applied biosystems

Complete Workflow Solutions for High- and Low-Density Canine Genotyping from Thermo Fisher Scientific

Manley[1], Lillian; Hoang[1], Quoc; Gunter[1], Calvin; Chadaram[1], Srinivas; Patil[2], Mohini A.; Lei[2], Tao

[1]Thermo Fisher Scientific, 2150, Woodward St, Austin, TX 78701

[2]Thermo Fisher Scientific, 3450 Central Expressway, Santa Clara, CA, 95051

ABSTRACT

Genotyping applications can be varied based on customer needs and requirements. High to low density genomic testing can be useful, whether it is for a veterinary disease research company or a canine breeding program. The Applied Biosystems™ Axiom™ Canine HD Array, a high-density genotyping platform, is useful for genome wide association studies and marker assisted selection for the research of traits and diseases. Applied Biosystems™ AgriSeq™ targeted GBS platform, a low density targeted GBS method which allows for marker assisted selection for the research of traits and diseases, identification of novel mutations, parentage verification and identification of individuals based on genotypes. Having one complete workflow solution from sample prep to genotyping for both platforms allows for more convenience and versatility.

Here, we demonstrate a single extraction used for various genotyping platforms. Using the Applied Biosystems™ MagMAX™ CORE DNA Extraction kit, DNA isolations from canine buccal swabs were tested on two different genotyping platforms. AgriSeq™ Canine SNP Parentage and ID Panel, was combined with AgriSeq™ Canine Traits and Disorders Panel, to target 535 total markers on the AgriSeq™ targeted GBS platform. The same extracted samples were also tested on the Axiom™ Canine HD Array, which provides over 712,000 markers. The data supports a complete solution from DNA extractions to genotyping for companion animals and livestock from Thermo Fisher Scientific.

INTRODUCTION

The **MagMax™ CORE AgGenomic DNA Extraction kit** is a highly-effective solution for purifying genomic DNA from diverse veterinary sample types. The universal chemistry is optimized for use on a variety of KingFisher magnetic particle processors and the kit's modular design enables flexibility to evolve with future testing needs.

The **Applied Biosystems™ Axiom™ Genotyping Platform** is ideal for identification, verification and screening of complex genetic traits in plants and animals. It offers customizable content to genotype any species, genome size or ploidy level. Axiom genotyping arrays have 100% fidelity with no dropped SNPs.

The **Axiom Canine HD array** was developed by analyzing over 2000 samples using the Axiom Canine Genotyping Array Sets A and B. The samples covered over 50 breeds and were carefully selected with appropriate pedigree to maximize polymorphic content. The array offers over 712,000 markers for verification and discovery of variants associated with specific phenotypes. It contains over 150 disease and trait specific markers.

The **AgriSeq targeted genotyping by sequencing (GBS) solution** utilizes a highly efficient multiplexed PCR chemistry where hundreds to thousands of markers can be targeted and uniformly amplified in a single reaction. The amplicon libraries are barcoded and pooled for simultaneous sequencing of hundreds of samples on the Ion Torrent suite of next-generation sequencing (NGS) instruments.

The **AgriSeq Canine SNP Parentage and Identification Panel** contains 381 SNP markers for parentage determination and identification of canines by targeted GBS. **AgriSeq Canine Traits and Disorders Panel** contains 154 markers targeting clinically important canine traits and disorders. Most of these markers are common to all breeds, while some are breed-specific for certain traits and conditions.

METHODS

MagMAX™ CORE AgGenomic DNA Extraction kit

Canine buccal swabs were collected using CytoSoft Cytology Nylon brush or PerformaGene DNA sample collection swab kits. DNA sample extraction were performed on 95 canine buccal swabs using the modular Applied Biosystems™ MagMAX CORE AgGenomic DNA Extraction kit. The AgGenomic Module is specifically designed for cost efficient high throughput extractions.

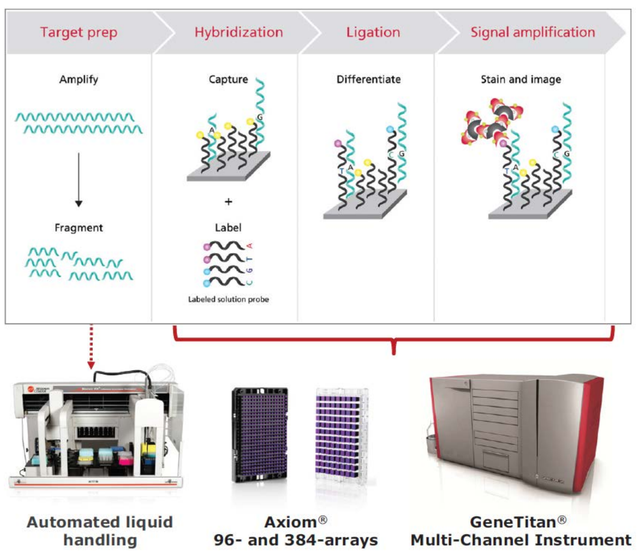
Extraction Workflow:

- Prepare processing plates
- Prepare PK/PK Buffer Mix
- PK treat sample
- Prepare Lysis/Binding /Bead Mix
- Combine PK treated samples with Lysis/Binding/Bead Mix
- Process samples on the instrument

Applied Biosystems™ Axiom™ Genotyping Solution

- Extracted genomic DNA is isothermally amplified and randomly fragmented into 25 to 125 base pair (bp) fragments.
- These fragments are purified, re-suspended, and hybridized to the Axiom™ Canine HD Array (712,331 markers).
- Following hybridization, the bound target is washed to remove non-specific background. Each polymorphic nucleotide is queried via a multi-color ligation event carried out on the array surface.
- After ligation, the arrays are stained and imaged on GeneTitan™ MC Instrument.
- The resulting data is analyzed using Applied Biosystems ™ Axiom™ Analysis Suite software following the Best Practices Workflow.

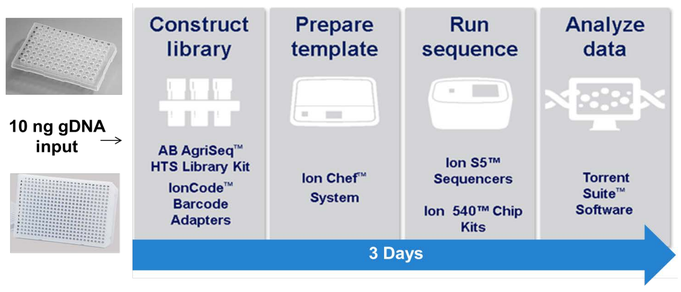
Figure 1. Axiom™ biochemistry and workflow



AgriSeq™ Targeted Genotyping By Sequencing

Extracted genomic DNA was also genotyped by AgriSeq targeted GBS using a combined panel of the AgriSeq™ Canine SNP Parentage and ID Panel and AgriSeq™ Canine Traits and Disorders Panel for a total of 510 markers.

Figure 2. Complete AgriSeq™ Targeted GBS Workflow



- Using the AgriSeq HTS Library Kit, DNA isolated from the buccal swabs was amplified with the combined AgriSeq Canine SNP panel in a 384-well format using a combination of automation and manual workflows
- Each sample was treated with a Pre-ligation Enzyme to remove residual primer dimers allowing for more efficient sequencing
- Samples were ligated with unique barcoded adapters allowing them to be pooled for subsequent clean-up and sequencing while retaining traceability to the original sample during analysis
- Libraries were cleaned-up by a two-round AMPure purification. A final bead-based normalization step helps ensure each library is at a consistent final concentration suitable for direct input into template prep on the Ion Chef™ instrument
- Libraries were placed on the Ion Chef overnight, for template preparation and chip loading, followed by sequencing on the Ion S5 system the next day
- Data was analyzed using the Torrent Variant Caller (TVC) plugin available as part of the Torrent Suite software package, to determine the genotype calls for each marker and sample tested
- Sample call rate and genotype concordance was determined between workflow methods and between different amount of DNA for each sample

RESULTS

The performance of Axiom™ genotyping was evaluated in four ways: Dish QC, QC call rate, sample pass rate, and SNP filtering.

Dish QC: Dish QC (DQC) evaluates the overlap between the two homozygous peaks (AT versus GC) in contrast space using normalized intensities of non-polymorphic probes from both channels. It is a measurement that is independent of the samples in the batch, unlike QC call rate discussed below.

Axiom Average DQC for canine samples processed = 0.96

QC call rate: The QC call rate is computed over a subset of previously tested SNPs that are known to perform well (sometimes referred to as the Step1 probe set list). The Canine HD Array Step 1 list contains 20K SNPs. The QC Call rate is the percentage SNPs called. Samples with a QC call rate value greater or equal to 97% are considered passing.

Average QC Call Rate for canine samples processed = 97.74

SNP filtering: After generating the genotypes for high quality samples, the SNPolisher tool is used to sort the best probe set for each SNP into one of 6 classifications as shown in Figure 3.

Figure 3. Axiom SNP categories

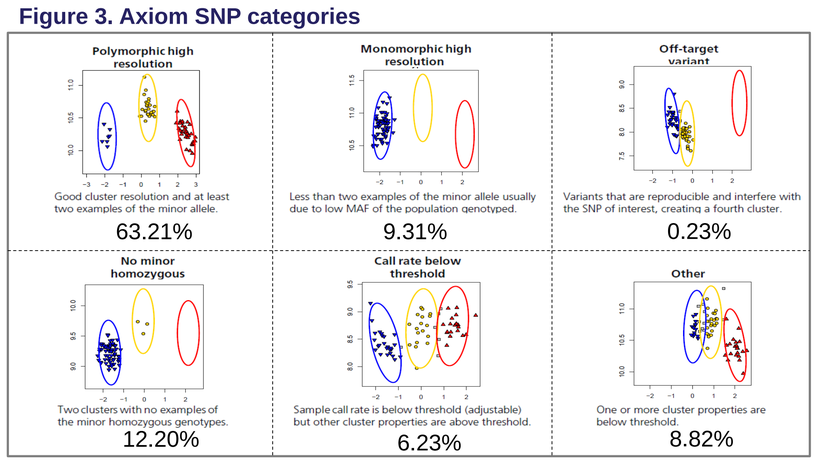


Figure 3 above shows examples of each of the 6 SNP classifications as categorized by SNPolisher. For the Axiom Canine HD Array, Polymorphic high resolution, Monomorphic high resolution, and No minor homozygous categories are recommended. Summed count for the recommended categories equals 81.6%

Reproducibility of 4 sets of replicates = 99.81%

Gender calling accuracy of passed samples = 96.97%

Average Sample Call Rate: 99.7%

Average Sample Reproducibility: 99.9%

Average Sample Concordance (across platform): 98.7%

Table 1. AgriSeq™ Workflow Run Summary DNA normalized to 1ng/µl DNA before amplification

The AgriSeq™ genotyping workflow runs are reviewed for run metrics and panel performance prior to evaluating the genotype calls. All samples were tested in triplicates and prepared on a 384 well plate with other canine samples.

Metric	Suggested Value	Actual Value	Comment
Total Reads	>60,000,000	73,842,438	Pass
% Enrichment	>95%	100%	Pass
Usable Reads	>50%	52%	Pass
% Polyclonal	<40%	31%	Pass
% Low Quality	<20%	12%	Pass
% Loading	>70%	94%	Pass
Aligned Bases	>98%	98%	Pass

The performance of AgriSeq™ panels are evaluated by mean depth/coverage, uniformity, On Target reads and Call rates for Markers and Samples in a run.

Mean Depth/Coverage: A measure of how many reads per amplicon were attained during sequencing. AgriSeq team recommends a 100X coverage with a minimum 20X coverage for making genotype calls.

Uniformity A percentage of the target bases that had at least 0.2 times mean depth. It is a measure of end-to-end coverage over a target region and the average value should be greater than 90% for a good panel.

On Target Reads is a percentage of mapped reads that were aligned correctly over a target region. This metric infers off-target alignments, which can be indicative of issues such as sample contamination and uncharacterized genetic variation.

Marker Call Rate Percentage of all samples tested generating a genotype call for a specific marker. We design panels with high performing markers with overall mean Marker call rates >90%.

Figure 4: AgriSeq™ Targeted GBS Results

CONCLUSIONS

The Applied Biosystems™ MagMax™ CORE AgGenomic DNA Extraction kit is very effective for extracting high quality DNA from canine buccal swabs. It can be used for a variety of applications, ranging from microarrays to targeted genotyping by sequencing. Hence, providing a complete solution from DNA extractions to genotyping for companion animals from Thermo Fisher Scientific.

ThermoFisher SCIENTIFIC