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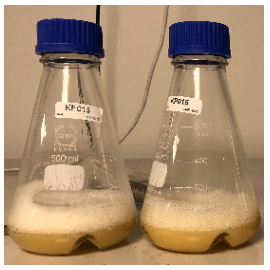
Protein expression in *Komagataella phaffii* (formerly *Pichia pastoris*)



In 1 collection

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

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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).

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

MATERIALS

- Yeast Extract **Catalog #**Y1625
- GS115, Pichia pastoris Yeast Strain **Thermo Fisher Catalog #**C18100
- YNB without Amino Acids **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y0626-1KG
- Biotin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**B4639-1G
- Peptone from soybean enzymatic digest **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**70178-500G
- Potassium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P0662-1KG
- Potassium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3786-1KG
- Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860-2.5L-M
- Glycerol **Merck MilliporeSigma (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.

Before start

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,** pH 6

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

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

> Note
>
> 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

> Note
>
> 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

Note

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

Note

The shelf life of this solution is approximately one year

4 **Prepare BMGY and BMMY media**

Ingredients (final concentrations):

[M] 1 Mass / % volume yeast extract

[M] 2 Mass / % volume peptone

[M] 100 millimolar (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)

Note

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

Note

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

Note

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

Note

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

Note

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 [M] 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 [M] 100 % volume methanol to 100 mL of BMMY culture) around 24 hours after last addition of methanol

Feeding - Day 6

15 Add 1% methanol to the expression culture (1 mL of [M] 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