seedlings tested, 150 were determined to be interspecific hybrids. Once the true hybrids were identified, 1,205 SSR markers were tested for polymorphism on six confirmed hybrid progeny and the two parents. Three hundred sixty markers were determined to be polymorphic and were subsequently screened across the first 94 progeny. The fragment data was genotyped and a linkage map was constructed using JoinMap 4.1.

MATERIALS AND METHODS

To compare Norton and Cynthiana, a total of 185 polymorphic markers—seven to ten from each of the 19 linkage groups in the Norton map—were randomly selected and screened across 8 total leaf samples. DNA was isolated from the leaf samples using a Qiagen kit and DNA fragments were amplified using PCR. The fragments produced from 185 primers were analyzed using capillary electrophoresis to determine fragment lengths. To improve the speed and cost efficiency of SSR genotyping, seven to twelve fluorescent-labeled microsatellite loci, depending on their size range, were multiplexed and evaluated simultaneously during capillary electrophoresis.

In order to construct a linkage map for a Chambourcin x Cabernet Sauvignon population, the population was developed at the Missouri State Fruit Experiment Station (MSFES) and true hybrids were identified using capillary electrophoresis following DNA extraction. A set of 1,205 SSR markers were tested for polymorphisms using six confirmed true hybrid progeny and the parents. Polymorphic markers were screened across the hybrid population and utilized for linkage map construction in JoinMap 4.1.

Plant Materials

Four Norton samples were obtained from Missouri Vineyards, three Cynthiana samples were obtained from Arkansas vineyards and one Cabernet Sauvignon sample was obtained from a vineyard in Missouri (Table 1). The original cutting source of the St. James 'Norton' leaf sample is Double A Vineyards in Fredonia, New York. The cutting

source of the ‘Norton’ sample from Missouri State Fruit Experiment Station (MSFES) is a block planted at Stone Hill Vineyard in Hermann, Missouri in the 1860s.

In May 2013, a cross was made between Chambourcin and Cabernet Sauvignon following the emasculation and pollination protocol described by Adhikari et al. (2014). Seeds were collected from the clusters during harvest and placed into a container of water. Any seeds which floated to the top were removed and discarded. The remaining seeds were cold stratified for three months at 4°C. Germination was performed as outlined in Adhikari et al. (2014).

DNA was extracted from plant leaf materials following the extraction protocol described by Adhikari et al. (2014). Liquid nitrogen was used to grind approximately 100 mg of grape leaf tissue until it became a fine powder. A DNeasy Plant Mini Kit (Qiagen, Valencia, CA) was used to isolate DNA following the protocol provided by Qiagen. DNA concentrations were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). DNA was diluted to 15 ng/µL and stored at 4°C when not in use.

PCR Amplification and Fragment Analysis

Microsatellite marker alleles were amplified using PCR following the protocol described by Adhikari et al. (2014). The total volume of the PCR reaction was 10 µL, consisting of:

- 2 µL of 15 ng of template DNA
- 1.8 µL of a primer mix containing 0.1 µM of forward and 2 µM of reverse primer
- 1 µL 2 µM WellRed M13 primer

- 0.2 µL 25 mM $MgCl_2$
- 5 µL AmpliTaq GoldR 360 Master Mix buffer (Life Technologies, Grand Island, NY)

The following touchdown PCR method was used to amplify the DNA:

- Initial denaturation: 10 min at 95° C,
- 10 touchdown cycles of:
    - Denaturation: 94° C for 30 sec
    - Annealing: Initial temperature of 62° C for 30 sec, decreasing by 1° C in each consecutive cycle
    - Extension: 72° C for 1 min where annealing temperature was decreased by 1° C at each cycle
- 24 cycles of:
    - Denaturation: 94° C for 30 sec
    - Annealing: 56° C for 30 sec
    - Extension: 72° C for 1 min
- Final extension: 72°C for 7 min.

Four µL of the resulting PCR products were loaded onto a 1.5% agarose gel to confirm the success of the reactions and evaluate the amount of PCR required for capillary electrophoresis (Bio-Rad, Hercules, CA).

A GenomeLab GeXP genetic analysis system, otherwise known as capillary electrophoresis (Beckman Coulter, Brea, CA), was used to determine allele sizes. The system uses a GenomeLab GeXP Genetic Analysis software, Fragment Analysis Module, to evaluate fragment sizes. Fragment lengths were analyzed and interpreted for all SSR markers utilized for the comparative assessment and linkage map construction. A control DNA size standard 400 ladder and Sample Loading Solution was combined with PCR

products prior to capillary electrophoresis. A multiplex capillary electrophoresis program was implemented to evaluate seven to twelve PCR products simultaneously.

Chambourcin x Cabernet Sauvignon Population Analysis

Following germination, seedlings were tested using fragment analysis to determine if they were F_1 interspecific hybrids. Leaf samples were collected from each seedling to be used for DNA isolation. PCR was performed on the extracted DNA using five different SSR markers (FAM15, FAM35, VrZAG62, VVS2, FAM75, and FAM115). Gel electrophoresis was then implemented in order to verify the presence of PCR product and to assess sample quantities to be used for capillary electrophoresis. The verified interspecific hybrids were then transferred into larger pots and eventually transferred to the vineyard. DNA from interspecific hybrids was stored at -20°C for later use in population analysis.

Prior to testing microsatellites for polymorphisms, a preliminary test was run to determine the presence or absence of a band by running PCR using the two parents. Gel electrophoresis was used to evaluate PCR products for band presence. SSR markers which displayed bands for both Chambourcin and Cabernet Sauvignon were tested for polymorphism using six of the confirmed interspecific hybrid progeny and the two parents. The confirmed polymorphic markers were utilized for population analysis on the first 90 Chambourcin x Cabernet Sauvignon hybrid progeny and the two parents. Capillary electrophoresis was used for allelic size determination during true hybrid identification, polymorphic marker testing and population analysis.

Linkage Map Construction

Microsatellite results from population analysis were genocoded following the JoinMap segregation codes for a cross pollinated (CP) population (<abxcd>, <abxac>, <abxab>, <abxaa>, <aaxab>). Genotyped results were transferred from MS-Excel to JoinMap 4.1 (Van Ooijen 2006) for mapping. Three hundred eighteen loci were evaluated across 90 individuals in the population. Loci genotype frequencies were sorted by amounts of missing data and those with a substantial amount missing were excluded from map construction. Markers were also evaluated for similarity and markers with a similarity greater than 0.97 were also excluded.

The 'recombination frequency' grouping parameter was used for map construction and confirmed through re-evaluation using the 'independence LOD parameter'. The recombination frequency threshold range began at 0.250 and ended at 0.050, decreasing stepwise by 0.05. Both the regression mapping algorithm and the maximum likelihood (ML) mapping algorithm were used to generate parental and consensus maps. Kosambi's mapping function was used with the regression mapping algorithm. Parental nodes were constructed for regression mapping using the "Create Maternal and Paternal Population Nodes" function in JoinMap. Parental maps were automatically constructed when using the ML algorithm. Chromosomes were assigned to linkage groups based on ESTs present in the linkage groups. A reference framework of *Vitis* was used to identify chromosome numbers for linkage groups which did not contain ESTs (Doligez et al. 2006). MapChart (Voorips 2002) was utilized to export all maps.

RESULTS

Norton and Cynthiana

Of the 185 markers (740 alleles) evaluated, Norton and Cynthiana fragment lengths were revealed to be identical for nearly all markers (Table 2). Ten alleles (1.4%) showed differences between Norton and Cynthiana. Differences in fragment sizes never exceeded one base pair. A comparison of the fragment peak patterns between Norton and Cynthiana revealed many similarities and few differences. Slight differences in peak height may have been caused by differences in sample disbursement. A further comparison of Norton and Cynthiana peak patterns to Cabernet Sauvignon peak patterns revealed significant differences in Cabernet Sauvignon (Fig. 1-3).

Chambourcin x Cabernet Sauvignon

Out of 215 Chambourcin x Cabernet Sauvignon seedlings tested, 150 were revealed to be true hybrids following fragment analysis. The results from the preliminary tests to determine band presence of microsatellite markers (n=1,205) using gel electrophoresis are as follows:

- Both parents—952
- Chambourcin only—20
- Cabernet Sauvignon only—24
- No band—209

Six hybrid progeny and the two parents were used to screen 952 markers for polymorphisms using capillary electrophoresis. Three hundred sixty-three polymorphic

markers were identified and deemed suitable for use in population analysis. Following population analysis, 318 of these polymorphic markers produced ratios suitable for linkage evaluation in JoinMap. The following totals (n=318) were recorded for CP marker segregation types and utilized for mapping in JoinMap:

- <abxcd>—85
- <abxac>—73
- <abxab>—17
- <abxaa>—91
- <aaxab>—52

Using the regression mapping algorithm, 276 markers were mapped in the consensus map, 214 in the map for Chambourcin, and 194 in the Cabernet Sauvignon map. These maps spanned 1160.0 cM, 999.3 cM, and 1076.5 cM, respectively. The parental maps were aligned along either side of the consensus map (Fig. 4). Markers not mapped were either ungrouped or excluded due to similarity or high amounts of missing data. The linkage group covering the largest distance in the consensus map was linkage group 18 and spanned 96.2 cM (Fig 4; Table 3). Linkage groups 9 and 7 were the largest in Chambourcin and Cabernet Sauvignon, respectively (Fig 4; Table 4, 5). The average gap in the consensus map was 4.20 cM. In the Chambourcin and Cabernet Sauvignon maps, the average gaps were 4.67 cM and 5.55 cM, respectively (Table 3-5). Twenty-three markers were excluded from the Chambourcin map, 21 from the Cabernet Sauvignon map, and 17 from the consensus map due to high similarity or distortion determined using Chi-square analysis (p=0.01).

Two hundred sixty-nine markers were mapped in the consensus, 226 markers in the Chambourcin, and 201 markers in the Cabernet Sauvignon maps produced using the maximum likelihood mapping algorithm. The maps covered a genetic distance of 1821.9 cM, 1774 cM, and 1643.4 cM, respectively. Both parental maps were able to be aligned with the consensus map (Fig. 5). Linkage group 14 spanned the largest distance in both the consensus map and the Cabernet Sauvignon map (Fig. 5; Table 6, 7). Linkage group 10 was the longest group in the map for Chambourcin. The average gaps in the consensus, Chambourcin, and Cabernet Sauvignon maps were 6.77 cM, 7.85 cM, and 8.18 cM, respectively (Table 6-8). Twenty-four markers were excluded from ML mapping due to high similarity or distortion determined using Chi-square analysis (p=0.01).

DISCUSSION

Comparative Assessment of Norton and Cynthiana

The use of 185 SSR markers spanning 19 linkage groups in this study proved to be a reasonable approach for the genetic analysis between Norton and Cynthiana. All of the PCR products were successfully amplified and the use of multiplex capillary electrophoresis allowed for a quick and efficient investigation of the microsatellite loci within two genomes. First testing the six standard markers on each sample provided an initial idea of the DNA quality, as well as the expected final results.

The fragment sizes and peak patterns for Norton and Cynthiana revealed undeniable similarities and very minute differences. Conversely, the data revealed significant differences between Norton/Cynthiana and Cabernet Sauvignon. Since the data collected did not show significant differences between the Norton and Cynthiana cultivars, this is solid evidence that the two cultivars are genetically identical within these 185 loci. The slight differences in fragment length observed between Norton and Cynthiana were likely the result of computational errors from the capillary array. Variation in fragment length for the same primer was never so markedly different between Norton and Cynthiana that it could be labeled significant. Quite often variations in base pair values were the result of rounding, though the fragment lengths may not have differed by more than a tenth of a base pair. Furthermore, it is not uncommon for clones to display some genetic variations. Clonal evaluations of Cabernet Sauvignon using SSR markers have revealed some fragment differences between the clones despite being the same cultivar (Moncada et al. 2006). It has also been suggested that differences seen in

clones may be caused by transposable elements in somatic cells (Carrier et al. 2012). Although Norton and Cynthiana were similar to one another, polymorphisms could be seen in Cabernet Sauvignon. These results support the initial hypothesis that Norton and Cynthiana are the same cultivar.

The identical results between Norton and Cynthiana were largely expected due to the results of isozyme analysis. The isozyme analysis data were identical at all five banding patterns tested, leading researchers to believe Norton and Cynthiana were indistinguishable (Reisch et al. 1993). Due to the advancement of technology and identification of hundreds of microsatellites, the results provided by isozyme analysis represent a low resolution comparison of the two genomes. A more detailed investigation of the genomes would leave less room for uncertainty. This comparative assessment using microsatellites provided an effective method for analyzing the genomes of the two cultivars by utilizing capillary electrophoresis. Capillary electrophoresis has proven to be a reliable method for DNA sequencing and sample identification (Huang et al. 1992). The high resolution results produced by capillary electrophoresis provided a more accurate and reliable conclusion than simply using isozyme or gel electrophoresis banding patterns.

Although many growers and wine makers have asserted that Norton and Cynthiana are different, the most recent documentation of these differences dates back to 1908 (Hendrick 1908). This source states:

> "The botanical differences between the two varieties are not greater than might be attributed to environment, soil, climate, and culture; but side by side the two grapes ripen at different times, and the quality of the fruit, and more particularly of the wine is such that the varieties must be considered as distinct. The distinction should be maintained for Cynthiana is the better of the two."

Furthermore, a publication by Husmann, the owner of the Missouri vineyard where Norton and Cynthiana were first planted side by side, stated in 1883:

> "[Cynthiana] resembled the Norton so much in growth and foliage, that I supposed it to be identical with it, until it bore fruit, and more especially when I made wine from it, when the difference became very apparent. This seeming identity has prevented dissemination…but the bunch is generally heavier, with broader shoulders, the berry somewhat larger, sweeter, and less astringent, and the wine is not quite as dark, less rough and astringent…"

In defense of these statements, a variety of hypotheses can be formed to explain the phenotypic differences between the two cultivars. For instance, the soil texture and/or quality could have differed between the locations where Norton and Cynthiana were grown within the same vineyard. Soil texture can affect water retention and thus water availability to the grapevine (Van Leeuwen et al. 2009). Water availability is important in grapevines because it can directly affect the amount of sugar and water held within the berry—factors which are key in wine-making (Tramontini et al. 2012). Soil quality can influence berry harvest and can affect phenolic components such as anthocyanin (de Andrés-de Prado et al. 2007). Anthocyanin differences could explain the color differences noted between Norton and Cynthiana wine by Husmann (1883) (Sacchi et al. 2005). Potassium levels in the soil also have the ability to affect pH in grapes and wine (Jackson and Lombard 1993).

Another hypothesis to explain the differences between the two cultivars is that the Norton and Cynthiana wines being compared were different ages. Norton wine is said to peak between 8 and 10 years after bottling (Pollack 2011). If the bottling dates differed, this could have resulted in differences in taste when comparing the two. Wines bottled in different years could also have variations caused by differing climate/environmental factors between the two years. Summers which are warmer than normal can result in a

lighter colored wine (Main and Morris 2007). Seasons with higher or lower than normal rainfall can also affect the wine produced as it has the ability to affect almost every aspect of berry quality including phenolics, degrees Brix, titratable acidity and pH (Jackson and Lombard 1993).

As stated above, there are a variety of factors and management techniques which could alter berry and wine quality. It is difficult to determine the factors which contributed to the differences noted between Norton and Cynthiana over 100 years ago. The main solution to this problem would be to perform a controlled evaluation comparing the two side by side while ensuring that the soil quality and texture are the same in all locations and that any wines used to compare the vines were produced in the same year.

The origin of Norton and Cynthiana is largely unknown, although it has been suggested that Norton originated prior to Cynthiana (Husmann 1883). A study on the origin of Norton presumed it to be the older of the two cultivars and thus the original cultivar if they are the same (Ambers and Ambers 2004). Despite hypothesized origins of the two cultivars, growers in Missouri are more prone to call the cultivar 'Norton' while Arkansas growers are likely to use the term 'Cynthiana' for what is now thought to be the same cultivar. These reasons may cause complications to arise when determining what the identical cultivars should be called. It is unlikely that either 'Norton' or 'Cynthiana' will be used universally when deciding how to name and market the products of these two cultivars. Consequently, if these results are taken into consideration, then the terms 'Norton' and 'Cynthiana' should be accepted as the same and used interchangeably.

‘Chambourcin’ x ‘Cabernet Sauvignon’ Linkage Map Construction

Linkage maps have become very useful in genomics research because they assist in and allow for QTL mapping, marker-assisted selection (MAS), and cultivar comparisons such as the one mentioned previously. The cross made between *V.* interspecific hybrid ‘Chambourcin’ and *V. vinifera* ‘Cabernet Sauvignon’ allowed for the production of the first genetic map developed from Chambourcin. Chambourcin is a French-American hybrid which was originally developed in France but has recently developed popularity in the Midwestern United States due to its wine quality and moderate disease resistance.

Prior to this study, very little work had been carried out to investigate the genetic information available in Chambourcin. The consensus maps produced here covered 1,160 cM and 1,821.9 cM, depending on the algorithm used, and utilized a maximum of 276 out of 318 total SSR markers. Fewer SSR markers were included in the parental maps than the consensus maps regardless of the algorithm used (Table 3-8). This could be explained by the exclusion of <abxab> markers for both maps, <aaxab> markers for Chambourcin, and <abxaa> markers for Cabernet Sauvignon during map construction. Markers with these segregation types were not used for parental map construction due to an inability to garner linkage information from these marker types when evaluating the parents individually. Grapevine chromosome numbers were determined for the linkage groups using EST markers which have known locations. Linkage groups within the consensus maps which did not contain any EST markers utilized the reference map for *Vitis* to determine chromosome numbers (Doligez et al 2006).

A comparison of the maps produced using the regression and ML algorithms revealed both similarities and differences. Marker order was conserved in many chromosomes although some differences were seen in chromosomes 1, 2, 8, 13, 15, and 16 (Fig. 6). Linkage groups in the maps produced using the ML algorithm revealed genetic distances which were larger than those seen in the maps produced from regression mapping (Table 9; Fig. 6). These differences were likely caused by the sensitivity the ML algorithm displays to errors within the dataset, which can cause linkage group distances to increase (Hackett and Broadfoot 2003; Cheema and Dicks 2009). The larger distances in the ML mapping also resulted in a larger average gap than what is seen in the regression map.

Another large difference between regression mapping and ML mapping was that the ML map showed better linkage group coverage on chromosome 18 in Chambourcin than the corresponding linkage group in the regression map (Fig. 4, 5). In the map produced from regression mapping, an extra linkage group was required to provide equivalent coverage and thus two separate linkage groups were needed for Chambourcin to align with the two ends of chromosome 18 in the consensus map (Fig. 4). This was likely because the linkage groups covered opposite ends of the chromosome and, as a result, the regression mapping algorithm was unable to identify substantial linkage between the two groups to incorporate them into one linkage group. However, the ML algorithm was able to identify linkage between the two ends of chromosome 18 and thus displayed better alignment with chromosome 18 in the consensus map (Fig. 5).

The map constructed using the ML algorithm contained 7 fewer SSRs than the regression map (Table 9). This was likely due to the removal of more distorted markers in

the ML map. Prior to the construction of the final consensus maps, the maps were evaluated with and without distorted markers (p=0.01) included. The separate maps were then compared to evaluate how the removal of distorted markers affected the marker order in the maps. Distorted markers which did not affect marker order were included in the final map. The map produced using the ML algorithm was highly sensitive to distorted markers than the regression map. As a result, the ML map had to be produced without distorted markers because of the large affect they had on marker order in the map.

The consensus map obtained using the regression algorithm was also used for comparison with a framework linkage map constructed for *Vitis* (Doligez et al. 2006). The comparison of these maps revealed consistencies between the consensus map and the reference framework map. However, the consensus covered only 0.86 of the distance that the framework map covered between the outermost markers shared between the maps (Table 10). The consensus map also covered a shorter total distance than the reference map, although it contained a higher total number of markers (Table 11; Doligez et al. 2006).

Despite containing more markers than the reference map, other linkage maps have been produced using *Vitis* which contain larger numbers of SSRs than the map produced here (Welter et al. 2007; Vezzulli et al. 2008). Since Chambourcin contains a substantial amount of *V. vinifera* within its pedigree, this could have affected the number of polymorphic SSR markers available for a population developed by crossing Chambourcin with another *V. vinifera* cultivar. The low marker numbers in the map also could have been caused by an insubstantial number of progeny. A larger population is always better

when linkage mapping because it provides more opportunities for recombination to occur and for the mapping program to identify linkage. The population used for map construction contained only 90 progeny. The plates used for capillary electrophoresis will hold 96 total samples, which limits the number of progeny that can be tested at one time. Ideally, 94 progeny and the two parents would have been screened using the identified polymorphic markers. However, four plants died while population analysis was being conducted and only 90 total samples had a complete data set. Fortunately, these 90 progeny represent only one part of the Chambourcin x Cabernet Sauvignon population and there are more progeny left to be tested. Additionally, this population is also being expanded to approximately 300 true hybrids. Once the remaining population is tested, the linkage map will likely improve because there will be more opportunities for recombination.

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Table 1. Location and age of leaf sample collections

Cultivar	Vineyard	Vineyard Location	Year planted
Norton	St. James	St. James, MO	1986
Norton	Les Bourgeois	Rocheport, MO	Late 1980s
Norton	McMurtrey	Mountain Grove, MO	1984
Norton*	Missouri State Fruit Experiment Station	Mountain Grove, MO	2011
Cynthiana	Post	Altus, AR	~1890
Cynthiana	Leding	Altus, AR	~1920
Cynthiana	University of Arkansas Fruit Research Station	Clarksville, AR	~1980
Cabernet Sauvignon	Missouri State Fruit Experiment Station	Mountain Grove, MO	2008

Table 2. Genome-wide comparison of 185 loci in Norton and Cynthiana

Chr.	Primer	Norton	Cynthiana	Cab. Sauv.
1	FAM79	146/148	146/148	146/156
	VMCNG2g7	91/112	91/112	110/110
	UDV-055	163/165	163/165	167/167
	VMC9D3	198/200	198/200	179/206
	VMC8D1	198/221	198/221	217/221
	VVIO61	228/231	228/231	234/234
	VVIF52	260/265	260/265	257/257
	VVIS21	276/290	276/290	282/282
	VMC9F2	289/313	289/313	214/289
	VVIP60	310/332	310/332	307/315
2	FAM24	266/270	266/270	270/277
	FAM140	256/265	256/265	251/266
	VMC3B10	86/121	86/121	123/127
	VMC7g3	119/149	119/149	119/136
	UDV-109	131/142	131/142	131/154
	VMC6F1	140/142	140/142	134/140
	VMC5G7	191/213	191/213	198/200
	VRIP93	199/208	199/208	197/199
	VVMD34	240/244	240/244	240/248
	VVIU20	366/384	365/383	366/388
3	FAM102	147/160	147/160	150/150
	FAM138	207/212	207/212	204/204
	FAM030	278/283	278/283	285/285
	VVIN54	100/106	100/106	100/100
	VMC3F3	127/135	127/135	131/137
	UDV-093	147/164	147/164	163/168
	VMC1G7	243/260	243/260	254/264
	VVMD36	278/291	278/291	253/262
	CF1608	281/293	281/293	284/284
	ctg0171	297/303	297/303	297/320
4	FAM46	143/148	143/148	143/143
	FAM126	202/222	202/222	192/201
	FAM02	203/226	203/226	193/203
	FAM38	224/232	224/232	226/230
	VMC2E10	53/55	53/55	57/59
	VMC7h3	131/143	131/143	135/161
	VVIP37	117/129	117/129	149/154
	VVIP77	180/186	180/186	186/191
	CTG6983	254/277	254/277	244/254
	VVIR46	377/381	377/381	379/385

Table 2 continued

Chr.	Primer	Norton	Cynthiana	Cab. Sauv.
5	FAM10	100/134	100/134	103/131
	FAM72	166/176	166/176	174/174
	FAM12	368/377	368/377	355/364
	VVC71	83/99	83/99	96/96
	VMC3B9	100/101	100/101	90/105
	ctg6305	158/163	158/163	161/175
	VRIP89	159/186	159/186	159/159
	PSCtg199_2	177/223	177/223	185/208
	ssrVrZAG79	250/254	250/254	246/246
	VVIN33	283/285	283/285	283/291
6	FAM110	287/311	287/311	287/287
	FAM78	296/306	296/306	306/306
	FAM40	315/318	315/318	316/326
	VVIM43	88/94	88/94	85/101
	VMC2F10	95/114	95/114	95/105
	VVC07	98/119	98/119	98/98
	VMC4G6	124/140	124/140	124/130
	UDV-085	128/152	128/152	133/138
	VMCNg1h11	238/261	238/261	238/238
	VVIP28	246/252	246/252	248/261
7	FAM13	191/196	191/196	197/197
	FAM115	315/336	315/336	327/330
	VVIV36	156/171	156/171	155/161
	VMC16F3	178/185	178/185	176/187
	ssrVrZAG62	181/205	181/204	189/195
	VVCS1H059O18F1-1	191/194	191/194	189/194
	Vamu111-CS	194/196	194/196	196/196
	Psctg45_2	203/211	203/211	202/204
	VVMD06	212/215	212/215	212/212
	VVMD7	236/246	236/246	239/239
8	FAM59	166/169	166/169	190/190
	FAM113	246/263	246/263	256/263
	FAM55	270/273	270/273	278/278
	FAM16	326/329	326/329	329/329
	FAM76	395/399	395/399	395/395
	UDV-125	112/116	112/116	97/138
	VMC1b11	175/177	175/177	185/185
	VMC5h2	195/212	195/212	195/195
	VMC2F12	193/232	193/232	212/249
	VMC1e8	225/229	225/229	224/224

Table 2 continued

Chr.	Primer	Norton	Cynthiana	Cab. Sauv.
9	FAM35	139/140	139/140	153/164
	EST2B07	246/252	246/252	246/246
	VMC6d12	147/162	147/162	181/183
	FAM42	370/398	370/398	380/395
	VMC2E11	86/94	86/94	94/98
	VMC3G8	160/164	160/164	162/172
	CD009354	183/185	183/185	183/195
	SC8_0141_028	219/229	219/229	223/223
	VVIU37	228/230	228/230	237/237
	VVIO52	375/377	375/377	384/384
10	FAM148	392/395	392/395	398/398
	VMC2E8	64/69	64/69	67/77
	VMCZAG67	136/138	136/138	123/136
	VRZAG67	142/144	142/144	129/142
	VRIP64	149/160	149/160	139/158
	VVIV37	147/163	147/163	166/166
	VmcSsrVrZAG025	239/242	239/242	228/239
	ctg5592	213/223	213/223	219/224
	VRIP25	239/242	239/242	228/239
	VVIH01	246/247	246/247	245/260
11	EST8c01b	151/154	151/154	156/171
	FAM149	342/345	342/345	345/351
	FAM07*	348/360	347/360	356/356
	FAM73	376/389	376/389	364/385
	VVS2	135/137	135/137	141/154
	ctg0393	195/197	195/197	195/199
	VVCS1H091D05F1-1*	259/271	258/270	270/270
	CF6881	280/293	280/293	268/288
	SC8_0118_063	307/320	307/320	300/303
	ctg3410	316/337	316/337	338/338
12	FAM71	181/184	181/184	180/182
	VVCS1H084C16R1-1	94/98	94/98	98/101
	VVCS1H078D22R1-1	97/112	97/112	97/97
	UDV-120	155/166	155/166	136/152
	VMC8g6	144/152	144/152	162/166
	VMCNG1G4	171/183	171/183	174/180
	ctg3230	193/198	193/198	202/215
	VMC2H4	203/214	203/214	214/221
	CTG0863	227/240	227/240	226/261
	C004	310/315	310/315	315/315

Table 2 continued

Chr.	Primer	Norton	Cynthiana	Cab. Sauv.
13	EST10h11	154/162	154/162	156/156
	SCE_0071_014	144/173	144/173	177/181
	VVIC51	175/177	175/177	166/186
	PSCTG231_2	179/187	179/187	193/193
	NS01	183/188	183/188	183/183
	VMC3D12	214/216	214/216	205/225
	SC8_0053_001	234/241	234/241	226/234
	VMCNG4el0.1	247/259	247/259	248/252
	VMC9H4	271/283	271/283	272/276
	CTG7356	279/298	279/298	279/279
14	FAM90	371/377	371/377	375/375
	VMC2H12	112/116	112/116	95/95
	VVCh14-9	101/104	101/104	106/106
	VMCNG1G1.1	192/200	192/200	175/229
	VVCh14-18	180/184	180/184	178/178
	VVC34	184/187	184/187	201/207
	VVC62	184/188	184/188	184/204
	VRIP112	235/240	235/240	224/229
	VVIN94*	273/280	273/279	294/294
	FAM44	110/116	110/116	116/116
15	FAM105	271/277	271/277	277/279
	VVIM42-2	87/89	87/89	85/85
	ctg4274	283/293	283/293	277/279
	VMC5G8	297/299	297/299	313/321
	SC8_0040_088	360/371	360/371	334/334
	VVIQ61	364/366	364/366	362/368
	VVIV67	335/337	335/337	369/377
16	FAM36	357/375	357/375	371/381
	UDV-009	143/158	143/158	141/177
	UDV-052	150/182	150/182	160/184
	VMC1E11	203/205	203/205	195/199
	GB007D01	214/222	214/222	205/205
	CTG7620	227/230	227/230	226/226
	VVMD5	233/247	233/247	231/240
	ctg7933	242/251	242/251	242/246
	ctg9366	266/269	266/269	263/266
	ctg2141	358/367	358/367	361/361

Table 2 continued

Chr.	Primer	Norton	Cynthiana	Cab. Sauv.
17	VMC2H3	77/79	77/79	81/87
	VVCS1H068D03F1-1	83/106	83/106	87/98
	VMC3C11.1	93/104	93/104	96/108
	CTG8270	141/149	141/149	157/157
	VMC9G4	153/160	153/160	170/172
	ctg9346	141/149	141/149	157/158
	ctg6954	229/235	229/235	222/230
	ctg5672	228/236	228/236	244/244
	ctg6344	268/276	268/276	271/279
	AF3283	249/254	249/254	265/267
18	FAM132	137/140	137/140	136/136
	FAM100	171/182	171/182	191/193
	FAM75	161/177	161/177	174/174
	FAM06	288/316	288/316	298/298
	VMC2B1	97/107	97/107	107/107
	UDV-130	125/133	125/133	138/153
	VVCS1H066N21R1-1*	156/160	156/159	159/163
	VVIU04	170/173	170/173	170/170
	UDV737	294/309	294/309	290/300
	B004	389/397	389/397	394/394
19	FAM15	119/124	119/124	109/115
	UDV029	77/91	77/91	85/97
	VMC3b7.2	97/128	97/128	103/103
	UDV023	205/224	205/224	195/201
	VMC5e9	201/208	201/208	195/218
	PSCTG196_2	284/309	284/309	289/297
	VVIN04	360/363	360/363	367/367
	VVIV33	351/373	351/373	344/356

Table 3. Summary of consensus map size and marker distribution constructed using the regression algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	52.8	21	2.51
2	68.1	13	5.24
3	15.5	7	2.21
4	90.0	23	3.91
5	65.3	19	3.44
6	45.2	10	4.52
7	84.4	14	6.03
8	73.1	11	6.65
9	67.6	12	5.63
10	93.8	20	4.69
11	59.6	12	4.97
12	44.6	12	3.72
13	31.0	9	3.44
14	69.3	29	2.39
15	48.9	6	8.15
16	36.8	11	3.35
17	71.8	17	4.22
18	96.2	18	5.34
19	46.0	12	3.83
Total	**1160.0**	**276**	**4.20**

Table 4. Summary of the Chambourcin (P1) map size and marker distribution constructed using the regression algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	39.1	18	2.17
2	66.4	8	8.30
3	13.5	5	2.70
4	70.8	16	4.43
5	64.5	12	5.38
6	43.9	9	4.88
7	23.8	5	4.76
8	73.9	11	6.72
9	82.5	12	6.88
10	77.0	17	4.53
11	61.1	11	5.55
12	39.1	9	4.34
13	31.9	9	3.54
14	64.5	21	3.07
15	47.4	6	7.90
16	28.6	10	2.86
17	61.9	12	5.16
18	57.0	13	4.38
19	52.4	10	5.24
Total	**999.3**	**214**	**4.67**

Table 5. Summary of the Cabernet Sauvignon map size and marker distribution constructed using the regression algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	83.7	13	6.44
2	57.3	9	6.37
3	12.4	4	3.10
4	80.2	16	5.01
5	70.7	17	4.16
6	45.5	7	6.50
7	103.1	13	7.93
8	23.8	5	4.76
9	70.0	14	5.00
10	76.2	15	5.08
11	46.0	8	5.75
12	54.0	10	5.40
13	40.3	6	6.72
14	73.1	14	5.22
15	32.8	5	6.56
16	40.3	10	4.03
17	67.2	12	5.60
18	97.7	12	8.14
19	2.2	4	0.55
Total	**1076.5**	**194**	**5.55**

Table 6. Summary of consensus map size and marker distribution using the Maximum Likelihood algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	127.5	21	6.07
2	68.9	12	5.74
3	19.0	7	2.71
4	143.8	22	6.54
5	115.1	18	6.39
6	61.5	10	6.15
7	146.0	14	10.43
8	97.1	11	8.83
9	110.9	13	8.53
10	156.0	19	8.21
11	72.8	12	6.07
12	63.5	12	5.29
13	34.0	9	3.78
14	196.0	28	7.00
15	51.7	5	10.34
16	48.0	11	4.36
17	98.9	17	5.82
18	150.4	18	8.36
19	60.8	10	6.08
Total	**1821.9**	**269**	**6.77**

Table 7. Summary of the Cabernet Sauvignon (P2) map size and marker distribution using the Maximum Likelihood algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	90.5	11	8.23
2	67.6	11	6.15
3	13.2	5	2.64
4	136.8	16	8.55
5	139.6	17	8.21
6	50.5	8	6.31
7	131.9	12	11.0
8	93.3	8	11.7
9	113.2	11	10.3
10	105.2	15	7.01
11	44.9	9	4.99
12	61.1	11	5.55
13	17.8	5	3.56
14	250.5	17	14.7
15	27.7	4	6.93
16	46.3	11	4.21
17	86.0	12	7.17
18	118.5	12	9.88
19	48.8	6	8.13
Total	**1643.4**	**201**	**8.18**

Table 8. Summary of the Chambourcin (P1) map size and marker distribution using the Maximum Likelihood algorithm

Linkage group	Length (cM)	Number of SSRs	Average gap (cM)
1	119.4	20	5.97
2	65.0	7	9.29
3	14.1	5	2.82
4	150.8	20	7.54
5	90.6	13	6.97
6	63.6	9	7.07
7	134.8	9	15.0
8	97.3	11	8.85
9	97.4	11	8.85
10	196.0	17	11.5
11	77.1	11	7.01
12	61.2	9	6.8
13	36.7	9	4.08
14	134.5	23	5.85
15	47.6	5	9.52
16	48.7	10	4.87
17	88.3	12	7.36
18	178.1	14	12.7
19	72.8	11	6.62
Total	**1774**	**226**	**7.85**

Table 9. Summary of the linkage group comparison using different mapping algorithms

	Regression		Maximum Likelihood	
Linkage Group	Total distance	Number of SSRs	Total distance	Number of SSRs
1	52.8	21	127.5	21
2	68.1	13	68.9	12
3	15.5	7	19.0	7
4	90.0	23	143.8	22
5	65.3	19	115.1	18
6	45.2	10	61.5	10
7	84.4	14	146.0	14
8	73.1	11	97.1	11
9	67.6	12	110.9	13
10	93.8	20	156.0	19
11	59.6	12	72.8	12
12	44.6	12	63.5	12
13	31.0	9	34.0	9
14	69.3	29	196.0	28
15	48.9	6	51.7	5
16	36.8	11	48.0	11
17	71.8	17	98.9	17
18	96.2	18	150.4	18
19	46.0	12	60.8	10
Total	**1160.0**	**276**	**1821.9**	**269**

Table 10. Comparison of the Chambourcin x Cabernet Sauvignon regression consensus map and a *Vitis* reference map using distances between common markers in the maps

Chromosome	First common marker	Last common marker	*Vitis* reference map distance (cM)	Consensus map distance (cM)	Ratio
1	VVIF52	VRZAG29	55.7	35.2	0.63
2	VVMD34	VMC8C2	50.9	58.8	1.16
3	UDV043	VVIB59	22.9	6.7	0.29
4	VMCNG1F1.1	VRZAG83	73.5	89.5	1.22
5	VVMD27	VMC4C6	65.5	50.6	0.77
6	VMC2H9	VVIM43	51.8	41.0	0.79
7	UDV011	VVIV04	83.3	72.4	0.87
8	VMC6G8	VVIB66	57.4	58.1	1.01
9	VMC3G8	VVIQ52	49.1	39.3	0.80
10	VVIH01	UDV063	65.0	59.0	0.91
11	VMCNG2H1	VVIB19	34.5	34.2	0.99
12	VMC4H9	VMC8G9	17.7	16.7	0.94
13	VVIC51	VMC9H4	20.3	11.3	0.56
14	VMC1E12	VVIN70	70.9	66.9	0.94
15	VVIP33	VMC4D9	21.2	15.6	0.74
16	VMC1E11	VVMD5	42.8	25	0.58
17	VMC3C11.1	VVIB09	43.5	41.8	0.96
18	VMC2A3	VMC7F2	98.6	73.8	0.75
19	VMC3B7	UDV127	27.8	24.6	0.88
Total			**952.4**	**820.5**	**0.86**

Table 11. Comparison of the Chambourcin x Cabernet Sauvignon regression consensus map and the *Vitis* reference map using total linkage group distances

Linkage Group	*Vitis* reference map distance (cM)	Consensus map distance (cM)	Ratio
1	83.6	52.8	0.63
2	78.0	68.1	0.87
3	59.2	15.5	0.26
4	82.3	90.0	1.1
5	65.5	65.3	0.97
6	69.1	45.2	0.65
7	99.7	84.4	0.85
8	90.6	73.1	0.81
9	90.3	67.6	0.75
10	80.1	93.8	1.2
11	72.5	59.6	0.82
12	75.9	44.6	0.59
13	83.5	31.0	0.37
14	92.3	69.3	0.75
15	32.6	48.9	1.5
16	76.9	36.8	0.48
17	53.7	71.8	1.3
18	127.0	96.2	0.76
19	72.3	46.0	0.64
Total	**1485.1**	**1160**	**0.78**

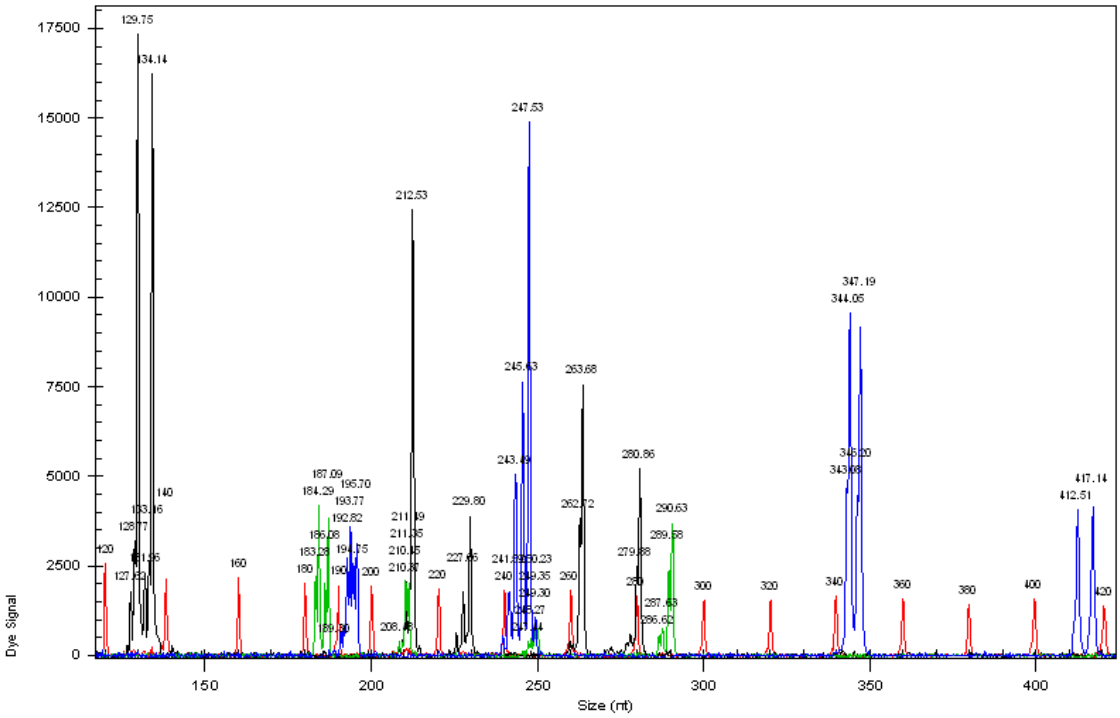


Fig. 1. Norton capillary electrophoresis chromatogram from Linkage Group 8

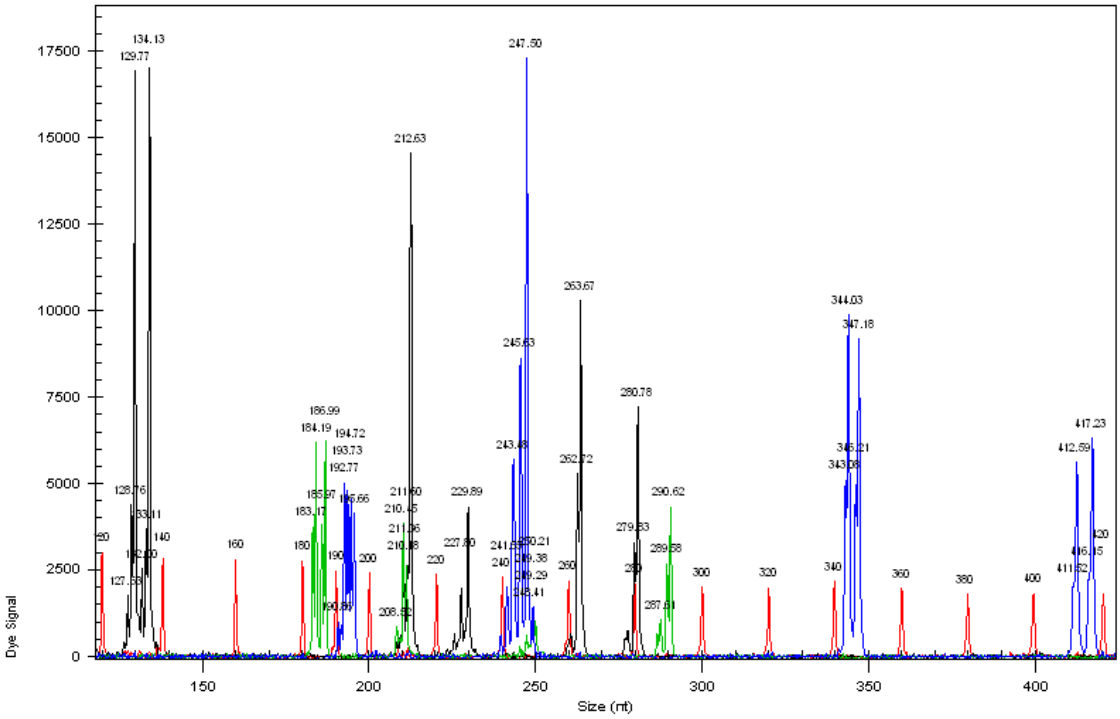


Fig. 2. Cynthiana capillary electrophoresis chromatogram from Linkage Group 8

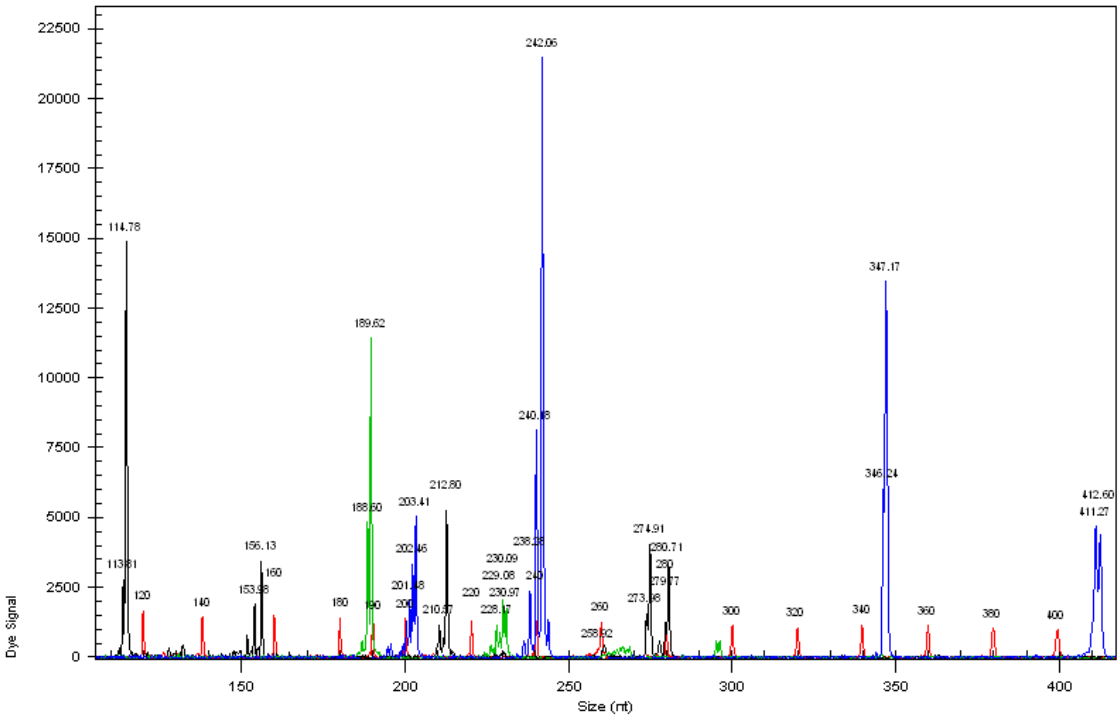


Fig. 3. Cabernet Sauvignon capillary electrophoresis chromatogram from Linkage Group 8

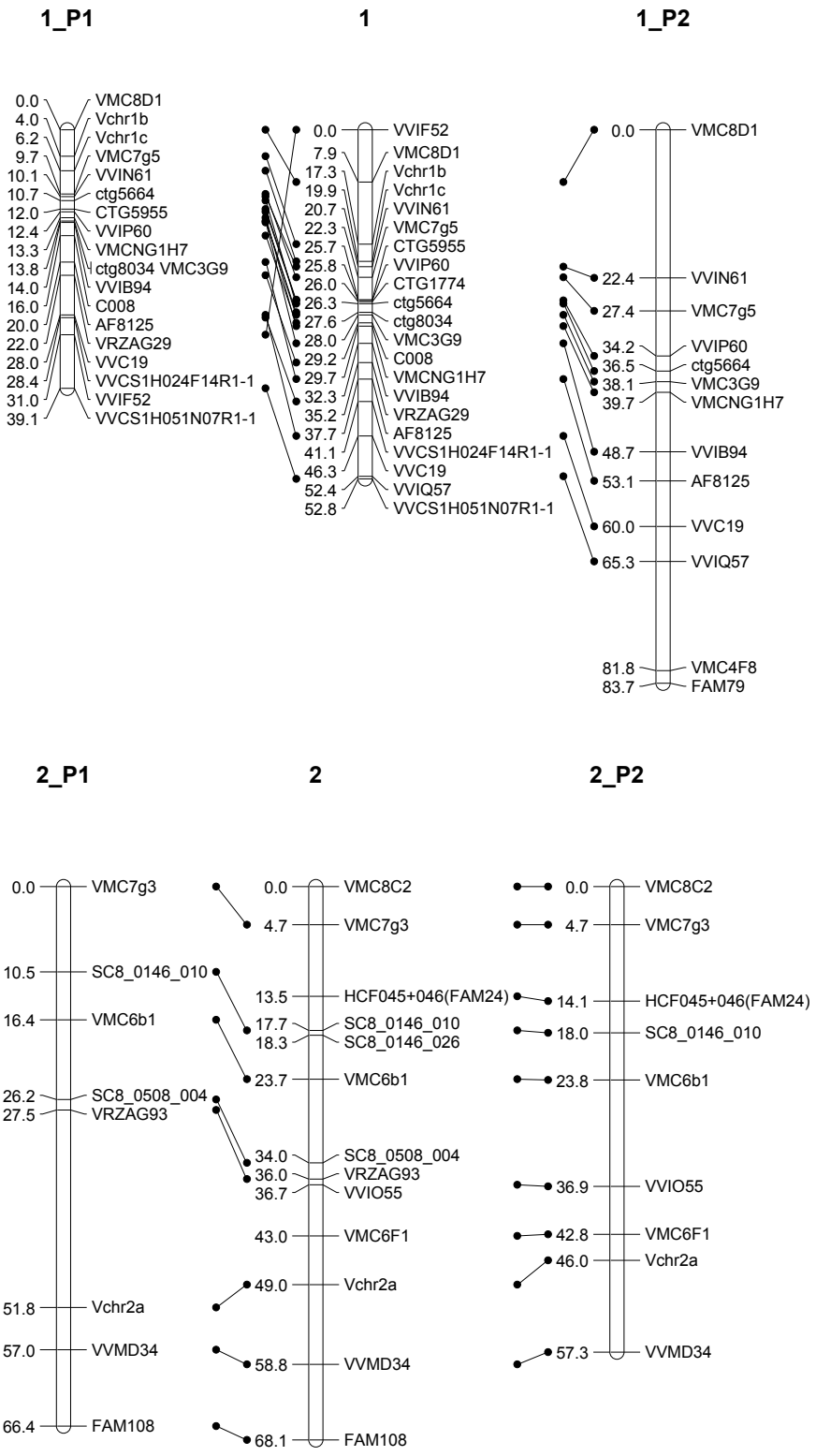
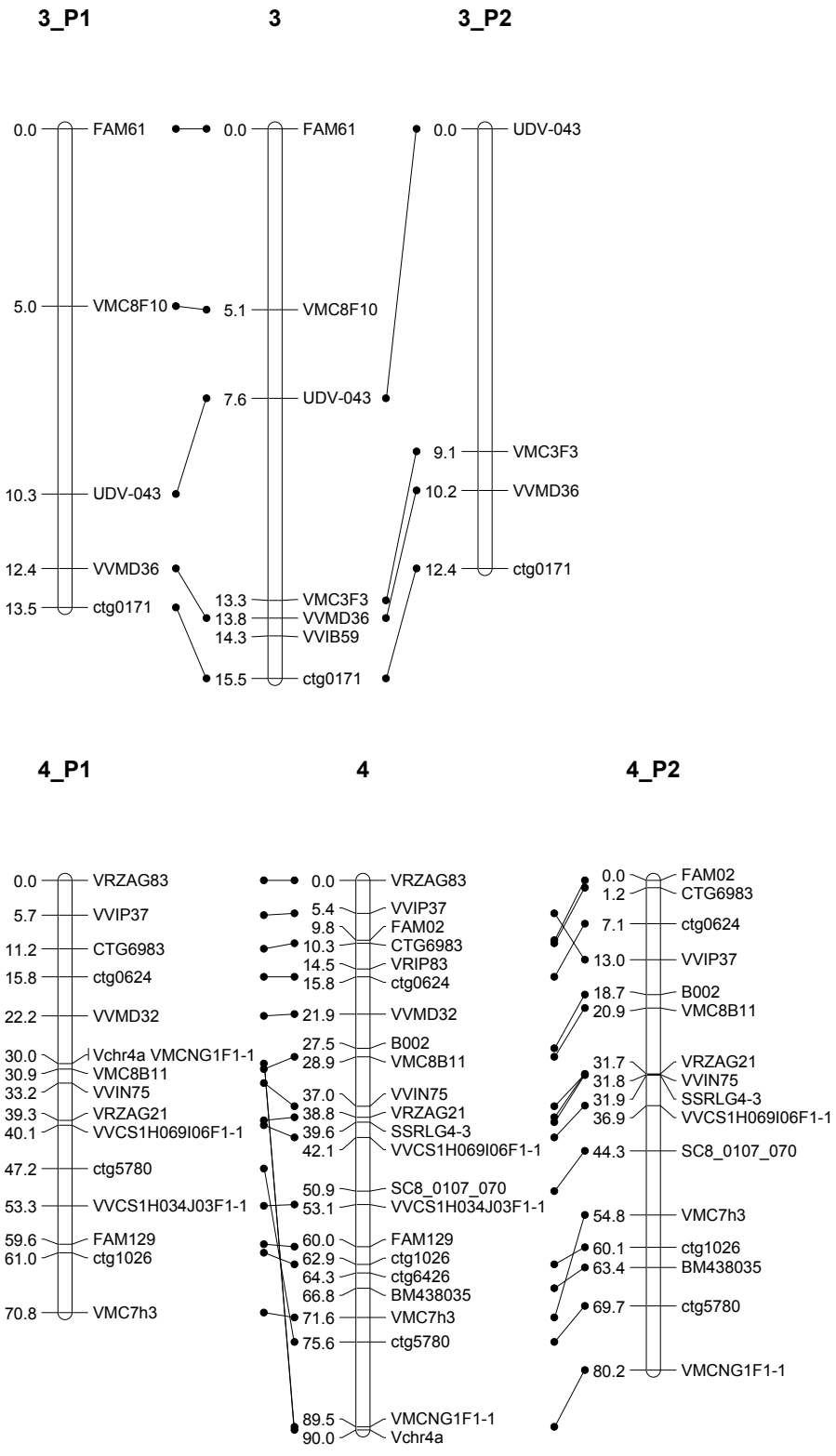


Figure 4. Genetic maps for Chambourcin (P1), Cabernet Sauvignon (P2), and the consensus using the regression algorithm



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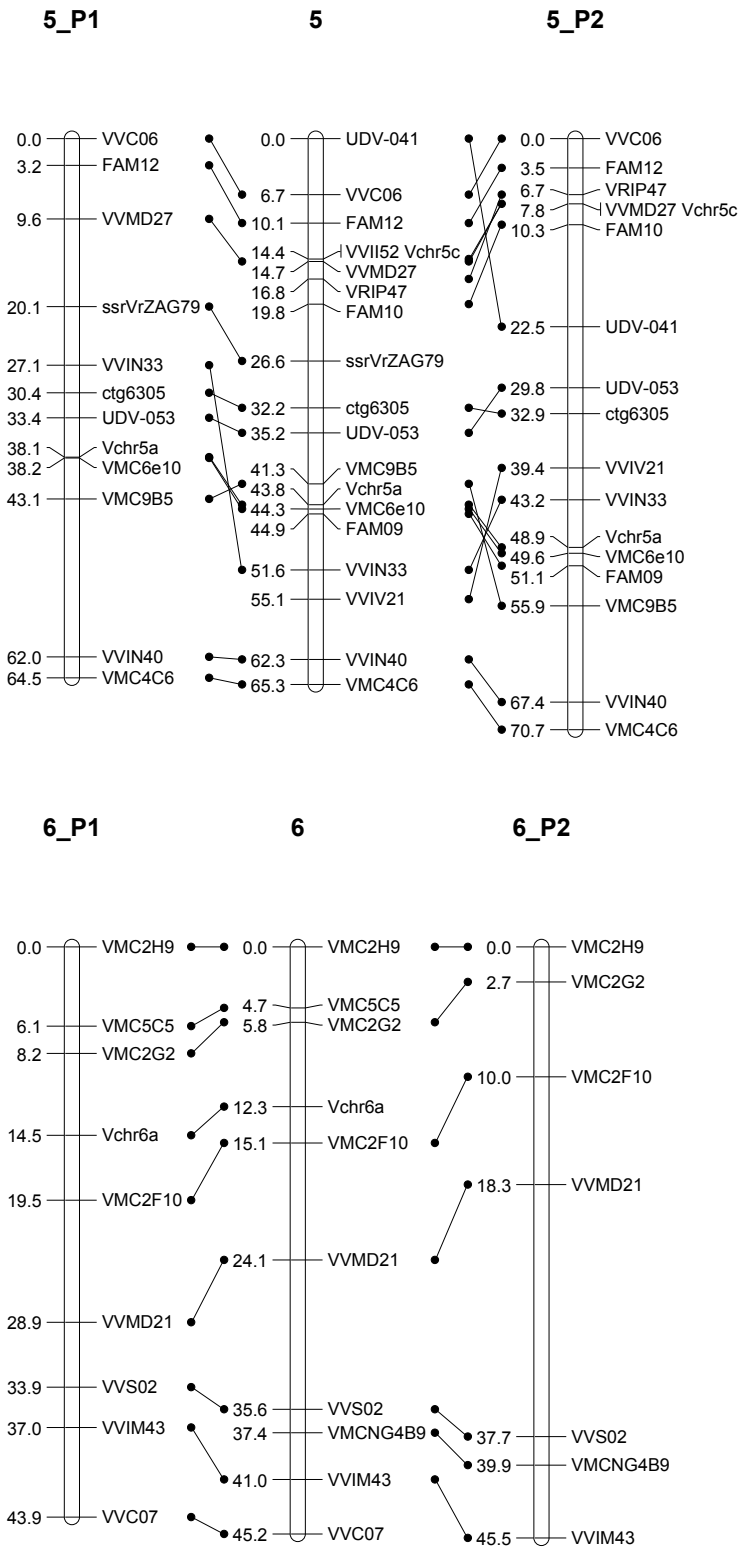

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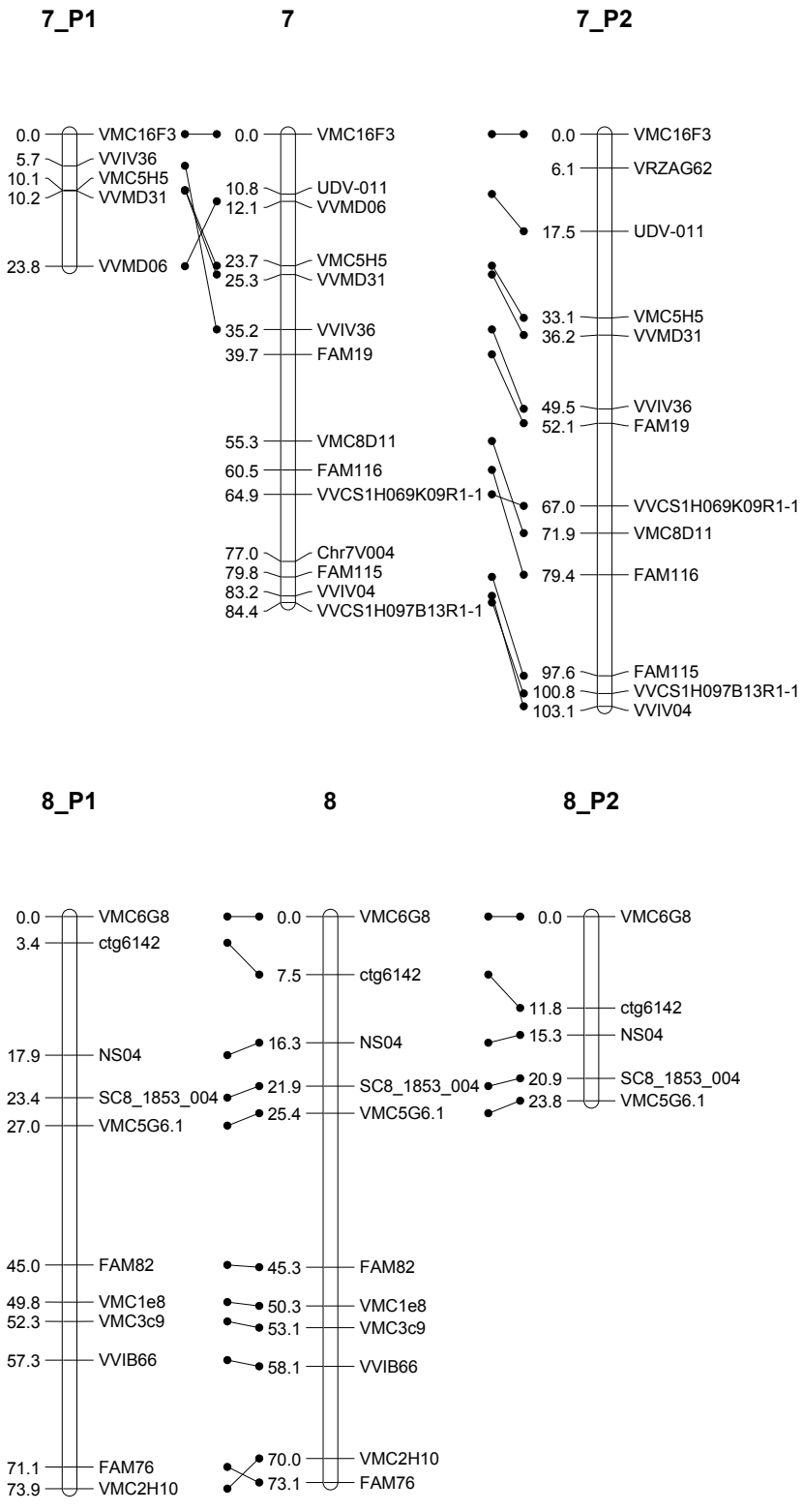

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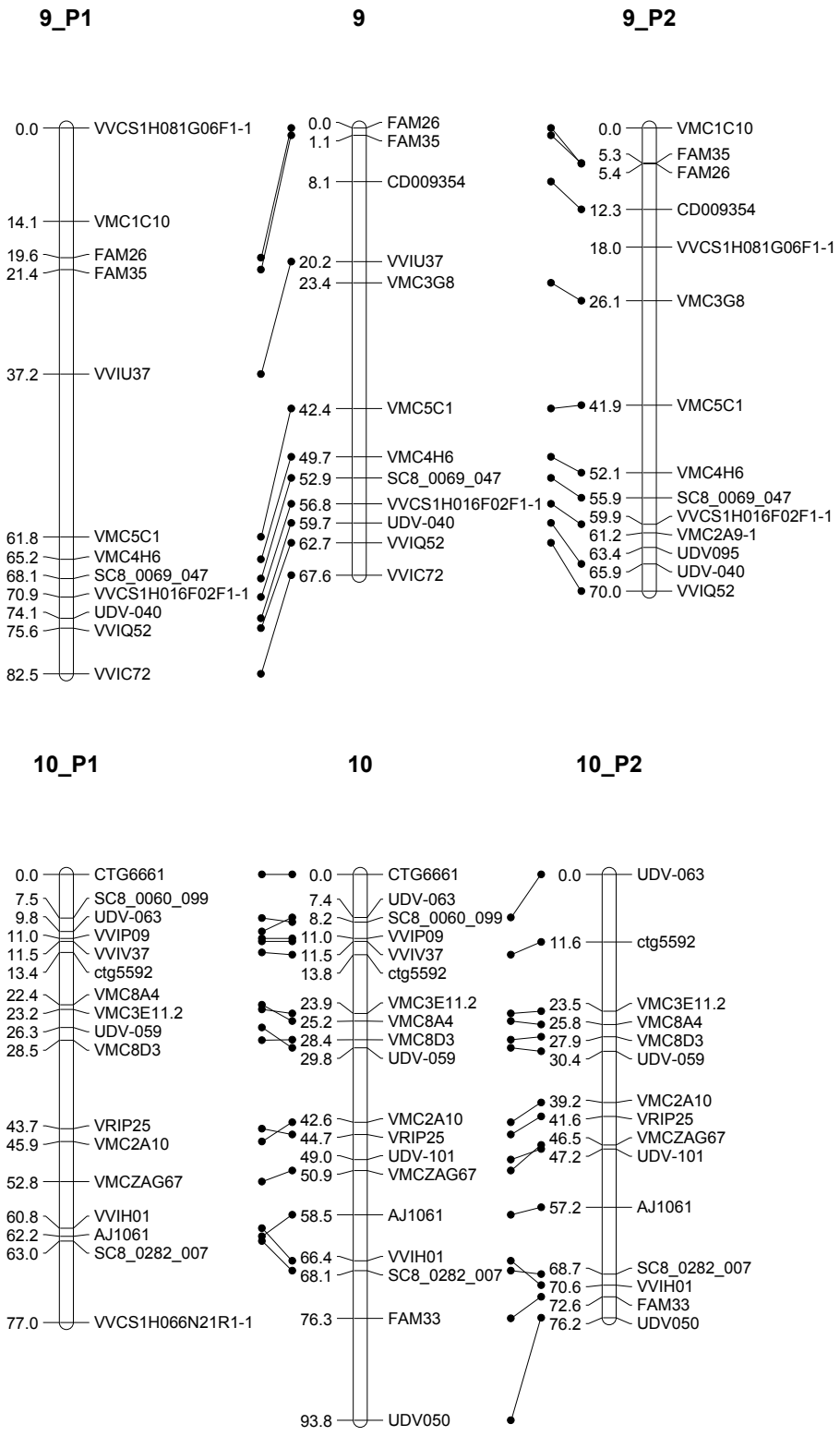

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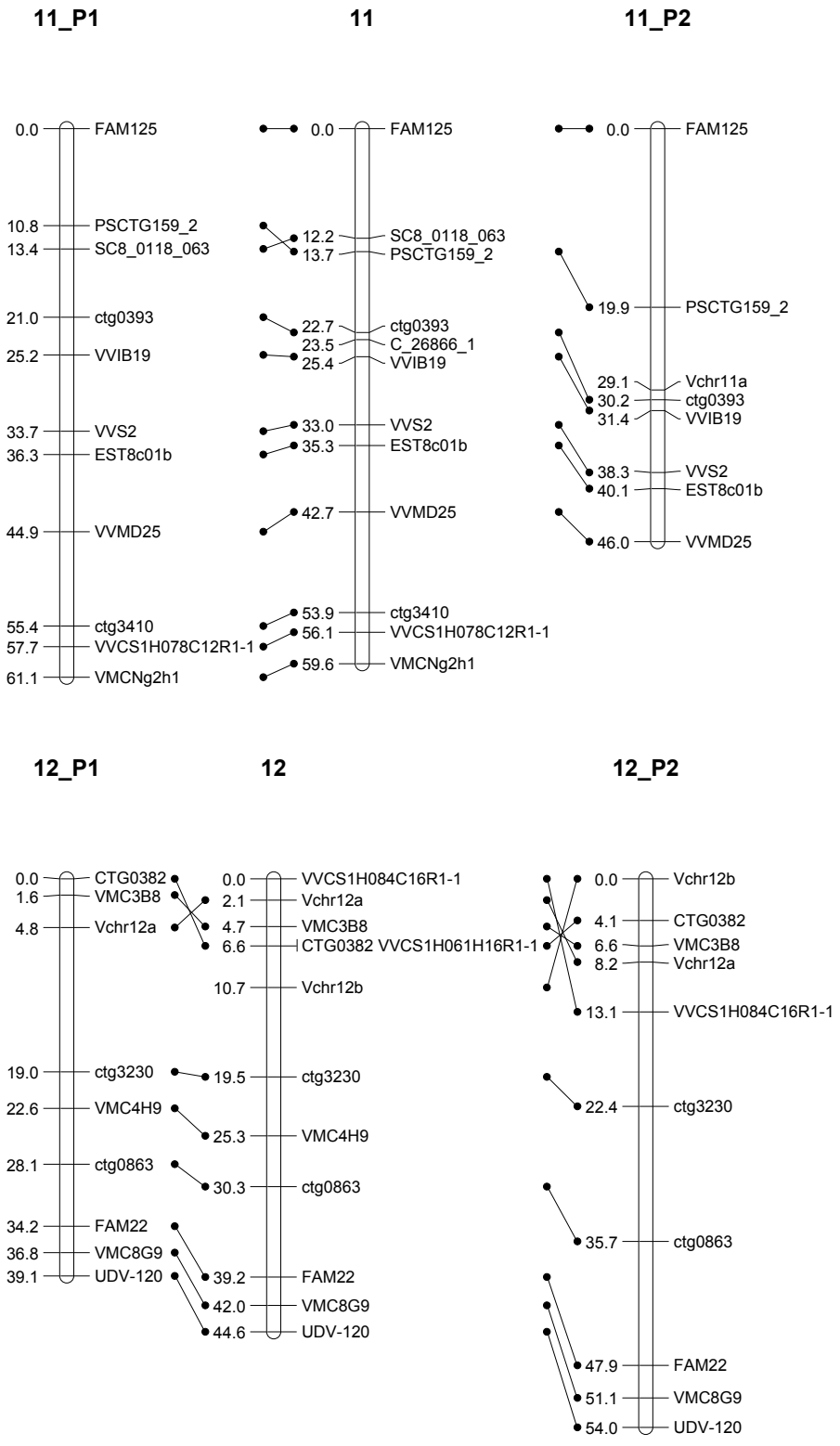

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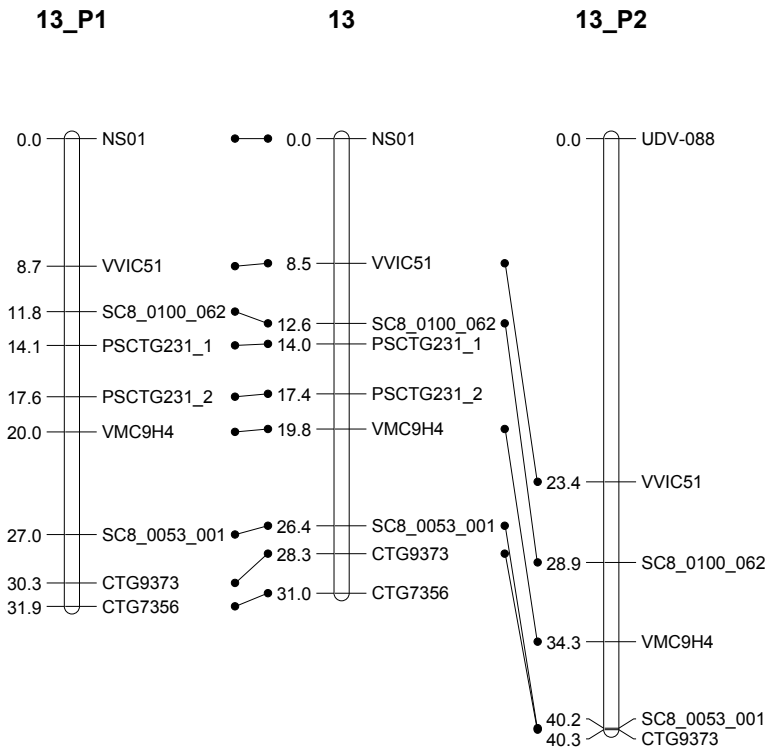

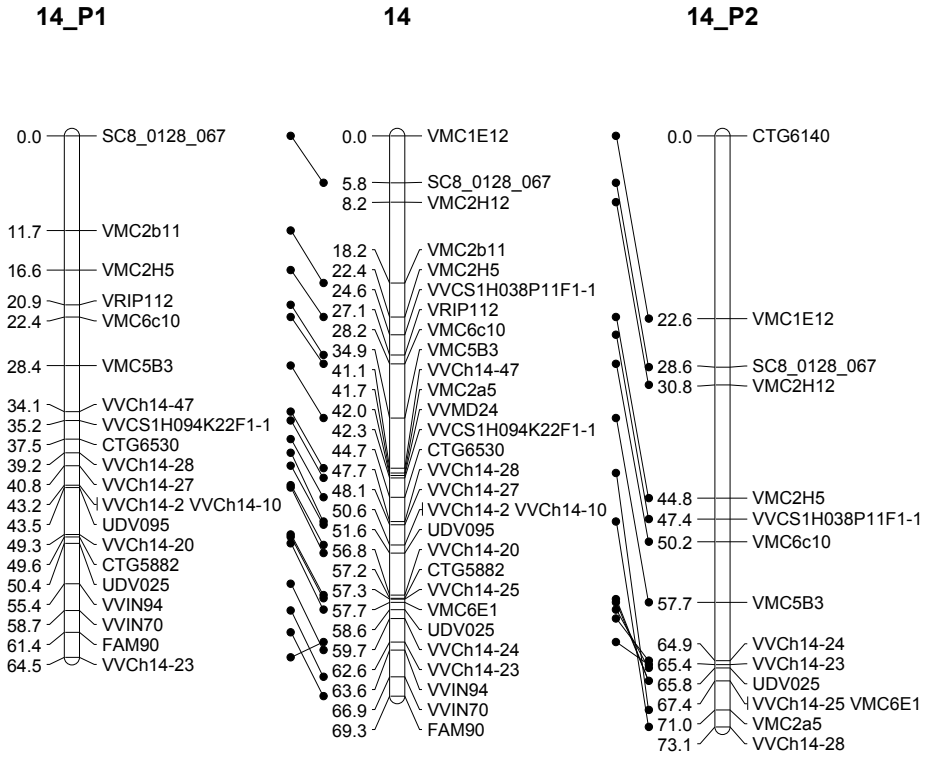

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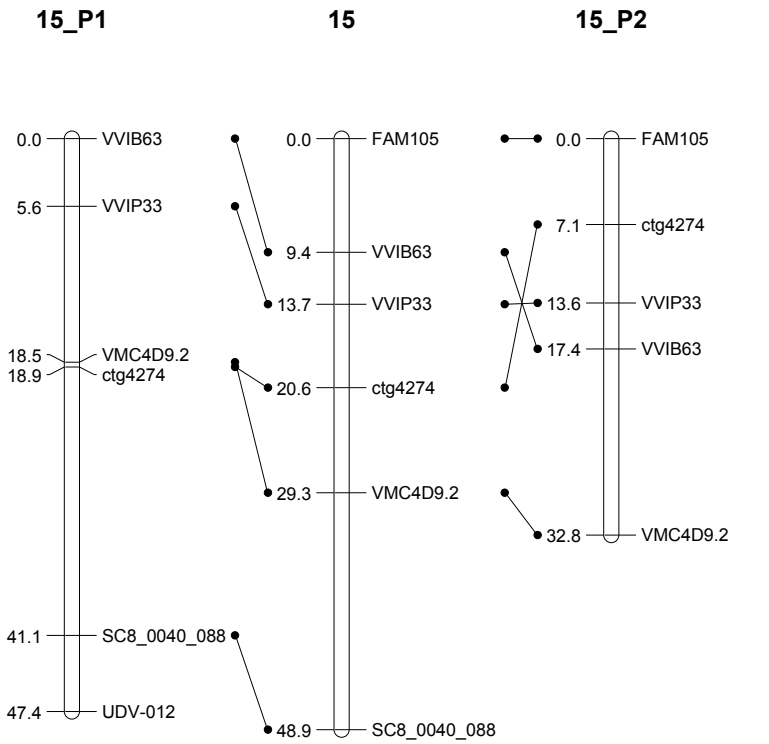

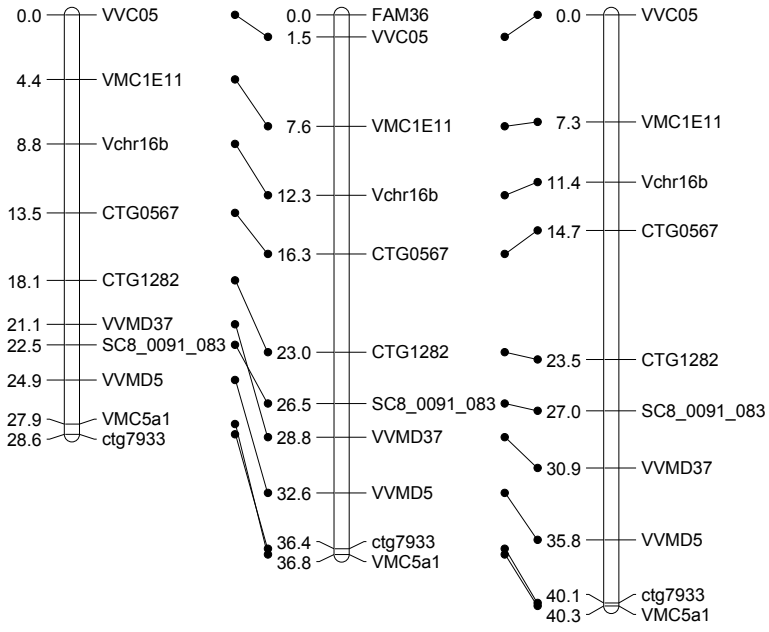

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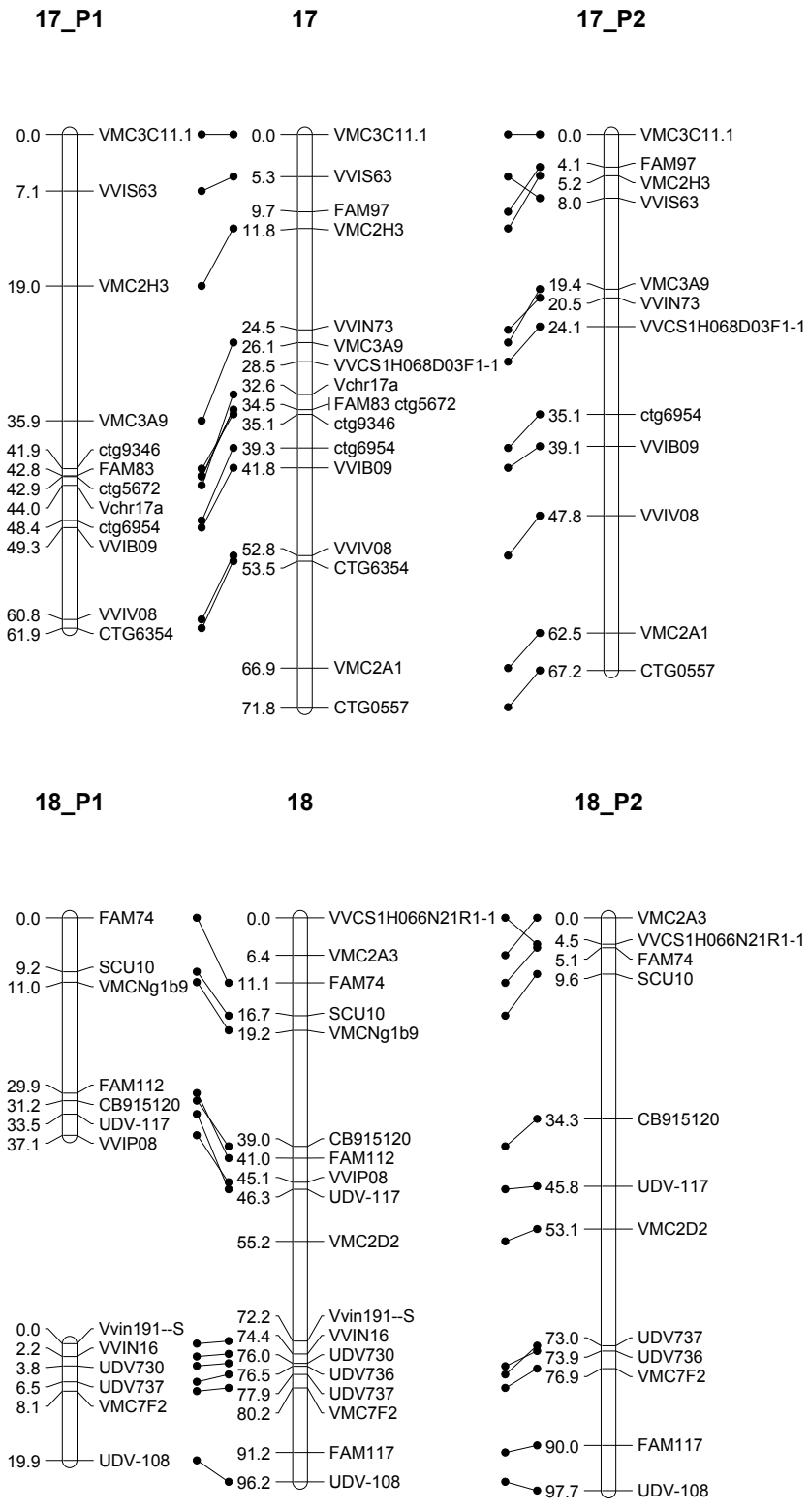

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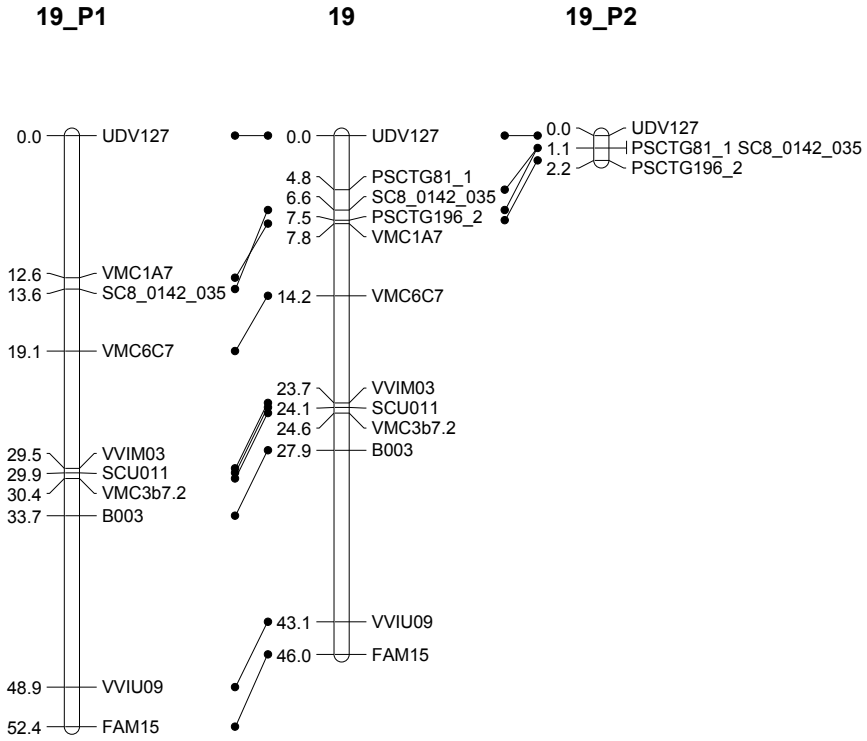

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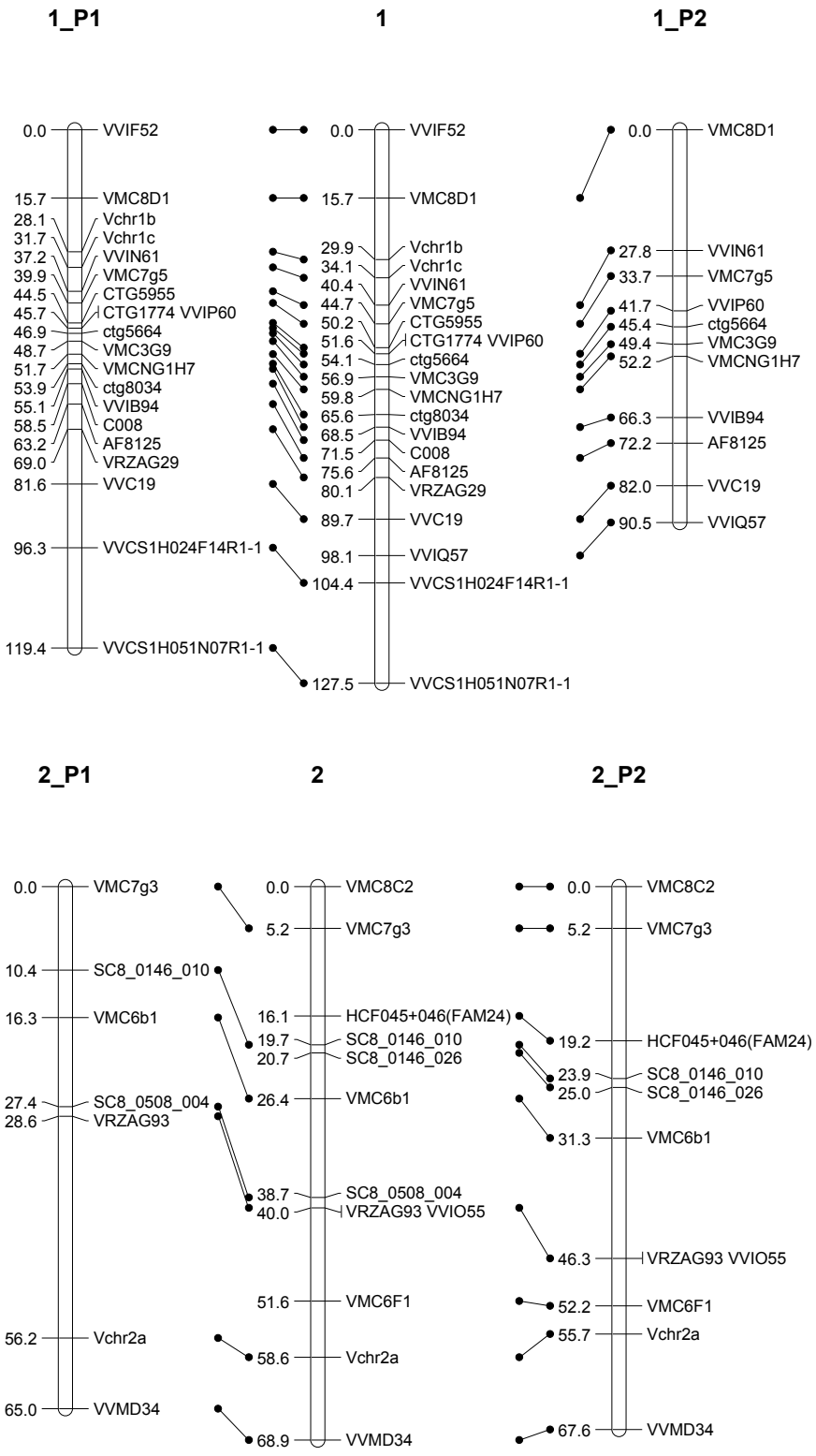
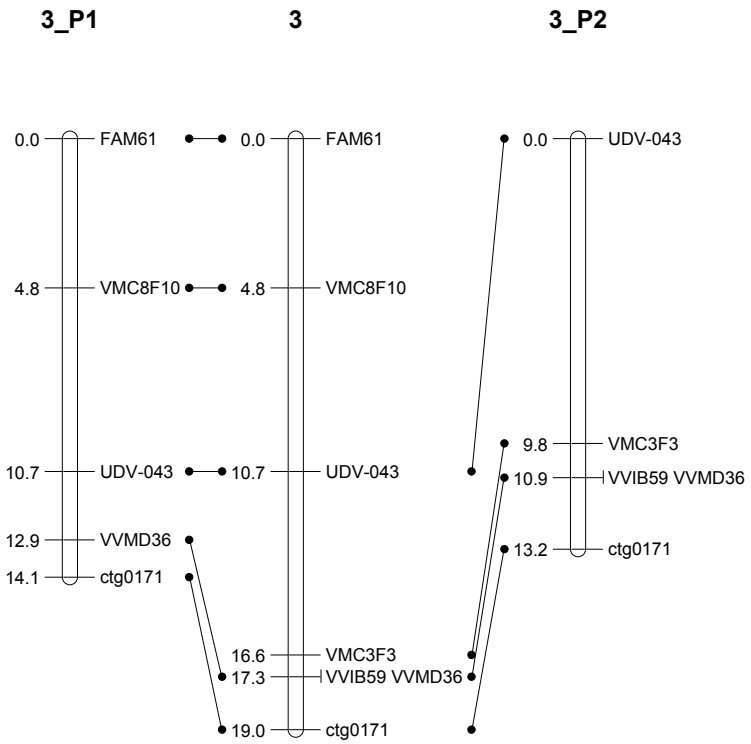
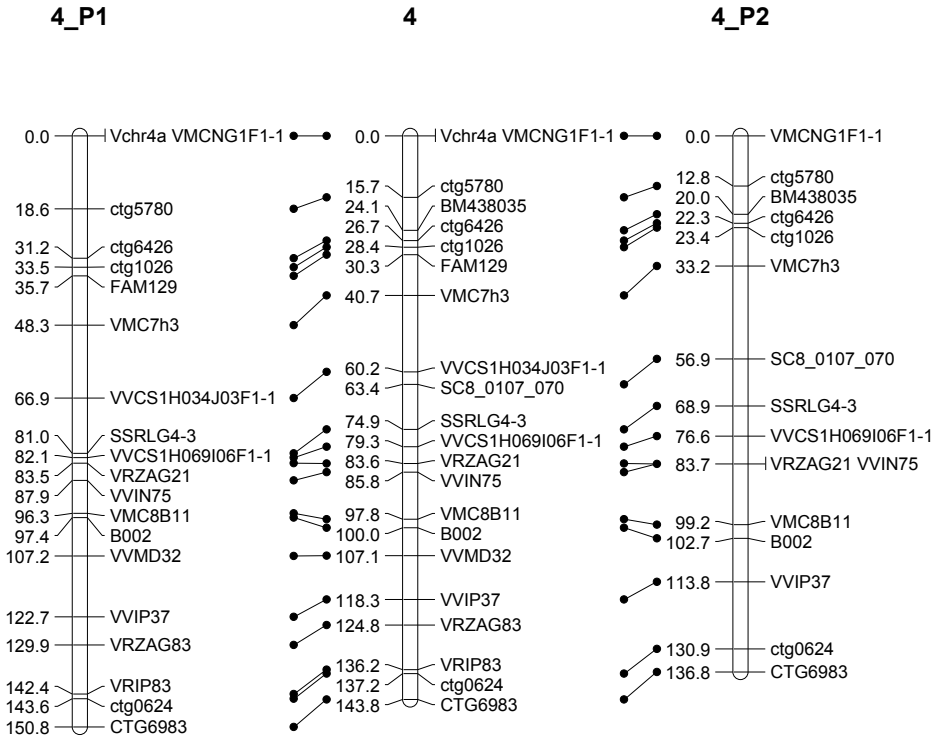


Figure 5. Genetic maps for Chambourcin (P1), Cabernet Sauvignon (P2), and the consensus using the ML algorithm





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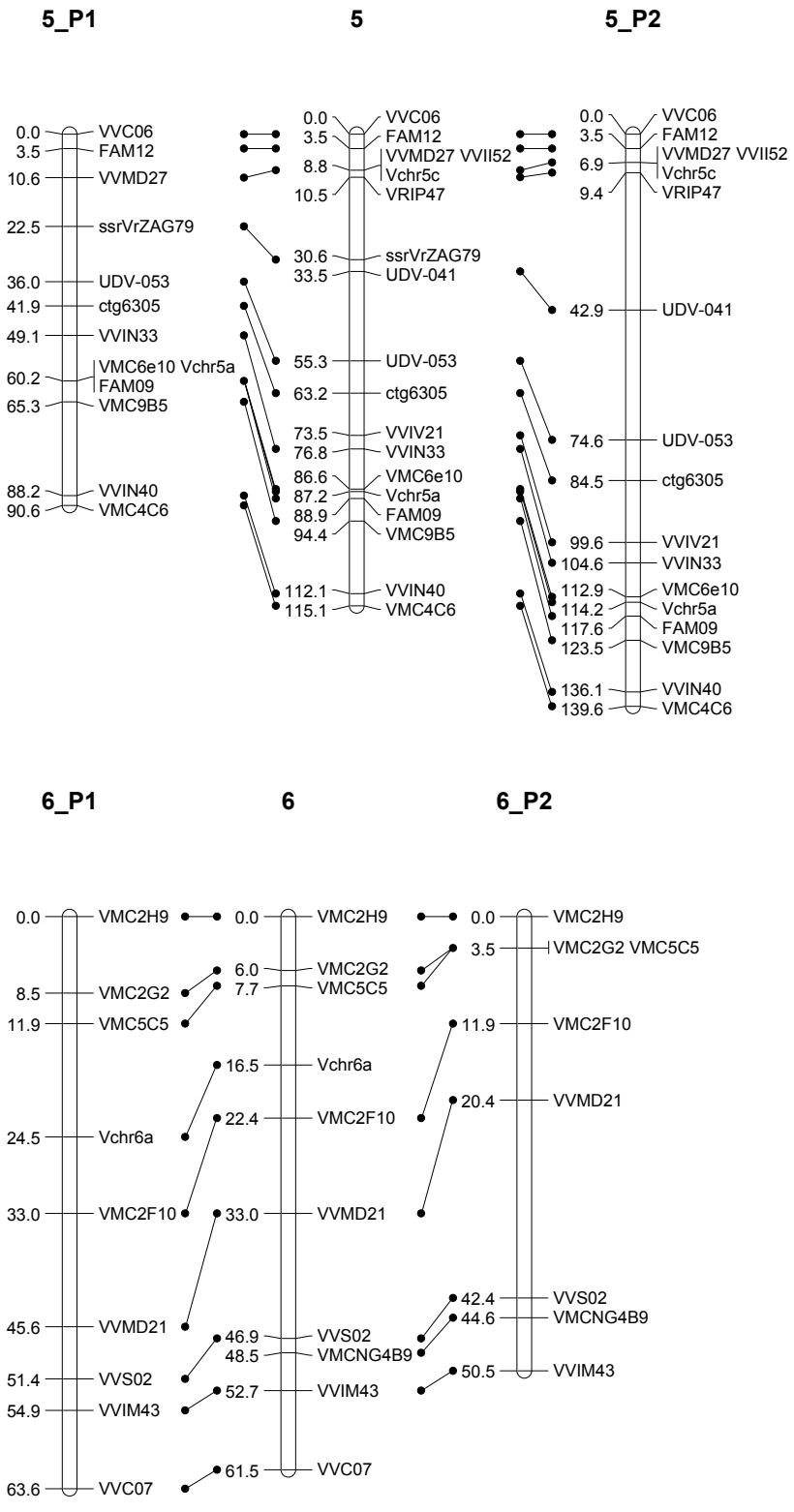

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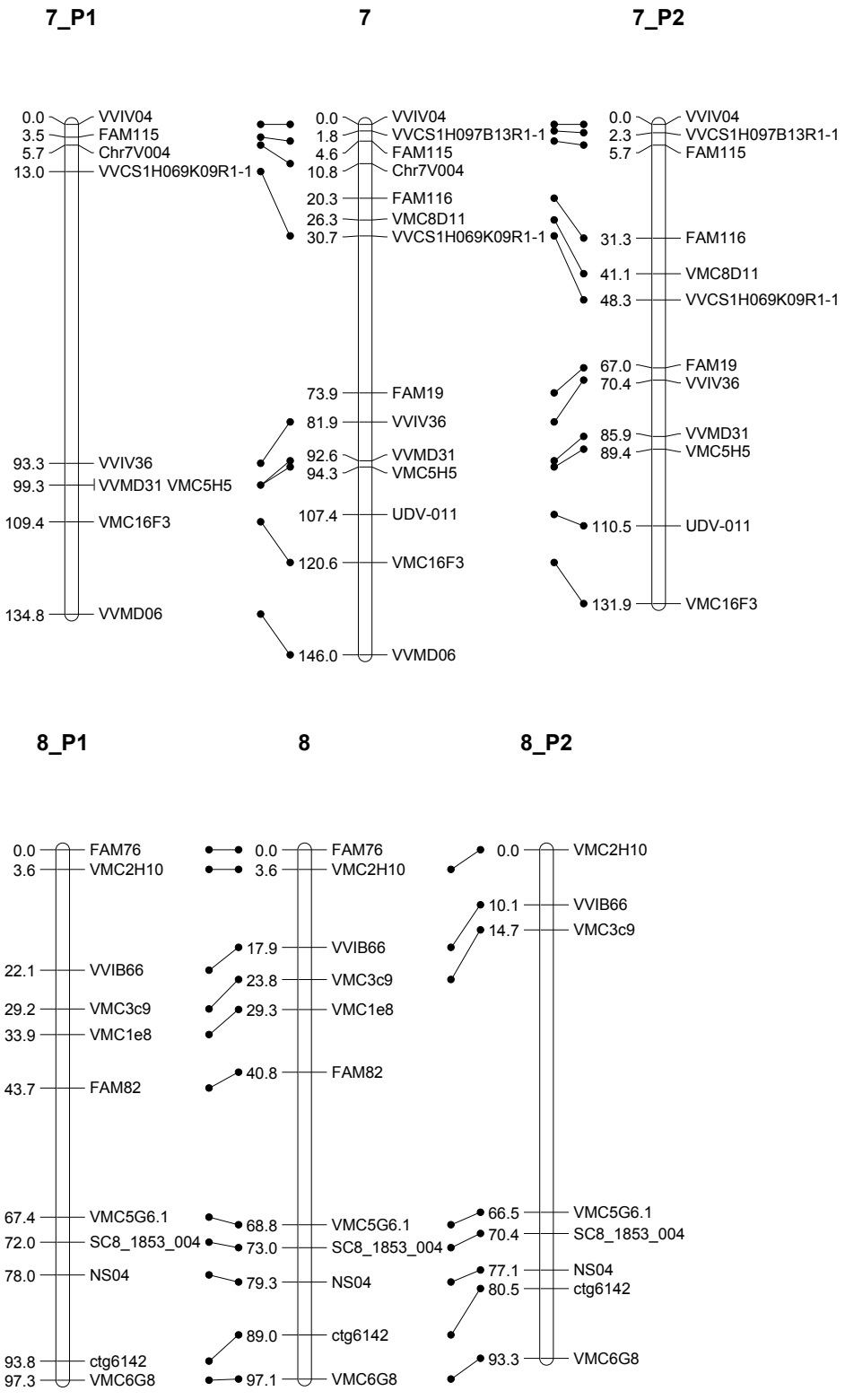

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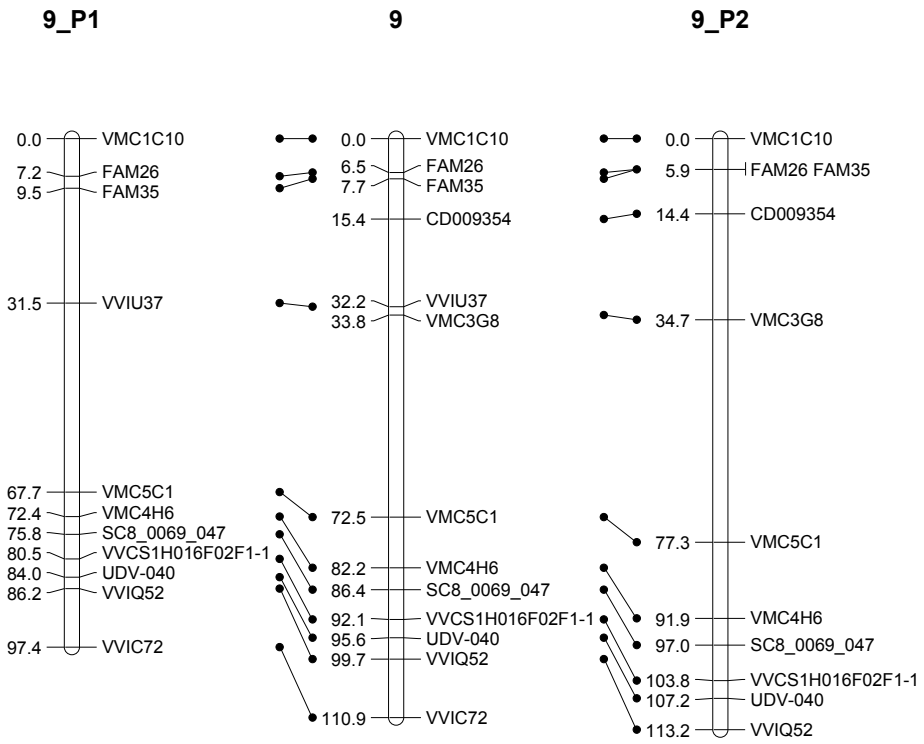

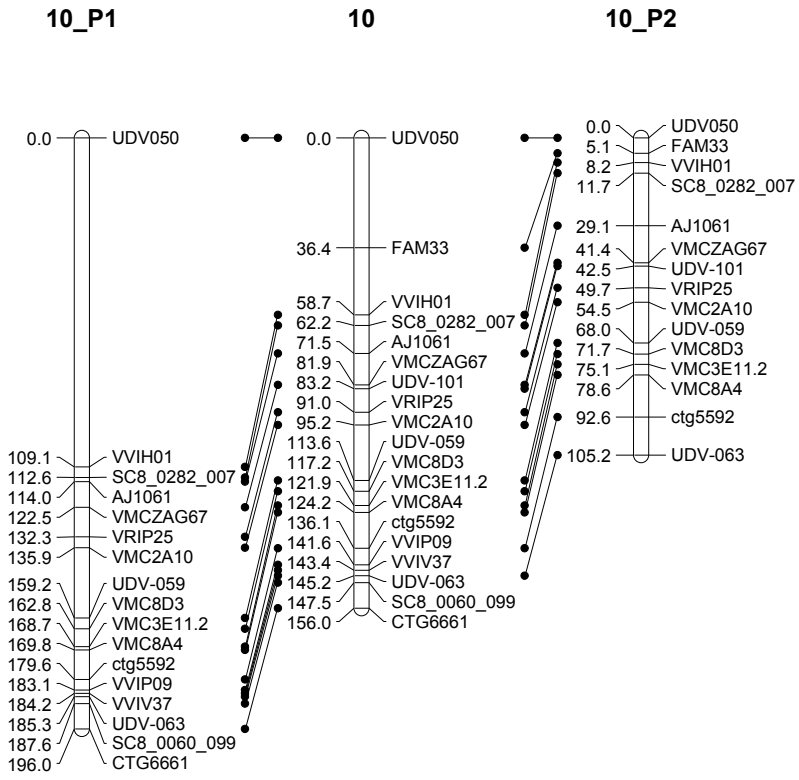

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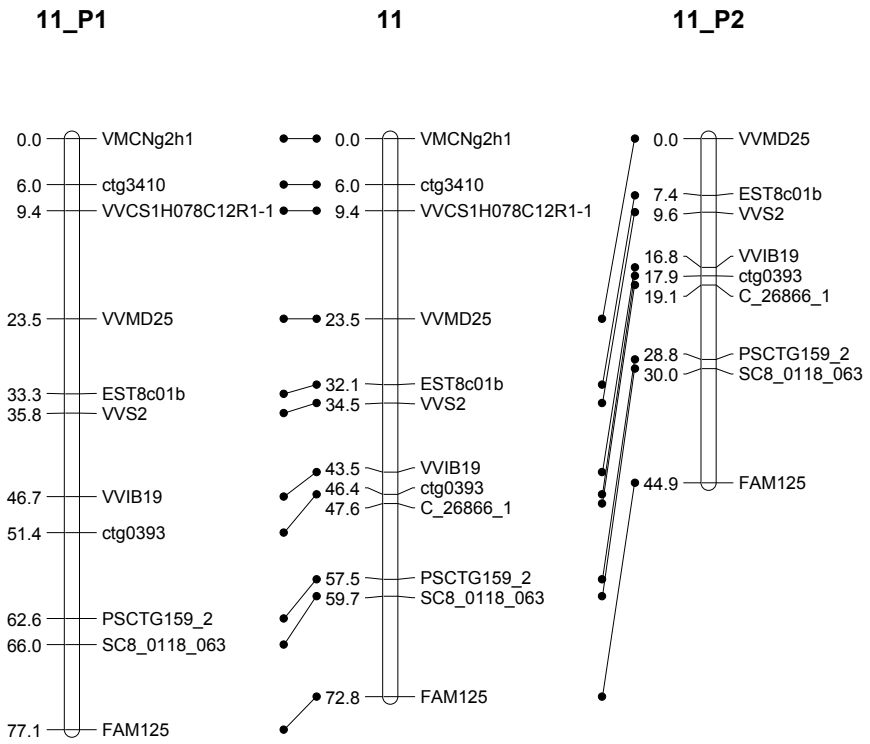

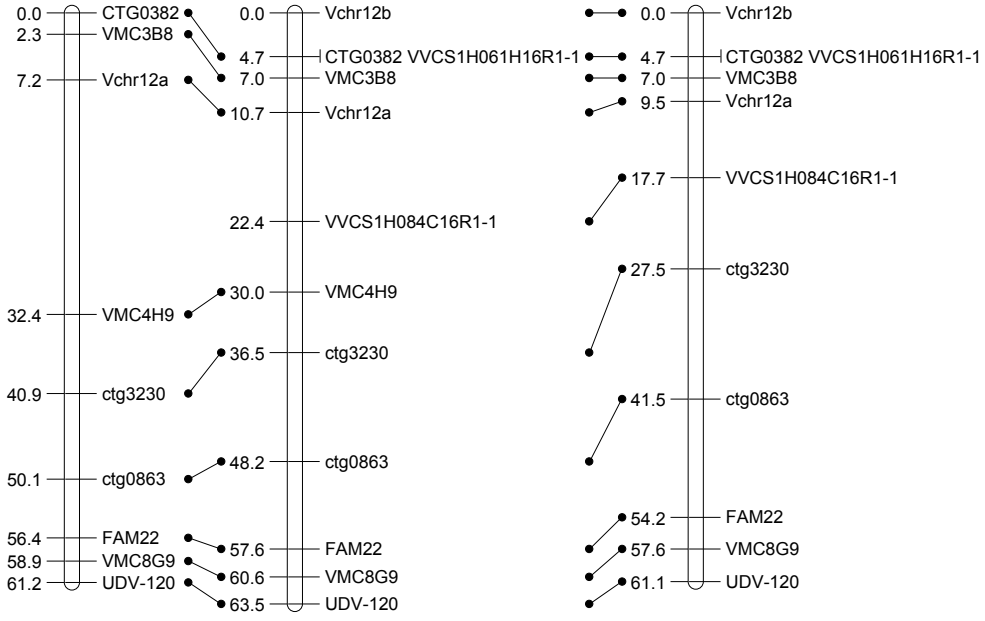

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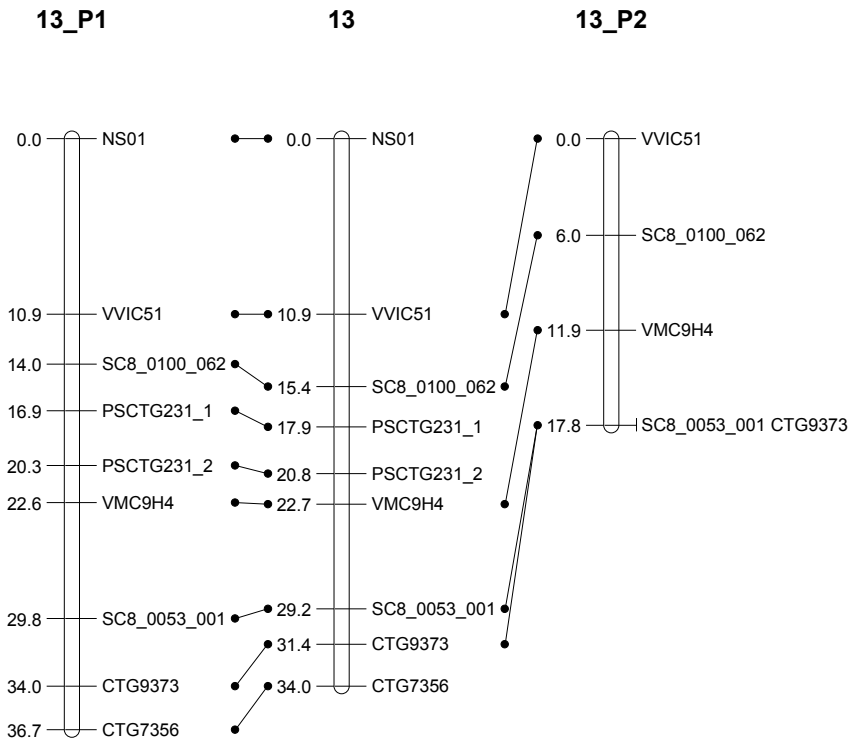

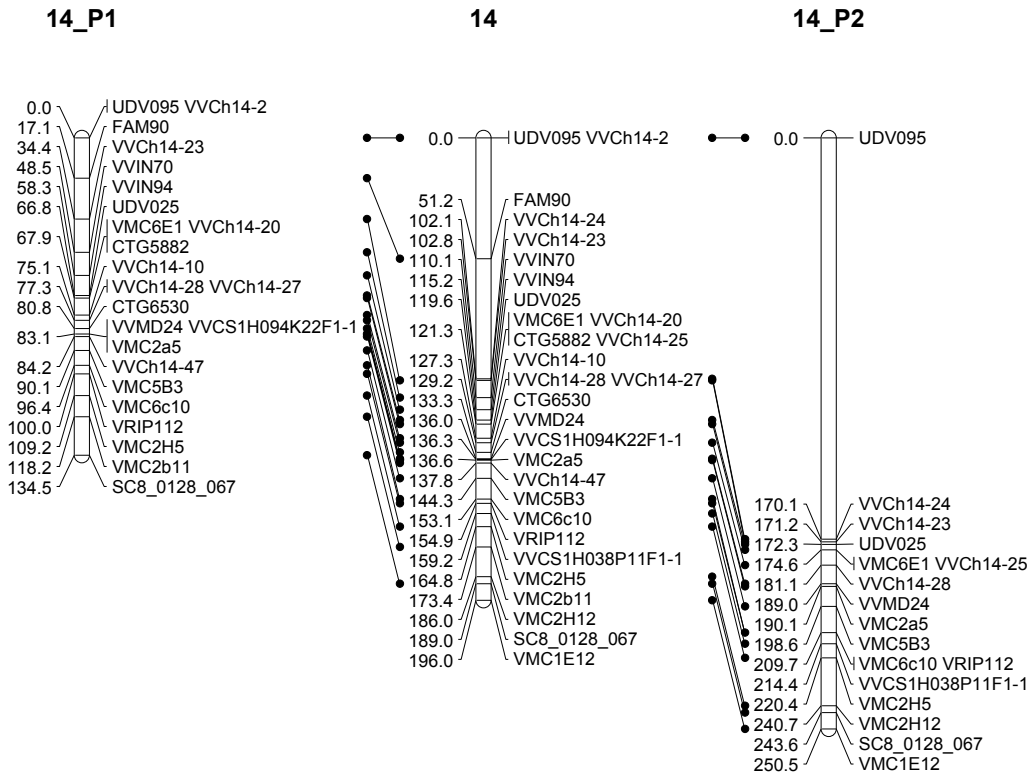

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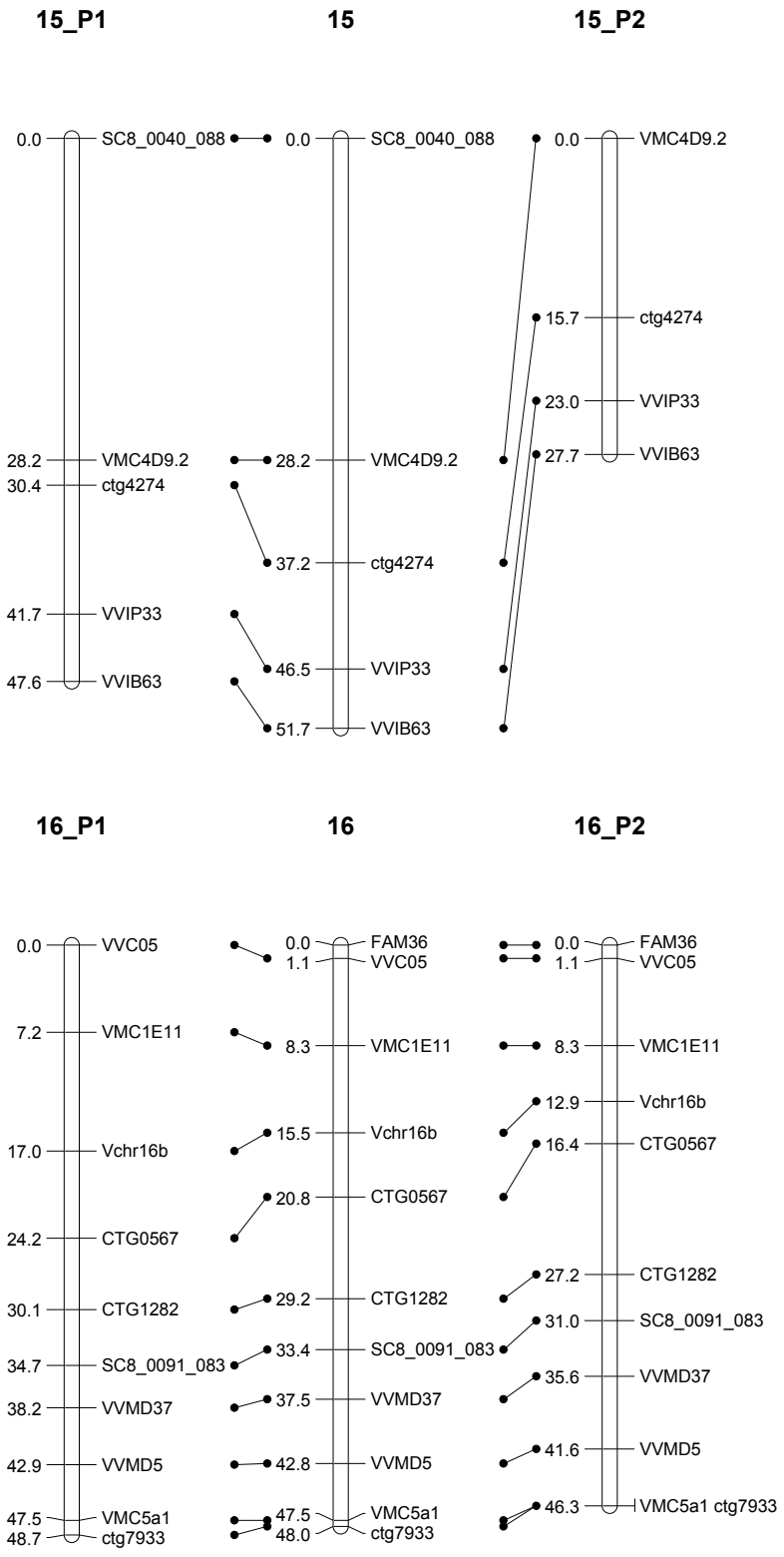

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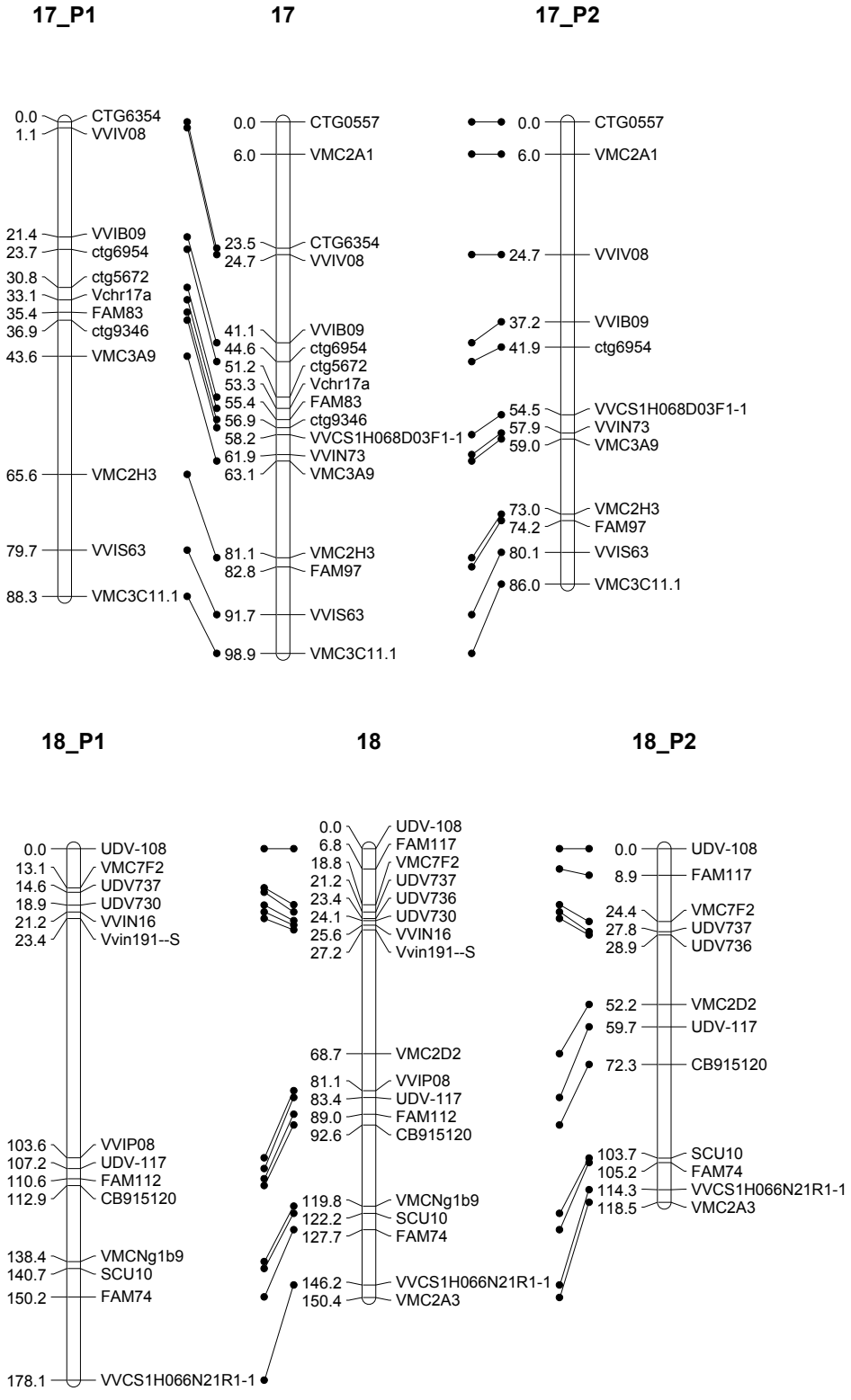

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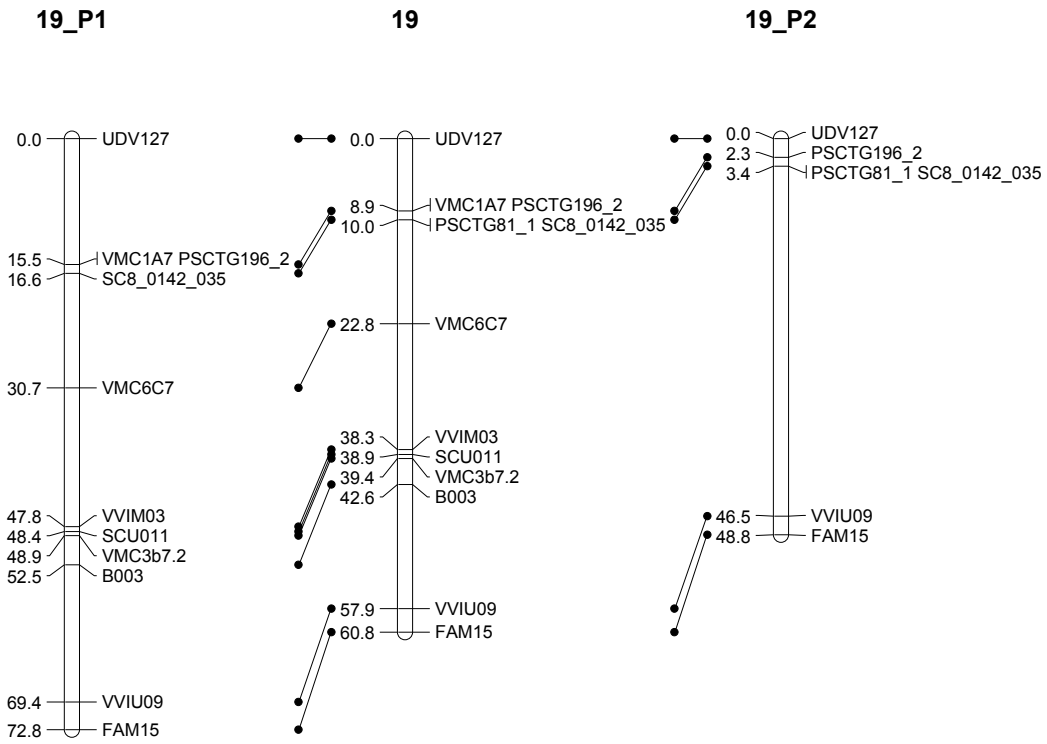

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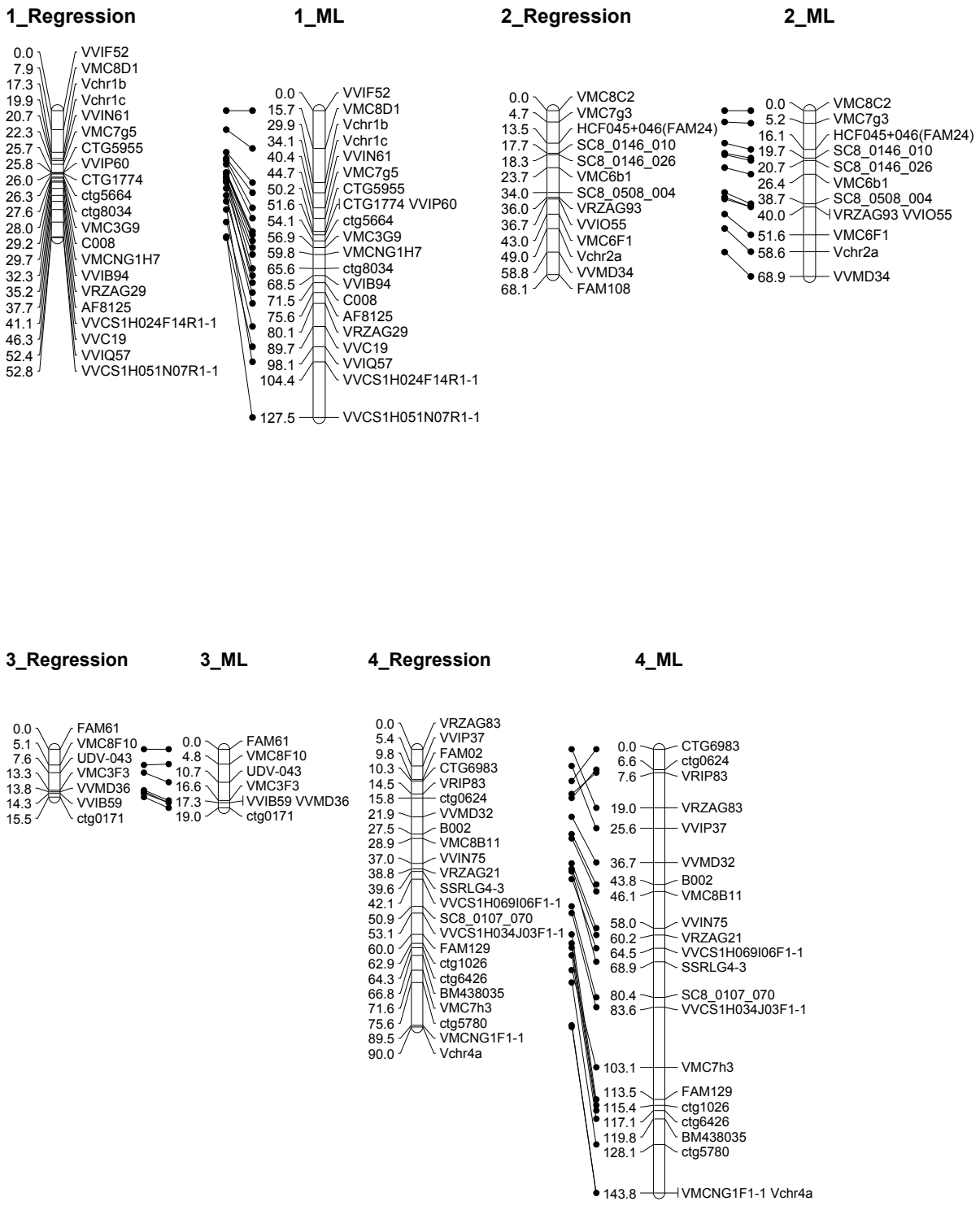
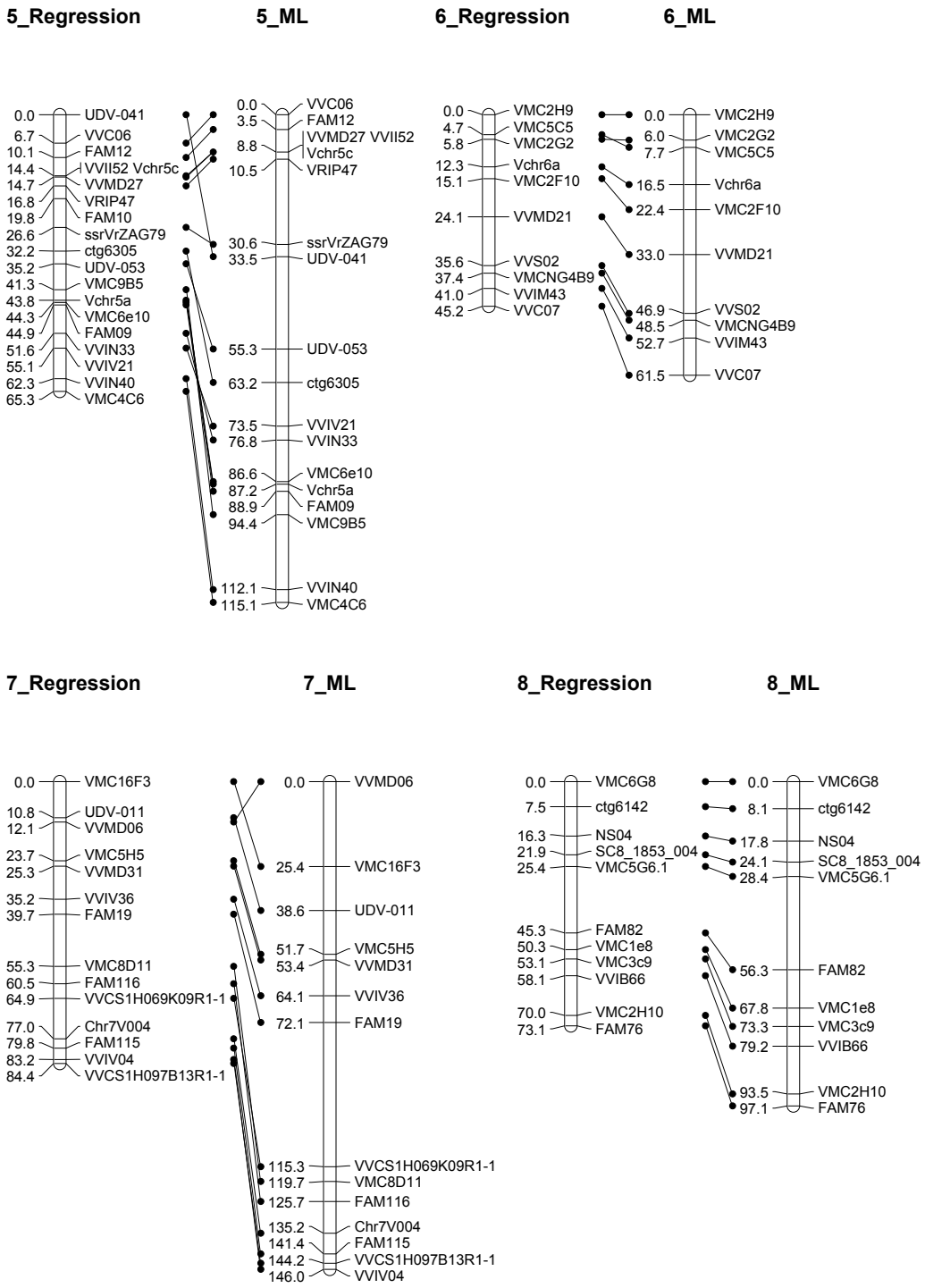


Figure 6. A comparison of linkage groups in the Chambourcin x Cabernet Sauvignon consensus maps generated using different mapping algorithms available in JoinMap 4.1



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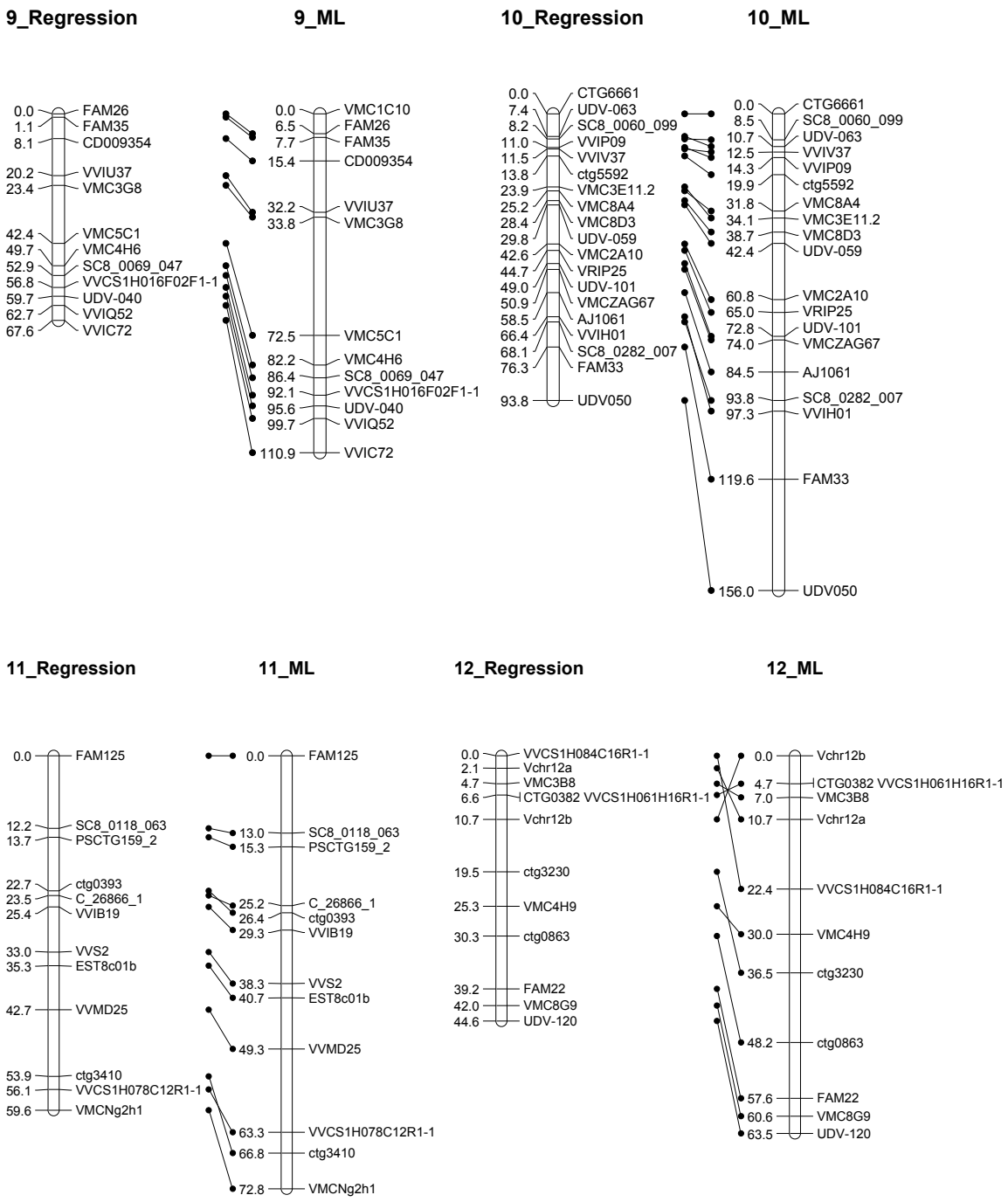

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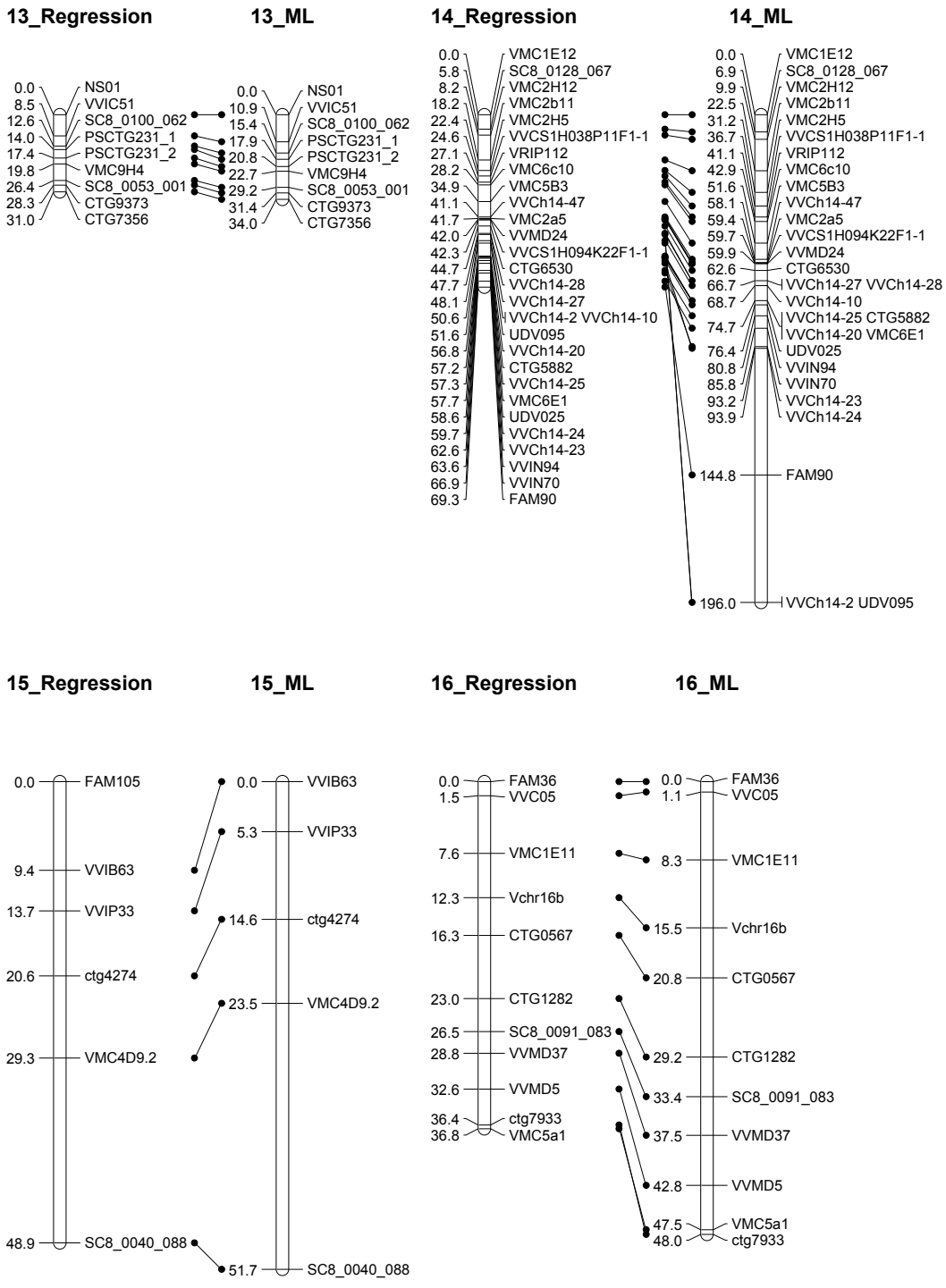

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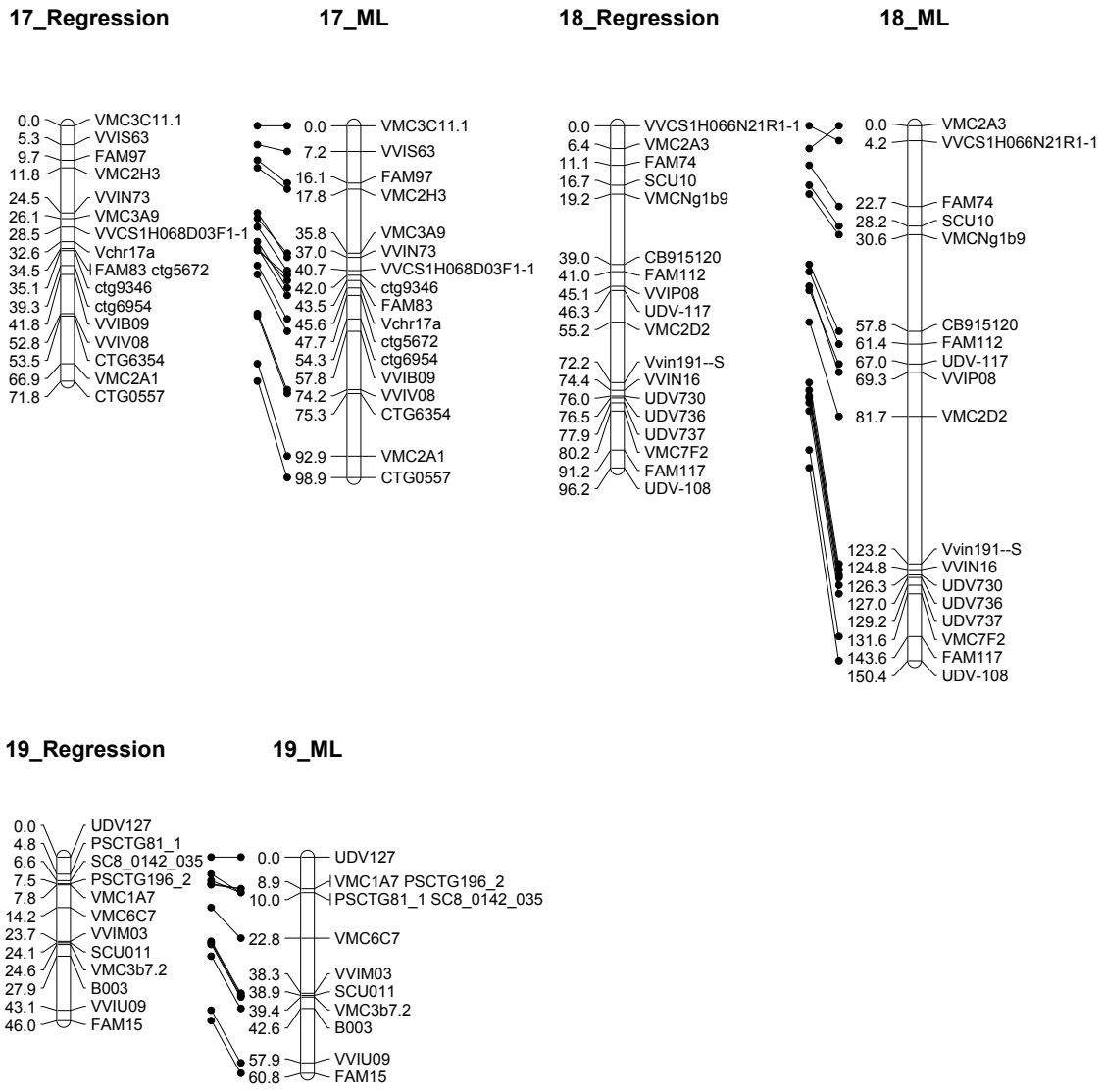

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