



Data Sheet

■ Affymetrix® Genome-Wide Human SNP Array 6.0

The new Affymetrix® Genome-Wide Human SNP Array 6.0 features more than 1.8 million markers of genetic variation, including single nucleotide polymorphisms (SNPs) as well as probes for the detection of copy number variation. The SNP Array 6.0 allows researchers to design highly powered association studies with large sample sizes in both initial scan and replication phases, thereby significantly increasing the overall genetic power of their studies.

The SNP Array 6.0 demonstrates industry-leading performance and represents more genetic variation on a single array than any other product, providing maximum panel power, coverage and the highest physical coverage of the genome.

Introduction

The new Affymetrix® Genome-Wide Human SNP Array 6.0 contains more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation. SNPs on the array are present on 200 to 1,100 base pairs (bp) Nsp I or Sty I digested fragments in the human genome, and are amplified using the Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0. This assay, which is also compatible with the SNP Array 5.0, now combines the Nsp and Sty fractions previously assayed on two separate arrays.

SNPs on the Genome-Wide Human SNP Array 6.0 were screened in more than 500 distinct samples, including 270 HapMap samples and separate diversity samples. Approximately 482,000 SNPs are derived from the previous-generation Mapping 500K and SNP 5.0 Arrays. The remaining 424,000 SNPs include tag SNP markers derived from the International HapMap Project, better representation of SNPs on chromosomes X and Y, mitochondrial SNPs, SNPs in recombination hotspots and new SNPs added to the dbSNP database after completion of the Mapping 500K Array.

The array also contains 202,000 probes targeting 5,677 known regions of copy number variation from the Toronto Database of Genomic Variants. These

regions resolve into 3,182 distinct, non-overlapping segments, each interrogated with an average of 61 probes. In addition to the interrogation of these regions of known copy number polymorphism, more than 744,000 probes were chosen, evenly spaced along the genome, to enable the detection of novel copy number variation.

The median inter-marker distance taken over all 1.8 million SNP and copy number markers combined is less than 700 bases.

THE WHOLE-GENOME SAMPLING ASSAY

The Affymetrix® Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0 [P/N 901152, 901015] is validated for use in conjunction with the Genome-Wide Human SNP Array 6.0. Briefly, total genomic DNA (500 ng) is digested with Nsp I and Sty I restriction enzymes and ligated to adaptors that recognize the cohesive 4 bp overhangs. All fragments resulting from restriction enzyme digestion, regardless of size, are substrates for adaptor ligation. A generic primer that recognizes the adaptor sequence is used to amplify adaptor-ligated DNA fragments. PCR conditions have been optimized to preferentially amplify fragments in the 200 to 1,100 bp size range. PCR amplification products for each restriction enzyme digest are combined and purified using polystyrene beads. The amplified

DNA is then fragmented, labeled and hybridized to a Genome-Wide Human SNP Array 6.0.

The Genome-Wide Human SNP Nsp/Sty Assay Kit contains validated and qualified reagents for the most critical steps in the assay. This includes the PCR primer and adaptors, reagents to fragment and label the PCR products and control reagents. Kits are available for either 50 or 100 reactions (see ordering information).

Whole-genome-amplified material prepared by the Qiagen REPLI-g® kits may also be used as the starting material for the Genome-Wide Human SNP Assay Kit.

Performance Data

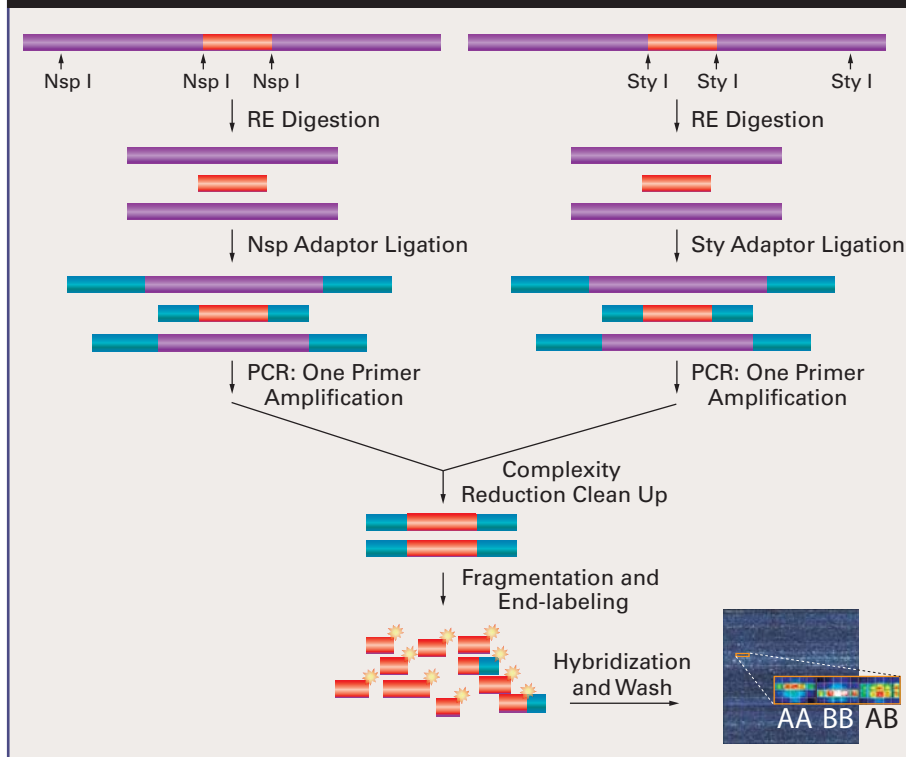
To test the performance of the SNP Array 6.0, Affymetrix ran the 270 samples from the International HapMap Project. In addition, two external sites ran a plate of 44 HapMap DNAs, which includes 30 unique samples, 10 trios and five samples with multiple replicates.

The arrays that passed the QC call rate threshold were analyzed using the Birdseed algorithm at the default setting of 0.1. The average call rate for each set was greater than 99 percent, and the concordance with HapMap genotypes was observed to be greater than or equal to 99.7 percent. For the 10 trios, the Mendelian inheritance consistency was found to be greater than 99.9 percent. Reproducibility was measured at 99.9 percent.

Data analyzed with Birdseed (0.01).			
	270 HapMap	Site 1	Site 2
Call Rate	99.8	99.7	99.7
HapMap Concordance	99.8	99.7	99.8
Mendelian Consistency	99.97	99.95	99.96
Reproducibility	NA	99.9	99.9
SNP Completeness*	99.9	99.7	99.8

*SNP completeness is defined as the proportion of SNPs with per-SNP call rate greater than 85 percent.

Figure 1: Overview of the Genome-Wide Human SNP Assay 5.0/6.0



GENOTYPE CALLS USING AFFYMETRIX

GENOTYPING CONSOLE

The Genome-Wide Human SNP Array 6.0 is used in conjunction with Affymetrix® Genotyping Console, which implements a novel genotype calling algorithm called Birdseed. Birdseed is an evolution of the RLMM genotype calling algorithm¹. It performs a multiple-chip analysis to estimate a signal intensity for each allele of each SNP, fitting probe-specific effects to increase precision (like the BRLMM-P algorithm developed for the Genome-Wide Human SNP Array 5.0). It then makes genotype calls by fitting a Gaussian mixture model in the two-dimensional A-signal vs. B-signal space, using SNP-specific models to improve accuracy.

Genotyping Console was designed to streamline genotyping calling and genotyping quality control. In addition to the algorithm, features include automated QC that sorts samples by QC call rate, visualization of QC metrics across samples, SNP cluster visualization and signature SNP list for

tracking. Seventy-two signature SNPs enable verification of a sample's identity by comparing the genotype calls to different technologies or other known reference. Flexible SNP filter and export are also included to enable downstream analysis. In addition to the Birdseed algorithm, Genotyping Console supports the BRLMM-P algorithm for the SNP Array 5.0.

Refer to the *Affymetrix® Genome-Wide Human SNP Nsp/Sty 5.0 Assay 5.0 or 6.0 Manual* (P/N 702419-2, P/N 702054) for details on the QC call rate thresholds, as well as procedures on DNA target preparation, target hybridization, fluidics setup, array scanning and data analysis.

FLUIDICS PROTOCOL REQUIRED

GenomeWideSNP6_450

LIBRARY FILES REQUIRED

GenomeWideSNP_6

Library files contain information about probe array design layout and other char-

acteristics, probe use and content and scanning and analysis parameters.

These files are unique for each probe array type. Library files are available from the Affymetrix website at www.affymetrix.com/support/technical/libraryfilesmain.affx.

Affymetrix products can be purchased directly from Affymetrix in the United States and many European countries. For all other territories, refer to our list of distribution partners located at www.affymetrix.com/site/contact/index.affx.

REFERENCES

1. Rabbee, N., *et al.* A genotype calling algorithm for Affymetrix SNP arrays. *Bioinformatics* **22**:7-12 (2006).
2. Affymetrix, Inc. BRLMM: An Improved Genotype Calling Method for the Mapping 500K Array Set.
3. Matsuzaki, H., *et al.* Genotyping over 100,000 SNPs on a Pair of Oligonucleotide Arrays. *Nature Methods* **1**:109-111 (2004).
4. Frayling, T. M., *et al.* A Common Variant in the FTO Gene is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science* (ePub April 12 2007).

Product Information

Number of SNPs on the array	906,600
Number of Non-polymorphic Probes for Copy Number Detection	946,000
Number of Arrays	1
DNA Required	500ng
Average Birdseed Call Rate (0.1)	>99 percent*
Average Minor Allele Frequency (MAF)	19.6% in HapMap Caucasians 18.2% in HapMap Asians 20.6% in HapMap Africans
Average Heterozygosity	26.7% in HapMap Caucasians 24.6% in HapMap Asians 28.5% in HapMap Africans
PCR Primers	1 per sample
Instrumentation	GeneChip® Scanner 3000 7G with AutoLoader
Throughput	>40 million genotypes per day, per scanner with three GeneChip® Fluidics Station 450s

* Average scan from four independent studies

Affymetrix® Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0 Components

Adaptor, Nsp I or Adaptor, Sty I	Two annealed oligonucleotides specific for ligation to the Nsp I or Sty I restriction site
PCR Primer 002	PCR primer to amplify ligated genomic DNA
Reference Genomic DNA, 103	Human genomic DNA control, with consensus genotypes
GeneChip® Fragmentation Reagent	DNase I enzyme, formulated to fragment purified PCR amplicons
10X Fragmentation Buffer	Buffer for fragmentation reaction
GeneChip® DNA Labeling Reagent (30mM)	Proprietary biotin-labeled reagent for end-labeling fragmented PCR amplicons
Terminal Deoxynucleotidyl Transferase	Enzyme used to end-label fragmented PCR amplicons with the GeneChip® DNA Labeling Reagent
5X Terminal Deoxynucleotidyl Transferase Buffer	Buffer for labeling reaction
Oligo Control Reagent, 0100	Mixture of five biotin-labeled oligonucleotides, which hybridize to control regions (gridding and array controls) on the SNP Array 5.0/6.0

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Ordering Information

Affymetrix® Genome-Wide Human SNP Array 6.0

Affymetrix® Genome-Wide Human SNP Array 6.0

901153 Contains 50 arrays

901150 Contains 100 arrays

Affymetrix® Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0

901152 Sufficient for 50 reactions

901015 Sufficient for 100 reactions

To Order

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