DNA Analyst Training
Laboratory Training Manual

Protocol 3.05
Chelex®100 Non-Differential Extraction

This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the National Forensic Science Technology Center. It has been included for training and example purposes only.

PRESIDENT'S DNA INITIATIVE

INTRODUCTION

Chelex® 100 is an ion-exchange resin composed of styrene divinylbenzene copolymers with paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions. Removing magnesium from the sample inactivates nucleases and DNA destroying enzymes, thus protecting the DNA molecules.

Chelex® 100 extraction procedures are provided for the following sample types:

- liquid blood and bloodstains
- epithelial and buccal cells
- hair

For liquid blood and bloodstains, the sample is extracted in sterile deionized water to lyse the red blood cells and remove heme, a PCR inhibitor, prior to the addition of the Chelex® 100.

For hair, bodily fluids and other contaminants are removed from the surface. The root material is then incubated in the Chelex® 100 suspension.

SAFETY CONSIDERATIONS

Refer to the Laboratory Safety Manual(s)

PREPARATIONS

5% Chelex® 100 Suspension

1. Add 50ml of ultra pure or sterile deionized water to capped bottle.
2. Add a sterile stir bar to bottle.
3. Weigh out 2.5 grams of Chelex® 100 into bottle.
4. Properly label and date the container. Store at room temperature. Expiration date is one year from preparation date.

Proteinase K (20 mg/ml)

1. 20 mg Proteinase K
2. 1 ml sterile DI water
3. Dissolve in a sterile microcentrifuge tube. Aliquot 100 µl into each of 10 sterile microcentrifuge tubes. Store at -20°C.
   Expiration date one year.

Stain Extraction Buffer Premix

1. 2.4 g Tris base
2. 9.26 g $Na_2EDTA \bullet 2H_2O$
3. 14.6 g NaCl
4. 1600 ml DI water
5. Dissolve and pH to 8.0. Adjust volume to 2 liters with DI water. Filter sterilize or autoclave. Store at 2° to 8°C.
   Expiration date one year.

Stain Extraction Buffer Working Solution

1. 320 µl Stain Extraction Buffer Premix
2. 40 µl 20% SDS
3. 40 µl 0.39 M DTT
4. Store at -20°C for up to 2 weeks.

TE-4 (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

1. 10 ml 1 M Tris-HCl, pH 8.0
2. 0.2 ml 0.5 M EDTA
3. 990 ml DI water
4. Adjust pH to 8.0 ± 0.2
5. Autoclave. Store at 2° to 8°C.
   Expiration date one year.

INSTRUMENTATION

- Centrifuge
- Heat block and/or wWater baths or heat blocks
- Pipettes
- Vortex
- Top loading balance

MINIMUM STANDARDS & CONTROLS

- Positive extraction control
- Extraction blank – contains reagents only

PROCEDURE OR ANALYSIS

Bloodstains and Liquid Blood

1. Place approximately 3 mm^2 swatch or $^1/_4$ to $^1/_3$ of a swab into a sterile 1.5 ml microcentrifuge tube. The size of the cutting may be adjusted based upon the amount of biological material or the possible presence of inhibitors. For liquid blood, use 3-10 µl.

2. Pipette 1 ml of sterile deionized water into the microcentrifuge tube. Vortex briefly. *This step may be omitted for extremely small samples containing little heme.*

3. Incubate for 15-30 minutes at room temperature. Mix occasionally by inversion or gentle vortexing.

4. Centrifuge at 10,000 to 15,000 x g for 3 minutes at room temperature.

5. Without disturbing the cell pellet, remove all but 20-30 µl of the supernatant and discard. Leave the substrate in the tube.

6. Add 200 µl of 5% Chelex® 100 (a large bore pipette tip may be necessary). The volume of 5% Chelex® 100 may be increased to ensure the substrate is completely submerged. Continue with general method described below.

Epithelial and Buccal Cells

1. Place a 3 mm^2 swatch or $^1/_4$ to $^1/_3$ of a swab into a sterile 1.5 ml microcentrifuge tube. The size of the cutting may adjusted based upon the amount of biological material or the possible presence of inhibitors.

   For cigarette butts, cut a strip of the paper covering the butt in the area that would be in contact with the mouth; approximately 5 mm wide. Remove the paper from the filter and cut into several small pieces. Place into a sterile 1.5 ml microcentrifuge tube.

   For envelope flaps or stamps, cut a 1 cm^2 area of the gummed flap or stamp and place into a sterile 1.5 ml microcentrifuge tube. An alternate collection method is to use a sterile cotton swab which has been slightly moistened with sterile distilled water to swab the gummed envelope flap or stamp. The swab is then removed from the stick and placed into a sterile 1.5 ml microcentrifuge tube.

2. Add 200 µl of 5% Chelex® 100 (a large bore pipette tip may be necessary). The volume of 5% Chelex® 100 may be increased to ensure the substrate is completely submerged.

3. Add 10-50 µl of 10mg/ml of Proteinase K per 200 µl of Chelex® 100. Continue with general method described below.

Hair

1. Hair samples should be handled with clean forceps. Determination of the presence of root material should be completed prior to extraction. It should be noted that other bodily fluids may be present on the hair.

2. Wash the hair to remove any extraneous bodily fluids and/or contaminants by immersing the hair in 200 µl of digest buffer, TE $^{-4}$ or sterile deionized water for 1-15 minutes. Note: longer times may lead to lysis of root material. Remove the hair from the wash. The wash may be retained and extracted as needed.

3. Using a sterile scalpel, remove approximately 1 cm of hair from the root end and place into a sterile 1.5 ml microcentrifuge tube. The shaft of the hair should be retained for possible future analysis (i.e. mtDNA typing).

4. Add 200 µl of 5% Chelex® 100 (a large bore pipette tip may be necessary). The volume of 5% Chelex® 100 may be increased to ensure the substrate is completely submerged.

5. Add 2 µl of 10 mg/ml of Proteinase K per 200 µl of Chelex® 100. Continue with general method described below.

General Method

1. Vortex at high speed for 10-30 seconds. Ensure substrate is completely submerged in the Chelex® 100 suspension.

2. Incubate at 55°-56°C for a minimum of 30 minutes, in general the incubation should not exceed 90 minutes. Hairs should be incubated for a minimum of 6 hours.

3. Vortex at high speed for 5 -10 seconds.

4. Heat for 8 minutes at 100°C in heating block or in a boiling water bath. Ensure substrate is completely submerged during this step.

5. Vortex at high speed for 5-10 seconds

6. Centrifuge for 3 minutes at approximately 10,000-15,000 x g.

7. The supernatant may be transferred to a sterile 1.5 ml microcentrifuge tube or centrifugal filter device (i.e. Microcon® filter units) for concentration and purification.

8. Store DNA extract at 4°C or proceed to quantification. For long term storage, samples should be frozen or freeze dried. .

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