ATF-LS-FB23
Low Template DNA Extraction using the QIAamp®
Investigator Kit on the QIAcube® Robotic Workstation

Authority: Technical Leader

Unofficial Copy; May Not Be Most Current Version

1. Scope

This protocol is used to extract and purify DNA from low template evidence samples and hair root material. DNA can be extracted from biological material efficiently using the QIAamp® DNA Investigator Extraction Kit on the QIAcube® Robotic Workstation using the custom ATF DNA purification protocol. First, the biological material is digested and the cells are lysed in a buffer containing a detergent and a protease. Next, the samples are placed on the QIAcube® and through automation, the lysate is passed through a membrane that binds the DNA. Contaminants are then washed off the membrane through three 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.

2. References

2.3. Qiagen, Developmental validation of the QIAamp DNA Investigator Kit, July 2015, available at: https://www.qiagen.com/us/resources/resourcedetail?id=1d9de4e8-cbd4-4be7-aec2-dd9f79f038fb&lang=en&autoSuggest=true.


2.15. ATF Laboratory Validation of the Custom ATF DNA Extraction Protocol on the QIAcube® Robotic Workstation.

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. QIAcube® Robotic Workstation containing the following:
   3.11.1. QIAcube® Reagent bottles
   3.11.2. QIAcube® Reagent rack with appropriate labeling strips
   3.11.3. QIAcube® Centrifuge rotor adapters
   3.11.4. QIAcube® 1000ul Filter tips
   3.11.5. QIAcube® 1.5 mL Microcentrifuge elution tubes
3.12. QIAamp® DNA Investigator Kit (Qiagen Catalog # 56504) containing the following:
   3.12.1. QIAamp® MinElute™ columns
   3.12.2. Collection Tubes
   3.12.3. Buffer ATL
   3.12.4. Buffer AL
   3.12.5. Buffer AW1
   3.12.6. Buffer AW2
   3.12.7. Buffer AE
   3.12.8. Carrier RNA
   3.12.9. Proteinase K (typically not used)
3.13. Invitrogen Proteinase K
3.14. 96-100% Ethanol (EtOH)
3.15. Dithiothreitol (DTT)
3.16. Pipette
3.17. Disposable aerosol-resistant pipette tips
3.18. Microcentrifuge tubes
3.19. NAO™ Baskets/QIAGEN™ Investigator Lyse and Spin Baskets and Tubes (OPTIONAL: DNA IQ™ Spin Baskets)
3.20. Bench top hood
3.21. TE-d (10mM Tris-HCl, 0.1mM EDTA, pH 8.0)
3.22. Bench Paper
3.23. Thermomixer
3.24. Centrifuge
3.25. Vortexer

4. Safety/Quality Assurance

4.1. 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.
4.2. Disposable gloves shall be worn when handling kit reagents and evidence.
4.3. Extraction steps performed outside of the QIAcube® robotic workstation should be performed in a hood to reduce the risk of contamination. Clean surfaces with 10% bleach solution prior to use. After exiting hood, turn on UV light (automatically set for 15 minutes of exposure).
4.4. Use aerosol-resistant pipette tips when transferring liquids containing DNA.
4.5. Change pipette 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 the appropriate number of reagent blanks as the final samples of the set of extractions.
4.8. Lab coat and eye protection must be worn at all times while performing this procedure.
4.9. When practical, 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 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 evidence examination.
4.12. The QIAcube® worktable shall be cleaned with 70% ethanol solution before and after use.
4.13. Final DNA extract tubes shall be labeled, at a minimum, with case number, item number, and analyst’s initials.
4.14. Samples thought to contain lower levels of DNA will be handled before those thought to contain large amounts of DNA.
4.15. 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 technical leader.

5. Procedure

5.1. 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, Buffer ATL must contain no precipitates. If precipitates have formed, gently heat the bottle prior to dispensing the reagent.

**Cell Lysis (performed manually off instrument)**

5.1.1. Lyse samples according to step 5.2 (touch evidence) or 5.3 (hair roots):

NOTE: 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 sample(s), and is the most sensitive volumes and steps used with the evidentiary sample(s). 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. Additionally, 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 it is possible that the three sets of swabbings from a firearm may be combined and concentrated at a later step of the analysis, then the analyst shall initiate three reagent blanks at the DNA extraction step.

NOTE: If batches containing greater than twelve samples are extracted using QIAcube® workstations at least one reagent blank shall be processed on each of the QIAcube® workstations employed. If, after quantitation, the analyst determines that evidence samples processed on separate QIAcube® workstations are to be combined for further processing, then at least one reagent blank processed on each QIAcube® shall be combined for further processing. If, after quantitation, the analyst determines that none of the samples will be combined, then at least one reagent blank from each QIAcube® workstation will be processed with the associated samples.

5.2. Touch Evidence

5.2.1. Sample the area of interest using the appropriate sampling method for the evidence, and place sample in a clean Qiagen® Investigator Lyse and Spin basket.

5.2.2. To initiate reagent blanks, cut the tips of two clean swabs using a clean pair of scissors (disposable scalpel, razor blade) and place them in a clean Qiagen® Investigator Lyse and Spin basket. Initiate the appropriate number of reagent blanks based on the sample set being processed.

5.2.3. Add 400 µL of Buffer ATL and 20 µL of ProK (20 mg/mL) to each sample and vortex for approximately 10 seconds. Continue at step 5.4.

5.3. Hair Roots

NOTE: Prior to processing any hairs for DNA, consult with trace evidence examiners.

5.3.1. Using a clean pair of forceps and/or scissors (or disposable scalpel/razor blade), cut off a 0.5-1 cm piece of the root end of the hair and place it in a clean 2 mL safe-lock microcentrifuge tube. Gently rinse hair with 70% ethanol followed by sterile water.

5.3.2. Initiate a reagent blank using a clean 2 mL safe-lock microcentrifuge tube.

5.3.3. Add 400 µL of Buffer ATL, 20 µL of ProK (20 mg/ml), and 20 µL of 1M DTT to each sample and vortex the tubes for approximately 10 seconds. Continue at step 5.4.

5.4. Place samples in a thermomixer and incubate at 56°C with shaking at 900 rpm for at least three hours. Samples may be incubated overnight; however, incubation times greater than 18 hours have been shown to decrease DNA yield.

NOTE: If NAO™ Basket/QIAGEN® Investigator Lyse and Spin baskets are being used to improve lysate recovery, then centrifuge samples for 1 minute at 10000xg. OPTIONAL: Centrifuge time can be increased up to 3 minutes and speed increased up to maximum if residual lysate is observed in the baskets. Baskets and substrate may be discarded at this point.

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

5.6. Add 1 µL of cRNA, vortex for approximately 15 seconds, and briefly centrifuge.

QIAcube® Workstation Setup

5.7. Fill both tip racks with 1000 µl filter tips.

5.8. Fill reagent bottles with current lot of Buffer AL, 100% EtOH, Buffer AW1, Buffer AW2, and TE-4. Place the filled reagent bottles in the correct position in the reagent bottle rack as marked on the QIAcube®.

NOTE: Upon receipt of new Qiagen® Investigator kits and prior to quality control
testing of the new kits, Buffers AW1 and AW2 will be prepared according to manufacturer specifications. Buffers AL, AW1, and AW2 will be individually combined to create one lot of each of the three buffers. When replenishing reagents on the QIAcube® workstations, the combined reagent bottles will be used. NOTE: Workstation setup will differ based on the number of samples being processed. Refer to the QIAcube® protocol sheet (Appendix A) for instructions on appropriate placement of loaded rotor adapters in centrifuge and sample tubes containing lysate into shaker rack.

NOTE: The QIAcube® cannot process 1 sample or 11 samples in a single run due to balance requirements of the centrifuge.

5.9. Remove the shaker rack from the QIAcube®. Place labeled sample tubes containing lysate into the shaker rack with the lids of the samples placed in the slots on the edge.

5.10. Place appropriate number of QIAamp® MinElute™ columns and labeled 1.5 mL microcentrifuge elution (collection) tubes into rotor adapters. NOTE: The QIAamp® MinElute™ column is placed in the rotor position with one corresponding lid holder with the lid in the slot and the microcentrifuge elution tube is placed in the rotor position with two corresponding lid holders with the lid placed in the lower left slot (Figure 1).

Figure 1: Appropriate placement of QIAamp MinElute™ column and Elution tubes in rotor adapter.

5.11. Place loaded rotor adapters in centrifuge buckets. Ensure that the lips of the QIAamp® MinElute™ columns and labeled 1.5 mL microcentrifuge elution tubes are flush with the top of the rotor adapter. NOTE: Rotor adapters only fit in the centrifuge buckets in one orientation to ensure correct instrument setup.

5.12. Place the loaded shaker rack in the shaker. Ensure that the lips of the 2.0 mL microcentrifuge sample tubes are flush with the top of the shaker rack.

Performing the ATF Custom DNA Purification Run on QIAcube®

5.13. Power on the QIAcube® workstation and allow the instrument to perform start-up procedure.

5.14. From the home menu, press DNA.

5.15. From the “DNA” menu, use the down arrow to highlight option 2 “QIAamp DNA Investigator” and press Select.

5.16. The option titled “Surface and buccal swabs” will be highlighted. Press Select.
5.17. Use the down arrow to highlight option 2 titled “Swab purification modified” and press Select.
5.18. Press Start then follow the instructions on the screen to ensure the instrument is set-up properly and initiate the custom ATF DNA purification protocol.
5.19. Upon completion of the protocol, carefully remove rotor adapters from the centrifuge and retain the labeled 1.5 mL microcentrifuge elution (collection) tubes containing the DNA extracts. Place caps on the elution tubes containing the DNA extracts. Discard the remaining rotor adapter and its contents.
5.20. The DNA extracts may be concentrated at this point or may proceed to quantification directly.
5.21. Discard the sample tubes from the shaker rack.
5.22. Place the appropriate lids on the reagent bottles.
5.23. Discard the used tips located in the waste drawer under the touch screen.
5.24. Discard any empty tip racks.
5.25. Wipe down the internal surface area of the workstation and the waste drawer using 70% EtOH.
5.26. Complete the appropriate instrument run log to include run date, analyst’s initials, number of samples on run, run type (casework, research, etc.), and any additional comments.
Appendix A: Loading the Centrifuge and Shaker Rack

<table>
<thead>
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<th># of Samples</th>
<th>Centrifuge Setup</th>
<th>Shaker Rack Setup</th>
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<td><img src="image2" alt="2 samples Shaker Rack Setup" /></td>
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<tr>
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<td><img src="image3" alt="3 samples Centrifuge Setup" /></td>
<td><img src="image4" alt="3 samples Shaker Rack Setup" /></td>
</tr>
<tr>
<td>4 samples</td>
<td><img src="image5" alt="4 samples Centrifuge Setup" /></td>
<td><img src="image6" alt="4 samples Shaker Rack Setup" /></td>
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Appendix A: Loading the Centrifuge and Shaker Rack

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<td><img src="image1" alt="Centrifuge Setup 5 samples" /></td>
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<td>6 samples</td>
<td><img src="image3" alt="Centrifuge Setup 6 samples" /></td>
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<tr>
<td>7 samples</td>
<td><img src="image5" alt="Centrifuge Setup 7 samples" /></td>
<td><img src="image6" alt="Shaker Rack Setup 7 samples" /></td>
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Appendix B: Internal View of QIAcube®
Components of Note:
1. Centrifuge Lid
2. Centrifuge with Loaded Rotor Adapters
3. Shaker with Loaded Shaker Rack
4. Reagent Rack with Reagents Bottles
5. Tip Rack with 1000µl Filter Tips
6. Disposal Slot for tips