Affymetrix Genome-Wide SNP Array 6.0

Affymetrix Genome-Wide SNP Array 6.0 产品的特点是超过 1,800,000 个遗传变异标志物，包括超过 906,600 个 SNP 和超过 946,000 个用于检测拷贝数变化的探针。大约有 482,000 个 SNPs 来自于前代产品 500K 和 SNP5.0 芯片。剩下 424,000s 个 SNP 包括了来源于国际 HapMap 计划中的标签 SNPs，X，Y 染色体和线粒体上更具代表性的 SNPs,以及来自于重组热点区域和 500K 芯片设计完成后新加入 dbSNP 数据库的 SNPs。SNP6.0 还包括了 202,000 个用于检测 5,677 个已知拷贝数变异区域的探针，这些区域来源的于多伦多基因组变异体数据库。该数据库中的每个 3,182 个非重叠片断区域分别平均用 61 个探针来检测。除了检测这些已知的拷贝数多态区域，还有超过 744,000 个探针平均分配到整个基因组上，用来发现求知的拷贝数变异区域。设计方面 SNP 6.0 芯片只取最佳 SNP 位点和每组等到位基因的一个完全匹配，并有三次重复检测，所以总共只有六个探针。

具体参数如下：

超过 906,600 个 SNP，包括：

Unbiased selection of 482,000 SNPs; historical SNPs from the SNP Array 5.0

Selection of additional 424,000 SNPs

Tag SNPs

SNPs from chromosomes X and Y

Mitochondrial SNPs

New SNPs added to the dbSNP database

SNPs in recombination hotspots

超过 946,000 的 用于检测拷贝数变化的探针，包括：

202,000 probes targeting 5,677 known CNV regions from the Toronto Database of Genomic Variants

Regions resolve into 3,182 distinct, non-overlapping segments; on average 61 probes per region744,000 probes,

evenly spaced along the genome

样品要求

1. 样品纯度：OD 260/280 值应在 1.7～2.0 之间；RNA 应该去除干净；不得有其它个体或其它物种的 DNA 污染。
2. 样品浓度：浓度不低于 55ng/μl。
3. 样品总量：每个样品总量不少于 1μg。
4. 样品溶剂：溶解在 Reduced TE (10mM Tris, pH 8,0，0.1mM EDTA)中。
5. 样品运输： DNA 低温运输（-20℃）；在运输过程中请用 parafilm 将管口密封好，以防出现污染。

试验流程

1. DNA抽提；
2. 样品质检：用琼脂糖凝胶电泳或LAB-ON-CHIP系统对样品DNA进行质量检测和定量；
3. 实验过程：

a、扩增，标记； b、杂交、洗脱（具体见左图）；

4. 图像扫描：用Affymetrix Scanner3000 7G激光共聚焦扫描仪对杂交结束后的芯片进行扫描；
5. 数据处理：确定检出的SNP位点相应的核苷酸。

数据分析流程

1. SNP 位点过滤

1）Nocall rate>=10%

2）最小等位基因频率，Minor allele frequency<0.05

3）不符合哈维平衡的位点，HWE P value<0.001 的位点

2. 频数统计：计算 case/control 组中各等位基因和基因型的频率

3. 关联分析

1）样品群的分层分析

2）卡方检验、Fisher 精确检验，比较各 allele 的频率分布在 case 和 control 两组中是否具有统计学差异

3）卡方检验、Fisher 精确检验和 Cochran-Armitage 趋势检验,比较各基因型频率分布在两组中是否具有统计学差异

4. 单倍型分析

1）选取包含显著的 SNP 的区段进行单倍型分析，以进一步定位候选区段

2）频率较高的几种单倍型各自的频率，在 case/control 两组中的分布状况，卡方统计量及 p 值，判断单倍型与疾病相关性

结果报告

1、实验数据：原始数据（cel 文件），基因芯片数据总表，SNP 位点信息。

2、实验文件：SNP 芯片实验操作方法。

3、实验图像：SNP 芯片扫描图（jgp 格式），所有样品的质检电泳图。

4、质检文件：DNA 质检报告，SNP Call rate。

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, nonoverlapping 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

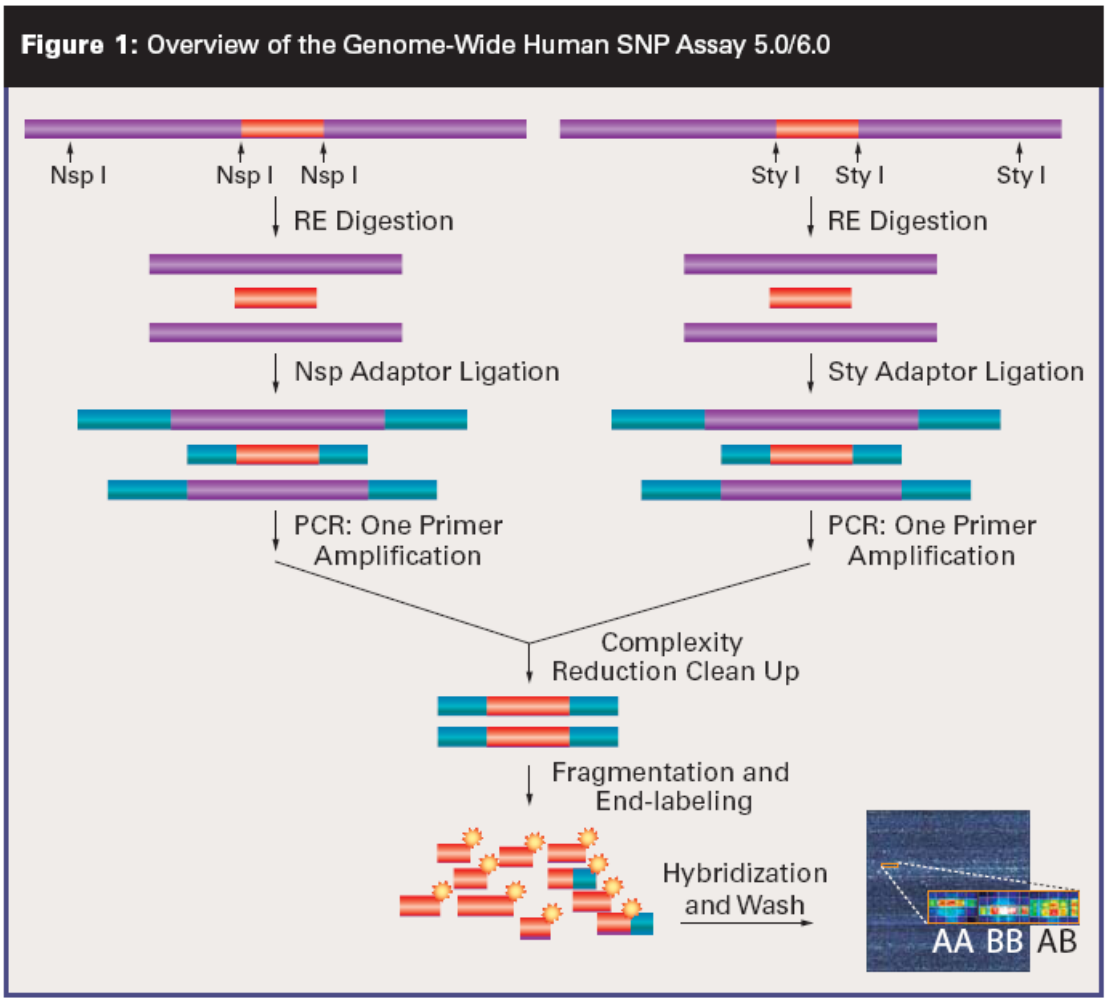
the most critical steps in the assay. This includes the PCR primer and adaptors, reagents to fragment and label the PCR products and control reagents. Kits are available for either 50 or 100 reactions (see ordering information). Whole-genome-amplified material prepared by the Qiagen REPLI-g? kits may 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.



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 BRLMMP 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.

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