

Whole genome amplification of poor quality DNA rescues SNP microarray assays

Cheng Eng Ang, Chris Jones, Jo McBride, Martin Yuille

MRC geneservice, The Wellcome Trust Genome Campus, Hinxton Cambridge CB10 1SB

Abstract

DNA samples received at MRC geneservice for microarray SNP analysis vary in quality. We investigated whether whole genome amplification might be of value in rescuing poor quality DNAs. Using Multiple Displacement Amplification and the Affymetrix 10K GeneChip® Mapping array on such samples and controls, we measured call rates exceeding manufacturer's specifications and concordance of 98.9%. These findings suggest that MDA can rescue large-scale genotyping studies where only poor quality DNA is available and that MDA may increase laboratory efficiency.

Introduction

MRC geneservice provides access to DNA sample management and microarray services and associated technology platforms. We provide whole genome amplification service based on multiple displacement amplification (MDA)¹. We are also providers for Affymetrix microarray products including the GeneChip® 10K and 100K Mapping Assays.

DNA samples received from users for microarray SNP analysis vary in both their quantity and quality. Whole genome amplification provides a means of overcoming limitations of DNA quantity. However, we wished to investigate whether MDA might also be of value in rescuing poor quality DNA samples.

Materials and Methods

Samples DNA samples were obtained from MRC geneservice users and from an EBV-transformed B-cell line MZ0048 (European Cell Culture Collection, Porton Down).

DNase treatment 1µg DNA was treated with 0.04 units of DNaseI (Promega UK) at 37 °C for 10 minutes. The product was then purified using a QIAquick PCR Purification kit (Qiagen Inc., Valencia, CA) and examined by agarose gel electrophoresis.

Whole genome amplification Whole genome amplification was performed using φ29 DNA polymerase following manufacturer's instructions (MSI Inc, New Haven). This method is termed Multiple Displacement Amplification (MDA). The yield ranged from 700-900µg MDA-DNA.

Affymetrix GeneChip® Mapping 10K Assay Samples were prepared and hybridised to Affymetrix GeneChip® Mapping 10K arrays (containing 11,765 assays) following protocols supplied by the manufacturer (Affymetrix Inc Santa

Clara CA). These protocols include quality controls (QC) on the initial sample and on PCR product made after fragmentation and ligation. Spectrophotometric absorbance of 1.0µl aliquots of initial sample was estimated at 260nm, 230nm and 280nm using a NanoDrop spectrophotometer (Nanodrop Technologies, Wilmington NC). 250ng of DNA was digested with *Xba*I, ligated with *Xba*I adaptors and PCR amplified. The purified PCR product was labelled and hybridised to a chip. After washing, data was accrued using an Affymetrix GeneChip Scanner 3000 and processed using GDAS. Call rate is calculated as (interpretable SNPs / total SNPs x100) %. The manufacturer specifies an 85% call rate as satisfactory. Concordance is calculated after identifying the common set of interpretable SNPs called on both treated and untreated DNA and expressing these numbers as a ratio (%).

Results and Discussion

If a DNA sample has $A_{260}/_{280} < 1.6 > 2.3$ and/or $A_{260}/_{230} < 1.6 > 3$, then its quality is inadequate for the Affymetrix 10K GeneChip® since there is a significant risk of microarray giving a low call rate (<85% of SNP assays yielding interpretable results) and the possibility of inaccurate calling (discordance).

MRCg receives DNA samples from customers that on occasion fail this quality control step. Table 1 provides data on absorbance in nine such samples from different customers. Seven samples failed the $A_{260}/_{280}$ test and all the samples failed the $A_{260}/_{230}$ test. The customers permitted us to undertake MDA on their samples to discover whether this might yield material that did pass the QC step. The table indicates that in all nine samples, MDA yielded material of good quality with no detected variation in either the $A_{260}/_{280}$ or $A_{260}/_{230}$ ratios.

When these nine MDA-DNA samples were used in the GeneChip® assay, call rates exceeded manufacturer's specifications with call rates >90% (>10,350 assays) in all cases.

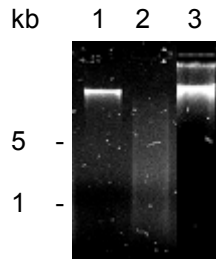
These results left open the possibility that some assays were discordant. To exclude this possibility, it is necessary to test a subject's DNA where both good and poor quality DNA is available.

To address this issue, we took DNA extracted from a cell line and treated an aliquot with DNase (Figure 1 lane 2). This degraded DNA was then amplified by MDA. Table 2 shows that the DNase-treated DNA failed the $A_{260}/_{230}$ QC step. It was therefore deemed to be poor quality. The other samples had satisfactory $A_{260}/_{280}$ and $A_{260}/_{230}$

ratios and yielded satisfactory call rates. Concordance of 98.9% was calculated for the DNA that had been degraded and then amplified.

This provides evidence that whole genome amplification by MDA can rescue poor quality

Figure 1: Electrophoresis of treated DNA samples



In each lane 5ul of DNA from a cell line was gel-fractionated. Lane 1: DNA; 2: DNA treated with DNase;

3: DNA treated with DNase and amplified by MDA.

DNA samples to give a high call rate with high concordance on the Affymetrix 10K GeneChip®. These findings suggest that in studies where only poor quality DNA is available, MDA can rescue the study by permitting accurate large-scale genotyping. They also suggest that by introducing an initial MDA step, data accrual can be accelerated without significant effect on data quality by circumventing potential repeat experiments on failed samples. Further studies will be undertaken to define more factors contributing to poor quality DNA that MDA can rescue.

Reference

1. Dean et al. Proc Natl Acad Sci (US) 2002;99:5621-5266.

Table 1: Effect of MDA on UV absorbance by DNA samples and resulting SNP call rate in GeneChip® assays

Sample	DNA		MDA-DNA		MDA-DNA call rate
	A260/280	A260/230	A260/280	A260/230	
1	1.4	0.8	1.7	2.0	95.65
2	1.4	0.74	1.7	2.0	97.31
3	0	0.33	1.7	2.0	95.72
4	0	0.44	1.7	2.0	91.77
5	0	0.38	1.7	2.0	91.48
6	0	0.33	1.7	2.0	92.82
7	3.8	0.47	1.7	2.0	90.81
8	1.8	0.63	1.7	2.0	96.11
9	2.1	0.71	1.7	2.0	93.17

The table gives results on UV absorbance of DNA samples before and after MDA and the SNP call rate in GeneChip® assays of DNA samples after MDA.

Table 2: Effect of MDA and DNase on SNP call rate and concordance

Sample	A260/280	A260/230	SNP call rate	Concordance
DNA	1.85	2.27	90	(100)
MDA on DNA	1.67	2.11	96.5	99.9
DNase-treated DNA	1.73	0	ND	ND
MDA on DNase-treated DNA	1.71	2.17	87.7	98.9

The table gives results for UV absorbance of cell line DNA samples, the SNP call rate (as %) after GeneChip® assay and concordance as % relative to untreated DNA. DNA samples were amplified by MDA or treated with DNase as indicated. ND: not done