MATERIALS AND METHODS FOR DETECTING SOURCE BODY FLUIDS

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a divisional application of U.S. application Ser. No. 15/921,079, filed Mar. 14, 2018, the disclosure of which is incorporated herein by reference in its entirety, including any figures, tables, and drawings.

GOVERNMENT SUPPORT

This invention was made with government support under 2015-R2-CX-0012 awarded by National Institute of Justice. The government has certain rights in the invention.

The Sequence Listing for this application is labeled "SeqList-08Mar18-ST25.txt", which was created on Mar. 8, 2018, and is 6 KB. The Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

DNA is used to match a sample retrieved from a crime scene with DNA retrieved from a suspect to identify a connection of the suspect to the crime scene. Current DNA analyses do not permit identifying the source of DNA from a suspect. However, certain forensic cases, such as sexual abuse require confirmation that a DNA from a suspect is from an intimate body fluid.

Techniques currently used for body fluid identification are not based on DNA analysis. For example, microscopic observation of sperms is used to identify semen as a source body fluid or histological staining of glycogen-rich cells is used to identify vaginal cells. However, these tests are not reliable. For example, if the male donor does not produce sperm, the source cannot be identified as semen. Similarly, false negatives can occur because the glycogen content of vaginal cells varies depending on the menstrual cycle and reproductive age; whereas, false positives can occur because buccal and urogenital skin cells (even from males) can have high glycogen.

Certain other methods are based on protein/enzyme reactivity or cell staining and are merely presumptive. These methods may have low sensitivity and render the portion of the sample useless for subsequent analysis. Therefore, forensic laboratories may be left to choose between isolating DNA to compare a suspect's DNA or determining body fluid of origin.

Certain other methods of identifying source body fluid are based on analyzing RNA transcripts. However, the need to identify a body fluid often arises after DNA is isolated. To perform an RNA transcript analysis, the laboratory technician would have to retrieve a new portion of the original sample (if available) and isolate RNA. However, the original sample may have already been consumed.

BRIEF SUMMARY OF THE INVENTION

The invention provides methods that avoid the problems and difficulties with current methods of detecting body fluids in a sample, particularly, a forensic sample. The methods of the invention depend on the analyses of levels of DNA methylation at specific genetic loci to detect specific body fluids.

In one embodiment, the body fluid and/or cells present in the sample comprise vaginal secretion or vaginal epithelial cell, semen or sperm, saliva or buccal epithelial cell, and blood or blood cell.

The level of methylation at specific loci in the genomic DNA isolated from a sample can be determined by high-resolution melt analysis (HRM) of amplicons produced using specific primers designed to amplify the specific loci.

A further embodiment of the invention provides a method for determining the level of methylation at specific loci in the genomic DNA isolated from a cell, for example, a cell suspected to be a vaginal epithelial cell, buccal epithelial cell, sperm, or blood cell isolated from a forensic sample.

Kits containing primers and reagents for carrying out the methods disclosed herein are also provided.

Assays for determining the level of methylation at specific loci in the genomic DNA isolated from a sample are also provided. In certain embodiments, the assays comprise HRM or pyrosequencing of amplicons produced using specific primers designed to amplify specific loci in the genomic DNA.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. **1** provides melt curves for the amplified region ZC3H12D, which is located on human chromosome 6. The blue lines represent semen samples and have a lower TM when compared to blood (red lines) and saliva (green lines). For this marker (ZC3H12D), the typical TM for semen is 75.5° C.±0.2° C., whereas the TM for blood is 78.2° C.±0.4° C. and saliva 78.1° C. with 0.3° C. SD.

FIG. **2** provides melt curves (−dF/dT) for the amplified region VE_8 (cg08751438). The pink lines represent DNA samples from vaginal epithelia, blue represents semen, red represents blood and green represents saliva.

FIG. **3** provides melt curves (normalized fluorescence versus temperature in degree Celsius) for the amplified region VE_15. The pink lines represent DNA samples from vaginal epithelia, blue represents semen, red represents blood and green represents saliva.

FIG. **4** provides melt curves (normalized fluorescence versus temperature in degree Celsius) for the amplified region INPP5D200_TM60. Orange lines represent DNA samples from vaginal epithelia, blue represents semen, red represents blood and green represents saliva.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1: Sequence of the locus specific for vaginal epithelial cell.

SEQ ID NO: 2: Sequence of a forward primer designed to amplify the locus specific for vaginal epithelial cell.

SEQ ID NO: 3: Sequence of a reverse primer designed to amplify the locus specific for vaginal epithelial cell.

SEQ ID NO: 4: Sequence of the locus specific for vaginal epithelial cell after bisulfite treatment assuming 100% unmethylation of all CpG sites.

SEQ ID NO: 5: Sequence of the locus specific for vaginal epithelial cell after bisulfite treatment assuming 100% methylation of all CpG sites.

SEQ ID NO: 6: Sequence of the locus specific for sperm.

SEQ ID NO: 7: Sequence of a forward primer designed to amplify the locus specific for sperm.