Arcis Hair Follicle Nucleic Acid Extraction Protocol

Forensic analysis of hair samples in order to extract DNA is a method commonly used for the purpose of identification in both criminal investigations as well as parental DNA testing. Genetic identification and profiling is commonly through analysis of nuclear DNA. Despite the possibility of remnants of nuclear DNA in the hair shaft (in cut hairs or naturally shed hairs), analysis with this specific part of the hair will likely not yield results. Hair follicles are usually required to yield DNA as they contain keratinocytes which contain nuclear DNA.

Methods to extract DNA from hair follicles have frequently involved complex enzymatic digestions in solutions ranging from proteinase K to laundry detergent! Here we describe a protocol using the Arcis Sample Prep Kit - a ready to use, room temperature kit comprising two reagents enabling pre-analytical processing of samples. In 3 minutes, with no prior sample preparation, the kit allows you to go from follicle to downstream nucleic acid investigations without the need for isolation or purification. The kit is suitable for untreated specimens and specimens that have been frozen. The DNA released is ready for immediate use in PCR or other molecular applications. The Arcis Sample Prep Kit is intended for in vitro identification of nucleic acids or diagnostic use.

The following protocol describes the extraction of DNA from single hair follicle samples and the subsequent analysis of the genetic material extracted by the Arcis Sample Prep Kit.

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

Samples tested were from multiple samples of single hair follicles.

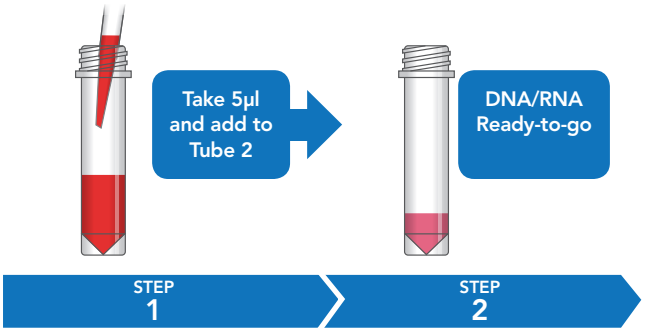
Method

- Single hair follicles were dropped into the buffer 1 Lysis solution (150µl) and agitated briefly to release the nucleic acids.
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to 20µl of Tube 2 reagent to give a 1:4 volume ratio and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec, 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC channel (540-580nm) at the annealing step.

hRNase P PCR Mix	Vol µl
Master Mix 2x	12.5
F Primer (50µM)	0.4
R Primer (50µM)	0.5
Probe (1µM)	0.5
Water	6.2
Template (tube 2)	5



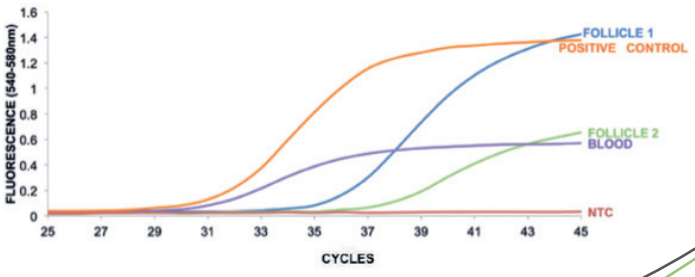
Results (Ct values averaged)

	Sample
Positive control	31.14
Hair follicle 1	35.26
Hair follicle 2	36.98
30µl blood	30.50
NTC	No amp

Conclusion

The Ct values obtained were between 35 and 37. The amplification curves were prominent and easily detected. This test may be near the limit for detection for this assay.

Hair Follicles



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