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Assigning forensic body fluids to DNA donors in mixed samples by targeted RNA/DNA deep seqeuncing of coding region SNPs using ion torrent technology

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ABSTRACT

Biological traces found at crime scenes can be analyzed to genetically identify the donor(s) but also to determine the body fluid composition of the stain. The latter can be accomplished with high specificity by mRNA profiling. In some mixed body fluid samples, it might be of probative value to directly associate each body fluid with each of the DNA donors. Associating a DNA profile to the contributing body fluid is not often possible, with the exception of simple binary mixtures that contain male and female specific body fluids. However genomic information is transferred from DNA to mRNA, and RNA-cSNPs (coding region SNPs) should be identifiable by MPS methods. Previously, we have performed proof-of-concept studies using a prototype Illumina MiSeq MPS cSNP assay to demonstrate the usefulness of this approach. Here, we have continued our work and introduce an MPS assay on the Ion S5 system comprising a set of 21 cSNPs in body fluid specific mRNA transcripts (7 blood (3 genes); 8 semen (4 genes); 6 saliva (4 genes)). Combined with optimized primer sets for body fluid identification, the assay can identify all forensically relevant body fluids and skin as well as differentiating blood, semen and saliva transcripts from different individuals.

The assay has been evaluated with numerous donors of each body fluid to evaluate the specificity and the discriminatory power of the cSNPs. The findings are promising as we were able to associate donors with body fluids in mixtures of body fluids. However, more cSNPs are needed to improve the discriminatory power, particularly for vaginal secretions and menstrual blood transcripts. Assigning body fluids to DNA donors is a realistic possibility with the continued development of MPS cSNP assays.

1. Introduction

Despite the ability to definitively identify the body fluids present in a mixture, it is currently not possible to associate the component DNA profiles with specific body fluids, a requirement in order to meet the goal of obtaining probative objective 'activity level' information in criminal investigations. Coding region SNPs (or RNA-SNPs), judiciously chosen to be present in the body fluid specific mRNA biomarkers targeted and sequenced as part of the body fluid identification assay will, for the first time, permit an association of a DNA profile with a specific body fluid or tissue in admixed samples. Previously, we have performed proof-of-concept studies using a prototype Illumina MiSeq MPS cSNP assay to demonstrate the usefulness of this approach [1]. Here, we have continued our work and introduce an MPS assay on the Ion S5 system comprising a set of 21 cSNPs in body fluid specific mRNA transcripts (7 blood (3 genes); 8 semen (4 genes); 6 saliva (4 genes)). Combined with optimized primer sets for body fluid identification, the assay can identify all forensically relevant body fluids and skin as well as differentiating blood, semen and saliva transcripts from different individuals.

2. Materials and methods

2.1. Body fluid samples

Body fluid samples were collected from volunteers using procedures approved by the University of Central Florida's Institutional Review board. Blood, semen, saliva, vaginal secretions and menstrual blood samples were collected as previously described [2]. Admixed samples

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were prepared by placing two dried stains in the same extraction tube and co-extraction. Additional mixtures were prepared by depositing a liquid body fluid on top of a dried body fluid or mixing liquid body fluids together prior to drying.

2.2. RNA extraction, DNase and quantitation

Total RNA was extracted from body fluid samples using an RNA organic extraction as previously described. Samples were DNase-treated and quantitated as previously described [2].

2.3. DNA extraction and quantitation

DNA was extracted from body fluid samples using the QIAGEN EZ1 DNA Investigator kit and the QIAGEN EZ1 Advanced XL instrument. DNA extracts were quantitated using the Quantifiler™ Trio DNA Quantification kit (ThermoFisher Scientific) according to the manufacturer's protocol.

2.4. Library preparation, quantification and sequencing

Libraries were prepared using 25 ng of total RNA (RNA cSNP assay) and 10 ng of DNA (corresponding DNA reference assay) and the Ion AmpliSeq™ kit for Chef DL8 (24 cycles; 4 min anneal; ThermoFisher Scientific). The pooled libraries were quantitated using the Ion Library TaqMan™ Quantitation kit according to the manufacturer's protocol (ThermoFisher Scientific). Automated template preparation was performed using 50 pM libraries and the Ion 510™ & Ion 520™ & Ion 530™ kit – Chef and Ion 520™ chips (ThermoFisher Scientific). Sequencing was performed using the Ion S5 instrument (200 bp reads, 500 flows, plugins: HID_SNP Genotyper_5_2_2 (DNA assay), Coverage Analysis and AmpliSeqRNA (RNA assay)).

3. Results and discussion

We have developed a new MPS assay on the Ion S5 system comprising a set of 21 cSNPs in body fluid specific mRNA transcripts (7 blood (3 genes); 8 semen (4 genes); 6 saliva (4 genes)). Combined with optimized primer sets for body fluid identification, the assay can identify all forensically relevant body fluids and skin as well as differentiating blood, semen and saliva transcripts from different individuals. The assay has been used to successfully perform body fluid identification on mumerous samples of each body fluid type and skin, as well as cSNP sequencing for blood, semen and saliva samples. All RNA genotypes were confirmed using the corresponding DNA cSNP reference assay. For blood, 11 samples have been genotyped and 11 unique cSNP genotypes were obtained (PD > 0.91). For semen, 19 samples have been genotyped and 19 unique cSNP genotypes were obtained (PD > 0.95). For saliva, 15 samples have been genotyped and 8 unique genotypes were obtained (PD > 0.82).

To demonstrate the usefulness of this assay in associating an individual donor with a contributing body fluid, 2-person reciprocal mixtures were prepared in which one mixture contained body fluid 1 from donor 1 and body fluid 2 from donor 2, and the second mixture contained body fluid 1 from donor 2 and body fluid 2 from donor 1. The samples consisted of blood-saliva, semen-saliva and blood-semen admixtures. Two donor sets for each type were prepared with the exception of the blood-semen mixture in which only one donor set was used. For each of the mixtures, both body fluids were correctly identified and cSNP RNA genotyping was performed. A cSNP genotype was obtained for each of the body fluid donors in the mixture. Comparison of the obtained cSNP genotypes to reference DNA genotypes permitted an association of each body fluid to the correct donor in each of the admixtures. These results demonstrate the unique and novel ability to now directly associate a DNA profile to a contributing body fluid in an admixed sample.

Continued work will be performed on this assay including an evaluation of additional mixture samples (including more than 2-person mixtures), population studies, an evaluation of allele specific expression variation and the development of a probabilistic framework for interpretation.

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Declaration of Competing Interest

None.

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