1. Scope

This protocol can be used to extract and purify DNA from reference samples and evidence samples containing biological material. DNA can be extracted from dried biological stains or liquid biological material efficiently using the QIAamp DNA Micro Kit and extraction protocol described below. The method is composed of four basic steps. First, the biological material is digested and the cells lysed in a buffer containing a detergent and a protease. Next, the lysate is passed through a membrane that binds the DNA. Contaminants are then washed off the membrane through two washing steps. Finally, the purified DNA is eluted off the membrane in a small volume of an appropriate buffer.

The DNA extract should be free of contaminants, but further purification methods may be used to remove inhibitory substances that remain, if necessary.

This method is recommended for use on biological samples where high levels of DNA are expected such as neat bloodstains or reference samples. Analysts should refer to ATF-LS-FB09 (General QIAamp Investigator DNA Extraction) or ATF-LS-FB35 (QIAamp Micro Double AL DNA Extraction) for extraction of low-level evidence and hair samples in order to maximize recovery of DNA.

2. References

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3. Equipment

3.1. Disposable gloves
3.2. Eye protection
3.3. Lab coat
3.4. Sterile swabs
3.5. Sterile water
3.6. Disposable scalpels or razor blades
3.7. Scissors
3.8. Forceps
3.9. 70% ethanol or alcohol wipes
3.10. 10% bleach solution
3.11. QIAamp® DNA Micro Kit (Qiagen Catalog # 56304) containing the following:
  3.11.1. QIAamp® MinElute™ columns
  3.11.2. Collection Tubes
  3.11.3. Buffer ATL
  3.11.4. Buffer AL
  3.11.5. Buffer AW1
  3.11.6. Buffer AW2
  3.11.7. Buffer AE (not used)
  3.11.8. Carrier RNA
  3.11.9. Proteinase K (typically not used)
3.12. Invitrogen Proteinase K
3.13. Dithiothreitol (DTT)
3.14. Pipettes
3.15. Disposable aerosol-resistant pipettor tips
3.16. Microcentrifuge tubes
3.17. NAO™ Baskets/QIAGEN® Investigator Lyse and Spin tubes
OPTIONAL: DNA IQ™ Spin Baskets
3.18. Benchtop hood
3.19. TE^{-4} (10mM Tris-HCl, 0.1mM EDTA, pH 8.0)
3.20. Bench paper
3.21. Thermomixer
3.22. Centrifuge
3.23. Vortexer

4. Safety/Quality Assurance

4.1. Disposable gloves shall be worn when handling kit reagents and evidence.
4.2. Perform extraction steps, where possible, in a hood to reduce the risk of contamination. Clean surfaces with 10% bleach solution before and after use. After exiting the hood, turn on the UV light (automatically set for 15 minutes of exposure).

4.3. When possible, reference samples will be processed in a separate dedicated area after the analysis of evidence samples has been completed.
4.4. Use aerosol-resistant pipettor tips when transferring liquids containing DNA.
4.5. Change pipettor tips after transferring any liquids potentially containing DNA.
4.6. Record the lot number of each reagent used in notes. Do not use the reagents after the expiration date.
4.7. Initiate at least one reagent blank as the final sample of the set of extractions.
4.8. Lab coat and eye protection must be worn at all times while performing this procedure.
4.9. Only tubes associated with one sample shall be open at a time.
4.10. Exercise caution when opening tubes.
4.11. The laboratory bench surface shall be cleaned before and after use with 10% bleach solution or other sanitizing agent and may be followed by 70% ethanol. Fresh bench paper shall then be placed on the surface prior to examination.
4.12. Final DNA extract tubes shall be labeled, at a minimum, with case number, item number, and analyst's initials.
4.13. Samples thought to contain lower levels of DNA will be handled before those thought to contain large amounts of DNA.
4.14. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Sample Preparation

5.1. Any utensils used to cut or manipulate the swabs or other types of evidence must be cleaned between uses with 10% bleach solution followed by 70% ethanol or alcohol wipe. Alternatively, single-use disposable razor blades may be used.
5.2. To maximize lysate recovery, cuttings of swab tips or evidence substrate should be placed in a clean NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket with tube. Alternatively, a clean microcentrifuge tube may be used.
5.2.1. Cuttings from biological stains/substrate (clothing, carpet, etc.) should be approximately 5 mm x 5 mm in size. Some specimens may require cuttings of differing sizes depending on the type, condition and concentration of the biological material. Larger sized cuttings may be cut into smaller pieces before placing them into the tube.
5.3. For liquid samples, place a portion of the liquid (1-100 µL) directly into a clean microcentrifuge tube.
5.4. For reference samples, cut a small portion of blood card (up to 5 mm^2), swab tip, or other reference material and place in a clean microcentrifuge tube (or in a clean NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket with tube if maximum recovery of lysate is a priority).

6. Procedure

6.1. Check the reagent logs or the reagent bottles to ensure that Buffer AW1, Buffer AW2, and carrier RNA have been appropriately prepared. Prior to use, Buffers ATL, AW1, and AW2 must contain no precipitates. If precipitates have formed, gently heat the bottles prior to dispensing the reagents.
6.2. A Reagent Blank shall be initiated as the last sample in the set of samples. The Reagent Blank shall contain all the liquid reagents contained in the evidentiary samples except for the biological material. The Reagent Blank shall be handled in a manner that is identical to the evidentiary samples and use the most sensitive volumes and steps used with the

evidentiary samples. For example, if carrier RNA is used with only a few of the evidentiary samples being extracted in a set, carrier RNA will be added to the Reagent Blank, as well.

6.2.1. If the analyst determines that two or more evidentiary extracts may be combined during the concentration step, the same number of reagent blanks shall be initiated at the DNA extraction step. For example, if the analyst determines that the extracts of three sets of swabs from a firearm may be combined and concentrated later in the analysis, then the analyst shall initiate three reagent blanks at the DNA extraction step.

6.2.2. If an NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket is used, the tips of two swabs should be placed in the Reagent Blank to facilitate the recovery of the entire volume of sample.

Cell Lysis

6.3. Add **300-400 µL of Buffer ATL** and **20 µL of ProK** (20 mg/mL) to the sample tube.

6.3.1. The volume of ATL buffer may be increased to ensure that the sample is completely immersed in the lysis solution. If the volume of Buffer ATL is increased, the volume of ProK should be increased proportionally.

6.4. Vortex for approximately 10 seconds.

6.5. Place the samples in a thermomixer and incubate at 56° C with shaking at 900 rpm for at least 1 hour.

NOTE: Samples may be incubated overnight; however, incubation times greater than 18 hours have been shown to decrease DNA yield.

6.6. If NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket is used for improved lysate recovery, centrifuge samples for 1 - 3 minutes at 18,000 x g. Baskets and substrate may be discarded at this point. Continue at step 6.8.

6.7. Briefly centrifuge the samples to remove condensation from inside the lid.

6.8. Add **300-400 µL of Buffer AL** (use a volume equal to the volume of Buffer ATL used in step 6.3).

6.9. **1 µL of carrier RNA** (1 µg/µL) can be added at this point if, due to the concentration or condition of the biological material, it is determined by the analyst that carrier RNA may aid in the recovery of the DNA.

6.10. Vortex for approximately 10 seconds.

6.11. Place the tubes in a thermomixer and incubate at 70° C with shaking at 900 rpm for at least 10 minutes.

NOTE: Any white precipitate that formed when the Buffer AL was added should disappear during this incubation step.

6.12. Briefly centrifuge the samples to remove condensation from the inside of the lid.

6.13. If still present, the solid substrate can be removed and discarded.

NOTE: If maximum recovery of lysate is a priority, and NAO™ Baskets/QIAGEN® Investigator Lyse and Spin baskets are unavailable, the solid substrate can be placed in a DNA IQ™ Spin Basket for centrifugation.

6.13.1. If DNA IQ™ Spin Baskets were used, centrifuge tubes for 2 minutes at full speed.

6.13.2. Discard basket and substrate after centrifugation.

Bind DNA

6.14. Carefully transfer up to 700 µL of the supernatant from the microcentrifuge tube to a QIAamp MinElute™ column without wetting the rim of the column.
6.15. Centrifuge the column at 6000 x g for 1 minute. Place the MinElute ™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
6.16. If any supernatant remains, repeat steps 6.14 and 6.15.

Wash DNA

6.17. Carefully open the QIAamp MinElute™ column and add **500 µL of Buffer AW1** without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute.
6.18. Place the QIAamp MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
6.19. Carefully open the QIAamp MinElute™ column and add **500 µL of Buffer AW2** without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute.
6.20. Place the QIAamp MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
6.21. Centrifuge at full speed for 3 minutes to dry the membrane completely.
6.22. Place the QIAamp MinElute™ column in a clean microcentrifuge tube and discard the collection tube containing the flow-through.

Elute Purified DNA

6.23. Carefully apply **20-50 µL of TE^{-4}** to the center of the membrane.
6.24. Incubate at room temperature for 0-5 minutes.
6.25. Centrifuge at full speed for 1 minute.
OPTIONAL: Repeat steps 6.23 through 6.25 to increase yield.

6.26. Remove the QIAamp MinElute™ column and cap the tube. The QIAamp MinElute™ column can be discarded. The DNA extract may be concentrated at this point or may proceed to quantitation directly.