Yeast Protocols[1]

[1]Compiled from various sources including internet, manuals and books

Contents

Chapter 1

Yeast transformation

1.1 Materials required

1.1.1 Solutions / reagents

1. YPD Plates
2. Plasmid DNA
3. Carrier DNA (Salmon /Herring sperm DNa in H_2O, 2 mg/ml stock, 500 μl aliquots, heat inactivated at 95 ℃ for 5–6 minutes and keep on ice (has to be done once))
4. 50 % Polyethylene glycol (PEG) MW 3350 solution (filter sterilized)
5. 1 M Lithium acetate (autoclaved)
6. Cells scrapped from plate
7. Plates containing appropriate dropout medium
8. ddH_2O

1.1.2 Equipment

1. 30 ℃ incubator
2. 4 ℃ centrifuge
3. Water bath or heat block set at 45 ℃
4. Sterile 1.5 ml microfuge tubes

1.2 Recipes

1.2.1 Yeast extract Peptone Dextrose (YPD)medium

1. Dissolve 10 g of BactoYeast extract in 800 ml water
2. Dissolve 20 g of BactoPeptone in the above solution
3. Add 20 g agar
4. q.s. to 900 ml with water
5. Autoclave
6. Add 100 ml of filter sterilized 20 % Dextrose solution after autoclaving and cooling down to ~ 55 ℃

1.2.2 SC dropout medium –1L

1. 6.7 g yeast nitrogen base (without amino acids)
2. 200 ml 10 % raffinose (Filtersterilized,add after autoclaving)
3. 100 ml 10 X Amino acid mix (without tryptophan and uracil)
4. 100 mg tryptophan (for SC-Uracil) or 100 mg Uracil (for SC-Tryptophan)
5. 2 % agar (for plates)

1.3 Procedure

1. Streak yeast culture from glycerol stock two days before the transformation date in YPD medium and incubate at 30 ℃. Takes 2 days to grow

2. On the day of transformation, measure out 50 μl of carrier DNA into a sterile 1.5 ml microfuge tube.
3. Add cells scrappped from plate.
4. Add in the following order
   (a) Add 1-2 μl of plasmid DNA
   (b) Add 240 μl of 50 % polyethylene glycol (PEG) solution.
   (c) Add 36 μl of 1 M Lithium acetate
5. Mix solution by pipetting up and down several times.
6. Incubate at 30 ℃ for atleast 30 minutes
7. Heat shock at 45 ℃ for 15 minutes
8. Centrifuge at maximum speed for 1 minute at room temperature, gently aspirate out the supernatant and discard it.
9. Add 100 μl of water
   (a) Resuspend pellet by vortexing / by shaking vigorously
   (b) Plate out resuspension onto plate containing appropriate selection medium

Chapter 2

PCR

2.1 Introduction

Confirmation of yeast colonies by PCR.

2.2 Materials required

1. 20 mM NaOH

2.3 Procedure

1. Aliquot 20μl NaOH in to PCR tubes
2. Pick colonies with a pipette tip and place the tip in the tube
3. Incubate at 95℃ for ~ 45 minutes
4. Centrifuge at max speed for 10 minutes
5. Use 1μl of supernatant as template in a 10μl PCR

Chapter 3

Expression of recombinant proteins in yeast

3.1 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*)

3.2 Materials required

3.2.1 Equipment required

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

3.2.2 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

3.3 Media recipes

3.3.1 20 % Galactose

Dissovle galactose at a concentration of 20% (wt/vol) in ddH_2O at room temperure while stirring. Galactose may take an hour or longer to dissolve. Filter sterilize; **do not autoclave** as galactose gets converted to glucose.

3.3.2 10 % Raffinose

Dissovle raffinose at a concentration of 10% (wt/vol) in ddH_2O at room temperure while stirring. Filter sterilize; **do not autoclave**. It is difficult to filter sterilize.

3.3.3 20 % Glucose

Dissolve glucose at a concentration of 20% (wt/vol) in ddH_2O at room temperure while stirring. Filter sterilize; **do not autoclave**.

3.3.4 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.

3.3.5 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)

3.3.6 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)

3.3.7 Induction media – 1L

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

3.3.8 Breaking buffer

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

5. pH 7.4

3.4 Protocol

3.4.1 Time course for expression of recombinant protein (Protocol specifically for pYES2)

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.

3.4.2 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.

Chapter 4

SDS-PAGE

4.1 Introduction

PAGE: Polyacrylamide gel electrophoresis (PAGE) is probably the most common analytical technique used to separate and characterize proteins. A solution of acrylamide and bisacrylamide is polymerized. Acrylamide alone forms linear polymers. The bisacrylamide introduces crosslinks between polyacrylamide chains. The 'pore size' is determined by the ratio of acrylamide to bisacrylamide, and by the concentration of acrylamide. A high ratio of bisacrylamide to acrylamide and a high acrylamide concentration cause low electrophoretic mobility. Polymerization of acrylamide and bisacrylamide monomers is induced by ammonium persulfate (APS), which spontaneously decomposes to form free radicals. TEMED, a free radical stabilizer, is generally included to promote polymerization.

SDS PAGE: Sodium dodecyl sulfate (SDS) is an amphipathic detergent. It has an anionic headgroup and a lipophilic tail. It binds non-covalently to proteins, with a stoichiometry of around one SDS molecule per two amino acids. SDS causes proteins to denature and dissassociate from each other (excluding covalent cross-linking). It also confers negative charge. In the presence of SDS, the intrinsic charge of a protein is masked. During SDS PAGE, all proteins migrate toward the anode (the positively charged electrode). SDS-treated proteins have very similar charge-to-mass ratios, and similar shapes. During PAGE, the rate of migration of SDS-treated proteins is effectively determined by molecular weight.

4.2 Materials required

4.2.1 Chemicals/solutions

1. 30 % Acrylamide/bisAcrylamide (37.5:1)
2. 10% SDS
3. 1.5M Tris pH 8.8
4. 10% APS
5. TEMED
6. DDH_2O

4.2.2 Equipment

1. Protein gel rig
2. Gel doc

4.3 Protocol

4.3.1 Acrylamide gel %

	6%	**8%**	**10%**	**12%**	**15%**
Acrylamide	1.6 μl	2.1 μl	2.7 μl	3.2 μl	4 μl
10% SDS	0.1 μl	0.1 μl	0.1 μl	0.1 μl	0.1 μl
1.5M Tris pH 8.8	2.6 μl	2.6 μl	2.6 μl	2.6 μl	2.6 μl
10% APS	0.1 μl	0.1 μl	0.1 μl	0.1 μl	0.1 μl
TEMED	0.01 μl	0.01 μl	0.01 μl	0.01 μl	0.01 μl
H2O	5.6 μl	5.1 μl	4.5 μl	4.0 μl	3.2 μl

Chapter 5

MUG assay

5.1 Introduction

The Fluorescent β-Galactosidase Assay (MUG) is a highly sensitive, fluorescent assay for determining the β-galactosidase activity in the lysates of cells transfected with a β-galactosidase expression construct. The study of the lac operon has played an important role in understanding the control of gene expression in bacteria. In prokaryotes, gene expression is controlled primarily at the level of transcription. For eukaryotes, the promoter activity can be analyzed by using fusion genes containing the promoter of interest attached to the bacterial β-galactosidase gene and be assayed by measuring β-galactosidase activity. Because -galactosidase has a high turnover rate and is absent in mammalian cells it serves as a very useful and sensitive reporting tool for gene expression.
Esters of the fluorescent compound, 4-methylumbelliferone (4-MU), provide a sensitive, quantitative assay for β-galactosidase. 4-methylumbelliferyl-β-D-galactopyranoside (4-MUG) is a substrate of β-galactosidase that does not fluoresce until cleaved by the enzyme to generate the fluorophore 4-methylumbelliferone. The assay can be used with extracts from different expression systems including mammalian, insect cells, yeast, and bacteria. The Fluorescent β-Galactosidase Assay (MUG) provides a 96 well assay format for galactosidase activity that is suitable for high throughput applications. The production of the fluorphore is monitored at an emission/excitation wavelength of 365/460nm.

5.2 Materials

5.2.1 Chemicals

1. 4-Methylumbelliferyl-β-D-glucuronide (MUG)
2. 4-Methylumbelliferone (4-MU)
3. Sodium carbonate (Na_2CO_3)
4. Dimethylsulfoxide (DMSO)

5.2.2 Equipment

1. Plate reader
2. 37 ℃ incubator
3.

5.3 Protocol

5.3.1 Preparation of stock solutions

1. 2mM MUG. Prepare MUG substrate stock solution by weighing out 7 mg MUG in a microtube. Add 40 μl DMSO to dissolve. Transfer to a 15 ml falcon tube and bring the final volume to 10 ml with ddH_2O.
2. 1 mM 4-MU. Prepare by weighing out 1.7 mg 4-MU in a microtube. Add 500 μl DMSO to dissolve. Transfer to a 15 ml falcon tube and bring up to a final volume of 10 ml in extraction buffer. Wrap in foil and store in 4℃ for a maximum of one month.

5.3.2 Preparation of working solutions

1. 10μM 4-MU. Prepare enough for use on the day of assay by diluting 4mM stock in extraction buffer.
2. Stop buffer. Dissolve 21.2 g Na_2CO_3 in ddH_2O and make up to 1 L. pH 10.5

5.4 Procedure

Chapter 6

Yeast plasmid extraction

6.1 Introduction

Extraction of plasmid from yeast.

6.2 Materials required

1. Qiagen buffers P1, P2, N3

6.3 Procedure

1. Grow culture to saturation in 4 ml selective media
2. Spin 1.5 ml for 10 min in microfuge tube, and remove supernatant
3. Add another 1.5ml of culture to pellet, spin, and remove supernatant
4. Resuspend in 500 μl sterile ddH_2O
5. Spin 10 min, remove supe
6. Resuspend pellet in 250 μl Qiagen buffer P1 (containing RNAse A)
7. Add 250 ul Qiagen buffer P2 and 250 μl acid washed glass beads
8. Vortex for 2 min. Let sit 5 min in P2. Do this at 4C.
9. Add 350 ul chilled Qiagen buffer N3
10. Mix by inverting, then incubate on ice for 5 min.
11. Spin 10 min
12. Place Qiaprep - spin column in 2 ml microfuge tube and apply supe (after the spin)
13. Spin 30-60 sec, drain tube
14. Wash column with 0.5 ml Qiagen buffer PB
15. Spin 30-60 sec, drain tube
16. Wash column with 0.75 ml Qiagen buffer PE
17. Spin 30-60 sec, drain tube, spin again
18. Place column in clean 1.5 ml microfuge tube
19. Elute DNA with 50-100 μl TE (10 mM Tris-HCl pH 8.5, 1 mM EDTA) or ddH_2O
20. Spin 30 sec
21. Transform a range of plasmid amounts into E. coli to amplify

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