Date: 09/01/13 People in lab: Emily Puleo, David Pohlman

Title: Making competent cells

Start Time: 4:00 PM

Purpose: Make chemically competent cells

Protocol: 1. Add 1 mL overnight DH5alpha culture to 250 mL SOC, incubate for 2 hrs 2. Chill on ice 10 min 3. Centrifuge 10 min, 3000 rpm 4. Resuspend in 100mL TB buffer (Inoue) 5. Incubate on ice 10 min 6. Centrifuge 10 min 7. Resuspend in 8.67 mL TB with 3.5 mL DMSO 8. Incubate on ice 10 min 9. Aliquot 50 uL per epi tube 10. Store at -80C

Notes: Centrifuge malfunctioned at step 3, cells were not made.

Stop Time: 7:05 PM

Next: Fix centrifuge, try again

Date: 09/14/13 People in lab: Hannah Frye

Title: PCR of norCB an nosZ for TOPO TA cloning

Start Time:

Purpose: Add iGEM prefix&suffix to the genes, then TOPO-TA clone to amplify

Protocol: 1. Resuspended P. aeru cells in0.5mL milliQ 2. Spun down Taq Master Mix 3. Mixed - 12.5 uL Taq MM, 7.5 uL P. Aeru, 2.5 uL F primer, 2.5 uL R primer 4. Spun down mixture 5. Annealing Temp: 65C, Elongation Time: 7 min

Products:

Sample Label	Description
norCB 9/14	norCB with prefix/suffix
nosZ 9/14	nosZ with prefix/suffix

Stop Time:

Next: Gel electrophoresis

Date: 09/16/13 People in lab: Blythe Ferriere

Title: Digestion of plasmids containing norV gene with EcoRI

Start Time: 9:04 AM

Purpose: To check for correct part in 8/9/13 MP1-9

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
9/16/13 D1	norV gene digested with EcoRI	8/9/13 MP1	9

Stop Time: 11:30 AM

Next:

Date: 09/19/13 People in lab: Levi Palmer

Title: Gel Electrophoresis of 9/14 PCR of nosZ norCB

Start Time: 1:30 PM

Purpose: To check for nosZ and norCB

Protocol: LTM ed. 2

Well	1	2	3	4	5	6	7	8
Sample		nosZ	Ladder	norCB				

Results: Possible norCB product, very weak band

Stop Time: 3:20 PM

Date: 09/19/13 People in lab: Emily Puleo, Kelsey Crossen

Title: Plating DH5alpha for seed plate

Start Time: 6:40 PM

Purpose: To grow a seed plate of DH5alpha to make competent cells with

Protocol: LTM ed. 2

Products:

Sample Label	Description
9/16/13 comp cells	comp cell seed stock from -80C freezer

Stop Time: 7:00 PM

Next: Inoculation

Date: 09/20/13 People in lab: Kelsey Crossen

Title: Inoculate 9/19 comp cells seed stock

Start Time: 3:45 PM

Purpose: To prepare the overnight culture for making chemically competent cells

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label
9/20/13 comp cells	Overnight seed culture for making chemically competent cells	9/19/13 comp cells

Stop Time: 3:55 PM

Next: Make competent cells

Date: 09/22/13 People in lab: Kelsey Crossen

Title: Inoculating comp seed cells

Start Time: 4:00 PM

Purpose: To prepare the overnight culture for making competent cells

Protocol: LTM ed 2

Products:

Sample Label	Description	Source Label
Comp cells 9/22/13	Comp cells 9/19/13	Comp cell seed stock in LB

Stop Time: 4:10 PM

Next: Making competent cells

Date: 09/23/13 People in lab: Kelsey Crossen, Emily Puleo

Title: Making chemically competent cells

Start Time: 3:05 PM

Purpose: To make chemically competent cells

Protocol: 1. Add overnight culture to 50 mL SOB, incubate 2 hrs 2. Chill on ice 10 min 3. Centrifuge 10 min 4500 rpm at 4C, decant supernatant 4. Resuspend in 5mL TB buffer (Inoue) 5. Incubate on ice 10 min 6.Centrifuge 10 mins, 4500 rpm, 4C 7. Resuspend in 930 uL TB and 70 uL DMSO 8. Incubate on ice 10 mins 9. Aliquot 50uL per epi tube 10. Store in -80C freezer

Products:

Sample Label	Description
9/23/13 Comp cells	Chemically competent DH5alpha

Stop Time: 4:38 PM

Date: 09/27/13 People in lab: Emily Puleo

Title: PCR hmp and norCB for TOPO TA cloning

Start Time: 10:15 AM

Purpose: To add prefix/suffix to hmp and norCB

Protocol: LTM ed. 2 **Exceptions:** Annealing Temp: 65C, Elongation time 7min

Products:

Sample Label	Description
9/27/13 A norCB	norCB with prefix/suffix
9/27/13 A hmp	hmp with prefix/suffix
9/27/13 hmp F primer	1:100 dilution of hmp F primer
9/27/13 hmp R primer	1:100 dilution of hmp R primer

Notes: See 8/6/13 for hmp calculations, 7/31/13 for norCB. Restarted at 12:35PM

Stop Time: 3:15 PM

Next: TOPO TA cloning and transformation

Date: 09/27/13 People in lab: Emily Puleo, Levi Palmer

Title: TOPO TA cloning of hmp and norCB, gel electrophoresis to check for products of PCR

Start Time: 4:45 PM

Purpose: To check for prescence of genes and amplify by TOPO-TA cloning

Protocol: LTM ed. 2, Invitrogen TOPO-TA cloning manual

Products:

Sample Label	Description
9/27/13 CT1 norCB	transformed, 20uL plated
9/27/13 CT2 norCB	transformed, 200uL plated
9/27/13 CT1 hmp	transformed, 20uL plated
9/27/13 CT2 hmp	transfored, 200uL plated

Results: hmp visible on gel, norCB had no product visible

Next: Check for hmp growth, miniprep if present. Redo PCR for norCB

Date: 09/27/13 People in lab: Emily Puleo, Levi Palmer

Title: Gel extraction and ligation of backbone

Start Time: 6:50 PM

Purpose: To extract the hmp from the gel, to convert the linear plasmid backbone to a circular backbone.

Protocol: LTM ed. 2

Products:

Sample Label	Description
9/27 hmp GE	hmp with iGEM prefix/suffix

Notes: Did not digest backbone first, cannot ligate.

Stop Time: 8:00 PM

Next: Digestion of backbone

Date: 09/28/13 People in lab: Levi Palmer

Title: Digest backbone, ligate into circular plasmid

Start Time: 1:16 PM

Purpose: To circularize the linear backbones

Protocol: LTM ed. 2

Products:

Sample Label	Description
D LP 9/28	Digested ampicillin backbone with E and P
L1 LP 9/28	Circular ampicillin backbone

Notes: IMPORTANT: Don't do this, it won't work. Must digest with S and X, not E and P! -LP 11/21/13

Stop Time: 3:30 PM

Date: 09/29/13 People in lab: Levi Palmer

Title: Transform backbone into competent cells

Start Time:9:25 AM

Purpose: To amplify the ampicillin backbone

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
9/29 Amp Backbone CT	E. coli with amp standard backbone	L LP 9/28	2

Notes: 9/30: transformation did not work.

Stop Time: 12:20 PM

Next: Try again

Date: 09/30/13 People in lab: Levi Palmer

Title: Transform backbone into competent cells

Start Time: 4:12 PM

Purpose: To test for competence of cells and amplify amp backbone

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
9/30 Amp Backbone CT	E. coli transformed with Amp backbone	L LP 9/28	2
9/30 pUC19 BB CT	E. coli transformed with pUC19 control plasmid	pUC 19 DNA	2

Results: 10/01: Transformations did not work.

Notes: Performed with both amp and pUC 19 plasmids

Stop Time: 7:00 PM

Next: Check for success, if succesful, inoculate amp cells into LB broth.