Exo-CIP PCR cleanup protocol

This protocol is used to clean PCR products for direct sequencing. It utilizes the action of two enzymes to remove free dNTP's and excess primers remaining from PCR. Exonuclease I (Exo) is an enzyme that will cut up single-stranded DNA such as primers and unfinished PCR products. Calf Intestinal Alkaline Phosphatase removes dNTPs that were not incorporated into the PCR DNA. This protocol is not suitable to prepare samples for A-overhang (TOPO-TA/pGEM) cloning.

Procedure

1. For each PCR reaction, add 0.2 µl of Exonuclease (ExoI) to a 1.5 ml tube.

2. Add an equal amount of Calf Intestinal Phosphatase (CIP) to the same tube.

3. Add 0.4 µl of the Exo-CIP mixture to each PCR reaction tube and mix by pipetting.

4. Incubate 15 min at 37° C followed by 15 min inactivation incubation at 85° C.

5. Dilute the PCR product 1:1 with sterilized DI H_2O. The sample is now ready for direct sequencing

Materials necessary

- Fresh PCR product in 0.2 ml PCR tube
- Exonuclease 1 (link)
- Calf Intestinal Phosphatase (link)
- 1.5 ml tube
- Sterilized DI H_2O

Equipment required

- 2 µl micropipette
- Thermocycler or heat block