

Axiom® Cotton Genotyping Array

Whole-genome high-density genotyping of cotton for diversity analysis and marker-trait association studies

Gossypium hirsutum (Upland cotton) and *Gossypium barbadense* (Pima cotton) account for the majority of global cotton production. Upland cotton is an important worldwide commercial crop used for producing fiber. Axiom® Cotton Genotyping Array was designed through Affymetrix' Expert Design Program, and the markers on the array were selected from public databases in collaboration with the National Botanical Research Institute, India. The array includes 35,550 markers that were discovered in *G. hirsutum* and *G. barbadense*.^{1,2}

Cotton has an allotetraploid genome and a narrow germplasm base, which have complicated advances in SNP discovery and genotyping because populations derived from a narrow genetic base exhibit fewer polymorphisms. Cotton exhibits one polymorphism every 2,474 bp (0.04%)³ and is consequently difficult to genotype using newer genotyping technologies such as genotyping-by-sequencing (GBS). A polyploid genome, narrow genetic base, and the absence of a reference genome make GBS inefficient and expensive. Axiom® Genotyping Solution can effectively overcome the challenges⁴ in genotyping and analyzing data from allopolyploids such as cotton.

Axiom Cotton Genotyping Array can be used to analyze samples from different populations by adding up to 380,000 custom markers or by transferring polymorphic markers with 100% fidelity onto the Axiom® 384HT myDesign™ breeders array. The Axiom® GT1 algorithm can automatically cluster, assign genotypes, and classify the markers into six different categories for easy visualization.

Highlights

Content

Axiom Cotton Genotyping Array includes a total of 35,550 markers:

- 28,158 intra-specific markers identified using gene-enriched genomic sequences of *G. hirsutum*¹
- 7,392 markers discovered using genome reduction methodology that is based on restriction site conservation (GR-RSC)²
 - 5,286 markers discovered in an inter-specific assembly of *G. hirsutum* and *G. barbadense*
 - 2,106 markers that are intra-specific to *G. hirsutum*

Diversity

Axiom Cotton Genotyping Array includes markers that were discovered in the following accessions:

- Six accessions used in breeding programs in Asia for varietal and mapping population development. These included four accessions

with superior fiber traits (JKC725, JKC770, LRA5166, and MCU5) and two with inferior fiber quality (JKC703 and JKC737).

- Four accessions that represent domestic and wild accessions of two species of allotetraploid cotton: *G. hirsutum* (Acala Maxxa and TX2094) and *G. barbadense* (Pima-S6 and K101).

Applications

Complex trait research

- Conduct GWAS and fine mapping
- Identify traits of economic importance

Molecular breeding

- Conduct association mapping
- Improve efficiency of breeding programs
- Create new varieties of cotton

SNP discovery

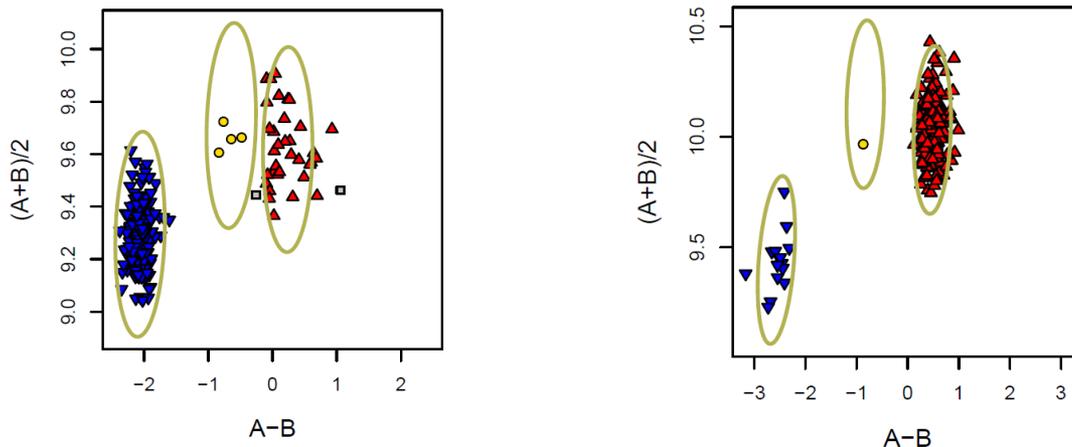
The markers on the array were identified from two separate SNP discovery efforts on the Roche 454™ pyrosequencing platform. The intra-specific markers were discovered in gene-enriched genomic sequences of *G. hirsutum* using a hypomethylated restriction-based genomic enrichment strategy. Sequencing was conducted on six diverse accessions of *G. hirsutum*. The inter-specific markers were identified by combining the GR-RSC assemblies of Maxxa, TX2094, Pima-S6, and K101 accessions.

A total of 110,095 putative markers from the two SNP discovery methods were submitted to Affymetrix to calculate *in silico* design scores. Markers that passed the SNP design pipeline were selected for design. The array was designed with a total of 35,350 markers that are represented by 42,668 probe sets.

Results

The performance of the array was evaluated by genotyping 192 cotton samples that represented the F2 mapping population. Samples were provided by Tropical Breeding and Genetics Ltd., BR. All samples were prepared using the guidance provided for the Axiom® 2.0 assay. A total of 191 samples passed the Axiom® best practices workflow. A total of 23,172 markers (65.18%) markers were automatically

Figure 1: Cluster plots showing automated clustering and genotype assignment using the Axiom® GT1 algorithm on data from Axiom® Cotton Genotyping Array. Each point on the cluster represents a sample. The BB genotypes are shown as blue triangles. AA genotypes are shown as red triangles. AB genotypes are shown as gold circles. No-calls are shown as gray squares.



identified as high-quality markers, with 8.81% identified as polymorphic high resolution and 52.4% as monomorphic high resolution. An additional 107 markers were put through supplemental filters to further classify the markers. The Axiom® GT1 algorithm automatically clusters and assigns genotypes to the markers as shown in Figure 1.

The data analysis and clustering were automated using Affymetrix® Genotyping Console™ Software and Affymetrix® SNPolisher™ package. SNPs were filtered as per the *Best Practice Supplement to Axiom® Genotyping Solution Data Analysis Users Guide* (P/N 703083).

Ordering information

Part number	Description	Details
550563	Axiom® Cotton Genotyping Array	Contains one plate with 96 arrays
901606	Axiom® GeneTitan® Consumables Kit	Contains all GeneTitan® Instrument consumables required to process one array plate
901758	Axiom® 2.0 Reagent Kit	Includes all reagents (except isopropanol) for processing 96 DNA samples

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