DNA: Organic Extraction

Samples

DNA can be extracted from samples, such as those listed below, using this procedure:

- concentrated DNA samples (e.g., blood, saliva, non-contact samples)
- keratinized samples (hair and fingernails)
- slides
- tissue
- contact samples
- samples containing sperm (for direct Y-STR analysis only)

Principle

The phenol-chloroform extraction method removes proteins and other cellular components from nucleic acids, resulting in relatively purified DNA preparations. This method results in double-stranded DNA that is suitable for amplification.

Safety

Phenol-chloroform reagent is corrosive, highly toxic, and a suspected teratogen. Avoid skin contact and inhalation. Use a fume hood and wear a lab coat, gloves, and protective eyewear when handling.

Equipment and supplies

The extraction procedures below use the following laboratory equipment and supplies:

- heat block
- microcentrifuge
- centrifuge
- pipettes and barrier pipette tips
- Vivacon 2 tubes or equivalent
- vortex
- microcentrifuge tubes

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DNA: Organic Extraction, Continued

Chemicals

The extraction procedures use the following chemicals:

- chloroform
- dithiothreitol
- EDTA
- isoamyl alcohol
- phenol
- Proteinase K
- sodium acetate
- sodium chloride
- sodium dodecyl sulfate (SDS)
- Tris HCl

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Prepared reagents

The extraction procedures use the following prepared reagents:

- 1 M Tris HCl, pH 7.5
- 0.5 M EDTA
- 5 M NaCl
- 20% w/v SDS (SERI)
- 1 M Tris HCl, pH 8.0
- 1 M sodium acetate, pH 5.2
- Buffered Phenol-Chloroform-Isoamyl Alcohol Solution (Sigma)
- Digest Buffer
  - For each liter, mix together
    - 10 mL of 1 M Tris HCl, pH 7.5
    - 20 mL of 0.5 M EDTA
    - 10 mL of 5 M NaCl
    - 100 mL of 20% w/v SDS
    - 860 mL of sterile water. Store at room temperature.
- Proteinase K Solution
  - Dissolve 100 mg Proteinase K in 10 mL sterile water. Aliquot the solution and store frozen at –20°C.
- Low TE Buffer
  - For each liter, mix together
    - 10 mL of 1 M Tris HCl, pH 8.0
    - 0.2 mL of 0.5 M EDTA
    - 990 mL sterile water. Autoclave the solution. Store at room temperature.
- 1 M Dithiothreitol
  - Dissolve 1.0 g dithiothreitol in 6.5 mL sterile water.
  - Add 65 uL of 1 M sodium acetate pH 5.2. Aliquot the solution and store at –20°C.

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Sample preparation

Certain types of evidentiary samples require preparation prior to extraction.

- For samples other than hair or slides,
  - no sample preparation is required. Proceed to *Sample digestion (non-keratinized)*.
- For hair,
  - use the following procedure to prepare hair samples for extraction.

Step	Action
1	Examine the hair under a microscope and note the presence of surface dirt, body fluids, or other contaminants. If necessary, wash the hair prior to extraction by placing the hair sample in a sterile microcentrifuge tube and adding sterile water.
2	Cut off about 1 cm of the proximal (root) end for digestion.
3	Cut off about 1 cm of the shaft adjacent to the root for separate analysis as a control, if appropriate.
4	Proceed to *Keratinized sample digestion*.

- For slides,
  - use the following procedure to remove samples from pre-mounted slides.

Step	Action
1	Clean the slide surfaces with alcohol.
2	Place the slide in a -20º C freezer for at least five minutes.
3	While in the freezer, remove the cover slip using a sterile scalpel.
4	Soak the slide in xylene or toluene at room temperature for about 10 minutes.
5	Remove the slide and rinse with additional solvent.
6	Use a sterile swab with a wooden handle to remove material from the slide for extraction. Place the swab in a sterile microcentrifuge tube and proceed to: • *Sample digestion (non-keratinized)* for samples needing organic extraction • *DNA: Differential Extraction, Differential lysis* for samples needing differential extraction

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Sample digestion (non-keratinized)

The following procedure is used to digest and lyse all non-keratinized samples.

Step	Action
1	Add an appropriate amount of sample to a sterile microcentrifuge tube. Pipette 0.5 mL of Digest Buffer into the tube with the sample.
2	Add 15 µL of Proteinase K Solution. Mix gently. If digesting a sexual assault sample containing sperm for Y-STR analysis, add 20 µL of 1 M dithiothreitol.
3	Incubate the sample at 56° ± 1°C for at least one hour.
4	Add 15 µL of Proteinase K Solution. Mix gently.
5	Incubate the sample one hour or overnight at 56° ± 1°C.
6	After digestion, remove any substrate with an autoclaved stick or an autoclaved disposable pipette tip. NOTE: If the sample has been consumed, retain the substrate.

Keratinized sample digestion

Use the following procedure to digest keratinized samples, such as hair and fingernails.

Step	Action
1	To the sterile microcentrifuge tube that contains the sample, add the following: • 0.5 mL of Digest Buffer • 20 µL of 1 M Dithiothreitol • 15 µL of Proteinase K Solution.
2	Incubate the sample at 56° ± 1°C for at least 6 hours. Hair will usually soften but not dissolve after this initial incubation.
3	Vortex the sample.
4	Add to the sample an additional 20 µL of 1 M Dithiothreitol and 15 µL of Proteinase K Solution.
5	Incubate the sample at 56° ± 1°C for 6-8 hours or overnight until completely dissolved.
6	Vortex the sample.

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DNA: Organic Extraction, Continued

DNA extraction

Use the following procedure to extract DNA from samples.

Step	Action
1	To the digested sample, add 0.5 mL of Buffered Phenol-Chloroform-Isoamyl Alcohol solution. Cap the tube and vortex the sample for 15 seconds or until an emulsion forms.
2	Centrifuge the sample in a microcentrifuge for 3-5 minutes on high speed and at room temperature to separate the two layers.
3	Remove the lower phenol-chloroform layer and discard into a chemical waste container.
4	Repeat steps 1-3 an additional two to three times until nothing is visible at the interface and the aqueous layer is clear.

DNA concentration and wash (Vivacon)

Use the following procedure to concentrate and wash the DNA from the samples using the Vivacon unit.

Step	Action
1	Label the Vivacon concentration unit.
2	Add approximately 1.5 mL of TE Buffer to the upper reservoir.
3	Transfer the entire (approximately 0.5 mL) aqueous (upper) layer containing extracted DNA to the TE Buffer in the unit.
4	Centrifuge the sample at room temperature for at least 10 minutes at approximately 2000 rcf.
5	Discard the effluent in the lower reservoir.
6	Add approximately 2 mL of TE Buffer to the concentrated DNA solution in the upper reservoir.
7	Centrifuge as in step 4.
8	Repeat steps 5-7 for a total of three washes.
9	After the last wash, collect the concentrated DNA sample by inverting the upper reservoir into the provided retentate cup and centrifuging for 2 minutes at approximately 2000 rcf to transfer the concentrate into the cup.
10	Transfer the entire sample to a new labeled microcentrifuge tube. Record the volume of DNA recovered. The sample is now ready for DNA quantitation and the PCR amplification process.
11	Refrigerate or freeze the sample until ready to perform quantitation.