Molecular Ecology Lab PCR Clean-up

Overview: This protocol covers the steps necessary to clean PCR products after they have been amplified, in order to remove extraneous primer and salts from the reaction tubes. These salts interfere with capillary sequencing, and the unincorporated primers can cause both directions of the PCR product to be sequenced at the same time. This protocol should be completed within one or two weeks of the PCR, in order to avoid sample degradation.

ExoSap-IT Protocol

1. The tube of premixed Exo-Sap-IT should be stored at -20ºC. Keep it chilled when following this procedure.
2. Prepare a 1:5 dilution of ExoSAP reagent in PCR-grade H_2O (1 ul ExoSAP, for every 4 ul ddH_2O).
3. Thoroughly mix on a vortex.
4. Add 1.5ul of this mixture to each PCR product (assuming there is ~20ul of PCR product). Change filter tips among samples, as PCR products are highly concentrated.
5. Put in thermocycler under a ExoSAP protocol (37 C for 30 minutes, 80 C for 15 minutes, 4 C hold).
6. Freeze tubes after reaction, or proceed directly to cycle sequencing.