Part I

Materials and Methods

1 Buffers

1.1 Growth Media

Prepare required volume of Luria Broth. Add appropriate antibiotics to following concentration

Antibiotic	**Concentration** ($\mu g\,ml^{-1}$)
Ampicillin	100
Chloramphenicol	39

1.2 Induction Media

Prepare growth media with appropriate antibiotic. Add appropriate inducers to following concentration

Inducer	**Concentration**
Arabinose	$100\,\mu g\,ml^{-1}$
IPTG	$25\,\mu mol\,dm^{-3}$

1.3 5X Isothermal Buffer

	Stock Concentration (mmol)	**Volume** (µl)
PEG-8000	25%	0.75 g
Tris-HCl pH 7.5	500	1500
$MgCl_2$	50	75
DTT	50	150
dATP	1	30
dTTP	1	30
dCTP	1	30
dGTP	1	30
NAD	5	300
Nuclease free water		...
Total	5X	3000

Use nuclease free water as necessary to make up to 3 ml.

1.4 Gibson Master Mix

Prepare on ice in the ligase tube.

	Stock Concentration ($U\mu l^{-1}$)	**Volume** (µl)
Taq ligase	40	50
5X Isothermal Buffer	5X	100
T5 Exonuclease	1	2
Phusion Polymerase	2	6.25
Nuclease free water		216.75
Total	1.33X	375

For best performance, aliquot 25 µl to 75 µl portions in 1.5 ml microcentrifuge tubes and store at $-20°$.

2 Cell Protocols

2.1 Overnight Cultures

Prepare in a 14ml shaky top tube 2 ml growth media with appropriate antibiotic. Inoculate a single colony in the growth media and grow overnight at 37°.

2.2 Inducing Cultures

Prepare 2 µl overnight culture. Prepare in a 14 µl shaky top tube 2 ml induction media with appropriate inducer. Inoculate induction media with 20 µl overnight culture.

2.3 Preparation of CaCl Competent Cells

Prepare 2 ml overnight culture. Meanwhile, prewarm 500 ml LB and two 1 l or one 2 l conical flasks and pre-cool a centrifuge to 4°. Also cool 16 1.5 ml microcentrifuge tubes. Add the overnight culture to the warmed 500 ml in the conical flask(s) and incubate in a shaking incubator for 2 h to 3 h. Incubate cells on ice for 10min. Spin down cells for 3 min at 6krpm. Resuspend cells in 10 ml 0.1 mmol dm^{-3} CaCl. Incubate cells on ice for 10 min. Spin down cells for 3 min at 6krpm. Resuspend cells in 5 ml 0.1 mmol dm^{-3} CaCl in 15% glycerol. Alliquot 300 µl into each of the cooled microcentrifuge tubes. Freeze at −80°.

2.4 Transformation of CaCl Competent Cells

Thaw 20 µl to 200 µl competent cells on ice in 1.5 ml microcentrifuge tubes. Add 1 µl plasmid DNA or 20 µl ligation assembly DNA or complete Gibson Assembly DNA. Incubate on ice for 30 min. Heat shock for 45 s to 120 s at 42° Incubate on ice for at least 2 min. Add 800 µl LB. Incubate for 1 h at 37° in a shaking incubator. If required, spin down cells and remove excess LB to concentrate. Plate out 10 µl to 100 µl on LB agar plates with appropriate antibiotic. Incubate plates overnight at 37°.

2.5 Miniprep

2.6 Glycerol Stock

Prepare 2 ml overnight culture with appropriate antibiotic. Add 250 µl to a 1.5 ml cryogenic tube. Add 1.15 µl overnight culture and mix well. Freeze in -80° freezer.

Source http://web.wi.mit.edu/sive/pub/Lab

3 DNA Protocols

3.1 Restriction Digest

Prepare on ice in order the following mixture.

	Volume(µl)
DNA	50
Buffer	6
Milli Q Water	1.4
Enzyme A	1
Enzyme B	1
Total	**60**

Incubate the mixture at 37° in a shaking incubator for 2-3 hours. If necessary, dephosphorylate the digested product.

3.2 Dephosphorylation

Prepare on ice in order the following mixture.

	Volume(µl)
DNA	15
SAP Buffer	2
Milli Q Water	2
SAP	1
Total	**60**

Incubate the mixture at 37° for 1-2 hours.

3.3 PCR

3.4 Gibson Assembly

3.5 DNA Precipitation