

## Date: 02/5/14 People in lab: Kelsey Crossen

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**Title:** PCR of nosZ, adding prefix/suffix

**Start Time:** 12:05 PM

**Purpose:** To amplify the nosZ gene and add the iGEM prefix/suffix

**Protocol:** LTM ed. 2 and Dr. Shannon's lab PCR protocol **Exceptions:** 1. Annealing temp: 50C, 2. Final primer conc. <0.1uM

**Products:**

Sample Label	Description	Source Label	Quantity
nosZ 1	LTM protocol amplified nosZ	P aeru 7//31	1
nosZ 2	Dr. Shannon lab protocol amplified nosZ	P aeru 7/31	1

**Notes:** Dr Shannon's Protocol: 7.5 uL template, 3 uL DreamTaq buffer, 3 uL dNTPs, 2.5 uL F primer, 2.5 uL R primer, 10.5 uL milliQ, 1 uL DreamTaq. Hot start (95C) then add Taq.

**Stop Time:**

**Next:** Gel electrophoresis of PCR product

**Date: 02/5/14 People in lab: Levi Palmer**

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**Title:** Gel electrophoresis of 2/5 PCR product

**Start Time:** 4:00 PM

**Purpose:** To check for successful PCR

**Protocol:** LTM ed. 2

Well	1	2	3	4	5	6	7	8
Sample	Ladder	nosZ1	nosZ2					

**Results:** No product visible

**Notes:** Only 30sec elongation time, extend time and try again.

**Stop Time:** 8:00 PM

## Date: 02/25/14 People in lab: Levi Palmer + helpers

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**Title:** PCR of hmp, gel electrophoresis, TOPO TA cloning

**Start Time:** 12:00PM

**Purpose:** To amplify hmp gene and put into a vector for further amplification

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

**Products:**

Sample Label	Description	Source Label	Quantity
hmp A 2/25	PCR of hmp with suffix&prefix	E. coli 8/6/13	1
2/25 LP ET hmp TOPO	hmp in TOPO-TA vector on xgal	hmp A 2/25	1

Well	1	2	3	4	5	6	7	8
Sample	Ladder	hmp						

**Results:** hmp visible on gel

**Notes:** Transformation arced b/c did not dilute the transformation before electroporating(TOPO has high salt content)

**Stop Time:** 6:00 PM

**Next:** Blue/white colony screening

## Date: 02/26/14 People in lab: Levi Palmer

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**Title:** Inoculation of possible hmp

**Start Time:** 5:00 PM

**Purpose:** To grow up cells for blue/white colony screening

**Protocol:** LTM ed 2

**Products:**

Sample Label	Description	Source Label	Quantity
Poss. hmp LP	Possible hmp colonies in LB broth	2/25 LP ET TOPO hmp	1

**Notes:** Only 2 colonies grew, expected due to arcing

**Stop Time:** 6:00PM

**Next:** cBlue/white colony screening

## Date: 02/27/14 People in lab: Levi Palmer

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**Title:** Blue white colony screening of hmp

**Start Time:** 11:00 AM

**Purpose:** To confirm colonies have hmp gene

**Protocol:** 1. Add 15 uL of 1000x X-gal to LB Amp plates 2. Plate 0.2 uL overnight culture 3. Add 15 uL SOC to plate to help spread cells 4. Spread with beads 5. Incubate overnight 37C

**Products:**

Sample Label	Description	Source Label	Quantity
2/27 Poss hmp Xgal LP	Transformed cells on amp+xgal	Poss hmp LP 2/26	1

**Results:** 2/28/14: Lawn growth, unusable for B/W colony screening

**Notes:**

**Stop Time:** 12:00 PM

**Next:** Inoculate any white colonies in LB Amp broth

## Date: 02/27/14 People in lab: Levi Palmer

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**Title:** norCB primer dilution

**Start Time:** 12:00 PM

**Purpose:** To prepare new primers for PCR, bring down to ~0.7 uM

**Protocol:** 1. To make 1 ug/uL stock, add 430 uL milliQ to 0.43 mg F primer, and 400uL to .4 mg R primer, 2. To make (1X) working solution, add 1uL stock to 99uL milliQ for final concentration of 10 ng/uL or ~0.7 uM

**Products:**

Sample Label	Description	Source Label	Quantity
2/27 F norCB primer	0.7 uM F primer norCB	IDT norCB F 2/24	1
2/27 R norCB primer	0.7 uM R primer norCB	IDT norCB R 2/24	1

**Notes:** Stored in -20C freezer in norCB box

**Stop Time:** 1:00 PM

**Next:** Inoculate any white colonies in LB Amp broth