Recovery of Spotted DNA Extracts from Whatman FTA Elute MicroCards		
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Recovery of Spotted DNA Extracts from Whatman FTA Elute MicroCards

1 Procedure:

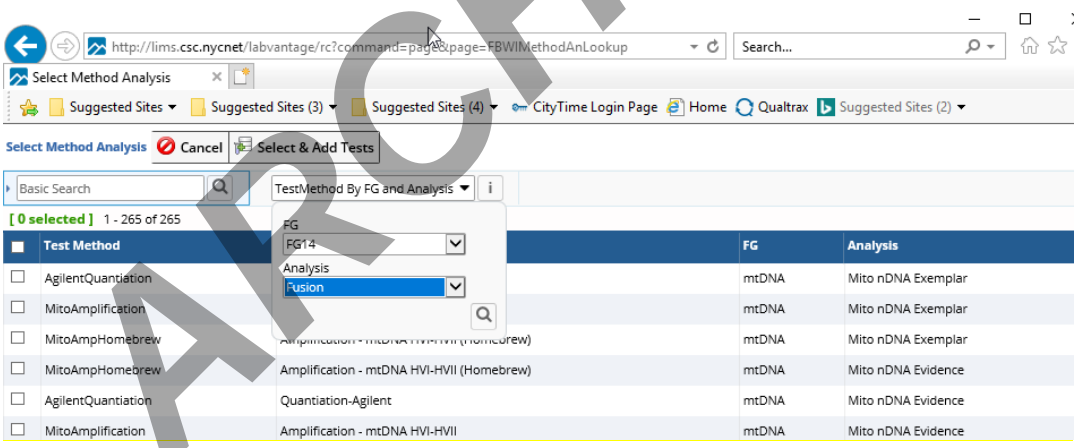
1.1 Obtain the spotted extract card(s) via the "Fbio_Spotted Extract Request" workflow in Qualtrax.

1.1.1 The spotted card for the original extraction negative should also be pulled and reconstituted with the spotted sample.

1.2 Ad-Hocing samples into LIMS (See Creating Ad Hoc Samples in the LIMS Process Manual):

1.2.1 If the samples were extracted pre-LIMS, ad-hoc them into LIMS as OC-Extracted DNA. List the original case number in the notes.

1.3 If the original extraction negative and sample are in LIMS, selected the Extracted DNA samples in the Manage Sample tram stop, click "Add Test" and choose the appropriate FG and Analysis (ie: FG14 – Fusion) and then select the appropriate Test Method (ie: Amplification Fusion).



1.4 Once all necessary samples are in LIMS, add them onto an "Extract-Recon" test batch for further processing.

1.5 The test batch should contain 1 new extraction negative for the recovery process (reconstitution extraction negative) and 1 for the spotted samples being reconstituted (if requested), unless that original extraction negative was already previously reconstituted. Print the input labels and label the appropriate number of 1.5 mL tubes.

1.6 Have a witness verify the samples, including the labels on the spotted extract cards against the test batch input samples.

1.7 Clean a 3mm hole puncher by punching a blank FTA Card 3 times, followed by wiping the puncher with a lint free wipe that has been prewet with 70% Ethanol.

1.8 Use the 3mm hole punch to punch out sample into a labeled 1.5 mL tube based on the chart below. The entire stain may be punched as needed, regardless of the concentration. The entire stain should be punched for spotted negative controls. All cards should be resealed in a kapak pouch and returned to storage, including those where the entire stain was punched.

Original Quantitation Value	*Recommended # of 3 mm punches for Recovery*
1 ng/uL and above	At least 8 punches
100 pg/uL to 999 pg/uL	At least 10 punches
Less than 100 pg/uL	Punch entire stain

A maximum of ten punches per 1.5mL tube is suggested for enhanced recovery. If more than ten punches are needed, separate the punches into multiple 1.5mL tubes. If multiple tubes are being used for one sample, the same LIMS input label may be used for each tube. A note should be added to the test batch notes to indicate the number of input tubes per sample. The extract from these samples can be combined during the microcon.

1.9 Heat the tubes with the punches at 80°C for 20 minutes to increase binding of the DNA to the card to prevent loss during washing.

1.10 Wash Step: Add 500 µL of sterile/ultrapure water to each tube, vortex for 5 seconds.

1.10.1 Note: Make sure the punches are moving around during the vortex step to ensure all punches are being washed. You may need to add more water if there are a large number of punches. Otherwise, there is the potential for PCR inhibitors to be left behind.

1.11 Briefly centrifuge the tubes, remove and discard the supernatant.

1.12 Add sterile/ultrapure water to each tube, 50 uL per hole punch. Briefly centrifuge the tubes to ensure the punches are all immersed in water.

1.13 Place tubes in thermomixer at 95°C for 30 minutes, shaking at 500 rpm. At the end of the incubation step, remove the tubes from the heat block and vortex for 60 seconds.

1.14 Briefly centrifuge the tubes.

1.15 Collect the eluted DNA solution with a pipette and transfer to a 1.5 mL tube labeled with the LIMS output label. Discard the tube containing the discs/hole punches.

1.16 All samples should then proceed to microcon via the Microcon testbatch. Samples should be microconned down to 25 uL. See Microcon DNA Fast Flow DNA Concentration and Purification.

1.16.1 Samples can be microconed to concentrate, however, for samples where color from the card is left behind (usually samples with a greater number of punches), microcon to clean and concentrate.

1.16.2 Filter a maximum of 400µL at one time. For samples with more than 400µL, transfer the microcon membrane to a new collection tube as needed. If samples were separated into multiple tubes during the reconstitution extraction, combine the samples using the same microcon membrane filter and continue to add the remaining extract to the microcon filter (up to 400µL each time).

1.17 After re-extraction, if sample testing will continue in Forensic Biology, a quantitation of the sample(s) is recommended. Store samples at 4C and update the storage location in LIMS.

1.18 If samples will be transferred outside of Forensic Biology, refer to QC710 - Locating and Processing of Retained and/or Spotted Extracts