Development of AgriSeq™ pre-designed Panels for Crop Genotyping

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ABSTRACT

Over the years our Team at Thermo Fisher Scientific has designed over 300 custom panels for over 70 crops for customers for use with the AgriSeq[™] targeted genotyping by sequencing (T-GBS) workflow on the Ion Torrent[®] platform. Here, we report the recent design of ready to use catalog crop panels for wheat, barley, maize, soybean, strawberry, tomato, pepper and lettuce. These panels have various marker densities for use in breeding programs for utilization in Marker Assisted Selection, Genomic Selection and screening of larger 5k panels for SNP performance evaluation and selection in specific breeding germplasm. Designs were completed for the major crops based on publicly available SNP content as well as relevant public content from our Catalog Axiom microarray platform utilizing the most up to date reference genome information at NCBI (Ruff et al. 2020, Ganal et al. 2011, Hardigan et al. 2021, Hulse-Kemp et al. 2016; Sims et al. 2012) (Table 1). SNP content was specifically chosen on the basis of minor allele frequency (greater than 5% where applicable), expert breeder review and public information from population screening and selection of SNPs of interest. Most of the crop panels include 20-200+ trait-based SNP marker designs for the most important economic traits of interest such as disease resistance, agronomic traits, crop quality, and yield component parameters. Results from initial screening with these panels show adequate performance for quickly screening breeding material for routine screening or to select the best performing SNP content for potential custom designs tailored to specific breeding programs. These sequencing panels can be used for routine screening of economic traits of interest or utilization in GS modelling to improve speed, accuracy and efficiency of selection in crop breeding programs.

Table 1. AgriSeq Catalog Crop Panels.

Number	Description	Description	# SNPs
1	USDA Wheat 5k	USDA Consortia 5k Equidistant / 212 Traits	5,174
2	Strawberry 4.5k	Selected from FanaSNP Axiom 50k / High MAF	4,515
3	Maize QAQC	Genetic differentiation (CIMMYT)	180
4	Maize Trait	Disease, Yield, Morphology, Sbe/ae, Protein	230
5	Maize 3.6k	Axiom 60k Equidistant Coverage high MAF	3,691
6	Lettuce QAQC/Trait	High MAF Disease, Yield, Leaf Morphology	1.7k + 215
7	Tomato 5k + Traits	Processing/Fresh/Disease Traits/High MAF	5k
8	Pepper 5k	High MAF from 16k Array Data	5k
9	Barley 3.5k	NDSU - Barley Traits - Protein, Malt, FHB	3559
10	Sunflower	EBDI / USDA Grain QTL Mapping and GS	700
11	Chickpea	ICRISAT / R. Varshney - Genetic Diversity + Traits	5k
12	Peanut	ICRISAT / M. Pandey - Genetic Diversity + Traits	5k
13	Pearl Millet	IIMR / Nepoleon T Genetic Diversity for GS	4k
14	Cotton	USDA MSU Dwayne Deng - High Density	4.5k
15	Soybean 5k	International Soybean Consortia - HD Williams82	5k
16	Melon QAQC + 1.9k	Disease, Flavor/Flesh Traits, MAF	40
17	Rice 2k	Axiom Rice Array Content	2k
18	Watermelon 2k (20 Traits)	Univ Georgia / Korea Ins Ag	2k
19	Cucumber	Nat Inst Hort Herb Sci-Korea	515
20	Canola 5k	Equidistant MAF / Disease Traits	4.7k
21	Pine 1k	NC State - Fikret Isik - GS and Pedigree QC	1.1k

MATERIALS AND METHODS

These panels were created by our Thermo Fisher Scientific Product Management and Bioinformatics Design Teams for each respective crop group from current publicly available Axiom Catalog Panels and publicly available journal articles published with consent to release information under the Creative Commons Attribute license. SNP and variant content from certain Consortia Groups like the Wheat (USDA), Strawberry (UC Davis) and Soybean Consortia Group was brought to us by the group or individual researchers with consent to include in a publicly available design whereby the content would be published in a journal and made publicly available for use by breeding groups. SNP content which was brought to us by these Consortia Groups or made available through various publications was collected and submitted to Thermo Fisher Scientific's Bioinformatics Design Team. For most crop groups high density 1k to 5k panels were made and for certain crop groups researchers were consulted and subsets of Trait based markers for use in Marker Assisted Selection and downstream breeding selection applications. For larger high density (HD) 3k to 5k panels content was chosen on the basis of minor allele frequencies (MAF) (above 5-10%), many panels maintained average MAF > 20%-40%.

Figure 3. Illustration of the Components of AgriSeq Workflow. Genomic DNA is isolated through various Sample Prep kits, library prep is carried out in one day to prepare amplicon fragments from the crop panel for sequencing, sequencing is carried out the next day with a 2.5 hr sequencing run, and data is analyzed through our AgriSum Tool kit plug-in within 24 hours after sequencing.



Figure 1. Illustration of the AgriSeq library preparation step. Genomic DNA is isolated, amplified with PCR, treated with an enzyme prior to ligating barcodes, after barcoding libraries are pooled, cleaned-up and normalized with a bead-based library normalization step.



RESULTS

This effort collected and collated SNP content from Consortia Groups and publicly available content published within the Creative Commons Attribute license. Panels for Wheat, Barley, Strawberry, Maize, Lettuce, Tomato, and Pepper have been developed with future panels planned in the coming year for Soybean, Melons, Watermelon, Rice, Cucumber and Canola (Table 1)¹. Results from initial screening with these panels show adequate performance for quickly screening breeding material, for routine screening or to select the best performing SNP content for potential custom designs tailored to specific breeding programs. These sequencing panels can be used for routine screening of economic traits of interest or utilization in GS modelling to improve speed, accuracy and efficiency of selection in crop breeding programs.

CONCLUSIONS

The authors concluded that the use of AgriSeq targeted sequencing pre-made crop panels are robust and readily available for quick screening and potential use in breeding applications for downstream selection for research purposes.

Please reach out to your local Thermo Fisher Scientific representative (at *Agriseq.gbspanel@thermofisher.com*) for more information regarding these panels or others you may be interested in developing for future projects.

Figure 2. Illustration of the entire AgriSeq workflow process conducted on the Ion Torrent Sequencer which takes around 3 days from library preparation to data analysis.

	Library construction	Templating	Sequencing	Data analysis	
10 ng of DNA	Sample-to-results in 3 days				
Hands-on time	<3 hr (manual) <1 hr (automated)	<15 min	<15 min	<15 min	
Total time	6–7 hr	Overnight	2.5 hr	6–24 hr	



Ion Torrent NGS Sequencer S5 GenStudio and Ion 550 Chip for sequencing.

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