Application Brief

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SNP genotyping using Affymetrix Axiom® Genotyping Solution

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Abstract

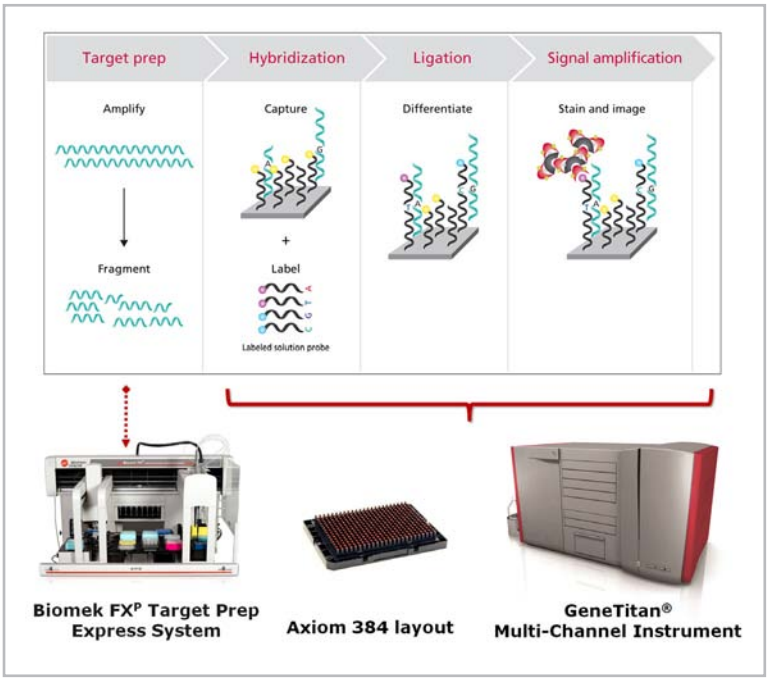
The use of DNA microarrays for easy, cost-effective genotyping of single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (indels) continues to play an important role in genotype-trait association studies and marker-assisted selection in both plant and animal breeding programs.

Axiom® Genotyping Solution enables complete automation of DNA target preparation, DNA amplification, and enzymatic fragmentation of post-amplification products on the Biomek® FX^P Target Prep Express platform. Following target preparation, arrays are processed using GeneTitan® Multi-Channel (MC) Instrument. Here we describe a new Axiom product in which 384 individual arrays, contained in the footprint of a standard microtiter plate, offer the capability to genotype approximately 50,000 variants in combination with a processing throughput of greater than 3,000 samples per week. The 384-array layout is ideal for high-throughput screening in molecular breeding or marker-assisted selection programs where ease of use, accuracy, and turnaround time are all essential. The Axiom 384-array layout retains full compatibility with the currently existing Axiom instrumentation platform and downstream data analysis stream. Genotyping performance metrics obtained with Axiom 384-array layout will be presented.

Axiom biochemistry and workflow overview

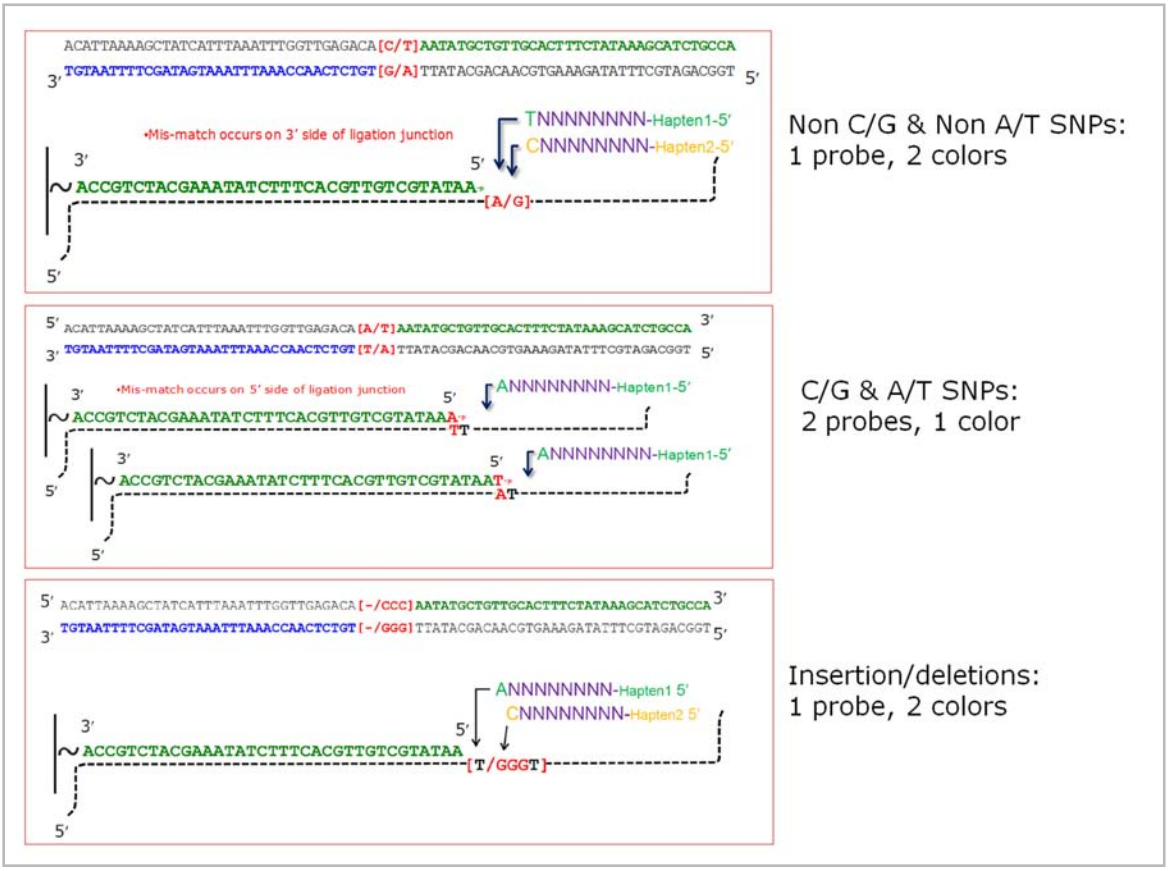
- Total genomic DNA (100 ng) is amplified and randomly fragmented into 25 to 125 base pair (bp) fragments
- These fragments are purified, re-suspended, and hybridized to customized Axiom® myDesign™ Arrays Plates
- Following hybridization, the bound target is washed under stringent conditions to remove non-specific background caused by random ligation events. Each polymorphic nucleotide is queried via a multi-color ligation event carried out on the array surface
- After ligation, the arrays are stained and imaged on GeneTitan MC Instrument

Figure 1: Axiom biochemistry and workflow overview



- Axiom 384-array layout can be processed on an existing in-market GTMC with only a software upgrade
- Axiom 384-array layout is capable of genotyping 100–45,000 SNPs from diploid species or up to 32,000 SNPs from polyploid species
- 8-plate workflow in 5 days offers a throughput in excess of 3,000 samples per week
- The SNP content on the Axiom 384-array layout can be selected from in-market agrigenomic arrays for plants and animals or from SNPs that have been discovered through next generation-sequencing

Figure 2: Probe design for interrogation of SNPs and indels.



- Axiom probes are typically 30 mers that contain a 5′-phosphate group
- Fragmented whole-genome amplified genomic DNA hybridizes to the array-bound probes
- The polymorphic nucleotides are detected using two differentially labeled sets of ligation probes

Results

Axiom 384-layout automated target prep (ATP) performance

An experiment was designed to uncouple Axiom 384-layout ATP performance from Axiom 384-layout array performance in order to independently assess Axiom 384 layout ATP and compare the performance with specifications established for the Axiom 96-layout ATP. DNA samples were prepared using Axiom 384-layout ATP executed on three different Biomek instruments and a subset of samples were tested on standard 96-array Axiom® GW CEU 1 Array Plate (genome-wide array that includes validated markers from human populations with northern and western European ancestry).

Table 1: Acceptance criteria and data generated from 384-layout automated target prep run on 96-array plates. All genotyping performance specifications pass acceptance criteria

Metrics	Acceptance criteria	384 ATP#1	384 ATP#2	384 ATP#3
Overall sample pass rate	≥97%	100%	100%	100%
DQC median		0.985	0.984	0.988
Average call rate	≥99.0%	99.7%	99.7%	99.8%
Average HapMap concordance	≥99.5%	99.7%	99.7%	99.7%
Average reproducibility	≥99.8%		99.9%	

Axiom 384-layout array performance

Once the 384-layout automated target prep passed genotyping performance specifications, the 384-layout array plate was evaluated. Axiom 384-layout ATP was used with HapMap270 DNA samples (4 blank wells excluded from analysis; T03 YRI samples in duplicate). Axiom 384 Test Array_1 was used to assess genotyping performance. The SNP content is from Axiom validated database and represents ~14K SNPs known to have MAF >5% in YRI samples.

Table 2: Acceptance criteria and data generated from 384-layout automated target prep run on 384-array plates. All genotyping performance specifications pass acceptance criteria.

Metrics	384 sample acceptance criteria	384 ATP_I
Number of input samples		380
Overall sample pass rate	≥97%	100%
DQC median		0.984
Average call rate	≥99.0%	99.7%
Average HapMap concordance	≥99.5%	99.8%
Average reproducibility	≥99.8%	99.9% (100 sets)

Conclusion

In summary, new Axiom® advances to DNA sample type compatibility, high-throughput sample processing enabled with laboratory automation, and the 384-array layout further extend the platform's capabilities to genotype thousands of samples per week with minimal manual intervention that is consistent with the needs of both animal and plant agrigenomics solutions.

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P/N DNA01918 Rev. 1