Nar / Cell-Free Lab Notebook

2/22/21

- Transformed NarL (173), NarX (174), pNar/GFP (263) plasmids in BL21

2/23/21

- Transformations successful, made glycerol stocks

3/9/21

- Grow BL21 on PLAIN plates
- Made wash buffer
    - Made 100mM tris base

3/10/21

- Restreak BL21 cells onto a new PLAIN plate

3/11/21

- Made and autoclaved 2X YT+P Media
- Autoclaved all the flasks
- Sixteen hours before (4 pm) inoculate BL21 cells in LB
- Warm 2X YT+P Media to 37˚C overnight (for next day)

3/12/21

- Cell-free extract extraction

Time (hr)	Flask 1 OD	Flask 2 OD
2	0.5	0.52
3	0.77	0.8
3.5	0.99	1.03
4	1.01	1.07
4.5	1.05	1.12

- Mass of pellet: 1.25 g
- After last centrifuge pellet mass: 0.7901
- Sonicated 6 times, 2 seconds each

3/23/21

- Made and autoclaved 2X YT+P Media
- Autoclaved all flasks
- Sixteen hours before (4 pm) inoculate BL21 cells in LB

3/24/21

- Cell-free extract extraction
- 120mL 2X YT+P Media in 2 flasks

Time (hr)	Flask 1 OD	Flask 2 OD

2	0.689	0.720
2.5	0.833	0.85
3	0.909	0.906
3.5	1.062	1.034
4	1.115	1.102

- Mass of pellet: 1.034 g
- Sonicated 6 times, 3 seconds on 10 seconds off

3/25/21

- Try growing cells to OD in LB
- Call with Dr. Silverman for troubleshooting and experimental design help

3/29/21

- Preparing for extraction
    - 3 500 mL flasks of 125 mL 2XYT
    - Sterilized flasks
    - Inoculated cells into 30 mL LB

3/30/21

- Cell-Free extraction
- 3mL starter culture to 3 flasks of 120 mL 2XYT+P

Time (hr)	Flask 1 OD	Flask 2 OD	Flask 3 OD
2	0.774	0.966	0.836
2.5	0.954	1.154	1.052
3	1.11	1.201	1.153
3.5	1.22	1.287	1.25
4	1.3	1.351	1.324

- Mass of pellet: 1.641g

4/21/21

- Made 500 mL 2XYT+P media
- Made 125 mL TB

4/22/21

- Inoculated 500 mL 2XYT+P with 10mL cells
- Inoculated 25 mL TB with 2.5mL cells

4/23/21

- Cell OD growth test

Time (hr)	2XYT OD	TB OD
2.5	0.52	0.53
3.5	1.28	1.29
4.5	1.5	1.9

- Conclusion: TB did seem to improve growth a little

4/26/21

- Inoculated ALL midiprep colonies (NarX-174, NarL-173, pNar/GFP-263, LacO/GFP-303, LacI-301, GFP-343) to liquid culture for midi-prep on Tuesday
- Made 2XYT+P and TB for media testing on Tuesday
- Inoculate BL21 from streaked plain plate for media testing into 30mL LB (4:00 pm)

4/27/21

- Test 1: 2L flask, 500 mL TB media, 20 mL cell culture
- Test 2: 500 mL flask 125 mL 2XYT+P, 5 mL cell culture
    - Growing test failed, inconclusive results
- Midi-prepped all colonies

Plasmid	Purity	Concentration (ng/µL)
173	1.62	21.4
174	1.93	10
263	2.09	7.6
301	8.31	6.8
303	0	-3
343	0	0.3

- No usable midi-prep, need to redo

4/28/21

- Inoculate for midi-preps (301, 303, 343, 173, 174, 263)
- Mase 125mL 2XYT+P media
- Made LB media
- Inoculate BL21 cells into 30 mL LB
- Autoclaved 2L baffled flasks and 500mL baffled flasks

4/29/21

- Media Testing
- Experiment 1: 2L flask, 500mL TB, 20mL cell culture
- Experiment 2: 500mL flask, 125mL 2XYT+P, 5mL cell culture

Time (hr)	TB OD	2XYT+P OD
2	0.844	1.268
2.5	1.146	1.199
3	1.373	1.419
3.5	1.435	1.554
4	1.527	1.68
4.5	1.62	1.76

- Not conclusive - no difference

4/30/21

- Media testing again
- Starting OD: 1.293
- Grew starter culture at 37˚C not shaking
- Added phosphate fugger to TB after autoclaved
- TB OD after 4 hr: 1.05
- 2XYT+P after 4 hr: 0.9
- Doubled amount of cells inoculated next time

5/10/21

- Planning for the week
- Make 250mL 2XYT+P media
    - pH media - 7.6
- Prepare for media testing

5/11/21

- Starter culture OD: 2.024
- OD after 4 hours: 1.648
- Experiment failed
- Think starter culture is the problem

5/13/21

- Planning
    - OD testing test 1
        - 20mL LB and inoculated
        - Grown at 37˚C shaking
        - OD after 2 hours = 1.0
    - OD testing test 2
        - 250mL LB, 12.5mL cell culture
        - 250mL LB, 25mL cell culture
- Glycerol stock of BL21 cells

- Liquid culture of 343, 303, 301, 173, 263, 174

5/14/21

- Starter culture: 2.198
- 25mL cell culture to 475 2XYT+P media

Time (hr)	OD
2	1.024
2.5	1.314
3	1.422
3.5	1.48
4	1.559
4.5	1.596

- OD seems to improve, doesn't seem to be growing by much

7/6/21

- Streak plain plate with BL21 cells
- Prepped 30mL LB to inoculate and grow tomorrow

7/19/21

- Streak plate w/ BL21 cells
- Document location of needed experimental reagents
- Made schedule

7/20/21

- Sterilize flasks for midiprep
- Streak plain plate w/ BL21 cells

7/21/21

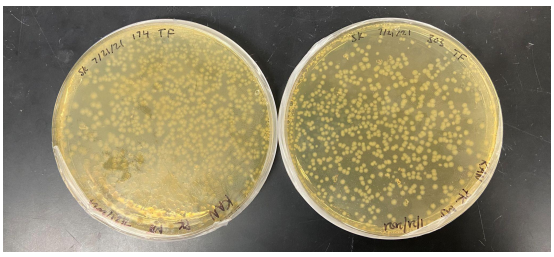
- Midi-prepped on 173 and 301

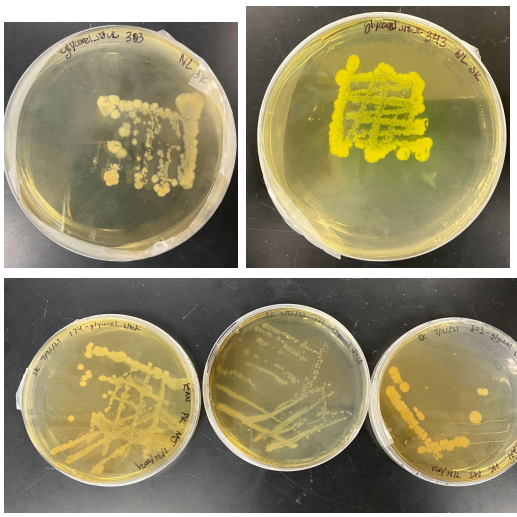
Plasmid	Concentration (ng/μL)	Purity
173	136.8	1.87
303	36.9	1.96

- 343, 303, 263, 174 didn't grow - retransform in BL21 cells / replate from glycerol stock

7/22/21

Transformations (174, 303)	Glycerol stock (174, 173, 303, 343, 263)





- Transformations all worked
- Sterilize flasks for media testing
- Make 2XYT+P

7/23/21

- OD growth testing

Time (hr)	2XYT+P	2XYT+P and Dextrose
3	1.265	1.217
3.5	1.585	1.639
4	1.831	1.735
4.5	1.83	1.859
5	1.974	1.872

- Inconclusive- dextrose made no difference

7/26/21

- Liquid culture 174, 173, 393, 263, 343 for midi-prep

7/27/21

- Midi-prep on 174, 173, 393, 263, 343
- Pellet didn't form after solution 3 - failed

8/2/21

- Prepare for extraction
- Made 2XYT+P media
- Inoculate starter culture 16 hours before extraction (BL21 cells)

8/3/21

- Cell-free lysate extraction (plain BL21 cells) - Megan McSweeny & Hutson Chilton from GT visited our lab to help

- Starter culture OD: 2.144

Time (hr)	Flask 1	Flask 2
2	0.56	0.561
2.5	Undiluted: 0.737 Diluted/calculated: 0.862	Undiluted: 0.722 Diluted/calculated: 0.898
3	Diluted/calculated: 1.22	Diluted/calculated: 1.134
3.5	Diluted/calculated: 1.832	Diluted/calculated: 1.974

- **Megan told us about diluting the samples to measure OD correctly
- Mass of pellet: 15.12 - 12.9202 = 2.2g
- Add 2.2 mL wash buffer to resuspend
- 6 1.5mL tubes with 500µL in each for sonication
- Sonication: 5-8 secs on, 15 sec off
- Extractions completed after final spin after sonication

8/5/21

- Inoculate for midi-prep (174, 173, 303, 343, 263) into 30mL LB
- Plan for experimental testing of normal (BL21) lysates

8/6/21

- Midiprep on 174, 173, 343
    - 303, 263 didn't grow

Plasmid	Concentration (ng/µL)	Purity
343	46.8	1.62
174	77.8	1.85
173	84.6	1.85

- 174 and 173 should be usable

8/9/21

- Restreak 303, 343, 263 on new plates

8/10/21

- Inoculate 343, 263, 303 into 30mL LB for midi-prep
- Experimental calculations for BL21 lysate testing with GFP

8/11/21

- Midiprep 343, 263, 303
    - Lost 263 in the middle

Plasmid	Concentration (ng/µL)	Purity
343	27.0	1.77

303	74.5	1.83

- 303 should be usable

8/12/21

- Run experiment on plain BL21 lysates with GFP (301, 303, 343)
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

Tests	**Master Mix**	**303 (LacI)**	**301 (LacO)**	**343 (GFP)**	**IPTG**	**Water**
Blank	18.84µL	0µL	0µL	0µL	0µL	15.16µL
343	13.60µL	0µL	0µL	4.204µL	0µL	16.20µL
301	13.60µL	0µL	0.984µL	0µL	0µL	19.42µL
301 + 303	13.60µL	2.007µL	0.984µL	0µL	0µL	16.39µL
343 + IPTG	13.60µL	0µL	0µL	4.204µL	3.40µL	12.80µL
301 + IPTG	13.60µL	0µL	0.984µL	0µL	3.40µL	16.02µL
301 + 303 + IPTG	13.60µL	2.007µL	0.984µL	0µL	3.40µL	12.99µL

-
- Incubated for 3 hours at 37˚C
- Lysates did not work, plasmids don't seem to be good

8/16/21

- Inoculate 263 and 343 for midi-prep

8/17/21

- Midiprep 263A, 263B, 343

Plasmid	Concentration (ng/µL)	Purity
263A	113.9	1.82
263B	73.1	1.87
343	19.0	1.64

- 263A is good, 263B also seems usable

8/18/21

- Inoculate 343 for midi-prep

8/29/21

- Midi-prep 343

Plasmid	Concentration (ng/µL)	Purity

343A	12.9	1.9
343B	28.1	1.89

- Midi-prep not good, need to redo

8/23/21

- Inoculate 343 for midi-prep

8/24/21

- Midi-prep 343

Plasmid	Concentration (ng/µL)	Purity
343A	31.0	1.85
343B	19.2	1.89

- 343A not great, should be usable

8/25/21

- Run experiment on plain BL21 lysates with new GFP (343)
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

Tests	**Master Mix**	**343 (GFP)**	**IPTG**	**Water**
Blank	18.84µL	0µL	0µL	15.16µL
343	13.60µL	4.204µL	0µL	16.20µL
343 + IPTG	13.60µL	4.204µL	3.40µL	12.80µL

- Incubated for 3 hours at 37˚C
- Did not work, lysates seem to be problem - will try lysate preparation with full protocol (run-off and dialysis after sonication)

8/30/21

- Prepare for BL21 cell-free extraction
- Make 2XYT+P
- Sterilize flasks
- Inoculate 16 hours before extraction

8/31/21

- Cell-free extraction (250mL-full protocol)

Time (hr)	OD
2	0.498 (induce with IPTG)
5	2.56

- Did all 4 wash steps this time
- Mass pellet: 15.23 - 12.978 = 2.252g
- Flash freeze for continued extraction on monday

9/1/21

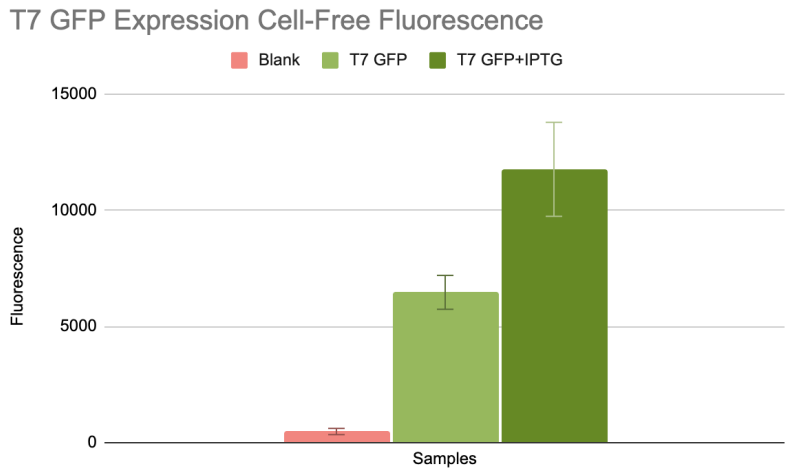
- Thaw pellet at 6:00am for an hour
- Pt. 2 of extraction: sonication, run-off reaction, dialysis
- Lysates look GOOD!

9/7/21

- Run experiment on plain BL21 lysates with GFP (343)
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

Tests	**Master Mix**	**343 (GFP)**	**IPTG**	**Water**
Blank	18.84µL	0µL	0µL	15.16µL
343	13.60µL	4.204µL	0µL	16.20µL
343 + IPTG	13.60µL	4.204µL	3.40µL	12.80µL

- Incubated for 3 hours at 37˚C
- Plate reader data shows GFP - lysates are working!



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- Will start NarX enriched lysates!

9/14/21

- Streak plate of NarX transformed BL21 cells (from glycerol stock) for enriched lysate prep

9/15/21

- Inoculate NarX cells in 30mL LB 16 hours before extraction + 30µL kan
- Make 2XYT+P
- Sterilize flasks

9/16/21

- NarX enriched lysate preparation
- Starter culture OD: 0.235
- OD at 6 hr: Flask 1 - 0.236 / Flask 2 - 0.264
- No extraction because cells didn't grow - try growing starter culture for longer to grow cells since starter OD was low

9/29/21

- Inoculate NarX cells in 30mL LB 24 hours before extraction + 30µL kan
- Make 2XYT+P
- Sterilize flasks

9/30/21

- NarX enriched lysate preparation

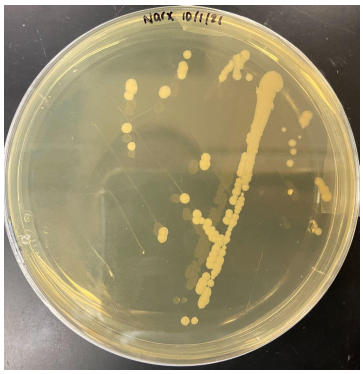
Time (hr)	Flask 1 OD	Flask 2 OD
2	0.67 (induce with IPTG)	0.633 (induce with IPTG)
5	2.18	2.18

- Mass of pellet: 2.5948
- 9 tubes with 500µL cells in each for sonication
- Sonication looked a little bad, stopped protocol with spin after
- Concluded that we oversonicated

10/1/21

- Streak NarX (174) onto new kan plate

10/4/21

- 
- Inoculate NarX cells (restreaked, shown above)in 30mL LB 24 hours before extraction + 30µL kan
- Make 2XYT+P
- Sterilize flasks

10/5/21

- NarX enriched lysate preparation (250mL full protocol)

Time (hr)	OD

2	0.5 (induce with IPTG)
5	1.536

- Mass of pellet: 1.067g
- Flash freeze for extraction pt 2 tomorrow

10/6/21

- Thaw pellet at 6:00am for an hour
- Pt. 2 of extraction: sonication, run-off reaction, dialysis
- Lysates look good, maybe slightly tinted yellow but should be usable

10/7/21

- Run experiment on NarX enriched lysates with NarX (174), NarL(173), pNar/GFP(263) with 0ppm nitrate, 100ppm, 170ppm nitrate
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

[NO_3^-] PPM	**Tests**	**Master Mix**	**NarX(173)**	**NarL(174)**	**pNAR/ sfGFP (263)**	**IPTG**	**NO_3^-**	**Water**
0	Blank	18.84µL	0µL	0µL	0µL	3.4µL	0µL	11.76µL
0	263	13.60µL	0µL	0µL	2µL	3.4µL	0µL	15µL
0	173 + 263	13.60µL	2.204µL	0µL	2µL	3.4µL	0µL	12.796µL
0	173 + 174 + 264	13.60µL	2.204µL	1.631µL	2µL	3.4µL	0µL	11.165µL
100	Blank	18.84µL	0µL	0µL	0µL	3.4µL	3.362µL	8.398µL
100	263	13.60µL	0µL	0µL	2µL	3.4µL	3.362µL	11.638µL
100	173 + 263	13.60µL	2.204µL	0µL	2µL	3.4µL	3.362µL	9.434µL
100	173 + 174 + 264	13.60µL	2.204µL	1.631µL	2µL	3.4µL	3.362µL	7.803µL
170	Blank	18.84µL	0µL	0µL	0µL	3.4µL	5.717µL	6.043µL
170	263	13.60µL	0µL	0µL	2µL	3.4µL	5.717µL	9.283µL

170	173 + 263	13.60µL	2.204µL	0µL	2µL	3.4µL	5.717µL	7.079µL
170	173 + 174 + 264	13.60µL	2.204µL	1.631µL	2µL	3.4µL	5.717µL	5.448µL

- Incubated for 3 hours at 37˚C
- Inconclusive data - may be lysates or low expression

10/11/21

- Run experiment on NarX enriched lysates with NarX (174), NarL(173), pNar/GFP(263) with 0ppm nitrate,100ppm nitrate, 170 ppm, 300ppm nitrate
- Did another set with half the PEP in master mix with extract (I - O) - same numbers with varied final master mix (half PEP, replace with extract for volume and concentrations)
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

Well Plate Key	**[NO_3^-] PPM**	**Tests**	**Master Mix**	**NarX (173)**	**NarL (174)**	**pNar (263)**	**IPTG**	**NO_3^-**	**Water**
A1-3	0	Blank	18.84µL	0µL	0µL	0µL	3.4µL	0µL	11.76µL
A5-7	0	pNar	13.60µL	0µL	0µL	2µL	3.4µL	0µL	15µL
A9-11	0	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	0µL	12.796µL
A13-15	0	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	0µL	13.369µL
A17-19	0	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	0µL	11.165µL
C1-3	100	Blank	18.84µL	0µL	0µL	0µL	3.4µL	3.362µL	8.398µL
C5-7	100	pNar	13.60µL	0µL	0µL	2µL	3.4µL	3.362µL	11.638µL
C9-11	100	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	3.362µL	9.434µL
C13-15	100	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	3.362µL	10.007µL
C17-19	100	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	3.362µL	7.803µL
E1-3	170	Blank	18.84µL	0µL	0µL	0µL	3.4µL	5.717µL	6.043µL
E5-7	170	pNar	13.60µL	0µL	0µL	2µL	3.4µL	5.717µL	9.283µL

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E9-11	170	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	5.717µL	7.079µL
E13-15	170	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	5.717µL	7.652µL
E17-19	170	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	5.717µL	5.448µL
G1-3	300	Blank	18.84µL	0µL	0µL	0µL	3.4µL	10.084µL	11.76µL
G5-7	300	pNar	13.60µL	0µL	0µL	2µL	3.4µL	10.084µL	4.916µL
G9-11	300	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	10.084µL	2.712µL
G13-15	300	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	10.084µL	3.285µL
G17-19	300	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	10.084µL	1.081µL

- Incubated for 3 hours at 37˚C
- Inconclusive data

10/12/21

- Set up experiments with plant pathogen *Fusarium* biosensor

10/15/21

- Run experiment with normal BL21 and plant pathogen *Fusarium* biosensor
  - Trigger only, Toehold+Trigger

Tests	**Master Mix**	**Trigger**	**Toehold**	**Fusarium DNA**	**Water**
Blank	18.84µL	0µL	0µL	0µL	15.16µL
Trigger	13.60µL	1.522µL	0µL	0µL	18.878µL
Trigger + Toehold	13.60µL	1.522µL	1.884µL	0µL	16.994µL
Trigger + Fusarium DNA (yellow tube)	13.60µL	0µL	1.884µL	3.251µL (yellow tube)	15.265µL

- Didn’t work - forgot to add IPTG

10/18/21

- Run experiment with normal BL21 and plant pathogen *Fusarium* biosensor
    - Trigger only, Toehold+Trigger (1:1), Toehold+Trigger (1:1.5), Toehold+*Fusarium* DNA (1:1), Toehold+*Fusarium* DNA (1:1.5)
    - Added 20 µL of water to visualize data better
- May have worked but data varies so much, want to re-run with more replicates
    - Bubbles affecting plate reader read

10/19/21

- Run experiment on NarX enriched lysates with NarX (174), NarL(173), pNar/GFP(263) with 0ppm nitrate,100ppm nitrate, 170ppm nitrate, 300ppm nitrate
- Made final master mix with salt solution, master mix, reagent mix, amino acids, PEP, and extract

Well Plate Key	[NO_3^-] PPM	Tests	Master Mix	NarX (173)	NarL (174)	pNar (263)	IPTG	NO_3^-	Water	Predicted results
A1-3	0	Blank	18.84µL	0µL	0µL	0µL	3.4µL	0µL	11.76µL	Clear
A5-7	0	pNar	13.60µL	0µL	0µL	2µL	3.4µL	0µL	15µL	Maybe green
A9-11	0	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	0µL	12.796µL	Maybe green
A13-15	0	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	0µL	13.369µL	Green
A17-19	0	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	0µL	11.165µL	Green
C1-3	100	Blank	18.84µL	0µL	0µL	0µL	3.4µL	3.362µL	8.398µL	Clear
C5-7	100	pNar	13.60µL	0µL	0µL	2µL	3.4µL	3.362µL	11.638µL	Clear
C9-11	100	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	3.362µL	9.434µL	Maybe green
C13-15	100	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	3.362µL	10.007µL	Green
C17-19	100	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	3.362µL	7.803µL	Green
E1-3	170	Blank	18.84µL	0µL	0µL	0µL	3.4µL	5.717µL	6.043µL	Clear
E5-7	170	pNar	13.60µL	0µL	0µL	2µL	3.4µL	5.717µL	9.283µL	Clear
E9-11	170	NarX + pNar	13.60µL	2.204µL	0µL	2µL	3.4µL	5.717µL	7.079µL	Maybe green
E13-15	170	NarL + pNar	13.60µL	0µL	1.631µL	2µL	3.4µL	5.717µL	7.652µL	Green
E17-19	170	NarX + NarL + pNar	13.60µL	2.204µL	1.631µL	2µL	3.4µL	5.717µL	5.448µL	Green

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- Incubated for 3 hours at 37˚C
- Inconclusive data - extract most likely not working

10/20/21

- Run experiment with normal BL21: plant pathogen *Fusarium* biosensor & plant pathogen *Phytophthora* biosensor
- Had difficulty with data collection with only 10 µL reaction in each well - 20µL in each well, 6 replicates
    - Blank
    - F. Toehold Only
    - F. Trigger Only

- F. Trigger+Toehold (1.5:1)
- F. DNA+Toehold (1.5:1)
- Ph. Toehold Only
- Ph. Trigger Only
- Ph. Trigger+Toehold (1.5:1)

Tests	**Master Mix**	**Trigger** (Fusarium)	**Toehold** (Fusarium)	**Fusarium DNA**	**Trigger** (Phytophthora)	**Toehold** (Phytophthora)	**IPTG**	**Water**
Blank	18.84µL						3.40µL	11.76µL
Toehold (Fusarium)	13.60µL		1.884µL				3.40µL	15.116µL
Trigger (Fusarium)	13.60µL	2.283µL					3.40µL	14.717µL
Trigger + Toehold (Fusarium) (1.5:1)	13.60µL	2.283µL	1.884µL				3.40µL	12.833µL
Fusarium DNA (yellow tube) + Toehold (1.5:1)	13.60µL		1.884µL	4.8765µL (yellow tube)			3.40µL	10.18µL
Toehold (Phytophthora)	13.60µL					1.942µL	3.40µL	15.058µL
Trigger (Phytophthora)	13.60µL				2.258µL		3.40µL	14.742µL
Trigger + Toehold (Phytophthora) (1.5:1)	13.60µL				2.258µL	1.942µL	3.40µL	12.8µL

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- 4x these amounts, 20µL in each well, 6 wells
- No success