1. Follow the Procedure for DNA Extraction using the EZ1 Advanced XL through 5.4.3.

QIAcube setup and Plasticware Prep

2. During the incubation period, label adaptors, the lids of clean microcentrifuge flip top tubes, and 2mL screw top tubes with a minimum of the spin bucket number. This will correspond with the workbook QIACube tab. Analysts may choose to close the lids until the incubation period has completed. If the analyst chose not to close the microcentrifuge tubes, it is recommended for the analyst to bend the hinge of each tube prior to adding samples. This will help the tube to sit flush in the adaptor.

3. Ensure the current lot of STR-SEB is available, fill the bottle to the line, and place in well 1 of the reagent block rack. Fill the diH2O to the line and place in well 2 of the reagent bottle rack. Confirm the tips are full and turn on the QIACube. When filling the tips, confirm the proper tips are within the rack.

4. Place the 2mL flat bottom tubes, without lids into the shaker which corresponds to the workbook set up. Place the rubber stoppers (tape can be used) in the shaker slot where the 2mL tubes have been placed. The solution within the 2mL flat bottom tubes will be considered as Fraction 1 (formerly non-sperm fraction or epithelial cell portion). The solution within the flip top microcentrifuge tubes located in the adaptor is considered as Fraction 2 (formerly the sperm fraction).

Sample Prep

5. Steps 2, 3, and 4 can be performed at any time that makes it easiest for the analyst.

6. Once the incubation period has completed, briefly spin in a microcentrifuge to force condensate to the bottom of the tube. Aseptically transfer the material to a basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at high speed for 30 seconds. Remove the basket and discard it into a biohazard waste container.

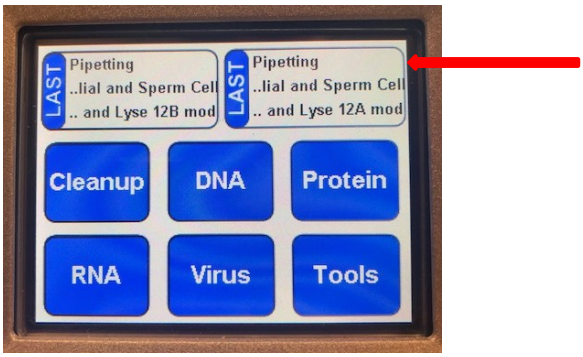
7. With the workbook QIACube form available, align each sample in bucket/adaptor order. Secure the flip top microcentrifuge tube in the corresponding adaptor and place in the convenience carry tray. Open the first incubated sample, if applicable discard the catch basket, and while pipetting up and down to dislodge any pellet that might have formed, aseptically transfer the solutions to the newly labeled microcentrifuge flip top tube and adaptor assembly. Continue this step until all items have been transferred. The baskets shall be discarded into a biohazard waste container.

8. If the QIACube lid is not open, open the lid, place the adaptors, starting with bucket 1, in the corresponding buckets. Bucket 1 is circled and may not always be in the same position to load at the beginning of each run.

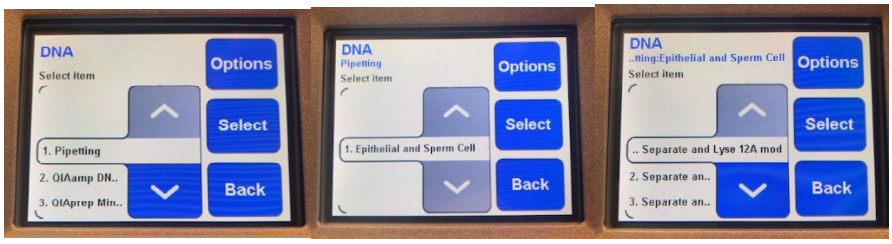
9. Make sure all lids on tubes for Fraction 1 (epithelial fraction, formerly non-sperm fraction) and the reagent bottles are removed, all tubes and adaptors are flush, and the tips are secure.

QIAcube Program

10. Select the desired program using the touch screen. The 12A/B program can be used for 1 up to 12 samples and the 6 program can be used for 1 up to 6 samples. If the desired run is listed at the top of the touch screen you may select from here.



- **12 A program** This program can be selected for 1 through 12 samples
  - I. On the touch screen select DNA
  - II. Pipetting, then press select
  - III. Epithelial and Sperm Cell, then press select
  - IV. Separate and Lyse 12A mod, then press select
  - V. Start
- **12 B program** This program can be selected for 1 through 12 samples
  - VI. On the touch screen select DNA
  - VII. Pipetting, then press select
  - VIII. Epithelial and Sperm Cell, then press select
  - IX. Separate and Lyse 12B mod, then press select
  - X. Start
- **6 program** This program can be selected for 1 through 6 samples
  - XI. On the touch screen select DNA
  - XII. Pipetting, then press select
  - XIII. Epithelial and Sperm Cell, then press select
  - XIV. Separate and Lyse 6 mod, then press select
  - XV. Start



11. The program estimated time is based off of the last run. When running at full capacity of the program, plan for the approximate times of completion: 6 Program - roughly 45 minutes, 12A Program – roughly 55 minutes, and 12B Program – roughly 45 minutes.

12. For all programs, the analyst shall stay at the instrument until after the initializing steps have been completed. This period is the only time in which an analyst may be permitted to opening the lid. At any other time during the protocol if the lid is opened the program will stop and will have to be re-started from step 1.

13. In instances where the Qiacube could not complete the separation process, either due to the lid being opened or any error messages present, the analyst shall remove all the adaptors, take out the 1.5mL or 1.7mL sample tube, spin in a microcentrifuge on high for 5 minutes, remove the supernatant leaving approximately 35-50µL in the tube, and continue to step 19. All instances of incomplete runs shall be reported to the DNA Technical Leader. Errors shall also be reported to the QC Officer or designee. A note shall be added to the workbook as well.

12A and 6 Program Complete

14. After the 12A Program is completed, open the lid, restock the tips, and analysts are allowed to remove Fraction 1. Analysts shall then follow the DNA Purification (Large Volume) protocol. Continue with the 12B Program.

15. Once the 6 Program has completed, remove the 2mL screw top tubes, Fraction 1, and continue with the DNA Purification (Large Volume) protocol.

16. Once the 12B and/or 6 Programs have completed, label slides with case number, item number, initials and date (or with a printed label).

17. Re-stock all tips and refill buffer and water bottles. Screw lids back on the bottles, wipe the deck down and empty and wipe the tip drawer, deck, and inside of centrifuge lid. Also wipe down the adaptor tray and turn the instrument off.

Master Mix and Slide

18. Use the workbook calculations for setting up the master mix. Vortex for approximately 10 second and spin briefly in a microcentrifuge. **(Do Not add master mix until the slide(s) has been made)**

    160µL STR-SEB
    10µL Proteinase K
    40µL DTT
    1µL carrier RNA

19. Turn hot plate on. If slides are not available with a designated area for a sample to be added, analysts may use an oil/wax pencil and draw a circle on a clean slide prior to adding the sample confirming case, item numbers, and initials.

20. With the workbook map available, remove adaptor 1 from the bucket. Remove the flip top microcentrifuge tube from the adaptor and discard the adaptor into a biohazard waste container. While holding the flip top microcentrifuge tube, pipette solution up and down to dislodge any pellet that may have formed and add 5µL to the corresponding slide. Place the slide on the hot plate until dry and add 211µL of the master mix from step 18 to the tube. Close the flip top microcentrifuge tube lid.

    Note: Slides are not required for extraction controls.

21. Continue until all samples have been removed, added to a slide, and master mix has been added.

22. Once the slides have dried, add the slides, per case number, to a slide transporter. Do not combine cases.

23. Vortex samples and incubate the samples for 10 minutes at 70 °C in a thermomixer set to 700 rpm.

24. Spin the sample tubes in a microcentrifuge to force all the condensation to the bottom of the tube and continue with the DNA Purification (Trace) protocol.