Cultivation of Yeast Cells and Induction of Autophagy

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METHOD

Preculture

1. Inoculate yeast cells (from a single colony) into 2 ml of liquid medium (YPD, SD/CA, or SD/DO medium) in a test tube.
2. Cultivate overnight at 30°C by using a rotator RT-50 at 50 rpm.

NOTE A saturation culture will reach nearly the same OD_{600} in the same medium.

NOTE This preculture can be stored at 4°C for ~3 weeks.

Cultivation of yeast cells

3. For a small-scale culture, inoculate 0.5–2.5 µl of the preculture into 5 ml of liquid medium in a test tube. For a large-scale culture, inoculate 5–25 µl of the preculture into 50 ml of liquid medium in a 200-ml flask.
4. Cultivate overnight at 30°C by using a rotator RT-50 at 50 rpm (small scale) or a rotary bioshaker BR-43FL-MR at 150 rpm (large scale).

NOTE A doubling time of yeast cells will be 90-120 min.

5. Measure OD_{600} of the yeast culture by pipetting 100 µl of the yeast culture into 900 µl of water.

NOTE A yeast culture containing 1×10^7 cells/ml will give the OD_{600} of 1.0.

Induction of autophagy by starvation

6. Harvest the yeast cells (OD_{600}=1.2–1.8) by using a centrifuge LC-100/LC-200 (small scale) or a centrifuge CF5RX/CF16RN (large scale) at 2,000 × *g* for 1 min at room temperature.

NOTE To induce autophagy efficiently, the OD_{600} should be over 1.0.

7. Remove the supernatant and add an equivalent volume of sterile water.

NOTE This sterile water should be prewarmed at 30°C.

8. Harvest the yeast cells as in step 6.
9. Remove the supernatant and add an equivalent volume of starvation medium (SD(–N), S(–C), or S(–NC) medium).

NOTE This starvation medium should be prewarmed at 30°C.

10. Incubate at 30°C by using a rotator RT-50 at 50 rpm (small scale) or a bioshaker BR-43FL-MR at 150 rpm (large scale).

Induction of autophagy by rapamycin treatment

11. Add 1/5,000 volume of 1 mg/ml rapamycin into the yeast culture (final 0.2 µg/ml rapamycin).

NOTE Rapamycin of LC Laboratories (200 mg/¥21,000) is considerably cheaper than that of SIGMA (1 mg/¥63,900).

12. Incubate at 30°C by using a rotator RT-50 at 50 rpm (small scale) or a bioshaker BR-43FL-MR at 150 rpm (large scale).

MATERIALS

REAGENTS

- Bacto yeast extract (BD, 212750)
- Bacto peptone (BD, 211677)
- Glucose/dextrose (nacalai tesque, 16806-54)
- Yeast nitrogen base w/o amino acids and ammonium sulfate (BD, 233520)
- Ammonium sulfate (nacalai tesque, 02620-04)
- Bacto casamino acids (BD, 223050)
- Agar (Matsuei Kanten, K986)
- Adenine sulfate (Ade; Wako, 010-19612)
- L-Histidine (His; Wako, 084-00682)
- L-Leucine (Leu; Wako, 124-00852)
- L-Tryptophan (Trp; Wako, 204-03382)
- Uracil (Ura; Wako, 212-0062)
- L-Arginine-HCl (Arg; Wako, 014-04622)
- L-Aspartic acid (Asp; Wako, 013-04832)
- L-Glutamic acid (Glu; Wako, 070-00502)
- L-Isoleucine (Ile; Wako, 121-00862)
- L-Lysine-HCl (Lys; Wako, 121-01462)
- L-Methionine (Met; Wako, 133-01602)
- L-Phenylalanine (Phe; Wako, 161-01302)

• L-Serine (Ser; Wako, 199-00402)
• L-Threonine (Thr; Wako, 204-01322)
• L-Tyrosine (Tyr; Wako, 202-03562)
• L-Valine (Val; Wako, 228-00082)
• MES (DOJINDO, 343-01626)
• KOH (Wako, 168-21815)
• G418 sulfate (Wako, 070-05183)
• ClonNAT (Werner BioAgents, 5.000 000)
• Hygromycin B (Wako, 085-06153)
• Zeocin (funakoshi, InvivoGen, ant-zn-1)
• Rapamycin (SIGMA, R0395)
• Rapamycin (funakoshi, LC Laboratories, R-5000)
• Tween 20 (nacalai tesque, 28353-85)

EQUIPMENT

• Vacuum filter system, 0.22-μm pore PES (Corning, 431097, etc.)
• Rotator RT-50 (TAITEC)
• Rotary shaker BR-43FL-MR (TAITEC)
• Centrifuge LC-100 or LC-200 (TOMY)
• Centrifuge CF5RX or CF16RN (Hitachi)

REAGENT PREPARATION

YPD medium

• 1% (w/v) yeast extract
• 2% (w/v) bacto peptone
• 2% (w/v) glucose

Dissolve 10 g of bacto yeast extract and 20 g of bacto peptone in 900 ml of distilled water (if preparing agar plates, add 20 g of agar). Dissolve 20 g of glucose in 100 ml of distilled water. Autoclave at 121°C for 20 min. Mix the autoclaved solutions. This medium can be stored at room temperature (if preparing agar plates, dry the plates for 2–3 days at room temperature and store at 4°C).

SD/CA medium (for Ade, Trp, and/or Ura prototrophy strain)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate
- 0.5% (w/v) ammonium sulfate
- 0.5% (w/v) casamino acids
- 2% (w/v) glucose

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate, 5 g of ammonium sulfate, and 5 g of bacto casamino acids in 900 ml of distilled water (if preparing agar plates, add 20 g of agar). Add 750 µl of 5 N NaOH to adjust the pH to ~6.2. Dissolve 20 g of glucose in 100 ml of distilled water. Autoclave at 121°C for 20 min. Mix the autoclaved solutions. Add 10 ml of 100× stock solutions (100× Ade, 100× Trp, and/or 100× Ura). This medium can be stored at room temperature (if preparing agar plates, dry the plates for 2-3 days at room temperature and store at 4°C).

SD/DO medium (for Ade, His, Leu, Trp, and/or Ura prototrophy strain)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate
- 0.5% (w/v) ammonium sulfate
- 1× dropout mix
- 2% (w/v) glucose

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate and 5 g of ammonium sulfate in 800 ml of distilled water (if preparing agar plates, add 20 g of agar). Add 750 µl of 5 N NaOH to adjust the pH to ~6.2. Dissolve 20 g of glucose in 100 ml of distilled water. Autoclave at 121°C for 20 min. Mix the autoclaved solutions and add 100 ml of 10× dropout mix. Add 10 ml of 100× stock solutions (100× Ade, 100× His, 100× Leu, 100× Trp, and/or 100× Ura). This medium can be stored at room temperature (if preparing agar plates, dry the plates for 2-3 days at room temperature and store at 4°C).

SD(–N) medium (nitrogen-starvation medium)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate
- 2% (w/v) glucose

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate in 900 ml of distilled water. Dissolve 20 g of glucose in 100 ml of distilled water.

Autoclave at 121°C for 20 min. Mix the autoclaved solutions. As needed, add 50 ml of 1 M MES-KOH pH 5.2 (final 50 mM MES-KOH pH 5.2). This medium can be stored at room temperature.

S(−C) medium (S/CA(−C), carbon-starvation medium)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate
- 0.5% (w/v) ammonium sulfate
- 0.5% (w/v) bacto casamino acids
- 1× Ade, Trp, and/or Ura

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate, 5 g of ammonium sulfate, and 5 g of bacto casamino acids in 1000 ml of distilled water. Autoclave at 121°C for 20 min. Add 10 ml of 100× stock solutions (100× Ade, 100× Trp, and/or 100× Ura). As needed, add 50 ml of 1 M MES-KOH pH 5.2 (final 50 mM MES-KOH pH 5.2). This medium can be stored at room temperature.

S(−C) medium (S/DO(−C), carbon-starvation medium)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate
- 0.5% (w/v) ammonium sulfate
- 1× dropout mix
- 1× Ade, His, Leu, Trp, and/or Ura

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate and 5 g of ammonium sulfate in 900 ml of distilled water. Autoclave at 121°C for 20 min. Add 100 ml of 10× dropout mix. Add 10 ml of 100× stock solutions (100× Ade, 10 ml of 100× His, 10 ml of 100× Leu, 10 ml of 100× Trp, and 10 ml of 100× Ura). As needed, add 50 ml of 1 M MES-KOH pH 5.2 (final 50 mM MES-KOH pH 5.2). This medium can be stored at room temperature.

S(−NC) medium (nitrogen- and carbon-starvation medium)

- 0.17% (w/v) yeast nitrogen base w/o amino acids and ammonium sulfate

Dissolve 1.7 g of yeast nitrogen base w/o amino acids and ammonium sulfate in 1000 ml of distilled water. Autoclave at 121°C for 20 min. As needed, add 50 ml of 1 M MES-KOH pH 5.2 (final 50 mM MES-KOH pH 5.2). This medium can be

stored at room temperature.

10× dropout mix stock solution

Dissolve 0.2 g of L-arginine-HCl, 1.0 g of L-aspartic acid, 1.0 g of L-glutamic acid, 0.3 g of L-isoleucine, 0.3 g of L-lysine-HCl, 0.2 g of L-methionine, 0.5 g of L-phenylalanine, 4.0 g of L-serine, 2.0 g of L-threonine, 0.3 g of L-tyrosine, and 1.5 g of L-valine in 1000 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at –30°C.

100× Ade stock solution

Dissolve 1.0 g of adenine sulfate in 500 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at 4°C.

100× His stock solution

Dissolve 1.0 g of L-histidine in 500 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at 4°C.

100× Leu stock solution

Dissolve 5.0 g of L-leucine in 500 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at 4°C.

100× Trp stock solution

Dissolve 1.0 g of L-tryptophan in 500 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at 4°C in a light-proof container.

100× Ura stock solution

Dissolve 1.0 g of uracil in 500 ml of distilled water and filtrate by using a vacuum filter system. This solution can be stored at room temperature.

1 M MES-KOH pH 5.2

Dissolve 106.6 g of MES in ~400 ml of distilled water. Adjust the pH to 5.2 with 5 N KOH. Fill up to 500 ml and filtrate by using a vacuum filter system. This

solution can be stored at room temperature.

25 mg/ml G418 sulfate (100× stock solution)

Dissolve 2.5 g of G418 sulfate in 100 ml of distilled water and filtrate by using a vacuum filter system. This reagent can be stored at –30°C. As needed, add 5 ml of 25 mg/ml G418 sulfate to 500 ml of YPD medium (final 250 µg/ml G418 sulfate).

10 mg/ml clonNAT (100× stock solution)

Dissolve 1.0 g of clonNAT in 100 ml of distilled water and filtrate by using a vacuum filter system. This reagent can be stored at –30°C. As needed, add 5 ml of 10 mg/ml clonNAT to 500 ml of YPD medium (final 100 µg/ml clonNAT).

30 mg/ml hygromycin B (100× stock solution)

Dissolve 3.0 g of hygromycin B in 100 ml of distilled water and filtrate by using a vacuum filter system. This reagent can be stored at –30°C. As needed, add 5 ml of 30 mg/ml hygromycin B to 500 ml of YPD medium (final 300 µg/ml hygromycin B).

20 mg/ml zeocin (100× stock solution)

Dissolve 2.0 g of zeocin in 100 ml of distilled water and filtrate by using a vacuum filter system. This reagent can be stored at –30°C. As needed, add 5 ml of 20 mg/ml zeocin to 500 ml of YPD medium (final 200 µg/ml zeocin).

1 mg/ml rapamycin (5,000× stock solution)

Dissolve 10 mg of rapamycin in 10 ml of 90% ethanol and 10% Tween 20. This reagent can be stored at –30°C.

TROUBLESHOOTING TIPS

1. No induction of autophagy.

   Make sure that the culture medium is completely washed away. If needed, the culture medium is removed by aspiration.