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Technical Note

Linking Whole-genome Amplification to SNP Genotyping

This technical note contains recommendations for whole-genome amplification of small amounts of genomic DNA for analysis using the Affymetrix GeneChip® Mapping 50K Xba Array, the Affymetrix® Genome-Wide Human SNP Array 5.0 and the SNP Array 6.0. These recommendations were developed by the systematic assessment of assay performance using different starting genomic DNA amounts and cleanup options.

INTRODUCTION

In this study, we systematically explored the use of whole-genome amplified (WGA) DNA for SNP genotyping on the Affymetrix GeneChip® Mapping 50K Xba Array, the Affymetrix® Genome-Wide Human SNP Array 5.0 and the SNP Array 6.0.

We compared amplified genomic DNA (gDNA) to non-amplified gDNA to determine the minimum amount of starting material that can be used without compromising data quality (see Table 1). We also investigated the impact of WGA cleanup on array performance. We found that WGA DNA can be used to obtain efficient and reproducible genotyping on Affymetrix mapping and SNP genotyping arrays. The results can be used as guidelines for analyzing limited quantities of gDNA samples for genome-wide assessments of SNP genotypes.

WHOLE-GENOME AMPLIFICATION

The Mapping 50K Xba Array and SNP Arrays 5.0 and 6.0 serve as SNP genotyping tools with increasing resolution, enabling the analysis of nearly 2 million markers of genetic variation on the SNP Array 6.0. To prepare templates for these arrays, sample gDNA is digested and ligated to adaptors, then amplified during a polymerase chain reaction (PCR) using a single universal primer set.

Next, PCR amplicons are fragmented, labeled and hybridized to the array (Kennedy *et al.*). Because the availability of gDNA is often limited, it may be necessary to amplify a small amount using WGA prior to genome-wide analysis (Paez *et al.*, Tzvetkov *et al.*). Today, WGA is commonly used to amplify gDNA from purified DNA samples or directly from clinical or case work samples without any DNA purification. In this study, we explored the use of WGA DNA for whole-genome SNP genotyping.

A common method of WGA is multiple displacement amplification, which is based on a mechanism using the Φ29 DNA polymerase. This polymerase combines high processivity with proofreading and strand displacement activity, resulting in large fragments (greater than 10 kilobases [kb]). Furthermore, it replicates DNA without changing the number of repetitive sequence elements.

The isothermal reaction is performed at 30°C without any change in temperature. The reaction starts with the annealing of multiple random primers to the target

Table 1: Summary of amounts of DNA used and resulting data quality from the application of WGA DNA to Affymetrix SNP genotyping arrays. For the SNP Arrays 5.0 and 6.0, a total of 59 HapMap samples were used for each array. For the GeneChip® Human Mapping 50K Array Xba, triplicates of the Ref103 DNA were used.

Array	Amount of gDNA Used for WGA	Amount of WGA DNA Used for Assay	Observed Average Call Rate	Observed Average Concordance
SNP Array 6.0	30 ng	500 ng	99.5%	99.7%
SNP Array 5.0	30 ng	500 ng	99.5%	99.3%
50K Xba Array	10 ng	250 ng	98.3%	99.8%

DNA and then the primers are elongated. Since Phi29 DNA polymerase is able to displace DNA strands in a 5' to 3' direction, the polymerase reaction is not blocked by downstream elongating DNA strands. The replicated DNA strand becomes an additional target for multiple primed elongation reactions so that the DNA template is amplified exponentially in a branch-like manner (Hosono *et al.*, 2003).

We report three conclusions from tests comparing WGA DNA with non-amplified DNA on various Affymetrix SNP genotyping arrays:

- Titration of input gDNA into WGA reactions indicated that starting with less than 10 ng gDNA could reduce performance of the sample on the array, as demonstrated using the Mapping 50K Xba Array.
- WGA DNA, amplified from 30 ng gDNA, provided high call rates and concordant genotypes to matching non-amplified gDNA on the SNP Arrays 5.0 and 6.0.
- A cleanup step does not appear to be required between WGA and the use of WGA DNA to the SNP genotyping assay, regardless of the final array used.

In summary, it is possible to use WGA DNA with the Affymetrix SNP genotyping WGSA assay for efficient and reliable genotyping. It is suggested that a minimum of 10 ng of gDNA be used in the WGA assay when the resulting amplified DNA will be applied to the Mapping 50K Xba Array, and 30 ng of gDNA be used in the WGA assay when the resulting amplified DNA will be applied to the SNP Arrays 5.0 and 6.0.

MATERIAL AND METHODS

WHOLE-GENOME AMPLIFICATION

A standard gDNA sample (Reference 103, Affymetrix) was used for direct analysis on Mapping 50K Xba Arrays. A total of 59 HapMap gDNA samples from all population types were used for pre- and post-WGA comparisons on the SNP Arrays 5.0 and 6.0. WGA was conducted using the REPLI-g® Midi Kit (QIAGEN) and was performed as described in the REPLI-g Mini/Midi handbook.

Briefly, gDNA was incubated with 2.5 µl Buffer D1 for three minutes followed by the addition of 5 µl N1. Both solutions are provided with the kit. A master mix containing 29 µl REPLI-g Midi Reaction Buffer and 1 µl REPLI-g Midi DNA-Polymerase was added to the preprocessed DNA. Amplification was performed for between eight to 16 hours at 30° C. The polymerase was deactivated by incubating the reaction mix at 65°C for three minutes. The amplified gDNA was frozen for storage.

DETERMINATION OF DNA CONCENTRATION

To determine yield and concentration of the amplified gDNA, the PicoGreen® reagent (Invitrogen) was used. Because the fluorophore PicoGreen binds specifically to double-stranded DNA, yield or concentration was not overestimated by residual single-stranded primers or nucleotides.

PURIFICATION OF WGA DNA

Purification of the WGA DNA was performed according to a modified QIAamp® Blood Protocol (QIAGEN). First, 150 µl of H2O and 50 µl WGA DNA were mixed. After the addition of 200 µl Buffer AL and 200 µl ethanol (96 to 100 percent), the QIAamp® blood column was loaded. After binding, the DNA was washed with AW1 and AW2 according to the handbook. Elution of WGA DNA was performed by using 1xTE, pH 8.0.

LABELING OF DNA FOR ANALYSIS AND HYBRIDIZATION

For the Mapping 50K Xba Array, amplified or non-amplified DNA was processed strictly according to the Mapping 100K assay manual. For the SNP Arrays 5.0 and 6.0, 500 ng amplified or non-amplified DNA was processed strictly according to the Genome-Wide Human SNP Nsp/Sty 5.0 and 6.0 User Guides.

GENOTYPE ANALYSIS

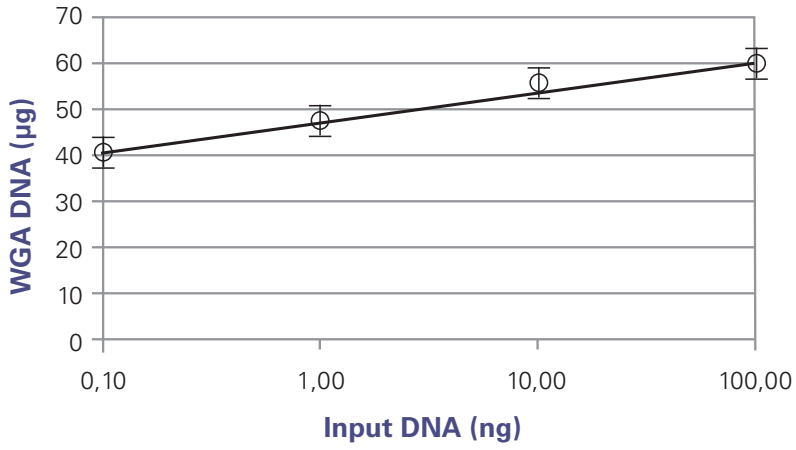
Default settings were used for all data analysis. The Mapping 50K Xba Array SNP call rates were determined using GTYPE with a *p*-value threshold of 0.25. The SNP Arrays 5.0 and 6.0 data were analyzed using the BRLMM-P and Birdseed v.2 algorithms (respectively) implemented in Genotyping Console™ 2.0.

RESULTS AND DISCUSSION

STARTING MATERIAL FOR WGA

To test whether different amounts of gDNA can be used for WGA with the REPLI-g

Figure 1: Yield of amplified DNA. The WGA was performed using different amounts of gDNA as starting material. The yield was determined by fluorescence measurement using the double-strand specific dye PicoGreen. Each input DNA was tested in triplicate.



Kit and applied to the arrays for reliable genotyping, 100 ng, 10 ng, 1 ng and 0.1 ng human gDNA were amplified in three independent reactions each. Various amounts of starting material resulted in different yields of WGA DNA, ranging from 40 µg to 60 µg and corresponding to the amplification factor of 6,000- to 400,000-fold for gDNA (Figure 1).

APPLICATION OF WGA DNA TO THE MAPPING 50K XBA ARRAY

Titration experiments were performed using the Mapping 50K Xba Array to determine the minimum amount of starting material for amplification of DNA targeted to the genotyping arrays. Input amounts for WGA ranged from 0.1 ng to 100 ng gDNA, with three technical replicates each.

Starting with 1 ng or more, we observed consistently high call rates (data not shown). In contrast, call rates dropped to an average of 85 percent for amplification products using 0.1 ng gDNA. Concordance rates between matching amplified and non-amplified DNA samples averaged 98.67 percent, 99.79 percent and 99.93 percent for samples in which 1 ng, 10 ng and 100 ng gDNA were used for WGA, respectively. Concordance levels dropped significantly to 84 percent for samples starting with 0.1 ng.

Overall, a large decrease in performance was observed for WGA DNA starting with 0.1 ng, and a small decrease was also observed for samples where WGA was performed on 1 ng gDNA. Therefore, a minimum of 10 ng was identified as the recommended input of gDNA for uncompromised genotyping array performance with the Mapping 50K Xba Array. For the SNP Arrays 5.0 and 6.0, 30 ng was identified as the recommended input of gDNA to the assay for uncompromised genotyping array performance (data not shown).

CLEANUP OF WGA DNA

Purified WGA DNA was compared with both non-purified WGA material and non-amplified gDNA on the Mapping 50K Xba Array. Using 10 ng gDNA for WGA and applying 250 ng amplified DNA through the assay, the call rates remained consistent with or without a cleanup step.

Table 2: Effect of WGA purification on SNP call rates and call concordance. WGA and reference samples were hybridized to Mapping 50K Xba Arrays. The SNP call rates were determined using GTYPE with a *p*-value threshold of 0.25.

Table 2A: SNP call rates determined from purified and non-purified WGA triplicates (and a single, non-amplified gDNA control) applied to Mapping 50K Xba Arrays.

Sample	gDNA input	WGA?	Purified?	SNP Call Rate
WGA 1	10 ng	yes	no	99.21%
WGA 2	10 ng	yes	no	98.38%
WGA 3	10 ng	yes	no	98.50%
WGA 1	10 ng	yes	yes	98.13%
WGA 2	10 ng	yes	yes	98.75%
WGA 3	10 ng	yes	yes	98.43%
Non-amplified Ref 103 gDNA	250 ng	no	no	98.19%

Table 2B: Call concordances determined by comparison of amplified versus non-amplified and purified versus non-purified gDNA applied to Mapping 50K Xba Arrays.

Sample 1	Sample 2	Call Concordance
WGA 1	WGA 2	99.96%
WGA 1	WGA 3	99.97%
WGA 1 purified	WGA 2 purified	99.91%
WGA 1 purified	WGA 3 purified	99.87%
WGA 1	WGA 1 purified	99.95%
WGA 2	WGA 2 purified	99.93%
WGA 3	WGA 3 purified	99.91%
Ref 103	WGA 1	99.93%
Ref 103	WGA 2	99.90%
Ref 103	WGA 3	99.90%
Ref 103	WGA 1 purified	99.87%
Ref 103	WGA 2 purified	99.93%
Ref 103	WGA 3 purified	99.89%

Table 3: Average call rate and concordance (to HapMap) for unpurified WGA gDNA samples applied to the SNP Arrays 5.0 and 6.0. N = 59 samples for the SNP Array 5.0 and 59 samples for the SNP Array 6.0.

Metric	SNP Array 5.0	SNP Array 6.0
Average Call Rate 30 ng	99.5% using BRLMP	99.5% using Birdseed-2
Average Concordance to HapMap	99.3%	99.7%

The average SNP call rate for the purified material was 98.44 percent, while the non-purified material showed an average call rate of 98.70 percent (Table 2A). Comparisons of amplified and non-amplified DNA from purified and non-purified samples revealed concordance measures of greater than 99.9 percent (Table 2B). Therefore, the purification of the WGA samples appears to be unnecessary for high genotyping efficiency or reliability on these arrays.

APPLICATION OF WGA DNA TO SNP ARRAYS 5.0 AND 6.0

Using 30 ng gDNA as input to WGA and without cleaning up the WGA product, 500 ng DNA of WGA and non-amplified samples were processed using the SNP geno-

typing assay and the resulting templates applied to the SNP Arrays 5.0 and 6.0. Performance of the WGA DNA exceeded specifications for the arrays, with an average call rate of 99.5 percent for both (Table 3). For comparison, the average expected call rate for standard high-quality, non-amplified DNA is greater than 99 percent for these arrays. The reliability of these genotype calls was demonstrated by the high concordance rates between WGA and non-amplified cell line DNA. Concordances calculated for the WGA material compared to HapMap data averaged 99.7 percent for the SNP Array 6.0 (Table 3).

CONCLUSION

The advancement of technologies to assess human genetic variation in a high-throughput, high-resolution format has enabled extensive analysis of the role of genetic changes in disease. However, it is often difficult to collect adequate amounts of DNA from scarce samples, and in many cases it is preferable not to deplete precious sources.

For these situations, we have outlined recommendations for amplifying small amounts of DNA to assess for SNP genotype. These recommendations include starting with a minimum of 10 ng of gDNA (depending on the final array to be used) for WGA and not cleaning up the amplified gDNA prior to use in the genotyping assay. For the genotyping assay, there are no changes to the amount of non-amplified or amplified gDNA used in the assay.

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