



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

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Background

- There is a need for a robust, repeatable, and unambiguous workflow needed for feline parentage and genetic trait testing.
- We developed a targeted sequencing panel, one for feline parentage/ID verification and genetic defect/trait identification.
 - The AgriSeq Feline PITD Panel
- Utilizes the AgriSeq workflow

AgriSeq™ Targeted GBS Solutions for Agriculture

AgriSeq is a powerful, customizable, flexible and cost-effective high throughput Targeted GBS workflow capable of rapidly genotyping 50 - 5000 markers across plant, aqua and animal species.

**>100 custom panel
designs for over 35
species and counting**

Plants

- Barley
- Cacao
- Canola
- Corn (maize)
- Cotton
- Cucumber
- Eucalyptus
- Lettuce
- Oats
- Onion
- Pine
- Potato
- Rice
- Sorghum
- Soybean
- Spinach
- Spruce
- Sunflower
- Tomato
- Wheat
- Watermelon

Animals

- Bovine
- Porcine
- Equine
- Canine
- Deer
- Ovine
- Feline
- Chicken
- Salmon
- Chicken
- Shrimp
- Tuna
- Trout
- Turbot
- Black Soldier Fly

Panel Background

- **AgriSeq Feline Parentage, ID, Traits & Defects (PITD) Panel***
(A43408)- 175 markers

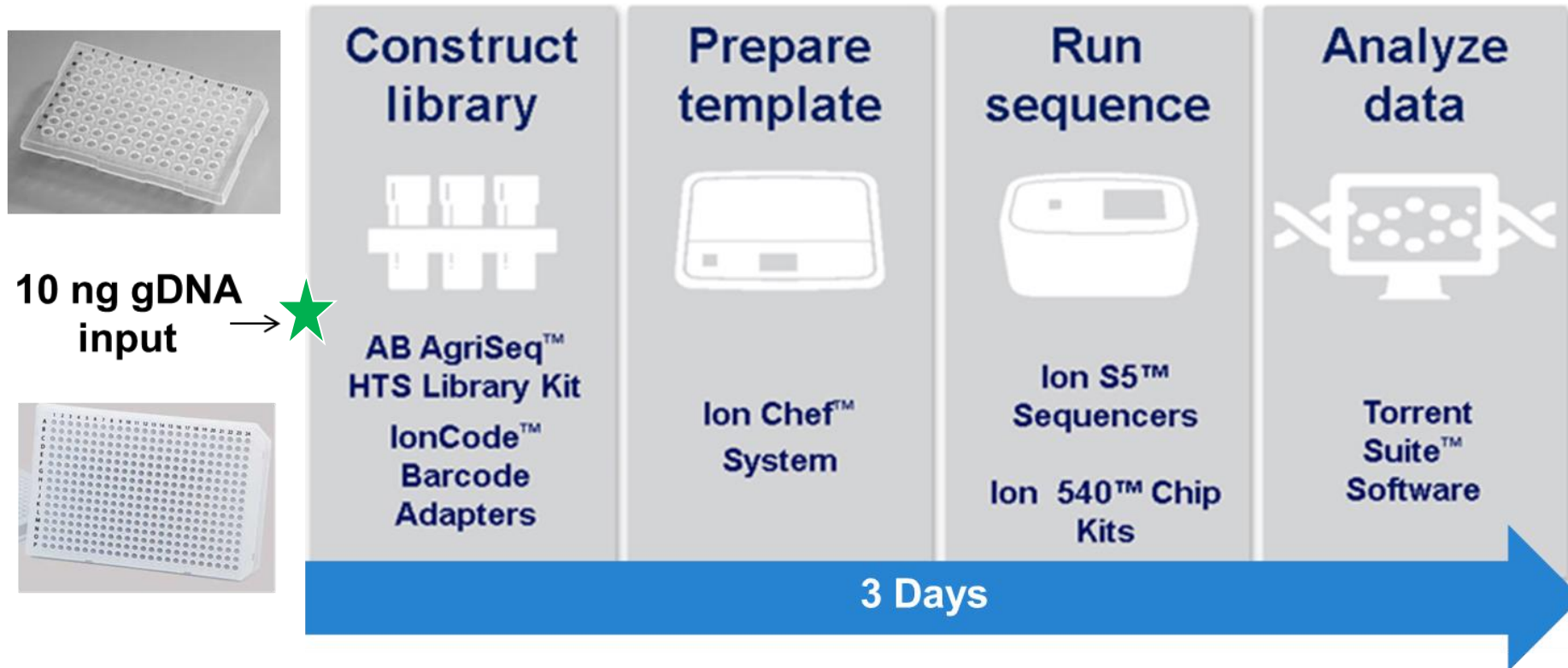
Marker Type	SNPs	MNPs	Insertions	Deletions	Total
Parentage & ID Markers	111	0	0	0	111
Traits & Disorders Markers	43	1	4	16	64
Total	154	1	4	16	175



Targeted primer panel for the combined detection of feline genetic disorders/trait detection and parentage verification.

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

AgriSeq Sequencing Workflow



★
AgriSeq Feline Parentage, ID, Traits and Disorders (PITD) panel addition.

TVC Browser Output – Data for Analysis

									View Allele Annotations	View Coverage Metrics	View Quality Metrics	
<input type="checkbox"/>	Position	Ref	Variant	Allele Call	Frequency	Quality	Subset Of	Variant Type	Allele Source	Allele Name	Gene ID	Region Name
<input type="checkbox"/>	chr3:364807	G	A	Heterozygous	46.0 %	661.7	---	SNP	Novel	tvc.novel.357	ASGA0100357	SP_2188.95472
<input type="checkbox"/>	chr3:364860	C	G	Heterozygous	46.5 %	677.5	---	SNP	Novel	tvc.novel.358	ASGA0100357	SP_2188.95472
<input type="checkbox"/>	chr3:364888	A	G	Heterozygous	50.6 %	926.1	---	SNP	Hotspot	ASGA0100357	ASGA0100357	SP_2188.95472
<input type="checkbox"/>	chr3:2882725	T	C	Homozygous	100.0 %	3759.9	---	SNP	Novel	tvc.novel.359	MARC0069764	SP_2190.153415
<input type="checkbox"/>	chr3:2882816	A	G	Heterozygous	51.5 %	962.3	---	SNP	Hotspot	MARC0069764	MARC0069764	SP_2190.153415
<input type="checkbox"/>	chr3:3490669	T	C	Heterozygous	51.7 %	972.2	---	SNP	Hotspot	MARC0029348	MARC0029348	SP_2193.195026
<input type="checkbox"/>	chr3:3640453	T	C	Homozygous	100.0 %	3741.6	---	SNP	Novel	tvc.novel.360	M1GA0025634	SP_2194.75717
<input type="checkbox"/>	chr3:3640507	C	T	Homozygous	100.0 %	3961.7	---	SNP	Hotspot	M1GA0025634	M1GA0025634	SP_2194.75717

							View Allele Annotations			View Coverage Metrics		View Quality Metrics	
<input type="checkbox"/>	Position	Ref	Variant	Allele Call	Frequency	Qual...	Coverage	Coverage +	Coverage -	Allele Cov	Allele Cov +	Allele Cov -	Strand Bias
<input type="checkbox"/>	chr3:364807	G	A	Heterozygous	46.0 %	661.7	400	171	229	184	87	97	0.546
<input type="checkbox"/>	chr3:364860	C	G	Heterozygous	46.5 %	677.5	400	163	237	186	76	110	0.501
<input type="checkbox"/>	chr3:364888	A	G	Heterozygous	50.6 %	926.1	399	170	229	202	91	111	0.525
<input type="checkbox"/>	chr3:2882725	T	C	Homozygous	100.0 %	3759.9	400	244	156	400	244	156	0.500
<input type="checkbox"/>	chr3:2882816	A	G	Heterozygous	51.5 %	962.3	400	225	175	206	106	100	0.548
<input type="checkbox"/>	chr3:3490669	T	C	Heterozygous	51.7 %	972.2	400	196	204	207	88	119	0.565
<input type="checkbox"/>	chr3:3640453	T	C	Homozygous	100.0 %	3741.6	398	203	195	398	203	195	0.500
<input type="checkbox"/>	chr3:3640507	C	T	Homozygous	100.0 %	3961.7	399	200	199	399	200	199	0.500

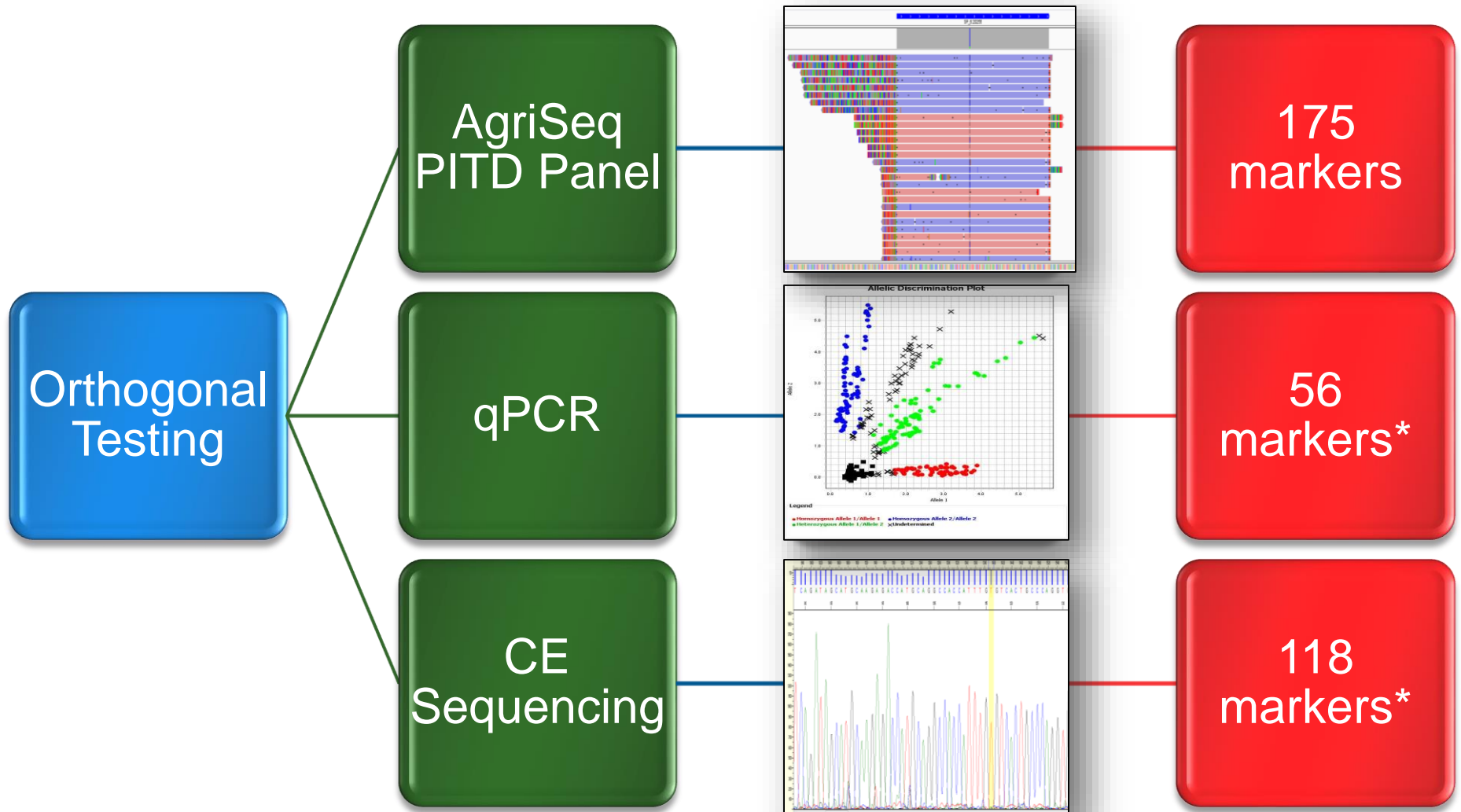
- Data analyzed by TVC (Torrent Variant Caller) Plugin
- All the information from the browser can be downloaded into a summary .XLS file.

- Three general experiments were performed to validate performance of the Feline PITD panel.
 - 1. Orthogonal Testing \Rightarrow Evaluation of panel accuracy.
 - 2. Robustness Testing \Rightarrow Evaluation of panel consistency.
 - 3. Field Sample Testing \Rightarrow Evaluation of panel performance.

Experiment 1: Orthogonal Testing

- Purpose: To confirm that genotypes generated with the AgriSeq Feline PITD panel were accurate by testing with a separate, orthogonal technology.

Orthogonal Testing Workflow



*One marker generated a No Call with both CE and qPCR and was omitted from calculations.

Orthogonal Genotype Concordance Results

- Of the 161 markers generating a call for both technologies, no markers were discordant.

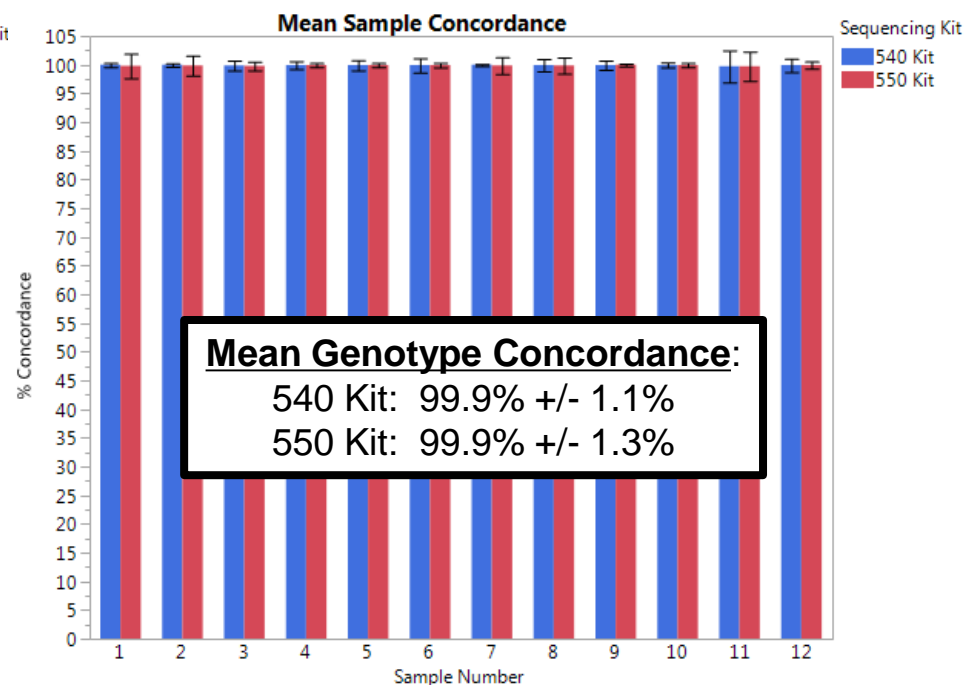
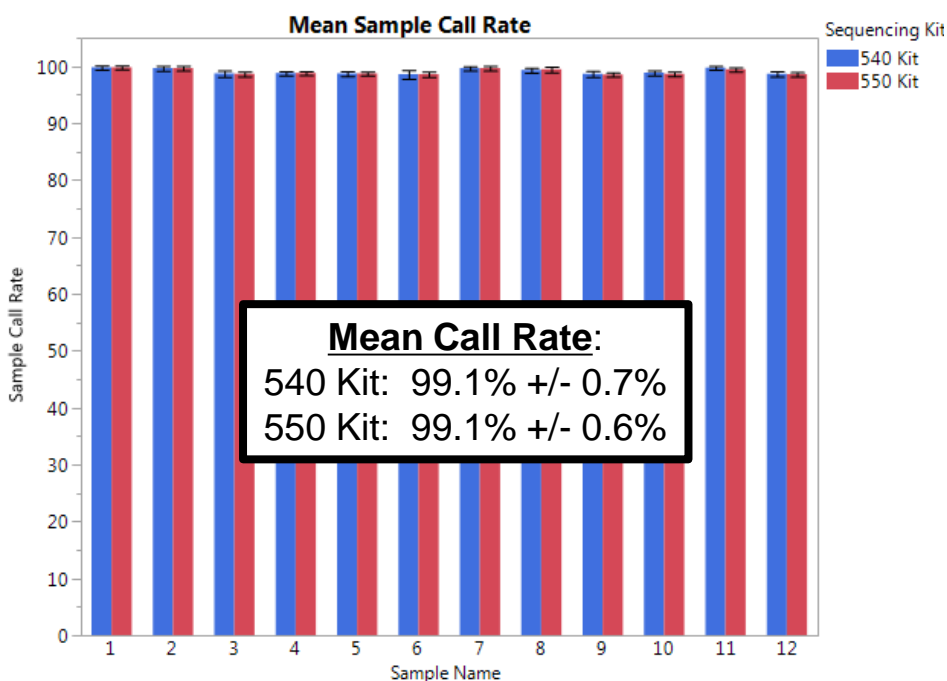
Orthogonal Method	# Concordant Markers to GBS	# Discordant Markers to GBS	# No Call Markers	Orthogonal Concordance
CE Sequencing	105	0	13 (CE only)	100%
qPCR	56	0	1 (CE and GBS)	

Experiment 2: Reproducibility and Repeatability

- Purpose: To test workflow robustness and genotype call consistency through multiple replicate reactions of a panel of samples.
 - 12 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.

Repeatability and Reproducibility

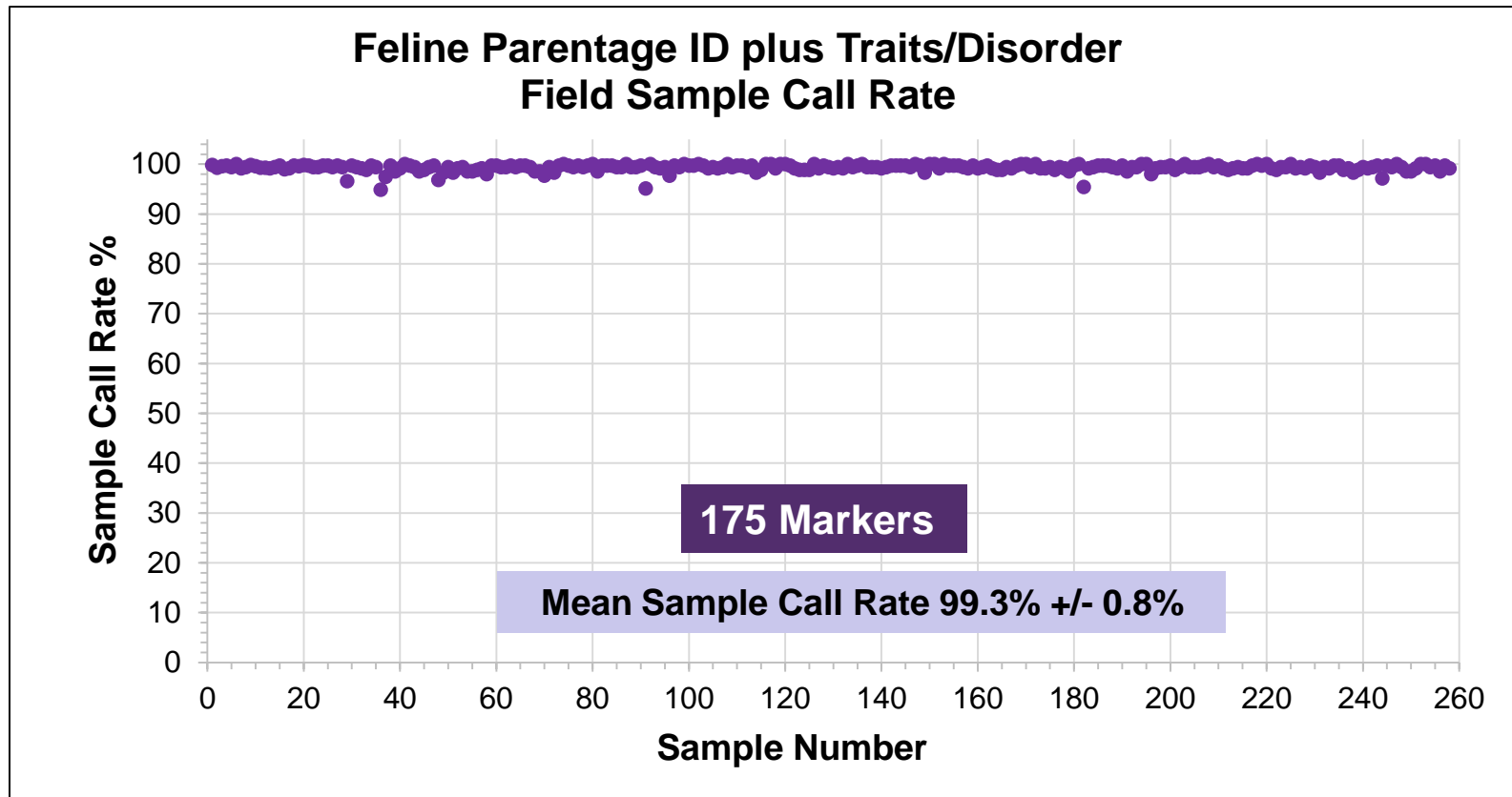
- Mean call rate was >99% with minimum variation between samples demonstrating the robustness of the genotype calls.
- The Feline PITD panel results were highly consistent with a mean genotype concordance of >99.9% for both sequencing kits between replicate samples.



- Purpose: To determine panel performance with a diverse set of sample.
- 258 feline oral swab DNA samples from 4 different labs were tested with the AgriSeq workflow using the Feline PITD kit.
- 1ng/rxn DNA was input into library prep

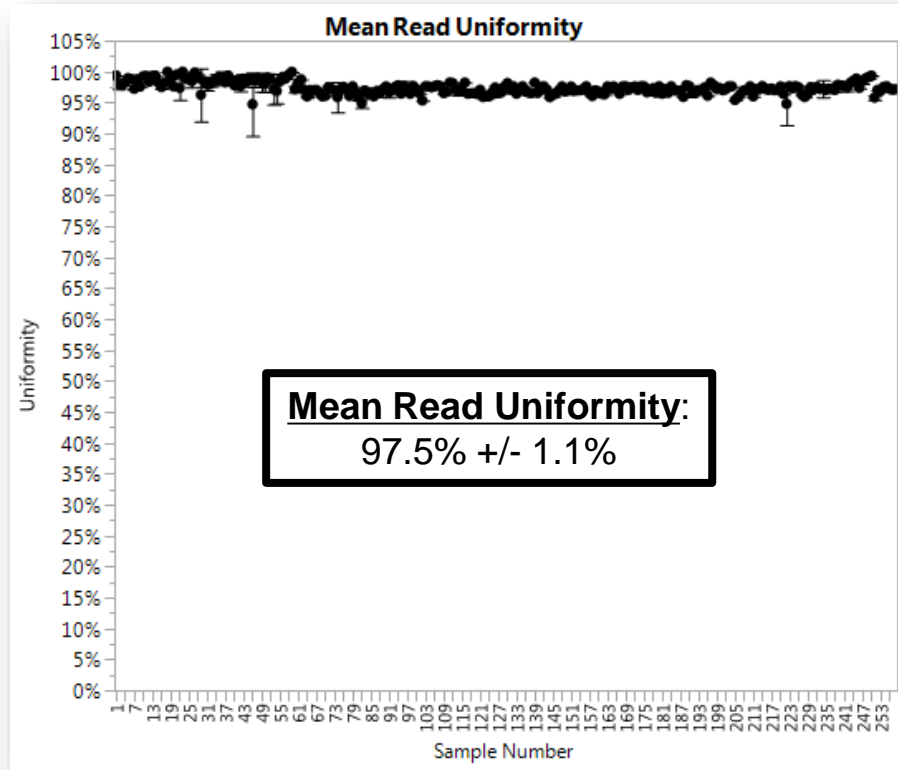
Sample Call Rates

- The mean call rate was 99.3% and all samples had call rates > 92.5% demonstrating the high performance obtained from field samples.



Read Uniformity

- Read uniformity measures 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 panel was excellent, even when testing a set of very diverse field samples (>97%).



Conclusions

- AgriSeq library prep kit and Feline PITD panel combine into a robust and efficient workflow for feline genotyping applications.
- Orthogonal Concordance 100%
- Mean Field Sample Call Rate $\geq 99\%$
- Replicate Genotype Concordance $\geq 99.9\%$



Experience the power of AgriSeq with 2 Enabling Options

Acknowledgements:

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Bruce Lau
Betsy Parker
Ravi Ramadhar

Visit Thermo Fisher Scientific (booth #1) to learn about:

GBS EASY Jump Start bundles



appliedbiosystems

Agrigenomics Ion GeneStudio instrument and starter kit promotion

The image shows a promotional banner for Applied Biosystems. It features a background of a green agricultural field with rows of crops. In the foreground, there are two pieces of laboratory equipment: a white Ion GeneStudio instrument and a black Ion GeneStudio instrument. To the left of the instruments, there are two dogs, a cat, and a small animal, all looking towards the camera.

See more - from STRs to SNPs



**Free genotyping of your sample using
AgriSeq GBS Panels**