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The risk of missing one allele with very low DNA quantities using PCR

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Abstract. TH01 typing was performed by semi-nested PCR using a very low concentration of DNA (TH01 type = 7–11) as a template. With 10 pg of template DNA, the probabilities of obtaining both of two alleles, only allele 7, only allele 11, or no PCR product, were 23.1%, 15.4%, 38.5% or 23.1%, respectively. Our findings suggest that these differences were found because the initial proportions of alleles 7 and 11 in the PCR tube were not equal. However, the allele distribution of TH01 types 7 and 11 using single cells considered to contain equal amount of the two alleles was similar to that obtained using a very low concentration of DNA. These results indicate that correct genotypes could not be obtained by using equal amount of both alleles, when the concentration of DNA was very low. To obtain a correct genotype, it was necessary to use 100 pg of DNA as the template for PCR amplification.

Keywords: Microsatellite; TH01; PCR; DNA

1. Introduction

In molecular studies using forensic samples, the amount of DNA available for genetic typing can be very low. Under these circumstances, it is possible for one allele of a heterozygous individual not to be detected, leading to the incorrect genotyping of this individual as a homozygote. In this study, we clarified the amount of template DNA required for detecting both of two alleles by semi-nested PCR and demonstrated that the cause of invalid genotyping results is not always pipetting errors or PCR samples containing in equal amounts of the alleles per PCR when using single cells for genetic typing.

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2. Materials and methods

2.1. Cell sources

Mononuclear cells were isolated from tonsils extracted from a patient (TH01 type = 7–11) with chronic tonsillitis using Ficoll Hypaque density-gradient separation. DNA was extracted from the tonsillar cells using the DNeasy Tissue Kit (QIAGEN, CA).

2.2. PCR amplification

TH01[AATG]$_n$ PCR primers were as follows (5′ → 3′); TH01F, att caa agg gta tct ggg ctc tgg; TH01R, gtg ggc tga aaa gct ccc gat tat [1]. TH01Ff, cca ttg gcc tgt tcc tcc ctt att, was also synthesized for semi-nested PCR [2]. Using single cells as a template, PCR amplification was performed as described by Zhang [3]. First- and second-round 30 amplifications consisted of a 45-s denaturation step at 94 °C, a 30-s annealing step at 60 °C, and a 30-s extension step at 72 °C. The PCR products were resolved on a 4% agarose gel in 1 × TAE.

3. Results and discussions

Four TH01 typing experiments were performed using a set of 10-fold serially diluted DNA by semi-nested PCR (Fig. 1). At lower concentrations of DNA (below 10 pg), the experiments failed to amplify both alleles (Table 1). At 10 pg of DNA as a template, the probabilities of obtaining both alleles, only allele 7, only allele 11, or no PCR product, were 23.1%, 15.4%, 38.5% or 23.1%, respectively (Fig. 2). This is believed to be because the initial proportions of allele DNA in the PCR tube are not equal [4]. To avoid random sampling of template DNA, whole cells were used for TH01 typing. However, the same variations of the amplified alleles could be observed when using single cells (Fig. 3). The results using single cells showed that the number of tubes with both alleles, only allele 7, only allele 11 or no PCR product, were 8 (36.4% of positive tubes), 7 (31.8%), 7 (31.8%), and 154, respectively.

These results indicate that correct genotypes could not be obtained at very low concentration, even if equal amounts of both allele DNAs were used for PCR amplification. When the amount of template DNA used per PCR reaction is below 10 pg, as is the usual practice, the risk of missing one allele is almost always present. To obtain the correct genotype, it is necessary to use 100 pg of DNA as a template. When the quantity of

Table 1
Results of four TH01 typing experiments at a heterozygous locus (TH01 type = 7–11) for five different concentrations of template DNA

Exp. no.	Amount of DNA				
	10 ng	1 ng	100 pg	10 pg	1 pg
1	7/11	7/11	7/11	not detected	not detected
2	7/11	7/11	7/11	11	not detected
3	7/11	7/11	7/11	not detected	7
4	7/11	7/11	7/11	7	7

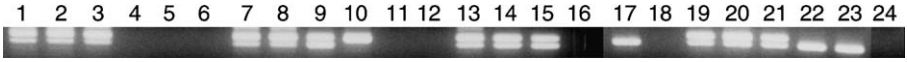

Fig. 1. Allele distribution among four experiments by semi-nested PCR. Lanes 1, 7, 13, 19 = 10 ng of template DNA; lanes 2, 8, 14, 20 = 1 ng; lanes 3, 9, 15, 21 = 100 pg; lanes 4, 10, 16, 22 = 10 pg; lanes 5, 11, 17, 23 = 1 pg; lanes 6, 12, 18, 24 = buffer control.

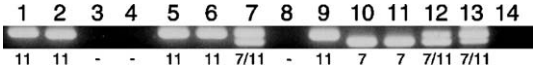

Fig. 2. Allele distribution of TH01 types 7 and 11 among 13 PCRs using 10 pg of template DNA by semi-nested PCR. Lane 14 = buffer control.

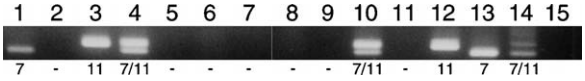

Fig. 3. Electrophoretic pattern of semi-nested PCR products using single cells as a DNA template. Lane 15 = buffer control.

extracted DNA is unknown and this quantity could be high enough to give a PCR product, but insufficient for a reliable genotyping, it is very difficult to determine genotypes correctly.

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