

Elimination template and PCR Clean-up

Elimination template

Materials

- FastCut DpnI (SidEnzyme,catalog no.FC017)
- Out smart buffer
- Target DNA(to be digested)

table 1

PCR product	50uL
Fast Cut buffer	5uL
Dpn I	1ul

Procedure

1. Prepare reaction mixture according to table 1. Multiple types of re-striction enzymes can be used in one mix. As a rule of thumb, 5 ptof10x buffer and 1 ptof each enzyme are added.
2. Mix components by resuspending with pipette . Incubate reaction mix for 2 h in a metal bath at 37 °C.

PCR Clean-up

Materials

- PCR amplified DNA of interest
- Axygen PCR-clean up kit
- Eluent

Procedure

1. Put PCR product into Centrifuge tube(1.5mL),than add 5-volume Buffer BB.
2. Centrifuged for 1 min with $10000 \times g$. Discardingthe filtrate.
3. 650ul Buffer WB was added into the tube and centrifuged for 1 min with $10000 \times g$.Discardingthe filtrate.
4. Repeat the last step once.
5. Centrifuged for 2 min with $10000 \times g$ with nothing added.
6. Let stand for 10min (volatile ethanol).
7. Add 50ul Eluent into the preparation tube, let stand for one minute, and centrifuge with $10000 \times g$ for 1min.

