GENOME-WIDE SPACED SIMPLEX SNP ASSAYS FOR MARKER-BASED INTERSPECIFIC GERMPLASM INTROGRESSION AND GENETIC MANIPULATION IN COTTON

A Thesis

by

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ABSTRACT

Upland cotton (*Gossypium hirsutum* L., $2n=4x=52$, $2[AD]_1$) is a major fiber and oilseed crop that contributes significantly to the livelihoods of millions of people, as well as the economies of the USA and many other countries. Genetic improvements of cotton are needed to enhance sustainability, address evolving pest and pathogen pressures, and meet industrial requirements. However, genetic diversity is low among elite Upland cotton cultivars and breeding germplasm, which constrains the effectiveness of breeding efforts, and renders the crop more vulnerable to emerging biotic and abiotic threats. Genetic diversification efforts involving interspecific hybridization, introgression, and genetic dissection would benefit greatly by the availability of the inexpensive assays for targeted genotyping. Single-nucleotide polymorphisms (SNPs) are the most abundant type of marker and most amenable to high-throughput technologies and can be used for marker-assisted selection (MAS) and efficient manipulation of important genes and genome regions, e.g., for genes that enhance yield, pest resistance, and drought tolerance. In this study, we have endeavored to develop a genome-wide panel of map-spaced (cM) simplex SNP marker assays.

Using the Illumina™ CottonSNP63K array, an associated interspecific linkage map, and new AD genome sequence assemblies, we identified candidate SNPs that were [1] low- or single-copy and polymorphic between Upland cotton and donor species, [2] positioned well (spaced) on the linkage map, and [3] possessed a sequence milieu that seemed amenable to PCR-based KASP/PACE assay development, based on *in silico*

analysis. We identified SNPs from 18,000 mapped interspecific SNPs on CottonSNP63K array that are biallelic and can distinguish Upland cotton, *Gossypium hirsutum* L., from the three germplasm donor species, *Gossypium mustelinum, Gossypium tomentosum* and *Gossypium barbadense*, and their corresponding F_1s. About 550 SNP assays, either "Kompetitive Allele Specific PCR" (KASP) or "PCR Allelic Competitive Extension" (PACE), were progressively identified according to their linkage map positions, contribution to genome coverage relative to neighboring SNP markers and their functionality in Phase-I evaluation of the assays for genotyping an Upland cotton inbred line 'TM-1', three donor species and F_1 hybrids. For each of the 26 linkage groups, we chose 10 or more spaced simplex assays that should collectively suffice for many interspecific breeding needs. While likely useful for much more, this SNP genotyping assay will be especially useful for efficient introgression of wild species germplasm into an Upland cotton genetic background, genetic dissection, analysis, and breeding manipulations. A set of 453 KASP/PACE assays were subjected to Phase-II evaluations using 88 interspecific F_2 hybrids (~30 per donor species) with Fluidigm 96.96 Dynamic Arrays™ on the BioMark™ platform. The results indicated about 70% of the assays enable genotyping of self-generations, e.g., for recovery of true-breeding BC_5S_1 chromosome segment substitution lines. A pipeline for in silico assembly-based targeted SNP assay development was developed and should facilitate future completion of genome-wide coverage with map-spaced interspecific AD-genome SNP assays.

DEDICATION

I dedicate my thesis research to my loving parents Nurcan and Ozcan Velioglu, my sisters, Nuray and Necibe, and my brother, Fatih, who has been the source of my strength to help make my dream come true throughout my scientific journey in the United States. I also dedicate this work to my aunt, Duriye Sargut, who always supports me. Thank you all those for the support, love, affection, and encouragement.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction of Cotton

Cotton is a versatile and widely cultivated crop. Cotton fibers are used in major industries, such as textile, home decorating, attire, and medical equipment, while cottonseeds are used for the production of vegetable oil, animal feed, and industrial materials such as soap. Cottonseed oil provides for 8% of the world's vegetable oil consumption, ranking it fifth in production amongst all vegetable oils worldwide (Zhang, Li, Wang, & Chee, 2008). More than 70 countries cultivate cotton. As of 2018, the world reached a record of about 123.7 million 480-pound bales of cotton produced ("Statista", 2018). India produces the most cotton (5.77 million metric tons), followed by China and the USA ("Statista," 2018). Production of Upland cotton accounts for 90% of the world's cotton, whereas 8% of the world's cotton is provided by American Pima cotton. The remaining 2% comes from Old World cottons, *G. herbaceum* (Levant Cotton), and *G. arboreum* (Tree Cotton), which are from Asian origin (Waghmare et al., 2005; Zhang et al., 2008). Due to the broad applicability of cotton and cottonseed, the crop is regarded as a major economic contributor.

Cottons belong to the genus of *Gossypium*, which includes 52 extant species, 45 diploid (2n=2x=26), and 7 allotetraploid (2n= 4x=52) (Wendel & Grover, 2015). The genomes of these species have been categorized into eight groups with designations A-G and K that relate to meiotic affinity, chromosome and genome size, ploidy, and

geographic location. Clades of these *Gossypium* genome groups relate closely to the various classifications of species among the three "gene pools", i.e., the prospective difficulty of using them in genetic improvement of Upland cotton. The primary gene pool contains all AD-genome cottons, including *G. hirsutum L.*, *G. barbadense* L., *G. tomentosum* Nuttall ex Seemann, *G. mustelinum* Miers ex Watt and *G. darwinii* Watt and two recently validated species (Gallagher, Grover, Rex, Moran, & Wendel, 2017; Grover et al., 2015). The secondary gene pool consists of diploid species with genomes of the A, B, D, and F genome clades, whereas the tertiary gene pool includes the diploid species with genomes in the Australian C, G, K and African/Arabian E genome clades. (Campbell et al., 2010; Zhang et al., 2008).

Early 20th-century scientists like Skovsted and Beasley determined that the 52-chromosome species *G. hirsutum* and *G. barbadense* arose from one or more hybridization-polyploidization events between two ancestral 26-chromosome species, one having a large A-like ancestral genome, i.e., similar to the genome of some extant Old World species, and the other having a relatively small ancestral D-like genome, i.e., similar to those found among a New World diploids (Skovsted, 1933; Beasley, 1942; Wendel, 1989; Wendel & Cronn, 2003). Based on phylogenic molecular investigations of extant species, the divergence between A-genome and D genome lineages was estimated to have occurred 5-10 mya (Wendel & Cronn, 2003). Recent "molecular clock" analyses placed the origin of the AD-genome allopolyploid cotton species at approximately 1-2 million years ago, i.e., well before the existence of *Homo sapiens* (Cronn, Small, & Wendel, 1999; Seelanan, Schnabel, & Wendel, 1997; Small, Ryburn,

Cronn, Seelanan, & Wendel, 1998; Wendel, 1989). Among extant species, the most comparable hybrid would involve either *G. herbaceum* (A1) or perhaps *G. arboreum* (A2) and *G. raimondii* (D5) (Wendel & Cronn, 2003).The A genomes (1,746 Mb/1C) are ~ 2 times larger than D genome (885 Mb/1C) (Li et al., 2014).

Cytogenetics observations led to early suggestions that the origin of cottons included a paleopolyploidization event, based on secondary associations on meiosis and comparatively high gametic chromosome number (Skovsted, 1933). Genetic hybridization and linkage events based on RFLP markers also indicated genetic redundancy and recombination per homologous chromosome associating with polyploidy (Reinisch et al., 1994). Further studies with molecular phylogenetic analyses exhibit the cotton lineage share a common ancestor having divergence from *Theobroma cacao* at least 60 million years ago, after which the cotton linage went through five- to six-fold ploidy increase (Paterson et al., 2012). These increases were on top of the very ancient paleohexaploidization ca. 300 mya that is common to all eu-dicots. Thus, modern Upland cotton is a partially diploidized tetraploid with disomic inheritance, but its genomes nonetheless retain complicated vestiges of multiple paleopolyploidizing and diploidizing events.

There are two cultivated allotetraploid cotton species, one being *G. hirsutum* (Long Staple Cotton, Upland Cotton, or Mexican Cotton) and the other being *G. barbadense* (Egyptian Cotton, American Pima, Extra Long Staple Cotton, or Sea Island Cotton). These two species, also known as New World Cottons, and are thought to have originated in Central America (*Gossypium hirsutum* L.) and western South America (*G.*

barbadense L.) (Waghmare et al., 2005; Zhang et al., 2008). Although cultivated cotton species are important for the economy and improvement of cotton breeding, the genetic base of Upland cotton is very narrow (Richmond, 1951; Van Esbroeck & Bowman, 1998). Natural and man-made "genetic bottlenecks" likely contributed to the low levels of genetic diversity, including the original polyploidization, selection associated with domestication, subsequent selection, and modern breeding with preferential reliance on highly related elite types as breeding parents. The reality of reduced genetic diversity has become more problematic in Upland cotton because of the high risks associated with disease susceptibility and vulnerability to climate alteration and pests. International travel and trade are now extensive and can lead to the introduction of invasive foreign species of pathogens and pests, plus changing environmental and climatic conditions demand the development of germplasm that contains beneficial alleles from existing cotton germplasm resources. However, current elite Upland cotton germplasm exhibits an extremely low amount of diversity and is thought to descend from only approximately twelve introgressions (Hinze et al., 2017; Hulse-Kemp et al., 2014; Richmond, 1951; Van Esbroeck & Bowman, 1998). Because of this, the genetic variation found within current elite breeding germplasm is very low, which limits the opportunities to genetically improve important traits such as resilience to abiotic stresses, tolerance, and resistance to pests and pathogens, yield, fiber properties, and other traits may be unable to be. Genetic improvement may be obtained via introgression of unique genes sourced from obsolete cultivars, wild *G. hirsutum* accessions, and other cotton species. Of these,

the other species harbor far greater amounts of genetic diversity (Fang et al., 2014; Robinson et al., 2007; Saha et al., 2006).

Improved fiber characteristics, disease, and insect resistance traits have been observed among diploid cotton species (Liu, Guo, Lin, Nie, & Zhang, 2006). Though cotton germplasm resources are available throughout the world, they have traditionally been characterized by breeders based only on phenotype. The use of phenotypic selection often involves extensive time, cost, and space for visual observations and measurements, so lower-cost alternative methods are desirable and often necessary, e.g., marker-based methods. Once valuable traits are associated with molecular markers, molecular screening methods typically enable cost- and time-efficient recovery of desired genetic types among segregating progeny, and collective screening with multiple genetic markers can be used to identify important differences amongst accessions and lines that are otherwise difficult to discern (Ai et al., 2017; Hinze et al., 2017; Hulse-Kemp et al., 2014).

Molecular Markers in Cotton

It is undeniable that genetic markers have become indispensable to plant breeding since the advent of effective DNA technologies in 1980s, e.g., RFLPs (Powell et al., 1996). Genetic markers are tightly associated with a target gene that can be categorized into two categories -- classical markers versus DNA/molecular markers. Phenotypic, cytological, and biochemical markers belong to the classical markers category. Generally, phenotypic markers have involved distinctive seed and plant characters such as seed and plant structure, pigmentation, flower color, et cetera.

Cytological differences have involved variations of chromosome numbers, banding patterns, sizes, shapes, and meiotic affinity. Detection of gene and genotypic frequencies can be analyzed by using biochemical markers (Collard, Jahufer, Brouwer, & Pang, 2005). However, the advantages of molecular markers over morphological, cytological and biochemical markers include higher numbers of polymorphisms, and independence from effects induced by the physiological stage of the plant and related to environmental conditions, and increasing amenability to high-throughput and decreasing costs. These advantages have led to molecular markers wide usage in breeding purposes, genetic applications, and investigations into genetic diversification and relatedness between wild parents and their cultivated offspring species (Bertini et al., 2006). The types of molecular markers that have been developed in various crop plant species include restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplification of polymorphic DNAs (RAPDs), and simple sequence repeats (SSRs) or microsatellites (Varshney, Graner, & Sorrells, 2005).

RFLP was the initial molecular marker technique, and a study of heterosis and varietal origins was conducted on Upland cotton by using RFLPs first-time in 1992 (Meredith, 1992; Schlötterer, 2004). The first linkage map of cotton derived from 705 restriction fragment length polymorphism (RFLPs) loci segregating among plants of an interspecific *G. hirsutum-G. barbadense* F_2 family; linkage analysis led to the assembly of 41 linkage groups with 4675 cM length, and the study showed that 64% of cotton RFLPs were co-dominant (Reinisch et al., 1994). Because there are 26 pairs of chromosomes, it was clear that the RFLP map, by far the best at the time, was

nonetheless incomplete. An RFLP consensus map was constructed from four different mapping populations of Upland cotton that showed about 31% of the total recombination, including 1,506 cM (Ulloa et al. 2002). Development of polymerase chain reaction (PCR) enabled improvement and utilization of PCR-based markers (RAPD, AFLP, SSR, ISSR) that required fewer DNA quantities than RFLPs and no radioactivity for visualization (Gupta, Varshney, Sharma, & Ramesh, 1999). In cotton, these markers were used in numerous studies; 86 random decamer primer used in the RAPD method to assess the genetic relation and differentiation of ten varieties of *G. hirsutum* L. (Lu & Myers, 2002); AFLP markers have been employed to prove genetic diversity and develop day-neutral cultivar in cotton (Iqbal, Reddy, El-Zik, & Pepper, 2001; Pillay & Myers, 1999; Zhao et al., 2012). SSRs have widely facilitated the construction of genetic maps in cotton. At present, 98561 microsatellites are listed in CottonGen ("CottonGen," 2018; Raveendren & Reviews, 2008). The first comprehensive SSR map was reported in a study using 138 BC1 plants developed from an interspecific cross of *G. hirsutum* TM-1/*G. barbadense* Hai 7124//*G. hirsutum* TM-1. Expressed sequence tag (EST) sequences were used to create most of these SSR markers (Guo et al., 2007).

Despite microsatellites being widely distributed in the genome, highly polymorphic, and exhibiting co-dominance as markers, all of which make them useful in genetic map construction, their use in the genetic improvement of cotton has been quite limited. Some attributed this to the absence of a complete genome sequence and scarcity of DNA polymorphisms (Wang et al., 2015). The quantity of identified and mapped

SSRs was quite limited for many years, and apart from a consensus map, thus far no map composed of greater than 3500 SSR markers has been constructed from a single mapping population (Blenda et al., 2012; Yu et al., 2011; Zhao et al., 2012). However, the biggest constraint of SSRs was their lack of amenability to automatable techniques that were ultra-high throughput and low-cost (Gonzaga, Aslam, Septiningsih, & Collard, 2015; Hamblin, Warburton, & Buckler, 2007).

The development of rapid and inexpensive DNA sequencing technologies led to an extensive reduction in sequencing costs and increased capacities and speed, which facilitated the discovery of single nucleotide polymorphism (SNP) markers and led indirectly and directly to their extensive implementation in plant breeding. SNPs were found to be abundant, genome-wide in distribution, bi-allelic nature, and amenable to low-cost high-throughput genotyping (Kuang et al., 2016; Li et al., 2014). Extremely high-quality SNP genotyping was achieved in ever-increasing capacities and lower costs, notably including Illumina GoldenGate, Infinium and then Infinium II assay platforms, and more recently with genotyping-by-sequencing (GBS) with Next Generation sequencing methods (Akhunov, Nicolet, & Dvorak, 2009; Davey et al., 2011; Huang, Poland, Wight, Jackson, & Tinker, 2014; Hyten et al., 2008; Morrell, Buckler, & Ross-Ibarra, 2012).

Advantages of SNPs include the ability to examine regions under selection and neutral variation. Once a high number of annotated markers are available, diverse applications become feasible. Methods that improve results from poor quality samples extended SNPs unequaled potential for genome-level screening of a high volume of

samples (Frascaroli, Schrag, & Melchinger, 2013). Some of the rapidity with which plant breeding has been propelled forward is due to SNP markers and other advanced technologies. SNPs have allowed the creation of super-saturated genetic maps, which earlier marker systems could not achieve. Finely mapped target regions, genome-wide tracking, improved cloning of genes/QTLs of interest, and expedited marker-trait associations have all been made possible through these improved genetic maps (Mammadov et al., 2012).

The identification of assay-suitable SNPs in polyploid species is much more difficult than in diploid species due to the repetitive nature of plant genomes (Ganal, Altmann, & Roder, 2009; Van Poecke et al., 2013). Remnants of paleo-polyploidy heritage can further complicate accurate SNP identification and assay development. There are several approaches to avoid repetitiveness. The DNA sequencing field was dominated by the first-generation Sanger's sequencing method and its variants for nearly 40 years. The length of Sanger sequence reads has been steadily increased from 450 bp to 900 bp over the past ten years of research (Hebert et al., 2018; Morozova & Marra, 2008; Prober et al., 1987; Sanger, Nicklen, & Coulson, 1977; Smith et al., 1986). Modern sequencing methods have enabled the experimental discovery of SNPs via resequencing of unique amplicons and computer-simulated SNP identification. One method in silico SNP identification relied on "mining" of EST databases for discovery, followed by validation at the DNA level using PCR (Batley, Barker, O'Sullivan, Edwards, & Edwards, 2003). Gene-based SNP discovery has been achieved by using these approaches; however, the frequency of SNPs is not high in genic regions.

Therefore, these approaches were not as useful in identifying SNPs positioned in low-copy non-coding regions and intergenic spaces. The process of amplicon resequencing required more labor and considerable financial investment as well (França, Carrilho, & Kist, 2002; Ganal et al., 2009).

The advent of "Next Generation" sequencing technologies ushered forth seismic shifts in genome sequencing, assemblies, and genome informatics, and brought forth various solutions to the challenges presented by high-cost and low-throughput of SNP discovery and analysis. Infinium assays and the iScan platform by Illumina (San Diego, CA) were especially robust for high-density SNP genotyping. Massively parallel short-read sequencing by Illumina and, to a lesser extent, a few other companies, e.g., Ion Torrent products (Life Technologies Corporation, Carlsbad, CA), provide immensely parallel short-read technologies which allow routine re-sequencing of genomes. Pacific Biosciences provides long-read technologies that make the rapid development of excellent quality reference genomes possible (Morozova & Marra, 2008; Thomson, 2014). Next-generation sequencing technologies facilitate re-sequencing of complete plant genomes, subsets of those genomes, and whole transcriptomes or subsets of the transcriptomes. The depth, efficiency, and economy of these techniques are unprecedented. Instead of simply sequencing individual genomes, there is now the tangible possibility of sequencing thousands of related genomes to assess the genetic diversity even between and within germplasm resources. Thousands of genetic variants may be successfully followed within large populations due to the precision and efficiency of modern sequencing (Varshney, Nayak, May, & Jackson, 2009).

The initial successes of high-throughput SNP genotyping were due to fixed sets of SNP markers, which were assayed via microarrays. Highly multiplexed SNP detection was facilitated by Illumina BeadArrays arranged into patterned microwells. These initially used the GoldenGate assay, which employs beads coated with specific oligos that hybridize with locus and allele-specific oligos. Subsequently, allele-specific extension and fluorescent detection are performed (Shen et al., 2005). Infinium assays expanded upon the BeadArray technology by increasing the density of the arrays. The two-color single-base extension is caused by a single hybridization probe per SNP marker in these arrays. Consequently, a detailed analysis of individuals or lines is possible via such large genotyping assays (Steemers et al., 2006; Steemers & Gunderson, 2007; Yan et al., 2009). However, there are some disadvantages to these fixed arrays, which have been a staple of genotyping for the past decade. Custom SNP arrays are expensive to design, which constricts the number of re-designs available to optimize the "chip". Additionally, to be cost-effective, a large initial commitment is necessary to obtain volume discounts. Thus, “universal” design is optimal because the fixed arrays can be extensively used for a broad range of germplasms. This also spreads the development costs across a larger audience of users and can make custom SNP chip design, both useful and accessible to the larger community via a consortium model. One noteworthy challenge to the universal design is that it can rapidly become too expensive and large to cover unusual SNPs across varying germplasm groups as well as generating high numbers of monomorphic loci for groups of non-target germplasm. The usage of multiple population-specific chips limits the demand for specific chips and increases

development costs. Another disadvantage of selecting informative SNPs for various germplasm resources is the inherent introduction of ascertainment bias. The SNP variants selected will no longer represent a collection of neutral, random loci but will display a biased perspective of the genetic relationships based on which choice criteria were used for the SNPs. Unfortunately, the pricing of fixed arrays per sample has not fallen as rapidly as that of other competing technologies. Even so, the high quality, throughput, and easy-to-analyze data from a set of stable SNP loci provided by fixed arrays will probably continue to provide a valuable role in genotyping for the foreseeable future. The convenience and high quality of array data often justify their cost. In contrast to chip technology, multiple other high-throughput technologies are accessible for running single or flexible sets of SNP markers. On one end of the continuum, TaqMan® and KASP™ markers, which are PCR-based fluorescent-labeled SNP assays, can be performed on individual markers at a time and subsequently scanned via real-time PCR equipment or fluorescent plate readers. The size of the PCR reaction volume greatly affects the cost of these methods, as the reagents are very expensive. Flexible SNP genotyping systems are especially valuable for targeted genotyping because the cost per data point is low, but are too expensive for large-scale genotyping, e.g., genome-wide applications (Thomson, 2014). Fluorescence-based marker systems like TaqMan®, KASP™, and recently developed PACE and RhAMP systems also provide time-saving advantages over gel-based systems, where PACE assays lower the cost of reagents (Toth et al., 2020).

In cotton, the development of SNP detection and marker assays has been complicated and thus impeded by polyploid complexity with low genetic diversity. The presence of homeologous sequence variants (HSVs) and paralogous sequence variants (PSVs) complicates efforts to characterize and utilize SNPs because both HSVs and PSVs can found together with allelic variations (inter-varietal SNPs) (Byers et al., 2012; Chen et al., 2014). Even though these challenges have made the discovery of SNPs difficult in cotton, many *Gossypium* genome research projects have been conducted by using SNP markers. The first large-scale SNP development of SNPs in cotton resulted in 1,000 SNPs and 279 InDels, and was based on resequencing ESTs that were derived from 24 Upland cotton genotypes (Van Deynze et al., 2009). About 200 of these SNPs were mapped in the TM-1 (*G. hirsutum*) × 3-79 (*G. barbadense*) genetic map (Fang & Yu, 2012; Deynze et al., 2009; Yu et al., 2012). Furthermore, whole-genome sequencing of *G. arboreum*, *G. raimondii* and *G. hirsutum* provided the first publicly available reference genomes for the cotton genus, and a number of shotgun sequencing analyses have moreover contributed to the superior understanding of variations in the cotton genome and manipulation of fiber and agronomic traits of cotton (Li et al., 2015; Li et al., 2014; Page et al., 2013; Paterson et al., 2012; Wang et al., 2012; Zhang et al., 2015).

The recent enhancement of next-generation sequencing (NGS) has accelerated the identification of an enormous number of SNPs in cotton for the development of high-throughput genotyping. Our laboratory led an international collaboration that developed the Illumina-Infinium CottonSNP63K Array as a tool in various applications such as genome-wide studies, examination of genetic diversity of both currently cultivated

cotton species and wild species, genome characterization and as a cotton breeding tool, e.g., for genomic selection. The CottonSNP63K Array targeted 70,000 putative SNP markers that were deduced from a combination of genomic and transcriptomic sequence data. These markers represent 50,000 SNPs identified for usage in intraspecific crosses between *G. hirsutum*. The other 20,000 SNPs were included primarily for use in interspecific crosses between *G. hirsutum* and other species such as *G. tomentosum*, *G. barbadense*, *G. mustelinum, G. armourianum*, and *G. longicalyx* (Hulse-Kemp et al., 2015). Of the 70,000 designed assays, about 90% were fabricated properly, and nearly 40,000 of the assays seem to be very functional. About 30,000 of the SNP loci have been linkage mapped; nearly all have been placed onto sequence assemblies, so many of the SNP loci in the CottonSNP63K are exceptionally useful as DNA markers.

The per-sample cost of genotyping with the CottonSNP63K was originally about $100 and remains $60 - $70 depending on processing costs and can be higher, especially if fewer than 96 or even 192 samples are processed. Given the costs, the attractiveness of the CottonSNP63K as an analytical tool can be significantly diminished for studies where only limited genotypic information is needed and/or sample numbers are low, as are often the case in research and breeding. Simplex and low-plex methods are thus needed for many types of genetic and breeding research needs. Wide-cross breeding research on Upland cotton will greatly facilitate the development of suitable simplex and low-plex genotyping resources; these tools are crucial for marker-assisted selection for backcross breeding, genetic dissection, and recovery of true-breeding homozygotes. In

my thesis, research focused on establishing genome-wide spaced new simplex and low-plex genotyping assays and information resources to meet these needs.

The overall strategy was, first, to utilize existing resources, especially the linkage-mapped CottonSNP63K markers and genome sequences, as much as possible to identify SNPs that would be strategically positioned, be highly amenable to PCR, yield readily interpretable genotypic assay differences between TM-1 *G. hirsutum* versus AD-genome species being used as interspecific germplasm donors, and the respective F1 hybrids. At the outset, follow-up efforts using anticipated genome sequence assemblies for multiple AD-genome species that were being developed but not yet complete; these assemblies would be resources to use in follow-up comparisons to "fill in gaps" that might not be adequately addressed using the CottonSNP63K approach. Subsequent to the phase-I testing above of prospective assays, phase-II characterization of assays would be extended to F2 plants, to assess the clarity of graphically displayed clusters for genotyping purposes and provide an account of the behavior. In general, the expectation was that Phase-I testing would reveal markers able to serve marker-based selection needs during hybridization and backcrossing stages of introgression, i.e. when introgressed germplasm is typically in heterozygous state. In these cases, the main need is to differentiate between backcross progenies homozygous for TM-1 markers versus heterozygous for donor markers. Analogously, Phase-II testing was expected to identify assays able to meet needs at later stages of the introgression efforts, i.e., when advanced backcross hybrids are "selfed" and it is necessary to identify and select individuals

homozygous for specific segments. In these cases, one must be able to differentiate between self progenies that are homozygous versus heterozygous for donor segments.

CHAPTER II

PHASE-I: GENOME-WIDE MAP-SPACED SIMPLEX PCR-BASED SNP ASSAYS THAT DIFFERENTIATE UPLAND COTTON FROM AD-GENOME DONOR SPECIES AND F1 HYBRIDS

Introduction

The "CottonSNP63K Array" has been used extensively for intra- and inter-specific SNP genotyping and linkage mapping. Data about the SNP sequences and their linkage map positions provided the basis for the development of simplex SNP assays. As part of the array's initial assessment, all of its ~63,000 putative SNP assays were tested against an interspecific F_2 population from a cross between the two cultivated tetraploid species, *Gossypium hirsutum* (L.) and *G. barbadense* (L.). In designing the array, 20,000 of the 70,000 targeted SNPs were computationally identified from interspecific comparisons of *G. hirsutum* versus other species, versus 50,000 intraspecific comparisons. The success rate of Infinium II assay synthesis was 90% (~63,000), as is typical, and thus the array contains about 18,000 assays for putative interspecifically discerned SNP between *G. hirsutum* and non-*G. hirsutum* species (Hulse-Kemp et al., 2015). In using the array and genotyping information as a resource for the development of targeted simplex SNP assays, we targeted only SNPs that were [*i*] biallelic between *G. hirsutum* versus AD-genome germplasm donors, [*ii*] had SNP-flanking sequences that allowed the design of PCR primers suitable for KASP or PACE assays (see below) and [*iii*] could collectively provide comprehensive genome coverage with spacing at ~15 cM

or less and provide at least 10 well-spaced markers per chromosome. Thus, given the haploid genome includes 26 chromosomes, a complete set of assays would ideally include 260 or more approximately evenly spaced assays for wide-cross introgression and breeding. The mean length of the 26-linkage group is at about 148 cM.

The Kompetitive Allele Specific PCR (KASP) genotyping chemistry or 3CR Bioscience PCR Allelic Competitive Extension (PACE) chemistry served as the foundation for this study ("LGC Group," 2018; "3CR Bioscience Limited," 2019). The KASP competitive allele-specific PCR system is popular because of its relatively cheap cost of development and application for simplex genotyping of various crop species. In maize, 1,250 KASP assays have been converted and deployed in the breeding programs at the International Maize and Wheat Improvement Center (CIMMYT). Their usage includes QC analysis, marker-assisted recurrent selection (MARS), QTL mapping, and allele mining (Semagn, Babu, Hearne, & Olsen, 2014). KASP assays have been used to validate 1,052 genome-specific SNP markers in cotton, based on genomic or transcriptomic sequence comparisons; realized success rates were about 30-35% (Byers et al., 2012). Of these, 367 SNPs were afterward run on 96.96 Dynamic Arrays™ on a Fluidigm EP1™ for linkage mapping (Byers et al., 2012). The same laboratory realized success rates about twice as high for amaranth, which was attributed as likely resulting from the diploid nature of amaranth. In allopolyploid wheat, KASP assays were developed for 1114 SNP markers from a selected pool of 1659 putative varietal SNPs from two sorts of transcriptomic data (Allen et al., 2011). In the intraspecific wheat

effort, the KASP system provided about 67% conversion rate setting standard PCR conditions, which is crucial for marker-assisted selection (Allen et al., 2011).

The KASPar assay is composed of one reverse primer, two competing allele-specific primers, and a master mix that includes Taq polymerase and a FRET cassette containing quenched fluorochromes. These reagents are available in order to run on-site or as a service in LGC's automated high throughput genotyping platform ("LGC Group," 2018). KASP provides flexibility to design targeted genotyping. Recently a similar FRET-based allele-specific PCR assay, the PACE assay, has become available through 3CR Bioscience; it presents an alternative to the KASP assay. As with KASP, the PACE genotyping master mix contains Taq polymerase and a universal fluorescent reporting cassette. According to 3CR Biosciences, the benefits of using PACE over KASP include reduced non-specific amplification, just one master mix (regardless of reaction volume) and reduced expense ("3CR Bioscience Limited," 2019).

In this research, each ideal assay detected one "allele" for the recurrent Upland cotton parent (*G. hirsutum* L.) and a different "allele" that is common to all three donor species - *G. mustelinum* Miers ex Watt, *G. tomentosum* Nuttall ex Seemann, and *G. barbadense* (L.). In essence, it relies on a mutation in G. hirsutum relative to the other AD-genome species. This strategy minimized the requisite number of assays to attain a given level of coverage. In each assay, the recurrent parent allele can be differentially amplified by one of two forward primers and detected by one of two detection fluorochromes, while the common allele of the donor species can be differentially amplified by the other forward primer and detected by another fluorochrome; a third

DNA-binding fluorochrome in the PCR mastermix was used as a passive reference. After initial amplification, each round of allele-specific amplification releases the "allele"-specific fluorochrome(s) from FRET-based quenching; after sufficient rounds of PCR (usually 38+), the fluorescence was measured, and the relative level of each fluorochrome was quantified. Whether or not the sample is homozygous or heterozygous for the SNP will determine where the data point lies in relation to the other tested samples.

Well-designed SNP assays would expectedly discriminate between the inbred (homozygous) Upland cotton recurrent parent from interspecific F_1 hybrids, as well as from backcross-derived F1 (BCnF1) heterozygotes. In general, each assay would also expectedly distinguish the F_1 heterozygotes from the respective donor parents. In breeding applications, differentiation of the recurrent parent's homozygous genotype from heterozygotes would be most useful for identification of heterozygotes among segregating seed/progenies during backcross-mediated introgression process, whereas the differentiation of heterozygotes from the homozygous donor genotypes among segregating self-progenies would be of greatest use for recovering true-breeding introgression products. By design, the assays would not be expected to differentiate among donor "alleles" coming from different AD-genome donor species.

Material and Methods

Primer Design

Allele-specific primers and common primers used for the KASP or PACE reactions were designed with the software BatchPrimer3 v1.0, which is available on

several servers ("BatchPrimer3", 2019). The primer type option "allele-specific primers and allele-flanking primers" was chosen, and DNA sequences containing the SNP (ideally in IUPAC ambiguous format) were put into FASTA format as required by the software. Primer size was set to 20 bp minimum, 25 bp optimum, and 30 bp maximum. The primer temperature must be a minimum of 53 ºC, a maximum of 63 ºC, and an optimum temperature of 57 ºC. The rest of the parameters were left at default. The software generally gives four allele-specific primers and two allele-flanking primers. Two allele-specific primers of the same orientation and an allele-flanking primer in the opposite orientation are required for the KASP assays to function properly. Primers were selected based upon several factors. BatchPrimer3 output shows a Q-Score, a melting temperature, and primer size. A high Q-Score of around 85 or higher was the first priority, followed by similar melting temperatures, and then size. Once the two allele-specific primer sequences were chosen, the fluorochrome-specific "tails" were appended to the 5' end of the respective allele-specific primer sequence, i.e., GAAGGTGACCAAGTTCATGCT for FAM or GAAGGTCGGAGTCAACGGATT for HEX. The allele-flanking primer ("reverse primer") was used as defined by BatchPrimer3. Primers were synthesized by Integrated DNA Technologies and were shipped on dry ice. Once received, the individual primer components were combined and diluted to a working solution. The working stock solution consisted of 30 µL allele-flanking primer (common reverse primer), 12 µL allele-specific primer (FAM), 12 µL allele-specific primer (HEX), and 46 µL dH2O, based on instructions of KASP (LGC Ltd) and PACE user guides (3CR Bioscience).

Initial Testing of Designed Primers

Primers designed for this study were initially tested against Upland cotton (TM-1), three donor species and corresponding Upland interspecific F_1 hybrids (see **Table 1** for plant-specific identities), plus a "non-template" control (NTC):

1) *G. hirsutum* line 'Texas Marker-1', or TM-1 (Parent 1),

2) *G. barbadense* line '3-79' (Parent 2),

3) *G. mustelinum* (Parent 2), and

4) *G. tomentosum* (Parent 2), and

5) *G. barbadense* X TM-1 (F_1),

6) *G. mustelinum* X TM-1 (F_1),

7) *G. tomentosum* X TM-1 (F_1),

8) "non-template" control (NTC)

Table 1.Pedigree information on parental types and corresponding F1s.

Species	Sample
Gossypium hirsutum L. (TM-1)	1608002.02
Gossypium hirsutum L. (TM-1)	1608001.07
Gossypium barbadense L.	1608004.05
Gossypium barbadense L.	1608004.03
Gossypium mustelinum	1408121.02
Gossypium mustelinum	1408121.03
Gossypium tomentosum	7179.01
Gossypium tomentosum	7179.03
G. barbadense X TM-1 (F_1)	7181.01

Table 1. Continued

Species	Sample
G. barbadense X TM-1 (F_1)	7181.02
G. mustelinum X TM-1 (F_1)	1408123.03
G. mustelinum X TM-1 (F_1)	1408122.08
G. tomentosum X TM-1 (F_1)	1408123.05
G. tomentosum X TM-1 (F_1)	1408123.04

These plant materials came from the Stelly Lab collection at Texas A&M AgriLife Research. Small unfurled leaves were sampled from each type of plant for DNA extraction. Extracts were obtained using Synergy^{TM} 2.0 Plant DNA Extraction Kits (OPS Diagnostics LLC). DNA was quantified spectrophotometrically with a Denovix DS-11 spectrometer. To make working solutions, aliquots of quantified DNA extracts were diluted to standard 10 ng/uL prior to amplification. To facilitate consistency of subsequent fluorescence reading with the plate reader, high-quality 96-well full-skirted FrameStar PCR plates (Phenix Research) were used to house the KASP reactions. In the "Non-Template Control" (NTC), 4 µL distilled water substituted for any DNA template. Other wells contained 4 µL of template DNA at roughly 10 ng/µL, 4 µL KASP 2x Master Mix (LGC), and 0.112 µL of working solution of the primer set being tested. Amplification by PCR was accomplished by thermocycling on either a GenePro 96-well thermocycler (Hangzhou Bioer Technology Co., Ltd.) or a HC-16 multi-plate hydrocycler (LGC Ltd.). Thermal cycling protocols for the initial 38 or 40 thermal cycles are shown in **Table 2,** and for additional thermal cycles in **Table 3**. Fluorescence

readings were taken from plates using a suitable plate reader and software, namely the PHERAstarPlus scanner (BMG Labtech) and KlusterCaller software (LGC Ltd.); the software was used to visualize results and facilitate analysis.

Table 2. KASP/PACE thermal cycling conditions.

Thermal Cycling Conditions	Temperature	Duration	Cycles K	Cycles P
Activation	94 °C	15 minutes	1	1
Denaturation Annealing/Elongation	94 °C 65 → 57 °C	20 seconds 60 seconds (-0.8 °C/ cycle)	10	10
Denaturation Annealing/Elongation	94 °C 57 °C	20 seconds 60 seconds	28	30

*K is KASP and P is PACE

Table 3. KASP/PACE thermal cycling conditions for further amplification.

Thermal Cycling Conditions	Temperature	Time	Cycles
Denaturation	94 °C	20 seconds	3
Annealing/Elongation	57 °C	60 seconds	

In Silico Mining of the Genome Assemblies for Remaining Loci

To identify assembly segments from which new SNPs could be identified, CottonSNP63K marker array ID sequences (Hulse-Kemp et al. 2015) from selected regions were aligned to the Joint Genome Institute (JGI) *G. hirsutum* v2.1, JGI *G.*

barbadense v1.0, JGI *G. tomentosum* v1.0, and JGI *G. mustelinum* v1.0 sequence assemblies using BLASTn (v2.6.0+) with an e-value cutoff of e1-10. Respective marker base pair (bp) positions were then used to retrieve flanking sequences consisting of 500000 bps from their respective sequence assembly using BEDtools (v2.26.0) (Quinlan, & Hall 2010) Retrieved sequences were digested into 110 bp fasta sequences and concatenated into species-specific multi-fasta files. Multi-fasta files were then aligned to their respective sequence assemblies using Burrows Wheeler Aligner (BWA v0.7.17-r1188 using the BWA-MEM algorithm) (Abuín, Pichel, Pena, & Amigo, 2015). Inter-species SNPs were called with SAMtools (v1.7) using mpileup and filtered using BEDtools (v2.26.0) (Quinlan, & Hall 2010). Called SNPs were further aligned to their respective sequence assemblies with BLASTn using the previous parameters. Only the SNP positions concordant between both alignment programs were retained. Markers and assemblies from the haplotypic block in chromosome 8 were excluded from the above processes.

BLASTn Analysis of Assembly-derived Assays

BLASTn analysis was performed for the assembly-based assays. Common primer and forward primers sequences of the tested assembly-based assays blasted using Phytozome v13 (“Phytozome”, 2020) using respective genome assemblies of each species of *G. hirsutum* v2.1, JGI *G. barbadense* v1.0, JGI *G. tomentosum* v1.0, and JGI *G. mustelinum* v1.0.

Results

Each designed primer set was initially tested against a screening panel that featured DNAs of the recurrent parent (TM-1), three donor species (*G. mustelinum, G. barbadense, G. tomentosum*) as well as their corresponding interspecific F_1 hybrids **(Figure 1)**. In the 8x12 format (rows x columns) of the 96-well plates, the 8-row screening panel allowed up to 12 SNPs to be tested per plate. Depending on results, each assay was assessed at one or more endpoints, starting with either 38 or 40 cycles; if initial results were deemed insufficient, additional 3-cycle increments were applied, up to a possible total of 50 cycles. Many assays were retested to confirm initial findings, especially if the initial screening seemed inconsistent across species or if experimental errors were considered as possibly influential.

Based on screening panel results, each primer set was classified into one of two groups, either "Successful" **(Figure 2)** or "Failed" **(Figure 3)**. Successful assays detected codominant **(Figure 2A)** or dominant donor alleles (**Figure 2B, TM-1 dominant over null *G. mustelinum* allele).** Successful assays showed distinct clusters that allowed for distinguishing parent 1, parent 2, and F_1 genotypes while failed primer sets indicated that there was amplification but no polymorphism detection **(Figure 3A)**, or that amplification was lacking or limited and incongruous with expected genotypes **(Figure 3B)**. Results of Phase-I testing for 960 primer sets indicated that the total number of "Successful" assays was 586, for 552 of which the donor allele was co-dominant **(Table A1)** and 34 were recessive to TM-1 or null allele **(Table A2).**

Well (row)	Sample Designation	Sample Source	Type of Sample
A	Gh	*G. hirsutum* (Gh)	Parent 1 (recurrent)
B	Gb	*G. barbadense* (Gb)	Parent 2 (donor)
C	Gm	*G. mustelinum* (Gm)	Parent 2 (donor)
D	Gt	*G. tomentosum* (Gt)	Parent 2 (donor)
E	Gb-F1	(Gb x Gh)F_1	Hybrid (F_1)
F	Gm-F1	(Gm x Gh)F_1	Hybrid (F_1)
G	Gt-F1	(Gt x Gh)F_1	Hybrid (F_1)
H	NTC*	-	Water
*Gb, Gm, Gt and Gh represents *G barbadense, G. mustelinum, G. tomentosum and G. hirsutum* respectively.			

Figure 1. Screening panel for initial evaluation of KASP/PACE primer sets. The screening panel included seven DNA samples and a non-template control (NTC). The DNAs were from the recurrent parent (Parent 1), three donor parents (Parent 2), three corresponding F1 hybrids samples (F_1).

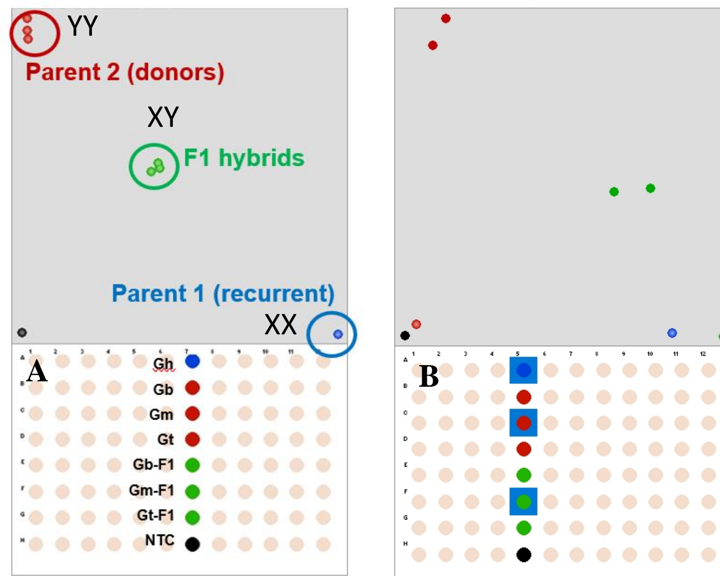

Figure 2. Two examples of "Successful" primer sets, one exhibiting species specificity. At left, an assay yielding exemplary results - three well-separated genotype-specific clusters of signals, with the donor parent cluster at one corner and the recurrent parent along the opposing corner, and the cluster of interspecific F1 hybrids positioned midway between the parental clusters; no signal occurred in the non-template control (NTC) **(A.)**. At right, a species-specific assay that was successful with *G. barbadense* and *G. tomentosum*, but *G. mustelinum* lacked signal **(B)**.

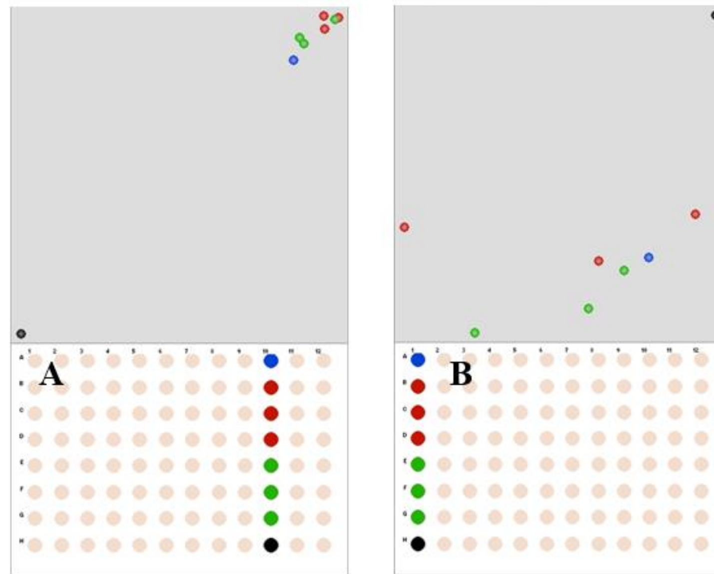

Figure 3. Examples of "Failed" primer sets. At left, example of strong amplification but no detectable polymorphism **(A)**. At right, example of assay with amplification that was variable and nonspecific to the genotypes **(B).**

The ability of each assay to differentiate P1, P2, and Het genotypes was recorded and characterized for each of the three donor species; rating were assigned and used to select assays that seem likely to be most useful. Ultimately, comprehensive genome coverage was desired with spacing less than or equal to ~15 cM based on the high-density CottonSNP63K-based F_2 interspecific linkage map (Hulse-Kemp et al., 2015). A total of 960 primer sets were tested based on linkage-mapped loci represented in the CottonSNP63K array (Hulse-Kemp et al. 2015). The screening tests yielded 552 "Successful" primer sets **(Table A1)** that enabled functional simplex SNP assays at ~371 positions **(Figure 4)**. Thus, more than one functional assay is potentially available at or near some locations. The ~371 locations are approximately evenly spaced across genome

according to the interspecific *G. barbadense* - *G. hirsutum* F_2 linkage map of Hulse-Kemp et al. (2015), with a 10.26 cM mean distance of separation. Based on that map, the spacing between neighboring pairs of simplex-assayable loci is less than or equal to ~15 cM. A histogram depicting the distribution of distances between the spaced assays provided a visual overview of coverage density **(Figure 5)**. A minimum of 10 markers per chromosome was successfully assayed with those specific primer sets. Another goal was to create simplex assays for loci near the ends of the chromosomes, i.e., for a SNP within 2 cM of each terminal SNP in each linkage group **(Table 4)**. Using CottonSNP63K-based information, successful primer sets were identified near 39 of the 52 total linkage-group termini, but for 13 linkage groups, the closest simplex-assayable locus for one terminus remained larger than 2 cM. After seemingly exhausting CottonSNP63K-based resources for KASP/PACE simplex assay development at targeted locations, there remain a total of 33 interstitial segments for which neighboring simplex-assayable loci are separated by more than 15 cM according to the interspecific map of Hulse-Kemp (2015) **(Figure 4)**. However, the 33 includes 10 segments for which alternative information will be required. For the other 19 segments and 4 termini location, perspective assays were identified using the CottonSNP63K, but we discovered after testing that the assays were mistakenly targeting unlinked loci so, efforts are underway to reorder and retest previously identified assays for these 19 segments, and 4 termini location.

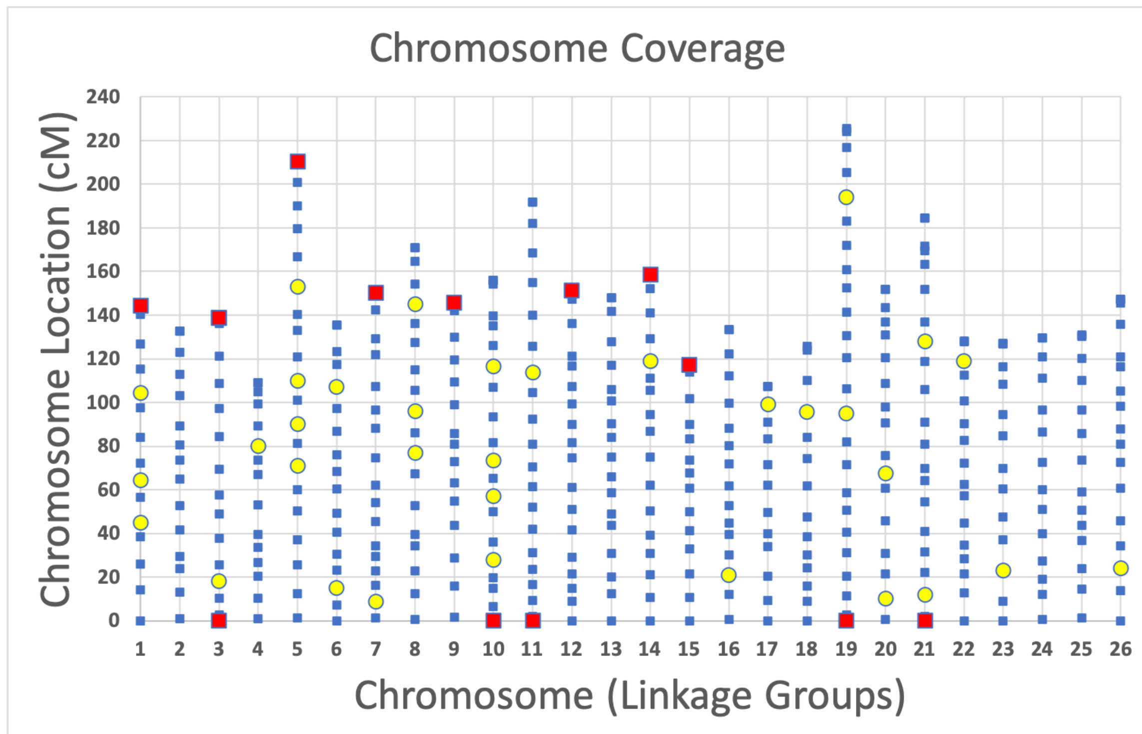

Figure 4. Linkage map positions of 371 KASP/PACE simplex-assayable SNP loci. Blue squares denote lower-to-upper marker spacing less than or equal ~15-cM. Yellow circles (33) indicate positions of 33 interstitial 15-cM or larger segments lacking an assay. Large red squares denote positions of 13 linkage group termini from which the nearest simplex-assayable SNP is farther than 2 cM.

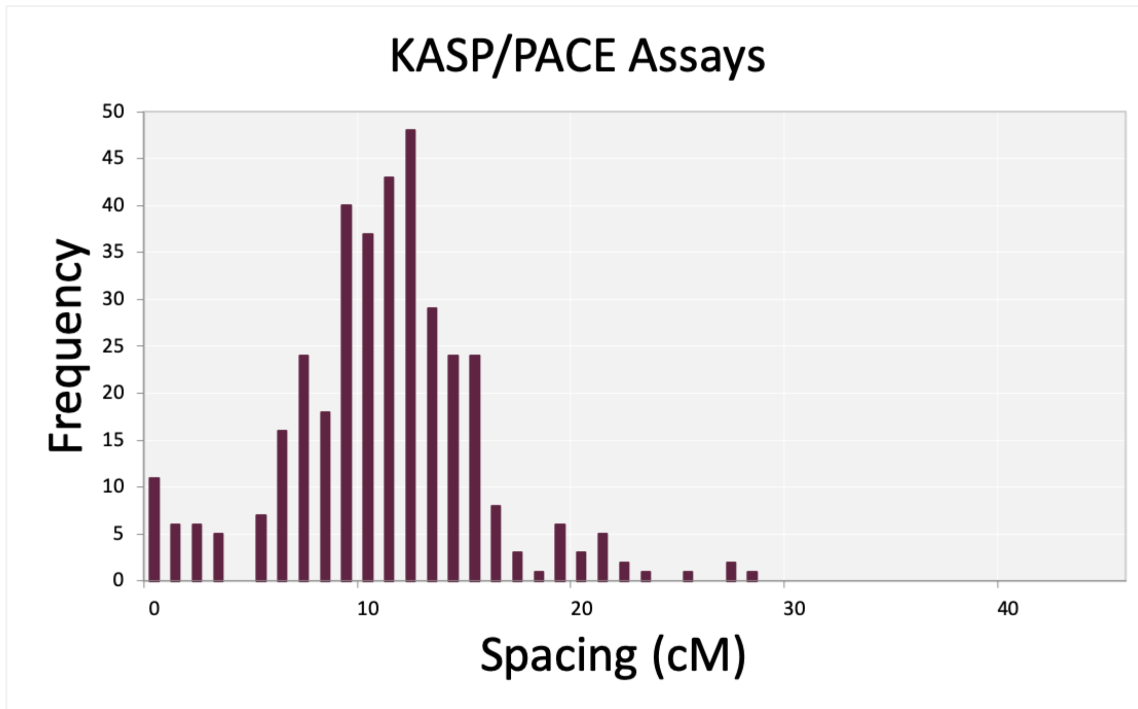

Figure 5. Distributions of distances between spaced assays. The figure provides coverage of density of KASP/PACE assays.

Table 4. Map distances between KASP/PACE-Assayable SNP loci versus both terminal markers of each CottonSNP63K-based linkage group.

Chromosome	Distance (cM)* from LG Origin	Length of LG (cM)*	Position (cM) of Distal-most SNP Assay	Distance (cM) from LG Terminus
1	0.00	144.31	140.47	3.84
2	0.85	132.73	132.73	0.00
3	2.56	138.70	136.14	2.56
4	0.43	109.21	109.21	0.00
5	1.28	210.35	202.67	7.68

Table 4. Continued

Chromosome	Distance (cM)* from LG Origin	Length of LG (cM)*	Position (cM) of Distal-most SNP Assay	Distance (cM) from LG Terminus
6	0.00	136.59	135.31	1.28
7	1.28	150.15	142.45	7.70
8	0.43	171.04	171.04	0.00
9	0.43	145.58	142.17	3.41
10	6.44	155.85	155.85	0.00
11	2.14	191.74	191.74	0.00
12	0.00	151.46	147.19	4.27
13	0.00	148.15	148.15	0.00
14	0.00	158.73	152.23	6.50
15	0.00	117.18	113.76	3.42
16	0.43	133.50	133.50	0.00
17	0.00	107.30	107.30	0.00
18	0.00	125.61	125.18	0.43
19	2.56	225.38	224.10	1.28
20	0.43	151.73	151.73	0.00
21	2.13	184.37	184.37	0.00
22	0.00	128.11	127.68	0.43
23	0.00	127.07	126.64	0.43
24	0.00	129.50	129.50	0.00
25	1.28	130.85	130.42	0.43
26	0.00	147.36	147.36	0.00

* Linkage group lengths according to Hulse-Kemp et al. (2015).

Mining Genome Assemblies to SNPs Assays at Remaining Loci

The work described above was based on linkage-mapped SNPs contained in the CottonSNP63K array, and demonstrated fairly complete cover the AD genome with spaced simplex assays that could differentiate between hybrid heterozygotes versus parental homozygous genotypes. Additional SNP coverage was needed to address the 10 interstitial segments longer than 15 cM and the 9 terminal segments longer than 2 cM. For each segment, the number of markers needed depended on the segment length. For interstitial segments over 15 cM, at least one more assay would be needed to bisect the segment and solve the issue; segments over 30 cM, two or more additional simplex assays would be needed to render all inter-assay segments under 15 cM. The DNA sequences for these segments were delimited from genome assemblies relative to the sequences of segment-relevant SNP markers in the *G. barbadense* - *G. hirsutum* F2 linkage map (Hulse-Kemp et al., 2015). *In silico* mining of the assemblies for *G. hirsutum*, *G. barbadense*, *G. tomentosum*, and *G. mustelinum* was conducted genome discovery.

Once SNP sequences were acquired, the closest bp position sequences were selected based on targeted remaining regions. Primers were designed using the parameters previously mentioned for KASP/PACE assays.

A batch of 14 primer sets from assembly-based SNP identification and selection were tested initially, of which only 4 (28%) failed completely. Fully successful assays were observed for 5 (~36%), i.e., observable codominance when tested with the aforementioned screening panel of parental species and three interspecific F1 hybrids.

Among the remaining 5, 4 (29%) worked well with two donors, 1 (7%) with just one donor or more species. Given a total of 14 x 3 = 42 potential assays across the three species, the collective success rate was (5x3)+(4x2)+(1x1)=24, or ~57%. Detailed information for the 14 tested markers, including linkage map and assembly position of targeted remaining loci as well as primer sequences are noted in **Table 5**.

Table 5. Chromosomal, linkage map, and assembly locations of 14 SNPs that were identified and targeted for KASP/PACE assays development based on assembly-referenced positioning and interspecific sequence alignment comparisons.

Chr. *	Map Position (cM)	Assembly Position (bp)	Common Reverse Primer	Allele-specific Primer	Allele-specific Primer	Assay Applicability **
1	143.88	38838	TATTCTC TATTACA TTGATTC ATCC	ATAAAA ACATTAC CGTGCA ATACG	ATAAAA ACATTAC CGTGCA ATACA	Fail
1	45.89	109714781	TGCTTAG AAACAT GTAGTA AAAGAT	GCTATTG TGTCTAT TGAGCA GTTTA	GCTATTG TGTCTAT TGAGCA GTTTT	Gm, Gt

Table 5. Continued

Chr. *	Map Position (cM)	Assembly Position (bp)	Common Reverse Primer	Allele-specific Primer	Allele-specific Primer	Assay Applicability **
3	138.7	80187	AGAAGAAGCATCACAATTACAT	CAAGATGGTTGGAAATAAAATCA	AAGATGGTTGGAAATAAAATCC	Gb, Gm, Gt
7	13.73	96939591	CTTTCCAGAGTTCTGCCGTTT	TTCTCAATGTCAGAGTAAATCCAC	TTCTCAATGTCAGAGTAAATCCAT	Fail
12	151.46	109427049	GAAAACCTTACCCCAATCTGATCTA	TAACCTAACGAGTCAGCAAGAAA	TAACCTAACGAGTCAGCAAGAAC	Fail
14	158.73	385	AACAAAAATAAAACAGAAACACCA	TCAAAATTTTTAGCTTTGGGTAAT	TCAAAATTTTTAGCTTTGGGTAAC	Gb, Gm
15	117.18	19693	TCATTCAATCAATAAAATAAATGGAAC	TAATTGTGAAAGCTGGTATTGTCA	TAATTGTGAAAGCTGGTATTGTCT	Gb, Gm

Table 5. Continued

Chr. *	Map Position (cM)	Assembly Position (bp)	Common Reverse Primer	Allele-specific Primer	Allele-specific Primer	Assay Applicability **
16	20.17	54974991	ATGAGGATAATGAAGACTTTGTAGA	GTAATGCCTTTACCTTTGTCTTTT	GTAATGCCTTTACCTTTGTCTTTG	Gb, Gm, Gt
19	81.86	35402201	TCCTTTGAGATTTGTCCTTTGA	CAGTCATCAATCACTTAACATCTACA	CAGTCATCAATCACTTAACATCTACT	Gb, Gm, Gt
20	67.49	53843333	CTAGACTTAGTGCAACACAAAAATG	GTTTTAACATTTGAAGTTCTCGC	ACGTTTTAACATTTGAAGTTCTCGT	Gb, Gm
21	124.26	54237088	AGGAGACTGGGAGGATTATGA	TAAGCAAAGAAACTACACCCTTCAT	TAAGCAAAGAAACTACACCCTTCAC	Gb
22	120.42	56827793	TCATTTTAGCGAATTTAATTGAG	AAACACAGTTATAAGAATGGAACCT	AACACAGTTATAAGAATGGAACCC	Fail

Table 5. Continued

Chr. *	Map Position (cM)	Assembly Position (bp)	Common Reverse Primer	Allele-specific Primer	Allele-specific Primer	Assay Applicability **
26	22.64	2013818	TTCCAAGCAACCAAAAGAATTAC	TCATCTGTTGAAACTACTGATCTA	TTCATCTGTTGAAACTACTGATCTT	Gb, Gm, Gt
26	112.74	58328384	AGAAAACTAAGTCCCATGAACC	TTTTACTAGAAGGGAACCTAACCA	TTTACTAGAAGGGAACCTAACCG	Gb, Gm, Gt

*Chr = Chromosome. Map Position = *Gossypium hirsutum - G. barbadense* linkage map positions (Hulse-Kemp et al. 2015. Assembly Position = base pair position in respective chromosome assembly in *G. hirsutum* v2.1 (Chen et al. 2020), available at CottonGen or JGI Phytozome v13 websites.
**Gm, Gt, and/or Gb indicate that an assay detects the donor allele of *G. mustelinum*, *G. tomentosum* and/or *G. barbadense*.

To better understand why 14 markers had different working status, their reverse primer and forward primers sequences blasted using Phytozome v13 online tools ("Phytozome", 2020). Failed markers either hit on different chromosomes (chromosome 1, bp-38838, chromosome 12, 109427049) or hit their homeologous chromosome as well but not having reverse primer or one of the allele-specific primers (chromosome 7, bp-96939591 or chromosome 22, bp-56827793).

Discussion

High-throughput genotyping is now possible with various genotyping systems. They enable powerful improvements in many plant breeding research applications, but in many others, they are not applicable, affordable, and/or suitable. Thus, there are many situations where SNPs genotyping at one location or in limited numbers of targeted regions is preferred and/or more cost-effective. KASP and PACE markers provide opportunities for running PCR-based fluorescent labeled SNP assays at relatively low cost. Inexpensive crude DNA extractions can often suffice, eliminating the need for laborious or expensive methods to extract high-quality DNA. The cost of individual assays is based largely on by the size of PCR reaction volume (Semagn, Babu, Hearne, & Olsen, 2014; Toth et al., 2020), but other factors beyond DNA extraction are significant too, e.g., plate costs, so assays per plate and cost per plate.

In this study, a genome-wide set of 586 functional simplex assays were developed using KASP and PACE chemistry. Phase-I testing showed codominance of 552 assay markers for all three donor species and their F1 hybrids, *i.e.*, they successfully resolved P1, Heterozygote (Het), P2 genotypes. Of the 552 assays, 371 were chosen to target 371 loci that are approximately evenly spaced according to linkage estimates. Thus, all of these 371 assays are able to distinguish *G. hirsutum* L. from the AD-genome species *G. barbadense, G. mustelinum and G. tomentosum* and their corresponding F1hybrids with Upland cotton. In addition to the 552 assays, 34 assays were found to be similarly functional for one or two donor AD-genomes, but exhibited a recessive donor marker or null allele for the other AD species.

These assays will be especially useful for germplasm introgression into the most important cotton, i.e., Upland cotton. While backcrossing, the assays can be used to detect and track donor segments. Because most of the assays are directly related to the CottonSNP63K array, all individual SNP and segment-specific results can be related to the CottonSNP63K-based linkage maps presented (Hulse-Kemp et al., 2015). Also, these simplex assays are suitable to use individually or low-plex formats and will be useful for diverse breeding and genetic research applications, e.g., transmission analysis, recovery of segregates in heterozygous or hemizygous states, or recombination research.

The pipeline was developed from new genome assemblies to provide primers for the region, which was not yielded by Cotton63K array (Hulse-Kemp et al., 2015). This pipeline showed overall ~71% success when it was thought five markers exhibited desired results with distinct clusters between P1, P2, and their F1s, and another set of five markers were useful for one or more species. However, more primers should be tested for developing this pipeline considering BLASTn results for reverse and forward allele-specific primers.

CHAPTER III

PHASE-II: MAP-SPACED SNP ASSAY PERFORMANCE ACROSS F2 POPULATIONS FROM *G. HIRSUTUM* L. HYBRIDIZED WITH *G. MUSTELINUM* MIERS EX WATT, *G. TOMENTOSUM* NUTTALL EX SEEMANN AND *G. BARBADENSE* (L.)

Introduction

Genetic or sequence-based marker systems can be employed for multiple purposes in research and breeding, and so a given use can entail one or any of a number of different capabilities. Thus, a given assay may be useful for some applications, but not others. In Phase-I of this project, KASP/PACE assay primer sets were screened against DNAs of four species and three interspecific F_1 hybrids. The Phase-I observations enabled selection among prospective SNP assays for the ability to differentiate *G. hirsutum* Upland (TM-1) from the other three tested AD species, the three F1 heterozygotes from the Upland (TM-1) homozygote, the three F1 heterozygotes from the respective AD-species homozygote, conformity of assay results across the three species (i.e., preferably *not* to differentiate among the three), and across the three F1 interspecific F1 hybrids (i.e., also preferably *not* to differentiate among the three). On the presumption that each species was homozygous, the ability of an assay to differentiate TM-1 from interspecific F1 hybrids would expectedly predict an ability to select heterozygotes segregated among backcross hybrid seed or plants, e.g., for marker-based or marker-assisted backcrossing phases of backcross-inbred breeding.

In contrast to Phase-I screening, a major goal of Phase-II screening would be to determine the expected ability of each assay for effective selection of homozygotes (or heterozygotes) among self-progenies, e.g., F_2 hybrids or backcross-inbreds (BC_nS_m). The challenge was to further assess each of hundreds of prospective SNP assays that passed Phase-I screening across segregating germplasm from three different donor species and do so in a financially frugal manner. The approach implemented in Phase-II was to screen the prospective assays against DNAs of three small interspecific F_2 populations, each involving Upland TM-1 crossed with a different AD-genome species, such that the resulting positions for all segregated genotypes could be compared across and within populations. Moreover, we employed a low-plex microfluidics platform to economize the screening. And to enable validation of at least some results from the microfluidics platform, some F2 samples were chosen because they had previously been genotyped with Infinium II assays of the CottonSNP63K array (Hulse-Kemp et al. 2015). As part of the assessment, we evaluated each assay and each individual to identify those yielding no or misleading data, such that they could be removed. Among the features assessed were cluster positioning, tightness, scorability (membership of datapoint to a given cluster).

Material and Methods

Plant Material

In Phase-II assessment, the simplex SNP assays were tested against 88 F_2 individuals comprising three individually small F_2 populations derived from crosses between TM-1 with *G. barbadense*, *G. mustelinum* or *G. tomentosum*, each with close to

30 F_2 individuals. Members of the *G. barbadense* F_2 population were genotyped previously using the CottonSNP63K, using remnant DNA samples **(Table 6)**. In contrast, the *G. mustelinum* and *G. tomentosum* F_2 populations were created anew. They were established from seed that had been produced previously based on open pollination of greenhouse-grown interspecific F1 plants during a winter season, i.e., free of cross-pollinating insects. They included 36 *G. mustelinum* X TM-1 F2 hybrids and 40 *G. tomentosum* X TM-1 F2 hybrids **(Table 7)**.

Table 6. Composition of the *Gossypium barbadense* x *Gossypium hirsutum* (TM-1) F2 population and characterization of remnant DNA samples from previous CottonSNP63K-based genotyping (Hulse-Kemp et al. 2015).

Sample Number	Plant Source	DNA Concentraration (ng/µL)	Absorbance Ratios (nm/nm) 260/280	Absorbance Ratios (nm/nm) 260/230
1	7146.13	257.51	1.8	1.9
2	7146.14	340.79	1.8	1.8
3	7146.52	252.48	1.9	1.8
4	7146.57	263.16	1.9	1.9
5	7146.61	251.03	1.9	1.9
6	7146.62	278.53	1.8	1.8
7	7146.63	277.67	1.9	1.9
8	7146.69	296.86	1.9	1.9
9	7146.70	317.95	1.9	1.9
10	7146.81	258.4	1.8	1.8
11	7146.82	375.11	1.8	1.8

Table 6. Continued

Sample Number	Plant Source	DNA Concentraration (ng/µL)	Absorbance Ratios (nm/nm) 260/280	Absorbance Ratios (nm/nm) 260/230
12	7146.83	291.35	1.8	1.8
13	7146.84	285.01	1.9	2.1
14	7146.88	258.78	1.9	1.9
15	7146.94	345.09	1.8	1.9
16	7146.97	235.18	1.8	1.9
17	7146.98	318.55	1.8	1.9
18	7147.04	462.12	1.8	2.0
19	7147.06	253.21	1.8	1.8
20	7147.08	309.04	1.8	2.0
21	7147.09	302.54	1.8	1.9
22	7147.10	757.05	1.8	1.8
23	7147.11	530.45	1.8	2.0
24	7147.12	281.99	1.8	1.8
25	7147.13	333.38	1.8	1.9
26	7147.14	472.98	1.8	1.9
27	7147.15	269.54	1.8	1.9
28	7147.17	326.87	1.8	1.9
29	7147.19	314.06	1.8	1.8

Table 7. Compositions of the *Gossypium mustelinum and G. tomentosum* F2 populations and F1 parent plant identities.

F2	F2 Identities	Parental F1	Origin of F1
1-5	7208.001-7208.005	200308064.10	(TM-1 x *G. mustelinum*)BC0F1

Table 7. Continued

F2	F2 Identities	Parental F1	Origin of F1
6-14	7208.006-7208.014	200308065.02	(*G. mustelinum* x TM-1)BC0F1
15-24	7208.015-7208.024	200308065.03	(*G. mustelinum x* TM-1)BC0F1
25-30	7208.025-7208.030	200308065.04	(*G. mustelinum* x TM-1)BC0F1
31-36	7208.031-7208.036	200308065.06	(*G. mustelinum* x TM-1)BC0F1
37-61	7208.037-7208.61	201808013.01	(TM-1 x *G. tomentosum*)F1
62-76	7208.062-7208.076	201808012.10	(TM-1 x *G. tomentosum*)F1

DNA Extraction and Quality Control

Young leaf tissues were collected from individual plants, and their genomic DNAs were extracted according to the 96-Well Synergy™ Plant DNA Extraction Kit (OPS Diagnostics LLC) protocol. DNA of each extract was quantified using QuBit procedure at TAMU Texas Institute for Genome Sciences and Society (TIGSS) laboratory.

SNP Assay Screening

KASP/PACE assays were run using the Fluidigm microfluidic 96.96 Dynamic Arrays™ on a BioMark™, which is available at the TIGSS laboratory. Eight samples were allocated to the controls - 4 parents, 3 F_1 hybrids and a non-template control (NTC). A total of 88 F_2s were analyzed, comprising samples of ~28-30 F_2 individuals from each of the same three respective interspecific combinations. The Fluidigm 96.96 Dynamic Arrays™ chip was prepared for genotyping of each sample (sample maps are listed in **Table A3**), including sample mix, containing 2.5 µL PACE reagent mix ("3CR

Bioscience Limited," 2019), 0.25 µL GT sample loading reagent (20X), 0.16 µL DNase-free water, and 2.09 µL (a concentration of 70 ng/µL) genomic DNA. Also, 4 µL of 10X assay mix was prepared for each SNP assay, including 2.0 µL 2X Assay Loading Reagent, 1.44 µL DNase-free water, and 0.56 PACE assay primer mix (30 µL common reverse primer and 12 µL allele-specific primers). Before loading assay mix and sample mix into chip inlets, control line fluid M96 was injected into each accumulator on the integrated fluid circuit (IFC), then the prime script (138x) was run in order to prime the control line using IFC controller software HX. After that, assay mix and sample mix were pipetted into chip inlets, and then chip was loaded using the load mix script (138x) so that samples and assays mixed into IFC by IFC HX. Reads were acquired using Fluidigm Biomark HD based on thermal cycling protocol with enough rounds of PCR (**Table 8**). Clustering results were assessed using Fluidigm SNP Genotyping Analysis Software ("Fluidigm," 2018).

Table 8. Thermal cycling conditions for 96.96 Dynamic Arrays™ on a Fluidigm BioMark™

Thermal Cycling Conditions	Temperature	Time	Number of Cycles
Thermal Mix	70 ^{o}C 25 ^{o}C 25 ^{o}C	30 minutes 5 minutes 5 minutes	1
Hot Start	94 ^{o}C	15 minutes	1

Table 8. Continued

Thermal Cycling Conditions	Temperature	Time	Number of Cycles
Touchdown (decreasing 0.8 ^oC per cycle starting from 65 ^oC with a final temperature of 57.8)	94 ^oC 65-57.8 ^oC	20 seconds 1 minute	10
Additional PCR Cycle	94 ^oC 57 ^oC	20 seconds 1 minute	26
Cool*	20 ^oC	1 minute	1
Denaturation* Annealing*	94 ^oC 57 ^oC	20 seconds 1 minute	3
*Repeat the cooling stage (1 cycle) and denaturation/annealing stage (3 cycles) for the desired total number of cycles.			

Recombination Rate Analysis

A maximum-likelihood method was used to estimate the recombination rate following the methods of Lorieux (1995). Loci that were targeted by assays that "failed" (63), resulted in "no-call" (4) or lacked codominance or performed differently in between wild species (38) were excluded from the analysis (total of 105 exclusions). Considering two dominant markers in coupling phase AB/ab X AB/ab, nine genotypic frequencies groups were projected in terms of SNP allele combinations **(Table 9)**. Observed genotypic frequencies numbers were calculated based on the F_2 genotypic

data. These data were converted into Parent-1(XX or YY genotypes based on HEX or FAM dye) as “TM-1”, Parent 2 (XX or YY genotypes based on HEX or FAM dye) as “Wild”, and heterozygous (XY genotype based on both FAM and HEX dye) as “Het”. With this information, nine genotypic frequency group numbers were calculated for each assay, and then the recombination rate fractions were estimated using maximum likelihood estimation (MLE) (Lorieux, 1995):

$$\frac{\partial L}{\partial r} = (a+i)\frac{2}{r-1} + (b+d+f+h)\frac{1-2r}{r(1-r)} + (c+g)\frac{2}{r} + e\frac{4r-2}{1+2r^2-2r} = 0.$$

To approximate the best estimation of recombination rate, values were assigned to “*r*” in small increments starting from 0.01 (increasing 0.01) and ending with 0.50, in search of a value of “0” for the derivative equation above.

Table 9. Designated parameters for specific genotypic combinations in an F_2 population from a parent with two codominant loci in coupling phase, i.e., A and B. For loci in repulsion, *r* is replaced by 1-*r*.

Genotypes	Observed Frequencies	SNP_A	SNP_B
AABB	*a*	TM-1	TM-1
AABb	*b*	TM-1	Het
AAbb	*c*	TM-1	Wild
AaBB	*d*	Het	TM-1
AaBb	*e*	Het	Het
Aabb	*f*	Het	Wild
aaBB	*g*	Wild	TM-1
aaBb	*h*	Wild	Het
aabb	*i*	Wild	Wild

Characterization of Genotypic Cluster Tightness and Separation

Microfluidic array data were used to graphically assess and numerically characterize the F2 performance of each assay, overall and at the species level. To enable potential users of the assays to gauge the applicability of each assay their research or breeding needs, each assay was rated separately "cluster scorability" and "tightness", where "scorability" largely reflects relative position and separation of genotypic clusters, as opposed to "tightness", which reflects dispersion among data points within a cluster. These ratings were extended to specific clusters (tightness) and specific pairwise cluster-to-cluster comparisons (scorability). Note that in some cases, these characteristics differed according among the three F2 populations, i.e., by donor species.

Cluster tightness was rated 1 to 5 for each cluster (typically three), i.e., P1, heterozygous and P2, according to the following criteria: "1"= perfect (nearly ideal), "2"=good (should be reliable in virtually all applications, but less robust than ideal), "3"=okay (should be usable and reliable in all or most applications, but perhaps prone to occasional error?), "4"=usable but not accurate for some genotypes (implying that detailed information should be consulted before use), "5"=unreliable.

Cluster scorability was characterized and rated 1 to 5 similarly for each of two comparisons:

1) the distinctiveness of the Parent-1 (P1) cluster containing TM-1 and nearby F2 segregates (presumptively similar homozygotes) versus the middle cluster (Het) that contained F1 hybrids, and nearby F2 segregates (presumptively similar heterozygotes), and

2) the distinctiveness of the Parent-2 (P2) cluster containing the species parents and presumptively similar F2 homozygotes versus the middle cluster (Het) that contained F1 hybrids and presumptive heterozygous F2s.

Results

Microfluidics Platform

A set of 576KASP/PACE assays was run on using Fluidigm 96.96 array system to genotype 88 F_2s individuals, 4 parents, 3 F1s, and non-template controls (NTCs). A total of six Fluidigm arrays were run. The first three arrays were each run total of 51 cycles; the last three arrays were each run a total of 57 cycles. An NTC was omitted from the first array but included in the subsequent five arrays.

A total of 55,296 genotyping data points were generated using the microfluidics platform and Fluidigm 96.96 IFC arrays. Numerical data were based on the Fluidigm SNP Genotyping Analysis Software. A total of 123 markers out of 576 markers were genotyped twice to assess the repeatability of the results. The fifth and sixth arrays included the retested SNPs. Overall, 453 different assays were evaluated based on the Fluidigm arrays. Four assays did not give any call due to the issues most associated with loading IFC. Of the remaining 449, 63 (14%) assays failed to give signal distributions that follow readily discerned simple genotypic clustering, and 386 (86%) assays worked completely or partially, as is described in more detail below.

The individual plant genotyping results were checked for each F2 across all 453 assays, and the frequency of "no-call" results was determined for each F2. Of the 88 F_2s,

83 F2s (94%) had very low "no-call" rates (0.88 to 3.31%), whereas 3 F2 individuals exhibited moderate "no-call" rates (4.19 to 5.52%), and two *G. mustelinum* F2s, “7208.001” and “7208.020”, had significant "no-call" rates of 8.17% and 24.06% respectively. Based on these results, data from the latter two individuals were excluded from analyses.

Genotypic Cluster Tightness and Separation

Based on the overall F2-based testing of 453 assays, 63 (14%) failed completely, and 4 (<1%) did not give any call, and 317 (70.1%) worked across F2 germplasm from all three species, albeit to varying degrees, with scorability ratings ranging from 1-4. The results were quite varied among the 317 functional assays, and some were deemed less than ideal for F2-like analyses. "Scorability" and "tightness" ratings for some assays were more or less uniform across all three species, but some assays exhibited species-specific differences. **Table 10** summarizes rating data on cluster scorability and cluster tightness for overall 449 assays except for 4 assays that yielded no call data. The species-specific variability of some assays is discernible in the ratings, and additional details are provided in tabulated annotations and in recorded graphics. To illustrate this variation and help describe the numerical ratings and annotations, several examples are detailed below.

A graphic depiction of results for an assay that yielded highly desirable results is shown in **Figure 6**; ratings for this assay were perfect, i.e., cluster tightness (rating = 1) and scorability (rating = 1), reflecting the expectation of a high degree of facility, robustness, and usefulness in diverse applications.

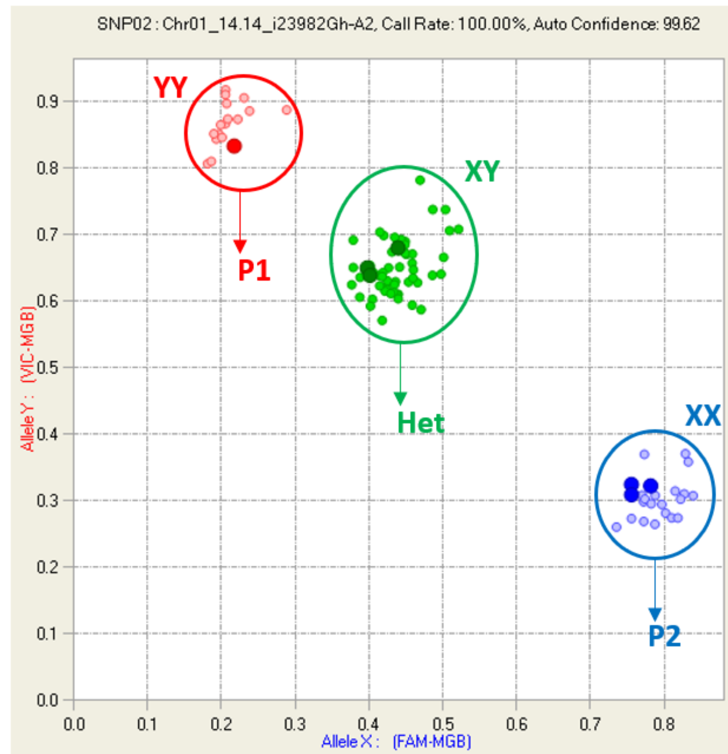

Figure 6. Cartesian plot of F2 fluorescence results for a high-performing SNP assay. Results were uniformly good for all three donor species, such that this marker (i23982) received perfect ratings, i.e., "1", for cluster scorability of both comparisons (P1-Het, and P2-Het) and cluster tightness of all three clusters (P1, Het, P2).

In addition to the 317 functional assays mentioned above, 17 markers (3.8%) worked well with one or more species, but not all three, as exemplified for chromosome-10 marker i00730 in **Figure 7**. The *G. barbadense* and *G. tomentosum* alleles function codominantly, whereas the allele of *G. mustelinum* functions recessively, with a 23:0:6 F2 segregation. Another 38 markers (8.4%) allowed accurate identification of homozygotes in one or more donor genotypes (P2) but, like i00730 for *G. mustelinum*, did not separate any of the heterozygous genotypes from Parent-1 as exemplified by

chromosome-1 marker i50196 (**Figure 8**). Another group comprising 14 markers (3.1%) was characterized by the existence of sub-clusters for one or more species, as exemplified by chromosome-17 marker i00195 (**Figure 9**). Thus, 386 assays (85.2 %) were partially to completely successful for the analyses of one or more these three AD-species interspecific F2 populations **(Table 11);** also see **Table A4.** Detailed ratings and annotations of the all markers is provided in **Table A5**.

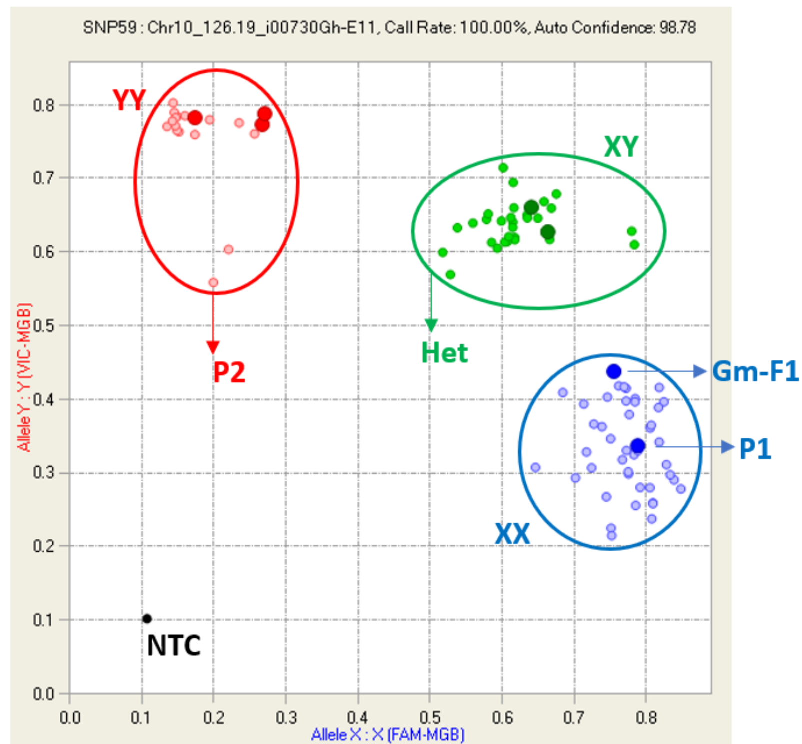

Figure 7. Cartesian plot of F2 fluorescence data for a SNP assay exhibiting species-specific behavior. Marker i00730 assays yielded readily interpreted codominant distributions for *G. tomentosum* and *G. barbadense*. The locations of the Gm-F1 (interspecific F1 hybrid of *G. mustelinum* X *G. hirsutum*) and 23 F2 segregates co-located in the P1 cluster with *G. hirsutum*, while 6 co-located in the P2 cluster, indicating recessiveness of the Gm allele relative to the P1 allele.

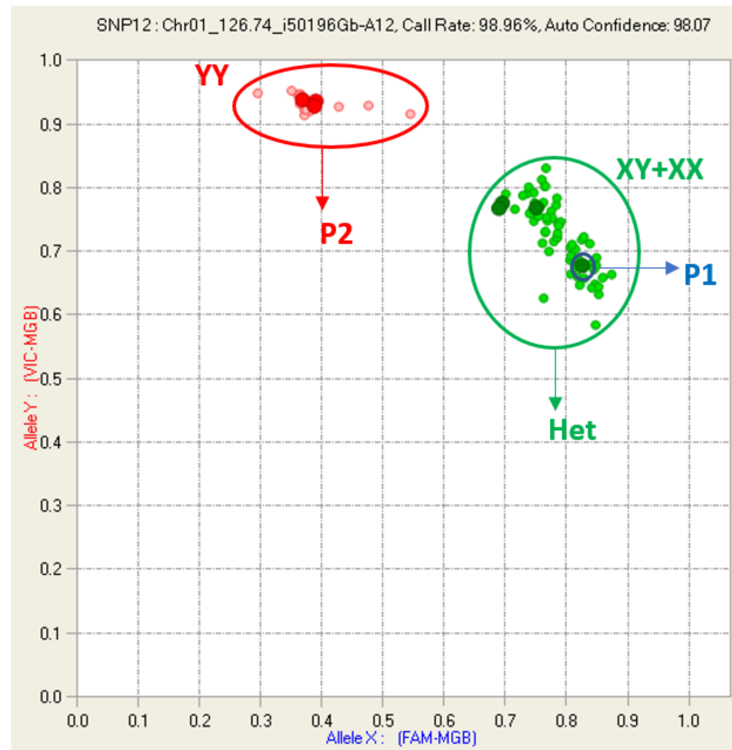

Figure 8. Cartesian plot of F2 fluorescence data for a SNP assay exhibiting inadequate separation of Het genotypes from P1 (TM-1). Marker i50196 assay yielded a P2 cluster well separated from heterozygous and P1 clusters, but the separation between the latter was insufficient for comprehensive genotyping**.**

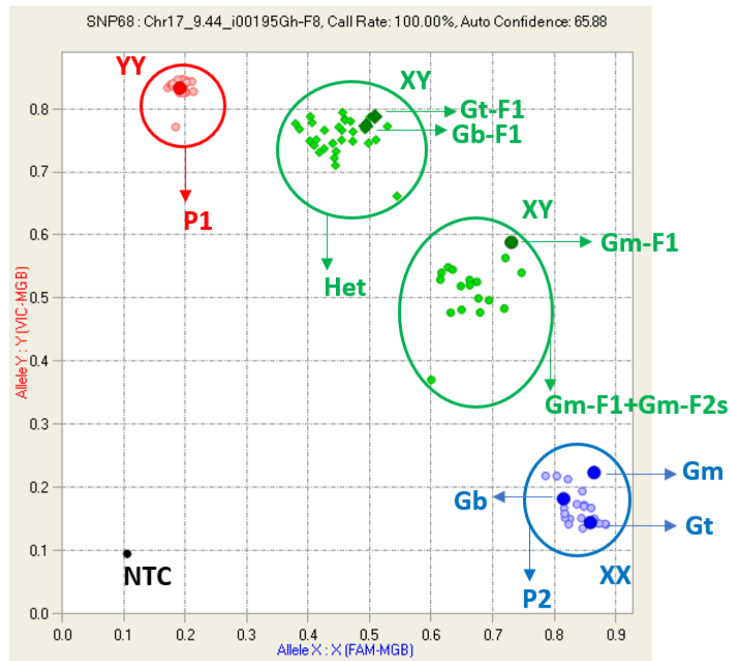

Figure 9. Cartesian plot of F2 fluorescence data for a SNP assay exhibiting species-specific heterozygote clustering. For marker i00195, a sub-cluster is formed by Gm-F1 and the heterozygous Gm-F2s, i.e., by heterozygotes between *G. mustelinum* and *G. hirsutum*.

Table 10. Ratings data on cluster scorability and cluster tightness for overall 449 assays

Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
1	Best	133	159	134	59	149
2		112	154	137	149	160
3		60	53	67	117	53
4		43	20	48	61	24
5	Failed	101	63	63	63	63
Total		449	449	449	449	449
Average		2.70	2.27	2.49	2.82	2.31

Table 11. Numeric summary of 386 assays categorized for applicability.

Number of Assays	Assay Applicability
317	Gm, Gb, Gt (generally)
17	One or more species, but not all
14	Sub-clustering, due to one or more species
38	Recovery of homozoygotes from one or more donor species
Total 386	Applicable generally or in specific situations

Chi-square Test

To assess the significance of differences between the observed and expected segregations, chi-square tests were performed for each species separately and collectively. Homogeneity tests were not conducted. Using associations of single data points and specific clusters as a guide, individuals were assigned genotypic classifications for each the 386 SNP assays across the overall F2 population (n=82), where two *G. mustelinum* F2 plants were excluded for poor PACE genotyping, as noted above, and four other *G. barbadense* F2 plants were excluded, as noted below, on the basis of low congruence rates between the PACE- and CottonSNP63K-based genotypes. Of the 386, 78 (20.2%) showed significant departure from a 1:2:1 ratio, based on a P value < 0.05 in chi-square test. Similarly, species-specific tests indicated significant segregation distortion for 66 (17.1%), 69 (17.9%), and 73 (18.9%) markers for *G. tomentosum, G. barbadense, and G. mustelinum*, respectively **(Table A6).** Additional chi-square tests performed for 38 markers with 2-cluster patterns to evaluate departure from an expected 3:1 distribution, P value < 0.05. Of the 38, just one (2.6%)

marker showed significant departure overall, and in *G. tomentosum* and *G. mustelinum* species-specific distributions, too. The chi-square test results are listed individually by assay in **Table A7**.

Comparing KASP/PACE Fluidigm Results to CottonSNP63K Results

PACE results were compared to CottonSNP63K genotyping as a means of assessing the accuracy of the Fluidigm F2 genotyping results. PACE assays were applied to remnant DNAs from 29 *G. barbadense* F_2 individuals that were previously genotyped with the CottonSNP63K (Hulse-Kemp et al., 2015). Of the 453 KASP/PACE assays, 82 markers were excluded due to being a failed marker (63), no-call data (4), absence from the available CottonSNP63K (12) data, or lack of genome assembly position data (3). Of the remaining 371 SNP assays, genotypic assignments were 100% identical for 306 SNPs (82.5 %), and 96.0-99.9% for 15 SNPs (4.1 %). A group of 18 (4.6 %) assays matched at a rate of 92.0-80.0%, while 29 markers (7.8 %) matched at 64.0-76.0%), and 3 (~1%) markers were not correlated (match rate of 44.0-48.0 %). The data from all 29 *G. barbadense* F2 hybrids were examined for potential sample-specific issues. The genotype calls of each individual were tallied for "match" versus "mismatch" results for each marker; tabulated results for each F2 showed overall differences in average mismatch frequencies; "no call" genotype were excluded from calculated percentages. Whereas the vast majority F2s, 25 of 29, showed a matching range (93.7-98.7%), 4 individuals (7146.62, 7146.63, 7146.83, and 7146.84) showed low match rates (69.5-73.9%). Data from those four individuals were excluded from comparative analyses.

Assessment of Repeatability Using the PACE Fluidigm Assays

To evaluate genotyping repeatability of the array, 84 markers were retested using two separate Fluidigm arrays, and the genotype results were compared. Results were identical for 42 markers (50%), and high (95.1-98.8%) for another 32 markers (38.1 %). Seven markers (8.3%) matched at lower rates (80.5-93.9 %), and three (3.6%) markers were very inconsistent (24.4-78.1% matching). Also, three SNP markers regenotyped using KASP/PACE assay platform with 88 same F2s, and call results compared with Fluidigm array call. The call results indicated an average 95.0% identical match with KASP/PACE assays. Furthermore, using the same *G. barbadense* data for 25 F_2s of CottonSNP63K data call checked with KASP/PACE assays genotype results. The sets of three assay comparisons showed range was about 93.2% identical match.

Recombination Rate Analysis

Linkage analysis was conducted as a possible means to assess the accuracy of the Fluidigm F_2 genotyping results, validate locations of assayed loci, and crudely compare linkage intensities across species combinations. Previously estimated distances between CottonSNP63K mapped loci were used as a reference and as a guide for expectations. The previously reported (Hulse-Kemp et al. 2015) and the recently observed recombination rates (%) between neighboring loci analyzed with PACE assays of F2 populations are detailed in **Table A8**. These were examined with respect to overall and species-specific results. A review of the recombination rates revealed considerable variation among rates, as might be expected for small populations and interspecific crosses, so the main focus was on whether any systematic perturbations were apparent. It

was noted that high recombination rates (~50%) with *both* flanking markers for 25 assays, which indicated a significant anomaly was present. Most of these (23) were among a set of misidentified assays that were subsequently traced back to two commercial orders for primer sets that contained significant errors and included primer sets for loci unrelated to the ones intended. Thus, the linkage analysis enabled the discovery and correction of this error. As a consequence, we at least temporarily eliminated the assays for 19 interstitial segments and 4 terminal segments. The correct primers are being ordered, and functional assays may result once the correct primer sets are obtained and tested, so it is hope coverage of all or most of these 23 segments will soon be restored. There were two remaining markers with unexplained high recombinations rates with flanking loci, as discussed below.

Unexpectedly high recombination with flanking loci was noted for the locus detected by the PACE assay targeting SNP marker i41274, which mapped to the c09 LG (A09), located about 15 cM from the origin by Hulse-Kemp et al. (2015). However, when analyzed using the PACE-based F2 results here, the marker i61099 that was mapped very close (0.43 cM) to the c09 origin in the Hulse-Kemp et al. study, exhibited a 50% recombination rate relative to the origin, as well as to a flanking locus (i50500) about 13 cM in the opposite direction. Thus, PACE results indicated zero linkage between the locus detected by the PACE assay labeled for i41274. BLASTn analysis of the 101-bp sequences associated with these CottonSNP63K markers conformed mostly to expectations, mapping with high identity (E-values $< 10^{-39}$) to A09 and D09; the i41274Gh sequence aligned quite well to a locus nearby in the assembly with only

slightly lower high identity and coverage, but given its physical proximity, it seems unlikely to have caused the observed lack of linkage. BLASTn of the primer sequences for these three assays (i61099, i41274, and i50500) to *G. hirsutum* v. 2.1 at Phytozome v13 online tools ("Phytozome", 2020) indicated that their positions were congruent with expectations for A09. One or both primers for i41274 and i50500 aligned well with both chromosome A09 and for i41274 also with D09, and i61099 primers aligned with A09. The alignments did not suggest a facile explanation as to the lack of observed linkage. At this time, it seems most likely that i41274 assay primer mixture integrity was compromised.

The loci detected by the PACE assays that target chromosome-14 adjacent markers of i05683 and i15563 showed a higher recombination rate with ~43%. Marker i05683 had only a 44% identical match with SNP chip data. Thus, together, the results suggest that the PACE assay intended for i05683 result may not be reliable.

Discussion

In phase II of this research effort to develop targeted simplex SNP assays, 88 interspecific F2 hybrids involving three different AD species related to cultivated Upland cotton were genotyped using Fluidigm 96.96 Dynamic Arrays and a set of 554 KASP/PACE assays. A major goal was to assess these assays for analysis of self-progenies from interspecific hybrids, where progeny segregation typically includes at least two homozygous and one heterozygous progeny genotypes, but where considerable complication can arise from segregation at one to many loci with related sequences.

Complications are especially common in disomic polyploids like cotton and exacerbated if they also have vestiges of paleopolyploidy, also true of cotton.

Results from the 88 F2 plants **(Table 11 and Table A5)** suggest that 317 of the 453 markers will enable reasonably accurate calling of P1, P2, and Het genotypes in most situations involving introgressed germplasm from the three donor species. A guarded approach would be wise for markers with scorability or cluster tightness ratings of "4" **(Table A5)**. In addition to the 317, another set of 38 SNP markers enables detection of a recessive donor allele of at least one species; i.e., in hybrids with Upland cotton, the marker can be used to identify segregates homozygous for the donor allele. The most likely explanation for this kind of assay behavior is the presence of a “dominant” SNP from Upland cotton; these dominant SNP primers amplify preferentially relative the other SNP primer in the heterozygous population skewing the results (Walsh, Erlich, & Higuchi, 1992). Another group of 14 markers for which one species formed a "sub-cluster"; the causes are unknown but, in some cases, could involve minor allelic differences that slightly alter amplification. Another set of 17 markers should suffice for one or two species, but not all three donors. Of the 453 markers, F2 analysis indicated that 63 markers (~15%) failed; this is attributed in part to the very limited Phase-I screening, which was not sensitive to the potential effects of other segregating sequences that could influence results.

Segregation distortion has been recognized in cotton for intra-specific and inter-specific populations (Hulse-Kemp et al., 2015; Byers et al., 2012; Kumar et al., 2019; Diouf et al., 2014). Chi-square tests of some markers deviated from 1:2:1 or 3:1

Mendelian patterns. Such distortions could be artifacts of incorrect genotyping, or if real, such segregation distortions could reflect a wide variety of possible perturbations, e.g., genetic and physiological factors such as pollen lethality, pollen tube competition, sterility, chromosome translocation (Kumar et al., 2019; Diouf et al., 2014). Either competition among gametes or abortion of the gamete/zygote may cause segregation distortion (Faris, Laddomada, & Gill 1998).

Comparisons between genotype calls using CottonSNP63K data versus PACE-based Fluidigm array data showed an average concordance rate of about 86.6% for the best 386 assays and 99.8% for the best 321 assays. These different results may be due to DNA degradation following long-term storage (5 years), differences in genotyping chemistry with low reagent volumes, differences in genotype-calling methods or improper maintenance of sample identities or purities, improper loading and/or microfluidic malfunctions. Errors in genotyping would unlikely be due to the Cotton SNP63K, but might be due to the inaccurate genotype calling based on the PACE-Fluidigm analysis. For comparison, concordance between two Fluidigm arrays was 88.1%. Also, the concordance between plate-based KASP and Fluidigm-based PACE assays was ~ 93.0%. In the Fluidigm system, call rates of individual markers and sample call rate showed variation. Therefore, optimization of PCR conditions may be necessary for different markers set (Hulse-Kemp, 2015). Some markers worked optimally at different numbers of cycles. Also, we did not use pre-amplification with a shorter common forward primer (SNP-less) for the Fluidigm analyses, which has been reported as sometimes enhancing genotyping robustness (Smith & Maughan, 2015).

Perhaps most importantly, the accuracy of the results for some assays was limited by the small population sizes. For those assays, bigger F2 populations would be especially desirably for each species - *G. tomentosum*, *G. mustelinum*, and *G. barbadense.* Additional insight might also be gleaned by running *G. tomentosum* and *G. mustelinum* DNAs on the CottonSNP63K to compare genotype calls with the results of Fluidigm Array genotype calls for checking DNA degradation with no long-term storage for the respective population.

Recombination rates were determined by maximum likelihood estimation using a published equation (Lorieux, 1995). For the most part, indicated recombination rates were not far different from what was expected based on previous linkage mapping of an F2 population between *G. hirsutum* and *G. barbadense*, especially given that two of the interspecific F2 populations involved species other than *G. barbadense*.

The main purpose of calculating the recombination rates and comparing them to previous results was to detect errors, especially instances where assays might be detecting homeologous locations rather than the intended locus, or for that matter, any other locus. This strategy worked very well, in that most of the assays mapping to non-intended locations were readily discerned as being totally or nearly unlinked to both flanking markers, providing a "red flag" that something was amiss. Of 25 such "flags", 23 were traceable to two orders for the commercial synthesis of assays targeted to linkage mapped CottonSNP63K loci that would prospectively bisect known interstitial (19) and terminal (4) gaps in coverage. However, by way of backward sleuthing, it was discovered that some sort of spreadsheet error led to the substitution of the intended

targets with CottonSNP63K loci from other chromosomes. While the new assays will be of some use, the assays for the 23 loci intended are yet to be tested. All data and graphs affected by this temporary setback were corrected, such that the information depicted in this thesis are presently accurate.

In addition to the above, there were two additional assays for which the detected SNP locus was "flagged" for excessively exhibited high recombination rates relative to both flanking markers. One of them was a marker near the origin of the c09 linkage group, which was 50% recombinant with flanking markers on both sides, whereas it was expected to show roughly 15% or less recombination with each. Given that the clustering of F2 signals and ratios among them indicated that the assay was indeed detecting a locus, the most reasonable explanation is that the assay presently labeled (i41274) is amplifying and detecting a SNP unlinked to the intended i61099 and i50500 loci detected by the CottonSNP63K array, e.g., perhaps a homeologous locus or some other locus. Further insight might be pursued in several ways. The PACE assay primers could be reordered and tested on some critical F2 individuals to see if they yield results obtained to date. To determine if the unlinked locus happens to be a homeologous locus in c23 (D09), F2 data from the assays of loci near the homeologous location could be entered into MLE of recombination frequencies to detect linkage. In principle, this same strategy could be implemented for all of the functional PACE assays tested with the Fluidigm devices to determine the approximate location of the unknown target. Another method would be to compare PACE results for this assay with the CottonSNP63K genotyping results for the best 25 *G. barbadense* F2 plants. Assuming that the primer

sequences of this assay match their design, which may or not be true, insight might be gained into potential off-target SNP amplification, e.g., to a homeologous sequence, by aligning the target SNP with flanking sequences all problematic loci primer sequences to the genome assembly to detect positions and evaluate respective alignment quality features. Analysis bigger population size should lead to more informative results as well but is unlikely to resolve this particular issue.

As a testing platform, F2 populations present perhaps the most stringent type of assay assessment due to the extreme degrees of segregation that occur, both at each assayed locus, as well as at any other loci for which allelic segregation influences results, e.g., allele-specific PCR-based amplification of the targeted locus. So, if an assay works well against the backdrop of an F2 population, it is likely to work as well or better when applied in genetic backgrounds that are more uniform, e.g., advanced-generation backcross inbreds such as BC5S1 families, as for example are being used to develop chromosome substitution lines and chromosome segment substitution lines.

The main goal of the F2 assessments was to determine the relative ease and robustness with which one could expect to use the assay for genotyping, especially the identification of self-progenies that are homozygous and thus true-breeding for chromosome segments from the three donor species. However, other valuable kinds of information were also sought. Phase-I testing included only one example of each of three interspecific F1 hybrid heterozygotes, and thus provided only a very limited opportunity to assess heterozygous SNP "phenotypes"; the analysis of F2 populations was expected provide opportunities to characterize the SNP "phenotypes" of multiple

heterozygous F2 individuals for each SNP, including any "noise" caused by extensive background segregation. The F2 analyses also provided an opportunity to determine the dominance versus codominance of each SNP assay.

A general limitation of the Phase-II F2-based assessments of PACE assays was the small sizes of the individual populations used in this study. That said, the procedure was reasonably cost-efficient and sufficed for evaluation in most regards and for most of the assays. It was clearly sufficient for all of the assays that performed ideally or really well, and performed similarly across all three F2 populations. It also sufficed to identify assays that "failed". In spite of size, the three populations provided a cost-efficient means of detecting major differences in behavior among SNPs from the three donor species, too, including failures and shifts in positions or differences in dominance.

Data from larger F2 populations would have facilitated assessment of the less robust and less consistent assays, e.g., those yielding middle to low cluster tightness, poor separation of clusters, sub-clusters, or inconsistencies across the three F2 populations. It is estimated that approximately 130 assays could potentially benefit from expanded F2 testing, e.g. if each of the three populations were increased to *ca.* 90-100. Cost, of course, is a very important concern, so having the knowledge of which assays most need expanded F2 analysis creates an opportunity to economize such an effort. In Phase-II, the cost per F2 plant for testing close to 500 assays was about $30 (about half of what it costs to use the CottonSNP63K, which gives much more data, and higher quality, too). However, if only 130 assays need to be run on an expanded F2 population, then the cost per F2 will be reduced to about $ 8 per plant; if we run another ~192 F2

plants, the cost savings will be ~$7,106 = (453-130 assays)x ($30-$8). Under such a scenario, the resulting data would provide better information on cluster tightness and separation, but typically lack data from flanking markers. Thus, it would be difficult to access the accuracy of genotype calls. Possible solutions would be to conduct PACE assays for flanking markers, too, or independently genotype at least some (e.g., *ca.* 30+) *G. tomentosum and G. mustelinum* F2 plants with a highly reliable platform, e.g., the CottonSNP63K. The cost would be about $2000 for every 30 F2 plants.

CHAPTER IV

CONCLUSION

SNP genotyping has become an attractive tool in molecular breeding research and many breeding applications. SNP arrays have been developed for many crops for utilization of plant breeding activities (Ganal et al., 2011; Chen et al., 2013). High-throughput genotyping platforms play important roles for whole-genome sequencing with rendering massive SNP discovery, enabling to discover informative markers for different sets of germplasm. Once developed, multiplexed fixed arrays can provide highly accurate genotypic data and economize powerful studies such as QTL, GWAS, and MAS. Genotyping-by-sequencing (GBS) is more demanding technologically, especially if not targeted and at high depth but can be applied in diverse manners and thus offers greater flexibility in target numbers and sample numbers. In general, GBS is complementary and, in cases, competitive with array-based platforms for genome-wide analyses. In addition to the array- and GBS-based approaches, there remains an important need for flexible and cost-effective platforms that can be applied for genotyping smaller numbers of SNPs across large (or small) breeding populations for targeted genotyping (Thomson, 2014). Two of the most common platforms are Taqman and KASP/PACE assays, which can be used simplex platforms, but scaled up to low-, medium or high-plex applications.

Public researchers have used Fluidigm dynamic arrays platforms that have enabled large-scale genotyping of cotton with low-cost (Byers et al., 2012). The nano-

fluidic chip technology reduced each reaction volume to 9.7 nL, and increased overall genotyping speed. Excluding instrument costs, the cost-effectiveness was thus high; the overall cost for consumables per data point genotyping cost was ~$0.05. For this experiment, each Fluidigm 96.96 IFC produced 9,216 PCR reactions per run (~3 hours). Perhaps most importantly, it greatly reduced pipetting and opportunities for pipetting errors. Moreover, it requires little additional technical expertise (Maughan, Smith, Fairbanks, & Jellen, 2011). Therefore, in Phase-II, 96.96 Fluidigm Array with PACE chemistry enhanced several aspects of this Phase-II screening of hundreds of prospective SNP assays.

Development of sequence-based markers such as SNPs and technologies that facilitate their scalable utilization is important to a wide variety of breeding and research applications (Paterson et al., 2012; Li et al., 2014; Wang et al., 2012; Hulse-Kemp et al., 2015). High-throughput techniques for SNP analysis lend themselves well to large-scale applications, especially those that are genome-wide in scope. SNP markers are the most abundant markers in the genome and are useful markers for targeted simplex genotyping (Fang et al., 2010; Rimbert et al., 2018). Relatively few simplex assays have been publicly reported for cotton (Byers et al., 2012; Islam et al., 2015; Zheng et al., 2015; Ashrafi et al., 2015). A high-density CottonSNP63K array has improved the genotyping of cotton in terms of genetic and molecular research in cotton (Hulse-Kemp et al., 2015). and created an opportunity to develop simplex SNP assays in a targeted manner. The goal of this project was to exploit this opportunity to develop, test, and validate a set of targeted SNP genotyping assays that would be widely applicable to the four tetraploid

Gossypium species predominately used in our wide-cross introgression populations at the Stelly Laboratory and elsewhere. The CottonSNP63K array provided polymorphism information, linkage map positions, and flanking sequences (Hulse-Kemp et al., 2015).

The initial part of this research was to use CottonSNP63K-based polymorphism information, linkage map positions and flanking sequences to identify potential candidates for simplex KASP/PACE assays that distinguish *G. hirsutum* from three other tetraploid cotton species, namely *G. mustelinum, G. tomentosum* and *G. barbadense* that are germplasm donors in wide-cross introgression If the chosen sequences proved amenable to the design of suitable sets of PCR primers for the assays, they were considered for testing. Generally, the testing included two phases.

Phase-I testing involved screening each assay against a panel of 4 species (*G. hirsutum* and the aforementioned three AD-genome donors) and the 3 corresponding F1 interspecific hybrids, plus a non-template control. This was designed to be cost-efficient and to contribute information that would be useful in the selection of heterozygotes during backcrossing of three interspecific F_1 populations of wild species crosses with Upland cotton (TM-1). Phase-I screening provided initial assessments for 586 genome-wide spaced simplex assays using KASP/PACE chemistry. Choices of assays were guided by the goal of creating usable assays approximately evenly spaced regions of the genome, at ~15 cM or less.

While the CottonSNP63K and associated diversity and mapping data are valuable resources that can facilitate and expedited targeted assay development, they are finite resources, and cannot suffice for all purposes, e.g., especially in regions where

SNP density per cM is low, and when targeted markers need to be physically close. In this work, it was necessary to develop assays in regions not successfully addressed using the CottonSNP63K-based resources. Thus, we endeavored to create a new sequence assembly-based pipeline to identify additional candidate SNPs using our recently published genome assemblies of *G. hirsutum*, *G. mustelinum, G. tomentosum, G. barbadense* (Chen et al., 2020). These efforts were quite successful, and given the abundance of in silico SNPs, the methods can provide a "segway" for future SNP assay development efforts.

Phase-II testing extended the analysis of 453 selected KASP/PACE assays to three small F2 populations involving the same three wild species. To economize, each F_2 population was small, *ca.* 30, but prospectively large enough collectively assess uniformity across the three species, or not, and, if so, to deduce segregation and clustering patterns. Results indicated that 317 markers of the tested 453 markers will generally allow genotyping of P1, P2 and heterozygotes. Additionally, 38 SNP markers can be useful for recovering of homozygous of three donor species. In addition, 14 markers showed species-specific applicability and 17 markers can be used for one or more species.

Using the SNP data sets from developed CottonSNP63K greatly facilitated targeted genotyping in this study. Most of the KASP/PACE simplex assays were successful for the initial test of primers. The combination of PACE chemistry on Fluidigm multiplex array reduced overall data point cost and time effort compared to KASP, making 96 primers testing with 96 sample at the same time. Genome-wide panel

of map-spaced (cM) simplex SNP marker assays will be important to facilitate cost-effective marker-assisted introgression, analysis, and manipulation of wild germplasm. Most of the assays will detect SNPs previously described in the CottonSNP63K array, and thus most SNP markers can be related to high-density linkage maps, QTLs, and AD-genome sequence assemblies, too.

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APPENDIX ADDITIONAL TABLES

TABLE A1

List of CottonSNP63K Array features adapted to KASP/PACE assays, and for each, the chromosome association, linkage map position (Hulse-Kemp et al., 2015), gap size, and primer sequences for respective assay.

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
1	i61225Gt	0.00	0.00	GAGGAAATGGCGAATATTGAA	CCTGATCTCTTCAACCTTACTTTCT	CCTGATCTCTTCAACCTTACTTTCC
1	i23982Gh	14.14	14.14	CACAATGTTTCAATGTGTATGACAG	GCACAAAGACAATCTCTACTTCCATAA	GCACAAAGACAATCTCTACTTCCATAG
1	i51041Gb	26.10	11.96	GGGCTCTCAAGTTTCTCCAA	CCATGAGGATAAAAGCGAAGAA	CCATGAGGATAAAAGCGAAGAC
1	i27626Gh	38.59	12.49	GACTAAGCGATCCAGCTTGTTT	GTTGATGAAAGTGCTAAATGAAAAAT	GTTGATGAAAGTGCTAAATGAAAAAG
1	i34731Gh	56.68	18.09	TTGGCCCAACACAAATATGA	ATTCAGGTCACGAGATTGAGAGTC	ATTCAGGTCACGAGATTGAGAGTT
1	i55595Gb	72.04	15.36	ACTTTCCATCTGCAAACCAT	TACCTGAATTACTCTCTCTGACTGC	TACCTGAATTACTCTCTCTGACTGA
1	i45642Gh	84.03	11.99	TCGTTCTCCGTTGAACTAACC	TCAAATTCATCGGAAACAGAGAC	TCAAATTCATCGGAAACAGAGAA
1	i47972Gh	97.67	13.64	AACAAAACCGACCCTTACTTGA	GTGACTGAGTGTTTTAGTGTTGTGAA	GTGACTGAGTGTTTTAGTGTTGTGAC
1	i02391Gh	115.19	17.52	CCATTGTAGCTTTTAGGCATTG	TTCAATAGCAATTCTTTAGTAGCCAA	TTCAATAGCAATTCTTTAGTAGCCAG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
1	i50196Gb	126.74	11.55	**GAGAGTAACTTATGGAGCTGCTTCT**	**GCATATAAGCGACCAACAAACG**	**GCATATAAGCGACCAACAAACA**
1	i00132Gh	140.47	13.73	**CGCATAATATGAAGCTTGTTG**	**TCATAAAGAGACTGGTGATAAGAACT**	**CATAAAGAGACTGGTGATAAGAACC**
2	i50655Gb	0.85	0.85	**ATTGCTCGAAGCCAATGAAC**	**GTCAGTTCCATCTTCTTCTTCTTTTC**	**GTCAGTTCCATCTTCTTCTTCTTTTT**
2	i52423Gb	13.26	12.41	**CCCAAACTAGCTATTCCGTCA**	**AGGGAATTCATTACATACTCCACTT**	**AGGGAATTCATTACATACTCCACTG**
2	i03174Gh	21.83	8.57	**TATTCGGTTTTCTATCTTGGTTTAG**	**GAACCATTCAGAGTGTGTGTG**	**TGAACCATTCAGAGTGTGTGTT**
2	i25210Gh	21.83	0.00	**CAGCATAGCTTTGTGTGCAA**	**CATCACCTAATCATCACCTACTACTAT**	**CATCACCTAATCATCACCTACTACTAC**
2	i50125Gb	23.96	2.13	**GGTAAGCCGTGGAGATCCATTAT**	**CTTACGTTCACCATCTTCTTCA**	**TTACGTTCACCATCTTCTTCG**
2	i01044Gh	29.51	5.55	**AACCCGGTATTTTTCCCAAC**	**CCAAGAAGCTGAAGTGGTCC**	**CCAAGAAGCTGAAGTGGTCT**
2	i43496Gh	41.50	11.99	**TTGTGCTAAATAAAATGTTGAAAAGC**	**CTTCCTCAAACAACACATTCCTC**	**CTTCCTCAAACAACACATTCCTT**
2	i22764Gh	50.90	9.40	**CAGTTTGTTTAGTCTTTGACG**	**TGAAAGTTTCGGCCAAAATT**	**TTGAAAGTTTCGGCCAAAATC**
2	i61080Gt	52.61	1.71	**TTAACATACTCTCCATCAATCTTCC**	**CTTTAAAAGATCGCCCTACC**	**CTTTAAAAGATCGCCCTACT**
2	i60741Gt	64.99	12.38	**AATCGAGATCAAGGCTGGAAT**	**TGTGCTACTCAACTAAATAGGAGAC**	**TTGTGCTACTCAACTAAATAGGAGAT**
2	i29065Gh	73.53	8.54	**ATAACACCCAAACACCCGTAAAT**	**AAGGTCAATCTCCGTATTAAGTTC**	**AAAGGTCAATCTCCGTATTAAGTTT**
2	i52292Gb	80.37	6.84	**ATTGTTGAAGAATGGATTTCTG**	**CCACATCAACTGATTTCCCTA**	**CACATCAACTGATTTCCCTC**
2	i62379Gt	80.37	0.00	**CCCTGATCTAGTGCTTTTCG**	**GAAGATCAATTATGAATGCCAG**	**GAAGATCAATTATGAATGCCAA**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
2	i57002Gb	89.33	8.96	GGCACCAAACCCTATCAAGA	GTATATATGAGTGCTTGAGGTGTTCC	AAGTATATATGAGTGCTTGAGGTGTTCT
2	i49510Gh	90.18	0.85	CTCCATAGAAGCGAGTAAAATCA	GGCTAAGTCGGATTTCAGTAGTTTTA	GCTAAGTCGGATTTCAGTAGTTTTG
2	i64451Gm	103.05	12.87	TCACTTGGAAGTTGAGGAAGG	ACTATATCATCAATCCTTGAACCACTC	ACTATATCATCAATCCTTGAACCACTT
2	i51254Gb	112.02	8.97	GCAGTAGAGGTTTTGGAGGTG	GGACAGATGATGGTATGGGA	GGACAGATGATGGTATGGGC
2	i63139Gt	112.87	0.85	TCTTCTTGACACACTGGACA	TATGCCGGTCTTGAAAAATCTG	TATGCCGGTCTTGAAAAATCTA
2	i38489Gh	122.79	9.92	AACAATCACCCCAAAATTCCT	GAAATGGTATTGCACCGTCG	GAAATGGTATTGCACCGTCA
2	i00543Gh	132.73	9.94	TGCTGGTTCGAGAACAAATG	TTACCAATAATAGACACCCTTGACTT	TTACCAATAATAGACACCCTTGACTC
3	i05735Gh	2.56	2.56	GCCATGAAAGCTCTGCTAGT	GTAAAGGAACAAAGGTGACACTAAT	GTAAAGGAACAAAGGTGACACTAAC
3	i05710Gh	10.32	7.76	CATATATCAACTCAGGCATAAATGA	GATGTGCACTGTTTGGGGTA	GATGTGCACTGTTTGGGGTG
3	i50850Gb	25.77	15.45	AAAGCTGGGCTAAAATGTGTAGATA	TGTTAACTTCACACCCATCA	TGTTAACTTCACACCCATCG
3	i05602Gh	37.80	12.03	GGATAATGATTACATTGTTGGTCTT	AAACCCTGGGTGGATAAAGA	AAACCCTGGGTGGATAAAGC
3	i19058Gh	38.22	0.42	ATCCTTCTAGCTATAAAAGAAGCTG	ATTCTCAGGCACCTTGATGTA	ATTCTCAGGCACCTTGATGTC
3	i00178Gh	48.93	10.71	CAGCTCATTCACATCCCAAA	GTGGAGAATACGTTGAGATAGAGAG	GTGGAGAATACGTTGAGATAGAGAT
3	i05459Gh	57.46	8.53	GAATGGGACGAAGCTCAAGA	TACAGTCTCGGCAGCTAGAGAAG	TACAGTCTCGGCAGCTAGAGAAA
3	i14041Gh	69.43	11.97	TGTGAACATTGGAATGGAGAA	ACATGAAGAAACATCATCACCATT	ACATGAAGAAACATCATCACCATC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
3	i14878Gh	84.36	14.93	GCTCCCTCAGTTAAGGAGTTTG	GAATAGCCACGAGCATCACAC	AATAGCCACGAGCATCACAA
3	i52480Gb	97.24	12.88	ACCATTTCAGCTCAGGGAAC	AGGGTACACCATTTTTTCAATCC	AGGGTACACCATTTTTTCAATCA
3	i14000Gh	108.78	11.54	CGATGACCGGAATCTAAAGC	TAAAAGGTCTTTCAAGATGAGCG	GTAAAAGGTCTTTCAAGATGAGCA
3	i43612Gh	121.18	12.40	GAGAAGGACACCGAAAGTTTG	GAAGCTTCCAAATCGCGTAG	GAAGCTTCCAAATCGCGTAA
3	i66018Ga	136.14	14.96	TGATCTTCAAATCTGCCATCAT	TCTTCTTCTTTTTCATGTTTGATTC	TCTTCTTCTTTTTCATGTTTGATTT
4	i00706Gh	0.43	0.43	CCAGCAGGTGAACCTAAAATG	TTGAGAACTCTACAAAGACTAGGAC	TTGAGAACTCTACAAAGACTAGGAT
4	i00466Gh	0.85	0.42	ACTTGTCCAATTGATCCTTGTAA	TAAGGCTAATATCGAGAAGATTGGA	TAAGGCTAATATCGAGAAGATTGGG
4	i24385Gh	0.85	0.00	ATTCGCTTCGTTTTGGTTTG	AGGATATTGTATGAAAATATGAGTGA	AGGATATTGTATGAAAATATGAGTGG
4	i53715Gb	9.40	8.55	TACCGGTGTTGGAAGTGGTT	CCTGTTGGCTGTAAGTTTGGTT	CCTGTTGGCTGTAAGTTTGGTC
4	i25377Gh	10.25	0.85	TCTTGACAATAAATCATCCAAGACA	GGTTCCAAGATATGAGGTATTCTAT	GGTTCCAAGATATGAGGTATTCTAC
4	i42893Gh	20.54	10.29	GGTTTATGTTCAACTAACCTTCTTCA	AAACCAACCCAAGAGAATAACTC	AAACCAACCCAAGAGAATAACTT
4	i49276Gh	21.40	0.86	GAGATTATGTGTTGGACTGTTGAAA	CCTCGATGGCATCATTCTTAT	CCTCGATGGCATCATTCTTAC
4	i50068Gb	21.40	0.00	TGGCAATGTTCGAGTCCATA	CCCTTCTATGTCTTGGCATT	CCCTTCTATGTCTTGGCATC
4	i49010Gh	26.62	5.22	CCCCTGACTGTGGCATATAAA	ATTATAGTCCACGTTTACTTTGTGC	ATTATAGTCCACGTTTACTTTGTGA
4	i10424Gh	33.46	6.84	TACCTATTTTCAGTGTGATCTTGTT	GAATGCTAGAGTTGGATATTCTTTA	GAATGCTAGAGTTGGATATTCTTTG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
4	i29272Gh	39.48	6.02	TTCCTTTAATCCTCAAACAACTTC	CAATATAAGCTAACAAGTAAGACAGCA	AATATAAGCTAACAAGTAAGACAGCG
4	i50228Gb	53.19	13.71	GTCGATCATTCTGCTGGTGATT	TAAGTCTGTCAACTGGATCGTACT	TAAGTCTGTCAACTGGATCGTACC
4	i32552Gh	66.86	13.67	ATTTGCCCTAAAACGTGCAG	TTACTTCGGTTTCTGATTTAGTTCT	TACTTCGGTTTCTGATTTAGTTCC
4	i47058Gh	73.73	6.87	ACCAAGAGGCCCAATCATTA	ATTGTAAATGTAAATGGGCTTATGA	ATTGTAAATGTAAATGGGCTTATGG
4	i52022Gb	89.10	15.37	TTAGGCCCCAGTCCCATATC	GATGAGTTCATTTATCTCTTCACAA	GATGAGTTCATTTATCTCTTCACAC
4	i43998Gh	92.09	2.99	ACCGCTCCTTCAAGTTTCCT	TGTGTTTCATTGTGCTTATTTACA	GTGTTTCATTGTGCTTATTTACG
4	i53431Gb	99.36	7.27	TGATCGACATGAAAGTAGAAAGA	CTACCACTATCAGGGTCTTTACTG	CCTACCACTATCAGGGTCTTTACTT
4	i51441Gb	101.92	2.56	CCATCATAATATTCCCAGTCTATGA	TTTTCGTTTAAATAACATCATACAGCA	TTCGTTTAAATAACATCATACAGCG
4	i60947Gt	104.94	3.02	TGAAGTAGCCGAAGAAGTCA	CTTAGTTGGTTTTCCTTTTGCT	TAGTTGGTTTTCCTTTTGCC
4	i50976Gb	109.21	4.27	TTCAACTAGAAGCGCGGAGT	AAGAGGTAACAACCCCAACTGAG	AGAGGTAACAACCCCAACTGAA
5	i12375Gh	1.28	1.28	TGTCAGAAAAAGCGAAAACA	ATTAAGATACTTCATTTCATTTCCG	AATTAAGATACTTCATTTCATTTCCA
5	i30358Gh	12.38	11.10	CAAGCAGACATACTAAACATTCAAG	TACTCCATGAATAAACAACTCTAAA	TACTCCATGAATAAACAACTCTAAG
5	i12489Gh	12.38	0.00	CCCTATAATCACTGTGTTTCTCCT	TTAAGCAAACCAGCAGATCAAA	TTAAGCAAACCAGCAGATCAAC
5	i61091Gt	17.09	4.71	CCTTAGAGCAGTCTACATCTACATC	AGTTGTTCCAGGAGGGAATGA	AGTTGTTCCAGGAGGGAATGG
5	i61414Gt	22.23	5.14	TGGTTTGAAATCCGTGGAAG	ATGGGTGAAAGCATGGAGAT	ATGGGTGAAAGCATGGAGAC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
5	i29617Gh	25.65	3.42	GAAAGAAGAGTTGTTGCTTTTACG	GAAGTTTGATTTAGTTCTTGACTTGT	AAGTTTGATTTAGTTCTTGACTTGC
5	i41855Gh	37.20	11.55	TCACCTGTGTCTATGCCAAAA	CAACTTATTTTTATTTTAATTCATGTCTG	CAACTTATTTTTATTTTAATTCATGTCTA
5	i00670Gh	50.35	13.15	ACCATTGAGATTGGATCTACAAATA	ACAAACTTTTGGGTGGTTCG	ACAAACTTTTGGGTGGTTCA
5	i00972Gh	50.35	0.00	GTCGTCTCTTAAGCAGAACTCAAT	GAATTTTCGCTGGTAAGGCG	GAATTTTCGCTGGTAAGGCA
5	i32528Gh	57.36	7.01	GAAACATTTCCAGTTTGATCTTTAT	GAGTCTCAAATGAGCTTACGAA	TGAGTCTCAAATGAGCTTACGAG
5	i12598Gh	59.92	2.56	CAGATCAATAACGGCGTGAA	CTTCCATCAGTTCTTGTAATTCCTT	CTTCCATCAGTTCTTGTAATTCCTC
5	i27980Gh	62.06	2.14	AATTGCTCAAGAATATTAAAAGGAG	CACAACACACAGGTCATAATAAAAT	CACAACACACAGGTCATAATAAAAG
5	i43462Gh	81.29	19.23	CAAATCGAAGATCACAAATCTTAT	AAATCTGCTCCCTTGTTACT	AAATCTGCTCCCTTGTTACC
5	i09845Gh	100.90	19.61	ATTGGATCCAGCTTGGACAG	CGCTTCAATTAGCTTAGCAGAAC	CGCTTCAATTAGCTTAGCAGAAA
5	i09743Gh	118.86	17.96	CTCAACATATAGAGTATTGGAAGCA	GTAGGTTATGGTAATCAACTTCCG	TGTAGGTTATGGTAATCAACTTCCT
5	i01007Gh	120.99	2.13	AACCGTAGCCAAAACTGAAG	TTTTTGTTCTTCTCGTTGTTCTC	TTTTTGTTCTTCTCGTTGTTCTT
5	i09556Gh	132.95	11.96	AGTCTTTTCGGTCATTTCAAGT	AGACGACTATCATTGCCGGG	AGACGACTATCATTGCCGGA
5	i37015Gh	140.24	7.29	CAAGCAAAGAAGAGCCCAAA	CTTTGTCTAGAGCCAACACTTTCTC	CTTTGTCTAGAGCCAACACTTTCTT
5	i09206Gh	166.74	26.50	AGCTCCATAACGAGTCAGATG	GGAACTCAGCAATCACTCAT	GGAACTCAGCAATCACTCAC
5	i48057Gh	179.55	12.81	CGTTCCCTCGTTAGCAACAC	GTGGAAAAAGAAACTGAAAACCAT	GTGGAAAAAGAAACTGAAAACCAG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
5	i08918Gh	189.79	10.24	**TATTTTTGAACTTGTTGGTTTCAT**	**ACTTCGTCATATCATCAACTCCT**	**TTCGTCATATCATCAACTCCC**
5	i61049Gt	189.79	0.00	**CTAAGATTTACAATACAATAGCCAGAA**	**ACCTCTCCATTGTTTCAAGC**	**CACCTCTCCATTGTTTCAAGA**
5	i51036Gb	190.22	0.43	**ATAAACAAGAATCCCCAAAATCT**	**GTCATTCCTTTTCTTGTTTTCTG**	**CAGTCATTCCTTTTCTTGTTTTCTA**
5	i50380Gb	200.96	10.74	**CACCTGAGACCAACTTCACA**	**GCTTATAATGTAGTCTTTGATGGC**	**TGCTTATAATGTAGTCTTTGATGGT**
5	i16528Gh	202.67	1.71	**AGACAATGAAGATGACGATAACTTT**	**TGTTGGAAGATGAGAAACAAGAT**	**TGTTGGAAGATGAGAAACAAGAC**
6	i49551Gh	0.00	0.00	**GCAGTTGCAGTGTTAAAATTAGTTA**	**ATCCAATATCCATCATCATCTTCAG**	**ATCCAATATCCATCATCATCTTCAA**
6	i11398Gh	7.26	7.26	**ATATTTTACAAGCATTTGAGTCCAT**	**TGTGGCATCTTTGTTCATTTT**	**TGTGGCATCTTTGTTCATTTC**
6	i11399Gh	7.26	0.00	**CAAAATATCACACAAAAGTACATGC**	**ATTAGAAAGAGCTTGCGATACCAAA**	**AAAGAGCTTGCGATACCAAC**
6	i11399Gh	7.26	0.00	**AGAGACCTCGTCGGACAAAA**	**ATTAGAAAGAGCTTGCGATACCAAA**	**AAAGAGCTTGCGATACCAAC**
6	i44308Gh	23.07	15.81	**CCGTCTACAGAGTTAATGTCTGCTT**	**GACTCAAATATGATTCTTGGTGAT**	**GACTCAAATATGATTCTTGGTGAC**
6	i11312Gh	30.33	7.26	**ACACAGTGAGAGGCTAGCAAT**	**CCTTGGCTGGTATTCAACAG**	**CCTTGGCTGGTATTCAACAA**
6	i43700Gh	40.58	10.25	**GGATTCTGTTTGGGTCATCG**	**AATGAAGTGTTTTGTCTTGGTCAAT**	**AATGAAGTGTTTTGTCTTGGTCAAC**
6	i39865Gh	49.13	8.55	**GAATGGGCCATGGAATTTTA**	**CAGTCGATGTAGAATTAGTAAATGAGAC**	**CAGTCGATGTAGAATTAGTAAATGAGAA**
6	i55732Gb	60.36	11.23	**TCGAGGTAGACCGCCTAGAA**	**CAGTCGTATCTTTGGTTACCCTCTA**	**AGTCGTATCTTTGGTTACCCTCTG**
6	i51763Gb	60.36	0.00	**GTTAGTGCCAACATCACGAATTA**	**GGGAGAAAATGATTCCCTATT**	**GGGAGAAAATGATTCCCTATG**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
6	i52583Gb	60.36	0.00	**AGAGAGAACCAAAGTGGAGCATAAC**	**GAGAGGAAGGTTGGGAGAAC**	**GAGAGGAAGGTTGGGAGAAT**
6	i51850Gb	68.47	8.11	**GTTCTTGTTAGATTTGTTGGATTC**	**GTCGCACTGCCTTTTTTACG**	**GTCGCACTGCCTTTTTTACA**
6	i53701Gb	71.45	2.98	**ACATAGATGTCAACAGGGACAAT**	**GTGGAGGATACTACCACTACTCG**	**GGTGGAGGATACTACCACTACTCA**
6	i10945Gh	76.15	4.70	**TGTCCGGTCCACAATTAGGT**	**GGTTCACTTGAAAATCAACTTCTGT**	**GTTCACTTGAAAATCAACTTCTGG**
6	i50838Gb	86.81	10.66	**TAATATAAGGTTAGGATGCCTCAAT**	**GCATATGATGGTAGGAAAACTGT**	**CATATGATGGTAGGAAAACTGC**
6	i51788Gb	96.21	9.41	**ACGACACAGGAAAGCCAAAC**	**TTAGCGTCGTCTTCTATTATATGTTT**	**TAGCGTCGTCTTCTATTATATGTTG**
6	i39178Gh	97.06	0.85	**AACCAGGTATAGGGTGTTACACAT**	**ATCATAGCCATTAGCTTGTTTTT**	**ATCATAGCCATTAGCTTGTTTTC**
6	i59235Gb	97.06	0.00	**ACTTTAAAAGGCCCAAACAGTA**	**CTTATTTAGGGTTTTCGTTGTTT**	**CTTATTTAGGGTTTTCGTTGTTC**
6	i44977Gh	97.91	0.85	**AATAGATAAATTTTAAACGGAACCTC**	**ATGACCCTTTACCAAGTAGCG**	**AATGACCCTTTACCAAGTAGCA**
6	i62896Gt	117.40	19.49	**CTAGCTTTGGGTTTTGCTTGT**	**AGAATAAAATCCCAAACAAAAATAG**	**AGAATAAAATCCCAAACAAAAATAA**
6	i51681Gb	123.37	5.97	**ACTTTCTTTGTGGTGGGTAAGAC**	**GCTGAACTTCACACAGATTATGA**	**GCTGAACTTCACACAGATTATGG**
6	i52154Gb	135.31	11.94	**CGACGTGCAAGATGGAGAT**	**CCATTAGCACCAGGTAAGGAAT**	**CCATTAGCACCAGGTAAGGAAC**
7	i49561Gh	1.28	1.28	**GAGGTTTGTTCCCCACTTCA**	**GAGTAAATGAAAGGAATGCTAGAAAAA**	**AGAGTAAATGAAAGGAATGCTAGAAAAG**
7	i49561Gh	1.28	0.00	**GGCTTGCCTCTATTTCTCTATAAC**	**GTAAATGAAAGGAATGCTAGAAAAA**	**GTAAATGAAAGGAATGCTAGAAAAG**
7	i02126Gh	16.30	15.02	**GCCTTGTTCCACAATTCGAC**	**CATCGGAGTAGTGCAAGACG**	**GCATCGGAGTAGTGCAAGACA**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
7	i44501Gh	21.45	5.15	GGAAACGTGATGTCTGGTGAGT	GTCTCTCCTCTTCCACCAGA	GTCTCTCCTCTTCCACCAGG
7	i41379Gh	22.73	1.28	TGTAATATCCGCTGAAACAAA	AAAAGGGTACAAATGTTATGATCC	AAAAAGGGTACAAATGTTATGATCT
7	i02083Gh	29.59	6.86	GCCATTGCTATTGAAAAAGG	TAGAGTACTGTGCATACAACATTGA	TAGAGTACTGTGCATACAACATTGG
7	i53793Gb	34.33	4.74	AGAGCATAGATCAATTAAAGATGG	AATCACAGACATAGTACCTGCAAT	AATCACAGACATAGTACCTGCAAC
7	i30393Gh	34.75	0.42	CCTAAAGAGAACAAATAGGTTTGAA	ATATTTTGGGTTTGTAGTTGTCC	TCATATTTTGGGTTTGTAGTTGTCT
7	i28496Gh	45.46	10.71	AGCATTGAGAACCTCGCTCT	TGTTACCATCTCTATGGGATACTCAA	TGTTACCATCTCTATGGGATACTCAG
7	i30200Gh	53.99	8.53	AAGGAAAAGATGGATGTGTCC	TCTCACCCATTTTTCTTGCC	TCTCACCCATTTTTCTTGCT
7	i51660Gb	59.54	5.55	CAACGAATACAACAAAGTTTATTTGAG	ATGTCTTGTTATGCTAGGTGGTCG	ATGTCTTGTTATGCTAGGTGGTCA
7	i28659Gh	62.09	2.55	CATCAGATTGCACCTAAGAAGA	TGAATAAATCCATAAACAGATTCAG	TGAATAAATCCATAAACAGATTCAA
7	i01705Gh	74.46	12.37	TGGATCCTGTCAAATAGTCGAG	TTCCTCGTTCGTAACATTGATTC	TTCCTCGTTCGTAACATTGATTT
7	i36899Gh	84.28	9.82	TTCTTGTACTGTGCAAATGAGTGT	TCAGCCAAAGAGATCCAAAG	TCAGCCAAAGAGATCCAAAA
7	i54160Gb	84.28	0.00	GATTGGGATAATGGAGTTAAA	ATGTCGAAGAAAGAGTTATCGAA	ATGTCGAAGAAAGAGTTATCGAG
7	i42454Gh	88.13	3.85	ATCGAAAGAAGAGAAATCTCAA	TAAATATGTCCATACCCTTTGCATT	TAAATATGTCCATACCCTTTGCATC
7	i56676Gb	88.13	0.00	ATCGCACCCTAAACAAGACAT	TGAGAGGGTAACCATCGAAA	TGAGAGGGTAACCATCGAAC
7	i57012Gb	88.13	0.00	ATCTGGAGTATGCTCTTCAACTTC	CGATCATGTTCGTACTGTTCTT	CGATCATGTTCGTACTGTTCTC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
7	i14360Gh	96.67	8.54	TTTAGGTGAGTAGGTAAAGACTGCT	GGATCTAAATCAACCTTCACG	TGGATCTAAATCAACCTTCACA
7	i51776Gb	106.50	9.80	AGAGTAAGGTTGCGGTATTATGG	ATTGATTGAAATCTAATCCCTT	ATTGATTGAAATCTAATCCCTG
7	i50472Gb	107.35	0.85	GATACCACATGTACATCAAACAGAC	TAAATACAGAAAGGTCAAATACAAT	TAAATACAGAAAGGTCAAATACAAG
7	i60984Gt	110.35	3.00	GCTAGCGGATTGGCTCATAG	GTTAATCATTGAACCAAGATACAAG	GTTAATCATTGAACCAAGATACAAA
7	i50320Gb	121.90	11.55	TCTGGCTATATTGAATGTTTTGG	CTAAAATAGGGGGAAACAATGTAGG	GCTAAAATAGGGGGAAACAATGTAGT
7	i35632Gh	122.33	0.43	TTTGGGAATGAAACGTCATA	AAATAATTGAAGCAAAAAGTGAAA	CAAATAATTGAAGCAAAAAGTGAAC
7	i01376Gh	129.15	6.82	ATTGAAGAAAAAGAAAACCTGTGT	GTGATCTCCCCCATTGTATT	GTGATCTCCCCCATTGTATC
7	i51470Gb	142.45	13.30	TTGTTGTACCTATACGAACTTATGTGA	GAGTGGTGAACACCTTTACTATCG	GGAGTGGTGAACACCTTTACTATCA
8	i61029Gt	0.43	0.43	TGGAGTACTTACCTGCAATGGT	CCATTTCCATTAACTGTTCTCTTGT	CATTTCCATTAACTGTTCTCTTGC
8	i60714Gt	12.46	12.03	TAAATAGCATCATCCACATCTTTTA	AGAATAAGAATTACACATCGAAAAAC	AGAATAAGAATTACACATCGAAAAAT
8	i50749Gb	13.74	1.28	TATTGCTCCTGGCCCTATTG	AGTTTACTTAAGCCGGGTGTATT	GTTTACTTAAGCCGGGTGTATC
8	i04669Gh	22.71	8.97	GTCAAGGATCACCATTAAGTAAGAT	ATGTCCCCGACTTTTCTTCA	ATGTCCCCGACTTTTCTTCG
8	i30796Gh	34.26	11.55	TCAATTGCTTCCCAACTTTGT	ACTGTGCAAGGTTAATTGAAGTAGAG	AACTGTGCAAGGTTAATTGAAGTAGAT
8	i62711Gt	39.37	5.11	ATTGGCCCAGAATACCTTGG	ATGGTACGTGGGTCACATTAC	AATGGTACGTGGGTCACATTAT
8	i00540Gh	39.37	0.00	GTAAACAAAGTAAAGAGCCCATATC	TGGGTGGATCTTGTTTGGTT	TGGGTGGATCTTGTTTGGTG

Chr.*	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
8	i31486Gh	52.80	13.43	TCAAAGAGCCTTGTCAAATCG	CAAAGTCATTTTTTCCTTGTCTC	AACAAAGTCATTTTTTCCTTGTCTA
8	i56645Gb	67.31	14.51	GTTCGCGATTTGGGGAAT	TGTGTTGTAATCGATGAACTTTCTG	CATGTGTTGTAATCGATGAACTTTCTA
8	i04134Gh	86.25	18.94	AATAATCTCCTCAAAATAGGAAGTG	CATTCCATCCAAAATCAGGTA	ATTCCATCCAAAATCAGGTG
8	i04229Gh	86.68	0.43	AGTTTTCAGGTAAGACCTTTTAAGC	TGACTGTATCTAAGCTATTTGAAGAAC	TGACTGTATCTAAGCTATTTGAAGAAT
8	i50119Gb	86.68	0.00	AAATAAATGATTCCGAGTACAAG	CTGTTCCTAATGGATGTGTAAAAC	TCTGTTCCTAATGGATGTGTAAAAT
8	i51034Gb	86.68	0.00	TCTTAAACACCTCTCTTCCCATA	CATTGGATTACAAGGATTCGT	ATTGGATTACAAGGATTCGC
8	i31221Gh	86.68	0.00	TTCCATTTCCACAATATTGCTAC	TTCAAATTAAGAAGGAGGATTTACA	TCAAATTAAGAAGGAGGATTTACG
8	i51390Gb	103.00	16.32	TGAGTTGCTGCCAGTGTTTC	GTCAACTGTTTTGGTGGATCG	AGTCAACTGTTTTGGTGGATCA
8	i58509Gb	105.57	2.57	TAGGAAGGGACACGGATGAC	ATGATTGTATGAAACAGTTATGCT	ATGATTGTATGAAACAGTTATGCC
8	i40138Gh	115.08	9.51	GAACGGGATCATTCAAGACT	ATTCAGTTTCTTGATTTTTTTCG	AGATTCAGTTTCTTGATTTTTTTCA
8	i48310Gh	115.08	0.00	TTTTATTTCTTAATTGTTTGACCTC	ACCGGAATGACTTATGGTGA	ACCGGAATGACTTATGGTGG
8	i64684Gm	127.46	12.38	AATATCGTAAAACCATCGTTGC	ACCAGGCTTTTAGTTCTGATTTT	CCAGGCTTTTAGTTCTGATTTC
8	i20535Gh	127.46	0.00	GATCTAACTTAAATAGGCCATGATA	GACCCTAATTCTAATCAACAAACAT	GACCCTAATTCTAATCAACAAACAC
8	i29120Gh	129.59	2.13	CATAAGAAACAAAATTCGAGGATTA	GAGATCACGAACTGTAAAGAGGA	AGATCACGAACTGTAAAGAGGC
8	i25939Gh	136.02	6.43	CCCGAACCAAACTTAATGGA	AGATTAACCAACTAAACCAACTGAAATA	TTAACCAACTAAACCAACTGAAATC

Chr.*	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
8	i61072Gt	154.39	18.37	CATGGGAACTTTGCTCAACA	GTTCTTTTCTCAGCTTTGATACCAT	GTTCTTTTCTCAGCTTTGATACCAG
8	i55286Gb	160.36	5.97	TTGATCCAAGGACTCTTTCGT	CTCTTGTACTACTAGCAAGCTGATG	ACTCTTGTACTACTAGCAAGCTGATT
8	i18599Gh	164.63	4.27	AATTGATTAGTCGTTTCAATTTCTT	CAACAACACCAGCCATGTAG	CAACAACACCAGCCATGTAA
8	i61692Gt	171.04	6.41	CCTTTTCCTCTCTTCTCCTTCC	AGGAAAGGGAAGTTGAGAGTTTAGA	GGAAAGGGAAGTTGAGAGTTTAGG
9	i61099Gt	0.43	0.43	ATCCAACCCTAAACCCCAAG	AATCAGCGCCTTCGGTATTA	AATCAGCGCCTTCGGTATTC
9	i52286Gb	1.71	1.28	AAGTACTTGTTCCTCAAGGTTCTAA	GCGGATGAGATCTTGAGTCTTTAAT	GCGGATGAGATCTTGAGTCTTTAAC
9	i50474Gb	2.99	1.28	CCTCTCCATTTGTAGTAGAAGCA	ACTCCCGTCAAGATCCCTAACTAAA	ACTCCCGTCAAGATCCCTAACTAAC
9	i56187Gb	2.99	0.00	CTAGAGATCCATATGAAATTCGG	AATTCAATTTAACCTATGCTTGA	AATTCAATTTAACCTATGCTTGG
9	i32438Gh	12.79	9.80	TTTGCATGTATTTTAAGGGGCTA	TGTTATATTATGTACGAGCCCTATTC	CTGTTATATTATGTACGAGCCCTATTA
9	i41274Gh	15.78	2.99	TGTTCCTTTTCACCCGAAAG	ACAGGATTCACATATCCTTTCTCT	AGGATTCACATATCCTTTCTCC
9	i49356Gh	23.93	8.15	TTGCTTGCCAATTGTTTAGAGA	GTATTCCAAGGATTGAACCTAAAA	GTATTCCAAGGATTGAACCTAAAG
9	i43775Gh	24.35	0.42	AAGTCACTTGGTGGCAATCG	AGTCATCTGTAAACAACAAAAAATA	AGTCATCTGTAAACAACAAAAAATC
9	i50500Gb	28.62	4.27	TGAAAGAGCAAATCAGAGCAGA	TAATTTGCCTAAGTAATCCTTTTCT	TAATTTGCCTAAGTAATCCTTTTCC
9	i25619Gh	43.58	14.96	CCCAGACAACATCACCAAGA	TAGATATGGCCATTGTGGGC	CTAGATATGGCCATTGTGGGT
9	i06016Gh	54.89	11.31	GTGGCTCCAGTTCTCCGTTA	ATGGAACCAACCAAACAAGAACTA	GGAACCAACCAAACAAGAACTG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
9	i59036Gb	62.70	7.81	AAAATTGATTATTAGACCATTACCA	CACATATACGTTCCAAAAAAATG	CCACATATACGTTCCAAAAAAATA
9	i52416Gb	63.13	0.43	GGAGGAAACTAAGGGAGACC	TATCCTTATCTTTCTTCTCCGAATA	TATCCTTATCTTTCTTCTCCGAATC
9	i50335Gb	70.40	7.27	GGAAAGACTCCTGCTTCCTG	GACTAATGATGGAGATAATCGGAA	CGACTAATGATGGAGATAATCGGAC
9	i06087Gh	72.95	2.55	ATGCGGTGGTAAAGTTCGTC	AGTTTGACAAGGTTACTCCTTTATATT	AGTTTGACAAGGTTACTCCTTTATATC
9	i25043Gh	81.06	8.11	GAACACAGATAGAAGACTGAGACAT	ACAAGATCACATCTTTTCATACGTG	CAAGATCACATCTTTTCATACGTT
9	i51639Gb	85.76	4.70	CGACCTTGGAAACCATCAAC	TGAAGATGTTGAAAAGCAATGAAT	TGAAGATGTTGAAAAGCAATGAAG
9	i50477Gb	88.77	3.01	CAACTGAAAGGTTCTCAACCAG	ATGTTGCAGCATTAAGAAAAGTGTA	TTGCAGCATTAAGAAAAGTGTC
9	i06319Gh	99.01	10.24	GCAGTAGAAAACATTTTGGAAAC	GATGAACTCTCACGATCATTTG	GGATGAACTCTCACGATCATTTA
9	i61111Gt	109.26	10.25	TGTACCAACACAAGCATCAGTG	CCCAACTCAACTCTTCCTTCAG	CCCAACTCAACTCTTCCTTCAA
9	i06474Gh	119.53	10.27	ATTGGCATCATTATCTACTCTTTTT	GTTCAAAAAGCTATGGAAAAACT	GTTCAAAAAGCTATGGAAAAACC
9	i50857Gb	120.81	1.28	TAGTAGCAGCATCAAGCATAAGA	CCTGTCTCATCTGCAGTCTCTTA	CTGTCTCATCTGCAGTCTCTTG
9	i51975Gb	129.78	8.97	TGCAAGATAATGTTATTGCTCAGA	TCTGGATTTAAACCACGTACAAG	CTGGATTTAAACCACGTACAAA
9	i51086Gb	142.17	12.39	GCTGCATGGTTTACCTTTCG	ATATATGCACGCAACCTTGTAATG	AATATATGCACGCAACCTTGTAATA
10	i12331Gh	6.44	6.44	GCAGCATTCCTTGATCTACCA	GTGTGGTGCAACCAGAACAA	GTGTGGTGCAACCAGAACAG
10	i12328Gh	6.87	0.43	AAGATGAACAGATGATTGAGATTG	CTGGCTCCAATTCTTCATCA	CTGGCTCCAATTCTTCATCG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
10	i49130Gh	10.29	3.42	AGCGATGGTTAAAGCTTTTTCTAAAT	AATATTGAAAGTGGGGTGATAAG	AATATTGAAAGTGGGGTGATAAA
10	i12285Gh	10.71	0.42	AAAATATAACTACAGGGTCTTACCTTT	TATATTACGTGGGCATTACAATACG	ATATTACGTGGGCATTACAATACA
10	i52506Gb	13.70	2.99	GCAATTCGGATTCTCTACGG	CTTTGAGAGAATGGATAAGGAAG	CTTTGAGAGAATGGATAAGGAAA
10	i12245Gh	14.98	1.28	GACCTTGGCTTTTCCAATGA	AACTTTGATTTTCTTTCACTTTATG	AACTTTGATTTTCTTTCACTTTATT
10	i17601Gh	19.68	4.70	GAAATGAGGATGTTCAAGATGG	CCTGTTGTTTACTACTTGTCCCG	TCCTGTTGTTTACTACTTGTCCCA
10	i51066Gb	35.91	16.23	GGCAAAGAGAGAGGGTATCG	TACCTATCATTACCTCTTGGAGCAG	TACCTATCATTACCTCTTGGAGCAA
10	i00406Gh	50.00	14.09	TCATACAAAAATGGCGGACA	CGGAAAGAATTAGCTATAAGAGGAC	CGGAAAGAATTAGCTATAAGAGGAT
10	i11901Gh	65.37	15.37	CCAGAAAGTGGAGATGATGG	TTGATTGTAGGCTGCCCTTTAC	TTGATTGTAGGCTGCCCTTTAT
10	i48101Gh	81.69	16.32	AATGACGTGATGGCAGCTT	AAGTTGAAAAAACTTGGATTGCTC	AAAGTTGAAAAAACTTGGATTGCTT
10	i24931Gh	81.69	0.00	CATTTAAGTTTTGTTCTGGAAAAGG	GACATCGTTGAAAAATTAGAAACTC	GACATCGTTGAAAAATTAGAAACTT
10	i42848Gh	84.25	2.56	AGAATTGACGGTCTCAAATATTCAT	TAACAGCGATGGTGTATTATGTTTC	TAACAGCGATGGTGTATTATGTTTT
10	i11978Gh	88.09	3.84	CGATTCTACAAATGTATAGCCAGTA	TCATTCTATCAATGTGTTATAAGTAGGT	CATTCTATCAATGTGTTATAAGTAGGC
10	i41571Gh	93.24	5.15	AATCTGAACTCGAGATGTGTTATATG	TTCCACAACACATATAAATCACATT	TTCCACAACACATATAAATCACATC
10	i00714Gh	93.24	0.00	AACTTCAGGAAGACAAGATCTGTAG	CAATTCTACAAATCATGTCCTCAA	CAATTCTACAAATCATGTCCTCAG
10	i17539Gh	93.24	0.00	ATATGAACTGAATATATCTGCTTGC	ATGTGAAAAAAGAATGCATAAGAAC	ATGTGAAAAAAGAATGCATAAGAAA

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
10	i51931Gb	103.49	10.25	ACTGTTTCCTCTACTTCCTTCTAAA	TTTTGTTTCCAGTAACAACACTTC	TTTTGTTTCCAGTAACAACACTTT
10	i17458Gh	106.92	3.43	CCTAAGTCAAAGGACTTAAGGAGTA	ACCAGTAGTCGGCACCATTT	ACCAGTAGTCGGCACCATTG
10	i00730Gh	126.19	19.27	TACTTAGTCATTGTGGTTATCTTGC	TACTCTGTTTTGCTCATAAAGTTCA	TACTCTGTTTTGCTCATAAAGTTCC
10	i39597Gh	135.21	9.02	TTTGGGAGCAATTTGTCTAGC	AGAAAACAATCGGACTAAAAAATGAC	AGAAAACAATCGGACTAAAAAATGAT
10	i60917Gt	139.49	4.28	TCAAGTGTGCGACTGAAAGAT	GCTATTTCGGGATAGCTGTATTC	GCTATTTCGGGATAGCTGTATTT
10	i11518Gh	151.58	12.09	CTTGAGAGTGATTATGATGAACCTA	TCTGCCTGTGTCTTTTCCAG	TCTGCCTGTGTCTTTTCCAA
10	i50887Gb	154.14	2.56	AACTCCGGGTGGTAGGATTT	GACATAAGATTAGACAACCCTTTG	TGACATAAGATTAGACAACCCTTTA
10	i51961Gb	155.85	1.71	GCAGTTGCTCGGCTTCTT	TTGAAGAAGATCGAAGAACCTGATA	GAAGAAGATCGAAGAACCTGATG
11	i00366Gh	2.14	2.14	GTGACCTCGACCTCAACTCC	TTAAACAAATTACTTCCATCACCATC	GTTAAACAAATTACTTCCATCACCATT
11	i06827Gh	16.69	14.55	GTCTTCGGACATGAGAATCATAAG	AGACTATTGGAACGAAACGCC	GACTATTGGAACGAAACGCT
11	i06885Gh	23.51	6.82	ATTCAGGCAATGGACCAGAG	AAGCAACAACTTCACTGGTAATCTC	CAAGCAACAACTTCACTGGTAATCTA
11	i52333Gb	31.18	7.67	AGGCAATAAAAGAACCAAGTAAAAC	CAGAACTAGTTACATATTTGGCTCTT	CAGAACTAGTTACATATTTGGCTCTC
11	i52531Gb	41.84	10.66	CTACCTTTGGCTGCCATTTG	AGATTGAAGTAGACACGAATAATGGAT	GATTGAAGTAGACACGAATAATGGAG
11	i50158Gb	52.11	10.27	TTTAATCAACATCTAATGGCTCAG	CGGAATAGGCATAACAGGAG	CGGAATAGGCATAACAGGAT
11	i07182Gh	61.51	9.40	ACTCTTAACTTTCCTCTTCTGTTCC	TGCAAGCTTTGATGGTGTTG	TGCAAGCTTTGATGGTGTTT

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
11	i00792Gh	70.47	8.96	AGTCACTGCCTACTGAAGTATATGA	TTTGAGGAGCACAGATTTACTG	TCTTTGAGGAGCACAGATTTACTA
11	i58197Gb	70.90	0.43	GGAGACTTGCTGACTAGACTTAAA	CAAATTCAGGACATTTTCGTAGT	AAATTCAGGACATTTTCGTAGC
11	i31411Gh	74.75	3.85	GGAGTTGAACAAAGTGATCCTG	ATGCTAGGCCTGTCAAATGT	ATGCTAGGCCTGTCAAATGG
11	i43779Gh	76.03	1.28	GGAAGAAAGTTTGACCATTAGAGC	ACTACCACGCTACCTCTTTAAATCT	ACTACCACGCTACCTCTTTAAATCC
11	i52034Gb	80.73	4.70	GAAGGTTGAACTGCATCTGGA	CTCGTCTGAGGAAGTAGAGGATGTA	CGTCTGAGGAAGTAGAGGATGTG
11	i34920Gh	92.27	11.54	ATGTGCTGTCATTTTGAATGTCC	GTGTCTCTCACACGGCCAAT	GTGTCTCTCACACGGCCAAC
11	i03278Gh	104.47	12.20	CCCCTGTGTATTCTGCAATG	TTTAATCATGTGGTGCAGTTATACG	TTTTAATCATGTGGTGCAGTTATACA
11	i03280Gh	104.47	0.00	GTGAATGAGCTTGTCCATACC	GCAACTAGTGAGCAACATAAACT	GCAACTAGTGAGCAACATAAACG
11	i52558Gb	122.44	17.97	GTAGCAACAGCAGCATTGAC	GTTGTTTAATGTACCGTGGAGACA	GTTGTTTAATGTACCGTGGAGACG
11	i57533Gb	122.44	0.00	CCAAGATATAATGCCCATGTAGTT	ATAACTCAATCTTGTGATCCACCTT	ATAACTCAATCTTGTGATCCACCTC
11	i50251Gb	123.72	1.28	AGACTGTTAGATTGCAAGAGATGT	CCGTAAGGACCCAAGTCAAAT	CCGTAAGGACCCAAGTCAAAG
11	i57155Gb	125.86	2.14	ACCATCGACTTTATTCCAACA	CATGATCAGCATTTCTTTATTCTTC	CATGATCAGCATTTCTTTATTCTTT
11	i19367Gh	139.94	14.08	ATTTCGCCATTGAACCAAAG	TTGTATTAAAATAAAATTCCGTTGGTT	TTGTATTAAAATAAAATTCCGTTGGTC
11	i07548Gh	154.91	14.97	GCTGCTACTACTGACTCTGAAAATA	AAAACAGTAGGCAGGTCATATCTC	AAAACAGTAGGCAGGTCATATCTT
11	i58276Gb	168.61	13.70	GATTGGAGTTGCTTGTTGCT	GAAGTCGATAAAGCCCACAC	GAAGTCGATAAAGCCCACAT

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
11	i29860Gh	181.91	13.30	TGCACGAGAGCAGTTTAAGG	AATGTTGCTTCTCTTTCAAATTAGC	CAAATGTTGCTTCTCTTTCAAATTAGT
11	i50720Gb	191.74	9.83	TTTTCCAACCTTTTTCTAAAGCA	ATCTGGTCAGAAGTTCAGGAGG	GATCTGGTCAGAAGTTCAGGAGA
12	i07754Gh	0.00	0.00	ATACCAACACAAGAAGAGATCAACT	TAGGGCGATCTTCTTCGTACAG	ATTTAGGGCGATCTTCTTCGTACAT
12	i28665Gh	0.00	0.00	ACAAAAGCCTTGACCCTAAGAAAT	CAAGGCAACCTTTTATGCTC	CAAGGCAACCTTTTATGCTT
12	i07809Gh	8.96	8.96	CAGCACAATTGAGACAAGTGG	TACATTACATGTAGCTTTTTGATGG	TTACATTACATGTAGCTTTTTGATGA
12	i49602Gh	14.94	5.98	TCCTTTTATTGTTTTTGCTTATTGC	CAACAACAATGACTGGTGAAGAG	CAACAACAATGACTGGTGAAGAA
12	i29317Gh	14.94	0.00	CACGCGTTATCACTCCTCAA	GATTTAGAGGTGATTGTTCTAGTGTC	GATTTAGAGGTGATTGTTCTAGTGTT
12	i16251Gh	29.07	14.13	CCTTCAAACCCCTTTTGTGA	CACTTTCCATGTCATTAGACCAAG	CACTTTCCATGTCATTAGACCAAA
12	i57388Gb	41.48	12.41	GTGTGGCTCTTGAAACTTGC	GAGCGAAAGACAAGTGAAAATTA	GCGAAAGACAAGTGAAAATTG
12	i07954Gh	41.90	0.42	GATCCTAGAGATTATGAATTTGGAA	TTTTTTCTCTTTTTCTCCTCCTC	TTTTTTCTCTTTTTCTCCTCCTT
12	i26734Gh	50.60	8.70	AAGCAATGAAACTACACCTTTAAGT	GATCATGAATGATATAACCTGTACTC	GGATCATGAATGATATAACCTGTACTT
12	i28903Gh	50.60	0.00	GCCATGGGGTGTACTATATG	ATATCAGAGTAGAACAACCCACGTC	ATATCAGAGTAGAACAACCCACGTT
12	i32547Gh	50.60	0.00	GTCCTAGCTACAATGAGTGATGAT	TTTATCAAACACTCTTATCGTATTCTT	TTTATCAAACACTCTTATCGTATTCTC
12	i34342Gh	50.60	0.00	TCTTACTGGACACTAAATTCACGTA	GTTATTGCATTGATTGATCCTG	ATGTTATTGCATTGATTGATCCTA
12	i36037Gh	50.60	0.00	GGTAAGCTACAATGGTATGAATGC	TCAAGTATTAAACCATTACACATTCA	CAAGTATTAAACCATTACACATTCG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
12	i41605Gh	50.60	0.00	AATGTTAAGAGTTGGAAAGGAGTTA	TTTTTATACAAAGCCTAAAACTATCA	TTTTTATACAAAGCCTAAAACTATCC
12	i56214Gb	51.03	0.43	CGTCCATGAATCCAACTTCT	ATCATATTAGGATTTGGGGAAA	ATCATATTAGGATTTGGGGAAG
12	i07978Gh	51.88	0.85	GAGAGAAAGGAACTAACTGAGATTT	TAACTTTCATCACATGGCCTCTC	TAACTTTCATCACATGGCCTCTT
12	i30336Gh	53.59	1.71	ATCGAGCAACAATATTTACCTTAG	CGTGGGATATGAAACAATACTTCTA	GTGGGATATGAAACAATACTTCTG
12	i60172Gb	53.59	0.00	ACACCATTTGCCTGTTCTACTTTA	GAACCTGATGAAGAGGAGCAT	AACCTGATGAAGAGGAGCAC
12	i45967Gh	56.15	2.56	CCTTATAGGTCACCATTGCACTAC	ATAAAAGGGAAAATATGGGGAGAGA	ATAAAAGGGAAAATATGGGGAGAGG
12	i52800Gb	60.93	4.78	TGAGAATTCCCAGTGAGCAA	AGTCATAGGACTTTCATGCCG	CAGTCATAGGACTTTCATGCCA
12	i50197Gb	74.59	13.66	ACGATATGTGGTGGCCTCAG	GTTGTAGTGGTAGCTGCTGTTGTT	GTTGTAGTGGTAGCTGCTGTTGTC
12	i08110Gh	81.42	6.83	GAGACAGCAATCAGAAGTCTTAATC	TTGATTTTGAAAAGATACAACTTACC	GTTGATTTTGAAAAGATACAACTTACT
12	i44466Gh	89.96	8.54	ACGAGATTTGTTTATCCTTGTCA	TTCACCTTATATGAATCTTGAATTGTTT	TTCACCTTATATGAATCTTGAATTGTTC
12	i38901Gh	92.53	2.57	CCAAGTGATCATTGTTGAAGTAAC	CCACCCTTATCTACGATTCTCA	CACCCTTATCTACGATTCTCG
12	i19468Gh	99.37	6.84	TTCACTGCATTTCAGGTATAATATG	TGTTGAATTTGTATTTATCAAAGGA	TGTTGAATTTGTATTTATCAAAGGG
12	i08345Gh	107.46	8.09	AGAGTGCGTTCAAGCTTATAGTAAT	ACCAAACACTCATCCAAAGCG	CCAAACACTCATCCAAAGCA
12	i08352Gh	107.46	0.00	GTCCATTGGATGAAGAATCAAC	ACTACCAGCATCGCCATCTC	ACTACCAGCATCGCCATCTA
12	i19491Gh	116.87	9.41	AGATGTGGTCACTACACGTTTTC	AGAAAAAAGAATTCATAACACTCAAT	AGAAAAAAGAATTCATAACACTCAAC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
12	i19492Gh	117.29	0.42	CTGTATGGATGGCTCTAGAAATACT	ATGCATTCACAAATACCACAATC	ATGCATTCACAAATACCACAATA
12	i54995Gb	117.72	0.43	GCTGAAGGGTTTGTGAGGAT	CTCATCTTCTCAATCTCCTTCAA	CTCATCTTCTCAATCTCCTTCAC
12	i08473Gh	118.15	0.43	AACACCTCAGCAGCATTTCA	GTTTTGGAGCGTAAAGTATTGAA	GTTTTGGAGCGTAAAGTATTGAG
12	i19497Gh	119.43	1.28	TCTCGTCTACCAAATTCCTCTG	GAACGAATTCCAGAAAAGTAGAAGAG	GAACGAATTCCAGAAAAGTAGAAGAA
12	i08493Gh	120.71	1.28	AACTCCTGGGTCTGGTTTCC	GTGACACACCACATTCTGAGTTAG	GTGACACACCACATTCTGAGTTAT
12	i08502Gh	121.14	0.43	ATAACCAAGGTTTGAAGAGATTAAA	GACCGCAACGTCTCAACAAT	GACCGCAACGTCTCAACAAG
12	i50471Gb	121.14	0.00	GTCCCTTTATTCAGGACCTC	GTGAAGCCAAATACGGGTCT	GTGAAGCCAAATACGGGTCC
12	i53894Gb	136.09	14.95	GAGAGATAGCAAAGCTTCTTCAAAT	GTGGTTAGAAGCTTATTTAAGGATAGGA	TGGTTAGAAGCTTATTTAAGGATAGGG
12	i08694Gh	147.19	11.10	TCCAACCAGACCTGAAGCAT	ATTTGCTGTCTCTGGTATTTCTCTA	TTGCTGTCTCTGGTATTTCTCTC
13	i54272Gb	0.00	0.00	TCAAGTTCTATAACTCTGCTACCATTC	TTTGATATATCGTCAACTAGAAACAAG	TTGATATATCGTCAACTAGAAACAAA
13	i12961Gh	0.00	0.00	AATGGCACATTTCAGGCATC	GGTGAAACCAAGTTCTTACATCT	GGTGAAACCAAGTTCTTACATCC
13	i13011Gh	12.47	12.47	GTAATACCAACCACGTTTTCTTC	TCCAATAGCCGTTGTATTTCTC	CTCCAATAGCCGTTGTATTTCTA
13	i51288Gb	16.31	3.84	ATGCCAGGGACATTAGCTTT	ATTGACACCCACACGTTTCTC	AATTGACACCCACACGTTTCTT
13	i30991Gh	16.74	0.43	CCAGCTACTTCAGGCCTTTG	ATCCCTTAACTAAAGAAATGACCAA	ATCCCTTAACTAAAGAAATGACCAC
13	i38620Gh	16.74	0.00	AAACAATTAGCTAGGCGTATGATT	GCTTGAAAATTAATCAAGAGAAATAGA	CTTGAAAATTAATCAAGAGAAATAGG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
13	i46139Gh	20.16	3.42	TGAAGCAGACCCTTTGTGC	GAAGAAATTGTGAGTTTTTCTTATACC	AGAAGAAATTGTGAGTTTTTCTTATACT
13	i54568Gb	30.90	10.74	ACCCCAACAGTGTTCTGAGC	GAGTCTATTGTTCTACTGTTATCACTGCT	GTCTATTGTTCTACTGTTATCACTGCG
13	i39407Gh	43.72	12.82	GTTGAATCGTAATTGAATGATGTTA	ACTCATATGTTCATTCATACCCAC	ACTCATATGTTCATTCATACCCAA
13	i54898Gb	43.72	0.00	TCTTCCTCTTCGTCTTCAATG	GACTTCACCATTCCTAAATACCAT	GACTTCACCATTCCTAAATACCAC
13	i61112Gt	48.85	5.13	ATTCCTTGTAGTGACTGGTGAAAG	AATTCCTTGGTAGGGAACTTTAC	AAATTCCTTGGTAGGGAACTTTAT
13	i56157Gb	58.67	9.82	CCACTCGAACTTAACATTTTTCTG	GGTTCATGAAAGGTTTCTCTATTG	GGTTCATGAAAGGTTTCTCTATTT
13	i24816Gh	65.95	7.28	ATTTGCTATTATAGATCAAGAGAAATG A	CCTAATCGTCAAATACTTTGTTCTG	CCTAATCGTCAAATACTTTGTTCTT
13	i35005Gh	74.92	8.97	AACGAATAGATGCTCAACAAGATT	GGTAAAATCTAGGCAAGTGAGACAA	GGTAAAATCTAGGCAAGTGAGACAG
13	i27466Gh	83.89	8.97	AAGTGTTACAACTCTCCTACCCTTA	TGCCCTAAGTTAAGGTAAAAAAT	TGCCCTAAGTTAAGGTAAAAAAG
13	i50603Gb	90.29	6.40	CCATCCAGAACACACCAAAA	GTATGTTCTCGGTTGGTTTAGCTC	GTATGTTCTCGGTTGGTTTAGCTT
13	i13517Gh	100.55	10.26	AACTTTAGATTCTGCTGTCCATAAC	AAGAAGAGTTCGATGAGAATGC	CAAGAAGAGTTCGATGAGAATGT
13	i13553Gh	106.09	5.54	ATGCATCATCAAGAGCACCA	GGAAAGGTGGACTTTCTATGTTTCTA	GAAAGGTGGACTTTCTATGTTTCTG
13	i13632Gh	117.20	11.11	AACGTCTAGATCTTTCAAGAGAGG	CAACTCGAGAAACCCCAAAC	CAACTCGAGAAACCCCAAAT
13	i52496Gb	117.20	0.00	GAAATAAACAGCAGCGCTTAAA	GAGGCTGTTGAGAGCGAAGA	GAGGCTGTTGAGAGCGAAGC
13	i61260Gt	125.35	8.15	TCAGTGTTTGTAATGGCGATG	CAAACAAAGCACAAATACAAACTAAGA	AAACAAAGCACAAATACAAACTAAGC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
13	i51136Gb	127.93	2.58	**GGCTCCACTGGATTCAACAT**	**AAAGCTAAACAAGAGGATTAAAAGA**	**AAAGCTAAACAAGAGGATTAAAAGG**
13	i13759Gh	141.73	13.80	**AATTAAAACAGAGGAAATCAAAGAG**	**GTTCGATCCATCTTCTCTTTACA**	**TCGATCCATCTTCTCTTTACG**
13	i13809Gh	144.30	2.57	**TAGGAGAAGTTTGAGGAGTCTGA**	**TCTAGAGTAGCTTTTGTTAGAACCG**	**CTTCTAGAGTAGCTTTTGTTAGAACCT**
13	i13873Gh	148.15	3.85	**CGATGTAGCCAAGCCATTCT**	**CACATCAACCCCATGATGAAT**	**CACATCAACCCCATGATGAAC**
14	i05721Gh	0.00	0.00	**AGTTCAGCAGCAACTTGCAC**	**AGTTGTCCGAATCTCTCTAAACTCC**	**AAGTTGTCCGAATCTCTCTAAACTCT**
14	i05712Gh	10.72	10.72	**GGTGTCGGAGAAGACGATTT**	**AAACAAATATGGAAGAGAAAGATGA**	**AACAAATATGGAAGAGAAAGATGC**
14	i05683Gh	13.71	2.99	**GAGTTTTCCAGACGGAATCG**	**CGAAGAAGGTTTTTCATTGTTA**	**CGAAGAAGGTTTTTCATTGTTG**
14	i62442Gt	13.71	0.00	**TGGTGTTTCCCAACCATTTT**	**AAAGCATTAACAGACCTTTTTTTTT**	**AAGCATTAACAGACCTTTTTTTTC**
14	i15563Gh	21.07	7.36	**TTCCGTATCGGGTTCAAGTG**	**CTGTATACCTGCTTTTCAGTTTCTC**	**CTGTATACCTGCTTTTCAGTTTCTA**
14	i19064Gh	30.96	9.89	**TGTTATTTTAGTGAAAACGAAACAG**	**AACAGGTTCCTTTTTGGAGC**	**AAACAGGTTCCTTTTTGGAGT**
14	i49624Gh	39.17	8.21	**AAGGATCTGGATTTGAAGTTACTC**	**AAGAGTGATGGTTCTTGGGA**	**AAGAGTGATGGTTCTTGGGG**
14	i52324Gb	39.17	0.00	**GTAGCCGTAGTCGGGGTTTGT**	**ACAGATAGTTTTCGGTGGTCG**	**ACAGATAGTTTTCGGTGGTCA**
14	i15512Gh	49.42	10.25	**CCGAAAACTGGGAATCCATA**	**GGATGCATACTAAGATCAAACAAG**	**GGATGCATACTAAGATCAAACAAA**
14	i05458Gh	50.27	0.85	**AAAAACAACCAGTCAGGCAAC**	**GCAGAAAAAATTTGGATGATAAAGT**	**CAGAAAAAATTTGGATGATAAAGC**
14	i05355Gh	60.52	10.25	**TGGAGTAGATGCTGCTGAGG**	**TCTGAGATTGTAGTCAAAGAGTTGT**	**CTGAGATTGTAGTCAAAGAGTTGC**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
14	i38261Gh	60.52	0.00	**TTTCCATGCACTGCAAACTC**	**AAACCCCTTATCAAGTCTCCTTAT**	**AACCCCTTATCAAGTCTCCTTAC**
14	i57074Gb	60.95	0.43	**CAGAATCAGATGTAGTTTCCTACCC**	**GGGATTGTTAGATATGGTGTGTAA**	**GGGATTGTTAGATATGGTGTGTAG**
14	i35706Gh	62.22	1.27	**GGGATGGATGCTAAAGAATTGA**	**CTAAGGGAGTACTAAATCCGAGACT**	**TAAGGGAGTACTAAATCCGAGACC**
14	i38921Gh	75.04	12.82	**TCTGATTTATCATATAGCGAAGAACC**	**CTTATTTCTCAACTCTTTGATCTCAT**	**CTTATTTCTCAACTCTTTGATCTCAC**
14	i29634Gh	75.46	0.42	**CGAAGGACACCAAGAGGAAA**	**TGATGTTCAAGTTTCTCATCATTTA**	**GATGTTCAAGTTTCTCATCATTTC**
14	i28274Gh	86.86	11.40	**CGGATGTTACTTAAAGCCATGT**	**AGCTATAGAGAAGTTTTTTGGAGAA**	**AGCTATAGAGAAGTTTTTTGGAGAG**
14	i58710Gb	94.55	7.69	**TCCATGGTGTAACCCTACAAAA**	**TGTTTATGAAATCCATGAGGAAC**	**TGTTTATGAAATCCATGAGGAAT**
14	i05256Gh	96.26	1.71	**GGCAAAAGAATGCAGGAAGT**	**GCTTCAGCTTTGGCATCTTT**	**GCTTCAGCTTTGGCATCTTC**
14	i05148Gh	105.43	9.17	**CAGGGCACGTACTGAGACAA**	**TCTTAAATCAATTCTTCTGCTTTTC**	**TCTTAAATCAATTCTTCTGCTTTTT**
14	i05146Gh	106.28	0.85	**AAGAATGCTCCTCAACAAGGTC**	**AGAGCTGTATGTAAAGATTGGTGTT**	**GAGCTGTATGTAAAGATTGGTGTC**
14	i36289Gh	110.98	4.70	**CCTCATTCTACTATATTGCTTAAATCC**	**TGCAGCTGAGTTAGGTTCCAG**	**TGCAGCTGAGTTAGGTTCCAA**
14	i49059Gh	128.29	17.31	**CTATACGGCCTGGGCACTT**	**AAATTCTGCAAAATAGGGTCACAC**	**AAATTCTGCAAAATAGGGTCACAT**
14	i57809Gb	129.14	0.85	**CTAAACCTTTGGACTTGAAGGT**	**AAAGTACAACACATGACACCTTG**	**AGAAAGTACAACACATGACACCTTA**
14	i04957Gh	141.10	11.96	**GGGTTTGCCATCTTCAGGTA**	**CTTTTCCATCAGATTAAGCTCTTTTT**	**CTTTTCCATCAGATTAAGCTCTTTTC**
14	i04870Gh	152.23	11.13	**AAACAAATTGGGAGCAGGTG**	**CATTGACCAATAAAATACCCTAAG**	**CATTGACCAATAAAATACCCTAAA**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
15	i13988Gh	0.00	0.00	TGCCCAAATCATAGAAAACA	AATTAATGGAAGTGTAGAAGAGGC	AAATTAATGGAAGTGTAGAAGAGGA
15	i14767Gh	9.42	9.42	GTTTTCTATCTCACATGTTCCTTTC	TCAAAAACTCTCCAAGCCTCT	CAAAAACTCTCCAAGCCTCC
15	i02942Gh	10.70	1.28	TGCATAGGGAGCTTTCCATT	GATCCTTAGACGGATCTATCATCA	GATCCTTAGACGGATCTATCATCG
15	i02942Gh	10.70	0.00	TTCCATTATACACACTGATCTGAAG	ATCCTTAGACGGATCTATCATCA	ATCCTTAGACGGATCTATCATCG
15	i50860Gb	21.39	10.69	GATCATCATCAGCAACAACC	TTATCTTGAATCACCTCTTTAAGTTT	TTATCTTGAATCACCTCTTTAAGTTC
15	i02862Gh	33.00	11.61	TTGAAGCTGAGAATCCCACA	GGATCTCTCCTCGTTTCTTCCT	GATCTCTCCTCGTTTCTTCCC
15	i48166Gh	33.86	0.86	GGGGTTTGATACTTCGTGTT	GATTAACCTAACTAACTCATTGCTGA	ATTAACCTAACTAACTCATTGCTGC
15	i14720Gh	41.11	7.25	TCATGGTGCAAGGGAGGATA	TATCAGAGAGCCATCTTCAAAGTAA	AATATATCAGAGAGCCATCTTCAAAGTAG
15	i50187Gb	50.06	8.95	TCTGCATCCCGAAATTTACA	GCTCTGCACGTTTCTTGTGAC	GCTCTGCACGTTTCTTGTGAT
15	i02576Gh	60.75	10.69	GTGCCAAAACAGGGTGAACT	GCTACTGTAATTTGGTCTGCTTGA	GCTACTGTAATTTGGTCTGCTTGG
15	i35660Gh	67.63	6.88	GGGGCTCACACTATCTGTTT	CTAGCTCAAAATGATCAAAAGTCTAT	TAGCTCAAAATGATCAAAAGTCTAC
15	i02512Gh	71.03	3.40	AGGGATTGTAGTATTTCTCTCTCTG	GACAAAGCATCTTTAGCAAGTC	GGACAAAGCATCTTTAGCAAGTT
15	i50340Gb	73.59	2.56	AGTGATGCGAATGAAACTTTTG	CACATGCCAACATAAAGGCG	CACATGCCAACATAAAGGCT
15	i02444Gh	83.43	9.84	TGAGAAGTTTTCAATTTCTATTTCC	CCAGATATACTTTCAGTCAATGGC	TCCAGATATACTTTCAGTCAATGGT
15	i18386Gh	89.83	6.40	TCGGCATTGACTATGCAAAT	GCATTTTTATTTTTGTTTCCACTTT	GCATTTTTATTTTTGTTTCCACTTC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
15	i14594Gh	98.79	8.96	ACTCAACCCTGCTCCAACTG	CATTATTGTATTGAGAAACGAAAGTT	ATTATTGTATTGAGAAACGAAAGTG
15	i13968Gh	101.78	2.99	GAATCTCTCAGTAATTCTGATTTTG	ATTTCAATCACTGCTTTTGTCA	TTCAATCACTGCTTTTGTCG
15	i02249Gh	112.48	10.70	CGGTTTAAGCACTGGTATGGA	GTACATTATTTAAAGTCATCCTCCG	GGTACATTATTTAAAGTCATCCTCCT
15	i56500Gb	113.76	1.28	TCTCTCCACCATTCTGTCATGT	GTGTTCAGATACTCTCTTGTCTTTT	GTGTTCAGATACTCTCTTGTCTTTG
16	i51425Gb	0.43	0.43	CCAGATCAACCTGGATTTGTT	AAAGATGAAAACTTCGGGTATAAGG	GAAAGATGAAAACTTCGGGTATAAGA
16	i43172Gh	11.97	11.54	GGAATTTTGGTGGAAACGTA	AAATTGCAGATATATTCAAAGAACTAAG	AATTGCAGATATATTCAAAGAACTAAA
16	i47062Gh	30.05	18.08	AAGATAAATCGAAGCTAATTCGTC	TTTATCTTCCAATCTCAATTTTACC	TTTTATCTTCCAATCTCAATTTTACT
16	i58337Gb	39.49	9.44	TCTTGCTTGGTAGGTAAGATGAC	TCTAGGGGATTTCTGACCTTCA	CTAGGGGATTTCTGACCTTCG
16	i24697Gh	44.62	5.13	CGAAGTTTCATTTAATTCGTCTAA	ACTATTATCCACTTCAGCATAGAGG	GACTATTATCCACTTCAGCATAGAGA
16	i46465Gh	44.62	0.00	AGAGCTAATCCAATCAATAATGTG	GAATGTATAGCTCTTTGGAGTATTTTC	GAATGTATAGCTCTTTGGAGTATTTTT
16	i30646Gh	44.62	0.00	TAAAATCCTTAAGAGATCCTCACAC	AATTTTGAAAAATCGACAAGCTAT	ATTTTGAAAAATCGACAAGCTAC
16	i34538Gh	52.74	8.12	GCGTCAAGGAATCTCATAGC	TGACGTCAACATCATCTTCC	TGACGTCAACATCATCTTCT
16	i44284Gh	61.70	8.96	CGGAAAGTAAGGTGGACATC	AATAGTTATCCATTTTAGGTATCCGT	ATAGTTATCCATTTTAGGTATCCGC
16	i01703Gh	71.95	10.25	TGCTGAAGATAGGTTTGTTGTCA	GTAGAGCTCCCAAAGTGAGTATCTT	GTAGAGCTCCCAAAGTGAGTATCTG
16	i01592Gh	80.07	8.12	ACATGAAAATCTCTAGCTCTTTGAG	CACAAGATCAAAAGCTGATAACC	TCACAAGATCAAAAGCTGATAACT

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
16	i37829Gh	80.50	0.43	**GGCAACGCAATACTATAAACTAGG**	**GAGACAAATTGGAAGGGAAT**	**GAGACAAATTGGAAGGGAAC**
16	i01516Gh	88.19	7.69	**CCTTTCATCACTAACAAAGCTAAAT**	**TAGTGCTTTCTTAGTGTGTTAGAGG**	**TTAGTGCTTTCTTAGTGTGTTAGAGA**
16	i14326Gh	99.74	11.55	**GGGTGACAAGGGAACAAGAA**	**TCAGCTACTTCATTCATTATTCCTTC**	**TTCAGCTACTTCATTCATTATTCCTTT**
16	i01404Gh	112.15	12.41	**GTACCCTATATTGCCGAGTCC**	**TTTGATTGCTACAAAAGCAAAA**	**TTTGATTGCTACAAAAGCAAAG**
16	i61313Gt	112.15	0.00	**ATAGATCGAAATAGCTAACTCAAAAA**	**AAGCTATACAGCAGTAAAAAGAACAC**	**AGCTATACAGCAGTAAAAAGAACAA**
16	i42554Gh	122.39	10.24	**ATGTCCTCAGGGCCTTCTTT**	**ACACCTATCCAAATAAACAAAACTA**	**ACACCTATCCAAATAAACAAAACTC**
16	i51754Gb	133.50	11.11	**TGATCTTCGACTCAGCTTCG**	**CAAGATCTTCAAGGGTTATGGTCT**	**AAGATCTTCAAGGGTTATGGTCC**
17	i53982Gb	0.00	0.00	**ATTGCTCGAAGCCAATGAAC**	**ATACTGAGTCAGAAGCTGAAACGAT**	**ATACTGAGTCAGAAGCTGAAACGAC**
17	i00195Gh	9.44	9.44	**GGCGAAGTGCGAATACTTTT**	**GTCGAGAAATTCCAAATATAATCCTC**	**GTCGAGAAATTCCAAATATAATCCTT**
17	i03187Gh	20.56	11.12	**ACCCGAAAACTTCCGTTCAT**	**GCACCTGAAGTTCACAGAAAAC**	**GCACCTGAAGTTCACAGAAAAA**
17	i51917Gb	33.79	13.23	**CAGTGTCGGCTTTTCTGCTA**	**TCTAAAGGACCATATCAACCATAAC**	**TCTAAAGGACCATATCAACCATAAA**
17	i58179Gb	39.78	5.99	**CCTGAACCACTTGCAGTTACA**	**ATCATATCAGAAAAATGAGAAGGTT**	**ATCATATCAGAAAAATGAGAAGGTC**
17	i62933Gt	39.78	0.00	**CCACATCGGGGACATACTG**	**GCTACTCAAAAGGTTAACAAACAC**	**TGCTACTCAAAAGGTTAACAAACAT**
17	i51043Gb	41.49	1.71	**TTCAAAGCTAGAGAGATTGTTTTTAGA**	**GATTTTAGTGCCACCCCCAC**	**GATTTTAGTGCCACCCCCAA**
17	i03371Gh	49.60	8.11	**CAACAAATGGCACAAACGTC**	**TAAGGCTGCATCAGCTACATTT**	**TAAGGCTGCATCAGCTACATTC**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
17	i14906Gh	62.07	12.47	ATTAAGTATGGCATCTGGGTAAGT	TATAAGGATTATGTCACAGGGCCAG	TATAAGGATTATGTCACAGGGCCAA
17	i03479Gh	71.66	9.59	ATCCTCCGAGTCCAAGAATAC	GGCACCAGGATTATAGAAGCA	GCACCAGGATTATAGAAGCG
17	i63519Gm	83.37	11.71	CCAGACAAGTGAACAACCTACC	GGGTTCTAGTTGTGTTGTATTGAA	AGGGTTCTAGTTGTGTTGTATTGAC
17	i63519Gm	83.37	0.00	GTCTCCTGTTCAAGAACACTCC	GGGTTCTAGTTGTGTTGTATTGAA	AGGGTTCTAGTTGTGTTGTATTGAC
17	i39989Gh	91.05	7.68	TGGTCTTTTCCCTTCTTTGG	CACAATCATAGGCAAAAATAGGAAC	CACAATCATAGGCAAAAATAGGAAT
17	i60810Gt	107.30	16.25	CCATTTGTTGAAACCGGAAT	TTACCGTTCCTGATTCCTTTACTTT	TACCGTTCCTGATTCCTTTACTTC
18	i12965Gh	0.00	0.00	GAGAATGTGAAGGCTGTTGG	CTCGAAAAAGAACTAAGCATTTGG	TCTCGAAAAAGAACTAAGCATTTGA
18	i21923Gh	8.22	8.22	GTATAAGACCTAATTTGCCCATTT	AGTACCATGTAGGCTCTTGTACTGA	AGTACCATGTAGGCTCTTGTACTGG
18	i12997Gh	9.08	0.86	TTTCTTCTTTCTCATTTCTTAGTCC	GAATCATCTTCCTTCTTACGCCTTT	GAATCATCTTCCTTCTTACGCCTTC
18	i49418Gh	15.95	6.87	TGCTTATCCCACACATCCTG	GATATTGCAAGTTCTCAAGTCAC	GGATATTGCAAGTTCTCAAGTCAT
18	i13068Gh	24.10	8.15	ACCAAGCCCTGTTAGCTTGA	ACTGTGAAAGTGAATGAACAAATGA	TGTGAAAGTGAATGAACAAATGG
18	i34219Gh	30.07	5.97	CATTGGACCAACGGATGTAA	GAGGACCTTCTTCTTCTCTATTTC	GGAGGACCTTCTTCTTCTCTATTTT
18	i34709Gh	38.61	8.54	AGAACCATTCGTTCAGAGCGTTA	AAAGAAGTTTGGAAACAGGTAATCT	AGAAGTTTGGAAACAGGTAATCC
18	i13189Gh	45.51	6.90	GAACGAAAGAAGGAAAGAGTAACTA	ACCTAAAACCCTCTTAAACTCTTCT	ACCTAAAACCCTCTTAAACTCTTCC
18	i52366Gb	47.65	2.14	GTGATGTAGCGCAAGCAAGA	AACCGGTAAGCTTCTCACTTTA	CCGGTAAGCTTCTCACTTTG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
18	i49235Gh	58.75	11.10	CAACCGAGACTGCAATGTGT	TCCCGACTAATAGCTTTATATCCTT	CCCGACTAATAGCTTTATATCCTC
18	i17987Gh	61.74	2.99	TTAAGAGAGCCACGTTCTTACAACT	CTGGGATCAAGGAGGACTTACTTT	CTGGGATCAAGGAGGACTTACTTC
18	i13504Gh	73.71	11.97	ATACATTGCCTACTACCATCTCATT	TGCCATAAAAAGGCAAAGTC	TTGCCATAAAAAGGCAAAGTA
18	i13519Gh	74.14	0.43	ATAGAAGAAACTGCTGAGAAATGTT	GATCTTCTAGCAAAGAAGTGTGC	GGATCTTCTAGCAAAGAAGTGTGT
18	i50412Gb	83.96	9.82	TGGGAACTCAGCATCAAACA	AGTGAAGAAGAGGGATCATTGC	CAGTGAAGAAGAGGGATCATTGT
18	i00473Gh	107.60	23.64	GCATTTGCTTTTATGGTCCTC	TTCTAGAAAATATGAAAGGATGAAACAG	TTCTAGAAAATATGAAAGGATGAAACAT
18	i13724Gh	110.16	2.56	TCTTAATCCTTTTCTTCATCATCTC	CCGGCAAATCAGACATACTT	CCGGCAAATCAGACATACTC
18	i47826Gh	123.90	13.74	GGATTGCTAATGTAGATAGACAGAAGA	TGTACTGCTTATTCAAACCTACCTAT	TGTACTGCTTATTCAAACCTACCTAC
18	i13806Gh	123.90	0.00	AAACCACCATTTCCAAGTGC	ACTAAAAGTTTTGTAGGAGAAAGATG	GACTAAAAGTTTTGTAGGAGAAAGATT
18	i13850Gh	125.18	1.28	GGTGTTGACAATTGCCTTCC	CCTCTGGACAGAAGAAATGACA	CTCTGGACAGAAGAAATGACG
19	i65047Gm	2.56	2.56	TGAGGAAGAAACATGCTGTG	AAGGTAGATACTAATAATCACCCAAG	AAGGTAGATACTAATAATCACCCAAA
19	i00196Gh	5.98	3.42	AAAGTCTCTGATGCTTGATTGTAG	ATTTGCCATTTAAAAACTCAACTT	ATTTGCCATTTAAAAACTCAACTC
19	i50131Gb	11.53	5.55	GTTGTTTCACTTAGTTCTTTATTTG	CCCCTAAAGCTACCTACTAACTAAG	CCCCTAAAGCTACCTACTAACTAAA
19	i65286Gm	11.96	0.43	AGATTACATACAACGAAGGTTTCC	GAAGAAGAAGAGGGTAAAGGTAGA	GAAGAAGAAGAGGGTAAAGGTAGG
19	i10421Gh	20.50	8.54	GATGACAAGGTCTTGAGTGCTG	TAGCCTGTTGTGATGAGACAGTG	TTTAGCCTGTTGTGATGAGACAGTA

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
19	i10319Gh	31.19	10.69	**ACAGAAAGAGAGAGAGAACCTTACA**	**GCTCTTAGTGAATCTAGGGAAGC**	**ATGCTCTTAGTGAATCTAGGGAAGT**
19	i10321Gh	31.19	0.00	**GACTCTGAAACCAATGATACTGTAG**	**TCCTTCTCTTCATACCAGTGC**	**ATCTCCTTCTCTTCATACCAGTGT**
19	i26414Gh	40.58	9.39	**TTCCTTAATAATGGATGATTCTAGC**	**CCACAATAAGAGAGGAGAAGAAAT**	**CCACAATAAGAGAGGAGAAGAAAG**
19	i00755Gh	50.53	9.95	**CTGTAATGCTAACAGCTTTTGTAAG**	**TACACCTAATGAACGCTGCTG**	**TACACCTAATGAACGCTGCTT**
19	i45829Gh	58.71	8.18	**AAATACCTACTCCTCCTCTTGC**	**ACAATCAATGTCAATCGTTTCTTAT**	**ACAATCAATGTCAATCGTTTCTTAC**
19	i26399Gh	71.62	12.91	**GATGCAATTTCTCTATAGCCTTCT**	**GTATCCCGTACCAGTACACTCCAAC**	**GTATCCCGTACCAGTACACTCCAAT**
19	i10126Gh	81.86	10.24	**ATGTGCTCCTTGACATCTGC**	**CTATGAATGACAGGCAAAGTCG**	**TTTCTATGAATGACAGGCAAAGTCT**
19	i09951Gh	106.32	24.46	**TTCCTACTACCTTGGACTCTCCTA**	**TCTTTAATGCTCCAATCCCTAC**	**TCTTTAATGCTCCAATCCCTAT**
19	i09954Gh	106.32	0.00	**ACATTGTCAGAGAGGAGGATCT**	**CATCTTCATCGTCACTTTCG**	**GTCATCTTCATCGTCACTTTCA**
19	i09898Gh	120.45	14.13	**GGAGGCTTAGCATCTGCTGT**	**ACAATCTTAATACTCAAACCACCAC**	**ACAATCTTAATACTCAAACCACCAT**
19	i50264Gb	130.71	10.26	**AAAAAGTAGCTGAATTTTGATTGAA**	**TCTATTTGCTTGGAGGGTTG**	**TTCTATTTGCTTGGAGGGTTT**
19	i09626Gh	141.39	10.68	**CCTAGAGGCACTTCCTTAGCA**	**TTTCTAGACGTTATTTTTATTGCAG**	**CTTTCTAGACGTTATTTTTATTGCAT**
19	i09572Gh	152.59	11.20	**CTGTTCGGAAACAAGCCTAC**	**CGGATAGTCCACTTGAGGAA**	**CGGATAGTCCACTTGAGGAG**
19	i09526Gh	160.78	8.19	**TCTCTGCTAGCATGGCTTGA**	**CATTAGTACTGATCACGGGTTTCA**	**ATTAGTACTGATCACGGGTTTCG**
19	i09340Gh	171.89	11.11	**GGAAAAGTGAAGATAACATTGTCAG**	**TTCTTCGTTTTCAGTTTAAGAGAGT**	**CTTCGTTTTCAGTTTAAGAGAGC**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
19	i09334Gh	172.74	0.85	**TTTGTTCGTTTAACTTATATTTTGG**	**CTTCTGCTTGATGAGGACCG**	**CTTCTGCTTGATGAGGACCA**
19	i09244Gh	183.05	10.31	**CTTCTGCATCACCCGAGATT**	**TTGGTTAGTGTGGATATTTTATTCCTT**	**TTGGTTAGTGTGGATATTTTATTCCTC**
19	i16569Gh	205.27	22.22	**TATAATCGCCATGGAATATTTTCTT**	**TAAATTTGAATTCCTATTGAGGC**	**GTAAATTTGAATTCCTATTGAGGT**
19	i52329Gb	205.27	0.00	**GGGGAAATTTTTCGTTACTTC**	**TTGAGCTCGAACTTTCATACTCT**	**TGAGCTCGAACTTTCATACTCC**
19	i61131Gt	216.82	11.55	**CAGATTTTGATGAGAACGATGT**	**AACCAAGTAACTATCCACGACA**	**ACCAAGTAACTATCCACGACG**
19	i16538Gh	217.25	0.43	**TGCTTATTACTTATTGGGTGTATCA**	**AAACATTTGGGCGGTAAACG**	**AAACATTTGGGCGGTAAACA**
19	i42912Gh	222.82	5.57	**ATAACCTCAATACACACATCCAAG**	**TAAATAGTCTGTATGGATTGTTTTC**	**TAAATAGTCTGTATGGATTGTTTTT**
19	i08802Gh	224.10	1.28	**AGATCAAGGTTCAAGTCTAGTTACC**	**ATGCTCGTCCCTTGGTTCTA**	**ATGCTCGTCCCTTGGTTCTC**
19	i08803Gh	224.10	0.00	**TGGAAGCCTTGAGAGCTTGT**	**CGCAAACATAGAAACTAAATAATAGCAG**	**CGCAAACATAGAAACTAAATAATAGCAA**
20	i17673Gh	0.43	0.43	**ATGAGGCTGTGTCGGTTGTT**	**GAGCAAGATCGTAATAAAGAGCAGA**	**AGCAAGATCGTAATAAAGAGCAGC**
20	i17604Gh	21.40	20.97	**GGTGTTAGTGTTGTTCATTTTAGTG**	**TACAGTGGTCTGGGATAAAAGTAAT**	**TACAGTGGTCTGGGATAAAAGTAAC**
20	i50202Gb	24.82	3.42	**GAGAGAAGGACCTAATCTTGTCG**	**CTTCAAATTTCTTCTGTGTTTTGT**	**TCAAATTTCTTCTGTGTTTTGC**
20	i12189Gh	30.82	6.00	**TCGGTTTATGGCATCTCTGG**	**TGTCATTTGCAGAAGCTTTTTTATT**	**TGTCATTTGCAGAAGCTTTTTTATC**
20	i20406Gh	45.81	14.99	**AGCAAGTCGGGCTAAGAAAA**	**CAACTATATTCCACACTATCACCTTCTG**	**CCAACTATATTCCACACTATCACCTTCTA**
20	i20107Gh	51.35	5.54	**CATTTTAGTCAATGTATCATTACGG**	**ATTCTTGTCACACGATATTTTTG**	**ATTCTTGTCACACGATATTTTTA**

Chr.*	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
20	i49481Gh	60.66	9.31	GGATAGGTGTAGGGGTGATTTTT	GACAGTTGAATAACTAAGCCCCA	GACAGTTGAATAACTAAGCCCCC
20	i25537Gh	75.70	15.04	TGAATTCGATTTAATTTTGGGAAT	ATAGAACTTGATGCTCAACCAACC	AATAGAACTTGATGCTCAACCAACT
20	i34452Gh	80.40	4.70	ATCTGTAATGTTAAGAAACAAACAGAA	TGATTTGGGTTGGCTGTGTG	TGATTTGGGTTGGCTGTGTA
20	i44471Gh	90.70	10.30	GACGTGCTCAATCACGTACAA	CAAAATATTACGCAAAACAAACAAC	CAAAATATTACGCAAAACAAACAAA
20	i11812Gh	97.96	7.26	CTTCTAGATTCCCGTACCTAAAGTT	ATGCTTACAAGGTTTTAGAAGAGAT	ATGCTTACAAGGTTTTAGAAGAGAC
20	i58775Gb	108.62	10.66	TGATGTTTCATCATCGGAAAA	TTCCAATTTAGTCCTTTTCTTTAAT	TTCCAATTTAGTCCTTTTCTTTAAC
20	i17444Gh	120.59	11.97	GAGATGTGGTCATGCCAGTTT	GCCTATCCTCTGAAAATATTGGTGT	CCTATCCTCTGAAAATATTGGTGC
20	i11623Gh	130.84	10.25	ATATCAGAAAAATGCTTTACTCCAT	AAATTTGTTCCCAGCTTCTCT	AATTTGTTCCCAGCTTCTCC
20	i11625Gh	130.84	0.00	GATTTTCAATAGAAAGACTCTCTGC	CTCTCTTCACGGCTCTCTAAGT	CTCTTCACGGCTCTCTAAGC
20	i11590Gh	136.81	5.97	GTGGAGTTCCATGCTTTGGT	AGGTAAGGTTAGCAATGGGTTTG	GTAAGGTAAGGTTAGCAATGGGTTTA
20	i50531Gb	143.64	6.83	AAGGGTGAACTCGAGGACTTT	AATTTGGATGATGATACTAACAGGT	ATTTGGATGATGATACTAACAGGG
20	i61483Gt	143.64	0.00	ATGGCAATTCGTCGTAATCC	TGTTATCATCAATATCGAACACACT	TTATCATCAATATCGAACACACC
20	i11470Gh	151.73	8.09	AACAAAACCTTCCTATGGACGA	GCAGCAATGATATCTTTCGATTTAG	GCAGCAATGATATCTTTCGATTTAT
21	i50608Gb	2.13	2.13	CGACTTTCTTGACGGGGTAA	CAATACCGAGAATAACCGATACG	CCAATACCGAGAATAACCGATACA
21	i55362Gb	22.21	20.08	TCTTCTTCATTTTCCATGTACCC	ATAGAGTTCGTGGAAGGAGAAA	ATAGAGTTCGTGGAAGGAGAAG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
21	i15938Gh	31.22	9.01	GTTCTGAAGACAACAAAGTCCTATT	GAGATCGCAGAAAAGAAGACC	AAGAGATCGCAGAAAAGAAGACT
21	i06916Gh	31.65	0.43	TTATTGTTTAGTCCCAGATTCAAA	GAGAGAAGCGACGATTAGGG	GAGAGAAGCGACGATTAGGA
21	i52139Gb	41.04	9.39	AAGGTGTCTGCTCAGGAAGC	AGCAATATGCTAGTAAAGGAGAAGA	AGCAATATGCTAGTAAAGGAGAAGG
21	i51304Gb	45.32	4.28	AGGGTTGCTGTAAAATTTGG	AATTTGTTACTGAATGTTGTGTGTA	AATTTGTTACTGAATGTTGTGTGTG
21	i07031Gh	54.36	9.04	GTGAGATGTAGGGGAAGTGG	CTCAGAACTAAAGTAACAAAATGAATG	GACTCAGAACTAAAGTAACAAAATGAATA
21	i07037Gh	54.36	0.00	AAATGCATCTGTATCACACGTATAG	AGAAGGGGCAAAAAACAACT	AGAAGGGGCAAAAAACAACC
21	i07089Gh	64.22	9.86	GAGTAATTTCAAGCATCATCCTTAT	GTACAAGGTAGTTGTATGGGGACT	GTACAAGGTAGTTGTATGGGGACC
21	i14095Gh	64.22	0.00	TGCTCTTCTGCAAGTAAGATATAAA	ATGTAACGTGTTGATCTTCCG	AGATGTAACGTGTTGATCTTCCA
21	i50923Gb	69.79	5.57	CCAGTAGGTAACGGGAAATCA	ACTCCAAGGAATACTGCAAAA	GACTCCAAGGAATACTGCAAAG
21	i60993Gt	80.89	11.10	AGAAGCTTGGAGTGGTGGTG	CCATATGAACTTCAATACTACCCG	TCCATATGAACTTCAATACTACCCT
21	i60559Gb	91.12	10.23	TTCAGAACTCTAGCTTATGAGAATG	ATCTTCTTATCATGCCACCGA	ATCTTCTTATCATGCCACCGC
21	i16036Gh	91.98	0.86	GAGATCGATCATCAAGTTCCTAC	AGACCCCAATTTTTTGCATC	AGACCCCAATTTTTTGCATT
21	i57476Gb	106.06	14.08	CATATGTGAGTTATCTCCGAATGTC	GAATCACATGTTTACTATCCCAACT	ATCACATGTTTACTATCCCAACG
21	i51903Gb	118.68	12.62	TGGAGCAGGTTTTGGATTCT	GTTAGGAATATGACAGAGGAAGAGAGA	TAGGAATATGACAGAGGAAGAGAGG
21	i40036Gh	136.90	18.22	TGATGACCTGTCACCCTAGAT	TAAACCATTACTATCAGGCAATTCA	TAAACCATTACTATCAGGCAATTCG

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
21	i07470Gh	138.61	1.71	**ATGAGCACTTGTGAATTATTGTAAG**	**GAAGAGACGAAGAATCAACATCAAT**	**GAAGAGACGAAGAATCAACATCAAC**
21	i37548Gh	139.46	0.85	**AAAGGTTCTGACATATAACCTAGCA**	**GTTACTTAACCTCATGACTCGTTCA**	**GTTACTTAACCTCATGACTCGTTCG**
21	i07568Gh	151.86	12.40	**AAATCTTGAACACGTTGATATTATG**	**GTTGTTATTGCTCAGTTTAGGG**	**GGTTGTTATTGCTCAGTTTAGGA**
21	i16110Gh	151.86	0.00	**ATATTTGAAGCTCTCCCATATGTT**	**CTTACTGATGGTTCTCAAGTGC**	**CCTTACTGATGGTTCTCAAGTGT**
21	i16137Gh	163.44	11.58	**TCAATTCATCCACACCCAAC**	**CGAAAGATTAAGGAATTGAAGATG**	**TCGAAAGATTAAGGAATTGAAGATA**
21	i50692Gb	169.41	5.97	**AGGACTAGCATTAGTCTTTTTATAAGC**	**TGCTAATAAACAAATGGTATTGCT**	**GCTAATAAACAAATGGTATTGCG**
21	i59747Gb	171.55	2.14	**GTCTTTGGGGATAATTTGGCATTAG**	**TACCATGGCTTTCTCTACATTCA**	**TACCATGGCTTTCTCTACATTCG**
21	i52000Gb	184.37	12.82	**TGTAAACAGAGCCAAAGCTACC**	**GATTTTCTTCTGGTAATTTAATTGGTGT**	**TTTCTTCTGGTAATTTAATTGGTGC**
22	i17680Gh	0.00	0.00	**AAACTTTTCCCCTCCTTTCG**	**TGGAAGATGCAGTGAAAGTAGAGTTA**	**GGAAGATGCAGTGAAAGTAGAGTTG**
22	i38556Gh	12.81	12.81	**CCTCTTTTCTTCATTACCACCA**	**GATCTTTTGACAAGTTTACTACGTTCC**	**GATCTTTTGACAAGTTTACTACGTTCA**
22	i20148Gh	26.17	13.36	**TTGGACATGGATGCTACACA**	**ATGTGAGCGTAACAGTCATTTCATC**	**ATGTGAGCGTAACAGTCATTTCATA**
22	i12526Gh	28.30	2.13	**AGAAGAAAAGAGCCATGACC**	**ATCCATTTAGGTCTGTGTCCA**	**ATCCATTTAGGTCTGTGTCCG**
22	i40262Gh	34.77	6.47	**CTCCTGTTTTGGTGGAAGTG**	**TAACTCCTTAACGTGAAAACTTACC**	**TAACTCCTTAACGTGAAAACTTACT**
22	i12566Gh	35.63	0.86	**TGATGATGCTTCCAACAAGC**	**GTTCTTTCTCAGGGAATCATCAA**	**GTTCTTTCTCAGGGAATCATCAG**
22	i61086Gt	44.59	8.96	**TAGTGGAAGGGGATTCCAAGT**	**GTGCTAATTTGTGATATGTCTGC**	**GTGTGCTAATTTGTGATATGTCTGT**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
22	i39605Gh	45.87	1.28	CCATCACATCTTCCATCTGA	CTTCTTGTTCCCGCTTCAAT	CTTCTTGTTCCCGCTTCAAC
22	i48531Gh	54.54	8.67	CATACGTGTCTTTTAGTAGGCATAA	GGAATGATCATCAACCACTCA	GAATGATCATCAACCACTCG
22	i12685Gh	57.10	2.56	AACCTCACCTCCTGTTACTTTATC	ATCCTCCTGCAGCTGTAATCT	ATCCTCCTGCAGCTGTAATCC
22	i12686Gh	57.10	0.00	CCTGTGATTTAGATGGAATGC	GTCTGGCCCAAACTTCCTTT	GTCTGGCCCAAACTTCCTTC
22	i10394Gh	62.32	5.22	AGCCGATGTCAAAGCTGATT	TTAATTTAGCCAAGAATCCCAACTAT	TTAATTTAGCCAAGAATCCCAACTAC
22	i12687Gh	72.15	9.83	CTCTCTGCACAAGGCAACC	TGAAGGTTACTTCCCCAGTAAAAAC	TGAAGGTTACTTCCCCAGTAAAAAT
22	i34388Gh	82.74	10.59	GCTTTCAAGCACAAAATTATTATCG	CTAAACGTTCTACCCCAGCATAC	CTAAACGTTCTACCCCAGCATAA
22	i56516Gb	90.44	7.70	AACAAATTCATGATTATTCATATCG	GAGAGGATGATGAGTTAGAGAGTGT	GAGGATGATGAGTTAGAGAGTGC
22	i12801Gh	100.71	10.27	GCGAAAGCTTCAAATCTGGT	ATTTCGTTAGTGAGGTTATGAGTGC	AATTTCGTTAGTGAGGTTATGAGTGA
22	i12895Gh	112.66	11.95	CCTAAGCGTGCTGAGAGAGTC	TTGTCAAAGTCAGATTGGAGTGTT	TTGTCAAAGTCAGATTGGAGTGTC
22	i12939Gh	127.68	15.02	GTGGGATGAGAGGGTGAGAA	TCTTCCATTACTCCCCTTGTTTT	TCTTCCATTACTCCCCTTGTTTC
23	i05794Gh	0.00	0.00	TGTATGTGGAGAAGAGATCGAGA	TTTCTCATTTCGCTTTTATCTGC	TTTTTCTCATTTCGCTTTTATCTGA
23	i05851Gh	8.97	8.97	CATTGGCAGGTCATTTTTCC	ATATTCAGTAACATCAGCAGAACAA	ATATTCAGTAACATCAGCAGAACAG
23	i44760Gh	9.82	0.85	TATGAGCTTCGGGGCTACTG	CTCCCTTAAAAATTCAAAATGCC	GCTCCCTTAAAAATTCAAAATGCT
23	i05936Gh	36.95	27.13	AGCTTTCCAATGTCCTGCAA	CATCTGTGATGATTCCAAGAGG	TTCATCTGTGATGATTCCAAGAGT

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
23	i35476Gh	47.63	10.68	CGAAAAGGACTTCATGTAGCA	GTTTATCGAGTTCATCCACAATAGA	GTTTATCGAGTTCATCCACAATAGG
23	i61300Gt	60.43	12.80	GCATTCATGTCCTGGTTCAA	TGGCTCCATTTCTTGAGGTC	GTGGCTCCATTTCTTGAGGTA
23	i15725Gh	69.81	9.38	AATCAACAATACTCTCATTGCTTCT	GAGGCAATCATCGGTCTAAA	GAGGCAATCATCGGTCTAAG
23	i06169Gh	71.95	2.14	GAAATGATGCTGCGGAGACT	CACATGTGTTAAGAATGTTGTCCAT	CACATGTGTTAAGAATGTTGTCCAC
23	i61516Gt	83.92	11.97	TCCCTCAGCTTCACTTCCTC	CCTAGAAATTAACAATATTAGAGGCGAC	CCTAGAAATTAACAATATTAGAGGCGAA
23	i62777Gt	84.77	0.85	CCCCCAATTTTTATTTCTCTAGC	GAGATATTCAGTCCTACAGCGTTT	AGATATTCAGTCCTACAGCGTTC
23	i23862Gh	94.60	9.83	GCTTTGGAGAAGGTAACTTGTGA	ATTATTGACCACGAATATCATCCAT	ATTATTGACCACGAATATCATCCAC
23	i34362Gh	108.28	13.68	AGGCCTTAACATGGGAACAA	TTTCATTGTCCAAGTTAGTTTCTG	TTTTCATTGTCCAAGTTAGTTTCTA
23	i06568Gh	116.39	8.11	TTTTTAGAGATTGCGTTCTTTATGT	TCTGCGCATGAGTTAGTCCC	ATACTTCTGCGCATGAGTTAGTCCT
23	i06632Gh	126.64	10.25	TCGGAACTAGGCGGTATCAC	CCACCATGATTTCGTTATGACT	CACCATGATTTCGTTATGACC
24	i51057Gb	0.00	0.00	TTCCGGTTTTATGTCAAGTGG	GGCAAACATAGGATAGTGTGAAGAT	GGCAAACATAGGATAGTGTGAAGAC
24	i54758Gb	0.43	0.43	TAATGGCGTCTCGCTTTACA	TTTGTTGTAAACCAAACCGAT	TTTGTTGTAAACCAAACCGAC
24	i04738Gh	12.03	11.60	TCCTCAGGAAAGAACCGAGA	TTGTCCGCAGCTTAAGGTAAAC	TTGTCCGCAGCTTAAGGTAAAT
24	i35512Gh	18.86	6.83	TATTGGGATTCGCAAAGGAA	TCTTTTTGAACATTAAACAATCTCC	TCTTTTTGAACATTAAACAATCTCT
24	i04633Gh	24.43	5.57	ATCGAGGGTCCAGGAAAGAA	AAACCCTAGTCCATGCTTGCTAT	AACCCTAGTCCATGCTTGCTAC

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
24	i04615Gh	27.41	2.98	**CTAGGACACCGGCGAGAGT**	**TCCACTTATAGTAGGAACCCTTTTT**	**TCCACTTATAGTAGGAACCCTTTTC**
24	i31467Gh	39.80	12.39	**TTCAAACTATTGGTTCTTTTCAAGG**	**CAAAGCTATTGGTTGCTATTCTATT**	**CAAAGCTATTGGTTGCTATTCTATC**
24	i36237Gh	50.91	11.11	**AAGGATAAGCAGTGGCAGAT**	**TGATTTTCACACAGCAACAAATA**	**GATTTTCACACAGCAACAAATG**
24	i50001Gb	50.91	0.00	**GGATAAAGAGACAAAGGTTGGTTA**	**CCTTAAAGAAGATACTCAAACCCTC**	**CCTTAAAGAAGATACTCAAACCCTT**
24	i49724Gh	59.88	8.97	**GCCTTGTTCCCACCAGAATA**	**GACAGATTTGCAGACAGGGAC**	**GACAGATTTGCAGACAGGGAT**
24	i04257Gh	72.68	12.80	**GCTGAGTTGGATGCGAAACT**	**ACTTGTGGGTATTAGTTTTTCTGCTTA**	**TTGTGGGTATTAGTTTTTCTGCTTG**
24	i50008Gb	86.35	13.67	**TTATTGGGAGTGTTCTGAAGAGTA**	**GGTTCAAGATCGCCAAAAAG**	**GGTTCAAGATCGCCAAAAAA**
24	i03938Gh	96.61	10.26	**CTCCTCAACGCCTTTATGTC**	**CGTTGGAGGTATCGTTGTTATT**	**CGTTGGAGGTATCGTTGTTATC**
24	i03855Gh	111.14	14.53	**AGTCAGCATCTGGTTCTAAGGT**	**AGAAGGCAAATCACATACAGCA**	**AGAAGGCAAATCACATACAGCC**
24	i03844Gh	113.71	2.57	**TCTGATACCCAAATGAAGTTATTG**	**CAACCTCGTGGTGATTTGTCT**	**CAACCTCGTGGTGATTTGTCC**
24	i03838Gh	114.56	0.85	**ATCGGTTGTTGCGGTGTT**	**CTACGACAGCATAAAATGAAGAAT**	**CTACGACAGCATAAAATGAAGAAC**
24	i51167Gb	120.96	6.40	**ATTCCACATTCATTGCTACAGTCT**	**GAAGAATATAGAAGGGCATCAGTT**	**AAGAATATAGAAGGGCATCAGTG**
24	i50055Gb	129.50	8.54	**ACAAGGACCTGCATGCATAA**	**CATTTCTAATTTTTCATCTCCGACA**	**ATTTCTAATTTTTCATCTCCGACG**
25	i51276Gb	1.28	1.28	**AACCCATTAGAGGTGCCAAA**	**TATTCCTATACATCGGTTTCACGTC**	**TATTCCTATACATCGGTTTCACGTT**
25	i11447Gh	2.56	1.28	**CAACAGACATTGAGGGGTTT**	**ACACCTGTTGTTTAGAATGTTTAGC**	**ACACCTGTTGTTTAGAATGTTTAGT**

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
25	i11372Gh	14.61	12.05	AACTTCATCTCCGGCTTGC	CTAAATAATTTGCGCCATGAAC	GCTAAATAATTTGCGCCATGAAT
25	i53437Gb	15.89	1.28	CCTCCTCATCTTCATCATCA	TGTAAGTAATAATCCGTATGTGAGG	CTGTAAGTAATAATCCGTATGTGAGA
25	i11316Gh	24.02	8.13	TTGACTGTGGAAGCCATTGA	TTAGCCTCATAAAGCTTCTTTTTTCT	TAGCCTCATAAAGCTTCTTTTTTCC
25	i11253Gh	36.84	12.82	TCACCTCTCCGATTTTCTCAA	GGTTGATCCAACTCTAAGCTCTTTT	GGTTGATCCAACTCTAAGCTCTTTC
25	i33715Gh	43.67	6.83	CGAGAAATTACAAATGCCTCT	TTACGACTATCTCTTTTGGGTATTC	TTTACGACTATCTCTTTTGGGTATTA
25	i11187Gh	47.97	4.30	TGCTGTTGATAATGAGGCAAA	TCTTCAAGAGCTTGTACCTTCTCC	TCTTCAAGAGCTTGTACCTTCTCA
25	i51081Gb	50.53	2.56	TAGTACCGAGGATAAGTCAGCTA	TTCTTAAGTGTGTTATTTTCAGCAG	TTCTTAAGTGTGTTATTTTCAGCAA
25	i17265Gh	59.06	8.53	TTTGCATGAAATGAGGGTGA	GAATAACGAAAGTGTCGAGATGATT	AATAACGAAAGTGTCGAGATGATG
25	i52068Gb	73.63	14.57	CAGCTGCTGAGGGAATAAGG	TAATTCCACAGTTTTGCTATTACAC	TAATTCCACAGTTTTGCTATTACAT
25	i60673Gb	84.32	10.69	AGTAACATTCTGAAGCAAAAAGTCT	TCAACATGCATCACCTCGAC	TCAACATGCATCACCTCGAA
25	i00963Gh	85.60	1.28	CATGAATTTGCAGTCCCTTTAG	TACCTTGTCTAGGATGTCCCTTTC	TACCTTGTCTAGGATGTCCCTTTT
25	i10803Gh	96.70	11.10	GAAGTTGCTCTTTGCGCTTC	AACTTGGAAGATATGAAATTGAGACA	ACTTGGAAGATATGAAATTGAGACG
25	i17168Gh	109.96	13.26	TGGAAGAGTCAGTCCACGTA	GCATAAATATTACTGGATCACGAA	GCATAAATATTACTGGATCACGAG
25	i10674Gh	120.19	10.23	AAAGACGCCACTACGAAGGT	GTTACTGATATGGGTATTGGTGTTG	GTTACTGATATGGGTATTGGTGTTT
25	i60789Gt	130.42	10.23	AGACTGCTATTGGGAAGACC	ACCTAGATAGTTAAATTCATTGCTG	TCACCTAGATAGTTAAATTCATTGCTA

Chr. *	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
26	i40991Gh	0.00	0.00	ACAGCAGCAGCAACAAAATG	CGATCCTAATAATTCCAGTTATGGTCT	GATCCTAATAATTCCAGTTATGGTCC
26	i30070Gh	13.63	13.63	GGCGTGTTCGGGTTGTATTA	TGAGGAACTTTTATTTTCCTGACTA	GAGGAACTTTTATTTTCCTGACTG
26	i07792Gh	14.06	0.43	TTCACTTGGTTTTGCCCTTT	ATGAATTCAGCTTCTGCATATCCTT	GAATTCAGCTTCTGCATATCCTC
26	i59951Gb	34.18	20.12	CCTGTCAGGTAGGTGCCACT	CCTATGAGGCACTGTTAATGG	CCCTATGAGGCACTGTTAATGA
26	i50506Gb	45.69	11.51	ATGCAAGACAACGTGGTTGA	CTTACAGTTCGGAGGATGTTAGG	GCTTACAGTTCGGAGGATGTTAGA
26	i47604Gh	60.61	14.92	CAGACAGATTGAGACCAACACAA	GTTGCATTCTATTCAAATCACCAG	GTTGCATTCTATTCAAATCACCAA
26	i08115Gh	72.59	11.98	CAGTCGGGGTGTTTTCTTTG	AAAGTAACTTCTTTGTGATGTGGTC	AAAGTAACTTCTTTGTGATGTGGTA
26	i08123Gh	74.29	1.70	CCTTCGCTATGGCAAATGTT	TTGATGTCTTGGTCTTCGTCTTC	TTGATGTCTTGGTCTTCGTCTTT
26	i19454Gh	80.73	6.44	TATGTCGTCATTAGTCTTTATGGAA	TCTGCTACTTCTGTCTGTGTGATA	CTGCTACTTCTGTCTGTGTGATG
26	i08217Gh	87.98	7.25	GAGAGATCTGAAAGAGGGAGGT	CTGAAATTCCTCAAACCAGG	CCTGAAATTCCTCAAACCAGT
26	i08312Gh	98.22	10.24	CTTCTTCATTTTGTTCATCAACTC	AGAAAAGGACTTGGGTTGAGA	GAAAAGGACTTGGGTTGAGG
26	i16420Gh	105.06	6.84	CCAATCGATCTGCTGTTGAA	TGATATCGAGCATGTAGTGGAAGT	GATATCGAGCATGTAGTGGAAGC
26	i14139Gh	116.15	11.09	CAACAACAACAACAACAAGG	CCAATTTTCTTTTAAGATTCCC	CCAATTTTCTTTTAAGATTCCA
26	i55509Gb	120.85	4.70	AAGAAGGGCCACTGTTTGTG	ATGCTTCTGCTAAAAGGAATGG	GATGCTTCTGCTAAAAGGAATGA
26	i08657Gh	135.83	14.98	TCTTTTTCTAAAACTTCAAGAATCG	AGGAACAGCTCTAAGTCCCA	AGGAACAGCTCTAAGTCCCG

Chr.*	Marker ID	Chr.* Location (cM)	Gap Distance between Primers (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer Sequence	Allele-specific (forward) Primer Sequence
26	i19526Gh	136.69	0.86	**GTACCCGAGCATTGCCATAG**	**CATCAATGGAATAAGCTCCTCAATA**	**ATCAATGGAATAAGCTCCTCAATG**
26	i47939Gh	145.65	8.96	**AAACACCACCCTTCCATGC**	**CTACATAAAATGAAACAAGAAATGC**	**CCTACATAAAATGAAACAAGAAATGA**
26	i08773Gh	147.36	1.71	**CAAAATAACCGGGCTTTTGA**	**ACTAGTTAATGAGTTGATTTCAGGC**	**ACTAGTTAATGAGTTGATTTCAGGT**

*Chromosome

TABLE A2

Chr. *	Marker ID	Chr.* Location (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer	Allele-specific (forward) Primer	Assay Applicability **
2	i54009Gb	65.00	**CCATTTGGAGATCACATAGGATT**	**AATTCTCTCTGCAGTACATCCAC**	**AATTCTCTCTGCAGTACATCCAT**	Gb
2	i55860Gb	77.80	**GTCATCTCTCTTGGACATGG**	**AAGGTTTCTTATAGGGTTTTTTCTA**	**AAGGTTTCTTATAGGGTTTTTTCTG**	Gb
2	i52679Gb	77.80	**ACAATAATAAAAATGAAAAGCATCC**	**ATCAATCTCACATCTCATCCTG**	**CATCAATCTCACATCTCATCCTA**	Gb
3	i52684Gb	18.50	**TGGACAGATCCCAAGTTCAA**	**ATATGGTTGGCTGTGATTCTCTG**	**ATATGGTTGGCTGTGATTCTCTA**	Gb
4	i38788Gh	54.04	**CAAATTATAACTGAGCCAAAATAAA**	**GAACATCATTGAAGATCACATTATTA**	**AACATCATTGAAGATCACATTATTC**	Gb, Gt
5	i57054Gb	80.86	**TTGTTAGGTTGATGTCCCAAAG**	**TTGTCTCCACAAACTCTTTCAA**	**TTGTCTCCACAAACTCTTTCAG**	Gb, Gm
5	i09202Gh	167.20	**ACATCCTGAATTGGATAGGTTACT**	**AGCATTCCTTTCTTTGGCAT**	**AGCATTCCTTTCTTTGGCAC**	Gb
6	i39819Gh	17.09	**GATGCAGACAAGCATGGTCA**	**AGAGAATACAGCACTCTGAAAACA**	**GAGAATACAGCACTCTGAAAACG**	Gb, Gm
8	i53234Gb	24.00	**ACCGAGACAAAGAAATTGTGG**	**AGTGACACCAAAGGTTGAAAAT**	**GTGACACCAAAGGTTGAAAAC**	Gb
8	i53187Gb	143.30	**AGTAACAAATGCTTCGAGCAAA**	**GACTGCAACCCATAGATAAAAAGT**	**TGCAACCCATAGATAAAAAGC**	Gb
9	i38265Gh	10.66	**CAGCTACCCCAAGATTCCAA**	**CTCCCATATGTTTTTTTAGTTGAA**	**CTCCCATATGTTTTTTTAGTTGAG**	Gb, Gt
10	i12344Gh	0.00	**ACTGTCAGTGAACACCATTAAAAC**	**AAAAGGGAGTGAAGCTATGAGA**	**AAAGGGAGTGAAGCTATGAGG**	Gb
11	i57772Gb	92.70	**GTGATGACTTGTGTTACTAGAATAGGA**	**TGTTCAAACTATTATACCAGTCAATTC**	**TGTTCAAACTATTATACCAGTCAATTT**	Gb
11	i35097Gh	168.61	**CAAGCTAACGCAACAATCCA**	**CTACGAATCCTAAACATAGACAAAC**	**CTACGAATCCTAAACATAGACAAAT**	Gb, Gm
12	i30183Gh	8.53	**TGTTGCTAGAGGATGTTGTGC**	**CATAATCTTCCAACATTTCCTATCT**	**ATAATCTTCCAACATTTCCTATCC**	Gb, Gm

Chr. *	Marker ID	Chr.* Location (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer	Allele-specific (forward) Primer	Assay Applicability **
12	i38424Gh	92.53	**TTTCAGAGTCCATTACTAAGAGTCA**	**GGGTTGGCCATATTTTAGCA**	**AGGGTTGGCCATATTTTAGCG**	Gb, Gt
13	i50597Gb	68.09	**CCCTTGTTAGACTTGTTTTCTTCTA**	**ACAAAAAACTCAACGCTGATACAT**	**AAAAAACTCAACGCTGATACAC**	Gm, Gt
13	i29895Gh	71.94	**CCATAGTGGGTGTCAAAAGATAATA**	**AGTTCCAGTTGGTATGTGTGTC**	**AGTTCCAGTTGGTATGTGTGTT**	Gb, Gt
13	i42126Gh	71.94	**GGCTTAGAACAAATGCTGAATTAC**	**CATTCATTCAGTCATTTCTTCTTG**	**ACATTCATTCAGTCATTTCTTCTTA**	Gb, Gm
13	i18161Gh	148.15	**TTCATTTTACCGAATAAAAGAGC**	**TTCTCTCTCCTCCGACGACC**	**TTCTCTCTCCTCCGACGACT**	Gb, Gm
14	i49372Gh	94.97	**CAACTCTCCCCTCATTAGGAA**	**CACAATAGACAAACCTTTTCACAG**	**CACAATAGACAAACCTTTTCACAT**	Gm, Gt
15	i41293Gh	51.35	**CCGAATATGACAGAGAAGAAATC**	**ATGTTACGATTGGGTTTTGATTT**	**ATGTTACGATTGGGTTTTGATTC**	Gb, Gm
15	i02476Gh	79.16	**ATATTGCATACTGGTTACCAACTG**	**ATCCCTGAAATACCTTGGAA**	**CATCCCTGAAATACCTTGGAG**	Gm, Gt
16	i23935Gh	41.63	**ACAGAATGGGCCTGTTAGTCT**	**ATCAGAGAATTGAACACAGGACTC**	**ATCAGAGAATTGAACACAGGACTT**	Gb, Gt
18	i29063Gh	38.61	**CCAATCCAAGGTCCAAGTTTTAAG**	**GGAATTCTGTTATAGTTCCCCTG**	**TGGAATTCTGTTATAGTTCCCCTA**	Gm, Gt
18	i18118Gh	110.16	**ATATTTTTCTGGTTTTCTTTTTCCT**	**CAGAGACAAGGTTGTACCAAG**	**CCAGAGACAAGGTTGTACCAAT**	Gb, Gm
19	i30712Gh	71.62	**AAAGTGTTATTTTTAGCACTGGATAC**	**ATTTTCTTGAATGCATCTTGTACCT**	**TCTTGAATGCATCTTGTACCC**	Gb, Gt
19	i09946Gh	105.47	**GAGTTCTTGCCCTGATATAAGTATT**	**GTATGCCATATTGGGTGTTG**	**GGTATGCCATATTGGGTGTTA**	Gb, Gm
19	i61307Gt	133.70	**GAATAAGAACAAGAGCAACAACAAG**	**GATCAAAGGGAAGATTAGGGTAGT**	**CAAAGGGAAGATTAGGGTAGC**	Gb, Gm
20	i35001Gh	97.10	**TACGATGATGTGTAATCGTATTGG**	**AAGTGGGTCTCAAGATTCTCATA**	**AGTGGGTCTCAAGATTCTCATC**	Gb, Gt
21	i55800Gb	158.25	**CCTTTAAGCCTGAGTTTGGA**	**TTTCTATGTTTTTGTGGAGTGAATA**	**TCTATGTTTTTGTGGAGTGAATG**	Gb, Gt

Chr. *	Marker ID	Chr.* Location (cM)	Common (reverse) Primer Sequence	Allele-specific (forward) Primer	Allele-specific (forward) Primer	Assay Applicability **
22	i12679Gh	57.10	TTCTACTTTGAATTCTTCCTTCATT	TCTGGTACAAGAGGGTTGGG	TCTGGTACAAGAGGGTTGGA	Gb, Gt
23	i28369Gh	6.41	ATTGATTACGAATAATCTTAGCATA	ACACTTTTAAGAGGTTATATCCAGG	GACACTTTTAAGAGGTTATATCCAGA	Gb, Gt
25	i59371Gb	50.53	GCGTCCCTTGTTCTTCTCTT	GTGATAGAAATCAAAAAGGGATAGG	GTGATAGAAATCAAAAAGGGATAGA	Gb

*Chromosome

**Gm, Gt, and/or Gb indicate that an assay detects the donor allele of *G. mustelinum*, *G. tomentosum* and/or *G. barbadense*

TABLE A3

Array Map for Fluidigm Plate 1											
7208.001	7208.009	7208.017	7208.025	7208.033	7208.002	7208.018	7208.026	7208.034	7208.003	7208.011	7208.019
7208.027	7208.035	7208.004	7208.020	7208.028	7208.036	7208.021	7208.029	7208.014	7208.022	7208.03	7208.007
7208.015	7208.031	7208.008	7208.016	7208.024	7208.032	7208.041	7208.049	7208.073	7208.042	7208.05	7208.058
7208.066	7208.074	7208.043	7208.051	7208.044	7208.052	7208.06	7208.068	7208.076	7208.037	7208.045	7208.053
7208.061	7208.069	7208.038	7208.062	7208.07	7208.047	7208.063	7208.071	7208.04	7208.048	7208.056	7208.064
7146.13	7146.14	7146.52	7146.57	7146.61	7146.62	7146.63	7146.69	7146.70	7146.81	7146.82	7146.83
7146.84	7146.88	7146.94	7146.97	7146.98	7147.04	7147.06	7147.08	7147.09	7147.10	7147.11	7147.12
7147.13	7147.14	7147.15	7147.17	7147.19	P1-TM-1	P2-Gb	P2-Gt	P2-Gm	Gb-F1	Gm-F1	Gt-F1

Array Map for Fluidigm Plates 2-6											
7208.001	7208.009	7208.017	7208.025	7208.033	7208.002	7208.018	7208.026	7208.034	7208.003	7208.011	7208.019
7208.027	7208.035	7208.004	7208.020	7208.028	7208.036	7208.021	7208.029	7208.014	7208.022	7208.03	7208.007
7208.015	7208.031	7208.008	7208.016	7208.024	7208.032	7208.041	7208.049	NTC	7208.042	7208.05	7208.058
7208.066	7208.074	7208.043	7208.051	7208.044	7208.052	7208.06	7208.068	7208.076	7208.037	7208.045	7208.053
7208.061	7208.069	7208.038	7208.062	7208.07	7208.047	7208.063	7208.071	7208.04	7208.048	7208.056	7208.064
7146.13	7146.14	7146.52	7146.57	7146.61	7146.62	7146.63	7146.69	7146.70	7146.81	7146.82	7146.83
7146.84	7146.88	7146.94	7146.97	7146.98	7147.04	7147.06	7147.08	7147.09	7147.10	7147.11	7147.12
7147.13	7147.14	7147.15	7147.17	7147.19	P1-TM-1	P2-Gb	P2-Gt	P2-Gm	Gb-F1	Gm-F1	Gt-F1

TABLE A4

Group of 317 Markers						
Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
1	Best	122	133	113	42	123
2		101	126	112	124	132
3		55	43	54	101	43
4		39	15	38	50	19
5	Failed	0	0	0	0	0
Total		317	317	317	317	317
Average		2.03	1.81	2.05	2.50	1.87

Group of 17 Markers						
Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
1	Best	2	3	3	4	3
2		7	6	6	4	6
3		4	5	4	6	5

Group of 17 Markers						
Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
4		4	3	4	3	3
5	Failed	0	0	0	0	0
Total		17	17	17	17	17
Average		2.59	2.47	2.53	2.47	2.47

Group of 14 Markers						
Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
1	Best	9	5	8	4	6
2		4	7	4	5	6
3		1	1	2	4	1
4		0	1	0	1	1
5	Failed	0	0	0	0	0
Total		14	14	14	14	14
Average		1.43	1.86	1.57	2.14	1.79

Group of 38 Markers						
Ratings		No. Assays Scorability		No. Assays Tightness		
		P1-Het	P2-Het	P1	Het	P2
1	Best	0	18	10	9	17
2		0	15	15	16	16
3		0	4	7	6	4
4		0	1	6	7	1
5	Failed	38	0	0	0	0
Total		38	38	38	38	38
Average		5.00	1.68	2.24	2.29	1.71

TABLE A5

F2-based ratings for assay applicability, pan-specific and species-specific genotype cluster tightness and scorability of Phase-I-selected KASP/PACE Assays run in microfluidic Fluidigm arrays.								
Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
1	i61225Gt	0.00	Gm, Gb, Gt	2	2	2	2	3
1	i23982Gh	14.14	Gm, Gb, Gt	1	1	1	1	1
1	i51041Gb	26.10	Gm, Gb, Gt	1	1	1	1	1
1	i27626Gh	38.59	Gm, Gb, Gt	1	2	1	3	2
1	i34731Gh	56.68	Gm, Gb, Gt	2	2	2	2	2
1	i55595Gb	72.04	Gb,Gt	2	3	2	2	3
1	i45642Gh	84.03	Gm, Gb, Gt	1	3	1	2	2
1	i47972Gh	97.67	Gm, Gb, Gt	1	1	1	2	1
1	i02391Gh	115.19	Fail	5	5	5	5	5
1	i50196Gb	126.74	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
1	i00132Gh	140.47	Sub-cluster-Gt	1	1	1	2	1
2	i50655Gb	0.85	Gm, Gb, Gt	3	2	2	2	2
2	i52423Gb	13.26	Sub-cluster-Gt	1	2	1	1	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
2	i03174Gh	21.83	Gm, Gb, Gt	1	1	1	2	1
2	i01044Gh	29.51	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
2	i43496Gh	41.50	Gm, Gb, Gt	2	2	3	3	2
2	i22764Gh	50.90	Fail	5	5	5	5	5
2	i61080Gt	52.61	Gm, Gb, Gt	2	2	2	3	2
2	i60741Gt	64.99	Gm, Gb, Gt	2	2	1	2	2
2	i29065Gh	73.53	Gm, Gb, Gt	1	2	1	2	2
2	i52292Gb	80.37	Gm, Gb, Gt	2	2	2	3	2
2	i57002Gb	89.33	Sub-cluster-Gm	2	1	2	2	1
2	i64451Gm	103.05	Fail	5	5	5	5	5
2	i63139Gt	112.87	Gm, Gb, Gt	2	1	2	2	1
2	i38489Gh	122.79	Gm, Gb, Gt	1	1	1	2	1
2	i00543Gh	132.73	Gm,Gt	2	2	2	1	2
3	i05735Gh	2.56	Gm, Gb, Gt	2	1	2	2	1
3	i05710Gh	10.32	Gm, Gb, Gt	2	2	2	2	2
3	i50850Gb	25.77	Gm, Gb, Gt	1	2	1	1	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
3	i19058Gh	38.22	Gm, Gb, Gt	4	3	4	3	3
3	i00178Gh	48.93	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	2
3	i05459Gh	57.46	Gm, Gb, Gt	3	2	2	3	1
3	i14041Gh	69.43	Gm, Gb, Gt	2	1	2	1	1
3	i14878Gh	84.36	Gm, Gb, Gt	1	1	1	1	1
3	i52480Gb	97.24	Gb,Gm	2	2	1	3	2
3	i14000Gh	108.78	Gm, Gb, Gt	3	2	3	3	2
3	i43612Gh	121.18	Gm, Gb, Gt	2	2	2	2	2
3	i66018Ga	136.14	Gm, Gb, Gt	2	1	1	2	2
4	i00706Gh	0.43	No Call					
4	i00466Gh	0.85	Gm, Gb, Gt	2	2	3	2	2
4	i24385Gh	0.85	Sub-cluster-Gb	1	1	1	1	1
4	i25377Gh	10.25	Sub-cluster-Gb	1	1	1	1	1
4	i49276Gh	21.40	Gm, Gb, Gt	1	1	1	2	1
4	i10424Gh	33.46	Gm, Gb, Gt	4	2	4	3	2
4	i29272Gh	39.48	Gm, Gb, Gt	3	1	3	1	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
4	i50228Gb	53.19	Gm, Gb, Gt	1	2	1	2	2
4	i32552Gh	66.86	Gb,Gm	3	3	3	3	3
4	i47058Gh	73.73	Gm, Gb, Gt	2	2	2	3	2
4	i52022Gb	89.10	Gm, Gb, Gt	2	2	2	3	2
4	i53431Gb	99.36	Gm, Gb, Gt	2	3	2	2	3
4	i50976Gb	109.21	Recovery of homozygous-Gm, Gb, Gt	5	2	3	3	2
5	i12375Gh	1.28	Gm, Gb, Gt	4	3	4	4	3
5	i12489Gh	12.38	Gm, Gb, Gt	3	3	3	4	4
5	i30358Gh	12.38	Fail	5	5	5	5	5
5	i29617Gh	25.65	Gm, Gb, Gt	2	1	2	2	1
5	i41855Gh	37.20	Gm, Gb, Gt	2	4	2	3	4
5	i00670Gh	50.35	Gm, Gb, Gt	2	1	2	3	1
5	i32528Gh	57.36	Gm, Gb, Gt	2	2	3	3	2
5	i12598Gh	59.92	Fail	5	5	5	5	5
5	i27980Gh	62.06	Gm, Gb, Gt	2	2	2	3	2
5	i43462Gh	81.29	Gm, Gb, Gt	2	2	2	3	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
5	i09845Gh	100.90	Gm, Gb, Gt	1	2	1	2	2
5	i09743Gh	118.86	Recovery of homozygous-Gm, Gb, Gt	5	2	2	2	1
5	i01007Gh	120.99	Gm, Gb, Gt	1	2	1	2	2
5	i09556Gh	132.95	Fail	5	5	5	5	5
5	i37015Gh	140.24	Gm, Gb, Gt	1	1	1	2	1
5	i09206Gh	166.74	Gm, Gb, Gt	1	1	1	1	1
5	i48057Gh	179.55	Gm, Gb, Gt	2	1	2	3	1
5	i08918Gh	189.79	Fail	5	5	5	5	5
5	i61049Gt	189.79	Gm, Gb, Gt	3	1	3	4	1
5	i51036Gb	190.22	Gm, Gb, Gt	3	3	4	3	4
5	i50380Gb	200.96	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
5	i16528Gh	202.67	Gm, Gb, Gt	3	2	3	2	1
6	i49551Gh	0.00	Fail	5	5	5	5	5
6	i11398Gh	7.26	Gm, Gb, Gt	3	1	3	3	1
6	i11399Gh	7.26	Fail	5	5	5	5	5
6	i44308Gm	23.07	Gm, Gb, Gt	2	2	2	2	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
6	i11312Gh	30.33	Gm, Gb, Gt	3	3	3	3	3
6	i43700Gh	40.58	Gm, Gb, Gt	1	1	1	2	1
6	i39865Gh	49.13	Gm, Gb, Gt	1	1	1	2	1
6	i51763Gb	60.36	Fail	5	5	5	5	5
6	i52583Gb	60.36	Fail	5	5	5	5	5
6	i55732Gb	60.36	Gm,Gt	1	1	1	1	1
6	i51850Gb	68.47	Gm, Gb, Gt	2	2	2	2	2
6	i10945Gh	76.15	Gm, Gb, Gt	2	1	1	2	1
6	i50838Gb	86.81	Gm, Gb, Gt	2	1	2	2	1
6	i51788Gb	96.21	Gm, Gb, Gt	1	1	2	2	1
6	i62896Gt	117.40	Fail	5	5	5	5	5
6	i51681Gb	123.37	Gm, Gb, Gt	1	1	1	1	1
6	i52154Gb	135.31	Gb,Gm	2	2	2	1	2
7	i49561Gh	1.28	Fail	5	5	5	5	5
7	i49561Gh	1.28	Fail	5	5	5	5	5
7	i02126Gh	16.30	Fail	5	5	5	5	5

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
7	i44501Gh	21.45	Gm, Gb, Gt	2	2	2	2	2
7	i41379Gh	22.73	Gm, Gb, Gt	2	1	2	3	1
7	i02083Gh	29.59	Gm, Gb, Gt	3	2	3	2	2
7	i53793Gb	34.33	Gm, Gb, Gt	3	2	3	3	2
7	i28496Gh	45.46	Gm, Gb, Gt	1	1	2	2	1
7	i51660Gb	59.54	Fail	5	5	5	5	5
7	i28659Gh	62.09	Gt	2	1	2	2	1
7	i01705Gh	74.46	Sub-cluster-Gm	2	2	2	3	2
7	i36899Gh	84.28	Gm, Gb, Gt	1	2	2	2	2
7	i42454Gh	88.13	Gm, Gb, Gt	2	1	2	3	1
7	i56676Gb	88.13	Gm, Gb, Gt	2	1	2	3	1
7	i57012Gb	88.13	Gm, Gb, Gt	1	1	2	3	1
7	i14360Gh	96.67	Gm, Gb, Gt	1	3	1	4	3
7	i51776Gb	106.50	Gm, Gb, Gt	4	4	4	4	4
7	i50472Gb	107.35	Gm, Gb, Gt	1	1	1	1	1
7	i35632Gh	122.33	Fail	5	5	5	5	5

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
7	i01376Gh	129.15	Gm, Gb, Gt	4	1	4	2	1
7	i51470Gb	142.45	Gm, Gb, Gt	1	1	1	1	1
8	i61029Gt	0.43	Gm, Gb, Gt	2	1	2	2	1
8	i60714Gt	12.46	Gm, Gb, Gt	2	2	2	2	2
8	i04669Gh	22.71	Gm, Gb, Gt	3	2	2	3	2
8	i30796Gh	34.26	Gm, Gb, Gt	3	2	2	3	2
8	i62711Gt	39.37	Fail	5	5	5	5	5
8	i00540Gh	39.37	Gm, Gb, Gt	3	3	3	3	3
8	i31486Gh	52.80	Sub-cluster-Gb	2	2	2	3	2
8	i56645Gb	67.31	Gm, Gb, Gt	1	2	1	2	2
8	i04134Gh	86.25	Gm, Gb	4	4	4	4	4
8	i04229Gh	86.68	Recovery of homozygous-Gm, Gb, Gt	5	4	4	4	4
8	i31221Gh	86.68	Gm, Gb, Gt	2	2	2	1	2
8	i50119Gb	86.68	Gm, Gb, Gt	1	2	1	3	2
8	i51390Gb	103.00	Gm, Gb, Gt	2	1	2	1	1
8	i58509Gb	105.57	Gm, Gb, Gt	1	2	1	2	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
8	i40138Gh	115.08	Fail	5	5	5	5	5
8	i48310Gh	115.08	Gm,Gt	4	4	4	4	4
8	i20535Gh	127.46	Gm, Gb, Gt	1	2	2	3	2
8	i29120Gh	129.59	Gm, Gb, Gt	1	1	1	1	1
8	i61072Gt	154.39	Gm, Gb, Gt	2	2	3	3	2
8	i55286Gb	160.36	Fail	5	5	5	5	5
8	i18599Gh	164.63	Gm, Gb, Gt	1	1	1	2	1
8	i61692Gt	171.04	Gm, Gb, Gt	1	1	1	1	1
9	i61099Gt	0.43	Gm, Gb, Gt	1	1	1	2	1
9	i41274Gh	15.78	Gm, Gb, Gt	1	2	1	2	2
9	i50500Gb	28.62	Gm, Gb, Gt	1	1	1	2	1
9	i25619Gh	43.58	Gm, Gb, Gt	1	1	1	2	1
9	i06016Gh	54.89	Gm, Gb, Gt	2	1	2	2	1
9	i59036Gb	62.70	Gm, Gb, Gt	2	3	2	2	3
9	i52416Gb	63.13	Gm, Gb, Gt	1	1	1	1	1
9	i50335Gb	70.40	No Call					

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
9	i06087Gh	72.95	Fail	5	5	5	5	5
9	i25043Gh	81.06	Gm, Gb, Gt	1	2	1	4	2
9	i50477Gb	88.77	Gm, Gb, Gt	1	1	1	1	1
9	i06319Gh	99.01	Sub-cluster-Gm	2	2	2	3	2
9	i61111Gt	109.26	Fail	5	5	5	5	5
9	i06474Gh	119.53	Gm, Gb, Gt	1	2	1	3	1
9	i50857Gb	120.81	Gm, Gb, Gt	2	2	2	2	3
9	i51975Gb	129.78	Gm, Gb, Gt	2	1	2	2	1
9	i51086Gb	142.17	Gm, Gb, Gt	1	1	1	2	2
10	i12331Gh	6.44	Gm, Gb, Gt	4	3	3	3	3
10	i12328Gh	6.87	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
10	i49130Gh	10.29	Gm, Gb, Gt	1	1	1	2	1
10	i12285Gh	10.71	Fail	5	5	5	5	5
10	i52506Gb	13.70	Gm, Gb, Gt	3	1	3	1	1
10	i17601Gh	19.68	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
10	i51066Gb	35.91	Gm, Gb, Gt	2	1	2	1	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
10	i00406Gh	50.00	Gm, Gb, Gt	2	3	2	3	3
10	i11901Gh	65.37	Gm, Gb, Gt	4	1	3	2	1
10	i24931Gh	81.69	Gm, Gb, Gt	3	2	3	4	2
10	i48101Gh	81.69	Gm, Gb, Gt	2	1	2	2	1
10	i42848Gh	84.25	Gm, Gb, Gt	1	2	1	3	2
10	i11978Gh	88.09	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	1
10	i41571Gh	93.24	Gm, Gb, Gt	2	3	2	3	3
10	i00714Gh	93.24	Gm, Gb, Gt	2	2	2	2	2
10	i51931Gb	103.49	Fail	5	5	5	5	5
10	i17458Gh	106.92	Fail	5	5	5	5	5
10	i00730Gh	126.19	Gb,Gt	1	3	1	2	3
10	i39597Gh	135.21	Gm, Gb, Gt	3	3	3	3	3
10	i60917Gt	139.49	Gm, Gb, Gt	2	2	2	2	2
10	i50887Gb	154.14	Gm, Gb, Gt	2	1	3	3	2
10	i51961Gb	155.85	Fail	5	5	5	5	5
11	i00366Gh	2.14	Gm, Gb, Gt	3	3	3	2	3

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
11	i06827Gh	16.69	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	1
11	i06885Gh	23.51	Fail	5	5	5	5	5
11	i52333Gb	31.18	Gm, Gb, Gt	2	2	2	3	2
11	i52531Gb	41.84	Gm, Gb, Gt	4	3	4	4	3
11	i50158Gb	52.11	Fail	5	5	5	5	5
11	i07182Gh	61.51	Recovery of homozygous-Gm, Gb, Gt	5	2	2	2	2
11	i00792Gh	70.47	Fail	5	5	5	5	5
11	i58197Gb	70.90	Gm, Gb, Gt	3	1	1	4	2
11	i43779Gh	76.03	Gm, Gb, Gt	1	1	1	2	1
11	i52034Gb	80.73	Gm, Gb, Gt	2	1	2	1	1
11	i34920Gh	92.27	Gm, Gb, Gt	1	2	1	2	2
11	i03278Gh	104.47	Gm, Gb, Gt	3	3	3	3	3
11	i03280Gh	104.47	Fail	5	5	5	5	5
11	i57533Gb	122.44	Fail	5	5	5	5	5
11	i50251Gb	123.72	Fail	5	5	5	5	5
11	i57155Gb	125.86	Gm,Gt	4	4	4	3	4

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
11	i19367Gh	139.94	Gm, Gb, Gt	2	2	2	2	2
11	i07548Gh	154.91	Gm, Gb, Gt	4	2	4	4	2
11	i58276Gb	168.61	Recovery of homozygous-Gm, Gb, Gt	5	2	3	3	1
11	i29860Gh	181.91	Gm, Gb, Gt	2	1	1	2	2
11	i50720Gb	191.74	Gm, Gb, Gt	1	3	1	3	3
12	i28665Gh	0.00	Gm, Gb, Gt	2	1	2	3	1
12	i07754Gh	0.00	Recovery of homozygous-Gm, Gb, Gt	5	1	3	1	2
12	i29317Gh	14.94	Gm, Gb, Gt	2	1	2	1	2
12	i16251Gh	29.07	Fail	5	5	5	5	5
12	i57388Gb	41.48	Gm, Gb, Gt	1	2	1	2	2
12	i07954Gh	41.90	Recovery of homozygous-Gm, Gb, Gt	5	2	3	3	2
12	i41605Gh	50.60	Fail	5	5	5	5	5
12	i56214Gb	51.03	Gm, Gb, Gt	1	2	1	2	2
12	i60172Gb	53.59	Gm, Gb, Gt	3	3	3	1	3
12	i45967Gh	56.15	Gm, Gb, Gt	1	1	2	1	2
12	i52800Gb	60.93	Fail	5	5	5	5	5

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
12	i50197Gb	74.59	Gm, Gb, Gt	2	2	3	2	2
12	i08110Gh	81.42	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	2
12	i44466Gh	89.96	Gm, Gb, Gt	1	2	1	1	2
12	i38901Gh	92.53	Gm, Gb, Gt	1	2	1	2	2
12	i19468Gh	99.37	Gm, Gb, Gt	4	3	4	4	3
12	i08345Gh	107.46	Gm, Gb, Gt	3	2	2	2	2
12	i08473Gh	118.15	No Call					
12	i08502Gh	121.14	Gm, Gb, Gt	3	2	3	4	2
12	i53894Gb	136.09	Gm, Gb, Gt	2	2	2	2	2
12	i08694Gh	147.19	Gm, Gb, Gt	3	1	1	3	3
13	i12961Gh	0.00	No Call					
13	i54272Gb	0.00	Gb,Gt	3	2	3	3	2
13	i13011Gh	12.47	Gm, Gb, Gt	2	2	2	3	2
13	i46139Gh	20.16	Gm, Gb, Gt	1	2	2	2	3
13	i54568Gb	30.90	Recovery of homozygous-Gm, Gb, Gt	5	2	2	2	2
13	i54898Gb	43.72	Gm, Gb, Gt	2	2	2	3	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
13	i56157Gb	58.67	Gm, Gb, Gt	3	1	3	2	1
13	i24816Gh	65.95	Fail	5	5	5	5	5
13	i35005Gh	74.92	Gm, Gb, Gt	3	1	3	1	2
13	i27466Gh	83.89	Gm, Gb, Gt	1	2	1	4	2
13	i50603Gb	90.29	Gm, Gb, Gt	1	1	1	2	1
13	i13517Gh	100.55	Gm, Gb, Gt	2	3	2	2	3
13	i13553Gh	106.09	Recovery of homozygous-Gm, Gb, Gt	5	1	1	2	2
13	i52496Gb	117.20	Gm, Gb, Gt	1	1	1	3	2
13	i61260Gt	125.35	Gm, Gb, Gt	4	1	4	4	2
13	i51136Gb	127.93	Gm, Gb, Gt	3	1	3	2	1
13	i13759Gh	141.73	Gm, Gb, Gt	3	2	3	4	3
13	i13873Gh	148.15	Fail	5	5	5	5	5
14	i05721Gh	0.00	Fail	5	5	5	5	5
14	i05712Gh	10.72	Recovery of homozygous-Gm, Gb, Gt	5	2	2	2	2
14	i05683Gh	13.71	Gm, Gb, Gt	2	1	2	3	1
14	i15563Gh	21.07	Gm, Gb, Gt	4	4	4	4	4

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
14	i19064Gh	30.96	Gm, Gb, Gt	1	2	3	3	2
14	i49624Gh	39.17	Recovery of homozygous-Gb and Gm	5	2	2	2	2
14	i52324Gb	39.17	Fail	5	5	5	5	5
14	i15512Gh	49.42	Fail	5	5	5	5	5
14	i05458Gh	50.27	Recovery of homozygous-Gm, Gb, Gt	5	3	3	4	3
14	i05355Gh	60.52	Gm, Gb, Gt	3	2	2	1	2
14	i57074Gb	60.95	Gm, Gb, Gt	1	2	1	2	1
14	i29634Gh	75.46	Gm, Gb, Gt	2	1	2	3	1
14	i28274Gh	86.86	Gm, Gb, Gt	3	3	3	3	3
14	i58710Gb	94.55	Gm, Gb, Gt	2	4	2	3	3
14	i05148Gh	105.43	Fail	5	5	5	5	5
14	i05146Gh	106.28	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	1
14	i36289Gh	110.98	Gm, Gb, Gt	2	2	2	1	1
14	i49059Gh	128.29	Fail	5	5	5	5	5
14	i04957Gh	141.10	Gm, Gb, Gt	1	3	1	2	3
14	i04870Gh	152.23	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
15	i13988Gh	0.00	Gm, Gb, Gt	4	4	4	4	4
15	i14767Gh	9.42	Recovery of homozygous-Gm, Gb, Gt	5	2	3	3	2
15	i02942Gh	10.70	Fail	5	5	5	5	5
15	i02942Gh	10.70	Gm, Gb, Gt	4	2	3	4	1
15	i50860Gb	21.39	Gm, Gb, Gt	2	2	2	2	2
15	i02862Gh	33.00	Recovery of homozygous-Gm, Gb, Gt	5	1	2	3	1
15	i48166Gh	33.86	Gm, Gb, Gt	1	2	1	4	2
15	i14720Gh	41.11	Gm, Gb, Gt	3	3	4	4	4
15	i50187Gb	50.06	Gm, Gb, Gt	4	3	4	3	3
15	i02576Gh	60.75	Fail	5	5	5	5	5
15	i35660Gh	67.63	Gm, Gb, Gt	3	2	3	2	2
15	i02512Gh	71.03	Recovery of homozygous-Gm, Gb, Gt	5	3	4	4	3
15	i50340Gb	73.59	Gm, Gb, Gt	4	2	4	3	2
15	i02444Gh	83.43	Gm, Gb, Gt	4	3	4	3	4
15	i18386Gh	89.83	Fail	5	5	5	5	5
15	i14594Gh	98.79	Gm, Gb, Gt	2	3	3	3	3

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
15	i02249Gh	112.48	Gm, Gb, Gt	1	2	2	4	2
15	i56500Gb	113.76	Gm, Gb, Gt	4	4	4	4	4
16	i51425Gb	0.43	Gb,Gm	3	2	3	1	2
16	i43172Gh	11.97	Gm, Gb, Gt	1	2	2	2	1
16	bp-54974991	bp	Gm, Gb, Gt	2	2	2	3	2
16	i47062Gh	30.05	Gm, Gb, Gt	2	4	2	3	4
16	i58337Gb	39.49	Gm, Gb, Gt	2	1	2	2	1
16	i30646Gh	44.62	Gm, Gb, Gt	2	1	2	2	1
16	i34538Gh	52.74	Gm, Gb, Gt	2	3	2	4	1
16	i44284Gh	61.70	Gm, Gb, Gt	1	2	1	3	2
16	i01703Gh	71.95	Gm, Gb, Gt	3	1	3	3	1
16	i01592Gh	80.07	Gm, Gb, Gt	3	1	3	4	1
16	i37829Gh	80.50	Gm, Gb, Gt	2	2	2	3	2
16	i01516Gh	88.19	Gm, Gb, Gt	3	2	2	3	2
16	i14326Gh	99.74	Gm, Gb, Gt	2	1	2	3	1
16	i01404Gh	112.15	Fail	5	5	5	5	5

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
16	i61313Gt	112.15	Gm, Gb, Gt	2	3	3	3	2
16	i42554Gh	122.39	Gm, Gb, Gt	1	2	1	2	2
16	i51754Gb	133.50	Gm, Gb, Gt	1	1	1	2	1
17	i53982Gb	0.00	Gm, Gb, Gt	3	3	2	3	2
17	i00195Gh	9.44	Sub-cluster-Gm	1	2	1	1	1
17	i03187Gh	20.56	Gm, Gb, Gt	1	1	1	2	1
17	i51917Gb	33.79	Gm, Gb, Gt	4	1	4	3	1
17	i51043Gb	41.49	Gm, Gb, Gt	1	1	1	2	1
17	i03371Gh	49.60	Gm, Gb, Gt	2	2	2	3	2
17	i14906Gh	62.07	Gm, Gb, Gt	4	2	4	4	2
17	i03479Gh	71.66	Gm, Gb, Gt	4	2	4	4	2
17	i63519Gm	83.37	Fail	5	5	5	5	5
17	i39989Gh	91.05	Gm, Gb, Gt	2	2	2	3	2
17	i60810Gt	107.30	Gm, Gb, Gt	3	2	3	3	2
18	i12965Gh	0.00	Gm, Gb, Gt	1	1	1	1	1
18	i21923Gh	8.22	Gm, Gb, Gt	2	1	2	1	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
18	i12997Gh	9.08	Fail	5	5	5	5	5
18	i13068Gh	24.10	Gm, Gb, Gt	3	2	3	3	2
18	i34219Gh	30.07	Gm, Gb, Gt	3	2	3	2	2
18	i34709Gh	38.61	Gm, Gb, Gt	2	2	2	2	3
18	i13189Gh	45.51	Gm, Gb, Gt	3	1	3	1	1
18	i52366Gb	47.65	Gm, Gb, Gt	1	1	1	2	1
18	i49235Gh	58.75	Fail	5	5	5	5	5
18	i17987Gh	61.74	Recovery of homozygous-Gm, Gb, Gt	5	2	2	2	2
18	i13504Gh	73.71	Fail	5	5	5	5	5
18	i13519Gh	74.14	Gm, Gb, Gt	4	3	4	4	3
18	i50412Gb	83.96	Gm, Gb, Gt	1	1	1	2	1
18	i00473Gh	107.60	Gm, Gb, Gt	2	1	2	2	1
18	i13724Gh	110.16	Gm, Gb, Gt	4	4	4	4	2
18	i13806Gh	123.90	Gm, Gb, Gt	2	1	2	2	1
18	i13850Gh	125.18	Gm, Gb, Gt	4	3	4	3	3
19	i65047Gm	2.56	Gm, Gb, Gt	1	1	2	3	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
19	i00196Gh	5.98	Gm, Gb, Gt	1	1	1	2	1
19	i50131Gb	11.53	Gm, Gb, Gt	1	2	1	1	2
19	i10421Gh	20.50	Recovery of homozygous-Gm, Gb, Gt	5	3	2	2	1
19	i10319Gh	31.19	Gm, Gb, Gt	4	4	4	4	3
19	i10321Gh	31.19	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	1
19	i26414Gh	40.58	Sub-cluster-Gm	3	3	3	3	3
19	i00755Gh	50.53	Gm, Gb, Gt	1	1	1	2	1
19	i45829Gh	58.71	Gm, Gb, Gt	1	1	2	2	2
19	i26399Gh	71.62	Gm, Gb, Gt	2	2	2	4	2
19	i10126Gh	81.86	Fail	5	5	5	5	5
19	bp-35402201	bp	Gm, Gb, Gt	4	2	4	4	1
19	i09954Gh	106.32	Gm, Gb, Gt	3	3	4	4	4
19	i09898Gh	120.45	Recovery of homozygous-Gm, Gb, Gt	5	2	4	4	1
19	i50264Gb	130.71	Recovery of homozygous-Gm, Gb, Gt	5	1	1	2	1
19	i09626Gh	141.39	Gm, Gb, Gt	4	3	4	4	3
19	i09572Gh	152.59	Gm, Gb, Gt	3	3	3	3	3

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
19	i09526Gh	160.78	Gm, Gb, Gt	1	2	1	3	2
19	i09340Gh	171.89	Gm, Gb, Gt	2	1	1	3	2
19	i09244Gh	183.05	Gm, Gb, Gt	4	2	4	4	2
19	i16569Gh	205.27	Gm, Gb, Gt	2	2	2	3	2
19	i61131Gt	216.82	Fail	5	5	5	5	5
19	i16538Gh	217.25	Gm, Gb, Gt	1	3	2	1	4
19	i42912Gh	222.82	Gm,Gt	4	3	4	4	3
19	i08803Gh	224.10	Gm, Gb, Gt	1	2	1	2	2
19	i08802Gh	224.10	Recovery of homozygous-Gm, Gb, Gt	5	2	1	1	2
20	i17673Gh	0.43	Gm, Gb, Gt	1	2	1	2	3
20	i17604Gh	21.40	Fail	5	5	5	5	5
20	i50202Gb	24.82	Gm, Gb, Gt	4	2	4	2	2
20	i12189Gh	30.82	Gm, Gb, Gt	2	1	2	3	1
20	i20406Gh	45.81	Gm, Gb, Gt	2	2	2	4	3
20	i20107Gh	51.35	Gm, Gb, Gt	1	1	1	1	1
20	i49481Gh	60.66	Sub-cluster-Gt	1	2	1	2	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
20	i25537Gh	75.70	Gm,Gt	3	2	3	3	2
20	i34452Gh	80.40	Gm, Gb, Gt	3	2	3	3	2
20	i44471Gh	90.70	Gm, Gb, Gt	1	2	2	3	2
20	i11812Gh	97.96	Gm, Gb, Gt	4	2	4	4	2
20	i58775Gb	108.62	Gm, Gb, Gt	2	2	2	3	2
20	i17444Gh	120.59	Gm, Gb, Gt	1	1	1	2	1
20	i11623Gh	130.84	Gm, Gb, Gt	4	2	3	4	2
20	i11590Gh	136.81	Gm, Gb, Gt	4	2	4	3	2
20	i11470Gh	151.73	Gm, Gb, Gt	1	1	1	2	1
21	i50608Gb	2.13	Gm, Gb, Gt	1	1	2	2	2
21	i55362Gb	22.21	Gm,Gt	2	3	2	3	3
21	i15938Gh	31.22	Gm, Gb, Gt	2	2	2	2	2
21	i06916Gh	31.65	Gm, Gb, Gt	1	1	1	1	1
21	i52139Gb	41.04	Fail	5	5	5	5	5
21	i51304Gb	45.32	Fail	5	5	5	5	5
21	i07031Gh	54.36	Gm, Gb, Gt	1	4	1	4	4

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
21	i07037Gh	54.36	Recovery of homozygous-Gm, Gb, Gt	5	1	2	2	1
21	i07089Gh	64.22	Gm, Gb, Gt	1	2	1	4	2
21	i50923Gb	69.79	Gm, Gb, Gt	3	2	3	3	2
21	i60993Gt	80.89	Gm, Gb, Gt	3	3	3	3	3
21	i60559Gb	91.12	Gm, Gb, Gt	2	2	2	3	2
21	i57476Gb	106.06	Gm, Gb, Gt	2	2	1	3	2
21	i51903Gb	118.68	Gm, Gb, Gt	1	1	1	1	1
21	i40036Gh	136.90	Gm, Gb, Gt	2	1	2	3	1
21	i07568Gh	151.86	Gm, Gb, Gt	4	1	4	3	1
21	i16110Gh	151.86	Fail	5	5	5	5	5
21	i16137Gh	163.44	Fail	5	5	5	5	5
21	i59747Gb	171.55	Gm, Gb, Gt	1	1	1	2	1
21	i52000Gb	184.37	Gm, Gb, Gt	2	1	2	2	1
22	i17680Gh	0.00	Gm, Gb, Gt	1	4	1	3	4
22	i38556Gh	12.81	Gm, Gb, Gt	1	1	2	2	1
22	i20148Gh	26.17	Gm, Gb, Gt	1	2	1	2	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
22	i12526Gh	28.30	Fail	5	5	5	5	5
22	i40262Gh	34.77	Gm, Gb, Gt	4	1	4	4	2
22	i61086Gt	44.59	Gm, Gb, Gt	2	3	2	2	3
22	i39605Gh	45.87	Fail	5	5	5	5	5
22	i48531Gh	54.54	Gm, Gb, Gt	2	3	2	4	4
22	i12685Gh	57.10	Recovery of homozygous-Gm, Gb, Gt	5	3	4	4	2
22	i12686Gh	57.10	Gm, Gb, Gt	4	2	4	4	2
22	i10394Gh	62.32	Gm, Gb, Gt	1	2	1	2	2
22	i12687Gh	72.15	Recovery of homozygous-Gm, Gb, Gt	5	2	4	4	3
22	i34388Gh	82.74	Gm, Gb, Gt	2	1	2	3	2
22	i56516Gb	90.44	Gm, Gb, Gt	4	3	4	4	3
22	i12801Gh	100.71	Gm, Gb, Gt	1	2	1	2	2
22	i12895Gh	112.66	Gm, Gb, Gt	1	1	1	1	1
22	i12939Gh	127.68	Gm, Gb, Gt	1	1	2	2	1
23	i05794Gh	0.00	Recovery of homozygous-Gm, Gb, Gt	5	1	1	1	2
23	i05851Gh	8.97	Gm, Gb, Gt	3	1	3	3	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
23	i05936Gh	36.95	Gm, Gb, Gt	1	1	1	2	1
23	i35476Gh	47.63	Gm, Gb, Gt	2	1	2	2	1
23	i61300Gt	60.43	Gm, Gb, Gt	1	1	1	2	1
23	i15725Gh	69.81	Gm, Gb, Gt	1	4	2	3	4
23	i61516Gt	83.92	Sub-cluster-Gm	1	1	1	2	1
23	i62777Gt	84.77	Sub-cluster-Gb	1	4	1	4	4
23	i23862Gh	94.60	Gm, Gb, Gt	1	1	1	2	2
23	i34362Gh	108.28	Gt	2	1	2	2	1
23	i06568Gh	116.39	Gm, Gb, Gt	1	3	1	3	3
23	i06632Gh	126.64	Gm, Gb, Gt	1	1	1	2	1
24	i54758Gb	0.43	Gm, Gb, Gt	3	1	2	2	1
24	i04738Gh	12.03	Gm, Gb, Gt	2	1	1	1	1
24	i35512Gh	18.86	Gm, Gb, Gt	1	2	1	3	2
24	i04615Gh	27.41	Gm, Gb, Gt	2	1	2	2	1
24	i31467Gh	39.80	Gm, Gb, Gt	1	1	1	3	1
24	i50001Gb	50.91	Gm, Gb, Gt	1	1	1	2	1

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
24	i49724Gh	59.88	Gm, Gb, Gt	2	2	2	3	2
24	i04257Gh	72.68	Gm, Gb, Gt	3	1	3	3	1
24	i50008Gb	86.35	Gm, Gb, Gt	1	1	1	1	1
24	i03938Gh	96.61	Fail	5	5	5	5	5
24	i03855Gh	111.14	Gm, Gb, Gt	1	1	1	2	1
24	i51167Gb	120.96	Gm, Gb, Gt	2	1	2	2	1
24	i50055Gb	129.50	Gm, Gb, Gt	1	1	1	2	1
25	i51276Gb	1.28	Fail	5	5	5	5	5
25	i11447Gh	2.56	Fail	5	5	5	5	5
25	i53437Gb	15.89	Gm, Gb, Gt	1	1	1	3	2
25	i11316Gh	24.02	Gm, Gb, Gt	1	1	1	1	1
25	i11253Gh	36.84	Recovery of homozygous-Gm, Gb, Gt	5	2	3	3	3
25	i33715Gh	43.67	Gm, Gb, Gt	2	2	2	4	2
25	i11187Gh	47.97	Gm, Gb, Gt	1	1	1	1	1
25	i51081Gb	50.53	Gm, Gb, Gt	1	1	1	1	1
25	i17265Gh	59.06	Gm, Gb, Gt	1	2	1	3	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
25	i52068Gb	73.63	Gm, Gb, Gt	1	1	1	2	1
25	i60673Gb	84.32	Gm, Gb, Gt	3	4	4	4	4
25	i00963Gh	85.60	Gm, Gb, Gt	1	1	1	1	1
25	i10803Gh	96.70	Gm, Gb, Gt	1	3	1	3	3
25	i17168Gh	109.96	Gm, Gb, Gt	3	2	3	3	2
25	i10674Gh	120.19	Fail	5	5	5	5	5
25	i60789Gt	130.42	Gm, Gb, Gt	4	2	4	4	2
26	i40991Gh	0.00	Recovery of homozygous-Gm, Gb, Gt	5	2	4	4	2
26	i30070Gh	13.63	Gm, Gb, Gt	2	2	2	2	2
26	bp-2013818	bp	Gm, Gb, Gt	1	2	1	3	2
26	i59951Gb	34.18	Gm, Gb, Gt	1	1	1	1	1
26	i50506Gb	45.69	Gm, Gb, Gt	1	1	1	2	1
26	i47604Gh	60.61	Gm, Gb, Gt	3	2	3	2	2
26	i08115Gh	72.59	Sub-cluster-Gm	1	2	3	2	2
26	i19454Gh	80.73	Gm, Gb, Gt	4	4	4	4	4
26	i08217Gh	87.98	Gm, Gb, Gt	2	2	2	4	2

Chr.*	Marker ID	Position (cM)	Assay Applicability.***	Scorability		Cluster Tightness		
				P1 vs Het.**	P2 vs Het	P1	Het	P2
26	i08312Gh	98.22	Gm, Gb, Gt	2	3	2	3	3
26	i16420Gh	105.06	Gm, Gb, Gt	1	1	1	2	1
26	i14139Gh	116.15	Gm, Gb, Gt	3	2	3	2	2
26	i55509Gb	120.85	Gm, Gb, Gt	1	1	1	2	1
26	i08657Gh	135.83	Gm, Gb, Gt	3	4	3	4	4
26	i19526Gh	136.69	Gm, Gb, Gt	1	1	1	2	1
26	i47939Gh	145.65	Gm, Gb, Gt	3	2	3	3	2
26	i08773Gh	147.36	Gm, Gb, Gt	4	1	3	4	3

*Chromosome

**Heterozygotes

***Gm, Gt, and/or Gb indicate that an assay detects the donor allele of *G. mustelinum*, *G. tomentosum* and/or *G. barbadense*

"1"= perfect (nearly ideal), "2"=good (should be reliable in virtually all applications, but less robust than ideal), "3"=okay (should be usable and reliable in all or most applications, but perhaps prone to occasional error?), "4"=usable but not accurate for some genotypes (implying that detailed information should be consulted before use), "5"=unreliable.

TABLE A6

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
1	i61225Gt	0.66	0.38	0.95	0.84
1	i23982Gh	0.25	0.87	0.12	0.58
1	i51041Gb	0.31	0.87	0.22	0.80
1	i27626Gh	0.37	0.32	0.42	0.71
1	i34731Gh	0.21	0.32	0.42	0.84
1	i55595Gb	0.00	0.00	0.32	0.71
1	i45642Gh	0.87	0.27	0.63	0.20
1	i47972Gh	0.50	0.68	0.57	0.20
1	i50196Gb	0.00	0.01	0.01	0.00
1	i00132Gh	0.24	0.09	0.83	0.44
2	i50655Gb	0.93	0.90	0.95	0.98
2	i52423Gb	0.80	0.65	0.86	0.98
2	i03174Gh	0.16	0.35	0.16	0.58
2	i01044Gh	0.00	0.01	0.00	0.01
2	i43496Gh	0.37	0.08	0.16	0.32
2	i61080Gt	0.76	0.09	0.28	0.68
2	i60741Gt	0.90	0.28	0.72	0.71
2	i29065Gh	0.52	0.21	0.86	0.32
2	i52292Gb	0.98	0.68	0.98	0.42
2	i57002Gb	0.66	0.87	0.42	0.68
2	i63139Gt	0.11	0.65	0.57	0.10
2	i38489Gh	0.12	0.87	0.22	0.05
2	i00543Gh	0.01	0.75	0.22	0.00
3	i05735Gh	0.45	0.68	0.75	0.03
3	i05710Gh	0.37	0.51	0.72	0.31

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
3	i50850Gb	0.78	0.73	0.25	0.84
3	i19058Gh	0.25	0.90	0.19	0.80
3	i00178Gh	0.00	0.00	0.00	0.00
3	i05459Gh	0.29	0.79	0.16	0.98
3	i14041Gh	0.37	0.28	0.36	0.52
3	i14878Gh	0.16	0.35	0.32	0.58
3	i52480Gb	0.00	0.40	0.00	0.52
3	i14000Gh	0.67	0.09	0.95	0.14
3	i43612Gh	0.05	0.05	0.83	0.17
3	i66018Ga	0.12	0.09	0.75	0.32
4	i00466Gh	0.74	0.54	0.75	0.98
4	i24385Gh	0.90	0.90	0.75	0.98
4	i25377Gh	0.05	0.00	0.24	0.71
4	i49276Gh	0.76	0.02	0.42	0.68
4	i10424Gh	0.54	0.03	0.42	0.68
4	i29272Gh	0.52	0.38	0.03	0.94
4	i50228Gb	0.37	0.38	0.01	0.10
4	i32552Gh	0.00	0.68	0.00	0.26
4	i47058Gh	0.67	0.87	0.63	0.52
4	i52022Gb	0.25	0.90	0.28	0.52
4	i53431Gb	0.23	0.51	0.19	0.52
4	i50976Gb	0.00	0.01	0.01	0.01
5	i12375Gh	0.37	0.01	0.25	0.20
5	i12489Gh	0.77	0.06	0.25	0.42
5	i29617Gh	0.45	0.21	0.42	0.94
5	i41855Gh	0.21	0.08	0.42	0.32

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
5	i00670Gh	0.38	0.04	0.48	0.84
5	i32528Gh	0.26	0.01	0.42	0.38
5	i27980Gh	0.29	0.02	0.72	0.72
5	i43462Gh	0.02	0.01	0.63	0.71
5	i09845Gh	0.00	0.00	0.63	0.07
5	i09743Gh	0.00	0.01	0.00	0.01
5	i01007Gh	0.01	0.22	0.27	0.03
5	i37015Gh	0.29	0.90	0.48	0.14
5	i09206Gh	0.76	0.65	0.42	0.36
5	i48057Gh	0.01	0.07	0.18	0.32
5	i61049Gt	0.09	0.08	0.75	0.09
5	i51036Gb	0.31	0.10	0.75	0.19
5	i50380Gb	0.00	0.00	0.01	0.01
5	i16528Gh	0.13	0.00	0.63	0.94
6	i11398Gh	0.32	0.42	0.43	0.12
6	i44308Gm	0.14	0.65	0.24	0.36
6	i11312Gh	0.45	0.96	0.83	0.20
6	i43700Gh	0.45	1.00	0.63	0.42
6	i39865Gh	0.80	0.28	0.63	0.58
6	i55732Gb	0.05	0.07	0.86	0.00
6	i51850Gb	0.93	0.08	0.12	0.84
6	i10945Gh	0.20	0.16	0.02	0.14
6	i50838Gb	0.09	0.42	0.04	0.71
6	i51788Gb	0.37	0.51	0.10	0.58
6	i51681Gb	0.13	0.09	0.25	0.03
6	i52154Gb	0.02	0.90	0.00	0.36

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
7	i44501Gb	0.45	0.90	0.72	0.03
7	i41379Gh	0.54	0.82	0.72	0.06
7	i02083Gh	0.12	0.04	0.28	0.14
7	i53793Gb	0.67	0.38	0.48	0.12
7	i28496Gh	0.03	0.07	0.63	0.01
7	i28659Gh	0.25	0.16	0.86	0.00
7	i01705Gh	0.10	0.34	0.48	0.00
7	i36899Gh	0.66	1.00	0.63	0.36
7	i42454Gh	0.29	0.51	0.86	0.26
7	i56676Gb	0.41	0.75	0.86	0.26
7	i57012Gb	0.29	0.51	0.86	0.26
7	i14360Gh	0.08	0.51	0.32	0.36
7	i51776Gb	0.14	0.44	0.36	0.18
7	i50472Gb	0.37	0.38	0.36	0.12
7	i01376Gh	0.07	0.09	0.01	0.06
7	i51470Gb	0.50	0.90	0.72	0.22
8	i61029Gt	0.08	0.98	0.01	0.68
8	i60714Gt	0.74	0.65	0.22	0.42
8	i04669Gh	0.81	0.51	0.95	0.68
8	i30796Gh	0.17	0.28	0.25	0.44
8	i00540Gh	0.29	0.51	0.25	0.68
8	i31486Gh	0.76	0.90	0.37	0.80
8	i56645Gb	0.95	0.54	0.75	0.17
8	i04134Gh	0.90	1.00	0.48	0.61
8	i31221Gh	0.98	0.51	0.83	0.61
8	i04229Gh	0.00	0.00	0.00	0.00

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
8	i50119Gb	0.73	0.68	0.65	0.61
8	i51390Gb	0.90	0.16	0.75	0.17
8	i58509Gb	0.67	0.16	0.95	0.14
8	i48310Gh	0.67	0.90	0.65	0.61
8	i20535Gh	0.80	0.65	0.57	0.61
8	i29120Gh	0.81	0.65	0.36	0.61
8	i61072Gt	0.37	0.65	0.12	0.44
8	i18599Gh	0.12	0.87	0.01	0.71
8	i61692Gt	0.14	0.65	0.00	0.58
9	i61099Gt	0.90	0.09	0.48	0.94
9	i41274Gh	0.37	0.56	0.37	0.12
9	i50500Gb	0.45	0.28	0.27	0.42
9	i25619Gh	0.52	0.28	0.25	0.20
9	i06016Gh	0.14	0.01	0.09	0.08
9	i59036Gb	0.61	0.02	0.06	0.14
9	i52416Gb	0.52	0.01	0.06	0.14
9	i25043Gh	0.29	0.22	0.16	0.32
9	i50477Gb	0.06	0.12	0.42	0.52
9	i06319Gh	0.25	0.02	0.72	0.36
9	i06474Gh	0.26	0.45	0.83	0.45
9	i50857Gb	0.25	0.42	0.83	0.36
9	i51975Gb	0.76	0.56	0.57	0.52
9	i51086Gb	0.90	0.87	0.63	0.98
10	i12331Gh	0.23	0.02	0.83	0.71
10	i12328Gh	0.00	0.00	0.00	0.01
10	i49130Gh	0.29	0.51	0.32	0.98

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
10	i52506Gb	0.45	0.65	0.36	0.80
10	i17601Gh	0.00	0.00	0.00	0.00
10	i51066Gb	0.11	0.42	0.65	0.20
10	i00406Gh	0.58	0.71	0.36	0.58
10	i11901Gh	0.37	0.16	0.05	0.71
10	i24931Gh	0.02	0.22	0.37	0.02
10	i48101Gh	0.52	0.18	0.57	0.44
10	i42848Gh	0.76	0.22	0.63	0.44
10	i11978Gh	0.00	0.01	0.00	0.01
10	i41571Gh	0.73	0.22	0.86	0.68
10	i00714Gh	0.67	0.18	0.86	0.68
10	i00730Gh	0.00	0.00	0.57	0.22
10	i39597Gh	0.37	0.65	0.75	0.52
10	i60917Gt	0.50	1.00	0.37	0.68
10	i50887Gb	0.41	0.12	0.95	0.68
11	i00366Gh	0.50	0.90	0.36	0.80
11	i06827Gh	0.00	0.00	0.00	0.01
11	i52333Gb	0.76	0.32	0.19	0.20
11	i52531Gb	0.94	0.51	0.23	0.42
11	i07182Gh	0.00	0.01	0.01	0.01
11	i58197Gb	0.52	0.34	0.75	0.20
11	i43779Gh	0.80	0.32	0.63	0.36
11	i52034Gb	0.80	0.90	0.37	0.58
11	i34920Gh	0.67	0.90	0.86	0.17
11	i03278Gh	0.37	1.00	0.83	0.17
11	i57155Gb	0.29	0.90	0.65	0.36

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
11	i19367Gh	0.41	0.75	0.42	0.98
11	i07548Gh	0.84	0.63	0.36	0.32
11	i58276Gb	0.00	0.01	0.00	0.01
11	i29860Gh	0.80	0.56	0.25	0.71
11	i50720Gb	0.76	0.38	0.42	0.71
12	i28665Gh	0.04	0.45	0.02	0.42
12	i07754Gh	0.00	0.01	0.01	0.01
12	i29317Gh	0.14	0.07	0.12	0.80
12	i57388Gb	0.00	0.12	0.02	0.26
12	i07954Gh	0.00	0.00	0.01	0.01
12	i56214Gb	0.25	0.42	0.37	0.94
12	i60172Gb	0.16	0.38	0.18	0.71
12	i45967Gh	0.10	0.38	0.18	0.68
12	i50197Gb	0.22	0.54	0.57	0.68
12	i08110Gh	0.00	0.01	0.00	0.01
12	i44466Gh	0.52	0.90	0.36	0.98
12	i38901Gh	0.67	0.75	0.83	0.98
12	i19468Gh	0.81	0.58	0.32	0.84
12	i08345Gh	0.54	0.87	0.22	0.52
12	i08502Gh	0.43	0.52	0.12	0.52
12	i53894Gb	0.20	0.75	0.38	0.01
12	i08694Gh	0.41	1.00	0.18	0.01
13	i54272Gb	0.00	0.00	0.18	0.20
13	i13011Gh	0.81	0.21	0.57	0.19
13	i46139Gh	0.90	0.38	0.18	0.58
13	i54568Gb	0.00	0.01	0.00	0.01

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
13	i54898Gb	0.81	0.68	0.49	0.52
13	i56157Gb	0.05	0.21	0.95	0.01
13	i35005Gh	0.73	0.90	0.98	0.51
13	i27466Gh	0.37	0.51	0.86	0.32
13	i50603Gb	0.63	0.56	0.86	0.84
13	i13517Gh	0.50	0.21	0.95	0.71
13	i13553Gh	0.00	0.00	0.00	0.01
13	i52496Gb	0.90	0.42	0.37	0.36
13	i61260Gt	0.67	0.42	0.72	0.20
13	i51136Gb	0.37	0.28	0.25	0.22
13	i13759Gh	0.33	0.61	0.19	0.84
14	i05712Gh	0.00	0.01	0.00	0.02
14	i05683Gh	0.03	0.11	0.48	0.00
14	i15563Gh	0.99	0.68	0.75	0.52
14	i19064Gh	0.89	0.04	0.63	0.14
14	i49624Gh	0.00	0.01	0.00	0.00
14	i05458Gh	0.00	0.00	0.00	0.00
14	i05355Gh	0.25	0.28	0.63	0.94
14	i57074Gb	0.52	0.51	0.63	0.98
14	i29634Gh	0.90	0.87	0.95	0.84
14	i28274Gh	0.76	0.87	0.86	0.84
14	i58710Gb	0.99	1.00	0.86	0.94
14	i05146Gh	0.00	0.00	0.01	0.01
14	i36289Gh	0.78	0.38	0.24	0.85
14	i04957Gh	0.19	0.65	0.25	0.80
14	i04870Gh	0.00	0.00	0.00	0.00

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
15	i13988Gh	0.27	0.12	0.42	0.00
15	i14767Gh	0.00	0.01	0.00	0.01
15	i02942Gh	0.67	0.42	0.95	0.84
15	i50860Gb	0.12	0.42	0.18	0.84
15	i02862Gh	0.00	0.01	0.01	0.00
15	i48166Gh	0.55	0.68	0.37	0.17
15	i14720Gh	0.45	0.90	0.37	0.01
15	i50187Gb	0.50	0.65	0.25	0.06
15	i35660Gh	0.37	0.68	0.57	0.68
15	i02512Gh	0.00	0.01	0.00	0.00
15	i50340Gb	0.09	0.42	0.63	0.31
15	i02444Gh	0.07	0.90	0.57	0.03
15	i14594Gh	0.04	0.22	0.48	0.00
15	i02249Gh	0.03	0.02	0.98	0.01
15	i56500Gb	0.34	0.68	0.95	0.01
16	i51425Gb	0.00	0.07	0.00	0.02
16	i43172Gh	0.45	0.51	0.12	0.20
16	bp position-54974991	0.59	0.85	0.16	0.23
16	i47062Gh	0.60	0.58	0.83	0.32
16	i58337Gb	0.19	0.65	0.72	0.03
16	i30646Gh	0.66	0.68	0.95	0.20
16	i34538Gh	0.45	0.51	0.48	0.20
16	i44284Gh	0.14	0.51	0.00	0.20
16	i01703Gh	0.17	0.90	0.00	0.36
16	i01592Gh	0.24	1.00	0.02	0.36
16	i37829Gh	0.34	0.90	0.02	0.36

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
16	i01516Gh	0.29	0.90	0.06	0.52
16	i14326Gh	0.02	0.73	0.00	0.58
16	i61313Gt	0.09	0.90	0.03	0.68
16	i42554Gh	0.04	0.87	0.07	0.20
16	i51754Gb	0.02	0.68	0.09	0.20
17	i53982Gb	0.19	0.68	0.63	0.19
17	i00195Gh	0.76	0.42	0.86	0.98
17	i03187Gh	0.90	0.38	0.42	0.80
17	i51917Gb	0.16	0.42	0.83	0.03
17	i51043Gb	0.02	0.32	0.49	0.05
17	i03371Gh	0.03	0.68	0.29	0.05
17	i14906Gh	0.03	0.03	0.42	0.32
17	i03479Gh	0.17	0.22	0.28	0.80
17	i39989Gh	0.22	0.70	0.10	0.84
17	i60810Gt	0.34	0.09	0.72	0.61
18	i12965Gh	0.45	0.16	0.75	0.80
18	i21923Gh	0.85	0.70	0.75	0.84
18	i13068Gh	0.15	0.90	0.08	0.58
18	i34219Gh	0.09	0.65	0.19	0.36
18	i34709Gh	0.17	0.90	0.04	0.52
18	i13189Gh	0.23	0.90	0.05	0.52
18	i52366Gb	0.17	0.87	0.05	0.52
18	i17987Gh	0.00	0.01	0.00	0.01
18	i13519Gh	0.24	0.45	0.61	0.20
18	i50412Gb	0.63	0.38	0.86	0.22
18	i00473Gh	0.37	0.32	0.83	0.84

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
18	i13724Gh	0.31	0.12	0.86	0.98
18	i13806Gh	0.34	0.22	0.95	0.14
18	i13850Gh	0.25	0.56	0.75	0.32
19	i65047Gm	0.74	0.65	0.57	0.42
19	i00196Gh	0.67	0.90	0.19	0.84
19	i50131Gb	0.34	0.54	0.28	0.84
19	i10421Gh	0.00	0.00	0.01	0.02
19	i10319Gh	0.16	0.02	0.37	0.84
19	i10321Gh	0.00	0.00	0.01	0.00
19	i26414Gh	0.45	0.03	0.25	0.61
19	i00755Gh	0.15	0.16	0.05	0.80
19	i45829Gh	0.37	0.21	0.11	0.26
19	i26399Gh	0.09	0.16	0.07	0.80
19	bp position-35402201	0.15	0.16	0.05	0.80
19	i09954Gh	0.86	0.50	0.48	0.80
19	i09898Gh	0.00	0.01	0.01	0.00
19	i50264Gb	0.00	0.00	0.01	0.00
19	i09626Gh	0.66	0.42	0.14	0.52
19	i09572Gh	0.54	0.12	0.01	0.68
19	i09526Gh	0.30	0.68	0.01	0.94
19	i09340Gh	0.64	0.02	0.05	0.84
19	i09244Gh	0.20	0.87	0.09	0.80
19	i16569Gh	0.41	0.87	0.18	0.52
19	i16538Gh	0.03	0.28	0.57	0.09
19	i42912Gh	0.39	0.27	0.32	0.71
19	i08802Gh	0.00	0.00	0.00	0.00

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
19	i08803Gh	0.16	0.71	0.18	0.80
20	i17673Gh	0.37	0.28	0.63	0.52
20	i50202Gb	0.01	0.03	0.86	0.04
20	i12189Gh	0.01	0.03	0.37	0.02
20	i20406Gh	0.11	0.68	0.90	0.02
20	i20107Gh	0.29	0.56	0.75	0.05
20	i49481Gh	0.18	0.68	0.83	0.16
20	i25537Gh	0.00	0.65	0.57	0.00
20	i34452Gh	0.25	0.68	0.98	0.14
20	i44471Gh	0.21	0.38	0.95	0.14
20	i11812Gh	0.22	0.42	0.63	0.17
20	i58775Gb	0.37	0.12	0.95	0.36
20	i17444Gh	0.21	0.28	0.75	0.26
20	i11623Gh	0.35	0.21	0.63	0.58
20	i11590Gh	0.29	0.02	0.37	0.58
20	i11470Gh	0.67	0.38	0.25	0.52
21	i50608Gb	0.31	0.38	0.18	0.80
21	i55362Gb	0.16	0.11	0.86	0.61
21	i15938Gh	0.04	0.01	0.36	0.68
21	i06916Gh	0.04	0.00	0.36	0.44
21	i07031Gh	0.00	0.00	0.16	0.36
21	i07037Gh	0.00	0.00	0.03	0.01
21	i07089Gh	0.01	0.01	0.16	0.52
21	i50923Gb	0.01	0.07	0.11	0.31
21	i60993Gt	0.50	0.68	0.72	0.94
21	i60559Gb	0.15	0.68	0.27	0.58

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
21	i57476Gb	0.22	0.61	0.42	0.38
21	i51903Gb	0.50	0.65	0.42	0.71
21	i40036Gh	0.81	0.38	0.48	0.80
21	i07568Gh	0.63	0.90	0.86	0.58
21	i59747Gb	0.80	0.42	0.63	0.94
21	i52000Gb	0.39	0.54	0.98	0.09
22	i17680Gh	0.31	0.04	1.00	0.23
22	i38556Gh	0.23	0.08	0.72	0.52
22	i20148Gh	0.37	0.04	0.48	0.32
22	i40262Gh	0.32	0.16	0.95	0.58
22	i61086Gt	0.80	0.65	0.49	0.84
22	i48531Gh	0.93	0.56	0.75	0.98
22	i12685Gh	0.00	0.01	0.00	0.01
22	i12686Gh	0.61	0.87	0.48	0.71
22	i10394Gh	0.60	0.61	0.75	0.71
22	i12687Gh	0.00	0.01	0.00	0.01
22	i34388Gh	0.85	0.70	0.75	0.71
22	i56516Gb	0.30	0.90	0.37	0.42
22	i12801Gh	0.47	0.63	0.95	0.58
22	i12895Gh	0.61	0.75	0.98	0.42
22	i12939Gh	0.85	0.61	0.86	0.44
23	i05794Gh	0.00	0.00	0.01	0.00
23	i05851Gh	0.24	0.38	0.43	0.31
23	i05936Gh	0.67	0.68	0.72	0.19
23	i35476Gh	0.67	0.90	0.86	0.32
23	i61300Gt	0.52	0.90	0.95	0.32

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
23	i15725Gh	0.69	0.29	0.86	0.20
23	i61516Gt	0.41	0.90	0.98	0.20
23	i62777Gt	0.09	0.16	0.98	0.20
23	i23862Gh	0.81	0.51	0.95	0.68
23	i34362Gh	0.81	0.16	0.57	0.38
23	i06568Gh	0.25	0.35	0.25	0.84
23	i06632Gh	0.25	0.07	0.28	0.22
24	i54758Gb	0.52	0.38	0.18	0.20
24	i04738Gh	0.61	0.65	0.16	0.17
24	i35512Gh	0.34	0.09	0.28	0.80
24	i04615Gh	0.52	0.51	0.28	0.94
24	i31467Gh	0.35	0.70	0.72	0.58
24	i50001Gb	0.00	0.90	0.00	0.06
24	i49724Gh	0.25	0.90	0.36	0.58
24	i04257Gh	0.31	0.38	0.83	0.58
24	i50008Gb	0.35	0.68	0.32	0.23
24	i03855Gh	0.21	0.18	0.83	0.52
24	i51167Gb	0.54	0.18	0.95	0.58
24	i50055Gb	0.81	0.75	0.83	0.44
25	i53437Gb	0.45	0.38	0.98	0.36
25	i11316Gh	0.31	0.65	0.95	0.17
25	i11253Gh	0.00	0.01	0.00	0.00
25	i33715Gh	0.81	0.65	0.95	0.23
25	i11187Gh	0.61	0.65	0.86	0.58
25	i51081Gb	0.66	0.90	0.86	0.58
25	i17265Gh	0.52	0.65	0.83	0.58

Chi-square Test for Segregation Distortion of Markers (1:2:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
25	i52068Gb	0.95	0.73	0.72	0.58
25	i60673Gb	0.66	0.65	0.51	0.84
25	i00963Gh	0.99	1.00	0.83	0.84
25	i10803Gh	0.52	0.75	0.72	0.68
25	i17168Gh	0.52	0.75	0.86	0.44
25	i60789Gt	0.80	0.56	0.25	0.32
26	i40991Gh	0.00	0.00	0.01	0.00
26	i30070Gh	0.07	0.28	0.75	0.20
26	bp position-2013818	0.05	0.05	0.11	0.58
26	i59951Gb	0.02	0.18	0.10	0.68
26	i50506Gb	0.02	0.18	0.27	0.32
26	i47604Gh	0.06	0.29	0.02	0.98
26	i08115Gh	0.53	0.79	0.07	0.36
26	i19454Gh	0.75	0.00	0.18	0.36
26	i08217Gh	0.70	0.22	0.27	0.84
26	i08312Gh	0.10	0.38	0.12	0.58
26	i16420Gh	0.30	0.90	0.24	0.38
26	i14139Gh	0.81	1.00	0.86	0.68
26	i55509Gb	0.90	0.61	0.63	0.71
26	i08657Gh	0.67	0.28	0.25	0.09
26	i19526Gh	0.37	0.42	0.27	0.03
26	i47939Gh	0.40	0.22	0.37	0.16
26	i08773Gh	0.25	0.09	0.37	0.20

TABLE A7

Chi-square Test for Segregation Distortion of Markers (3:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
1	i50196Gb	0.85	1.00	0.19	0.30
2	i01044Gh	0.70	0.38	0.16	0.91
3	i00178Gh	0.17	0.56	0.22	0.56
4	i50976Gb	0.16	0.66	0.45	0.20
5	i09743Gh	0.00	0.19	0.04	0.08
5	i50380Gb	0.90	0.08	0.24	0.73
8	i04229Gh	0.30	0.01	0.91	0.56
10	i12328Gh	0.52	0.38	0.91	0.91
10	i17601Gh	0.37	0.66	0.59	0.56
10	i11978Gh	0.52	1.00	0.33	0.91
11	i06827Gh	0.25	0.19	0.33	0.73
11	i07182Gh	0.70	0.66	0.75	0.91
11	i58276Gb	0.52	1.00	0.91	0.20
12	i07754Gh	0.10	1.00	0.11	0.20
12	i07954Gh	0.95	0.74	0.75	0.91
12	i08110Gh	0.70	0.66	0.91	0.73
13	i54568Gb	0.75	0.38	0.19	0.91
13	i13553Gh	0.90	0.38	0.91	0.20
14	i05712Gh	0.65	0.91	0.91	0.42
14	i49624Gh	0.80	0.66	0.91	0.40
14	i05458Gh	0.06	0.38	0.33	0.13
14	i05146Gh	0.90	0.38	0.75	0.73
14	i04870Gh	0.18	0.74	0.33	0.30
15	i14767Gh	0.37	0.19	0.91	0.73
15	i02862Gh	0.37	0.38	0.24	0.56

Chi-square Test for Segregation Distortion of Markers (3:1)					
Chromosome	Marker ID	Population	Gm	Gt	Gb
15	i02512Gh	0.52	0.19	0.33	0.13
18	i17987Gh	0.90	0.66	0.59	0.91
19	i10421Gh	0.48	0.66	0.38	0.42
19	i10321Gh	0.90	0.38	0.24	0.56
19	i09898Gh	0.69	1.00	0.24	0.56
19	i50264Gb	0.28	0.22	0.75	0.30
19	i08802Gh	0.10	0.66	0.07	0.56
21	i07037Gh	0.22	0.38	0.19	0.08
22	i12685Gh	0.80	0.50	0.91	0.91
22	i12687Gh	0.70	0.38	0.91	0.91
23	i05794Gh	0.70	0.38	0.24	0.30
25	i11253Gh	0.52	0.66	0.91	0.13
26	i40991Gh	0.25	0.08	0.45	0.30

TABLE A8

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr01_000.00_i61225Gt	Chr01_014.14_i23982Gh	Gm, Gb, Gt	14.14	20	22	19	33
Chr01_014.14_i23982Gh	Chr01_026.10_i51041Gb	Gm, Gb, Gt	11.96	14	7	31	18
Chr01_026.10_i51041Gb	Chr01_038.59_i27626Gh	Gm, Gb, Gt	12.49	11	7	25	18
Chr01_038.59_i27626Gh	Chr01_056.68_i34731Gh	Gm, Gb, Gt	18.09	7	3	N/A	18
Chr01_056.68_i34731Gh	Chr01_072.04_i55595Gb	Gb, Gt	15.36	20	N/A	11	20
Chr01_072.04_i55595Gb	Chr01_084.03_i45642Gh	Gm, Gb, Gt	11.99	23	38	31	8
Chr01_084.03_i45642Gh	Chr01_097.67_i47972Gh	Gm, Gb, Gt	13.64	14	19	12	17
Chr01_097.67_i47972Gh	Chr01_140.47_i00132Gh	Sub-cluster-Gt	42.80	41	36	50	41
Chr02_000.85_i50655Gb	Chr02_013.26_i52423Gb	Sub-cluster-Gt	12.41	11	17	8	16
Chr02_013.26_i52423Gb	Chr02_021.83_i03174Gh	Gm, Gb, Gt	8.57	9	9	16	6
Chr02_021.83_i03174Gh	Chr02_041.50_i43496Gh	Gm, Gb, Gt	19.67	22	18	29	29
Chr02_041.50_i43496Gh	Chr02_052.61_i61080Gt	Gm, Gb, Gt	11.11	4	5	5	14
Chr02_052.61_i61080Gt	Chr02_064.99_i60741Gt	Gm, Gb, Gt	12.38	10	9	15	23
Chr02_064.99_i60741Gt	Chr02_073.53_i29065Gh	Gm, Gb, Gt	8.54	4	3	1	15
Chr02_073.53_i29065Gh	Chr02_080.37_i52292Gb	Gm, Gb, Gt	6.84	6	9	6	6
Chr02_080.37_i52292Gb	Chr02_089.33_i57002Gb	Sub-cluster-Gm	8.96	10	13	9	12
Chr02_089.33_i57002Gb	Chr02_112.87_i63139Gt	Gm, Gb, Gt	23.54	24	24	34	21

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr02_112.87_i63139Gt	Chr02_122.79_i38489Gh	Gm, Gb, Gt	9.92	11	11	14	10
Chr02_122.79_i38489Gh	Chr02_132.73_i00543Gh	Gm, Gt	9.94	11	3	N/A	N/A
Chr03_002.56_i05735Gh	Chr03_010.32_i05710Gh	Gm, Gb, Gt	7.76	6	7	5	16
Chr03_010.32_i05710Gh	Chr03_025.77_i50850Gb	Gm, Gb, Gt	15.45	15	24	15	24
Chr03_025.77_i50850Gb	Chr03_038.22_i19058Gh	Gm, Gb, Gt	12.45	9	7	11	10
Chr03_038.22_i19058Gh	Chr03_057.46_i05459Gh	Gm, Gb, Gt	19.24	11	5	20	23
Chr03_057.46_i05459Gh	Chr03_069.43_i14041Gh	Gm, Gb, Gt	11.97	7	3	10	24
Chr03_069.43_i14041Gh	Chr03_084.36_i14878Gh	Gm, Gb, Gt	14.93	15	14	18	19
Chr03_084.36_i14878Gh	Chr03_097.24_i52480Gb	Gb, Gm	12.88	18	6	N/A	18
Chr03_097.24_i52480Gb	Chr03_108.78_i14000Gh	Gm, Gb, Gt	11.54	30	30	45	22
Chr03_108.78_i14000Gh	Chr03_121.18_i43612Gh	Gm, Gb, Gt	12.40	16	19	14	20
Chr03_121.18_i43612Gh	Chr03_136.14_i66018Ga	Gm, Gb, Gt	14.96	18	15	28	23
Chr04_000.85_i24385Gh	Chr04_000.85_i00466Gh	Gm, Gb, Gt	0.00	N/A	1	N/A	N/A
Chr04_000.85_i00466Gh	Chr04_010.25_i25377Gh	Sub-cluster-Gb	9.40	16	25	15	27
Chr04_010.25_i25377Gh	Chr04_021.40_i49276Gh	Gm, Gb, Gt	11.15	5	5	7	8
Chr04_021.40_i49276Gh	Chr04_033.46_i10424Gh	Gm, Gb, Gt	12.06	8	11	6	18
Chr04_033.46_i10424Gh	Chr04_039.48_i29272Gh	Gm, Gb, Gt	6.02	9	13	22	2
Chr04_039.48_i29272Gh	Chr04_053.19_i50228Gb	Gm, Gb, Gt	13.71	9	7	13	11

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr04_053.19_i50228Gb	Chr04_066.86_i32552Gh	Gb, Gm	13.67	11	7	N/A	10
Chr04_066.86_i32552Gh	Chr04_073.73_i47058Gh	Gm, Gb, Gt	6.87	17	5	29	14
Chr04_073.73_i47058Gh	Chr04_089.10_i52022Gb	Gm, Gb, Gt	15.37	23	26	43	26
Chr04_089.10_i52022Gb	Chr04_099.36_i53431Gb	Gm, Gb, Gt	10.26	8	7	10	17
Chr05_001.28_i12375Gh	Chr05_012.38_i12489Gh	Gm, Gb, Gt	11.10	9	11	15	40
Chr05_012.38_i12489Gh	Chr05_025.65_i29617Gh	Gm, Gb, Gt	13.27	16	15	16	45
Chr05_025.65_i29617Gh	Chr05_037.20_i41855Gh	Gm, Gb, Gt	11.55	11	13	14	14
Chr05_037.20_i41855Gh	Chr05_050.35_i00670Gh	Gm, Gb, Gt	13.15	12	11	26	8
Chr05_050.35_i00670Gh	Chr05_057.36_i32528Gh	Gm, Gb, Gt	7.01	4	2	7	7
Chr05_057.36_i32528Gh	Chr05_062.06_i27980Gh	Gm, Gb, Gt	4.70	1	N/A	6	2
Chr05_062.06_i27980Gh	Chr05_081.29_i43462Gh	Gm, Gb, Gt	19.23	19	9	36	37
Chr05_081.29_i43462Gh	Chr05_100.90_i09845Gh	Gm, Gb, Gt	19.61	12	9	14	32
Chr05_100.90_i09845Gh	Chr05_120.99_i01007Gh	Gm, Gb, Gt	20.09	22	22	38	21
Chr05_120.99_i01007Gh	Chr05_140.24_i37015Gh	Gm, Gb, Gt	19.25	26	42	27	37
Chr05_140.24_i37015Gh	Chr05_166.74_i09206Gh	Gm, Gb, Gt	26.50	33	35	24	49
Chr05_166.74_i09206Gh	Chr05_179.55_i48057Gh	Gm, Gb, Gt	12.81	11	9	20	18
Chr05_179.55_i48057Gh	Chr05_189.79_i61049Gt	Gm, Gb, Gt	10.24	10	13	12	11
Chr05_189.79_i61049Gt	Chr05_190.22_i51036Gb	Gm, Gb, Gt	0.43	1	3	N/A	2

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr05_190.22_i51036Gb	Chr05_202.67_i16528Gh	Gm, Gb, Gt	12.45	10	13	8	16
Chr06_007.26_i11398Gh	Chr06_023.07_i44308Gm	Gm, Gb, Gt	15.81	15	19	15	15
Chr06_023.07_i44308Gm	Chr06_030.33_i11312Gh	Gm, Gb, Gt	7.26	9	10	14	5
Chr06_030.33_i11312Gh	Chr06_040.58_i43700Gh	Gm, Gb, Gt	10.25	11	10	21	14
Chr06_040.58_i43700Gh	Chr06_049.13_i39865Gh	Gm, Gb, Gt	8.55	10	7	21	12
Chr06_049.13_i39865Gh	Chr06_060.36_i55732Gb	Gm,Gt	11.23	10	3	1	N/A
Chr06_060.36_i55732Gb	Chr06_068.47_i51850Gb	Gm, Gb, Gt	8.11	17	11	20	30
Chr06_068.47_i51850Gb	Chr06_076.15_i10945Gh	Gm, Gb, Gt	7.68	6	1	16	21
Chr06_076.15_i10945Gh	Chr06_086.81_i50838Gb	Gm, Gb, Gt	10.66	11	9	23	6
Chr06_086.81_i50838Gb	Chr06_096.21_i51788Gb	Gm, Gb, Gt	9.40	9	13	1	10
Chr06_096.21_i51788Gb	Chr06_123.37_i51681Gb	Gm, Gb, Gt	27.16	20	24	15	41
Chr06_123.37_i51681Gb	Chr06_135.31_i52154Gb	Gb, Gm	11.94	20	15	N/A	19
Chr07_021.45_i44501Gh	Chr07_022.73_i41379Gh	Gm, Gb, Gt	1.28	1	1	N/A	2
Chr07_022.73_i41379Gh	Chr07_029.59_i02083Gh	Gm, Gb, Gt	6.86	6	8	14	4
Chr07_029.59_i02083Gh	Chr07_034.33_i53793Gb	Gm, Gb, Gt	4.74	10	15	10	8
Chr07_034.33_i53793Gb	Chr07_045.46_i28496Gh	Gm, Gb, Gt	11.13	12	11	14	18
Chr07_045.46_i28496Gh	Chr07_062.09_i28659Gh	Gt	16.63	14	N/A	23	N/A
Chr07_062.09_i28659Gh	Chr07_074.46_i01705Gh	Sub-cluster-Gm	12.37	10	11	19	9

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr07_074.46_i01705Gh	Chr07_084.28_i36899Gh	Gm, Gb, Gt	9.82	15	18	14	22
Chr07_084.28_i36899Gh	Chr07_088.13_i42454Gh	Gm, Gb, Gt	3.85	6	5	5	8
Chr07_088.13_i42454Gh	Chr07_088.13_i56676Gb	Gm, Gb, Gt	0.00	N/A	1	N/A	N/A
Chr07_088.13_i56676Gb	Chr07_088.13_i57012Gb	Gm, Gb, Gt	0.00	N/A	1	N/A	N/A
Chr07_088.13_i57012Gb	Chr07_096.67_i14360Gh	Gm, Gb, Gt	8.54	5	N/A	12	16
Chr07_096.67_i14360Gh	Chr07_106.50_i51776Gb	Gm, Gb, Gt	9.83	10	18	10	30
Chr07_106.50_i51776Gb	Chr07_107.35_i50472Gb	Gm, Gb, Gt	0.85	1	2	N/A	2
Chr07_107.35_i50472Gb	Chr07_129.15_i01376Gh	Gm, Gb, Gt	21.80	31	36	45	27
Chr07_129.15_i01376Gh	Chr07_142.45_i51470Gb	Gm, Gb, Gt	13.30	18	20	22	18
Chr08_000.43_i61029Gt	Chr08_012.46_i60714Gt	Gm, Gb, Gt	12.03	10	7	14	27
Chr08_012.46_i60714Gt	Chr08_022.71_i04669Gh	Gm, Gb, Gt	10.25	11	18	10	12
Chr08_022.71_i04669Gh	Chr08_034.26_i30796Gh	Gm, Gb, Gt	11.55	15	13	38	18
Chr08_034.26_i30796Gh	Chr08_039.37_i00540Gh	Gm, Gb, Gt	5.11	6	9	6	6
Chr08_039.37_i00540Gh	Chr08_052.80_i31486Gh	Sub-cluster-Gb	13.43	14	15	21	12
Chr08_052.80_i31486Gh	Chr08_067.31_i56645Gb	Gm, Gb, Gt	14.51	11	7	17	15
Chr08_067.31_i56645Gb	Chr08_086.25_i04134Gh	Gm, Gb	18.94	19	18	N/A	22
Chr08_086.25_i04134Gh	Chr08_086.68_i31221Gh	Gm, Gb, Gt	0.43	4	5	7	N/A
Chr08_086.68_i31221Gh	Chr08_086.68_i50119Gb	Gm, Gb, Gt	0.00	1	3	N/A	N/A

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr08_086.68_i50119Gb	Chr08_103.00_i51390Gb	Gm, Gb, Gt	16.32	13	11	32	13
Chr08_103.00_i51390Gb	Chr08_105.57_i58509Gb	Gm, Gb, Gt	2.57	3	N/A	5	6
Chr08_105.57_i58509Gb	Chr08_115.08_i48310Gh	Gm, Gt	9.51	10	7	16	N/A
Chr08_115.08_i48310Gh	Chr08_127.46_i20535Gh	Gm, Gb, Gt	12.38	8	5	18	16
Chr08_127.46_i20535Gh	Chr08_129.59_i29120Gh	Gm, Gb, Gt	2.13	3	N/A	12	4
Chr08_129.59_i29120Gh	Chr08_154.39_i61072Gt	Gm, Gb, Gt	24.80	25	33	27	49
Chr08_154.39_i61072Gt	Chr08_164.63_i18599Gh	Gm, Gb, Gt	10.24	12	11	22	8
Chr08_164.63_i18599Gh	Chr08_171.04_i61692Gt	Gm, Gb, Gt	6.41	5	7	6	10
Chr09_000.43_i61099Gt	Chr09_015.78_i41274Gh	Gm, Gb, Gt	15.35	50	50	50	50
Chr09_015.78_i41274Gh	Chr09_028.62_i50500Gb	Gm, Gb, Gt	12.84	50	50	50	50
Chr09_028.62_i50500Gb	Chr09_043.58_i25619Gh	Gm, Gb, Gt	14.96	4	N/A	8	18
Chr09_043.58_i25619Gh	Chr09_054.89_i06016Gh	Gm, Gb, Gt	11.31	12	15	10	20
Chr09_054.89_i06016Gh	Chr09_062.70_i59036Gb	Gm, Gb, Gt	7.81	5	3	5	12
Chr09_062.70_i59036Gb	Chr09_063.13_i52416Gb	Gm, Gb, Gt	0.43	N/A	1	N/A	N/A
Chr09_063.13_i52416Gb	Chr09_081.06_i25043Gh	Gm, Gb, Gt	17.93	20	28	19	17
Chr09_081.06_i25043Gh	Chr09_088.77_i50477Gb	Gm, Gb, Gt	7.71	13	11	35	8
Chr09_088.77_i50477Gb	Chr09_099.01_i06319Gh	Sub-cluster-Gm	10.24	11	5	14	27
Chr09_099.01_i06319Gh	Chr09_119.53_i06474Gh	Gm, Gb, Gt	20.52	19	25	26	20

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr09_119.53_i06474Gh	Chr09_120.81_i50857Gb	Gm, Gb, Gt	1.28	N/A	1	N/A	N/A
Chr09_120.81_i50857Gb	Chr09_129.78_i51975Gb	Gm, Gb, Gt	8.97	8	7	9	14
Chr09_129.78_i51975Gb	Chr09_142.17_i51086Gb	Gm, Gb, Gt	12.39	20	28	33	19
Chr10_006.44_i12331Gh	Chr10_010.29_i49130Gh	Gm, Gb, Gt	3.85	10	15	7	12
Chr10_010.29_i49130Gh	Chr10_013.70_i52506Gb	Gm, Gb, Gt	3.41	3	5	10	2
Chr10_013.70_i52506Gb	Chr10_035.91_i51066Gb	Gm, Gb, Gt	22.21	28	38	39	20
Chr10_035.91_i51066Gb	Chr10_050.00_i00406Gh	Gm, Gb, Gt	14.09	18	21	17	20
Chr10_050.00_i00406Gh	Chr10_065.37_i11901Gh	Gm, Gb, Gt	15.37	17	14	22	23
Chr10_065.37_i11901Gh	Chr10_081.69_24931Gh	Gm, Gb, Gt	16.32	14	9	14	28
Chr10_081.69_24931Gh	Chr10_081.69_i48101Gh	Gm, Gb, Gt	0.00	6	N/A	6	18
Chr10_081.69_i48101Gh	Chr10_084.25_i42848Gh	Gm, Gb, Gt	2.56	1	1	1	N/A
Chr10_084.25_i42848Gh	Chr10_093.24_i00714Gh	Gm, Gb, Gt	8.99	1	1	5	2
Chr10_093.24_i00714Gh	Chr10_093.24_i41571Gh	Gm, Gb, Gt	0.00	N/A	1	N/A	N/A
Chr10_093.24_i41571Gh	Chr10_126.19_i00730Gh	Gb, Gt	32.95	32	N/A	33	21
Chr10_126.19_i00730Gh	Chr10_135.21_i39597Gh	Gm, Gb, Gt	9.02	20	32	21	14
Chr10_135.21_i39597Gh	Chr10_139.49_i60917Gt	Gm, Gb, Gt	4.28	3	3	6	5
Chr10_139.49_i60917Gt	Chr10_154.14_i50887Gb	Gm, Gb, Gt	14.65	19	25	19	29
Chr11_002.14_i00366Gh	Chr11_031.18_i52333Gb	Gm, Gb, Gt	29.04	29	31	36	44

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr11_031.18_i52333Gb	Chr11_041.84_i52531Gb	Gm, Gb, Gt	10.66	13	13	11	15
Chr11_041.84_i52531Gb	Chr11_070.90_i58197Gb	Gm, Gb, Gt	29.06	37	39	49	39
Chr11_070.90_i58197Gb	Chr11_076.03_i43779Gh	Gm, Gb, Gt	5.13	4	9	1	2
Chr11_076.03_i43779Gh	Chr11_080.73_i52034Gb	Gm, Gb, Gt	4.70	6	9	8	4
Chr11_080.73_i52034Gb	Chr11_092.27_i34920Gh	Gm, Gb, Gt	11.54	8	7	7	10
Chr11_092.27_i34920Gh	Chr11_104.47_i03278Gh	Gm, Gb, Gt	12.20	10	13	16	4
Chr11_104.47_i03278Gh	Chr11_125.86_i57155Gb	Gm,Gt	21.39	7	9	1	N/A
Chr11_125.86_i57155Gb	Chr11_139.94_i19367Gh	Gm, Gb, Gt	14.08	9	5	12	28
Chr11_139.94_i19367Gh	Chr11_154.91_i07548Gh	Gm, Gb, Gt	14.97	11	6	14	31
Chr11_154.91_i07548Gh	Chr11_181.91_i29860Gh	Gm, Gb, Gt	27.00	37	50	39	29
Chr11_181.91_i29860Gh	Chr11_191.74_i50720Gb	Gm, Gb, Gt	9.83	1	1	1	N/A
Chr12_000.00_28665Gh	Chr12_014.94_i29317Gh	Gm, Gb, Gt	14.94	22	35	26	17
Chr12_014.94_i29317Gh	Chr12_041.48_i57388Gb	Gm, Gb, Gt	26.54	20	16	33	23
Chr12_041.48_i57388Gb	Chr12_051.03_i56214Gb	Gm, Gb, Gt	9.55	11	9	20	21
Chr12_051.03_i56214Gb	Chr12_053.59_60172Gb	Gm, Gb, Gt	2.56	1	1	1	5
Chr12_053.59_60172Gb	Chr12_056.15_i45967Gh	Gm, Gb, Gt	2.56	N/A	N/A	N/A	2
Chr12_056.15_i45967Gh	Chr12_074.59_i50197Gb	Gm, Gb, Gt	18.44	22	23	42	29
Chr12_074.59_i50197Gb	Chr12_089.96_i44466Gh	Gm, Gb, Gt	15.37	12	14	20	14

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr12_089.96_i44466Gh	Chr12_092.53_i38901Gh	Gm, Gb, Gt	2.57	3	1	7	N/A
Chr12_092.53_i38901Gh	Chr12_099.37_i19468Gh	Gm, Gb, Gt	6.84	6	8	10	8
Chr12_099.37_i19468Gh	Chr12_107.46_i08345Gh	Gm, Gb, Gt	8.09	12	17	12	12
Chr12_107.46_i08345Gh	Chr12_121.14_i08502Gh	Gm, Gb, Gt	13.68	15	21	21	12
Chr12_121.14_i08502Gh	Chr12_136.09_i53894Gb	Gm, Gb, Gt	14.95	22	18	49	28
Chr12_136.09_i53894Gb	Chr12_147.19_i08694Gh	Gm, Gb, Gt	11.10	7	7	12	9
Chr13_000.00_i54272Gb	Chr13_012.47_i13011Gh	Gm, Gb, Gt	12.47	20	38	16	10
Chr13_012.47_i13011Gh	Chr13_020.16_i46139Gh	Gm, Gb, Gt	7.69	10	9	12	12
Chr13_020.16_i46139Gh	Chr13_043.72_i54898Gb	Gm, Gb, Gt	23.56	17	19	30	12
Chr13_043.72_i54898Gb	Chr13_058.67_i56157Gb	Gm, Gb, Gt	14.95	13	9	19	28
Chr13_058.67_i56157Gb	Chr13_074.92_i35005Gh	Gm, Gb, Gt	16.25	11	13	8	25
Chr13_074.92_i35005Gh	Chr13_083.89_i27466Gh	Gm, Gb, Gt	8.97	11	15	10	15
Chr13_083.89_i27466Gh	Chr13_090.29_i50603Gb	Gm, Gb, Gt	6.40	8	9	10	8
Chr13_090.29_i50603Gb	Chr13_100.55_i13517Gh	Gm, Gb, Gt	10.26	9	7	8	28
Chr13_100.55_i13517Gh	Chr13_117.20_i52496Gb	Gm, Gb, Gt	16.65	15	19	17	18
Chr13_117.20_i52496Gb	Chr13_125.35_i61260Gt	Gm, Gb, Gt	8.15	10	11	12	26
Chr13_125.35_i61260Gt	Chr13_127.93_i51136Gb	Gm, Gb, Gt	2.58	3	3	5	2
Chr13_127.93_i51136Gb	Chr13_141.73_i13759Gh	Gm, Gb, Gt	13.80	13	16	10	14

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr14_013.71_i05683Gh	Chr14_021.07_i15563Gh	Gm, Gb, Gt	7.36	40	41	43	50
Chr14_021.07_i15563Gh	Chr14_030.96_i19064Gh	Gm, Gb, Gt	9.89	9	17	5	10
Chr14_030.96_i19064Gh	Chr14_060.52_i05355Gh	Gm, Gb, Gt	29.56	32	17	46	50
Chr14_060.52_i05355Gh	Chr14_060.95_i57074Gb	Gm, Gb, Gt	0.43	1	1	N/A	2
Chr14_060.95_i57074Gb	Chr14_075.46_i29634Gh	Gm, Gb, Gt	14.51	5	5	7	8
Chr14_075.46_i29634Gh	Chr14_086.86_i28274Gh	Gm, Gb, Gt	11.40	9	7	11	29
Chr14_086.86_i28274Gh	Chr14_094.55_i58710Gb	Gm, Gb, Gt	7.69	5	3	3	18
Chr14_094.55_i58710Gb	Chr14_110.98_i36289Gh	Gm, Gb, Gt	16.43	17	18	35	17
Chr14_110.98_i36289Gh	Chr14_141.10_i04957Gh	Gm, Gb, Gt	30.12	27	40	21	32
Chr15_000.00_i13988Gh	Chr15_010.70_i02942Gh	Gm, Gb, Gt	10.70	20	24	21	21
Chr15_010.70_i02942Gh	Chr15_021.39_i50860Gb	Gm, Gb, Gt	10.69	16	15	39	20
Chr15_021.39_i50860Gb	Chr15_033.86_i48166Gh	Gm, Gb, Gt	12.47	4	1	1	12
Chr15_033.86_i48166Gh	Chr15_041.11_i14720Gh	Gm, Gb, Gt	7.25	5	3	N/A	21
Chr15_041.11_i14720Gh	Chr15_050.06_i50187Gb	Gm, Gb, Gt	8.95	8	13	6	17
Chr15_050.06_i50187Gb	Chr15_067.63_i35660Gh	Gm, Gb, Gt	17.57	21	13	36	22
Chr15_067.63_i35660Gh	Chr15_073.59_i50340Gb	Gm, Gb, Gt	5.96	7	9	5	12
Chr15_073.59_i50340Gb	Chr15_083.43_i02444Gh	Gm, Gb, Gt	9.84	13	22	5	12
Chr15_083.43_i02444Gh	Chr15_098.79_i14594Gh	Gm, Gb, Gt	15.36	13	9	29	15

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr15_098.79_i14594Gh	Chr15_112.48_i02249Gh	Gm, Gb, Gt	13.69	13	13	23	10
Chr15_112.48_i02249Gh	Chr15_113.76_i56500Gb	Gm, Gb, Gt	1.28	5	14	1	6
Chr16_000.43_i51425Gb	Chr16_011.97_i43172Gh	Gm, Gb, Gt	11.54	25	26	37	8
Chr16_011.97_i43172Gh	Chr16_bp position-54974991	Gm, Gb, Gt	bp	9	5	9	16
Chr16_bp position-54974991	Chr16_030.05_i47062Gh	Gm, Gb, Gt	bp	11	15	21	8
Chr16_030.05_i47062Gh	Chr16_039.49_i58337Gb	Gm, Gb, Gt	9.44	8	6	17	8
Chr16_039.49_i58337Gb	Chr16_044.62_i30646Gh	Gm, Gb, Gt	5.13	11	13	18	4
Chr16_044.62_i30646Gh	Chr16_052.74_i34538Gh	Gm, Gb, Gt	8.12	8	7	14	8
Chr16_052.74_i34538Gh	Chr16_061.70_i44284Gh	Gm, Gb, Gt	8.96	8	7	11	8
Chr16_061.70_i44284Gh	Chr16_071.95_i01703Gh	Gm, Gb, Gt	10.25	10	15	8	14
Chr16_071.95_i01703Gh	Chr16_080.07_i01592Gh	Gm, Gb, Gt	8.12	11	13	8	17
Chr16_080.07_i01592Gh	Chr16_080.50_i37829Gh	Gm, Gb, Gt	0.43	N/A	1	N/A	N/A
Chr16_080.50_i37829Gh	Chr16_088.19_i01516Gh	Gm, Gb, Gt	7.69	5	7	6	6
Chr16_088.19_i01516Gh	Chr16_099.74_i14326Gh	Gm, Gb, Gt	11.55	9	9	5	19
Chr16_099.74_i14326Gh	Chr16_112.15_i61313Gt	Gm, Gb, Gt	12.41	6	11	3	8
Chr16_112.15_i61313Gt	Chr16_122.39_i42554Gh	Gm, Gb, Gt	10.24	11	17	10	10
Chr16_122.39_i42554Gh	Chr16_133.50_i51754Gb	Gm, Gb, Gt	11.11	10	13	21	7
Chr17_000.00_i53982Gb	Chr17_009.44_i00195Gh	Sub-cluster-Gm	9.44	17	22	27	10

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr17_009.44_i00195Gh	Chr17_020.56_i03187Gh	Gm, Gb, Gt	11.12	12	9	20	14
Chr17_020.56_i03187Gh	Chr17_033.79_i51917Gb	Gm, Gb, Gt	13.23	15	13	29	13
Chr17_033.79_i51917Gb	Chr17_041.49_i51043Gb	Gm, Gb, Gt	7.70	14	11	9	28
Chr17_041.49_i51043Gb	Chr17_049.60_i03371Gh	Gm, Gb, Gt	8.11	6	9	7	6
Chr17_049.60_i03371Gh	Chr17_062.07_i14906Gh	Gm, Gb, Gt	12.47	16	13	20	15
Chr17_062.07_i14906Gh	Chr17_071.66_i03479Gh	Gm, Gb, Gt	9.59	5	5	1	18
Chr17_071.66_i03479Gh	Chr17_091.05_i39989Gh	Gm, Gb, Gt	19.39	18	18	22	35
Chr17_091.05_i39989Gh	Chr17_107.30_i60810Gt	Gm, Gb, Gt	16.25	26	40	34	17
Chr18_000.00_i12965Gh	Chr18_008.22_i21923Gh	Gm, Gb, Gt	8.22	7	7	N/A	27
Chr18_008.22_i21923Gh	Chr18_024.10_i13068Gh	Gm, Gb, Gt	15.88	16	18	17	15
Chr18_024.10_i13068Gh	Chr18_030.07_i34219Gh	Gm, Gb, Gt	5.97	3	5	5	4
Chr18_030.07_i34219Gh	Chr18_038.61_i34709Gh	Gm, Gb, Gt	8.54	15	22	16	14
Chr18_038.61_i34709Gh	Chr18_045.51_i13189Gh	Gm, Gb, Gt	6.90	4	3	5	4
Chr18_045.51_i13189Gh	Chr18_047.65_i52366Gb	Gm, Gb, Gt	2.14	N/A	1	N/A	N/A
Chr18_047.65_i52366Gb	Chr18_074.14_i13519Gh	Gm, Gb, Gt	26.49	27	28	32	40
Chr18_074.14_i13519Gh	Chr18_083.96_i50412Gb	Gm, Gb, Gt	9.82	5	1	5	10
Chr18_083.96_i50412Gb	Chr18_107.60_i00473Gh	Gm, Gb, Gt	23.64	23	29	16	35
Chr18_107.60_i00473Gh	Chr18_110.16_i13724Gh	Gm, Gb, Gt	2.56	5	9	1	8

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr18_110.16_i13724Gh	Chr18_123.90_i13806Gh	Gm, Gb, Gt	13.74	15	20	12	40
Chr18_123.90_i13806Gh	Chr18_125.18_i13850Gh	Gm, Gb, Gt	1.28	5	9	8	2
Chr19_002.56_i65047Gm	Chr19_005.98_i00196Gh	Gm, Gb, Gt	3.42	6	9	3	10
Chr19_005.98_i00196Gh	Chr19_011.53_i50131Gb	Gm, Gb, Gt	5.55	8	9	14	12
Chr19_011.53_i50131Gb	Chr19_031.19_i10319Gh	Gm, Gb, Gt	19.66	15	15	35	16
Chr19_031.19_i10319Gh	Chr19_040.58_i26414Gh	Sub-cluster-Gm	9.39	12	22	7	12
Chr19_040.58_i26414Gh	Chr19_050.53_i00755Gh	Gm, Gb, Gt	9.95	6	5	8	10
Chr19_050.53_i00755Gh	Chr19_058.71_i45829Gh	Gm, Gb, Gt	8.18	6	5	8	8
Chr19_058.71_i45829Gh	Chr19_071.62_i26399Gh	Gm, Gb, Gt	12.91	6	5	5	16
Chr19_071.62_i26399Gh	Chr19_bp position-35402201	Gm, Gb, Gt	bp	1	3	N/A	N/A
Chr19_bp position-35402201	Chr19_106.32_i09954Gh	Gm, Gb, Gt	bp	13	10	21	21
Chr19_106.32_i09954Gh	Chr19_141.39_i09626Gh	Gm, Gb, Gt	35.07	29	30	40	26
Chr19_141.39_i09626Gh	Chr19_152.59_i09572Gh	Gm, Gb, Gt	11.20	14	21	7	19
Chr19_152.59_i09572Gh	Chr19_160.78_i09526Gh	Gm, Gb, Gt	8.19	4	5	N/A	16
Chr19_160.78_i09526Gh	Chr19_171.89_i09340Gh	Gm, Gb, Gt	11.11	13	13	25	14
Chr19_171.89_i09340Gh	Chr19_183.05_i09244Gh	Gm, Gb, Gt	11.16	14	15	27	10
Chr19_183.05_i09244Gh	Chr19_205.27_i16569Gh	Gm, Gb, Gt	22.22	23	24	35	29
Chr19_205.27_i16569Gh	Chr19_217.25_i16538Gh	Gm, Gb, Gt	11.98	7	11	12	6

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr19_217.25_i16538Gh	Chr19_222.82_i42912Gh	Gm, Gt	5.57	8	10	1	N/A
Chr19_222.82_i42912Gh	Chr19_224.10_i08803Gh	Gm, Gb, Gt	1.28	8	8	7	14
Chr20_000.43_i17673Gh	Chr20_024.82_i50202Gb	Gm, Gb, Gt	24.39	31	39	32	25
Chr20_024.82_i50202Gb	Chr20_030.82_i12189Gh	Gm, Gb, Gt	6.00	3	N/A	10	7
Chr20_030.82_i12189Gh	Chr20_045.81_i20406Gh	Gm, Gb, Gt	14.99	18	24	30	12
Chr20_045.81_i20406Gh	Chr20_051.35_i20107Gh	Gm, Gb, Gt	5.54	7	1	21	10
Chr20_051.35_i20107Gh	Chr20_060.66_i49481Gh	Sub-cluster-Gt	9.31	8	9	5	15
Chr20_060.66_i49481Gh	Chr20_075.70_i25537Gh	Gm, Gt	15.04	11	1	5	N/A
Chr20_075.70_i25537Gh	Chr20_080.40_i34452Gh	Gm, Gb, Gt	4.70	13	1	14	31
Chr20_080.40_i34452Gh	Chr20_090.70_i44471Gh	Gm, Gb, Gt	10.30	4	3	1	12
Chr20_090.70_i44471Gh	Chr20_097.96_i11812Gh	Gm, Gb, Gt	7.26	4	5	6	12
Chr20_097.96_i11812Gh	Chr20_108.62_i58775Gb	Gm, Gb, Gt	10.66	9	9	14	8
Chr20_108.62_i58775Gb	Chr20_120.59_i17444Gh	Gm, Gb, Gt	11.97	10	9	8	17
Chr20_120.59_i17444Gh	Chr20_130.84_i11623Gh	Gm, Gb, Gt	10.25	12	11	9	36
Chr20_130.84_i11623Gh	Chr20_136.81_i11590Gh	Gm, Gb, Gt	5.97	7	18	8	N/A
Chr20_136.81_i11590Gh	Chr20_151.73_i11470Gh	Gm, Gb, Gt	14.92	15	24	11	10
Chr21_002.13_i50608Gb	Chr21_022.21_i55362Gb	Gm, Gt	20.08	23	40	23	N/A
Chr21_022.21_i55362Gb	Chr21_031.22_i15938Gh	Gm, Gb, Gt	9.01	7	7	15	18

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr21_031.22_i15938Gh	Chr21_031.65_i06916Gh	Gm, Gb, Gt	0.43	1	1	N/A	5
Chr21_031.65_i06916Gh	Chr21_054.36_i07031Gh	Gm, Gb, Gt	22.71	12	17	21	18
Chr21_054.36_i07031Gh	Chr21_064.22_i07089Gh	Gm, Gb, Gt	9.86	7	10	3	14
Chr21_064.22_i07089Gh	Chr21_069.79_i50923Gb	Gm, Gb, Gt	5.57	5	3	5	10
Chr21_069.79_i50923Gb	Chr21_080.89_i60993Gt	Gm, Gb, Gt	11.10	19	24	23	25
Chr21_080.89_i60993Gt	Chr21_091.12_i60559Gb	Gm, Gb, Gt	10.23	14	11	29	15
Chr21_091.12_i60559Gb	Chr21_106.06_i57476Gb	Gm, Gb, Gt	14.94	3	N/A	3	6
Chr21_106.06_i57476Gb	Chr21_118.68_i51903Gb	Gm, Gb, Gt	12.62	14	11	21	24
Chr21_118.68_i51903Gb	Chr21_136.90_i40036Gh	Gm, Gb, Gt	18.22	8	5	3	15
Chr21_136.90_i40036Gh	Chr21_151.86_i07568Gh	Gm, Gb, Gt	14.96	24	20	34	26
Chr21_151.86_i07568Gh	Chr21_171.55_i59747Gb	Gm, Gb, Gt	19.69	20	22	22	39
Chr21_171.55_i59747Gb	Chr21_184.37_i52000Gb	Gm, Gb, Gt	12.82	20	34	16	29
Chr22_000.0_i17680Gh	Chr22_012.81_i38556Gh	Gm, Gb, Gt	12.81	13	15	16	16
Chr22_012.81_i38556Gh	Chr22_026.17_i20148Gh	Gm, Gb, Gt	13.36	10	16	6	17
Chr22_026.17_i20148Gh	Chr22_034.77_i40262Gh	Gm, Gb, Gt	8.60	11	23	14	5
Chr22_034.77_i40262Gh	Chr22_044.59_i61086Gt	Gm, Gb, Gt	9.82	12	26	8	5
Chr22_044.59_i61086Gt	Chr22_054.54_i48531Gh	Gm, Gb, Gt	9.95	6	3	6	20
Chr22_054.54_i48531Gh	Chr22_057.10_i12686Gh	Gm, Gb, Gt	2.56	3	3	1	4

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr22_057.10_i12686Gh	Chr22_062.32_i10394Gh	Gm, Gb, Gt	5.22	5	7	1	12
Chr22_062.32_i10394Gh	Chr22_082.74_i34388Gh	Gm, Gb, Gt	20.42	3	3	10	N/A
Chr22_082.74_i34388Gh	Chr22_090.44_i56516Gb	Gm, Gb, Gt	7.70	10	13	6	29
Chr22_090.44_i56516Gb	Chr22_100.71_i12801Gh	Gm, Gb, Gt	10.27	12	22	8	8
Chr22_100.71_i12801Gh	Chr22_112.66_i12895Gh	Gm, Gb, Gt	11.95	18	28	20	8
Chr22_112.66_i12895Gh	Chr22_127.68_i12939Gh	Gm, Gb, Gt	15.02	14	13	17	19
Chr23_008.97_i05851Gh	Chr23_036.95_i05936Gh	Gm, Gb, Gt	27.98	25	15	29	50
Chr23_036.95_i05936Gh	Chr23_047.63_i35476Gh	Gm, Gb, Gt	10.68	12	15	20	6
Chr23_047.63_i35476Gh	Chr23_060.43_i61300Gt	Gm, Gb, Gt	12.80	10	11	12	12
Chr23_060.43_i61300Gt	Chr23_069.81_i15725Gh	Gm, Gb, Gt	9.38	8	11	8	12
Chr23_069.81_i15725Gh	Chr23_083.92_i61516Gt	Sub-cluster-Gm	14.11	14	22	21	12
Chr23_083.92_i61516Gt	Chr23_084.77_i62777Gt	Sub-cluster-Gb	0.85	2	7	N/A	N/A
Chr23_084.77_i62777Gt	Chr23_094.60_i23862Gh	Gm, Gb, Gt	9.83	10	7	12	24
Chr23_094.60_i23862Gh	Chr23_108.28_i34362Gh	Gt	13.68	14	N/A	24	N/A
Chr23_108.28_i34362Gh	Chr23_116.39_i06568Gh	Gm, Gb, Gt	8.11	16	25	24	12
Chr23_116.39_i06568Gh	Chr23_126.64_i06632Gh	Gm, Gb, Gt	10.25	9	12	10	25
Chr24_000.43_i54758Gb	Chr24_012.03_i04738Gh	Gm, Gb, Gt	11.60	12	17	14	17
Chr24_012.03_i04738Gh	Chr24_018.86_i35512Gh	Gm, Gb, Gt	6.83	11	14	8	24

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr24_018.86_i35512Gh	Chr24_027.41_i04615Gh	Gm, Gb, Gt	8.55	5	9	3	8
Chr24_027.41_i04615Gh	Chr24_039.80_i31467Gh	Gm, Gb, Gt	12.39	9	11	12	8
Chr24_039.80_i31467Gh	Chr24_050.91_i50001Gb	Gm, Gb, Gt	11.11	21	24	20	23
Chr24_050.91_i50001Gb	Chr24_059.88_i49724Gh	Gm, Gb, Gt	8.97	21	15	31	29
Chr24_059.88_i49724Gh	Chr24_072.68_i04257Gh	Gm, Gb, Gt	12.80	14	15	22	17
Chr24_072.68_i04257Gh	Chr24_086.35_i50008Gb	Gm, Gb, Gt	13.67	15	7	18	29
Chr24_086.35_i50008Gb	Chr24_111.14_i03855Gh	Gm, Gb, Gt	24.79	20	18	31	38
Chr24_111.14_i03855Gh	Chr24_120.96_i51167Gb	Gm, Gb, Gt	9.82	11	11	21	14
Chr24_120.96_i51167Gb	Chr24_129.50_i50055Gb	Gm, Gb, Gt	8.54	11	15	10	14
Chr25_015.89_i53437Gb	Chr25_024.02_i11316Gh	Gm, Gb, Gt	8.13	9	9	8	20
Chr25_024.02_i11316Gh	Chr25_043.67_i33715Gh	Gm, Gb, Gt	19.65	29	20	49	33
Chr25_043.67_i33715Gh	Chr25_047.97_i11187Gh	Gm, Gb, Gt	4.30	5	N/A	12	10
Chr25_047.97_i11187Gh	Chr25_050.53_i51081Gb	Gm, Gb, Gt	2.56	1	1	N/A	4
Chr25_050.53_i51081Gb	Chr25_059.06_i17265Gh	Gm, Gb, Gt	8.53	1	1	1	N/A
Chr25_059.06_i17265Gh	Chr25_073.63_i52068Gb	Gm, Gb, Gt	14.57	12	14	25	19
Chr25_073.63_i52068Gb	Chr25_084.32_i60673Gb	Gm, Gb, Gt	10.69	6	3	9	6
Chr25_084.32_i60673Gb	Chr25_085.60_i00963Gh	Gm, Gb, Gt	1.28	1	3	5	N/A
Chr25_085.60_i00963Gh	Chr25_096.70_i10803Gh	Gm, Gb, Gt	11.10	13	11	25	18

Adjacent Markers		Assay Applicability	Marker Distance	Recombination Rate (%)			
				Population	Gm	Gt	Gb
Chr25_096.70_i10803Gh	Chr25_109.96_i17168Gh	Gm, Gb, Gt	13.26	10	15	8	22
Chr25_109.96_i17168Gh	Chr25_130.42_i60789Gt	Gm, Gb, Gt	20.46	20	23	32	25
Chr26_013.63_i30070Gh	Chr25_bp position-2013818	Gm, Gb, Gt	bp	7	5	17	6
Chr26_bp position-2013818	Chr26_034.18_i59951Gb	Gm, Gb, Gt	bp	15	13	16	29
Chr26_034.18_i59951Gb	Chr26_045.69_i50506Gb	Gm, Gb, Gt	11.51	10	3	30	13
Chr26_045.69_i50506Gb	Chr26_060.61_i47604Gh	Gm, Gb, Gt	14.92	13	15	24	16
Chr26_060.61_i47604Gh	Chr26_072.59_i08115Gh	Sub-cluster-Gm	11.98	18	17	27	15
Chr26_072.59_i08115Gh	Chr26_080.73_i19454Gh	Gm, Gb, Gt	8.14	11	18	5	16
Chr26_080.73_i19454Gh	Chr26_087.98_i08217Gh	Gm, Gb, Gt	7.25	9	12	14	10
Chr26_087.98_i08217Gh	Chr26_098.22_i08312Gh	Gm, Gb, Gt	10.24	12	16	10	26
Chr26_098.22_i08312Gh	Chr26_105.06_i16420Gh	Gm, Gb, Gt	6.84	13	19	14	9
Chr26_105.06_i16420Gh	Chr26_116.15_i14139Gh	Gm, Gb, Gt	11.09	16	17	20	10
Chr26_116.15_i14139Gh	Chr26_120.85_i55509Gb	Gm, Gb, Gt	4.70	7	11	5	2
Chr26_120.85_i55509Gb	Chr26_135.83_i08657Gh	Gm, Gb, Gt	14.98	13	12	12	36
Chr26_135.83_i08657Gh	Chr26_136.69_i19526Gh	Gm, Gb, Gt	0.86	2	3	1	5
Chr26_136.69_i19526Gh	Chr26_145.65_i47939Gh	Gm, Gb, Gt	8.96	8	5	12	10
Chr26_145.65_i47939Gh	Chr26_147.36_i08773Gh	Gm, Gb, Gt	1.71	N/A	1	N/A	N/A

N/A: Recombination rate could not be calculated for marker pairs where only three genotypic classes (a, e, i) existed, or when the assay for a marker was nonfunctional, e.g. species-specific.