OCT 20, 2019

🌐 Pichia pastoris transformation through electroporation

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

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

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8heht3e

Protocol Citation: Igem Dusseldorf 2019. Pichia pastoris transformation through electroporation.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.8heht3e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Oct 20, 2019

Last Modified: Oct 20, 2019

PROTOCOL integer ID:
 28934

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 Transfer 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