Exo/Sap PCR primer inactivation - Based on Blaxter Lab Exo/SAP protocol and probably not useful for NGS approaches due to Exo1 activity

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Abstract

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Protocol

Defrost your PCR products

Step 1.

The following steps assume you are doing 96 x 15 ul PCR products. If you've performed a 20 ul PCR and used approximately 5 ul for a gel this will be perfect for the remaining protocol steps.

Mix your Exo/SAP master mix - IMPORTANT KEEP ON ICE AT ALL TIMES!

Step 2.

For each PCR well you need to add 1U of SAP and 1.5U of Exo1 and dilute to 2.5ul with either the CutSmart Buffer supplied with the SAP or the Exo1 reaction buffer supplied with the Exo1 (they seem to be the same buffer).

For one reaction:

Reagent	Volume
Exo1	1 ul
SAP	0.075 ul
Buffer	1.425 ul

For 96 reactions, make 100 samples:

Reagent	Volume
Exo1	100 ul
SAP	7.5 ul
Buffer	142.5 ul

REAGENTS

- Exonuclease I Reaction Buffer - 6.0 ml B0293S by New England Biolabs
- CutSmart Buffer - 5.0 ml B7204S by New England Biolabs
- Exonuclease I (E.coli) - 3,000 units M0293S by New England Biolabs
- Shrimp Alkaline Phosphatase (rSAP) - 500 units M0371S by New England Biolabs

Molecular Biology Grade Water 10154604 by Fisher Scientific

Add 2.5 ul of Exo/Sap Mix to each PCR well, mix thoroughly and seal your PCR plate

Step 3.

Put in PCR machine with following profile

Step 4.

1. 37°C 30 min
2. 80°C 10 min
3. cool to 4°C

You now have 17.5 ul of cleaned PC product ready for use in a dye terminator reaction.

Step 5.