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Transforming pJC8 into HB101

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Protocol status: Working
We use this protocol and it's working

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Abstract

Taken from *Mix and Go E. coli* Transformation Kit (see attachment).
Transforming *E. coli* HB101 with pJC8-empty cosmid to use as a control strain.

Attachments

PDF

_t3001_t3002_mix_go_...

870KB

Materials

Mix and Go E. coli Transformation Kit (Zymo Research, T3001/T3002)

Contents of SOB medium:
20g Bacto Tryptone
5g Yeast Extract
0.58g Sodium Chloride
0.19g Potassium Chloride
1L sterile H20

Before starting

1 Prepare tetracycline LB agar plates

Protocol

NAME

Making tetracycline LB agar plates

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Preview

2 Warm plates in 37 °C incubator

Note

Chilled plates will decrease *Mix & Go* cell transformation efficiency.

3 Prepare SOC medium

3.1 Get SOB medium and 20 % glucose from media kitchen

3.2 Add 2 mL 20 % glucose to 100 ml SOB medium

Transformation

4 Isolate pJC8 cosmid from *E. coli* DH5α

Protocol

NAME

Qiagen QIAprep Spin Miniprep for cosmids from metagenomic library

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Preview

5 Thaw 100 uL aliquot of *E. coli* HB101 *Mix & Go* competent cells on ice

6 Add 5 uL pJC8 cosmid

7 Mix by tapping the tube and shaking downwards once

8 Place on ice for 5 minutes

9 Add 400 uL SOC medium

10 Incubate at 37 °C with shaking at 200 rpm for 1 hour

Note

An outgrowth in SOC medium is required for efficient transformation when selecting with tetracycline.

11 Pipette all (505 uL) of the transformation mix onto a tetracycline LB agar plate and spread using plating beads.

12 Dry under laminar flow for a few minutes

13 Incubate plate at 37 °C overnight

Expected result

If the transformation is successful, you will have individual colonies.