NON-DIFFERENTIAL CHELEX DNA EXTRACTION FROM SEMEN STAINS OR SWABS		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 1 OF 2

Non-differential Chelex DNA Extraction from Semen Stains or Swabs

NOTE: For very large cuttings 200 µL of Chelex might not be enough to provide enough suspension of the sample. The reaction can be scaled up and reconcentrated using Microcon concentrators.

Sample sizes for non-differential Chelex extractions depend on the circumstances of the case. Regularly 1/3 of a swab or a 3x3mm cutting of a stain should be used. For cases where semen is present but no sperm cells were detected, the sample size can be increased.

1. Remove the extraction rack from the refrigerator. Obtain tubes for the extraction negatives and label them.
2. Have a witness confirm that the tube label and entire LIMS input sample ID match for each sample and that the samples are in the correct order.
3. Obtain reagents and record lot numbers.
4. To each tube add: 200 µL of 5% Chelex (from a well-resuspended Chelex solution).
   1 µL of 20 mg/mL Proteinase K
   7 µL of 1 M DTT
5. Use the pipette tip when adding the DTT to thoroughly mix the contents of the tubes.
6. Incubate at 56°C for approximately 2 hours.
7. Vortex at high speed for 10 to 30 seconds.
8. Incubate at 100°C for 8 minutes using a screw down rack.
9. Vortex at high speed for 10 to 30 seconds.
10. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).
1. Place the LIMS output sample labels on the proper tubes. Confirm that the tube label and entire LIMS output sample ID match for each sample.
12. 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 (refer to the current Quantitation procedure in the STR manual).
13. Store the extracts at 2 to 8°C or frozen.

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