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Isolating Microsatellite DNA Loci*

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Glenn & Schable_update4. Last updated in 2007.

Materials and Solutions:

Home-made ExoAP: Combine equal volumes of Exonuclease I (NEB Catalog #M0293L; 20 units/μL) with Antarctic Phosphatase (NEB Catalog #M0289L; 5 units/μL]. Keep this in the -20°C freezer as a stock solution.

1. Quantify PCR product concentration and size. For a single sequencing reaction, the desired amount of template is 10ng of PCR product per 100 bp of length (i.e., for a 500 bp product, 50 ng is needed). Generally, we purify enough PCR product for at least 2 sequencing reactions. Use the imaging results saved from step VIII.12 above (see Figure 3).

2. When ready to purify reactions for a plate, combine:

22 µL	10X AP buffer (10x PCR buffer may be used instead)
44 uL	ExoAP (from above)
374 µL	dH_2O
54 µL	of this mix into each tube of an 8-tube strip
4.0 µL	Mix from the strip tubes in each well of a 96-well plate
4.0 µL	PCR product from colony PCR (spin plate to remove condensation from the lid.)

(Note: volumes can be adjusted to account for differences in amplification & lengths of the PCR products, so that the molar concentrations are approximately equal, however, in practice we find this recipe generally works fine without such adjustment).

3. Incubate the samples at 37°C for 15 minutes, 80°C for 15 minutes then hold at 15°C. The samples are now purified, and ready for use as sequencing reaction template.