



Cost effective and informative genotyping by sequencing using AgriSeq targeted sequencing for genotyping in the livestock industry.

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Brenda Murdoch, University of Idaho

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Ovine Targeted Genotyping-By-Sequencing Project

- Advancements in sequencing technology have led to decreased sequencing cost
- AgriSeq™ targeted Genotyping By Sequencing (GBS) is a cost effective and flexible genotyping system for Ovine
- Design a cost effective panel that uses amplicon targeted GBS to facilitate the application of genomics in the sheep industry



Objectives

- Evaluate the AgriSeq™ Targeted GBS solution as a genotyping system for Ovine
- Evaluate panel performance on field and control samples
- Panel design on *Ovis aries* Oar_v3.1, evaluate the panel against a new reference genome - Oar_rambouillet_v1.0
- Explore the novel genotypes based on the Oar_rambouillet_v1.0 reference



Materials and Methods: 1K Marker Panel Design

- Causative variants manually curated from publicly available information

	Phenotype	Gene	Type
Defects/ Disorders	Chondrodysplasia, Spider lamb	<i>FGFR3</i>	SNP
	Chondrodysplasia, Texel	<i>SLC13A1</i>	SNP
Disease predispositions	Resistance/susceptibility to lentivirus	<i>TMEM154</i>	SNP
	Resistance/susceptibility to spongiform encephalopathy	<i>PRNP</i>	SNP
Coat color	Coat color, agouti	<i>ASIP</i>	SNP
	Coat color, brown	<i>TYRP1</i>	SNP
Production traits	Fecundity	<i>B4GALNT2, BMP15, BMPR1B, GDF9</i>	SNP
	Muscular hypertrophy (double muscling)	<i>MSTN, DLK1</i>	SNP

Materials and Methods: 1K Marker Panel Design

- Causative variants manually curated from publicly available information
 - Defects and disorders
 - Disease predispositions
 - Production traits
- Parentage panel (Heaton et al., 2014)
- Remaining markers
 - dbSNP information retrieved from Ensembl
 - Sorted by minor allele frequency identified by **Axiom** 50K
 - Genome divided into 1000 evenly distributed intervals
 - SNPs preferentially chosen if in a transcript or QTL

Materials and Methods:

AgriSeq™ Targeted GBS Sequencing Workflow

10 ng gDNA
input

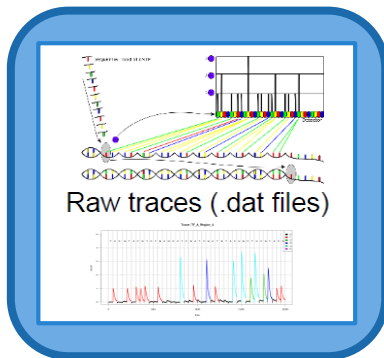


- Samples acquired from 7 different Superior Farms producers (n = 384)
 - DNA extracted from blood, tissue, semen, blood cards
- DNA samples for 4 rams are added as controls
- Samples were quantified using the Quant-It dsDNA High Sensitivity Assay kit
- Samples were normalized to 3.3 ng/uL
- Samples processed using the AgriSeq workflow

Materials and Methods: Analysis

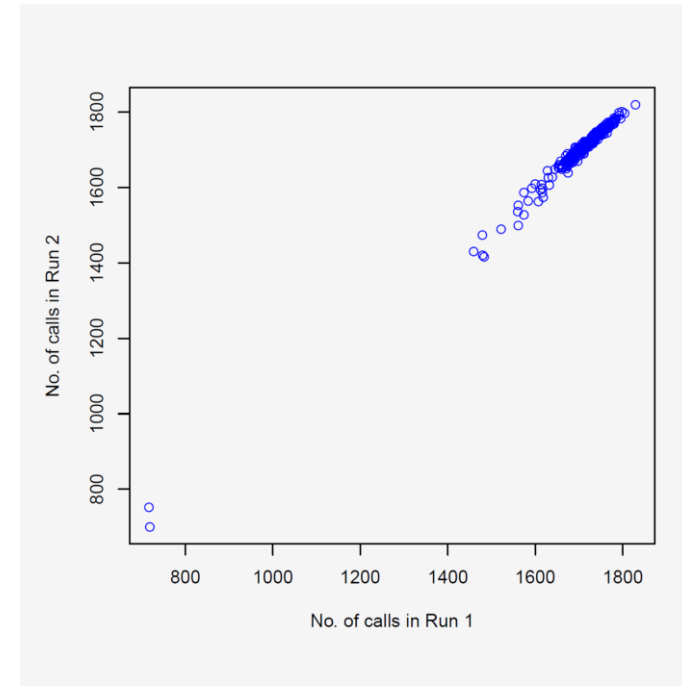
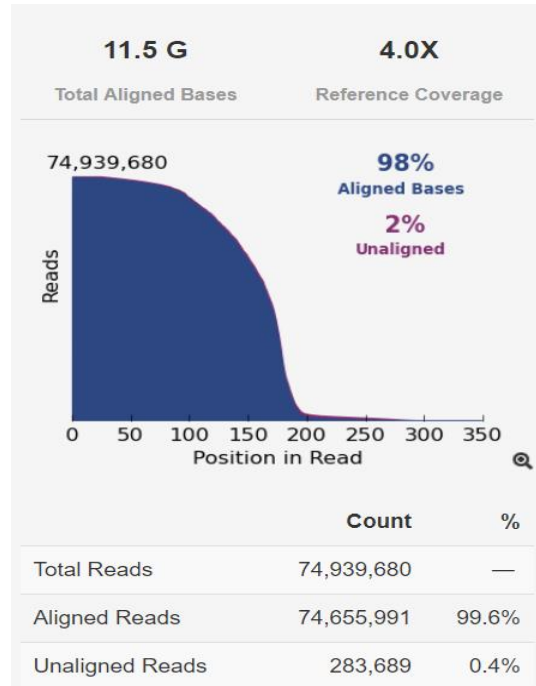
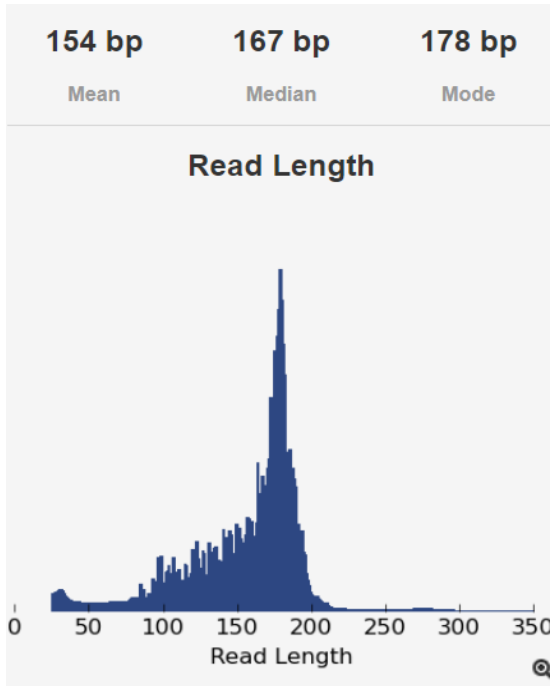
Ion Torrent Suite Software (TSS) Analysis Workflow

Raw data
processing



Results: Sequencing Summary

- The average read length is 154 bp
- 98% of the reads aligned
- High call reproducibility between replicates



Reference Genomes Comparison

- Ensemble version: *Ovis aries* Oar_v3.1.dna_sm.toplevel.fa
- GenBank version:
GCA_002742125.1_Oar_rambouillet_v1.0_genomic.fna

Sequence Entries	Oar_v3.1	Oar_rambouillet_v1.0
Genome Size	2,534,344,180	2,869,914,396
Number of chromosomes	1-26, X	1-26, X
Number of scaffolds	5,677	2,641

Poster # P163

Results: Sequencing Summary

	Oar_v3.1	Oar_rambouillet_v1.0
Panel Size (Targets)	999	999
Samples Tested*	384	384
Samples > 50X Read Depth	328	334
Reads Per Chip	73M	85M
Sample Call Rate	98.0%	97.2%
Sample Uniformity	97.8%	97.3%
Sample on Target	93.6%	92.5%
Average Coverage*	175.6X	181X

* Samples below the minimum threshold of 50X read depth are dropped from the analysis

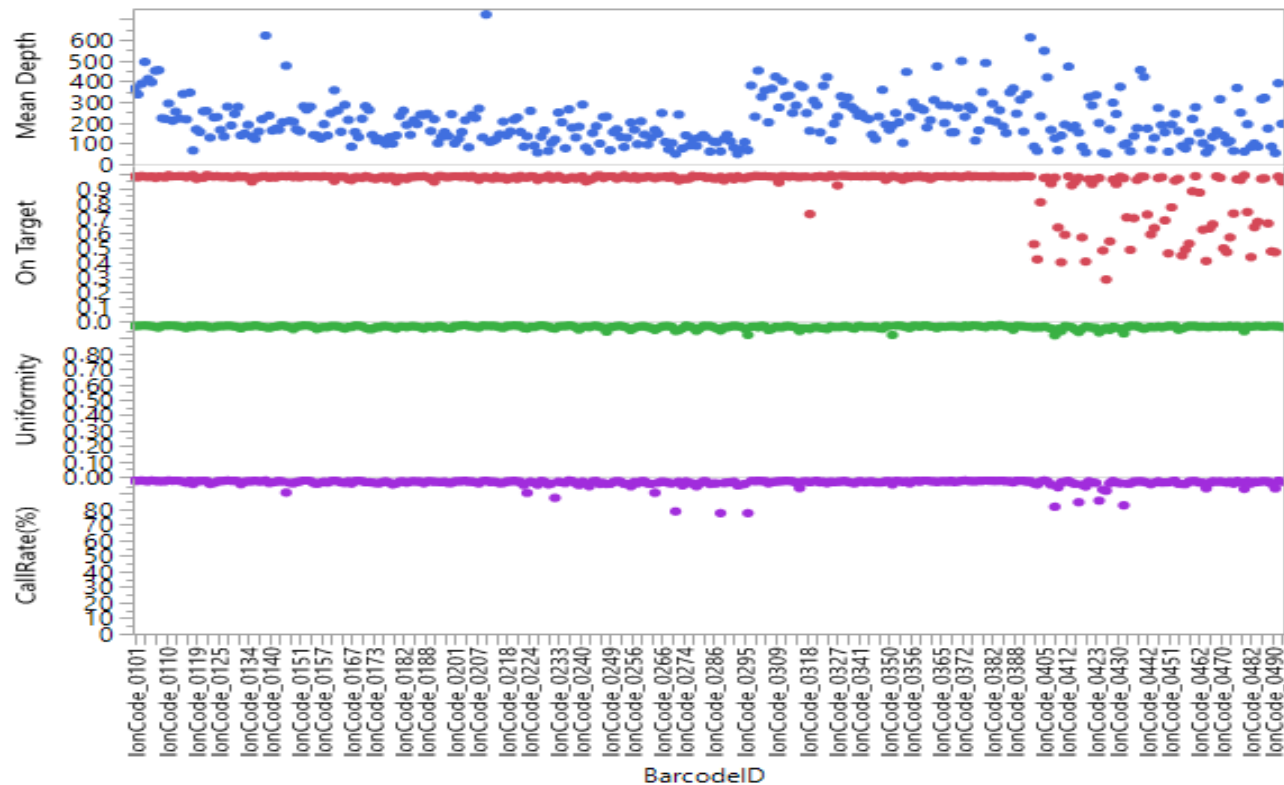
Results: Sequencing Summary

● Mean Depth
181 X

● On Target
92.5%

● Uniformity
97.3%

● Call Rate (%)
97.2%

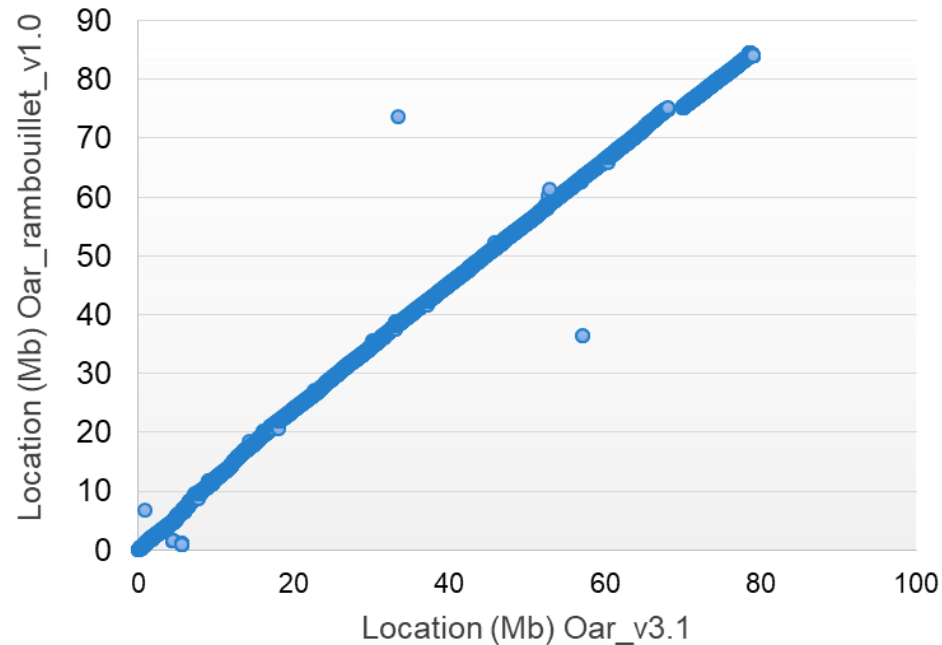


Reference Genomes Comparison

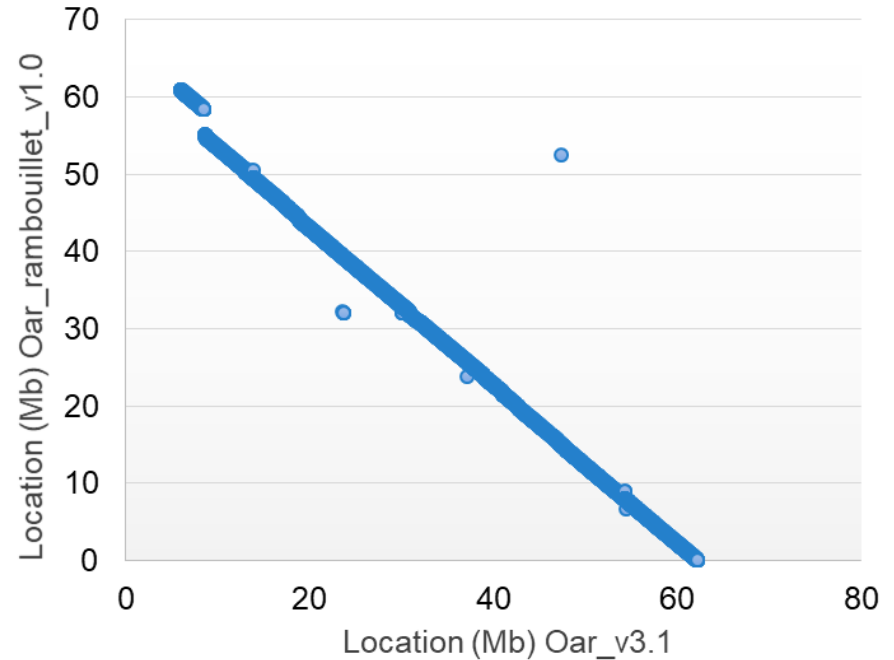
- New positions were obtained by uniquely mapping the amplicon sequences to the Oar_rambouillet_v1.0 genome
- 16 markers (multi-mapping) were dropped
- Alleles changed for 40 markers based on the amplicon mapping
 - Genotypes are called based on the new alleles from forward strand
- No major issues for the remaining 943 markers

Reference Genomes Comparison

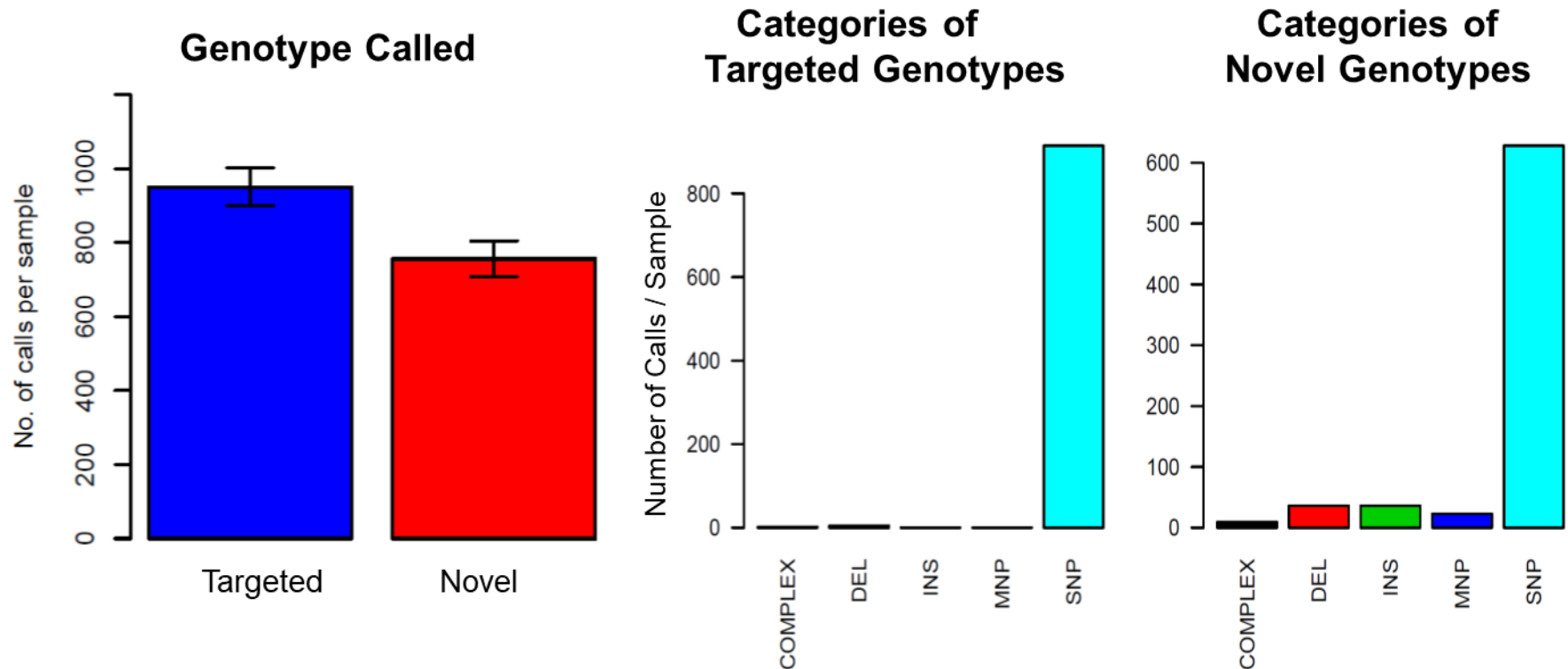
Ovine Chromosome 12



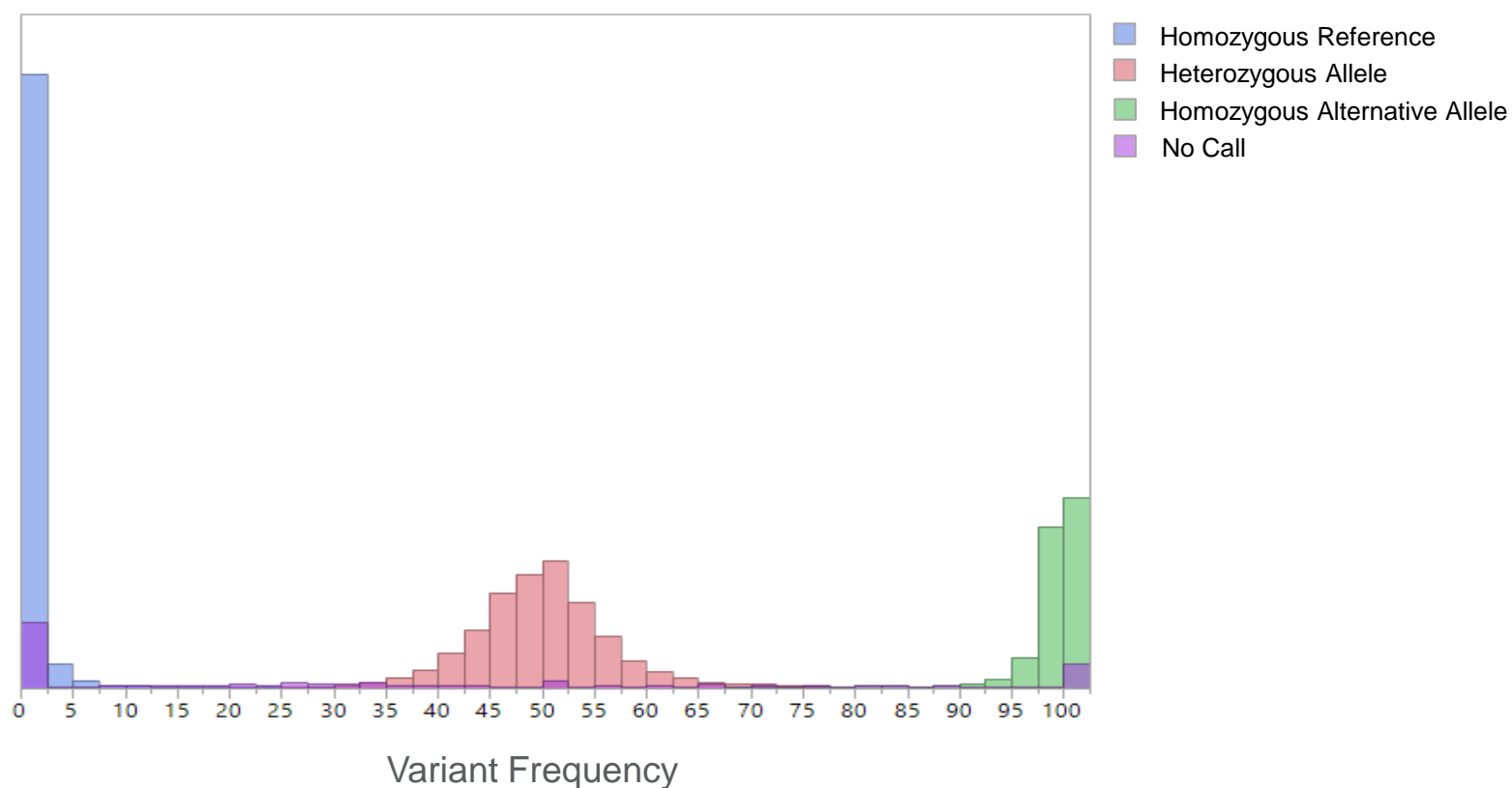
Ovine Chromosome 11



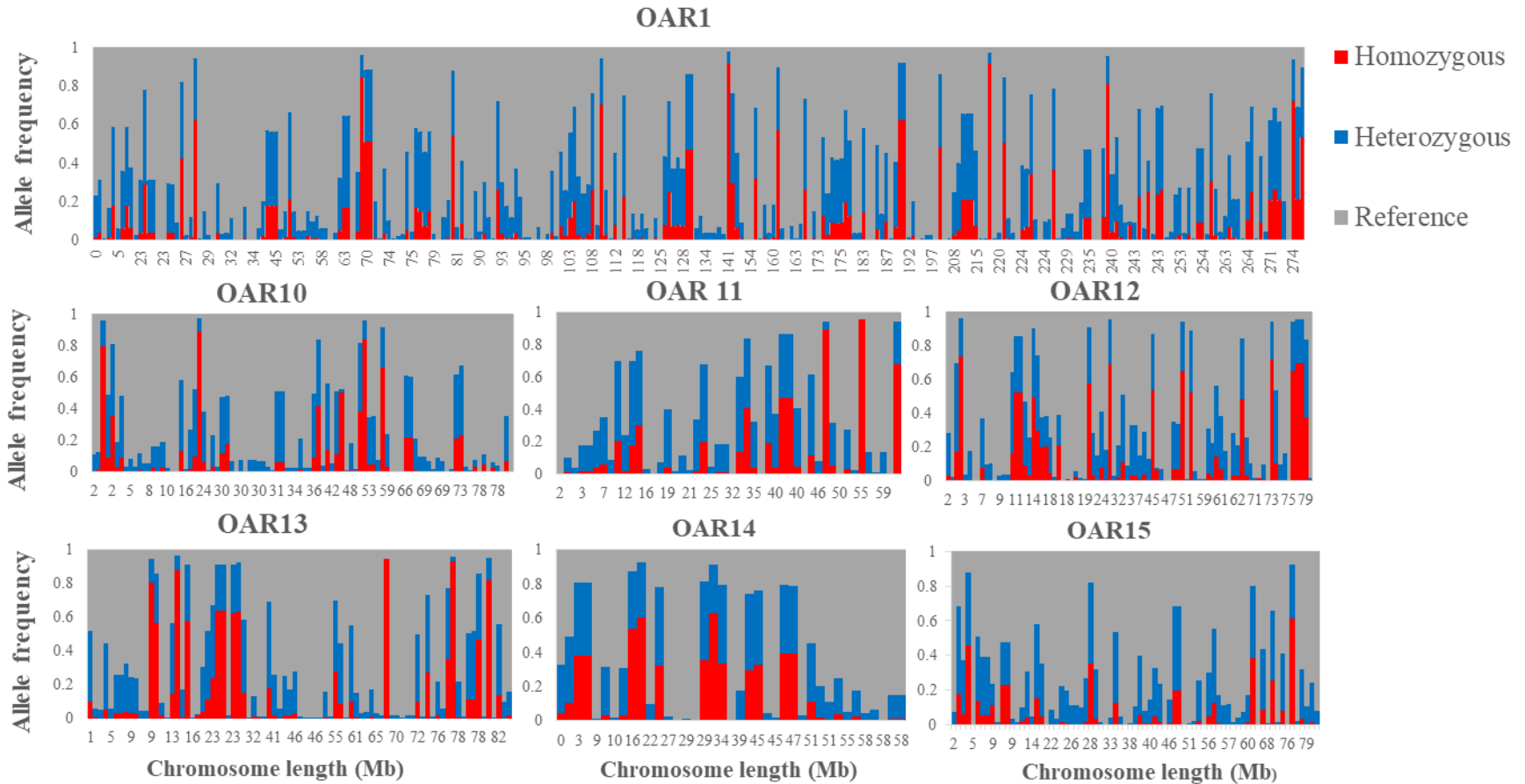
Results: Targeted & Novel Genotyping Calls



Results: Allele Frequencies for Targeted Variant

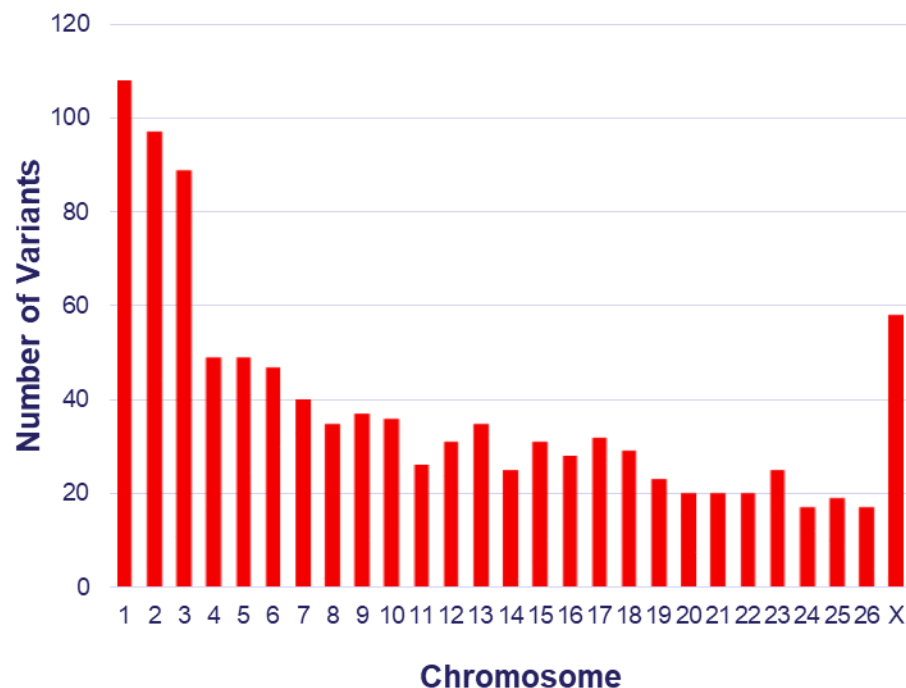


Results: Number and Frequency of Novel Variants

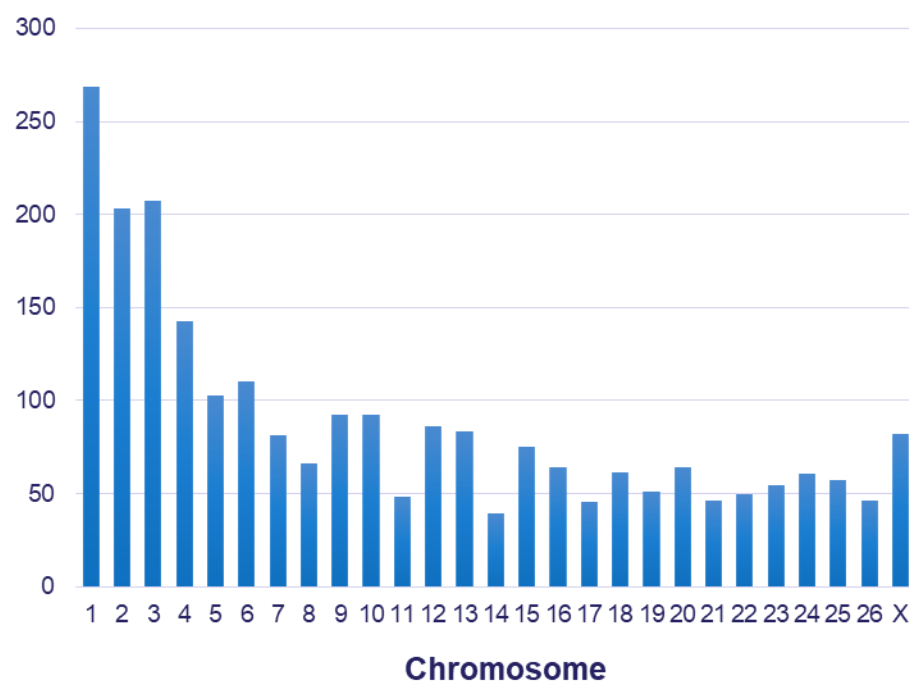


Results: Number of Variants on Each Chromosome

Number of Target Variants Identified per Chromosome



Number of Novel Variants Identified per Chromosome



Rickets study

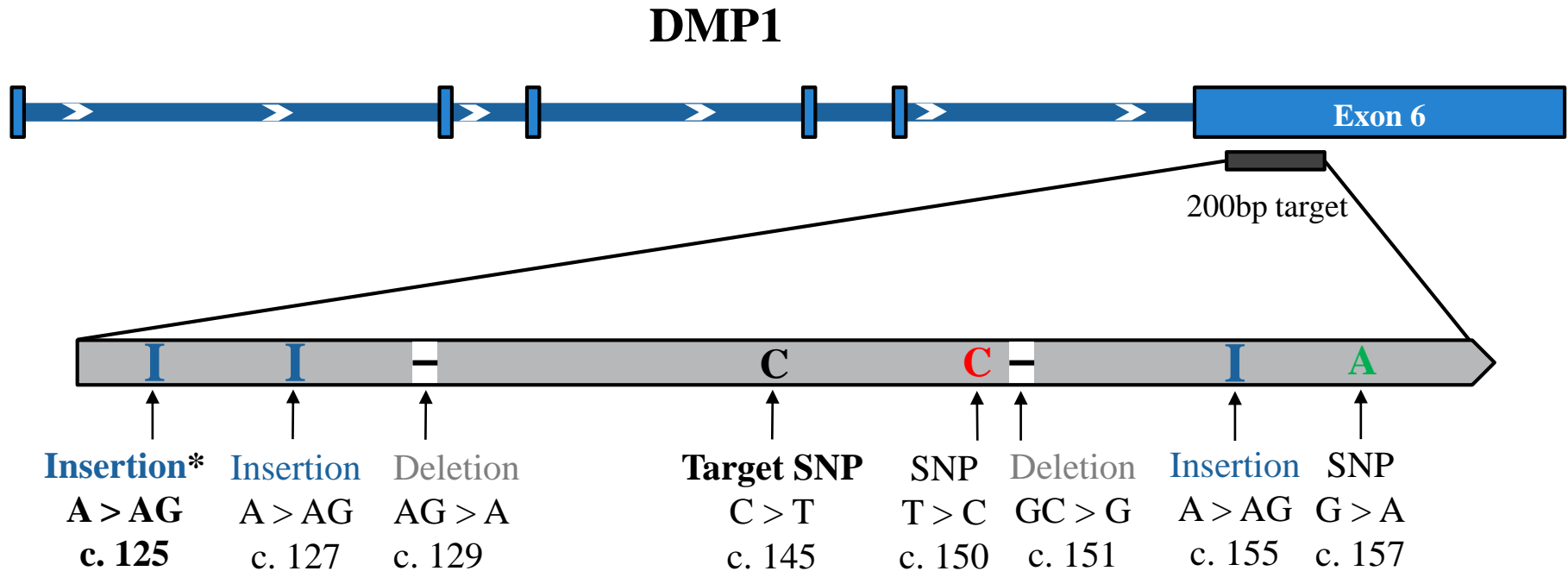
DMP1

- Previous studies identified a premature nonsense mutation, SNP in codon 145, in exon 6 of dentin matrix protein 1 (*DMP1*) that is associated with an inherited form of rickets in Corriedale sheep (Zhao, et al., 2011)

Animals

- Samples for gene (*DMP1*) test
 - Blood samples from North Dakota (n = 59), 6 that exhibited the rickets phenotype
 - Blood samples from Wyoming (n = 99), 5 that exhibited the rickets phenotype

Results: Rickets study



* $P = 0.041$

- The result of (A > AG) insertion located at codon 125 is a frameshift creating in a premature stop codon at codon 137

Conclusion

- Marker and sample call rates are very high - 97%
- Genotypes are consistent between replicate sequencing runs with concordance of 98.5% for target and 97% for novel markers
- Panel designed against the *Ovis aries* Oar_v3.1 reference genome and verified against the Oar_rambouillet_v1.0 genome
- Data analysis with the Oar_rambouillet_v1.0 genome had minimal impact on the panel performance
- More novel calls are made from the amplicon sequences



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Questions?

The Flock54 Ovine panel is available to everyone and can be purchased through Superior Farms