

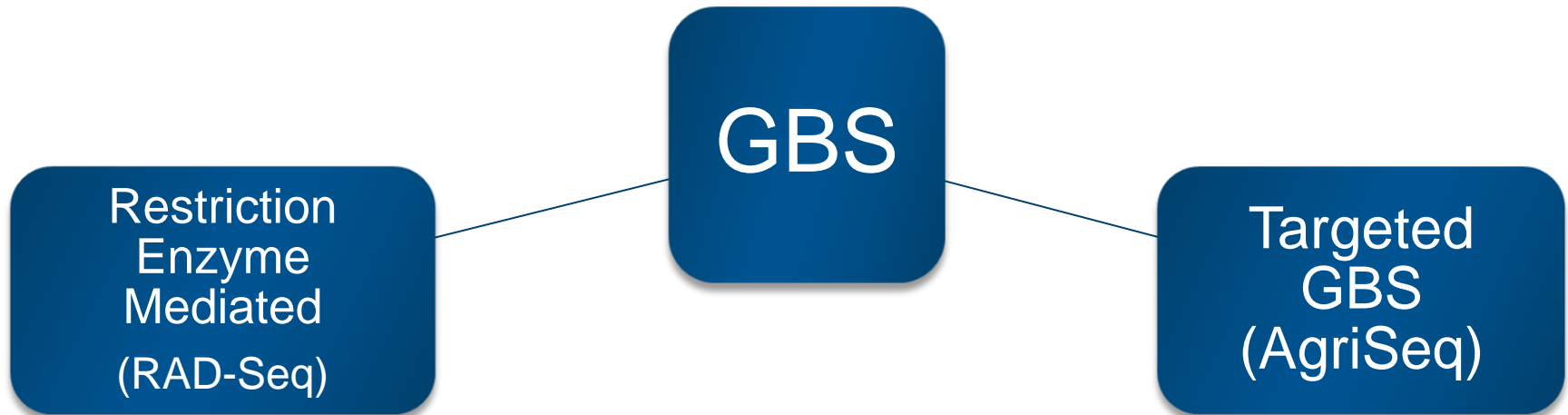


# Development of targeted GBS panels for breeding and parentage applications in cattle and swine

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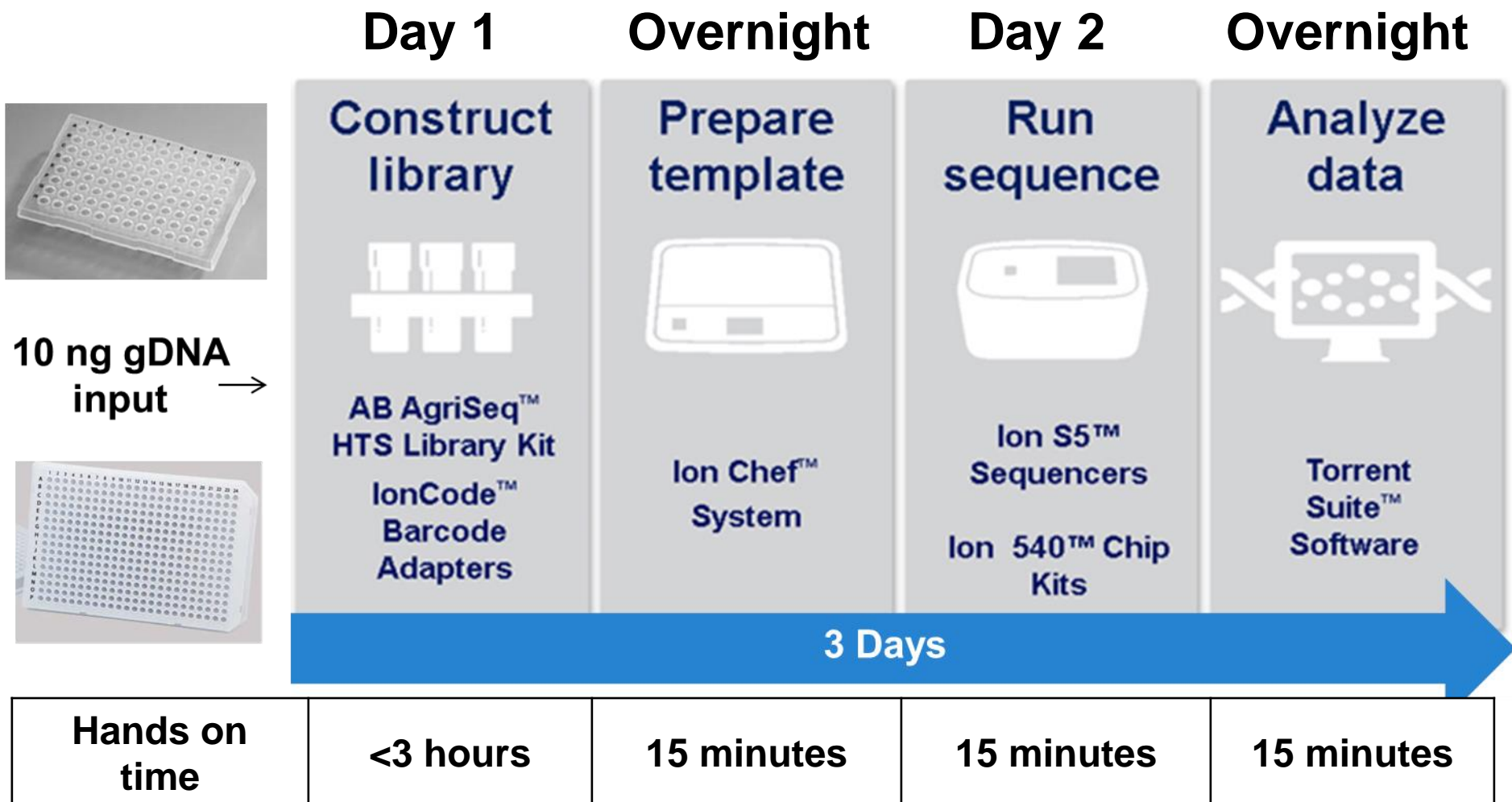
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# Genotyping by sequencing can represent different approaches



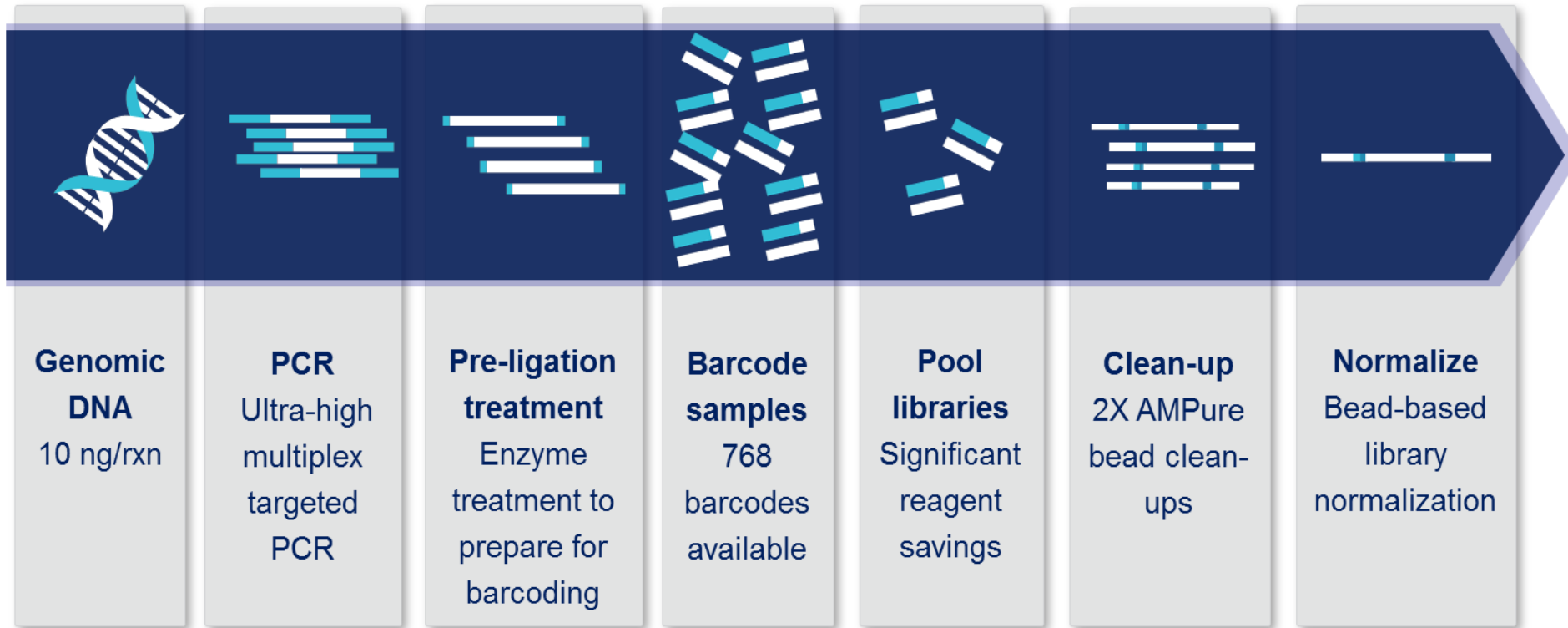
Key Differences		
Restriction enzyme gDNA digestion and sequencing	Workflow	Ultra-high multiplex amplicon re-sequencing
Random	Types of markers identified	Targeted – user selected
Up to 100,000s	Number of SNPs interrogated/sample	Up to 5,000
Med (20-80%)	Consistency of markers called between samples	High >95%
Discovery	Best fit for ...	Mapping/Screening

# AgriSeq Targeted GBS Sequencing Workflow



***Up to 1536 samples can be processed/time***

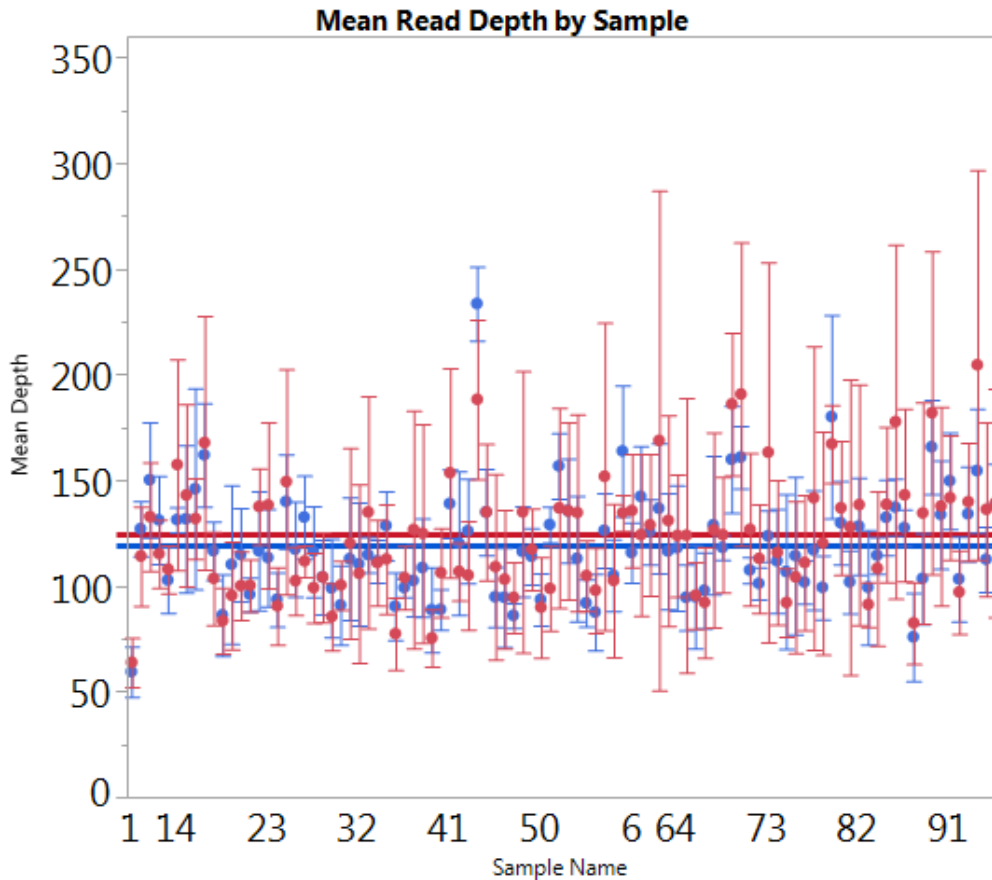
# AgriSeq Library Prep Workflow



- Custom Amplicon Panels- Multiplex 50-5,000 markers (SNPs, MNPs, and InDels) in a single tube.
- 96 and 384-well protocols that can be performed manual or automated on liquid handlers.
  - 6-7 total time (manual <3 hours hands on-time, automated: ~1 hour hands on time)

- The AgriSeq workflow was validated with two different custom GBS panels:
  - Porcine Genotyping Panel
    - 1500 amplicon SNP panel
    - Markers chosen by a collaborator for porcine genotyping
    - 96 samples, n=4 replicates,
  - Bovine ISAG SNP Parentage Panel (2013)
    - 200 amplicon panel
    - Marker chosen by ISAG for bovine parentage verification
    - 115 samples, n=4 replicates

# Balanced Read Depth Across Samples Facilitate Multiplexing

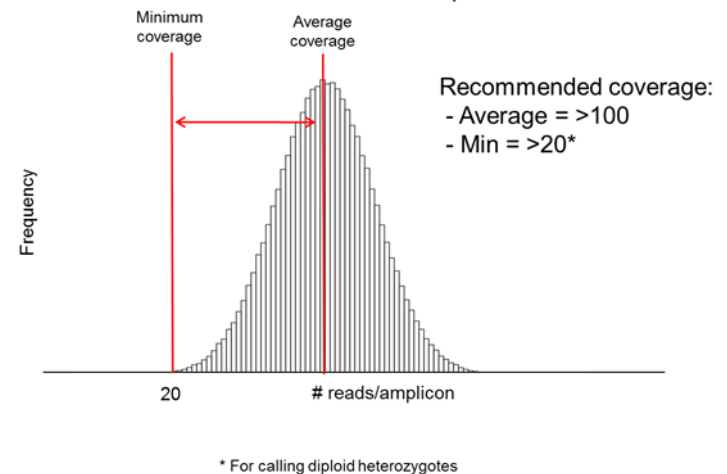


## Mean Call Rate

**AgriSeq 384-well: 119X +/- 31X**

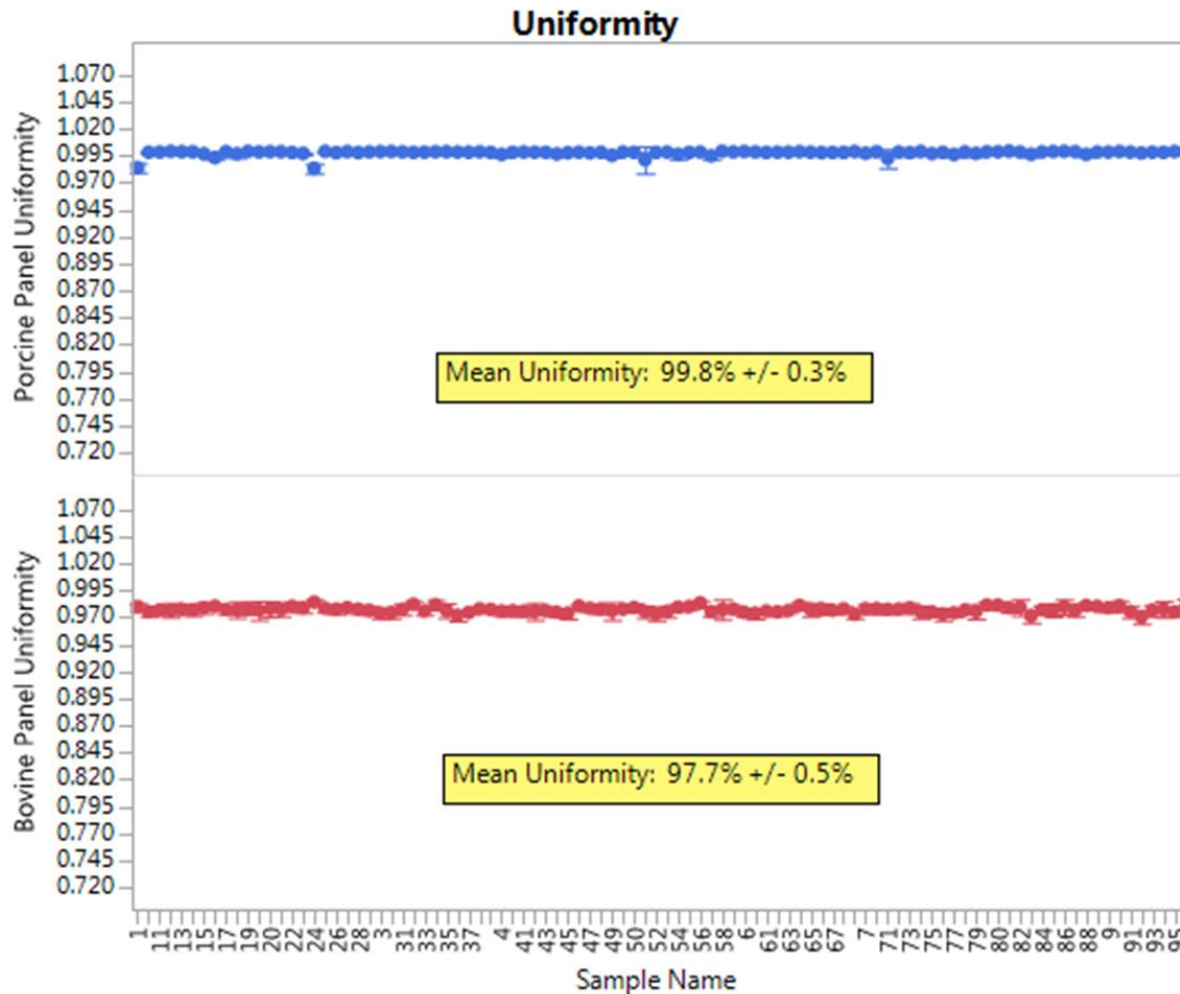
**AgriSeq 96-well: 124X +/- 45X**

How to calculate average coverage:  $\frac{\text{Reads/chip}}{\text{Samples} \times \text{SNPs}}$



- Sufficient read coverage depth (20X) is required for the software to make a genotype call.
- Targeting >100X coverage ensure that virtually all of your markers have sufficient coverage.

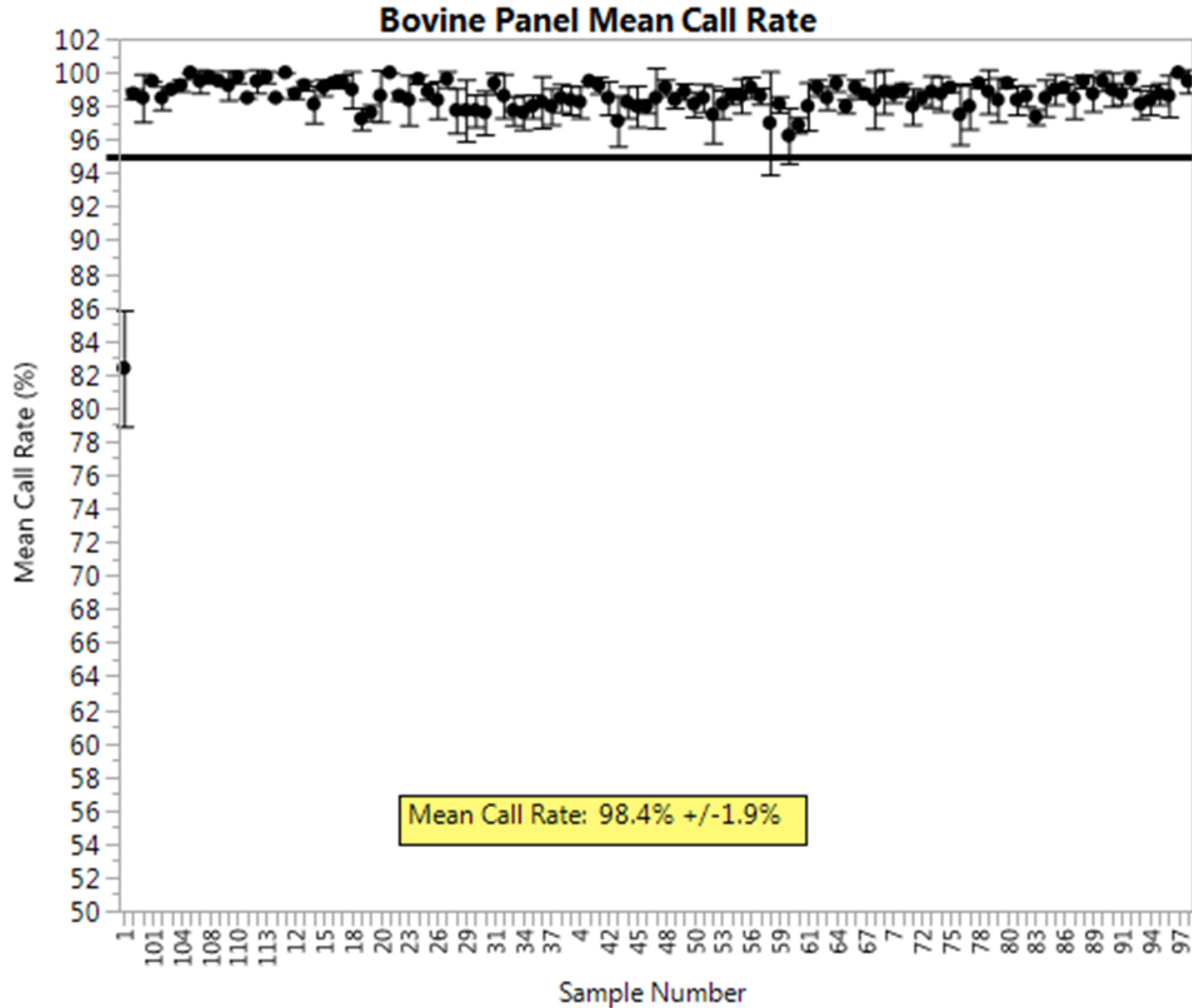
# Mean Read Uniformity



- Read uniformity is the % 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.

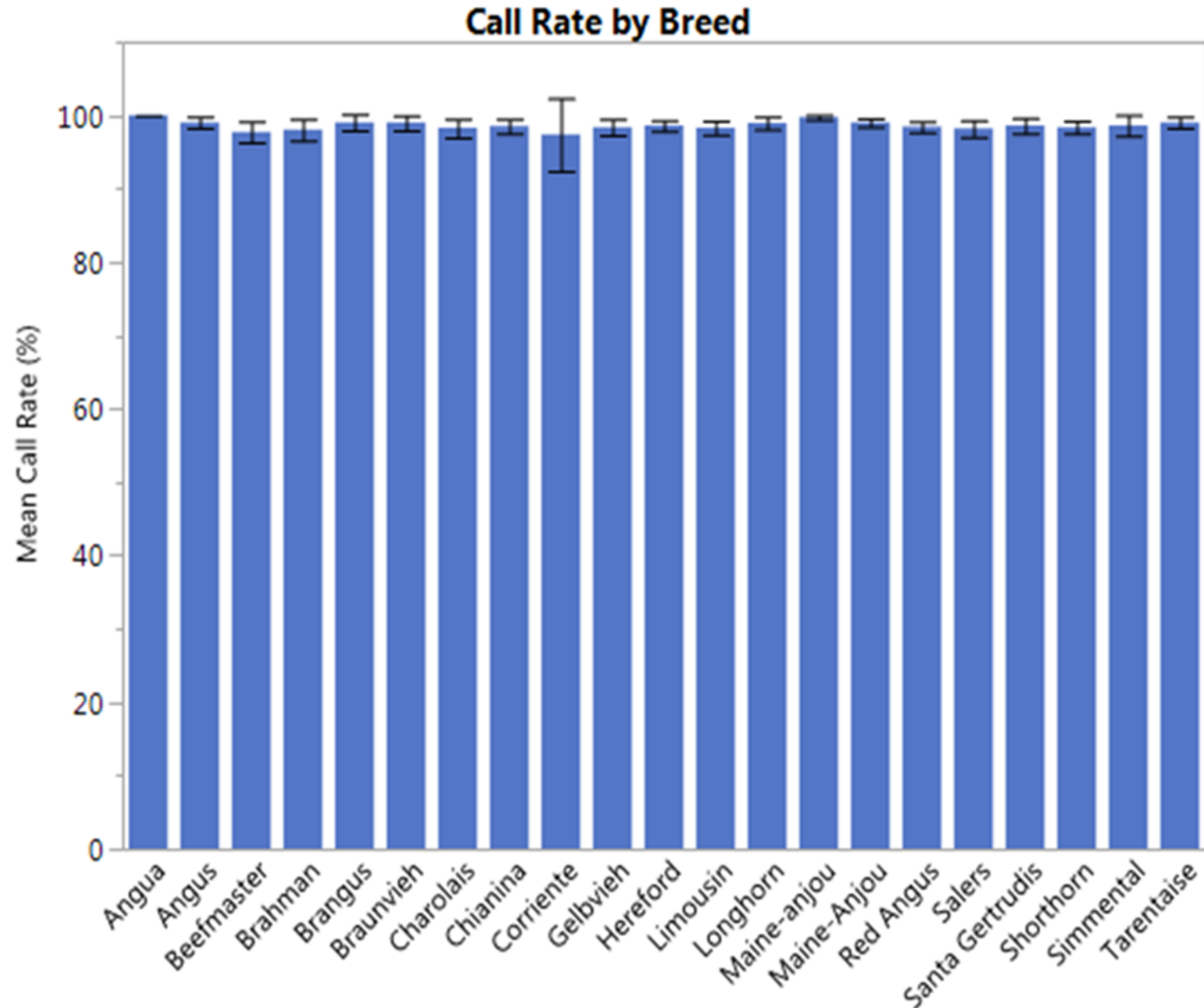


# Bovine ISAG SNP Parentage Panel Mean Sample Call Rate





# Call Rate Across Breed Comparison



# High Replicate Genotype Concordance

Panel	Metric	Mean Genotyping Call Concordance	Stdev
Bovine ISAG	Intra-run Concordance	99.96%	0.13
	Inter-run Concordance	100.00%	0.00

- To determine genotype concordance we ran a single sample in 96 replicates through the AgriSeq workflow.
- Genotype concordance was calculated between the replicates on the same sequencing run and between replicates on separate sequencing runs to determine intra-run and inter-run genotype concordance.
- Both inter-run and intra-run concordance was >99.9%.

# 2015 ISAG/ICAR Parentage Results

	Thermo Fisher Scientific Results	2015 ISAG/ICAR Reported Results	Results
Bovine 9 and 21	Parentage Verified	Parentage Verified	✓
Bovine 17 and 21	Parentage Excluded	Parentage Excluded	✓

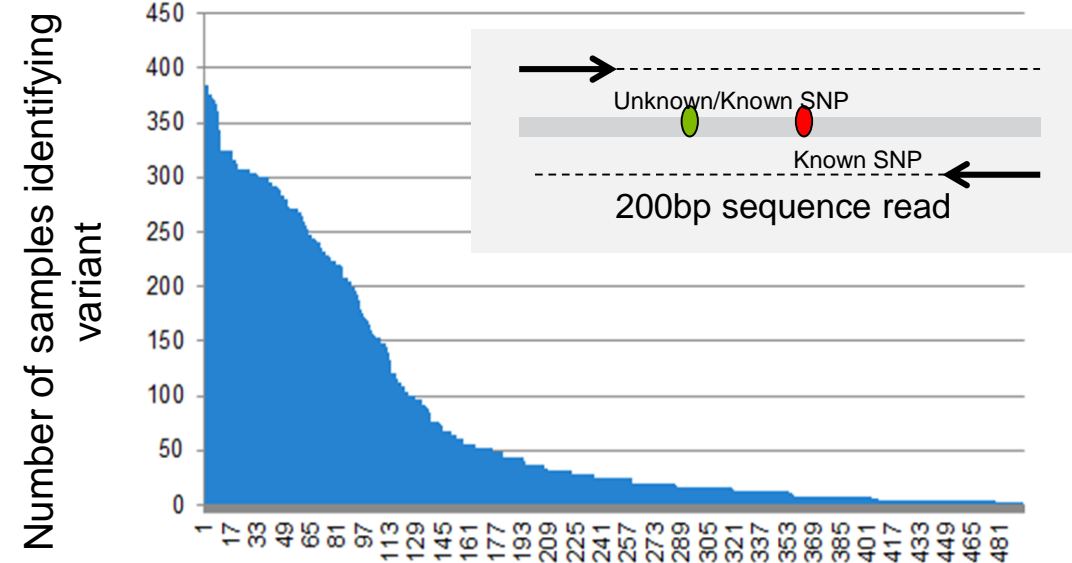
- Two pairs of samples in the 2015 ISAG/ICAR 3<sup>rd</sup> SNP Typing Bovine Comparison Test were tested for parentage validation and compared to the reported results.
- Using the AgriSeq workflow and ISAG criteria for parent verification (July 2012), both pairs of samples were tested.
- Results were completely concordant with the 2015 ISAG/ICAR reported results.

# Orthogonal Technology Call Concordances

Number of Samples	44
Total number of markers	8800
Number of concordant calls	8751
Concordance (%)	99.4%

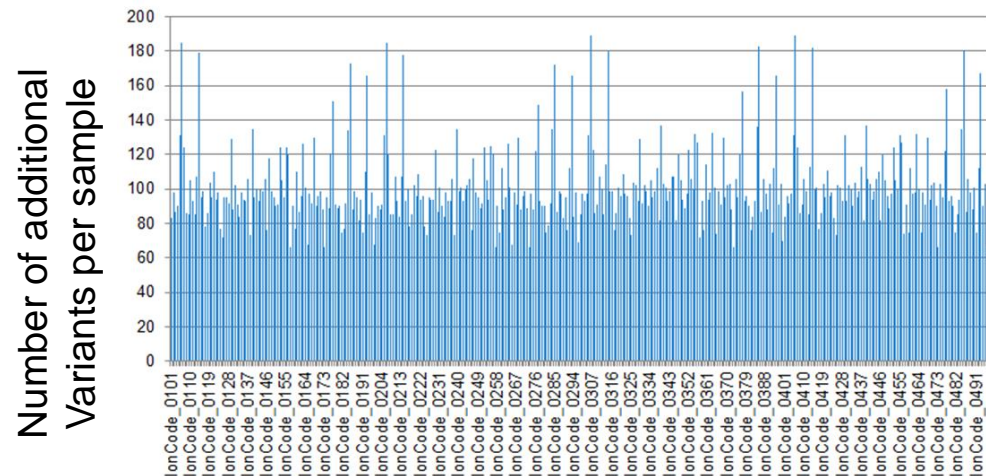
- Orthogonal technology genotyping calls not available for the porcine samples, analysis performed on Bovine samples only.
- Bovine samples were hybridized to six microarrays in order to obtain consensus genotype calls for the microarray data.
- Concordance was calculated as the number of times the genotype call matched between samples run on the two different technologies divided by the total number of markers (200).

# Sequencing-based SNP Detection Allows for Identification of Novel SNPs



- The AgriSeq workflow can identify additional SNPs that fall within the targeted amplicon regions.
- Utilized for linkage analysis and the generation of microhaplotypes for improved marker specificity or enhanced discrimination in parentage/traceability analysis.

- Above, 495 additional variants identified with varying frequency (4-384).
- Average 101 additional variants identified across samples (1088-1423) .



# Conclusions

- The AgriSeq library prep workflow along with custom GBS panels provide a streamlined, cost-effective method for animal 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.
- High call rates (98.4%), replicate call concordance (>99.9%), and high concordance with orthogonal microarray data (99.4%) demonstrate the utility of The AgriSeq workflow for robust and efficient animal genotyping.

# Acknowledgements

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