DIFFERENTIAL CHELEX DNA EXTRACTION FROM SEMEN STAINS OR SWABS		
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Differential Chelex DNA Extraction from Semen Stains or Swabs

Approximately 1/3 of a swab or a 3x3mm cutting of a stain should be used for this type of extraction.

1. LIMS Pre-Processing

   a. In the **Analytical Testing » Test Batches** tram stop, select the appropriate extraction assay and **Click Edit**

   Note: If you are creating a new extraction test batch use the New Test Batch tram stop followed by the create new test batch wizard. In that wizard include the following information: description, functional group, analysis, batch configuration, and test batch type (case test batch).

   b. If necessary, **Click Add Unknowns** and select any samples that need to be included on the test batch. Controls are present in the batch configuration. If additional controls are needed, **Click Add QC Samples**

   c. Select All Input Samples » Click Add Output Sample » » Diff Ext SWR» Click Select and Return » Click Ok» Click Create

2. Review batch setup.

3. Remove the samples from the refrigerator. Obtain two tubes for the sperm cell fraction (**SF**) extraction negatives and label them.

4. Have a witness confirm the names and order of the samples (from the Input Samples in LIMS).

5. Obtain reagents and record lot numbers.

6. Pipette 1 mL of PBS into each sample tube, including tubes for SF extraction negative controls, in the extraction rack.

7. Mix by inversion or vortexing.

8. Incubate at room temperature (25ºC) overnight or for a minimum of 1 hour using a shaking platform (at approx. 1000 rpm).

9. Label the SF extraction negative tubes and re-label all SF sample tubes with the DNA extract

output labels.

10. Obtain tubes for the epithelial cell fraction (**EC**) samples, epithelial cell fraction extraction negatives and substrate remains fraction (**SR**) samples. Label all with DNA extract output labels.

11. Vortex or sonicate the substrate or swab for at least 2 minutes to agitate the cells off of the substrate or swab.

12. Have a witness confirm the names and order of the samples (from the Output Samples in LIMS)

13. Sterilize tweezers with 10% bleach, distilled water, and 70% ethanol before the removal of each sample. Remove the swab or other substrate from the SF sample tube(s), one tube at a time, using sterile tweezers and close tube. Place swab/ substrate in the sterile labeled SR fraction tube. Attempt to remove as much liquid as possible from the swab or substrate and transfer this liquid back to the SF sample tube. This can be done by pressing down on the material with a pipette tip and drawing up any liquid remaining in the material. Set the SR and EC tubes aside.

14. Spin the SF sample tubes and SF extraction negative tubes in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g (13,200 rpm).

15. Without disturbing the pellet, remove and discard all but 50µL of the supernatant from the SF sample tubes and SF extraction negative tubes into a waste container containing 10% bleach.

16. Add 150µL sterile or UltraPure deionized water (final volume of 200µL) to the approximately 50µL of cell debris pellet in the SF sample tubes and SF extraction negative tubes.

17. Add 1µL of 20 mg/mL Proteinase K to SF sample tubes and SF extraction negative tubes. Vortex briefly to re-suspend the pellet.

18. Incubate SF sample tubes and SF extraction negative tubes at 56°C for about 60 minutes to lyse epithelial cells, but for no more than 75 minutes, to minimize sperm lysis.

19. Spin the SF samples and SF extraction negative tubes in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.

20. During the spin, add 50µL of 20% Chelex (from a well-suspended Chelex Solution) to each EC sample tube and EC extraction negative tube using a P1000 pipettor; close tubes.

21. Add 150µL of the supernatant from each SF sample and the SF extraction negatives to its respective EC sample or EC extraction negative tube. Store at 4°C or on ice until step 20. **Do not disturb pellet. If disturbed by accident, re-centrifuge the tube at 10,000 to 15,000 x g (13,200

rpm) for 5 minutes**

22. Wash the sperm pellet in the SF sample tubes and the SF extraction negative tubes with Digest Buffer as follows:
    a. Resuspend the pellet in 0.5 mL Digest Buffer.
    b. Vortex briefly to resuspend pellet.
    c. Spin in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.
    d. Remove all but 50 µL of the supernatant and discard the supernatant.
    e. Repeat steps a-d for a total of 5 times.

23. Wash the sperm pellet in the SF sample tubes and the SF extraction negative tubes once with sterile or UltraPure dH_2O as follows:
    f. Resuspend the pellet in 1 mL sterile or UltraPure dH_2O.
    g. Vortex briefly to resuspend pellet.
    h. Spin in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.
    i. Remove all but 50 µL of the supernatant and discard the supernatant.

24. To the approximately 50µL SF sample tubes, the SF extraction negative tubes, and to SR sample tubes, add 150µL of 5% Chelex, 1µL of 20 mg/mL Proteinase K, and 7µL of 1M DTT.

25. Vortex both the EC and SF sample tubes as well as the extraction negative tubes.

The following steps apply to all fractions.

26. Incubate samples at 56°C for approximately 60 minutes.

27. Vortex at high speed for 5 to 10 seconds.

28. Incubate samples at 100°C for 8 minutes using a screw down rack.

29. Vortex at high speed for 5 to 10 seconds.

30. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

31. Sperm Fractions (SF) and Substrate Remains Fractions (SR) must be microconned prior to quantitation with Quantifiler Trio. To avoid excess re-quantitation, elute SFs to approximately 25uL and SRs to approximately 50uL. Skip this step if Quantifiler Trio will not be used.

32. As needed, pipette aliquots of neat and/or diluted extract (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration.

33. Store the extracts at 2 to 8°C or frozen.

34. In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and indicate which samples are completed.

35. Ensure all required fields in the test batch have been filled out and review the assay.