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

Created: Mar 05, 2024

Transforming pJC8 into HB101

Bonnie Evans^{1,2}

¹MRC Laboratory of Medical Sciences; ²Imperial College London



Bonnie Evans

MRC Laboratory of Medical Sciences, Imperial College London

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

_t3001_t3002_mix_go_e._c oli_transformation_kit_buff er_set.pdf

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

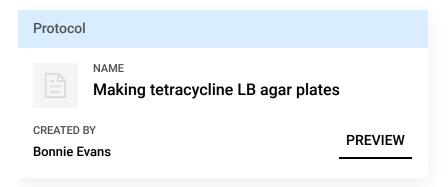
1L sterile H20

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Before starting

1 Prepare tetracycline LB agar plates



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

CREATED BY

Bonnie Evans

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

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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.