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Low copy number: Interpretation of evidence results

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Abstract. Crime scene evidence and samples from decomposed or skeletal remains have usually low copy number (LCN), with templates containing less than 100 pg of DNA. The choice of the best extraction methods as well as sensitive and robust STRs is crucial to obtain good results. However, in many situations, it is also necessary to make adaptations to standard protocols, such as increase in the number of amplification cycles (even when commercial kits are used) or the injection time in capillary sequencers. A set of four amplifications was made, with 28 (two of them), 32 and 34 cycles. To the samples with 28 PCR amplification cycles was added more enzyme and two other amplifications with 4 and 6 cycles was performed. Results were analysed and discussed.

Keywords: Low copy number; STR; Allelic drop out; Heterozygous balance; Spurious allele

1. Introduction

Low copy number (LCN) samples reveal sometimes difficulties at results interpretation because DNA is present at very low amounts (few or even one or single cell) or is coming from degraded or decomposed material. One of the most common modifications to improve those results is changing the number of amplification cycles, with an increment of 6 or more cycles; however, stochastic effects such as allele drop-out, heterozygote imbalance, spurious alleles and increased stutters are some of the most common problems [1–3].

Five different cases with DNA templates between 2 and 140 pg were tested, with some modifications to the amplification protocols.

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Table 1
Cases 1 to 5—number of identified loci vs. number of extra peaks (allelic drop in)

	Case 1			Case 2	Case 3	Case 4	Case 5
	0.02 g	0.04 g	0.06 g				
DNA template	2 pg	20 pg	32 pg	19 pg	25 pg	108 pg	140 pg
28 cycles	0/0	7/1	13/0	3/0	8/0	6/0	7/0
32 cycles	3/0	14/9	16/5	16/1	15/1	11/1	8/0
34 cycles	6/2	16/6	15/7	13/5	16/1	12/3	10/2
28+4 cycles	6/0	11/2	16/4	16/1	15/1	16/1	14/2
28+6 cycles	9/2	13/7	16/9	15/4	15/10	14/3	16/7

2. Materials and methods

In Case 1, DNA was extracted from two molar teeth (12 years old remains), using 0.02 g, 0.04 g and 0.06 g of dentin powder according to Puregene™ DNA Isolation Tissue Kit (Gentra Systems). In Case 2, DNA extraction was carried out with the previous extraction kit from the dentin of two molar teeth (3 years old remains at least), one of them filled and the other rotten. DNA extraction with the Chelex™ method [4] was performed in Cases 3 (crime scene evidence), 4 and 5 (decomposed bloodstains).

DNA quantification was made with the Quantifiler™ Human DNA Quantification Kit at the ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

Amplification of 16 genomic DNA loci was made using the AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) with 28, 32 and 34 cycles. To the samples with 28 PCR amplification cycles, 0.8 μl of AmpliTaq Gold® (Applied Biosystems) was added and other amplification was made using four PCR cycles in one sample and six PCR cycles [5] in the other one.

Extraction and amplification negative controls were made despite contamination.

All amplification products were performed using the ABI PRISM™ 310 Genetic Analyser (Applied Biosystems).

3. Results

All extraction as well as amplification negative controls were good. Results of the 16 loci (Amelogenin and 15 STRs) at five different amplifications are presented in Tables 1 and 2. Amplification was considered only when results were above background level.

Allelic drop-in refers to spurious alleles and stutters over 25% of the peak area of the associated allele. Allelic drop-out of heterozygous loci includes deletion of one or both alleles.

Table 2
Cases 1 to 5—Number of identified heterozygous loci vs. number of loci with heterozygote imbalance

	Case 1			Case 2	Case 3	Case 4	Case 5
	0.02 g	0.04 g	0.06 g				
DNA template	2 pg	20 pg	32 pg	19 pg	25 pg	108 pg	140 pg
28 cycles	0/0	5/4	11/4	2/1	5/2	4/1	3/2
32 cycles	3/2	12/11	14/8	14/10	12/10	9/5	4/2
34 cycles	5/4	14/11	13/11	11/9	13/12	10/6	6/1
28+4 cycles	5/2	9/9	14/8	14/12	12/8	14/7	10/3
28+6 cycles	8/7	11/9	14/8	13/11	12/8	12/5	12/5

Heterozygote imbalance was defined as $(\phi a - \phi b)/\phi a \geq 0.2$ where ϕa and ϕb are the areas of the larger and smaller peaks (irrespective of molecular weight) [1].

4. Discussion and conclusions

In our experiments, some spurious alleles appeared, suggesting that there are laboratory contaminants that could affect LCN samples, especially when the number of amplification cycles is increased.

At very low levels of DNA template (Case 1—0.02 g of dentin powder and 2 pg of DNA), the increment of amplification cycles allowed the definition of more STRs but not the complete profile. DNA extraction of more dentin powder (0.04 g) revealed a full profile at 34 amplification cycles; however, heterozygote imbalance and allelic drop-in make the interpretation of results difficult. At 32 pg of DNA template (0.06 g of dentin powder), the sample is also completely genotyped and the better results were obtained with the addition of more Taq followed by four amplification cycles, since there is a prevalence of real peaks and reduction of contaminating alleles.

Only in Case 3, the single amplification with 34 cycles provided a better profile. The other cases, even those with DNA templates near or above 100 pg (Cases 4 and 5), showed greater results with the addition of more Taq and increment of more amplification cycles, which also reduced some of the stochastic effects.

Samples containing <100 pg of DNA may be difficult to genotype, so extraction and amplification protocols must be improved to reach the best results. Our casework samples were tested in five different amplification conditions and showed that 34 cycles of amplification is not always the best choice since stochastic effects increase, especially the incidence of spurious alleles. In fact, in the majority of cases, better results were obtained with the following procedures: 28 cycles of amplification, typing, addition of Taq and other amplification with 4 or 6 more cycles.

We consider that when the final volume of the extraction method is sufficient, several amplifications should be made in order to compare results and achieve the consensus profile. Once it is desirable to keep the number of PCR cycles to a minimum, the amplification with only 32 cycles or with 28 cycles plus 4 more cycles should be done since it is enough to type some of the samples.

One of the disadvantages of making a second amplification is the manipulation of PCR products. The advantages are, besides the diminution of extra peaks, to reduce time and costs at the new amplification.

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