Expression of recombinant proteins in yeast

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Contents

1 Introduction

This protocol is for the production of recombinant proteins in yeast. Purpose of this experiment is to find out the best time after induction of protein expression to isolate the recombinant proteins from yeast (Sacchromyces cerevisiae)

2 Materials required

2.1 Equipment required

1. 30℃ shaker
2. 4℃ Centrifuges
3. Sterile 1.5 ml microfuge tubes

2.2 Chemicals required

1. Yeast Nitrogen Base
2. 10% Raffinose stock – filter sterilized
3. 20% Galactose stock – filter sterilized
4. 10X amino acid stock without uracil and tryptophan
5. Uracil — Dropout for pYES2
6. Tryptophan — Dropout for pGBKT7
7. Ultra Pure Water

3 Media recipes

3.1 Yeast Extract Peptone Dextrose Medium (YPD) – 1L

1. ☐ 10 g Yeast extract
2. ☐ 20 g Peptone
3. ☐ 100 ml 20% Dextrose solution (filter sterilized and add after autoclaving)
4. ☐ 20 g Agar (For plates only)

3.2 SC dropout media – 1L

1. ☐ 6.7g Yeast nitrogen base
2. ☐ 200 ml of 10% Raffinose – Add after autoclaving
3. ☐ 100 ml 10X amino acid dropout solution
4. ☐ 100 mg tryptophan (for SC-Uracil)

3.3 10X amino acid dropout mix

Aminoacid	Amount
Adenine	1000 mg
Arginine	1000 mg
Cysteine	1000 mg
Leucine	1000 mg
Lysine	1000 mg
Threonine	1000 mg
Aspartic acid	500 mg
Histidine	500 mg
Isoleucine	500 mg
Methionine	500 mg
Phenylalanine	500 mg
Proline	500 mg
Serine	500 mg
Tyrosine	500 mg
Valine	500 mg

Add all aminoacids to ddH_2O and make it up to 1000 ml. Either autoclave or fiter sterilize.

3.4 Induction media – 1L

1. ☐ 6.7 g Yeast nitrogen base
2. ☐ 100 ml of 20% Galactose – Add after autoclaving
3. ☐ 100 ml of 10% Raffinose – Add after autoclaving
4. ☐ 100 ml 10X amino acid dropout solution
5. ☐ 100 mg tryptophan (for SC-Uracil)

3.5 Breaking buffer

1. ☐ 50 mM Sodium phosphate
2. ☐ 1 mM EDTA
3. ☐ 5% Glycerol
4. ☐ 1 mM PMSF
5. ☐ pH 7.4

4 Protocol

4.1 Time course for expression of recombinant protein

1. Incoculate single colony of yeast containing the desired construct into 15ml SC-Uracil medium with 2% raffinose. Grow overnight at 30℃ with shaking at 150 RPM.
2. Determine OD_{600} of the overnight culture. Calculate the amount of overnight culture necessary to obtain a OD_{600} of 0.4 in 50 ml of induction solution.

   eg. Assume OD_{600} of the culture is 3 OD_{600} per ml.

$\frac{(0.4\ OD/ml)(50ml)}{3\ OD/ml} = 6.67$ ml

3. Remove the required amount (6.67 ml) and pellet cells by centrifuging at 1500 g for 5 minutes @ 4℃.
4. Re-suspend the cells in 1-2 ml of induction medium (SC-Uracil with 2% galactose) and inoculate 50 ml of induction medium. Grow at 30℃in a shaker at 150 rpm.
5. Harvest 5 ml of culture at 0, 2, 4, 6, 8, 10 hours of culture. Determine the OD of each sample.
6. Centrifuge and pellet the cells at 1500 x g for 5 minutes @ 4℃.
7. Resuspend the cells in 500 μl of sterile water in a sterile 1.5 ml microfuge.
8. Centrifuge cells at max speed for 30 seconds and remove the supernatant.
9. Cells can be stored at -80℃ until use.

4.2 Preparation of cell lysates

1. You can prepare cell lysates from either frozen or fresh cells. You need to know the OD_{600} of the cell samples before begining.
2. Resuspend cells in 500 μl of breaking buffer. Pellet at 1500 x g for 5 minutes @ 4℃
3. Remove supernatant and resuspend the cells in a volume of breaking buffer to obtain an OD_{600} of 50-100. Use the OD determined before to calculate the appropriate volume of beaking buffer to use.
4. Add equal volume of acid-washed glass beads and vortex for 30 seconds followed by 30 seconds on ice. Repeat 4 times.
5. Centrifuge for 10 minutes at max speed.
6. Remove supernatant and transfer to a new microcentrifuge tube. Assay for protein concentration using BSA as a standard.