

## Affymetrix<sup>®</sup> Mouse Diversity Genotyping Array



**The Affymetrix Mouse Diversity Genotyping Array features more than 623,000 single nucleotide polymorphisms (SNPs), enabling researchers to accurately characterize a broad range of mouse strains and their crosses as well as uncover genetic changes in mouse models of disease.**

**With more than 120 times the number of SNPs of competing products, and an average of 4.3 kilobases (kb) between SNPs, the Mouse Diversity Genotyping Array has greater density and distribution than any other publicly available mouse SNP array.**

### Introduction

The Mouse Diversity Genotyping Array, designed by The Jackson Laboratory and the University of North Carolina, contains more than 623,000 SNPs and more than 916,000 non-polymorphic copy number probes that are targeted to functional elements and regions known to harbor segmental duplications. The SNPs on this array originated from multiple sources, in order to capture maximum diversity in laboratory mouse strains while enabling discrimination among classical inbred strains. These sources primarily stem from the National Institute of Environmental Health Sciences (NIEHS)/Perlegen, the Ken Paigen lab, B6 and MSM strain singletons, wild-derived strains, chromosomes X and Y, and mitochondria. SNPs on the array are present on 200 to 1,100 base pairs (bp) Nsp I or Sty I digested fragments in the mouse genome, and are amplified using the Affymetrix<sup>®</sup> Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0.

### The whole-genome sampling assay

The Genome-Wide Human SNP Nsp/Sty Assay Kit (P/N 901152, 901015, and 901192) is validated for use in conjunction with the Mouse Diversity Genotyping Array.

Total genomic DNA (500 ng) is digested with Nsp I and Sty I restriction enzymes and ligated to adaptors that recognize the cohesive 4 base pair (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 Mouse Diversity Genotyping Array (see Figure 1, pg. 2).

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. Manual processing kits are available for 30, 50, and 100 reactions. Whole-genome-amplified material prepared by Qiagen REPLI-g<sup>®</sup> Kits may also be used as the starting material for the Genome-Wide Human SNP Assay Kit.

### Performance data

To validate the performance of the Mouse Diversity Genotyping Array, 148 distinct samples were processed by The Jackson Laboratory. These included 98 samples of classical inbred laboratory strains, nine F1 crosses of classical laboratory strains, and 41 F1 crosses of classical strains with wild-derived strains.

The arrays were analyzed by Affymetrix using a modified BRLMM-P algorithm within Affymetrix Power Tools. Table 1 (pg. 2) shows performance metrics for the classical inbred laboratory strains and their F1 crosses. Please refer to the supplementary Table 2 in Yang, H., *et al.* (*Nature Methods*, 2009) for performance data for all classical and wild-derived inbred strains tested.

As shown in Table 1, the average call rate was greater than 98.6 percent, and the concordance with reference genotypes obtained from The Jackson Laboratory was greater than or equal to 99.5 percent. For the nine trios, the Mendelian inheritance consistency was greater than 99.95 percent, and reproducibility for biological replicates was 99.9 percent. The average sample heterozygous rate (which is equivalent to an error rate) was 0.33 percent.

**Genotype calls using Affymetrix® Genotyping Console™ (GTC) Software**

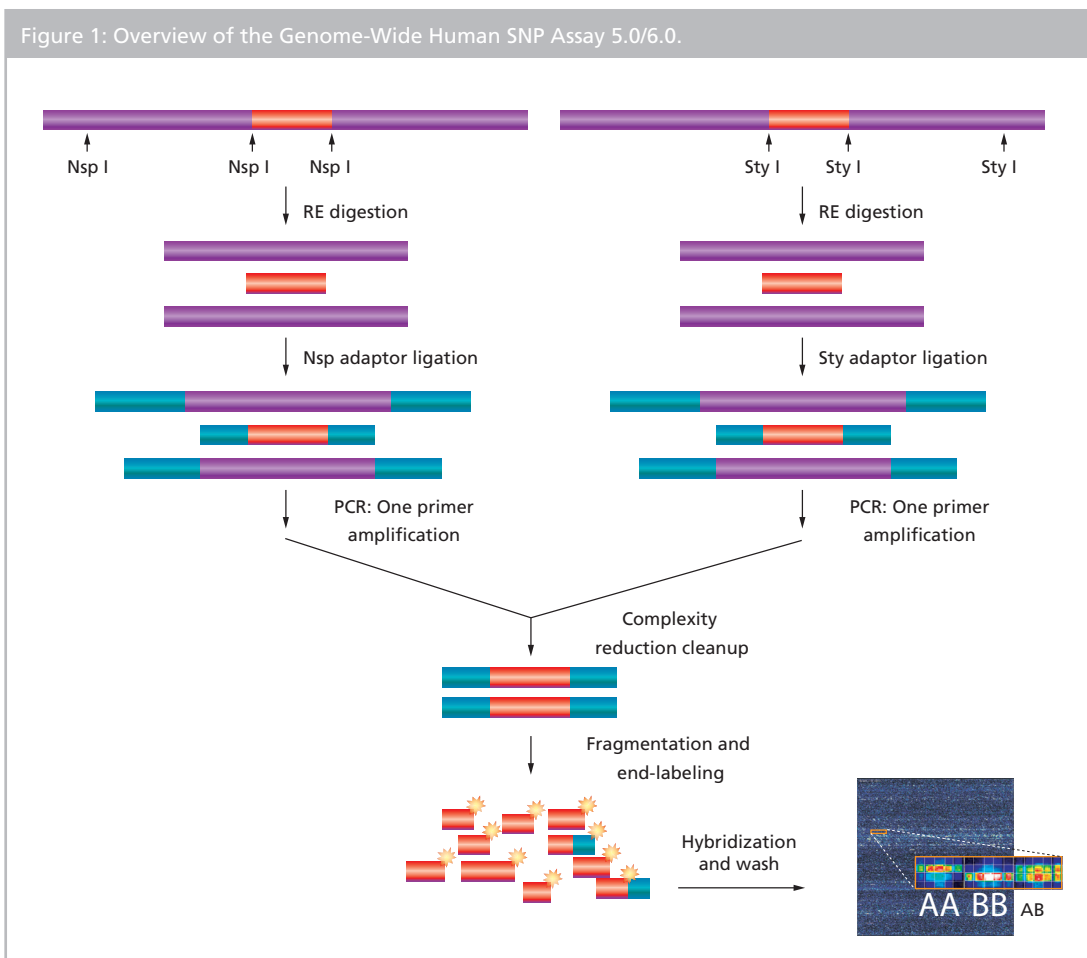
Genotype calls are made using Genotyping Console (GTC) Software. A modified version of the BRLMM-P algorithm is used for SNP genotyping analysis related to association studies and other applications.

**Table 1: Array performance.**

	Test set (The Jackson Lab)
Call rate	98.6%
Jackson reference concordance	99.5%
Homozygous concordance*	99.7%
Heterozygous concordance	98.9%
Mendelian consistency	99.95%
Reproducibility	99.9%
Inbred heterozygous rate	0.33%
SNP completeness**	98.5%

\*Homozygous concordance is the fraction of the genotype calls for a given homozygous SNP, which are called homozygous.

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



Detailed instructions for downloading and using GTC, along with sample CEL files, can be found on the Affymetrix website.

Refer to the Genome-Wide Human SNP Nsp/Sty Assay 5.0 or 6.0 User Guide (P/N 702419-2, 702504-3) for procedures on DNA target preparation, target hybridization, fluidics setup, and array scanning.

### Copy number probes

The Mouse Diversity Genotyping Array also contains more than 916,000 non-polymorphic copy number probes that are targeted to functional elements and regions known to harbor segmental duplications. Because so little information on copy number variation in mice exists, the copy number application is for discovery use only. Copy number analysis is not supported by Affymetrix because there are no known commercially available informatics tools for this purpose.

### Fluidics protocol required

GenomeWideSNP6\_450

### Library files required

MouseDIV

Library files contain information about probe array design layout, probe use and content, scanning and analysis parameters, and other characteristics.

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](http://www.affymetrix.com/support/technical/libraryfilesmain.affx).

### References

Yang, H., *et al.* A customized and versatile high-density genotyping array for the mouse. *Nature Methods* **6**(9):663-6 (2009).

### Specifications

Number of SNPs on the array	623,124
Number of non-polymorphic probes	916,269
DNA required	500 ng
Average BRLMM-P call rate	98.6%
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 450 instruments

### 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 (30 mM)	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)

<b>Ordering information</b>	
Part number	Description
<b>Affymetrix<sup>®</sup> Mouse Diversity Genotyping Array</b>	
901615	Contains 30 arrays (minimum initial order of 60 arrays; 30-array minimum for subsequent orders)
<b>Affymetrix<sup>®</sup> Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0/6.0</b>	
901013	Sufficient for 30 reactions
901152	Sufficient for 50 reactions
901015	Sufficient for 100 reactions

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P/N DS105 Rev. 2

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