International Congress Series 1288 (2006) 666–668

www.ics-elsevier.com

STR loci analysis of buccal cavity cells captured by laser microdissection

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Abstract. We intended to analyze short tandem repeat (STR) loci in low copy-number DNA from buccal cavity cells. The cell smears were stained with Harris' hematoxylin and eosin for the STR analysis. Individual cells were captured by laser microdissection using a PALM Microlaser System. After the whole genome was amplified using the improved primer extension pre-amplification PCR (I-PEP-PCR) method, 15 loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1339, D19S433, vWA, TPOX, D18S51, D5S818, and FGA, as well as the Amelogenin locus were amplified using the AmpFlSTR Identifiler PCR amplification kit. We resolved the 15 STR loci and the Amelogenin locus from 20 buccal cavity cells, 13 STR loci and the Amelogenin locus from 10 buccal cavity cells, nine STR loci and the Amelogenin locus from five buccal cavity cells, and five STR loci and the Amelogenin locus from two buccal cavity cells. This is a feasible method for analyzing STR loci and the Amelogenin locus of low copy-number DNA.

Keywords: Short tandem repeat; Buccal cavity cell; Laser microdissection; Improved primer extension pre-amplification PCR

1. Introduction

Laser microdissection has enabled the selective capture of a single targeted cell from a tissue slice used for histopathologic examination. To amplify low copy-number DNA,

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0531-5131/
doi:10.1016/j.ics.2005.09.015

whole genome amplification (WGA) methods using I-PEP-PCR [1] are available. These methods are powerful tools for a single cell or a small number of cells. Therefore, we attempted to analyze the DNA of buccal cavity cells on glass slides using laser microdissection with the PALM™ Microlaser System and I-PEP-PCR. The 15 short tandem repeat (STR) loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1339, D19S433, vWA, TPOX, D18S51, D5S818, and FGA, as well as the Amelogenin locus were amplified using the AmpFlSTR Identifiler PCR amplification kit.

2. Materials and methods

Saliva samples were obtained from healthy male and female volunteers. The samples were smeared on PALM™ membrane slides (PALM Microlaser Technologies, Bernried, Germany), fixed by drying, and stained using Harris' hematoxylin and 10% eosin. The stained membrane slides were placed on the equipment stage of the microdissection system of a PALM™ Microlaser System. To remove the buccal cavity cells, the membrane slides were irradiated with a laser microbeam (337 nm UV) under a microscope. We captured 20, 10, 5, and 2 buccal cavity cells. DNA from each captured cell was extracted with a DNA Extractor FM Kit (Wako, Osaka, Japan), and WGA of the extracted DNA was performed using I-PEP-PCR [1]. The AmpFlSTR Identifiler kit (Applied Biosystems, Foster City, CA, USA) was used for the analysis of the 15 STR loci from the WGA samples. The PCR products were separated by electrophoresis using an ABI PRISM® 310 genetic analyzer (Applied Biosystems). The product size and genotype were determined using a Genescan™ (Applied Biosystems) and Genotyper™ (Applied Biosystems), respectively.

3. Results and discussion

We resolved the 15 STR loci and the Amelogenin locus from 20 buccal cavity cells, 13 STR loci and the Amelogenin locus from 10 buccal cavity cells, nine STR loci and the Amelogenin locus from five buccal cavity cells, and five STR loci and the Amelogenin locus from two buccal cavity cells. The number of detected loci increased in relation to the number of cells captured by the PALM™ Microlaser System (Table 1).

In general, a single cell contains approximately 6 pg of DNA [2]. DNA analysis using specific PCR methods requires more than 1 ng of DNA, which is equivalent to the DNA from about 200 cells. Therefore, I-PEP-PCR must be performed for WGA before performing specific PCR [1]. Schneider et al. [3] reported that the WGA method produces allele dropout and a loss of peak

Table 1
Detectable rate of STR loci and Amelogenin from buccal cavity cells

	Number of buccal cavity cells analyzed			
	20	10	5	2
15 STR loci and Amelogenin	50%	25%	12.5%	0%
5–14 STR loci and Amelogenin	50%	75%	87.5%	62.5%
1–4 STR loci and Amelogenin	0%	0%	0%	37.5%
Average number of detected loci	14.4 ± 1.9	13.1 ± 2.3	9.3 ± 3.6	5.9 ± 2.2

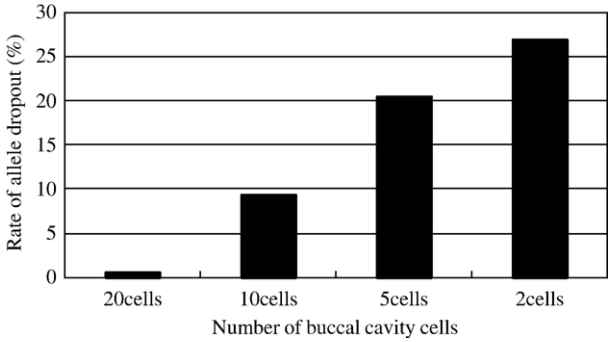

Fig. 1. Rate of allele dropout vs. cell number for WGA of DNA from buccal cavity cells.

balance. As shown in Fig. 1, their results are in agreement with our data for the WGA. The rate of allele dropout in our experiment was 26.9% in two cells (Fig. 1), and this is regarded as acceptable for the analysis of low copy-number DNA using the WGA method. It is feasible to use this method to analyze the STR loci and the Amelogenin locus of low copy-number DNA. After technical improvements, we should be able to perform the STR loci analysis on a single cell from mixed seminal/vaginal secretion stains and tissue slice specimens in future studies.

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