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Rachel R Redman

# A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of 

Master of Science

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# ABSTRACT <br> Oat SNP Marker Discovery and Mapping Based on 454 Pyrosequencing of Genome-Reduced Avena magna Murphy et Terrell 

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The size and complexity of the oat genomes (Avena L., $x=7$ ) have made genetic studies, including the discovery of molecular markers, difficult. Recent attention to these species has resulted in the development of many DArT -based markers in the tetraploid A. magna Murphy et Terrill ( $2 n=28$, CCDD genomes), along with numerous RFLP's, SSR's, DArT's, and EST-based SNPs in hexaploid A. sativa L. $(2 n=42$, AACCDD). Here we report the first SNP markers for tetraploid oat based on genome reduction and high-throughput pyrosequencing in two inbred lines of A. magna: A-169 (wild) and Ba 13-13 (domesticated). Initially, the genomes were reduced using restriction digests with EcoRI and BfaI and sequenced to produce 706,426 reads for both genotypes that were subsequently assembled into 57,048 contigs with an average read length of 345 bp . Comparisons of the contigs between the two lines resulted in the detection of 31,304 in silico SNPs. High Resolution Melt (HRM) and KASPar assays were used to validate 1,108 of these in silico SNPs across a panel of diploid, tetraploid, and hexaploid oats. Of the assays, 119 were validated using HRM and 384 using KASPar genotyping in the Fluidigm EP1 system. Both sets of assays were then mapped on a population of $117 \mathrm{~F}_{2: 8}$ recombinant inbred lines (RILs) developed from the A-169 x Ba 13-13 cross. A map of the A. magna genome was then constructed. The markers and map provide a new set of genomic tools for tetraploid and hexaploid oat breeding and allow for tracking of genes controlling traits of economic importance and other interesting genes through the evolution of Avena.

Keywords: SNP, pyrosequencing, Bio-Rad, Fluidigm, Avena, oat, genetic map, genome reduction

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## INTRODUCTION

Cultivated oat (Avena sativa L. and A. byzantina C. Koch, $2 \mathrm{n}=6 \mathrm{x}=42$, AACCDD genomes) was the world's seventh most important cereal crop with 11.3 million harvested acres in 2008 (UN-FAO, faostat.fao.org). Although oat acreage worldwide has been declining over the past 100 years with the demise of the agrarian horse culture, the nutritional benefits of common oat are beginning to make oat a desired component of the human diet. In turn, the demand for high-quality commercial oat is increasing primarily due to its whole grain soluble fiber content. The ability of beta glucan to lower serum LDL cholesterol has been medically documented and led the Food and Drug Administration to allow the labeling of whole-oat products as heart healthy beginning in 1998 (Cervantes-Martinez 2001). Additionally, oat has a favorable fatty acid composition and higher and more complete protein composition than other cereals (Holland 2001). Oat is also used in some brands of dog and chicken feed (Magness 1973).

Avena magna $(2 n=4 x=28, \mathrm{CCDD})$ is a weedy tetraploid species native to heavy clay soils in agricultural areas of northern Morocco. The species is of increasing interest to oat breeders due to its high protein content (up to $25 \%$ of the groat mass), large caryopses, and exceptional crown rust and powdery mildew resistance (Ladizinsky 1995; Ladizinsky 2000). This wild oat species has potential to improve hexaploid oat through gene transfer because it appears to be closely related to A. insularis, the tetraploid ancestor of hexaploid oat (Ladizinsky 1998; Jellen and Ladizinsky 2000). In order to facilitate genetic studies in A. magna, a recombinant inbred line (RIL) population derived from a cross between two A. magna genotypes, Ba 13-13 and $\mathrm{A}-169$, was developed. The parental line Ba 13-13 is a phenotypically
uniform, fertile, and cytogenetically stable oat derived from dual-backcross hybridization with hexaploid $A$. sativa, followed by repeated selfing, to transfer domestication syndrome genes (non-shattering, yellow lemma, glabrous, reduced awns) from A. sativa into A. magna (Ladizinsky 1995).

Recently, Oliver et al. (2011) reported the development of the first genetic map for $A$. magna using the A-169 x Ba 13-13 RIL population. Their map was based on EST-SNP and DArT markers. Since these new markers were derived either from cDNAs (EST-SNPs) or PstI-digested genomic sequence clones (DArTs), they are expected to be biased for coding regions, leaving gaps on the chromosomes of the genetic map. The development of genomic-based markers would serve to fill these gaps and extend the existing linkage groups.

Since Avena chromosomes - and therefore, the Avena genome - are massive (comparable in size to those of the Triticeae grasses), a stringent genome reduction- reduced complexity sequencing (GR-RSC) protocol might be useful to identify genomic SNPs for a fraction of the cost of alternative methods like whole-genome sequencing (Figure 1). Maughan et al. (2009) developed a genomic reduction approach based on restriction digest and restriction-site conservation to dramatically reduce the size and complexity of four Amaranthus genomes and produce the first SNP-based linkage map in this genus. Large genomes can theoretically be reduced by $>90 \%$ using this restriction site conservation and biotin-streptavidin paramagnetic bead separation method. Multiplex identifier (MID)-barcodes were then attached to the target genomes. These barcodes allowed for pooling of the DNA samples followed by parallel, high-throughput DNA sequence analysis in matching genomic contigs from the four genotypes
to identify single-base differences. Assays for these interparental SNPs were then designed without further genotyping.

Single-nucleotide polymorphisms were chosen as the marker of interest as they demonstrate lower mutation rates than tandem repeats (Xu et al. 2005). They are also the most frequent form of DNA sequence variation in eukaryotic genome sequences (Garg et al. 1999), allowing for dense genetic mapping. This type of high density, SNP-based linkage map affords the potential of identifying causal mutations (Rafalski 2002). Genome reduction and 454-pyrosequencing was used for SNP discovery on cattle (Van Tassell et al. 2008); however, due to pooled sampling of the restriction fragments, the individual alleles could not be assigned without further genotyping. Maize SNP discovery encountered similar problems (Barbazuk et al. 2007).

Putative SNPs generated from sequencing must be validated and translated into working PCR-based assays. A powerful technique called high resolution melting (HRM) was developed in 2003 and is capable of detecting polymorphisms, mutations, deletions, insertions or epigenetic differences in double-stranded DNA (Reed 2007). The method uses high data-density acquisition, and detects small sequence differences in PCR fragments, simply by direct melting and reannealing of the double helix. Melting curves can thus be used for mutation scanning, sequence matching, and mutliplex genotyping - analyses that traditionally required processing of PCR products by electrophoresis or other non-homogeneous means (Gundry 2003).

Here we report the use of GR-RSC to discover SNPs in our tetraploid oat mapping parents, eliminating the need for additional genotyping and providing novel genomic SNP markers to further populate the A-169 x Ba 13-13 linkage map. These genomic-based SNPs were further validated through polymorphism screening using a panel of tetraploid and hexaploid mapping-population parents. In addition, we compared the value of HRM versus KASPar/Fluidigm SNP assay platforms.

## MATERIALS AND METHODS

## Plant Materials

Deoxyribonucleic acid from A. magna lines Ba 13-13 and A-169 were used for genome reduction, sequencing, SNP identification and assay validation. Strain Ba 13-13 is a domesticated, tetraploid A. magna line originating in Israel that is morphologically similar to common hexaploid oat. The line originated from a cross between hexaploid A. sativa (cv. 'Ogle', '86-4189', '86-4467' or '86-5698') and a wild tetraploid A. magna line, A-169. The pentaploid progeny from this cross were then backcrossed twice with a wild tetraploid parent, with selection in the offspring for individuals that were fertile, tetraploid, and carried the domestication traits (Ladizinsky 1995). An $\mathrm{F}_{2}$ population was developed by crossing Ba 13-13 by A-169 at BYU (E. Jellen, personal communication). A single-seed descent approach was used to advance the population to the $\mathrm{F}_{8}$ generation at BYU and USDA-ARS (Aberdeen, ID), resulting in 117 recombinant inbred lines (RILs). Ploidy levels were cytologically inspected at both the $\mathrm{F}_{2}$ and $\mathrm{F}_{8}$ stages. Plants were grown in a $22-30^{\circ} \mathrm{C}$ greenhouse with a 16 hour photoperiod.

## DNA Extraction

Genomic DNA was extracted as described in Maughan et al. (2009). Approximately 4 cm of young leaf tissue from each sample was placed in individual 2 ml tubes and ground into powder by submerging the tubes in the liquid $\mathrm{N}_{2}$ and emaceration using a plastic tube pestle. A cetyltrimethylammonium bromide (CTAB) extraction procedure was performed (Kidwell and Osborn 1992). In brief, $600 \mu 1$ of extraction buffer [0.35 M sorbitol, $0.3 \mathrm{M} \mathrm{TrisHCl} \mathrm{pH} 8.0,5$
mM EDTA pH 8.0, $2 \mathrm{M} \mathrm{NaCl}, 2 \%$ CTAB, $5 \%$ (w/v) $N$-lauroylsarcosine, $2 \%$ (w/v)
Polyvinylpyrrolidone (PVP40, K29-32), and 0.5 \% (w/v) sodium metabisulfite] was added and mixed with the powder. The solution was incubated for 60 min at $65^{\circ} \mathrm{C}$ then mixed with $600 \mu \mathrm{l}$ chloroform. After mixing, the solution was centrifuged at $10,000 g$ for 20 min and the aqueous phase was transferred to a new 2 mL tube. Chilled isopropanol ( 600 ul ) was added to the aqueous layer and the solution was mixed by inversion to precipitate the DNA. The samples were centrifuged at $10,000 \mathrm{~g}$ for 30 min . and supernatant was discarded. The DNA pellet was rinsed twice with $70 \%$ ethanol, dried, and then suspended in 1xTE buffer and quantified using the NanoDrop ND 1000 Spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

## Genome Reduction

We reduced the genomes of Ba 13-13 and $\mathrm{A}-169$ using the techniques of Maughan et al. (2009). Genomic DNA was subjected to a double digestion with four- and six-base specific restriction endonucleases BfaI and EcoRI, respectively. Double stranded adapters labeled with a 5'-biotin molecule were ligated to the 6-base recognition sites, while the four-base recognition sites were ligated to unlabeled adapters. Streptavidin paramagnetic beads were used to separate the four-base unlabeled fragments from the labeled six-base fragments. The MID barcodes were incorporated onto the remaining DNA fragments via complementary PCR primers. A PCR process allowed for the annealing of these barcodes into the remaining amplified DNA fragments. Parental genotypes Ba 13-13 and A-169 were labeled with their own unique 10-base MID sequence to allow for post-sequencing bioinformatic separation. Prepared samples were then pooled and electrophoresed to select for the 500-650 base pair fragments, which were
excised from the gel.

## 454 Pyrosequencing, Assembly, and SNP Detection

The 454 pyrosequencing protocol was performed as described in Maughan et al. (2009).
A single micro-bead sequencing run was performed as a service at the Brigham Young University DNA Sequencing Center (DNASC) using a Roche-454 GS FLX instrument and Titanium reagents (Roche, Branford, CT, USA). The DNA from lines Ba 13-13 and A-169 were uniquely labeled with separate MID barcodes. After sequencing, each parent was separated into respective MID- barcode pools bioinformatically using CLCBio Workbench (v. 3.5.1;

Katrinebjerg, Aarhus N, Denmark). Contigs were assembled for each pool de novo. Roche Newbler assembler (v. 2.0.00; Branford, CT, USA) assembled these contigs following the parameters of minimum overlap length of 50 bp , with minimum overlap identity at $95 \%$. Newbler's gsAssembler allowed for de novo assembly of reads into contigs for each parental line. A custom PerlScript (SNP_Finder 3.0; Maughan et al. 2009) created at Brigham Young University was used to identify SNPs between the parental reads within large contigs (>200 bp). Putative SNPs were identified based on the following criteria: 1) at least 10x read coverage; 2) MID-barcode alleles were $90 \%$ identical for each parent; and 3) $40 \%$ minimum allele frequency.

## High Resolution Melt

Genotyping was performed by High Resolution Melt (HRM) analysis as described in Oliver et al. (2011), using a Bio-Rad C1000 thermal cycler with a CFX96 optics module. Bio-Rad's 1x SsoFast EvaGreen Supermix was mixed with 55 ng genomic DNA in each
reaction. For each reaction $0.5 \mu \mathrm{M}$ forward and reverse primers (Supplemental Table 1) were used in a $12.5 \mu \mathrm{l}$ reaction volume. The thermocycling protocol used was as follows: 1 ) denaturation at $98^{\circ} \mathrm{C}$ for 2 min ; 2) 46 cycles of $98^{\circ} \mathrm{C}$ for 2 sec and $55^{\circ} \mathrm{C}$ for $\left.5 \mathrm{sec}, 3\right)$ melt gradient from $65^{\circ} \mathrm{C}$ to $95^{\circ} \mathrm{C}$, increasing in $0.2^{\circ} \mathrm{C}$ increments every 10 sec . Melt curves were analyzed using Bio-Rad Precision Melt Analysis Software Version 1.0.534.0511. The differences in relative fluorescence units as a function of melting temperatures allowed for differentiation of primary polymorphic alleles as well as insertions, deletions and null alleles.

## False Discovery Validation

Thirteen primers (contig5030, contig5075, contig6122, contig6183, contig6404, contig6465, contig6923, contig7003, contig7325, contig7662, contig7937, contig8269, contig11641) were randomly chosen from the robust HRM reactions to validate SNPs via Sanger sequencing. These primers were used to PCR-amplify the genomic regions of Ba 13-13 and A-169. Qiagen HotStart Taq Master Mix (Qiagen, Valencia, California, USA) was used for the PCR amplification. The thermocycling conditions were as follows: $95^{\circ} \mathrm{C}$ for 15 min followed by 34 cycles of $94^{\circ} \mathrm{C}$ for $30 \mathrm{sec}, 55^{\circ} \mathrm{C}$ for 1 min , and $72^{\circ} \mathrm{C}$ for 1 min . The final $10-\mathrm{min}$ extension step was done at $72^{\circ} \mathrm{C}$. PCR products were visualized using $1.2 \%$ agarose gel. The amplified PCR products were then extracted from the gel, and purified using a QIAquick PCR Purification Kit with QIAquick spin columns in a microcentrifuge (Qiagen, Valencia, CA, USA).

PCR-purified DNA was transformed into the $\mathrm{pGEM}^{\circledR}$ - T-Easy Vector system using JM109 competent cells, following the manufacturer's protocol (Promega, Madison, WI, USA). The plasmid containing the DNA insert using the GenElute plasmid Miniprep Kit (Sigma, St.

Louis, MO, USA) followed by enzymatic cleavage. Plasmid DNA was then quantified using Nanodrop (ND 1000 Spectrophotometer, Nanodrop Technologies Inc., Montchanin, DE, USA) and 300-400 ng of it was amplified using Big Dye cycle sequencing and T3 forward (5'AATTAACCCTCACTAAAGGGA 3') and T7 reverse (5’TAATACGACTCACTATAGGG 3') primers. The sequencing reaction profile included 25 cycles of $96^{\circ} \mathrm{C}$ for 10 sec followed by $50^{\circ} \mathrm{C}$ for 6 sec , and $60^{\circ} \mathrm{C}$ for 4 min . Amplified PCR product was purified with Sephadex G-50 protocol (GE Healthcare) and sequenced with an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, California). Sequenced vectors were screened using the NCBI VecScreen. Sequences which were conserved with the genomic regions of grass families were included in the studies.

Sequences were aligned using MEGA 4.1 software (Tamura et al. 2007).

## RESULTS AND DISCUSSION

Genome reduction with methylation-sensitive and -insensitive enzymes allows the interrogation of areas of the chromosome neglected by EST-based markers. Maughan et al. (2009) reported that digestion with the BfaI-EcoRI restriction enzyme cocktail produced a continuous smear with Amaranthus DNA following electrophoresis. Although the restriction-digested A. magna DNAs in our study likewise presented a continuous smear following electrophoresis, post GR-RSC in our case resulted in an unusually large fraction of sequences ranging from $200-400 \mathrm{bps}$ - considerably smaller than the $500-650 \mathrm{bp}$ fragments initially excised from the reduction gel. We attributed this disparity to sub-optimal 454 sequencing performance and/or inefficient size selection (Figure 2).

Table 1 presents the results of the GR-RCS procedure with A. magna DNA. Pyrosequencing returned a total of 706,426 reads. These were then assembled into 57,048 large contigs (> 300 bp ), producing 27,200,520 total bases of sequence. Average read length was 345 bp with most reads greater than 40x quality. The average read depth per contig (Figure 3) was 16x. Barcodes MID1 (A-169) and MID2 (Ba 13-13) were removed prior to sequence assembly using Newbler's de novo assembler. As expected, reads from A-169 and Ba 13-13 were found in almost equal proportions, specifically $47 \%$ and $53 \%$, respectively (Table 1).

Sequence classifications (gene ontology), as determined using BLAST2Go, are presented in Figure 4. The presence of a diverse distribution of sequences in terms of molecular function, biological process and cellular components indicates that the pool of GR-RSC treated A. magna DNAs included a wide range of transcribed sequences (Figure 4). As expected, SNP composition
was predominantly in the form of $\mathrm{C} / \mathrm{T}$ and $\mathrm{A} / \mathrm{G}$ transitions (Figure 5).

SNP_Finder detected 31,304 total sequence variants, with most contigs containing only a single SNP and at an average 16X coverage at each SNP. SNP_Finder filtered these SNPs using strict parameters, resulting in 12,642 SNPs and 6,502 contigs containing true SNPs (Table 2). Of the 13 SNPs chosen for validation, only four verified the SNP-identified assembly from Newbler, while the remainder appeared to be amplified PCR products of orthologous and/or paralogous sequences-likely a result of the tetraploid nature of A. magna. This finding suggests that the relatedness of the two subgenomes may complicate the development of future SNP assays.

## Conclusion

This paper emphasizes the development of rapid marker discovery in the oat genus Avena using the wild Moroccan tetraploid A. magna (Murphy 1968) as a source of new DNA sequence-based markers. Genetic marker discovery in tetraploid oat by single nucleotide polymorphisms (SNPs) should provide at least four major benefits for improvement of cultivated oat. First, it provides a new set of CCDD genome specific markers for application in cultivated hexaploid oat breeding assuming tetraploid markers are transferable to hexaploid oat. Second, it provides a basis for genetic map development in A. magna, and potentially also A. murphyi (Ladizinsky, 1995) and A. insularis Ladizinsky, all of which are potential genetic resources for exotic alleles to improve cultivated hexaploid oat. Third, the A. magna-derived SNPs may potentially be used to track the genetic heritage of genes controlling traits of economic importance and other chromosome segments of interest back through the evolutionary ancestors
of oat. Lastly, these markers may be used to create a SNP panel that can be used to screen global diversity in all Avena species. Species-specific SNPs may be detected and analyzed for evolutionary patterns and divergences when Avena species of diverse genome composition are screened.

CHAPTER 2: SINGLE NUCLEOTIDE POLYMORHISM DEVELOPMENT AND GENETIC MAPPING IN TETRAPLOID AVENA MAGNA

## INTRODUCTION

Common cultivated oat, Avena sativa L. and A. byzantina C. Koch, have attracted very little attention until recently, despite being the world's fifth of sixth most significant cereal crop. Current research and industry support have uncovered and promoted a series of unique health benefits from regular oat consumption. One of the most valuable findings was the ability of oat soluble fiber (beta-glucans) to lower serum LDL cholesterol (Braaten et al. 1994). Dietary protein from plant sources has also been shown to have profound health benefits (Nutall et al. 1984; Wang et al. 2008). The good quantity and quality of oat seed protein, combined with high oil content, anti-itch properties, antioxidants, and soluble fiber all make oat an attractive commodity for various industries including breakfast cereals, agronomy, and cosmetology (Eggum et al. 1989). While other cereal crops have comparable protein content, oats have been shown to have higher levels of the limiting amino acid lysine (Young and Pellett 1994). The health benefits from oat are attracting more attention and therefore increasing protein content is a valuable objective (Jones et al. 1948).

While common oat has a diploid chromosome number of $2 n=6 x=42$ (AACCDD genome composition), A. magna Murphy et Terrell (syn. A. maroccana Gdgr) has $2 n=4 x=28$ (CCDD genomes). The latter species inhabits disturbed field sites on heavy alluvial clays in northern Morocco. While seed protein percent in common oat ranges to near 17\%, A. magna seed has been found to exceed $30 \%$ protein, making it a potentially valuable resource for improving common oat's protein content (Ladizinsky and Fainstein 1977). Besides high seed protein content, A.
magna carries other desirable qualities such as resistance to crown rust (Puccinia coronata f . sp. avenae) and powdery mildew (Erysiphe graminis; Ladizinsky 1995; Ohm and Shaner 1992).

Crosses between A. magna and A. sativa have been attempted for introgression purposes; however, hybrid progeny of such crosses are male-sterile pentaploids (Harlan et al. 1973; Ladizinsky 1995; Ladizinsky and Fainstein 1977; Thomas 1992). Pollination of these hybrids with either tetraploid or hexaploid pollen will rescue the sterility by converting it back to 4 x or 6 x , respectively, thus allowing traits of interest to be transferred in the process.

Ladizinsky (1995) took the novel approach of trying to transfer the 'domestication syndrome' traits from A. sativa to create a novel crop, A. magna subsp. domestica. He described the domestication syndrome in A. magna as being controlled by four loci: a partially dominant gene for large, geniculate awns $(A)$; a dominant lemma color gene, with black being dominant to yellow $(L c)$; a dominant gene for pubescent versus glabrous lemma ( $L p$ ); and a dominant gene for non-shattering spikelets (basal articulation, $B a$ ). One of the second-backcross $A$. magna lines having the domestication syndrome from A. sativa was named Ba 13-13 and was crossed with a wild A. magna line, A-169, to make an $\mathrm{F}_{2}$ mapping population (Jellen 2000). Oliver et al. (2011) advanced these lines to the $\mathrm{F}_{8}$ via single-seed descent to make a recombinant inbred line (RIL) mapping population and reported tight coupling linkage between the $A$ (prominent awn) allele and a heterochromatic knob at the telomere of one of the C-genome chromosomes, with the $L p$ locus mapping to a different chromosome. This telomeric knob had previously been noted on apparently homologous chromosomes in A. magna, A. sativa, A. insularis Ladizinsky, and on chromosome 5C in the wild hexaploid A. sterilis L. (Jellen and Ladizinsky 2000; Jellen et al. 1993). Its
segregation in the Ba 13-13 x A-169 progenies was also verified in the $\mathrm{F}_{2}$ (Jellen 2000) and RIL populations (Oliver et al. 2011).

The creation of molecular genetic maps in cultivars and wild relatives of economically important allopolyploid crops like oat can provide powerful tools for marker-assisted selection (MAS), to evaluate breeding value of these genetic resources, and to resolve questions related to genome origins and evolution. Molecular markers that have been used for mapping in oat include sequence characterized amplified regions or SCARs (Chong et al. 2004; Orr and Molnar 2008); amplified fragment length polymorphisms or AFLPs (Jin et al. 2000; Yu and Wise 2000); restriction fragment length polymorphisms or RFLPs (O'Donoughue et al. 1995; Kremer et al. 2001); simple sequence repeats or SSRs (Li et al. 2002; Pal et al. 2002); diversity array technology or DArT markers (Tinker et al. 2009); and SNPs. (Groh et al. 2001).

The first genetic map created for tetraploid oat was an AFLP-based map in A. barbata, a weedy AABB-genome tetraploid (Gardner and Latta, 2006; Latta and Gardner 2009). Nineteen linkage groups were reported and 129 loci mapped. Oliver et al. (2011) reported the first complete linkage map of tetraploid oat in A. magna. This map was constructed of DArT markers, small numbers of SNPs and SSRs, domestication syndrome genes $A$ and $L p$, and the telomeric 5CL knob (Jellen 2000). The Oliver et al. (2011) map is potentially biased toward genic regions because it was based on DArT markers derived from cloned, PstI-digested - and therefore, hypomethylated oat genomic fragments.

Single-nucleotide polymorphisms are potentially the most abundant, and generally the most informative, genetic markers for linkage mapping - short of mapping by sequencing. The two main problems with SNP markers are the initial requirement of DNA sequence data - which can be very expensive to generate - to identify the SNPs, and design of precise assays that can discriminate among homologous, paralogous, and orthologous SNPs. Paralogous SNPs can be an obstacle in species with large, highly duplicated genomes, like Avena. Orthologous SNPs can be especially problematic in allopolyploid species like A. magna (4x) and A. sativa (6x). Highly sensitive SNP assay methods like high-resolution melt-curve analysis (HRM) have proven useful in the mapping of selected SNPs in hexaploid oat populations (Oliver et al. 2011). Although HRM is capable of detecting point mutations, deletions, insertions or epigenetic differences in double stranded DNA (Wittner et al. 2003), it can prove time-intensive and costly, In contrast, other SNP assaying chemistries like TaqMan (Applied Biosystems, Foster City, CA, USA), KASPar (KBiosciences, Hoddesdon, UK), and Golden Gate (Illumina, San Diego, CA, USA) can be run on high-throughput platforms like Illumina's BeadXpress Reader or the Fluidigm 96.96 EP1 instrument (Fluidigm, South San Francisco, CA, USA).

Here we report the development of 436 new A. magna genomic SNPs derived from genome-reduced restriction site conservation (GR-RSC) methodology. These SNPs allowed for the refinement of linkage groups in the Oliver et al. map (2011) by filling in gaps and extending linkage groups. We compared two alternative methodologies for detecting these genomic SNPs in an $\mathrm{F}_{8}$ RIL-based mapping population: namely, high-resolution melting (HRM) analysis on a Bio-Rad instrument and KASPar assays detected on a Fluidigm 96.96 EP1 platform. This work
provides a genetic foundation for further domestication of the tetraploid oat A. magna and for the transfer of economically useful genes from this species to common hexaploid oats.

## MATERIALS AND METHODS

## Plant Materials

A total of 117 A. RILs were previously developed by crossing A. magna subsp. domestica var. Ba 13-13 with wild $\mathrm{A}-169$, then selfing the $\mathrm{F}_{2}$ plants to the $\mathrm{F}_{8}$ by single-seed descent to form the BAM population (Ladizinsky 1995; Oliver et al. 2011). Seed was provided by Dr. Eric Jackson (USDA-ARS, Aberdeen, ID, USA). Sixteen oat lines (Table 3) were selected for validation purposes while an additional 65 wild tetraploid lines and four hexaploid lines were selected to determine the level of diversity across the SNP loci (Table 4). These lines were provided by Dr. Rick Jellen at Brigham Young University and from the USDA-ARS germplasm bank at Aberdeen. All plants were grown in 4-inch square pots, in a greenhouse with an approximately $16-\mathrm{h}$ photoperiod and a daytime temperature ranging from $22-30^{\circ} \mathrm{C}$.

## SNP Primer Development

A total of 1,208 previously identified GR-RSC SNPs were chosen for genotyping. RepeatMasker (v.3.2.9 Tritticae) database was used to eliminate sequences having significant homology to the Triticum cytoplasmic genomes. A primer design program, PrimerPicker (KBiosceinces 2009), processed the sequences using default parameters. Primers were then randomly selected and synthesized by Bioneer Inc. (Alameda, CA, USA).

## HRM and KASPar Genotyping

Single-nucleotide polymorphism marker screening and genotyping was performed on two different platforms. Small-scale, HRM genotyping was performed using a Bio-Rad C1000 thermal cycler with a CFX96 optics module (Hercules, CA, USA) as previously described by Oliver et al. (2011), while large-scale genotyping of SNPs was performed using the Fluidigm (San Francisco, CA, USA) 96.96 Dynamic Array IFC's on the EP1 System. Protocols recommended by KBioscience and Fluidigm were followed.

In brief, Bio-Rad's 1x SsoFast EvaGreen Supermix was mixed with 55 ng genomic DNA in each reaction. In addition to the genomic DNA, $0.5 \mu \mathrm{M}$ forward and reverse primers were used in a $12.5 \mu \mathrm{l}$ reaction volume. The thermocycler protocol used was as follows: 1) denaturation at $98^{\circ} \mathrm{C}$ for 2 min ; 2) 46 cycles of $98^{\circ} \mathrm{C}$ for 2 sec and $55^{\circ} \mathrm{C}$ for 5 sec ; 3) melt gradient from $65^{\circ} \mathrm{C}$ to $95^{\circ} \mathrm{C}$, increasing in $0.2^{\circ} \mathrm{C}$ increments every 10 sec . Melt curves were analyzed using Bio-Rad Precision Melt Analysis Software Version 1.0.534.0511. The differences in relative fluorescence units as a function of melting temperatures allowed for genotyping.

The KASPar (KBioscience Ltd., Hoddesdon, UK) assay was used to validate a portion of the identified SNPs. Assays were designed for SNPs where coverage was between 12-20X and SNP flanking sequences were at least 100 bp long. All assay primer sets were designed using PrimerPicker using default parameters.

The KASPar reactions produce fluorescence intensities at two unique wavelengths, each corresponding to the presence of a an alternate nucleotide at the SNP. Fluorescence intensities
were measured with the Fluidigm EP1 reader and plotted two-dimensionally. Genotype calls based on EP1 measurements were made using the Fluidigm SNP Genotyping Analysis (Fluidigm 2011) program. All calls were manually checked for accuracy and ambiguous data points were left uncalled. The Fluidigm assay is based on KASPar genotyping chemistry, but using a nano-scale reaction volume. Each 96.96 Fluidigm chip accommodates 96 primer pairs x 96 genotypes, producing a total of 9,216 genotypic data points at a cost of $\sim \$ 0.05 / \mathrm{dpt}$. Genetic maps based on KASPar genotyping data were constructed in JMP Genomics v. 5.1 (SAS, Cary, NC, USA) using a regression mapping algorithm.

## SNP Diversity Data Analysis

Alleles for each segregating RIL in the Bio-Rad assays were scored based on the melting curve profile of the mapping parents. Different alleles melt at different temperatures and the Bio-Rad software colors these as green or red. Missing data, or those RILs that did not amplify, were colored black. The alleles were exported into a spreadsheet where the colors were then converted into 0 's and 1's for the biallelic data. A numerical value of 2 was assigned to designate missing data and were disregarded for mapping purposes. Fluidigm assays were scored and converted similarly, based on segregation of the parent's florescence. Both datasets were then converted into a binary matrix. Those reactions that had greater than $10 \%$ failure of RIL amplification were disregarded for both platforms.

## Map Construction

Genotype calls for the 117 BAM RILs at each locus were determined automatically by the Fluidigm progarm and then verified via visual inspection upon comparison with the parental alleles. Using this information, preliminary mapping of linkage groups was performed in MapManager QTX v.1.1 (Rockefeller University, New York City, NY, USA). This framework map was constructed for consensus and reference using markers from the tetraploid map developed by Oliver et. al (2011). Further linkage analysis and map construction were performed using JMP Genomics v. 5.1. Multilocus ordering was determined using an algorithm based on the evolutionary optimization strategy (Mester et al. 2003; Mester et al. 2004), with maximum likelihood estimation to calculate pairwise recombination fractions (rf) for all marker pairs. Preliminary clustering and assignment of markers to a linkage group (LG) was evaluated at a rf = 0.05 threshold. Markers were then attached to the framework scaffold

## Diversity Panel and Assay Validation

A panel of 16 oat lines representing various Avena genome combinations, including diploids and allopolyploids, was surveyed for SNP assay validation across the same 330 primers selected for the Bio-Rad analysis. Another 69 oat lines were selected for the tetraploid diversity analysis and assayed across the 768 primer sets selected for the Fluidigm EP1 analysis. The same platform protocols were used as described above. Each allele was scored as described above and converted to a binary matrix. The JMP Genomics v. 5.1 program was used to create a dendrogram via Neighbor-Joining analysis.

## RESULTS AND DISCUSSION

## SNP Assay Validation

A panel of 16 oat lines representing various Avena genome combinations, including diploids and allopolyploids, was surveyed for SNP assay validation across the same 330 primers selected for the Bio-Rad analysis. Another 69 oat lines were selected for the tetraploid diversity analysis and assayed across the 768 primer sets selected for the Fluidigm EP1 analysis. The same platform protocols were used as described above. Each allele was scored as described above and converted to a binary matrix. The JMP Genomics v. 5.1 program was used to create a dendrogram via Neighbor-Joining analysis.

For our purposes, any SNP with a >10\% amplification failure rate was considered incomplete for mapping and discarded. The $65.3 \%$ and $58.6 \%$ attrition rates for HRM and KASPar markers, respectively, can possibly be attributed to a number of factors. In the case of the KASPar assays on the Fluidigm platform, DNA template concentrations may have been sub-optimal, given the very large size, and duplication, of the A. magna genome. Poor DNA quality might also be an issue and interfere with both assay types, as other researchers have noted that high-quality oat DNA is unusually difficult to purify owing to its high polysaccharide content (E. Jellen, personal communication). The failure of large portions of several reactions to separate from the origin was probably indicative of poor amplification.

Figure 6 demonstrates a robust Bio-Rad assay. RILs that contained the allele from the Ba

13-13 parent were designated green. Alleles from the A-169 parent were labeled red. The differences in melting temperatures between the two alleles are visualized by a shift or space in the melt curves. Similarly, Figure 7 shows strong segregation of alleles for a KASPar assay. Alleles from one parent fluoresced red, while alleles from the other parent fluoresced green. The SNP may be visualized by individual clustering of the RILs. Figure 8 displays the markers run on the two platforms and their BAM genetic map distributions. Whereas markers assayed via KASPar chemistry on the Fluidigm system were randomly distributed along the length of all 16 linkage groups, the HRM-assayed SNPs showed some clustering. This was particularly true on linkage groups 14 and 15 . We cannot conceive of a rational explanation for this clustering effect with the HRM marker set.

## Linkage Map Construction

Delineation of linkage groups, selection of framework markers, and resolution of marker order were performed using JMP Genomics mapping package, with algorithms based on marker order and incrementally-increasing recombination thresholds (Korol et al. 2009; Mester et al. 2004). Figure 9 illustrates a high degree of segregation distortion in the mapping data, with markers skewed toward the wild A-169 parent predominating on linkage groups 2, 4, 6, and 14 . In contrast, linkage groups 5,15 , and 16 were heavily skewed toward markers from domesticated Ba 13-13. Linkage group 1 was unique in having only minor segregation distortion. Interestingly, linkage group 9, which harbors the telomeric knob in A-169 and several domestication syndrome genes originally contributed to Ba 13-13 by A. sativa, showed evidence of A-169 marker
distortion at one end. Though the knob was not included as a marker for this map, cytological analysis of the $\mathrm{F}_{8}$ BAM RILs verified a $41: 59$ skewed ratio in favor of lines homozygous for the knob (R. Jellen, personal communication). This region is likely represented by the three markers having >50\% distortion toward A-169 at the "bottom" of the BA_09 column in Figure 9.

We expected 14 linkage groups for the 14 chromosomes in tetraploid oat. MapManager recovered 14 linkage groups (Figure 10). However, recombination analysis of each marker in JMPGenomics created 16 linkage groups (Figures 11 and 12). We believe increasing marker density would resolve the discrepancy between the two maps. The average distance of markers on the linkage groups was 12.1 cM , and ranged from 8.4 cM on linkage group 6 to just under 20 cM on linkage group 2. The largest gap on any linkage group was 35.8 cM on BAM 7 and the average across the linkage groups was 25.3 cM , suggesting the linkage groups were fairly sound (Table 5). In addition, the heat plot in Figure 12 detected a marker "island" at one end of linkage group 12 showing strong correlation with markers from linkage group 4 [red "lines" in the lower left (horizontal line) and upper right (vertical line) quadrants of the grid]. Whether this is indicative of synteny between these two linkage groups, the presence of a reciprocal translocation in these regions, or some other phenomenon remains to be seen.

An allotetraploid oat genome was recently resolved into 14 linkage groups for the first time with both C and D genome classes distinguished (Oliver et al. 2011). Figure 13 illustrates the importance of genomic-based markers to supplement existing maps created by EST-SNP and DArT markers. The red markers in Figure 13 clearly demonstrate how the GS-RSC SNPs from this study extended linkage groups and filled in "gaps", especially in gene-poor regions of the
chromosomes.

## Avena Diversity Validation Panel

Thirty-two taxonomic entities have been distinguished among oats; however, there are discrepancies reported in the classification of some of the species (Jellen and Leggett 2006). Four basic genomes (A, B, C, and D) have been identified, with potentially a fifth genome (M) in A. macrostachya. Cytogenetic analysis, including C-banding, genomic and fluorescent in situ hybridization (GISH and FISH), provided the primary tools for identifying the individual chromosomes within the genus.

To further validate the accuracy of our results, we ran each of the 330 Bio-Rad assays across a selected diversity panel of 16 lines encompassing most known genome combinations and diversity within the genus Avena (Table 3). Melt curve analysis was scored based on differences in melting temperatures. Results were converted into binary matrix format and run through JMP Genomics v. 5.1 software to create a dendrogram (Figure 14). The resulting dendrogram formed four major clades. All six A. magna lines fell into the same clade, as expected. The diploid species formed two clades, with the CC genomes separating from the AA/DD genomes, which concurs with previous cytogenetic research (Jellen et al. 1994). The hexaploids, A. insularis, and A. murphyi constituted a fourth clade. The data confirmed what is most likely the correct relationship among the species. Avena insularis ( $4 x, \mathrm{CCDD}$ ) is the progenitor of wild $A$. sterilis ( $6 x$, AACCDD), which in turn is the progenitor of domesticated $A$.
sativa (Jellen and Ladizinsky 2000; Zhou et al. 1999). The results further indicate that $A$. murphyi (AACC or CCDD) might either be a progenitor of $A$. insularis, or both tetraploids participated in the hybridization event that gave rise to $A$. sterilis. This latter scenario would have to invoke a partial restituion mechanism and stabilization of the amphidiploid nucleus with only two copies of the C genome.

## Tetraploid Diversity Panel

A panel of tetraploids was created to determine genetic diversity based on the SNP markers interrogated. The diversity panel consisted of 65 wild tetraploid lines and four domesticated hexaploid lines (Table 4), representing seven oat species: A. agadiriana (AAAA or $\mathrm{AABB})$; A. barbata (AABB); A. magna (CCDD); A. murphyi (AACC or CCDD); A. sativa (AACCDD); A. sterilis (AACCDD); and A. vaviloviana (AABB). A total of 318 SNP markers (636 alleles) were polymorphic on the Fluidigm EP1 platform. These polymorphisms created clear genotypic clusters for scoring.

A dendrogram created from biallelic scoring of these markers (Figure 15) produced six distinct clades. As expected, the majority of the A. magna accessions grouped together in two clades (red and green branch lines). Closely related in the adjoining clade was the $A$. murphyi group (blue branch lines). This supports the cytogenetic data that both species have at least one subgenome in common (the C). Interestingly, A. vaviloviana (AABB) grouped among $A$. murphyi accessions, suggesting they have an ancestral relationship or possibly share subgenomes (common variants of the A). The A. agadiriana (AAAA or AABB) accessions grouped together
in a fourth clade (turquoise branch lines), along with several A. murphyi accessions, possibly indicating a molecular relationship between $A$. murphyi and $A$. agadiriana. As expected, the $A$. sativa (AACCDD) oat cultivars grouped together in their own clade (orange branch lines). However, PI 657271 (hexaploid A. sterilis) fell into the group with A. agadiriana and A. murphyi (turquoise). A sixth, small clade contained a mixture of species (purple branch lines). It should be noted that discrepancies in the clades may be the result of misclassification of USDA-ARS materials. Some of these misclassifications were confirmed by seed morphology analysis. Not only does the dendrogram further validate the accuracy of the KASPar/Fluidigm SNP assay method, but also it may potentially be used to extract species-specific SNPs, although polymorphisms arising from indel mutations would not be scorable using this marker platform in contrast to the HRM method (Wittner et al. 2003).

The introduction of molecular markers has revolutionized genetics. Technology is enabling the study of species that were not previously viewed as economic priorities, being too remote, expensive or complex for consideration in plant breeding communities (Eathington et al. 2007). The array of polymorphisms and molecular techniques that are available is increasing, and the arrival of low-cost genomic sequencing is a source of an escalating set of available markers (Cullis 2002). As more genetic information becomes available, the application of molecular markers to other experimental methods will become simpler, allowing for novel genetic analysis that is currently impossible to undertake.

This study reports on the production and utilization of a toolbox of genomic sequence-based SNP markers and their application for genetic mapping and diversity analyses in
an obscure secondary germplasm resource, A. magna. Although the GR-RSC technique has allowed for marker development in a species that could someday be commercialized into an important high-protein oat crop, its broader relevance is to potentially facilitate molecular genetic marker development in a wide range of minor crops and wild crop relatives (Maughan et al. 2009).

As cost and time requirements are decreased, scientists will view the functions of plants with incredible opportunity for innovative research. Unknown mutations will be identified, along with increased understanding of structure-function relationships (Bernardo 2008). Molecular markers can be used in either marker-assisted selection or marker assisted introgression. However, as sequencing supplies increased information, molecular properties may reduce the need for introgression, thereby removing the need for growing or rearing plants in order to measure phenotype.

Plant breeding programs can take advantage of this knowledge to increase crop yield, disease resistance, and a multitude of other qualities (Eathington et al. 2007). The understanding of the interaction between genes and environmental factors, including other organisms, also allows for discovering chromosomal conservation and evolution (Bernardo 2008). Such genetic variation, both within and outside specific plant species, augments transgenic possibilities, or the transfer of genes between species by molecular techniques (Gelvin 2003).

CHAPTER 3: LITERATURE REVIEW

## INTRODUCTION

Here we emphasize the utility of rapid marker discovery in the oat genus Avena using the wild Moroccan tetraploid A. magna (Murphy, 1968) as a source of new DNA sequence-based markers. Genetic marker discovery in tetraploid oat by single nucleotide polymorphisms (SNPs) should provide at least four major benefits for improvement of cultivated oat. Firstly, it provides a new set of markers for application in cultivated hexaploid oat breeding. Secondly, it provides a basis for genetic map development in A. magna, and potentially also A. murphyi (Ladizinsky, 1995) and A. insularis Ladizinsky, all of which are potential genetic resources for exotic alleles to improve cultivated hexaploid oat. Thirdly, the A. magna-derived SNPs potentially allow for tracking the genetic heritage of genes controlling traits of economic importance and other chromosome segments of interest back through the evolutionary ancestors of oat. Finally, these markers also provide for creation of a SNP panel that can be used to screen global diversity in all Avena species. Consequently, species-specific SNPs may be detected and analyzed for evolutionary patterns and divergences when Avena species of diverse genome composition are screened.

Common cultivated oat (Avena sativa L. and A. byzantina C. Koch, $2 n=6 x=42$, AACCDD genomes) was the world's seventh most important cereal crop, at 11.3 million harvested acres in 2008. (UN-FAO, faostat.fao.org). Although oat acreage worldwide has been declining over the past 100 years with the demise of the agrarian horse culture, the nutritional benefits of the common oat are beginning to make substantial contributions to the human diet. Therefore, the demand for high-quality commercial oats is increasing, due to the oat groat's
elevated soluble fiber content. The ability of soluble beta glucan fibers to lower serum LDL cholesterol has been medically documented and led the Food and Drug Administration to approve whole-oat product labeling as a health benefit beginning in 1998 (Cervantes-Martinez, 2001). Additionally, these crops have higher protein and oil contents than the other cereal grains (Holland, 2001). While oats are suitable for human consumption as oatmeal and rolled oats, one of the most common uses is as livestock feed. Oats are also used in some brands of dog and chicken feed (Magness, 1973).

The species composition of the oat genus Avena has been extensively studied cytogenetically and taxonomically, the latest review being that of Jellen and Leggett (2006). Prior to C-banding homoeologous chromosome groups in oat were unable to be differentiated by physical identification (Rajhathy 1963, Thomas 1974). In the late 70's Yen and Filton (1977) reported the first differences in heterochromatin detected by Giemsa stained C-banding in diploids. In 1988, Fominaya et al. performed the same method on diploids and tetraploids. The C-genome chromosomes were found to have significantly darker staining heterochromatin than the A genome. Hutchinson and Postoyko (1986) and Jellen (1994) published similar results on hexaploid A. sativa. The seven C-genome chromosomes were easily distinguished from the others as a consequence of their darker staining. However, the A- and D- genome chromosomes were difficult to be separated from one another. Linares et al. (1992) later attempted to assign the A- and D-genome chromosomes in A. byzantina based on euchromatin staining intensity and prominence and location of telomeric and interstitial bands. The analysis by Jellen et al. (1993) on C-banding patterns in AA diploids, AABB tetraploids and AACC tetraploids indicated significant alterations to the A- and D-genomes which prompted further investigation to
positively distinguish the two. In 1994, Jellen et al. provided the information necessary to differentiate the A- and D-genome using a powerful application of fluorescent microscopy known as fluorescent in situ hybridization (FISH). This technique allows for the detection of RNA or DNA sequences in a variety of cells, tissues and tumors. More specifically, FISH is a cytogenetic technique that is used to detect and localize a target nucleic acid sequence. FISH patterns combined with chromosome size and arm ratios identified by previous karyotyping analyses resulted in the development of a uniform nomenclature system to describe each chromosome in hexaploid oat. The A-genome comprises of the $8 \mathrm{~A}, 11 \mathrm{~A}, 13 \mathrm{~A}, 15 \mathrm{~A}, 16 \mathrm{~A}, 17 \mathrm{~A}$, and 19A chromosomes. The C-genome comprises of the $1 \mathrm{C}, 2 \mathrm{C}, 3 \mathrm{C}, 4 \mathrm{C}, 5 \mathrm{C}, 6 \mathrm{C}$, and 7 C chromosomes. Finally, the D-genome contains the 9D, 10D, 12D, 14D, 18D, 20D, and 21D chromosomes. Correct and uniform identification facilitates the ability to perform further oat cytogenetic research. Homeologous relationships may be evaluated between the three genomes, subgenome origins may be determined, alien genes may be introduced, and genes and molecular markers may be correctly identified and anchored.

Avena magna $(2 \mathrm{n}=4 \mathrm{x}=28)$ is a rather obscure, weedy tetraploid oat species native to heavy clay soils in agricultural areas of northern Morocco. It is of increasing interest to oat breeders due to its high protein content (up to $25 \%$ of the groat mass), large caryopses, and exceptional crown rust and powdery mildew resistance (Ladizinsky 1995; Ladizinsky 2000). This wild oat species has dramatic implications for genetic improvement of hexaploid oat because it is one of three possible ancestor-tetraploids of cultivated oat (Ladizinsky 1998; Jellen and Ladizinsky 2000). In order to facilitate genetic studies in A. magna, our lab made a recombinant inbred line (RIL) population derived from a cross between two A. magna
genotypes: Ba 13-13 and A-169. Parent Ba 13-13 is a phenotypically uniform and cytogenetically stable line derived from dual-backcross hybridization with hexaploid $A$. sativa, followed by repeated selfing, to transfer Ladizinsky's domestication syndrome (non-shattering, yellow lemma, glabrous, reduced awns) into A. magna (Ladizinsky, 1995).

Since essentially nothing was previously known about the molecular nature of A. magna, we used a genomic complexity-reduction and pyrosequencing protocol for rapid marker discovery using Ba 13-13 and A-169. We followed a recently developed genomic reduction approach based on restriction-site conservation using unique multiplex identifier (MID)-barcodes (Maughan et al. 2009) to dramatically reduce the size of the two parental genomes in preparation for 454-pyrosequencing. The resulting sequence-based contigs were assembled and screened for Single Nucleotide Polymorphisms (SNPs). Putative SNPs are being validated by a low-throughput genotyping technique called High Resolution Melting (HRM), which is capable of detecting polymorphisms, mutations, deletions, insertions and epigenetic differences in double-stranded DNA (Reed, 2007). As these A. magna-based SNPs are validated, they are being mapped onto the tetraploid population and are also being screened for polymorphism using a panel of hexaploid mapping-population parents. Once validated, the panel of SNPs can be screened on other species for global diversity of all other genome combinations. Species-specific SNPs may be derived to facilitate gene transfer from wild species for breeding purposes and to clarify evolutionary relationships among subgenomes within Avena.

## PLANT BREEDING

Plant breeding is a scientific art that has been practiced for thousands of years. Initially, selecting plants with desirable traits for propagation was standard for breeders. More complex molecular techniques have since evolved. Regardless of the breeding technique used, the goals of plant breeding programs remain largely unchanged. Improvements in disease and pest resistance, yield, quality and durability are among the qualities that are aggressively being explored (Eathington et al. 2007). Molecular techniques generate the fragments of DNA sequences that may represent variation in genomes. Genome variation between two lines within a species can be measured. These DNA fragments are called molecular markers (Tanksley 1983). Gene mapping is produced as the chromosomal location or distance between markers is discovered. Genetic maps are created based on recombination frequencies. Partial exchange of homologous chromosomes during meiosis is referred to as recombination. The frequency of analysis may be determined by statistical analysis. Higher rates of recombination imply greater distances between molecular markers on the chromosome. Plant breeders may take advantage of these gene maps by using marker assisted selection. Marker assisted selection allows for the indirect selection of traits of interest based on the location of the molecular markers (Collard 2007). The goal of each molecular method is to generate dense, repeatable, accurate molecular marker maps. Sequencing methods are quickly emerging as the technique of choice for developing these maps, but its applications have yet to be fully realized (Rudd et al. 2005). As present limitations are diminished, and future applications and sequencing procedures are presented, the revolutionary effects of whole genome sequencing on plant breeding programs will be more fully recognized.

## HISTORY OF MOLECULAR MAPPING

Gregor Mendel opened the door to modern genetics with his pea plant experiments in the mid-1800s. His discovery of patterned inheritable traits became the foundation of molecular mapping (Weiling 1991). In fact, until recently genetic linkage maps predominately contained markers for alleles with major phenotypic effects, or macromutations (Tanksley 1983). Many molecular techniques have emerged, contributing to denser, more accurate, rapid mapping. These methods include random fragment length polymorphisms (RFLPs; Tanksley et al. 1989), random amplified polymorphic DNAs (RAPDs; Martin et al. 1991), amplified fragment length polymorphisms (AFLPs; Blears et al. 1998), simple sequence repeats (SSRs; Oetting et al. 1995), diversity arrays technology (DArTs; Wenzl et al. 2004) and inter simple sequence repeats (ISSRs; Ratnaparkhe et al. 1998). Each of these marker systems has distinct disadvantages. Consequently, biotechnology has turned to sequencing to revolutionize molecular mapping.

Frederick Sanger successfully sequenced the phi X 174 bacteriophage genome in 1975 by enzymatic synthesis. His "shotgun" sequencing method commenced with utilizing random fragments of genomic DNA as primers to polymerase chain reaction (PCR) amplify the whole genome. The amplification products were overlapped and assembled based on overlapping contiguous transcripts, or contigs. Any gaps remaining between these contigs were resolved using custom primers (Sanger et al. 1977). Sequence segments, or reads, between 800-1000 nucleotides in length are capable. The method dramatically improved earlier DNA sequencing techniques developed by Allan Maxam and Walter Gilbert, as well as Sanger and Alan Coulson's own 'plus and minus' technique presented 2 years earlier (Sanger and Coulson 1975).Sanger's method enabled unprecedented speed in sequencing projects, expanding the scope of realistic
sequencing endeavors in all areas of biotechnology. Additionally, the use of radioisotopes and other toxic substances was limited, solidifying Sanger sequencing as the principle platform for nearly three decades (Sanger et al. 1977).

Perhaps the greatest accomplishment of the Sanger method was the complete sequencing of the human genome in 2000 (Waterston et al. 2002). This endeavor quickly drove the development of increasingly efficient automated procedures and process parallelization. New methods emerged to improve the speed, cost, throughput, and ability to process complex genomes for sequencing. This new wave of technology has become known as next-generation sequencing.

Next-generation sequencing was introduced by Pal Nyren and Mostafa Ronaghi in 1996 with their Pyrosequencing method (Nyren 2007). Unlike Sanger sequencing, which detects chain termination with dideoxynucleotides (Sanger et al. 1977), Pyrosequencing observes nucleotide incorporation by pyrophosphate release. Single strands of DNA act as templates while complementary strands are synthesized (Ronaghi et al. 1996). The DNA polymerase and chemiluminescent enzyme activity is monitored. Nucleotide solutions of A,C,G, and T are added and removed sequentially, producing light as the solutions complement the order of the unpaired template (Nyren 2007). The need for labeled primers, gel-electrophoresis and labeled nucleotides are thus eliminated. Despite the additional advantages of accuracy, flexibility, parallel processing, rapid analysis of large sample sizes and relatively simple automation, Pyrosequencing produces shorter DNA sequence read lengths of 300-500 nucleotides (Ronaghi et al. 1996). Consequently, genome assembly may prove more difficult, especially in the
presence of repetitive DNA. The history of biotechnology has proven that limitations soon lead to improvements, with no exception here.

Pyrosequencing was first commercialized by Roche's 454 Life Sciences in 2005. Their GS20 sequencing machine and GS FLX series were the first next-generation sequencing methods on the market. As many as- 400-600 million base pairs are capable of being sequenced within hours (Wheeler 2008). Advances in speed, read lengths, higher accuracy, and lower cost allowed the first competitive alternative to Sanger sequencing

Despite the progress in genetic research by next-generation sequencing, the limitations in utilizing these methods remains historically unchanged. Cost, time, effectiveness, and reproducibility still remain the principle determining factors of any research-based method (Coombs 2008). New technology brings novel concerns as well. Whole genome sequence analyzers have generated unprecedented amounts of data in a short period of time. In addition to the storage of this massive quantity of data, there is a need for bioinformatics programs and computers capable of processing prodigious amounts of information (Rudd et al. 2005). These programs increase the cost and complexity of such methods because of the need to hire or train personnel to run these programs and interpret their output. Notwithstanding, genome sequencing has profoundly impacted plant breeding programs. Genetic markers and maps are being developed with unprecedented accuracy, speed and depth. The properties and functions of genomes are being discovered, as well as the ability to view the original transcriptome expression (Eathington et al. 2007).

## EFFECTS OF MAPPING ON PLANT BREEDING

The introduction of molecular markers has revolutionized genetics. Technology is enabling the study of species that were not previously viewed as economic priorities, being too remote, expensive or complex for consideration in plant breeding communities (Eathington et al. 2007). The array of polymorphisms and molecular techniques that are available is increasing, and the arrival of genomic sequencing is a source of an escalating set of available markers (Cullis 2002). As more genetic information becomes available, the application of molecular markers to other experimental methods will become simpler, allowing for novel genetic analysis that is currently impossible to undertake.

As cost and time requirements are decreased, scientists will view the functions of plants with incredible opportunity for innovative research. Unknown mutations will be identified, along with increased understanding of structure-function relationships (Bernardo 2008). Not only will genes be sequenced, but the expression of genes will continue to be found. Epigenomic understanding will increase plant breeders' knowledge of desirable traits. Molecular markers can be used in either marker-assisted selection or marker assisted introgression. However, as sequencing supplies increased information, molecular properties may reduce the need for introgression, thereby removing the need for growing or rearing plants in order to measure phenotype.

Characteristics that involve a large number of genes, or traits that are complicated to select due to genotype-environment interactions have been difficult to analyze. As sequencing allows for the direct monitoring of genotypes, the efficiency for selecting such traits is enhanced.

Plant breeding programs can take advantage of this knowledge to increase crop yield, disease resistance, and a multitude of other qualities that fall into this category (Eathington et al. 2007). The understanding of the interaction between genes and environmental factors, including other organisms, also allows for discovering chromosomal conservation and evolution (Bernardo 2008). Such genetic variation, both within and outside specific plant species, augments transgenic possibilities, or the transfer of genes between species by molecular techniques (Gelvin 2003). The transgenic properties alone, indicated by genomic sequencing of molecular markers, are invaluable to plant breeders.

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## TABLES

Table 1. Newbler Assembler results. All assembled contigs and unassembled singletons were compared to the NCBI non-redundant monocot database by BLASTx.

| Total Number of Reads | 706,426 |
| :--- | ---: |
| Total Number of Bases | $27,200,520$ |
| Number of Aligned Reads | $1,177,520$ |
| Number of Aligned Bases | $363,482,847$ |
| Number of Contigs | 57,048 |
| Number of Bases | $31,911,706$ |
| Average read length per contig | 345 bp |
| Average reads depth per contig | 16 x |

Table 2. SNP markers identified by SNP Finder. Number of SNPs found and coverage. True SNP results are reported for strict parameters.

| Total number of SNPs found | 31,304 |
| :--- | ---: |
| Average coverage | $16 x$ |

True SNP Results for 10x, 40\% MAF, 100\% match within accession

| Total true species SNPs found | 12642 |
| :--- | ---: |
| Total contigs containing true SNPs | 6502 |
| Total true SNPs/assembly length | 0.000268 |

Table 3. A list of 16 Avena diversity lines for SNP assay validation.

## VALIDATION <br> PANEL

|  | ID | AVENA SPECIES | GENOME DESIGNATION |
| :---: | :---: | :---: | :---: |
| $\mathbf{1}$ | PI 657427 | damascena | AdAd or DdDd |
| $\mathbf{2}$ | CN 23017 | canariensis | AcAc or DcDc |
| $\mathbf{3}$ | PI 657352 | wiestii | AsAs |
| $\mathbf{4}$ | PI 657576 | eriantha | CdCd |
| $\mathbf{5}$ | PI 657337 | ventricosa | CvCv |
| $\mathbf{6}$ | PI 657606 | murphyi | AACC or CCDD |
| $\mathbf{7}$ | PI 657271 | sterilis | AACCDD |
| $\mathbf{8}$ | PI 657514 | magna | CCDD |
| $\mathbf{9}$ | PI 657613 | magna | CCDD |
| $\mathbf{1 0}$ | PI 657620 | magna | CCDD |
| $\mathbf{1 1}$ | Cc 7070 | magna | CCDD |
| $\mathbf{1 2}$ | Cc 7071 | magna | CCDD |
| $\mathbf{1 3}$ | Cc 7073 | magna | CCDD |
| $\mathbf{1 4}$ | BYU 210 | insularis | AACCDD |
| $\mathbf{1 5}$ | BYU 661 | sativa | AACCDD |
| $\mathbf{1 6}$ | BYU 284 | sativa | AACCDD |

Table 4. The list of Avena genotypes used for the tetraploid-SNP diversity analysis.

| Accession | Species | Origin | Locality |
| :---: | :---: | :---: | :---: |
| A-169 | magna | Morocco | unknown |
| BA 13-13 | magna | Israel | cultivated |
| GS7 | sativa | Indiana | cultivated |
| Ogle | sativa | Illinois | cultivated |
| PI 412765 | vaviloviana | Ethiopia | Shewa |
| PI 412767 | vaviloviana | Ethiopia | Shewa |
| PI 412768 | vaviloviana | Ethiopia | Shewa |
| PI 657271 | sterilis | Morocco | Merchouch |
| PI 657274 | barbata | Morocco | Merchouch |
| PI 657351 | barbata | Morocco | Ain Aouda |
| PI 657355 | murphyi | Morocco | Tangier |
| PI 657357 | murphyi | Morocco | Tangier |
| PI 657358 | murphyi | Morocco | Tangier |
| PI 657361 | murphyi | Morocco | Tangier |
| PI 657364 | murphyi | Morocco | Tangier |
| PI 657366 | murphyi | Morocco | Tangier |
| PI 657367 | murphyi | Morocco | Tangier |
| PI 657368 | murphyi | Morocco | Tangier |
| PI 657370 | murphyi | Morocco | Tangier |
| PI 657371 | murphyi | Morocco | Tangier |
| PI 657372 | murphyi | Morocco | Tangier |
| PI 657373 | murphyi | Morocco | Tangier |
| PI 657374 | murphyi | Morocco | Tangier |
| PI 657375 | murphyi | Morocco | Tangier |
| PI 657379 | murphyi | Morocco | Tangier |
| PI 657381 | murphyi | Morocco | Tangier |
| PI 657394 | barbata | Morocco | Larache |
| PI 657514 | magna | Morocco | Maaziz |
| PI 657515 | magna | Morocco | Maaziz |
| PI 657519 | magna | Morocco | Had Brachoua |
| PI 657522 | magna | Morocco | Rommani |
| PI 657528 | magna | Morocco | Rommani |
| PI 657534 | magna | Morocco | El Gara |
| PI 657535 | magna | Morocco | El Gara |
| PI 657536 | magna | Morocco | El Gara |
| PI 657538 | magna | Morocco | El Gara |
| PI 657539 | magna | Morocco | El Gara |
| PI 657541 | magna | Morocco | Ben Slimane |
| PI 657544 | magna | Morocco | Ben Slimane |
| PI 657546 | magna | Morocco | Rommani |
| PI 657548 | magna | Morocco | Rommani |
| PI 657551 | magna | Morocco | Rommani |
| PI 657555 | magna | Morocco | Had Moualine el Oued |


| PI 657557 | magna | Morocco | Had Moualine el Oued |
| :--- | :--- | :--- | :--- |
| PI 657585 | agadiriana | Morocco | Tifnit |
| PI 657590 | agadiriana | Morocco | Tamri |
| PI 657591 | agadiriana | Morocco | Tamri |
| PI 657592 | agadiriana | Morocco | Tamri |
| PI 657594 | agadiriana | Morocco | Tamri |
| PI 657595 | agadiriana | Morocco | El Jadida |
| PI 657596 | agadiriana | Morocco | El Jadida |
| PI 657598 | murphyi | Morocco | Tangier |
| PI 657600 | murphyi | Morocco | Tangier |
| PI 657601 | murphyi | Morocco | Tangier |
| PI 657602 | murphyi | Morocco | Tangier |
| PI 657604 | murphyi | Morocco | Tangier |
| PI 657605 | murphyi | Morocco | Tangier |
| PI 659373 | magna | Morocco | Maaziz |
| PI 659376 | magna | Morocco | Had Brachoua |
| PI 659378 | magna | Morocco | Had Brachoua |
| PI 659380 | magna | Morocco | Rommani |
| PI 659383 | magna | Morocco | Rommani |
| PI 659385 | magna | Morocco | Rommani |
| PI 659388 | magna | Morocco | Maaziz |
| PI 659390 | magna | Morocco | Maaziz |
| PI 659399 | magna | Morocco | Rommani |
| PI 659406 | magna | Morocco | Had Moualine el Oued |
| Provena | sativa | Idaho | cultivated |
| TAM-0301 | sativa | Texas | cultivated |

Table 5. Marker distribution and lengths of linkage groups in a genetic map constructed from a wild x cultivated A. magna RIL population. The map was constructed using JMP Genomics v.5.1.

| Linkage <br> Group | Total <br> no. <br> markers | Total length <br> (cM) | Largest <br> gap (cM) | Ave. marker <br> distance (cM) |
| :---: | :---: | :---: | :---: | :---: |
| BAM 1 | 16 | 137.8 | 29.3 |  |
| BAM 2 | 11 | 122.1 | 26.1 | 15.4 |
| BAM 3 | 13 | 122.2 | 28.4 | 19.9 |
| BAM 4 | 18 | 149.8 | 30.7 | 16.4 |
| BAM 5 6 | 13 | 73.9 | 17.8 | 14.5 |
| BAM 6 | 19 | 88.4 | 31.3 | 10.8 |
| BAM 7 | 15 | 128 | 35.8 | 8.4 |
| BAM 8 | 24 | 150 | 22.8 | 13.9 |
| BAM 9 | 22 | 110.6 | 16.4 | 11.2 |
| BAM 10 | 22 | 133.5 | 24.9 | 9.3 |
| BAM 11 | 18 | 119.8 | 29 | 10.9 |
| BAM 12 | 23 | 155.8 | 25.9 | 11.8 |
| BAM 13 | 24 | 145.3 | 18.3 | 12.2 |
| BAM 14 | 20 | 124.8 | 17.1 | 11 |
| BAM 15 | 25 | 122.9 | 31.1 | 11.5 |
| BAM 16 | 19 | 95.6 | 19.2 | 8.9 |
| Total | 302 | 1980.5 | Ave | 25.3 |

## FIGURES



Figure 1. Alternative strategies for developing molecular genetic markers in oat. The cost of sequencing remains a concern, especially in large or complex genomes, or orphan crops. Transcriptome analysis and DArT arrays have been used to overcome cost and complexity obstacles. Genome reduction is a novel, relatively inexpensive method for generating genetic markers for such crops.

Figure 2. Frequency of pyrosequencing read lengths (in bp, x-axis) plotted against number of library reads.(y-axis).


Read Lengths (bp)


Figure 3. SNP detection and coverage results. 31,304 SNPs were identified. (A) Number of contigs sorted by SNP quantity. (B) Number of "true" SNPs with 6X coverage and above.
A.

## Molecular Function


B.

## Biological Process



## C.

## Cellular Components



Figure 4. Putative gene ontology (GO) pie charts for the pool of GR-RSC DNAs from two $A$. magna lines, as determined by BLAST2Go, showing homology to genes for (A) molecular functions, (B) biological processes, and (C) cellular components.


Figure 5. Pie chart depicting SNP classification results from the GR-RSC treated A. magna DNAs. As expected, C/T and A/G SNPs derived from pyrimidine/pyrimidine and purine/purine transition mutations were more prevalent than purine/pyrimidine transversions.


Figure 6. Bio-Rad Precision Melt analysis of contig5030 ran across a subset of the BAM RIL population. Differences in melting temperatures, indicated by red and green respectively, validate putative SNPs.


Figure 7. Fluidigm analysis. Example of SNP assays using the KASPar genotyping chemistry on Fluidigm access array on the F8 RIL mapping population. SNP loci grs_ 54878 demonstrates dominance. The no template control (NTC) is located at the origin of the Cartesian graph.


Figure 8. Marker groupings are demonstrated for both Fluidigm (red) and Bio-Rad (blue) genotyping platforms. While Bio-Rad appears to have minor clustering along a few linkage groups, the majority of the markers appear to have random distribution across all linkage groups, supporting the efficacy of both chemistries.


Figure 9. The genetic distances (cM) of markers on each linkage group is shown in addition to segregation distortion. The blue spectrum indicates predominance of the A. magna A-169 allele at a given locus, while red indicates the predominance of the Ba 13-13 mapping parent's allele at a given locus.

| BA_01 |  |
| :---: | :---: |
| $\begin{gathered} 0.0 \\ 14.2 \\ \text { 亿_ } \\ \text { BA_grs_62747_143 } \\ \text { oPt-4447 } \end{gathered}$ |  |
|  |  |
| 20.8 GMI_c2391_5 |  |
| 23.4 |  |
| 29.1 OPt-1197 |  |
| 41.9 | BA_grs_55797_98 |
| 58.3 | - BA_grs_54715_292 |
|  |  |
| 70.6 | - BA_grs_66690_231 |
| 82.9 | - BA_grs_61983_229 |
| 92.6 | - BA_grs_83796_309 |
| 103.6 | - BA_grs_24640_298 |
| 111.5 | - BA_grs_15424_351 |
| 119.5 | - BA_grs_39974_421 |
| 126.1 | - oPt-7910 |
| 135.2 | - oPt-15432 |
| 143.4 | - BA_grs_42747_240 |
| 158.8 | - BA_grs_11279_261 |
| 169.3 | - BA_grs_46256_397 |
| 179.1 | - BA grs 60221_323 |
| 188.1 | - BA_grs_33469_357 |

BA_02


## BA_3

| 0.0 - BA_grs_45736_154 |  |
| :---: | :---: |
| 10.6 | BA grs_59767_194 |
|  |  |
| 25.0 - BA_grs_72129_148 |  |
|  | BA_grs_71963_165 |
|  | - BA_grs_106931_323 |
| 49.6 | BA_grs_108904_232 |
|  | BA_grs_26639_367 |
|  | BA_grs_14756_221 |
|  | BA_grs_16610_132 |
|  | BA grs 59490_137 |
|  | - BA_grs_89279_219 |
|  | BA_grs_71868_375 |
| 112.4 | BA_grs_39719_264 |
| $2.6$ | 521_213 |
| 132.3 | BA_grs_48717_114 |
| 141.5 | BA_grs_35480_424 |
| 148.5 | BA_grs_108332_177 |
| 158.1 | BA_grs_103172_292 |
| 165.5 | BA_grs_34088_196 |
| 174.3 | BA_grs_44890_322 |
| 183.0 | BA_grs_82325_251 |
| 187.9 | Pt-7690 |
| 192.0 | BA_grs_55135_245 |
| 196.1 | BA_grs_17472_329 |
| 207.0 | BA grs 25202404 |
| 214.1 | BA grs 29062 294 |
| 223.1 | BA grs 43373_424 |
| 232.8 | AB_AM_796 |

BA_04



BA_07


BA_9


BA_10

| 0.0 |  |
| :---: | :---: |
| 18.9 | BA_grs_41650_119 |
| 27.5 |  |
| 29.2 | BA_grs_47079_104 |
| 34.9 | oPt-2569 |
| oPt-8259 |  |
| oPt-9063 |  |

BA_11


BA_12

BA_grs_-77857_-106 BA_grs_72874_297
BA -grs 88253 - 382 oPt-10908
oPt-13070 oPt-1460
oPt-17170 oPt-7104
$1 \begin{aligned} & \text { oPt-17170 } \\ & \text { OPt-795210 }\end{aligned}$

Unlinked
GMI_c1361_1 GMI_c7461_1 BA_grs_103930_312 BA_grs_106369_131 BA grs 12266-246 BA_grs_17145_115 BA-grs_18812_249 BA_grs_17145_115 BA_-grs_29453_94 BA _grs_ 34954 _398
 BA_grs_ 43752 _328 BA_grs_ 48810 - 294
BA grs 54542 325 BA grs_54542- 325 BA_gr_ $56411-156$
BA_grs_ 58549127 BA grs $66238-275$ BA_-grs_58549_127 BA_grs_66238_275
BA_grs_70857_106 BA_grs_72874_297

Figure 10. Genetic map of A. magna A-169 x Ba 13-13 RIL population, including GR-RSC SNP markers (BA_grs_XXX) generated from Fluidigm assays. Other markers are as follows: GMI_XXX, hexaploid EST-SNPs; oPt-XXX, DArTs; AB_AM_XXX, SSRs. Distance shown is in centiMorgans (cM) in Multipoint.


## 



Figure 11. 302 markers were mapped across 16 linkage groups using JMP
Genomics. Colors indicate magnitude of segregation distortion for individual markers


Figure 12. Heat Plot generated by JMP Genomics v. 5.1 (SAS, Cary, NC). Plot is based on marker-to-marker correlation coefficients. The key indicates decreasing levels of correlation between markers, i.e. dark red indicates $100 \%$ of the markers are shared between two RILs, dark purple indicates $21.43 \%$ shared markers.



Figure 13. Linkage Groups. The SNP markers indicated in red are some of the genome complexity reduced SNPs added to linkage
groups by Multipoint analysis. Their map locations demonstrate both linkage group extension and filling in "gaps" between EST based SNPS and Dart markers.

Figure 14. Sixteen Diversity Line Validation Panel Dendrogram generated from 330 primers run across diploid, tetraploid and hexaploid oat species having most known genome combinations. Bio-Rad's HRM platform was utilized to validate panel.


## SUPPLEMENTAL DATA

Supplemental Table 1. List of 330 PCR primers used for High Resolution Melt analysis.

| Forward Primer |  | ${ }_{2}^{\text {Allele }}$ | Reverse Primer |
| :---: | :---: | :---: | :---: |
| contig00740_F, AAATCCCAAAAATGAAAGAGG | C | T | contig00740_R GATCCACTTCAATTGGGTAGATAAT |
| contig00741_F, TTTGTACTATGTGTCATGTG | G | T | contig00741_R, TTCTCTTTCAGCAATTCCTTTTC |
| * contig01058_F, СGTCTCCACCCTCTCTTC | C | T | contig01058_R, CCCTTTGAAGATGTCGT |
| * contig02122_F, AGCAAGGCAGCCAACACT | C | T | contig02122_R, CATACGGACCTGAAAGC |
| contig00813_F, CCCATTGGTGACTAAACTTGC | C | T | contig00813_R, CCAGAGCAGTGATGCGTCTA |
| contig01530_F, GCTCAATCCGATGTGCAGAG | C | T | contig01530_R, AATGATTGGGAAAGTTGCTG |
| * contig01667_F, TTGTGGGTTAACAATGG | G | A | contig01667_R, CCGTGTTGAATGCTAACGTC |
| contig02239_F, AAGTTCCTCTCGATAAGATTGGTG | A | T | contig00239_R, GCAGATTAGGCAGAGGCAAG |
| contig02735_F, TCGGGATTAGAAGGGCAA | T | C | contig02735_R, TGATCTTGTTTTATGTGGCGT |
| contig03399_F, TGTCCCCTACCGACCAGT | A | G | contig03399_R, AATGACTTTGGCATTCACGTC |
| contig03486_F, GACAACGTATCCGTCGAACC | A | G | contig03486_R, CTATTCTATTCCGCAGGCTTC |
| contig03659_F, TCCGACGACAAATATGGTGA | T | C | contig03659_R, GAGTGGATGCGCAAAGTG |
| contig04271_F, CTCGAATCTGAAGAAGATCGT | T | G | contig04721_R, TATTGATGCGTGTGCCTGA |
| * contig04507_F, AATTCCGCCTGGATAGTAGC | T | C | contig04507_R, CGTTAAACTCTCAGTAACCCAGAA |
| contig04646_F, TTCCCTCTGCATCAGTCCTAA | G | A | contig04646_R, CTTCCTTTCAATCCGCTCAT |
| contig04737_F, CCTCGGGGTATCCTAAAACC | A | G | contig04737_R, AAAATTCCTGCTTCTCTACTTCG |
| contig04846_F, TCGTTCACCACACCTTACGA | C | A | contig04846_R, TTGCATTGTCCGCTGGTA |
| contig05114_F, TTGATGCAAAGGTAAGAGTTCA | C | T | contig05114_R, AACCTTGGCTTATGTTCTTTCC |
| contig05335_F, GAGTACTGAAAGTTTAACGACCAAC | C | T | contig05335_R, TGGAGACGGGTCGATAACTA |
| contig05451_F, CCGGGAGTGCATAAGTAGAT | G | A | contig05451_R, TGTTCGTGCGAGACAACG |


| contig05553_R, CAAGGCCTGCGATTTGAT |
| :--- |
| contig06414_R, TGAAACTGCGAGTGTCCTTG |
| contig06685_R, CTGCCTCGAATTGTGCTTG |
| contig07523_R, AAGCCAATCCTTCTTTGGA |
| contig07554_R,T TCTTCAAAGGTTGATTTTTATTCC |
| contig07611_R, AGCCACAATCCATGTGACATAC |
| contig07686_R, AGGTGTATTCGGTCGAGGTG |
| contig07813_R, CTCCAAACCCTGCATTCATT |
| contig07940_R, CTGGGTGGGGGTAGAAGG |
| contig08001_R, TCCACCGATTCTGAACGTCTA |
| contig05030_R, TCCTCCCTATGGGAGTAGCC |
| contig05049_R, TTCGCAAGTGTCTCGAAGTC |
| contig05063_R, TGGCCAATAGTTGGTTTCAA |
| contig05075_R, CCTGACTCTCTACCGATGTAGGAC |
| contig05084_R, GTGTTGCCGCAATTTAATGA |
| contig05100_R, TGCCCAAATCCAACTACTCA |
| contig05146_R, TGGAATCGCCCTACTTCTTC |
| contig05169_R, CTAAGAAAGGATTGGGTCCAC |
| contig05185_R, GCGCGGAGTTCACAGGT |
| contig05195_R, CCTCGTTAAATCCCATTGATTC |
| contig05264_R, AGGGCGAGACGTGATCTACA |
| contig05374_R, TGTGATCATTTGTGAGGACTAAA |
| contig05406_R, GTTGACGAGTGGAGGCTTG |
| contig05435_R, TGTTTAGAAGGCGTCTTTGG |
| contig05524_R, CGTACCGCCAATTTGAGATTA |
| contig05572_R, GGGCAAGTTGATGTTTGTGA |
| contig05573_R, ATGACCCACCGGAAGGA |
| contig05594_R, ATCAGCGTCATGGTGGACTT |
| contig05634_R, TCCCAAGATACTCCGCTGAC |



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| C | A | contig05664_R, CTTCCCGATCCGCTACAA |
| A | C | contig05715_R, ACCAACTCACCCATTCTTCTAA |
| G | A | contig05772_R, TTCATGACCCACTTCCTTGAC |
| A | G | contig05805_R, GACGACTACGGTGCTACGAA |
| C | T | contig05845_R, TCACACGACTTCGTCGACAC |
| G | A | contig05851_R, TGGCATCTTGGGTTATTTCA |
| A | G | contig05853_R, CAACACATGCCACAAAGCAT |
| G | A | contig05916_R, TTGTTGATATAATATTCACGAAGTACTGTT |
| G | C | contig05961_R, CATGGGCATACCAACATAATAAA |
| T | C | contig05965_R, GCCGCATAACGACCAACTAT |
| T | C | contig05966_R, GTTTGGGAGGAGAGCTTCG |
| G | C | contig06020_R, TCCGAATGCAAAGCTTGTTT |
| T | A | contig06043_R, TCAGAGTTTCTTTCACTAACCATAGG |
| G | C | contig06056_R, GGTCGATCGATCTGCCTAAC |
| G | C | contig06057_R, GGTCCTTCCCGAACCTTACT |
| T | C | contig06058_R, CCGCCCATGCTGTCT |
| G | A | contig06060_R, TTGAGATATGACATAGAACCATTCAA |
| G | T | contig06070_R, TGATGCACTTGTGGAAGAACA |
| G | A | contig06113_R, TAGCTGTTGCGTCGCTCTT |
| G | T | contig06120_R, TGGTCTCCTTAATTCCTACAGTTTG |
| T | C | contig06122_R, GTGGCAAGAGTTGAACCAAA |
| T | G | contig06142_R, CCGGGTGGATTTGTAATGAT |
| G | A | contig06158_R, AAGGCCACGAGCATAAGGT |
| A | G | contig06183_R, CGGTTGACCCATAGTCAAGA |
| T | C | contig06229_R, TGATGAACCCAAGTCAATTCC |
| G | A | contig06239_R, TGTATGGTTTGTAATGGATGATGT |
| A | G | contig06241_R, CGGATACCATGGTGCTCTAAA |

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contig05664_F, CCCAAGATGGCATAAGAAGAA
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contig06279_F, CAGGGTGGACTCACCACCT contig06323_F, TGCTTGCTTCGTTCTCGTATT contig06329_F, GGAGAACCAAATAAAGACCCAAA contig06330_F, GCAACCTCTTAGGCAAATCG contig06372_F, GGACGCCTTCAAGAGGAAC contig06376_F, CAGCTACATGGTTGCAGGATT contig06401_F, AAACAATCAACCCTCCATCC contig06404_F, TGTCCATGGATTTGGTGACT contig06427_F, CCAAACCGAAGCCTCGAC contig06436_F, CAAGAATTATTGATCGTGTTATGGA contig06465_F, CCACGGCTTACTAAACCTGAA contig06471_F, TGGAAACTCACATCGACGAA contig06526_F, CGTTTGTTCGTTGTCTTTATTACTTG contig06565_F, ATGGCAACCATCTCACCAAG contig06565_F, ATGGCAACCATCTCACCAAG
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contig07623＿F，TATGCAAGAAGCGTTCACCA contig07634＿F，CGCCTTTAAGTTAGGCATGA contig07650＿F，TCGAATCTGAAGAAGATTGTGTTG contig07652＿F，GGAAGATGAAATCGAACTCACA contig07662＿F，TGGGCAGTCCGATAGAGA
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contig08123_F, CGATCAGGTTCGGGATTAAC
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contig08577_F, CCTAAGAAGGACTGGCTCCAT
contig08585_F, ACATGTTGCGGATGCTCTT
contig08597_F, GACCCTCTCCGCCGTTAC
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 contig09887_F, CCCAACCGGATAAGAAACAA contig09894_F, ACCGTGGCCGATGGA * contig09912_F, CCCAACTATATGGGCGGAAT
 contig09941_F, TTTACTCCGGCGATCAATTC contig09945_F, TTGGACAAGAATTTGGTGTTTC * contig09953_F, TGAAAGTTTAGCACTAATAATAACATGAAA contig09959_F, CCCTTCGCCACTCTCTCTATC
contig 10021_F, GAACTTAGCATTCAAATTAGCAACA contig10044_F, CTGGTGTGGTGTTTCCCTTC contig10049_F, TCATAAATTCGTGGCATGGA contig10054_F, GGCCTCTTAGGCCATTTGT contig 10062_F, GCAACTCCTTTACTTGACGAGA contig10066_F, GCGGAGAGGGAGAGGTTTA contig10085_F, AAGGCTGGACACTGTGCTCT contig10110_F, TGCTTGCAAATTATAGGATGG contig 10161_F, GTAGGCAGAGGTCGTTCTGG contig 10197_F, CCACCTTTAAATGACCTCCA contig 10260_F, AGAAGTGCGTAGACATCAAGTCTTT contig 10318_F, TGCCACTGACTGGAACTGTC contig 10320_F, TGCATTAATGATTTCCTTGCAG contig10323_F, GTCCTTCTTTGGCGAGGTG contig 10327_F, TCATGGACACTGCATCTAACA

| C | G | contig10364_R, GAGGCAAATGCAAATAATGG |
| :---: | :---: | :---: |
| G | T | contig 10421_R, AATCCTTTGGACGTGCTTTG |
| C | T | contig 10493_R, TCCAACCTACAGATCTTCCTAAA |
| A | C | contig 10509_R, GCCTTCCGTCGTGGTGT |
| C | T | contig10538_R, TTTCTTGTTCCTACACCTCAACC |
| C | T | contig 10539_R, TTGCATTTAATTGGCGTTCA |
| G | A | contig 10555_R, CCCTTTGTGTTTGACAGGTTT |
| A | T | contig 10556_R, CCATTCAAATATTACACATGACTATGC |
| A | G | contig 10577_R, TGATGGAGCATCTCAAGACAA |
| A | T | contig 10582_R, AAACGCAAATGTTCCTCCAC |
| A | G | contig 10592_R, ATGAGGCGTGACGTGGA |
| A | G | contig 10600_R, TGACAAGGCCCAGAAGAGTT |
| T | C | contig 10608_R, ATTCCAATACGGTGCCAATC |
| G | A | contig 10611_R, CTCAATCACCACCTGAACGA |
| T | C | contig 10624_R,TTTAGACCACAAGTATCTAATACAATATGA |
| C | G | contig10626_R, GAGGCACGGATGGGTTAATA |
| A | G | contig 10635_R, TTACAGGAAGCGAGGAAGGA |
| G | C | contig 10669_R, GGCACAAGCAAGCAAATAAA |
| C | T | contig 10684_R, ACGTACTGATCGGTTGTCGAT |
| G | T | contig 10685_R, ACGACAGCACAAATTTCCAT |
| G | A | contig 10729_R, TGCCATGAACGTGTGGAATA |
| T | C | contig 10730_R, CAAGGCCCTATTGGAGGACT |
| C | A | contig 10736_R, AAACCTTGGTTTCATAACTGAGGA |
| A | G | contig 10773_R, CTCCGAAATGTTGTGTCCAA |
| C | T | contig 10778_R, AAATAACTTGAACCTAATAATAAATGTCG |
| A | G | contig 10798_R, CGCGAGTCTTTGGATAATGA |
| C | T | contig10820_R, AAGAATGTTGATGGTATTCTTCCA |
| C | T | contig10836_R, AATGCCGCATGTTTAATGTT |
| G | A | contig 10844_R, TGAGCATATTGCATGTTTCAA |

contig 10364_F, GATTTGAGCAATATAAGGTCTTCG contig10421_F, CTCCTTTGACCGCGTAGTTC contig10493_F, CAGTGGTATGCTGGGTCAC contig10509_F, ACGTCGTCGAGGTCGAAAT contig10538_F, CATGATGGAATGATACACAATTTACA contig 10539_F, GGGTGGCACTACTCGACCTA contig 10555_F, GCGTCCATAACACATTCCAG contig 10556_F, GGAGATAGGCCAGGGTTGA contig 10577_F, TGGCTGTTTGGCCTTATTAAA contig10582_F, CAACAAACTCCAATAGTTGAAGCA contig10592_F, GCATATAAAGACCATCGGGATT contig10600_F, CGGCTACGTTACAGAGTCGAT contig10608_F, GGCTTGGTACTGCTACACTCC contig 10611_F, AGGTTCCTGGTGCAACATTC contig 10624_F, ACAAGTTTAGGACACATCAAAGC contig10626_F, TTAAGAACTCGGGTCCTTGGT contig10635_F, TACCTTGAGCTTCGCCACA contig 10669_F, TTAGGTCTCACGGATTTGCAT contig 10684_F, TGCGCGTCGTCTTCAA contig 10685_F, AAGATCCCAGTCACCAAAGAA contig10729_F, GTGCATGACCTTTCGAGGTG contig 10730_F, TTGACGGGAGAGGTAGGAGA contig10736_F, TGTAGAGCTCGTTGGGAACC contig10773_F, TGCATTCTTGTGCGATTACC contig 10778_F, TGATGCATGCCATGGTAATA contig 10798_F, ATGCTCACCGATGCCTATTT contig 10820_F, TGTTTGCAATACACAAAGAATCAA contig 10836_F, TGTGCCTCTGCATTATTTACC

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Indicates robust primer sets used for genetic mapping
Allele 1 and 2 indicates the SNP being interrogated
contig10846_F, ATTAGCAGCGTCCTTGGAGA
contig 10863_F, GATGCTCCTGCAGTGCTAAG contig 10881_F, CGATGGAACCACAGAAACAT contig 10884_F, TTCAATTAAGACATAATGCATCTCA contig10914_F, CGGTGGAACCAACTATACGG contig 10925_F, CACATGAAGGTTCCGAAGGT contig 10937_F, TTTGCTATATTCTTCTCTTGTGATAAA contig 10969_F, TTCCGACTTTCCTGGAACC contig11010_F, TGTCATCTTGATCACTTATTAATGTTT contig 11012_F, CTTGCAGCTTGCTCTTCACA * contig11013_F, GGCTCGCGCTACAATTATG contig11017_F, AATGTAAGATGTTGGTAATTTACATTGG contig11032_F, CCTTACTGGGCAATAATGGTTT contig11053_F, GGTACGTATAAGCGTCCCTGT * contig11059_F, TCTAAAGAACTTGCCGCTCA contig 11097_F, GACAAGCTTACGGCCAACTC contig11107_F, ATCAAGCACAACGGTCTGAA contig11129_F, AGGGAAGACGTGATGGAATG contig11147_F, TGGCAATCAAAGTTTGGAATC
Supplemental Table 2. List of 768 SNP primer pairs used in KASPar assays on the Fluidigm EP1 platform.

## Forward Primer 2

 GAAGGTCGGAGTCAACGGATTGCTGATCAAGCACCTGCTTCG gatgitcgaatcaacgaattatccaagtatccttctccacaatca GAAGGTCGGAGTCAACGGATTGGTTGTGGTGGTTGTTGTACTTTC GAAGGTCGGAGTCAACGGATTGGATCCTGCCTATGATTTCATTGC GAAGGTCGGAGTCAACGGATTCCACTCTCGACATGCTTGCAC gaAgGtcgGagtcaacgGattgctacacttcggctgacttcta Common Reverse Primer | $\begin{array}{l}\text { Common Reverse Primer } \\ \text { CCTAACCTTGGTCGTAGACTCTTCTT }\end{array}$ |
| :--- |
| TGGGCTATtTGGGGGGTTTTGGAT |
| AGGGTGAGAAGGTTCGGTTCCAT |
| TAGTTTTGATAAGAACAGCTCGGGATCAT |
| TGCCATAGATGGCCCGGTTACTAT | tgCcatagatgacceggitactat ACGATGGTGATCACAGGCACGG


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| Al_BA_grs_14015_350 | GAAGGTGACCAAGTTCATGCTTTCTTTCAGGACTACTAAAGACTACG | GAAGGTCGGAGTCAACGGATTCTTTCTTTCAGGACTACTAAAGACTACA | tTTGGGTTAAGTTAGTAACTTACTAATTTT |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_109705_162 | GAAGGTGACCAAGTTCATGCTTTATTGTGTTTTCACCCTTTTAAATTATTTCG | GAAGGTCGGAGTCAACGGATTTATTGTGTTTTCACCCTTTTAAATTATTTCA | CGACTCGGGGAGGTCGGCAA |
| Al_BA_grs_17079_189 | GAAGGTGACCAAGTTCATGCTTGGCGTACTGCACAATACTTCG | GAAGGTCGGAGTCAACGGATTAGTTGGCGTACTGCACAATACTTCA | CTCCTGGATGTTTCGGTTGCGCTT |
| Al_BA_grs_106931_323 | GAAGGTGACCAAGTTCATGCTTCAGGAGTTTGTATAATGGTGCGG | GAAGGTCGGAGTCAACGGATTTTCAGGAGTTTGTATAATGGTGCGT | GATGCAGTCAGTCGGTTCGTAAGAT |
| Al_BA_grs_36836_433 | GAAGGTGACCAAGTTCATGCTGTCAATAGAGTGCCCCACAGAC | GAAGGTCGGAGTCAACGGATTGGTCAATAGAGTGCCCCACAGAT | ACGCTTCAGTTCTGTGGTGACCAT |
| Al_BA_grs_13904_433 | GAAGGTGACCAAGTTCATGCTGGAGTGGAGGCCAATGAAGAAAG | GAAGGTCGGAGTCAACGGATTGGAGTGGAGGCCAATGAAGAAAT | GCTGTTGCACCAAGCCCTTGCAT |
| Al_BA_grs_69245_242 | GAAGGTGACCAAGTTCATGCTGCCGATTAATTACTCCCCTGCG | GAAGGTCGGAGTCAACGGATTGGCCGATTAATTACTCCCCTGCT | GTGAGGATTTTGTAACTGCCACGCA |
| Al_BA_grs_30550_248 | GAAGGTGACCAAGTTCATGCTGAACTCCCTGTCGGACCTCTC | GAAGGTCGGAGTCAACGGATTGAACTCCCTGTCGGACCTCTT | CCAGGCCCTAATGGCATTATCACAA |
| Al_BA_grs_33956_162 | GAAGGTGACCAAGTTCATGCTCTCCAATGCTCAGATTCTCCAC | GAAGGTCGGAGTCAACGGATTCTCTCCAATGCTCAGATTCTCCAT | tTCGGTCATATCCGGAGTAATCTTGAAA |
| Al_BA_grs_16159_326 | GAAGGTGACCAAGTTCATGCTCGTCGAGTGGGCCTTCCG | GAAGGTCGGAGTCAACGGATTGTCGTCGAGTGGGCCTTCCA | tataccctacgagtacccaatgita |
| Al_BA_grs_25521_213 | GAAGGTGACCAAGTTCATGCTCGGTGTGAGCTCGAGAACGG | GAAGGTCGGAGTCAACGGATTGCGGTGTGAGCTCGAGAACGT | CCATATTTCCCCCTCAAAACCCCAT |
| Al_BA_grs_41770_204 | GAAGGTGACCAAGTTCATGCTCATCATGTTGCCTGTACTACTACG | GAAGGTCGGAGTCAACGGATTCCATCATGTTGCCTGTACTACTACA | TGGGAAATACTCTTGTGAATTGTTCGGTA |
| Al_BA_grs_43509_155 | GAAGGTGACCAAGTTCATGCTCAGTGAGTAGTTGCAGAGTGACG | GAAGGTCGGAGTCAACGGATTACAGTGAGTAGTTGCAGAGTGACA | GAGTCTATTTTTCCTTCTGTTTTCGTACTT |
| Al_BA_grs_25242_300 | GAAGGTGACCAAGTTCATGCTCACAATGGTTCAGGTTTATTAAGTGC | GAAGGTCGGAGTCAACGGATTGTCACAATGGTTCAGGTtTATtAAGTGT | TTAGTGATTAAAGCTCTCACGAAACTTGAA |
| Al_BA_grs_20690_314 | GAAGGTGACCAAGTTCATGCTATTGAATAATTCTTGTAAACAAAAATCACCAG | GAAGGTCGGAGTCAACGGATTCATTGAATAATTCTTGTAAACAAAAATCACCAA | CCATGTTTTCCCAACAAGACACCAATATT |
| Al_BA_grs_107791_242 | GAAGGTGACCAAGTTCATGCTATTATGTTGGAGATGGAACTGTCC | GAAGGTCGGAGTCAACGGATTACTATTATGTTGGAGATGGAACTGTCT | CCCGCATAATTCGTGTATAAAGAGCAAAT |
| Al_BA_grs_38330_329 | GAAGGTGACCAAGTTCATGCTATCGTGGTATCCACCGCTG | GAAGGTCGGAGTCAACGGATTGCTATCGTGGTATCCACCGCTA | CGACAAACGCGACGACAACCACAA |
| Al_BA_grs_29915_265 | GAAGGTGACCAAGTTCATGCTATATCTATACGGCTACAACTAATACGG | GAAGGTCGGAGTCAACGGATTCTATATCTATACGGCTACAACTAATACGA | GGTACAGAGGGTTTGATGACCAACTA |
| Al_BA_grs_107247_238 | GAAGGTGACCAAGTTCATGCTACGCTGACTCGATGCACTTTCG | GAAGGTCGGAGTCAACGGATTGACGCTGACTCGATGCACTTTCA | TCGCAGACTCGAGTGAAGACTCAT |
| Al_BA_grs_16672_120 | GAAGGTGACCAAGTTCATGCTAATTGAAGACTCTATCAGATAATCTTAAATAC | GAAGGTCGGAGTCAACGGATTGAATTGAAGACTCTATCAGATAATCTTAAATAT | TGAGTTCTTCTTCATCCCCTAAAAGCTTA |
| Al_BA_grs_15840_171 | GAAGGTGACCAAGTTCATGCTAAGGCGACGATGATCGACAGC | GAAGGTCGGAGTCAACGGATTGAAGGCGACGATGATCGACAGA | GGACCTTGGCCCGCACAGCAT |
| Al_BA_grs_37706_409 | GAAGGTGACCAAGTTCATGCTGTGAGTCCAGGAGAGCACCGA | GAAGGTCGGAGTCAACGGATTGAGTCCAGGAGAGCACCGG | GCTGTGCGACTCGCCGGTGTA |
| Al_BA_grs_72887_279 | GAAGGTGACCAAGTTCATGCTGTCGTGGCAGAGCCGGACTT | GAAGGTCGGAGTCAACGGATTCGTGGCAGAGCCGGACTC | AAAGGCCCGTTTCTCTGCCACG |
| Al_BA_grs_33787_381 | GAAGGTGACCAAGTTCATGCTGTCATGACTCCGATCTGAGCGT | GAAGGTCGGAGTCAACGGATTGTCATGACTCCGATCTGAGCGA | aCtTTCAGATCCGGAACCATAGGTTATAT |
| Al_BA_grs_20427_213 | GAAGGTGACCAAGTTCATGCTGTACGTGGTGAGCTGGTAGGTT | GAAGGTCGGAGTCAACGGATTACGTGGTGAGCTGGTAGGTG | agcagatgattgiccaagctagcat |
| Al_BA_grs_21612_226 | GAAGGTGACCAAGTTCATGCTGGGCGATACTTTGGTGTATGATGA | GAAGGTCGGAGTCAACGGATTGGCGATACTTTGGTGTATGATGG | ATGAGAACCGCAAGCTTCGCATGAT |
| Al_BA_grs_46950_332 | GAAGGTGACCAAGTTCATGCTGGAGGTTCTTGTCTTAGGCTACAT | GAAGGTCGGAGTCAACGGATTGAGGTTCTTGTCTTAGGCTACAC | CGACAGTAGGTGTATCAACATCCCAA |
| Al_BA_grs_53297_269 | GAAGGTGACCAAGTTCATGCTGATTGACCTGGCGAGTGATGGA | GAAGGTCGGAGTCAACGGATTGACCTGGCGAGTGATGGC | GATCCAAAAGGGTCGCACCTCCAA |
| Al_BA_grs_42195_300 | GAAGGTGACCAAGTTCATGCTGAGCATATCAATTCAGTTATCTTGGTGAT | GAAGGTCGGAGTCAACGGATTGCATATCAATTCAGTTATCTTGGTGAC | GTCCTTCTCCATAATCGAGAAGGCTT |
| Al_BA_grs_29391_265 | GAAGGTGACCAAGTTCATGCTGACGAGGTCGGAGTCGCCA | GAAGGTCGGAGTCAACGGATTACGAGGTCGGAGTCGCCG | CGAGCAGCCAAGGAAATGAACATCAA |
| Al_BA_grs_62899_192 | GAAGGTGACCAAGTTCATGCTGAAAACAAGATGAAACAAGTTGAGAGCAA | GaAGGTCGGAGTCAACGGATTAAACAAGATGAAACAAGTTGAGAGCAG | CCCGAGTTGTGCTTCTCAAAGCTAT |
| Al_BA_grs_105608_246 | GAAGGTGACCAAGTTCATGCTCTCAATAAGATTGGTGTCTTGCTT | GAAGGTCGGAGTCAACGGATTCTCTCAATAAGATTGGTGTCTTGCTG | atgTagaggcanganatanagatgitggat |
| Al_BA_grs_62300_111 | GAAGGTGACCAAGTTCATGCTCGTGGGGAAGGTACATAGTGCA | GAAGGTCGGAGTCAACGGATTGTGGGGAAGGTACATAGTGCG | GCAATGACGCCCCGTGGTTGAA |
| Al_BA_grs_21506_179 | GAAGGTGACCAAGTTCATGCTCGAAGTTCTCGGAGATCCTGCA | GAAGGTCGGAGTCAACGGATTGAAGTTCTCGGAGATCCTGCG | GGCCCGTGCGCATGAAGCTTT |
| Al_BA_grs_29453_94 | GAAGGTGACCAAGTTCATGCTCCACCCTTGTTGATCATATCGTGT | GAAGGTCGGAGTCAACGGATTCACCCTTGTTGATCATATCGTGC | tttatgatgccaicttgggtgtaggattt |
| Al_BA_grs_80443_334 | GAAGGTGACCAAGTTCATGCTCAAACATATTTGCAAATAAACCATATGAAAGAT | GAAGGTCGGAGTCAACGGATTAAACATATtTGCAAATAAACCATATGAAAGAG | CTCTCGTATTGTTGTTGCACCAAACATTA |


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|  | Al_BA_grs_83796_309 | GAAGGTGACCAAGTTCATGCTGAAGAGATGAAAGGCCAGACTACAA | GAAGGTCGGAGTCAACGGATTAAGAGATGAAAGGCCAGACTACAG | CATCTGGATGATCCTGACTCACGAA |
| :---: | :---: | :---: | :---: | :---: |
|  | A1_BA_grs_27756_174 | GAAGGTGACCAAGTTCATGCTGAAAGAATCCAACGGTCAGTCTTGT | GAAGGTCGGAGTCAACGGATTAAAGAATCCAACGGTCAGTCTTGG | GGTTTAAAAGAACTCCTTGAGAGGAA |
|  | Al_BA_grs_49498_297 | GAAGGTGACCAAGTTCATGCTCTGCTTGTCCAAGTAGATTTGTGTTT | GAAGGTCGGAGTCAACGGATTCTGCTTGTCCAAGTAGATTTGTGTTC | CCTGTCACCTATAGCAGAAAAATACTCAA |
|  | Al_BA_grs_15999_324 | GAAGGTGACCAAGTTCATGCTCCTAAGCTTGATTGCGGACCGT | GAAGGTCGGAGTCAACGGATTCTAAGCTTGATTGCGGACCGC | GCTATTGGAGAGGTACCGAGTACAA |
|  | Al_BA_grs_31313_342 | GAAGGTGACCAAGTTCATGCTCCGTTTATCCAAGTACATTAGTGAGAAA | GAAGGTCGGAGTCAACGGATTCCGTTTATCCAAGTACATTAGTGAGAAT | GTCTTGTTAGTCCTTTCTATGAACTTCTTT |
|  | Al_BA_grs_18812_249 | GAAGGTGACCAAGTTCATGCTCCCTGTTCTGGCGGTATTCGTT | GAAGGTCGGAGTCAACGGATTCCTGTTCTGGCGGTATTCGTC | ACGATGGAAACACGGGACCAAGTTA |
|  | Al_BA_gr_109268_225 | GAAGGTGACCAAGTTCATGCTCATAATCTACAACTCAAATTCTTGAAGTGA | GAAGGTCGGAGTCAACGGATtATAATCTACAACTCAAATTCTTGAAGTGG | GGAAAGACCATAAGCCGAGACTGAT |
|  | Al_BA_grs_39719_264 | GAAGGTGACCAAGTTCATGCTCAACCAAGGCTTATCTAACCAAGCT | GAAGGTCGGAGTCAACGGATTCAACCAAGGCTTATCTAACCAAGCA | gTaCaAGATGGATTAAGGCTAAGCCAT |
|  | Al_BA_grs_54156_270 | GAAGGTGACCAAGTTCATGCTATTAAAACTAATTAAATTACTGCAGTAACCACA | GAAGGTCGGAGTCAACGGATTAAAACTAATTAAATTACTGCAGTAACCACG | GTAGAATCATCTTATCAGAATAGATCCAA |
|  | Al_BA_grs_42747_240 | GAAGGTGACCAAGTTCATGCTATACGTGTGAAATCAGAGGCGCT | GAAGGTCGGAGTCAACGGATTACGTGTGAAATCAGAGGCGCC | TACCATAACAAAAAAAAATCCTTGCAGTAT |
| * | Al_BA_grs_48442_249 | GAAGGTGACCAAGTTCATGCTAGTTCTATGGAAGGATGTGGAGGT | GAAGGTCGGAGTCAACGGATTGTTCTATGGAAGGATGTGGAGGC | TAAACTCACGGGAGGAAACCAGATTT |
| * | Al_BA_grs_66238_275 | GAAGGTGACCAAGTTCATGCTAGCATCCAGCTGAAAGGATACCTA | GAAGGTCGGAGTCAACGGATTGCATCCAGCTGAAAGGATACCTG | TGCTGAGAGGGAGAAGTTGTTCGAA |
| * | Al_BA_grs_107263_216 | GAAGGTGACCAAGTTCATGCTACTGCTCATGTCGGCAGACGA | GAAGGTCGGAGTCAACGGATTCTGCTCATGTCGGCAGACGG | CGACCCGGCAAGCGTCATAGAT |
|  | Al_BA_grs_37795_404 | GAAGGTGACCAAGTTCATGCTACACTGGAATACATGTATTATTTCAACCA | GAAGGTCGGAGTCAACGGATTCACTGGAATACATGTATTATTTCAACCG | CTTGGAGATATGCCACTACAACTTCTTTA |
|  | Al_BA_grs_86450_346 | GAAGGTGACCAAGTTCATGCTAATAACGACAAGACAATCAAGCATGCT | GAAGGTCGGAGTCAACGGATTAACGACAAGACAATCAAGCATGCC | CTTGCCATGCTACATTATTGCCATGTAAA |
|  | Al_BA_grs 55181_284 | GAAGGTGACCAAGTTCATGCTAACCCTCTTTGGGTAATGATCCCT | GAAGGTCGGAGTCAACGGATTAACCCTCTTTGGGTAATGATCCCA | CCGAGATAGGCCAAGCTTGGCAA |
| * | Al_BA_grs_104898_197 | GAAGGTGACCAAGTTCATGCTAACAAGATTGGTGTGTTGCTGGGA | GAAGGTCGGAGTCAACGGATTCAAGATTGGTGTGTTGCTGGGC | TTATGCAGAGGCAAGAAGTAAAGATGGTA |
| * | A1_BA_grs_21636_110 | GAAGGTGACCAAGTTCATGCTGAACGAAATTTGGTCAAATTTTGAGTCG | GAAGGTCGGAGTCAACGGATTGAACGAAATTTGGTCAAATTTTGAGTCC | CTCTAACATCGCACCGCCTTCAAAT |
| * | Al_BA_grs_26396_267 | GAAGGTGACCAAGTTCATGCTTGTTGAATTGTTTCGTTGATTTTGAATGC | GAAGGTCGGAGTCAACGGATTTTTGTTGAATTGTTTCGTTGATTTTGAATGT | AAAAAAGAGCAGAGGCGCTCGATGA |
| * | Al_BA_grs_24713_153 | GAAGGTGACCAAGTTCATGCTTGGGTAATACACTTAAGGAAACTTGC | GAAGGTCGGAGTCAACGGATTCTTGGGTAATACACTTAAGGAAACTTGT | CAGTTGTTGGGCCTCCCAAGTGTA |
|  | Al_BA_grs 55489_309 | GAAGGTGACCAAGTTCATGCTGTTTTTCACTGTTTACCTATATGGTAACG | GAAGGTCGGAGTCAACGGATTGTTTTTCACTGTTTACCTATATGGTAACA | CACTGTATAAACTCCTGTTTGCTCTATCTT |
|  | Al_BA_grs_68273_327 | GAAGGTGACCAAGTTCATGCTGTCTCACGAATGCTATATCATCAATG | GAAGGTCGGAGTCAACGGATTAATGTCTCACGAATGCTATATCATCAATA | GGCAAGTTAATGATGCAAACTATGTACGAA |
|  | Al_BA_grs_76755_240 | GAAGGTGACCAAGTTCATGCTGTATTGTCACAAGAGCTTCCAAGTAC | GAAGGTCGGAGTCAACGGATTGTATTGTCACAAGAGCTTCCAAGTAA | TGAAAGAGCAAGTGGTCCTTATGTTTGTT |
| * | Al_BA_grs 52899_364 | GAAGGTGACCAAGTTCATGCTGGTTTGGCATGGTTGCGACAG | GAAGGTCGGAGTCAACGGATTGGTTTGGCATGGTTGCGACAT | TATTCCTTGCACTGAGCAAGAACATGAT |
|  | Al_BA_grs_23029_265 | GAAGGTGACCAAGTTCATGCTGCCTCTGCCAGTACTGGACAG | GAAGGTCGGAGTCAACGGATTGCCTCTGCCAGTACTGGACAT | GCATCATCTCCCTCAAGGCCTAAT |
| * | Al_BA_grs_51174_295 | GAAGGTGACCAAGTTCATGCTGCACTGTCAGTCACGTATTTGCC | GAAGGTCGGAGTCAACGGATTGCACTGTCAGTCACGTATTTGCT | ACCCGACACTTTGCCGAGTATCTAA |
|  | Al_BA_grs_45013_235 | GAAGGTGACCAAGTTCATGCTGAGCTTCATGATGAGTGTTGGTAC | GAAGGTCGGAGTCAACGGATtTGAGCTTCATGATGAGTGTTGGTAA | GGCCAAGGCCACCTTATATAAGAGTA |
| * | Al_BA_gr_104239_330 | GAAGGTGACCAAGTTCATGCTGAACTCACATAAGAAGAATGGTGAGG | GAAGGTCGGAGTCAACGGATTAGAACTCACATAAGAAGAATGGTGAGA | GCTTTATTTATAAAGCAGGACGAAAGCCTA |
|  | A1_BA_grs_31972_334 | GAAGGTGACCAAGTTCATGCTGAACTATTTCTCTAATTGTAGTACCAAATATG | GAAGGTCGGAGTCAACGGATTAGAACTATTTCTCTAATTGTAGTACCAAATATT | CGATTGGAATCGACGTCATGTTTAACTAA |
| * | Al_BA_grs_42785_298 | GAAGGTGACCAAGTTCATGCTCGTTATTGTTGAGGTTGAGGAGC | GAAGGTCGGAGTCAACGGATTGTCGTTATTGTTGAGGTTGAGGAGA | CCACCATAATGGGGACCAACAAGTT |
|  | Al_BA_grs_33681_351 | GAAGGTGACCAAGTTCATGCTCGAGCAATCCCTGACATCACC | GAAGGTCGGAGTCAACGGATTGCGAGCAATCCCTGACATCACT | GCAGAGAAGCTCAACGTGGTTGTAA |
|  | Al_BA_grs 57629_198 | GAAGGTGACCAAGTTCATGCTCCGTCTGCCGACATAAAGCAG | GAAGGTCGGAGTCAACGGATTGCCGTCTGCCGACATAAAGCAA | AGGTTACAGAGAGATATGCCGGCAA |
|  | A1_BA_grs_30011_326 | GAAGGTGACCAAGTTCATGCTCCCGTTGGCAGTTCTCTATGC | GAAGGTCGGAGTCAACGGATTCCCCGTTGGCAGTTCTCTATGA | CTACTCAACTTCAGTCATAAGTAAGCTCTT |
|  | A1_BA_grs_11721_341 | GAAGGTGACCAAGTTCATGCTCCAGATACAGTGGAAGCCCAC | GAAGGTCGGAGTCAACGGATTGTCCAGATACAGTGGAAGCCCAT | GTCCGTAGGCCACTCGAAGTCTT |
| * | Al_BA_grs_29686_290 | GAAGGTGACCAAGTTCATGCTCACATCGCTGCACTCGCCG | GAAGGTCGGAGTCAACGGATTCTCACATCGCTGCACTCGCCA | AGTGGTGACCTTCTTGCTGCCG |
|  | Al_BA_grs_108332_177 | GAAGGTGACCAAGTTCATGCTCACACTAAAGAGGTTCACAATCGG | GAAGGTCGGAGTCAACGGATTGACACACTAAAGAGGTTCACAATCGT | GTGTTGATGGCGAGCAAGATGATGAT |


| Al_BA_grs 55725_301 | GaAGGTGACCAAGTTCATGCTATAATCACATTATTATGAAAATAGTGTTTTCTG | GAAGGTCGGAGTCAACGGATTATAATCACATTATTATGAAAATAGTGTTTTCTA | tgcanatccagtgitctcatcgect |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_14646_143 | GAAGGTGACCAAGTTCATGCTAAGCCAAGACTAATCCGTTGGATTC | GAAGGTCGGAGTCAACGGATTATAAGCCAAGACTAATCCGTTGGATTA | TTCTTGCCTCTGCATAATCTACAACTCAA |
| Al_BA_grs_15500_194 | GAAGGTGACCAAGTTCATGCTAAATCTAATCTTGGAGATTGCGGGC | GAAGGTCGGAGTCAACGGATTAAAAATCTAATCTTGGAGATTGCGGGA | CGGAAGGCTTTAGCAACCAACGAT |
| Al_BA_grs_15562_330 | GAAGGTGACCAAGTTCATGCTTTTCATGAGTTCTTCTTCTTCTTTTCCTA | GAAGGTCGGAGTCAACGGATTCATGAGTTCTTCTTCTTCTTTTCCTG | ACCACCCCCAGAATTGAAGACTCTA |
| Al_BA_grs_47963_340 | GAAGGTGACCAAGTTCATGCTTTGGGAAACCTGAGTGGCACA | GAAGGTCGGAGTCAACGGATTTGGGAAACCTGAGTGGCACC | CAAGTATCATTTCATGACTGAATGATCAAA |
| Al_BA_grs_82362_346 | GAAGGTGACCAAGTTCATGCTTGTCTATTGGATATTGATGCTTTGTTGAT | GAAGGTCGGAGTCAACGGATTGTCTATTGGATATTGATGCTtTGTTGAA | GGAAATTGTTCAAACTATTTTTTGTTGCAA |
| Al_BA_grs_17512_343 | GAAGGTGACCAAGTTCATGCTTGGAAACAAGTATATCGAGGCACA | GAAGGTCGGAGTCAACGGATTGGAAACAAGTATATCGAGGCACG | GTATACCTTTTCACCAACTTTGTGTTCTTT |
| Al_BA_grs_37686_300 | GAAGGTGACCAAGTTCATGCTTCTCCTCCCTTCAAAGTTTCATCAT | GAAGGTCGGAGTCAACGGATTCTCCTCCCTTCAAAGTTTCATCAC | GTCGGCTGAAGGAAAGCTTACCAAT |
| Al_BA_grs_53832_345 | GAAGGTGACCAAGTTCATGCTTATAGTGATTTATACCAGGAGTAGGATTT | GAAGGTCGGAGTCAACGGATTAGTGATtTATACCAGGAGTAGGATTC | CGAGGTAACACATCTTACCCAGGTT |
| Al_BA_grs_17654_297 | GAAGGTGACCAAGTTCATGCTTAACTGTAGTTACAAATTTTCATCCTCCA | GAAGGTCGGAGTCAACGGATTACTGTAGTTACAAATTTTCATCCTCCG | GCAAAACACAGTTGGTTACCCTACTTAAA |
| Al_BA_grs_4743_286 | GAAGGTGACCAAGTTCATGCTGTTTAGAGAGGTGGAGGAAGGCA | GAAGGTCGGAGTCAACGGATTTAGAGAGGTGGAGGAAGGCG | ACCTGGGTAAGCTGTGTTACCTCAT |
| Al_BA_grs_19468_239 | GAAGGTGACCAAGTTCATGCTGTACGGGAAACCTCTCGTCAGA | GAAGGTCGGAGTCAACGGATTACGGGAAACCTCTCGTCAGG | CCTTCCTTTCTGGCTTTAATGTACCAATT |
| Al_BA_grs_46375_216 | GAAGGTGACCAAGTTCATGCTGGGTCGGCTGTCCGAGT | GAAGGTCGGAGTCAACGGATTGGGTCGGCTGTCCGAGC | CTCACCCCGAAGAACCGAGCTA |
| Al_BA_grs_27368_222 | GAAGGTGACCAAGTTCATGCTGGGACCACTTCAAAGGTCATGCA | GAAGGTCGGAGTCAACGGATTGGACCACTTCAAAGGTCATGCG | GGCGAGATTAAACCTCGACATCAATAATT |
| Al_BA_gr_ 87512 _320 | GAAGGTGACCAAGTTCATGCTGGCAATGATGCGATCCTTTTTGTCAT | GAAGGTCGGAGTCAACGGATTGCAATGATGCGATCCTTTTTGTCAC | CGACCCTCGCCATACTTGCGAA |
| Al_BA_grs_76379_100 | GAAGGTGACCAAGTTCATGCTGGAATGGACTGGATGACTAAGCAA | GAAGGTCGGAGTCAACGGATTGAATGGACTGGATGACTAAGCAC | AGTGAGACAGTATGGTTTGCACAATCTAT |
| Al_BA_grs_5883_168 | GAAGGTGACCAAGTTCATGCTGCGAAATCATCACACGCTGCAGT | GAAGGTCGGAGTCAACGGATTCGAAATCATCACACGCTGCAGC | ACTACGAATGTGAGCGCTACACGTT |
| Al_BA_grs_106036_155 | GAAGGTGACCAAGTTCATGCTGCCGAGCAGCTTCATCACGA | GAAGGTCGGAGTCAACGGATTGCCGAGCAGCTTCATCACGG | GCGGCCATGGCGCAGAGCT |
| Al_BA_grs_106092_190 | GAAGGTGACCAAGTTCATGCTGCCATTCATATATCCAATATGACAAGCAT | GAAGGTCGGAGTCAACGGATTCCATTCATATATCCAATATGACAAGCAC | CTTAAGGAATAGTCGATCCCATTTGTGAA |
| Al_BA_grs_49262_176 | GAAGGTGACCAAGTTCATGCTGCCACCAATTTAGCCTGGCACA | GAAGGTCGGAGTCAACGGATTCCACCAATTTAGCCTGGCACG | ATGGAGGCCTCAGCAAATCTGTCTT |
| Al_BA_grs_81051_272 | GAAGGTGACCAAGTTCATGCTGCCAAGAAGATCTACAACAAGGCT | GAAGGTCGGAGTCAACGGATTCCAAGAAGATCTACAACAAGGCC | TCATGAGAGATTCAGGGGCGAGAA |
| Al_BA_grs_50029_251 | GAAGGTGACCAAGTTCATGCTGAAATCGGTACCACTTTCTGGCA | GAAGGTCGGAGTCAACGGATTGAAATCGGTACCACTTTCTGGCC | GGAGCAGGGAGGTGGTGAGATA |
| Al_BA_grs_13399_237 | GAAGGTGACCAAGTTCATGCTGAAAGAGCAATCAAAGCAAAATCTTTTTGA | GAAGGTCGGAGTCAACGGATTAAAGAGCAATCAAAGCAAAATCTTTTTGG | Ctttgattigcgicatccttttiagcti |
| Al_BA_grs_49013_332 | GAAGGTGACCAAGTTCATGCTCGTGGCTCAGGATTTGTTTTTAAAAACAT | GAAGGTCGGAGTCAACGGATTGTGGCTCAGGAtTTGTTTTTAAAAACAG | GGTGTGTATCAAAAGTTATGAATTTTGCAA |
| Al_BA_grs_57545_273 | GAAGGTGACCAAGTTCATGCTCGGTCAAGCTTTCCGACTTGCAT | GAAGGTCGGAGTCAACGGATTGGTCAAGCTTTCCGACTTGCAC | CTTCCAGTCCTCGATAAGCTTTGGAA |
| Al_BA_grs_28284_295 | GAAGGTGACCAAGTTCATGCTCCCAATGCACTTGGCCTCCA | GAAGGTCGGAGTCAACGGATTCCCAATGCACTTGGCCTCCG | ATCTGGAGTGTTATGGATCTTGATGAGAA |
| Al_BA_grs_20229_336 | GAAGGTGACCAAGTTCATGCTCCATAACCATTATCAACACCAAGACAAT | GAAGGTCGGAGTCAACGGATTCATAACCATTATCAACACCAAGACAAG | AGTAGTTTAGGTATAATTTTTATCCCTCTT |
| Al_BA_grs_108819_315 | GAAGGTGACCAAGTTCATGCTCAGGCCATCCAAGGGTGTATCA | GAAGGTCGGAGTCAACGGATTAGGCCATCCAAGGGTGTATCG | AGGTGAACTCGCCCCTCTCCTT |
| Al_BA_grs_41506_242 | GAAGGTGACCAAGTTCATGCTCAAGTTGTCCACCTCTGAGCA | GAAGGTCGGAGTCAACGGATTCAAGTTGTCCACCTCTGAGCG | GAGCCTCGGGGCTCCACCAA |
| Al_BA_grs_33475_433 | GAAGGTGACCAAGTTCATGCTCAAGTACATCCAGAATTTCACAATCATCA | GAAGGTCGGAGTCAACGGATTAGTACATCCAGAATTTCACAATCATCG | CACACTCGAAACCCCTTACGAGATT |
| Al_BA_grs_77365_188 | GAAGGTGACCAAGTTCATGCTCAACAAACATAAGGACCACTTGCTCT | GAAGGTCGGAGTCAACGGATTCAACAAACATAAGGACCACTTGCTCA | GGAAGCAAAAACAACCAGGGTGCAA |
| Al_BA_grs_104834_200 | GAAGGTGACCAAGTTCATGCTCAAATCACCTTGAAACGATATAGCATCT | GAAGGTCGGAGTCAACGGATTAAATCACCTTGAAACGATATAGCATCC | CTTCAGGGCTCTTAGCTCAATTTGCTT |
| Al_BA_grs_26569_191 | GAAGGTGACCAAGTTCATGCTATTCTTGAAGTAAAAGGATCCAACGGT | GAAGGTCGGAGTCAACGGATTCTTGAAGTAAAAGGATCCAACGGA | GTTTAAAAAGAACTCCTCGAGAGGAAGTT |
| Al_BA_grs_87873_178 | GAAGGTGACCAAGTTCATGCTAGATGCTCATTGTCTGCTTGCCTTA | GAAGGTCGGAGTCAACGGATTATGCTCATTGTCTGCTTGCCTTG | CCCCTTTGCACATGATATAGGAGCTA |
| Al_BA_grs_38692_386 | GAAGGTGACCAAGTTCATGCTAAAGCTCGAATTGATAATAAGAAGGAGA | GAAGGTCGGAGTCAACGGATTAAAGCTCGAATTGATAATAAGAAGGAGT | GGCCTCTTATTTAGTTTTGCAAAGTGGAA |
| Al_BA_grs_22275310 | GAAGGTGACCAAGTTCATGCTAAAATTCTTGAAGTAAAAGGATCCAACGA | GAAGGTCGGAGTCAACGGATTAATTCTTGAAGTAAAAGGATCCAACGG | CAGATCGTGATGGTTTAAAAGAACTCCTT |




| Al_BA_grs_22020_338 | GAAGGTGACCAAGTTCATGCTGAAAATTCCACTCAAGATTAAAATTTTCATGTA | GAAGGTCGGAGTCAACGGATTAAAATTCCACTCAAGATTAAAATTTTCATGTG | Cattegrangang iactitgitatttagta |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_108511_161 | GAAGGTGACCAAGTTCATGCTGAAAAGAATCCAAAGGATCAGTCTTGA | GAAGGTCGGAGTCAACGGATTAAAAGAATCCAAAGGATCAGTCTTGG | CTCTTGTTATAAATTTTGTTCCTATtTAGT |
| Al_BA_grs_46464_325 | GAAGGTGACCAAGTTCATGCTCTCCTACATACAACAAGAGACCGT | GAAGGTCGGAGTCAACGGATTCCTACATACAACAAGAGACCGG | GCTCAAGTGGAATGCTCAAGGCAAA |
| Al_BA_grs_26548_143 | GAAGGTGACCAAGTTCATGCTCGTTTACGAGAATTATTAAATGAATTAAGGTT | GAAGGTCGGAGTCAACGGATTCGTTTACGAGAATTATTAAATGAATTAAGGTC | CAACAAAGACTTCTTCTCTTTTAGAGGAAT |
| Al_BA_grs_43072_300 | GAAGGTGACCAAGTTCATGCTCGCCCCTGCGCACAACACTA | GAAGGTCGGAGTCAACGGATTGCCCCTGCGCACAACACTG | CGCAGGTCGGTGTCGCGCAT |
| Al_BA_grs_77838_125 | GAAGGTGACCAAGTTCATGCTCCTAATTGCTGGTGATGATAATTTCTCA | GAAGGTCGGAGTCAACGGATTCTAATTGCTGGTGATGATAATTTCTCG | CCATATATAATGCAGAGGTCAGGGAAATA |
| Al_BA_grs_71750_324 | GAAGGTGACCAAGTTCATGCTCCGCAACCTGAGTCGAACCAT | GAAGGTCGGAGTCAACGGATTCCGCAACCTGAGTCGAACCAC | CGAATACAGAAGCAAAAGAAGAGACGAAA |
| Al_BA_grs_48295_384 | GAAGGTGACCAAGTTCATGCTCCGAGACCACCTTGGTGACCA | GAAGGTCGGAGTCAACGGATTCGAGACCACCTTGGTGACCG | GTGGTCCTCGGTGAAGGTGCAA |
| Al_BA_grs_54151_274 | GAAGGTGACCAAGTTCATGCTCCCTGCAAACTGTCCATGTGAGT | GAAGGTCGGAGTCAACGGATTCCTGCAAACTGTCCATGTGAGC | TCCTACTTCTTCAAGGGCCGTCAAT |
| Al_BA_grs_39304_279 | GAAGGTGACCAAGTTCATGCTCCACGCGCAATAGTAAATAAAGT | GAAGGTCGGAGTCAACGGATTCTCCACGCGCAATAGTAAATAAAGC | CTAATGTAATGCATGCATGCAATGCTCAA |
| Al_BA_grs_104526_322 | GAAGGTGACCAAGTTCATGCTCCACACCACAAGCTTATTGATTACATT | GAAGGTCGGAGTCAACGGATTCACACCACAAGCTTATTGATTACATC | TCTTGCATGTGTGCAACTTTAACTGACA |
| Al_BA_grs_56627_343 | GAAGGTGACCAAGTTCATGCTCATTTTGACTGAGAGTAAAATTATGCATTTAAA | GAAGGTCGGAGTCAACGGATTCATTTTGACTGAGAGTAAAATTATGCATTTAAT | ATGAAACAATGATATGGTAACACTCGCATA |
| Al_BA_grs_22311_214 | GAAGGTGACCAAGTTCATGCTCACCAGGACTAAAGTTCCTCTCAA | GAAGGTCGGAGTCAACGGATTCACCAGGACTAAAGTTCCTCTCAT | GCAGTGTCGATGTTTGCCCAGCAA |
| Al_BA_grs_18921_322 | GAAGGTGACCAAGTTCATGCTCAATCATCAGCATCCAATGGCTCTTT | GAAGGTCGGAGTCAACGGATTAATCATCAGCATCCAATGGCTCTTC | GAACTTTCAGAACACCAATCCACCCAT |
| Al_BA_grs_32531_244 | GAAGGTGACCAAGTTCATGCTCAATATATAGGGGAAACTCCACCAAATA | GAAGGTCGGAGTCAACGGATTAATATATAGGGGAAACTCCACCAAATC | GGAGAGACTCCACATAATTATGTGCATAA |
| Al_BA_grs_25202_404 | GAAGGTGACCAAGTTCATGCTCAAGGGAGAAGAAGTCTATGTTGTTA | GAAGGTCGGAGTCAACGGATTCAAGGGAGAAGAAGTCTATGTTGTTG | GCACTTACCATTTGCTTATTCGGGGAT |
| Al_BA_grs_38572_283 | GAAGGTGACCAAGTTCATGCTATTACCGAGATTATCCCTTCTCACATT | GAAGGTCGGAGTCAACGGATTACCGAGATTATCCCTTCTCACATA | TCGGGTTGTTCAGTCTGCTAATTTTGTTT |
| Al_BA_grs_108554_271 | GAAGGTGACCAAGTTCATGCTATAGCAATGCTCCCCCTGACTA | GAAGGTCGGAGTCAACGGATTATAGCAATGCTCCCCCTGACTG | CCATCTACTACCTATCGTGCATGCTT |
| Al_BA_grs_38812_220 | GAAGGTGACCAAGTTCATGCTATACGGCCATAATTTTAATTTCCGCCA | GAAGGTCGGAGTCAACGGATTACGGCCATAATTTTAATTTCCGCCG | CAATTTCTTTCCCAGGTACGGAGAGTT |
| Al_BA_grs_28723_184 | GAAGGTGACCAAGTTCATGCTATAATTTTGTCAAGTTCTCAAGGATCCTA | GAAGGTCGGAGTCAACGGATTAATTTTGTCAAGTTCTCAAGGATCCTG | TGAAAAGCAGCGAGGGAGATAGCAT |
| Al_BA_grs_110381_96 | GAAGGTGACCAAGTTCATGCTAGTTTCTTATCTGCATTTCTACGGTGT | GAAGGTCGGAGTCAACGGATTGTTTCTTATCTGCATTTCTACGGTGC | GGAGAAATGAAGAAAAAGTC |
| Al_BA_grs_15007_124 | GAAGGTGACCAAGTTCATGCTAGGTCCCACCTTTATCGAAGAACT | GAAGGTCGGAGTCAACGGATTAGGTCCCACCTTTATCGAAGAACA | CCTCGCAACAACTATAGGGGATCAA |
| Al_BA_grs_70372_127 | GAAGGTGACCAAGTTCATGCTAGGACGATGAGATATGTGAGGTCT | GAAGGTCGGAGTCAACGGATTGGACGATGAGATATGTGAGGTCC | GCCTGGAAAGCATTGCCGAAGATAT |
| Al_BA_grs_75416_305 | GAAGGTGACCAAGTTCATGCTAGCATCTGGATGATCCTGACTCAT | GAAGGTCGGAGTCAACGGATTGCATCTGGATGATCCTGACTCAC | CGAAAGGCCAGATTACAGGTATGGTT |
| Al_BA_grs_65058_130 | GAAGGTGACCAAGTTCATGCTAGCAACACCATCAGATGCATGCT | GAAGGTCGGAGTCAACGGATTGCAACACCATCAGATGCATGCG | GTTGGAGACATCCGCTAACTCTGTA |
| Al_BA_grs_45261_178 | GAAGGTGACCAAGTTCATGCTACTGCCACATGGCTATCGTAAAGAA | GAAGGTCGGAGTCAACGGATTGCCACATGGCTATCGTAAAGAG | GatGAGATTATTCGGCACTTATATTTTATT |
| Al_BA_grs_32215_258 | GAAGGTGACCAAGTTCATGCTACTCGCCAAGTCAACTTGGCGA | GAAGGTCGGAGTCAACGGATTCTCGCCAAGTCAACTTGGCGC | CACTGCGGTACTCAGTGGTGGAA |
| Al_BA_grs_54474_354 | GAAGGTGACCAAGTTCATGCTACTCAGGATGGGGAGGAGAGT | GAAGGTCGGAGTCAACGGATTCTCAGGATGGGGAGGAGAGC | TCTTCTTCTTTCCTGGAGCGGCAAT |
| Al_BA_grs_34366_403 | GAAGGTGACCAAGTTCATGCTACGGATCCTCTGTTCTATCCCTTA | GAAGGTCGGAGTCAACGGATTCGGATCCTCTGTTCTATCCCTTG | CCTGATGGGTATGACATTAACCCCAT |
| Al_BA_grs_81977_212 | GAAGGTGACCAAGTTCATGCTACATTTTTATCTCTTCTTATTCCAAATTGTCA | GAAGGTCGGAGTCAACGGATTCATTTTTATCTCTTCTTATTCCAAATTGTCC | CAGTGAGAGCAGCGTTTTTTAGATGTAAT |
| Al_BA_grs_89672_241 | GAAGGTGACCAAGTTCATGCTACAATACAAGATACTTGGAAGCTCTCA | GAAGGTCGGAGTCAACGGATTCAATACAAGATACTTGGAAGCTCTCG | ATGTTTTGTGTGCAACCATAATGCCACTA |
| Al_BA_grs_70258_392 | GAAGGTGACCAAGTTCATGCTAAGTGCATGGTGGATTGTGGAAGT | GAAGGTCGGAGTCAACGGATTGTGCATGGTGGATTGTGGAAGC | tTCATCTTGTGAGTGTATCCAGGTACAT |
| Al_BA_grs_103686_196 | GAAGGTGACCAAGTTCATGCTAAGGGTGAGCTAAGTTGGCTTGAT | GAAGGTCGGAGTCAACGGATTGGGTGAGCTAAGTTGGCTTGAC | TTGGTTTCGGACCGTGAAGCTGAAA |
| Al_BA_grs_107365_310 | GAAGGTGACCAAGTTCATGCTAAGAATCAAAAGTCTAAGAGTAAAAGGTAATA | GAAGGTCGGAGTCAACGGATTGAATCAAAAGTCTAAGAGTAAAAGGTAATG | ATGACCGAAATTGTACGAGTACTCAAGTA |
| Al_BA_grs_57497_370 | GAAGGTGACCAAGTTCATGCTAACCCCTGACCAAAGTTTATCACCA | GAAGGTCGGAGTCAACGGATTCCCCTGACCAAAGTTTATCACCG | GTCAATTAGCGCGGTCTGTGATGAA |
| Al_BA_grs_34495_308 | GAAGGTGACCAAGTTCATGCTAAAATTTCCCAAGTCACTAAACTGGAAAA | GAAGGTCGGAGTCAACGGATTAATTTCCCAAGTCACTAAACTGGAAAG | TGGCCCACCAATAATTATCTCAATTCGTT |


| Al_BA_grs_24003_202 | GAAGGTGACCAAGTTCATGCTTATTTGGACAATAATGTTGAGCTACTTTG | GAAGGTCGGAGTCAACGGATTATTTGGACAATAATGTTGAGCTACTTTC | AGAAATACCGACTGGACAAGCATAGATTT |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_82447_112 | GAAGGTGACCAAGTTCATGCTGGTGGATCCATATGTGCGTG | GAAGGTCGGAGTCAACGGATTCTGGTGGATCCATATGTGCGTC | ACATATGGATCCACCAGAGTTAATTGCTT |
| Al_BA_grs_60346_283 | GAAGGTGACCAAGTTCATGCTGAAACCAGGATAACCATGGCTCC | GAAGGTCGGAGTCAACGGATTGAAACCAGGATAACCATGGCTCG | GCATCTAATGGCTCTTCAGACTTACTAAA |
| Al_BA_grs_72874_297 | GAAGGTGACCAAGTTCATGCTACCGAAAACATCAGGCACCTGC | GAAGGTCGGAGTCAACGGATTACCGAAAACATCAGGCACCTGG | CTTTCCTTGATGATACTGGGATGGGAT |
| Al_BA_grs_11463_236 | GAAGGTGACCAAGTTCATGCTAAGCCCGAGGAAGATGATCCTC | GAAGGTCGGAGTCAACGGATTAAGCCCGAGGAAGATGATCCTG | CTTCATCCCCTACCAAAATCTCCTCAT |
| Al_BA_grs_87637_89 | GAAGGTGACCAAGTTCATGCTTTATTTCTCCAATAACTGTCAAGGCG | GAAGGTCGGAGTCAACGGATTCTTTTATTTCTCCAATAACTGTCAAGGCA | aACACATCATCAATGCTAAAGAACAGATAT |
| Al_BA_grs_29681_335 | GAAGGTGACCAAGTTCATGCTTTATGAAGAACTTCGCAACAACTTAC | GAAGGTCGGAGTCAACGGATTACTTTATGAAGAACTTCGCAACAACTTAA | aCaAtttantantagtctctaccggitcaa |
| Al_BA_grs_36345_222 | GAAGGTGACCAAGTTCATGCTTACTACCTTAAATCCAAAAATTATCGTAATG | GAAGGTCGGAGTCAACGGATTGTtactaccttanatccananattatcgtanta | GACAAGTACTCAGTAACGAATCTGGTAAT |
| Al_BA_grs_16864_246 | GAAGGTGACCAAGTTCATGCTTAAGCCAAACCGGTCGGTTGG | GAAGGTCGGAGTCAACGGATTGTTAAGCCAAACCGGTCGGTTGA | CTCTCGGTCAGAAGCAGTTGGTTT |
| Al_BA_grs_110839_129 | GAAGGTGACCAAGTTCATGCTGTTACCGCAAGGAGGAACATTGG | GAAGGTCGGAGTCAACGGATTATGTTACCGCAAGGAGGAACATTGT | ATTTGTCAAGCCAAGTCAAGTGGATTATAT |
| Al_BA_grs_30486_344 | GAAGGTGACCAAGTTCATGCTGTGACATCTTGAAATAGTCGATGAGG | GAAGGTCGGAGTCAACGGATTAGTGACATCTTGAAATAGTCGATGAGA | GTCGCCAGTCCAGGGGAGCTT |
| Al_BA_grs_27764_303 | GAAGGTGACCAAGTTCATGCTGTCTACGCACTTCTATTCTTGTAGAC | GaAGGTCGGAGTCAACGGATTGTCTACGCACTTCTATTCTTGTAGAT | GCTACTGTTCTACAAACCTCTGCACTT |
| Al_BA_grs_29207_269 | GAAGGTGACCAAGTTCATGCTGGTTTTATATTCTTGAGTATTTTGCTGC | GAAGGTCGGAGTCAACGGATTGTGGTTTTATATTCTTGAGTATTTTGCTGA | GAGGCCTAAACATGCATCAAAACAAATTTT |
| Al_BA_grs_38319_99 | GAAGGTGACCAAGTTCATGCTGGTTCTATCGTGTATGCATGAAGG | GAAGGTCGGAGTCAACGGATTAATGGTTCTATCGTGTATGCATGAAGA | TATCGCCCCAAGATTATCTGGCCTT |
| Al_BA_grs_31368_179 | GAAGGTGACCAAGTTCATGCTGGTGAAGCATACGCTTTGGTTCG | GAAGGTCGGAGTCAACGGATTGGTGAAGCATACGCTTTGGTTCA | AGGTTGGTTCCAATCGAGGGTGTT |
| Al_BA_grs_71867_257 | GAAGGTGACCAAGTTCATGCTGGGAGATGATGGCTCAATGCAC | GAAGGTCGGAGTCAACGGATTATAGGGAGATGATGGCTCAATGCAT | aCGagctctacatcancactgccat |
| Al_BA_grs_15779_266 | GAAGGTGACCAAGTTCATGCTGGCTTCATAAAGTCCCCAGTCG | GAAGGTCGGAGTCAACGGATTAAAGGCTTCATAAAGTCCCCAGTCA | CTGCAGGGTATACCCATGTCAGTA |
| Al_BA_grs_44822_218 | GAAGGTGACCAAGTTCATGCTGGATGTTATCGACACACAGTATGATG | GAAGGTCGGAGTCAACGGAtTGGATGTtatcgacacacagtatgata | GCGCTGGTCGCGCACCCTA |
| Al_BA_grs_33630_324 | GAAGGTGACCAAGTTCATGCTGGAGCTCAGGTGATGGTCAGC | GAAGGTCGGAGTCAACGGATTGGAGCTCAGGTGATGGTCAGT | GTCGACTTCATCATTCACCCCCAT |
| Al_BA_grs_11756_153 | GAAGGTGACCAAGTTCATGCTGCAAGAGATCATCTACATCAACCAC | GAAGGTCGGAGTCAACGGATTGGCAAGAGATCATCTACATCAACCAT | ACATACCCTTGAAGACTGACAAAGCTTAA |
| Al_BA_grs_43513_297 | GAAGGTGACCAAGTTCATGCTGAGTCCCGAGGGCTACTGAC | GAAGGTCGGAGTCAACGGATTGGAGTCCCGAGGGCTACTGAT | CTTAGGGTAAATAATCATAGGGACCGATA |
| Al_BA_grs_71868_375 | GAAGGTGACCAAGTTCATGCTGAGGGATAATGTTCCCACAAACAAAC | GAAGGTCGGAGTCAACGGATTAGAGGGATAATGTTCCCACAAACAAAT | actgatcatattegtcgicctigacgat |
| Al_BA_grs_11718_249 | GAAGGTGACCAAGTTCATGCTGAGGCAAGAAATAAAGATGTTAGATGC | GAAGGTCGGAGTCAACGGATTAGAGGCAAGAAATAAAGATGTTAGATGA | CCAGGACTAAAGTTCCTCTCAATAAGATT |
| Al_BA_grs_22046_207 | GAAGGTGACCAAGTTCATGCTGAGCTATGTGTTGAAGGGTGGC | GAAGGTCGGAGTCAACGGATTGAGCTATGTGTTGAAGGGTGGA | AATGTTGCAGATCTCGCAAATGTTGGTTT |
| Al_BA_grs_105670_336 | GAAGGTGACCAAGTTCATGCTGAGCCAGGAGAATGAGTCGCC | GAAGGTCGGAGTCAACGGATTGAGCCAGGAGAATGAGTCGCT | CCAACAGACCAGCGGATGGTC |
| Al_BA_grs_31301_210 | GAAGGTGACCAAGTTCATGCTGAAGGAGTAATCAGAAATATCTATGTCTC | GAAGGTCGGAGTCAACGGATTGAAGGAGTAATCAGAAATATCTATGTCTT | GGAAATCTCGAGGCATATCAACAATATGAA |
| Al_BA_grs_107213_257 | GAAGGTGACCAAGTTCATGCTGAACCCGCTCGCCACCG | GAAGGTCGGAGTCAACGGATTGTGAACCCGCTCGCCACCA | GGCGGAGCGGCTTCGGCAA |
| Al_BA_grs 51382_260 | GAAGGTGACCAAGTTCATGCTCCCTATTGAGGTTGGATACACG | GAAGGTCGGAGTCAACGGATTCTCCCTATTGAGGTTGGATACACT | GGTAGAACCGGATCCGAAGCGAA |
| Al_BA_grs_13816_313 | GAAGGTGACCAAGTTCATGCTCCCACCTTGCATATAATGAACTTGC | GAAGGTCGGAGTCAACGGATTCCCACCTTGCATATAATGAACTTGT | GAGTCTAATCAAGGGATCAAAGGAAAGAA |
| Al_BA_grs_45736_154 | GAAGGTGACCAAGTTCATGCTCCACGCAATATGTCCACCTC | GAAGGTCGGAGTCAACGGATTCCTCCACGCAATATGTCCACCTT | ATAATTATTACGAAAAATGAGGGGTCGACA |
| Al_BA_grs_70857_106 | GAAGGTGACCAAGTTCATGCTCATGGTGCGGACATCCTCCC | GAAGGTCGGAGTCAACGGATTATCATGGTGCGGACATCCTCCT | GTTTCATTGGGCCAGAGACAACTGAA |
| Al_BA_grs_22268_229 | GAAGGTGACCAAGTTCATGCTCAGTGAATGGTTAGAGGTTACGG | GAAGGTCGGAGTCAACGGATTCTCAGTGAATGGTTAGAGGTTACGA | CAGCTCGCCGCTCCTTCTGTTT |
| Al_BA_grs_18172_140 | GAAGGTGACCAAGTTCATGCTCAGGTGTTTGGGGGCTTACTG | GAAGGTCGGAGTCAACGGATTGCAGGTGTTTGGGGGCTTACTA | ACCACCAAAAATAAAGACGTCCCATGTT |
| Al_BA_grs_110873_224 | GAAGGTGACCAAGTTCATGCTCACTTTCGCTGCAGCACGC | GaAGGTCGGAGTCAACGGATTACTCACTTTCGCTGCAGCACGT | GCGGATCTCAAGCTGTAACCACAA |
| Al_BA_grs_47795_198 | GAAGGTGACCAAGTTCATGCTCACCGAAGCAGTCCTCTACGG | GAAGGTCGGAGTCAACGGATTCACCGAAGCAGTCCTCTACGA | TTTGAAGCTGGCATCTTCGAGGCAT |
| Al_BA_grs_70422_294 | GAAGGTGACCAAGTTCATGCTCACAACTGAATATTGAAGTTGTAGAAAAAC |  | GCAATGCATCTGTCACGGATTGATTAAAT |


| Al_BA_grs 43090_295 | GAAGGTGACCAAGTTCATGCTCAAGTATTGTTAAATCAATtTTTTGAACCTCG | GAAGGTCGGAGTCAACGGATTCAAGTATTGITAAATCAATtTTTTGAACCTCA | gTCTACCTTGAttcgGctttittaactia |
| :---: | :---: | :---: | :---: |
| Al_BA_grs 45750 306 | GAAGGTGACCAAGTTCATGCTCAAGAGGAAGTCAAAGGAGAGGG | GAAGGTCGGAGTCAACGGATTCAAGAGGAAGTCAAAGGAGAGGA | CTTATATGGCTAAGTCTGGTGCctgta |
| Al_BA_grs_36187_311 | GAAGGTGACCAAGTTCATGCTATTGCAATCAAGATAGTGCCCTTG | gatggtcgangtcaacgiattctattccaatcaigatagtgccetta | GGGCTTTTCGAACATTTTAGGCATGTT |
| Al_BA_grs_42271_316 | GAAGGTGACCAAGTTCATGCTATGGTGGGTGGAACGTCTATCG | GAAGGTCGGAGTCAACGGATTGATGGTGGGTGGAACGTCTATCA | GTGTAAGAGCCGGGTAGACTATCTT |
| 1_BA_grs_1793_142 |  |  | antagttttctaatatccttgtctta |
| Al_BA_zrs_66231_310 | GAAGGTGACCAAGTTCATGCTATCCTCATAGCATGTCTCCTATCC | GAAGGTCGGAGTCAACGGATTGTATCCTCATAGCATGTCTCCTATCT | CCAGTAGGCCATGGGCAATGCTA |
| Al_BA_grs_18010_210 | GAAGGTGACCAAGTTCATGCTATCACCGGGACTAAAAGTTCCTTTC | GAagGtcgaatcancgaattaatcaccgagactanaigttccttt | CAGTGTCCATGATTTCCCAGCAAGAT |
| Al_BA_grs_39607_166 | GAAGGTGACCAAGTTCATGCTATATTGCTATTGTTGCAAATCATATGACG | gangGtcgaagtcancgaattcatattcctattgitccaantcatatgac | gGtcacatgattttaccgigtattcacat |
| Al_BA_grs_2979, 91 | GAAGGTGACCAAGTTCATGCTATAAGAGGAAACTATTTCACTGAAGTTTG |  | CTCTTCTTTAGTTTGGCGAAGGGGAA |
| Al_BA_grs_106476_114 | GAAGGTGACCAAGTTCATGCTAGTAAAACACAATACTCGCAACAAGTC | GaAGgTCGGAgTCAACGGATTGAAGTAAAACACAATACTCGCAACAAGTA | GTGACCGTTACACCATGCAAAAAACTAA |
| 1_BA_grs_50105_166 | GGTGACCAAGTTCATGCTAGGACCCACCTATGTCTGTAGG | GaAGGTCGGAGTCAACGGATTCAGGACCCACCTATGTCTGTAGA | CatGttgagtccacatagangtgcatatt |
| Al_BA_-rs_40519-355 | GAAGGTGACCAAGTTCATGCTAGCGGCTCGGCCAGAGAC | GaAGGTCGGAGTCAACGGATTGAGCGGCTCGGCCAGAGAT | CCTCCtTTGGCGATGGCagcaa |
| Al_BA_grs 56328 -393 | GAAGGTGACCAAGTTCATGCTACTGTGAGTATTGATTTTGTTACTGC |  | Cagttatccatattgganacaahgtancaa |
| BA_zrs_13164_197 | GAAGGTGACCAAGTTCATGCTAATCACCAGGACTAAAGTTCCTCTC | a aggtcgaagtcaacgaattaanatcaccaggactaangtt | gatacaatatclatgittccciaccaa |
| Al_BA_rs 24802 _137 | GaAGGTGACCAAGTTCATGCTAATAATTTGAACAACTTCATTTACTTCCCG | GAAGGTCGGAGTCAACGGATTAAAATAATTTGAACAACTTCATTTACTTCCCA | ATGTGTAACTCCAATTCGAAAATTTGAAAA |
| Al_BA_grs_11627_418 | GAAGGTGACCAAGTTCATGCTAAGGTGAATCTATTGTGCACCACC |  | CTTAAGGGTGAATCGGTGGATCCTT |
| BA grs 2302 | ;GTGACCAAGTTCATGCTAAGATCCCTCAATTCTTC | GAAGGTCGGAGTCAACGGATTGAAGATCCCTCAATTCTTCATGATGTAT | actigatcantaggtcitt cGacagcat |
| Al_BA_grs_25255_270 |  |  | CTTCATCATGACCTTGAGGTAGGCTT |
| Al_BA_rs_14263 283 | GAAGGTGACCAAGTTCATGCTAACTATATATTACAAGTAAATTATAGGTTGCTC | GAagGtcgaagtcaacgaattaactatatattacaagtanattataggttgcta | GTTGTCAAAACTCTCGGTTGTTTCTCAAT |
| Al_BA_grs 84979 _305 | GGTGACCAAGTTCATGCTAAATCAACGCCGCTGCAGCTG | GAAGGTCGGAGTCAACGGATTCAAATCAACGCCGCTGCAGCTA | GCAACCGCAGTCCACATAGTATTCAT |
| Al_BA_grs_2809\%_287 | gatgatgaccaagttcatgcttttatctancacgiangatantgagtca | GaAGgTCGGAGTCAACGGATTATCTAACACGGAAGATAATGAGTCG | gCGCCACGGGGTGGTGTGA |
| Al_BA_grs_49516.365 | GAAGGTGACCAAGTTCATGCTTTGGACCCAATATGTTTGGGGAA | GAAGGTCGGAGTCAACGGATtTGGACCCAATATGITTGGGGAC | CCACTTCTTCAATATTGAGAGACAAGCAT |
| Al_BA_grs 55748.286 | TGACCAAGTTCATGCTTGTTGTGGTTGCTGGTTGATCAGA | AGGTCGGAGTCAACGGATTGTTGTGGTI | gangancangattaggiclagcancatant |
| Al_BA_grs $50960 \_123$ | GAAGGTGACCAAGTTCATGCTTGTTACTCCCTCCAATCCATATTACT | GAagGTCGGAGTCAACGGATTGTTACTCCCTCCAATCCATATTACC | GGAACTACTTCCCTCCGATCCATAT |
| Al_BA_rsf_ 63693 _289 | GAAGGTGACCAAGTTCATGCTTGTGCTTATCAAAGTGATGCTTCCTA | GAAGGTCGGAGTCAACGGATTGTGCTTATCAAAGTGATGCTTCCTC | GCCACCATCTTGGAAAGCAAGA |
| Al_BA_zrs_70822_144 | GAAGGTGACCAAGTTCATGCTTGGCCGGGAATCTGTTTGTCTTA | GAAGGTCGGAGTCAACGGATTGGCCGGGAATCTGTTTGTCTTG | GCTGTGGTCTCGTCTCGCTCTA |
| Al_BA_grs_75304_237 | GAAGGTGACCAAGTTCATGCTTGATGCTTTGTGTCTTGCCACAAAAT | GAAGGTCGGAGTCAACGGAtTGATGCTtTGTGTCTTGCCACAAAAA | TCAAAATGGCACTATGGTTGCACACAAA |
| Al_BA_grs_23792 245 | GAAGGTGACCAAGTTCATGCTTCCTGGGCAAACATGGACACTA | GAAGGTCGGAGTCAACGGATTCCTGGGCAAACATGGACACTG | tTGCAGATTATGCAGAGGCAGGAAATA |
| Al_BA_grs_73682-333 | gaAGGTGACCAAGTTCATGCTTCATTCAAAGTAGGTAAGATGCtaAgaat | gaiggtcgangtcancgiattcattcanagtaggtangatgctangang | CtTCCATGAACTTGGTACAATCTtTCATTA |
| Al_BA_grs 66690 _31 | GAAGGTGACCAAGTTCATGCTTCATGACACTAATCGGTGATGCGT | aggtcgaagtcaacggattcatgacactaitcggtgatgcgi | ACCTGGTCGTCCTGAAGCCGTT |
| Al_BA_grs_21436_323 | GAAGGTGACCAAGTTCATGCTTATTTATTCCAGGCATTTGGGAGCAA | GAAGGTCGGAGTCAACGGATTTATTCCAGGCATTTGGGAGCAG | AATCGCTAAAATTGAAGTAGACCTTACCTT |
| Al_BA_grs 3947\% 320 | GAAGGTGACCAAGTTCATGCTTACTCTGTTAAGAGTTTAAACTTATGATGTTA | gaaggtcgaagtcaacgiattctctgttangagtteaacttatgatgttg | GGAAGATCACAGTATGATTCGGTAACATT |
| Al_BA_grs 38928_159 | GAAGGTGACCAAGTTCATGCTGTGTTGCATTTGTTGATCTCTATGTGTA | baAGGTCGGAGTCAACGGATTGTTGCATtTGTTGATCTCTATGTGTG | TACAATCCTCACAATCTACACGAAAGCAA |
| Al_BA_grs_2094_153 | GAAGGTGACCAAGTTCATGCTGTCCAGATATGGTGGAAGCCCAT | GAAGGTCGGAGTCAACGGATTCCAGATATGGTGGAAGCCCAC | CGTGGGCCACTCGAAGTCTTCAT |
| Al_BA_rss_20446_192 | GAAGGTGACCAAGTTCATGCTGTATGCATAATGATAGTGTTTTGTTGTATGTT |  | CaCTCACTTGCTACTCCACAAACCTA |
| Al_BA_grs 54317-241 | GAAGGTGACCAAGTTCATGCTGGTTAAATCAGGCACGCACATCAAT | GAAGGTCGGAGTCAACGGAtTGTtaAATCAGGCACGCACATCAAC | TGAATGAGTATATTTTCGACTGCTCTGTTT |


| Al_BA_grs_7468_252 | GaAGGTGACCAAGTTCATGCTGGTGTCATGAAGCACTTGGTGCA | GAagGtcgaagtcaacgiattgigtcatgaagcacttcgigch | acgcttcatgcagacaatgganaicata |
| :---: | :---: | :---: | :---: |
| A1_BA_grs 35480_424 | AGGTGACCAAGTTCATGCTGGGTTTAACATAACTGTTGTAGCTTCTA | GGTCGGAGTCAACGGATtGGTtTAACATAACTGTTGTAGCTTCTG | Cagctacaatttantccacaicticta |
| Al_BA_grs_3059_-114 | GAAGGTGACCAAGTTCATGCTGGCTTTCTATGTCTTGTGTTCCTCT | GAAGGTCGGAGTCAACGGATTGCTTTCTATGTCTTGTGTTCCTCC | CTATGAAATTAAATTCAACCAACACACAAT |
| Al_BA_grs_10525_144 | GAAGGTGACCAAGTTCATGCTGGATCCGGAGTGGATTGCGCT | GAAGGTCGGAGTCAACGGATTGATCCGGAGTGGATTGCGCG | CTCGGTCCCTCATTCCAGGCAT |
| Al_BA_grs_81326_338 | GAAGGTGACCAAGTTCATGCTGGAGCGGTGATCTGTGGTCGT | GaAGgTCGGAGTCAACGGATTGAGCGGTGATCTGTGGTCGC | agtcactctatccatgtcccagat |
| Al_BA_grs_105739_119 | GAAGGTGACCAAGTTCATGCTGCAAGTGGCCACACTTGAGAGA | GAAGGTCGGAGTCAACGGATTCAAGTGGCCACACTTGAGAGC | TTGGTGTTCCGCGTGAACTTCACTT |
| Al_BA_grs_57018_162 |  | GAAGGTCGGAGTCAACGGATTATGGCTTGAAGAATCAGAGTGTCAG | gTaAGATGCTTGAACTAAGTtTGTCGGTT |
| Al_BA_grs_44314_197 | gangGtgaccaagttcatgctgatanaacacatagttctcacacgagtt | Gatggtcgantcancgaattatanaicacatagttctcacacgagtc | tTGTGCCTGCGAGATACTATtTGCTATtT |
| Al_BA_grs $89103-286$ |  | GAAGGTCGGAGTCAACGGATTGAGCATGTATTATCATGAGAGCTTCC | gTGGTCCTTATGTtTGTTGCAACAACTAT |
| Al_BA_grs_10303_214 | GAAGGTGACCAAGTTCATGCTGAGCAAATGGAAATTAGTGTAAAACCCA | GaAggtcgaagtcancgaattagcaatggaattagtgtanacccg | CAGGAACGTTGTCCGTTGCGCTT |
| Al_BA_grs_106652_25 | GAAGGTGACCAAGTTCATGCTGAGAATCGTTCAGGAACATTGTCCA | GaAggTcGGagtcaicgiattagaitcgitcagGaicattgtcci | GGATTCAAACCCTGCATCAGAGTGTT |
| Al_BA_grs_4417_203 | gatggtaccaagttcatgctaatataagttcttatcgatatctttacca | GAagGtcgaagtcancgaattgantataagttcttatcgatatgitteacct | CTTCCAAGGTGAAACTTGCCCCTTT |
| Al_BA_grs_108904_232 | GAAGGTGACCAAGTTCATGCTGAAGATGTCTGGTACCATGCCTT | GaAGGtCgGagtcaacgeattgangatgictggtaccatgccta | CTTTTTCTATTCCATCACATACGTGTTCTT |
| Al_BA_grs S2581_191 | GAAGGTGACCAAGTTCATGCTGAAAGTACAAAACAGGTAAAGAAACCCT |  | tganaggtattattanccagcatctcaatt |
| Al_BA_grs_8059_119 | GAAGGTGACCAAGTTCATGCTCTATGCAAGACAGGTCTGTCGT | GAAGGTCGGagtcaacgaattctatgcangacaggtctatcic | GTGGTTCCTCCATCTACCCGACAA |
| Al_BA_grs_106905397 | GaAGGTGACCAAGTTCATGCTCGATtTCCACTGTCAGAAATTGCCT | GAAGGTCGGAGTCAACGGATtTTCCACTGTCAGAAATTGCCC | tgaatantattiattcgangctcctccaaa |
| Al_BA_grs_1742_329 | GAAGGTGACCAAGTTCATGCTCCGCTCATAGCAGTTCTGGCTA | gaaggtcgaagtaacgiattcgctcatagcagttctggcta | Aattttccttctacttaagaatttgagata |
| Al_BA_grs_7987-167 | GAAGGTGACCAAGTTCATGCTCCCGTTGAATCATGTCGACCATA | GaAGGTCGGAGTCAACGGATTCCCGTTGAATCATGTCGACCATG | GCTGTGTACCCATTGCGTGATATGAT |
| Al_BA_grs_3621_-169 | GAAGGTGACCAAGTTCATGCTCCCCAAGAAATAAAAGTGTGAATCAAGA |  | tTCCTTGAATATGCGCACTATCCACATA |
| Al_BA_grs_40585163 | GAAGGTGACCAAGTTCATGCTCCCAGCATAACAAAATACGGAAACAA | gatggtcgaagtcaacgiattcccagcatancanaatacgaancag | GTAGCTTGTAACCTATATGCACATTCACAA |
| Al_BA_grs 24451_323 | GAAGGTGACCAAGTTCATGCTCCATCATGGACTAATGTGTAATGGTA | GAAGGTCGGAGTCAACGGATtCCATCATGGACTAATGTGTAATGGTC | CCATGCACTTTTGTGCGGGTGGAA |
| Al_BA_grs 75715_300 | GaAGGTGACCAAGTTCATGCTCCACAAAAATGTTGAACAATTTCTCTTTCT | GAAGGTCGGAGTCAACGGATTCACAAAAATGTTGAACAATTTCTCTTTCC | aAtacatcctcataitgicattatgctt |
| Al_BA_grs 69833-156 | GaAGGTGACCAAGTTCATGCTCATGTTGACGTCATCGTGGTCATT | gaagitcgaagtcancgiattatgttaacgicatcgtgatcatc | GACGCGACCGCCGAGGCAA |
| Al_BA_grs_104369.94 | GAAGGTGACCAAGTTCATGCTCATCTACCGACATAAGCAACGGT | gaAggtcggagtcaacgiattcatctaccgacataagcancgic | actatgicanaggitacagacagacat |
| Al_BA_grs_2268_300 | GaAGGTGACCAAGTTCATGCTCATCAGAGTGTTGAGAAGTGCAACA | GAAGGTCGGAGTCAACGGAtTATCAGAGTGTTGAGAAGTGCAACG | atcattaattctticctitgicangacant |
| Al_BA_grs_14647345 | GAAGGTGACCAAGTTCATGCTCATAGGCAAGACACCAATACACCTT | gaaggtcgaagtcancgaattataggcangacaccantacacttc | ataa aiaat |
| Al_BA_grs 38953_122 | GAAGGTGACCAAGTTCATGCTCATACAAGGAAGGGTGGAtTTTGGAT |  | agctactactacticactaccatacta |
| Al_BA_grs 32165_167 | GAAGGTGACCAAGTTCATGCTCAGTGAGAAGATCGGGCGT | GAAGGTCGGAGTCAACGGATTCTCAGTGAGAAGATCGGGCGA | gTgGattgtggatgctcaccaagTa |
| Al_BA_grs_6867_101 | GAAGGTGACCAAGTTCATGCTCAGGCTCATCATCTTGCTCAGAT | GaAGgtcgaagtcancgaattcaggctcatcatcttgcteagac | agasgatgattccgaggangangatgatt |
| Al_BA_grs 39720_296 | GAAGGTGACCAAGTTCATGCTCACTTATGTTAATAAGCAAGCTTTGTTCT |  | AAATCCAACAGATGAAGGTCACGAGAAA |
| Al_BA_grs_2569_149 | GaAGGTGACCAAGTTCATGCTCACACACATAATTGAACAAGATATGAACA |  | Cagttgitchacttitatgatcatticta |
| Al_BA_grs_1736_249 | GAAGGTGACCAAGTTCATGCTCAATGCTGCAAATCTTGCAAATGTTTGT |  | GGCTAAGTTGGCTTGATGTTTGGCT |
| Al_BA_grs 62588_348 | GAAGGTGACCAAGTTCATGCTCAAGCTATTCATGTGGCTGGCAT | GaAGgTCGGAGTCAACGGATtCAAGCtattcatctggctgacag | GTCTAAAGGGTATGTCCGCAGTTCAA |
| Al_BA_grs_43399330 | GaAGGTGACCAAGTTCATGCTCAAGAGAAGAAATGACTTCTGAAGTACA |  | GCTTCGAGGTTTCCTTTTGCCTCAT |
| Al_BA_grs_105572_134 | GAaGGTGACCAAGTTCATGCTCAAGAACCTGCGGCCGAAGTTT |  | GCCCCGTCGCCACGTCACAT |
| Al_BA_grs_2578_310 | GaAGGTGACCAAGTTCATGCTATtTTTCCTTCGTTCTATATAAACACACTTATT | GAAGGTCGGAGTCAACGGAttTCCTTCGTTCTATATAAACACACTTATG | gacgagactgicatgccgatatana |




|  |  |  |  |  | S <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 |  | E <br> E <br> E <br> 4 <br> 4 <br> 0 <br> 0 <br> 0 |  |  |  |  |  |  |  |  | 1 |  | 気 U 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |  |  |  |  |  |  |  | $\begin{aligned} & 4 \\ & 0 \\ & 4 \\ & 4 \\ & 4 \\ & 4 \\ & 4 \\ & 4 \\ & 0 \\ & 0 \\ & 0 \\ & 0 \\ & 4 \\ & 0 \\ & 0 \\ & 0 \end{aligned}$ |  |  |  |  | $\begin{array}{l\|} \substack{4 \\ 4 \\ 0 \\ 0 \\ 4 \\ 0 \\ 0 \\ 4 \\ 0 \\ 4 \\ 0 \\ 0} \\ 0 \\ 0 \\ 4 \\ 0 \end{array}$ |  |  |  |  |
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|  | GAAGGTCGGAGTCAACGGATTCAAATTCTTGAAGTAAAAGGATCCAACAT |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | U <br> 0 <br> E <br> 0 <br> 0 <br> 0 |  |  |  |  |  |  |  |  |  |
| 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 4 0 0 0 |  |  |  |  |  |  | 0 <br> $\mathbf{S}$ <br> $\mathbf{S}$ <br> 0 <br> 0 <br> 0 <br> 0 |  |  |  |  |  |  |  | E |  |  | gaAGGTGACCAAGTTCATGCTAATGACCGTAGCAGCAAATACCG |  |  | GaAGGTGACCAAGTTCATGCTAAGGAAACAAAATGAGATATACAGCGC |  |  | U <br> 0 <br> 4 <br> 0 <br> 0 <br> 4 <br> 5 |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | (icl |
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| Al_BA_grs 58830_200 | GAAGGTGACCAAGTTCATGCTCCATGGCACAGATCTGGGACA | GAAGGTCGGAGTCAACGGATTCCATGGCACAGATCTGGGACG | GACTCAGGATGGGGAGGAGGA |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_73447_285 | GAAGGTGACCAAGTTCATGCTCCATCTCCGCCTTCCCCTCA | GAAGGTCGGAGTCAACGGATTCATCTCCGCCTTCCCCTCG | GTCTGGACCTTACCTGACATCGATT |
| Al_BA_grs_56980_100 | GAAGGTGACCAAGTTCATGCTCATTATGAGAAGAAAATGAGTGCTGTCAT | GAAGGTCGGAGTCAACGGATTATGAGAAGAAAATGAGTGCTGTCAG | GTGCTCATCATAATTCATAAGTGTGCCAA |
| Al_BA_grs_46633_285 | GAAGGTGACCAAGTTCATGCTCATTACAAGGATTTAGTAGGTAAATAACATATT | GAAGGTCGGAGTCAACGGATTACAAGGATTTAGTAGGTAAATAACATATG | tttgtacattgicattgatgatagcatata |
| Al_BA_grs_21036_210 | GAAGGTGACCAAGTTCATGCTCATGTCGTTAGAAACCAATACCTCT | GAAGGTCGGAGTCAACGGATTCATGTCGTTAGAAACCAATACCTCG | AGTTTCGTGAGGATCCTACAGCCAT |
| Al_BA_grs_103043_193 | GAAGGTGACCAAGTTCATGCTCATGCAAAAATAGATGAAATAACATAAGTATCA | GAAGGTCGGAGTCAACGGAtTATGCAAAAATAGATGAAATAACATAAGTATCG | GGGAGGGGGTGATACGTCCATT |
| Al_BA_grs_11338_127 | GAAGGTGACCAAGTTCATGCTCACCACTAACAAGGATTCTTTCTGCA | GAAGGTCGGAGTCAACGGATTACCACTAACAAGGATTCTTTCTGCG | GCGATCATGGACATCAACCCTTGTT |
| Al_BA_grs_35877_183 | GAAGGTGACCAAGTTCATGCTATTTTTCTAATCTTCTTTTGTATCTTCTTCCA | GAAGGTCGGAGTCAACGGATTTTCTAATCTTCTTTTGTATCTTCTTCCG | GAATAGGGAGGGAAGGTGGGGTA |
| Al_BA_grs_107962_149 | GAAGGTGACCAAGTTCATGCTATTCGTTGTATACCGGTT | GAAGGTCGGAGTCAACGGATTCGTTGTATACCGGTG | CTACGGAAACAAACACCCCAAACAATATA |
| Al_BA_grs_45108_445 | GAAGGTGACCAAGTTCATGCTATTATCCCTCTTTCTACCTATTTTATATTTCT | GAAGGTCGGAGTCAACGGATTATCCCTCTTTCTACCTATTTTATATTTCC | GAGGTTCCAGGGAAGTATAAAGGAGTA |
| Al_BA_grs_26197_264 | GAAGGTGACCAAGTTCATGCTATTACTTCAGTAATGACCTCTCCTTATTA | GAAGGTCGGAGTCAACGGATTACTTCAGTAATGACCTCTCCTTATTC | TCTCATTAGAGGTCATTCTTGGCACAAA |
| Al_BA_grs_19842_193 | GAAGGTGACCAAGTTCATGCTATCGCCACCTGGTATAGGCGA | GAAGGTCGGAGTCAACGGATTCGCCACCTGGTATAGGCGG | ttTGAGGAAGTACACACGACCAAGTAATT |
| Al_BA_grs_53256_289 | GAAGGTGACCAAGTTCATGCTATCCGCTGTTGCGGTTGTCGTT | GAAGGTCGGAGTCAACGGATTCCGCTGTTGCGGTTGTCGTC | AGGACCGCGAAGAGGTCGACAT |
| Al_BA_grs 37642_255 | GAAGGTGACCAAGTTCATGCTATCAATTCGACGAAAGTAGATCCTCAA | GAAGGTCGGAGTCAACGGATTCAATTCGACGAAAGTAGATCCTCAC | agGatccgatcganagttctgattcttat |
| Al_BA_grs_52644_193 | GAAGGTGACCAAGTTCATGCTATAGATTTCAATATAACTTGTCCATTAAAGGT | GAAGGTCGGAGTCAACGGATTATAGATTTCAATATAACTTGTCCATTAAAGGA | CCACCGGTGCCTCTGCATTAGTT |
| Al_BA_grs_80127_217 | GAAGGTGACCAAGTTCATGCTATACGAGGAGGCTCCACAAGGT | GAAGGTCGGAGTCAACGGATTATACGAGGAGGCTCCACAAGGA | AATATCGAGGGAGTGAGGATGCCAT |
| Al_BA_grs_86814_283 | GAAGGTGACCAAGTTCATGCTAGTCTGGGACAATAAACATTGAACACA | GAAGGTCGGAGTCAACGGATTGTCTGGGACAATAAACATTGAACACG | CCAACTATCAGGTTGATGATGAAATCCTT |
| Al_BA_grs_59767_194 | GAAGGTGACCAAGTTCATGCTAGGGAGAAGTTGTTCGAAGAGCA | GAAGGTCGGAGTCAACGGATTGGGAGAAGTTGTTCGAAGAGCG | GagCatcheanamatccagccanaigat |
| Al_BA_grs_47079_104 | GAAGGTGACCAAGTTCATGCTAGCAGCGAGGAAGAGCACCAA | GAAGGTCGGAGTCAACGGATTGCAGCGAGGAAGAGCACCAG | CTCGATGAGAGATAGAGGAGGACAA |
| Al_BA_grs_11530_300 | GAAGGTGACCAAGTTCATGCTAGATTTATCTAAATTTTGACAAATCTAAGACAT | GAAGGTCGGAGTCAACGGATTAGATTTATCTAAATTTTGACAAATCTAAGACAA | CGACCGCTACTCGCTCTGTtTTAAA |
| Al_BA_grs_53772_252 | GAAGGTGACCAAGTTCATGCTAGATCTCGAGCAAGATCGCTCTAT | GAAGGTCGGAGTCAACGGATTGATCTCGAGCAAGATCGCTCTAC | tactGgctittanctattgcacgatcgat |
| Al_BA_grs_44524_221 | GAAGGTGACCAAGTTCATGCTAGAGTGGCAGATACATTGGTGTCAA | GAAGGTCGGAGTCAACGGATTAGTGGCAGATACATTGGTGTCAG | ACATTGCTCGGGTTCGCTAACTCAT |
| Al_BA_grs_33480_316 | GAAGGTGACCAAGTTCATGCTAGAGCTGCCTATAATGATCCAGCTA | GAAGGTCGGAGTCAACGGATTAGCTGCCTATAATGATCCAGCTG | TAGGGGACTACATCAATTCAACTGCATTA |
| Al_BA_grs_41650_119 | GAAGGTGACCAAGTTCATGCTAGACATTTACATGGTTGAAAGAAGTTTGA | GAAGGTCGGAGTCAACGGATTGACATTTACATGGTTGAAAGAAGTTTGC | GGAATAAGCAGTGCCTAAAGTAGCAAA |
| Al_BA_grs_41971_299 | GAAGGTGACCAAGTTCATGCTAGACATATTATCGCGTCGGGCAT | GAAGGTCGGAGTCAACGGATTGACATATTATCGCGTCGGGCAC | CAGCAACTCTAACAGCCAAACAAGGTA |
| Al_BA_grs_83083_209 | GAAGGTGACCAAGTTCATGCTACTTTCCTAATTGGACCACCCCA | GAAGGTCGGAGTCAACGGATTCTTTCCTAATTGGACCACCCCG | TGGACTGTGTCTTGGAGCCCATTA |
| Al_BA_grs_105440_108 | GAAGGTGACCAAGTTCATGCTACTTGGACAAGCGGAGGAGT | GAAGGTCGGAGTCAACGGATTACTTGGACAAGCGGAGGAGC | TTTGCTGCTGAGATTTGGAGCATTTGAT |
| Al_BA_grs_51204_398 | GAAGGTGACCAAGTTCATGCTACTGGTTGCAGACCACGGAGA | GAAGGTCGGAGTCAACGGATTCTGGTTGCAGACCACGGAGG | CCAAATAATGGGTGGCAAACAGTACTTT |
| Al_BA_grs_89134_157 | GAAGGTGACCAAGTTCATGCTACTGGTTCTGTCACAAAGATACAAATTAT | GAAGGTCGGAGTCAACGGATTACTGGTTCTGTCACAAAGATACAAATTAA | CATTACATTGTGCCACCCCATAGTTTAAA |
| Al_BA_grs_72129_148 | GAAGGTGACCAAGTTCATGCTACTGGTTCCAACGGAACATTCTGTT | GAAGGTCGGAGTCAACGGATTGGTTCCAACGGAACATTCTGTG | CGGTACGCCGTATACTGACCCTT |
| Al_BA_grs_87629_432 | GAAGGTGACCAAGTTCATGCTACTGGAAGCCAATGAGAAGGTACTA | GAAGGTCGGAGTCAACGGATTGGAAGCCAATGAGAAGGTACTG | CAAGCTCTTGCATCTCCTACCAGAT |
| Al_BA_grs_31176_165 | GAAGGTGACCAAGTTCATGCTACCCATCAGGGAGAATATCATTTTGT | GAAGGTCGGAGTCAACGGATTCCCATCAGGGAGAATATCATTTTGC | GTGATTAAAATCAACTCGGGTACCACATT |
| Al_BA_grs_31932_150 | GAAGGTGACCAAGTTCATGCTACCAATCCACCAATGCGAATGTCAT | GAAGGTCGGAGTCAACGGATTCAATCCACCAATGCGAATGTCAC | TCATCCAACACTTACGTTACGAAAGCAA |
| Al_BA_grs_23681_127 | GAAGGTGACCAAGTTCATGCTACATGGAGCACTAAAAGCATATTCGA | GaAGGTCGGAGTCAACGGATTCATGGAGCACTAAAAGCATATTCGG | CTTTTCGACTGCTGTCATTTTTTGGTTTTA |
| Al_BA_grs_47354_274 | GAAGGTGACCAAGTTCATGCTACATGCATAGAAGTTTTTGATGCAATAGT | GAAGGTCGGAGTCAACGGATTCATGCATAGAAGTTTTTGATGCAATAGG | CCTCTTCAATTGACAAACAAGAATTAATAA |
| Al_BA_grs_60258_411 | GAAGGTGACCAAGTTCATGCTACATGAAGGACTTTTGCATAAGCCATT | GAAGGTCGGAGTCAACGGATTCATGAAGGACTTTTGCATAAGCCATC | CATTCTCTAATGGATGCTTTGGTATTTGTT |



| Al_BA_grs_ 30365191 | gaaggtgaccaagttcatccttcatggicttctcaatccga | GAAGGTCGGAGTCAACGGATTGCTTGATGGTCTTGTCAATCCGA | gGacctgcaatacgatccgia |
| :---: | :---: | :---: | :---: |
| Al_BA_grs 52811 _326 | gaAGgTGACCAAGTTCATGCTTGAAGTGGCatGaccatgicatc | GAAGGTCGGAGTCAACGGATTGTTGAAGTGGCATGACCATGTCATT | gaatangtcaitcatctcactccaccata |
| Al_BA_grs 32880_174 | GAAGGTGACCAAGTTCATGCTTCTCCGAGGTGTAATGGACGC | GAAGGTCGGAGTCAACGGATTTCTCCGAGGTGTAATGGACGT | GACAAATCTCTTCATCCTTCCGAGAAAT |
| rs- | GGTGACCAAGTTCATGCTTCCACACCTTTCGTATTAATAAG | gatgitcgaatcancgaattcttccacacctitcgtattaatangitatai | CaAAttangtgctcgancagtanaactt |
| Al_BA_grs 52683_312 | GAAGGTGACCAAGTTCATGCTTCATCTTTGGTTCTCTTCCAGAC | GAAGGTCGGAGTCAACGGATTCTTCATCTTTGGTTCTCTTCCAGAT | GAAGGAAGAGGAAAGCATGCCAGAA |
| Al_BA_grs_ 53746 _224 | GAAGGTGACCAAGTTCATGCTTCAAGTGAGAGATTGCCATGATTTG | GAAGGTCGGAGTCAACGGATTGTTCAAGTGAGAGATTGCCATGATTTT | attctantcctatagaagctccttacait |
| Al_BA_grs 35289_349 | gaaggtgaccaagttcatGcttatttggaggtgagcteactcta | GAAGGTCGGAGTCAACGGATTGTTATTTGGAGGTGAGCTCACTCTT | gatgitcactacangictcgtagga |
| Al_BA_grs_23002_337 | GAAGGTGACCAAGTTCATGCTGTGGCCTCCCAAACATAACACG | gaiggtcgaagtcancggattcgtcgcctcccaaicataicaca | Aattcatttatcatcaatctattattect |
| Al_BA_grs_1138_-159 | GAAGGTGACCAAGTTCATGCTGTGCTGGATTCTGTTGTTGTGAATC | GAAGGTCGGAGTCAACGGATTGTGCTGGATTCTGTTGTTGTGAATA | GTGAAAATTTGCAACATGGTTCAAAGGAAT |
| Al_BA_grs_4766_114 | gaaggtgaccaagttcatGctgigatantcatttgiccatgattittgic | GAAGGTCGGAGTCAACGGATTGTGATAATCATtTGTCCATGATTTTTGGA | GTGCAAACCAACCATTGGAAAGAGAAATT |
| Al_BA_grs 47188.142 | GaAGGTGACCAAGTTCATGCTGTCCGTATATCATGTTCTCGTCG | GAAGGTCGGAGTCAACGGATTAGGTCCGTATATCATGTTCTCGTCA | CAGCTCATAGTCCTCCGAAACGAA |
| Al_BA_grs_ 22224318 | GAAGGTGACCAAGTTCATGCTGTCACTCACACGGCCACGG | GAAGGTCGGAGTCAACGGATTGGTCACTCACACGGCCACGA | CTGCTGGCCAGTCGTGGCGAT |
| A1_BA_grs 24550 366 | GAAGGTGACCAAGTTCATGCTGTATCAAGGTGACAGTGAGCTCG | GAAGGTCGGAGTCAACGGATTGTATCAAGGTGACAGTGAGCTCA | a ACAGACCAGCCGCCGtTGCTA |
| BA_grs_11448_330 | gtacactaaccganatcaccc | GAAGGTCGGAGTCAACGGATtCGTACACTAACCGGACATCACCT | Gacagtccgatctuctgaaia |
| Al_BA_grs 78633_101 | gatagtaaccaagttcatgctagtactatcgigaggtcgac | GAAGGTCGGAGTCAACGGATTGGTGCTGTCGTGAGGTCGAT | CAGCTTAATTCTAAGTTCTAATGCTGGCAT |
| Al BA grs 32742_102 | GAAGGTGACCAAGTTCATGCTGGTGCctGacgatgactci | GAAGGTCGGAGTCAACGGATTCTGGTGCCTGACGATGACTCA | tCcGGGagctatacticcica |
| AI_BA_grs_16269_315 | GAAGGTGACCAAGTTCATGCTGGTCGACTACATACTCGACGG | GAAGGTCGGAGTCAACGGATTATGGTCGACTACATACTCGACGA | CCTCTGGGGTCTCAAGGTCCAT |
| Al_BA_grs 82583 373 |  | GAAGGTCGGAGTCAACGGATTGGGTCCACAATGAGCAAGTCCA | ССатСGтTGCTTCGTCATCтTCTGTT |
| Al_BA_grs_27250 399 | GAAGGTGACCAAGtTCATGctggtatcagttanccacctiaggtg | GAAGGTCGGAGTCAACGGATTCGGTATCAGTTAACCACCTTAG | CCGGGGACTCAAATTCGAGTTAGAT |
| Al_BA_grs 59169.329 | GAAGGTGACCAAGTTCATGCTGGGTTACCGGTATtCTTCGTCG | GAAGGTCGGAGTCAACGGATTGGGGTTACCGGTATTCTTCGTCA | CGTTCCCGTCCACCTCCGCAA |
| Al_BA_grs_14011_86 | gaAGgTGaccaagttcatgctaggacgitchcctanctang | GAAGGTCGGAGTCAACGGATTAGGGGGGGTTCGCCTAACTAAA | Catacaigttagacatgcattccgactt |
| Al_BA_grs 32174 _235 | GaAGGTGACCAAGTTCATGCTGGGGCCTtTGGAAGCATtTCTTC | GAAGGTCGGAGTCAACGGATTGGGGCCTTTGGAAGCATTTCTTT | ATTGGATAGAGAAAATGGCACCACCAA |
| Al_ BA_grs 78039 - 332 |  | GAAGGTCGGAGTCAACGGATTATGGGGATATGAGTATCACGAGCA | gttGcaacaacatangaactacttgctt |
| Al_BA_grs 12696405 | GAAGGTGACCAAgTTCATGCTGGCCTCCTCGCTAACC | GAAGGTCGGAGTCAACGGATTGCTGGCCTCCTCGCTAACT | ttgtagctacgitgicgactgatt |
| A1_ BA _grs 27804_162 | GaAGGTGACCAAGTTCATGctGgcanatatcgacacticattci | GAAGGTCGGAGTCAACGGATTGGGCAAATATGGACACTGCA | taAGCCAAGACTAATCCGTTGGAtTCTTT |
| BA_-ris 48665 _240 | gigaccaagttcatgctggatgitacaagtgatccttag | GAAGGTCGGAGTCAACGGAtTGGGATGTTACAAGTGGTCCTTAGA | CTAACCCCGTGCTGGTCTCCAA |
|  | gatggtaaccaagttcatgctagacaagtacgccaccaccg | GAAGGTCGGAGTCAACGGATTGGACAAGTACGCCACCACC | gGcattgatcgtccaccacgat |
| A1 BA grs 55573159 | gaAGgTGaccaagttcatgctganagagtcacagaigcgicag | TCAACGGATTGGAAGAGTCACAGA | tCCCtttcgicagtgaagcac |
| Al_BA_grs $59466 \_289$ | GGTGACCAAGTTCATGCTGCCTGACAATGACTTTTATGATGCG | GAAGGTCGGAGTCAACGGATTGGCCTGACAATGACTTTTATGATGC | gGtaAtaattgittcattgatcgatccgra |
| Al_BA_grs 58339,94 | gaAGgTGaccaagttcatcctocctctatchaacaccgtac | GAAGGTCGGAGTCAACGGATTGGCCTCTGTCCGACACCGTAT | ataatahtgtteagatataggiaggtccat |
| Al_BA_grs 97232 _198 | GAAGGTGACCAAGTTCATGCTGCCGAGCAGGTTCGTCCAC | GAAGGTCGGAGTCAACGGATTGGCCGAGCAGGTTCGTCCAT | aAATCTGTGGGGCGTCACAGCAAT |
| Al_BA_grs 33469.357 | GAAGGTGACCAAGTTCATGCTGCCCGAACCAAAGAAGTAGTCG | GAAGGTCGGAGTCAACGGATTGCCCGAACCAAAGAAGTAGTCA | GaСТСтTССТССастССстССа |
| Al_BA_grs_46578_124 | GAAGGTGACCAAGTTCATGCTGCCAGGCCATCAGATGATATCC | GAAGGTCGGAGTCAACGGATTGCCAGGCCATCAGATGATATCT | GCaCCTCCGCGGTGACAAACAT |
| Al_BA_grs 48157-183 | GAAGGTGACCAAGTTCATGCTGCAGCTGCTTGCCAGCGAC | GAAGGTCGGAGTCAACGGATTGGCAGCTGCTTGCCAGCGAT | Caggcccacatancctttcgatctt |
| Al_BA_grs_71494_201 | gaaggtgaccaagttcatgctgcacacaatacatcagaahgagaattg | GaAGGTCGGAGTCAACGGATtGCaCaCaAtacatcagaangagaatta | gTCTCGTTGCtattcgitattaatGctit |
| Al_BA_grs_12452-228 | GAAGGTGACCAAGTTCATGCTGCACAAGGACCAGGAGGAGAC | GAAGGTCGGAGTCAACGGATTGCACAAGGACCAGGAGGAGAA | CGTGGGTTGAAGTAGTCGAACTTGTA |


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| :---: | :---: | :---: | :---: |
|  |  |  |  |
| LVLODLOVLDOPLLLLLLVDOLJLLJVV |  | DJVODSLVDVDVDDVDLLLVLVOLODLVOLLDVVOJVDLDOVVD | $0 \varepsilon 1^{-} 29 L 6 \varepsilon^{-8 . s i s}{ }^{-1} \mathrm{Vg}^{-}$IV |
| LVDOVDVDODVVDLLDOJVLDıכد |  |  |  |
| VFOOVDOLLVVVYOLDODIVOLLVLLLLLI |  | OLLVODLLVODLLVVLOLVODVLVOLVOLODLVOLLDVVOJVOLDOVVD |  |
| LLVODLVLLODDLODLDVYODVVLLLDLL |  |  |  |
|  | VLLDLVOVODOVODDLVODLVOLLVDDOVYOLDVDDSLDOVY |  |  |
| LLLDOVOVDVLDLVLDLVDDOLLVDVLJL |  | OOSLOVLDOLLVOLVLOLDLDLVOLODLVOLLDVVOJVDLOOVVD |  |
| LLDLDOVDDODOLLVEDVVILSLLS |  |  |  |
| LLDLLOLVOJOOSLVVLVODLLLVILOV |  |  |  |
| LLDVYOLVOLVDVVOVVDLLVYDOLVDLL |  |  |  |
| VVDOLDVODOVOLDVOLDOLVLVVVFL |  | DOLVYDOLDVOL.DLDVDOOLODLVOLLDVYOJVOLDOVVD |  |
| VFODVLDOVLDOVVYVJVYOLVFVYODVLD |  |  |  |
| LLVVLDVYODLSLYOOLLOLLOLVLVVDLS |  | OLDLLDVDLVDLLVDOLVVOLOJOLODLVOLLDVVOJVOLDOVV | $99 \varepsilon^{-16+1 I^{-s . s i d} 8^{-} \mathrm{Vg}^{-} \mathrm{IV}}$ |
|  |  |  |  |
| LVLVJLLVLLDDJVDVDLDOLIDVJVJL |  | OVVOVVDOL.DVVVVLVOLLVOLVVVVVLVVDJLODLVOLLDVVJJVDLDSVVD |  |
| LVOLVVLLOOVDOVOOLDVLOVOVDLLVV |  | OLDVLLVVODVDOLLVDOVLDLVVDOLODLVLLDVVOJVOLDOVVD | 281-612t9 -ssis ${ }^{-1} \mathrm{Vg}^{-} \mathrm{IV}$ |
| VLDDOLOLLLVDDDOLDOLVLDVVLS |  |  |  |
| LVOTVILOULLLDODSDOLVID |  | TVDODLDSVODVLDDOLVDOLODLVOLLDVVOJVDLDSVVD | $86 \tau^{-5 z z o s-s s i s}{ }^{-1} \mathrm{Vg}^{-} \mathrm{IV}$ |
|  | YODOLDYDOL.LY |  |  |
|  |  |  |  |
| LLDOLDOLIOOLDLVDVVLDVDPVVI |  |  |  |
| LLLDLLVVLLLVOLLLOLLOLDODVVJDว |  |  |  |
| LLDLLLOOVODOOLDOLOVLOLLV |  | ODLLVOVDOYVDVVLLOODLDVYDLODLVOLLDVYOJVDLODVV | $16 \varepsilon^{-}$- $200 \varepsilon^{-5 \mathrm{ssid}^{-}-\mathrm{vg}^{-} \text {IV }}$ |
| LLDOLOVVLVLOLLLOOVVLVOLVJLLJVD |  |  |  |
| LLLVOLLLLDVLVOVLLDLJVYVLDODSLD |  | OLLDVLVLDOLVDOLLODOLLLLVYDLOOLVOLLDYVOJVDLDOVVD |  |
| LVDODOVDDLDOLDLDSLLVLVOLV |  |  |  |
|  |  |  |  |
| VLIVVVDVLVLLOVOVVLVOVOJOVTDLD |  |  |  |
| VLVLDOVODLLOOOVOOLLDLLVOD | LDLVVVLDLVLLכפV\%OวLDOD. |  |  |
| LLOLLLVYOVLDLYOLDJOLVYOLYOJVD |  |  |  |
| VLILODLVVVDLDVOLVOLLDOVDVSOVL |  |  |  |
| LLLDVEVOVVVDVVVOVVLVDVOLVLDLDS |  | OVFOVVOVOVLVLDVVLVLVVLVVODVDLLVDLODLVOLLOVVOJVDLODVVD |  |
| VVVDDOSDOODLLLLVOLVLVVOLDVLLLL |  |  |  |
| OLVLOJVVLDLVLVVVVVJLVVLDILVL | VDDLLOPV |  |  |
|  | VOVDVว | OJVDVILVLDOOLVOJLDVYODLOOLVOLLDVVJJVDLDOVV | E9\% ${ }^{-} 0 \varepsilon 89^{-\mathrm{ssid}^{-1} \mathrm{Vg}^{-} \mathrm{IV}}$ |


| Al_BA_grs_25906_286 | GAAGGTGACCAAGTTCATGCTCACTTCATGTGCGCCGTTATCC | GAAGGTCGGAGTCAACGGATTCCACTTCATGTGCGCCGTTATCA | GCGGAGGTGTCGCCTGCGT |
| :---: | :---: | :---: | :---: |
| Al_BA_grs_65050_207 | GAAGGTGACCAAGTTCATGCTCACTCGCCCATGAACTTTTGGG | GAAGGTCGGAGTCAACGGATTGCACTCGCCCATGAACTTTTGGA | GCCGTAGAGAAACAGTGTGTCGAAA |
| Al_BA_grs_48971_177 | GAAGGTGACCAAGTTCATGCTCACTACTTCTTTGTACTGTATTTGTTCG | GAAGGTCGGAGTCAACGGATTGCACTACTTCTTTGTACTGTATTTGTTCA | CGTCACATCTGGCTAATATGTCATAAGAT |
| Al_BA_grs_48040_202 | GAAGGTGACCAAGTTCATGCTCAATCCTGCATAAAAGGATTGGATAG | GAAGGTCGGAGTCAACGGATTAATTCAATCCTGCATAAAAGGATTGGATAA | GGGAAAGAATGAAATCTTTAGTGAGGAATT |
| Al_BA_grs_11460_254 | GAAGGTGACCAAGTTCATGCTCAAGTTTTCTGGAGAGATGACTTCG | GAAGGTCGGAGTCAACGGATTGCAAGTTTTCTGGAGAGATGACTTCA | GCAAATTGTTCAAGTGTTAAAGGATTTGAT |
| Al_BA_grs_44566_218 | GAAGGTGACCAAGTTCATGCTCAAGTAAGGTCGGTACATTAGCAC | GAAGGTCGGAGTCAACGGATTCCAAGTAAGGTCGGTACATTAGCAT | TGATTAATCTTCTTTGGTTGGATAAGTGTT |
| Al_BA_grs_55135_245 | GAAGGTGACCAAGTTCATGCTCAAATTGACCGTATGGCCCTCAG | GAAGGTCGGAGTCAACGGATTCAAATTGACCGTATGGCCCTCAA | GGTAGACGGTTTTCTAATATTGCCAGAAT |
| Al_BA_grs_55523_149 | GAAGGTGACCAAGTTCATGCTCAAAGCATCAATATCCAATGGCAACG | GAAGGTCGGAGTCAACGGATTACAAAGCATCAATATCCAATGGCAACA | CCTGTGGTTTGCTTGCAAGCATGTT |
| Al_BA_grs_34451_197 | GAAGGTGACCAAGTTCATGCTATTTTAACAGAGGCAAAACATTAAACAC | GAAGGTCGGAGTCAACGGATTAACTATTTTAACAGAGGCAAAACATTAAACAT | TATCAATGGAATCAGGAGAGAGAATCATTT |
| Al_BA_grs_46767_204 | GAAGGTGACCAAGTTCATGCTATTTGAGAATATGGAAATTAAGCCAAGAC | GAAGGTCGGAGTCAACGGATTCATTTGAGAATATGGAAATTAAGCCAAGAT | CTTACCTTAACACCAGTTGACAAAAGCTA |
| Al_BA_grs_66329_305 | GAAGGTGACCAAGTTCATGCTATTGCCACGGTTGCTTTTTCATTGC | GAAGGTCGGAGTCAACGGATTATTGCCACGGTTGCTTTTTCATTGT | CTTATTGCCACGAAATGATGTTCATTGGAA |
| Al_BA_grs_39022_147 | GAAGGTGACCAAGTTCATGCTATTGAAGAACAAGACTATCTATTTAGTTGC | GAAGGTCGGAGTCAACGGATTAATATTGAAGAACAAGACTATCTATtTAGTTGT | GCAACTTAAATTGCAGGCATAAGTGAAGTA |
| Al_BA_grs_49490_162 | GAAGGTGACCAAGTTCATGCTATTCTCGCCCCTGCTCCGG | GAAGGTCGGAGTCAACGGATTCTCGCCCCTGCTCCGA | GGGCGCTGAGTGAGGGGAAAT |
| Al_BA_grs_105835_262 | GAAGGTGACCAAGTTCATGCTATTCCAATCATTCACTTAAATATAAAGTGAAC | GAAGGTCGGAGTCAACGGATTATTCCAATCATTCACTTAAATATAAAGTGAAT | tCCCAAGTTTTAGATTTATGATAGGTATAA |
| Al_BA_grs_59440_334 | GAAGGTGACCAAGTTCATGCTATGTTATACCATGCAATTGTGCATC | GAAGGTCGGAGTCAACGGATTGCTATGTTATACCATGCAATTGTGCATT | TATGCAACAAGACAAAACAAGGTATGCCA |
| Al_BA_grs_106231_245 | GAAGGTGACCAAGTTCATGCTATGTGAAATTGAATTTCATGCACAACTTAG | GAAGGTCGGAGTCAACGGATTGATGTGAAATTGAATTTCATGCACAACTTAA | GGCATGCTTGCTTTCTTTGATCCAATATA |
| Al_BA_grs_49269_122 | GAAGGTGACCAAGTTCATGCTATGTAAAAGTGTTATGATATGGTTCTTGC | GAAGGTCGGAGTCAACGGATTCATATGTAAAAGTGTTATGATATGGTTCTTGT | TATATTATCACAGCCATCAATGAACAAGAT |

Indicates robust primer sets used for

* genetic mapping


[^0]:    contig 10844_F, AGTTTAACCCTTACCTCATCGAC

