

Jul 08, 2024

Yeast transformation

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5xp65g1b/v1>

is Sparrow¹

¹UIUC

Yeast Protocols, Tools, a...



is Sparrow

UIUC

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5xp65g1b/v1>

Protocol Citation: is Sparrow 2024. Yeast transformation. protocols.io
<https://dx.doi.org/10.17504/protocols.io.8epv5xp65g1b/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: January 10, 2024

Last Modified: July 08, 2024

Protocol Integer ID: 93353

Keywords: yeast transformation protocol, peg method, transformation, efficient liac

Abstract

Protocol was obtained from Ulschan Bathe and Kristen Van Geller from UF on May 2023 and optimized by myself.

This protocol is a variation on the highly efficient LiAc/SS/PEG method from Gietz et al., 2007

DOI: [10.1038/nprot.2007.13](https://doi.org/10.1038/nprot.2007.13)



Materials

Materials

Solutions

10x TE

A.

100mM

Tris, pH 7.5-8

B.

10mM

EDTA, autoclave or filter sterilize

10x LiAc

A.

10.2g

Lithium acetate

B.

100mL

MilliQ water, autoclave or filter sterilize

50% PEG 3350

A.

50g

PEG 3350

B.

30mL

MilliQ water, dissolve with stirring and heat, bring volume to 100mL with MilliQ water.

Keep tightly capped to prevent evaporation.

Filter sterilize in aliquots of 10 mL.

ssDNA 11mg/mL

A.

Dissolve

in MilliQ water, boil at 95°C, aliquot and freeze at -20°C

MilliQ water

A.

Sterile

via autoclave or filter



PEG mixture – this can be made and stored in fridge

A.

80% of

50% PEG 3350

B.

10% of

10x TE

C.

10% of

10x LiAc, store at 4°C

Media

YPD media

A.

10g

yeast extract

B.

20g

tryptone

C.

950mL

MilliQ water, autoclave

D.

Add

50mL 40% glucose once cooled

40% glucose

A.

40g

glucose

B.

70mL MilliQ

water, dissolve with stirring and heat and then bring volume up to 100mL with more MilliQ water, filter sterilize

SC -Leu

A.

1.675g

YNB with Ammonium acetate

B.

0.35g

Drop-out His, Leu, Trp and Ura



C.

235mL

MilliQ water, pH 6.1

D.

5g

agar and autoclave

E.

Added

12.5mL 40% glucose, 2.5mL each of 100x URA, 100x TRP and 100x HIS

100x URA

A.

0.076g

uracil

B.

10mL

MilliQ water, add drops of 5M NaOH till dissolves, filter sterilize

100x TRP

A.

0.076g

tryptophan

B.

10mL

MilliQ water, add drops of 5M NaOH till dissolves, filter sterilize

100x His

A.

0.076g

histidine

B.

10mL

MilliQ water, filter sterilize

Equipment

125mL flask

Culture tubes

Falcon tubes

Eppendorf tubes



Prep

- 1 2 days before yeast transformation, start 3mL pre-culture of your yeast strain in appropriate selective media. Growth in SC is fine for this step.
- 2 If the DNA to be transformed is for **integration**, linearize your plasmid the night before and store in the fridge or freezer the morning of the transformation. If you do, heat inactivate the enzyme.

Note: for integrations, a high amount of DNA is needed for efficient transformation. Make the following digestion:

55.5 uL plasmid (aim for a concentration of 90-300 ng/uL, more is better)

6.5 uL CutSmart/r3.1/NEB buffer

3 uL Restriction enzyme (BE SURE TO DOUBLE CHECK WHICH ENZYME YOU ARE USING)

Double check the expected cut patterns on a gel by running 1 uL of digest.

Growth of yeast to exponential phase

6h 10m 15s

- 3 Dilute pre-culture, **1:35 into**  30 mL **YPD** per 3 transformations (875uL of pre-culture into 30mL YPD). **Grow until OD600 of 0.6 (it is acceptable between 0.6 and 1.5) is reach (approximately**  06:00:00)

6h

Note: For slow growing strains, start the cultures the night before. For example, JH45 takes 9-12 hours to reach OD 0.6 from a starter dilution of 0.13.

Note: in my experience, addition of antibiotic is necessary at this step especially for slow growing yeast strains as this helps prevent contamination.

Note: when transforming plasmids, the OD matters less and as long as it is somewhere between 0.4 and 1.5 you will get transformants, but when you are looking for integration, the ideal range is between 0.6 and 0.8.

Note: Yeast should have at least 2 divisions to maximize transformation efficiency, but not more than 4. Do not start a very dilute starter culture the night before unless you know that the yeast genotype is slow to grow and will reach desired OD overnight from 1:35~ dilution.



4 When yeast reaches OD600 of 0.6, pour into  50 mL falcon tube and spin at

5m

 1500 x g, 4°C, 00:05:00

During this step, put your ssDNA in a  95 °C water bath/heat block for 5 minutes, and immediately plunge in ice. Mark on the vial how many times the ssDNA has been boiled. Discard after 3 boils.

Note: pellet should be whitish, or off-white. A spot of brown at the center of the pellet is expected, but yellowish or brownish color overall is indicative of contamination.

5 Decant supernatant

6 Resuspend in  30 mL sterile milliQ water

7 Spin at  1500 x g, 4°C, 00:05:00

5m

8 Resuspend in  900 µL of **TE/LiAc/water solution** (make  1.5 mL :  150 µL 10x TE,  150 µL 10x LiAc and  1200 µL sterile water) and transfer to  1.5 mL tube. See materials for recipes.

9 Spin at max speed  Undetermined, 4°C, 00:00:15

15s

10 resuspend in  300 µL **TE/LiAc/water solution**

Note: a  300 µL suspension will actually yield more than 300, so you may instead choose to resuspend in less volume (~  233 µL) to yield a total volume of  300 µL , which serves to concentrate the cells more.

Addition of DNA

11 Aliquot  100 µL per transformation to individual tubes



Note: because of the volume of the cells, resuspension in 300 uL from previous step will yield more than actually 300 uL. 100 uL of this, even though not truly 1/3rd of resuspension is OK to use, or more can be used, but adjust the additives amount accordingly.

12 Add, in the following order:

12.1  20 μ L of [M] 5 mg/mL salmon sperm ssDNA

Note: high quality ssDNA is critical - don't overuse your ssDNA

12.2 DNA

For linear DNA: add 65 uL of overnight, heat-inactivated digest. You want upwards of 5 ug, and I have had success with 12 ug, but I do not know if there is an upper cap.

For plasmid DNA: around 1 ug of plasmid is sufficient.

If you are cotransforming with linear dsDNA (e.g., as a repair fragment for Cas9) generated from PCR, there is no need to PCR cleanup the fragment. Add the entirety of the PCR reaction alongside the plasmid DNA.

12.3 Mix gently by flicking the tube

12.4  600 μ L of "PEG mix" (see materials for recipe)

The PEG mixture must be made the day of with freshly sterilized PEG (do not autoclave, filter sterilize it)

12.5 Mix gently by pipetting until homogeneous

Incubation and outgrowth

1h 10m

13 Incubate at  30 °C for  00:45:00 with rotation

45m

This can be allowed up to 3 hrs if necessary

14 Incubate at  42 °C for  00:20:00

20m

This must be stuck to as close as possible





15 Centrifuge at  700 x g, 00:05:00

5m

16 Resuspend in  1 mL sterile water

Can also resuspend in less if low efficiency expected

Note : be certain of the sterility of your water!!! I suggest autoclaving fresh MilliQ water the morning of the transformation and stick it in the cold room

17 Plate unto appropriate selection plates

Note

If selection is antibiotic, cells should be resuspended in 600ul of YPD and rotated at 30°C for at least 5 hours but can be left overnight if necessary. Alternatively, transformations can be plated onto YPD plates and replica-plated the next day onto appropriate antibiotic containing plates.

Auxotrophic selection is OK to plate as is.

Ensure that your plating method will keep everything sterile: use L spreaders or sterile glass beads.

Note: do not plate the entire volume of cells unto a plate. High plating density decreases growth and may also obscure selection if using minimal plates. Instead, I recommend pouring 3 plates for each transformation and plating 3:2:1 and saving some resuspension in the cold room just in case. This is especially important when selecting with auxotrophy, in which case 1/10-20th of the total resuspension buffer is typically enough to yield some transformants. You will not see any transformants if you plate all your cells in one plate, even if it worked.

18

Expected result

CEN/ARS plasmid colonies can be expected in 2-3 days, genomic integrations take between 3-5. p1 integrations can take 4-7 days to appear.