

Alkaline Phosphatase

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[Abstract] Alkaline phosphatase removes 5' phosphate groups from vector so that prevents self-ligation of the vector and facilitates the ligation of other DNA fragments into the vector.

Materials and Reagents

1. Vector DNA
2. Restriction enzymes (New England Biolabs)
3. Alkaline phosphatase: Calf intestinal alkaline phosphatase (CIP) (New England Biolabs, catalog number: M0290) or Shrimp Alkaline Phosphatase (SAP) (Promega Corporation, catalog number: M8201)

Procedure

1. Cut the vector (5 µg) in 100 µl digestion reaction.
2. Separate the digestion reaction to 2 tubes, 50 µl each.
3. Add 2.5 µl CIP enzyme or 1 µl SAP enzyme to 50 µl digestion (normally NEB digestion buffer is good for CIP enzyme, if not, purify the digestion and resuspend DNA in appropriate buffer).
4. Incubate at 37 °C for 1 h.
5. Heat inactivate the reaction at 65 °C for 30 min (for CIP) or 10 min (for SAP).
6. Gel purify the vector.

Because CIP removes phosphate group, it is better to add some ATP during ligation step.