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REVIEW ARTICLE

Multiple Displacement Amplification To Create a Long-Lasting Source of DNA for Genetic Studies

Lovisa Lovmar* and Ann-Christine Syvänen

Molecular Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden

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In many situations there may not be sufficient DNA collected from patient or population cohorts to meet the requirements of genome-wide analysis of SNPs, genomic copy number polymorphisms, or acquired copy number alternations. When the amount of available DNA for genotype analysis is limited, high performance whole-genome amplification (WGA) represents a new development in genetic analysis. It is especially useful for analysis of DNA extracted from stored histology slides, tissue samples, buccal swabs, or blood stains collected on filter paper. The multiple displacement amplification (MDA) method, which relies on isothermal amplification using the DNA polymerase of the bacteriophage Φ29, is a recently developed technique for high performance WGA. This review addresses new trends in the technical performance of MDA and its applications to genetic analyses. The main challenge of WGA methods is to obtain balanced and faithful replication of all chromosomal regions without the loss of or preferential amplification of any genomic loci or allele. In multiple comparisons to other WGA methods, MDA appears to be most reliable for genotyping, with the most favorable call rates, best genomic coverage, and lowest amplification bias. Hum Mutat 27(7), 603–614, 2006.

KEY WORDS: multiple displacement amplification; MDA; whole genome amplification; WGA; Φ29 DNA polymerase; genotyping; comparative genomic hybridization

INTRODUCTION

Due to the combination of the annotation of the human genome and recent advances in technology, studies to identify disease-related genetic variants on a genome-wide scale have become possible [Altshuler et al., 2005; Pinkel and Albertson, 2005a; Syvänen, 2005]. Since a prerequisite for genome-wide studies is that sufficient amounts of genomic or tumor DNA is available for study, efforts have previously focused on collecting large amounts of biological specimens. Because of the substantial effort devoted to the collection of DNA samples from well-characterized patient- or population-based cohorts for genetic association studies, sample sets should serve as a long-lasting resource for current and future genetic studies. Although many of the currently used genotyping methods are based on PCR amplification (for a review see [Syvänen, 2001]), the amount of DNA may be insufficient for SNP genotyping on a very large scale using widely available technologies for medium-scale genotyping. Some of the new systems for genome-wide SNP genotyping use PCR for signal amplification instead of target amplification [Fan et al., 2003; Hardenbol et al., 2005; Oliphant et al., 2002], and for these assays the availability of large amounts of genomic DNA of good quality is crucial for successful genotyping. Ample amounts of genomic DNA are required for quantitative microarray-based methods for analysis of genomic copy number polymorphisms or acquired copy number alternations in tumor samples [Pinkel and Albertson, 2005a; Sharp et al., 2005]. Moreover, the amount of DNA is often limiting in studies in which the only available source of DNA is stored tumor or other tissue samples, buccal swabs, or blood stains collected on filter paper. For some genetic analyses there is a need to increase the amount of genomic DNA available; for example in forensic analysis or in preimplantation diagnosis of single cells, in which a sufficient DNA amount is essential to enable reanalysis in the case of ambiguous results.

A traditional approach to create an infinite DNA source is the immortalization of peripheral lymphocytes by transformation with Epstein-Barr virus. However, this technique is labor intensive and expensive to apply on a large scale. Moreover, it is not applicable to already existing DNA samples. A technically more feasible approach for increasing the amount of DNA is to faithfully amplify the genome using a whole-genome amplification (WGA) method. The main challenge of WGA methods is to obtain balanced and faithful replication of all chromosomal regions without the loss of genomic regions or preferential amplification of genomic loci or alleles.

PRINCIPLES OF WHOLE-GENOME AMPLIFICATION (WGA) METHODS

An early approach for amplifying large regions of a genome was to use primers directed at repeated interspersed sequences, such as the human Alu-repeats [Nelson et al., 1989], and later also the

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*Correspondence to: Lovisa Lovmar, Molecular Medicine, Department of Medical Sciences, Entrance 70, 3rd Floor, Research Department 2, Uppsala University Hospital, SE-75185 Uppsala, Sweden. E-mail: lovisa.lovmar@medsci.uu.se

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mouse B1-repeats [Himmelbauer et al., 2000], and perform a PCR amplification with Taq polymerase. In the linker-adaptor based amplification method, a representation of a part of the genome is generated by fragmentation using restriction enzyme cleavage and ligation of adaptors used as binding sites for universal PCR primers [Klein et al., 1999; Lucito et al., 1998; Matsuzaki et al., 2004; Vos et al., 1995]. An alternative strategy was to randomly shear the genome instead of using restriction digestion [Tanabe et al., 2003]. The OmniPlex technology (Sigma Aldrich; www.sigmaaldrich.com) relies on nick translation to produce a library of DNA fragments of amplifiable size, combined with adaptor ligation to allow PCR amplification with universal primers [Kamberov et al., 2002].

Another subgroup of amplification strategies apply random or partly degenerate primers and cyclic amplification. These techniques have been used for single-cell amplification for preimplantation genetic diagnosis [Jiao et al., 2003; Kristjansson et al., 1994; Paunio et al., 1996; Sermon et al., 1996; Wells and Delhanty, 2000]. The primer extension preamplification (PEP) technique applies random femtomer primers [Zhang et al., 1992] and has been further refined by using a high-fidelity PCR system and slightly modified thermocycling conditions [Dietmaier et al., 1999]. The improved PEP protocol was found to dramatically increase SNP genotyping performance using buccal swab samples [Zheng et al., 2001]. The degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) [Telenius et al., 1992] and its further development aimed at long products from low DNA quantities [Kittler et al., 2002] are similar to PEP, but use partially degenerate primers. A more "brute force" approach employed by Perlegen Sciences (www.perlegen.com) relies on long-range PCR reactions; in this system roughly 300,000 long-range PCRs are generated prior to genome-wide genotyping on microarrays and the resulting genomic coverage of this process is approximately 92% [Hinds et al., 2005].

The multiple displacement amplification (MDA) method for WGA takes advantage of isothermal amplification using the Φ29 bacteriophage DNA polymerase. MDA was first described in 2002 [Dean et al., 2002]. As can be seen in the diagram in Figure 1, the number of publications on WGA methods has increased substantially during the past 3 years (Fig. 1). A large proportion of the publications on WGA methods during 2003–2005 evaluated the fidelity and feasibility of MDA for amplifying small amounts of scarce DNA templates in a variety of applications and by using different techniques for genetic analysis. Since MDA appears to represent a major breakthrough in the application of WGA methods, this review will focus on studies describing the technical performance of MDA and its applications to a variety of genetic analyses.

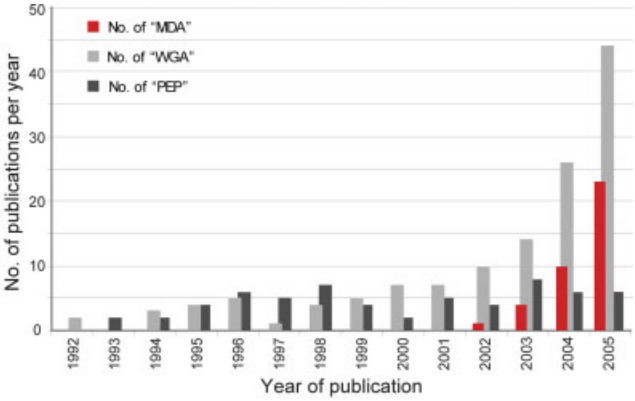

FIGURE 1. **Number of publications per year on whole genome amplification over the past 14 years. The statistics are based on a crude search of NCBI PubMed (www.ncbi.nlm.nih.gov/entrez) in December 2005 with the search criteria "multiple displacement amplification" ("MDA"), "whole genome amplification" ("WGA"), or "primer extension preamplification" ("PEP") and year of publication. A marked increase in the number of publications is seen after 2002 when the original study on MDA by Dean et al. [2002] was published. During the same time the number of publications matching the search criteria "primer extension preamplification" has remained stable. A search like this is by necessity incomplete because it depends on the phrasing used in each original article; hence some important publications may have been omitted or only included in one of the resulting search groups. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].**

MULTIPLE DISPLACEMENT AMPLIFICATION (MDA)

The MDA procedure relies on a high degree of processivity and fidelity using the strand displacement activity of the Φ29 DNA polymerase [Blanco et al., 1989; Esteban et al., 1993; Paez et al., 2004]. The bacteriophage Φ29 from *Bacillus subtilis* contains a single double-stranded 19,285-bp-long DNA genome [Vlcek and Paces, 1986]. Gene 2 of the Φ29 phage encodes the DNA polymerase that harbors multiple enzymatic activities required for replication of the genome [Blanco and Salas, 1996]. The error rate of Φ29 DNA polymerase has been estimated to be less than 3×10^{-6} [Esteban et al., 1993; Nelson et al., 2002] in contrast to 3×10^{-5} for *Taq* DNA polymerase, or 9×10^{-6} for *Taq* polymerase in combination with the *Pwo* polymerase used in high fidelity PCR systems.

In 1998, Lizardi et al. [1998] presented an isothermal procedure for the rolling circle amplification (RCA) of circular DNA templates using the Φ29 bacteriophage DNA polymerase. RCA does not, in contrast to PCR, require thermal cycling or a thermostable polymerase. In these early studies, only short circular molecules were amplified with RCA using specific primers. Lizardi et al. [1998] introduced RCA for signal amplification, which consequently has been applied in other assays [Alsmadi et al., 2003; Baner et al., 1998; Chen et al., 2005]. By using random hexamer primers with 3′-thiophosphate-modified ends to protect the primers from degradation by the proofreading 3′-5′ exonuclease activity of the Φ29 DNA polymerase, a hyperbranched amplification, denoted "multiple-primed RCA" can be achieved. This technical improvement increased the yield of RCA approximately 40-fold compared to standard, nonmodified, random hexamers when applied to a double-stranded circular M13 DNA template; it has also made it possible to use larger circular templates [Dean et al., 2001]. The amplification of a circular DNA molecule is useful for producing template for sequencing of vector constructs on a large scale and of even whole bacterial genomes, perhaps up to 5 Mb in size [Detter et al., 2002]. Multiple-primed RCA on BAC clones has also been applied to produce whole-genome microarrays for comparative genomic hybridization [Smirnov et al., 2004].

Replication of linear templates is also possible with Φ29 DNA polymerase [Dean et al., 2002]. In the MDA reaction, random hexamers anneal to multiple sites along the target molecule and serve as initiation sites for the Φ29 DNA polymerase-mediated DNA replication. As the replication proceeds along the template molecule, it reaches the initiation site for other replication events that are processed in parallel. These DNA strands are displaced, which allows DNA replication to continue. The displaced DNA strands then serve as new template molecules for random hexamers for initiation of subsequent DNA replication events

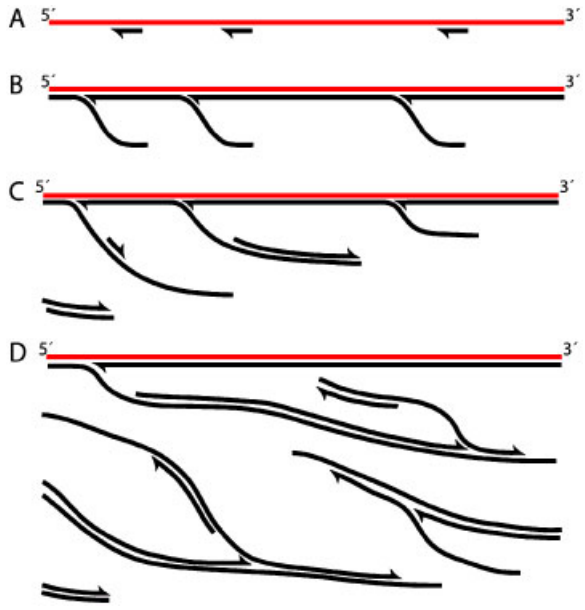

FIGURE 2. **Principle of multiple displacement amplification (MDA). The random hexamers anneal to the single stranded target molecule (A). As the DNA polymerase elongates the primer, the upstream DNA strands are displaced (B). The displaced DNA strands can then serve as templates for new priming events, which results in primer elongation in the opposite direction (C). The MDA reaction continues, and new DNA strands are displaced to produce new templates and a hyperbranched structure (D), generating an abundance of copies of the original DNA molecule. Scheme adapted from Lage et al. [2003]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]**

catalyzed by the Φ29 DNA polymerase. This mechanism results in a "hyperbranched" amplification of the target molecules during the process and enables amplification of large genomes (Fig. 2).

The MDA process generates products that are on average >10 kb in size and hence are well suited for further analysis, as initially shown by Dean et al. [2002]; who presented data for restriction site analysis and ligation-based SNP genotyping, quantitative real-time PCR analysis, and comparative genomic hybridization to metaphase chromosomes. PCR fragments of 10 and 12 kb in size, with GC-contents up to 80%, have been successfully amplified from MDA product, but with a lower PCR amplification efficiency for the 12 kb fragments. The MDA process appears to have an advantage over PCR-based WGA procedures in that sequence-dependent differences in amplification efficiency between regions due to GC-content are less significant [Yan et al., 2004].

The yield of a MDA reaction is less dependent on the amount of input DNA, but because the reaction is self-limited, the yield will depend on the reaction conditions and amount of reagents and hence on the reaction volume [Dean et al., 2002]. Consequently, varying DNA concentrations in the initial sample will plateau during MDA, which is a potential benefit for MDA in large-scale genotyping applications because it unifies and increases the DNA concentrations of the samples. The Infinium assay (Illumina; www.illumina.com) for genome-wide genotyping of SNPs utilizes MDA as the first step of the genotyping procedure [Gunderson et al., 2005; Steemers et al., 2006]. Also, when a pooling strategy is used to decrease the genotyping effort, MDA may be used to equalize the concentrations of the samples prior to genotyping but detailed assessment of concentration of each sample is required prior to creating the pools [Zhao et al., 2005]. MDA can be performed directly on crude samples, such as Guthrie cards, and eliminates the need for an initial DNA purification step [Hosono et al., 2003]. The reports on MDA yields vary between studies, but typically 3–6 μg of DNA is produced per 10 μl of MDA reaction volume [Cardoso et al., 2004; Dean et al., 2002; Hellani et al., 2004; Hosono et al., 2003; Rook et al., 2004].

The performance of MDA is dependent upon the quality of the input DNA [Lage et al., 2003]. In this regard, the quality of the DNA yield from degraded DNA templates is poor and often not suitable for genotype analyses. A degraded DNA template has fewer primer binding sites per DNA molecule for initiation of replication, and will thus undergo fewer hyperbranching events. In this case, the high processivity of the MDA reaction will not be utilized fully, which lowers the yield. This effect, which has been observed experimentally, is in concordance with predictions by mathematical modeling [Lage et al., 2003]. By combining isothermal Φ29 DNA polymerase-mediated amplification with restriction enzyme cleavage and ligation, a solution to the problematic amplification of degraded samples, such as for example formalin-fixed tissue, has been suggested [Wang et al., 2004b]. In the restriction and circularization-aided rolling circle amplification (RCA-RCA) method, the DNA is first cleaved by a restriction enzyme that optimally cuts each DNA fragment at least twice. Treatment with a ligase results in circularization of the fragmented DNA, and with subsequent treatment with an exonuclease, uncircularized material is removed. The resulting circles are denatured, followed by hyperbranched rolling circle amplification mediated by the Φ29 DNA polymerase. The RCA-RCA strategy was compared to MDA, and it was found to be equally efficient for high molecular mass DNA and markedly superior for DNA extracted from formalin-fixed tissue [Wang et al., 2004b]. The RCA-RCA method was evaluated using quantitative real-time PCR, array–comparative genomic hybridization (array-CGH), and analysis of microsatellite instability. Interestingly cDNA was successfully amplified; however, in this case a drawback is that the ends of the cDNAs are lost [Wang et al., 2004b]. The RCA-RCA method requires further evaluation prior to use on a larger scale, but it appears promising, especially for fragmented DNA. The OmniPlex technology has been shown to improve array-CGH performance as compared to DOP-PCR when 5 ng of DNA from formalin fixed paraffin embedded samples was used. Unfortunately, this study did not evaluate MDA products as template [Little et al., 2005].

In the absence of DNA template, MDA can occasionally produce spurious products that are visible by agarose gel electrophoresis. Recently, it has been shown that these products contain a substantial amount of single-stranded DNA [Bergen et al., 2005a]. The products usually do not yield PCR-products, and thus do not interfere with subsequent genotyping. Introduction of two 5′-terminal nitroindole residues [Lage et al., 2003] or attachment of a additional 5′-nucleotide preceded by a C3 phosphoramidite or 18-atom hexaethylene glycol spacer [Brukner et al., 2005] to the random primers have been shown to limit spurious amplification, although the mechanism for this phenomenon is not fully understood.

COPY NUMBER DETECTION BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

Balanced amplification of all genomic loci is essential for determination of copy number alterations. Especially in cancer samples, in which only a few cells may be extracted by

TABLE 1. Key Studies on Multiple Displacement Amplification for Array-Based Comparative Genomic Hybridization

Type and number of samples	Amount of DNA (No of cells)	Type and number of probes on arrays	Detectable copy number alteration[a]	Note	Reference
Yeast cultures with known deletions. DNA from a human cancer cell line with chromosomal duplications.	500–1000	6135 yeast ORFs 4600 human cDNAs	3-fold	Both control and test DNA amplified by MDA.	Lage et al. [2003]
Laser-capture microdissected parenchymal cancer cells.	1,000	3600 BAC-clones	1.5-fold	Result based on statistical corrections.	Cardoso et al. [2004]
Leukocytes from a patient with trisomy 21.	1	2600 BAC-clones	1.5-fold	Trisomy in MDA product from single cells.	Hellani et al. [2004]

[a]Detection of copy number variations was only shown for a low total number of copies, such as three copies as compared to two copies in trisomy.
ORF, open reading frame; BAC, bacterial artificial chromosome.

microdissection, the potential of a WGA strategy could be valuable for CGH. MDA produces longer products from each priming event than PCR-based methods, and thus theoretically generates equal representation of loci. In the original publication on MDA, Dean et al. [2002] used CGH on metaphase chromosomes with identical results for MDA products and unamplified samples, provided that repetitive sequences were suppressed with *Cot 1* DNA. They also suggest the possibility of using MDA to prepare probes for CGH or for karyotyping by chromosome painting. The precursor to MDA, multiple-primed RCA [Dean et al., 2001], has been applied for this purpose to BAC-clones for array-CGH [Smirnov et al., 2004].

During recent years, CGH technology has been developed and, currently, arrays can be constructed with genomic clones, complementary DNA (cDNA) clones, PCR products, or oligonucleotides as the immobilized hybridization probes. These new arrays permit detection of DNA copy number aberrations at higher resolution metaphase chromosomes [Pinkel and Albertson, 2005b]. Table 1 presents summary statistics on the studies on array-CGH using the MDA products discussed below. Array-CGH can detect a three-fold copy number increase using DNA amplified by MDA with both human and yeast cDNA clones as immobilized probes [Lage et al., 2003]. Several studies report differences in amplification between loci, with over- or under-representation of especially telomeric and centromeric regions of the chromosomes. These differences have been found to be largely reproducible, and may hence be corrected for by also amplifying the reference sample [Lage et al., 2003], or by applying a correction in the statistical analysis [Cardoso et al., 2004]. Notably, Lage et al. [2003] found that the amplification bias between loci was more pronounced when employing the Φ29 DNA polymerase than when using the *Bst* DNA polymerase. The *Bst* polymerase does not have the comparably high proofreading capacity as the Φ29 DNA polymerase does, and would hence only be useful in methods such as array-CGH in which single-base alterations are not a crucial issue. The more accurate genomic representation for the chromosomal ends obtained by using the *Bst* polymerase may be due to template-switching by the *Bst* polymerase, also described with *Taq* DNA polymerase [Lage et al., 2003]. It has been argued that the amplification bias of the Φ29 DNA polymerase may have been due to the relatively low amount of Φ29 DNA polymerase used in the study cited above [Hosono et al., 2003].

Laser-capture microdissected tissue from as little as 1,000 cells has been subjected to MDA and subsequent analysis by array-CGH with BAC clones [Cardoso et al., 2004]. By applying statistical models to the analysis of the results, single-copy variations were accurately detected. In the study by Lage et al. [2003], 1,000 cells were required to obtain reproducible results without amplification bias. Single cells have been subjected to MDA prior to array-CGH with promising results in a test for trisomy of chromosome 21 [Hellani et al., 2004].

When performing array-CGH, especially with a small amount of initial DNA in the MDA reaction, an underrepresentation of genome-derived sequences was observed in the MDA samples, probably due to spurious nongenomic DNA produced during MDA, as discussed above. This underrepresentation became more pronounced at lower amounts of MDA, and may have been caused by a relative increase in the number of primers available for producing spurious products [Rook et al., 2004].

In conclusion, MDA has the potential to provide sufficient amounts of DNA for array-CGH even from small amounts of original cells. There is, however, some uncertainty regarding the genomic coverage by MDA and in addition, an amplification bias is apparent for different chromosomal regions. For analyzing genomic copy number alterations in degraded DNA samples, such as formalin-fixed and paraffin-embedded samples, balanced PCR has been suggested as a better alternative than MDA. However, if good quality DNA is available, MDA is preferable since it produces products of higher molecular weight with a more complete coverage of the genome than balanced PCR [Wang et al., 2004a]. A special case of copy number alteration is loss of heterozygosity (LOH), which often occurs in cancer tissue samples; LOH is discussed further in the section entitled Genotyping and Genomic Coverage Using Genome-Wide SNP Genotyping Assays below.

QUANTITATIVE REAL-TIME PCR

Amplification bias between genomic loci may be assessed more accurately by real-time quantitative PCR than using CGH [Heid et al., 1996]. A three-fold bias between genomic loci was observed in MDA products using the TaqMan assay (Applied Biosystems;www.appliedbiosystems.com) to analyze eight genes, of which two are located close to a centromere and a telomere, respectively. This result should be compared to a 10^3- to 10^6-fold amplification bias using DOP-PCR and a 10^2- to 10^4-fold bias using PEP [Dean et al., 2002]. When 47 loci were analyzed, the representation of genomic loci in the MDA product ranged from 0.5 to three times that of the original DNA, which results in maximally a six-fold

amplification bias between loci. Similar levels of amplification bias between loci were also found when applying MDA directly to whole blood, buccal swabs, finger-stick blood, buffy coats, or Guthrie cards [Hosono et al., 2003], as well as when analyzing laser-capture microdissected cells [Rook et al., 2004].

Similar to the observations reported for array-CGH data, real-time PCR reveals underamplification of repetitive sequences, which results in enrichment of coding sequences [Dean et al., 2002; Hosono et al., 2003]. This underrepresentation of repetitive sequences may be due to the rapid consumption of random primers that match the repetitive sequences. Long and short interspersed repetitive sequences (LINEs and SINEs) have been found to have a 1.4-fold underrepresentation in MDA product relative to the starting genomic DNA when analyzed by the TaqMan assay [Hosono et al., 2003].

GENOTYPING OF SNPS AND SHORT TANDEM REPEATS (STRS)

The central issue in using WGA DNA for genotype analysis is adequate representation of alleles, not only for SNP analysis but also for short tandem repeats (STRs). Imbalanced amplification can lead to genotyping errors by interpreting heterozygous genotypes as homozygous due to allelic dropout or due to low quality genotype data, which would result in low genotyping success rates. Table 2 provides summary statistics from a number of key studies on SNP and STR genotyping MDA products discussed below.

Ten SNPs were assayed by ligation assays in the original study on MDA by Dean et al. [2002], with complete concordance with results from unamplified DNA. Genotyping of 45 SNPs in 15 individuals on a single microarray showed >99% concordance between original DNA and MDA material, as compared to 89% for PEP (Fig. 3). In this study, the success rate of genotyping was identical (97%) for MDA product and genomic DNA [Lovmar et al., 2003]. In the same study it was also shown that at least 3 ng of DNA, corresponding to 1,000 human genome equivalents, is needed to ensure equal amplification of both alleles. The requirement of 1,000 sequence copies to avoid amplification bias is concordant with results from the determination of stochastic variation in signal ratios after competitive PCR [Stenman et al., 2003], and also to results using CGH, as discussed above. When only a small number of cells or a sample with low DNA concentration is available, application of a pooling strategy or analysis of multiple-cell samples in parallel, as has been applied for PEP [Paunio et al., 1996], may be used to correct for stochastic variations between the amplification of two SNP or STR alleles.

The advantage of pooling MDA products from replicate DNA samples was shown for analysis of STRs and SNPs in as little as 100 microdissected cells [Rook et al., 2004]. In this study, two to three MDA products from 33 cells were pooled and genotyped, with an accuracy that ranged from 82 to 100%. Discordant genotypes were found to be caused mainly by allelic dropout, and the pooling procedure clearly increased the call rate. In this study, alkaline lysis and protein-K lysis of the cells were also compared. The protein K procedure showed a genotype concordance rate of >88% between genomic DNA and MDA product, while the alkaline lysis protocol gave a concordance rate as low as 33%. Pooling replicate MDA products was also shown to improve the genotyping results in a study in which 24 SNPs were genotyped in 59 epidemiological samples with low amounts of DNA or poor quality of the extracted DNA [Silander et al., 2005].

Recently, a more thorough assessment of genotyping results with MDA-amplified DNA was performed for a 5cM STR-map with 768 genome-wide STR markers. High quality genomic DNA was used as MDA template and the genotyping call rate (95.0%) was similar to that of genomic DNA (96.5%). A small increase of LOH was observed in the MDA samples, but no marked tendency for under- or overamplification of larger or smaller STR alleles was observed. Analogously to the results by Rook et al. [2004], pooling of three replicate MDA reactions gave a small improvement in both call rate and genotyping accuracy. Overall, 34 STRs (4.4% of all markers) showed higher genotype failure rates or discrepant genotypes in the MDA samples, and these markers were responsible for a large proportion of the total failures or discrepancies. Hence, the authors emphasize the importance of controls to identify problematic markers [Dickson et al., 2005].

As discussed earlier, a prerequisite for successful downstream applications of MDA is amplification without biased or imbalanced genomic representation, either of which can contribute to amplification of alleles resulting in genotype failures or errors. Moreover, input DNA is an important variable in the MDA reaction. This was the subject of a study by Bergen et al. [2005b], who found that the required amount of genomic DNA template was even higher than expected, and recommended that at least 10 ng of lymphoblastoid DNA should be used prior to SNP analysis, and over 100 ng for optimal STR genotyping. These results do not imply that MDA cannot be performed on smaller amounts of DNA, but the downstream analysis and interpretation of results should be made with caution. For example, in forensics, DNA is often available only in trace amounts. Instead, MDA may supply larger amounts of DNA than what is possible to retrieve, for example from a crime scene. The utility of MDA for forensic purposes has been tested on DNA extracted from fingerprints [Sorensen et al., 2004]. This study showed that MDA improved the frequency of successful alu-PCR, as compared to unamplified samples.

Highly accurate single-cell analysis would enable preimplantation diagnostics, but in analysis of single cells there is a large risk for allelic dropout or imbalanced amplification between alleles. Analysis of five MDA reaction products from single lymphocytes showed allelic dropout in 28% (9/32) and 40% (4/10) of known heterozygous STRs and SNPs sites, respectively. Additionally, partial imbalance between the alleles was observed frequently. By increasing the number of cells subjected to MDA to two, five, 10, and 20, respectively, the allelic dropout and imbalance was progressively reduced [Handyside et al., 2004]. Allelic dropout was observed in 1 of 20 parallel single-cell MDA reactions from one individual for one STR, while 10 other STR assays showed no dropout [Hellani et al., 2004]. So far, PEP has been used for genotyping single-sperm DNA to study recombination and linkage disequilibrium patterns [Cullen et al., 2002; Zhang et al., 1992]. Recently, single sperm cells have been successfully amplified by MDA followed by analysis of SNPs, STRs, and DNA sequences [Jiang et al., 2005].

MDA product from clinical samples, i.e., from whole blood, finger-stick blood, and buccal swabs, has been shown to give accurate genotyping results for a small number of SNPs and STRs. The two STR alleles were almost equally amplified, and the STR analysis on MDA product showed no increase in the number or amount of stutter bands compared to genomic DNA [Hosono et al., 2003]. Stutter bands may occur if there is a slippage of the polymerase on the repetitive sequence, and could thus occur when applying a PCR-based WGA strategy. Replication slippage of the DNA polymerase is inversely related to strand displacement efficiency; and Φ29 DNA polymerase, which has a high strand

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TABLE 2. Key Studies on Multiple Displacement Amplification for Genotyping SNP- or STR Markers

Type of sample	Amount of DNA (ng)	No. of samples	No. of markers	Genotyping method	Concordance rate (%)[a]	Failure rate (%)[b]	Reference
Cell line	0.3–300	4	10 SNPs	Ligation assay	100	0	Dean et al. [2002][c]
Cell lines of family members	0.003–3	15	45 SNPs	Minisequencing on arrays	>99	3	Lovmar et al. [2003]
LCM prostate or colon cancer cells	0.2–20 (33–3,000 cells)	10	12 SNPs	TaqMan	82–100	2–25	Rook et al. [2004][d]
			45 STRs	Size analysis	91–100	0–32	
Peripheral blood	5–10	216	4 SNPs	Allele specific amplification	100	0	Bannai et al. [2004]
Single blastomeres and lymphocytes	0.006 (single cell)	10	5 SNPs	Restriction cleavage or minisequencing	84	0	Handyside et al. [2004]
		5	15 others	Size analysis	70	11	
Lymphoblasts, mouthwash, and buffy coats	4	20	49 SNPs	Hybridization probes	>99	4	Bergen et al. [2005a][e]
			15 STRs	Size analysis	93	2	
Cell lines	1–200	27	49 SNPs	Hybridization probes	>99	<1–3	Bergen et al., [2005b][f]
			15 STRs	Size analysis	80–99	0–5	
Cell lines	10	5	2,320 SNPs	Illumina Golden Gate assay	>99	2	Barker et al., [2004][g]
Cell lines	10	14	≈10,000 SNPs	Gene Chips, Affymetrix	>99	8	Paez et al. [2004]
Cell lines	20	86	345 SNPs	Illumina Golden Gate assay	99	0.2	Pask et al. [2004]
Buccal swabs, whole blood, Guthrie cards	≈1	5–19	1,260 SNPs	Illumina Golden Gate assay	85 to >99	<1–10	Park et al. [2005]

[a]Genotyping results from MDA product compared to genomic DNA.
[b]Proportion of failed genotype calls of total number attempted.
[c]At the time of publication of the study, some of the authors were representatives of Molecular Staging, Inc., which markets products for MDA commercial companies.
[d]Results are from 2–3 pooled MDA products.
[e]STR results given for allele height >50 relative fluorescence units (RFU).
[f]SNP results given for input of 20 ng of MDA product to genotyping assay.
[g]Some of the authors are representatives of Illumina, Inc., which markets products for WGA and genotyping.
STR, short tandem repeat; SNP, single nucleotide polymorphism; LCM, laser-capture microdissected.

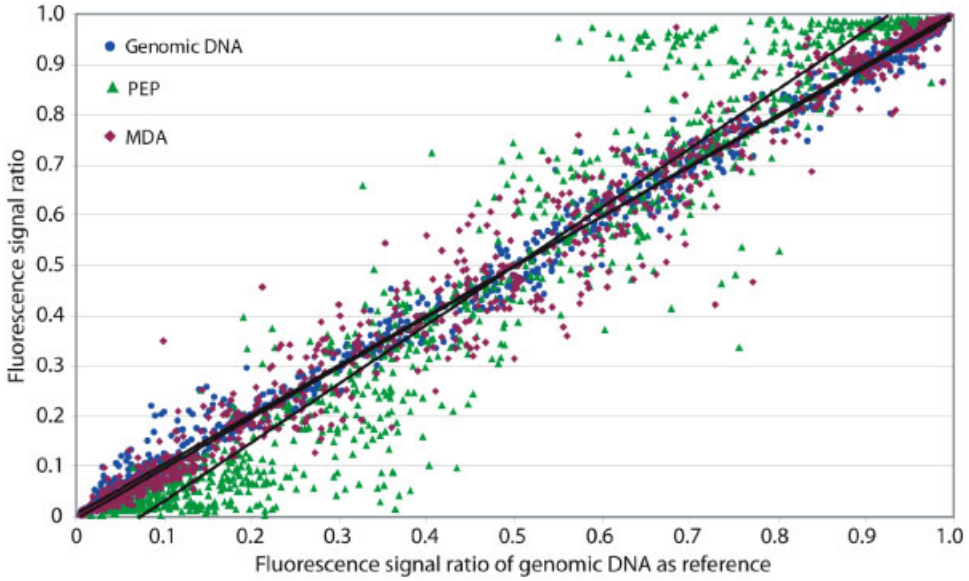

FIGURE 3. **Correlation between fluorescence signal ratios obtained in SNP genotyping by four-color tag-microarray minisequencing of WGA products and genomic DNA in a single microarray experiment. The signal ratios obtained when genotyping PCR products from a reference genomic DNA template are plotted on the x-axis and the signals from a duplicate genomic DNA template (filled circles), MDA-product (diamonds), and PEP-product (triangles) from the same DNA sample are plotted on the y-axis. The linear trend lines are indicated and the pairwise Pearson correlation coefficients are 0.997 for genomic DNA, 0.991 for MDA products, and 0.962 for PEP products. Data from Lovmar et al. [2003]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]**

displacement activity, does not show slippage [Canceill et al., 1999]. Hence, MDA does not infer additional stuttering prior to PCR amplification. This result is also supported by two other studies [Bergen et al., 2005a; Hellani et al., 2004]. In another study to assess the performance of clinical samples in MDA, >99.95% accuracy was achieved for six SNPs in 352 samples, including buccal cells [Tranah et al., 2003]. Preliminary studies of SNPs and STRs in DNA extracted from blood spots and amplified by either MDA or an improved PEP protocol generated correct genotypes [Sun et al., 2005]. However, in that study the PEP protocol showed allelic imbalance to a lower extent than MDA, indicating that the PEP strategy may be advantageous on some occasions. Thompson et al. [2005] showed that SNP genotyping of a few SNPs on MDA product from buccal cells gave equal results as buccal cell DNA without amplification. In another study, 15 STRs and 49 SNPs were genotyped in MDA product from 4 ng of genomic DNA extracted from mouthwash samples, buffy coat cells and lymphoblasts [Bergen et al., 2005a]. Discrepant genotyping results between MDA product and genomic DNA were observed, mainly due to an amplification bias between alleles, which led to heterozygote dropout across separately-generated MDA products. The MDA products from mouthwash samples exhibited higher discordance between SNP genotypes compared to genomic DNA and MDA products than MDA products from lymphoblasts. There were also indications that SNP genotyping performance with the TaqMan assay system could be problematic in regions characterized by high GC sequence content (%GC). In the study by Bergen et al. [2005a], the results from MDA were compared to the OmniPlex method, and generally, MDA worked more satisfactorily.

GENOTYPING AND GENOMIC COVERAGE USING GENOME-WIDE SNP GENOTYPING ASSAYS

The preliminary reports for genome-wide SNP genotyping assays [Steemers et al., 2006; Syvänen, 2005] are promising for analysis of DNA samples amplified by an MDA technique. Both the genomic coverage and possible imbalance in amplification between loci and alleles in MDA products can be assessed when applying dense and genome-wide SNP assays.

The ability to detect allelic imbalance due to LOH and copy number alterations in cancer tissue samples after MDA was evaluated with encouraging results using the Affymetrix 10 K GeneChip SNP genotyping assay (Affymetrix; www.affymetrix.com) [Matsuzaki et al., 2004]. For detection of LOH, the MDA product showed a false-positive rate of 13.5% and a false-negative rate of 19.9% compared to genomic DNA [Wong et al., 2004]. Detection of LOH in the same MDA samples using STR markers was 78% (14/18) concordant with the 10 K assay. These discrepant results may be due either to erroneous heterozygous SNP genotype calls or due to the fact that the boundary of the LOH region lies between the SNP and microsatellite loci. When analyzing copy number changes along part of chromosome 6, a Pearson correlation coefficient of 0.72 was calculated between MDA product and unamplified DNA [Wong et al., 2004].

In a separate study, which also used the 10 K array, SNP genotyping call rates for MDA product were comparable to the call rates for genomic DNA (92 vs. 93%) [Paez et al., 2004]. The genotype concordance between MDA product and reference genomic DNA was >99%. Also in this study, LOH analyses of heterozygous loci were 99.6% concordant with those from genomic DNA, and estimates of relative copy numbers in cancer tissues were 87% concordant between results from MDA product and genomic DNA [Paez et al., 2004]. A third study found similar call and concordance rates to those found by Paez et al. [2004], but also that 7% of the heterozygous SNPs failed when MDA was applied [Tzvetkov et al., 2005]. As shown by Tzvetkov et al. [2005] (and also by Rook et al. [2004]), a pooling strategy of several MDA reactions for each sample increased both call rate and concordance. The genomic coverage of MDA product was estimated to be 99.8% by Paez et al. [2004], but six regions, with a maximal total size of 5.6 Mb, consistently failed. Interestingly,

Tzvetkov et al. [2005] did not see this feature, and found that the amplification failure was restricted to single SNPs. Thus, it remains unclear whether amplification failure is due to repetitive sequences or to other technical problems with the 10 K assay.

The Illumina SNP genotyping platform has been used to assess the quality of MDA reactions using a panel of 2,000 SNPs distributed over the genome. When the call rates were adjusted to 98 to 99% by genotyping quality requirements, the concordance rate between genomic DNA and MDA product was determined to be >99.8%, and, in general, amplified and unamplified DNA sample performed equally well [Barker et al., 2004]. When a smaller number of SNPs, but a larger number of samples (Table 2) were analyzed after MDA by the same SNP genotyping assay, a slightly lower concordance rate (98.8%) was observed. A larger number of MDA samples (10.5%) than genomic DNA samples (5.7%) were failed in this analysis [Pask et al., 2004]. MDA product SNP genotyping using the Illumina platform for DNA from Guthrie blood spots extracted using three different protocols, showed a decreased (90.3% as best) success rate compared to buccal swabs (97.7%) and whole blood DNA (99.5%). Also, the genotyping concordance as compared to genomic DNA was lower for DNA extracted from Guthrie cards. The size of DNA from Guthrie cards ranges from 2 to 10 kb, while DNA extracted from buccal swabs and whole blood has a size >20 kb. This is probably the reason for the differences in success rates [Park et al., 2005]. It is notable that irradiation of DNA samples with an electron beam (e.g., as is presently used to sterilize mail in the United States) causes a reduction in genotype success and concordance rates after MDA probably due to degradation of the DNA [Bergen et al., 2005c].

DNA SEQUENCING

Since clinical samples are often available only in small quantity, and sequencing often requires a large number of amplification reactions, a WGA procedure would be beneficial prior to sequencing. A 100% concordance between the α and β hemoglobin gene sequences retrieved from MDA product from 1 μl of whole blood and from purified genomic DNA was observed using a protocol from routine clinical practice [Mai et al., 2004]. PCR product from MDA amplified DNA obtained from micro-dissected cortical motor neurons has been also successfully sequenced in a small study [Pamphlett et al., 2005].

In a larger study to assess the genomic coverage and sequence fidelity of the Φ29 DNA polymerase, ~500,000 bp of DNA was sequenced. A total of 234 sequence variants were detected both in MDA products and original DNA, four variants were detected only in original DNA, and five variants were detected only in MDA product. This result indicates a relatively low risk of sequencing errors when using MDA products as PCR template for germline sequencing [Paez et al., 2004].

The MDA strategy for WGA prior to PCR has been tested in a SNP discovery study of the melanocortin 1 receptor (MC1R) locus. The results from MDA were compared to genomic unamplified DNA, and 9% (12/134) of the heterozygous positions were misinterpreted as homozygous in the amplified material. This allelic loss resulted in addition of false haplotypes in the MDA material using the PHASE software (www.stat.washington.edu/stephens/software.html) [Murthy et al., 2005]. As indicated by recent studies, these results may be due to the relatively low amount of genomic DNA used as MDA template [Bergen et al., 2005b]. This observation stresses the caution needed in the interpretation of MDA genotype results in studies in which a low amount of template DNA has been used.

One strategy for direct assessment of haplotypes is to dilute a DNA sample to the concentration of a single DNA molecule and follow that by MDA amplification. Using this approach, MDA has been shown to improve the downstream PCR performance as compared to DOP-PCR for haplotype analysis by sequencing of human leukocyte antigen (HLA) genes [Paul and Apgar, 2005]. An interesting feature of the dilution process to assess haplotypes is that it circumvents the problem of uneven amplification of alleles since only one DNA molecule is initially present. The ataxia-telangiectasia mutated (ATM) gene was sequenced in single neoplastic cells isolated from paraffin-embedded tissue by microdissection and displaying LOH. The results indicated a splice-site mutation possibly responsible for a loss of expression of ATM protein in some neoplastic cells showing LOH [Lespinet et al., 2005].

ATYPICAL APPLICATIONS OF MDA

MDA has recently been applied in several practical applications with large future potential; these applications are not covered above. For example, MDA has been shown to enable PCR analysis of sequences at low concentrations and in the presence of PCR inhibitors that are common in environmental samples [Gonzalez et al., 2005]. Moreover, MDA has been applied to characterize bacteria collected from their natural environment [Erwin et al., 2005]. MDA has also been useful for obtaining the sequences of unculturable microorganism such as *Epulopiscium* [Nelson, 2003] and has been shown to enable sequencing of DNA from single bacterial cells [Raghunathan et al., 2005]. MDA has also been applied directly to bacterial cells in colonies of *Salmonella enteritidis* [Kwon and Cox, 2004], to DNA extracted from single fungi spores [Gadkar and Rillig, 2005], and to crude DNA samples from the filamentous fungi *Penicillium paxilli* and *Epichloë festucae* [Foster and Monahan, 2005]. Further, MDA has been shown to improve the sensitivity of detecting bacterial DNA in an excess of host DNA, as compared to direct high-fidelity PCR [Jeyaprakash and Hoy, 2004]. Amplification of total DNA from human rectum and colon biopsies [Monstein et al., 2005] and human gallstones [Nilsson et al., 2005] has also been achieved using MDA.

Application of MDA to DNA from whole or parts of *Aedes aegypti* mosquitoes has been successful, and has given concordant results to genomic DNA [Gorrochotegui-Escalante and Black, 2006]. Insect genomics often requires repeated analysis on individual genomes, and hence MDA constitutes a promising solution to supply sufficient DNA for such studies.

There are many demands on the limited resources of nonhuman primate genetic resources that are available for medical genetic studies, comparative genetics, biodiversity assessment, and wildlife forensics. Recently, MDA has been applied to rare and limited primate samples and was found to be a reliable approach for genetic studies of a diversity of sample types using several genotyping methods used in this field of research [Rönn et al., 2006].

Studies on methylation patterns are performed to gain additional knowledge about epigenetic modifications of genomic DNA. DNA synthesized by a DNA polymerase does not contain methylated cytosines, which introduces the possibility of using DNA generated by the Φ29 DNA polymerase as universal unmethylated control DNA for optimization of assays for epigenetic modifications [Umetani et al., 2005]. MDA has an

TABLE 3. Comparison of Multiple Displacement Amplification With Other Whole Genome Amplification Techniques for SNP- Genotyping*

WGA methods evaluated				Concordance rate (%)[a]				Failure rate (%)[b]				
MDA	PEP	DOP-PCR	OmniPlex	MDA	PEP	DOP-PCR	OmniPlex	MDA	PEP	DOP-PCR	OmniPlex	Reference
X	X	X	–	100	n.d.	n.d.	–	0	n.d.	n.d.	–	Dean et al. [2002][c]
X	X	–	–	>99	89	–	–	3	10	–	–	Lovmar et al. [2003]
X	X	X	–	100	100	100	–	0	0	0	–	Bannai et al. [2004]
X	–	–	X	>99	–	–	>99	2	–	–	2	Barker et al. [2004][c,d,e]
X	–	–	X	>99	–	–	>99	10[f]	–	–	26[f]	Park et al. [2005]
X	–	–	X	>99	–	–	>99	4	–	–	7	Bergen et al. [2005a]

*For details on study design, see Table 2.
[a]Genotyping results from MDA product compared to genomic DNA.
[b]Proportion of failed genotype calls of total number attempted.
[c]At the time of publication of the study, some of the authors were representatives of Molecular Staging, Inc., which markets products for MDA commercial companies.
[d]Some of the authors were representatives of Illumina, Inc., which markets products for WGA and genotyping.
[e]Call rate fixed to >98%.
[f]Mean results from the different sample sources.
WGA, whole genome amplification; MDA, multiple displacement amplification; PEP, primer extension preamplification; DOP-PCR, degenerate oligonucleotide primer polymerase chain reaction; n.d., not determined; n.s., not specified.

advantage over PCR-based WGA methods since MDA does not result in underamplification of the GC-rich regions.

CONCLUDING REMARKS

Currently, MDA is the most promising method for WGA of genomic DNA. Although many of the reviewed studies used either small sample sizes or analyzed a small number of genetic markers (Tables 1 and 2), the overall picture is the same. In comparison to other WGA methods, MDA has provided the most reliable genotypes, highest call rates, best genomic coverage, and lowest amplification bias [Bergen et al., 2005a; Dean et al., 2002; Lovmar et al., 2003; Park et al., 2005; Paul and Apgar, 2005] (Table 3). From Table 3, it is evident that other techniques such as OmniPlex technology may be a favorable alternative to MDA when degraded samples are being used as template. The advantages of MDA are most probably due to the specific properties of the Φ29 DNA polymerase in combination with the isothermal non-PCR-based hyperbranched amplification using this enzyme. In addition to the examples on applications presented briefly above, an important application of WGA is to replenish depleted DNA stocks collected and consumed in human genetic studies. In this case, MDA may provide a suitable alternative for studies that would not be possible without WGA [Holbrook et al., 2005].

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