Accelerating genetic improvement in sheep by increased pedigree accuracy

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ABSTRACT

Genetic management is a key driver for increased revenue in agrigenomics production systems. The amount of genetic information required for a decision depends on the application. At the low end is genotypes on a few hundred markers for targeted SNP parentage and the associated reduction in pedigree errors and more efficient herd improvement. Correct parentage assignment increases the success of any breeding program by facilitating linkage of production performance to the correct families to improve estimates of breeding values. At the high end is full genome sequence for high value individuals.

The Applied Biosystems™ Eureka™ Ovine Parentage Panel is a comprehensive parentage panel for sheep and provides superior power to accurately verify parentage. It provides an affordable next-generation sequencing (NGS)-based panel for both parentage testing and traceability in diverse sheep breeds. The availability of over 4,000 barcodes enables processing of over 4,000 samples in a single sequencing run for fast turnaround time. Thus this genotyping panel may be used as a tool in an ovine breeding and production system that has the potential to increase overall revenue.

METHODS AND RESULTS

The Applied Biosystems™ Eureka™ Genotyping Solution is a modified version of a simple ligation-dependent PCR that is variant multiplexed by using barcodes to identify both allele and locus and sample indices to identify sample. Each locus is interrogated with a probe triplet (left, left-prime, and right hybridization sequences). Tens to thousands of triplets are combined to create a Eureka genotyping panel. The Eureka genotyping workflow is shown in Figure 1. Sample DNA/crude lysate is mixed with the Eureka genotyping panel (Figure 1a) and allowed to hybridize overnight (Figure 1b), followed by ligation (Figure 1c). Because crude lysates can be used, the cost and time to purify genomic DNA are removed. This completed ligation reaction is used as template for the sample indexing PCR, which adds sequencing reaction compatible adapters as well as a unique sample ID barcode (Figures 1d and e). Amplicons are combined, purified on a silica-based column, quantified, and sequenced (Figure 1f). On day three, each sequence read is assigned to appropriate sample index and allele + locus barcode (or not assigned if there is no match). The tabulated reads are normalized and are plotted in size vs contrast space. The genotype of each sample and locus is inferred with our proprietary genotype caller which is contained in the Eureka™ Analysis Suite and is an extension of the BRLMM-P clustering algorithm. This algorithm adapts pre-positioned genotype cluster locations called "priors" to the sample data in a Bayesian step and computes three posterior cluster locations (Figure 2).

Eureka Ovine Parentage Panel is intended to provide genotypes of up to 163 loci for use in candidate parent qualification (or disqualification) of globally diverse breeds of sheep. The panel has various subsets of loci that are predicted to be useful in many globally diverse breeds of sheep [Heaton et al. (2014) PLOS ONE Apr 16 ePub:94851]. This catalog panel can be customized to interrogate additional content.

Eureka Genotyping Solution has 4,224 sample indices to allow high- throughput generation of genotypes in a two-day turnaround. Eureka Analysis Suite software manages samples, ensures sample index combination integrity, and enables data analysis and genotype generation with a single software package.

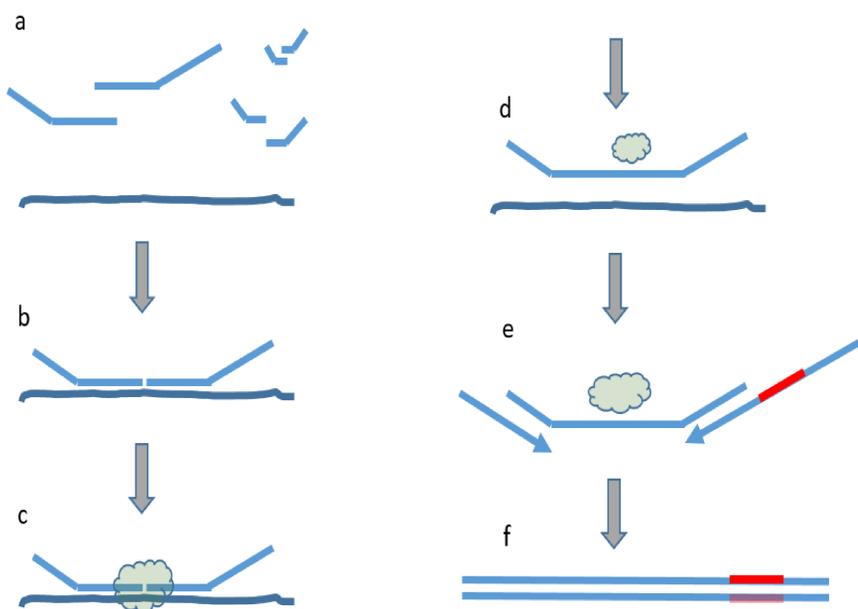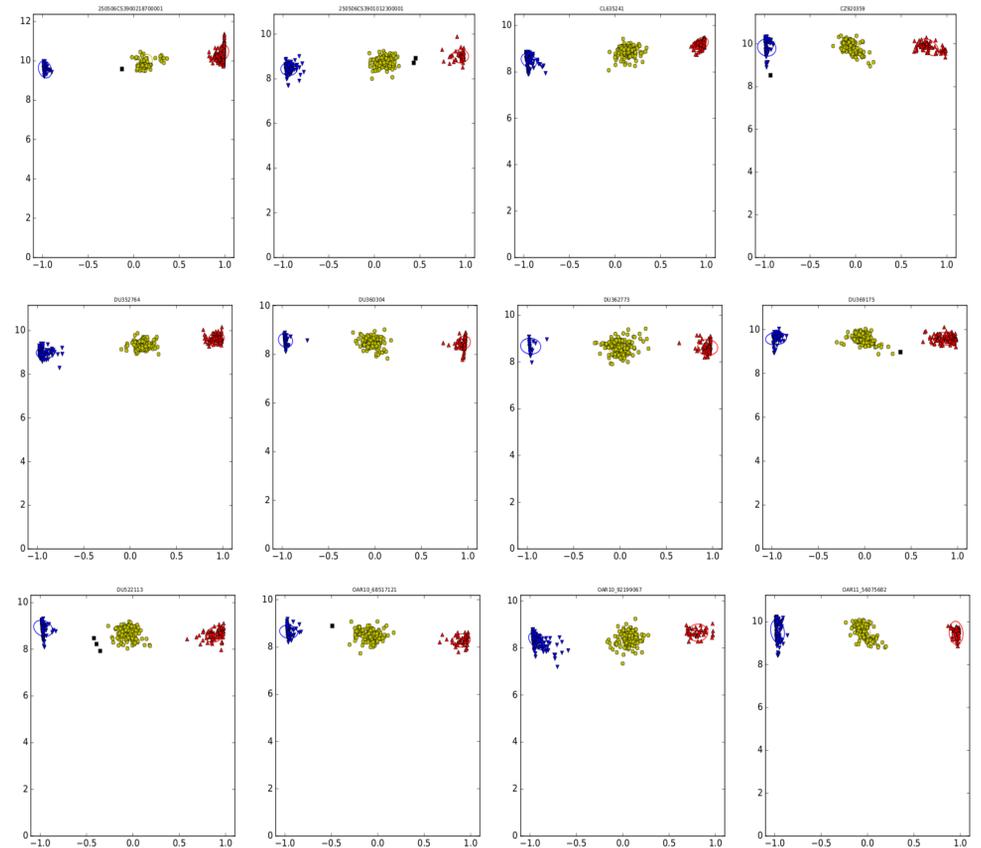### Figure 1. Eureka Genotyping Assay

Figure 2. Typical cluster plots from Eureka Genotyping Solution

Each plot is a single locus and each sample is a single point in the plot. The x-axis is contrast and the y-axis is size. The BB (blue), AB (yellow), and AA (red) genotype of each sample is shown. 324 samples were pooled (post-PCR) into a single Eureka Genotyping Library.

CONCLUSION

Rapid genotyping of sheep en masse is needed by researchers and companies to swiftly guide breeding and culling decisions and determine successful outcomes. Parentage assignments are particularly important in sheep breeding operations. The ability to qualify or disqualify candidate rams and ewes as the parents of a particular lamb has the potential to change sheep farming practices and increase revenue.

Eureka Genotyping Solution can provide rapid time to answer of genotype of biallelic variants (SNPs, insertion/deletions large and small) with up to 4,224 samples processed at a time. Custom content can be added to the Eureka Ovine Parentage Panel. Custom panels can be designed for other species. Acceptable samples include dried blood cards, hair follicle cards, ear tags, and semen straws.

Additional applications of the Eureka Genotyping Solution are shown in ISAG Posters MT85 and MT329 and ISAG talk 118 on Tuesday, July 18th, 2017.

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