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Yeast transformation

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Yeast Protocols, Tools, an… burgess-lab-uiuc

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Protocol status: Working
We use this protocol and it's working

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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**

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 1500 x g, 4°C, 00:05:00

5m

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 µL 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 µL 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.

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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.