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Pichia pastoris strain and growth condition

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1 Works for me dx.doi.org/10.17504/protocols.io.8hcht2w

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MATERIALS TEXT

Pichia Stock Solutions:

10x YNB:
- 134 g od yeast nitrogen base with ammonium sulfate (no amino acids)
- fill up to 1000 mL with water
- heat to dissolve if necesary

--> 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 *3H20 (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

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 Vortext 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 µL 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

From now on: use plastic tubes

8 Store harvested cells at -20°C