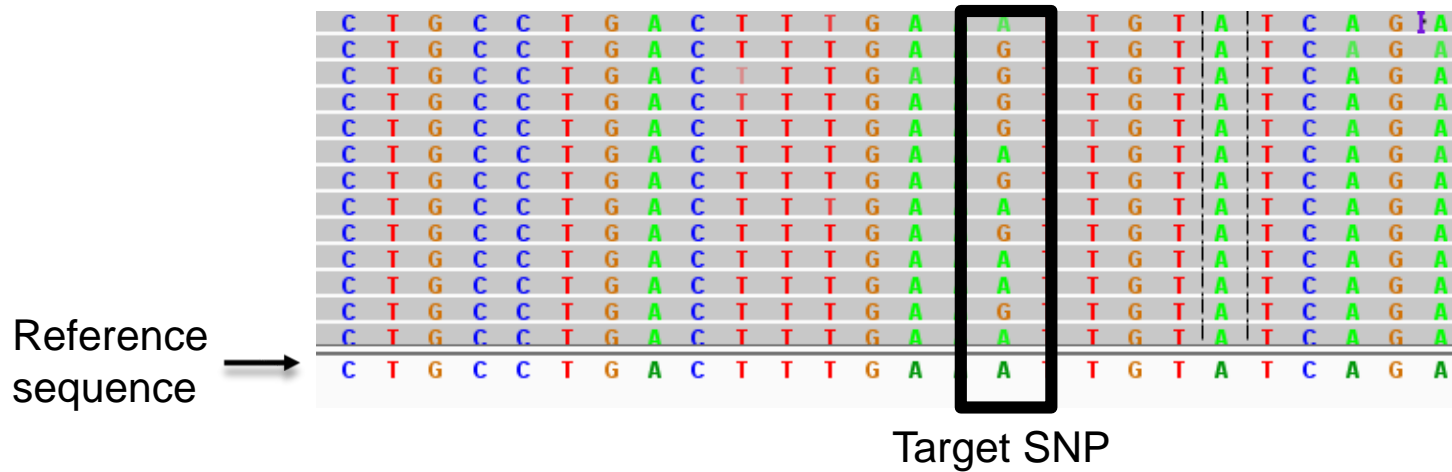**ThermoFisher**
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Modular automation solution for targeted genotyping by sequencing for animal breeding

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The world leader in serving science

AgriSeq™ targeted GBS is a flexible, powerful genotyping system

Reference sequence →

Target SNP

Select targets

Custom BFx design targeting 50-5000 SNPs, MNPs, or In/Dels

Construct library

Single tube amplicon based multiplexing

Run sequence

Barcode samples & combine different panels or species in same run

Analyze data

Automated genotype calling & flanking 200 bp SNP identification

Low sample cost $5-15/sample

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AgriSeq targeted GBS generates consistent, complete results

Metrics

- Sample call rates: ave number of markers called across diverse sample set
- Uniformity: % of bases covered at least 0.2X of the average coverage
- % On Target Read: Specificity of panel to targeted amplicons

Species	Markers	Sample Call Rate	% Uniformity	% on Target reads
Bovine	190	99.7%	98%	98%
Canine	229	99.2%	99%	99%
Feline	62	99.8%	99%	97%
Porcine	3000	96.2%	98%	99%
Salmon	3152	93.9%	94%	99%
Equine	204	99.2%	99%	99%
Cucumber	2804	91.4%	97%	100%
Maize	1079	87.5%	87%	98%
Soybean	1134	98.3%	97%	99%

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Ion™ 550 Chip & GeneStudio™ Systems

- Reads: 100 -130M
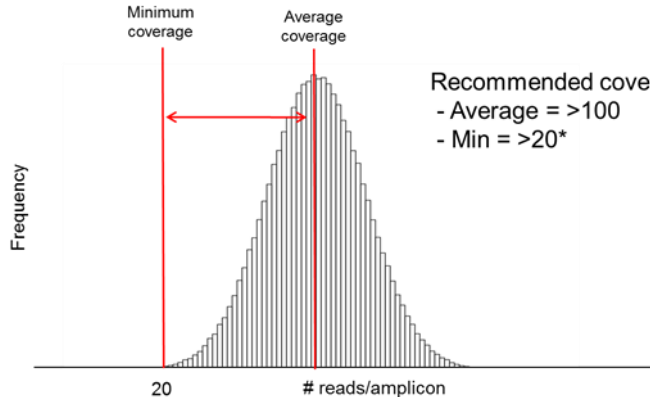
- Read-length: Up to 200 bp

- Run time: 2.5 hours

100X average coverage to ensure maximal genotyping

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

Minimum coverage

Average coverage

Recommended coverage:
- Average = >100
- Min = >20*

Frequency

20 # reads/amplicon

Flexible to scale different marker vs. sample combinations

		Number of Samples		
		per Chip	per Day	per Week
Number of makers	1500	768	1536	7680
	2500	460	920	4600
	5000	230	460	2300

2-3 day AgriSeq™ workflow with minimal hands on time

10 ng of DNA →

	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

2-3 days
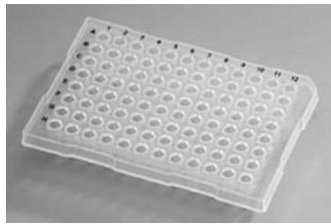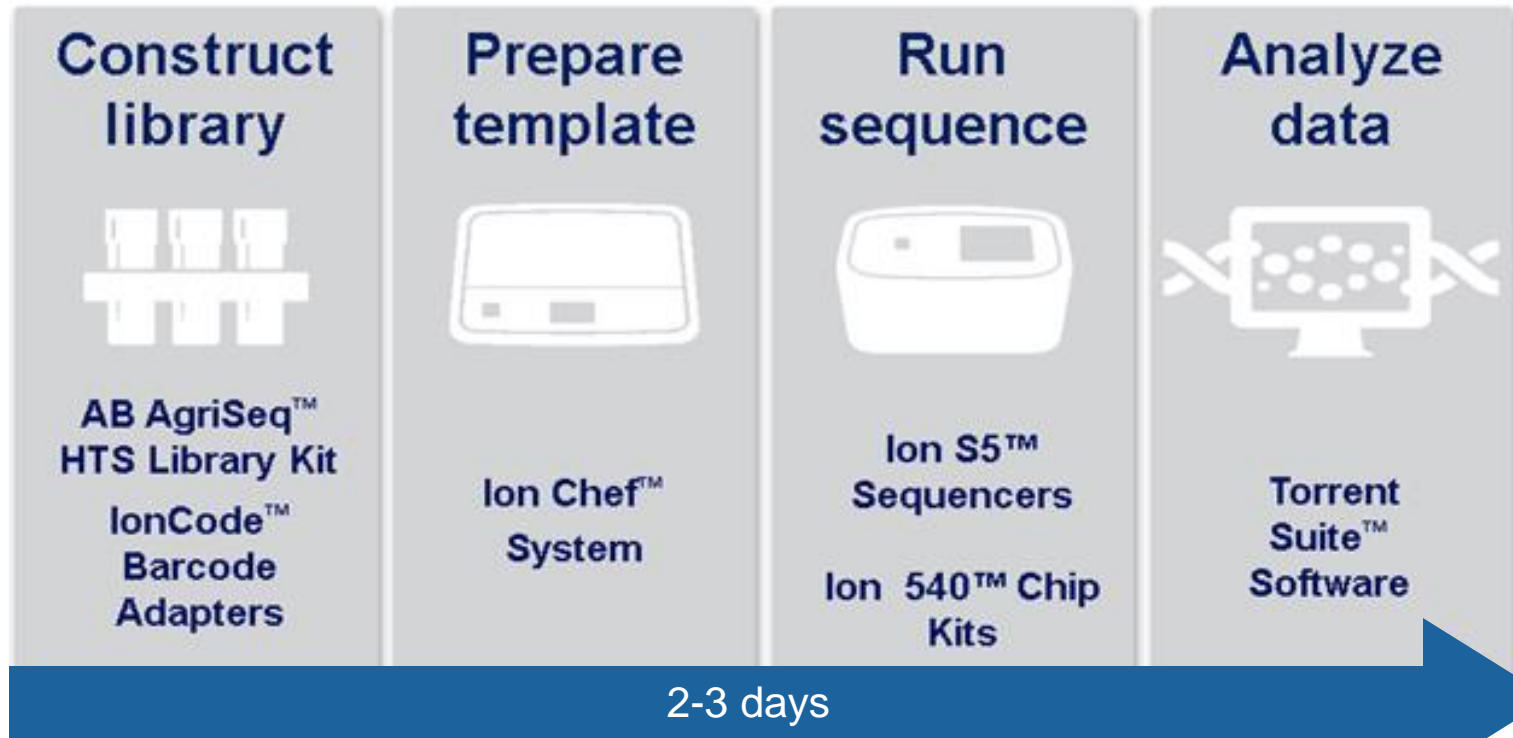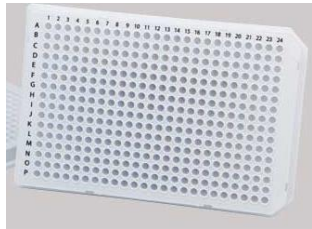
	Construct library	Prepare template	Run sequence	Analyze data
Total time	6-7 hours	overnight	2.5 hours	6-24 hours
Hands on time	<3 hours	<15 minutes	<15 minutes	<15 minutes

Workflow is achievable with 1 FTE, but opportunity for automation to reduce hands-on-time, operator fatigue and potential for error

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AgriSeq Library Prep Workflow

Genomic DNA	PCR	Pre-ligation treatment	Barcode samples	Pool libraries	Clean-up	Normalize
10 ng/rxn	Ultra-high multiplex targeted PCR	Enzyme treatment to prepare for barcoding	768 barcodes available	Significant reagent savings	2X AMPure bead clean-ups	Bead-based library normalization
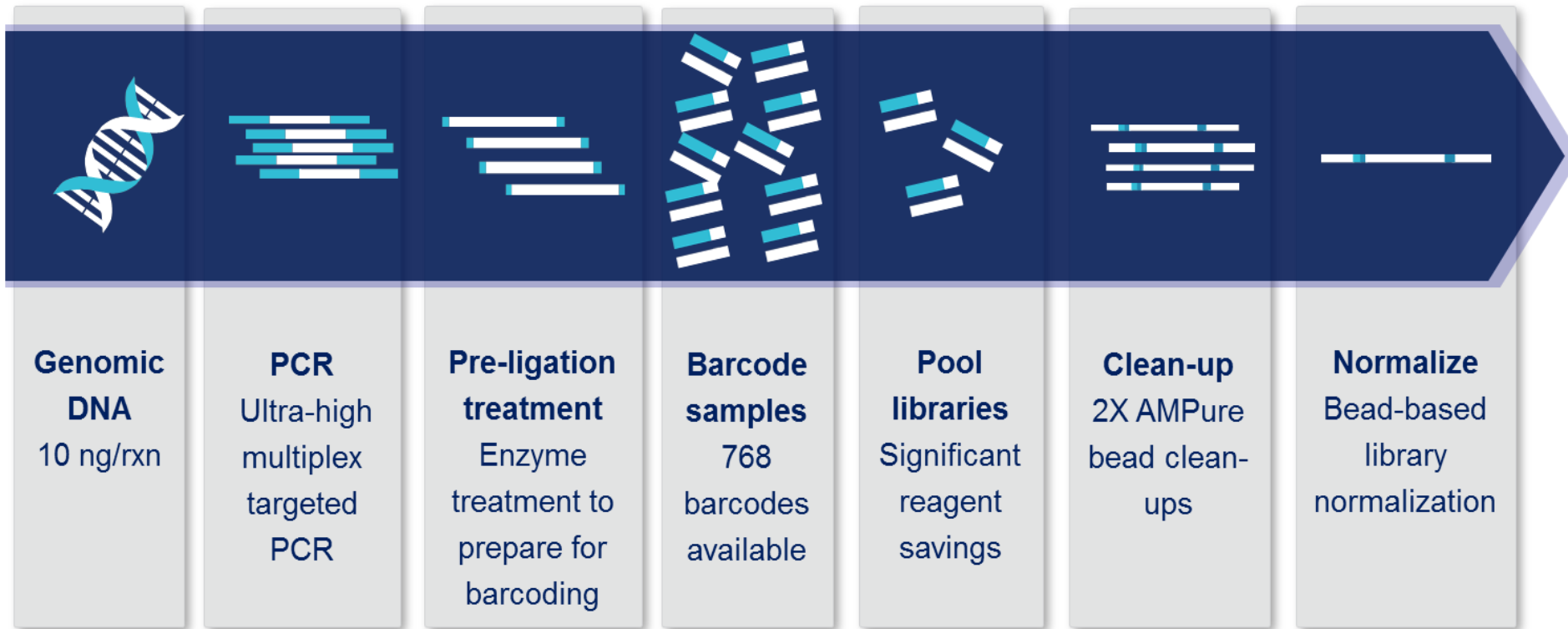
- 96 and 384-well protocols
- Best performance with normalized DNA
- Requires thermocycler, plate magnet and AMP Pure beads
- Pool samples post ligation to simplify sample handing, reduce tip usage and clean up reagent costs (<75% AmpPure Bead usage)
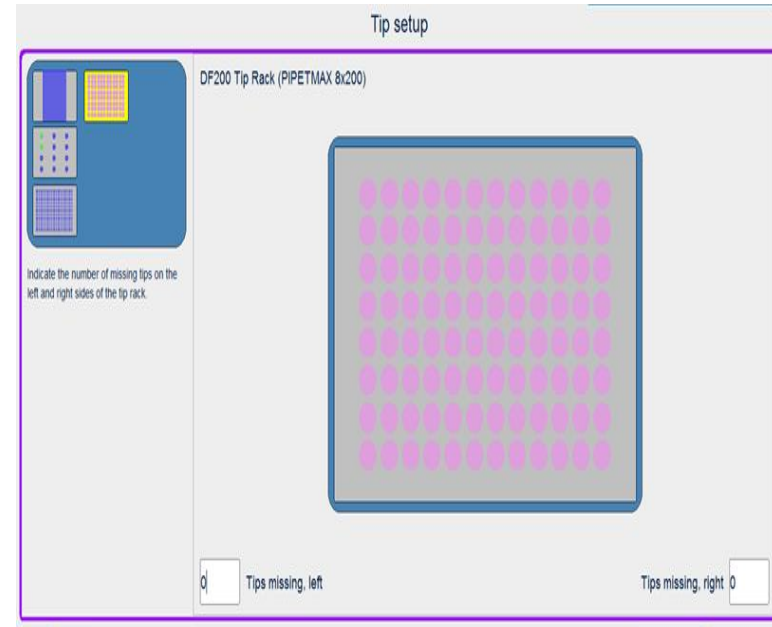
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S C I E N T I F I C

- Small footprint: Easily fits on most benchtops

- Low cost: less than $30,000

- Gilson PipetMan technology: precise, accurate pipetting

- Throughput: Moderate

- Normalization assistant available

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AgriSeq PipetMAX Script Modules

- Amplification Setup
- Sample Addition
- Pre-Ligation Enzyme Addition
- Barcode Addition
- Barcode Enzyme Mix Addition
- Post Ligation Pooling
- Library Purification
- Normalization Cleanup

Tip setup

DF200 Tip Rack (PIPETMAX 8x200)

Indicate the number of missing tips on the left and right sides of the tip rack

Tips missing, left 0

Tips missing, right 0

- Modular workflow allows for the separation of processes, flexibility and ease of use

- Step by step setup guide for easy tracking

- Scripts developed for processing either 2 -96 well plates or 1-384 plate

- Hands-on time reduced from 3 hours to 1 hour

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- Each AgriSeq automated workflow was validated with two different custom GBS panels:

    1. Bovine ISAG SNP Parentage Panel (2013)
    - 200 amplicon panel
    - Marker chosen by ISAG for bovine parentage verification
    - 96 diverse samples

    2. Porcine Imputation Panel
    - 1500 amplicon SNP panel
    - Markers chosen by a collaborator for porcine genotyping
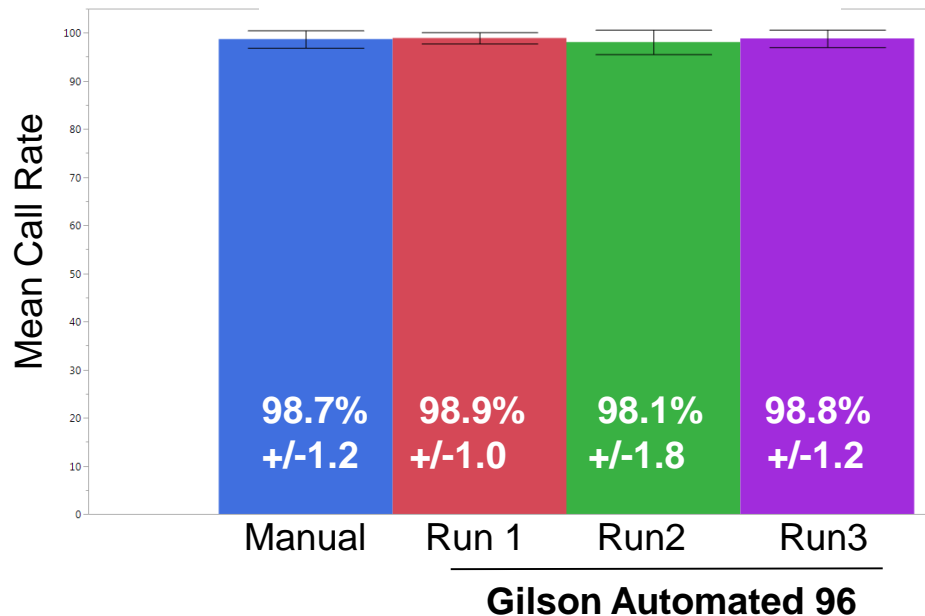   - 96 diverse samples

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- 96 diverse samples were tested with the AgriSeq 384-well and AgriSeq 96-well protocols in replicates (n=4).
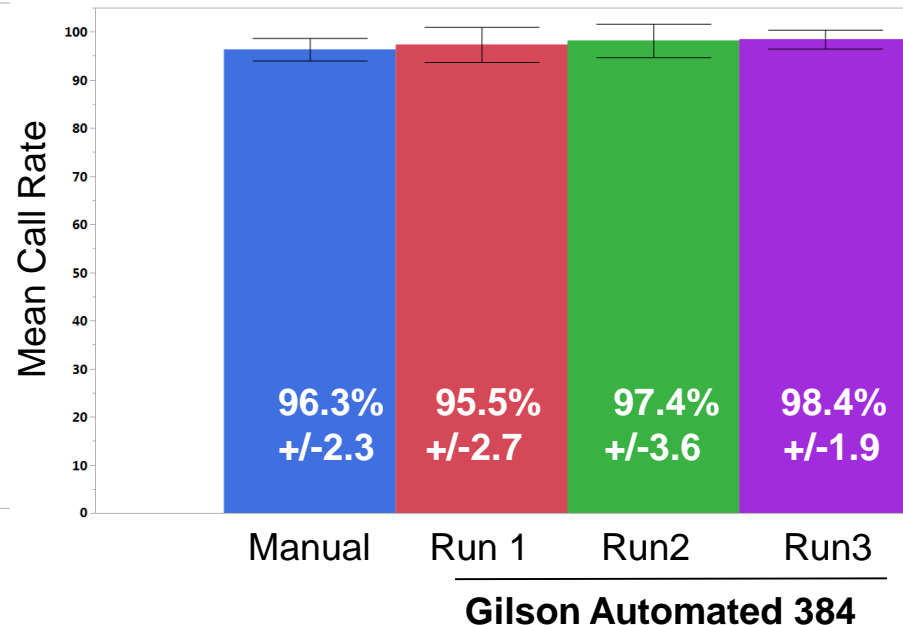

Bovine 200 Marker Panel

98.4+/-1.9%

Porcine 1500 Marker Panel

96.3+/-2.4%
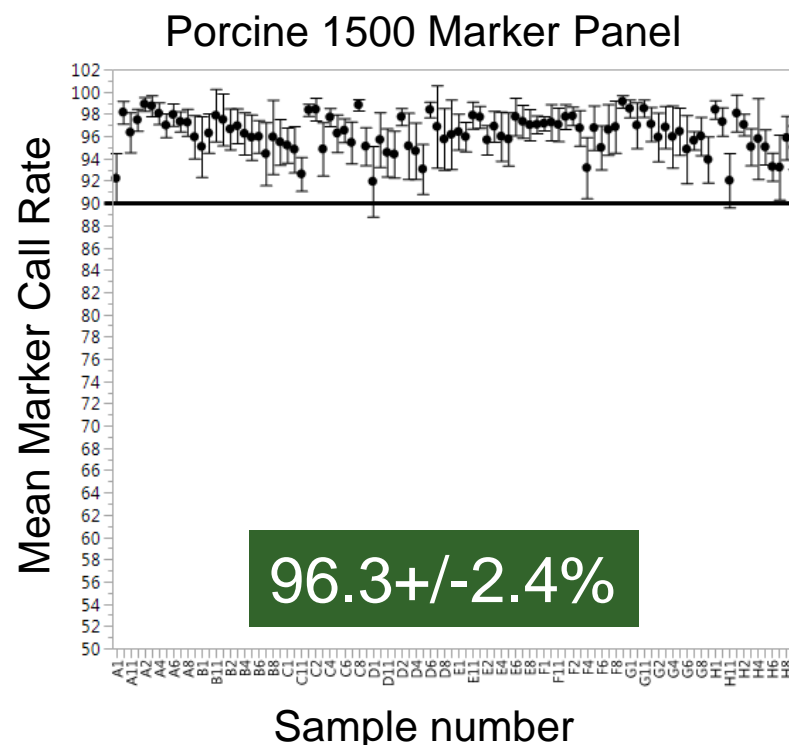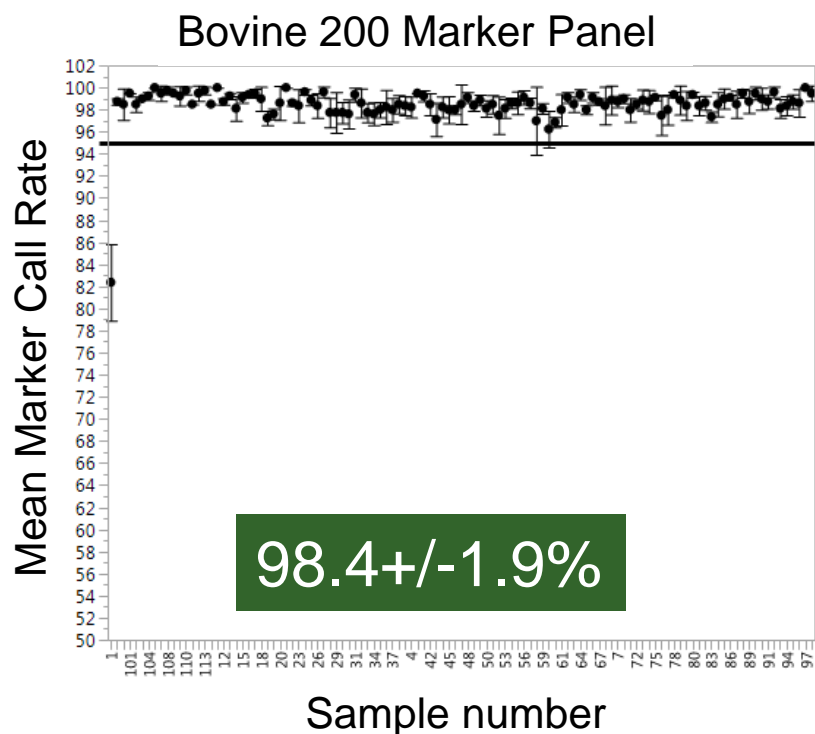
- 99.9% reproducible genotype calls between runs
- 99.4% concordance with Illumina Array Data (n=8800 datapoints)

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Similar performance between manual and automated workflows

Bovine 200 Marker

Mean Call Rate

| Manual | Run 1 | Run2 | Run3 |
| 98.7% +/-1.2 | 98.9% +/-1.0 | 98.1% +/-1.8 | 98.8% +/-1.2 |

Gilson Automated 96

Porcine 1500 Marker

Mean Call Rate

| Manual | Run 1 | Run2 | Run3 |
| 96.3% +/-2.3 | 95.5% +/-2.7 | 97.4% +/-3.6 | 98.4% +/-1.9 |

Gilson Automated 384

Run	Mean Genotyping Call Concordance	Stdev
Manual run	99.7%	0.7%
Gilson Agriseq-96 Run1	99.7%	0.7%
Gilson AgriSeq-96 Run2	99.1%	1.7%
Gilson AgriSeq-96 Run3	99.4%	1.2%

Run	Mean Genotyping Call Concordance	Std Dev
Manual run	99.5%	1.0%
Gilson Agriseq-384 Run1	99.1%	2.0%
Gilson AgriSeq-384 Run2	98.3%	2.5%
Gilson AgriSeq-384 Run3	98.5%	2.1%

*No significant difference was observed in other sequencing metrics included % on target reads and uniformity between manual and automated processing

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AgriSeq™ GBS generates ~200 bp of flanking sequence

Unknown/Known SNP

Known SNP

200bp sequence read
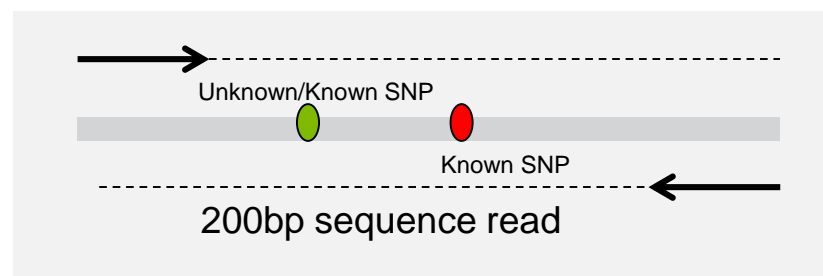
	Bovine	Porcine
Number of Targeted Markers	200	1500
Number of Samples	96	96
Number of Non-Hot Spot Variants	38,888	468,864
Additional Unique Markers	495	1221

If desired, additional variants can utilized for additional analysis
- Discovery of novel variants
- Utility for additional linkage analysis
- Generation of micro-haplotypes for improved marker specificity or enhanced discrimination in parentage/traceability analysis
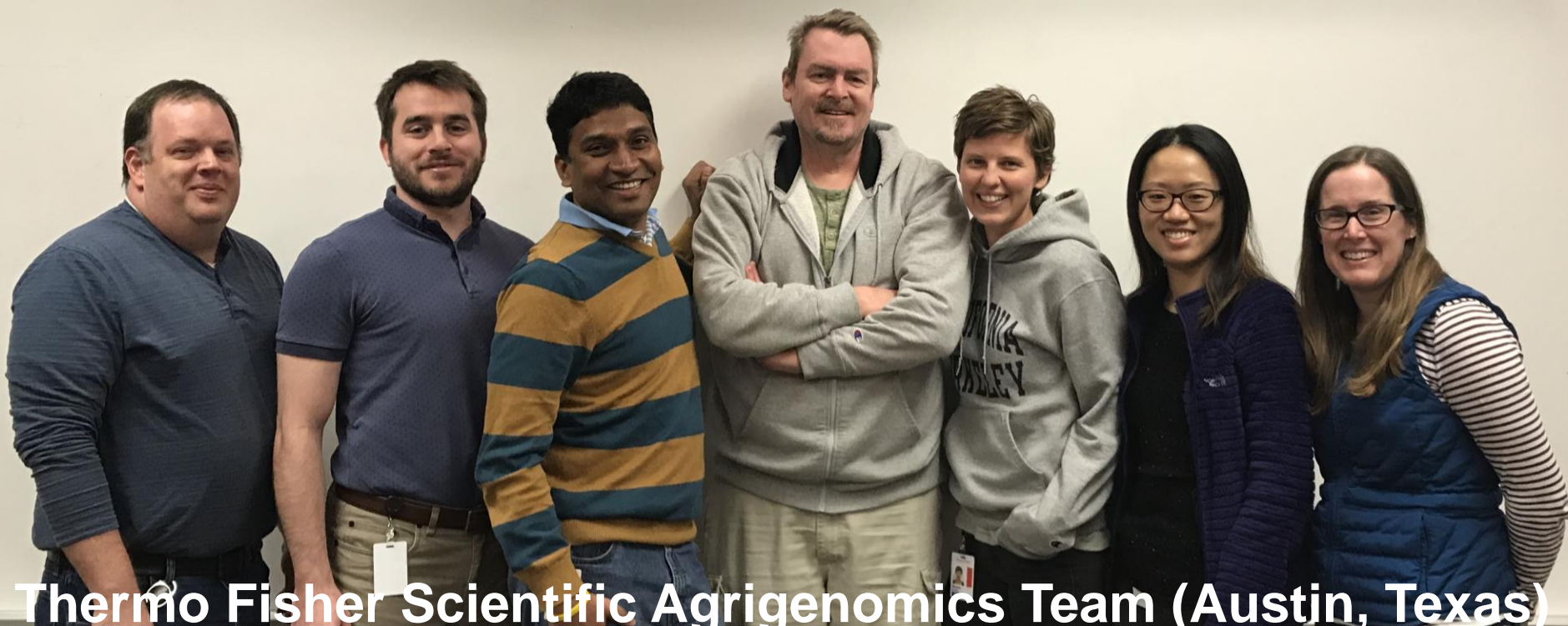
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Conclusions

- The AgriSeq library prep workflow along with custom GBS panels provide a high throughput, cost-effective method for animal genotyping.

- AgriSeq library prep can be easily automated on the Gilson PipetMax (or other platforms) to improve lab efficiency while reducing risk of technical errors while maintaining high call rates & genotyping concordance.

- Thermo Fisher Scientific offers bioinformatics services, wet lab validation and custom automation support for AgriSeq GBS.

- Visit our booth to learn more about our portfolio of genotyping tools to advance your research.

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Thermo Fisher Scientific Agrigenomics Team (Austin, Texas)

| Chris Willis | Haktan Suren | Prasad Siddavatam | Rick Conrad | Michelle Swimley | Jie Lu | Angela Burrell |

Ryan Ferretti (Neogen Corporation) - Concordance Data

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