One Step Procedure for Screening Recombinant Plasmids by Size

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Abstract

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Guidelines

MATERIALS

1) Overnight cultures of the bacterial recombinants to be screened
2) Agarose beads
3) Phenol:Chloroform (1:1)
4) Microfuge tubes

Reference

E. Beuken, C. Vink, and C.A. Bruggeman. (1998.) One-Step Procedure for Screening Recombinant Plasmids by Size. Biotechniques **24**: 748-750

Protocol

Step 1.
Inoculate 3.5 mL aliquots of LB media containing the appropriate antibiotic with single colony isolates of the bacteria containing the plasmid in question.

Step 2.
Incubate overnight at 37°C.

DURATION
18:00:00

Step 3.

Place 150 µL of each culture into a microfuge tube.

Step 4.
Centrifuge in the microfuge for 30 seconds to pellet the bacteria.

DURATION
00:00:30

Step 5.
Discard the supernatants.

Step 6.
Add 40 µL of agarose beads to each sample.

Step 7.
Add 14 µL of phenol:chloroform (1:1) to each sample and vortex for 5-10 sec.

DURATION
00:00:10

Step 8.
Centifuge the samples in the microfuge for 5 min to separate the phases.

DURATION
00:05:00

Step 9.
Layer 10 µL of each of the aqueous phases onto a 1.2% agarose horizontal gel made up with 1X TPE buffer.

Step 10.
In the outside lanes run lambda DNA digested with HindIII and/or StyI for size markers.

Step 11.
Also run the original vector to show the size of the plasmid without insert DNA. Gels may be double combed to facilitate running more samples.

Step 12.
Electrophorese the gel in 1X TPE buffer for approximately 400-450 volt-hours.

Step 13.
Stain the gel with ethidium bromide at a concentration of 0.5 µg/mL in d-H_2O for 30 minutes.

DURATION
00:30:00

Step 14.
Destain the gel with d-H_2O.

Step 15.
Observe the gel on a UV light box and photograph the gel.

Step 16.
For further analysis, plasmid DNA may be isolated from those samples that indicate that they have inserts of the appropriate size.