**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 plof10x buffer and 1 plof each enzyme are added.

2. Mix components by resuspending with pipette . Incubate reaction mix for 2 h in a metal bath at 37 ℃.

**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 × g. Discardingthe filtrate.

3. 650ul Buffer WB was added into the tube and centrifuged for 1 min with 10000 × g.Discardingthe filtrate.

4. Repeat the last step once.

5. Centrifuged for 2 min with 10000 × 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 × g for 1min.