Aug 12, 2022

Transforming Yeast (Instructor Protocol)

Brian Teague[1]

[1]University of Wisconsin - Stout

This protocol is published without a DOI.

Yeast ORFans CURE

Brian Teague
University of Wisconsin - Stout

ABSTRACT

This is the instructor protocol for

Transforming yeast
by Brian Teague,
University of Wisconsin - Stout

PREVIEW RUN

The yeast transformation is adapted from Geitz and Schiestl:

Gietz RD, Schiestl RH (2007). Frozen competent yeast cells that can be transformed with high efficiency using the LiAc/SS carrier DNA/PEG method.. Nature protocols.

PROTOCOL CITATION

Brian Teague 2022. Transforming Yeast (Instructor Protocol). **protocols.io** https://protocols.io/view/transforming-yeast-instructor-protocol-ce8cthsw

IMAGE ATTRIBUTION

Masur, Public domain, via Wikimedia Commons

CREATED

Aug 12, 2022

LAST MODIFIED

Aug 12, 2022

PROTOCOL INTEGER ID

68580

GUIDELINES

We use dropout media components from Sunrise Science, but I'm sure media from other vendors is fine. Double-check, though, that your "yeast nitrogen base" doesn't contain amino acids and other things.

MATERIALS TEXT

Equipment

- Shaking incubator
- Spectrophotometer for measuring OD600
- 500 ml baffled flask (the baffles improve the aeration)
- 3x Mr Frostys (or a large styrofoam container)
- Thermocycler

Materials

Bacto™ Peptone **Thermo Fisher**

- **Scientific Catalog #211677** Step 1

BD Bacto™ Yeast Extract **BD**

- **Biosciences Catalog #212750** Step 1

α-D-Glucose **Sigma**

- **Aldrich Catalog #492-62-6** Step 4

Yeast Nitrogen Base (YNB) **Sunrise**

- **Science Catalog #1500-100** In 2 steps

CSM-Leu-Ura Powder **Sunrise**

- **Science Catalog #1038-010** In 2 steps

Ammonium sulfate **Sigma**

- **Aldrich Catalog #A4418** In 2 steps

Uracil Yeast Culture Grade **Sunrise**

- **Science Catalog #1906-010** Step 16

L-Leucine Yeast Culture Grade **Sunrise**

- **Science Catalog #1980-010** Step 17

Agar, bacteriological

- grade **Amresco Catalog # J637** In 2 steps

Glycerol **Thermo**

- **Scientific Catalog #17904** Step 5

DMSO (dimethyl sulfoxide) **Sigma**

- **Aldrich Catalog #D8418** Step 5
-

Polyethylene Glycol (PEG) 3350 **Electron Microscopy Sciences Catalog #19760** Step 19

Salmon Sperm DNA **Research Products International**

- **Corp Catalog #D52150**

SAFETY WARNINGS

Lithium acetate causes eye and skin irritation. Wear appropriate PPE, including a lab coat, gloves and safety glasses.

DMSO is a minor irritant. It also readily penetrates skin and may significantly enhance the absorption of numerous chemicals. Wear appropriate PPE, including a lab coat, gloves and safety glasses, and remove contaminated gloves and clothes promptly.

Prepare 250 ml of 2xYPD media 30m

1 Put **250 mL** of deionized water in a 250 ml or 500 ml bottle, then add:

Bacto™ Peptone **Thermo Fisher**

- **10 g Scientific Catalog #211677**

BD Bacto™ Yeast Extract **BD**

- **5 g Biosciences Catalog #212750**

2 Cap the bottle tightly and shake to resuspend the powdered components.

You don't need to dissolve the powder, just get it off the bottom.

30m

3 Autoclave at **121 °C** for **00:30:00** on a liquid cycle.

4 Cool, then using good sterile technique, add **25 mL** of a **[M]40 Mass / % volume** solution of

α-D-Glucose **Sigma Aldrich Catalog #492-62-6** .

You need to cool the media before doing this or it will boil over.

Prepare frozen competent cell (FCC) solution 30m

5 To a 15 ml conical centrifuge tube, add:

- Glycerol **Thermo** **0.5 mL** **Scientific Catalog #17904**
- DMSO (dimethyl sulfoxide) **Sigma** **1 mL** **Aldrich Catalog #D8418**
- Deionized water to a total volume of **10 mL**

6 Filter-sterilize the solution into a new 10 ml tube.

I like to mount a filter on a syringe, pull the plunger out the back, pour the solution into the syringe, then re-insert the plunger.

Prepare competent yeast cells 15m

7 The day before, transfer 5 ml of 2xYPD media into two round-bottomed test tubes. Pick a colony of yeast off of the plate into each test-tube and grow, overnight, on the roller drum or orbital shaker at **30 °C** . This is your starter culture.

Also put the bottle of 2xYPD into the 30 degree incubator to warm it up overnight.

8 The morning of the prep, measure the OD600 of the starter cultures. Pour **250 mL** of the 2xYPD media into a 500ml *baffled* flask. Add enough starter culture so that the final OD600 is 0.5.

9 Shake **200 rpm, 30°C** until the OD600 reaches 2.0.

Starting at 2 hours, check the OD600 with a spec every 30 minutes. This may take 4-5 hours

Starting at 3 hours, check the OD600 with a spec every 30 minutes. This may take 4-5 hours.

10 Harvest the cells by centrifugation **2900 x g, 00:05:00** at room temperature in a swinging bucket (or fixed-angle highspeed) centrifuge. 5m

11 Decant the media from the centrifuge tubes back into the baffled flask. Resuspend the pellet(s) in **125 mL** of sterile water (vortexing is okay). Then, centrifuge **2900 x g, 00:05:00** . 5m

12 Decant the water back into the baffled flask. Resuspend the pellet(s) in a total of **0 mL** l of sterile water. Again, centrifuge **2900 x g, 00:05:00** 5m

13 Aspirate or decant the water. Resuspend the pellet in FCC for a *total volume* of **2.5 mL**

Because the volume of cells is pretty large (almost 2 ml!), you'll have to use substantially less than 2.5 ml of FCC. Use the markings on the side of the conical tube to guide you.

14 Transfer **50 µL** aliquots to sterile microcentrifuge tubes. Load into Mr Frosty's (or the styrofoam container) and freeze overnight at **-80 °C**

The Mr. Frostys or styrofoam container freeze the cells slowly, which is important to maintain their viability.

15 The next morning, transfer to a freezer box and store at **-80 °C**

Prepare dropout media 15m

16 Prepare 500 ml of synthetic defined media without leucine: 30m

Yeast Nitrogen Base (YNB) **Sunrise**

- **0.855 g** **Science Catalog #1500-100**

CSM-Leu-Ura Powder **Sunrise**

- **0.335 g** **Science Catalog #1038-010**

Ammonium sulfate **Sigma**

- **2.5 g** **Aldrich Catalog #A4418**
- **5 mL** of 100X uracil solution **2 mg/mL**

Uracil Yeast Culture Grade **Sunrise**

Science Catalog #1906-010

Agar, bacteriological

- **10 g** grade **Amresco Catalog # J637**

Make up to 475 ml with deionized water and autoclave **121 °C** **00:30:00** on a liquid cycle.

Cool to **60 °C** , then add **25 mL** **40 Mass / % volume**

α-D-Glucose **Sigma**

Aldrich Catalog #158968 and pour or pipette into petri dishes.

17 Prepare 500 ml of synthetic defined media without uracil: 30m

Yeast Nitrogen Base (YNB) **Sunrise**

- **0.855 g** **Science Catalog #1500-100**

CSM-Leu-Ura Powder **Sunrise**

- **0.335 g** **Science Catalog #1038-010**

Ammonium sulfate **Sigma**

- **2.5 g** **Aldrich Catalog #A4418**
- **5 mL** of 100X leucine solution **12 mg/mL**

L-Leucine Yeast Culture Grade **Sunrise**

Science Catalog #1980-010

Agar, bacteriological

- **10 g** grade **Amresco Catalog # J637**

Make up to 475 ml with deionized water and autoclave **121 °C** **00:30:00** on a liquid cycle.

Cool to **60 °C** , then add **25 mL** **40 Mass / % volume**

α-D-Glucose **Sigma**

Aldrich Catalog #158968 and pour or pipette into petri dishes.

Miscellaneous preparation 5m

18 Prepare the salmon sperm: 5m

- If necessary, dissolve the salmon sperm in sterile TE buffer at a concentration of 2 mg/ml. (This is best done in the cold room on a magnet stir plate.)
- Make **55 µL** aliquots in PCR tubes. (Strip tubes and a repeat pipettor make this easy.)
- Denature by setting a thermocycler to hold at **95 °C** and putting the PCR tubes in for **00:05:00** , then moving immediately into an ice/water bath.
- Store at **-20 °C**

19 Prepare the

Polyethylene Glycol (PEG) 3350 **Electron Microscopy Sciences Catalog #19760**

solution:

- Put **2 mL** of deionized water in a 15 ml conical microcentrifuge tube
- Add **5 g**

  Polyethylene Glycol (PEG) 3350 **Electron Microscopy Sciences Catalog #19760**
- Add more deinized water until the total apparent volume is **10 mL**
- Shake vigorously to suspend the PEG evenly
- Put on a roller (or nutator or rocker) until the PEG dissolves completely.
- Add more deionized water to a final volume of **10 mL** .

Instructor Tips & Common Student Errors

20 **Instructor Tips**

- Particularly after transforming E. coli, students usually find this lab to be pretty straightforward.
- Students still struggle with the unit analysis required to "compute the volume containing XXXX amount of DNA". I usually provide a basic example, along the lines of "if I have a solution that is 5 g/ml and I need 10 g total, how much solution do I need?" and then suggest they use the same reasoning. I also offer to double-check their answers if they'd like.
- A common issue is "not enough PCR", especially if there have been a failed transformation or two. (There's usually plenty of plasmid.) If there's more than 100 ng of PCR DNA, I usually tell students to go ahead and try transforming anyway, with the warning that things may not be efficient and they might consider retrying their PCR while the incubation is going.
- Wait three full days before trying to pick colonies. Yeast double every 2-3 hours on dropout

media like this, so it will take time before colonies are visible. They're usually visible (but small) after 2 days -- too small to do anything with.

- When interpreting their results, I usually ask students to think about why we plated on both the leucine and uracil dropout plates. What does it mean when cells are growing on one or the other? (FYI: the leucine plate is the transformation control -- it tells us whether we were successful at getting the DNA into the cells. The colonies on the uracil plate, though, necessitated both successful transformation and successful genome editing -- they're the colonies we want to move forwards with.)

21 Common student errors

- Didn't immediately thaw the cells in the hot water bath and remove the freezing medium. Yeast likes to freeze slowly and thaw quickly!
- Students still struggle with the unit analysis required to "compute the volume containing XXXX amount of DNA" (see above).
- Not enough plasmid or PCR (see above)