Extraction to Electrophoresis – Verifying Identity of Remotely Collected Blood Samples

Emma Fischer[1], Jennifer Mook[1], James Rudge[2]

[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711; [2]Neoteryx LLC, 421 Amapola Ave, Torrance, CA 90501

1. Introduction

Remote sample collection is a growing market that provides a convenient alternative to the traditional collection of biological samples in a variety of areas, such as drug monitoring and development, toxicology and infectious disease research, and numerous clinical applications. This method introduces the ability to collect anywhere, saving substantial time for the user and researchers. However, with sample collection taking place outside of the traditional, controlled setting, a need arises for authenticating sample identity. Here we present a straightforward workflow of DNA extraction, quantification, and STR (short tandem repeat) amplification with the *GenePrint*® 24 System to verify the identity of blood samples collected remotely on Neoteryx® Mitra® microsampling devices containing VAMS® technology.

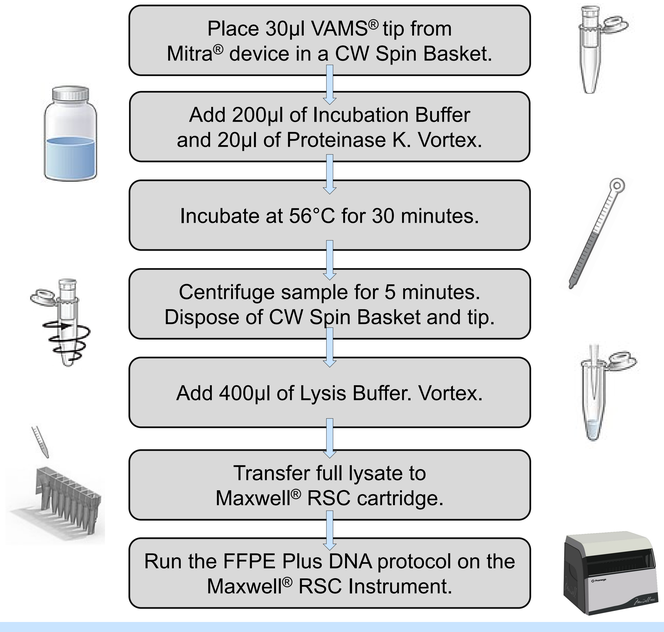
2. Remote Sampling with Mitra® Devices

Mitra® devices are ideal for easy, at-home collection of blood and other biological fluid samples by individuals or their caregivers. One device provides sufficient sample volume for both analyzing a wide range of analytes and confirming a genetic profile in applications where human identity testing is necessary and/or useful.

End-users receive Mitra® devices in a collection kit that contains all supplies needed for precise self-collection of samples. Collected samples are mailed directly to a laboratory for processing and analysis. Mitra® microsamples are transported and stored at ambient temperatures, eliminating the need for cold shipping/storage. At the lab, the absorptive VAMS® tip is easily removed from the Mitra® device to make it compatible with DNA extraction workflows.

3. DNA Purification

DNA purifications completed using the Maxwell® RSC FFPE Plus DNA Kit:



To serve as reference samples, buccal swabs were collected and purified using the Maxwell® RSC Buccal Swab DNA Kit. Alternative reference samples, such as blood, may also be used.

4. DNA Quantification

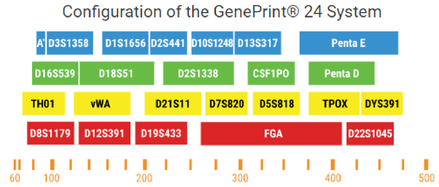
Determine DNA concentration using a fluorescent dye-based or amplification-based method. For the purposes of this poster, DNA eluates were quantified with both methods.

Fluorescent dye-based Quantification:
DNA eluates were quantified using the QuantiFluor® ONE dsDNA System on a Quantus™ Fluorometer. This system uses a fluorescent double-stranded DNA binding dye. Dye-based quantification is cost effective but is not specific to human DNA.

Amplification based Quantification:
DNA eluates were quantified using the ProNex® DNA QC Assay. This system is a multiplex qPCR-based method that amplifies 75, 150, and 300bp human DNA targets and an internal positive control. For complex samples (ex: microbiome), a qPCR-based human-specific quantification method may be more appropriate.

5. The *GenePrint*® 24 System

The *GenePrint*® 24 System is designed to generate a multi-locus human DNA profile from a variety from human-derived biological samples. 22 polymorphic STR loci and two loci for sex determination are co-amplified and visualized using capillary electrophoresis.



2.5ng of DNA input from each sample was PCR amplified in a single multiplex reaction using the *GenePrint*® 24 System. Following amplification, samples were analyzed by capillary electrophoresis using the Spectrum Compact CE System. The ranges and dye colors of the included loci are shown, along with the included size standard.

6. Spectrum Compact CE System

Preparing the CE instrument for electrophoresis
Prior to capillary electrophoresis, a spectral calibration was performed on the Spectrum Compact CE System using the *GenePrint*® 5C Matrix Standard and Polymer7.

Preparing samples for electrophoresis
Fluorescently-labeled PCR products were mixed with an internal sizing standard and heat-denatured with Hi-Di™ Formamide to prepare for capillary electrophoresis.

Capillary electrophoresis
Prepared samples were injected on the Spectrum Compact CE System using the pre-loaded fragment analysis conditions for Promega's 5-dye chemistries with Polymer7.

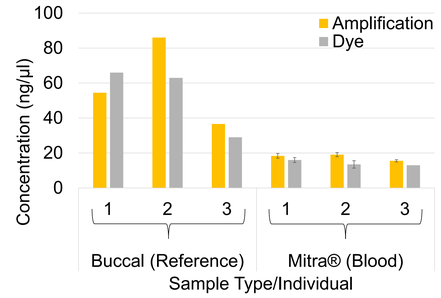
Injection voltage	1.5 kV
Injection time	9 seconds
Run voltage	13 kV
Run time	1290 seconds
Oven temperature	60°C

Data analysis
Capillary electrophoresis data was analyzed with the GeneMapper™ IDX v1.6 software using the appropriate panel, bin, and analysis files available on Promega.com.

7. DNA Quantification Data

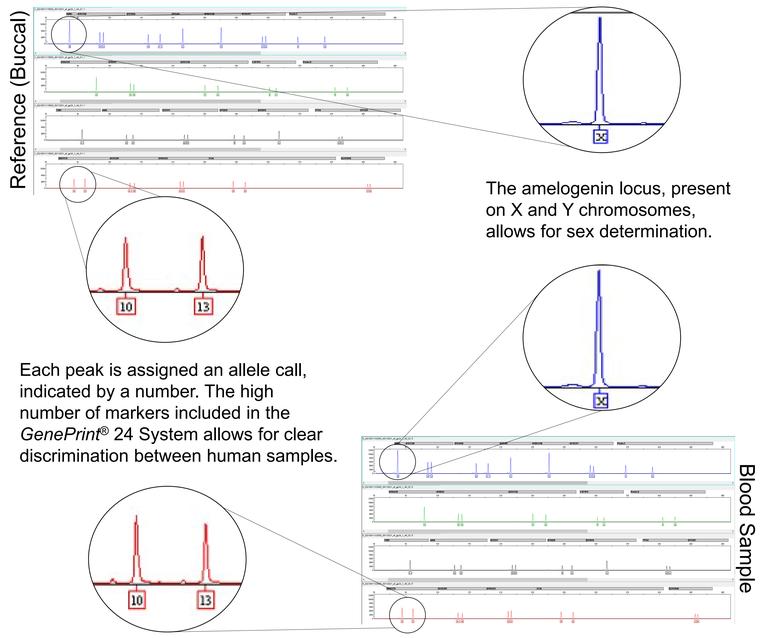
DNA was extracted from a single reference buccal swab and duplicate Mitra® blood microsamples as stated in section 3.

DNA eluate quantification was similar using QuantiFluor® ONE dsDNA (dye-based) or the ProNex® DNA QC Assay (amplification-based). Consistent DNA concentrations were observed across the Mitra® microsamples.



8. Representative Electropherograms

GenePrint® 24 PCR products of each purified DNA sample were separated on the Spectrum Compact CE System. The loci and allele calls were concordant when compared between the reference sample and Mitra® microsamples.



The amelogenin locus, present on X and Y chromosomes, allows for sex determination.

Each peak is assigned an allele call, indicated by a number. The high number of markers included in the *GenePrint*® 24 System allows for clear discrimination between human samples.

9. Conclusions

We have provided a full workflow including DNA extraction, DNA quantification, STR amplification, and capillary electrophoresis that can be used to confirm the identity of blood samples collected remotely on Neoteryx® Mitra® microsampling devices.

- Consistent high-quality DNA was purified using the Maxwell® RSC FFPE Plus DNA Kit from 30µl blood samples collected using Mitra® devices.
- Concordant loci and allele calls were observed using the *GenePrint*® 24 System for each paired reference buccal swab and Mitra® blood microsample.

scientificapplications@promega.com jamesr@neoteryx.com