

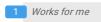


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Pichia pastoris strain and growth condition

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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

- **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

2.5	incubate at 30 C at 225rpm overnight (§ 24:00:00 % 30 C
Day t	wo
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 30° C
Day t	hree
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 f	our
5	Cell harvesting

5.1	Transfer cells to 50 mL falcon tubes from second day and harvest by centrifuging at <a>32000 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 310000 x g 2 min
Altern	atives
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	x g instead of 2.000 x g and only 20 μL of sterile 100 % methanol were added for methanol induction Grow liquid cultures with starting volume of 3 mL in similar procedure
7	
7	Grow liquid cultures with starting volume of 3 mL in similar procedure
8 (cc) BY	Grow liquid cultures with starting volume of 3 mL in similar procedure From now on: use plastic tubes Store harvested cells at -20°C