Semi-Automated Extraction of DNA

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Semi-Automated Extraction of DNA

1 INTRODUCTION

These procedures describe the process for extraction and purification of deoxyribonucleic acid (DNA) using the QIAcube® and/or EZ1® Advanced XL (EZ1).

2 SCOPE

These procedures apply to DNA personnel that perform semi-automated extraction and purification of DNA and DNA personnel that perform the associated quality control procedures.

3 EQUIPMENT

3.1 Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, vortex, centrifuge)
- Incubator (Thermo MaxQ 4450 or 4000, Thermo 6841, Labline Imperial III, Heratherm IGS 100, or equivalent) or thermomixer (Eppendorf Thermomixer 5350s or equivalent)
- Costar® spin baskets, or equivalent (*for differential extractions*)
- Qiagen® Lyse & Spin Baskets and Collection Tubes, or equivalent (*for normal extractions*)
- QIAcube®
- QIAcube® consumables (e.g., 1000 µL wide-bore filter-tips, reagent bottles, rotor adapters, QIAcube®-compatible 1.5 mL microcentrifuge tubes)
- EZ1® Advanced XL
- EZ1® DNA Investigator Kit (e.g., filter tips, tip holders, elution tubes, reagent cartridges)

3.2 Reagents

- Buffer ATL (for EZ1 female fraction)
- Buffer G2 (for EZ1 normal lysis and EZ1 male fraction)
- 1M Dithiothreitol (DTT)
- Proteinase K (Pro K)
- Reagent Grade Water

4 STANDARDS AND CONTROLS

At least one extraction control (i.e., reagent blank) must be processed in parallel with each extraction batch. The reagent blank(s) will be processed as the last sample(s) in the batch.

For evaluation of the extraction controls, refer to the appropriate DNA interpretation procedure (i.e., BIO-570, BIO-571, BIO-572).

5 PROCEDURE

Refer to the DNA Procedures Introduction (i.e., BIO-100) for applicable general precautions and cleaning instructions.

Ensure the appropriate fields (i.e., instruments, reagents) in the Sample Tracking and Control Software (STACS) are completed, as necessary.

5.1 Normal Lysis

The samples should generally be in Lyse & Spin baskets in corresponding tubes when using an incubator. Some samples do not require the use of a basket. If using a thermomixer, the Lyse & Spin baskets should not be used.

1.	Create master mix using the recipe below.

Normal Lysis Master Mix

Reagent	µL per sample
Buffer G2	470
Pro K	15
DTT	15

2.	Add 450 µL master mix to each tube.
3.	Incubate the tubes at 56ºC with agitation (generally in an incubator at 200 rpm) for ~1 hour.
4.	Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes). Discard the basket, if applicable.

If the lysate does not completely flow through the basket, additional spins may be added. If necessary, lysate remaining in the basket may be manually transferred to the sample tube and/or a new basket may be used for additional spins. These additional manipulations will be recorded in the case notes. If after additional manipulations, the volume in the sample tube is significantly different than the expected volume a case note will be made.

5.	Process the lysates on the EZ1 following the steps in section 5.3.

5.2 Differential Lysis and QIAcube® Fractionation

The samples should be in QIAcube® compatible tubes. Lyse & Spin baskets must NOT be used for differential extractions.

1.	Create the epithelial (F) fraction master mix using the recipe below.

Female Fraction Master Mix

Reagent	µL per sample
Buffer ATL	160
Reagent Grade Water	320
Pro K	20

If precipitate has formed in the Buffer ATL, heat it, generally at 56°C, until precipitate is no longer visible.

2.	Add 450 µL master mix to each tube.
3.	Incubate the tubes at 56°C with agitation (generally in a thermomixer at 900 rpm) for ~1.5 hours.
4.	• If necessary, quick spin and transfer the substrate to a basket. • Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes). Discard the basket.
5.	Ensure consumables, reagent grade water, and lysate tubes are properly loaded onto the QIAcube® instrument.

Consumables include: bottles containing reagent grade water, 1000 µL wide-bore tips, rotor adapters, and F fraction collection tubes. A shaker rack plug must be in place next to each F fraction collection tube.

Appendix A *has additional guidance for loading the QIAcube®.*

6.	Initiate the "Separate and Lyse 12A Mod" protocol on the QIAcube®.

The bottle in position 1 will contain reagent grade water, not Buffer G2 as prompted.
Be aware that lysates are 450 µL, not 500 µL as referenced in the QIAcube® prompt.

7.	• At the completion of the run, tubes containing the F fraction should be removed and capped. • Refill consumables and reagent grade water as necessary.

The M fractions continue processing on the QIAcube®. Processing of the F fractions resumes at section 5.3, either independently or with the M fraction lysates.

8.	Initiate the “Separate and Lyse 12B Mod” protocol on the QIAcube®.

The bottles will contain reagent grade water, not Buffer G2 as prompted.

9.	At the completion of the run, remove and close the tubes containing the M fraction(s).

The M fraction tubes should contain approximately 50 µL of liquid following this run. If excess liquid remains, the tubes may be centrifuged (generally between 9,000 and 13,000 rpm) for 5 minutes and the excess supernatant manually removed and discarded while avoiding the pellet. If this step is necessary, a case note will be made.

10.	A slide for microscopy may be prepared from the M fraction according to Serology procedure for Christmas tree staining (i.e., BIO-421).
11.	Ensure the M fraction master mix is created.

Male Fraction Lysis Master Mix

Reagent	µL per sample
Buffer G2	375
Pro K	24
DTT	94

12.	Add 450 µL master mix to each M fraction tube.
13.	Vortex, quick spin, and incubate the tubes at 70ºC with agitation (generally in a thermomixer at 900 rpm) for ~10 minutes.
14.	Vortex the tubes vigorously (~10 seconds) and quick spin.
15.	Process the lysates on the EZ1 following the steps in section 5.3.

Ensure waste is disposed of properly.

The water bottles on the QIAcube® should be emptied at the end of each day of use.

5.3 Processing Lysates on the EZ1® Advanced XL

1.	Ensure the EZ1 is UV irradiated for 20 minutes prior to initial use each day.

If necessary, transfer the lysate to an EZ1 compatible tube.

2.	If appropriate, two lysates may be combined prior to loading on the EZ1.
3.	Ensure consumables, lysate tubes, and barcoded elution tubes are properly loaded onto the EZ1. See Figure 1.

If precipitate has formed in the EZ1 cartridges, heat them, generally at 56°C, until precipitate is no longer visible.

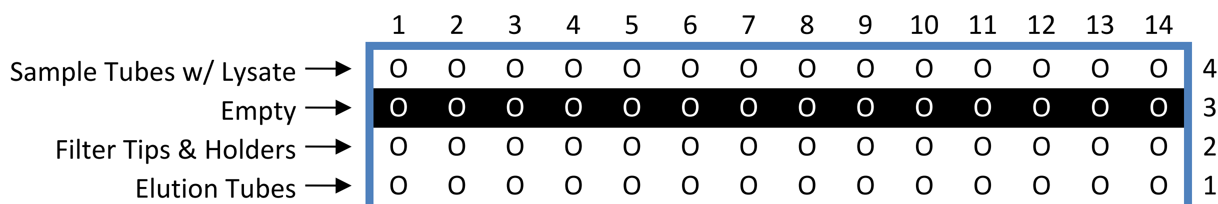


Figure 1 - Loading the EZ1® Advanced XL

4.	Ensure the Large Volume protocol with elution into 50 µL of water has been selected and start the EZ1.

Disregard the reference to MTL Buffer in the prompts on the EZ1.

5.	At the completion of the run, remove and cap elution tubes.

If the volume in the elution tube is significantly different than the expected volume a case note will be made.

6.	If necessary, combine appropriate extracts.

A maximum of eight 50 µL extracts may be combined into one tube and concentrated using the Speed-Vac or Vacufuge.

7.	• Ensure waste is disposed of properly. • Ensure the piercing units on the EZ1 are cleaned after daily use and clean the tray, worktable, and racks, if needed.

Bleach should not be used on the QIAcube® or the EZ1® Advanced XL. *70% ethanol should be used to clean instrument surfaces.*

5.4 Combining/Concentrating Extracted Samples Using a Microcon Filter

For concentrating samples using the Speed-Vac or Vacufuge, refer to the procedures for quantification of nuclear DNA (i.e., BIO-520).

1.	Vortex and quick spin the extract tubes. Transfer the extract for each sample being combined/concentrated into a labeled microcon assembly.

Corresponding reagent blanks must also be combined/concentrated using a Microcon.

2.	Spin the tubes to draw the fluid through the membrane (generally between 6,000 and 8,000 rpm for 10 minutes).

Speed and time may be increased to draw fluid through membrane, but to avoid damage to the membrane, excess speed and time should not be used.

If additional spins do not reduce the volume, the affected sample(s) may continue with processing at step 4. Record the final volume.

3.	Add reagent grade water (generally 15 µL).
4.	Invert microcon into a new, labeled tube.
5.	Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes).

6 Limitations

The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

7 Safety

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.
- Procedural Specific Chemical Hazards:
    - Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
    - EZ1 reagent cartridges contain ethyl alcohol and guanidine salts which are hazardous materials. Solutions containing guanidine salts will generate toxic fumes when combined with bleach. Use appropriate care and wear appropriate protective clothing and eyewear when handling. Be careful not

to expose face or hands to splashes. Dispose of EZ1 consumables in appropriate waste containers.

8 References

Qiagen®. *QIAcube® User Manual*, June 2008.

Qiagen®. *EZ1® Advanced XL User Manual*. May 2009.

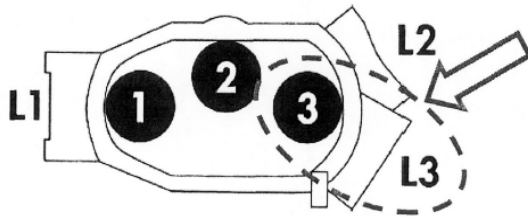
Qiagen®. *EZ1® DNA Investigator Handbook.* April 2009.

9 Revision History

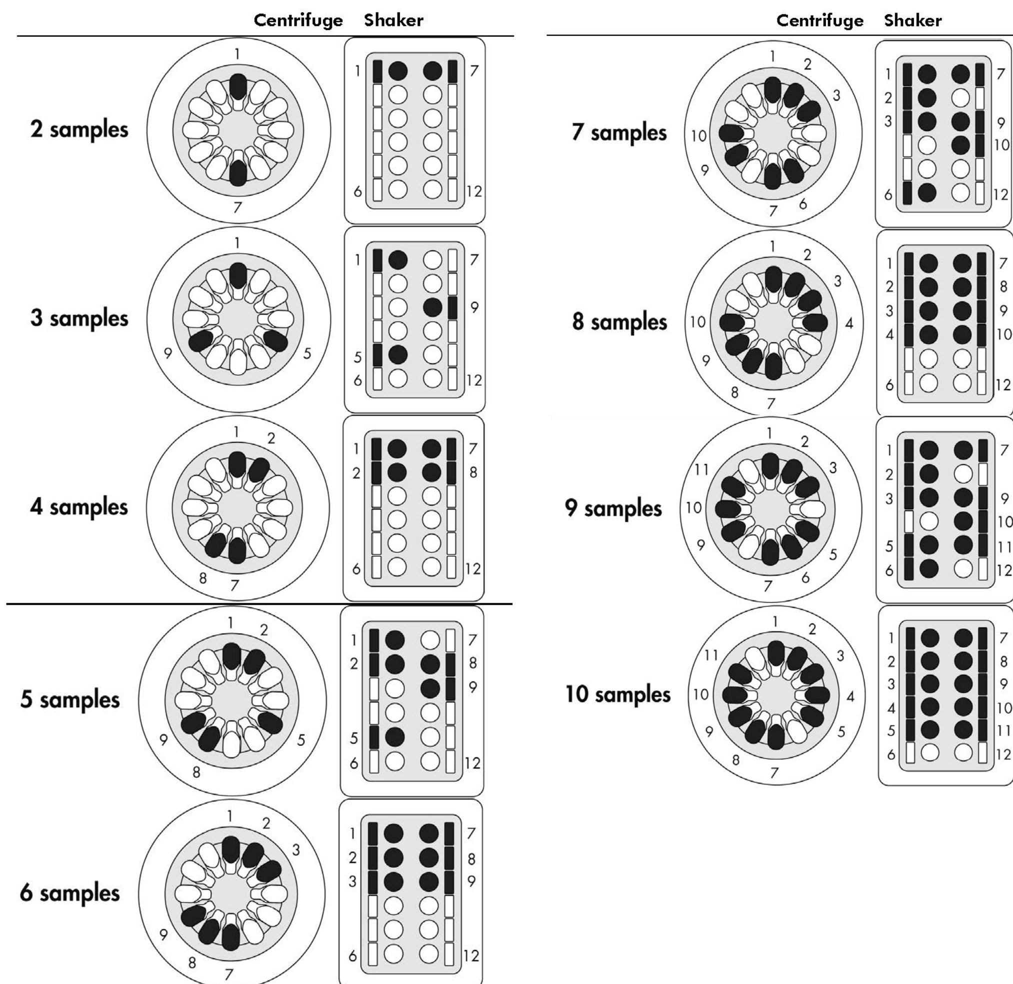
Revision	Issued	Changes
00	06/15/2022	Reformatted DNA 232-4 into new template and assigned new Doc ID.

10 APPENDIX A: LOADING THE QIACUBE®

The lysate tubes fit into position 3 of the rotor adaptors, with their caps folded back and inserted into position L3.

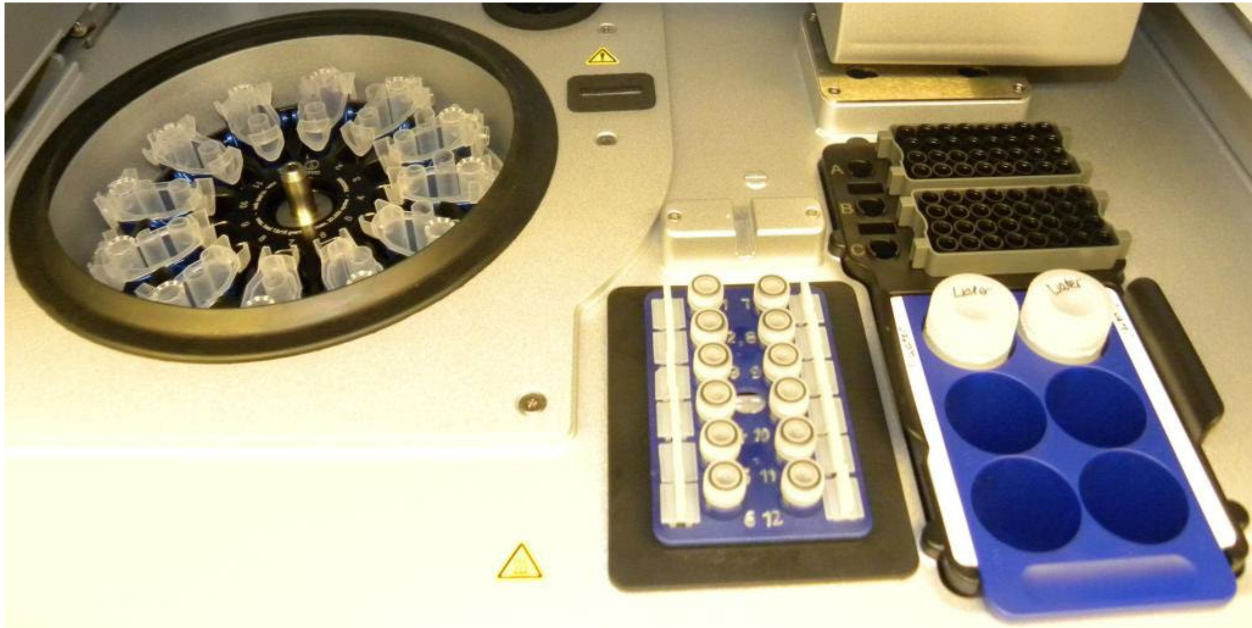


Rotor adaptors with lysate tubes must be distributed on the centrifuge for balance.



Appendix A: Loading the QIAcube®(continued)

An overview of the loaded QIAcube® . (All caps must be removed prior to starting a run)



11 Appendix B: Instrument General Maintenance and Performance Verification Procedures

Refer to the DNA procedure for instrument calibration and maintenance (i.e., BIO-104) for minimum frequency of performance verifications and additional requirements.

1. QIAcube® Robotic System (Extraction)

A. General Maintenance

There is no general maintenance required for the QIAcube®.

B. Performance Verification

A portion of a swab containing 5 µL female blood and 1 µL of a 10x dilution of semen (semen diluted with reagent grade water) with known typing results will be processed using the QIAcube® differential lysis procedure followed by extraction using the appropriate DNA standard operating procedure (SOP).

The mixture must yield a distinguishable M fraction containing predominantly male DNA with minimal carryover from the F fraction as determined by quantification and amplification. The mixture is expected to yield a distinguishable F fraction containing predominantly female DNA but may contain a mixture of male and female DNA depending on the sample.

If the sample does not produce a distinguishable M fraction the process should be repeated with a new sample. If a predominately male DNA containing M fraction is not accomplished after 2 attempts the Technical Leader (TL) will be consulted.

2. EZ1® Advanced XL Robotic System (Extraction)

A. General Maintenance

Quarterly: Inspect the O-rings and grease as needed.
Test Heating Block at 70°C.

B. Performance Verification

A swab containing 5 µL blood from a known donor will be processed using the Normal lysis procedure followed by the EZ1 extraction procedure for each of the 14 channels of the EZ1 robot. Alternately, individual channels may be tested to verify performance as needed.

A sample from each channel must yield a concentration, determined by quantification, greater than the minimum value established for the specific donor batch on the EZ1 using a 50 µL elution volume. A channel, or channels, may be repeated if necessary.

If a channel, or channels, does not yield the appropriate concentration after 2 attempts the TL will be consulted.

3. Establishing the minimum value for Extraction Robot PV

Use an in service instrument and the appropriate extraction procedure to extract several samples from a blood card (i.e., FTA card) or swabs spotted with donor blood. The minimum quantitation value will be established by calculating 50% of the average of the quantitation values of the extracted samples.