

DNA Analyst Training Laboratory Training Manual

Protocol 3.02
DNA Isolation: Differential Isolation of DNA
from 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

1. Place the sample into an extraction tube. Add:
500 μ l Stain Extraction Buffer
5 μ l Proteinase K (20 ug/ μ l)

Add sufficient reaction mixture to have excess liquid visible if substrate is too absorbent. Mix gently and incubate two hours at 37°C.

2. Place the sample in the basket of the extraction tube. Centrifuge for five minutes at 10,000 x g.
3. Divide the sample into three portions for extraction of the F1, F2, and F3 fractions.

F3 fraction: Place the material retained in the basket into a new extraction tube. Further processing of the F3 fraction is at the analyst's discretion.

F1 fraction: Carefully transfer the liquid above the sperm cell pellet into a microcentrifuge tube.

F2 fraction: The sperm cell pellet should be left in the original extraction tube.

4. F2 fraction:

A. Add the following to the sperm cell pellet:

500 μ l Stain Extraction Buffer

5 μ l Proteinase K (20 μ g/ μ l)

Mix gently and incubate 30 minutes at 37° C.

B. Centrifuge for five minutes at 10,000 x g.

C. Carefully remove the liquid from the sperm cell pellet and discard.

D. Rinse the sperm cell pellet with 1 ml TNE, vortex and centrifuge at maximum speed for ten minutes. Remove and discard the TNE buffer. Be careful not to disturb the sperm cell pellet. (Optional: After wash, 1 μ l of the sperm cell pellet may be removed for KPIC).

F2 and F3 fractions: Add:

350 μ l Stain Extraction Buffer

40 μ l 390 mM DTT

10 μ l Proteinase K (20 μ g/ μ l)

5. Add sufficient reaction mixture to have excess liquid visible if the substrate is too absorbent. Mix and incubate at 37°C for two hours.

6. Place the F3 sample in the basket of the extraction tube. Centrifuge for five minutes at 10,000 x g. Transfer the liquid into a microcentrifuge tube.

7. Organic Extraction: Add 500 μ l 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 μ l PCI or 500 μ l chloroform/isoamyl alcohol.

8. Place the aqueous phase from the organic extraction into a Microcon 100 tube. Following the manufacturer's recommendations for centrifugation speed, spin to dryness.

9. Add 50-100 μ l of TE⁻⁴ to the filter in order to wash residual extraction components from the DNA. Centrifuge to dryness.

10. Add the appropriate volume of TE⁻⁴ (depending on anticipated DNA recovery), invert filter, vortex, and spin out liquid.

11. Incubate the sample to resolubilize the DNA for at least one hour at 56°C.

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