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Fluorescent labeling and isolation of male cells

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Abstract. This study was prompted by the well-known problem concerning unsuccessful detection of male DNA profiles in unfavorable mixtures of male and female cells. Different mixtures of male and female cell samples were stained, using a fluorescence-in-situ-hybridisation kit (Vysis), which includes different color probes for the X- and the Y-chromosome. The male cells were isolated via the SL μCut LMD system from Molecular Machines and Industries AG (MMI). STR profiling of hybridized cells enabled a full profile to be obtained from samples containing at least 30 cells. Samples with 20 cells yield a partial profile with one or two locus dropouts. © 2005 Published by Elsevier B.V.

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1. Introduction

Laser capture microdissection (LMD) is a relatively new technique for the isolation of single cells. In forensic science, for example, LMD is used for tissue specific selection of fetal and maternal components from fetal products of conception for parentage testing [1], as well as the selection of sperm out of Haematoxylin/Eosin stained vaginal smears [2,3]. In particular in cases with low sperm numbers, aspermatozooic perpetrator, or individuals with vasectomies, it could be profitable to isolate male cells in general, rather than of focusing on sperm only. In previous studies we therefore used a Y-specific, digoxigenin labeled probe for the detection of male cells in mixtures with female cells [4]. In this study we exchanged the Y-specific probe for the fluorescent labeled X/Y-probe CEP X SpectrumOrange™/Y SpectrumGreen™ DNA-probe (Vysis), which includes probes for both the X- and the Y-chromosome. The X-specific probe, labeled with a fluorescent red dye, hybridizes to multicopy alphoid DNA located at the centromere. The Y-specific probe

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hybridizes to Satellite-III-DNA located on Yq12 and is labeled with a green fluorescence dye. The simultaneous detection of the X- and the Y-chromosome may be seen as an internal positive control for the success of hybridization. False negatives can now been distinguished from female cells. Moreover, since male and female cells show different signals, this method can be extended to all mixtures containing biological material from different genders.

Different mixtures of male and female buccal and blood cell samples were stained, and the male cells were isolated by means of the SL μCut LMD system from Molecular Machines and Industries AG (MMI, Glattburg, Zurich, Switzerland).

DNA was isolated from the LMD separated cells and a STR profiling was performed. In parallel we determined the overall content of male DNA of the different mixtures using the Quantifiler Human and Quantifiler Human Male DNA Quantification Kits (AB, PE Corporation, Forster City, CA, USA).

Simulating casework samples, the method was also optimized for air-dried samples.

2. Materials and methods

2.1. Sampling

Blood and buccal cell samples were taken from a male and a female individual, and mixtures of different portions of these samples were added to the same pretreated (poly-L-lysine solution P 8920, Sigma) slide. The solutions were spread out over an area of 1 cm^2. Some of such prepared slides were used directly for further treatment; others were air-dried for 1 week, and the cells were then transferred to another slide to simulate handling of dried bloodstains. After drying at 37 °C, the slides were incubated in 0.8% Na-citrate buffer for 30 min at 37 °C, fixed in glacial acetic acid/methanol 3:1 for 10 min at −20 °C and air-dried.

2.2. Enzymatic digestion and hybridization

Samples were treated with pepsin solution (0.1% in 0.1N HCL) at 37 °C up to 2 h. 8 μl of the fluorescent labeled X/Y-probe CEP X SpectrumOrange™/Y SpectrumGreen™ DNA-probe (Vysis, Downers Grove, IL, USA) were applied on the sample area of the slide, covered with a glass coverslip, and hybridization was carried overnight.

2.3. LMD and DNA profiling

From hybridized slides, 10, 20, 30 and 40 cells were isolated using the SL μCut LMD system (MMI). DNA was extracted using the QIAamp® DNA Micro Kit from Qiagen (Hilden, Germany), according to the manufacturer's instructions. PCR profiling was performed using the AmpFlSTR SGM Plus Kit (Applied Biosystems, PE Corporation, Foster City, CA, USA) and a 34-cycle program. The PCR products were analyzed on an ABI PRISM 3100 Avant capillary electrophoresis system (Applied Biosystems, PE Corporation, Foster City, CA, USA).

2.4. Quantification of male DNA

The quantification of the male DNA in the different mixtures was carried out using the Quantifiler™ Human and Quantifiler™ Human Male DNA Quantifikation Kits (Applied

Biosystems, PE Corporation, Foster City, CA), according to the user manual and a 7300 real time PCR sequence detection system from Applied Biosystems.

3. Results and discussion

Male cells show a green and a red signal and can clearly be distinguished from female cells, which show two red signals. Definite signals facilitate detection. Thus, this method can be extended to all mixtures containing biological material from different genders, which is a real advantage over the digoxigenin method [4]. The hybridization could also be optimized for dried stains, which is an essential forensic application.

LMD of hybridized cells made is possible to get a full STR profile from samples containing at least 30 cells. Samples with 20 cells yield a partial profile with one or two locus dropouts. These results are comparable with earlier published studies [2–4]. Thus, the entire digestion and hybridization procedure does not significantly influence the sensitivity of DNA profiling. In contrast, DNA profiling of the entire mixed samples without LMD and simultaneous quantification of the male component with real time PCR revealed that a definite male profile could clearly be derived from the mixture down to a ratio of 5%. Higher dilutions showed profiles with no male alleles at all or profiles in which the male alleles could not clearly be distinguished from stutter peaks and/or the female ones.

In summary, we believe that the results of our study reveal that fluorescent staining in combination with LMD seems to offer a real advantage when dealing with unfavorable male/female cell mixtures. The success of the method, of course, depends on the total amount of cells from the minor component in the mixture. However, a great deal of extremely optimization work is needed because, based on our investigation, it would appear difficult to find standard protocols for all different kinds of biological material and all kinds of stains differing in age and carrier material.

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