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 We use this protocol and it's working

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Pichia pastoris strain and growth condition

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MATERIALS

Pichia Stock Solutions:

10x YNB:

- 134 g of yeast nitrogen base with ammonium sulfate (no amino acids)
- fill up to 1000 mL with water
- heat to dissolve if necessary

--> filtrate

500x B:

- Dissolve 20 mg biotin in 100 mL water

--> filtrate

10x D:

- 200 g of Dextrose (D-glucose)
- fill up to 1000 mL with water
- heat to dissolve (30 min)

--> autoclave

10x M:

- 2.5 mL methanol
- fill up to 50 mL with water

--> filtrate

10x GY:

- 100 mL glycerol
- 900 mL water

--> autoclave

1 M PB pH 6.0:

- 30.125 g of K₂HPO₄ *3H₂O (228.22 g/mol)
- 118.126 g of KH₂PO₄ (136.09 g/mol)
- Fill to 900 mL with water and dissolve
- Read pH (initially 5.5) and adjust to 6.0 ± 0.1 by

- adding ~ 30 mL of 5 M KOH
- Fill to 1000 mL with water

--> autoclave

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

For 100 µg/mL Zeocin (variable) selection on plates:

- Melt agar in microwave, cool to ~60°C
- Add 250 µL of 100 µg/ml, pour plates

BMGY (200mL):

- 2 g yeast extract
- 4 g peptone
- Fill to 140 mL with water

--> autoclave

Cool to ~25°C, then add:

- 20 mL of 1 M PB with pH 6.0
- 20 mL of 10x YNB
- 0.4 mL of 500x B
- 20 mL of 10x GY

BMMY (200 mL):

2 g yeast extract 4 g peptone

Fill to 140 mL with water

--> autoclave

Preparation

- 1 Grow *Pichia pastoris* cells on agar plate containing desired antibiotic (non-shaking 30°C 72 hours)

Day one

2 Cultivation

- 2.1 Add growth medium (BMGY) to baffled flasks

Note

3ml-10ml growth medium in 30mL-100mL flasks to ensure sufficient aeration

- 2.2 Inoculate growth medium with single positive colony

- 2.3 Incubate at 30°C at 225rpm overnight 24:00:00 30 °C

Day two

3 Gene expression

- 3.1 Transfer cultures to sterile 50mL falcon tubes

- 3.2** Centrifuge and decant supernatant 2000 x g , 5 min
- 3.3** Add 10mL of sterile BMMY to used baffled flasks. Swirl and transfer to corresponding falcon tube
- 3.4** Vortex falcon tube
- 3.5** Centrifuge falcon tube at 2000 x g , 5 min
- 3.6** Decant supernatant and add 10 mL of fresh BMMY
- 3.7** Vortex solution and transfer to original flasks
- 3.8** Incubate at 225 rpm overnight 24:00:00 30 °C

Day three

4 Continued expression

4.1 Add 50 μ L sterile 100% methanol to flask

4.2 Incubate at 30°C at 225 rpm overnight 24:00:00 30 °C

Day four

5 Cell harvesting

5.1 Transfer cells to 50 mL falcon tubes from second day and harvest by centrifuging at 2000 x g , 5 min

5.2 Decant supernatant and resuspend cell wall containing pellet in 1 mL water.

5.3 Transfer to pre-weighed 2 mL tube

5.4 Centrifuge, decant water and use cells for desired experiments 10000 x g , 2 min

Alternatives

6 For each step where media was added, only 3 mL instead of 10 mL were added. All centrifugation steps were performed at 3.000 x g instead of 2.000 x g and only 20 µL of sterile 100 % methanol were added for methanol induction

7 Grow liquid cultures with starting volume of 3 mL in similar procedure

Note

From now on: use plastic tubes

8 Store harvested cells at -20°C