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AgriSeq targeted genotyping-by-sequencing solutions

Helping to accelerate plant and animal genetic screening and research

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 than ever before across large populations. 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 or less per data point, AgriSeq targeted GBS is capable of generating up to 2.6 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 consistent high marker call rates
- **Simple workflow of 2–3 days**—automated data analysis
- **Experienced bioinformatics support**—receive consultation service on panel design and validation

ThermoFisher SCIENTIFIC

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 Torrent™ chips that can generate 100–130 million reads per run, AgriSeq genotyping is able to produce up to 1.3 million 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 550™ Chip. The maximum number of samples that can be run per chip is restricted to 768, which is the number of barcodes currently available through our catalog. Additional barcodes are available through our custom program to further increase sample throughput and decrease sequencing cost per sample.

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 550 Chip, assuming an average of 115 million reads per chip to achieve 100x average amplicon coverage. Numbers are based on a single Ion Chef™ Instrument and Ion GeneStudio™ S5 Prime System with one operator working a standard 8-hour shift, 5 days per week.

	Maximum number of samples		
Markers	**Per chip**	**Per day**	**Per year**
5,000	230	460	119,600
2,500	460	920	239,200
1,500	768	1,536	399,360
1,000	1,150	2,300	598,000
500	1,536*	3,072	798,720

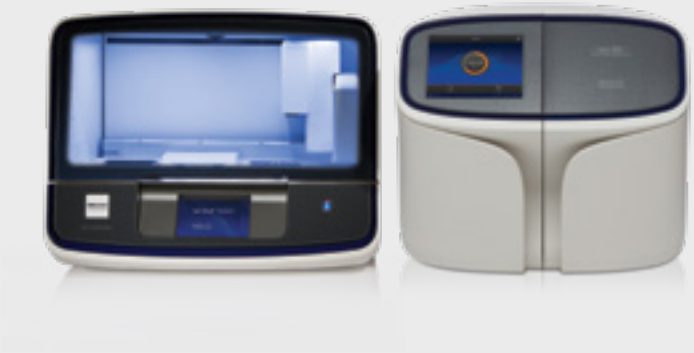
* Additional barcodes are available as early access.

Ion Torrent NGS systems

The simplicity, scalability, and speed of the Ion Chef™ and Ion GeneStudio™ S5 systems are 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
- Flexible chip compatibility, enabling throughput of 2.6 million genotypes per day with 2 chips (130 million reads/chip)
- As little as 2.5 hr sequencing run time per chip
- Simplified NGS data analysis and variant calling with easy-to-use Torrent Suite™ Software and Torrent Variant Caller Plugin



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 (Figure 1 and Table 2).

The AgriSeq targeted GBS data maintain high concordance (>99%) when compared to the orthogonal genotyping technologies such as DNA microarrays; and unlike non–sequencing-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].

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

With low all-in sample pricing offered exclusively to the agricultural customer, the 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 ~2–3 days with less than 4 hours of hands-on time (Figure 2). 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 Instrument automates template preparation and chip loading, requiring only 15 minutes of hands-on time, followed by an overnight run. Sequencing is then performed in as few as 2.5 hours on an Ion GeneStudio S5 series NGS system with less than 15 minutes of hands-on time. Standard data analysis processing includes automated quality assessment, alignment, and genotype calling using the included Torrent Suite Software and Torrent Variant Caller plug-in without the need for a bioinformatician, expensive data storage, or third-party programs.

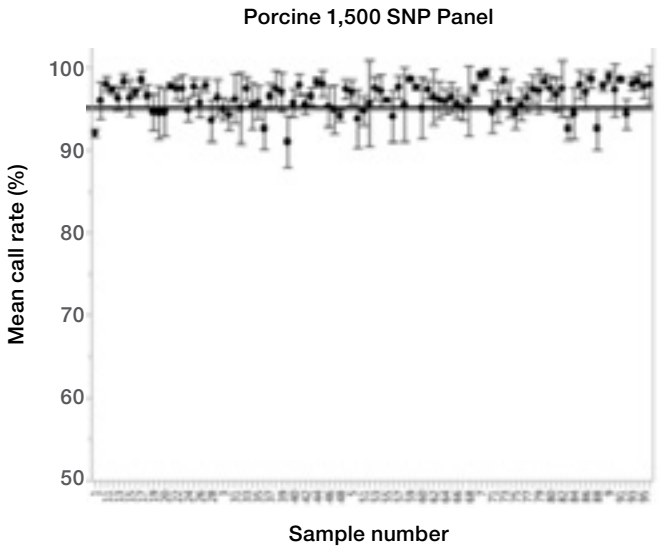


Figure 1. A total of 96 unique porcine samples were tested in quadruplicate using AgriSeq high-throughput screening (HTS) chemistry on a 1,500-marker porcine panel. The average marker call rate across all samples was 96.3 ± 2.5%.

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

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

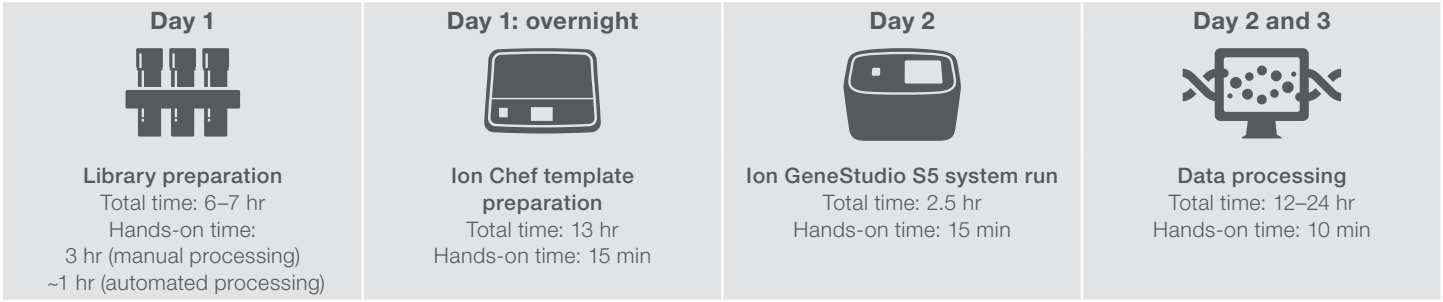


Figure 2. AgriSeq targeted GBS workflow—from library preparation to data analysis, or sample to results, in 2–3 days.

New Applied Biosystems™ AgriSeq™ companion animal and livestock genotyping solutions

The need for highly consistent detection of informative genetic markers is critical for genetic trait detection. Targeted GBS methods, like the AgriSeq technology, have the advantage over nontargeted GBS approaches (e.g., RAD-seq) that are highly susceptible to allele dropouts and missing data. Using the AgriSeq workflow, we can target hundreds to thousands of markers simultaneously in a highly reproducible manner across diverse sample sets. In collaboration with multiple key opinion leaders and International Society of Animal Genetics (ISAG)-member laboratories, we have developed and validated the performance of panels with SNPs and complex markers such as multi-nucleotide polymorphisms (MNPs) and insertions or deletions (indels) for companion animals and livestock.

Key features of Applied Biosystems™ AgriSeq™ ready-to-use panels include:

- Reproducible genotyping of ISAG-recommended markers for all species with high call rates
- Simple, high-throughput workflow
- Customizable approach to combine with compatible parentage, traits or disorders, or additional markers of interest

Applied Biosystems™ AgriSeq™ Canine Traits and Disorders Panel

The AgriSeq Canine Traits and Disorders Panel (Cat. No. A43406) contains 154 markers targeting clinically important genetic traits and disorders in canines. The panel includes 97 SNPs and complex markers such as 6 MNPs, 13 insertions, and 38 deletions. Most of these markers are common to all breeds, while some are breed specific for certain traits and conditions. The performance of the panel was verified using orthogonal testing, robustness testing, and field testing with diverse DNA samples from canine oral swabs. The panel enables multiplexed simultaneous genotyping of 154 markers to deliver reliable genotyping results for 136 genetic disorders and 18 traits using the AgriSeq targeted GBS workflow. The CanFam 3.1 reference genome is used for the analysis.

Applied Biosystems™ AgriSeq™ Canine SNP Parentage and Identification Panel

The AgriSeq Canine SNP Parentage and Identification Panel (Cat. No. A43407) contains 379 SNPs and 2 deletion markers designed to deliver reliable genotyping results for parentage determination and identity verification of canines by targeted GBS. The panel enables multiplexed simultaneous genotyping using the AgriSeq targeted GBS workflow. The performance of the panel was verified using orthogonal testing, robustness testing, and field testing with diverse DNA samples from canine oral swabs. The panel was also qualified using the 2019 ISAG/ICAR 2nd SNP Typing Canine Comparison samples. The CanFam 3.1 reference genome is used for the analysis.

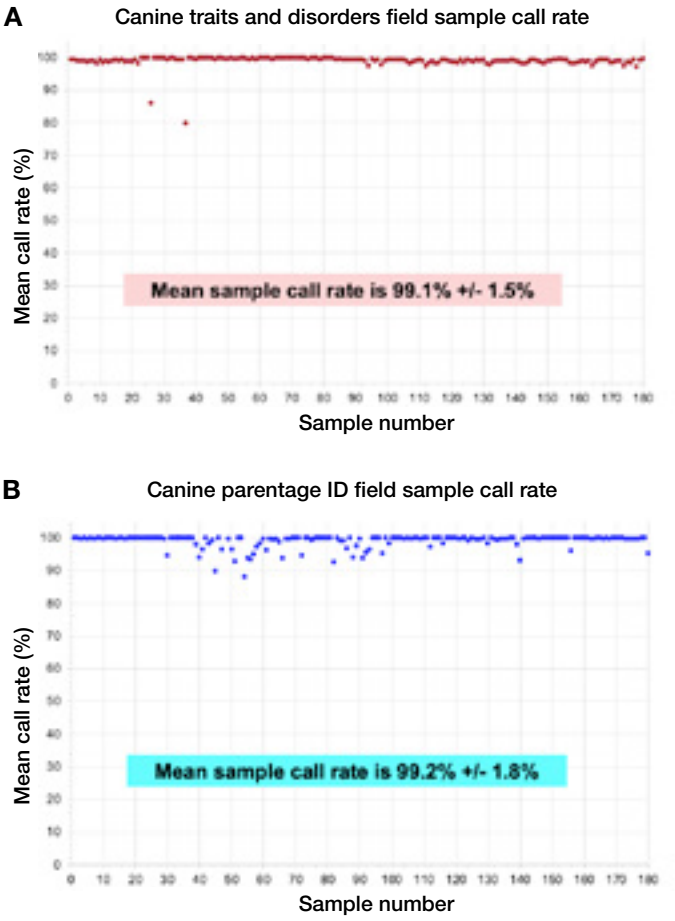


Figure 3. Performance of AgriSeq canine panels. Call rates were calculated for both panels after testing >180 diverse DNA field samples from canine oral swabs with the AgriSeq workflow. The mean call rate for the AgriSeq Canine Traits and Disorders Panel was 99.1% **(A)**, and for the AgriSeq Canine SNP Parentage and Identification Panel was 99.2% **(B)**, demonstrating the high performance obtained from customer samples.

These two canine panels can be combined and run as a single panel of 525 markers when desired, and additional custom markers referencing the CanFam3.1 can be added if compatible. Figure 3 shows the comparison in call rates obtained from >180 diverse DNA field samples from canine oral swabs with the two AgriSeq canine panels.

Applied Biosystems™ AgriSeq™ Feline Parentage and ID Plus Traits and Disorders Panel

The AgriSeq Feline Parentage and Identification Plus Traits and Disorders (PITD) Panel (Cat. No. A43408) is a combined panel for determining parentage and identification (111 SNP markers) plus targeting 64 clinically important traits and disorders (43 SNP, 1 MNP, 4 insertion, and 16 deletion markers) in felines by targeted GBS. The performance of the panel was verified using orthogonal testing, robustness testing, and field testing with diverse DNA samples from feline oral swabs (Figure 4). The 111 SNP parentage and ID markers were also qualified using the 2019 ISAG/ICAR 2nd SNP Typing Feline Comparison samples. The comprehensive AgriSeq Feline PITD Panel is designed to deliver reliable genotyping results for parentage determination, including identity verification and prediction of 42 genetic disorders and 22 traits that are clinically important. When desired, additional custom markers referencing the Felis_catus_8.0 can be added if compatible.

Applied Biosystems™ Bovine ISAG SNP Parentage Panel (2013)

The Applied Biosystems Bovine ISAG SNP Parentage Panel (2013) (Cat. No. A35297) contains primers targeting the 200 ISAG-recommended SNPs available for simple multiplexed analysis using the AgriSeq targeted GBS workflow. The ISAG has standardized a set of 100 core SNPs and 100 additional SNPs with sufficient minor allele frequency (MAF) and genomic spacing for accurate bovine parentage determination across a wide range of breeds (Figure 5). You can download SNP information from the ISAG website [3]. Qualified using the 2015 ISAG/ICAR 3rd SNP Typing Bovine Comparison samples, our comprehensive Bovine ISAG Parentage Panel is designed to deliver reliable genotyping results for animal parentage testing. The *Bos taurus* reference genome is needed for analysis of the Bovine ISAG SNP Parentage Panel and can be obtained by contacting our technical support specialists.

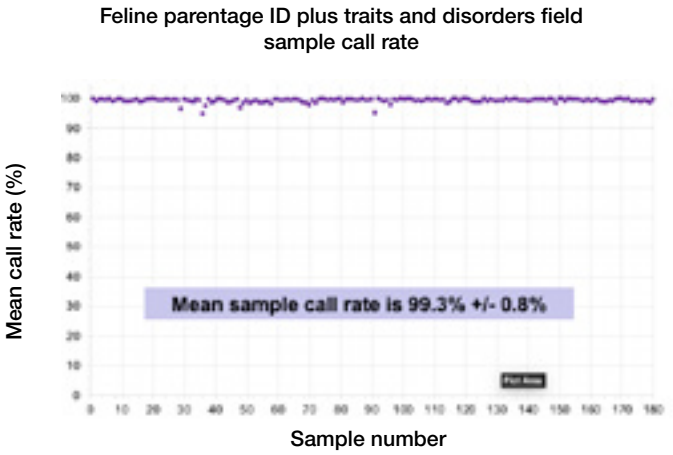


Figure 4. A total of 258 feline oral swab DNA samples were tested either in duplicate or in quadruplicate with the AgriSeq workflow using the AgriSeq Feline PITD kit. The call rate, the number of markers generating a genotype call for each sample, was calculated for all samples with at least 100x mean read depth, the minimum recommended. The mean call rate was 99.3% and all samples had call rates >92.5%, demonstrating the high performance obtained from field samples.

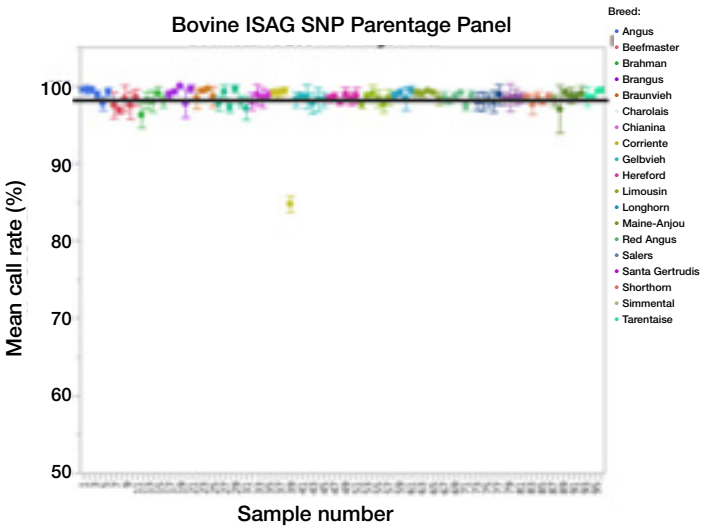


Figure 5. Evaluation of 96 diverse bovine gDNA samples from the United States Department of Agriculture (USDA), representing 19 different breeds with the Bovine ISAG SNP Parentage Panel (2013), with 4 replicates each. All 384 individually barcoded libraries were pooled onto a single Ion 540™ Chip and 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%.

Applied Biosystems AgriSeq custom panels for plants, aquaculture, and livestock

The Applied Biosystems™ AgriSeq™ Sunflower 700 SNP Panel is designed for QTL mapping, marker-assisted backcrossing, and genomic selection. A medium-density panel was developed and validated in collaboration with Eurofins BioDiagnostics Institute (EBDI) to help sunflower breeders. The panel consists of 768 SNP markers with high MAF >0.25 and high polymorphic information content (PIC) scores >0.25 that are very reproducible. The genotype calls were highly concordant between runs, between sites, and in comparison to the Illumina 10K array (Figure 6).

Custom AgriSeq SNP panels are being used for genotyping Chinook, coho, and chum salmon, trout, shrimp, and eulachon. Genotypes at all available SNPs for an individual are assembled to provide a multi-locus individual genotype.

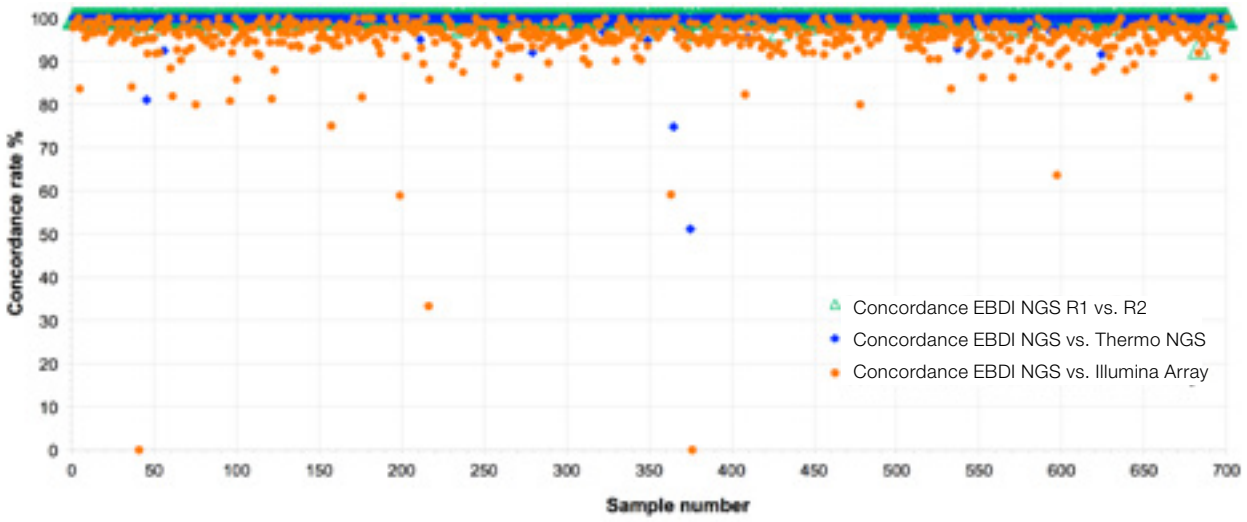


Figure 6. Evaluation of AgriSeq Sunflower 700 SNP Panel performance. A total of 96 samples acquired from Germplasm Resources Information Network (GRIN), which were previously analyzed on the Illumina 10K array, were selected for the study. DNA was extracted from a pool of 5 seeds and genotyped with the AgriSeq Sunflower 700 SNP Panel in replicates at EBDI and the Thermo Fisher Scientific research lab. The quality of the panel and overall genotype concordance was assessed. The concordance was 99.95% between EBDI replicates R1 and R2; 99.72% between EBDI and Thermo Fisher Scientific research labs; and 96.81% between historical Illumina 10K array and EBDI replicates.

Customized panels designed and validated to target your relevant markers

Since informative markers can vary widely between applications, genetic backgrounds, and intended uses, the AgriSeq solution includes a complementary custom panel design service. Our dedicated team of professionals helps 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 designs with high marker call rates and performance across a wide variety of relevant agricultural species and panel sizes (Table 3). 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 concordance verification of the replicates.

Table 3. Performance of AgriSeq panels designed by the bioinformatics team and analyzed internally. Mean sample call rate is defined as the percentage of markers generating a genotype call, averaged across all samples. Mean uniformity is the average of the percentage of bases covered by at least 0.2x of the average base read depth. Mean 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
	412	98.1	97.0	99.0
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
Equine	204	99.2	99.0	98.6
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
Salmon	3,153	94.1	94.4	99.0
Tomato	5,500	95.9	95.2	99.8

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 7), 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. They can help you successfully navigate the validation process required to bring new technologies into your lab.

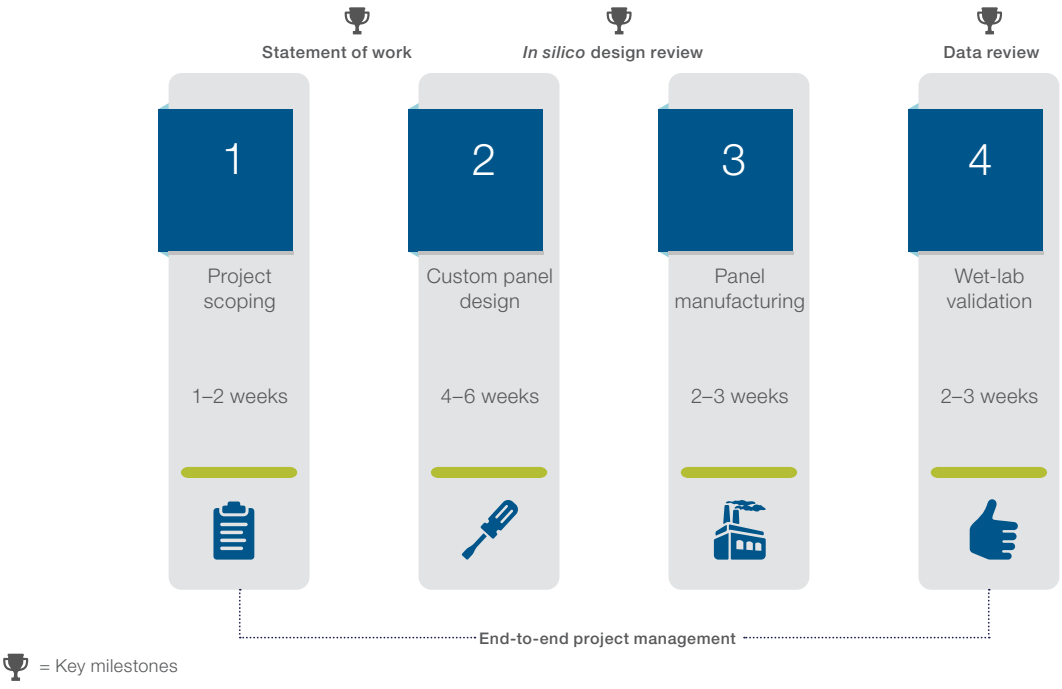


Figure 7. A typical pilot project can be completed in about 12 weeks.

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Ordering information

Product	Quantity	Cat. No.
Companion animal and livestock genotyping solutions		
AgriSeq Canine Traits and Disorders Panel (154 markers)	960 reactions	A43406
AgriSeq Canine SNP Parentage and ID Panel (381 SNP markers)	960 reactions	A43407
AgriSeq Feline Parentage and ID Plus Traits and Disorders Panel (64 markers for TD, 111 markers for PI)	960 reactions	A43408
Bovine ISAG SNP Parentage Panel (2013)	960 reactions	A35297
AgriSeq Custom Panel Pilot (for plant, livestock, and aquaculture)	Custom	A31975
Library kits		
AgriSeq HTS Library Kit	9,600 reactions	A34143
	960 reactions	A34144
Ion Torrent accessories		
IonCode Barcode Adapters, 1–384 (for Agrigenomics)	3,840 reactions	A31173
IonCode Barcode Adapters, 385–768	20 µL/barcode	A36546
IonCode Barcode Adapters, 0501–0596	20 µL/barcode	A36524
IonCode Barcode Adapters, 0601–0696	20 µL/barcode	A36525
IonCode Barcode Adapters, 0701–0796	20 µL/barcode	A36526
IonCode Barcode Adapters, 0801–0896	20 µL/barcode	A36527
Ion 540 Chip Kit for Agrigenomics	4 chips	A43540
	8 chips	A42849
Ion 540 Kit for Agrigenomics—Chef	8 reactions	A43541
Ion 550 Chip Kit for Agrigenomics	4 chips	A43562
	8 chips	A42962
Ion 550 Kit for Agrigenomics—Chef	8 reactions	A43542
Ion 550 Single Chip Supplemental Kit for Agrigenomics	4 reactions	A43559
Ion PI Chip Kit v3 for Agrigenomics	8 chips	A43730
Ion PI Hi-Q Chef Kit for Agrigenomics	8 reactions	A43729
Ion Torrent instruments		
Ion Chef Instrument for Agrigenomics	1 instrument	A43558
Ion GeneStudio S5 Plus System for Agrigenomics	1 system	A43557
Ion GeneStudio S5 Prime System for Agrigenomics	1 system	A43543
Ion GeneStudio S5 System for Agrigenomics	1 system	A44671

References

1. Cariou M, Duret L, Charlat S (2016) How and how much does RAD-seq bias genetic diversity estimates? *BMC Evol Biol* 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 cattle core + additional SNP panel 2013. International Society for Animal Genetics (ISAG). isag.us/Docs/Cattle-SNP-ISAG-core-additional-panel-2013.xlsx.

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