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Protein expression in *Komagataella phaffii* (formerly *Pichia pastoris*)

Maja Rennig[1], Kristoffer Bach Falkenberg[1], Cristina Hernandez Rollan[1], Andreas Birk Bertelsen[1], Morten Norholm[1]

[1]Technical University of Denmark

1 Works for me dx.doi.org/10.17504/protocols.io.bd32i8qe

Maja Rennig
Technical University of Denmark

ABSTRACT

The methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*) is the most commonly used yeast species in the production of recombinant proteins. This is most likely due to its ability to grow to high cell density, to express recombinant genes in a tightly controlled manner and to efficiently secrete proteins. Despite its biotechnological importance and wide use in industry, relatively few genetic tools are readily available for academic research. Here, we present a protocol for production of proteins in *K. phaffii.*

The protocol found here is modified from the protocol provided in the *Pichia* Expression Kit (Catalog Number K1710-01, Invitrogen).

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KEYWORDS

LyGo, LPMO, K. phaffii, P. pastoris, protein production

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GUIDELINES

Transformation and integration into *K. phaffii* GS115 can be done utilizing the *Pichia* EasyComp Kit (Catalog no. K1730-01, Invitrogen).

Recipes for all media and solutions can also be found in the manual for the *Pichia* Expression Kit (Catalog Number K1710-01, Invitrogen).

MATERIALS TEXT

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MATERIALS

Yeast Extract **Contributed by users Catalog #Y1625**

GS115, Pichia pastoris Yeast Strain **Thermo Fisher Catalog #C18100**

YNB without Amino Acids **Sigma Aldrich Catalog #Y0626-1KG**

Biotin **Sigma Aldrich Catalog #B4639-1G**

Peptone from soybean enzymatic digest **Sigma Aldrich Catalog #70178-500G**

Potassium phosphate monobasic **Sigma Aldrich Catalog #P0662-1KG**

Potassium phosphate dibasic **Sigma Aldrich Catalog #P3786-1KG**

Methanol **Sigma Aldrich Catalog #34860-2.5L-M**

Glycerol **Sigma Aldrich Catalog #15523-1L-M**

SAFETY WARNINGS

This protocol describes the use of GMO classified organisms. Make sure that the local GMO and safety legislations are respected.

This protocol involves the use of 100 % methanol. Make sure to store and handle 100 % methanol accordingly.

ABSTRACT

The methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*) is the most commonly used yeast species in the production of recombinant proteins. This is most likely due to its ability to grow to high cell density, to express recombinant genes in a tightly controlled manner and to efficiently secrete proteins. Despite its biotechnological importance and wide use in industry, relatively few genetic tools are readily available for academic research. Here, we present a protocol for production of proteins in *K. phaffii.*

The protocol found here is modified from the protocol provided in the *Pichia* Expression Kit (Catalog Number K1710-01, Invitrogen).

BEFORE STARTING

Make sure to have your expression strain streaked on an YPD agar plate

Prepare media and stock solutions

1 **Prepare [M]1 Molarity (M) potassium phosphate buffer, pH6**

1.1 Combine **132 mL** of **[M]1 Molarity (M)** K_2HPO_4 and **868 mL** of **[M]1 Molarity (M)** KH_2PO_4

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1.2 Confirm that the pH **6.0** and adjust using phosphoric acid or KOH

1.3 Sterilize by autoclaving for **00:20:00** on liquid cycle program and store at **Room temperature**

The shelf life of this solution is greater than one year

2 Prepare [M] 13.4 Mass / % volume YNB with ammonium sulfate without amino acids

2.1 Dissolve **134 g** of yeast nitrogen base (YNB) with ammonium sulfate and without amino acids in **1.000 L** of water.
The solution can be heated to dissolve YNB completely in water

Alternatively, use **34 g** of YNB without ammonium sulfate and amino acids and **100 g** of ammonium sulfate.

2.2 Sterilize by filteration and store at **4 °C**

The shelf life of this solution is approximately one year

3 Prepare [M] 0.2 Mass / % volume biotin solution

3.1 Dissolve **20 mg** biotin in **100 mL** of water

3.2 Sterilize by filteration and store at **4 °C**

The shelf life of this solution is approximately one year

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4 **Prepare BMGY and BMMY media**

Ingredients (final concentrations):

[M]**1 Mass / % volume** yeast extract

[M]**2 Mass / % volume** peptone

[M]**100 Milimolar (mM)** mM potassium phosphate, pH 6.0

[M]**1.34 Mass / % volume** YNB

[M]**0.0004 Mass / % volume** biotin

[M]**1 % volume** glycerol (BMGY) or [M]**0.5 % volume** methanol (BMMY)

K. phaffii cells exhibit optimal growth with higher YNB concentrations than *S. cerevisiae*

4.1 Dissolve **10 g** of yeast extract, **20 g** of peptone in **700 mL** of water

4.2 Autoclave for **00:20:00** on liquid a cycle program

4.3 Let cool down to room temperature, then add the following:

100 mL [M]**1 Molarity (M)** potassium phosphate buffer, pH**6** (autoclaved stock solution)

100 mL [M]**13.4 Mass / % volume** YNB with ammonium sulfate without amino acids (filter sterilized stock solution)

2 mL [M]**0.02 Mass / % volume** biotin (filter sterilized stock solution)

4.4 For BMGY add **100 mL** [M]**10 % volume** glycerol

For BMMY add **100 mL** [M]**5 % volume** methanol

If not used directly, store the media at **4 °C** for up to 2 months

Inoculation - Day 1

5 Inoculate **25 mL** of BMGY in a 250 mL shake flask with a single colony of *K. phaffii* GS115 with the desired construct integrated on the genome.

6 Incubate the culture at **28 °C** with 250RPM shaking until saturation

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It is also possible to grow the cells at **30 °C**

Pre-culture - Day 2

7 Transfer **2.5 mL** of the saturated culture into **100 mL** BMGY in a 500 mL shake flask with low baffles and grow overnight at **28 °C**

It is also possible to grow the cells at **30 °C**

Expression - Day 3

8 Measure OD_{600} of the overnight culture

9 Pellet **50 mL** of the culture in a 50 mL falcon tube at **4000 x g, Room temperature , 00:10:00**

10 Discard the supernatant and resuspend the pellet in BMMY medium to a final OD_{600} of 50. The appropriate amount of media can be calculated as:

$$V_{BMMY} = \frac{OD_{600,measured} \cdot V_{pre-culture}}{50}$$

11 Inoculate **100 mL** BMMY medium with **2 mL** of the normalized pre-culture, giving a start OD_{600} of 1.

12 Grow the cells at **28 °C** and 250 RPM of shaking

It is crucial that the temperature does not exceed **28 °C** when producing protein. It is advised to double-check the incubator's temperature reading with a thermometer.

Feeding - Day 4

13 Add 1% methanol to the expression culture (**1 mL** of **100 % volume** methanol to **100 mL** of BMMY culture) around 24 hours after inoculation

Feeding - Day 5

14 Add 1% methanol to the expression culture (**1 mL** of **100 % volume** methanol to **100 mL** of BMMY culture) around 24 hours after last addition of methanol

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Feeding - Day 6

15 Add 1% methanol to the expression culture (**1 mL** of **100 % volume** methanol to **100 mL** of BMMY culture) around 24 hours after last addition of methanol

Harvesting - Day 7

16 Harvest the cells by centrifugation at **4000 x g, 4°C, 00:10:00** and discard the pellets

17 Keep the sample **On ice** when working with it and at **4 °C** or **-20 °C** for storage

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