NITRATE SENSOR MODULE: LAB NOTEBOOK WEEKLY SUMMARY

Construction and Testing

The construct was designed and constructed by Nikola Dyulgyarov. Testing and characterization was conducted by Nikola Dyulgyarov and Ken Groszman.

Experimental protocol:

1. Inoculate 3 mL overnight M9 preculture of strain with proper antibiotics
    a. Grow to saturation
2. Back-dilute to OD .001 in tubes with 3 mL M9, antibiotics, and the corresponding aTc and $NaNO_3$ concentrations
3. Grow at 37 C and 250 rpm to OD600 ~ .1
4. Take tubes out and place them on ice for at least 15 minutes
5. Add ~100 uL of each sample to a pre-iced 1 mL aliquot of phosphate-buffered saline (PBS)
6. Analyze samples on Cytek flow cytometer

Week 1 (1/6/2015-5/6/2015)

1. Literature research, identification of NarXL, PdcuS promoter
2. Designed pND16, ordered primers to construct system

Week 2 (8/6/2015-12/6/2015)

1. Prepared genomic DNA from E. coli MG1655
2. Planned out assembly:

PCR Reactions

Name	Template	Primer Fd	Primer Rv	Length
PCR6.0	pRS334	ND37	ND38	3661
PCR6.1	MG1655	ND13	ND36	97
PCR6.2	pRS334	RS242	ND41	274
PCR6.3	MG1655	ND40	ND39	675

3. Ran PCR reactions, confirmed on agarose gels:

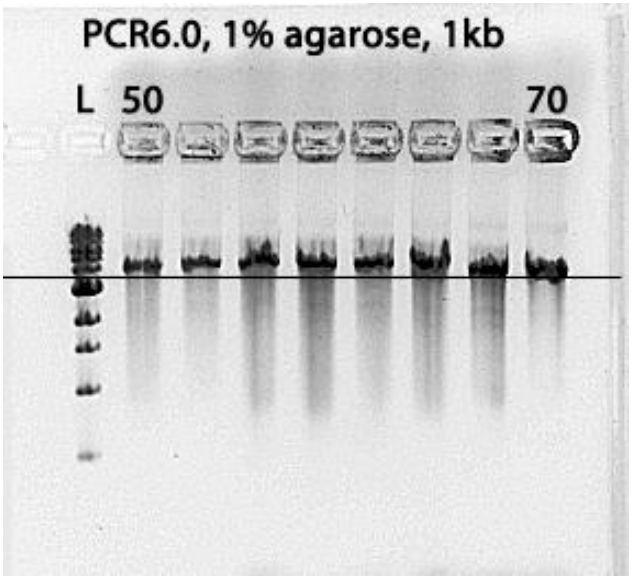
PCR6.0	Phusion	PCR Program
H20	106	**98|3:00**
Buffer: Phusion	36	**98|0:5**
4mM dNTP	9	**50/70|:15**
ND37	9	**x25**
		72|1:00
ND38	9	**72|5:00**
pRS334(1ng/uL)	9	**4|00:00**
Polymerase: Phusion	1.8	
	9x20uL	

PCR6.1	Phusion	PCR Program
H20	29.5	**98|2:00**
Buffer: Phusion	10	**98|0:5**
4mM dNTP	2.5	**56:15**
ND13	2.5	**x24**
		72|0:15
ND36	2.5	**72|5:00**
MG1655 (gDNA100ng/ul)	2.5	**4|00:00**
Polymerase: Phusion	0.5	
	50uL	

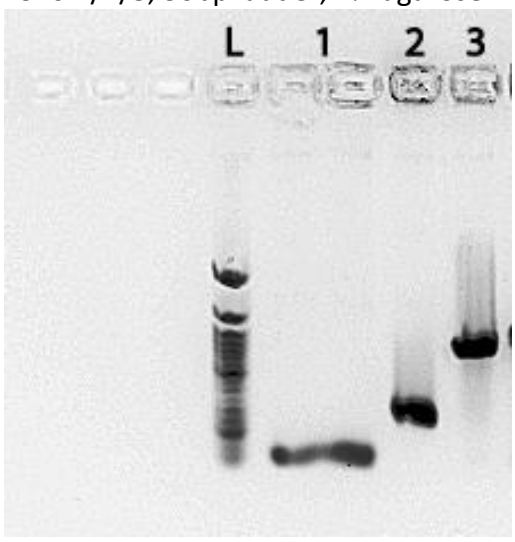
PCR6.2	Phusion	PCR Program
H20	29.5	**98|2:00**
Buffer: Phusion	10	**98|0:5**
4mM dNTP	2.5	**58:15**
RS242	2.5	**x24**
		72|0:15
ND41	2.5	**72|5:00**
pRS334(1ng/ul)	2.5	**4|00:00**
Polymerase: Phusion	0.5	
	50uL	

PCR6.3	Phusion	PCR Program
H20	29.5	**98|5:00**
Buffer: Phusion	10	**98|0:5**
4mM dNTP	2.5	**60:15**
ND40	2.5	**x24**
		72|0:15
ND39	2.5	**72|5:00**

MG1655(gDNA, 100ng/uL)	2.5	**4|00:00**
Polymerase: Phusion	0.5	
	50uL	



PCR6.1/2/3, 50bp ladder, 1% agarose



GG.pND16		**Thermocycler Program**
H20 to 15uL	7.20	**37|3:00**
100ng PCR6.0	1.89	**16|4:00** **x25**
PCR6.1	0.05	**50|5:00** **Exclude if construct**
PCR6.2	0.14	**80|5:00**
PCR6.3	0.22	**contains cut sites**
10x NEB T4 Ligase Buffer	1.5	
10 fold dilution BSA	1.5	
NEB T4 Ligase	1.5	
Type IIS RE (BsaI/BbsI/...)	1	
	15uL	

Week 3 (15/6/2015-19/6/2015)

1. Gel purified PCRs, set up a golden gate digest-anneal-ligate reaction:
2. Transformed 2uL of pND16 golden gate reaction in 25uL Neb10b competent cells and plated dilutions on selective (chloramphenicol 35ug/ml) LB plates.
3. Picked colonies and colony PCR'd to confirm plasmid integration. Made glycerol stocks.

Testing and Characterization

Week 4 (6/7/2015-10/7/2015)

1. Initial characterization of system with 5 different aTc levels, 5 nitrate concentrations
    a. Revealed that nonzero aTc values saturated the system with NarL
    b. For experimental procedure, see Experimental Procedure section below

Week 5 (3/8/2015-7/8/2015)

1. Characterization of system with only 1 aTc condition, 12 nitrate concentrations
    a. Gives sigmoidal results and allows us to determine relevant system parameters
    b. For experimental procedure, see Experimental Procedure section below

Experimental Procedure

Experimental protocol:

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    a. Grow to saturation
2. Back-dilute to OD .001 in tubes with 3 mL M9, antibiotics, and the corresponding aTc and $NaNO_3$ concentrations
3. Grow at 37 C and 250 rpm to OD600 ~ .1
4. Take tubes out and place them on ice for at least 15 minutes
5. Add ~100 uL of each sample to a pre-iced 1 mL aliquot of phosphate-buffered saline (PBS)
6. Analyze samples on Cytek flow cytometer