Rootless Hair DNA Purification Kit

Hair is common evidence found at crime scenes and a new sample type for medical research inquiries. It has been employed in criminology for statistics-based population work and DNA-based analysis. The most valuable DNA testing approach is a short repeat analysis of nuclear DNA, and it is conceivable when the hair's root and adherent tissue are present.

Hair's advantages in genetic genealogy

In forensic circumstances, rootless hair has distinct advantages. Hair is insoluble, storing DNA for centuries. The extracted DNA is less susceptible to microbial contamination than bone and teeth samples. Notably, a single hair is a distinct biological entity. Thus, sample mixes, a major stumbling block in DNA-based forensics studies, are not a concern for DNA generated from a single hair.

Hair structure

The hair follicle and the hair shaft (rootless hair shafts, Fig.1) are the two sections of hair from which DNA can be extracted and purified. The hair follicle can isolate cellular DNA (nuDNA) and mitochondrial DNA (mtDNA). However, the hair shaft often contains mtDNA and may have little nuclear material. Shed hair accounts for up to 90% of hair samples obtained at crime scenes. The shed hair shaft undergoes keratinization, stiffening, and inducing nucleus breakdown. Unwashed hair has a higher DNA output due to surrounding cells adhering to the hair shafts, which can be a source of nuclear DNA. Nonetheless, some nuclear DNA is known to remain in rootless hair shafts, albeit in small quantities and with highly varied quality. On the other hand, mtDNA is sufficiently intact to be isolated and used downstream.

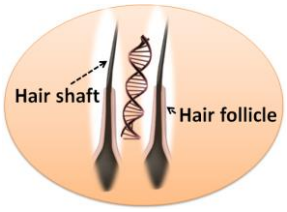

Fig.1 Hair structure

Sequencing of Mitochondrial DNA

MtDNA analysis can be utilized to connect human hair to a suspect. However, unlike nuclear DNA (nuDNA), mtDNA lacks selective potential due to its ubiquity within the maternal lineage. With a few rare circumstances where the father adds to the profile, mtDNA is inherited from the mother's side. As a result, the mitochondrial genetic composition would not distinguish a grandmother from her daughter or grandchildren, leaving the differentiation aspect to the nuDNA. When investigating a variety of hair samples, MtDNA sequencing can also provide important information in species identification. Because not all hair samples are human, this approach identifies the animal that provided the hair.

NuDNA for short tandem repeat (STR) typing

Typically, forensic laboratories will first examine hair evidence for the presence of root material. If the sample lacks root material, forensic laboratories frequently do not process it or send it for mitochondrial DNA (mtDNA) sequencing, which has historically proven more successful than nuDNA on hair shafts. Standard approaches such as STR typing may be used if nuclear DNA (nuDNA) profiling is present. Recently, Brandhagen et al. showed that, despite being fragmented, nuclear DNA could be retrieved from shed hair. Surprisingly, nuclear DNA accounts for most of the total DNA, eliminating the assumption that only mitochondrial DNA can be recovered from rootless hair and demonstrating that nuDNA is abundant compared to mtDNA. Thus, this method enabled the characterization of nuDNA in telogen hairs that would not have been achievable using conventional techniques, demonstrating yet again that nuDNA occurs in large quantities but in poor quality.

DNA extraction is the first step in the forensic analysis of rootless hair. Only picograms of ultrashort DNA can be extracted from a single rootless hair. Due to practical problems and technical reasons, optimizing the settings to maximize the yield and purity of DNA collected from diverse samples using various procedures is critical. Thus, finding an efficient, stable, and simple technique to extract DNA and amplify nuDNA targets is a major challenge in hair shaft DNA extraction and amplification. A streamlined method for extracting DNA from hair

shafts eliminates DNA contamination while significantly reducing analysis time, which would be valuable to forensic and population-based research communities.

The BcMag™ **Rootless Hair DNA Purification Kit** is designed to extract pigment-free (PCR inhibitor) nucleic acids from single rootless hair efficiently and sequentially (Fig1). The kit uses our unique proprietary lysis buffer to efficiently lyse hair cells and magnetic beads to purify hair shaft DNA.

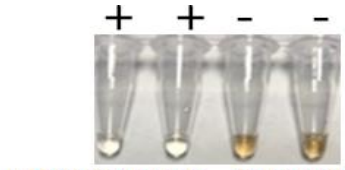

Fig.1 Hair Pigment Removal

Workflow (Fig.2)

Fig.1 Workflow of Hair Shaft DNA purification

1. Lyse the hair at 95°C for 2 hours.
2. Add magnetic beads to bind the DNA.
3. Wash the beads.
4. Elute DNA from the beads.

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	Cat #: AD101 (50 Preps)	Cat #: AD102 (100 Preps)
BcMag™ HO-DNA Beads	4°C	0. 5 ml	1.0 ml
BcMag™ Hair Pigment Removal Beads	4°C	0.3 ml	0.6 ml
1x Lysis Buffer	4°C	5 ml	10 ml
10x Binding buffer	4°C	1.5 ml	3.0 ml
1x Elution Buffer	4°C	0.75 ml	1.5 ml
Proteinase K	-20°C	10 mg	20 mg
Proteinase K Suspension Buffer	4°C	0.5 ml	1.0 ml
DTT	-20°C	75 mg	150 mg

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA Yield: Varies (depends on sample size and type. Typically, 5cm hair shaft has 100 pg)
- DNA Size: Varies (depends on the quality of starting material
- Quantification of the nucleic acids: Since there is a trace amount of DNA from the sample, use fluorescent dye to quantify. OD260 methods such as Nanodrop are not suitable.
- For long-term storage, store the extracted nucleic acids at -20°C.

A. Materials Required by the User

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- 95–100% ethanol
- 80% isopropyl alcohol
- 10x Triton X-100 (1% Triton X-100)
- 10x NaCl (5M NaCl)
- Digital Multi Heat Block
- Microcentrifuge tubes, 2 ml
- Aerosol-resistant micropipette tip
- Magnetic rack: Based on sample volume, the user can choose one of the following magnetic Racks:
  BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01)
  BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02)
  BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03)
  BcMag™ Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)

Sample	Example sample input
Rootless Hair	Up to 1mg- 5mg

A. Master mix Preparation

IMPORTANT!

- Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. For example, 10 mg dissolved in 500µl of Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
- DTT solution preparation: Provide DTT as powder and make 20% solution with ultrapure water. For example, 60 mg dissolved in 300µl of ultrapure water. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.

1. Preheat the Thermocycler or digital multi-heat block to 95°C.
2. Prepare a fresh Master Mix following Table 1 for the number of samples to be processed, plus 10% more (e.g., if you have 10 samples, prepare Master Mix for 11). Add the following components to the reservoir.

Table 1

Component	1 well (75 µL reaction volume)
1x Lysis Buffer	67.5 µL
20% DTT (add fresh)	7.5 µL

3. Use 1-5mg (~ 10 cm - 50 cm) and cut hair into small pieces.
4. Add sample to 0.2 PCR tube.
5. Add 100 uL of 1x Lysis buffer and incubate for 5 minutes at room temperature.
6. Centrifuge 14000 rpm for 5 minutes and remove the supernatant.
7. Add 75 uL of Master Mix (based on Table 1) and mix well.
   Note: Ensure the hair sample is completely covered by lysis buffer by a brief centrifuge.
8. Incubate at 95°C for 2 hours or until the hair is wholly dissolved.
9. Add 247.5 uL of 1x Binding buffer and mix well.
10. Add 10 uL of Proteinase K (20 mg/ml), mix well, and incubate at 65°C for 30 minutes.
11. Transfer all the solution to a 2 ml centrifuge tube.
12. Centrifuge at 14000 rpm for 5 minutes and transfer the supernatant to a new 1.5 ml centrifuge tube.
13. Immediately add 1.42 mL of 80% Isopropyl alcohol, 10 uL of BcMag™ HO-DNA Beads and mix well.
    Note:

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- Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
- Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.

14. Incubate at room temperature for 15 minutes with gentle rotation.
15. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack. Add 500 uL of 85% Ethanol and wash the beads by slowly pipetting up and down 20-25 times. Again, place the tube on the magnetic Rack for 1-3 minutes and remove the supernatant completely while the tube remains on the Rack.
16. Repeat step 13 once.
17. Remove the tube from the magnetic Rack.
18. Air dry for 30 minutes or dry them at 65°C for about 10 minutes (Ensure the Ethanol is completely evaporated.)
19. Add 10 uL of elution buffer for 1mg hair and 15 uL of elution buffer for 5mg hairs to elute DNA by slowly pipetting up and down 20-25 times.
20. Place the tube on the magnetic Rack for 1-3 minutes. Transfer the supernatant to a new centrifuge tube while the tube remains on the Rack.
21. Add 1µl of 10x Triton x-100 and 1µl 10x of NaCl to the eluted DNA mix well (Final concentration: 0.1% Triton x-100 and 0.5 M NaCl).
22. Transfer 6µl BcMag™ Hair Pigment Removal Beads to a centrifuge tube and place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack.
23. Combine the beads with the DNA solution and mix by slowly pipetting up and down 20-25 times. Again, place the tube on the magnetic Rack for 1-3 minutes.
24. Transfer the supernatant to a new centrifuge tube while the tube remains on the rack.
25. Store the extracted nucleic acids at -20°C.

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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