Analyzing E. coli. Colonies by PCR

Revised on December 9, 2012

Introduction

This protocol is designed to quickly screen for plasmid inserts directly from E. coli colonies, and can be used to determine insert size and/or orientation in the vector.

Platinum Blue PCR SuperMix contains recombinant Taq DNA polymerase, Platinum hot-start antibodies, dNTPs, magnesium, buffers, and an easy-to-track blue loading dye. All reagents are conveniently pre-mixed in one vial. Just add your template and primers. After completing the PCR cycling, simply load your PCR product directly onto the agarose gel. No extra steps for mixing reagents or adding loading/tracking dye.

Materials

· 8-well PCR strip tubes & caps (Axygen scientific, 22-705)
· LB plates with appropriate antibiotics
· Platinum Blue PCR SuperMix (Invitrogen, 12580-015)
· Primers, 40 pmol/µl

Procedure

1. In a sterile 0.5-ml microfuge tube, mix in the following order:

Reagent	Final con.	Quantity (µl)
Platinum Blue PCR SuperMix	~1x	49
Sense primer 40 pmol/µl (40 µM)	400 nM	0.5
Antisense primer 40 pmol/µl (40 µM)	400 nM	0.5

2. Use a sterile pipette tip to transfer a small amount of an E. coli colony to the reaction mixture (10-20 µl), then streak it onto a fresh replicate agar plate.
3. Incubate tubes in a thermal cycler at 94°C for 2 min to completely denature the template.
4. Perform 20 cycles of PCR amplification as follows:

   Denature 94°C for 30 s
   Anneal 55°C for 30 s
   Extend 72°C for 1 min per kb
5. Followed by a final incubation of 72°C for 10 min.
6. Analyze the PCR products (2-10 µl) by electrophoresis through an agarose gel, using markers of suitable size.

Tips: It was reported that if specific primers for the insert are used for colony PCR, false positive might occur

(Dallas-Yang et al., 1998).

Reference

Q Dallas-Yang, G Jiang, and FM Sladek (1998). Avoiding false positive in colony PCR. Biotechniques *24*. 580-582.