Date: 8/1/13 People in lab: Emily Puleo, Kelsey Crossen

Title: Gel of norCB PCR

Start Time: 1:15 PM

Purpose: Run a gel of norCB PCR to extract the DNA and sequence it.

Protocol: LTM ed. 2 pg. 45-47

Well	1	2	3	4	5	6	7	8
Sample	Ladder	7/31/13 A norCB 1	7/31/13 A norCB 2					

Results: None.

Notes: Gel streaked, no samples were extracted.

Stop Time: 3:00 PM

Next: Run PCR again, optimize salts

Date: 08/02/13 People in lab: Emily Puleo, Kelsey Crossen

Title: PCR of norCB

Start Time: 4:30 PM

Purpose: To PCR norCB and add iGEM prefix&suffix

Protocol: LTM ed. 2 pg 45-47 **Exceptions:** 1. Final primer concentration <0.1 uM 2. Decreased annealing temperature to 63C 3. Increased elongation time to 5 minutes

Products:

Sample Label	Description	Source Label	Quantity
8/2/13 A norCB 1	norCB gene from P. aeruginosa with iGEM prefix and suffix using 7.5 uL template P. aeruginosa	P. aeruginosa 7/30	1
8/2/13 A norCB 2	norCB gene from P. aeruginosa with iGEM prefix and suffix using 7.5 uL template P. aeruginosa 2x concentration	P. aerguginosa 7/30 2x in H2O	1

Results: N/A

Notes:

Stop Time: 8:35 PM

Next: Run samples on a gel. Extract, purify, sequence the better resultant gene product.

Date: 08/3/13 People in lab: Emily Puleo, Kelsey Crossen (1/2)

Title: Gel electrophoresis of 8/2/13 A norCB 1 and 2

Start Time: 2:05 PM

Purpose: Confirm norCB gene with iGEM prefix & suffix. Use the better sample to purify and sequence.

Protocol: LTM ed. 2 pg 45-47

Well	1	2	3	4	5	6	7	8
Sample	Ladder	8/2/13 A norCB 1	8/2/13 A norCB 2					

Products: N/A

Results: PICTURE

Notes:

Stop Time: 3:30 PM

Next: The ~1900 bp fragment from 8/2/13 A norCB 2 should be extracted and purified.

Date: 08/3/13 People in lab: Emily Puleo, Kelsey Crossen (2/2)

Title: Gel extraction of ~2000bp fragment from today's gel

Start Time: 3:50 PM

Purpose: To extract the PCR product of the norCB gene + iGEM prefix & suffix

Protocol: IBI Gel extraction **Exceptions:** 1. Milli-Q used instead of Elution Buffer

Products:

Sample Label	Description	Source Label	Quantity
8/3/13 GE norCB	norCB w/ iGEM prefix/suffix (~1900 bp) from well 3	Well 3 8/3/13 Gel	1

Results: N/A

Notes:

Stop Time: 4:45 PM

Next: 8/3/13 GE norCB should be ligated into the iGEM vector.

Date: 8/5/13 People in lab: Emily Puleo

Title: Chemical transformation of chloramphenicol resistant cells

Start Time: 3:10 PM

Purpose: To transform cells with the standard iGEM vector for use in digest and ligation with norCB gene.

Protocol: LTM ed. 2 pg. 42-43 **Exceptions:**

Products:

Sample Label	Description	Source Label	Quantity
Entry	Entry	Entry	#
Entry	Entry	Entry	#

Results:

Notes:

Stop Time:

Next:

Date: 08/06/13 Blythe Ferriere, Annie Goo

Title: People in lab: Dilute primers plus PCR

Start Time: 2:15 PM

Purpose: Dilute primers for PCR reaction of norV gene, PCR norV gene from E. coli K-12 add iGEM prefix-suffix

Protocol: Dilution: Forward Primer: 0.39mg, To make 1 ug/uL: add 390 uL milliQ, To make 10ng/uL: add 1 uL of 1 ug/uL solution to 100 uL milliQ, Final Solution: ~??.?? uM Reverse Primer: 0.38mg, To make 1ug/uL: add 380 uL milliQ, To make 10ng/uL: add 1 uL of 1 ug/uL solution to 100 uL milliQ, Final Solution: ~??.?? uM

Products:

Sample Label	Description	Source Label	Quantity
8/6/13 norV A 1	Amplified norV gene w/ iGEM prefix & suffix using 7.5 uL of template DNA	template DNA 7/31/13	3
8/6/13 norV A 4	Amplified norV gene w/ iGEM prefix & suffix using 5 uL of template DNA	Template DNA 7/31/13	3

Results: N/A

Notes: Changed programs about 15 minutes in

Stop Time: 5:00 PM

Next: Ligtate into iGEM backbone

Date: 08/06/13 Emily Puleo, Alie Abele

Title: Digestion of 8/3/13 GE norCB with EcoRI and PstI

Start Time: 6:17 PM

Purpose: Prep norCB for ligation with iGEM standard plasmid.

Protocol: LTM ed. 2 pg 37-39. **Exceptions:** 1. Master Mix: MilliQ - 39uL, EcoRI - 0.3uL, PstI - 0.3uL, 10x Tango buffer - 6uL, use 5uL DNA per reaction

Products:

Sample Label	Description	Source Label	Quantity
8/6/13 D	norCB digested with EcoRI and PstI	8/3/13 GE norCB	1
8/6/13 Master	Master mix for digestion	N/A	1

Results: N/A

Notes:

Stop Time: 9:00 PM

Next: Digestion of plasmids and ligation

Date: 08/06/13 Emily Puleo, Alie Abele

Title: PCR nosZ gene from P. aeruginosa to add iGEM prefix & suffix

Start Time: 7:32 PM

Purpose: To PCR amplify nosZ and add iGEM prefix and suffix

Protocol: LTM ed. 2 pg 48 **Exceptions:** 1. Final primer concentration <0.1 uM PCR run conditions: Annealing Temp: 65C, elongation time: 5min, template P. aeru 7/30/13

Products:

Sample Label	Description	Source Label	Quantity
8/6/13 A nosZ	nosZ gene with iGEM prefix&suffix using 7.5 uL template in 0.5 mL H2O	P. aeruginosa 7/30/13	2
8/6/13 F primer nosZ	0.77 uM nosZ forward primer	nosZ Forward Primer	1
8/6/13 R primer nosZ	0.8 uM nosZ reverse primer	nosZ Reverse Primer	1

Results: N/A

Notes: See 7/31/13 notes for calculations

Stop Time: 9:00 PM

Next: Gel electrophoresis and sequencing

Date: 08/06/13 Emily Puleo, Alie Abele

Title: PCR hmp gene from E. coli to add iGEM prefix & suffix

Start Time: 8:10 PM

Purpose: To PCR amplify hmp and add iGEM prefix and suffix

Protocol: LTM ed. 2 pg 48 **Exceptions:** 1. Final primer concentration <0.1 uM PCR run conditions: Annealing Temp: 65C, elongation time: 5min, template washed and re-suspended E. coli 7/30/13

Products:

Sample Label	Description	Source Label	Quantity
8/6/13 A hmp	hmp gene with iGEM prefix&suffix using 7.5 uL template in 0.5 mL H2O	E. coli 7/30/13	2
8/6/13 F primer hmp	10 ng/uL hmp forward primer	hmp Forward Primer	1
8/6/13 R primer nosZ	10 ng/uL hmp reverse primer	hmp Reverse Primer	1

Results: N/A

Notes: 0.39 mg of F primer + 390 uL of milliQ, diluted 1:100 with milliQ = 10 ng/uL F primer, repeated with 0.36 mg R primer and 360 uL milliQ. NOTE: Corrected math when entered into online notebook, recheck to confirm numbers

Stop Time: 9:00 PM

Next: Gel electrophoresis and sequencing

Date: 08/07/13 People in lab: Blythe Ferriere

Title: Gel Electrophoresis of 8/6/13 A1-A6 PCR reactions

Start Time: 8:45 AM

Purpose: To check for norV gene product with prefix&suffix from PCR amplification.

Protocol: LTM ed. 2 pg 45-47

Well	1	2	3	4	5	6	7	8
Sample	Ladder	8/6/13 A1	8/6/13 A2	8/6/13 A3	8/6/13 A4	8/6/13 A5	8/6/13 A6	Ladder

Products: N/A

Results: PICTURE

Notes:

Stop Time: 12:33 PM

Next: TOPO cloning of 8/6/13 PCR products

Date: 08/07/13 People in lab: Blythe Ferriere

Title: TOPO cloning of PCR product and chemical transformation

Start Time: 2:45 PM

Purpose: To TOPO clone 8/6/13 A1 norV PCR product and chemically transform to amplify gene product

Protocol: TOPO cloning: Fresh PCR product 4uL, PCR TOPO vector 1uL. 1. Mix gently and incubate for 5 minutes at room temperature. Transformation: LTM ed. 2 pg. 42 **Exceptions:** 1. 2uL of TOPO cloning reaction product 2. 1uL pUC19 control standard 3. Plate on amp+Xgal

Products:

Sample Label	Description	Source Label	Quantity

Notes: PCR product was a day old. 1 hour 35 min recovery time.

Stop Time: 3:40 PM

Next: TOPO cloning of 8/6/13 PCR products

Date: 08/08/13 People in lab: Blythe Ferriere

Title: Blue/white screening for norV TOPO-TA cloned colonies

Start Time: 4:10PM

Purpose: Plate screened for blue white colonies white colonies should have plasmid.

Protocol: Colonies from plate CT1 8/7/13 were inoculated into LB broth + ampicillin tubes

Products:

Sample Label	Description	Source Label	Quantity
8/8/13 I 1	Isolation and inoculation of white colony	8/7/13 CT1	8
8/8/13 I 9	Isolation and inoculation of light blue colony	8/7/13 CT1	1
8/8/13 I 10	Isolation and inoculation of blue colony	8/7/13 CT1	3

Notes: Ampicillin re-done in I1-I8

Stop Time: 5:41 PM

Next: Pick tubes with growth from white colonies for further analysis for check for norV gene

Date: 08/08/13 People in lab: Emily Puleo

Title: Gel electrophoresis of 8/7/13 PCR samples

Start Time: 4:37 PM

Purpose: To check for successful PCR reaction.

Protocol: LTM ed. 2 pg. 44

Well	1	2	3	4	5	6	7	8
Sample	Ladder	8/7/13 A norCB	8/7/13 A nosZ 1	8/7/13 A nosZ 2	8/7/13 A hmp 1	8/7/13 A hmp 2		

Results: Product from hmp only. PICTURE

Notes: Used 70 uL sample+LoadingDye per well

Stop Time: 5:20PM

Date: 08/08/13 People in lab: Emily Puleo

Title: TOPO-TA cloning of 8/7/13 PCR samples

Start Time: 5:05 PM

Purpose: To put genes into vectors and transform into bacteria to amplify.

Protocol: Invitrogen TOPO-TA cloning manual

Products:

Sample Label	Description	Source Label	Quantity
8/8/13 hmp L1	hmp in TOPO-TA cloning vector	8/7/13 hmp A1	1

Notes: Samples were 1 day old only one was completed because only one ampicillin plate was ready

Stop Time: 6:15 PM

Date: 08/08/13 People in lab: Emily Puleo

Title: Transformation of 8/8/13 hmp L1

Start Time: 6:10 PM

Purpose: To put the hmp-TOPO vector into DH5alpha cells for growth and selection.

Protocol: LTM ed. 2 pg 41

Products:

Sample Label	Description	Source Label	Quantity
8/8/13 hmp T	hmp in TOPO-TA cloning vector in DH5alpha plated on LB+amp	8/8/13 hmp L1	1

Notes:

Stop Time: 9:30 PM

Date: 8/09/13 People in lab: Blythe Ferriere

Title: Mini-prep of norV broth cultures from 8/8/13

Start Time: 1:00 PM

Purpose: To purify the plasmid containing norV from cultures 8/8/13 I1-I13

Protocol: LTM ed. 2 pg. ?? **Exceptions:** 1. MilliQ water was used instead of Elution Buffer

Products:

Sample Label	Description	Source Label	Quantity
8/9/3 MP 1	purified plasmid containing norV	8/8/13 I 1	8
8/9/13 MP 9	CORRECTION LP 3/21/14: purified TOPO-TA vector, NO norV	8/8/13 I 9	5

Results:

Notes:

Stop Time: 3:37 PM

Next: Transformation of purified plasmids containing norV

Date: 8/20/13 People in lab: Kelsey Crossen, Emily Puleo, Levi Palmer

Title: Making X-Gal

Start Time: 9:30 AM

Purpose: Making 1000x x-gal stock

Protocol: 1. Add 1 mL of DMF to epi tube 2. Add 40 mg X-Gal to epi 3. Cover and shake to dissolve 4. Store in fridge(4 degrees)

Products:

Sample Label	Description	Source Label	Quantity
X-Gal 8/20	40 mg/mL xgal (1000x), 1 mL	X-Gal, solid	1

Stop Time: 10:15 AM

Date: 8/20/13 People in lab: Kelsey Crossen, Emily Puleo, Levi Palmer

Title: PCR nosZ, norCB, and hmp to add iGEM prefix and suffix

Start Time: 3:21PM

Purpose: Make samples for GE and TOPO-TA cloning

Protocol: LTM ed. 2 pg 48 PCR run conditions: Final primer concentration <0.1 uM, annealing temp: 65 C

Products:

Sample Label	Description	Source Label	Quantity
8/20/13 norCB A	norCB w/ iGEM prefix&suffix using 7.5uL template in 0.5uL h20	P. aeru 7/30	1
8/20/13 nosZ A	nosZ w/ iGEM prefix&suffix using 7.5uL template in 0.5uL h20	P. aeru 7/30	1
8/20 hmp A	hmp w/ w/ iGEM prefix&suffix using 7.5uL template	E. coli 8/6	1

Notes: Products placed in freezer, no TOPO cloning

Stop Time: 7:20 PM

Date: 8/21/13 People in lab: Kelsey Crossen, Emily Puleo

Title: Gel electrophroresis and extraction of 8/20/13 PCR products

Start Time: 12:55PM

Purpose: Prepare PCR samples for digestion and ligation

Protocol: LTM ed. 2 pg 44

Products:

Sample Label	Description	Source Label	Quantity
	Entry	Entry	#
Entry	Entry	Entry	#

Well	1	2	3	4	5	6	7	8
Sample								

Results:

Notes:

Stop Time:

Next:

Date: 08/22/13 People in lab: Emily Puleo, Hannah Frye

Title: Gel electrophoresis of PCR products from 8/21/13

Start Time: 3:00 PM

Purpose: To confirm presence of amplified genes, check for successful PCR

Protocol: LTM ed. 2

Well	1	2	3	4	5	6	7	8
Sample	Ladder	8/21/13 norCB A	8/21/13 nosZ A	8/21/13 hmp A				

Results: Product visible for hmp, no product visible for nosZ or norCB

Notes: PCR hmp for TOPO-TA cloning, change temp of nosZ and norCB

Stop Time: 3:40 PM

Next: See notes

Date: 08/26/13 People in lab: Emily Puleo, Hannah Frye

Title: PCR norCB and nosZ

Start Time: 1:00 PM

Purpose: Adjust PCR program to attempt to get product

Protocol: LTM ed. 2 **Exceptions:** 1. Annealing Temp: 67C 2. Elongation Time: 7 min

Products:

Sample Label	Description
8/24 norCB A	norCB with iGEM prefix&suffix
8/24 nosZ A	nosZ with iGEM prefix&suffix

Notes: See 7/31 notes for calculations

Stop Time: 6:15 PM

Next: Gel electrophoresis

Date: 08/27/13 People in lab: Emily Puleo, Hannah Frye

Title: GE of norCB and nosZ

Start Time: 3:00 PM

Purpose: Check if PCR worked

Protocol: LTM ed. 2

Products:

Well	1	2	3	4	5	6	7	8
Sample	Ladder	8/26 norCB A	8/26 nosZ A					Ladder

Results: No product visible

Stop Time: 4:25 PM

Next: Try again with different temperatures

Date: 08/28/13 People in lab: Emily Puleo

Title: Prepare norCB and nosZ primers

Start Time: 11:50 AM

Purpose: To increase the concentration of primer

Protocol: Dr. Westenberg's own protocol

Products:

Sample Label	Description
8/28/13 norCB F 0.58	norCB forward primer, .58 uM concentration
8/28/13 norCB R 0.52	norCB reverse primer, .52 uM concentration
8/28/13 nosZ F 0.62	nosZ forward primer, 0.62 uM concentration
8/28/13 nosZ R 0.64	nosZ reverse primer, 0.64 uM concentration

Notes: See 7/31 notes for example calculations

Stop Time:

Next: PCR of norCB and nosZ

Date: 08/28/13 People in lab: Hannah Frye

Title: PCR of norCB and nosZ for TOPO cloning

Start Time: 1:00 PM

Purpose: Add iGEM prefix&suffix to the genes, then preparing samples for TOPO-TA cloning

Protocol: LTM ed. 2 **Exceptions:** 1. Annealing temp: 65C 2. Elongation time: 7 min 3. Final primer conc. <0.1uM

Products:

Sample Label	Description
8/28/13 norCB A	norCB plus prefix&suffix
8/28/13 nosZ A	nosZ plus prefix&suffix

Stop Time: 7:45 PM

Date: 08/30/13 People in lab: Kelsey Crossen

Title: Gel electrophoresis of 8/28 PCR products

Start Time: 9:00 AM

Purpose: Check if PCR was successful

Protocol: iGEM LTM ed. 2

Well	1	2	3	4	5	6	7	8
Sample	Ladder	norCB A 8/28	nosZ A 8/28					

Results: No product visible

Stop Time:

Next: Adjust PCR and try again

Date: 08/30/13 People in lab: David Pohlman

Title: Making competent cell seed plates

Purpose: To grow fresh DH5alpha cells for making competent cells

Protocol: iGEM LTM ed. 2 - Streaking from frozen stock

Products: 2 Seed plates - DH5alpha 8/30/13

Next: Inoculate into LB broth

Date: 08/31/13 People in lab: David Pohlman, Emily Puleo

Title: Inoculating DH5alpha into LB broth

Start Time: 3:30 PM

Purpose: Prepare to make competent cells

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
BC DH5alpha 8/31/13 DRP	Inoculated DH5alpha, use to make competent cells	Seed DH5alpha 8/30/13 DRP	2

Stop Time: 4:10 PM

Next: Make competent cells