



AgriSeq targeted genotyping by sequencing solutions

Helping to accelerate plant and animal genetics

Molecular breeding can significantly reduce the cost and time required to deliver improved plant and animal species for agricultural use. Advancements in genomic technologies are accelerating these breeding programs by enabling higher-throughput genotyping across large populations than ever before. Applied Biosystems™ AgriSeq™ targeted genotyping by sequencing (GBS) solutions are our latest innovation that helps our customers economically deliver high-throughput plant and animal genotypes.

The AgriSeq targeted GBS solution utilizes a highly efficient multiplexed PCR chemistry, where hundreds to thousands of markers can be targeted and uniformly amplified in a single reaction. The amplicon libraries can then be barcoded and pooled for simultaneous sequencing of hundreds of samples on the Ion Torrent™ suite of next-generation sequencing (NGS) instruments. At a cost of pennies-per-data point, AgriSeq targeted GBS is capable

of generating up to 1.6 million genotypes per day from high-quality NGS data. Whether you're looking to achieve more accurate marker-assisted breeding selection, or to improve your livestock parentage discrimination and trait monitoring, our solutions can help advance your plant and animal breeding research.

Key features include:

- **Flexible genotyping system**—target up to 5,000 relevant markers
- **Reproducible results**—achieve high marker call rates
- **Simple, 2-day workflow**—automated data analysis
- **Expert bioinformatics support**—receive consultation service on panel design and validation

Scalable, flexible, high-throughput genotyping

AgriSeq genotyping is performed on the Ion Torrent NGS platform and is currently validated for analysis of panels between 100 and 5,000 markers per sample. Analyzed on Ion 540™ Chips that generate 60–80 million reads per run, AgriSeq genotyping is able to produce up to 800,000 unique genotype results per chip at an average read depth of 100x to achieve maximal genotype call rates. To help maximize lab efficiency and turnaround time, our

flexible system allows for numerous experimental designs of sample and marker combinations to be simultaneously processed on the same run, including combinations of different panels and species. Table 1 summarizes the number of samples that can be analyzed at different marker densities on a single Ion 540 Chip. The maximum number of samples that can be run per chip are restricted to 768, which is the number of barcodes currently available through catalog and early access programs.

Table 1. The maximum number of samples that can be analyzed at different marker densities per chip, per day, or per year on an Ion 540 chip, assuming an average of 70 million reads/chip to achieve 100x average amplicon coverage. Numbers are based on a single Ion Chef™ and Ion S5™ System with one operator working a standard 8-hour shift, 5 days per week.

Markers	Maximum number of samples		
	Per chip	Per day	Per year
5,000	140	280	72,800
3,645	192	384	99,860
1,822	384	768	199,780
1,215	576	1,152	299,580
911	768	1,537	399,560

Ion Torrent NGS systems

The simplicity, scalability, and speed of the Ion Chef and Ion S5 sequencing systems is enabling NGS to move from research to applied markets. Integrated with the AgriSeq targeted GBS workflow, these systems are designed to deliver results with minimal hands-on time so you can spend less time doing repetitive labwork.

Key features include:

- Automated template preparation and chip loading
- Cartridge-based reagent systems
- 60–80 million reads per chip
- 2.5 hr sequencing run time
- Simplified NGS data analysis and variant calling with easy-to-use Torrent Suite™ software and Variant Caller plug-in



Consistent performance across samples with high marker call rates

Application of agricultural genomics in production requires consistent genotyping performance and high marker call rates to ensure accurate selection. Unlike nontargeted GBS approaches such as restriction site-associated DNA sequencing (RAD-seq), which are highly susceptible to

allele dropouts and missing data [1], AgriSeq targeted GBS is designed to deliver high marker call rates with greater reproducibility across diverse sample sets. For well-designed panels, average marker call rates can exceed 96% with >99% inter- and intra-run reproducibility (Figures 1 and 2, Table 2).

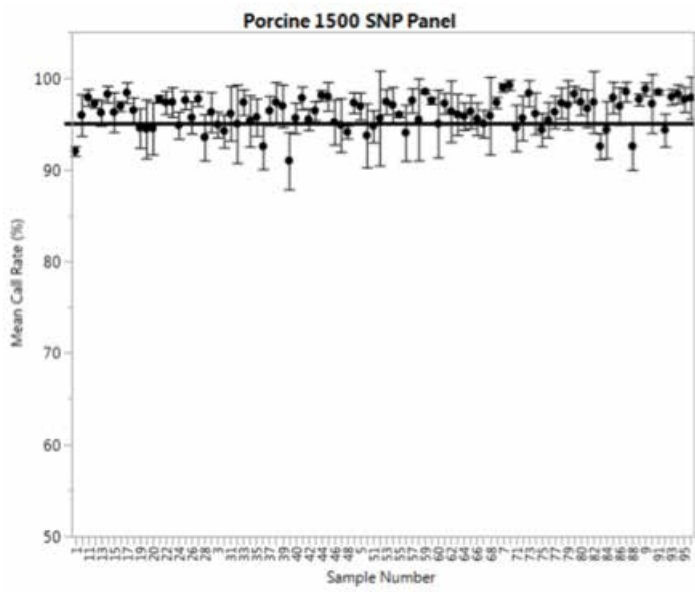


Figure 1. Ninety-six unique porcine samples were tested in replicates of $n = 4$ using AgriSeq high-throughput screening (HTS) chemistry on a 1,500-marker porcine panel. The average marker call rate across all samples was $96.3 \pm 2.5\%$.

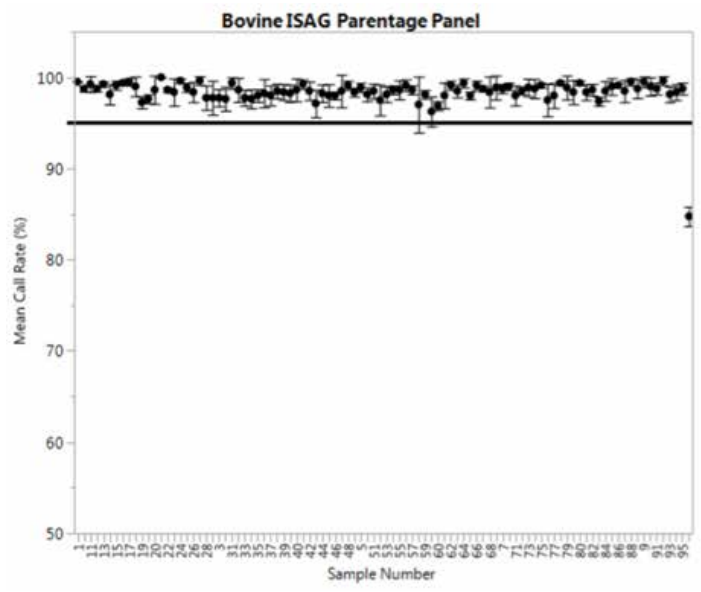


Figure 2. Ninety-six unique bovine samples were tested in replicates of $n = 4$ using AgriSeq HTS chemistry on a 200-marker bovine panel. The average call rate across all samples was $98.5 \pm 1.5\%$

Table 2. Intra-run concordance was determined by analyzing genotype calls of 96 replicate samples per panel analyzed on the same sequencing run. Inter-run concordance was determined by analyzing the genotyping of 96 replicates across 4 different sequencing runs.

AgriSeq panel	Intra-run concordance		Inter-run concordance	
	Mean (%)	Standard deviation (%)	Mean (%)	Standard deviation (%)
Bovine ISAG Parentage Panel	99.97	0.13	99.99	0.07
Porcine 1,500 SNP Panel	99.94	0.05	99.94	0.05

Consistent performance across samples with high marker call rates (continued)

The AgriSeq targeted GBS data maintains a high concordance (>99%) when compared to the orthogonal genotyping technologies such as DNA microarrays (Table 3); and unlike nonsequencing-based approaches,

AgriSeq technology can also discover additional novel variants in the amplicons of targeted single-nucleotide polymorphisms (SNPs). Additional variants and microhaplotype information can provide new markers for linkage analysis or enhance discrimination in parentage and traceability applications [2].

Table 3. Forty-four samples were tested with both the Applied Biosystems™ AgriSeq™ Bovine ISAG 200 Parentage Panel and the Illumina™ BeadChip (LDv2) DNA Microarray. Concordance, the percentage of identical genotyping calls between platforms, was greater than 99%.

Samples run on both technologies	44
Total number of markers	200
Total number of calls	8,800
Number of concordant calls	8,751
Concordance (%)	99.4%

Complete end-to-end workflow for cost-effective high-throughput genotyping

With low all-in sample pricing offered exclusively to the agricultural customer, AgriSeq targeted GBS is the most economical way to generate high-throughput genotyping data to advance your breeding programs. The AgriSeq workflow includes library generation, automated template preparation, sequencing, and data analysis, which can be completed in ~2 days with <4 hours of hands-on time (Figure 3). Requiring only 10 ng of genomic DNA (gDNA) input per sample, a single operator can easily process up to four 96- or 384-well plates manually per day. The library generation step also can be easily automated

on most standard automation platforms for even more efficient processing. The Ion Chef System automates template preparation and chip loading, requiring only 15 min of hands-on time, followed by an overnight run. Sequencing is then performed in only 2.5 hr on the Ion S5 or Ion S5™ XL NGS system with less than 15 min hands-on time. Standard data analysis processing includes automated quality assessment, alignment, and genotype calling using the included Torrent Suite software and Variant Caller plug-in without the need for a bioinformatician, expensive data storage, or third-party programs.



Figure 3. AgriSeq targeted GBS workflow—from library preparation to data analysis in ~2 days.

Customized panels designed and validated to target your relevant markers

Since informative markers can vary widely between applications, genetic background, and intended use, the AgriSeq solution includes a complementary custom panel designed to suit the applications of your relevant markers. Our dedicated team of professionals help design panels to your targets of interest for any agricultural species. Through careful analysis of your markers, and local DNA sequencing context and polymorphism information, primer panels are designed by our bioinformatics team using proprietary algorithms to maximize amplicon specificity, efficiency, and multiplex compatibility in AgriSeq sequencing reactions.

Formatted as multiplexed primer pools, or available as plated individual assays, custom primer sets are easy to use and can be reformatted or combined with additional compatible content or pools if project requirements should change over time. We have demonstrated successful panel design with high marker call rates and performance across a wide variety of relevant agricultural species and panel sizes (Table 4). To further ensure confidence in your design, we offer wet-lab validation services for a nominal fee to confirm the performance of custom-designed panels, providing marker call rate, panel uniformity, and replicate concordance verification.

Table 4. Performance of AgriSeq panels designed by the bioinformatics team and analyzed internally. Mean sample call rate is defined as the percent of markers generating a genotype call averaged across all samples. Mean uniformity is the average of the percent of bases covered by at least 0.2x of the average base read depth. Mean percent on-target reads is the average percentage of mapped reads that align over a desired target region (amplicon).

Species	Markers	Mean sample call rate (%)	Mean uniformity (%)	Mean on-target reads (%)
Bovine	190	99.7	98.0	97.6
	215	98.5	97.3	85.7
Canine	229	99.2	99.2	98.8
Feline	62	99.8	98.6	96.6
Porcine	1,500	96.3	99.7	95.8
	3,000	96.2	98.2	99.3
Soybean	1,134	98.3	96.7	98.9
Cucumber	2,804	91.4	96.8	99.7
Maize	1,079	87.5	87.2	97.7



Bovine ISAG SNP Parentage Panel (2013)

Parentage determination has applications in herd management as well as breed registration. The International Society of Animal Genetics (ISAG) has standardized a set of 100 core SNPs and 100 additional SNPs with sufficient minor allele frequency and genomic spacing for accurate bovine parentage across a wide range of breeds [3]. The Applied Biosystems™ Bovine ISAG SNP Parentage Panel (2013) contains primers targeting the 200 ISAG-recommended SNPs available for simple multiplexed analysis using the AgriSeq targeted GBS chemistry. Qualified using the 2015 ISAG/ICAR Bovine Comparison samples, our comprehensive Bovine ISAG Parentage Panel enables reliable genotyping results for animal parentage testing.

Key features include:

- Reproducible genotyping of 200 ISAG-recommended markers, >98% genotyping call rate across breeds
- Simple, high-throughput workflow
- Customizable approach to combine with compatible trait or markers that detect genetic defects

>98% genotyping call rate across breeds

Genotyping coverage and reproducibility are imperative to accurately determine parentage relationships within closely related populations. Ninety-six highly diverse bovine gDNA samples representing 19 different cattle breeds were evaluated using the AgriSeq workflow, and a 98.5% mean call rate was obtained between samples with >99.6% genotyping concordance between replicates (Figure 4).

Simple, high-throughput workflow

The Bovine ISAG SNP Parentage Panel (2013) has been optimized with the AgriSeq targeted GBS workflow. Up to 768 unique animals can be pooled per chip—greatly reducing the sequencing cost and labor per animal. Additionally, this workflow has shown compatibility with several simple DNA extraction kits, including Applied Biosystems™ DNA Extract All lysis chemistry, a simple 5 min, 2-step workflow that can further reduce time and labor in generating results (Figure 5).

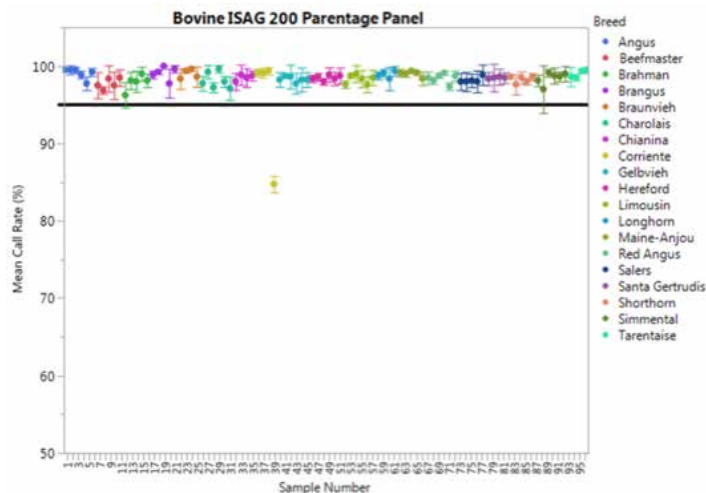


Figure 4. Evaluation of 96 diverse bovine gDNA samples from the United States Department of Agriculture (USDA), representing 19 different breeds with the Bovine ISAG 200 Parentage Panel (2013), n = 4. All 384 individually barcoded libraries were pooled onto a single Ion 540 Chip, and sequenced on the Ion S5 XL System. All but one of the samples tested gave a mean sample call rate of >95%. The mean call rate across all samples was 98.5%.

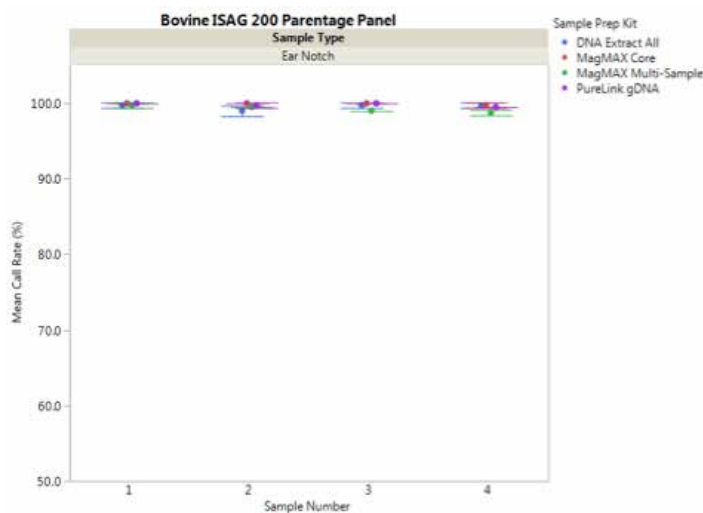


Figure 5. Four bovine ear punch samples (3 mm each) from different individuals were processed through either traditional sample prep methods (Applied Biosystems™ MagMax™ Core, PureLink™ gDNA Extraction, MagMax™ DNA Multi-Sample Kits) or the simple DNA Extract All lysis procedure in triplicate. Samples were then analyzed using the Bovine ISAG 200 Parentage Panel and the AgriSeq HTS chemistry. The average call rate for each chemistry exceeded 99%.

Pilot projects and custom targeted GBS panel design and validation

Agricultural genomics can be challenging for a number of reasons, including sequence complexity, high genetic diversity, and sometimes limited sequence availability. We offer end-to-end project management services through panel design, wet-lab validation, and data analysis to help ensure delivery of high-performance panels for your project. A typical pilot project can be completed in ~12 weeks (Figure 5), generating data for up to 768 customer-supplied samples.

These low cost, proof-of-concept pilot projects are a great way to confirm the robustness and relevance of AgriSeq targeted GBS for your research.

Service and support

More than 3,700 global sales, service, and technical support specialists are available to assist you in person, by phone, or online—that can help you successfully navigate the validation process required to bring new technologies into your lab.

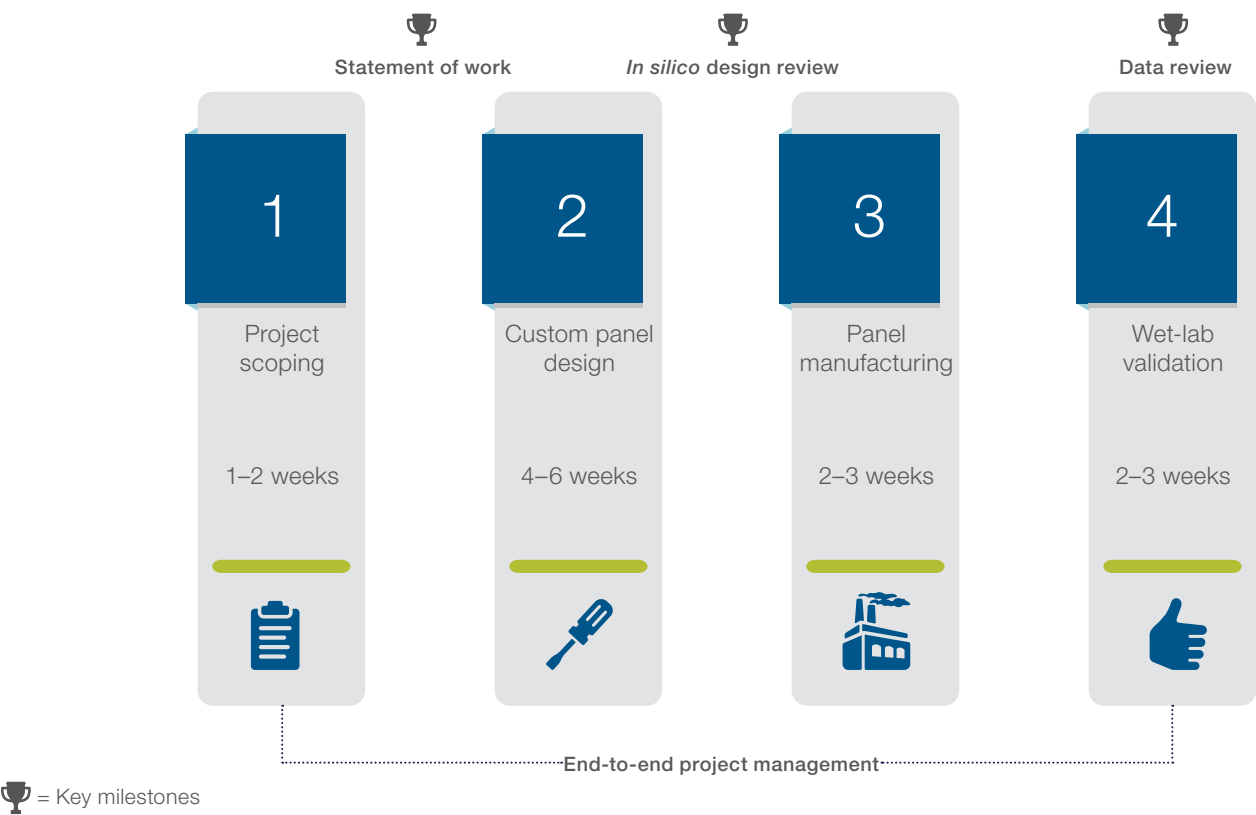


Figure 5. A typical pilot project can be completed in about 12 weeks.

Ordering information

Product	Quantity	Cat. No.
AgriSeq HTS Library Kit	9,600 reactions	A34143
AgriSeq HTS Library Kit	960 reactions	A34144
Bovine ISAG SNP Parentage Panel (2013)	960 reactions	A35297
IonCode Barcode Adapters		A31173
Ion 540 Kit-Chef		A27759
Ion 540 Chip Kit		A27766
Instruments		
Ion Chef		4484177
Ion S5 Sequencer		A27212
Ion S5 XL System		A27214

References

1. Cariou M, Duret L, Charlat S (2016) How and how much does RAD-seq bias genetic diversity estimates? *BMC Evolutionary Biology* 16:240.
2. Kidd KK, Pakstis AJ, Speed WC et al. (2014) Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic Sci Int Genet* 12:215–224.
3. isag.us/Docs/Cattle-SNP-ISAG-core-additional-panel-2013.xlsx

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