Growth media for Chlamy

Project: Cambridge-JIC iGem Shared Project
Authors: CJ Ong
Date: 2016-08-01

MONDAY, 1/8

Stock solutions

Phosphate stock K2HPO4 14.34 g OR K2HPO4●3H2O 18.78 g
KH2HPO4 7.26 g
DIH2O to 1 L

2M Tris stock Trizma Base 242.2 g
HCl (conc.) 149 mL
DIH2O to 1 L

1M Acetate stock NaAc●3H2O 27.2 g (136.1 g L-1) OR NaAc 16.4g in 200mL
DIH2O to 200 mL

Beij stock NH4Cl 8 g
CaCl2●2H2O 1 g
MgSO4●7H2O 2 g
DIH2O to 1 L

1L Acetate Medium 1 mL trace elements
10 mL Tris stock
10 mL phosphate stock
10 mL acetate stock
50 mL Beij stock
919 mL DIH2O
1 L Minimal Medium Idem Acetate Medium 1 mL trace elements
10 mL Tris stock
10 mL phosphate stock
50 mL Beij stock
929 mL DIH2O
Solid Medium add 15 g of Bacto Agar to 1 L of Medium (~20 x 100 mL Petri dishes)
Final solid medium should have 1.5% Bacto Agar (1% = 1 g / 100 mL)
Trace Elements Solution (see Hunter)
Pour gels when medium is still hot (~70 oC, right after autoclaving is done). Condensation should be removed after the next day, before turning plates upside down. When dry (~5 weeks), wrap a column of 20 dished in cling film and store in dust free, contamination free cupboard, away from heat and draft.

Notes:

- HCl is concentrated, handle with care in fume hood.
- Check salts hydration level
- Tris stock's salts take up significant volume, therefore, dissolve it in a (ideally 2L) beaker. Once solutes are dissolved, make up to correct volume in a (1L) volumetric flask
- Leave bottle cap slightly loose, and use at most ¾ full bottles of medium when autoclaving. (Explosion risk)
- Bacto Agar final concentration is 1.5% weight (1.5 g in 100 mL solvent). For 1 L of medium, add 15 g of Bacto Agar.

Procedure for stock medium (E.g. 1L Tris stock solution):

1. Using a weighing boat, measure 242.2 g of Trizma Base (done in multiple batches as weighing boat isn't big enough) and pour it into a 2 L beaker.
2. Using a measuring cylinder in a fume chamber, carefully measure out 149 mL concentrated HCl.
3. Pour the conc. HCl into the beaker in the fume chamber, and add some DIH2O into the beaker (do not make up to 1 L yet). This is to reduce the concentration of HCl.

4. Place a magnetic stirrer into the beaker and let it stir for 10-15 min to allow the solutes to resuspend.
5. Pour the solution into a 1 L volumetric flask and carefully add DIH2O to make up to exactly 1 L.
6. Pour the stock solution into a bottle.

Procedure for Acetate medium (final growth medium, e.g. 1 L Acetate medium):

1. Using a pipette, measure out the following:
    a. 1 mL trace elements
    b. 10 mL Tris stock
    c. 10 mL phosphate stock
    d. 10 mL acetate stock
    e. 50 mL Beij stock
2. Place the ‘ingredients’ in a bottle.
3. Using a measuring cylinder, measure out 919 mL DIH2O and add them to the bottle.
4. Before autoclaving to sterilise the medium, make sure the bottle is at most ¾ full (risk of explosion due to pressure build up).
5. Follow instructions for autoclaving.

Genomic extraction protocol

Project: Cambridge-JIC iGem Shared Project
Authors: Mie Monti
Date: 2016-08-03

WEDNESDAY, 3/8

Method to isolate chloroplast DNA from nuclear DNA by Saul Purton full protocol (might need an extra email)
http://link.springer.com/article/10.1007%2FBF02669846 (http://link.springer.com/article/10.1007/BF02669846)

For crude extraction of Chlamydomonas DNA

-Harvest 5-10 mL of exponencial growing cultures of Chlamy by centrifugation at 4000 rpm for 10 minutes. Aspirate supernatant.

- Resuspend biomass in 0.5ml of **Resuspension Buffer** (10mM EDTA, 50mM Tris-HCL, pH 8.0).

- Add 0.6 grams of 400 to 600 um **Glass Beads** (sigma), 25ul 10% **SDS**, 250ul **Chloroform** and 250ul **Phenol**.

- Vortex in agitator 1 minute, incubate on ice for 1 minute. Repeat 4 times.

- Centrifuge at 14,000 rpm for 10 minutes, at 4^{o}C. Transfer aqueous (top) phase to a clean 2ml eppendorff tube.

- Add 500ul **chloroform**. Vortex for 5 seconds, centrifuge at 14,000 rpm for 3 minutes, at 4^{o}C. Transfer aqueous (top) phase to clean eppendorff tube.

- Precipitate DNA with 1/10 volume **Sodium Acetate** (2-3M, pH 4.8-5.2) and 2.5 volumes of ice cold **ethanol (100%)**. Mix well, incubate at -80oC for 30 minutes to overnight.

- Centrifuge at 14,000 rpm for 10-30 minutes, at 4^{o}C, aspirate supernatant.

- Wash pellet (which might be translucent) gently with ice cold **70% ethanol**. Centrifuge at 14,000 rpm for 10 minutes. Carefully remove supernatant, air dry pellet.

- Resuspend DNA in 35uL nuclease free water, diluting to approximately 100ng/uL.

Preparation of CaCl2 chemically competent cells

Project: Cambridge-JIC iGem Shared Project
Authors: Mie Monti
Date: 2016-08-04

THURSDAY, 4/8

Reality: Don't bother. Just buy them.

Media/Solutions needed:
LB
0.1M $CaCl_2$, 10% glycerol (COLD)

1. Streak strain from stock onto LB agar plate, incubate at 37°C overnight. If pRARE is required, transform into cells prior to plating.
2. Inoculate a single, well-isolated colony from the plate into 5 ml LB in a 15 ml culture tube. If pRARE is transformed, add chloramphenicol (30ug/ml).
3. Incubate at 37°C overnight with shaking.
4. Inoculate 200 ml LB in a 1000 ml Erlenmeyer flask with 2ml of overnight culture.
5. Grow at 37°C, with shaking until OD_{600} reaches 0.6-0.8.
6. Transfer culture into 4 50 ml tubes, place on ice for 10 min (or up to an hour).
7. Centrifuge tubes at 4 000 rpm for 10 min at 4°C.
8. Discard supernatant, resuspend pellet gently (using cut 1 ml pipette tip) in 10 ml of ICE COLD 0.1M $CaCl_2$, 10% glycerol. Put the tubes back on ice for 15 min.
9. Centrifuge tubes again at 4 000 rpm for 10 min at 4°C.
10. Discard supernatant, resuspend pellet gently in 1 ml ICE COLD 0.1M $CaCl_2$, 10% glycerol.
11. Prepare 50ul aliquots in individual Eppendorf tubes on ice. Flash freeze aliquots in liquid N_2 and store at -80°C.

Competent Cells

Project: Cambridge-JIC iGem Shared Project
Authors: Anran Chen
Date: 2016-08-08

MONDAY, 8/8

HD5α: can be used to replicate igem standard backbone (Chloramphenicol)

subcloningefficiencydh5alpha_man.pdf

XL10-Gold: can be used to replicate Purton's backbone (ampicillin). CANNOT be used to replicate igem standard backbone since it is already resistant to chloramphenicol.

200314.pdf

E.coli Innoculation

Project: Cambridge-JIC iGem Shared Project
Authors: Anran Chen
Date: 2016-08-12

FRIDAY, 12/8

Do everything in the sterilized fume hood!

Better to do it on afternoon because it requires overnight culture

culture prepare:
5mL of LB with antibiotic in each 14mL flacon tube (do not need to be very accurate, can just use the marks on the falcon tube)
antibiotics:
For **Purton's** backbones: Ampicillin in water in the freezer
2000X wanted concentration in LB:50ug/ml
For **iGEM** backbones: Chloramphenicol in ethanol in the fridge
1000X wanted concentration in lB: 25ug/ml
LB+Chloramphenical stock is stored in the 50ml tube in the clean bench, can be used directly.

Innoculation
Remember to label the tubes with bacteria strains!
Solid media plates are labelled and cultivated in the incubator/stored in the fridge
use sterialised P200 tip to gently pick a colony, and release the whole tip into the LB medium in the falcon tube.
Purton's backbone to Ampicillin LB
iGEM backbone to Chloramphenicol LB

Incubation
Use the **37C** shaker on 2nd floor. Ask Linda if not sure. Leave them overnight

external protocols (Gibson Assembly, Biolistics, Vector construction, Golden gate)

Project: Cambridge-JIC iGem Shared Project
Authors: Lucie Studená
Date: 2016-08-22

MONDAY, 22/8

gibson protocol.pdf	The one Farhat recommended
Biolistics manual.pdf	The official one
Greg's protocol - ParticleBombardment.pdf	Biolistics from Greg. It's good it says things specific to our machine (for example the plate which holds microcarriers and stopping screens needs to go by writing (handwritten) up and towards you)
Vector construction.docx	Protocol on ligation, etc. grom Farhat

http://barricklab.org/twiki/bin/view/Lab/ProtocolsGoldenGateAssembly - Golden Gate, at the bottom there is an attachment which is for some calculator. That also says the cycling times.

Blue White test

Project: Cambridge-JIC iGem Shared Project
Authors: Alexander Mayorov
Date: 2016-08-26

FRIDAY, 26/8

To check whether the insertion of a part into the P10500 backbone is successful, we need to use blue white test. The **succeful insertion** will result in **white** bacteria colonies. The un **successful insertion** will result in **blue** colonies because of the the X Gal

How to make X Gal plate
Prepare LB agar plates containing the appropriate antibiotic to select for your recombinant plasmid. Onto each plate to be used for blue white screening, spread:
100 µl of 20 mg/ml x-gal stock (in the freezer)
100 µl of 10 mM IPTG (in the freezer)
allow the plates to dry with the lid slightly open before use. This can be performed in a laminar flow hood. Using a hood may dry out the plates if they are left for too long.

To make more stock solution
X Gal: 20 mg solid X-Gal (in the fridge) in 1 ml of **DMSO** (NOT water)
IPTG: 23.8mg of solid IPTG in 1ml of water

For innoculation
select the **white** colonies (**recommanded to pick the ones in the middle of the plates**, because the edge of the plate may not contain x-Gal, whcih results in false positinve)

Checklist for biolistics

Project: Cambridge-JIC iGem Shared Project

Authors: Mie Monti

Date: 2016-08-26

FRIDAY, 26/8

- [ ] bioloKeys + biolistics box from Hibberds lab (101 I believe)
- [ ] P200 tips
- [ ] P200 + P20 pipette
- [ ] Target plates
- [ ] Cylinder
- [ ] Isopropanol
- [ ] Filter syringe

Transformation & how to get more plasmid

Project: Cambridge-JIC iGem Shared Project
Authors: Alexander Mayorov
Date: 2016-08-26

FRIDAY, 26/8

DAY 1
transformation
bring these to Linda's lab
plasmids for transforamtion
P1000 pipette and tips
P20 pipette and tips
ice bucket (get ice on 2nd floor)

In Linda's lab

- get TOP10 cells from a blue box in the -80 freezer.(ask linda). Each tube should have 50 ul of TOP10 cells
- add 1ul plasmid(or more, for example 4ul for ligation products) to the cells. remember to label the TOP10 tubes what plasmid is added.
- for classical ligation, add all of your ligation mixture (usually 20 µl) to the cells
- leave it on ice for 30min, keep it still, do not move it. Set up 42C waterbath, put the SOC medium(ask linda) in to the water bath to preheat
- after 30 min on ice, put the bacteria in 42C waterbath for **exactly 1 min**
- take the tube out and put it on ice for 2 min
- add 250ul preheated SOC medium to the tube.
- leave the bacteria tube in 37C shaker for 1 hour

In the clean bench

- take the bacteria from the shaker in Linda's lab after 1 hour incubation
- spread 50ul of bacteria on the plate and spread them
- for classical ligation, spread 200 µl of bacteria
- If it is from ligation and needs blue white test, see the blue white test protocol(https://benchling.com/camigem/f/eLNxOkyi-protocol-files/etr-4MPQdvT9-blue-white-test/edit)
- spread the bacteria on the plate, label the plasmid, gene and date
- put it into the 37C incubator in the teaching lab overnight

DAY2
Morning: take the plate from the incubator and put it into the fridge
Afternoon: innoculation https://benchling.com/camigem/f/eLNxOkyi-protocol-files/etr-IsKlqmup-e-coli-innoculation/edit
Re-streak a colony on another plate when innoculating

DAY3
For miniprep
take the falcon tubes from the shaker in linda's lab and do miniprep
test concentration using nanodrop
https://benchling.com/camigem/f/eLNxOkyi-protocol-files/prt-pUpfmk6i-nanodrop/edit

For midiprep
in the clean bench
put 50ml of LB into a sterilised conical flask, add the anibiotic using the same way for the first innoculation on DAY2
add 1ml of the bacteria culture from the falcontube on DAY2
seal it with sponge and aluminium foil and put it in the 37C shaker in Linda's lab overnigh

DAY4(for midiprep only)
Do midiprep flow the protocol in the kit

test concentration using nanodrop
https://benchling.com/camigem/f/eLNxOkyi-protocol-files/prt-pUpfmk6i-nanodrop/edit