Xu_UMBC lab Standard Operation Procedure (SOP) (Cell culture)

(updated 06/01/2018)

Be meticulous about everything you do, don't rush, be slow and ask around when you are not sure or just starting a new protocol. Try to improve/optimize your experiment every time you do, don't just follow the protocol. Think how you could do it better and faster. We are working with nanogram of DNA, a few microliters of enzymes and cells. Be patient, confident and demonstrate your fine-motor skills to pipette and transfer liquids. You will form a good habit and enjoy the benchwork. This is the basic training as a molecular biologist and microbiologist.

One of the important skill is parallel or multiplexed experimenting. Clear your mind and keep tracking/labelling every DNA and cell you have worked, plan ahead and use your notes/spreadsheets to keep tracking which plasmid goes to which cell, which cell goes to which plate/media. Rush and carelessness is the enemy of molecular experiment.

Meida is critical to do genetic screening. Be careful of the concentration.

Symbol

● Steps should work under sterile conditions with gas burner or in biosafety hood

◘ Be careful of pipetting carry-over and cross-contamination

Ӝ suggestions (you may follow these suggestions to optimize your results)

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1. LB agar plate

(1) Label a clean bottle, and add the amount of DI water required. (maximally you can fill 80% of the bottle volume for autoclave safety practice)

(2) Lauria Broth power 16 g/L

(3) Agar 18 g/L

(4) Securely close the cap and shake vigorously until there is no visible nutrient chunk.

(5) Loose the cap and autoclave at 121C (250F) for 20 min (we only have one autoclave, arrange your experiment wisely, use liquid cycle 2, program 10)

(6) Wait until the liquid is not very hot (about 75C) and add appropriate amount of antibiotics if needed (this is the selective agent that will be used to screen the antibiotic marker carried by the bacteria, untransformed *E. coli* won't grow on this media). By convention, Ampicillin 100 ug/ml, streptomycin 50 ug/ml, Kanamycin 40 ug/ml and Chloramphenicol 25 ug/ml (chloramphenicol stock solution should be made with pure ethanol, all other antibiotics could be dissolved in DI water). You should briefly shake the bottle in case the media will solidify.

(7) Under biosafety hood, pour media to petri dishes. Keep plates at biosafety hood for a few hours (preferably overnight, turn off the blower in the hood) to make media completely solidify. Label each plate, wrap in plastic bag and keep in 4C fridge.

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2. Yeast Complete Synthetic Media (CSM) Agar Plate

(1) Label a clean bottle, and add the amount of DI water required. (maximally you can fill 80% of the bottle volume for autoclave safety practice)

(2) Glucose **20 g/L**

(3) Yeast nitrogen base without AA without Ammonium sulphate (YNB-AA-AS) **1.7 g/L**

(4) Ammonium Sulfate: **5.0 g/L**

(5) CSM-X, normally **0.67 g/L~0.74 g/L** (this is the selective/dropout media that will be used to screen the auxotrophic marker carried by the yeast, untransformed yeast won't grow on this media). (Be careful of weighting carry-over and cross-contamination)

(6) Agar **18 g/L** (ONLY for agar plate. No agar if you are making liquid media.)

(7) Securely close the cap and shake vigorously until there is no visible nutrient chunk.

(8) Loose the cap and autoclave at 121C (250F) for 20 min (we only have one autoclave, arrange your experiment wisely, use liquid cycle 2, program 10)

(9) Wait until the liquid is not very hot (about 70C). You should briefly shake the bottle every three or five minutes in case the media will solidify.

(10) Add other relevant component (5-FOA 1 mg/mL; Canavanin 50 μg/mL; or 200 μg/mL Hygromycin or G418 300 μg/mL) if you will work special genetic screening.

(11) Under biosafety hood, pour media to petri dishes. Keep plates at biosafety hood for a few hours (preferably overnight, turn off the blower in the hood) to make media completely solidify. Label each plate, wrap in plastic bag and keep in 4C fridge.

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3. Yeast Complete Synthetic Media regular media for seed culture

(1) Label a clean bottle, and add the amount of DI water required. (maximally you can fill 80% of the bottle volume for autoclave safety practice)

(2) Glucose **20 g/L**

(3) Yeast nitrogen base without AA without Ammonium sulphate (YNB-AA-AS) **1.7 g/L**

(4) Ammonium Sulfate: **5.0 g/L**

(5) CSM-X, normally **0.67 g/L~0.74 g/L** (this is the selective/dropout media that will be used to screen the auxotrophic marker carried by the yeast, untransformed yeast won't grow on this media). (Be careful of weighting carry-over and cross-contamination)

(6) Securely close the cap and shake vigorously until there is no visible nutrient chunk.

(7) Loose the cap and autoclave at 121C (250F) for 20 min (we only have one autoclave, arrange your experiment wisely, use liquid cycle 2, program 10)

(8) Wait until the liquid is not very hot (about 70C). You should briefly shake the bottle every three or five minutes in case the media will solidify.

(9) Add other relevant component (5-FOA 1 mg/mL; Canavanin 50 μg/mL; or 200 μg/mL Hygromycin or G418 300 μg/mL) if you will work special genetic screening.

(11) Keep your autoclaved media in room temperature or 4C fridge.

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4. Yeast Complete Synthetic Liquid Media, Nitrogen-limited media (C/N = 80)

(1) Label a clean bottle, and add the amount of DI water required. (maximally you can fill 80% of the bottle volume for autoclave safety practice)

(2) Glucose **40 g/L** (Adjust glucose if you try to control C/N ratio, see the notes below **$$$**)

(3) Yeast nitrogen base without AA without Ammonium sulphate (YNB-AA-AS) **1.7 g/L**

(4) Ammonium Sulfate: **1.1 g/L** (Adjust nitrogen if you try to control C/N ratio, check notes below **$$$**)

(5) CSM-X, normally **0.67 g/L~0.74 g/L** (this is the selective/dropout media that will be used to screen the auxotrophic marker carried by the yeast, untransformed yeast won't grow on this media). (Be careful of weighting carry-over and cross-contamination)

(6) Securely close the cap and shake vigorously until there is no visible nutrient chunk.

(7) Loose the cap and autoclave at 121C (250F) for 20 min (we only have one autoclave, arrange your experiment wisely, use liquid cycle 2, program 10)

(8) Wait until the liquid is not very hot (about 70C). You should briefly shake the bottle every three or five minutes in case the media will solidify.

(9) Add other relevant component (5-FOA 1 mg/mL; Canavanin 50 μg/mL; or 200 μg/mL Hygromycin or G418 300 μg/mL) if you will work special genetic screening.

(10) Under biosafety hood, pour media to petri dishes. Keep plates at biosafety hood for a few hours (preferably overnight, turn off the blower in the hood) to make media completely solidify. Label each plate, wrap in plastic bag and keep in 4C fridge.

$$$--Notes: A quick table to adjust C/N ratio to make Nitrogen-limited fermentation media

C/N ratio indicates the molar ratio of carbon element relative to nitrogen element

C/N = 100; Glucose 50 g/L, Ammonium Sulfate 1.1 g/L

C/N = 80; Glucose 40 g/L, Ammonium Sulfate 1.1 g/L

C/N = 60; Glucose 30 g/L, Ammonium Sulfate 1.1 g/L.

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5. Yeast rich YPD plate (yeast extract-peptone-dextrose)

(1) Label a clean bottle, and add the amount of DI water required. (maximally you can fill 80% of the bottle volume for autoclave safety practice)

(2) Yeast extract 10 g/L

(3) Peptone 20 g/L

(4) Dextrose (which id D-Glucose) 20 g/L

(5) Agar 18 g/L (ONLY for agar plate. No agar if you are making liquid media.)

(6) Securely close the cap and shake vigorously until there is no visible nutrient chunk.

(7) Loose the cap and autoclave at 121C (250F) for 20 min (we only have one autoclave, arrange your experiment wisely, use liquid cycle 2, program 10)

(8) Wait until the liquid is not very hot (about 70C). You should briefly shake the bottle every three or five minutes in case the media will solidify.

(9) Keep your autoclaved media in room temperature or 4C fridge..

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6. Luciferase reporter gene assay for yeast Yarrowia lipolytica

(1.) Harvest 1.0 mL cell culture from each sample at 24, 48, 72, 96 and 120 hour, measure cell density for each time point before harvesting (This is very important, we need to test Nluc/OD when we report the results). Transfer to 1.5 mL microcentriguge tube and centrifuge at 10,000 rpm for 3 minutes.

(2) Take supernatant and directly measure Nluc expression, follow Nano-glo kits

(3) Freeze each cell pellets at -20°C freezer before cell lysis.

(4) To prepare cell lysates, thaw frozen cell pellets on ice. Prepare cell lysis buffer as follows:

Component	Volume (mL)
Luciferase Cell Culture Lysis 5x Reagent	2
26 mg/mL Zymolyase	0.38
Sterile DI water	8
1 Roche mini EDTA-free protease inhibitor cocktail tablet	- (1 tablet)
Total volume	10

Luciferase Cell Culture Lysis 5x Reagent could be found in -20C fridge (this is cell lysis buffer, not the Nluc reaction buffer)
, and protease inhibitor could be found in 4C fridge at the corner.

(5) Resuspend each cell pellet in 500µL of lysis buffer and incubate at room temperature for 15 minutes.

(6) Transfer each reaction into a 1.5mL centrifuge tube containing 250µL of 0.5mm glass beads. Vortex for 2 minutes to shear the cells.

(7) Centrifuge the samples in a microcentrifuge at 4°C for 45 seconds, 15000 rpm. Transfer the supernatant to a new microcentrifuge tube on ice.

(8) Proceed to Nluc activity assay, follow Nano-glo kits