Yeast materials

Transformation

1M LiOAc stock solution (500 mL)
51g LiOAc
450 mL dH_2O

Dissolve LiOAc in the water and adjust the volume to 500mL with dH_2O. Filter sterilize or autoclave.

EDTA 0.5 M stock solution (100 mL)
18.61 g $Na_2EDTA*2H_2O$
80 mL dH_2O

Stir with magnetic stirrer until it's dissolved. Adjust pH to 8.0 and adjust volume to 100 mL. Sterile filer.

Tris*HCl 1 M stock solution (100 mL)
15.76g Tris*HCL
80 mL dH_2O

Stir with magnetic stirrer until it's dissolved. Adjust pH to 8.0 and adjust volume to 100 mL. Sterile filer.

10X TE (100 mL)
2 mL 0.5 M EDTA
10 mL 1M Tris-HCl (pH 8.0)
88 mL dH_2O

Mix in a 100 mL bottle.

1X LiOAc buffer (100 mL)
10 mL 1 M LiOAc
10 mL 10X TE
80 mL dH_2O

Mix in a 100 mL bottle.

PEG solution (100 mL)
Heat 50 mL ddH_2O and add

50 g PEG 3350
10 mL 10X TE
10 mL 1M LiOAc

Adjust the volume to 100 mL and filer sterilize. Store in tightly caped container.

Solutions needed for “Crude yeast genomic extraction prep for PCR”

20 % SDS stock solution (100 mL)
20g Sodium dodecyl sulfate (SDS)
80 mL dH_2O

Mix in a 250 ml bottle. The solution will be milky white and foamy and it will look like a lot more than 100mL. Let it stir for about a day until dissolved, if it has not dissolved in a day, add a bit more water. Fill up to 100 mL with dH_2O.

1M LiOAc stock solution (500 mL)
51g LiOAc
450 mL dH_2O

Dissolve LiOAc in the water and adjust the volume to 500mL with dH_2O. Filter sterilize or autoclave.

Crude yeast genomic DNA prep lysis buffer (100 mL)
5 mL 20% SDS
20 mL 1 M LiOAc
75 mL dH_2O

Mix in a 100 mL bottle

Solutions for colony PCR

1M NaOH stock solution (50 mL)

10 mL dH_2O

2.000 g NaOH (pellets)

Important add the pellets **to** the water. Be careful and in the ventilated hood. Always use a glass bottle or a beaker. Carefully and slowly adjust volume to 50 mL while stirring.

20mM NaOH (10 mL)

200µL 1M NaOH
9.8 mL dH_2O

Media for yeast

I usually make 5X or 2X media batches to dilute into the concentration needed and it allows me to add whatever carbon source, antibiotics, extra amino acid etc that I want. This way you can also easily make plates by mixing 2X media with 2X agar.

2X YNB (500 mL)

YNB is not a buffered media. If a minimal buffered media is needed, use Delft medium.

6.9 g Yeast nitrogen base ***without amino acids****
790 mg CSM complete*,**

Fill up to 500 mL with dH20. Adjust after autoclaving if needed with sterile dH2O.

*Different brands may vary in amount this is based on FORMEDIUM.

** For auxotrophic selective media use CSM dropouts. The amount varies between drop out type. Always double check! The correct amount is usually stated 1X/L on the label.

2X Agar (250 mL)

10g Agar

Fill up to 250 mL with dH20. Adjust after autoclaving if needed with sterile dH2O.

Double check that the bottle you use fitts into the microwave. Never fill the container more than half way (unless you like staring into the microwave while melting it very slowly and stirring it every 10 seconds).

40% Glucose (100 mL)

40g glucose (use the big weighing ship)

Fill up to 100 mL with dH2O. Adjust after autoclaving if needed with sterile dH2O.