Development of AgriSeq™ targeted GBS panels for breeding and parentage applications in cats

Michelle Swimley[1], Angela Burrell[1], Haktan Suren[1], Chris Willis[1], Prasad Siddavatam[1], Krishna Gujjula[1], and Rick Conrad[1]
[1]Thermo Fisher Scientific, 2130 Woodward Street, Austin, TX, USA, 78744.

ABSTRACT

Parentage testing and genomics-assisted breeding are critical aspects of successful veterinary management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high throughput workflow starting from either extracted nucleic acid or crude lysis samples.

We developed a targeted sequencing panel, one for the combined detection of feline genetic disorders/trait detection and parentage verification. Utilizing the AgriSeq™ HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, the panel's performance was tested on a panel of diverse DNA samples. Libraries were sequenced on the Ion S5™ using an Ion 540™ chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin.

The mean genotype call rate of markers across the samples was >95%. Concordance across replicate library preparations and independent sequencing runs was >99% for both panels. Panel results also were compared with genotyping results from qPCR, and/or CE sequencing for orthogonal confirmation of accuracy and the genotype calls were 100% concordant with the AgriSeq workflows.

The data demonstrates the utility of the AgriSeq targeted GBS approach for feline SNP genotyping applications.

For Research Use Only. Not for use in diagnostic procedures.

INTRODUCTION

Veterinary management applications require consistent genotyping performance and high call rates to ensure accurate parentage verification and genetic trait detection. Unlike non-targeted GBS approaches (e.g. RADSeq) that are highly susceptible to allele drop-outs and missing data, AgriSeq targeted GBS panels are designed to deliver reproducibly high marker call rates across diverse sample sets.

We have developed a feline primer panel designed to work with the AgriSeq sequencing workflow, a high-throughput, targeted GBS workflow designed to amplify and sequence thousands of genetic markers in a single multiplexed reaction. The AgriSeq Feline PITD (Parentage, Identification, Traits, and Disorders) panel combines markers for parentage verification, as well as genetic trait and disorders detection in a single combined panel. The panel consists of 181 makers, 113 of which target markers associated with feline parentage verification and 68 markers targeting clinically important genetic traits and disorders. The library prep workflow utilizing the Feline PITD panel can be automated on most standard liquid handling platforms for decreased hands-on time and increased throughput. Following library prep, all sample libraries can be pooled and sequenced on the Ion S5™ sequencing system.

MATERIALS AND METHODS

The AgriSeq Feline PITD panel performance was validated by three methods: Orthogonal Testing, Robustness Testing, and Field Testing in order to verify performance of the panels met all requirements.

Orthogonal testing was completed by running up to six samples with independent TaqMan genotyping assays on the ViiA 7 Real-Time PCR System and/or by CE sequencing. Samples were also run using the AgriSeq workflow (Figures 1 and 2) and genotype concordance was calculated between orthogonal technologies.

Figure 1. AgriSeq Library Prep workflow

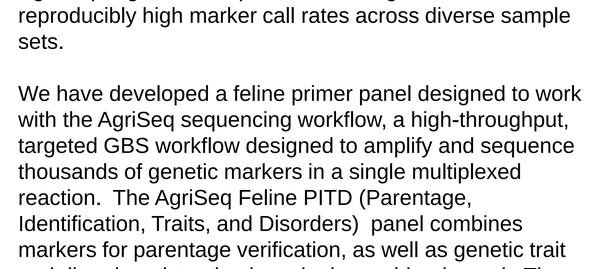


Figure 1. Using the AgriSeq HTS Library Kit , 10ng/rxn of feline DNA was amplified using The AgriSeq Feline PITD panel. Each sample was then treated with a Pre-ligation Enzyme to remove residual primer dimers allowing for more efficient sequencing. Samples were ligated with unique barcoded adapters allowing them to be pooled for subsequent clean-up and sequencing while retaining traceability to the original sample during analysis for significant cost savings. 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. All libraries were pooled 1:1 for templating in a single reaction.

AgriSeq libraries were sequenced on the Ion S5™ sequencing system using an Ion 540™ or Ion 550™ chip. Data was analyzed using the Torrent Variant Caller (TVC) plugin as part of the Torrent Suite™ software package to determine the genotype call for each marker and sample (Figure 2).

Figure 2. Complete AgriSeq Sequencing Workflow

Figure 2. Following library prep, libraries were pooled into a single tube and run overnight on the Ion Chef instrument for template prep. The following day, libraries were sequenced on the Ion S5 XL instrument and data was analyzed using the Torrent Suite Software v5.10. Genotypes for all markers were obtained from the Torrent Variant Caller plugin.

To test robustness of our sequencing results we tested 12 high-quality commercial DNA samples in replicates of n=64 for a total of 768 barcoded samples with the AgriSeq panel using our standard workflow (Figures 1 and 2). Each library was sequenced twice on an Ion 540 and Ion 550 chip (Figure 2). Replicate genotype concordance, the percent of genotype calls across all replicate samples that are identical, was also determined

The performance of the AgriSeq Feline PITD panel was also tested using 42 diverse feline oral swab DNA samples. The libraries were prepared in replicates of n=2 using the AgriSeq HTS Library Kit (Figure 1) and sequenced on the Ion S5 XL instrument using a 540 chip (Figure 2). Call rate, replicate genotype concordance and read uniformity, were calculated for all libraries.

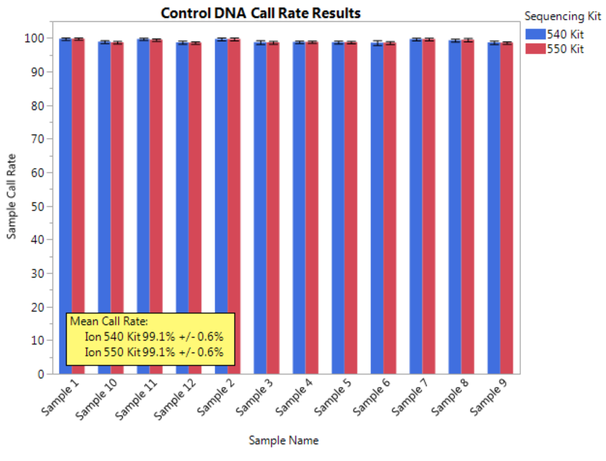
RESULTS

Figure 3. Orthogonal Testing Results Summary

Orthogonal Method	# Concordant Markers to GBS	# Discordant Markers to GBS	# No Calls	Orthogonal Concordance
CE Sequencing	109	0	14	100%
qPCR	58	16 (confirmed to be concordant by CE sequencing)	7 (confirmed to be concordant by CE sequencing)	

Figure 3. 81 markers were tested by independent qPCR genotyping assays on the ViiA 7 Real-Time PCR System using the 2X TaqMan Genotyping Master Mix with 6 unique feline DNA samples. All sample were also run through the AgriSeq workflow with the Feline PITD panel to generate a genotype to compare to the orthogonal results. 58 markers generated a concordant genotype to AgriSeq, 16 markers generated a discordant genotype and 7 generated a no call by qPCR. The 23 markers that generated a discordant call or no call were re-tested by CE sequencing to resolve the discrepancy and determine the final "truth" genotype along with the 100 remaining markers for a total of 123 markers. All of the discordant and no call qPCR markers were confirmed to be concordant with AgriSeq when tested by CE sequencing. Initial discordance with qPCR was likely due to an error in the qPCR genotyping algorithm in borderline calls. Of the remaining 100 markers tested only by CE, 14 were unable to be genotyped due to poor CE results and the remaining were all concordant with the AgriSeq genotype call. Of the 167 markers where orthogonal genotypes were available, genotyping results were 100% concordant to AgriSeq genotypes.

Figure 4. Robustness Testing Mean Sample Call Rate Results

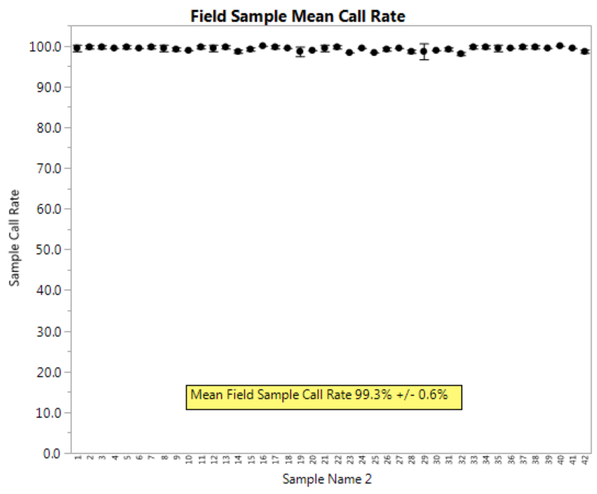


Figures 4. Twelve commercially available feline DNA samples were tested in replicates (n=64) for a total of 768 barcoded libraries with the AgriSeq Feline PITD panel. Libraries were sequenced twice on the Ion 540 and Ion 550 chips to look at genotype call robustness and consistency. Mean call rate for the panel utilizing both sequencing kits was >99% with minimum variation between samples demonstrating the robustness of the genotype calls.

Figure 5. Robustness Testing Replicate Genotype Concordance

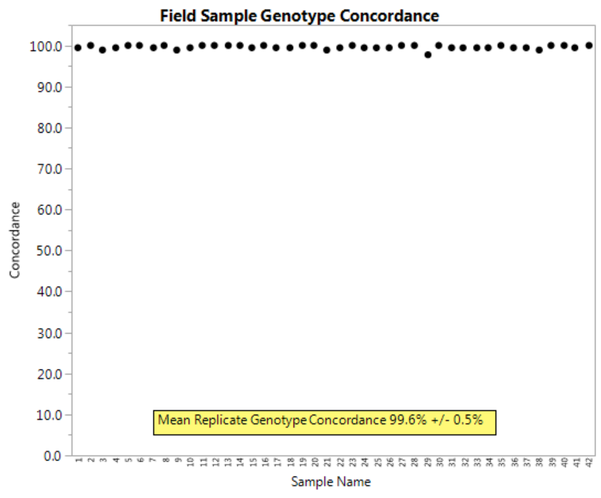
Figures 5. Genotype concordance is calculated as the percent of markers that give identical genotypes for replicate samples. The graph above (Figure 5) shows the Feline PITD panel had a mean genotype concordance of >99.9% for both sequencing kits between replicate samples even when n=64 replicates are tested demonstrating the high robustness and consistent results obtained with the panel.

Figure 6. Field Sample Call Rate



Figures 6. 42 feline oral swab DNA samples were tested in replicates of n=2 with the AgriSeq workflow using the Feline PITD kit. The call rate, the number of markers generating a genotype call for each sample, was calculated. The mean call rate for all samples was 99.3% demonstrating the high performance obtained from field samples.

Figure 7. Field Sample Concordance



Figures 7. Genotype concordance was calculated between technical replicates for all 42 field samples processed through the sequencing workflow. Mean genotype concordance was 99.6% demonstrating highly robust and repeatable results obtained through the AgriSeq workflow with a diverse set of field samples.

Figures 8. Field Sample Read Uniformity

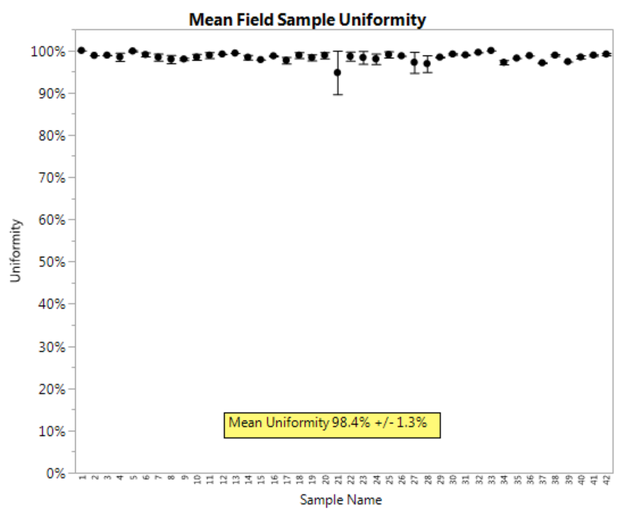


Figure 8. Read uniformity is the percentage of target bases covered by at least 0.2X of the average base read depth. It is a measure of how evenly you are covering target amplicons with reads. Low uniformity (<90%) can lead to marker drop-off and poor call rates. The mean read uniformity for the AgriSeq Feline PITD panel was excellent, even when testing a set of very diverse field samples. The panel had a mean uniformity of >98%

CONCLUSIONS

The AgriSeq library prep workflow along with the AgriSeq Feline PITD panel provide a streamlined, cost-effective method for feline parentage verification and trait genotyping. Up to 4X 384-well plates can be processed in a single day and full sequencing results can be obtained in as little as three days. The flexibility of AgriSeq allows hundreds of samples to be pooled together into a single sequencing run targeting hundreds to thousands of markers.

Our method yields calls for the vast majority of markers (>99% call rate). Replicate genotype concordance is >99.5% and calls were highly concordant with orthogonal data (100%). While we demonstrated the utility of AgriSeq sequencing technology for assessing parentage and genetic trait testing in cats, our approach can be applied to other agricultural genotyping problems as well.

In conclusion, the AgriSeq library prep kit and feline GBS panel combines into a robust and efficient workflow for animal genotyping and parentage applications.

REFERENCES

1. Cariou M, Duret L, Charlat S. How and how much does RAD-seq bias genetic diversity estimates? BMC Evolutionary Biology. 2016;16:240. doi:10.1186/s12862-016-0791-0.

2. Kidd, Kenneth K. et al. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics . Forensic Science International: Genetics , Volume 12 , 215 – 224.

ACKNOWLEDGEMENTS

We would like to acknowledge Maarten de Groot at VHL Genetics for supplying the feline field sample DNA used in this study.

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC