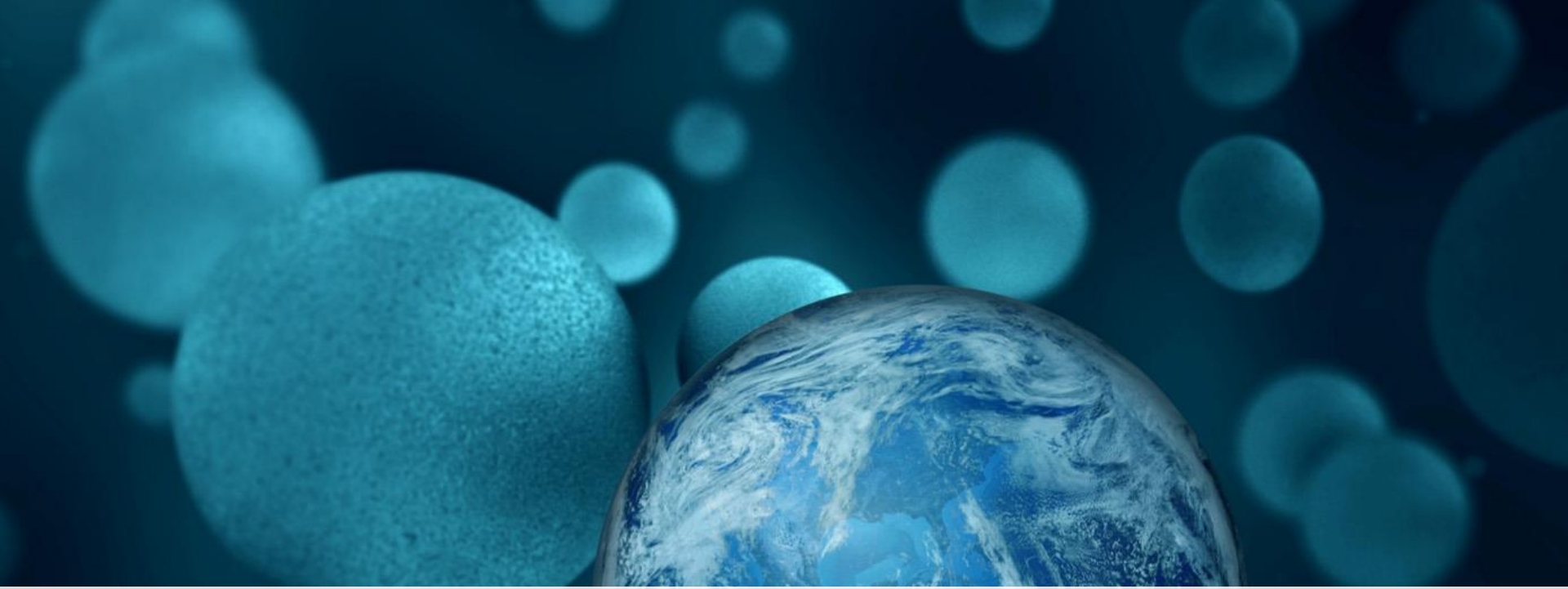


ThermoFisher SCIENTIFIC

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

Angela Burrell

9 July 2019

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

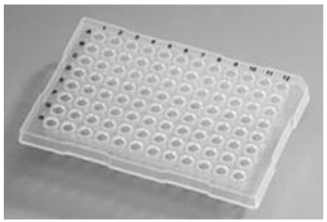
- **AgriSeq Feline Parentage, ID, Traits & Defects (PITD) Panel*** (A43408)- 175 markers

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

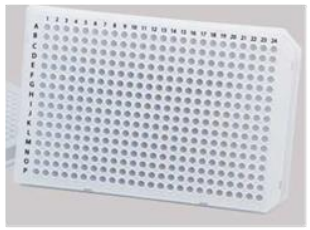
Targeted primer panel for the combined detection of feline genetic disorders/trait detection and parentage verification.

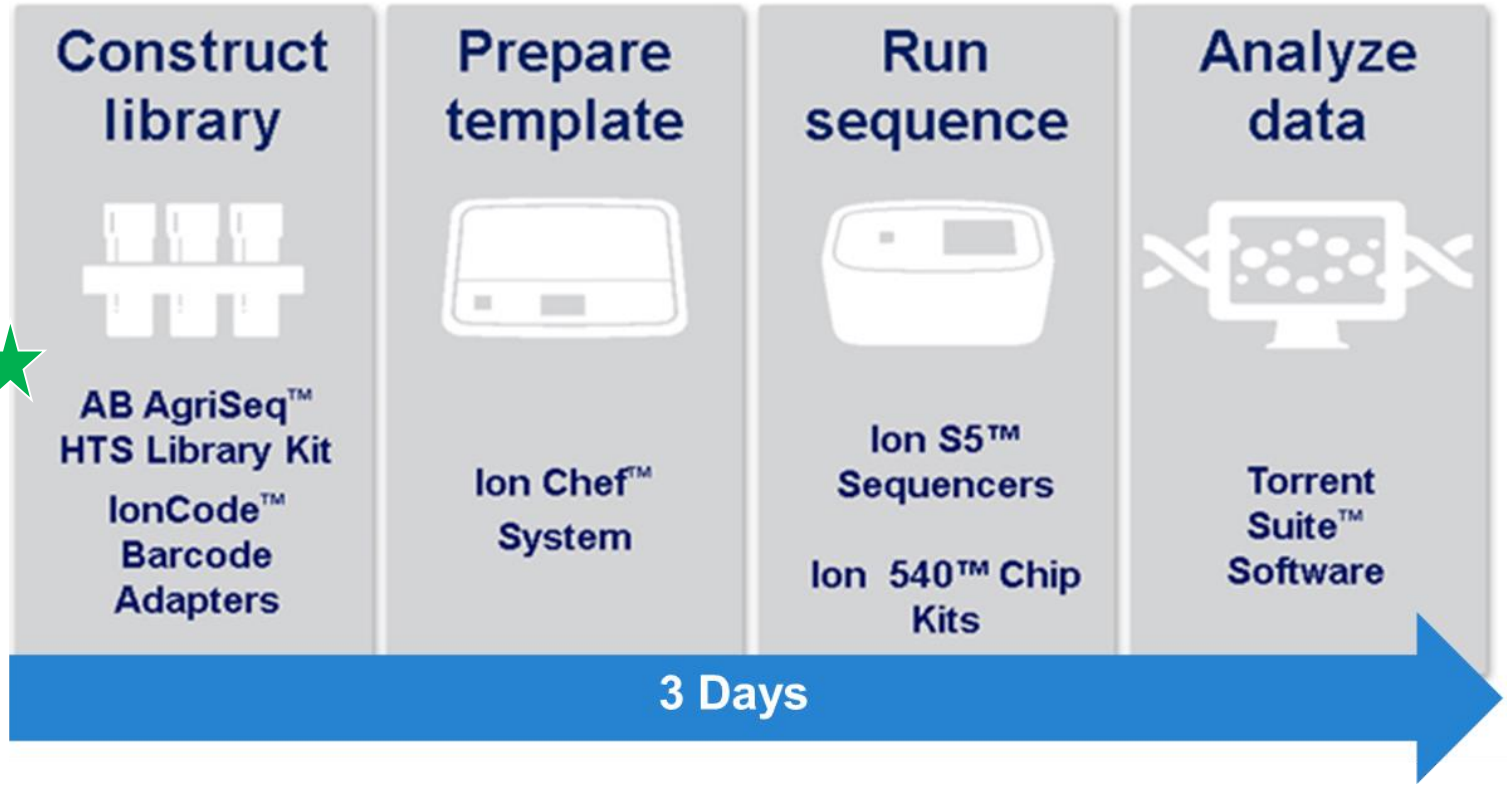
*For Research Use Only. Not for use in diagnostic procedures

AgriSeq Sequencing Workflow



10 ng gDNA input





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

ThermoFisher SCIENTIFIC

TVC Browser Output – Data for Analysis

View Allele Annotations | View Coverage Metrics | View Quality Metrics

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

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

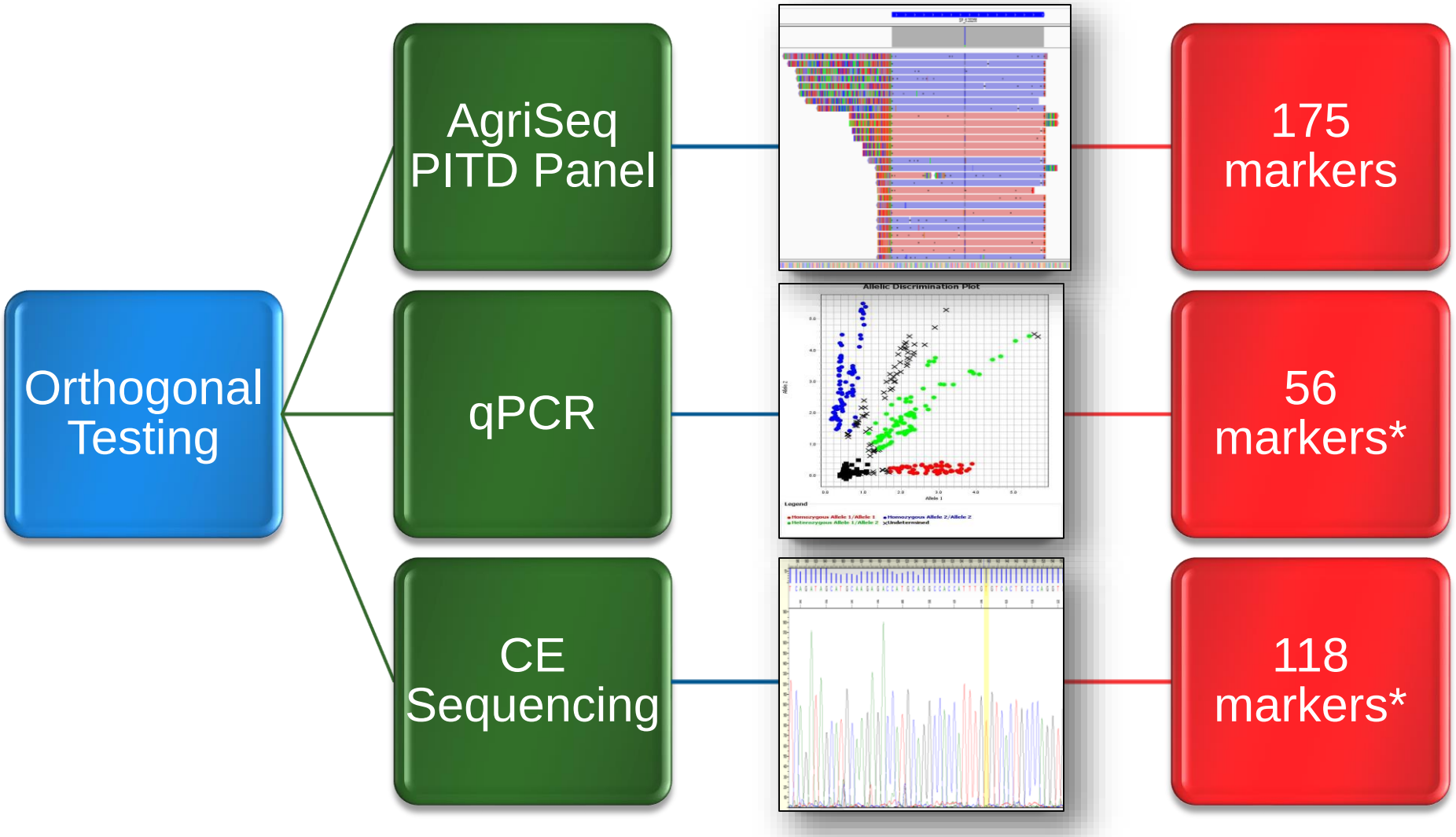
Experimental Overview

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

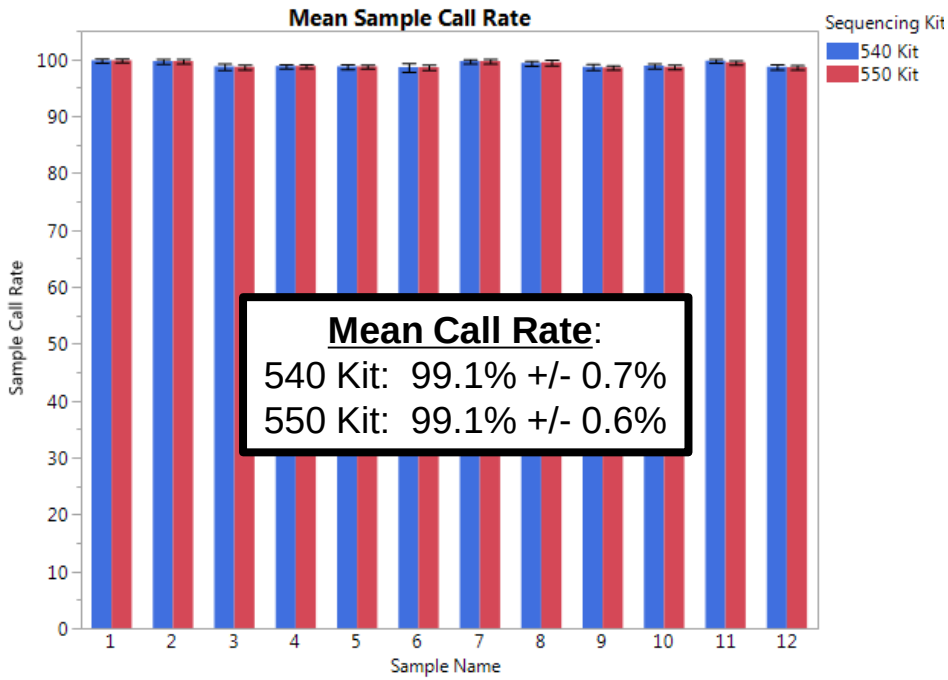
Experiment 2: Reproducibility and Repeatability

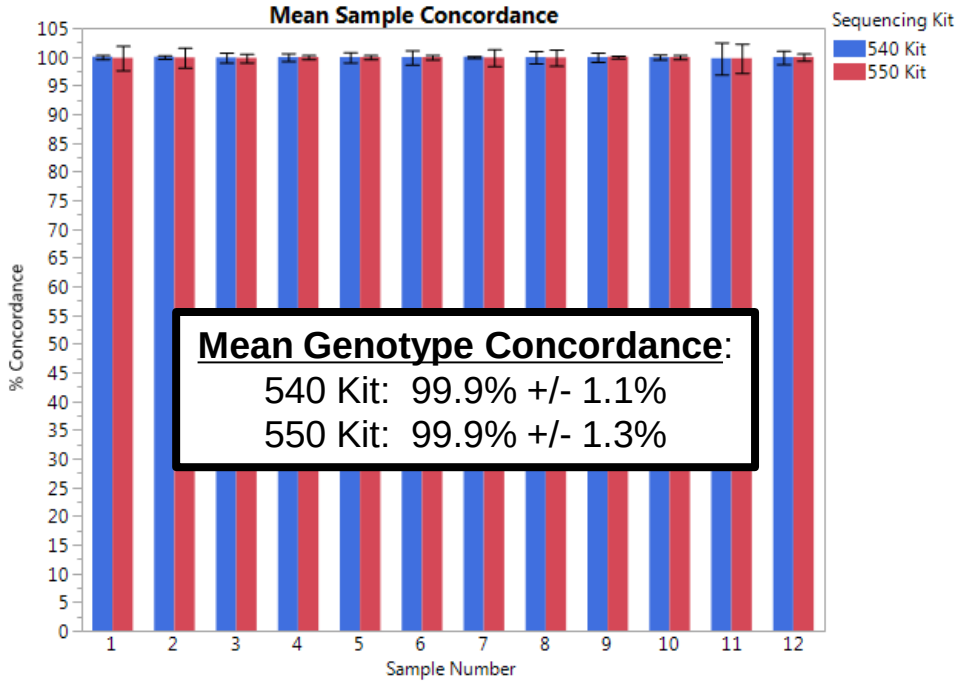
- Purpose: To test workflow robustness and genotype call consistency through multiple replicate reactions of a panel of samples.

  - 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.





ThermoFisher SCIENTIFIC

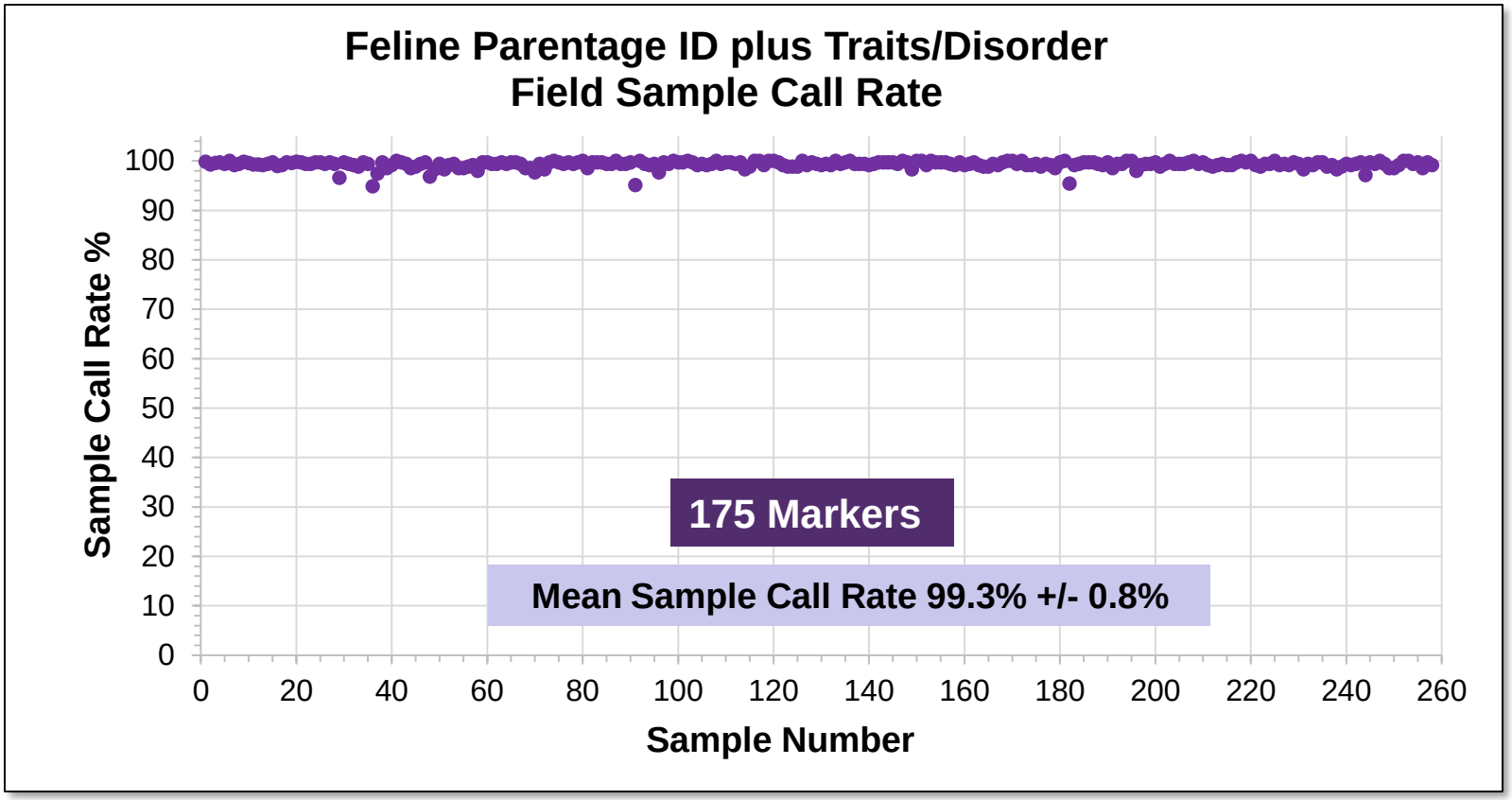
Experiment 3: Field Sample Performance

- 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

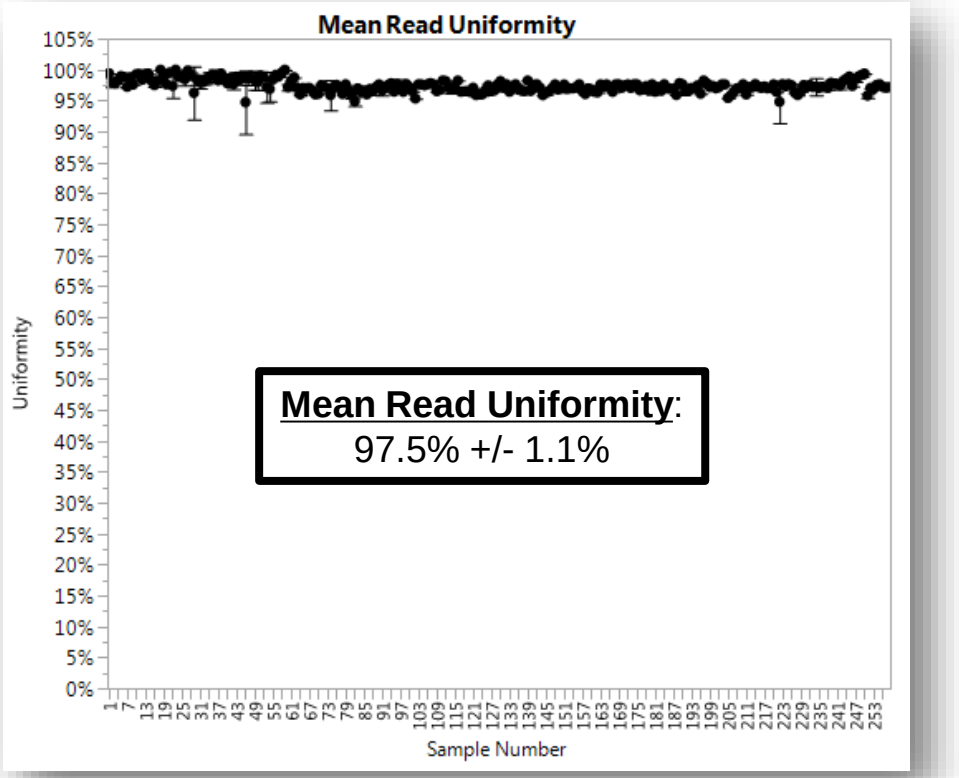
Sample Call Rates

- 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%).



ThermoFisher SCIENTIFIC

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 ≥99%
  - Replicate Genotype Concordance ≥99.9%

Experience the power of AgriSeq with 2 Enabling Options

Acknowledgements:

R&D Team
Rick Conrad
Michelle Swimley
Chris Willis

Bioinformatics Team
Prasad Siddavatam
Krishna Gujjula
Haktan Suren
Chunchao Zhang

Extended Team
Stacie Bush
Srinivas Chadaram
Mike Garguilo
Bruce Lau
Betsy Parker
Ravi Ramadhar

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

GBS EASY Jump Start bundles

See more - from STRs to SNPs

Free genotyping of your sample using AgriSeq GBS Panels

ThermoFisher SCIENTIFIC