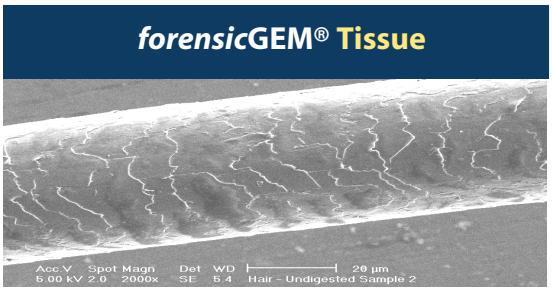

SEM Image of human hair shaft

Extraction of DNA from Hair using *forensic*GEM® Tissue

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Introduction

Hairs found at crime scenes can be important trace evidence, and in some cases are the one and only link to a suspect. As a source of DNA, hair can prove vital in forensic human identification. Nevertheless, most methods for the extraction of DNA from hair involve a lengthy process that requires extensive manual manipulation. The quality of forensic DNA profiles obtained from samples relies on the critical step of DNA extraction to produce sufficient template. The typical DNA amount extracted from plucked hair (with root) ranges from 1-750 ng/root and from shed hair (with root) from 1-10 ng/root[1].

Here we present a quick method for the extraction of DNA from hair using ZyGEM *forensic*GEM® Tissue. This method has been used successfully in our laboratory and it has proven to be a better alternative to other, more time-consuming protocols (for example phenol-chloroform extraction).

Methods

Preparation of samples

Hair samples were collected from donors in the laboratory by pulling hair, either manually (scalp) or with forceps (eyebrows). Samples to be extracted were placed in individual microcentrifuge tubes. The following steps are recommended:

1. In order to reduce the amount of contaminants that may be on the surface of the hair, it may necessary to thoroughly wash the hair as follows:
   - For evidentiary hairs, which are loose, fill a clean container (beaker or tube) with a sufficient volume of sterile deionized water. Using clean forceps, immerse one hair into the water and wash gently (wash each hair separately in fresh water).
   - For mounted hairs it may be necessary to freeze the slide at approximately -20ºC for 20 minutes. Pry off the cover slip with a scalpel. Using a glass disposable pipette, wash away the mounting medium by squirting with xylene. Using clean forceps, place the hair into a microcentrifuge tube containing absolute ethanol. Gently wash the hair and then place it in a clean microcentrifuge tube, which contains sterile, deionized water. After gentle washing, the hair is ready for microscopic examination.
2. Using a stereozoom microscope, the hair should be examined to ensure that it is clean and does not have any extraneous biological material adhering to its surface. Microscopic examination should be conducted to document the hair roots growth phase. Hair roots that are in the active growing phase (anagen) contain an abundance of nucleated cells in the root and in the surrounding sheath material. Telogen hairs without follicular tissue may not be amenable to nuclear DNA analysis because of the lack of nucleated cells[2].
3. If the hair is clean, cut off apporximately 5 to 10 mm of the root end for digestion.

Extraction

1. 89 µl water, 10 µl ZyGEM 10x Buffer and 1 µl of *forensic*GEM were added to each sample.
2. The samples were vortexed for approximately 15 seconds and then were spun in a microcentrifuge for 15 seconds 10,000 – 15,000 rpm.
3. The samples were heated to 75°C for 15 minutes in a heat block.
4. The samples were then transferred to a 95°C heat block for 5 minutes.

Quantitation and profiling

Extracts were quantified using the Applied Biosystems Quantifiler® Human Kit[3] and Applied Biosystems PRISM® 7000 Real-Time PCR System. Extracts were amplified using the Applied Biosystems AmpFlSTR® Identifier® Kit[4]. Amplification products were run electrophorethically in the ABI PRISM® 310 Genetic Analyzer.

Results

Eleven of the twenty samples gave full profiles. Seven produced partial profiles. Two samples did not produce any profile.

Description	Sample	Total DNA (ng)	310 Results
Hair	Batch 1 H1	0.0565	13 loci
Hair	Batch 1 H2	0.0201	14 loci
Hair	Batch 1 H3	4.46	16 loci
Hair	Batch 1 H4	2.01	16 loci
Hair	Batch 2 H1	2.21	16 loci
Hair	Batch 2 H2	0.00655	7 loci
Hair	Batch 2 H3	0.51	13 loci
Hair	Batch 2 H4	0.79	15 loci
Hair	Batch 3 H1	0.47	16 loci
Hair	Batch 3 H2	0.095	16 loci
Hair	Batch 3 H3	0.0139	9 loci
Hair	Batch 3 H4	1.245	16 loci
Hair	Batch 4 H1	1.64	16 loci
Hair	Batch 4 H2	0	No profile
Hair	Batch 4 H3	0	No profile
Hair	Batch 4 H4	0.0545	16 loci
Hair	Batch 5 H1	0.0067	14 loci
Hair	Batch 5 H2	1.925	16 loci
Hair	Batch 5 H3	0.01065	16 loci
Hair	Batch 5 H4	0.38	16 loci

No concordance results could be conducted with hair samples since they are consumed during the extraction process. A reproducibility study was conducted using the same hair donor and 6 hairs were extracted in four different batches. Each produced quantifiable DNA with correct typing results.

Description	Sample	Total DNA (ng)	Results	Correct Profile
Hair	Batch 1 H3	4.46	16 loci	Yes
Hair	Batch 1 H4	2.01	16 loci	Yes
Hair	Batch 2 H1	2.21	16 loci	Yes
Hair	Batch 2 H3	0.51	13 loci	Yes
Hair	Batch 3 H1	0.47	16 loci	Yes
Hair	Batch 4 H1	1.64	16 loci	Yes

Discussion

Profiles were obtained on samples that we expected to fail using our current method. The hair samples from Batch 4 were observed under a stereoscope for root material on the hairs to predict whether we expected a DNA profile or not. For the samples analyzed, a profile was observed when expected. The profiles that produced 'no profile' were supported by the quantitation values.

Our results indicate that the *forensic*GEM® Tissue extraction kit is accurate and reliable for extracting DNA from hair samples in casework. The method is faster than our current protocol and allows one-tube extraction of DNA in approximately 20 minutes.

References

1. Butler, John M. Fundamentals of Forensic DNA Typing. Academic Press, 2010, p.101.
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3. Quantifiler™ Kits User Manual ©2005 Applied Biosystems. Part number 4344790 Rev C 5/2005
4. AmpF*l*STR® Identifiler® PCR Amplification Kit User Manual ©2006, 2010, 2011 Applied Biosystems Part number 4323291 Rev F 4/2011