

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

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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. 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 175 makers, 111 of which target markers associated with feline parentage verification and 64 markers targeting clinically important genetic traits and disorders. Utilizing the AgriSeq™ HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, the panel's performance was tested on 258 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%. 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.

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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

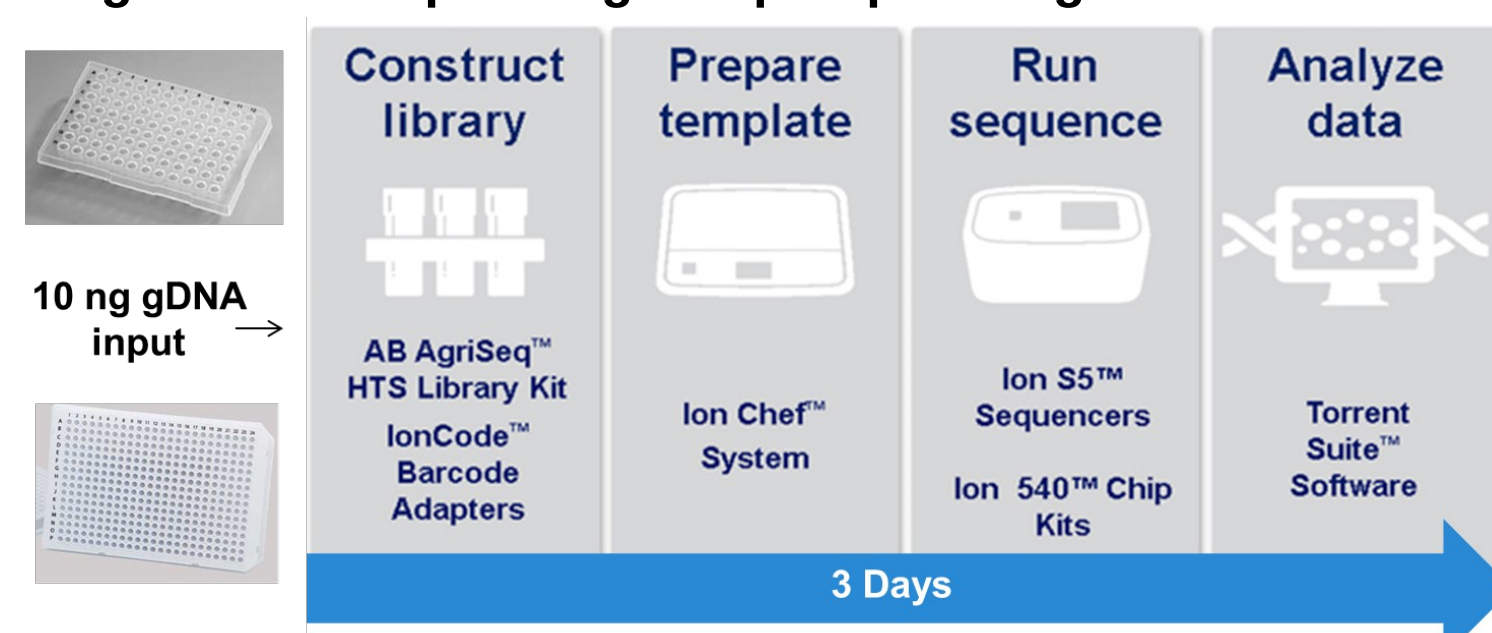


Figure 2. Following library prep, libraries were pooled into a single tube and run overnight on the Ion Chef instrument for template prep. The following day, libraries were sequenced on the Ion S5 XL instrument and data was analyzed using the Torrent Suite Software v5.10. Genotypes for all markers were 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 258 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 Call Markers	Orthogonal Concordance
CE Sequencing	105	0	13 (CE only)	100%
qPCR	56	0	1 (CE and GBS)	

Figure 3. 56 markers were tested by independent qPCR genotyping assays on the Viia7 Real-Time PCR System using the 2X TaqMan Genotyping Master Mix with 6 unique feline DNA samples. In addition, 118 markers were tested orthogonally using CE sequencing. All sample were also run through the AgriSeq workflow with the Feline PITD panel to generate a genotype to compare to the orthogonal results. 55 of the 56 qPCR markers generated concordant results with the AgriSeq Feline PITD workflow. One marker generated a no call for both qPCR and GBS and was removed from the calculation. No markers generated discordant results. 105 of the 118 markers tested by CE were concordant with the genotypes obtained from the AgriSeq Feline PITD workflow. The remaining 13 markers generated poor CE sequencing results and a genotype call could not be obtained. These markers were removed from the concordance calculation. No markers generated discordant results.

Figure 4. Robustness Testing Mean Sample Call Rate Results

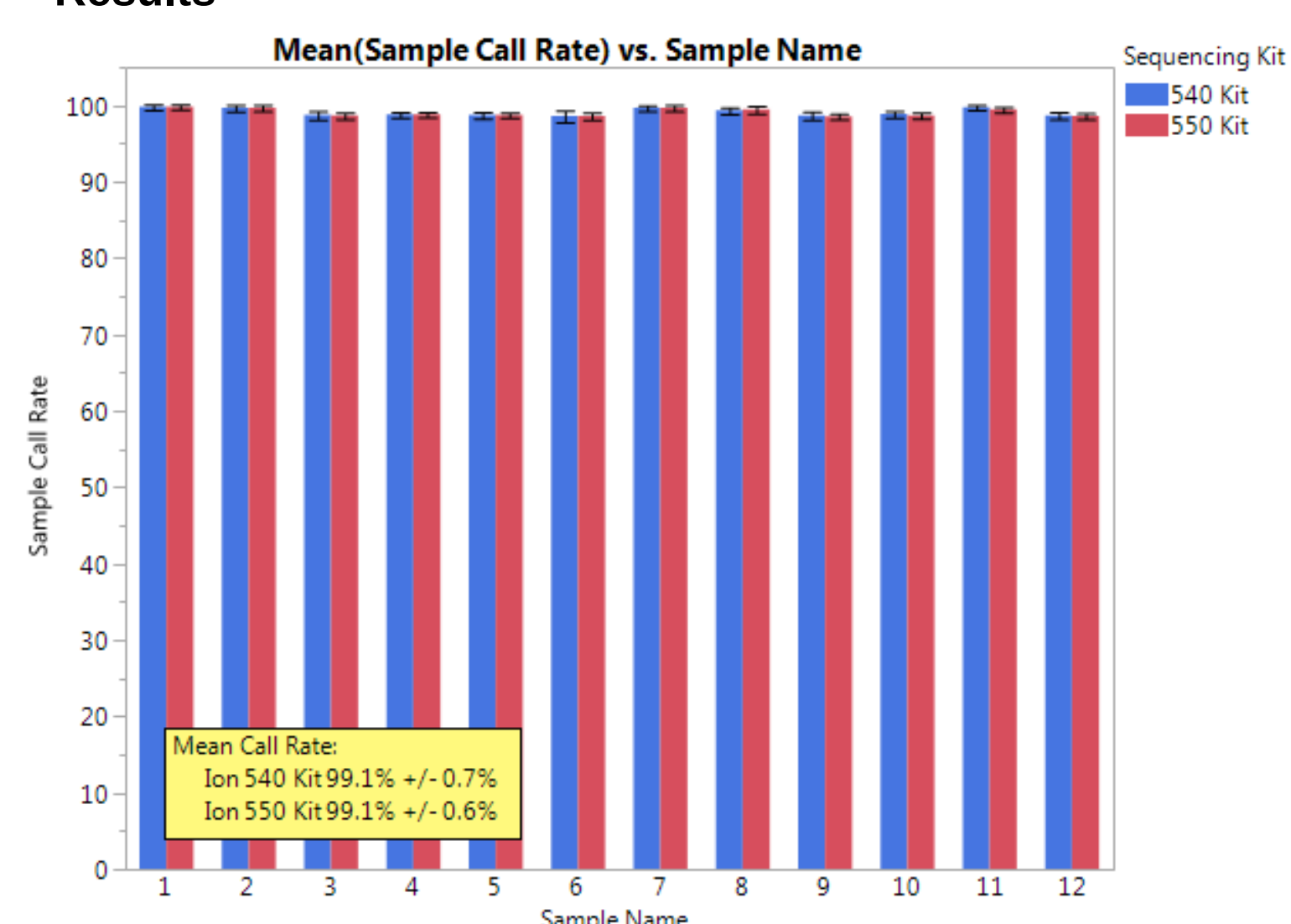


Figure 4. Twelve commercially available feline DNA samples were tested in replicates (n=64) for a total of 768 barcoded libraries with the AgriSeq Feline PITD panel. Libraries were sequenced twice on the Ion 540 and Ion 550 chips to look at genotype call robustness and consistency. Mean call rate for 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

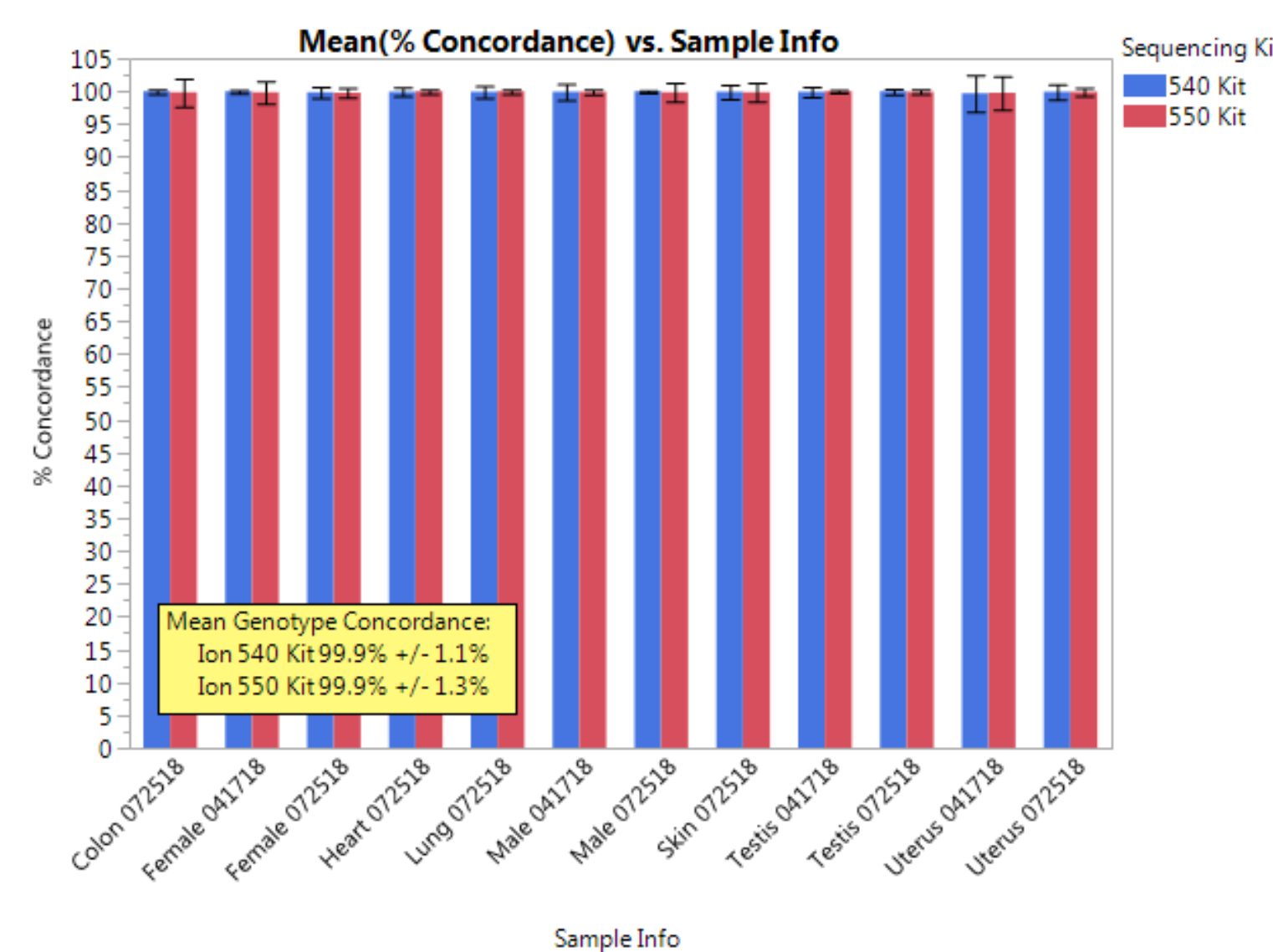


Figure 5. Genotype concordance is calculated as the percent of markers that give identical genotypes for replicate samples. The graph above (Figure 5) shows the Feline PITD panel had a mean genotype concordance of >99.9% for both sequencing kits between replicate samples even when n=64 replicates are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

Figure 6. Field Sample Call Rate

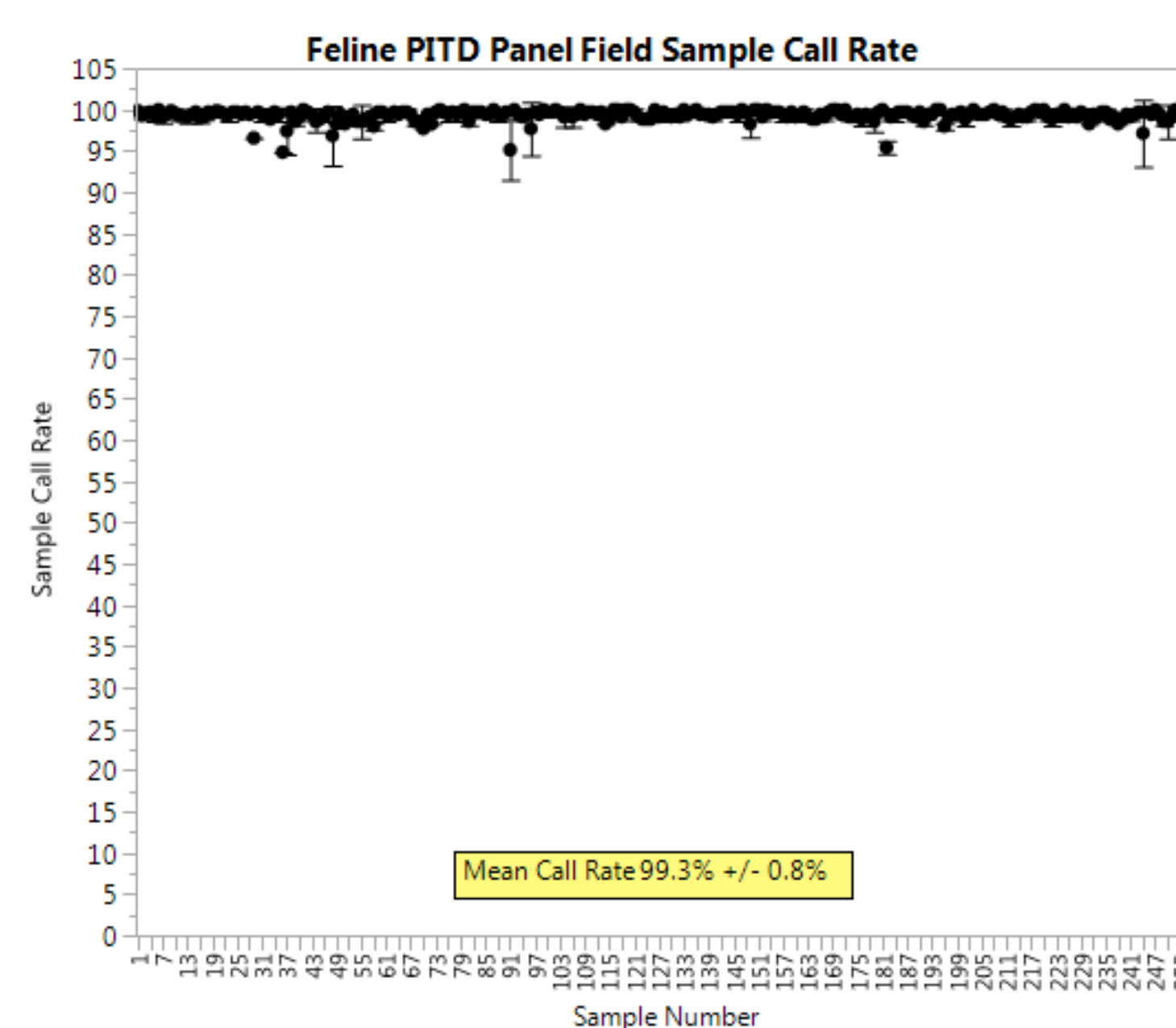


Figure 6. 258 feline oral swab DNA samples were tested in replicates of either n=2 or n=4 with the AgriSeq workflow using the Feline PITD kit. The call rate, the number of markers generating a genotype call for each sample, was calculated for all samples with at least 100X mean read depth, the minimum recommended. The mean call rate was 99.3% and all samples had call rates > 92.5% demonstrating the high performance obtained from field samples.

Figure 7. Genotype Concordance

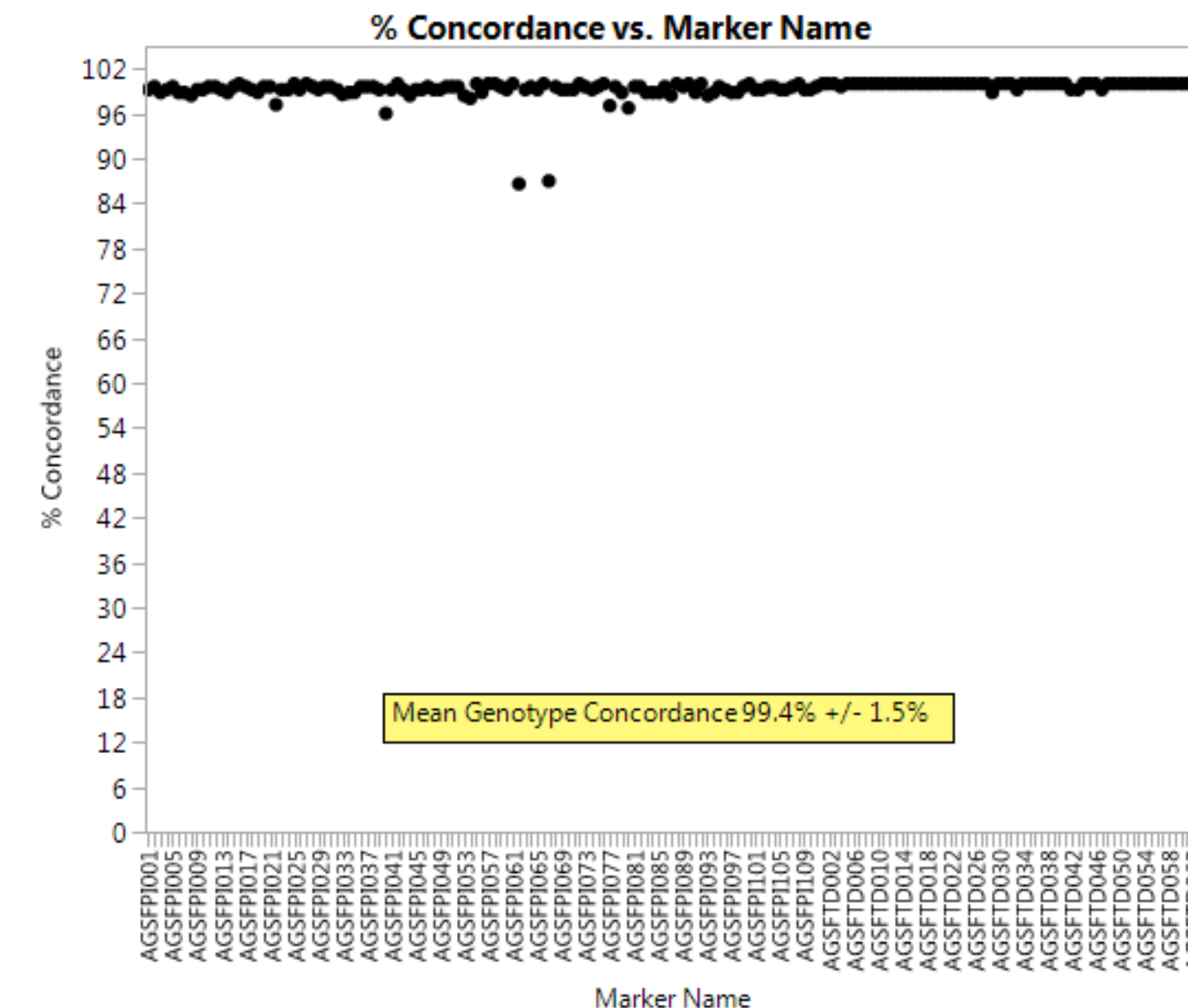


Figure 7. Genotype concordance was calculated between technical replicates for all field samples processed through the sequencing workflow. Mean genotype concordance was 99.4% demonstrating highly robust and repeatable results obtained through the AgriSeq workflow with a diverse set of field samples. The majority of the discordant calls were localized to three samples likely pointing to cross-contamination as a cause.

Figure 8. Field Sample Read Uniformity

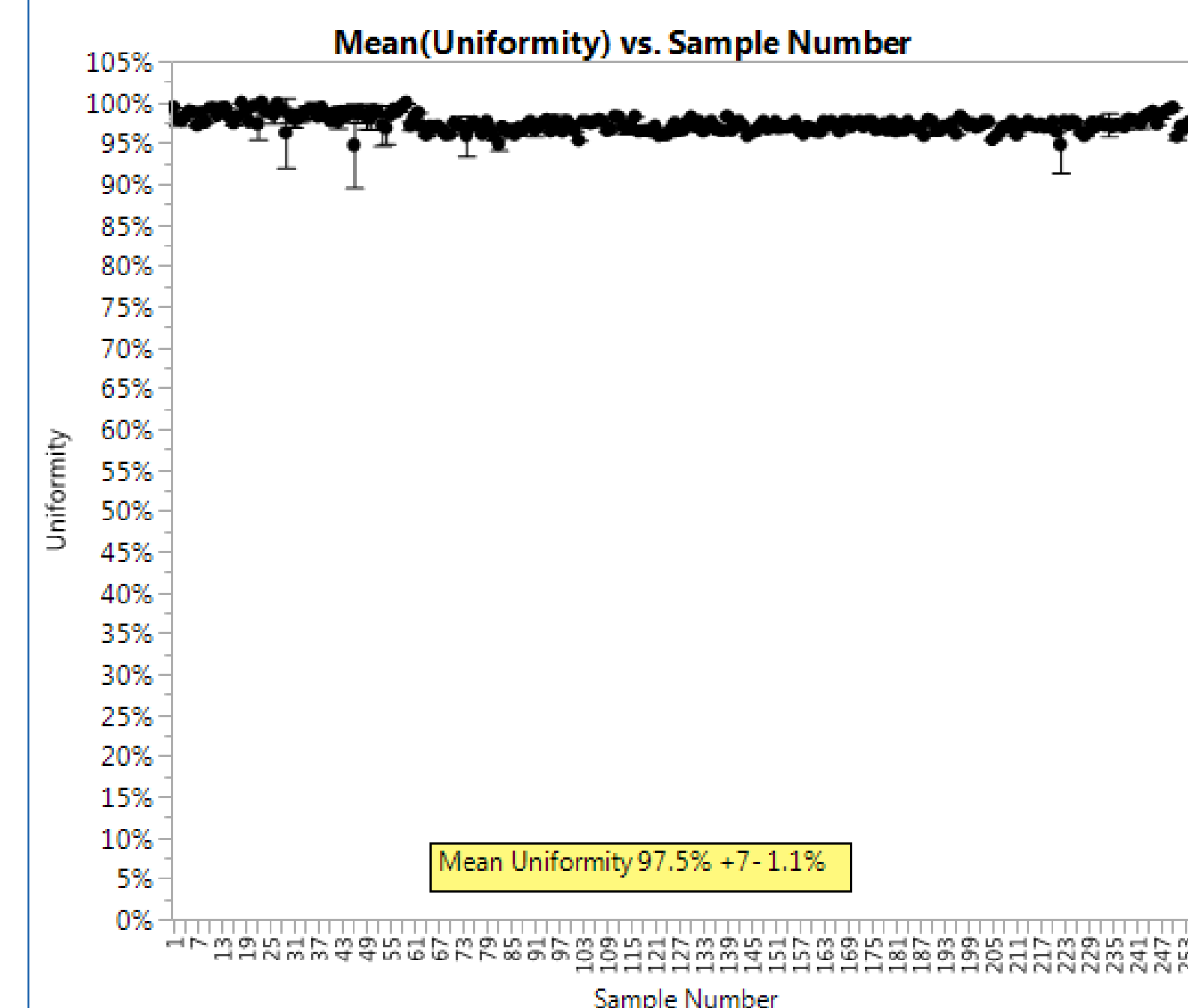


Figure 8. Read uniformity is the percentage of target bases covered by at least 0.2X of the average base read depth. It is a measure of how evenly you are covering target amplicons with reads. Low uniformity (<90%) can lead to marker drop-off and poor call rates. The mean read uniformity for the AgriSeq Feline PITD panel was excellent, even when testing a set of very diverse field samples. The panel had a mean uniformity of >97%.

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 99.4% 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

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TRADEMARKS/LICENSING

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