

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Centri-Sep Sample Filtration		
Status:Published		Document ID: 1175
DATE EFFECTIVE 09/14/2017	APPROVED BY mtDNA Technial Leader	PAGE 1 OF 1

Centri-Sep Sample Filtration

1 Purpose

- 1.1 Prior to sample electrophoresis, sequenced products must be purified in order to remove unincorporated dye terminators.

2 Procedure for Centri-Sep 8 Strips

- 2.1 ****It is important that the Centri-Sep Strips are at room temperature before being used for filtration.**
- 2.2 Determine how many strips are necessary to filter the amplified samples. Separate the desired number of strips by cutting the foil between the strips with scissors.
- 2.3 Open the well outlets on each strip by cutting off the bottom edge with scissors. Cut at the narrowest part of the bottom of the tube.
- 2.4 Peel off the top foil and arrange the strips evenly on deep-well centrifuge plates. Spin the plates at 750 rcf for 2 minutes to remove the liquid.
- 2.5 Arrange the newly drained strips on a new 96-well plate, labeled with the plate name, date, and analyst's initials.
- 2.6 Add the amplified sample to each column, taking care not to touch the gel with the pipet tip.
- 2.7 Once all of the samples are loaded, place the 96-well plate with the Centri-Sep 8 Strips into the centrifuge, and spin at 750 rcf for 2 minutes.
- 2.8 Confirm that all of the samples passed through the strip into the wells of the 96-well plate, and discard the Centri-Sep 8 Strip.
- 2.9 Evaporate the samples in the 96-well plate at 75 °C in a thermalcycler with the lid open. Evaporation may take 1-2 hours.
 - 2.9.1 The plate should not evaporate for more than 3 hours.
- 2.10 If the samples are not going to be loaded immediately, they should be stored as dried pellets at 4 °C for no longer then 14 days. When ready, proceed to 3130x/ setup.