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Multiple displacement amplification of blood and saliva samples placed on FTA® cards

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Abstract. We performed multiple displacement amplification (MDA) of 50 blood and 50 saliva samples placed on FTA cards. A 1.2-mm disk was punched out of the FTA cards. The disk was washed, dried and used as target for the GenomiPhi DNA amplification kit. On average, the Phi29 polymerase produced 2 μg whole genome amplified DNA (wgaDNA) with DNA fragment sizes ranging from a few hundred bp to 12 kbp. A total of 1–2 ng wgaDNA was typed using the AmpF*l*STR® SGM Plus™ amplification kit and the resulting STR profiles analysed and compared to genotypes obtained from genomic DNA. Only 25 of the 100 wgaDNA samples gave concordant STR profiles. Locus drop-outs, missing alleles, allele imbalances or false alleles were observed in 11, 27, 61 and 10 wgaDNA samples, respectively. The same FTA disk was used successfully in five consecutive rounds of MDA. However, after the 4th and 5th round of MDA, a further increase in the number of allele drop-outs as well as system and allele imbalances were observed.

Keywords: Whole genome amplification; Multiple displacement amplification; FTA card; Short tandem repeat

1. Introduction

Multiple displacement amplification (MDA) has become a popular method for whole genome amplification because the MDA products are long (up to 100 kb) [1], the mutation rate is low [2], the genome coverage is comprehensive [3] and the amplification bias is much lower compared to other methods of whole genome amplification [1,4–6]. MDA reactions with 5–10 ng genomic DNA was sufficient to minimize amplification bias [4,6]

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and obtain genotype concordance rates of approximately 95% for STRs [5,7] and 99% for SNPs [3,5,8,9]. With less than 5 ng genomic DNA in the MDA reaction, the amplification bias and the number of genotyping errors increased [1,6], thus, MDA is not useful in typical forensic case work but may be fundamental to large genomic studies involving thousands of loci.

FTA cards are an excellent medium for collection, shipping and storing of DNA samples. Cells lyse when they come in contact with the coating on the FTA cards and the DNA is released from the cells and irreversibly bound to the filter matrix from where the DNA can be assayed directly. We have tested if samples on FTA cards can be used as genomic DNA (gDNA) target for MDA, and if the same FTA disk can be used repeatedly in MDA reactions and, consequently, if one small FTA disk can be used as an infinite source of gDNA.

2. Materials and methods

Fifty Danes donated blood and saliva samples collected on FTA cards (Whatman). The Danish Ethical Committee (KF-01-037/03) approved the work presented here. A 1.2 mm disk of the FTA card was punched out using a BSD600-duet (BSD Robotics). The FTA disks were washed three times with 150 μl water using the THEONYX robotic system (MWG) and incubated overnight at room temperature in 150 μl water to remove all inhibitors. When the same disk was used for repetitive MDA reactions, the disk was washed twice with 100 μl water and air dried after each MDA reaction.

MDA was performed by adding 9 μl sample buffer (Amersham Biosciences) to the washed and air dried 1.2 mm FTA disk and incubating the mixture for 3 min at 95 °C. Nine microliter reaction buffer and 1 μl enzyme mix (Amersham Biosciences) was added and the mix was incubated at 30 °C overnight. The Phi29 enzymes were inactivated by incubation at 65 °C for 10 min.

PCR was performed by adding 1 μl diluted wgaDNA (diluted 1:40 or 1:100 in water) or 1 μl 1 ng/μl Chelex-purified gDNA to a mixture of 4 μl AmpF*l*STR PCR reaction mix (Applied Biosystems), 2 μl AmpF*l*STR SGM Plus primerset (Applied Biosystems), 0.2 μl AmpliTaq Gold (Applied Biosystems) and 3 μl dH_2O. A total of 6 blood and 11 saliva samples were amplified a second time with 4 μl diluted wgaDNA in the PCR reaction. The following cycle program was used: denaturation and enzyme activation at 95 °C for 11 min, 28 cycles of 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min, followed by 45 min at 60 °C. All PCR reactions were performed in the ABI 9700 thermal cycler (Applied Biosystems).

All PCR products were analysed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

3. Results

MDA was performed on 50 blood and 50 saliva samples placed on FTA cards. A total of 1–2 ng wgaDNA or gDNA from each sample was typed using the AmpF*l*STR SGM Plus™ amplification kit and the results compared. In summary, wgaDNA from 21 blood and 4 saliva samples gave acceptable and correct allele calls for all 10 STR loci. The remaining wgaDNA samples had locus drop-outs, allele imbalances, missing or false alleles in one or more STR loci (Table 1).

Table 1
STR typing of wgaDNA using the AmpF/STR SGM Plus amplification kit

	Blood samples	Saliva samples
Approved and concordant allele calls in all 10 STR loci	21	4
1 locus drop-out	0	8
>1 locus drop-out	0	3
1 missing allele	7	11
>1 missing allele	0	9
1 allele imbalance[a]	22	11
>1 allele imbalance[a]	4	24
1 false allele	1	7
>1 false allele	2	0

[a] The peak height of one allele was less than one third of the peak height of the other allele in the same locus.

Five different FTA disks were used successfully in five consecutive rounds of MDA. After the 4th and 5th round of MDA, an increase in the number of allele drop-outs as well as system and allele imbalances were observed (data not shown).

4. Discussion

The amplification bias observed in these experiments is difficult to explain. If the FTA disk was washed thoroughly, it did not inhibit the MDA reaction (this work and [6]). Furthermore, we estimated that more than 10 ng gDNA was bound to the 1.2 mm disk based on the highly efficient PCR amplification of samples placed on FTA cards [10], and thus, there were sufficient amounts of gDNA on the FTA cards to avoid strong amplification bias [4,6]. The gDNA was covalently bound to the FTA filter matrix, and therefore, we speculate if only a small fraction of the gDNA was amplified due to frequent interruptions of the MDA reactions.

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