| EXO-SAP-IT SAMPLE CLEANUP | | |
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# EXO-SAP-IT Sample Cleanup

PURPOSE: Prior to cycle sequencing, unincorporated primers and nucleotides present in the amplification reaction are deactivated by the addition of ExoSAP-IT.

PROCEDURE:

1. Create a new ExoSAP-It test batch in the LIMS system, and fill in the necessary documentation.

2. Confirm the tube label and sample description for each sample. Every run should include a positive control and an amplification negative control. **Note: It is very important for these entries to be in 3130xl format; do not use spaces or the following characters: \ / : * " > < | ? ')**

3. Based on each sample's previous runs, the appropriate values for each column in the ExoSAP-It batch will be automatically filled in. The "Vol, Misc" must be entered by the analyst, and then the batch should be saved. This will trigger the automatic values to populate in the data, and the calculations for the ExoSAP-It volume and the new concentration will automatically execute.

   ***For a detailed description of the calculations performed in this spreadsheet, refer to Appendix D – Detailed CycSeq/3130xl Spreadsheet Calculations***.

   **There should be 1ul of ExoSAP-IT added for every 5ul of sample in the amplification tube.**

4. Use the following settings to incubate the samples:

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| 9700 Thermal Cycler<br><br>User: mtDNA<br><br>File: exosap-it | The ExoSAP-IT file is as follows:<br><br>- Soak at 37°C for 15 minutes<br>- Soak at 80°C for 15 minutes<br><br>Storage soak at 4°C indefinitely |

5. Place the tubes in the tray in the heat block, slide the heated lid over the tubes, and fasten the lid

by pulling the handle forward.

6. Start the run by performing the following steps:

   a. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key directly under that menu option.

   b. Verify that the user is set to "mtDNA." If not, select the USER option (F5) to display the "Select User Name" screen.

   c. Use the circular arrow pad to highlight "mtDNA." Select the ACCEPT option (F1).

   d. Select the "exosap-it" file, and press the RUN button (F1).

   e. Verify that the reaction volume is set to 50 µLand the ramp speed is set to **9600 (very important)**.

   f. If all is correct, select the START option (F1).

   The run will start when the heated cover reaches 37ºC. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed; the hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

   Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Kimwipe and pull the lid closed to prevent dust from collecting on the head block. Turn the instrument off.

7. When the batch is complete, the new concentration value must be "pushed" to the ExoSAP-It'd DNA sample within the LIMS system. From this point forward, the ExoSAP-It'd DNA sample will be the point of all cycle sequencing testing for mtDNA analysis.