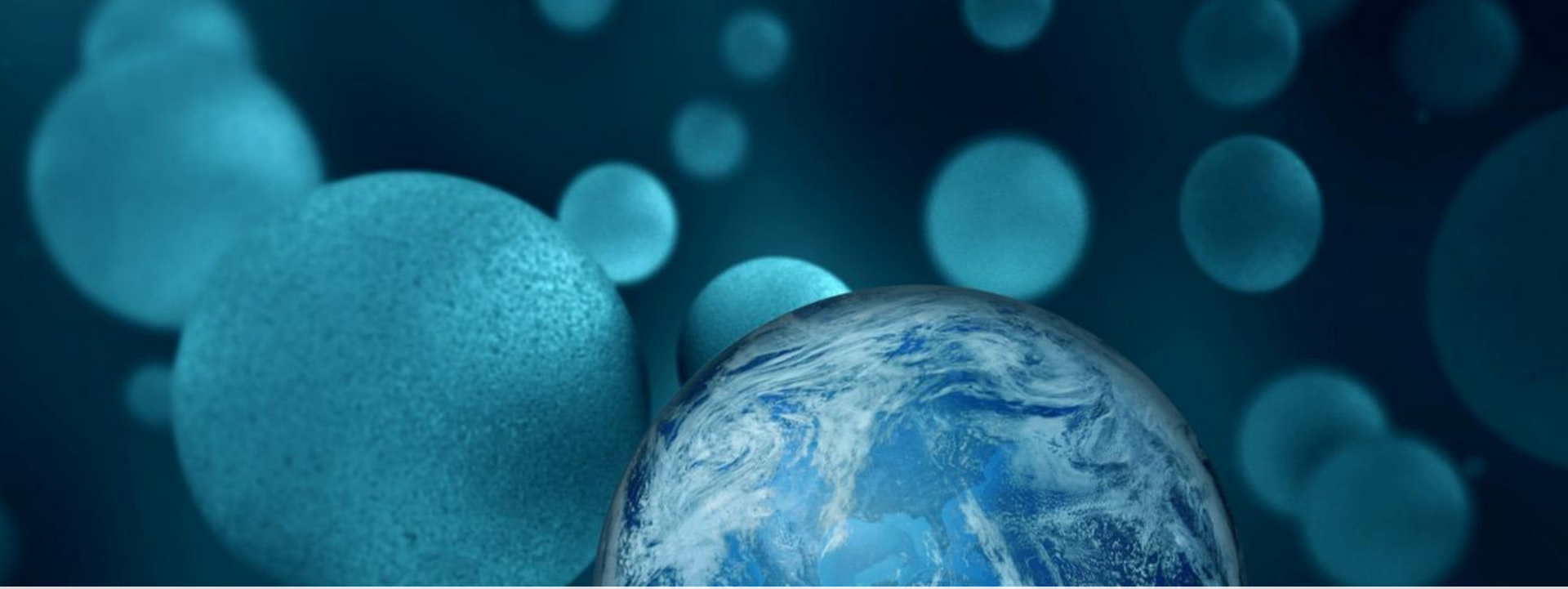

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Development of targeted GBS panels for breeding and parentage applications in dogs

Angela Burrell

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Background

- We recognize that there is a need for a robust, repeatable, and unambiguous workflow needed for canine parentage and genetic trait testing.
- We developed two targeted sequencing panels, one for canine parentage/ID verification and one for canine genetic defect/trait identification.
  - The AgriSeq Canine SNP Parentage & ID Panel
  - The AgriSeq Canine Traits & Disorders Panel
- Utilizes the AgriSeq workflow

Panels

- Evaluated performance of two panels:
  - 1. **AgriSeq Canine SNP Parentage and ID Panel*** (A43407)- 381 markers

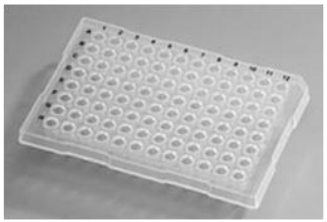
Panel	SNPs	MNPs	Insertions	Deletions
Parentage & ID	379	0	0	2

  - 2. **AgriSeq Canine Traits and Disorders Panel*** (A43406)- 154 markers

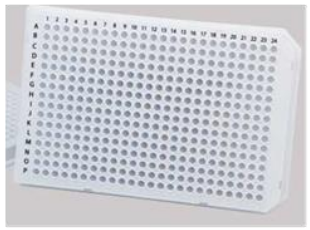
Panel	SNPs	MNPs	Insertions	Deletions
Traits & Disorders	97	6	13	38

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

AgriSeq Sequencing Workflow



10 ng gDNA input →



Construct library	Prepare template	Run sequence	Analyze data
AB AgriSeq™ HTS Library Kit IonCode™ Barcode Adapters	Ion Chef™ System	Ion S5™ Sequencers Ion 540™ Chip Kits	Torrent Suite™ Software

3 Days

★ AgriSeq Canine SNP Parentage & ID Panel and/or AgriSeq Canine Traits & Disorders Panel addition.

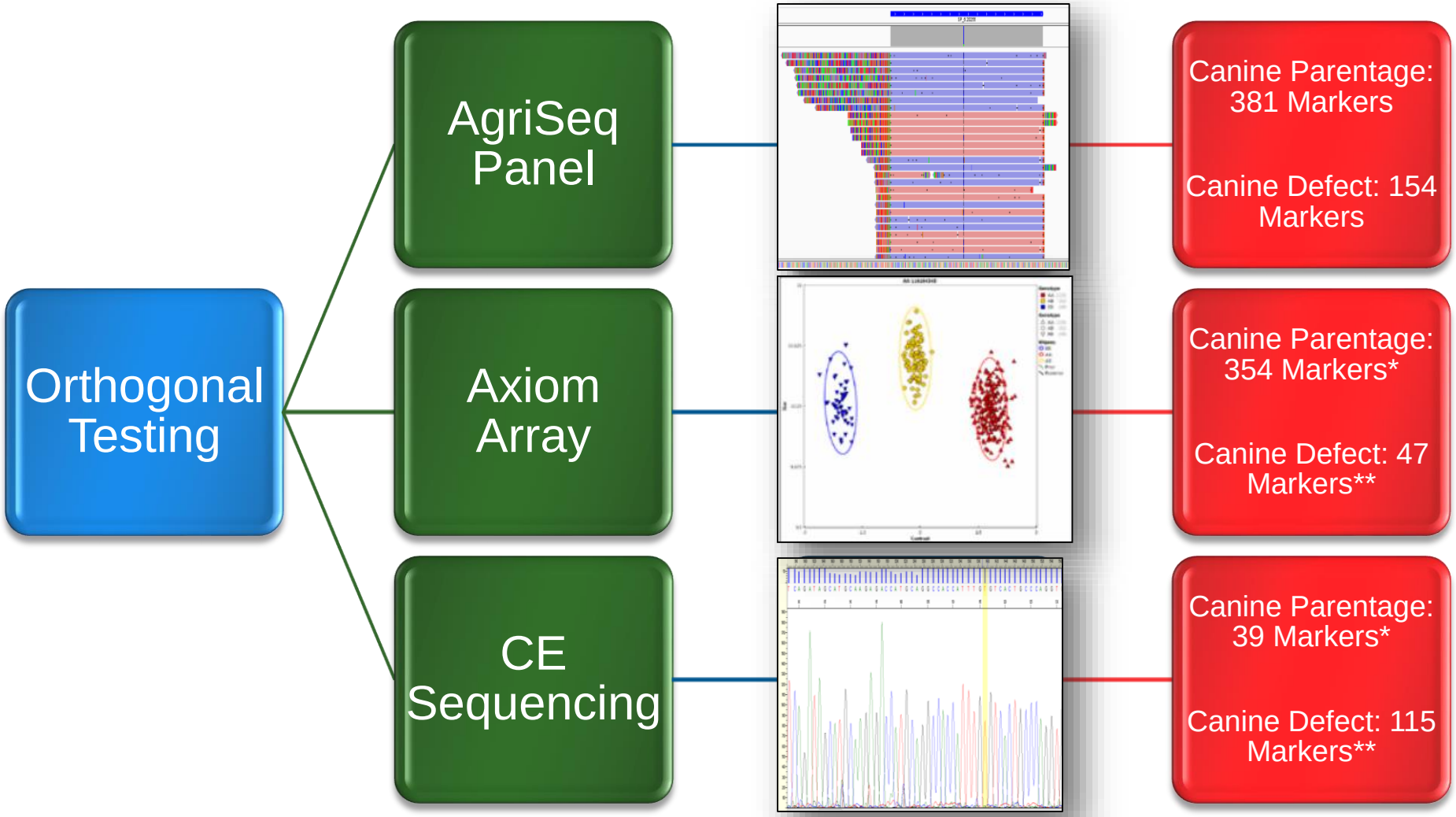
Experimental Overview

- Three general experiments were performed to validate performance of each of the canine panels.
  - 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 workflow were accurate by testing with a separate, orthogonal technology.

Orthogonal Testing Workflow- Canine Parentage & ID



Canine Parentage: *12 markers were tested with both the Axiom Array and CE Sequencing.
Canine Defect: **8 markers were tested with both the Axiom Array and CE Sequencing.

Canine Parentage and ID Orthogonal Testing

- Of the 381 markers tested, >99% of genotype calls were concordant with AgriSeq calls.
- *3 discordant markers by Array were tested by CE. CE Results for these markers matched AgriSeq results.

Orthogonal Method	# Concordant Markers to GBS	# Discordant Markers to GBS	# No Calls	Concordance
CE Sequencing	36	0	3	>99%
Axiom Array	349	3*	2	

Canine Traits & Disorders Orthogonal Testing

- 5 markers that were unable to be genotyped by CE testing due to poor sequencing quality and were omitted from calculations
- Of the 149 markers that were able to be genotyped by an orthogonal method, concordance to the AgriSeq workflow was 100%.

Orthogonal Method	# Concordant Markers to GBS	# Discordant Markers to GBS	# No Calls	Concordance
CE Sequencing Only	102	0	5	100%
Axiom Array Only	39	0	0	
Both CE and Array	8	0	0	

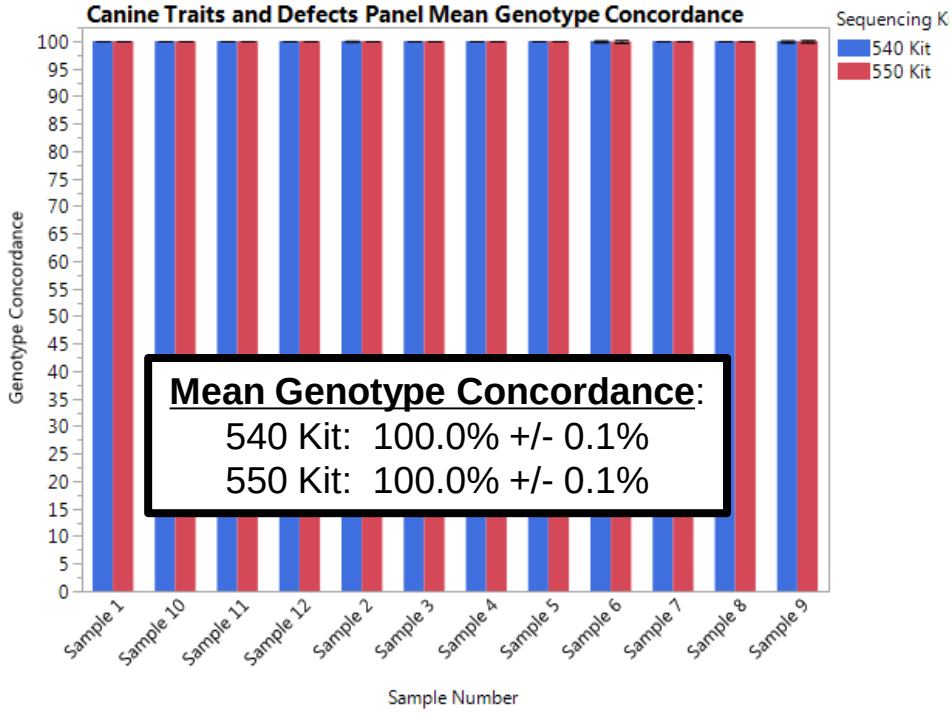
Experiment 2: Reproducibility and Repeatability

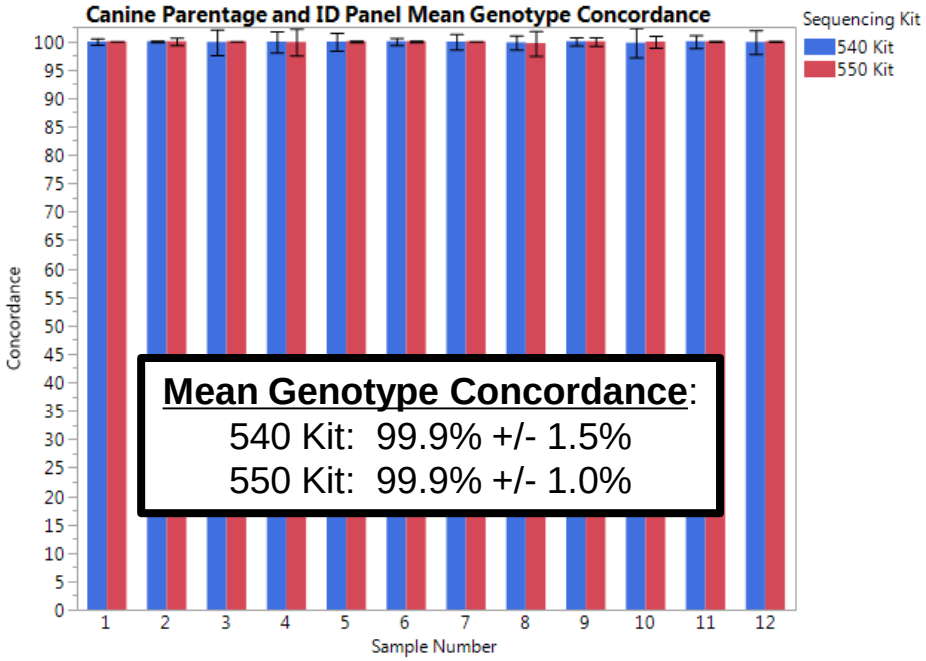
- Purpose: To test workflow robustness and genotype call consistency through multiple replicate reactions of a panel of samples.

- 12 DNA samples were tested in replicates of n=64 using the AgriSeq workflow for a total of 768 barcoded libraries.
- Each library pool was sequenced twice on a 540 and 550 chip.

Repeatability and Reproducibility

- Replicate genotype concordance was calculated.
- Both panels had ≥99.9% replicate concordance for all sequencing chips.





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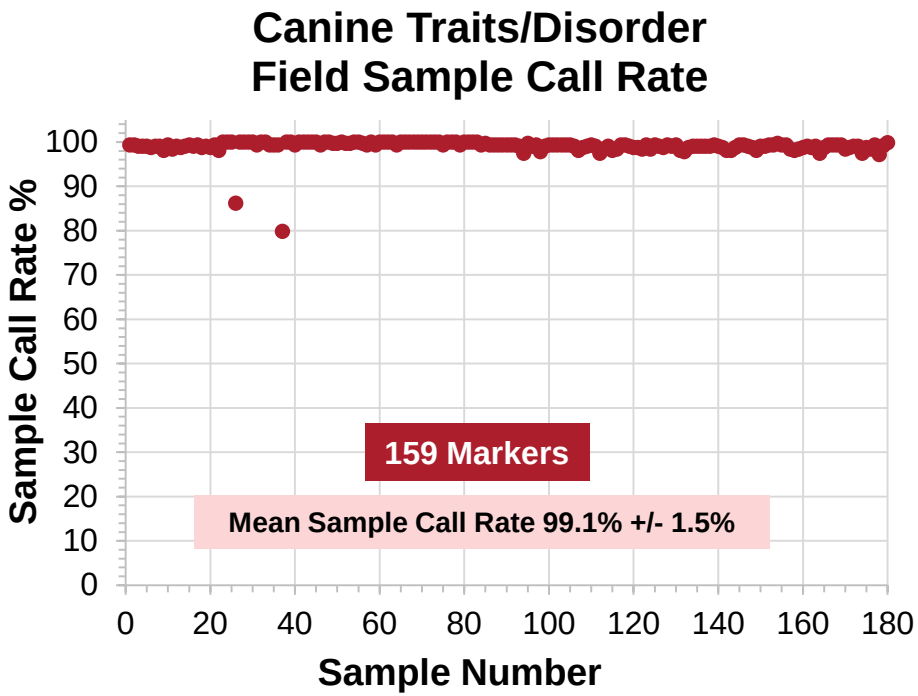
Experiment 3: Field Sample Performance

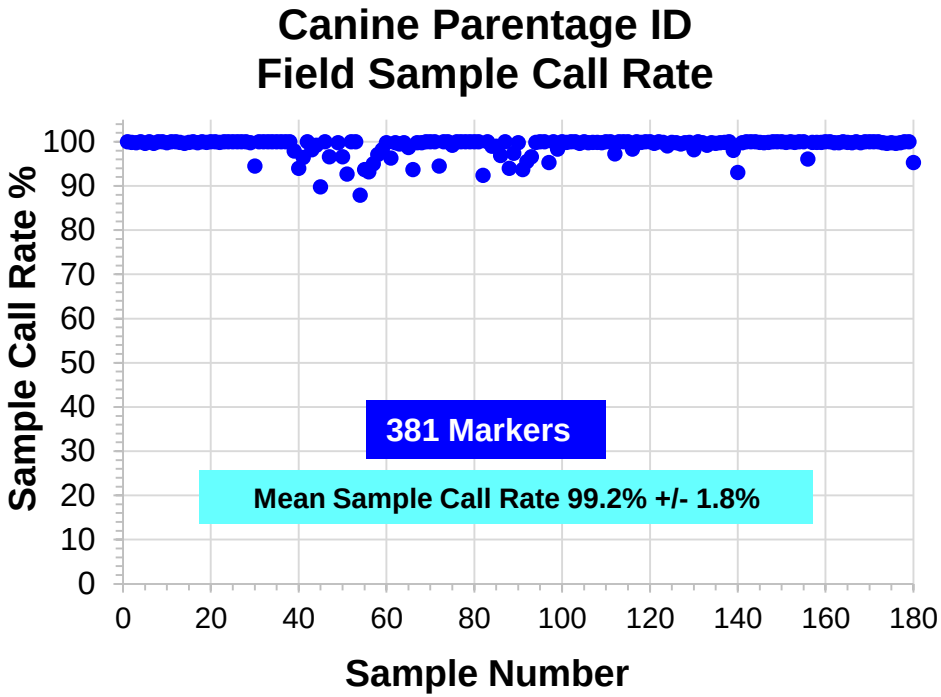
- Purpose: To determine panel performance with a diverse set of sample.

- Tested panel of 180 samples (oral swabs) in replicates (n=2) with each panel of the AgriSeq workflow.
- 1ng/rxn DNA was input into library prep.

Sample Call Rates

- ≥180 diverse canine oral swab DNA field samples with the AgriSeq workflow.
- The mean call rate for the Canine Trait/Disorders panel was 99.1% and the mean call rate for the Canine Parentage panel was 99.2%.





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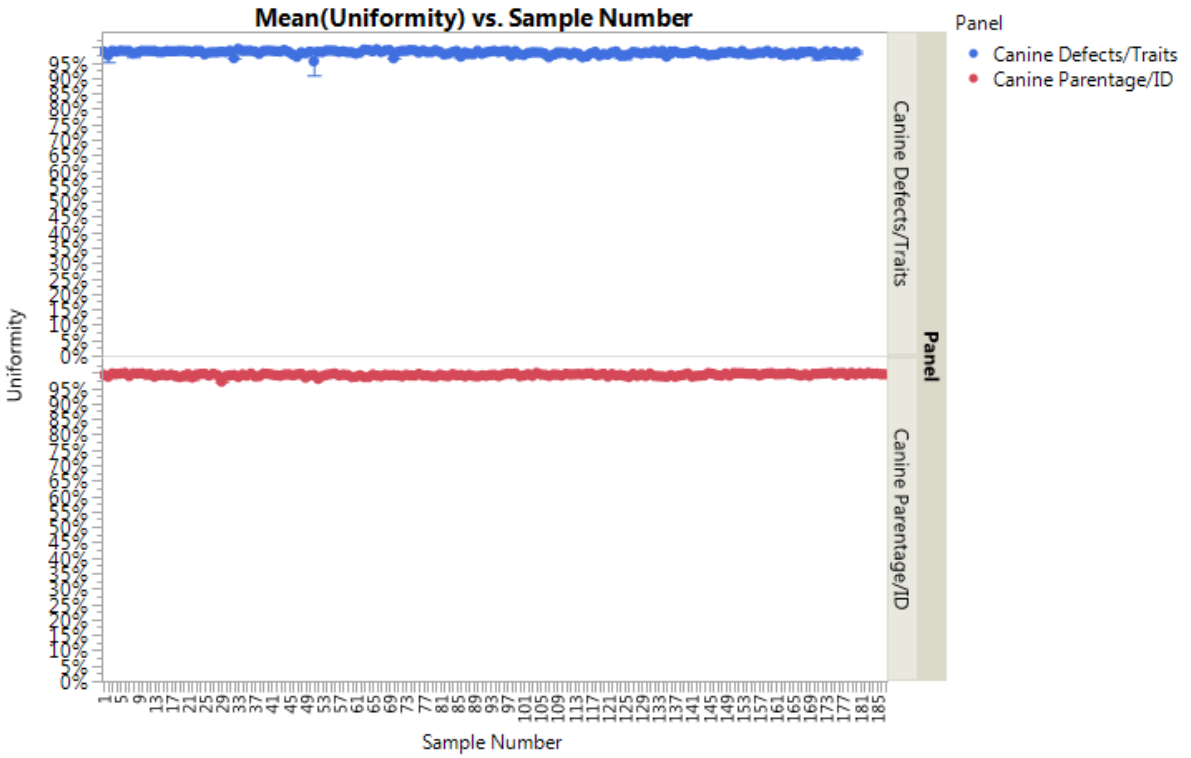
Read Uniformity

- 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 both panels was excellent, even when testing a set of very diverse field samples (>98%).



Panel	Mean Uniformity	stdev
Canine Parentage/ID	99.3%	0.4%
Canine Trait/Defect	98.2%	0.7%

Conclusions

- AgriSeq library prep kit and canine GBS panels combine into a robust and efficient workflow for canine genotyping applications.
  - Orthogonal Concordance ≥99.9
  - Mean Field Sample Call Rate ≥99%
  - Replicate Genotype Concordance ≥99.9%

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Experience the power of AgriSeq with 2 Enabling Options

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Ravi Ramadhar

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