Evaluation of genome coverage and fidelity of multiple displacement amplification from single cells by SNP array

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ABSTRACT: The scarce amount of DNA contained in a single cell is a limiting factor for clinical application of preimplantation genetic diagnosis mainly due to the risk of misdiagnosis caused by allele dropout and the difficulty in obtaining copy number variations in all 23 pairs of chromosomes. Multiple displacement amplification (MDA) has been reported to generate large quantity of products from small amount of templates. Here, we evaluated the fidelity of whole-genome amplification MDA from single or a few cells and determined the accuracy of chromosome copy number assessment on these MDA products using an Affymetrix 10K 2.0 SNP Mapping Array. An average coverage rate (86.2%) from single cells was obtained and the rates increased significantly when five or more cells were used as templates. Higher concordance for chromosome copy number from single cells could be achieved when the MDA amplified product was used as reference (93.1%) than when gDNA used as reference (82.8%). The present study indicates that satisfactory genome coverage can be obtained from single-cell MDA which may be used for studies where only a minute amount of genetic materials is available. Clinically, MDA coupled with SNP mapping array may provide a reliable and accurate method for chromosome copy number analysis and most likely for the detection of single-gene disorders as well.

Key words: chromosomal abnormality / genome coverage / multiple displacement amplification / PGD / SNP array

Introduction

Accurate analysis from a minute amount of DNA has been a challenge for geneticists. Several PCR-based protocols for whole-genome amplification (WGA) have been established and their applications evaluated, including primer extension preamplification (PEP) (Zhang et al., 1992; Xu et al., 1993) and degenerate oligonucleotide-primed PCR (DOP-PCR) (Telenius et al., 1992; Wilton et al., 2001). However, these protocols all have their limitations, including limited yield, strong biases and/or low genome coverage defined as gene representation (Cheung et al., 1996; Paunio et al., 1996; Kittler et al., 2002; Coskun et al., 2007). In the past few years, a non-PCR-based WGA called multiple displacement amplification (MDA) has been introduced (Dean et al., 2002). Taking advantage of the phi29 DNA polymerase, an enzyme with higher processivity and better proofreading activity compared with Taq DNA polymerase, MDA shows some unique advantages over PCR-based WGA, including better fidelity as it has: an error rate of less than 3 x 10^{-6} (Nelson et al., 2002), compared with 3 x 10^{-5} for Taq DNA polymerase; a higher average yield from a single cell varying from 1.7 to over 35 μg (Handyside et al., 2004; Jiang et al., 2005; Spits et al., 2006); larger amplified DNA fragments (>10 kb); and more uniform representation of sequences (Paez et al., 2004). For these reasons, MDA is now increasingly used in many studies including preimplantation genetic diagnosis (PGD) where molecular and cytogenetic diagnosis is based on the analysis of one cell only, in majority of cases, or a few cells from human embryos. Nevertheless, the scarce amount of DNA contained in a single cell as templates still poses a challenge for wide use of PGD.
because of a phenomenon called ‘allele dropout’ or ADO, defined as the loss of heterozygosity due to amplification failure of one of the two alleles. ADO rates of MDA products from single cells are reported to be from 0 to 60% (Handyside et al., 2004; Hellani et al., 2004; Spits et al., 2006; Ren et al., 2007; Renwick et al., 2007) and appear to be affected by multiple factors when PCR is used to amplify single-cell DNA (Pyamongkol et al., 2003). However, these studies are based on the analysis of small numbers of markers (up to 64 polymorphic microsatellite markers) and on a limited number of chromosomes (seven different chromosomes). A comprehensive profile of MDA genome coverage is not available.

Although different types of chromosomal abnormalities in all 23 pairs of chromosomes have been successfully detected by array-based comparative genomic hybridization (aCGH) using MDA products from single cells (Hellani et al., 2004; Le Caignec et al., 2006), several critical issues need to be addressed before it can be safely used for clinical PGD. First, the extent of over- and/or under-representations in the final MDA products is unclear and the accuracy of aCGH from MDA products requires further evaluation (Paez et al., 2004; Le Caignec et al., 2006). Second, aCGH has yet to be tested for detecting copy number aberrations and single-gene disorders simultaneously. And finally, the current aCGH protocol is a time-consuming process that does not seem to fit easily into all clinical PGD schedule, particularly if specimens are required to be shipped to a reference laboratory, though a promising report on this has been published recently (Hellani et al., 2008). Looking for alternative platforms and potentials for the detection of both chromosome copy number abnormalities and single-gene disorders, the present study focuses on the analysis of fidelity of MDA in terms of whole-genome analysis of ADO and the genome coverage of MDA from single cells. This study, however, does not focus on the detection of any single-gene disorders though potentially feasible. Initially used for linkage analysis (Middleton et al., 2004), an SNP mapping array can not only provide a reliable method to evaluate DNA products on genotype, but is also a potential tool for the detection of chromosome copy number aberrations with high accuracy and reproducibility. Our objective is, therefore, to evaluate single-cell DNA amplified by MDA using SNP array, which may overcome above-mentioned limitations of aCGH. In this study, the Affymetrix 10 K 2.0 SNP mapping array was chosen because this platform contained over 10,000 SNPs and was reported to perform accurately for the analysis of small quantities of DNA (Paez et al., 2004; Tzvetkov et al., 2005; Corneveaux et al., 2007).

Materials and Methods

MDA sample preparation

Two cell lines, a trisomy 18 (Tri-18; GM02 732, 47, XY, +18) and a chromosome 4 segment deletion [sDel-4; GM00 343, 46,XY,4(del)(qter->p14)] from Cornell Cell Repositories/NIGMS (Camden, NJ), were used for the study. Cells were collected as described before (Spits et al., 2006) with minor modifications. Briefly, prior to cell collection, cell cultures were treated with 0.25% trypsin–EDTA and twice washed in PBS. Genomic DNA was extracted from cells using QIAamp DNA Blood Mini Kit (QIAGEN Sciences, MD). Different number of cells (1, 2, 5 and 10) were picked up under a dissection microscope by a glass pipette and placed into PCR tubes containing 5 μl alkaline lysis buffer (200 mM KOH, 50 mM dithiothreitol) and the tubes were then stored at −80°C before use. Aliquots of 5 μl lysis buffer without cells were used as negative controls. Lysis was performed at 65°C for 10 min followed by addition of 5 μl neutralization buffer (400 mM Tricine). All necessary steps were taken to avoid contamination during the whole procedure.

MDA and experimental groups

Whole-genome amplification by phi29 polymerase-based MDA was performed using REPLI-g Midi Kit (QIAGEN Sciences, MD) with a final volume of 50 μl. MDA reactions were carried out at 30°C for 8 h and terminated by incubation at 65°C for 10 min. The products were purified using QIAamp DNA Micro Kit (QIAGEN Sciences, MD), quantified using ND-1000 spectrophotometer (NanoDrop Technologies, Denmark) and then stored at −20°C before next step. Considering the amount of specimens potentially available from clinical PGD, a total of seven experimental groups, using cells or extracted DNA from Tri-18 cell line, were established for genotype analysis. These included MDA products from: (1) a negative control with no templates (Neg); (2) a single cell (1C); (3) pooled from two separate single-cell MDA reactions (1 + 1C); (4) two cells (2C); (5) five cells (5C); (6) ten cells (10C); and (7) unamplified genomic DNA (gDNA). In a separate experiment, single cells were obtained from the sDel-4 cell line and MDA was performed in the same manner as in group 2 (1C-sDel-4). Three replicates were included in each group; except group 2 (1C) and group 3 (1 + 1C), both of which had six replicates, respectively. Detailed numbers of array used for each group are listed in Table I. For aneuploidy detection, a mean and standard deviation (SD) of all log2 signal intensity ratios of each array (10 M smooth ing treatment by CNAT4.0) was calculated, and the array-specific threshold to define euploidy was determined as the mean plus or minus three times of the SD. The average log2 ratio of each chromosome on a certain array was compared with this threshold and ratios exceeding the threshold indicated aneuploidy or euploidy otherwise.

The MDA products were treated as previously described (Tzvetkov et al., 2005). Briefly, 250 ng of MDA products or unamplified genomic DNA was digested with Xbal, ligated to a universal adaptor and amplified using primers complementary to the adaptor sequence. After purification, the products, which were less than 1 kb in size, were digested to the sizes of about 50 bp with DNase I, end-labeled with biotin, and hybridized to the mapping array for 16 h at 48°C. The arrays were then washed and stained with R-Phycoerythrin Streptavidin using the Fluidics Station 450 and scanned using the GeneChip Scanner 3000 from Affymetrix.

Genotyping and copy number analysis

The 10 K 2.0 SNP mapping arrays, containing over 10,000 SNPs, and assay kits were purchased from Affymetrix along with 45 normal reference DNA data sets from blood samples (Affymetrix, Santa Clara, CA). The probes on this array were 25-mer each and the mean intermarker distance was 210 kb on 22 autosomes and the X chromosome. Data were analyzed using GeneChip® Genotyping Analysis Software (GCOS 1.2), GeneChip® Genotyping Analysis Software (GTYPE 4.1) and Copy Number Analysis Tool (CNAT 4.0) from Affymetrix. The GC content of each SNP segment was obtained from NetAffx™ Analysis Center of Affymetrix. A window of 250,000 bases to each side of that SNP was used and the fraction of bases that were G and C was calculated for each SNP in a chromosome. For coverage and fidelity analysis, the call rate on the array was defined as the SNP that could be correctly read and the call rate was used as the index of genome coverage in the study.

An algorithm called relative allele signal (RAS) was used for calculation of genotye from the signals measured (Kennedy et al., 2003). Briefly, the genotye of a locus is determined by the ratio of allele A signal to the sum of signals of A and B [A/(A + B)], and RAS should be 0, 0.5 and 1 for BB, AB and AA, respectively. However, artifacts such as ADO, PA
Results

Yield and genome coverage of MDA

Following the MDA, the yield of double-stranded DNA in each group was 19.3 ± 10.9, 12.1 ± 6.0 and 20.9 ± 4.1 for single cells, respectively. The average genome coverage following whole-genome amplification was estimated based on the GC content of each sample. The genome coverage of each experimental group (IC, 2C, 5C and 10C) was compared with the control group (1C) (Fig. 1a and b). No significant difference was observed between any of the experimental groups (Fig. 2). The mean number of uncalled SNPs was 35 per sample, ranging from 375 to 1420, the number of consistent uncalled SNPs was about 10% (from 35 to 185) (Fig. 1a and b). To further investigate whether GC content influenced the occurrence of these uncalled SNPs, the genome coverage was calculated for each group. The number of consistent uncalled SNPs was obtained for each sample. The number of consistent uncalled SNPs was then compared between each group and the control group (1C) (Fig. 2). The number of consistent uncalled SNPs was significantly lower in the experimental groups than in the control group (1C) (Fig. 2).

Table I An overview of each array on the expected aneuploidy identification

| Group | 1C | 2C | 5C | 10C |
|-------|----|----|----|-----|
| Array no. | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| G Mean log | 0.0006 | 0.0012 | -0.0002 | -0.0001 | -0.0001 | -0.0001 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 | -0.0002 |
| SD | 0.21 | 0.18 | 0.18 | 0.17 | 0.21 | 0.21 | 0.19 | 0.19 | 0.19 | 0.19 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Average ratio of expected abnormal chromosome | 0.59 | 0.34 | 0.25 | 0.27 | 0.46 | 0.23 | 0.24 | 0.23 | 0.23 | 0.33 | 0.23 | 0.32 | 0.37 | 0.18 | 0.26 | 0.32 | 0.28 | 0.26 | 0.29 |

G: gDNA data set as reference. M: single-cell MDA–DNA as reference. Mean and SD: mean and standard deviation of log2 intensity ratio of all probes on the array (results from the Affymetrix CNAT4.0 with 10 M smoothing treatment). When single-cell MDA product was used as reference, the results from the abnormal chromosomes were excluded to avoid observing false trisomy. The threshold to define euploidy or aneuploidy was determined as the overall average ratio plus or minus three times of the standard deviation of the average log2 ratio. The average ratios of the expected abnormal chromosome (trisomy 18) were compared with the threshold. Values exceeding the threshold would indicate the trisomy and are shown in bold italics. Otherwise, regular values mean that the expected trisomy could not be identified from the results.
SNPs, we compared the GC content of the segments from which these consistent SNPs stood in each group to that of the overall SNPs on the array. Only those consistent uncalled SNPs in a single-cell group (group 1C) showed a significantly higher GC content (42.15% versus 39.86%, \( P < 0.05 \)), but no correlation of GC content related to uncalled SNPs was seen in other groups (\( P > 0.05 \)).

Fidelity of MDA for SNP analysis
Allele dropout was assessed in each group. Only those SNPs giving 'calls' in both unamplified and replicate pairs were included for further analysis. Three SNP genotypes among MDA amplified and unamplified groups were defined in this study: loss of heterozygosity (LOH, AB→AA or BB), gain of heterozygosity (GOH, AA or BB→AB) and change of homozygosity (COH, AA→BB or BB→AA). All these changes (LOH, GOH and COH) decreased as the cell number increased. The most dramatic difference was observed in the LOH type which decreased significantly with the increasing number of cells in the MDA reaction (\( P < 0.05 \), respectively), whereas GOH was observed in much smaller number of SNPs and only four SNPs were COH in four replicates (three in group 1C and one in group 2C) (Fig. 2a). Similar to LOH, the rates of ADO and PA decreased dramatically with an increasing number of starting cells (Fig. 2b). ADO rates varied greatly in Group 1C (19.46 ± 9.13%, with a range from 4.71 to 29.5%) and only a very small number of consistent ADO and PA SNPs existed (from 0 to 2) in each group. Further comparison between the GC content of the segments for which these consistent SNPs were located and those of the overall SNPs on the array showed no correlation (\( P > 0.05 \)).
Detection of chromosomal abnormalities from different number of cells

A total of 24 arrays were used for the chromosome copy number detection (Table I). Concordance in chromosome copy number analysis was significantly higher when data from the single-cell MDA product was used as reference than that when the gDNA data were used as reference in group 1C and 1 + 1C (P < 0.05), but not in other groups (P = 0.05) (Fig. 3). Although both the trisomy 18 and chromosome 4 segment deletion could be detected by this 10K platform from a single cell, a few specific chromosomes showed a higher tendency of PA than others, including chromosome 16, 17, 19 and 22, when gDNA data were used as reference (Fig. 4a). PA disappeared, however, when data from the single-cell MDA product were used as reference (Fig. 4b).

Discussion

MDA genome coverage

Compared with other PCR-based WGA methods, MDA is reported to be a robust and less biased procedure for genome coverage (Nelson et al., 2002; Handyside et al., 2004; Paez et al., 2004; Jiang...
et al., 2005; Spits et al., 2006). No accurate and comprehensive assessment of MDA products from single or a small number of cells is yet available on its fidelity, particularly, by high-resolution genetic analysis. Though a previous study has shown that MDA could generate a large amount of DNA product (Jiang et al., 2005), the nature of the products has not been tested. Recently using SNP arrays a profile of MDA bias in single cells was reported (Iwamoto et al., 2007), however, only one SNP for each chromosome was chosen to define the allele threshold of amplification in the study. Moreover, the knowledge of unspecific products of MDA on the genotyping accuracy (Handyside et al., 2004) is still limited. The present study confirmed the previous finding that a large quantity of DNA, close to 20 μg, can be obtained from single cells after 8 h of MDA. The quantity of DNA did not increase as more cells (up to 10) were included as templates. When high-density SNP arrays were used to assess the performance of MDA from single cells and a small number of cells, we found that the call rate, as an index of genome coverage, was very low in the negative control group (5.3%), suggesting that these were indeed non-specific products and would have a limited impact on SNP signals. The call rate showed no differences between MDA amplification using 1C, 1 + 1C or 2C (86.2, 85.9 and 86.7%, respectively, P > 0.05). The genome coverage from a single cell in the present study is lower than that reported from previous studies, which ranged from 92 to 95% (Handyside et al., 2004; Hellani et al., 2004; Jiang et al., 2005; Renwick et al., 2007). The difference could be due to the fact that a limited number of markers (up to 64 SNPs on seven chromosomes) were involved in estimating the genome coverage in those studies whereas more than 10 000 SNPs were assessed in the present study. Compared with the call rate of the one-cell group, significant differences were observed when 5 (90.4%) or 10 (96.3%) cells were used (P = 0.03 and 0.02). With the benefit from the progression of blastocyst cultivation in recent years, more cells can be removed from an embryo (blastocyst biopsy). Our study showed that when 10 cells were used as the template, the call rate could be similar to that of unamplified DNA, indicating that a much more reliable result could be achieved by blastocyst biopsy.

In our study, only a small number of SNPs (n = 35) were not called consistently in the one-cell group (Fig. 1b), suggesting a random and not locus-specific failure of amplification after MDA reaction from single cells. Further analysis showed that the genomic regions where these consistent uncalled SNPs located had a higher GC content than the overall level through the array. This may be due to the higher tendency for these GC rich regions to form secondary structures, which in turn may hamper the DNA synthesis from the template during MDA. Although such a correlation could not be observed in other groups (group 2C, 5C and 10C), the small sample numbers in these groups (n = 3 for each) might contribute to the result. Further research with more samples is warranted.

**SNP array for MDA fidelity**

If MDA is applied to PGD or other critical genetic analysis, the extent of ADO must be evaluated. Handyside et al. (2004) reported an ADO
rate of 31% on the analysis of 20 STRs with single-cell MDA. A similar study suggested the ADO rate to be 10.25% for the β-globin gene and 5% for a total 16 STRs (Hellani et al., 2004). The variable ADO rates observed may be due to the limited number of loci involved. A recent study reported an ADO rate of 38.9% for MDA in single cells using an SNP array (Iwamoto et al., 2007). To define whether or not the alleles A and B were amplified, 23 SNPs (one from each autosome and X chromosomes) were genotyped using a Taqman assay to set up the threshold. Again, only one SNP was chosen for each chromosome. The present study is the first in which ADO from single-cell MDA products is estimated throughout the whole genome and thus represents a more comprehensive assessment of ADO. In our study, a much higher rate of LOH than that of GOH and COH was observed in each group (Fig. 2a), suggesting that MDA rarely introduces new alleles. Nevertheless, the possibilities that some calls in the experimental groups may be due to the non-specific products cannot be ruled out since the negative group also showed 5.3% call signals.

The ADO rates varied greatly from 4.71 to 29.5% among group the one-cell group, which may be due to several reasons. Because only one copy of template was involved, the state of the DNA might have a great impact on the final result. For example, the ADO rate of replicate no. 3 in 1C group was much higher than those of other replicates in the same group but close to the results of 2C group. It is possible that the status of cell cycle may have contributed to the variation. For example, if a cell that had just finished the DNA replication but not yet divided was picked up so that actually two copies of DNA were present as templates. Furthermore, as fibroblast cells lines were cultured for number of passages, apoptosis or DNA fragmentation might have contributed to this variability. The overall ADO and PA rates decreased dramatically from 17.9 and 18.2% with a single cell to 0.1 and 0.05% with 10 cells (Fig. 2b), in agreement with a previous report (Handyside et al., 2004). It is important to emphasize that the present study obtained a more comprehensive profile for the impact of cell number on the genotyping compared with a previous study (Handyside et al., 2004) in which the fidelity of MDA from small numbers of cells was evaluated based on one locus as index only.

Interestingly, unlike the results of coverage analysis, in which the pooled DNA from two single-cell (1 + 1C) MDA reactions showed a similar performance as that from two cells (Fig. 1b), the LOH rate of the 2C group was much less than that of 1 + 1C (Fig. 2a). This might indicate that unspecific products were able to hybridize to the SNP array, but they were not able to have the same impact on signal capture or program analysis as the LOH rate is higher. In other words, slightly higher LOH from the 1C group may have limited impact on accuracy. The implication for clinical PGD is that biopsy of two cells may not provide a huge advantage. Separate MDA reactions should be performed when two single blastomeres are available, whereas pooled two cells would be recommended for MDA if LOH was to be minimized.

Finally, when the SNP array is used for the detection of the fidelity of any amplification methods, we should not regard LOH as ADO because some allelic PA will be easily mistaken as LOH by the RAS algorithm. As our method has successfully been used to distinguish the ADO from LOH in this 10K SNP mapping array, it can likely be extended to the analysis of WGA products using other SNP mapping array platforms.

**SNP array for copy number analysis**

As an alternative to FISH, array-CGH has been successfully used for chromosomal imbalances detection in whole-genome amplified DNA
products from single cells (Le Caignec et al., 2006; Fiegler et al., 2007). It has been known, and was demonstrated in the present study, that MDA will introduce background noise in the absence of template DNA. Whether these non-specifically amplified DNA will array randomly or specifically hybridize to the array remains unclear (Paez et al., 2004; Le Caignec et al., 2006). Controversy still exists regarding what sources of reference should be used. Paez et al. (2004) suggested that an amplified product using the same method should be used as reference because the errors caused by MDA seemed to be systematic. Le Caignec et al. (2006), however, held a different view based on their findings on a BAC array to detect the chromosomal imbalance from single cells. After comparing the copy number concordance between the results using these two different references, we found that in each group the mean copy number concordance was much higher when the data from a single-cell MDA product were used as reference (93.1% to 99.9%) than that from when normal gDNA was used as a reference (82.8–89.8%) (Fig. 3). A statistical difference was observed in the 1C and 1 + 1C groups (P < 0.05) but not in others (P = 0.05), most likely due to the small number of samples involved in the present study.

Two explanations could be provided for this difference. First, unlike what we found in the genotype analysis, most of the copy number variations caused by MDA seem to be not randomly but systematically distributed. From the copy number profiling (Fig. 4a and b), it appeared that the PA was consistent in some specific chromosomes (i.e. chromosome 16, 17, 19 and 22). This supports previous findings that these chromosomes had poorer representation compared with others (Paez et al., 2004). Although we could not find any correlation between GC content and PA on specific chromosomes as suggested by others (Teo et al., 2008), the different gene density may explain this phenomenon. We speculate that there are two types of chromatin locating inside the interphase nuclei: a highly condensed form called heterochromatin and a much less condensed form called euchromatin. Most DNA that is folded into heterochromatin does not contain genes (Alberts, 2002). Those chromosomes with higher gene density (Renwick et al., 2007), such as chromosome 16, 17, 19 and 22, are believed to have a higher proportion of euchromatin, whose loose structure would allow more efficient amplification which might produce some ‘artifacts’. Amplified controls from a similar low amount of starting DNA may help to level up these errors and increase the concordance rate. Second, as other oligonucleotide-CGH platforms, an Affymetrix SNP mapping array cannot make a definite call for a loss or gain for a specific SNP, signals of a few adjacent oligonucleotides are required to be analyzed as a whole for a reliable call (Ylstra et al., 2006). For this purpose, the Affymetrix copy number analysis tool integrates the loci within a certain smoothing size as one by the hidden Markov model, and one identical copy number was given to all these loci in the final result to reduce random errors. All these may contribute to the high copy number concordance when MDA amplified products were used as reference. More rigorous comparison between the two types of references is required before it can be used in clinical applications.

Here, in combination with MDA and an Affymetrix 10K 2.0 SNP mapping array, we were able to successfully detect chromosomal abnormalities such as trisomy and monosity. Though this relatively early version of an array can produce reproducible results, it has some obvious disadvantages, such as incomplete genome coverage (as it does not include Y chromosomes). SNP arrays with higher density such as a 500K array may overcome this limitation. It is also possible when an array with a much higher density of probes is used, the copy number variation (CNV) along the genome may further complicate the analysis. The optimized density of probes of array for PGD aneuploidy detection needs to be further studied and some analytic software must be improved.

Another disadvantage of this as a CGH system is the period required for MDA and hybridization, which may not be a problem for those facilities with an IVF laboratory and PGD laboratory, but could limit the clinical application for transport PGD. One strategy to overcome this problem is to cryopreserve the embryos after biopsy, and transfer the embryos in a subsequent cycle, as has been done with metaphase CGH (Wilton et al., 2003). The main drawback is that the embryo implantation potential would be reduced by the freezing and thawing procedure. An alternative approach is the investigation of the first polar body, which will provide sufficient time to perform transport PGD using aCGH system, but the presence of meiosis II or a paternally or post-zygotically derived error increases the misdiagnosis risk significantly. After all, further research on how to shorten the time required for MDA and hybridization may provide the best hope for resolving the problem.

In conclusion, the present study indicates that satisfactory genome coverage can be obtained from single-cell MDA. The assessment of the fidelity of MDA for single cells or a small number of cells and the comprehensive profile using the SNP mapping array revealed a reasonable accurate representation of MDA from a minute amount of DNA template. Furthermore, a high concordance of copy number estimation could be obtained when the MDA amplified product was used as reference. With a high-density SNP array, MDA products from single cells can accurately and reliably be used for chromosome copy number analysis and most likely for genotyping as well.

Acknowledgement

We are grateful to Dr. Jenny Z. Xiang, Director of Microarray Core Facility, Weill Cornell Medical College of Cornell University for processing the SNP array.

Funding

This study was partly supported by Guangdong Natural Science Fund (No. 06021276) and Guangdong Research Fund for Medical Science and Technology (No. B2006027).

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