

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

SDS Cleanup		
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SDS Cleanup

1 Purpose

- 1.1 To help separate the primers from the cycle-sequenced DNA with the addition of 2% SDS to the samples, prior to Centri-Sep filtration.

2 Notes

- 2.1 Do not refrigerate the 2% SDS tubes. This will cause the SDS to precipitate out of solution. Store the 2% SDS tubes at room temperature. Ensure that there is no precipitate in the tube before adding to samples.
- 2.2 **If necessary, transfer 1 mL of 2% SDS to a 1.5 mL microcentrifuge tube and place the tube into a thermomixer set at 37°C to ensure the solution is at least at room temperature.**
- 2.3 Allow the cycle-sequenced DNA to equilibrate to room temperature before adding SDS.
- 2.4 **If necessary, place the plate containing your samples on a thermocycler set to 37°C for 5-10 minutes to ensure that the samples are at least at room temperature.**

3 Cleanup

- 3.1 Using a multi-channel pipette, add 2 µL of 2% SDS to each well of cycle-sequenced DNA in a 96-well plate.
- 3.2 Use the 8-strip caps to re-seal each well after the SDS has been added.
- 3.3 Vortex and spin down the plate(s) in a centrifuge.
- 3.4 Place the tubes in a thermal cycler, using the following conditions-

9700 Thermal Cycler	The 2% SDS incubation file is as follows:
User: mtDNA	Soak at 98 °C for 5 minutes
File: SDS	Storage soak at 25 °C for 10 minutes

- 3.5 When the tubes are back to room temperature following the 25 °C soak, proceed to the Centri-Sep purification.