Illuminating Evidence

The Development of a Method for Labelling Forensically Relevant Cells

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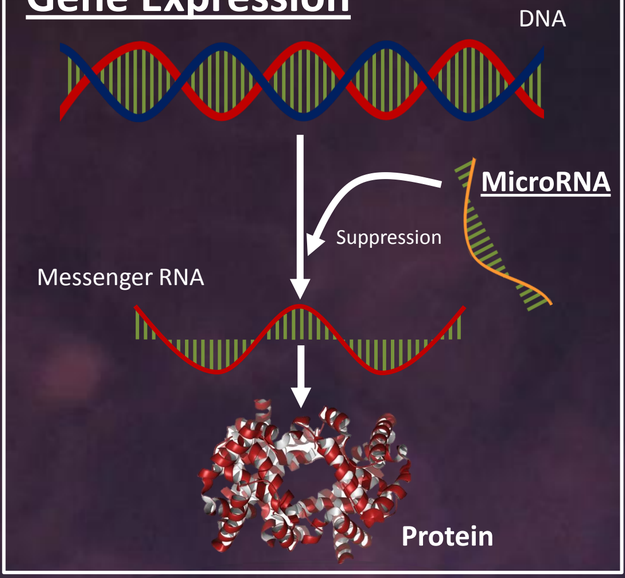
Why label cells?

Accurate, specific and sensitive testing for body fluid identification is an important part of forensic testing [1]. Genetically based messenger RNA profiling has come to the forefront to identify biological material [2-4], but becomes complex when mixtures of fluids from multiple donors are present[5].

This may be overcome by looking at the individual cells present, using targeted fluorescent labelling in a technique known as fluorescent *in situ* hybridisation, or FISH [6]. FISH uses a fluorescent probe designed to be complementary in sequence to a target nucleic acid, that will bind to the target molecule. The bound probe will be visible as a fluorescent signal using fluorescence microscopy.

By targeting molecules specific to forensically relevant cell types, the cells can be biologically identified, separated and undergo DNA analysis. To date this research has successfully developed a method for FISH labelling saliva, blood, vaginal and seminal cells by targeting a microRNA molecule.

Gene Expression



Why microRNA?

MicroRNAs are small, non-coding nucleic acid molecules. The active form of a microRNA is only 20-24 nucleotides in length [7]. MicroRNAs are a relatively recent discovery in genetics and their structure, sequence and function are still being explored, with novel microRNAs discovered all the time [8,9].

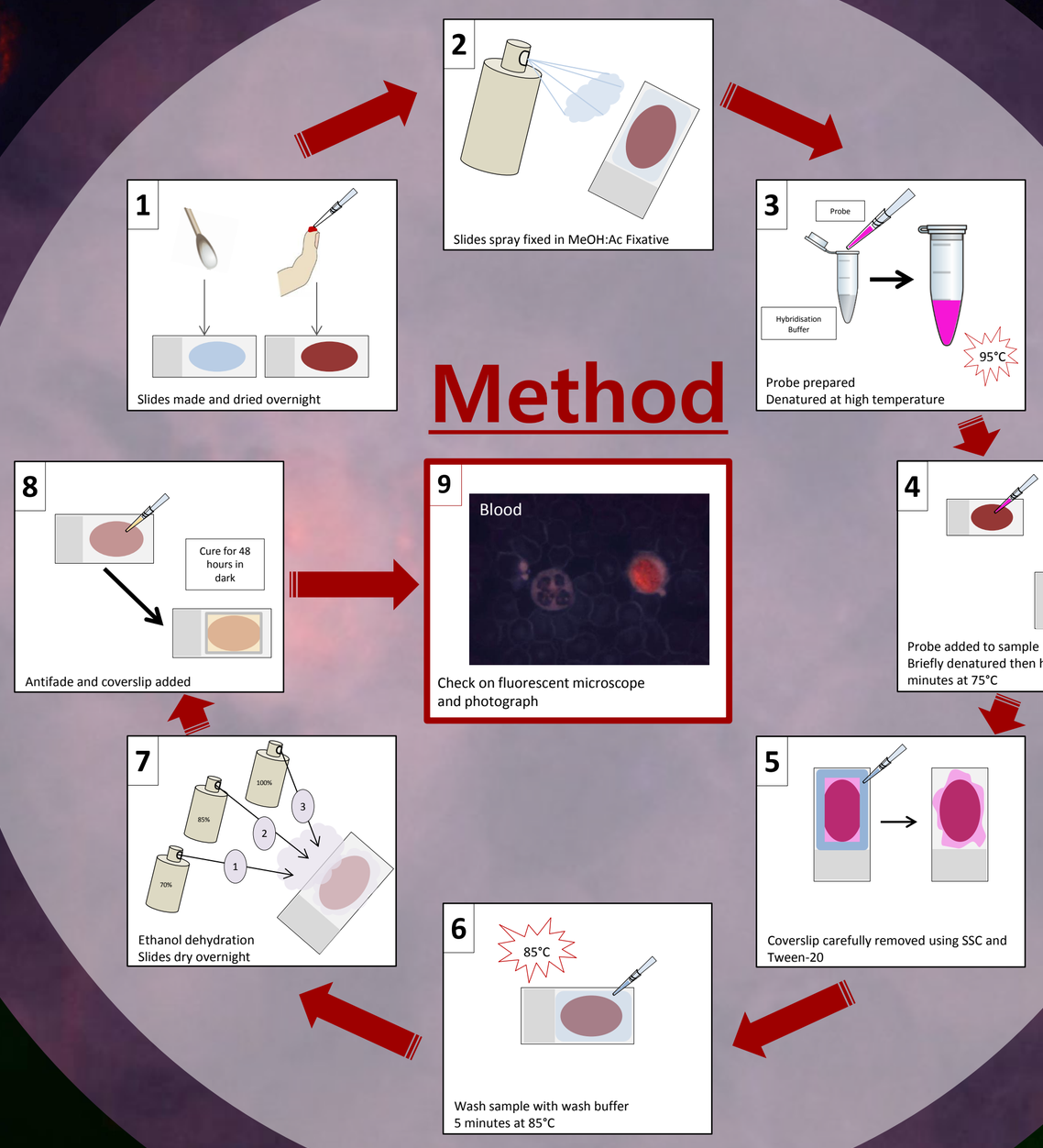
The main role of microRNAs is the control of gene expression [7]. MicroRNAs can bind to messenger RNA molecules, and when bound will prevent the mRNA sequence being used as a template for protein synthesis. This means that microRNA expression may vary between different kinds of cells and thus be utilised for cell identification [7, 10].

From a forensic perspective, microRNAs show potential as their small size means that they are more resistant to degradation than larger messenger RNA molecules [10]. This could be critical for cases where the samples are degraded, have been exposed to the environment, harsh cleaning chemicals, or are aged.

Blood

Vaginal Fluid

Method



Sperm

Saliva

Results

The methodology developed and outlined above was used to successfully label the four target cell types using slide FISH. The probe used for this FISH protocol was designed to bind to microRNA 891a, and the Alexa Fluor® 594 was attached to the probe, causing the bound probe to fluoresce red when excited and viewed through a filter on a fluorescent microscope. Examples of this successful labelling are shown above Top Left, Blood; Lower Left, Semen; Top Right, Vaginal Fluid; Lower Right, Saliva.

References

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Where to from here?

With the methodology optimised for the labelling of all four cell types with the microRNA target, the next stage of this research will be considering specific microRNA markers for leucocytes, spermatozoa and salivary and vaginal epithelial cells.

MicroRNAs for body fluid identification using profiling polymerase chain reaction or next generation sequencing is an area being extensively investigated at the moment[7-15], and this literature will form the basis for the design of targeted FISH probes for specific microFRNAs for these body fluids.

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