A14 Single Channel Simultaneous Analysis of DNA and MicroRNA

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After attending this presentation, attendees will be aware of a new proposed protocol that will analyse DNA and miRNA simultaneously.

This presentation will impact the forensic science community by exhibiting a new protocol that will expand the capability of forensic science laboratories with minimal modifications and expenses.

It is often necessary to establish the identity of the body fluid from which a DNA profile was obtained. This issue is frequently encountered in sexual offences where it can be necessary to distinguish between saliva and vaginal material or where there is a very small amount of the body fluid present, such as trace amounts of blood on a dark surface. This issue is currently being resolved by the use of mRNA and microRNA (miRNA) analysis utilising a co-isolation technique to separate out the DNA and the miRNA phases for subsequent analysis.

This study presents an alternative protocol in that the DNA and miRNA are co-isolated without physical separation. All downstream processes are carried out simultaneously through a single channel process resulting in a single electropherogram indicating both the DNA profile obtained from the body fluid stain and the identity of the body fluid.

A number of blood and saliva samples were collected from volunteers (with informed consent). Blood was collected using the finger prick method and depositing blood onto a filter paper. Saliva was collected using buccal swabs. Dual extraction was carried out using the QIAamp DNA Mini Kit with no modifications. miRNA was present in the eluents due to their small size and abundance within each cell. The DNA/miRNA sample then underwent cDNA synthesis by carrying out stem-loop reverse transcription PCR, which reverse transcribes and lengthens the miRNA marker prior to amplification.

Amplification was carried out using a modified version of the Amp*f*STR NGMSElect kit (ABI). The modification was the incorporation of labelled miRNA primers. The primers used are complementary to miR-205 and miR-451 markers which are specific to saliva and blood, respectively. No other modifications to the amplification protocol were made.

Following capillary electrophoretic separation on the ABI Prism 310 genetic analyser, full DNA profiles were obtained matching the volunteer DNA profiles. In all blood samples, a peak indicating the presence of miR-451 was generated and in all saliva samples, a peak indicating the presence of miR-205 was generated indicating a 100% correct identification rate (n=15). Small peaks were observed in the miR-205 bins for blood and vice versa; however, such peaks were considerably smaller than the expected peaks and in many cases were less than 150 rfu.

Reverse transcription negative controls were carried out by using sterile water in place of the MMLV reverse transcriptase. In all cases, no discernable peaks were obtained within the miRNA bin sets. The DNA profiles were unaffected.

In all cases, the correct DNA profiles and the correct body fluid identity were obtained, thus demonstrating the proof of principle in that a single stream simultaneous analysis of DNA and miRNA is possible and robust. The advantages of this technique are that only minor modifications are required to install the BFID capability into a forensic science laboratory. The single work stream element means that there are reduced opportunities for contamination, and it is cost-effective. Finally, this single stream simultaneous process means that it may be possible to definitively associate a DNA profile with a particular body fluid.

Future work will involve the identification and characterization of further body fluid markers with a view to developing a full BFID panel multiplexed with a DNA profiling system.

DNA, miRNA, Co-isolation

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