

7 DNA Extraction

This section details numerous procedures for the extraction of DNA from forensic evidence and reference samples, for the purpose of STR analysis. It is at the discretion of the analyst to determine which of these procedures is most useful for a particular sample. Evidentiary samples should not be extracted along-side reference samples to help avoid potential contamination between what is typically a higher level DNA sample and a lower level DNA sample. If the extraction of evidentiary and reference samples cannot be separated by time, the evidentiary sample should be handled prior to the reference sample and efforts to separate them by location should be made.

These procedures indicate recommended amounts of sample to be processed. Evidence samples may be in limiting supply. The analyst should add as much evidentiary sample to the tube as possible, up to the quantities specified in the procedure, while retaining sufficient sample for replicate analysis if possible. These amounts are recommendations, and the actual amount used for any sample is up to the discretion of the analyst. Examination documentation should reflect the quantity of sample consumed during DNA extraction.

Swabs and cuttings should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.

When the sample is expected to be limiting, or when quantification indicates that the amount of DNA is limiting, DNA extracts may be concentrated to as low as 15 μ L, to maximize the concentration of DNA in subsequent amplifications.

Ensure sample is completely submerged prior to incubation. This may require a spin prior to loading samples onto heat blocks. Alternatively, additional digestion reagents may be added to ensure the sample is submerged. If any additional reagents are added to a sample, the same must be done to the associated reagent blank. This must be noted on the extraction documentation.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Phenol, or solutions made from phenol (including the Phenol:Chloroform:Isoamyl alcohol used in the organic extraction procedures in this section), may be fatal if swallowed, inhaled, or absorbed through the skin in sufficient quantities. Phenol solutions should be used only in a chemical fume hood; avoid ingestion, inhalation, or skin contact.

Standards, Control, and Calibration

One reagent blank must be processed for each extraction batch as a negative control. The extraction method used must be recorded on the DNA extraction worksheet.

7.1 DNA Extraction Using Digest Buffer – Organic Method

The procedure uses digest buffer along with other reagents to digest and extract DNA. Extracts are purified using phenol-chloroform-isoamyl alcohol, followed by Microcon concentration.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- digest buffer
- Phenol:Chloroform:Isoamyl alcohol (v/v 25:24:1)
- TE Buffer
- 10 mg/ml Proteinase K
- Microcon concentrator
- tubes – 1.5 ml, microcentrifuge
- microcentrifuge with rotor for 2 ml tubes at room temperature
- water bath or dry bath ~56 °C
- vortex

Procedure

1. Add one of the following to a 1.5 ml microcentrifuge tube:
 - a. Blood samples- stains or liquid - 1 cm² bloodstain, 10 to 50 µl whole blood, or 2 to 10 µL buffy coat (approximately 105 white blood cells).
 - b. Saliva samples- including but not limited to oral swabs, filter paper, stamps, envelope flaps, cigarette butts, oral contact swab: 1 cm² stain (including filter paper, stamps, and envelope flaps), 1 cm strip of paper covering end of cigarette butt, or 0.25 to 1 swab.
 - c. Hair samples- Approximately 1 cm of root end of hair in one tube and a separate tube for a 1 cm portion of the adjacent shaft of each hair as a control. Hairs should be gently cleaned in sterile distilled water prior to extraction.
 - d. Tissue samples- including but not limited to skin, muscle, and body organs. Approximately 3-5 mm². It is helpful to mince the tissue prior to adding to digest buffer.
 - e. Bone and Teeth - One tooth or approximately 2 cm³ of bone (preferably flat bone in adults-i.e. pelvis, sternum, ribs). The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a dremel tool to remove debris. Teeth may be cleaned with sterile distilled water and bleach unless there are surface fractures in which case only sterile water is used. Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or using a single-use coffee grinder. Approximately 0.5g of sample will be placed into each 1.5 ml tube (this may take several tubes).
 - f. Miscellaneous- including but not limited to items containing shed skin cells, sweat or other body fluids that may contain sufficient quantities of recoverable DNA. Amounts to be used will be at the analyst's discretion.

7 DNA Extraction

2. Add 500 µl of Digest Buffer.
3. Add 15 µl of 10 mg/ml Proteinase K solution (to a final concentration of 0.3 mg/ml). Mix gently. Incubate at ~56°C for at least 1 hour. For evidence samples it is recommended that digestion continue for a minimum of 6 hours. Digestion may be performed overnight, but more than 24 hours is not recommended.
4. Briefly spin tubes. After digestion, substrate may be removed with a disposable pipette tip or tweezers. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Alternatively, the substrate and supernatant may be placed in a spin basket in a separate tube. Centrifuge the tube for 5 minutes at maximum RCF. Discard the substrate and the spin basket.
5. Separate DNA from proteins.
 - a. To ~500 µl of lysed and digested cells, add 500 µl of buffered phenol-chloroform solution. Cap tube and vortex until a complete emulsion forms.
 - b. Spin in a microcentrifuge for 5 minutes at maximum RCF at room temperature to separate the two phases.
 - c. Transfer the top aqueous phase to a 1.5 ml microcentrifuge tube (if additional extractions are needed) or an assembled Microcon tube.
 - d. Repeat Steps a-c an additional 1 to 3 times, if necessary, until the interface is clean and the aqueous (upper) phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.
6. Microcon concentration.
 - a. Using a sterile pipet tip, add up to 500 µl of sample to sample reservoir. If sample volume is less than 500 µl, add TE to bring to a final volume of 500. Seal with attached cap.
 - b. Centrifuge 8 minutes at 9,000 RCF. Continue to centrifuge if an insufficient amount of filtrate has passed through filter.
 - c. If sample volume is greater than 500 µl, repeat steps a and b until all sample has been added to the Microcon.
 - d. Add 500 µl TE Buffer to sample reservoir to wash DNA and centrifuge as in step b. Additional TE Buffer washes may be performed to assist in removal of pigments or other contaminants.
 - e. Invert sample reservoir into a fresh sample recovery tube. It may be necessary to add TE Buffer to the filter prior to inversion
 - f. Centrifuge inverted unit for 3 minutes at 800 RCF to recover DNA sample. Recovery volume should be 10-40 µl for samples expected to contain low levels of DNA. Samples that are expected to yield high concentrations of DNA, such as the epithelial cell fraction of a vaginal swab, may be eluted at a larger volume, such as 100 µl. The final eluate volume of the reagent blank cannot exceed the volume of any of its associated samples.
 - g. Store samples refrigerated or frozen until ready to perform qPCR.

7.2 DNA Extraction Using Stain Extraction Buffer – Organic Method

The procedure uses stain extraction buffer along with other reagents to digest and extract DNA. Extracts are purified using phenol-chloroform-isoamyl alcohol, followed by Microcon concentration.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- microcentrifuge tubes, 1.5 ml, or spin basket extraction tubes
- microcentrifuge with rotor for 2 ml tubes, at room temperature
- vortex
- ~56°C water bath or dry bath
- Microcon® concentrator
- Phenol:Chloroform:Isoamyl Alcohol (v/v 25:24:1)
- Stain Extraction Buffer
- 10mg/ml Proteinase K
- TE Buffer

Procedure

1. Add sample to a 1.5 ml microcentrifuge tube. Use same sample amounts/procedures found in section 7.1.
2. Add 500 µl of Stain Extraction Buffer.
3. Add 10 µl of 10 mg/ml Proteinase K solution. Mix gently. Incubate at ~56°C for at least 1 hour. For evidentiary samples, it is recommended that digestion continue for a minimum of 6 hours. Digestion may be performed overnight, but more than 24 hours is not recommended.
4. Briefly spin tubes. After digestion, substrate may be removed with a disposable pipette tip or tweezers. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Alternatively, the substrate and supernatant may be placed in a spin basket in a separate tube. Centrifuge the tube 5 minutes at maximum RCF. Discard the substrate and the spin basket.
5. Separate DNA from proteins.
 - a. To ~500 µl lysed and digested cells, add 500 µl buffered phenol-chloroform-solution.
 - b. Cap tube and vortex until a complete emulsion forms.
 - c. Spin in centrifuge for 5 minutes at maximum RCF, to separate the two phases.
 - d. Remove the top aqueous phase to a 1.5 ml microcentrifuge tube (if additional extractions are needed) or an assembled Microcon tube.
 - e. Repeat Steps a-c an additional 1 to 3 times, if necessary, until the interface is clean and the aqueous (upper) phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.

6. Microcon concentration.
 - a. Using a sterile pipet tip, add up to 500 μ l of sample to sample reservoir. If sample volume is less than 500 μ l, add TE to bring to a final volume of 500. Seal with attached cap.
 - b. Centrifuge 8 minutes at 9,000 RCF. Continue to centrifuge if an insufficient amount of filtrate has passed through filter.
 - c. If sample volume is greater than 500 μ l, repeat steps a and b until all sample has been added to the Microcon.
 - d. Add 500 μ l TE Buffer to sample reservoir to wash DNA and centrifuge as in step b. Additional TE Buffer washes may be performed to assist in removal of pigments or other contaminants.
 - e. Invert sample reservoir into a fresh sample recovery tube. It may be necessary to add TE Buffer to the filter prior to inversion.
 - f. Centrifuge inverted unit for 3 minutes at 800 RCF to recover DNA sample. Recovery volume should be 10-40 μ l for samples expected to contain low levels of DNA. Samples that are expected to yield high concentrations of DNA, such as the epithelial cell fraction of a vaginal swab, may be eluted at a larger volume, such as 100 μ l. The final eluate volume of the reagent blank cannot exceed the volume of any of its associated samples.
 - g. Store samples refrigerated or frozen until ready to perform qPCR.

7.3 DNA Extraction – QIAamp®

This procedure uses the QIAamp® DNA Mini Kit to digest and extract DNA. The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A body fluid extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted. Due to PCR inhibitors present in urine, this procedure is not recommended for urine samples.

Additional Safety Information

Buffers AL and AW1 are irritants and are incompatible with bleach. Buffer AW2 contains sodium azide, which is highly toxic and may react explosively with lead and copper drainpipes. Ethanol is an irritant and is flammable.

Equipment, Materials, and Reagents

- microcentrifuge with rotor for 2-ml tubes, at room temperature
- vortex
- water baths or dry baths at ~56°C and ~70°C
- QIAamp® DNA Mini Kit (QIAGEN) kit, containing the following:
 - QIAamp spin columns and 2-ml collection tubes
 - Buffer AL
 - Buffer ATL
 - Buffer AW1 concentrate
 - Buffer AW2 concentrate
 - Buffer AE
 - Proteinase K (20 mg/ml, 600 mAU/ml solution or 40 mAU/mg protein)
- 1 M DTT
- ethanol, denatured

General Instructions

- Do not wet the rim of the spin column when transferring liquid.
- Do not touch the membrane with the pipette tip.
- Swabs should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.
- Equilibrate samples and solutions to room temperature before use. Dissolve any precipitate in Buffer ATL by incubating at ~56°C.
- Prepare Buffer AW1 and Buffer AW2 by adding denatured ethanol at the volume noted on the bottle when the kit is first used. These solutions are good for 1 year at room temperature.
- All centrifugation steps should be performed at maximum RCF.

Procedure

1. Add sample to a 1.5 ml microcentrifuge tube. Use same sample amounts/procedures found in section 7.1.
2. Lyse cells and suspend.
 - a. Add 200 μ l Buffer ATL (\sim 56°C), 8 μ l 1 M DTT, and 20 μ l Proteinase K.
 - b. Incubate at \sim 56°C for at least an hour; 6 hours to overnight is recommended for evidence samples (**more than 24 hours is not recommended**).
3. Preheat AE Buffer for elution to \sim 70°C.
4. Adsorb nucleic acids to membrane:
 - a. Briefly spin tubes. After digestion, substrate may be removed with a disposable pipette tip or tweezers. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Alternatively, the substrate and supernatant may be placed in a spin basket in a separate tube. Centrifuge the tube 5 minutes at maximum RCF. Discard the substrate and the spin basket.
 - b. Gently shake Buffer AL and add 200 μ l to liquid in sample tube. Incubate at \sim 70°C for 10 minutes. Briefly spin tubes.
 - c. Add 210 μ l denatured ethanol. Vortex vigorously. Briefly spin tubes.
 - d. Transfer tube contents (including any precipitate) to a labeled QIAamp spin column in a collection tube.
 - e. Centrifuge for 1 minute or until all solution has passed through the membrane.
5. Wash membrane:
 - a. Transfer the column to a new collection tube. Add 250 μ l Buffer AW1 to the column.
 - b. Centrifuge for 1 minute or until all solution has passed through the membrane.
 - c. Add 250 μ l Buffer AW2 to the column and centrifuge for 3 minutes, or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.
6. Elute nucleic acids.
 - a. Transfer the column to a new collection tube.
 - b. Add 50–100 μ l Buffer AE (\sim 70°C) to the column depending on the expected quantity of DNA. Incubate at \sim 70°C for 10 minutes.
 - c. Centrifuge 1 minute.
 - d. Transfer liquid from collection tube to a labeled storage tube.
7. Optional: Repeat elution step using the recovered DNA solution to increase yield.
8. Optional: Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.
9. Store samples refrigerated or frozen until ready to perform qPCR.

7.4 DNA Extraction - QIAGEN BioRobot EZ1 Advanced XLs

This procedure uses the EZ1 DNA Investigator Kit and the EZ1 Advanced XL to digest and extract DNA. This procedure uses magnetic beads, which are coated with silica and contain grooves. The silica has an affinity for nucleic acids under certain buffer and temperature conditions and the grooves increase the surface area of the beads so they hold more nucleic acids. The EZ1 Advanced XL is an automated instrument that is programmed to extract DNA from samples using the magnetic beads in the EZ1 DNA Investigator Kit. DNA is isolated from lysates in one step through its binding to the silica surface of the beads in the presence of a chaotropic salt. The beads are separated from the lysates using a magnet and the DNA is then washed and eluted.

Additional Safety Information

The reagent cartridges contain ethanol, guanidine hydrochloride, and guanidine thiocyanate, which are highly flammable, harmful, and irritant. The guanidine salts can form highly reactive compounds when combined with bleach.

Equipment, Materials, and Reagents

- Vortex
- Water baths or heat blocks at ~56°C
- EZ1 DNA Investigator Kit (QIAGEN), containing the following:
 - Reagent Cartridges
 - Disposable Tip Holders
 - Disposable Filter-Tips
 - Sample Tubes (2ml)
 - Elution Tubes (1.5ml)
 - Buffer G2
 - Proteinase K
 - Carrier RNA
- MTL Buffer
- Sterilized water
- TE Buffer
- Sample tubes and spin baskets from Fitz Co.

General Instructions

- Swabs or stains should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections, which are not to be analyzed immediately, should be stored frozen.
- Equilibrate samples to room temperature before use.
- Carrier RNA (cRNA) must be prepared from the EZ1 DNA Investigator kit and aliquoted prior to use. Carrier RNA comes in the kit as a 310µg solid and a solution of cRNA must be made by:
 - Adding 310µl of water to the tube containing cRNA (310µg) to obtain a solution of 1 µg/µl.

- Dissolve the cRNA thoroughly
- Divide it into single use aliquots each containing 20µl of dissolved cRNA.
- Label with lot # and expiration date and store frozen. The expiration date is whatever is earliest: the expiration date of the EZ1 kit or the water being used.
- Buffer G2 should be diluted with sterilized water before use with the Tip Dance protocol. This is accomplished by diluting the Buffer G2 in sterilized water using a ratio of 1:1 (i.e. one volume of Buffer G2 to one volume of sterilized water). **NOTE: This is done only when using the Tip Dance Protocol. The Trace and Large Volume protocols use neat Buffer G2.**
- Invert the reagent cartridges before loading into the EZ1 instrument to ensure that the beads are mixed up for proper performance. Note that the particles are completely resuspended.
- Substrates should be removed prior to loading on the instrument in order to prevent jamming. This should be performed after digestion and before loading onto the EZ1.
- When using the Fitz Co. tubes, it is necessary to remove the snap tops completely from the tube before loading it onto the EZ1 instrument.
- Trace Protocol should be utilized for evidence samples in which the portion of sample taken is 1 swab or less.
- Tip Dance Protocol should be utilized for reference or high level DNA samples.
- Large Volume Protocol should be utilized for evidence samples in which the portion of sample taken is 2 swabs or more. IF one sample in a run has to use the Large-Volume than all samples in the run need to use that same protocol.

Procedure

1. Add sample to a 1.5 ml microcentrifuge tube. Use same sample amounts/procedures found in section 7.1.

When using the Trace Protocol (Evidence samples consisting of one swab or less (or the equivalent size of another substrate)):

2. Lyse cells and suspend DNA:
 - a. Place the sample into a 1.5 ml microcentrifuge tube.
 - b. Add 190µl Buffer G2, 10µl Proteinase K, and 1 µl of aliquoted and thawed cRNA. If the substrate has absorbed some or most of the reagents and is not fully submerged, proceed to the Large-Volume protocol which adds more Buffer G2.
 - c. Mix thoroughly by vortexing and incubate at ~56°C for 6 hours to overnight, as recommended for evidence samples (**more than 24 hours is not recommended**).
 - d. Briefly spin tubes. Transfer the supernatant and substrate to a fresh 2ml Fitz Co. tube containing a basket, **or the equivalent (e.g., Costar 2.0ml tubes)**. Centrifuge the tube 5 minutes at maximum RCF. Discard the substrate and the spin basket. **If using a tube other than a Fitz Co. tube, transfer all of the liquid to an EZ1 sample tube for step 3.i.iii.**
3. EZ1 Advanced XL Setup and Run:
 - a. Make sure the Instrument is turned on.
 - b. Press "START" to start the protocol set-up.
 - c. Press "ESC" to not create a report file.
 - d. Press "1" for Trace protocol.

- e. Choose the elution buffer and volume:
 - i. Press “2” to elute in TE.
 - ii. Press “1” to elute to a final volume of 40µl or “2” to elute to a final volume of 50µl. A 40µl elution will be appropriate in most instances.
- f. Press “ENT” to proceed through the text shown on the display and start the worktable setup below.
- g. Open the Instrument door.
- h. Invert the reagent cartridges and load one cartridge per sample into the cartridge rack, ensuring that the cartridge clicks into place.
- i. Under an extraction hood, set-up the tip rack as follows:
 - i. Load opened elution tubes into the first row (Row 1) of the tip rack, putting aside the caps for each elution tube.
 - ii. Load the tip holders containing filter-tips into the second row (Row 2) of the tip rack.
 - iii. Load opened sample tubes containing digested samples into the back row (row 4) of the tip rack.
- j. Place the set-up tip rack into the EZ1 instrument and close the instrument door.
- k. Press “START” to start the purification procedure.
- l. When the protocol ends, open the instrument door, remove the tip rack, and under an extraction hood, recap the elution tubes. Discard the sample preparation waste in the appropriate waste container.
- m. Proceed to step 8 below.

When using the Tip Dance Protocol (Reference or high level DNA samples):

4. Lyse cells and suspend DNA:
 - a. Place the sample into a 1.5 ml microcentrifuge tube or a 2ml sample tube from the EZ1 DNA Investigator Kit.
 - b. Add 290µl **diluted** Buffer G2 and 10µl Proteinase K to the sample. If the substrate has absorbed some or most of the reagents and is not fully submerged, proceed to the Large-Volume protocol which adds more Buffer G2.
 - c. Mix thoroughly by vortexing and incubate at~56°C for at least 15 min; 6 hours to overnight is recommended for evidence samples (**more than 24 hours is not recommended**).
 - d. Briefly spin tubes. Transfer the supernatant to an EZ1 tube.
5. EZ1 Advanced XL Setup and Run:
 - a. Make sure the Instrument is turned on.
 - b. Press “START” to start the protocol set-up.
 - c. Press “ESC” to not create a report file.
 - d. Press “2” for Trace Tip Dance (TD) protocol.
 - e. Choose the elution buffer and volume:
 - i. Press “2” to elute in TE.

- ii. Press "1" to elute to a final volume of 40µl or "2" to elute to a final volume of 50µl. A 40µl elution will be appropriate in most instances.
- f. Press "ENT" to proceed through the text shown on the display and start the worktable setup below.
- g. Open the Instrument door.
- h. Invert the reagent cartridges and load one cartridge per sample into the cartridge rack, ensuring that the cartridge clicks into place.
- i. Under an extraction hood, set-up the tip rack as follows:
 - i. Load opened elution tubes into the first row (Row 1) of the tip rack, putting aside the caps for each elution tube.
 - ii. Load the tip holders containing filter-tips into the second row (Row 2) of the tip rack.
 - iii. Load opened sample tubes containing digested samples into the back row (row 4) of the tip rack.
- j. Place the set-up tip rack into the EZ1 instrument and close the instrument door.
- k. Press "START" to start the purification procedure.
- l. When the protocol ends, open the instrument door, and under an extraction hood, recap the elution tubes. Discard the sample preparation waste in the appropriate waste container.
- m. Proceed to step 8 below.

When Using the Large-Volume Protocol (Evidence samples consisting of two or more swabs (or the equivalent size of another substrate)):

6. Lyse cells and suspend DNA:
 - a. Place the sample into a 1.5 ml microcentrifuge tube.
 - b. Add 490µl Buffer G2 to the sample, 10µl Proteinase K, and 1 µl of aliquoted and thawed cRNA. Check to see if the sample has absorbed some or all of the buffer. If necessary, the sample may be divided between two tubes to ensure complete submersion and combined later with a Microcon concentration. However, the reagent blank must be subjected to the same manipulations.
 - c. Mix thoroughly by vortexing and incubate at~56°C for 6 hours to overnight as recommended for evidence samples (**more than 24 hours is not recommended**).
 - d. Briefly spin tubes. Transfer the supernatant and substrate to a fresh 2ml Fitz Co. tube containing a basket, **or the equivalent (e.g., Costar 2.0ml tubes)**. Centrifuge the tube 5 minutes at maximum RCF. Discard the substrate and the spin basket. **If using a tube other than a Fitz Co. tube, transfer all of the liquid to an EZ1 sample tube for step 7.i.iii.**
 - e. Add 400µl of MTL buffer to each sample tube.
7. EZ1 Advanced XL Setup and Run:
 - a. Make sure the Instrument is turned on.
 - b. Press "START" to start the protocol set-up.
 - c. Press "ESC" to not create a report file.

- d. Press “3” for Large-Volume protocol.
 - e. Choose the elution buffer and volume:
 - i. Press “2” to elute in TE.
 - ii. Press “1” to elute to a final volume of 40µl or “2” to elute to a final volume of 50µl. A 40µl elution will be appropriate in most instances.
 - f. Press “ENT” to proceed through the text shown on the display and start the worktable setup below.
 - g. Open the instrument door.
 - h. Invert the reagent cartridges and load one cartridge per sample into the cartridge rack, ensuring that the cartridge clicks into place.
 - i. Under an extraction hood, set-up the tip rack as follows:
 - i. Load opened elution tubes into the first row (Row 1) of the tip rack, putting aside the caps for each elution tube.
 - ii. Load the tip holders containing filter-tips into the second row (Row 2) of the tip rack.
 - iii. Load opened sample tubes containing digested samples and MTL buffer into the back row (row 4) of the tip rack.
 - j. Place the set-up tip rack into the EZ1 instrument and close the instrument door.
 - k. Press “START” to start the purification procedure.
 - l. When the protocol ends, open the instrument door, and under an extraction hood, recap the elution tubes. Discard the sample preparation waste in the appropriate waste container.
8. Optional: Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.
9. Store samples refrigerated or frozen until ready to perform qPCR.
10. Perform the needed maintenance as listed in the Equipment Quality Control and Maintenance SOP (#6).
11. To run another sample set, press “ESC” and follow the procedure from step 3 onward. Otherwise, press “STOP” twice to return to the first screen of the display.

7.5 Differential Extraction of Semen Stains Using TNE

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid containing spermatozoa. The differential lysis procedure separates DNA into a sperm fraction and a non-sperm (epithelial cell) fraction. Following the differential lysis of the cellular material, the DNA fractions are further purified using organic extraction (phenol-chloroform).

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- tubes – microcentrifuge
- microcentrifuge with rotor for 2-ml tubes, at room temperature
- vortex
- dry bath (~56°C)
- TNE
- 20% Sarcosyl
- Proteinase K (10 mg/ml)
- 0.39 M DTT
- Phenol:Chloroform:Isoamyl alcohol (v/v 25:24:1)
- TE Buffer

Procedure

1. Place one of the following evidentiary semen stains into a 1.5 ml microcentrifuge tube:
 - a) 0.25 - 1 swab
 - b) 0.1 - 0.5 cm² dried stain
2. Add:
 - a) 400 µl Tris/EDTA/NaCl (TNE)
 - b) 25 µl 20% sarcosyl
 - c) 75 µl sterile diH₂O
 - d) 5 µl Proteinase K

Mix tube contents (a final volume of 505 µl) and incubate at ~56°C for 2 hours, +/- 5 minutes.

It is recommended to create a master mix of digestion reagents and distribute 505 µl of the master mix to each sample tube.
3. Briefly spin tubes. Place the substrate and supernatant in a spin basket in a separate tube. Centrifuge the tube 5 minutes at maximum RCF. Discard the substrate and the spin basket. Transfer the supernatant (digested epithelial cell fraction) to a fresh 1.5 ml microcentrifuge tube for organic extraction starting with step 6. This fraction should be stored refrigerated until step 6.
4. To obtain a cleaner sperm fraction, re-suspend sperm pellet in 500µl TNE. Centrifuge for 5 minutes at the maximum RCF. Discard the supernatant. This may be repeated one to three more times.
5. To the sperm pellet add:
 - a) 150 µl TNE
 - b) 50 µl 20% sarcosyl

7 DNA Extraction

- c) 40 μ l 0.39 M DTT
- d) 150 μ l sterile water
- e) 10 μ l Proteinase K

Mix tube contents and incubate at $\sim 56^{\circ}\text{C}$ for at least one hour. It is recommended to create a master mix of digestion reagents and distribute 400 μ l of the master mix to each sample tube.

Note: for evidentiary material, it is recommended that digestion continue for a minimum of 6 hours. It is appropriate at this point to allow samples to incubate overnight (not more than 24 hours).

Optional: Prior to the addition of 20% sarcosyl, 0.39 M DTT, sterile water, and Proteinase K, aliquot 10 μ l on microscope slide. Fix and stain slide using the Spermatozoa Examination protocol from the Biology Section SOP manual. Record the number of sperm on the DNA Extraction worksheet.

6. Separate DNA from proteins.

- a) To ~ 500 μ L of lysed and digested cells, add 500 μ L of buffered phenol-chloroform solution. Cap tube and vortex until a complete emulsion forms.
- b) Spin in a microcentrifuge for 5 minutes at maximum RCF at room temperature to separate the two phases.
- c) Transfer the top aqueous phase to a 1.5 ml microcentrifuge tube (if additional extractions are needed) or an assembled Microcon tube.
- d) Repeat Steps 6a-c an additional 1 to 3 times, if necessary, until the interface is clean and the aqueous (upper) phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.

7. Microcon concentration.

- a) Using a sterile pipet tip, add up to 500 μ l of sample to sample reservoir. If sample volume is less than 500 μ l, add TE Buffer to bring to a final volume of 500. Seal with attached cap.
- b) Centrifuge 8 minutes at 9,000 RCF. Continue to centrifuge if an insufficient amount of filtrate has passed through filter.
- c) If sample volume is greater than 500 μ l, repeat steps a and b until all sample has been added to the Microcon.
- d) Add 500 μ l TE Buffer to sample reservoir to wash DNA and centrifuge as in step b. Additional TE Buffer washes may be performed to assist in removal of pigments or other contaminants.
- e) Invert sample reservoir into a fresh sample recovery tube. It may be necessary to add TE Buffer to the filter prior to inversion.
- f) Centrifuge inverted unit for 3 minutes at 800 RCF to recover DNA sample. Recovery volume should be 10-40 μ l for samples expected to contain low levels of DNA. Samples that are expected to yield high concentrations of DNA, such as the epithelial cell fraction of a vaginal swab, may be eluted at a larger volume, such as 100 μ l. The final eluate volume of the reagent blank cannot exceed the volume of any of its associated samples.
- g) Store samples refrigerated or frozen until ready to perform qPCR.

7.6 Microcon® Concentration of DNA Solutions

Microcon® microconcentrators can be used for concentrating DNA solutions. This procedure is required following organic extractions, as it also serves to remove salts. It is optional for other extraction procedures.

Related Forms - Because it is optional in extractions other than organic extractions, its use in these situations should be documented on the DNA Extraction Worksheet.

Equipment, Materials, and Reagents

- Microcon® microconcentrators: consists of two components: filtration unit (sample reservoir) and filtrate/recovery tube
- Variable speed microcentrifuge with rotor for 2 ml tubes, at room temperature
- TE Buffer

General

- Do not touch filtration membrane with pipet tip or other object.
- Extended centrifugation can lead to dryness. If this should occur, add at least 10 µl TE Buffer to the sample reservoir, agitate gently for 30 seconds, then proceed with recovery.
- Excessive g-force by centrifugation at >9,300 RCF may result in leakage or damage to the membrane.
- The associated reagent blank sample must also be subjected to any additional manipulations, such as concentration via the Microcon® microconcentrators

Procedure

1. Using a sterile pipet tip, add up to 500 µl of sample to sample reservoir. If sample volume is less than 500 µl, add TE to bring to a final volume of 500. Seal with attached cap.
2. Centrifuge 8 minutes at 9,000 RCF. Continue to centrifuge if an insufficient amount of filtrate has passed through filter.
3. If sample volume is greater than 500 µl, repeat steps 1 and 2 until all sample has been added to the Microcon.
4. Add 500 µl TE Buffer to sample reservoir to wash DNA and centrifuge as in step 2. Additional TE Buffer washes may be performed to assist in removal of pigments or other contaminants.
5. Invert sample reservoir into a fresh sample recovery tube. It may be necessary to add TE Buffer to the filter prior to inversion.
6. Centrifuge inverted unit for 3 minutes at 800 RCF to recover DNA sample. Recovery volume should be 10-40 µl for samples expected to contain low levels of DNA. Samples that are expected to yield high concentrations of DNA, such as the epithelial cell fraction of a vaginal swab, may be eluted at a larger volume, such as 100 µl. The final eluate volume of the reagent blank cannot exceed the volume of any of its associated samples.
7. Store samples refrigerated or frozen until ready to perform qPCR.

7.7 Purification of Extracted DNA using QIAmp

Some DNA extraction procedures may yield DNA solutions containing inhibitors, identified during quantitation and/or STR analysis. This procedure uses the QIAamp® spin columns to remove PCR inhibitors from DNA extracts. Note that this procedure does not work well in removing inhibitors found in urine samples.

The associated reagent blank sample must also be subjected to any additional manipulations, such as purification using QIAamp.

Related Forms - The use of this optional procedure should be documented on the DNA Extraction worksheet.

Safety and Equipment, Materials, and Reagents - See **Section 7.3 DNA Extraction – QIAmp**

Procedure

Add extracted DNA to 200 µl ATL buffer. Incubate at ~56°C for at least one hour. Centrifuge briefly. Proceed to step 3 of **Section 7.3 DNA Extraction – QIAmp**.