

DNA Analyst Training Laboratory Training Manual

Protocol 3.03 DNA Isolation: Isolation of DNA from Non-Semen Stains



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Refer to the General Information on DNA Isolation section.

SAFETY CONSIDERATIONS

Refer to the General Information on DNA Isolation section.

PREPARATIONS

Refer to the General Information on DNA Isolation section.

INSTRUMENTATION

Refer to the General Information on DNA Isolation section.

MINIMUM STANDARDS & CONTROLS

Refer to the General Information on DNA Isolation section.

PROCEDURE OR ANALYSIS

A. Non-Semen Body Fluid Stains.

1. Place the sample into an extraction tube.

Add: 400 :l Stain Extraction Buffer
10 :l Proteinase K (20 mg/ml)
5 :l 390 mM DTT

Mix and spin briefly to force the sample into the liquid.

2. Incubate questioned source samples at 56°C overnight. Bloodstain standards or buccal swab standards may be incubated for two hours at 56°C.
3. Place the sample in the basket of the extraction tube. Centrifuge for five minutes at 10,000 x g. Transfer the liquid into a microcentrifuge tube.
4. Organic Extraction: Add 500 µl phenol/chloroform/isoamyl alcohol (PCI) to the liquid. This step must be done in the fume hood. Vortex for approximately one minute to achieve a milky emulsion. Centrifuge for two minutes at maximum speed.

At the analyst's discretion, the aqueous and interface may be re-extracted using 500 µl PCI or 500 µl chloroform/isoamyl alcohol.

5. Place the aqueous phase from the organic extraction into the filter of a Microcon 100 tube. Following the manufacturer's recommendations for centrifugation speed, spin to dryness.
6. Add 50-100 µl of TE⁻⁴ to the filter in order to wash residual extraction components from the DNA. Centrifuge to dryness.
7. Add the appropriate volume of TE⁻⁴ (depending on anticipated DNA recovery), invert filter, vortex and spin out liquid.
8. Incubate the sample to resolubilize the DNA for at least one hour at 56°C.

B. Hairs with Roots:

1. Use one of the following methods to remove mounted hairs from a slide.

Freezing Method Place slide in freezer for a few minutes. Pry off the cover slip. Add a drop of Xylene (or Xylene Substitute) to the hair to dissolve the mounting medium.

Xylene Method Soak the slide in Xylene or Xylene Substitute for several hours until the cover slip can be pried from the slide.

Remove the hair and soak in about 10-20 ml Xylene (or Xylene Substitute) for a few minutes to remove residual mounting medium. Rinse the hair briefly in ethanol to remove xylene.

2. Place the hair in a microcentrifuge tube with sterile distilled H₂O (or equivalent) and shake for one hour at room temperature. This will remove any debris adhering to the hair.
3. Rinse the hair briefly in fresh sterile distilled H₂O (or equivalent).
4. Cut a 2 cm segment from the base of the hair and place in a microcentrifuge tube.
Add: 500 µl stain extraction buffer
50 µl 390 mM DTT
15 µl proteinase K (20 mg/ml)
Incubate at 56EC overnight.

5. Vortex the hair/stain extraction buffer mixture for 30 seconds.
Add: 50 μ l of 390 mM DTT
15 μ l of proteinase K (20 mg/ml)
Incubate at 56EC overnight.
6. Spin the microcentrifuge tube at maximum speed to sediment the pigments.
Transfer the supernatant to a fresh tube.
7. Follow Section A, Steps 4 through 8.

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