Hackspace Workshops-29th, 30th, 31st August

Wednesday Workshop

- Making shaker!
- Day 1 Making competent cell protocol
- PCR of PSB1C3 (plasmid backbone containing cholorophenicol resistance)

Generating $CaCl_2$ Competent Bacteria:

FAQs:

What are competent cells? Why generate competent cells?

'Competence' is the ability of a cell to take up DNA from their environment. Bacteria can be naturally competent in the environment but this is highly regulated in bacteria.

Artificial competence is a laboratory procedure in which cells are passively made into permeable to DNA. This is necessary so the cells can uptake plasmids which contain genes which we wish 1) express to make proteins or want to use the bacterial machinery to 2) generate multiple copies of the plasmids containing our gene of interest. The process of direct DNA uptake is called 'transformation'.

Chilling cells in the presence of divalent cations such as $CaCL_2$ and/or $MgSO_4$ prepares the cell walls to become permeable to plasmid DNA. Cells are incubated with the DNA. During transformation, the cells are briefly heat shocked (37-42°C for 60-120 seconds), which causes the DNA to enter the cell.

Before Starting

Materials Needed:

Pour minimal media plates.
5x M9 salts
Prepare 100ml LB per strain.
Prepare 50ml ice cold 0.1M $CaCl_2$ / 15% glycerol per strain
Pre-chill eppendorf tubes

Minimal media plates *(Already prepared)

In 50 ml Falcon:

Reagent	**Volume (ul)**
20% (w/v) D-glucose	1000 (1ml)
2mg ml-1 thiamine	50
1M CaCl2	5
1M MgSO4	100
melted Bacteriological Agar solution (≤50°C)	39000 (39ml)
5x M9 salt solution* ***(Must be added LAST as they cause precipitation)***	10000(10ml)

0.1M CaCl2 / 15% glycerol* (Already prepared)
In 50 ml Falcon;

Reagent	**Volume (ml)**
1M CaCl2	**5**
100% glycerol	**7.5**

5x M9 salts in 500ml dH20 *(Already prepared)

Reagent	**Mass (g)**
Na2HPO4	32

KH2PO4	7.5
NaCl	1.25

Day 1	
1	Streak cells on **minimal agar plate**. Incubate 37°C overnight.
Day 2	
2	Pick a colony into 5ml **LB** + 100µl 1M **$MgSO_4$**
3	Incubate 37°C in shaker overnight.
Day 3	
4	Inoculate 100ml **LB** in pre-warmed conical with 1ml of the 5ml **O/N culture** from Day 2.
5	Incubate 2hrs in 37°C shaker until the cells at early log phase of growth curve (A_{600} ~ 0.3)
6	Transfer to chilled, sterile 50ml Falcon tube and incubate on ice 10min
7	Cf 3300g 5min in benchtop RmT. Cf.
8	Resuspend in 10ml ice cold 0.1M $CaCl_2$ / 15% glycerol and incubate on ice 30min
9	Centrifudge 3300g 5min in benchtop RmT. Cf.
10	Resuspend in 1ml ice cold 0.1M **$CaCl_2$ / 15% glycerol**. Transfer 100µl aliquots to pre-chilled, pre-labelled eppendorf tubes. Store -70°C.

PCR (Polymerase Chain Reaction)
-Amplification of plasmid backbones

We will be amplifying PSB1C3. This is a high-copy plasmid carrying the Cholorphenicol resistance. *All biobrick parts have to submitted in this plasmid backbone. The final biobrick construct will be ligated onto this plasmid backbone.*

For more information about the sequence of this plasmid, visit: http://partsregistry.org/wiki/index.php?title=Part:pSB1C3.

Protocol below is adopted from NEB website. (Phusion polymerase is from NEB.)

- Mix up the following reagents. Add DNA template and polymerase last.
- **Add oil into PCR tube to avoid sample evaporation or parafilm? Consult biohackers (Nick & Tom) → can do multiple PCR with and without oil**
- Gently tap on tube to mix (since no centrifuge). Avoid bubbles.

PCR Components	**Volume for single run (µl)**	**Volume (µl)**
H2O (Autoclaved RO)	31	Master Mix
5 x Phusion HF Buffer	10	
10mM dNTP Mix	1	
10mM Forward Primer	2.5	
10mM Reverse Primer	2.5	
DNA Template	1	
DM50	1.5	
Phusion DNA Polymerase	0.5	
Total Volume	**50**	

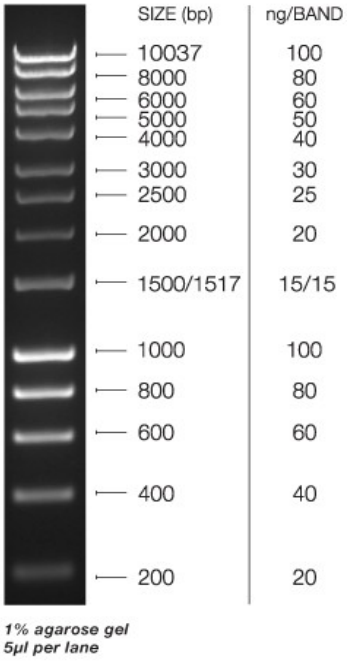
PCR conditions	**Temp (C)**	**Time**
Initial Denaturation 1 cycle	95	30s

(30 Cycles) Denaturation Annealing Extension (15-30s/kb)	95 55 72	10s 25s 1.5 min
Final Extension 1 cycle	72	10 min
Hold 1 cycle	4	∞

Success of PCR reaction will be verified by 1% agarose gel electrophoresis.

Should see very bright bands around 2000bp.

- Load 5ul of DNA ladder into well 1
- Load 3ul of PCR product with 2ul of loading dye (can try to add DIY loading dye-food colouring)
- Store DNA at -20C freezer



Hyperladder I (DNA ladder-store at -20C)

The expected sizes of all the other plasmid backbones which will be used for subsequent ligation steps are also around 2000bp.

Backbone	Expected Size(bp)

PSB1K3	2204
PSB1C3	2070
PSB1T3	2461

Depending on success of PCR of PSB1C3, we will be PCR and digesting the other backbones with restriction enzymes (EcoRI and PstI) on Thursday for 3A assembly (subsequent ligation steps)