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Pichia pastoris transformation through electroporation

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Igem Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

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

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Materials

linearized vector DNA with electrocompetent Pichia cells
1 mL of 1:1 1 M sorbitol

YPD Liquid Medium or Plates (500 ml):

- 5 g yeast extract
- 10 g peptone
- 10 agar (if preparing plates)
- Fill to 450 mL with water

→ autoclave

YPDS + antibiotic Plates (1 liter):

- 10 g yeast extract
- 20 g peptone
- 182.2 g sorbitol
- Fill to 800 mL with water
- Mix until dissolved
- Transfer 400 mL two 1 L bottles
- pre-filled with 10 g BactoAgar
- Fill each bottle to 450 mL with water

⟶ autoclave

- Cool bottles to ~60°C, add 50 mL of 10x D
- Mix well, then aliquot in 4 bottles of 250 mL

Prior

1 *Prior to transformation plasmid DNA containing the gene(s) of interest was linearized in restriction digests using an enzyme that only cuts once following the manufacturers manual !*

Transformation

2 Mix approx. 150 ng of linearized vector DNA with electrocompetent Pichia cells

3 Tranfer mixture to ice-cold 0.2 cm electroporation cuvette

4 Incubate cuvette for 2 min

5 Electroporate using a BioRad MicroPulser with a charging voltage of 1.5 kV

6 **Immediately** add 1 mL of 1:1 1 M sorbitol and YPD (v/v) to recover the cells

7 Transfer mixture to a plastic culture tube and incubated at 30°C for 1 hour at 225 rpm.

8 Centrifuge culture tube at 3.000 x g for 5 min to pellet the cells

9 Resuspend pellet in 200 µL YPD media

10 Plate out cells on YPDS plates containing desired antibiotic and incubated at 30°C for 3 days