Protocol: 300 mL Yeast culture preparation

Equipment and Reagents needed:

Autoclaved toothpicks
Shaker Incubator set at 30°C
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60 mm^2 sterile petri dishes
Autoclaved glass test tubes
Autoclaved caps for glass test tubes
500 mL Erlenmeyer flasks (autoclaved)
Cuvettes
Spectrophotometer
500 mL centrifuge tubes with caps (autoclaved)
50 mL tubes
15 mL tubes
25 mL and 10 mL pipettes (sterile)
Automatic Pipettor
Permanent Marker or Label System

Media:
SC-ura/Glucose Agar
SC-ura/Glucose Liquid
SC-ura/Raffinose Liquid
40% Galactose
Lysis Buffer for Yeast Expression (50mM Tris, pH 7.5, 100 mM NaCl, 1 mM EGTA,

10% glycerol, 0.1% TritonX-100). Stored at 4°C.
500 mL deionized H_2O stored at 4°C.

Recipes for Media and Solution Preparation
SC-ura/Glucose Agar Dishes (20mL/dish, enough for 8 clones)

Reagent	Amount (for 1 L)
YNB (without amino acids)	6.7g
Glucose	20g
SC-ura dropout mix	2g
Agar	20g
Deionized H_2O	To 1L

Autoclave. Cool the bottle in 65°C water bath for 15 minutes. Pour petri dishes (~20 ml/dish). Store the dishes at 4°C in a sealed bag or box. Use within 2 months.

SC-ura/Glucose Liquid

Reagent	Amount (for 1 L)
YNB (without amino acids)	6.7g
Glucose	20g
SC-ura dropout mix	2g
Deionized H_2O	To 1L

Autoclave.

SC-ura/Raffinose

Reagent	Amount (for 1 L)
YNB (without amino acids)	6.7g
Raffinose	20g
SC-ura dropout mix	2g
Deionized H_2O	To 1L

Autoclave.
40% Galactose Dissolve 400g galactose in 1 L boiling water. Use within 1 month.

Procedure

(Note: The following procedure describes the culture of one (1) yeast clone. For culturing multiple clones at the same time, follow the same procedural steps, and adjust the amount of initial media and buffers to prepare as needed).

Working Clone Preparation
1. First thing in the morning, streak out yeast clone (~ 2 inches in length) from -80°C stock plate onto a previously prepared SC-ura/glucose agar dishes using autoclaved toothpicks.
2. Set plates inside 30°C incubator for 2 days.
(Note: It is recommended to do this step on a Friday so that the plate is left to grow over the weekend).
Seed Culture Preparation

1. After 2 days of growth on SC-ura/glucose agar, use a sterile toothpick to inoculate all the yeast cells (single colonies are not necessary) in 9 ml SC-ura/glucose liquid medium.
2. Incubate at 30°C with shaking at 240 rpm for 24 hours
(Note: When growing multiple clones, the agar dishes can be sealed with Parafilm and stored at 4°C for 1-2 weeks. However, results are optimal with fresh yeast clones).

300 mL Culture Preparation and Induction

1. After 24 hrs of culture, dilute 100 µL of the seed culture in 900 µL of SC-ura/glucose liquid medium inside a cuvette.
2. Measure the optical density (O.D.) at 600 nm, using SC-ura/glucose liquid medium as reference.
3. If $O.D._{600} > 0.6$ (the higher the better), pour 9 mL of the seed culture into 300 mL of SC-ura/raffinose liquid medium.
4. Incubate at 30°C with shaking at 240 rpm for 6-8 hours.
5. After 6-8 hours of culture, measure the optical density (O.D.) at 600nm by taking 1 mL sample directly from the 300 mL culture. Do not dilute this sample. Use SC-ura/raffinose liquid medium as reference.
6. When the $O.D._{600}$ reaches 0.8-1.2 (the higher the better), add 16 mL of 40% Galactose to the 300 mL culture. The final concentration of galactose should be 2%.
7. Incubate at 30°C with shaking at 240 rpm overnight.

Cell Harvesting

1. The next morning stop the growth by transferring the entire contents of the culture into a labeled 500 mL centrifuge tube.
2. Centrifuge at 4000 rpm for 5 minutes at 4°C.
3. Discard the supernatant (media) by decanting.
(Note: From this moment on, place all the tubes on ice to maintain cold temperature).
3. Add 20 mL of ice-cold deionized water to resuspend the cell pellet.
4. Transfer the resuspended yeast cells to a labeled 50 mL tube.
5. Centrifuge at 4000 rpm for 5 minutes at 4°C.
6. Discard the supernatant (water) by decanting.
7. Resuspend the pelleted cells by adding 10 mL of cold Lysis Buffer without any inhibitors to the 50 mL tube. Once the cells have been resuspended transfer the entire contents to a labeled 15 ml tube.
8. Centrifuge at 4000 rpm for 5 minutes at 4°C.
9. Discard the supernatant (lysis buffer) by decanting.

10. The cell pellets can be used for protein purification immediately (see Yeast Protein Purification Protocol). If the cells are not going to be used for protein purification immediately freeze them at -80°C.

Pellet Storage

Store labeled 15 mL tubes at -80°C for up to 8 weeks