Toxin-Antitoxin Work

TUESDAY, 21/05/2019

HICA/HICB work

1. Received IDT pLac-HicA-AraC-pBad-HicB gBlock (iGEM-002)
2. Inoculated 10B containing **pGem backbone** into 50mL tube of LB + chloramphenicol overnight @ 37 deg cel (source: David's -80 stock).

Purpose: Preparation for Gibson assembly with genes-of-interest (HicA/HicB)

WEDNESDAY, 22/05/2019

1. pGEM backbone (obtained from David today) transformation

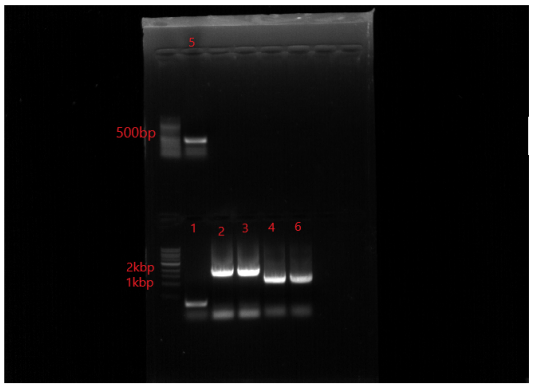
Purpose: make more pGEM (stock up)

2. Miniprep yesterday's backbone-transformed cells (pointer 2 above)
pGEM conc: 225.3ng/uL

3. **HICA/B work**

- Attempted to assemble HicA and HicAB plasmids
- Assembly primers for HicAB gblock and pGEM backbone plasmid arrived today
- Ran PCR to get fragments for Gibson (5 fragments in total)
- Ran gel to confirm size (3/5 fragments) are correct, **tomorrow 2 new pair of primers arrive then re-run PCR to get 2 failed fragments**
- Succeeded tubes: 2, 3, 5, 6 (note 2 & 3 are the same fragment, but diff conc therefore used as separate fragment DNA)
- Failed tubes: 1, 4

(HicAB and HicA plasmid assembly) Amplification of respective PCR fragments



Fragment 1 failed (1573bp); fragment 4 failed (2123bp); others correct sizes - 5 (448bp), 3 - (2122bp), 6 - (1414bp), HicA plasmid fragments all ok can proceed to assemble HicA plasmid first. Fragment 1 (pGEME backbone 2) and fragment 4 (HicA-HicB fragment) faulty, have to re-run to assemble HicAB plasmid. Fragment 2 for HicAB plasmid assembly size is ok (2122bp).

- Note: 3,5,6 can be used to assemble HicA plasmid first. HicAB plasmid assembly gotta wait for tube 1 and 4 to be successful before it can be assembled.

THURSDAY, 23/05/2019

1. Two new pair of primers designed yesterday has arrived. **Re-ran PCR** to get both fragments (1 and 4)

Tube 1:
New E6k_Fwd
E6k*k Rev (2)
Template: pGEME

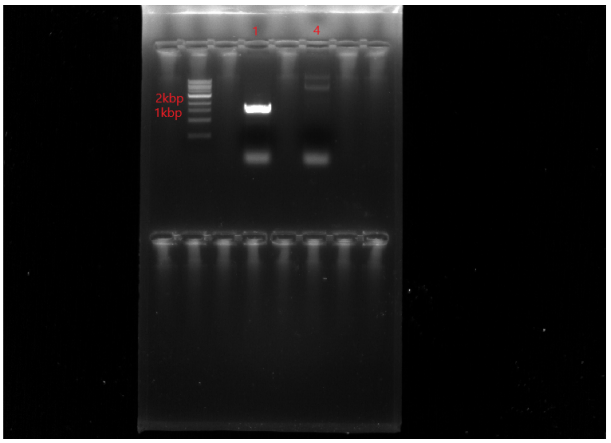
SUCCEEDED, band of 1573bp observed on gel

Tube 4:
HicAB Fwd
New_HicAB_Rev
Template: HicAB gblock
FAILED (no band of 2123bp observed on gel)

2. RE-RUN Tube 4 overnight, this time with 2 different annealing temperature settings: 68.2 deg cel & 72 deg cel (max it), elongation time: 1 min 15 s

3. **HicA plasmid assembly**:
(a) Performed gel extraction on yesterday's tubes of excised gel for 3, 5 and 6 incubated in QG buffer at 4 deg cel (frag 1 & 4 DNA extracted too)

HicAB assembly Fragment 1 HicAB bb 2(on pGEM E) and HicAB frag 4 (on gblock)



Correct band size for fragment 1 (1573bp) but wrong band size for fragment 4 (2123bp)

(b) Nanodrop to measure DNA conc of fragment 3, 5, 6 (and today's frag 1 and yesterday's frag 2 as well)

Concentration of fragments for plas...

	Fragment number	DNA conc (ng/uL)
1	1 (HicAB plasmid BB2)	30.8
2	2 (HicAB plasmid BB1)	46.7
3	3 (HicA plasmid BB1)	73.9
4	5 (HicA fragment)	63.3
5	6 (HicA plasmid BB2)	73.4

(c) Gibson assembly (total vol: 20uL; 10uL gibson mix, 1.5uL frag 3, 1.5uL frag 5, 1.5uL frag 6, topped up with 5.5uL dH2O)
(d1) Transformed competent 10B cells with 5uL of assembled HicA plasmid (Gibson end product) -> plate cells on LB+K agar
(d2) Stored remaining 15uL assembled HicA plasmid in -20 deg cel (in pcr tube)

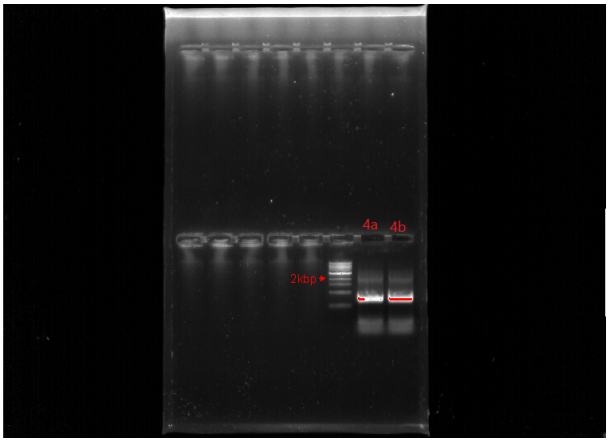
FRIDAY, 24/05/2019

1. **Deliveries**

Doc/Phd gblock
MqsR/MqsA gblock
ARRIVED

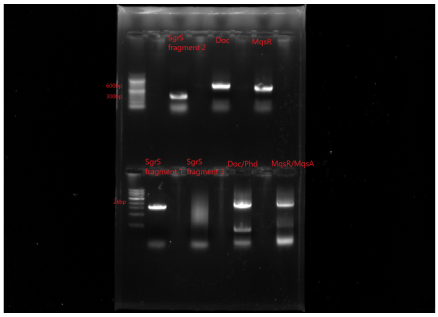
2. **Assembly of pGEME-Doc/PhD, pGEME-MqsR/MqsA, pGEME-Doc only, pGEME-MqsR only, pGEME-HicA/HicB**
(a) PCR (Doc/Phd & MqsR/MqsA only; HicA fragment and **all pGEME plasmid backbone** and **HicA/B insert** already obtained yesterday 23/5)
- Seven samples
(b) Gel extraction
- Nine samples: Additional HicAB fragment 4a (Tm=68.2) and 4b (Tm=72) from yesterday's overnight run

Gel for HicAB assembly HicAB fragment 4a and 4b



Gel showing **CORRECT band size** for HicAB fragment 4a & 4b

Gel for SgrS, Doc/Phd, MqsR/MqsA, Doc only, Mqr S only plasmid fragments



Gel showing correct band size for all

(c) Nanodrop
- Total 6 samples - DP, MRA, D only, MR only, HicAB fragment 4a, HicAB fragment 4b
(d) Gibson
- Total 5 reactions - Doc/Phd, MqrS/A, Doc only, MqrS only, HicAB plasmids to be assembled
- **NOTE: Used fragment 4a to assemble HicAB because fragment 4b's DNA conc is too low**
(e) Transformation (RTP over the weekend)
- Total 6 plates

PCR + gel size + DNA conc of assembly fragments (for SgrS, DP, MRA, D, MR and HicAB)

	Assembly of plasmid:	Fragment name	Primers used	Length of fragment (bp)	Final annealing temperature (deg cel)	Gel size (OK or FAIL)	Nanodrop DNA conc (ng/uL)
1	pBbs8c-SgrS	S1	pBbs8c Fwd (1)	1744	72	OK	19.8
2			pBbs8c Rev (1)				
3			Template: pBbs8c				
4		S2	Bba_k581005 SgrS Fwd	295	70.9	OK	39.1
5			Bba_k581005 SgrS Rev				
6			Template: SgrS plasmid (seq 1)				
7		S3	pBbs8c Fwd (2)	2937	72	FAILED (long stretch of smear; try two-step PCR); still excised gel to try assembling plasmid	11.2
8			pBbs8c Rev (2)				
9			Template: pBbs8c				
10	Toxin-Antitoxin plasmid: Doc/Phd	DP	HicAB Fwd	2089	72	OK	40.5
11			New_HicAB Rev				
12			Phd/Doc gblock				
13	Toxin-Antitoxin plasmid: MqrS/MqrA	M.RA	HicAB Fwd	2179	72	OK	18.9
14			New_HicAB Rev				
15			MqsR/MqsA gblock				
16	Toxin-only plasmid: Doc	D only	HicAB Fwd	609	70.5	OK	50.3
17			HicA end rev				
18			Phd/Doc gblock				
19	Toxin-only plasmid: MqrS	M.R only	HicAB Fwd	528	70.5	OK	59
20			HicA end rev				
21			MqsR/MqsA gblock				
22	Toxin-Antitoxin plasmid: HicA/HicB	Fragment 4a (ran PCR ytd), Tm=68.2	HicAB Fwd	2123	68.2	OK	23.5
23			New_HicAB_Rev				
24			HicA/HicB gblock				
25		Fragment 4b (ran PCR ytd), Tm=72	HicAB Fwd		72	OK	4.7
26			New_HicAB_Rev				
27			HicA/HicB gblock				

(a) Toxin-antitoxin plasmids supposed to use fragment 1 (pGEME bb2) and 2 (pGEME bb1) but accidentally used fragment 4a (HicAB fragment) and fragment 2 with the respective toxin fragments instead. **Note: Both Doc/Phd and MqsR/MqsA gibson assembly failed -> no colonies formed on plate (27 May)**

(b) Unsure why Doc-only toxin with correct backbone used (fragment 3 and 6) did not have any colony, also unsure why HicA/HicB plasmid (post-transformation recovery at 37degC have no colony but 50degC have one colony)

(c) Only two out of seven plates formed colonies: HicA/HicB plasmid (one and only sample with post-transformation recovery at 50degC) and MqsR-only plasmid (normal 37 degC)

MONDAY, 27/05/2019

1. Long-term GFP expression experiment

- repeated same single-GFP read protocol of same plate layout at 1.30pm only (measuring 72h time point)

2. Sleep-wake experiment: successful plates with colonies follow-up
(a) 3 colonies from MqsR-only plate and
(b) 1 and only colony from 50degC HicA/HicB plate inoculated in LB **only** media (supposedly in LB+K media)

4. Sleep-wake experiment: re-assembly of **high prioritized plasmids**
- Re-ran Gibson for:
1. HicA/HicB plasmid
2. MqsR/MqsA plasmid

(Doc/Phd and Doc-only plasmid to be assembled tomorrow, 28 May)

Gibson assembly mix preparation

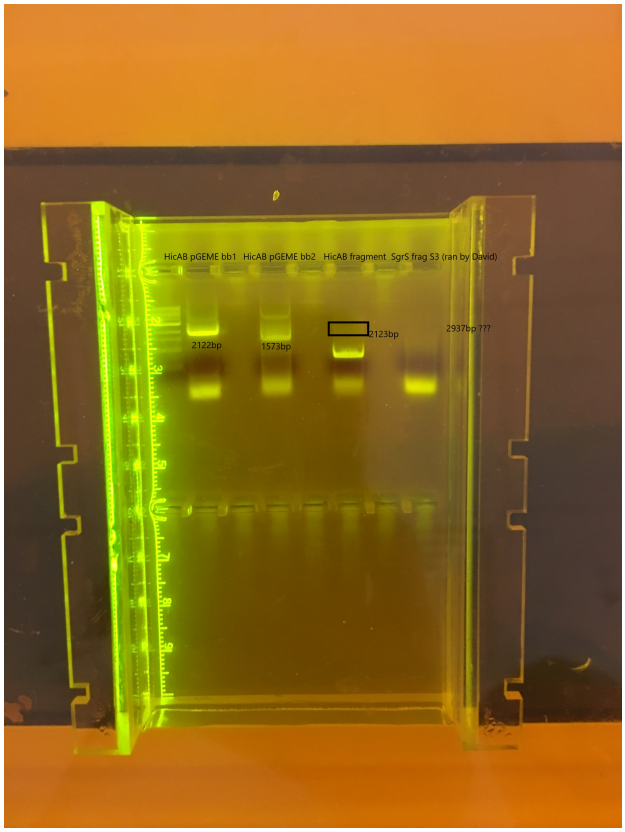
	Plasmid assembled	Fragment name	Length (bp)	Conc (ng/uL)	Volume to add to Gibson rxn mix	Top up H2O to mix?	Transformation results
1	HicA/HicB plasmid	HicAB frag 4a	2123	23.5	4.5		One or few colonies (all with GFP)
2		pGEME bb 2	2122	46.7	2.5		
3		pGEME bb 1	1573	30.8	2.5		
4	MqsR/MqsA plasmid	MqsRA (MRA)	2222	18.9	5		One or few colonies (all with GFP)
5		pGEME bb 2	2122	46.7	2		
6		pGEME bb 1	1573	23.5 (supposed to be 30.8 BUT molar diff is still accurate)	3		
7	pBbs8c-SgrS plasmid	S1	1744	19.8	1.5		No colony at all (halt pBbs8c SgrS for now, use new E8K bb)
8		S2	295	39.1	2		
9		S3	2937	11.2	5	Top up 1.5uL	

TUESDAY, 28/05/2019

Sleep-wake experiment

1. Assuming yesterday's failure to have non-fluorescing colony was due to transformation and Gibson assembly was done correctly (gibson ratio checked with David), **re-transformed HicA/HicB and MqsR/MqsA on kanamycin plate @ 37degC overnight**. (Re-transformed HicAB and MqsR/A plates labeled 10B-pGEME-HicA/HicB 2**3**5 & 10B-pGEME-MqsR/MqsA 2**3**5)
2. Also re-ran PCR for HicAB frag, pGEME bb1 and pGEME bb2 from HicAB gblock and pGEME template straight --> trying to get new backbone fragments (assuming fluorescing colony from yesterday was due to backbone problem; David checked primers on benchling, theoretically speaking primer is fine, fragment should be fine) --> Later gel extraction (done by David), nanodrop, Gibson and transformation (Newly assembled HicAB and MqsR/A plates labeled 10B-pGEME-HicA/HicB 2**8**5 & 10B-pGEME-MqsR/MqsA 2**8**5)

Gel for new HicAB frag, new pGEME bb 1, new pGEME bb2 (re-ran by David)



New pGEME bb1 and bb2 have strong bands at correct size;
HicAB fragment has **faint band** at size 2123bp

PCR + gel size + DNA conc of assembly fragments (for HicAB only)

	Assembly of plasmid:	Fragment name	Primers used	Length of fragment (bp)	Final annealing temperature (deg cel)	Gel size (OK or FAIL)	Nanodrop DNA conc (ng/uL)
1	pGEME-HicA/HicB plasmid	BB1	E6k*k Fwd (1)	2122	70.3	OK	26
2			E6k Rev (1)				
3			Template: pGEME				
4		BB2	New_E6k Fwd	1573	68.2	OK	14.7
5			E6k*k Rev (2)				
6			Template: pGEME				
7		HicAB fragment	HicAB Fwd	2123	72	Faint but have	14
8			New_HicAB_Rev				
9			Template: HicA/B gblock				

Gibson assembly mix reaction for new HicA/B (new 3 frags) and MqsR/MqsA (old MqsRA, new bb)

	Plasmid assembled	Fragment name	Length (bp)	Conc (ng/uL)	Volume to add to Gibson rxn mix	Top up H2O to mix?	Transformation results
1	HicA/HicB plasmid (285)	pGEME bb 1 (new)	2122	26	3		No successful colony
2		pGEME bb 2 (new)	1573	14.7	3		
3		HicAB frag (new)	2123	14	4		
4	MqsR/MqsA plasmid (285)	pGEME bb 1 (new)	2122	26	3		No successful colony
5		pGEME bb 2 (new)	1573	14.7	3		
6		MqsR/A (MRA)	2222	18.9	3	1uL H2O	

3. 23 May assembled HicA plasmid transformed into agar plate (stopped). Today, finished the loop by inoculating three colonies into LB+K to grow overnight (tomorrow to miniprep and send for seq)

4. Miniprepped MqsR-only and 50degC post-transformation-incubated HicA/B today (seq primers used for Toxin-antitoxin delivered late; tomorrow then send for sequencing along with HicA-only samples from pointer 3).
Conc:
MqsR colony 1: 37.9ng/uL
MqsR colony 2: 49.1
MqsR colony 3: 65.2
HicAB 50degC colony: 51.3

WEDNESDAY, 29/05/2019

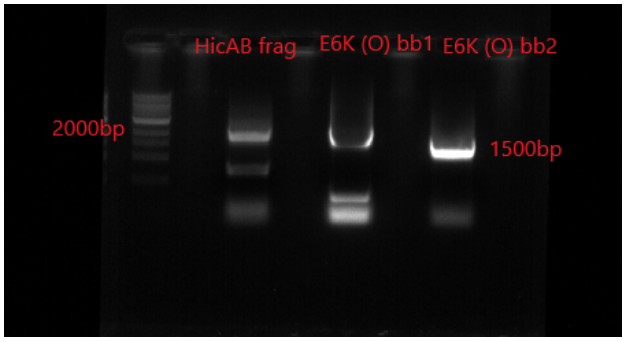
(Meeting with Prof Kitney for the whole day)
Chun yang:
- Miniprepped HicA-only colony 1, 2 and 3 --> sent to sequencing along with MqsR-only colony 1, 2, 3 and also HicA/HicB 50degC
- Discovered that 235 HicA/HicB is the only plate with few colonies; 238 HicA/HicB, MqsR/MqsA and 235 MqsR/MqsA all three have no colonies formed
- Inoculated 235 HicA/HicB into LB+K media
- Transformed E6k (o) plasmid from Jing Yun and E8k plasmid from David into 10b, plated.

THURSDAY, 30/05/2019

1. Sequencing results:
    a. HicA successful (Colony 3 picked and used for **transformation into MG1655**)
    b. MqsR successful (Colony 2 picked and used for **transformation into MG1655**)
    c. HicA/HicB 50degC failed
2. Miniprepped HicA/HicB 235 --> sent for sequencing (check if David's backbone E6K is ok for toxin-antitoxin plasmid)
3. Inoculated E6K (o) and E8K plasmid transformed yesteday in LB+k overnight
4. Gibson assembly for doc-only plasmid **since toxin-only plasmid using David's E6K bb have successful sequencing result**
5. Overnight PCR for MqsR/A and Doc/Phd gblock - redone to get more. **UPDATE: discontinued bef gel extraction because no point. 31/05/19 showed that seq result for E6K-HicA/HicB is unsuccessful (HicB's seq from sequencing result is actually the GFP seq on David's original backbone template; we can't use these two MqsR/A and Doc/Phd fragment anymore because it won't bind well with new E6K (o) backbone provided by Jing Yun.**

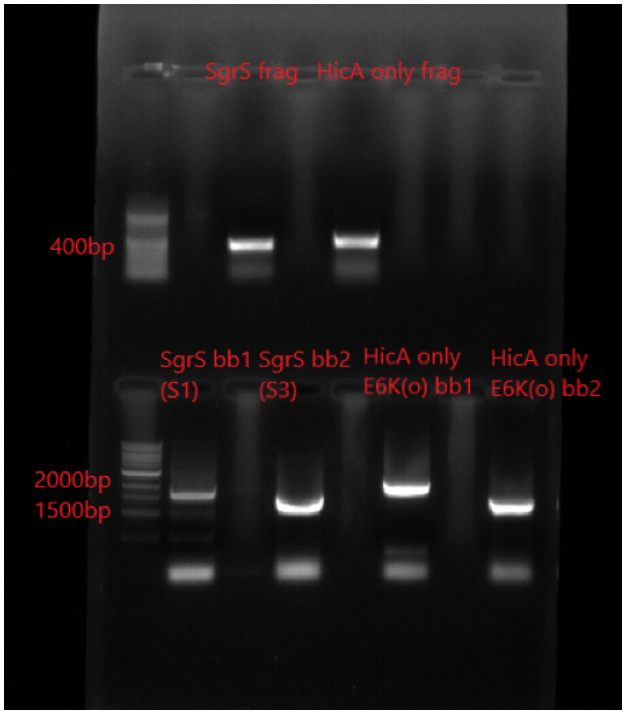
6. PCR for HicA/B and HicA in new E6K (original) backbone, **stopped after running gel (continuing with gel extraction tomorrow)**

HicAB frag, E6K (o) bb1, E6K (o) bb2



All of correct size

SgrS fragment (S2), E8K bb1 (S1), E8K bb2 (S3), Hi cA frag, E6K (o) bb1, E6K (o) bb2



All band sizes correct. (Note: later, HicA plasmid transformation yielded no growth. Decided to stick toxin-only plasmid backbond to old E6K bb (David's); **PCR frag for HicA frag and its bb1 and 2 seems correct**

PCR + gel extraction + nanodrop for new E6K (o) bb in HicA/HicB and HicA-only plasmid

	Assembly of plasmid:	Fragment name	Primers used	Length of fragment (bp)	Final annealing temperature (deg cel)	Gel size (OK or FAIL)	Nanodrop DNA conc (ng/uL)
1	pBbE6k(original) HicA-HicB	BB1	E6k*k Fwd (1)	2122		OK (strong but little band)	52.8
2			E6k Rev (1)				
3			Template: E6K (o)				
4		BB2	E6K Fwd (1)	1567		OK	54
5			E6k*k Rev (2)				
6			Template: E6K (o)				
7		HicAB fragment	HicAB Fwd	2123		OK	14
8			Hic AB_end*Rev				
9			Template: HicA/B gblock				
10	pBbE6k(original) HicA	BB1	E6k*k Fwd (1)	2122		OK	45.3
11			E6k Rev (1)				
12			Template: E6K (o)				
13		BB2	E6K Fwd (1)	1414		OK	39.7
14			E6k*k Rev (2)				
15			Template: E6K (o)				
16		HicA	HicAend	448		OK	52.1
17			Hic AB_end*Rev				
18			Template: HicA/B gblock				

FRIDAY, 31/05/2019

1. Sequencing results:
   a. HicA/HicB 235 (proper Gibson assembly) unsuccessful -> **shows that David's E6K bb is incompatible with toxin-antitoxin plasmid assembly**
2. Miniprepped E6K (o) and E8K plasmid from liquid culture, remaining culture stored in glycerol @ -80degC

Miniprep E6k (o) and E8k plasmid (la...

	Plasmid name	Conc (ng/uL)
1	E6K (o)	155.6
2	E8K	136.6

3. Transformed plate (with many relatively larger-sized colonies for HicA-3 and MqsR-2 only plasmid in **MG-1655** was successful) --> plate kept in fridge 4degC (until over the weekends)
4. [Continue] Gel extraction, nanodrop, Gibson assembly and transformation for HicAB and HicA-only in new pBbE6K (original) backbone (conc showed in table above on 30/05/19). **Also helped to transform Doc-only plasmid with David E6k bb assembled yesterday 30/05/19.**
Total number of plasmids assembled/plates transformed: 4 (HicA-only and HicA/HicB in new E6k (o) bb, Doc-only in old E6k bb; all in kanamycin plate)

MONDAY, 03/06/2019

1. Transformation result for HicAB and Hic-A only in new pBbE6K (original) bb is UNSUCCESSFUL **(we stopped trying to insert HicA into new E6K (o) plasmid anymore since HicA in old E6K is ok)**; Doc-only in old pBbE6K (David's) bb is UNSUCCESSFUL.
2. Also inoculated 3 colonies for HicA-only and MqsR-only transformed MG1655 (originally kept in fridge): HicA-3(1), HicA-3(2), HicA-3(3) and MqsR-2(1), MqsR-2(2), MqsR-2(3); tomorrow use only HicA-3(1) and MqsR-2(1); others store in -80degC for now.
3. Inoculated 3uL of MG1655 competent cell into 5mL LB only --> used as control for tomorrow's toxin characterization (for HicA and MqsR)
4. Re-transformation of HicAB in new pBbE6K (original) bb and Doc-only in old pBbE6K (David's) bb into **David's 10b competent cell** (suspected failure to grow colonies in point 1 could be due to poor competent cell prepared)
5. Overnight PCR run 2 rxn worth of HicAB fragment from gblock (later to be combined for more concentrated fragment during gel extraction step); note: both PCR mix provided by David. Primer used: HicAB Fwd and HicAB_end* Rev --> **HicAB is compatible for new E6K (o) bb only**

TUESDAY, 04/06/2019

1. Overnight liquid culture for: (a) E8K-SgrS (1) - (3): 400uL stored in 4degC, (b) HicA-3(1), HicA-3(2), HicA-3(3) and MqsR-2(1), MqsR-2(2), MqsR-2(3), as well as WT untransformed MG1655 all stored in -80degC with 200uL cells + 500uL 100% glycerol. (c) Some volume of cells for WT untransformed MG1655, HicA-3(1) only and MqsR-2(1) only are used for toxin characterization. (d) Leftover HicA-3(1) only and MqsR-2(1) only are miniprepped (**conc of plasmid** for HicA-3(1) only = 97ng/uL, MqsR-2(1) only = 96.8ng/uL) (e) FINALLY, samples are sent for sequencing.

2. Toxin characterization of HicA-3(1) and MqsR-2(1):

Toxin characterization (0h, 1h, 2h, 3h time point induction w/ IPTG)

	1	2	3	4	5	6	7	8	9	10	11	12
A	HicA-3(1), 25uM IPTG, 0h time point induction	HicA-3(1), 25uM IPTG, 0h time point induction	HicA-3(1), 25uM IPTG, 0h time point induction	HicA-3(1), 25uM IPTG, 1h time point induction	HicA-3(1), 25uM IPTG, 1h time point induction	HicA-3(1), 25uM IPTG, 1h time point induction	HicA-3(1), 25uM IPTG, 2h time point induction	HicA-3(1), 25uM IPTG, 2h time point induction	HicA-3(1), 25uM IPTG, 2h time point induction	HicA-3(1), 25uM IPTG, 3h time point induction	HicA-3(1), 25uM IPTG, 3h time point induction	HicA-3(1), 25uM IPTG, 3h time point induction
B	HicA-3(1), 50uM IPTG, 0h time point induction	HicA-3(1), 50uM IPTG, 0h time point induction	HicA-3(1), 50uM IPTG, 0h time point induction	HicA-3(1), 50uM IPTG, 1h time point induction	HicA-3(1), 50uM IPTG, 1h time point induction	HicA-3(1), 50uM IPTG, 1h time point induction	HicA-3(1), 50uM IPTG, 2h time point induction	HicA-3(1), 50uM IPTG, 2h time point induction	HicA-3(1), 50uM IPTG, 2h time point induction	HicA-3(1), 50uM IPTG, 3h time point induction	HicA-3(1), 50uM IPTG, 3h time point induction	HicA-3(1), 50uM IPTG, 3h time point induction
C	HicA-3(1), 100uM IPTG, 0h time point induction	HicA-3(1), 100uM IPTG, 0h time point induction	HicA-3(1), 100uM IPTG, 0h time point induction	HicA-3(1), 100uM IPTG, 1h time point induction	HicA-3(1), 100uM IPTG, 1h time point induction	HicA-3(1), 100uM IPTG, 1h time point induction	HicA-3(1), 100uM IPTG, 2h time point induction	HicA-3(1), 100uM IPTG, 2h time point induction	HicA-3(1), 100uM IPTG, 2h time point induction	HicA-3(1), 100uM IPTG, 3h time point induction	HicA-3(1), 100uM IPTG, 3h time point induction	HicA-3(1), 100uM IPTG, 3h time point induction
D	MqsR2(1), 25uM IPTG, 0h time point induction	MqsR2(1), 25uM IPTG, 0h time point induction	MqsR2(1), 25uM IPTG, 0h time point induction	MqsR2(1), 25uM IPTG, 1h time point induction	MqsR2(1), 25uM IPTG, 1h time point induction	MqsR2(1), 25uM IPTG, 1h time point induction	MqsR2(1), 25uM IPTG, 2h time point induction	MqsR2(1), 25uM IPTG, 2h time point induction	MqsR2(1), 25uM IPTG, 2h time point induction	MqsR2(1), 25uM IPTG, 3h time point induction	MqsR2(1), 25uM IPTG, 3h time point induction	MqsR2(1), 25uM IPTG, 3h time point induction
E	MqsR2(1), 50uM IPTG, 0h time point induction	MqsR2(1), 50uM IPTG, 0h time point induction	MqsR2(1), 50uM IPTG, 0h time point induction	MqsR2(1), 50uM IPTG, 1h time point induction	MqsR2(1), 50uM IPTG, 1h time point induction	MqsR2(1), 50uM IPTG, 1h time point induction	MqsR2(1), 50uM IPTG, 2h time point induction	MqsR2(1), 50uM IPTG, 2h time point induction	MqsR2(1), 50uM IPTG, 2h time point induction	MqsR2(1), 50uM IPTG, 3h time point induction	MqsR2(1), 50uM IPTG, 3h time point induction	MqsR2(1), 50uM IPTG, 3h time point induction
F	MqsR2(1), 100uM IPTG, 0h time point induction	MqsR2(1), 100uM IPTG, 0h time point induction	MqsR2(1), 100uM IPTG, 0h time point induction	MqsR2(1), 100uM IPTG, 1h time point induction	MqsR2(1), 100uM IPTG, 1h time point induction	MqsR2(1), 100uM IPTG, 1h time point induction	MqsR2(1), 100uM IPTG, 2h time point induction	MqsR2(1), 100uM IPTG, 2h time point induction	MqsR2(1), 100uM IPTG, 2h time point induction	MqsR2(1), 100uM IPTG, 3h time point induction	MqsR2(1), 100uM IPTG, 3h time point induction	MqsR2(1), 100uM IPTG, 3h time point induction
G	WT 100uM IPTG, 0h time point induction	WT 100uM IPTG, 0h time point induction	WT 100uM IPTG, 0h time point induction	WT 100uM IPTG, 1h time point induction	WT 100uM IPTG, 1h time point induction	WT 100uM IPTG, 1h time point induction	WT 100uM IPTG, 2h time point induction	WT 100uM IPTG, 2h time point induction	WT 100uM IPTG, 2h time point induction	WT 100uM IPTG, 3h time point induction	WT 100uM IPTG, 3h time point induction	WT 100uM IPTG, 3h time point induction
H	LB media ctrl	LB media ctrl	LB media ctrl	HicA-3(1) 0M IPTG ctrl	HicA-3(1) 0M IPTG ctrl	HicA-3(1) 0M IPTG ctrl	MqsR-2(1) 0M IPTG ctrl	MqsR-2(1) 0M IPTG ctrl	MqsR-2(1) 0M IPTG ctrl	WT 0M IPTG ctrl	WT 0M IPTG ctrl	WT 0M IPTG ctrl

Preparation:
(a) Prepared a working conc IPTG from stock (100mM): 10x dilution into 10000uM and futher 2x dilution into 5000uM and further 2x dilution into 2500uM.
To add directly to well containing 100uL of cells,
_____ x 10000 = 100uL x 100uM, vol of 10000uM IPTG to add into well = 1uL
_____ x 5000 = 100uL x 50uM, vol of 5000uM IPTG to add into well = 1uL
_____ x 2500 = 100uL x 25uM, vol of 2500uM IPTG to add into well = 1uL

(b) Starting OD = 0.08
WT OD600 = 0.1 (converted into 0.08, final vol = 2mL) -> diluted with LB only
HicA-3(1) OD600 = 0.08 (directly added 100uL into each well)
MqsR-2(1) OD600 = 0.09 (converted into 0.08, final vol = 5mL) -> diluted with LB+K

Round 1 of toxin characterization results:

040619 0h timepoint induction for HicA3(1), MqsR 2(1), WT MG1655, 25uM 50uM 100uM IPTG.xlsx

040619 1h timepoint induction for HicA3(1), MqsR2 (1), WT MG1655, 25uM 50uM 100uM IPTG.xlsx

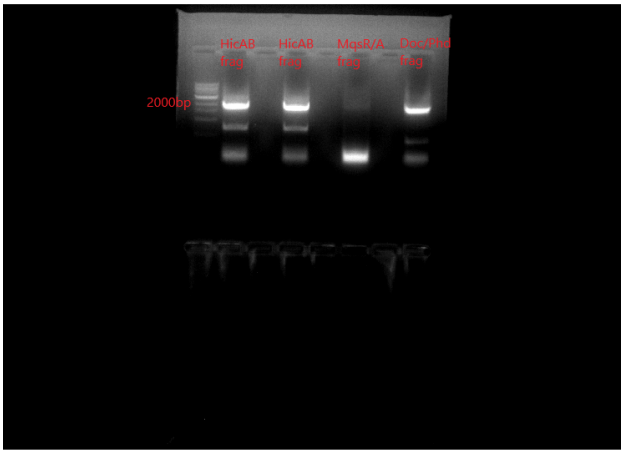
040619 2h timepoint induction for HicA3(1), MqsR 2(1), WT MG1655, 25uM 50uM 100uM IPTG.xlsx

040619 3h timepoint induction for HicA3(1), MqsR 2(1), WT MG1655, 25uM 50uM 100uM IPTG.xlsx

3. Cells transformed/plated with HicA/HicB in new E6K (o) bb and Doc-only in old E6K bb HAVE COLONIES (3 and ~8 colonies respectively); note this re-transformation done on 3 June used David's 10b competent cell --> plates parafilmed and kept in 4degC.

4. PCR-ed to get MqsR/MqsA fragment and Doc/Phd fragment in the morning. Later run gel for both samples + 2 pcr tubes of identical HicA/HicB fragments ran overnight yesterday (Total 4 samples loaded in gel) **ALL THREE SAMPLE FRAGMENTS ARE COMPATIBLE WITH NEW E6K (O) BB ASSEMBLY.**

PCR fragment size for HicA/HicB, MqsR/MqsA and Doc/Phd in new E6K (o) backbone



Faint band for MqsR/A (eluted with 20uL EB only). All four bands of **correct band size** - 2123bp, 2123bp, 2222bp, 2123bp

Later, combined both HicA/HicB band into one tube at start of gel extraction. MqsR/MqsA is the only fragment eluted with only 20uL elution buffer due to faint band; HicA/HicB and Doc/Phd eluted with 30uL elution buffer

Nanodrop to get conc:
HicA/HicB frag (for E6K (o) bb) = 84.5ng/uL
MqsR/MqsA frag (for E6K (o) bb) = 10.9ng/uL
Doc/Phd frag (for E6K (o) bb) = 97.8ng/uL

Gibson performed to assemble:

1. MqsR/MqsA plasmid (in new E6K (o) bb)
2. Doc/Phd plasmid (in new E6K (o) bb)

MqsR/A frag 2222bp, 10.9ng/uL, 7.5uL to add for Gibson
E6k (o) new bb1 2122bp, 52.8ng/uL, 1.5uL to add for Gibson
E6k (o) new bb2 1567bp, 54ng/uL, 1uL to add for Gibson
No need add dH2O
7TH JUNE UPDATE: NO COLONIES FORMED

Doc/Phd frag 2123bp, 97.8ng/uL, 2uL to add for Gibson
E6k (o) new bb1 2122bp, 52.8ng/uL, 3.5uL to add for Gibson
E6k (o) new bb2 1567bp, 54ng/uL, 2.5uL to add for Gibson
Topped with 2uL of dH2O
7TH JUNE UPDATE: NO COLONIES FORMED

Transformed with **David's competent cell (10B)**

THURSDAY, 06/06/2019

1. Inoculated cells from glycerol stock for: WT MG1655, HicA-3(1) MG1655, MqsR-2(1) MG1655 into LB and LB+K (for toxin characterization)
2. Inoculated 3 colonies for Doc-only (in old E6k bb) and HicA/HicB (in new E6k (o) bb) into LB+K media (to be miniprepped and sent for sequencing)

FRIDAY, 07/06/2019

1. For **second round** of toxin characterization work: Overnight culture for WT MG1655, HicA-3(1) MG1655 and MqsR-2(1) MG1655 refreshed 2% cells into two 50mL tubes (+ IPTG and - IPTG) -> +IPTG tubes induced with 100uM IPTG (50uL) in their respective tubes after 3h incubation, then incubate for another 3h (OD=0.1) before plating cells; -IPTG tubes to be refreshed 2% (OD=~0.1) then loaded; a portioned of cells serve as control for +IPTG experiment, another 2 portions to be induced (1uL from 100**u**M IPTG stock, 2uL from 100**m**M IPTG stock) directly in well after loading at 0h, for both 100uM & 2mM IPTG conc
2. HicA/HicB and Doc-only tubes (x3 each): (a) 400uL stored in 4degC; (b) leftover miniprepped (HicAB-1 = 175.1ng/uL, HicAB-2 = 145ng/uL, HicAB-1 = 147.1ng/uL, Doc only-1 = 142.6ng/uL, Doc only-2= 174.5ng/uL, Doc only-3 = 177.8ng/uL), (c) sent for sequencing
   SEQ RESULT: Doc-only (all three colonies) failed, turn out to contain RFP gene (which is weird because it's supposed to be containing David's E6K bb which is GFP not the new E6K (o) bb template containing RFP hmm); HicAB all three colonies either only contain HicA or HicB (AraC gene is not detected when sequenced)

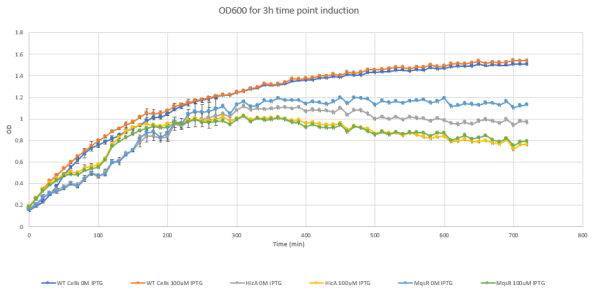
MONDAY, 10/06/2019

1. Re-transformed HicA-3 and MqsR-2 plasmid from -20degC into **Aiying's MG1655** || also inoculated two other colonies on previously transformed MG1655 plate for HicA-3 and MqsR-2 (aka HicA-3(2) and HicA-3(3) and MqsR-2(2) and MqsR-2(3).
2. Doc-only results failed (refer to 7 Jun seq result), picked a non-RFP colony and inoculated (to send for seq again)
3. HicA/HicB seq result were weird, among all three colonies either have HicA or HicB, all without AraC gene detected; **hence, re-transformed HicA/B gibson mix (from 31 May) into our own MG1655 (not Aiying's) to re-sequence**.
4. Analyzed 07/06/2019 results

070619__3h_timepoint_induction_for_HicA31_MqsR21_WT_MG1655_100uM_IPTG.xlsx

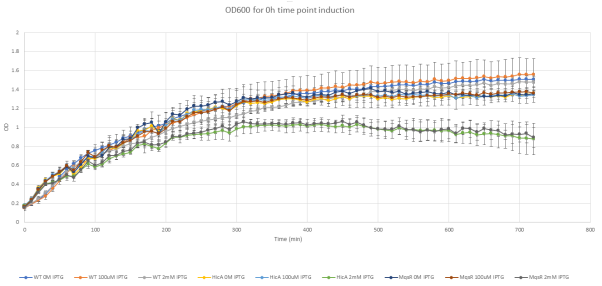
070619__0h_timepoint_induction_for_HicA31_MqsR21_WT_MG1655_100uM_2mM_IPTG.xlsx

image.png



- Induced has lower endpoint, but uninduced grew slower at the start

image.png

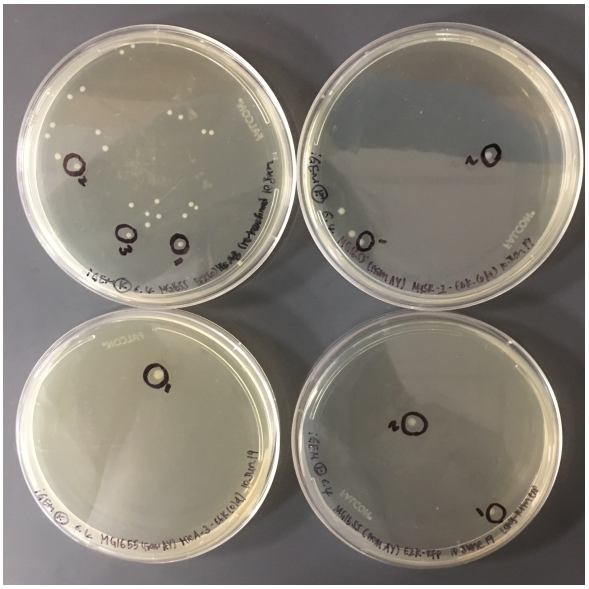


- 2mM induction has significantly stronger impact compared to 100uM and no induction

TUESDAY, 11/06/2019

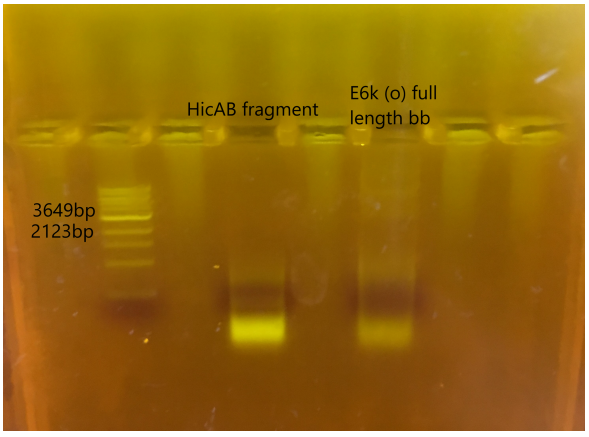
1. Inoculated colonies from MG1655-HicA/HicB (3 colonies) + Aiying's MG1655-HicA-3 (1 colony) + Aiying's MG1655-MqsR-2 (2 colonies) + Aiying's MG1655-E2K-RFP (2 colonies) into LB+K overnight

How the plates with colonies looked like



2. Miniprepped and sent 5 samples for sequencing: HicA-3(2), HicA-3(3), MqsR-2(2), MqsR-2(3) ALL FROM MG1655, also Doc-only from 10b
3. Overnight PCR results from yesterday -> NO HicAB frag detected at 2123bp position, faint 3+kbp band for E6K (o) bb. Only gel extracted backbone, 6.5ng/uL. Gibson assembled with 84.5ng/uL HicAB frag prepared on 4 Jun in the vol of 5uL each (independent of molar ratio), transformed into both 10b and Top10 (new competent strain) and plated onto kanamycin plate together.

Gel photo for HicAB frag and full length E6K (o) bb

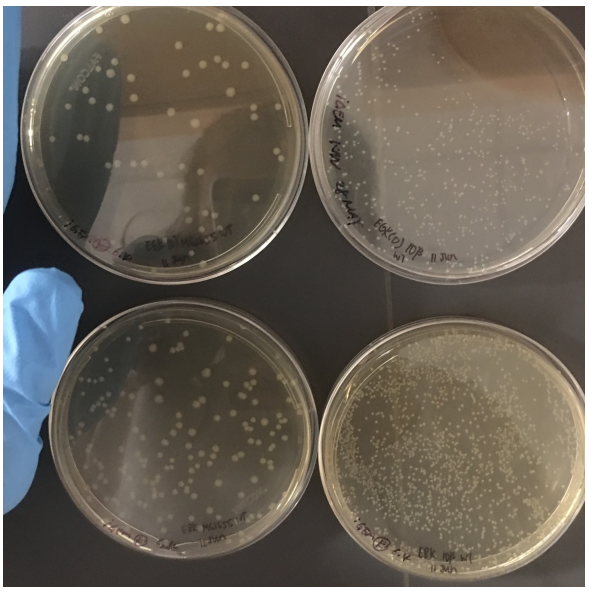


4. Re-ran PCR for E6k (o) full length backbone (labeled 3k bb) again, this time changes in Tm (68.5 to 72degC), 1min50s to 2min elongation time
5. Transformed HicA-3 plasmid into 10b competent cells -> to include characterization of toxin in 10b as well, not just MG1655. Next, also transformed empty but common backbone of E6K and E8K into 10b and MG1655 to serve as control

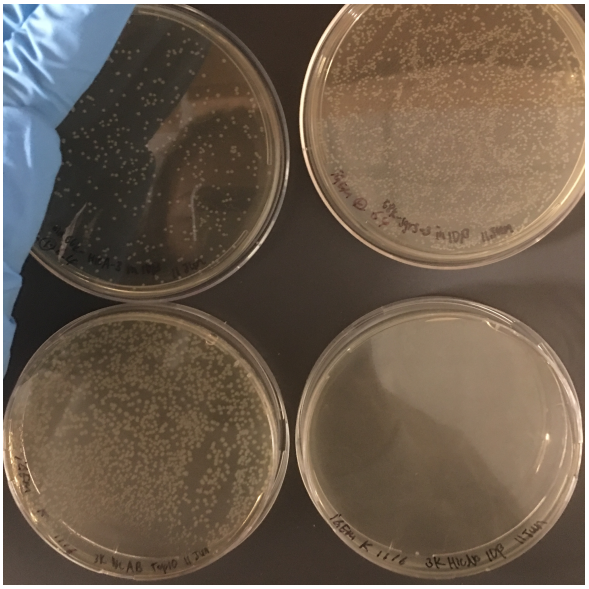
WEDNESDAY, 12/06/2019

1. Miniprepped re-transformed HicAB 1, 2, 3 + re-transformed HicA-3-1 + retransformed MqsR-2-1, 2-2 + E2K-RFP 1 + 2 (conc in respective orders: 244.5ng/uL, 474.4, 311.3, 242, 814.8, 922.6, 216.7, 688.4) --> SENT FOR SEQUENCING
2. Inoculated colonies from transformed plates from last night into LB+K media

MG1655 E6K (o) bb, 10b E6K (o) bb, MG1655 E8 K bb, 10b E8K bb

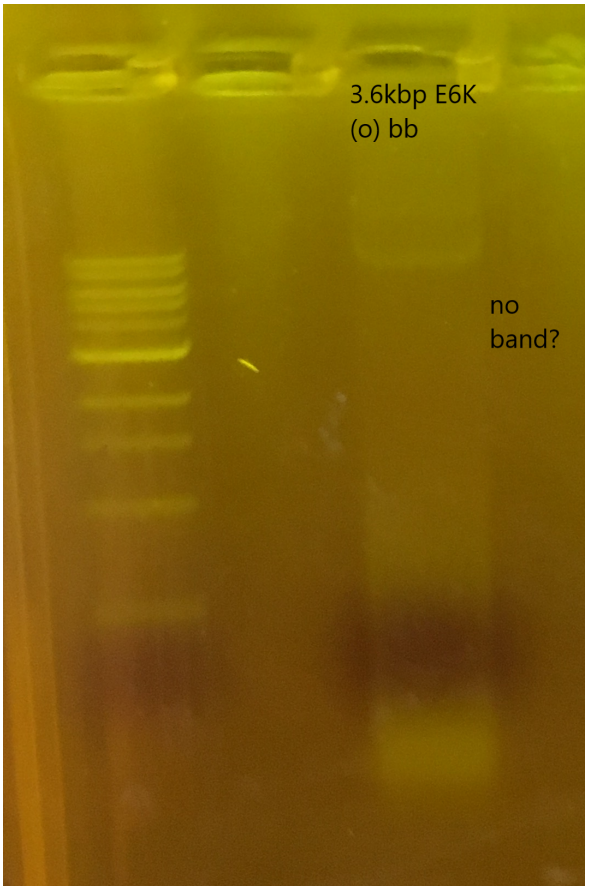


10b old E6k-HicA-3, 10b E8K-SgrS-3, Top 10 3kb p E6K (o) HicAB



3. Inoculated 3 non-RFP colonies from Doc-only plate into LB+K (to be miniprepped and re-sequenced to see if point mutation persist, based on 12 Jun seq result)
4. Gel result for overnight re-running of full length 3kbp E6K-(o) , TERRIBLE result:

Gel result for full length E6K (o), no band at 3.6 kbp



THURSDAY, 13/06/2019

1. MqsR-2 plasmids (previously seq ok) transformed into Aiying's MG1655 form colonies (MqsR-2(1) and -2(2)) are successfully sequenced! --> SHOWS THAT previous failure was likely due to lousy first batch of MG1655 that's why all sequencing results are nonsensical
2. Miniprepped and sequenced
   - HicAB 1 and 3 (colony 2 is RED unsure why but 2 is discontinued)
   - 10b HicA-3(1)
   - 10b E6k (o) plasmid
   - MG1655 E6k (o) plasmid **(TAKE NOTE THIS FIRST BATCH MG1655 IS BEING USED TO CHARACTERIZE TOXIN TOMORROW, SEE HOW RESULTS FARE, by right it shouldn't be used because this strain is problematic)**
3. Inoculated Doc-only colony 5, 6, 7 overnight in LB+K media -> tomorrow miniprep, send for seq
4. Inoculated cultures overnight that will be used for toxin characterization in the afternoon 3pm tomorrow, inclusive of:
   - MG1655 WT
   - 10b WT
   - MG1655 w/ E6k (o)
   - 10b w/ E6k (o)
   - MG1655 HicA-3(1) SEQ OK (in Aiying's MG1655)
   - 10b HicA-3(1)
   - MG1655 MqsR-2(1) SEQ OK (in Aiying's MG1655)
   - 10b MqsR-2 previously SEQ OK

FRIDAY, 14/06/2019

1. Miniprepped doc5, 6 and 7 (in old E6K bb) --> sent for sequencing.

2. Transformed MqsR/MqsA and Doc/Phd plasmids into TOP10 and 10B (left on bench over weekend)
   Note: MqsR/MqsA and Doc/Phd plasmids were obtained from existing gibson product kept in -20degC PCR rack

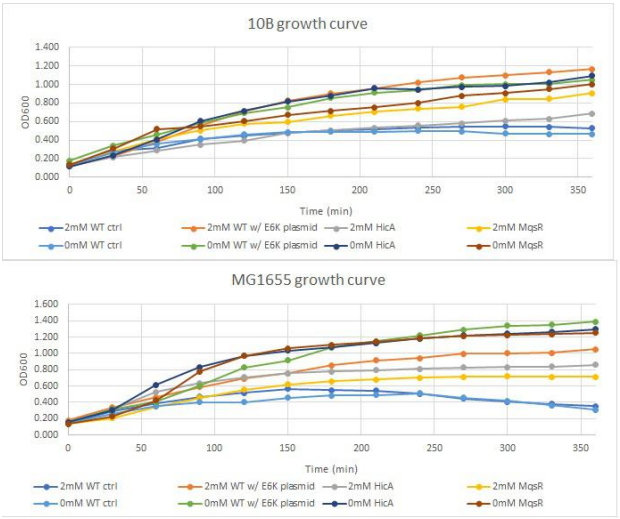
3. Toxin characterization
   0h incubation
   0h induction
   OD600 = 0.1
   IPTG = 2mM and 0mM
   Used both 10b and MG1655 strains to characterize toxin
   Both WT MG1655 (w/o plasmid) and MG1655 ctrl with E6K (o) have **FAILED sequencing result/weird strain (not a good control) = POOR CONTROL FOR THIS DATA SET**

Plate layout for toxin characterization on 15 June 2019

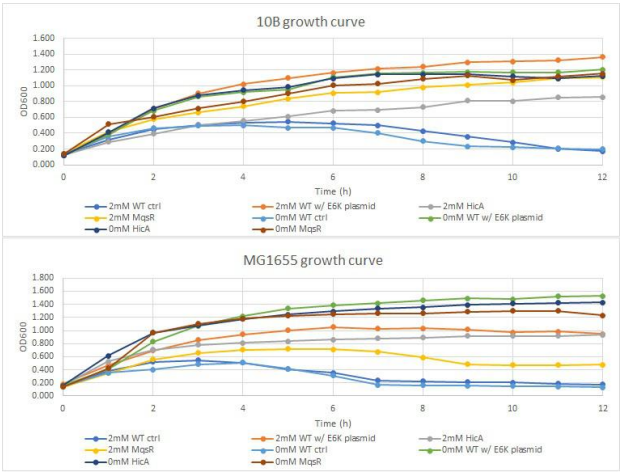
	1	2	3	4	5	6	7	8	9	10	11	12
A	10b WT ctrl (no plasmid)	10b WT ctrl (no plasmid)	10b WT ctrl (no plasmid)	10b WT ctrl (no plasmid)	10b WT ctrl (no plasmid)	10b WT ctrl (no plasmid)	LB media	LB media	LB media			
B	10b WT ctrl w/ E6k (o) plasmid	10b WT ctrl w/ E6k (o) plasmid	10b WT ctrl w/ E6k (o) plasmid	10b WT ctrl w/ E6k (o) plasmid	10b WT ctrl w/ E6k (o) plasmid	10b WT ctrl w/ E6k (o) plasmid		Green = 2mM IPTG	White = 0mM IPTG			
C	10b HicA-3(1)	10b HicA-3(1)	10b HicA-3(1)	10b HicA-3(1)	10b HicA-3(1)	10b HicA-3(1)						
D	10b MqsR-2	10b MqsR-2	10b MqsR-2	10b MqsR-2	10b MqsR-2	10b MqsR-2						
E	MG1655 WT ctrl (no plasmid)	MG1655 WT ctrl (no plasmid)	MG1655 WT ctrl (no plasmid)	MG1655 WT ctrl (no plasmid)	MG1655 WT ctrl (no plasmid)	MG1655 WT ctrl (no plasmid)						
F	MG1655 WT ctrl w/ E6k (o) plasmid	MG1655 WT ctrl w/ E6k (o) plasmid	MG1655 WT ctrl w/ E6k (o) plasmid	MG1655 WT ctrl w/ E6k (o) plasmid	MG1655 WT ctrl w/ E6k (o) plasmid	MG1655 WT ctrl w/ E6k (o) plasmid						
G	MG1655 HicA-3(1)	MG1655 HicA-3(1)	MG1655 HicA-3(1)	MG1655 HicA-3(1)	MG1655 HicA-3(1)	MG1655 HicA-3(1)						
H	MG1655 MqsR-2-1	MG1655 MqsR-2-1	MG1655 MqsR-2-1	MG1655 MqsR-2-1	MG1655 MqsR-2-1	MG1655 MqsR-2-1						

Results:

140619 6h time point for 10B and MG1655, HicA and MqsR.jpg



140619 12h time point for 10B and MG1655, HicA and MqsR.jpg



140619 10b WT ctrl, WT w E6kO, HicA, MqsR AND MG1655 WT ctrl, WT w E6kO, HicA, MqsR, 2mM and 0mM, 0h incubation & induction, 12h continuous read.xlsx

140619 6h results 10b WT ctrl, WT w E6kO, HicA, MqsR AND MG1655 WT ctrl, WT w E6kO, HicA, MqsR, 2mM and 0mM, 0h incubation, 12h continuous read.xlsx

140619 12h results 10b WT ctrl, WT w E6kO, HicA, MqsR AND MG1655 WT ctrl, WT w E6kO, HicA, MqsR, 2mM and 0mM, 0h incubation, 12h continuous read.xlsx

MONDAY, 17/06/2019

1. Checked transformed plates from friday:
   Top 10 - MqsR/MqsA: many colonies BUT RED, picked three for seq
   Top 10 - Doc/Phd : many colonies BUT RED, picked three for seq
   10B - MqsR/MqsA: no colonies
   10B - Doc/Phd: no colonies

2. Sequencing result for HicAB plasmid (2 frag assembled), HicA-3(1) in 10b is bad, no elongation -> likely primer problem (could be due to wrong preparation since lab-ready primers weren't ordered, primer seq should be fine; CK will re-order the primers)

TUESDAY, 18/06/2019

1. TOP10 competent cells CONTAMINATED --> All inoculated cultures thrown away/ABORTED
2. Send HicA-3(1) in 10B for seq again; previously failed due to ck seq primer error
3. Transformed cells
   a. Two-fragment E6K(o)-HicAB gibson mix into newly prepared **10B** (made today) **-> RESULT: NO COLONY**
   b. Doc-only gibson mix into newly prepared 10B (made today) **-> RESULT: NO COLONY**

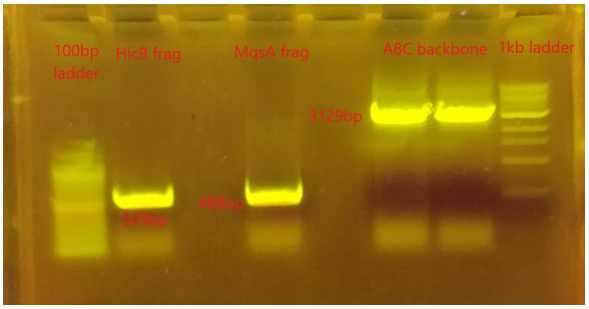
WEDNESDAY, 19/06/2019

1. Seq result for HicA-3(1) in 10b is SUCCESSFUL!

THURSDAY, 20/06/2019

1. PCR using new antitoxin primers to clone HicB and MqsA into pA8C-pBad (**chloramphenicol resistant**), fwd & rev (1) for cloning gblock, fwd & rev (2) for cloning A8C backbone -> gel electrophoresis -> gel extraction -> nanodrop -> gibson

Antitoxin only pcr fragments



All bands of correct size! Can proceed to assemble HicB and MqsA into A8c bb separately, later co-transform with HicA and MqsR plasmids to characterize toxin and antitoxin

2. Transform Doc/Phd gibson product from 4 Jun into newly made 10B, plated onto agar + **0.2% arabinose** (try to get colonies forming)

FRIDAY, 21/06/2019

1. Transformed plates:
   - 10B Doc/Phd in 0.2% arabinose agar plate --> NO COLONIES :(
   - MG1655 pE6k(o) --> MANY COLONIES -> kept in 4degC
2. Liquid culture of Top10 containing pA8C-pBad: some stored in -80degC, remaining miniprepped -> sent for seq = **BAD RESULT**
3. Transformed HicB only and MqsA only into 10B, rtp over the weekend

MONDAY, 24/06/2019

1. pA8c-HicB and pA8c-MqsA transformed into 10B **HAS MANY COLONIES**! ---> Picked 3 from each for inoculation
2. Also inoculated 3 colonies from our newly transformed pE6K(o) into new batch MG1655 (serving as ctrl for toxin characterization assay)
3. Cell culture for pA8c passed by David on Friday found to have nonsence sequence -> likely passed wrong cells to us; instead **TRANSFORMED pA8C plasmid into 10B** today.
4. Overnight PCR to clone pCon-GFP into toxin-only plasmid for HicA-3 and MqsR-2 (previously cloned in 10B), using primers originally ordered by David (check out toxin-antitoxin folder > HicA-HicB-GFP assembly > primers)

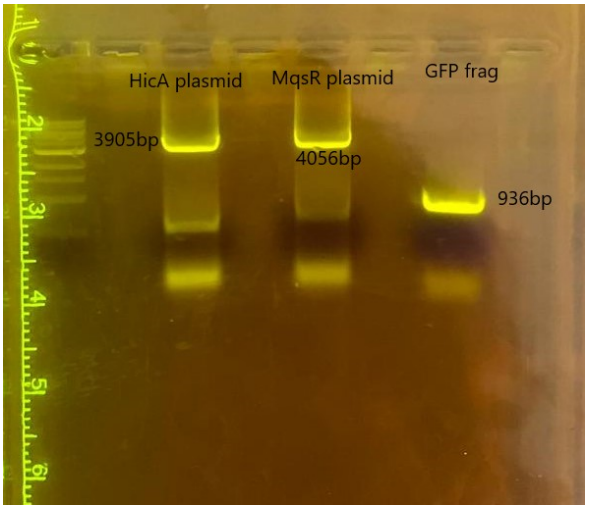
TUESDAY, 25/06/2019

1. Miniprepped antitoxin plasmids (pA8C-HicA, pA8C-MqsR) & also new MG1655 pE6K(o) -> SENT FOR SEQ
   10B MqsA **c1** - 87.3ng/uL **-> SEQ result: CHOSEN ONE; stocked in -80degC + used to co-transform (26 Jun)**
   10B MqsA c2 - 345.8ng/uL
   10B MqsA c3 - 181.6ng/uL **-> SEQ result: contain deletion = discarded**
   10B HicB c1 - 112.9ng/uL
   10B HicB c2 - 471.6ng/uL **-> SEQ result: contain deletion = discarded**
   10B HicB **c3** - 266.0ng/uL **-> SEQ result: CHOSEN ONE; stocked in -80degC + used to co-transform (26 Jun)**
   MG1655 E6K(O) c1 - 935.5ng/uL
   MG1655 E6K(O) c2 - 347.4ng/uL **-> SEQ result: contain deletion = discarded**
   MG1655 E6K(O) c3 - 537.2ng/uL

   E6K(O) seq using plac fwd and pColE rev; Antitoxin plasmid using universal pBad FP and CamR seq rev (from david)

2. Toxin-GFP cloning: Gel electrophoresis, gel extraction, nanodrop, gibson assembly, **transform into 10B** (along with two frag HicAB plasmid, retransform again)

Toxin-GFP cloning fragments



Seems to be of correct sizes

HicA - 33.9ng/uL
MqsR - 20.5ng/uL
GFP frag - 60.4ng/uL

Gibson:
HicA plasmid: 6uL HicA + 3.5uL GFP frag + 0.5uL dH2O + 10uL Gibson mix
MqsR plasmid: 6uL MqsR + 2uL GFP frag + 2uL dH2O + 10uL Gibson mix
Have colonies BUT, not fluorescing (pCon-GFP); checked back and discovered that the primer used to clone GFP out from the template did not include the first four base of promoter, David redesigned & ordered today

3. Toxin characterization preparation work: Inoculate new MG1655, MG1655 HicA-3(1), MG1655 MqsR-2(1) from -80degC into respective media (LB & LB+K), MG1655 w/ pE6K(O) inoculated by David for us

4. Overnight PCR Doc colony 4 plasmid (from -20degC) using Doc-corrected Fwd & Rev primers as well as E6k*K Fwd (1) and Rev (2)

WEDNESDAY, 26/06/2019

1. Miniprepped antitoxin pA8c c1, c2, c3 + sent for sequencing

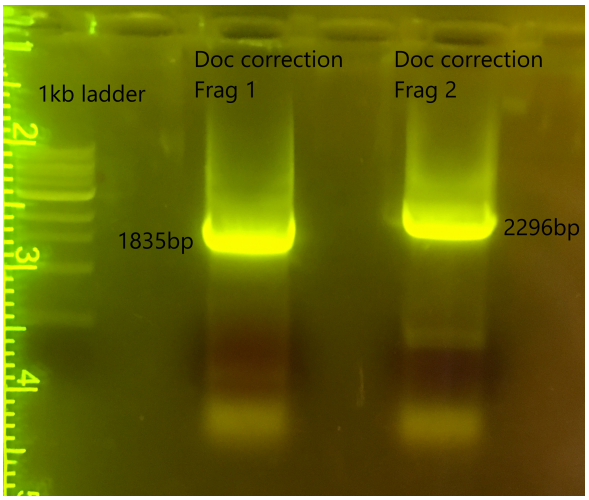
2. Antitoxin plasmid seq result came back; MqsA c1 and HicB c3 is ok -> **CO-TRANSFORMED WITH MqsR c2 and HicA c3 into:**
   - **10B**
   - **MG1655**

   PLATED ONTO K PLATE ONLY, EVEN IF COLONIES GROW, MAY NOT CONTAIN pA8C-HicB or MqsA (SEND SEQ TO COVER ANTITOXIN GENE REGION)!!! Is there a need to RE-TRANSFORM?

3. Toxin-GFP colonies not green; aborted.
   a. David redesigned and ordered new primer: GFP_Fwd_New (tomorrow 27 Jun re-run PCR)
   b. Transformed GFP plasmid into 10B -> to check if colonies fluoresce tomorrow (supposed to because pCon-GFP)

4. Doc plasmid correction: Ran gel for overnight pcr product (F1 and F2), gibson and transformed into 10B, K plate

Gel for Doc correction fragments - F1 and F2



Seemingly correct sizes

Doc pcr F1: 61ng/uL
Doc pcr F2: 56.2ng/uL

5. Toxin characterization:
   0h incubation
   0h induction
   OD600 = 0.1
   IPTG = 0mM, 100uM, 500uM, 2mM
   Used only MG1655 strains to characterize toxin
   All samples are of correct sequence/good forms
   USED DAVID'S SEQ OK MG1655 E6K(O) FOR ONE OF THE CONTROL

First DECENT toxin characterization

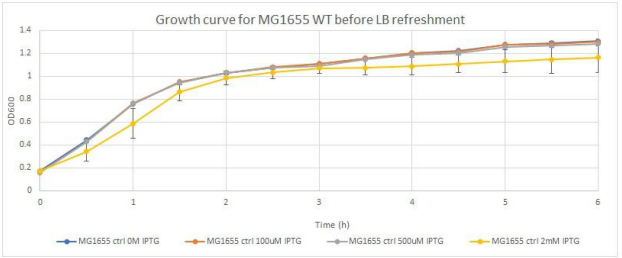
	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)
C	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3
D	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2
E												
F				100uM IPTG			500uM IPTG			2mM IPTG		
G												
H	LB blank	LB blank	LB blank									

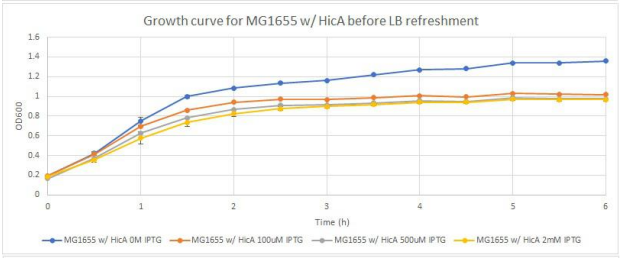
Results:

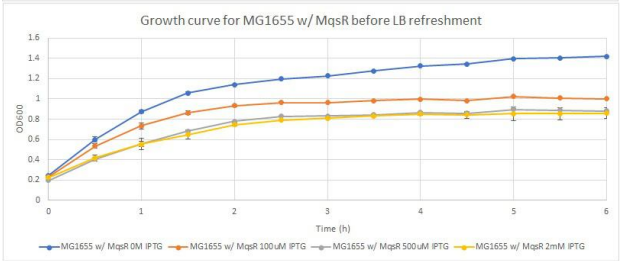
260619 New MG1655, David's MG1655 w 6h results (bef refresh) E6ko, MG1655 HicA and MG1655 Mqs R, 100uM, 500uM, 2mM IPTG.xlsx

260619 New MG1655, David's MG1655 w 12h results (aft refresh) E6ko, MG1655 HicA and MG1655 Mqs R, 100uM, 500uM, 2mM IPTG.xlsx

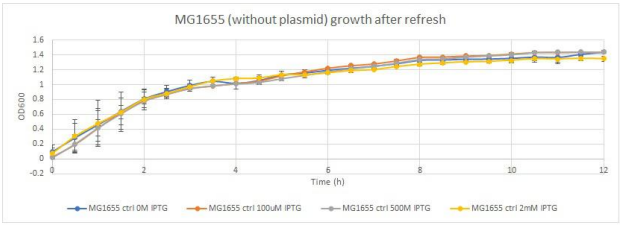
260619 (bef refreshment) 6h time point for MG1655 WT, HicA and MqsR, 0uM, 100uM, 500uM and 2mM IPTG.jpg

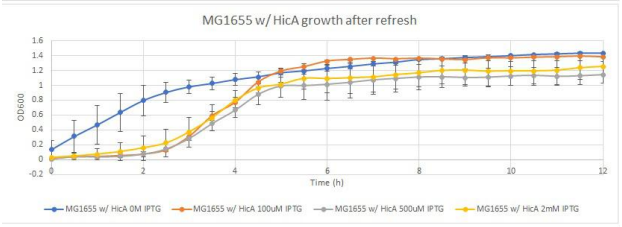


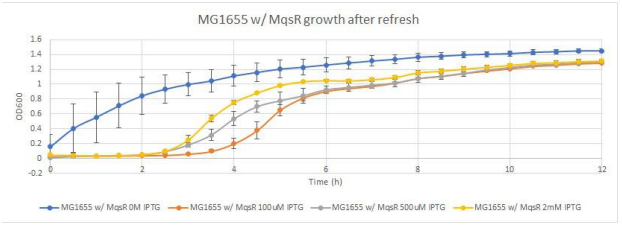




260619 (after refreshment) 12h time point for MG1655 WT, HicA and MqsR, 0uM, 100uM, 500uM and 2mM IPTG.jpg







THURSDAY, 27/06/2019

1. Cloning of GFP into toxin plasmids:
   (a) PCR-ed GFP frag w/ newly ordered primer: GFP_Fwd_New
   (b) Gel electrophoresis

(c) Gel extraction -> 88.2ng/uL
(d) Gibson to get HicA-GFP and MqsR-GFP plasmid
(e) Transformed into 10B --> **RESULT: HAVE COLONIES AND MOST ARE GREEN! (The rest seem to be pale red under blue light)**

2. Repeated co-transformation of:
(a) pE6K-HicA c3 with pA8C-HicB c3 **--> For 10B and MG1655 have colonies yay**
(b) pE6K-MqsR c2 with pA8C-MqsA c1 **--> For 10B and MG1655 have colonies yay**
INTO C+K plate

3. Inoculated 3 colonies from 10B **corrected doc** plate

FRIDAY, 28/06/2019

1. Co-transformed cells in C+K media for:
(a) pE6K-HicA c3 with pA8C-HicB c3 **--> HAVE COLONIES in 10B and MG1655 YAY**
(b) pE6K-MqsR c2 with pA8C-MqsA c1 **--> HAVE COLONIES in 10B and MG1655 YAY**

Transformed cells for toxin-GFP plasmids **--> HAVE COLONIES AND FLUORESCING YAY**

All 5 plates parafilmed and kept in 4degC

2. Miniprepped all three colonies for corrected Doc colonies -> sent for sequencing

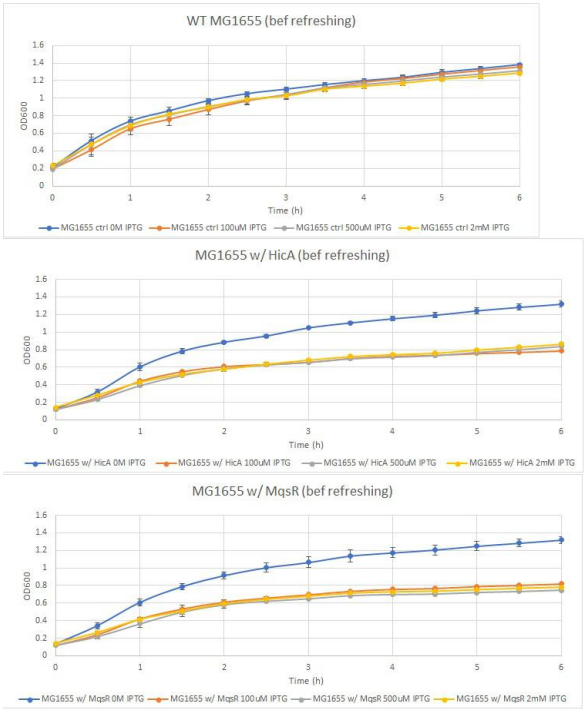
3. Second round of decent toxin characterization => **REPEAT**
0h incubation
0h induction
OD600 = **0.08** **(Difference 1: tried incubating refreshed cells (60uL in 5mL media) for 1.5 hour before standardizing O.D/inducing)**
IPTG = 0mM, 100uM, 500uM, 2mM
Used only MG1655 strains to characterize toxin
All samples are of correct sequence/good form --> MG1655 E6K (o) c3 ctrl has good seq result
Difference 2: USED OUR NEWLY TRANSFORMED MG1655 E6K(O) C3 FOR ONE OF THE CONTROL; SEQUENCING OK
After refreshing, WITH and WITHOUT induction performed!

Second round of DECENT toxin characterization (REPEAT "kinda" EXACTLY THE SAME)

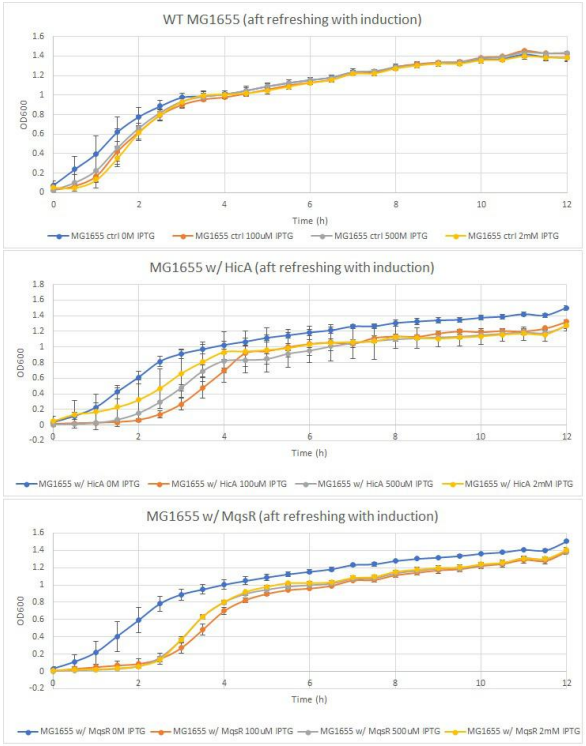
	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)	MG1655 w/ e6k(o)
C	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3
D	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2
E												
F				100uM IPTG			500uM IPTG			2mM IPTG		
G												
H	LB blank	LB blank	LB blank									

Results:

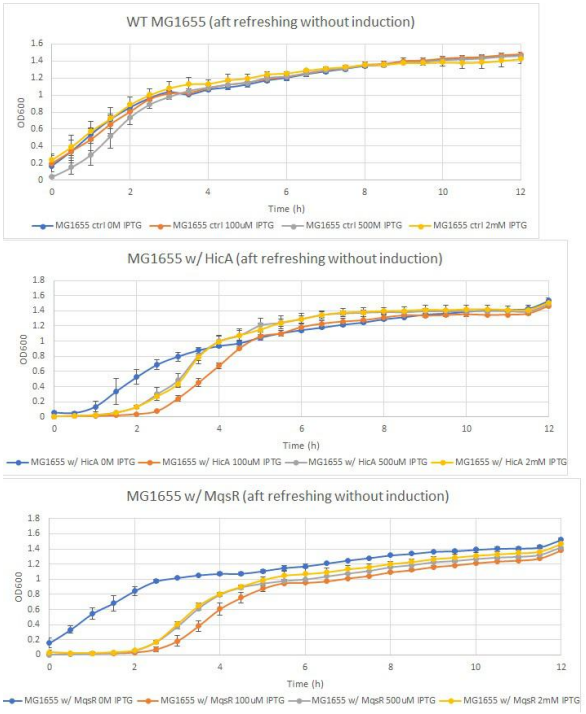
280619 (bef refreshment) 6h time point for New MG1655, MG1655 HicA and MG1655 MqsR, 0.08 OD, 100uM, 500uM, 2mM IPTG.jpg



280619 (after refreshment WITH induction) 12h time point for New MG1655, MG1655 HicA and MG1655 MqsR, 100uM, 500uM, 2mM IPTG.jpg



280619 (after refreshment WITHOUT induction) 12h time point for New MG1655, MG1655 HicA and MG1655 MqsR, 100uM, 500uM, 2mM IPTG.jpg



280619 6h results for New MG1655, MG1655 HicA and MG1655 MqsR, 0.08 OD to plateau (bef refresh), 100uM, 500uM, 2mM IPTG.xlsx

280619 12h results for New MG1655, MG1655 HicA and MG1655 MqsR, (aft refresh WITH induction), 100uM, 500uM, 2mM IPTG.xlsx

280619 12h results for New MG1655, MG1655 HicA and MG1655 MqsR, (aft refresh WITHOUT induction), 100uM, 500uM, 2mM IPTG.xlsx

MONDAY, 01/07/2019

1. Inoculated:
   one colony from 10B and MG1655 co-transformed with HicA/HicB and MqsR/MqsA respectively (in LB+CK media)
   two colonies from corrected Doc - colony 4 and 5 (since colony 1, 2 and 3 seq result still contain TAG mutation)
   three colonies from HicA-GFP and MqsR-GFP in 10B

TUESDAY, 02/07/2019

1. Miniprepped all 12 plasmid samples from yesterday --> sent for sequencing
2. Transformed HicA-GFP c1 and c2, MqsR-GFP c1 and c2 and also David's pBR322-pCon-GFP into MG1655 (for toxin-GFP characterization on thursday)

WEDNESDAY, 03/07/2019

1. Inoculated MG1655: HicA-GFP c2, MqsR-GFP c2 and pBR322-pCon-GFP in LB+K overnight

2. Stocked HicA-GFP and MqsR-GFP colonies (seq ok) in -80degC

THURSDAY, 04/07/2019

1. Stocked both 10B and MG1655 HicA/HicB and MqsR/MqsA co-transformed in -80degC (Seq ok)
2. Inoculated WT MG1655, MG1655 HicA-3(1) and MG1655 MqsR-2(1) from glycerol stock into LB and LB+K respectively -> for tomorrow's toxin-characterization (using <100uM IPTG).
3. First round of decent **toxin-GFP characterization**
   Key question: What is the effect of toxin on GFP expression?
   0h incubation
   0h induction
   OD600 = **0.1**
   IPTG = 0mM, 100uM, 500uM, 2mM (To see if GFP trend is different from OD for diff inducer conc)
   Used only MG1655 strains to characterize toxin
   All three samples seq ___

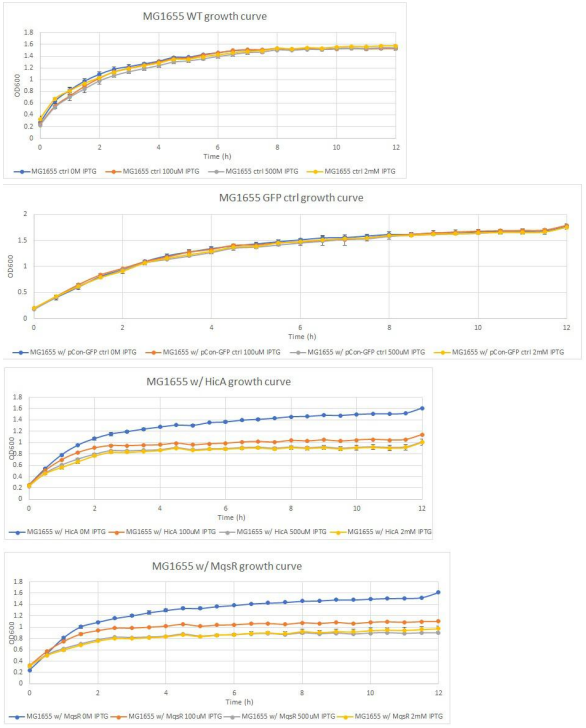
First round of Toxin-GFP characterization

	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP	MG1655 w/ pCon-GFP
C	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP	MG1655 HicA-3-GFP
D	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP	MG1655 MqsR-2-GFP
E										LB blank	LB blank	LB blank
F				100uM IPTG			500uM IPTG			2mM IPTG		
G												
H												

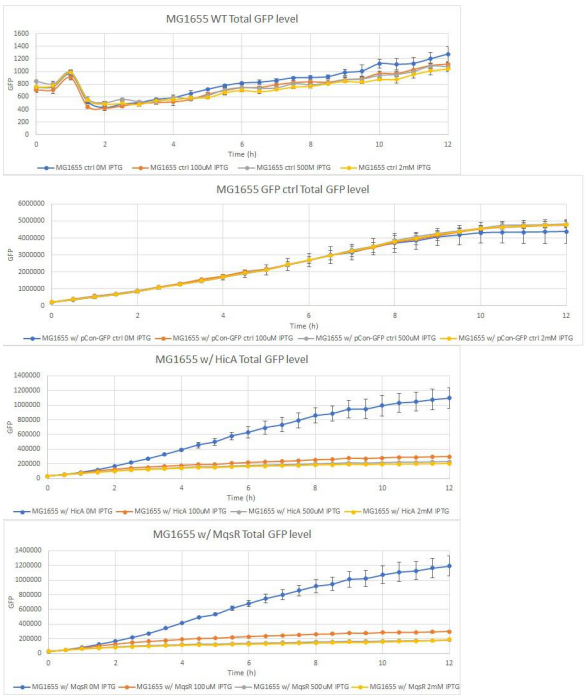
Results:

070419 12h read for New MG1655, pBR322 GFP ctrl, MG1655 HicA, MG1655 MqsR, 0.1 OD, 100uM, 500uM, 2mM, OD600 and GFP.xlsx

070419 12h OD600 read for New MG1655, pBR322 GFP ctrl, MG1655 HicA, MG1655 MqsR, 0.1 OD, 100uM, 500uM, 2mM, OD600 and GFP.jpg

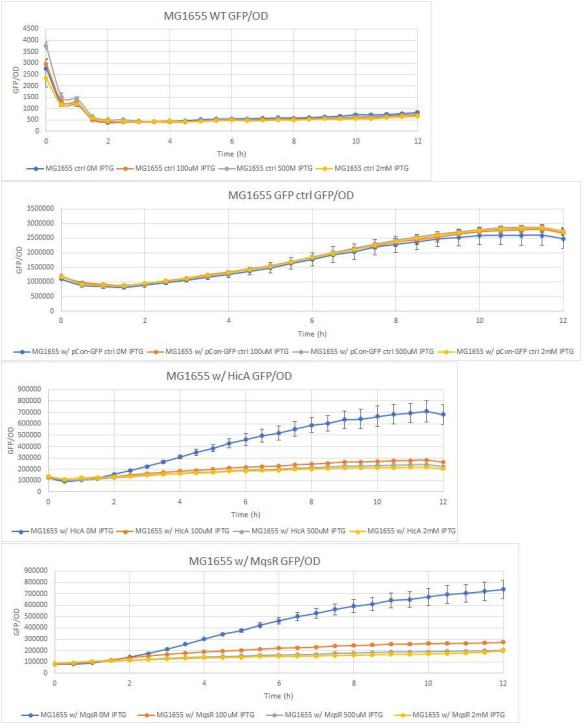


070419 12h Total GFP read for New MG1655, pBR322 GFP ctrl, MG1655 HicA, MG1655 MqsR, 0.1 OD, 100uM, 500uM, 2mM, OD600 and GFP.jpg



070419 12h Total GFP read for non-toxin containing controls, 0.1 OD, 100uM, 500uM, 2mM, OD 600 and GFP.jpg

070419 12h Average GFP read for New MG1655, pBR322 GFP ctrl, MG1655 HicA, MG1655 MqsR, 0.1 OD, 100uM, 500uM, 2mM, OD600 and GFP.jpg



FRIDAY, 05/07/2019

1. Stored 4degC, **miniprep and seq**:
   - MG1655 pBR322-pCon-GFP
   - MG1655 HicA-3-GFP C2(1)
   - MG1655 MqsR-2-GFP C2(1)

2. Toxin characterization (10uM, 25uM, 50uM, 75uM, 100uM, 500uM IPTG)
   Key question: Whether lower inducer conc (mainly <100uM) result in shorter lag phase after refreshing
   0h incubation
   0h induction
   OD600 = **0.1**
   IPTG = 0mM, 10uM, 25uM, 50uM, 75uM, 100uM, 500uM (To see if GFP trend is different from OD for diff inducer conc)
   Used only MG1655 strains to characterize toxin
   All three samples seq OK!

After refreshing, WITH induction performed!

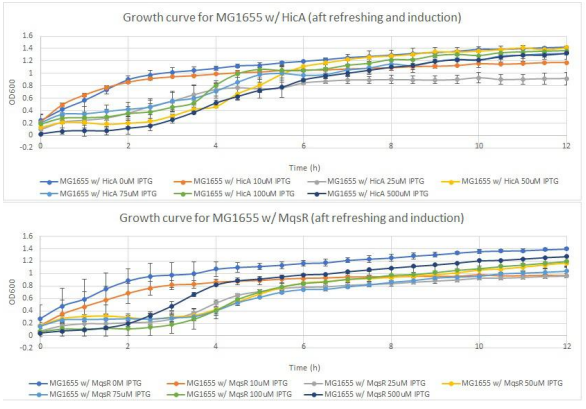
Third round of toxin characterization (mainly <100uM IPTG)

	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3
C	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2
D	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl			
E	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3	MG1655 HicA-3			
F	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2	MG1655 MqsR-2			
G												
H	LB blank	LB blank	LB blank	10uM IPTG	25uM IPTG	50uM IPTG	75uM IPTG	100uM IPTG	500uM IPTG			

Results:

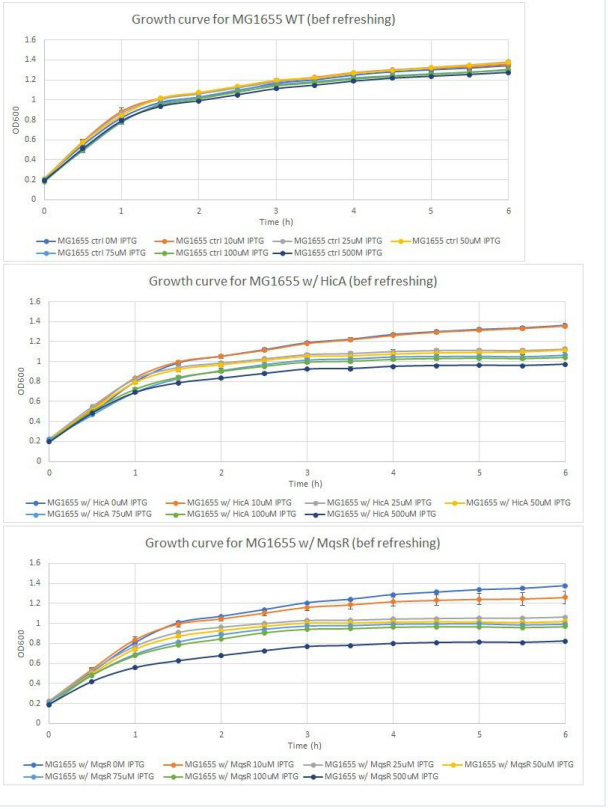
070519 12h results AFTer induction for New MG 1655, MG1655 HicA and MG1655 MqsR, 10uM, 25 uM, 50uM, 75uM, 100uM, 500uM.xlsx

070519 12h results AFT induction for MG1655 Hi cA and MG1655 MqsR, 10uM, 25uM, 50uM, 75u M, 100uM, 500uM.jpg



070519 6h results BEF induction for New MG165 5, MG1655 HicA and MG1655 MqsR, 10uM, 25uM, 50uM, 75uM, 100uM, 500uM.xlsx

070519 6h results BEF induction for New MG1655, MG1655 HicA and MG1655 MqsR, 10uM, 25uM, 50uM, 75uM, 100uM, 500uM.jpg



MONDAY, 08/07/2019

1. Inoculated from -80degC:
   - WT MG1655
   - MG1655 w/ HicA
   - MG1655 w/ MqsR
   - MG1655 co-transformed w/ HicA and HicB
   - MG1655 co-transformed w/ MqsR and MqsA

   into LB, LB+K and LB+KC respectively, for tomorrow's antitoxin characterization

TUESDAY, 09/07/2019

1. First antitoxin characterization:
   Key question: Whether cell growth is faster (steeper gradient and higher max OD) in cells treated with both antitoxin and toxin vs cells treated with toxin only
   OD600 = **0.1**
   IPTG = 0mM, 10uM, 25uM, 50uM, 75uM, 100uM, 500uM (To see if GFP trend is different from OD for diff inducer conc)
   Used only MG1655 strains to characterize toxin
   All samples seq OK!
   No refreshing performed

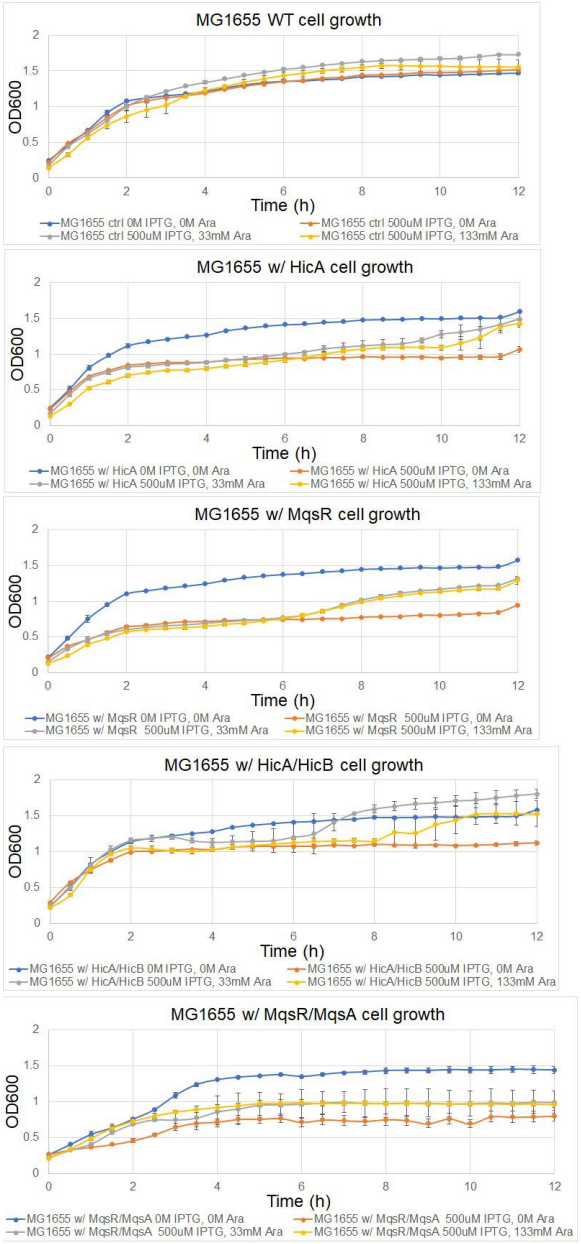
FIRST antitoxin characterization

	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA	MG1655 HicA
C	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR	MG1655 MqsR
D	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB
E	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA
F	0M IPTG 0M ara			500uM IPTG 0M ara			500uM IPTG 33mM Ara			500uM IPTG 133mM Ara		
G												
H	LB blank	LB blank	LB blank									

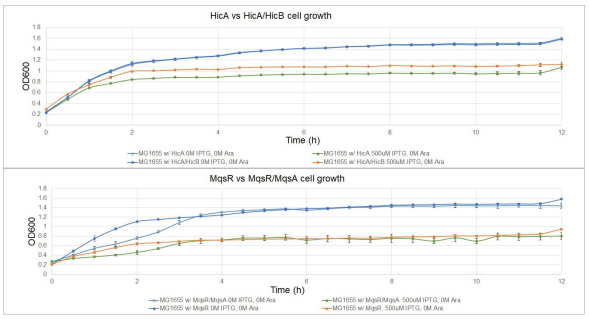
Results:

070919 12h antitoxin results for New MG1655, MG1655 w HicA, MG1655 w MqsR, MG1655 w HicA HicB, MG1655 w MqsRMqsA, OD0.1.xlsx

070919 12h antitoxin results for New MG1655, MG1655 w HicA, MG1655 w MqsR, MG1655 w HicA HicB, MG1655 w MqsRMqsA, OD0.1.jpg



070919 Effect of extra antitoxin gene block on cell growth.jpg



FRIDAY, 12/07/2019

1. Transformed MqsR only, MqsR and MqsA, MqsR-GFP only, MqsR-GFP and MqsA into MG1655-MqsR/A k.o competent cells (prepared by Chun yang yesterday)
2. Second antitoxin characterization:
   Key question: Finding the optimal arabinose conc and the effect on cell growth.
   OD600 = **0.1**
   IPTG = 500uM
   Arabinose = 0.0266M, 0.133M, 0.266M
   Used only MG1655 strains to characterize toxin
   All samples seq OK!
   No refreshing performed

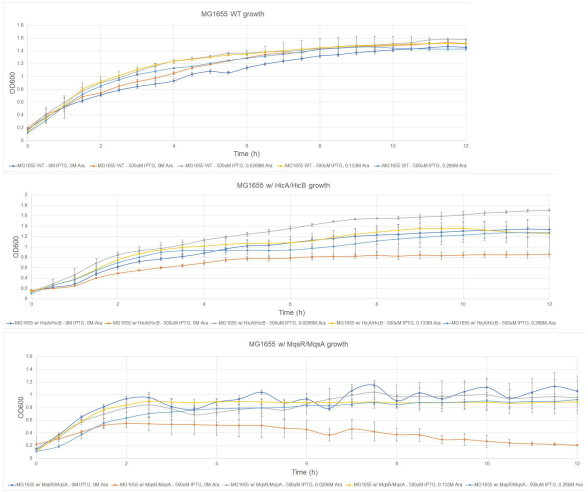
Second antitoxin characterization

	1	2	3	4	5	6	7	8	9	10	11	12
A	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
B	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB
C	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA
D	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl	MG1655 ctrl
E	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB	MG1655 HicA/HicB
F	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA	MG1655 MqsR/MqsA
G						**A-C: With 100uM IPTG**			**D-F: Without IPTG**			
H	LB blank	LB blank	LB blank		0.0266M Ara			0.133M Ara			0.266M Ara	

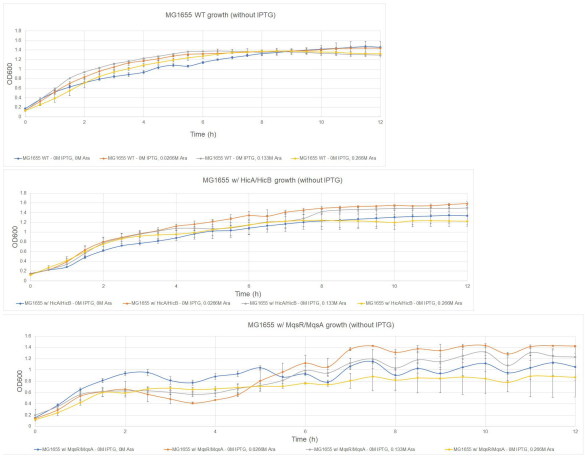
Results:

071219 12h results New MG1655, MG1655 HicAHicB, MG1655 MqsRMqsA, 500uM IPTG vs 0M IPTG, 0.0266M Ara 0.133M Ara 0.266M Ara, 0.1OD.xlsx

071219 12h results New MG1655, MG1655 HicAHicB, MG1655 MqsRMqsA, 500uM IPTG, 0M Ara 0.0266M Ara 0.133M Ara 0.266M Ara.jpg



071219 12h results New MG1655, MG1655 HicAHicB, MG1655 MqsRMqsA, 0M IPTG, 0M Ara 0.0266M Ara 0.133M Ara 0.266M Ara (effect of Ara).jpg



MONDAY, 15/07/2019

1. Inoculated the only few colonies in MqsRA k.o MG1655 transformed with E6K-MqsR in LB+K
2. Inoculated MG1655 WT, RES-CmR slab, HicAB k.o (no antibiotic resistance) into LB, LB + C and LB respectively
3. Re-transformed (a) MqsR-GFP and MqsA and (b) MqsR and MqsA into MG1655 k.o strain = **NO COLONIES AT ALL.**

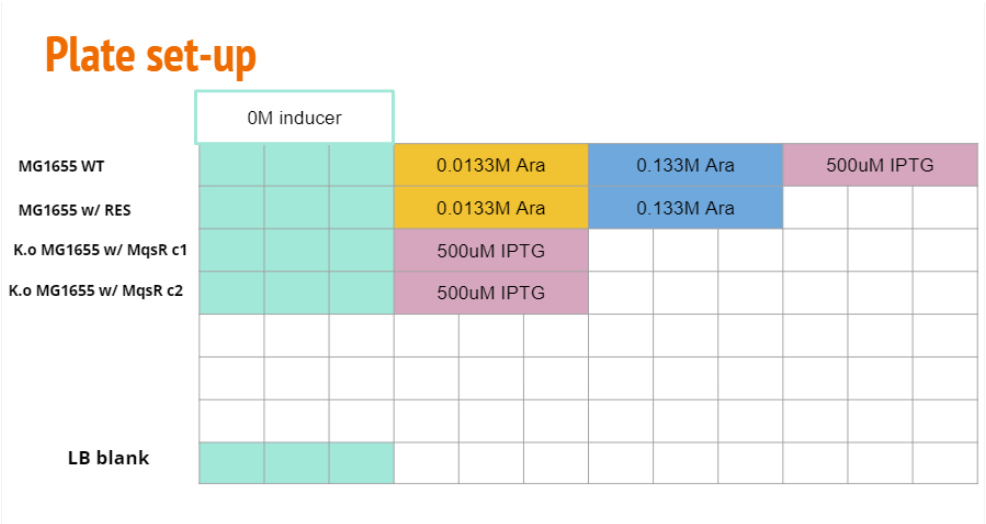
TUESDAY, 16/07/2019

1. Stored native MG1655 w/ RES (cmR) and k.o MG1655 w/ E6K-MqsR c1 and c2 in 4 degC -> send for seq
2. Stored HicAB k.o MG1655 strain in -80degC, leftover make competent cells
3. Inoculated:
    - MG1655 WT
    - MG1655 pCon-GFP
    - MG1655 E2K-SgrS c2
    - MG1655 E2K-SgrS-GFP c1

4. Transform MqsR-GFP into k.o MG1655 competent strain
   Transform HicA-GFP and HicA-GFP/HicB into k.o MG1655 competent strain

5. FIRST (a) **RES** and (b) **MqsR in k.o strain** characterization:
   Key question: What is the extent of growth arrest by (a) orthologous TA system and (b) in a k.o strain?
   OD600 = **0.1**
   IPTG (MqsR toxin inducer) = 500uM
   Arabinose (RES toxin inducer) = 0.0133M, 0.133M
   Used only MG1655 strains to characterize toxin
   RES sample seq acceptable (few point mutations)
   MqsR c1 and c2 seq FAIL!!!
   No refreshing performed

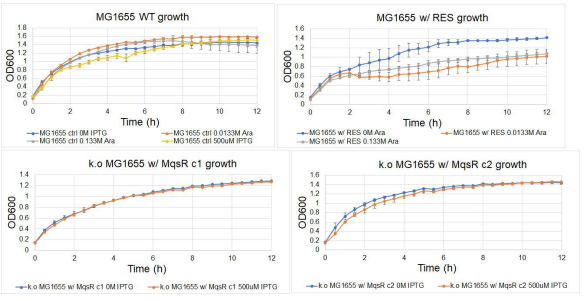
plate set-up



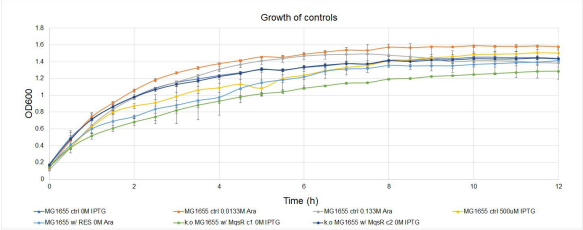
Results:

071619 12h results for New MG1655, MG1655 w RES, ko MG1655 w MqsR c1, ko MG1655 w MqsR c2, OD0.1, 0.0133M Ara and 0.133M Ara, 500uM IPTG.xlsx

071619 12h results for New MG1655, MG1655 w RES, ko MG1655 w MqsR c1, ko MG1655 w MqsR c2, OD0.1, 0.0133M Ara and 0.133M Ara, 500uM IPTG.jpg



071619 12h control growth results for New MG1655, MG1655 w RES, ko MG1655 w MqsR c1, ko MG1655 w MqsR c2, OD0.1, 0.0133M Ara and 0.133M Ara, 500uM IPTG.jpg



WEDNESDAY, 17/07/2019

1. Re-transform HicA-GFP into MG1655 k.o strain = HAVE COLONIES AND ARE GREEN YAY

THURSDAY, 18/07/2019

1. Inoculated 3 GREEN colonies into LB+K - HicA-GFP c1, c2, c3 (all in ko MG1655)
2. Inoculated WT MG1655 and MG1655 pBR322-GFP cells in LB and LB+K (tomorrow characterize HicA-GFP in ko strain)
3. Prepared RES competent MG1655
4. Stored 4degC and miniprepped Xre + seq (with RES too)
5. Transform Xre into RES competent MG1655 -> CA plate
6. Transform iLux pQE and iLux pGEX into 10B (C plate and A plate respectively)
7. Second **RES** characterization:
   Key question: Pattern of growth arrest/lag phase by orthologous RES after refreshing + which Ara conc is optimal
   OD600 = **0.1**
   Arabinose (RES toxin inducer) = 0.1%, 0.2%, 0.5%, 1%, 2%
   Used only MG1655 strains to characterize toxin
   All samples seq ok (except RES with two point mutations in RES gene)
   WITH refreshing performed

Plate set-up

Plate set-up

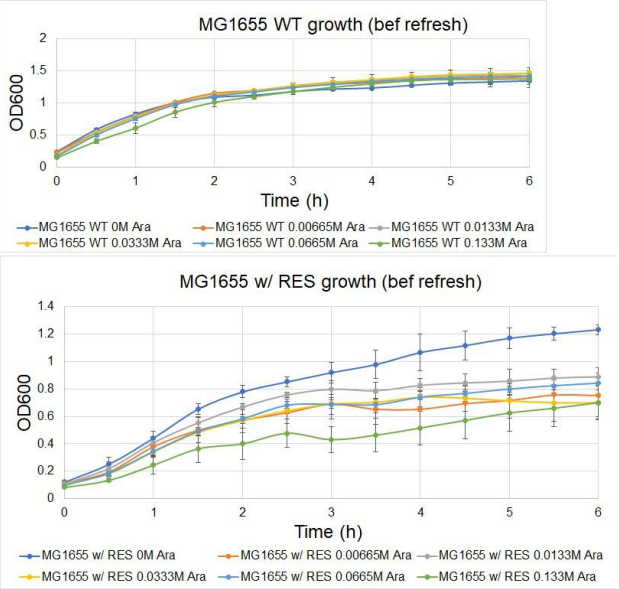
MG1655 WT	0% Ara						0.1% Ara				
MG1655 w/ RES	0% Ara						0.1% Ara				
MG1655 WT	0.2% Ara						0.5% Ara				
MG1655 w/ RES	0.2% Ara						0.5% Ara				
MG1655 WT	1% Ara						2% Ara				
MG1655 w/ RES	1% Ara						2% Ara				
LB blank											

Results:

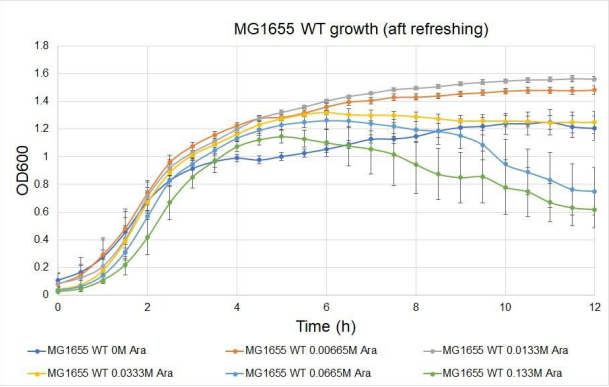
071819 6h results bef refreshing New MG1655 and native MG1655 RES, 0, 0.1, 0.2, 0.5, 1, 2% Ara, 0.1OD.xlsx

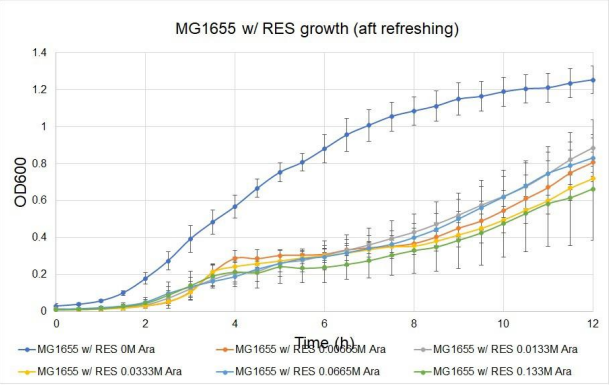
071819 12h results aft refreshing New MG1655 and native MG1655 RES, 0, 0.1, 0.2, 0.5, 1, 2% Ara, 0.1OD.xlsx

071819 12h OD600 results bef refreshing New MG1655 and native MG1655 RES, 0, 0.1, 0.2, 0.5, 1, 2% Ara, 0.1OD.jpg



071819 12h OD600 results aft refreshing New MG1655 and native MG1655 RES, 0, 0.1, 0.2, 0.5, 1, 2% Ara, 0.1OD.jpg

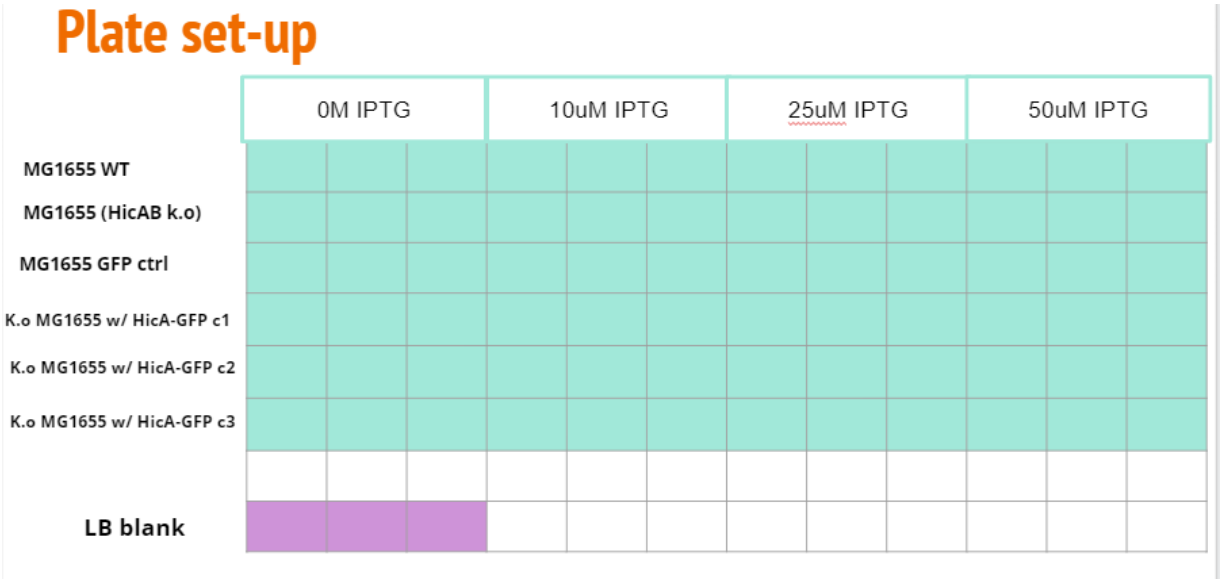




FRIDAY, 19/07/2019

1. Re-transformed Xre into RES competent, HicB into HicAB ko MG1655 and MqsA into MqsRA ko MG1655
2. Transform HicB into newly made HicA-GFP c1, c2 and c3 competent MG1655 (endogenos HicAB ko)
3. First characterization of HicA-GFP in k.o strain
   OD600 = **0.1**
   IPTG = 10uM, 25uM, 50uM
   Used only MG1655 strains to characterize toxin
   All samples seq _____
   NO refreshing performed

Plate layout

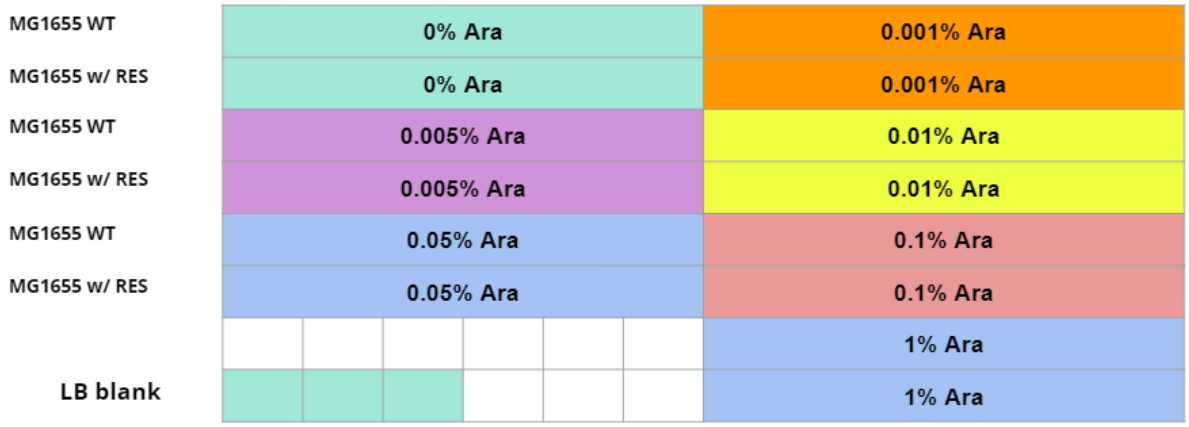


RESULTS: ABORTED BECAUSE SEQ RESULT SHOWS C1-C3 HAVE NO HICA GENE

TUESDAY, 23/07/2019

1. Stored in 4degC, miniprepped and sent HicA-GFP c4, c5, c6, c7, c8 (inoculated from plate yesterday) for sequencing
2. Make competent cells for Villu's ko MqsRA, Brodersen's ko HicAB, RES and Xre (all from valve sent over)
3. RES characterization (FIRST hourly single reading)
   OD600 = **0.1**
   Arabinose (RES toxin inducer) = 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 1%
   Used only MG1655 strains to characterize toxin
   All samples seq ok (except RES with two point mutations in RES gene)
   NO refreshing performed

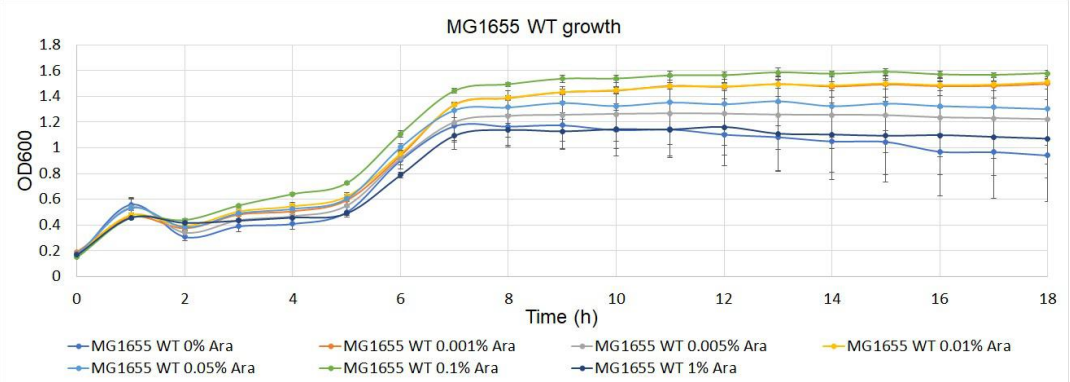
Plate layout

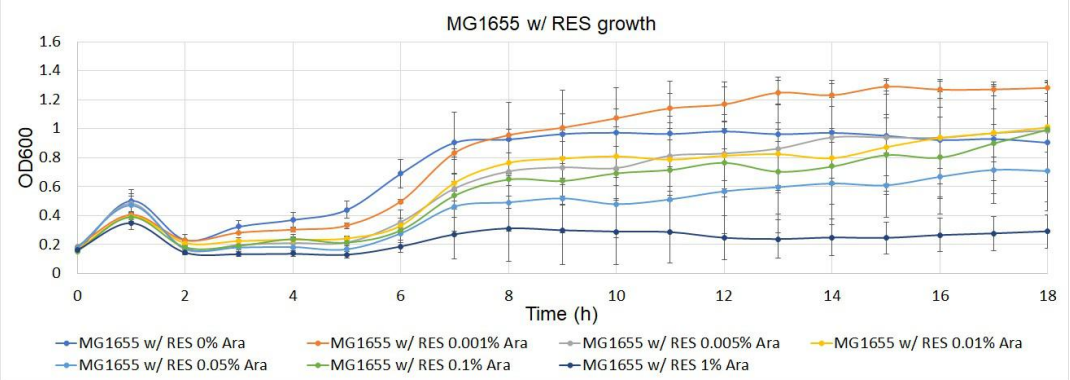


Results:

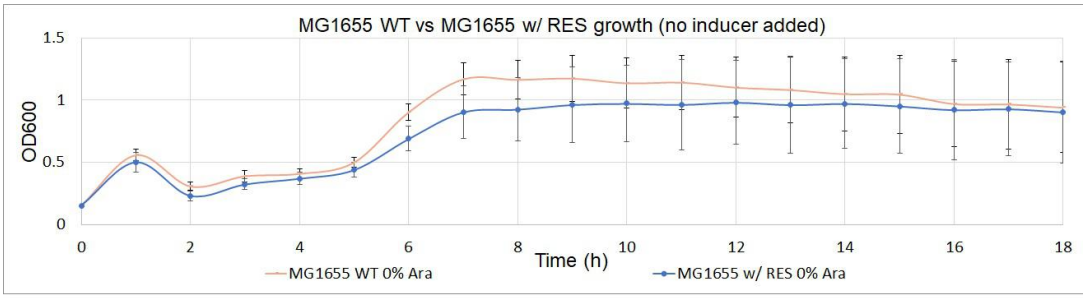
072319 6+12h results for WT MG1655 and pBad RES MG1655, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 1% Ara, single time point plus 12h cont read.xlsx

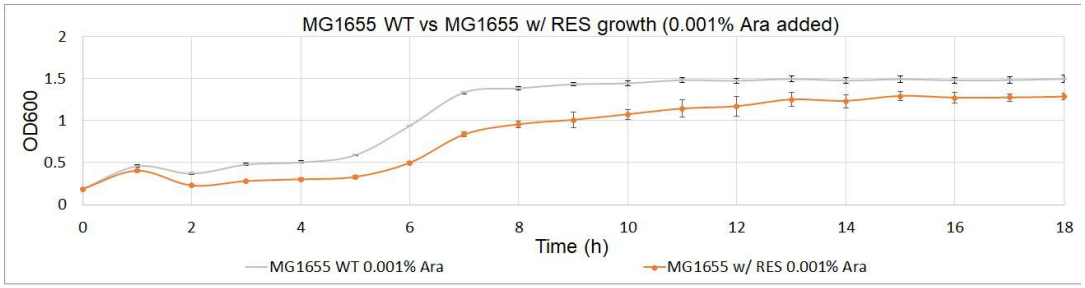
072319 18h results for WT MG1655 and pBad RES MG1655, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 1% Ara, single time point plus 12h cont read.jpg

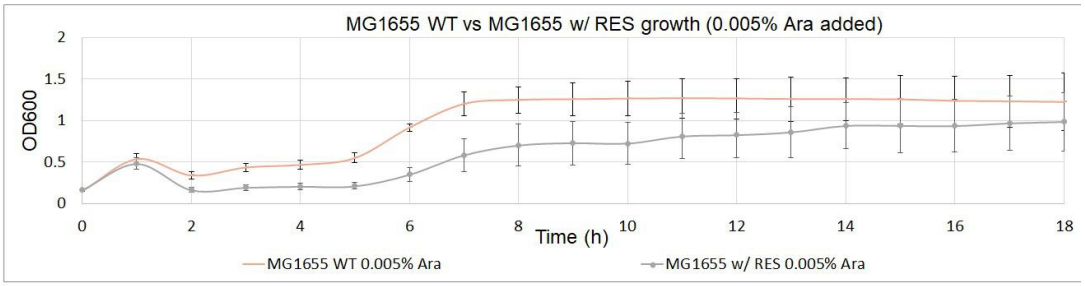


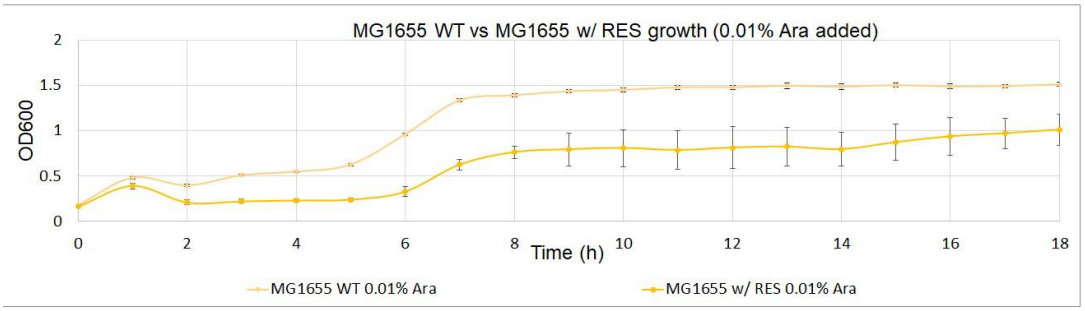


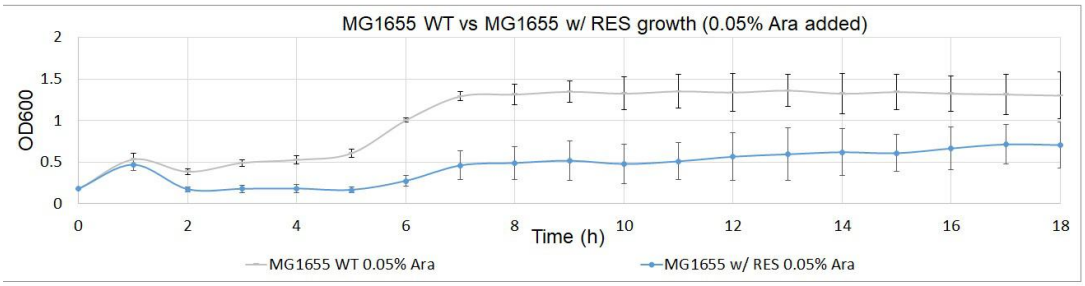
072319 18h results for WT MG1655 VS pBad RES MG1655, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 1% Ara, single time point plus 12h cont read.jpg

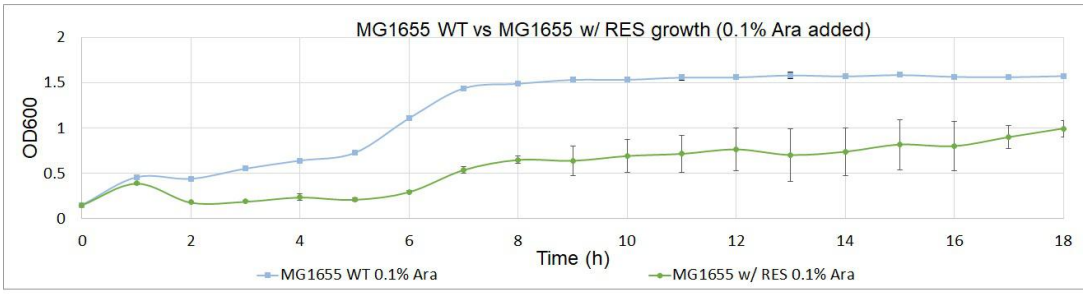

MG1655 WT vs MG1655 w/ RES growth (no inducer added)
OD600
Time (h)
MG1655 WT 0% Ara
MG1655 w/ RES 0% Ara

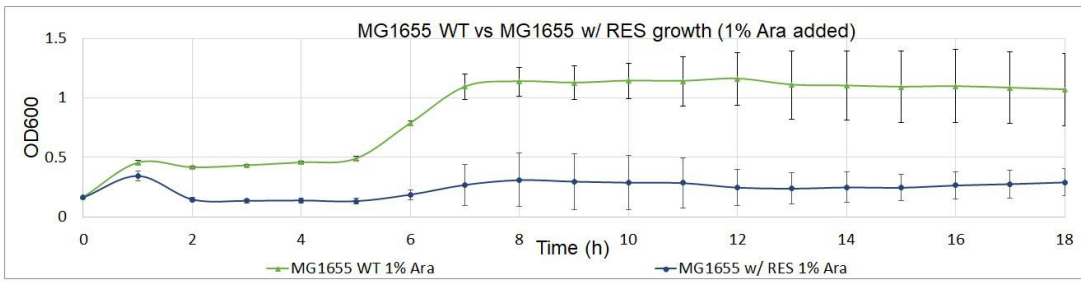

MG1655 WT vs MG1655 w/ RES growth (0.001% Ara added)
OD600
Time (h)
MG1655 WT 0.001% Ara
MG1655 w/ RES 0.001% Ara


MG1655 WT vs MG1655 w/ RES growth (0.005% Ara added)
OD600
Time (h)
MG1655 WT 0.005% Ara
MG1655 w/ RES 0.005% Ara


MG1655 WT vs MG1655 w/ RES growth (0.01% Ara added)
OD600
Time (h)
MG1655 WT 0.01% Ara
MG1655 w/ RES 0.01% Ara


MG1655 WT vs MG1655 w/ RES growth (0.05% Ara added)
OD600
Time (h)
MG1655 WT 0.05% Ara
MG1655 w/ RES 0.05% Ara


MG1655 WT vs MG1655 w/ RES growth (0.1% Ara added)
OD600
Time (h)
MG1655 WT 0.1% Ara
MG1655 w/ RES 0.1% Ara


MG1655 WT vs MG1655 w/ RES growth (1% Ara added)
OD600
Time (h)
MG1655 WT 1% Ara
MG1655 w/ RES 1% Ara

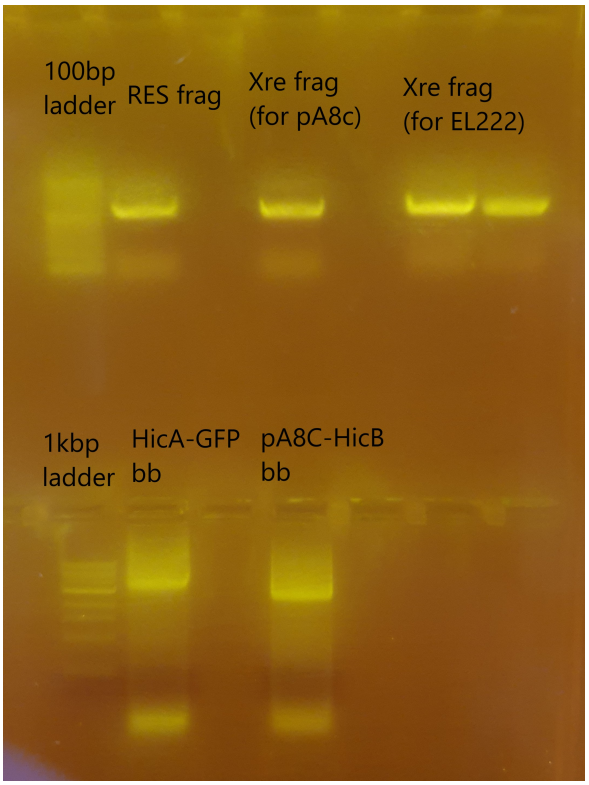
Variability was due to one or two replicates out of six replicates

WEDNESDAY, 24/07/2019

1. Inoculated 2 colonies from Res in Xre cells (RX c1, c2) and Xre in Res cells (XR c1, c2)
2. Re-streaked brodersen's hicAB ko strain transformed with HicB plasmid because of overcrowding
3. Seq iLux pGEX and pQE with both iLuxC_Rev, iLux_AmpR rev
4. Inoculated 10B pA8C HicB c3 and 10B pA8C MqsA c1 from -80degC (to miniprep and restock plasmids)
5. Prepared competent cells for Villu's MqsRA k.o and HicAB k.o MG1655, then co-transformed MqsR-GFP/MqsA and HicA-GFP/HicB respectively (C+K plate)
6. PCR -> gel/extract -> gibson -> transformed (C and K plate respectively) for:
    - E6K RES-GFP plasmid
    - A8C-Xre plasmid

   (standardizing TA systems)

Gel photo



~500bp for all three insert fragments, 4000+bp and 3000+bp for HicA-GFP bb and pA8C-HicB bb respectively

7. Inoculated WT MG1655 and MG1655 GFP-ctrl from -80degC for characterization tomorrow (in case)

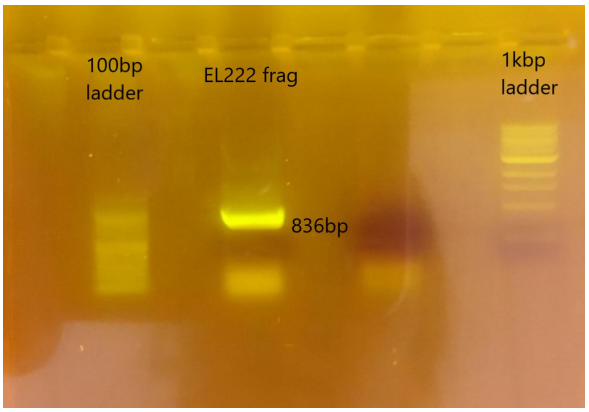
THURSDAY, 25/07/2019

1. Stored + miniprep + send for seq:
    - 10B pA8C-HicB c3 (not stored because already have stock in -80degC)
    - 10B pA8C-MqsA c1 (not stored because already have stock in -80degC)
    - Res in Xre (RX) c1
    - Res in Xre (RX) c2
    - Xre in Res (XR) c1
    - Xre in Res (XR) c2
2. Inoculated colonies:
    - Brodersen HicAB k.o - HicB plasmid (streaked plate) - c1

- Villu HicAB k.o- HicA-GFP and HicB - c1 and c2
- 10B RES-GFP c1, c2, c3
- 10B A8c-Xre c1, c2, c3

3. Blue light construct:
   a. EL222 frag
   b. A8C (EL222) bb

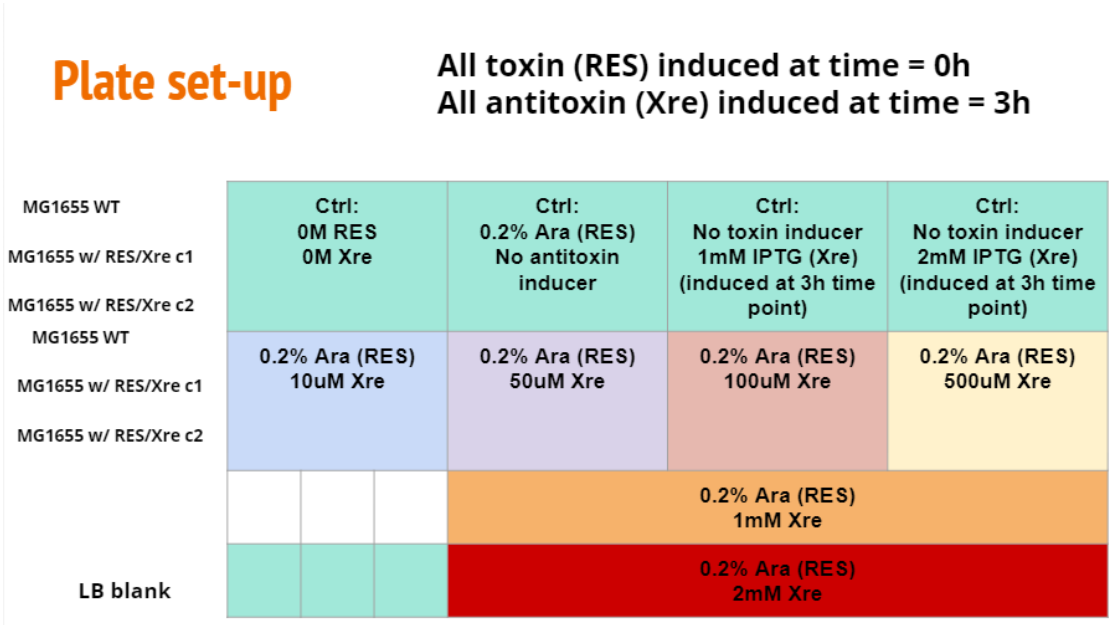
Gel photo



First PCR run, EL222 correct band size but A8c bb (2412bp) is not present. Note the A8C-HicB template for c3 ran out so we took the newly miniprepped c3 from -80degC (not seq yet tho); RE-RUN pcr for A8c

4. First RES/XRE characterization (co-transformed but seq unknown yet)
   Brodersen plasmid- pBad-RES-plac-XRE
   Single hourly reading for 8h then 12h continuous overnight
   Ara inducer: 0.2%
   IPTG: 0.01mM, 0.05mM, 0.1mM, 0.5mM, 1mM, 2mM
   Both RX c1 and XR c1 seq acceptable (perfect Xre gene, some point mutations in Res but acceptable)

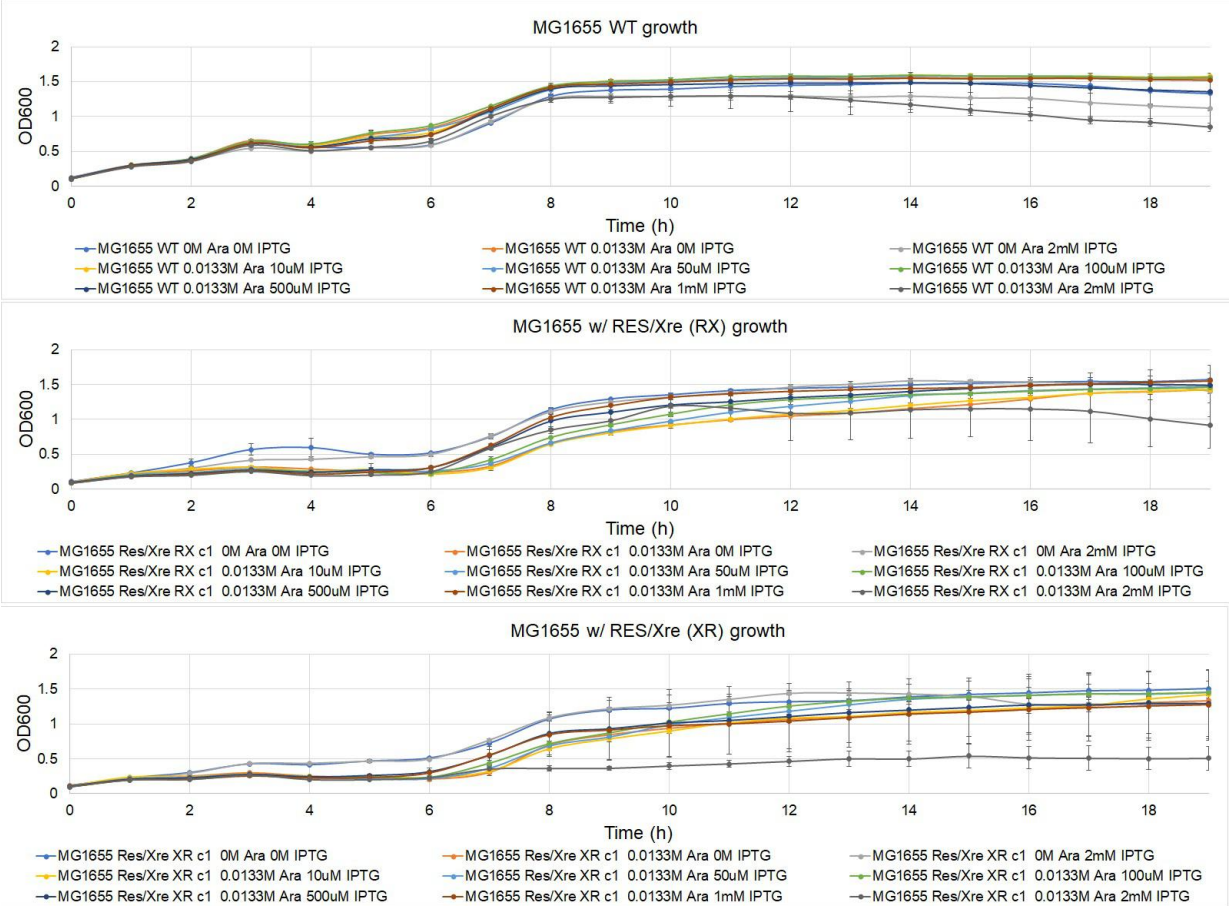
Plate set-up



Results:

072519 6h+12h results for RES-Xre single read hourly, 0h 0.2% Ara induction, 3h 0.01 0.05 0.1 0.5 1 2mM IPTG, OD0.1.xlsx

072519 6h+12h OD600 results for RES-Xre single read hourly, 0h 0.2% Ara induction, 3h 0.01 0.05 0.1 0.5 1 2mM IPTG, OD0.1.jpg



Variability mainly due to one of the three replicates

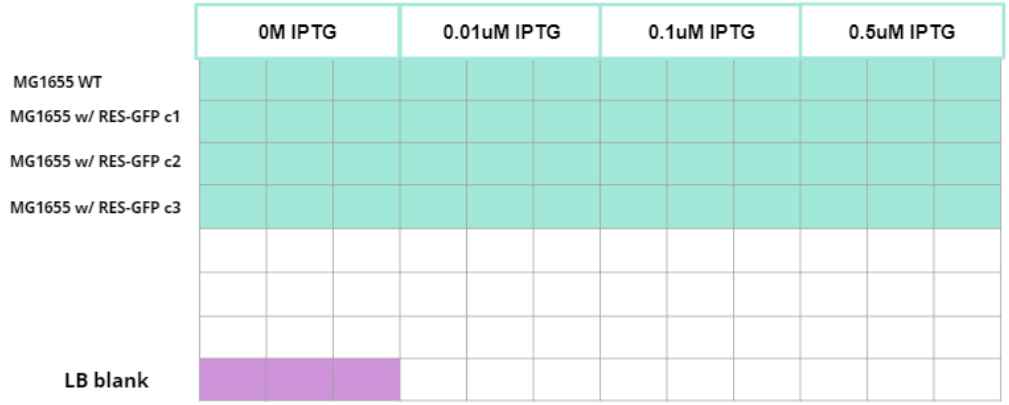
FRIDAY, 26/07/2019

1. Stored, miniprep and seq:
   - 10B RES-GFP C1
   - 10B RES-GFP C2
   - 10B RES-GFP C3
   - 10B A8C-Xre C1
   - 10B A8C-Xre C2
   - 10B A8C-Xre C3
   - Brodersen hicAB ko - w/ HicB c1
   - Villu hicAB ko- w/ HicA-GFP and HicB c1
   - Villu hicAB ko- w/ HicA-GFP and HicB c2
2. Gel extracted EL222 fragment from yesterday (note: A8c bb frag still failed)
3. Characterized pLac-RES-GFP (c1, c2, c3) for 9h (0h to 8h time point)
   10B strain
   Used lower IPTG conc (since its not found in native strain)
   Objective:
   a. visualize effect of toxin on GFP

b. effect of IPTG on growth vs Ara (past data)

Sequence _____

image.png



ABORTED BECAUSE ADDED WRONG CONC; SHOULD BE mM NOT uM

4. Characterized HicA-GFP and HicB (c1, c2, c3) in villu HicAB ko MG1655

Objective:

a. study effect in ko strain

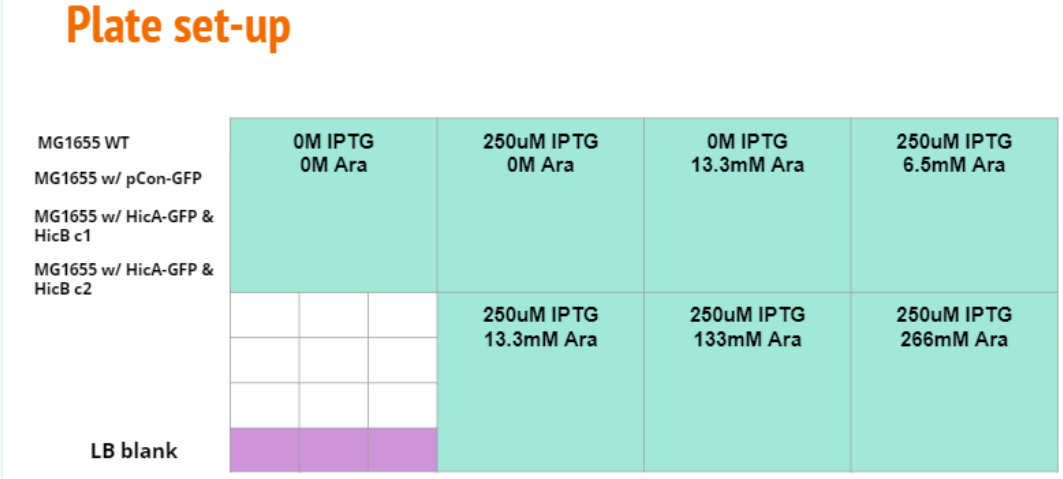
b. study effect on GFP and growth

Used lower IPTG conc (since its not found in native strain, shouldn't have AT interfering)

First induced toxin at 0h then induce antitoxin at 3h time point

Sequence not ok. C1 and C2 have perfect HicA-GFP seq result but POOR HicB seq result.

image.png



Results:

Link:

https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g5e1275b8ed_0_26

MONDAY, 29/07/2019

1. **Blue light construction:** Re-run PCR for EL222-A8c bb frag with A8c-EL222 fwd/rev primer, A8c-HicB c1 plasmid (72degC, 1min20s) -> failed no band :((faint band but negative conc)

TUESDAY, 30/07/2019

1. Re-ran PCR for plasmid construction of:
   - pLac-RES-GFP
   - pBad-EL222-HicB

   PCR used old primer pair for RES-GFP (used correct Tm this time) and new primer pairs ordered for EL222-HicB plasmid

2. Re-transformed villu HicAB MG1655 k.o strain with:
   - HicA-GFP c2 and A8c-HicB c1
   - HicA-GFP c2 and A8c-HicB c3

3. Transformed 10B with HicA-GFP c2 (make more copies)

4. Inoculated c3 from villu's HicAB knockout MG1655-HicA-GFP & HicB

5. Overnight PCR for RES frag (repeat same 67.5degC Tm)

6. (Brodersen's) RES/Xre characterization:
   MG1655 strain
   XR c2 (seq ok)
   18h continuous run
   0h toxin induction (ara); 3h antitoxin induction (iptg)

Plate layout

MG1655 WT MG1655 w/ RES/Xre XR c2	0M Ara 0M IPTG	0.0133M Ara 0M IPTG	0.0665M Ara 0M IPTG	0M Ara 2mM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	0.0133M Ara 50uM IPTG	0.0133M Ara 100uM IPTG	0.0133M Ara 500uM IPTG	0.0133M Ara 1mM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	0.0133M Ara 2mM IPTG	0.0665M Ara 50uM IPTG	0.0665M Ara 100uM IPTG	0.0665M Ara 500uM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	LB blank		0.0665M Ara 1mM IPTG	0.0665M Ara 2mM IPTG

Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g41a2cc5a65_2_0

WEDNESDAY, 31/07/2019

1. PCR sample RES frag have correct band -> PROCEEDED to Gibson (a) plac-RES-GFP and (b) pA8c-EL222-HicB plasmid -> Transformed into 10B (either one or both of them are David's) -> plated on k and c plate respectively

2. Inoculated:
    a. one colony for each re-transformed plate for villu HicAB ko w/:
        - HicA-GFP c2 and HicB c1
        - HicA-GFP c2 and HicB c3 (newly thawed replacement) in C+K media
    b. two colonies from 10B-HicA-GFP c2 (to re-stock HicA-GFP plasmid) in K media

3. Stored 4degC + miniprep + seq:
    - villu HicAB ko existing plate (HicA-GFP c2 and HicB c1(colony3))
    - 10B HicB c4
    - 10B HicB c5 (both colonies from existing HicB plate)

4. Inoculated:
    a. MG1655 WT
    b. MG1655 pCon-GFP
    c. MG1655 XR c2 (RES/Xre), backup characterization tomorrow

THURSDAY, 01/08/2019

1. Successful transformation for:
    a. 10B pE6K-RES-GFP
    b. 10B pA8C-EL222-HicB
    inoculated 3 colonies for each in LB+K and LB+C respectively

2. Miniprep and send for seq:
    a. 10B HicA-GFP c2(1)
    b. 10B HicA-GFP c2(2)
    c. villu's HicAB k.o MG1655 with HicA-GFP c2 and HicB c1
    d. villu's HicAB k.o MG1655 with HicA-GFP c2 and HicB c3

3. **RE-RUNNING** (Brodersen's) RES/Xre characterization:
    MG1655 strain
    XR c2 (seq ok)
    18h continuous run
    0h toxin induction (ara) and antitoxin induction (iptg); **induced together!**

Plate layout

MG1655 WT MG1655 w/ RES/Xre XR c2	0M Ara 0M IPTG	0.0133M Ara 0M IPTG	0.0665M Ara 0M IPTG	0M Ara 2mM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	0.0133M Ara 50uM IPTG	0.0133M Ara 100uM IPTG	0.0133M Ara 500uM IPTG	0.0133M Ara 1mM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	0.0133M Ara 2mM IPTG	0.0665M Ara 50uM IPTG	0.0665M Ara 100uM IPTG	0.0665M Ara 500uM IPTG
MG1655 WT MG1655 w/ RES/Xre XR c2	LB blank		0.0665M Ara 1mM IPTG	0.0665M Ara 2mM IPTG

Results:

https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g41a2cc5a65_2_54

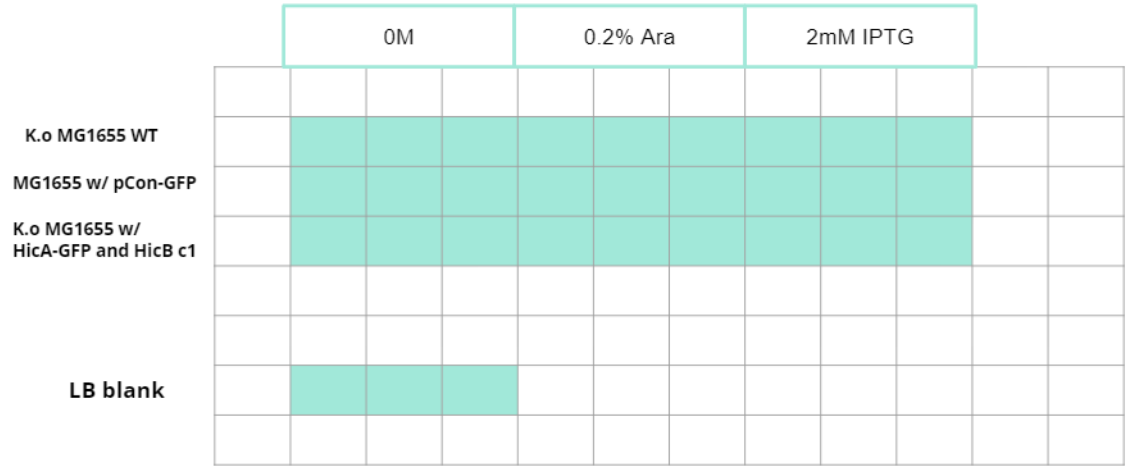
FRIDAY, 02/08/2019

1. Miniprepped 8 samples:
   a. 10B RES-GFP c1
   b. 10B RES-GFP c2
   c. 10B RES-GFP c3
   d. 10B A8C-EL222 HicB c1
   e. 10B A8C-EL222 HicB c2
   f. 10B A8C-EL222 HicB c

2. Overnight PCR for A8C-EL222-HicB c1, c2, c3

3. HicAB characterization in KNOCKOUT strain
   MG1655 strain
   All seq ok
   18h continuous run
   OD=0.1 at 0h reading
   1h induction for toxin and antitoxin respectively

Plate layout



Results:https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g5e2954e85f_0_1

SATURDAY, 03/08/2019

1. Ran gel, gel extraction, gibson and transformation in 10B for final plasmid - A8C-EL222-Xre

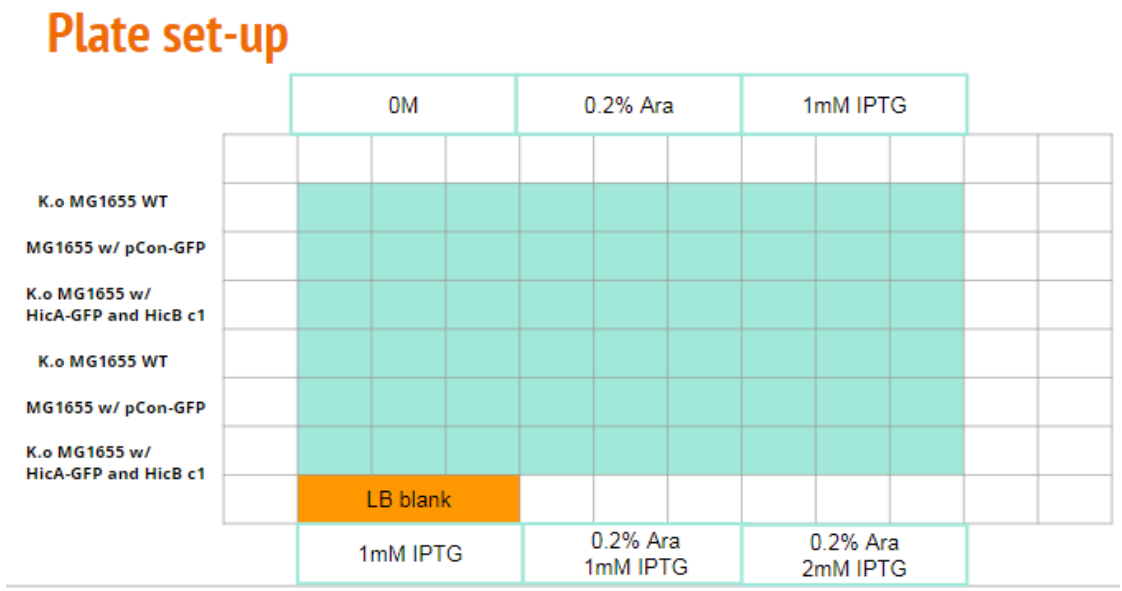
2. Co-transformed pE6K-RES-GFP c1, c2 and c3 with pA8C-pBad-Xre c3

3. Co-transformed pE6K-HicA-GFP c2 with pA8C-EL222-HicB c1, c2 and c3

4. Transformed pBR322-pCon-GFP into villu's HicAB k.o competent MG1655 strain

5. FIRST ROUND OF HicA/HicB characterization
   MG1655 strain
   All seq ok
   18h continuous run
   OD=0.1 at 0h reading
   1h toxin induction (IPTG)
   2.5h antitoxin induction (Ara)

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g5e33e6819a_0_0

SUNDAY, 04/08/2019

1. Re-ran HicA/HicB characterization - exact same layout as 8/3
   Results:
   https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g5e4836fd0f_0_70

2. Picked and inoculated colonies for all plates from 8/3
   a. 1 colony each from co-transformed plates, 3 colonies each for EL222-Xre clones

TUESDAY, 06/08/2019

1. Cloning for:
   a. pLac-HicA-pBad-LuxABCDE
   b. pBad-EL222-HicB
   Two new primer pairs for (a) and one new primer to clone out A8c-HicB bb

2. Inoculated:

a. One tube of villu HicAB ko MG1655 WT
b. One tube of MG1655 pCon-GFP c3 (latest)
c. TWO tubes of villu HicAB ko MG1655 C1 (one for characterization with a and b; another for module demonstration tomorrow)

WEDNESDAY, 07/08/2019

1. Transform gibson product for
   a. pBad-EL222-HicB
   into chemical competent 10B

2. Inoculated (a) pCon-GFP c3 (b) k.o HicAB c1

3. Performed sleep-wake module demonstration **DAY 0**:
   - Used HicAB k.o c1 cells
   - Split into three tubes - uninduced, induced with toxin, induced with antitoxin
   - OD600 = 0.1 at 0h
   - 1mM IPTG induced at 1h
   - Glucose-glo reading at 0h right after refreshing
   - Live dead staining at 5h (cells aliquoted out at this timing)
   - GFP reading at 7h

Results:

image.png

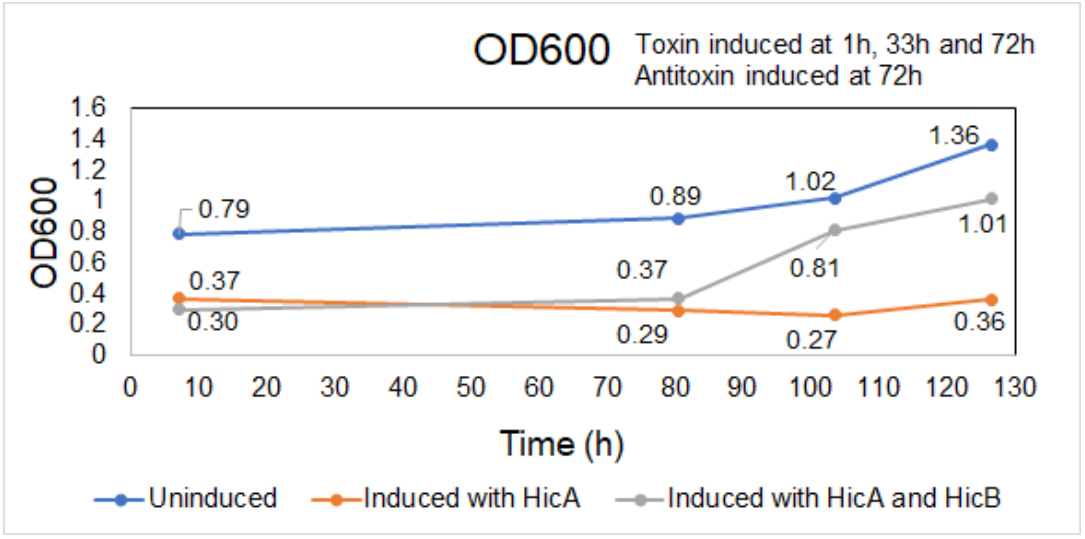


image.png

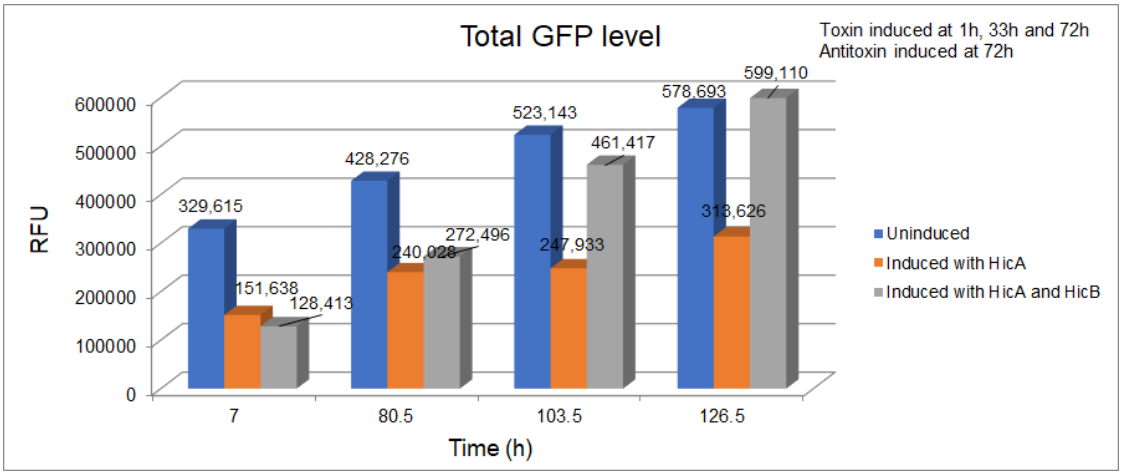


image.png

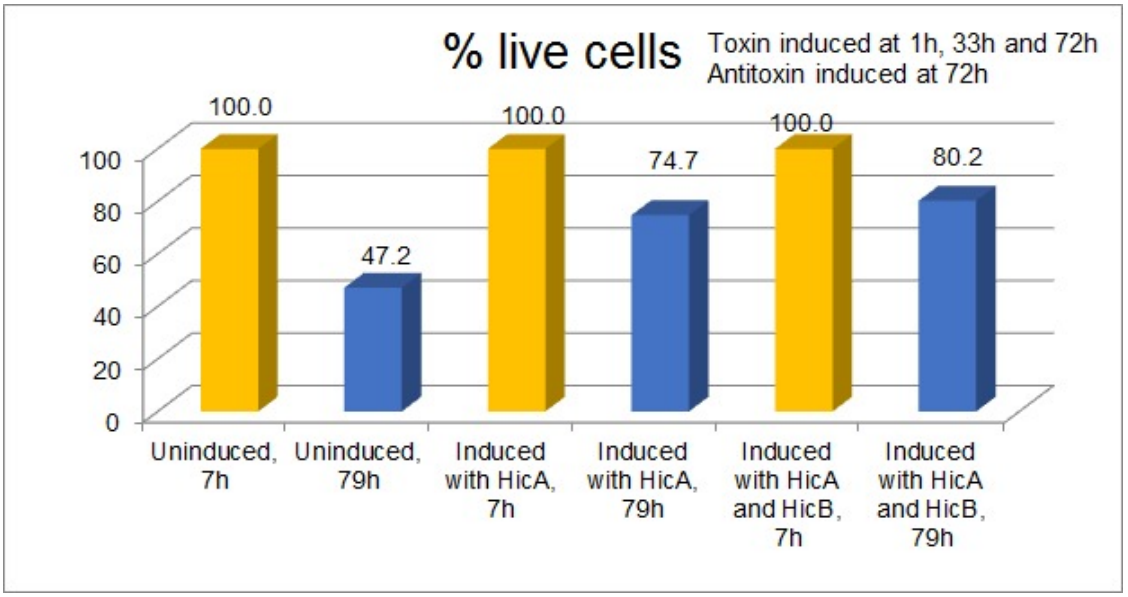
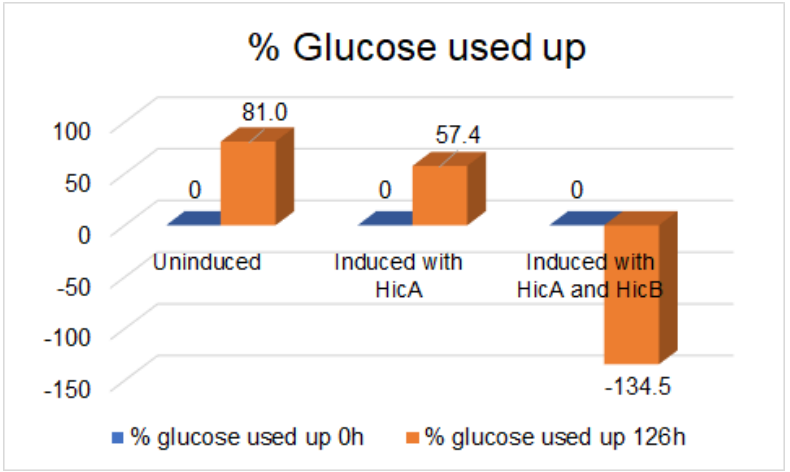
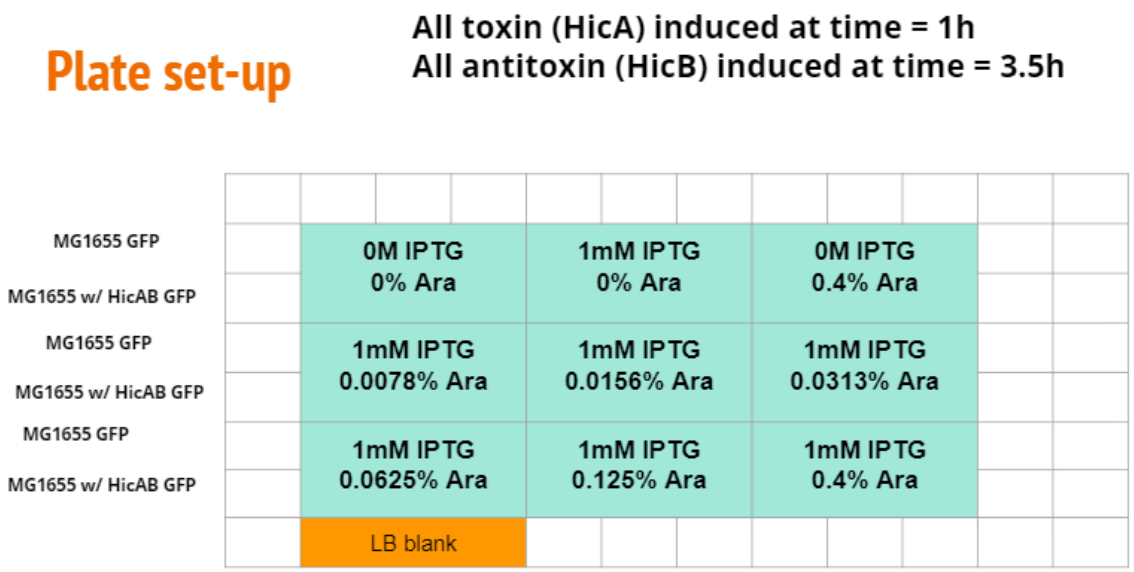


image.png



4. Perform HicAB characterization in ko strain
   MG1655 strain
   All seq ok
   18h continuous run
   OD=0.1 at 0h reading
   1h toxin induction (IPTG)
   3.5h antitoxin induction (Ara)

image.png



Results:
10 Aug (wed) vs 11 Aug (thurs) results:

- wed shows ara-only inhibiting growth of GFP control but thurs shows ara-only promoting growth of GFP control (higher OD), I'd like to trust thurs more because it's more consistent with what we've been observing but this time round the ara is 0.4% and not 0.2% so I can't say for sure it's growth promoting. I'd suggest repeating this characterization again.
- weirdly both wed and thurs show IPTG at 1mM affecting GFP control growth when past two other characterization of HicAB on 3rd and 4th Aug didn't see such an obvious growth arrest in the control. Not sure if GFP ctrl cells are problematic or inducer got problem hmm
- Re-run again on tues (mon was PH)

TUESDAY, 13/08/2019

1. Send sat and sun's miniprepped plasmid for seq:
   a. 10B EL222 HicB c1
   b. 10B EL222 HicB c2
   c. 10B EL222 HicB c3
   d. 10B RES-GFP c1
   e. 10B RES-GFP c2
   f. 10B RES-GFP c3
   g. 10B HicA-LuxABCDE c1
   h. 10B HicA-LuxABCDE c2
   i. 10B HicA-LuxABCDE c3
2. Co-transformed HicA-GFP c1 with EL222 HicB c1, c2, c3 into villu's hicAB ko MG1655 (kc plate)

3. SECOND repeated HicAB characterization in ko strain **(first two were weird to see pCon-GFP cells significantly affected by 1mM IPTG hmm)**
   MG1655 strain
   All seq ok
   18h continuous run
   OD=0.1 at 0h reading
   1h toxin induction (IPTG)
   3.5h antitoxin induction (Ara)

image.png

Plate set-up

All toxin (HicA) induced at time = 1h
All antitoxin (HicB) induced at time = 3.5h

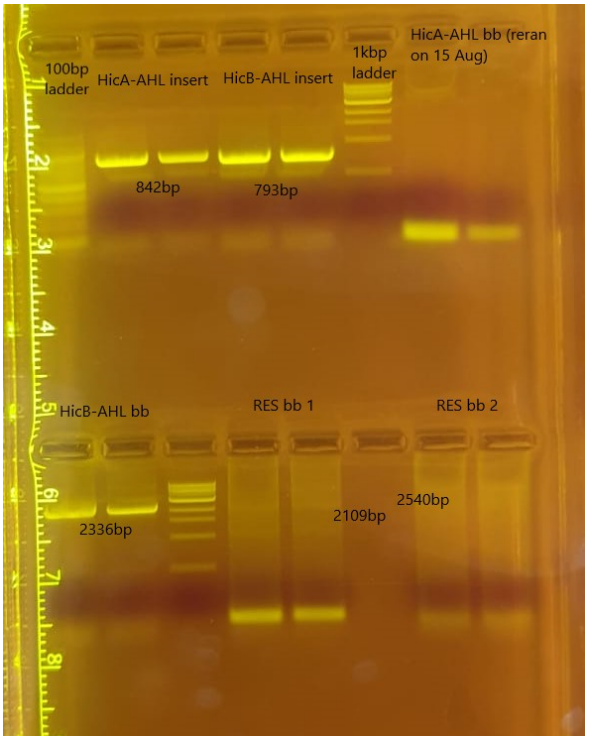
MG1655 GFP MG1655 w/ HicAB GFP	0M IPTG 0% Ara	1mM IPTG 0% Ara	0M IPTG 0.4% Ara
MG1655 GFP MG1655 w/ HicAB GFP	1mM IPTG 0.0078% Ara	1mM IPTG 0.0156% Ara	1mM IPTG 0.0313% Ara
MG1655 GFP MG1655 w/ HicAB GFP	1mM IPTG 0.0625% Ara	1mM IPTG 0.125% Ara	1mM IPTG 0.4% Ara
	LB blank		

Results:
weird again, could be due to pCon-GFP c3 being problematic because 1 and 2 Aug run we used pCon-GFP c2. Otherwise, if pCon-GFP c3 is ok means all along we have been missing out IPTG effect on normal control cells WHICH IS BAD.

WEDNESDAY, 14/08/2019

1. PCR cloning work for:
   a. HicA/HicB x AHL system (used jingyun's QSR1 template, HicA-GFP template and A8C-pBad-HicB template)
   b. **THREE-FRAGMENT** RES-GFP bb (used HicA-GFP template)

image.png



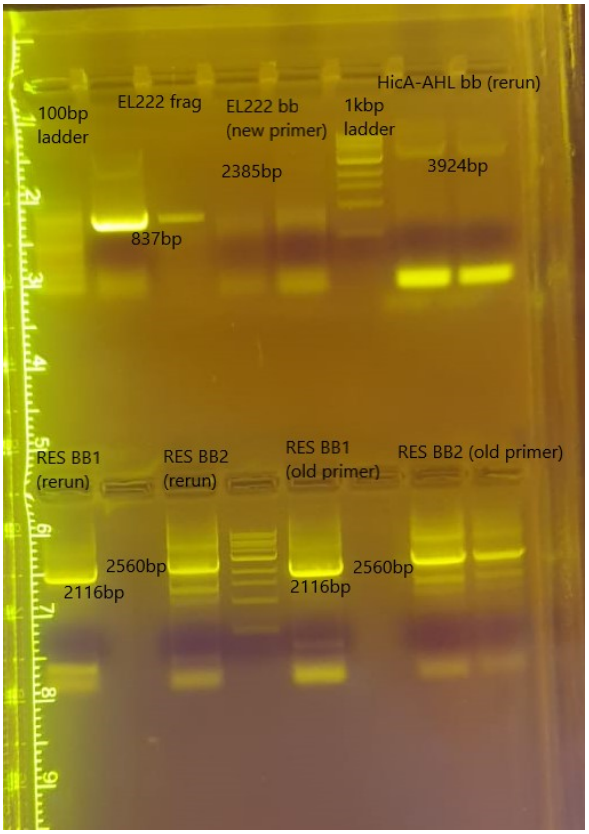
(1) HicA-AHL bb have faint band near 4kbp, (2) RES bb2 have no visible band near 2.5kbp --> re-run again on 15Aug, The rest all ok

2. Inoculated single colony for pECJ3-toggle-kan plasmid into LB+K
3. Inoculated new pBR322-pCon-GFP colony (C4) into LB+K -> try characterization tomorrow to see if IPTG does affect cell growth
4. Chemically transformed HicA-LuxABCDE and co-transformed HicA-LuxABCDE-A8C-pBad-HicB into villu k.o HicAB MG1655 on K and CK plate respectively

THURSDAY, 15/08/2019

1. PCR run:
    a. HicA-AHL bb
    b. RES bb 1 and 2 (repeat, same primers)
    c. OLD RES bb 1 and 2 (one of the primer for each PCR product is old primer)
    d. EL222 frag and EL222 bb (new primer pairs)

Gel photo



All about the right band, except EL222 bb faint band

Gibson:
(1) HicA-AHL plasmid
(2) HicB-AHL plasmid
Co-transform plasmid into villu k.o HicAB MG1655 (C+K)
Transform plasmid into 10B (K)
Transform plasmid into 10B (C)

Gibson:
(1) RES frag (existing)
(2) RES bb1
(3) RES bb2
into RES-GFP plasmid
Transform plasmid into 10B (K)

Gibson:
(1) RES frag (existing)
(2) RES bb1 old
(3) RES bb2 old
into RES-GFP (old) plasmid
Transform plasmid into 10B (K)

Gibson:
(1) EL222 frag
(2) EL222 bb
into EL222-HicB plasmid
Transform plasmid into 10B (C)

2. Inoculated:
    a. villu's MG1655 w/ HicA-Lux C1

   b. villu's MG1655 w/ HicA-Lux C2
   c. villu's MG1655 w/ HicA-Lux and pBad-HicB c1

   into LB+K and LB+KC respectively

3. Inoculated newly-picked pCon-GFP c4 and ran characterization to study IPTG effect on its growth

FRIDAY, 16/08/2019

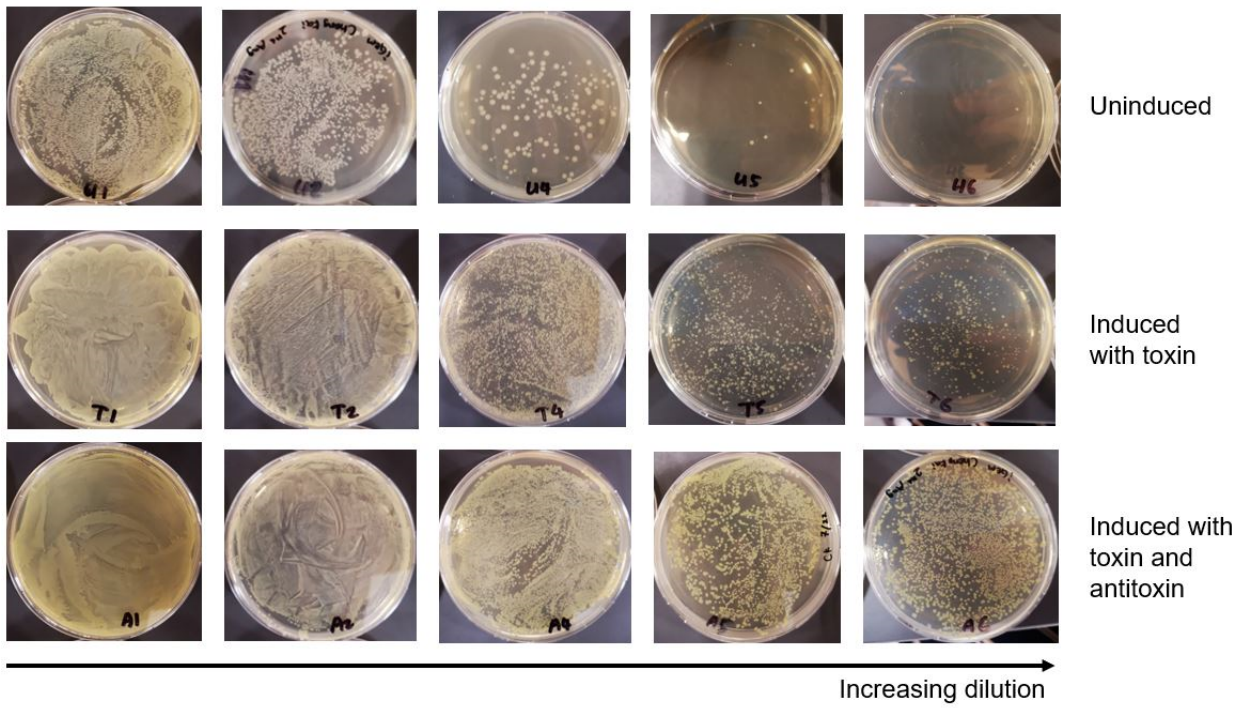
1. Inoculated all transformed colonies (except MG1655 co-transformed with HicA-AHL and HicB-AHL plasmids, none of them are non-green colonies)
2. Measured glucose level in 0.2% ara, LB, demo samples (U, T, AT) to study the effect of LB and 0.2% ara on glucose glo assay sensitivity
3. Refreshed villu's HicAB k.o MG1655 transformed with:
   a. HicA-Lux C1
   b. HicA-Lux C2
   c. HicA-Lux and pBad-HicB c1

   induced at OD ~0.4 - 0.5 with 0.2% arabinose
4. Sequenced Addgene pECJ3 toggle plasmid using biobasic
5. First round sleep-wake demo samples - performed CFU (plated at 1x dilution to 6x dilution)

SATURDAY, 17/08/2019

1. Miniprepped all 14 cloned colonies:
   a. 3 of 10B EL222-HicB
   b. 3 of 10B RES-GFP
   c. 3 of 10B RES-GFP old
   d. 3 of 10B HicB-AHL
   e. 2 of 10B HicA-AHL
2. Repeat HicAB c1 and new pCon-GFP c4 characterization:
   a. 1mM IPTG
   b. Various ara: 0.0156%, 0.125%, 0.4%
3. Inoculated:
   a. HicA-Lux - pBad-HicB co-transformed villu knockout MG1655 -> C1
   b. villu hicAB ko MG1655 pECJ3 toggle c1
   c. pCon-GFP c4 and hicAB c1 (to repeat characterization tomorrow) -> **ABORTED because sat characterization of GFP ctrl c4 (supposedly new) and HicAB k.o c1 show much lower total GFP in control than test - no point continuing**
4. Sleep-wake demo: Counted CFU for 6x dilution samples - uninduced, induced with T only and induced with T and AT (best dilution is 6x)
   Took pic of other dilution plates:

image.png



At 6x dilution, it was observed that both induced cells have higher CFU than uninduced cells.
Hypothesis: dormant cell with growth arrest undergo less number of division.

SUNDAY, 18/08/2019

1. Transformed pBR322-pCon-GFP plasmid into:
   a. 10B - to restock plasmid
   b. MG1655 WT (originated from david's) - to get a new fresh plate of GFP control colonies

2. Testing **functionality of Addgene toggle switch (pECJ3)** in villu k.o MG1655
   Starting fluorescence: green (gfp under ptet)
   Refreshed overnight culture to OD600= 0.1 at 0h, add 2mM IPTG at 1h. Add 3h time point...
   (a) observe if color change from green to red under blue light
   (b) read RFP and GFP level using microplate
   Microplate reading volume: 200uL

3. Inoculated from -80degC:
   a. HicAB k.o c1 (first used on 2nd aug)
   b. HicAB k.o c3 (first use today, 18 Aug)
   Both seq ok, trying to see if k.o c1 with multiple freeze thaw will show altered growth and behavior compared to new c3

MONDAY, 19/08/2019

1. Miniprepped villu MG1655 w/ HicA-LuxABCDE and pBad-HicB, sent for seq

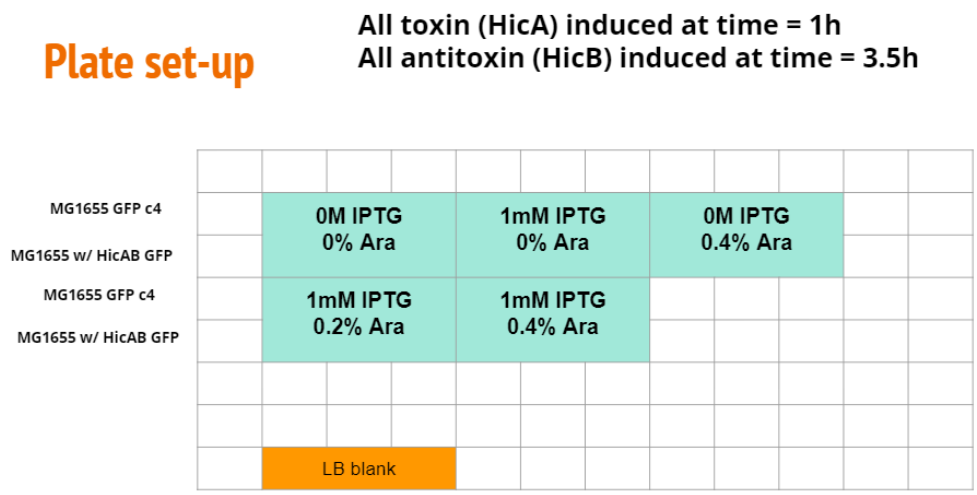
2. Send miniprepped samples from 17 Aug for sequencing -> biobasic

3. Characterized older and new HicAB ko samples' effect on growth when induced with 1mM IPTG and ara conc of 0.2% and 0.4%
   18h cont run
   OD600 = 0.1 at 0h

IPTG added at 1h
Ara added at 3.5h
Mainly **trying to replicate 3rd and 4th Aug** perfect data for HicAB c1 (only diff, this time avoided all borders and loaded 200ul of cells into each well)

PS top row is villu MG1655 hicAB c1 and bottom row is new villu MG1655 hicAB c3

image.png



4. Inoculated transformed 10B and MG1655 WT with pBR322-pCon GFP (choose one GREEN colony each) in LB + K

5. Inoculated both HicAB ko c1 and c3 (depending on characterization results tomorrow 20 Aug to determine characterize with hicAB ko c1 or c3)

6. Inoculated villu MG1655 w/ HicA-LuxABCDE and pBad-HicB in LB+KC (tomorrow start sleep-wake/Lux demo)

7. Toggle switch:
   Cells became visibly red
   Spun down cells, removed existing LB containing remnant of IPTG
   Added fresh LB+K
   Supposed to become red tomorrow, toggled to pTet and maintained there

TUESDAY, 20/08/2019

1. **STARTED SLEEP-WAKE + LUX demo**
   Eight tubes (4 uninduced control vs 4 induced with IPTG)
   Day 0, day 1, day 6, day 10 induce **2% arabinose** at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain?)
   2mM IPTG was added 1h post 0.1 OD **IN ALL FOUR TUBES** on day 0 and maintained thereafter
   Microplate reading volume: 200uL

2. Miniprepped 10B pCon-GFP c1 (new plate)

3. Add 500nM ATC into villu MG1655 containing pECJ3 toggle plasmid at 0h -> visualize + read next day

4. **Repeated characterization**: Studying HicAB effect on growth when induced with 1mM IPTG and ara conc of 0.125%, 0.2% and 0.4%
   18h cont run
   OD600 = 0.2 at 0h
   IPTG added at 1h
   Ara added at 3.5h
   THIS TIME, new pcon-GFP sample (newly transformed plate 2) and new HicAB c3 (seq ok)

5. Seq result for samples ok:
   a. Co-transform RES-GFP c2 w/ A8c-Xre c3 into villu HicAB ko MG1655
   b. Co-transform HicA-AHL c2 with pLasI-HicB-RFP c1 into villu HicAB ko MG1655

WEDNESDAY, 21/08/2019

1. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP, observe if color change (yep became redder), leave it and see if RFP degrade and GFP dominate tomorrow

2. Inoculated co-transformed cells and also QsR1 JY's AHL plasmid in MG1655 (2 colonies for each)

3. Inoculated new p2c1 pCON-GFP from -80degC for RES-GFP characterization tomorrow

4. Inoculated 2 new colonies from existing villu MG1655 HicA-Lux w/ HicB plate **1** - c2 and c3

5. Co-transform HicA-LuxABCDE and pBad-HicB into villu hicAB ko MG1655, **plate 2** (because seq can't tell if primer didn't bind or HicB is absent)

6. PCR cloning for:
   a. EL222-HicB bb (one of the primer is new)
   b. Toggle switch part 1 (cloning HicB into pECJ3)
   Tried sLice instead of Gibson to assemble EL222-HicB plasmid (latest EL222 insert frag) and Toggle switch part 1 plasmid
   Transformed EL222-HicB into 10B (c plate) and Toggle switch part 1 into 10B (k plate)

THURSDAY, 22/08/2019

1. **SLEEP-WAKE + LUX demo DAY 2**
   Eight tubes (4 uninduced control vs 4 induced with IPTG)
   4% arabinose was added to **DAY 2 tubes** at 29degC -> post 4h induction...
   (a) take picture of entire bottle - **Induced glow but uninduced glow**
   (b) read luminescence using microplate (gain= 118) - **Induced cells produced comparable level of lux as uninduced in D0, measured OD and the OD is still the same range 0.3 as D0, does that mean induced cells are still sleeping? How can they produce lux proteins when asleep?! But its good thing that by D2, uninduced control cells already cease to produce lux!**
   Microplate reading volume: 200uL

2. Store all inoculated cells in 4degC:
   a. villu ko MG1655 - RES-GFP w/ A8C-Xre c1 (green)
   b. villu ko MG1655 - RES-GFP w/ A8C-Xre c2 (green)
   c. villu ko MG1655 - AHL HicA HicB c1
   d. villu ko MG1655 - AHL HicA HicB c2
   e. MG1655 w/ Jy's QsR1 AHL c1
   f. MG1655 w/ Jy's QsR1 AHL c2
   g. 10B Re-transformed G*J* c1 (dual plasmid retention)
   h. 10B Re-transformed G*J* c2 (dual plasmid retention)
   i. 10B Re-transformed G*J* c3 (dual plasmid retention)

j. villu ko MG1655 - **plate 1** HicA-Lux w/ HicB c2
k. villu ko MG1655 - **plate 1** HicA-Lux w/ HicB c3

3. Miniprep and send above for sequencing

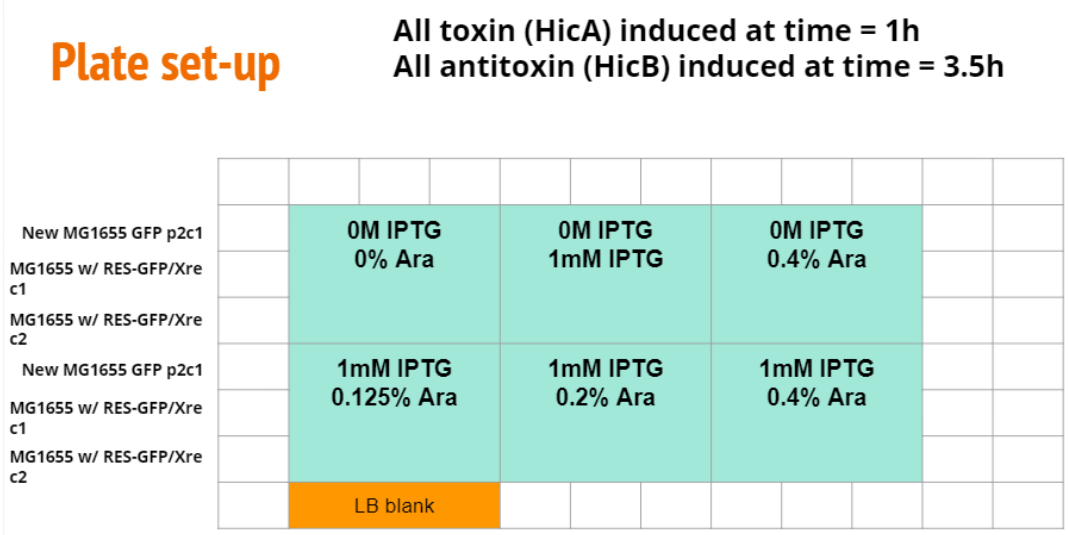
4. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP, observe if color change - not really, still red

5. Inoculate villu hicAB knockout - HicAB c3 to repeat characterization tomorrow

6. Inoculate transformed colonies (all to be non-green):
   a. 10B Toggle switch - pECJ3-HicB c1
   b. 10B Toggle switch - pECJ3-HicB c2
   c. 10B Toggle switch - pECJ3-HicB c3
   d. villu HicAB ko MG1655 - HicA-Lux and HicB plate 2 c1

7. **FIRST STANDARDIZED PLASMID: RES-GFP and Xre characterization IN HICAB KNOCKOUT STRAIN.**
   18h cont run
   OD600 = 0.1 at 0h
   1mM IPTG added at 1h
   0.125%, 0.2%, 0.4% Ara added at 3.5h
   Running both c1 and c2 because seq result not out yet (turned out both seq ok)

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g5fc39f50b8_2_0

FRIDAY, 23/08/2019

1. Store and miniprep inoculated cultures:
   a. 10B Toggle switch - pECJ3-HicB c1
   b. 10B Toggle switch - pECJ3-HicB c2
   c. 10B Toggle switch - pECJ3-HicB c3
   d. villu HicAB ko MG1655 - HicA-Lux and HicB plate 2 c1

send for sequencing (biobasic)

2. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP, observe if color change (3 days post 500nM ATC addition) - **nope color didn't change much, RFP dropped a little and GFP increased by a little but is still half of original fully toggled form**

3. **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 0**
   Eight tubes (4 uninduced control vs 4 induced with 2mM IPTG)
   4% arabinose was added to **DAY 0 tubes** at 29degC -> post 2.5h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   + **GLUCOSE GLO READ FOR DAY 0, Induced and uninduced with IPTG sample (0h read), gain = 190**

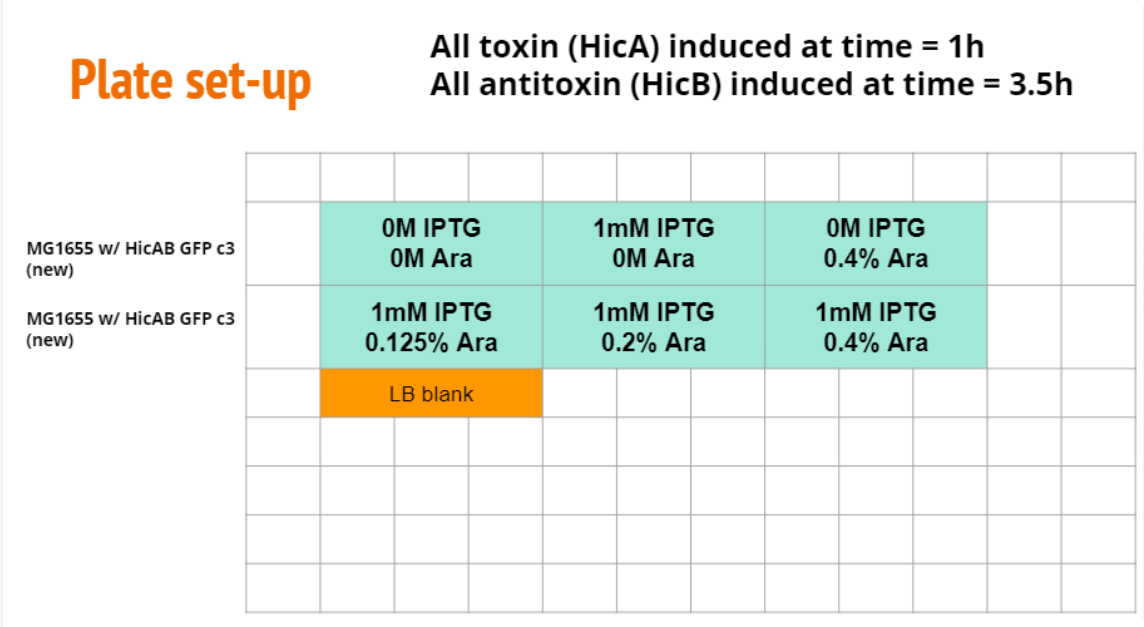
   Also induced LB lux demo D6 and D10 tubes with 2mM IPTG

4. Store seq-ok samples into -80degC:
   a. villu k.o MG1655 with HicA-Lux and HicB c2 (of plate 1)
   b. villu k.o MG1655 with RES-GFP and Xre c1
   c. villu k.o MG1655 with RES-GFP and Xre c2

5. If samples seq ok:
   a. Repeat demo for HicA/B Lux cells -> Friday = Day 0
   b. ~~AHL demo for HicA/B cells -> Friday = Day 0 (induce with 100nM AHL, 4 hour post induction read RFP)~~ (not ok)
   c. ~~Plasmid retention cells -> start passaging = Day 0~~ (not ok)

6. **(Supposedly) FINAL chemical system characterization**: Studying **HicAB** effect on growth when induced with 1mM IPTG and ara conc of 0.125%, 0.2% and 0.4%
   18h cont run
   OD600 = 0.2 at 0h
   IPTG added at 1h
   Ara added at 3.5h
   THIS TIME use only new HicAB c3 (seq ok)

image.png



Results:

https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g60d9f15761_1_1

SATURDAY, 24/08/2019

1. (a) **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 1**
   Eight tubes (4 uninduced control vs 4 induced with 2mM IPTG)
   4% arabinose was added to **DAY 1 tubes** at 29degC -> post 2.5h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   (b) ALSO, performed CFU of 10^5 - 10^7 dilution for **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 0 cells**

2. Toggle switch part I cloning work seq failed; picked another three colonies from the same plate: c4, c5, c6 in LB+K

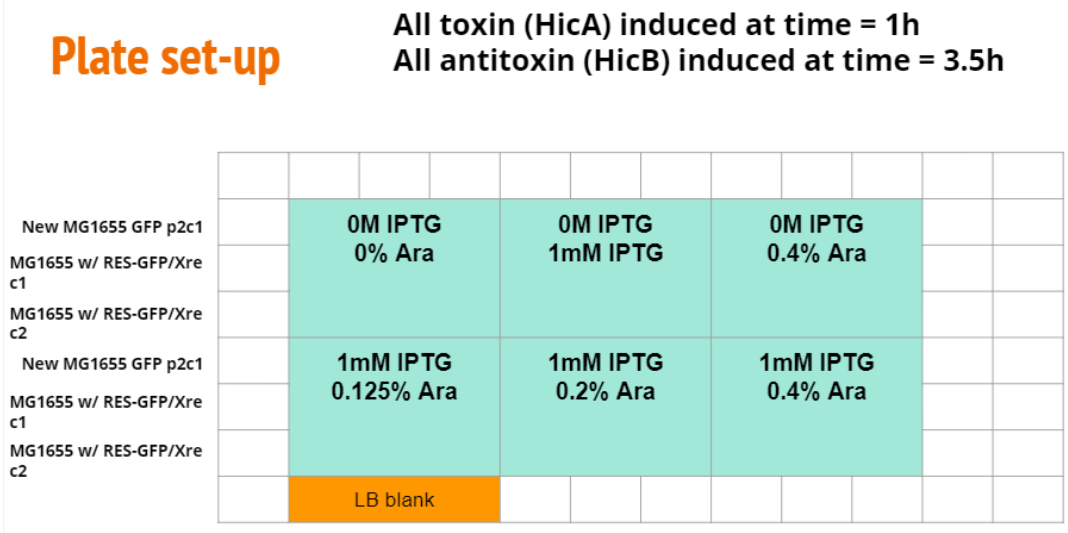
3. Co-transformed AHL HicA c2 w/ AHL HicB c1 into villu ko MG1655 (CK plate)

4. Inoculated WT MG1655 tranformed with RES/Xre C1, C2

5. Inoculated P2C1 GFP and k.o RES/XRE c1 and c2 from -80degC (maybe run another characterization tomorrow)

6. **SECOND STANDARDIZED PLASMID: RES-GFP and Xre characterization IN HICAB KNOCKOUT STRAIN.**
   18h cont run
   OD600 = 0.1 at 0h
   2mM IPTG added at 1h
   0.125%, 0.2%, 0.4% Ara added at 3.5h
   Running both c1 and c2 which are seq ok

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g60ce4263a8_2_0

SUNDAY, 25/08/2019

1. CFU of 10^5 - 10^7 dilution for **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 0 cells --> cell counting**

Results:
Induced cells have much fewer colonies than uninduced cells at a countable number. Induced cells also have much bigger colony size than uninduced cells.

Hypothesis 1: Growth-arrested cells grow slower and divide slower -> lesser cells
Hypothesis 2: Growth of IPTG-induced cells were arrested much earlier than uninduced which kept growing = lesser cells to begin with

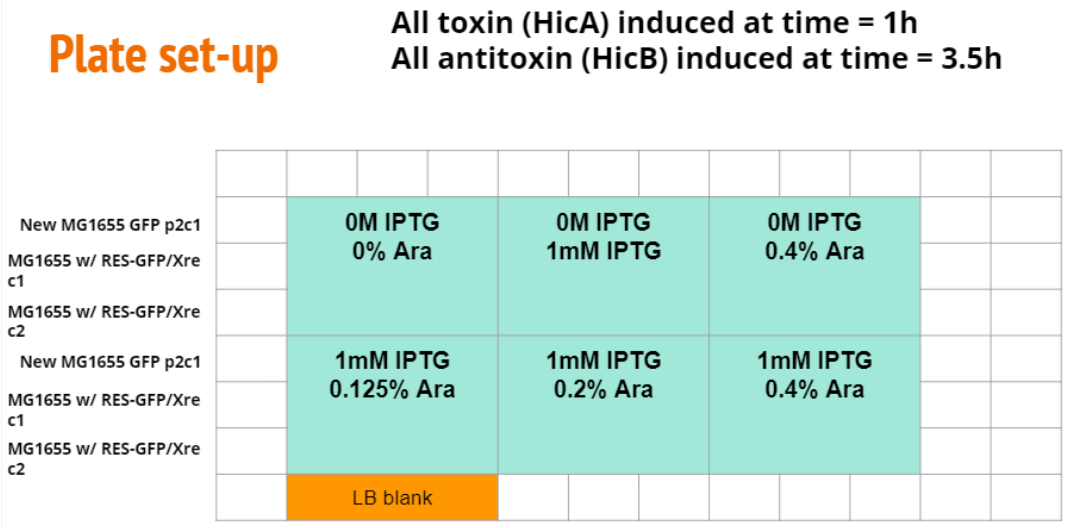
2. Re-plated co-transformed villu ko MG1655 AHL HicA c2 w/ AHL HicB c1 because no colony formed on the plate

3. Also re-transformed AHL HicA c2 w/ AHL HicB c1 into villu ko MG1655 - ck plate

4. Store cells in 4degC and miniprep:
    a. Toggle switch pECJ3-HicB c4
    b. ~~Toggle switch pECJ3-HicB c5~~ (red color when supposed to be green, aborted)
    c. ~~Toggle switch pECJ3-HicB c6~~ (red color when supposed to be green, aborted)
    d. WT MG1655 RES/Xre c1 (store 4degC)
    e. WT MG1655 RES/Xre c2 (store 4degC)

5. **STANDARDIZED PLASMID: FIRST RES-GFP and Xre characterization IN MG1655 WT STRAIN.**
    18h cont run
    OD600 = 0.1 at 0h
    2mM IPTG added at 1h
    0.125%, 0.2%, 0.4% Ara added at 3.5h
    Running both c1 and c2 which are... SEQ...

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g60eaaeddb7_0_0

6. Inoculated two new villu ko HicA-GFP and HicB colonies to run characterization for tomorrow
    a. villu ko MG1655 HicA/HicB c4
    b. villu ko MG1655 HicA/HicB c5

7. Inoculated four new plasmid retention G*J* colonies in LB+CK:

a. 10B G*J* c4
b. 10B G*J* c5
c. 10B G*J* c6
d. 10B G*J* c7

8. **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo IPTG REFRESHMENT**
   Eight tubes (4 uninduced control vs 4 induced with 2mM IPTG)
   2mM IPTG was added to **DAY 5 and DAY 10 induced tubes** --> post 2.5h induction...
   (a) read OD using microplate (gain= 118, lum read protocol used)
   (b) plate read overnight for 20h in H1 synergy microplate reader to study DAY 5 and DAY 10 induced and uninduced growth pattern
   Microplate reading volume: 200uL

9. Toggle switch villu's MG1655 w/ pECJ3 (still red) - spin down -> wash once with LB -> added 5mL LB+K and added 500nM ATC

MONDAY, 26/08/2019

1. villu ko MG1655 co-transformed AHL HicA/HicB - **both plates no colonies** (send individual plasmid for seq again)

2. Store 4degC and miniprep four new plasmid retention G*J* colonies and also 2 new villu ko HicAB c4 and c5 colonies:
   a. ~~10B G*J* c4~~ (no growth)
   b. ~~10B G*J* c5~~ (no growth)
   c. 10B G*J* c6
   d. ~~10B G*J* c7~~ (no growth)
   e. villu ko MG1655 HicA/HicB c4
   f. villu ko MG1655 HicA/HicB c5

3. Send plasmids for sequencing:
   a. Toggle switch pECJ3-HicB c4
   b. WT MG1655 RES/Xre c1 (store -80degC)
   c. WT MG1655 RES/Xre c2 (store -80degC)
   d. 10B G*J* c6
   e. villu ko MG1655 HicA/HicB c4
   f. villu ko MG1655 HicA/HicB c5
   g. 10B AHL-HicA c2
   h. 10B AHL-HicA c1

4. **LB SLEEP-WAKE + LUX demo DAY 6**
   Eight tubes (4 uninduced control vs 4 induced with IPTG)
   4% arabinose was added to **DAY 6 tubes** at 29degC -> post 3.5h induction...
   (a) take picture of entire bottle -
   (b) read luminescence using microplate (gain= 118) -
   Microplate reading volume: 200uL
   RESULTS: Induced cells have two fold higher Lux expression than uninduced!!! CFU PERFORMED AS WELL

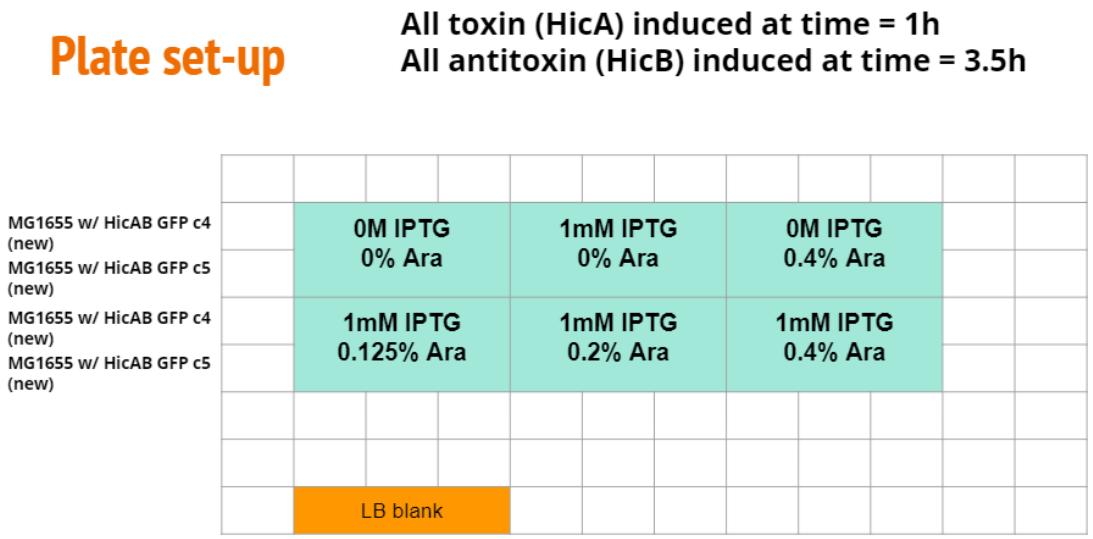
5. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP via microplate reader at 10.40am (one day post washing + addition of 500nM ATC)

6. **Chemical system characterization**: Studying **New HicAB** effect on growth when induced with 1mM IPTG and ara conc of 0.125%, 0.2% and 0.4%
   18h cont run
   OD600 = 0.1 at 0h
   IPTG added at 1h

Ara added at 3.5h

THIS TIME use new HicAB c4 and c5 only (seq?)

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g60eaaeddb7_0_38

TUESDAY, 27/08/2019

1. Seq ok:
   a. Transform AHL-HicB c1 into villu MG1655
   b. Re-do SLICE for Toggle switch part 1 fragments (1:3 ratio to make DNA amount the same; top up with water) --> transform into 10B

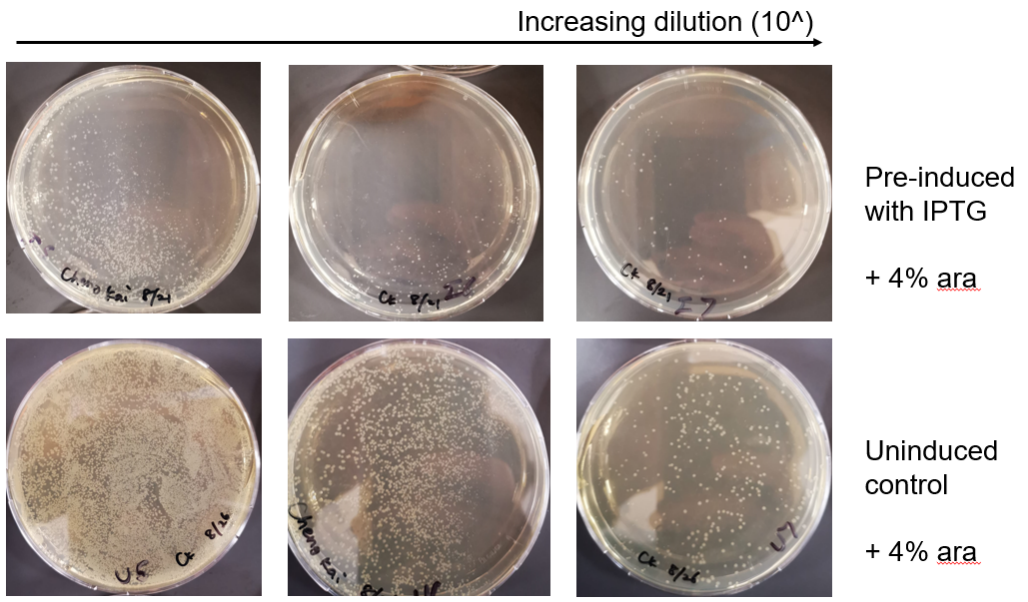
2. Transform jingyun's Ara-EL222 plasmid into 10B

3. Aborted EL222-HicB cloning because overnight EL222 bb 1 is unsuccessful (negative conc); other two pcr fragments kept in -20degC PCR box

4. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP via microplate reader

5. **LB SLEEP-WAKE + LUX demo DAY 6 CFU counting (plated 3.5h after ara induction)**
   10^7 plate:
   Uninduced with 364 colonies vs induced with 76 colonies
   Size of colonies are similar

image.png



6. Performed CFU for Day 6 plates 27h after inducing ara
7. Inoculate prof Brodersen's RES cells (tip labeled) for characterization on wednesday
8. Streak villu ko HicAB c1 and c3 from -80degC onto CK plate - characterize on thursday

WEDNESDAY, 28/08/2019

1. Streaked WT RES/Xre c1 and c2 onto CK plate - characterize on Friday
2. **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 5**
   Eight tubes (4 uninduced control vs 4 induced with 2mM IPTG)
   4% arabinose was added to **DAY 5 tubes** at 29degC -> post 2.5h induction... **@1.30pm**
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

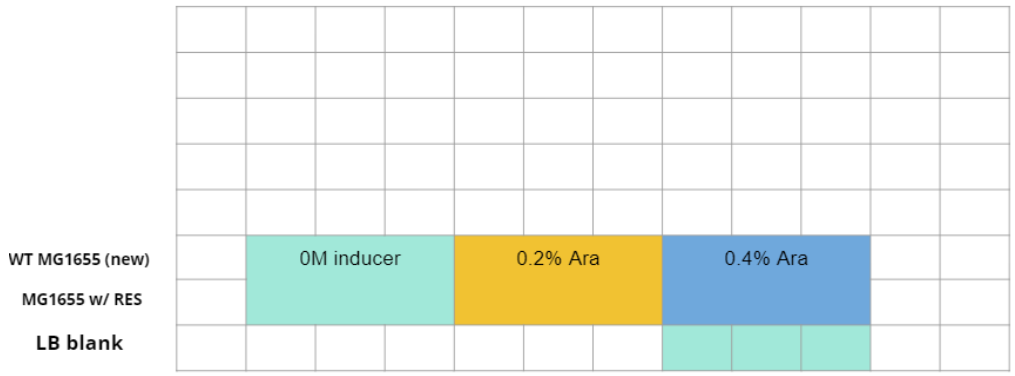
   Also do CFU **before** adding ara **@1pm;** 10^6 - 10^8 (three 5 is to 45 tubes)
3. **LB SLEEP-WAKE + LUX demo DAY 6 CFU counting (plated 27h after ara induction)**
   10^7 plate:
   Uninduced with 364 colonies vs induced with 76 colonies
   Size of colonies are similar (maybe CK plated wrongly or induced cells need longer time for resumption?)
4. Make new competent cells for villu HicAB ko MG1655
5. Re-streaked AHL-HicB onto C plate because colonies are all cluttered together
6. Inoculate three toggle switch part 1 colonies (supposedly HicB to be present)- plate 2 c1, c2, c3
7. Inoculate villu ko HicAB c1 and c3 from streaked CK plate

8. Inoculate one colony for 10B transformed with Jingyun's Ara-EL222 plasmid

9. Toggle switch villu's MG1655 w/ pECJ3 - read RFP and GFP via microplate reader - GFP dropped from 200k to 100+k

10. Third Prof Brodersen's **RES only** characterization (for more than 12h):
    Key question: Do cells auto resume growth some time after addition of IPTG?
    OD600 = **0.1**
    Induce at 1h time point
    Arabinose (REStoxin inducer) = 0.0133M, 0.0266M
    RES sample seq acceptable (few point mutations)
    WT MG1655 control (previously got from David)

image.png

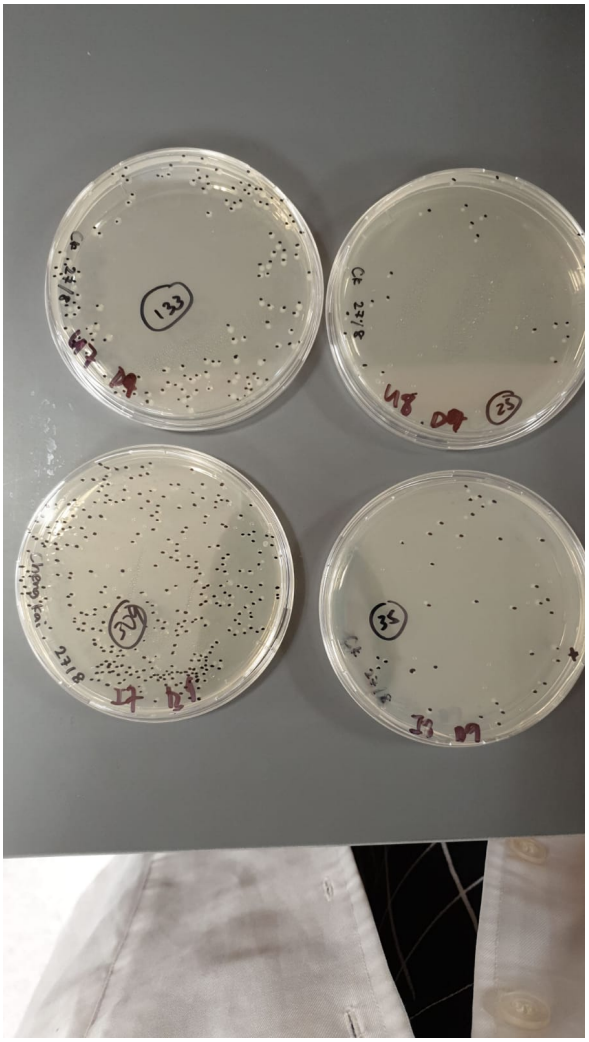


Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g60f2e6424a_0_34

THURSDAY, 29/08/2019

1. **LB SLEEP-WAKE + LUX demo DAY 7**
   Eight tubes (4 uninduced control vs 4 induced with IPTG)
   4% arabinose was added to **DAY 10 tubes (run in advance)** at 29degC -> post 3.5h induction...
   (a) take picture of entire bottle -
   (b) read luminescence using microplate (gain= 118) -
   Microplate reading volume: 200uL
   RESULTS: TEARS Uninduced cells are glowing but induced aren't.
   CFU PERFORMED AS WELL - more cells in induced than uninduced

image.png



2. **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 5; yesterday's pre-ara addition CFU counting (no point because induced have no colonies at all from ^6 to ^8 dilution while uninduced have many relatively large colonies)**

3. Inoculate colony for villu ko MG1655 containing AHL-HicB c1 plasmid

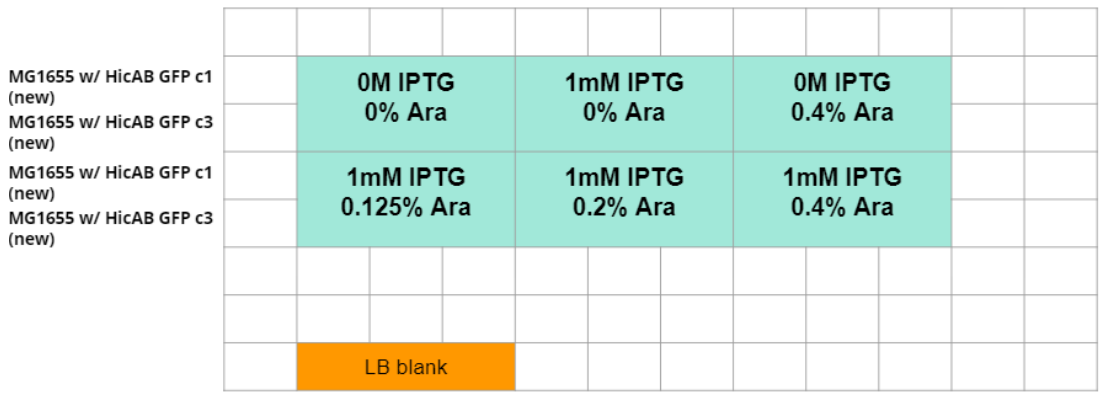
4. Inoculate WT RES/Xre c1 and c2 for characterization tomorrow

5. Stored 4degC and miniprep + send for seq:
    a. Three toggle switch part 1 colonies (supposedly HicB to be present)- plate 2 c1, c2, c3

6. Store -80degC for 10B transformed with Jingyun's Ara-EL222 plasmid + miniprep to stock up plasmid (store one tube for JY too)

7. **Chemical system characterization**: Studying **REFRESHED HicAB c1 and 3** effect on growth and protein expression when induced with 1mM IPTG and ara conc of 0.125%, 0.2% and 0.4%
   18h cont run
   OD600 = 0.1 at 0h
   IPTG added at 1h
   Ara added at 3.5h
   THIS TIME use restreaked + isolated colony of HicAB c1 and c3

image.png



Results:

FRIDAY, 30/08/2019

1. **0.4% glucose M9 MEDIA SLEEP-WAKE + LUX demo DAY 7**
   Eight tubes (4 uninduced control vs 4 induced with 2mM IPTG)
   2% arabinose was added to **DAY 10 tubes** at 29degC -> post 2.5h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

2. Make competent cells for villu ko MG1655 containing AHL-HicB c1 plasmid -> transform HicA-AHL c2 into competent cell

3. Inoculate villu ko HicAB c1 and c3 for characterization tomorrow **(using 500uM IPTG instead)**

4. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 0**
   Eight tubes (4 uninduced control vs 4 induced with **1mM IPTG**)
   2% arabinose was added to **DAY 0 tubes** at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   Results:

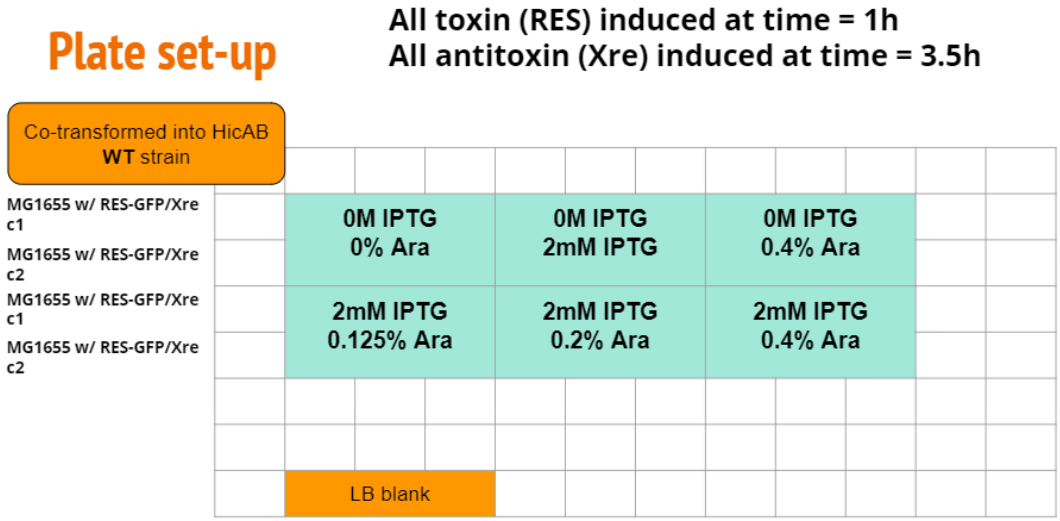
5. **NEW LB BATCH 3 (with refresh) SLEEP-WAKE + LUX demo D0**
   2% refreshing from overnight culture, 1h time point induce toxin for D1 and D5 induced samples
   Four tubes (2 uninduced control vs 2 induced with **1mM IPTG**)

   Results:

6. **OLD LB SLEEP-WAKE + LUX demo BATCH 1 DAY 7 induced samples**
   Refreshed 2% then 1h later **added 2% Arabinose** in the tube (10mL)
   Aliquoted 200ul into three wells -> read at 29degC using H1 synergy (20h cont)

7. **STANDARDIZED PLASMID: SECOND RES-GFP and Xre characterization IN MG1655 WT STRAIN.**
   18h cont run
   OD600 = 0.1 at 0h
   2mM IPTG added at 1h
   0.125%, 0.2%, 0.4% Ara added at 3.5h
   Running both c1 and c2 which should be SEQ ok (ck might have messed up during miniprep but prev characterization shows an effect of growth arrest and resumption)

image.png



Results:
https://docs.google.com/presentation/d/19EY5f5YrQjUSfEhHwoDvQxBUGTpSX03ELCqJ_bLYUHo/edit#slide=id.g41a2cc5a65_2_182

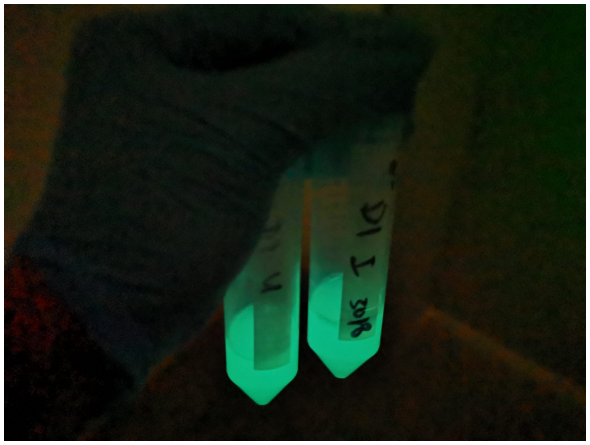
SATURDAY, 31/08/2019

1. Miniprep one of the G*J c8xc9 MG1655 samples - labeled G*J c8x9 c1 (accidentally labeled G*J c6)

2. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 1**
   Eight tubes (4 uninduced control vs 4 induced with **1mM IPTG**)
   2% arabinose was added to **DAY 1 tubes** at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   Results:

image.png



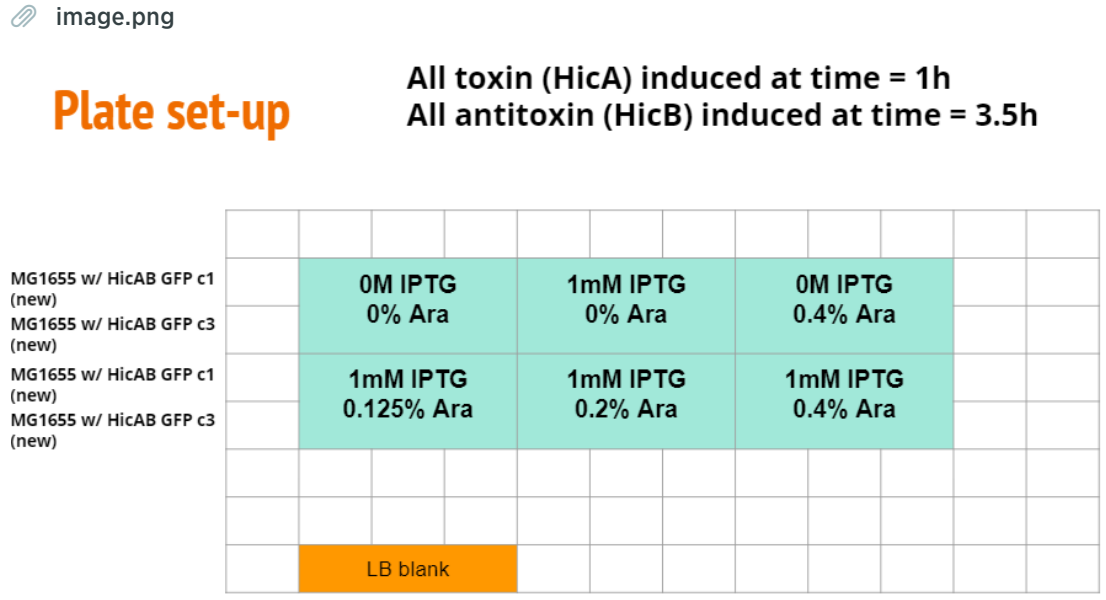
Induced is relatively higher in luminescence compared to uninduced (microplate result)

3. **NEW LB BATCH 3 (with refresh) SLEEP-WAKE + LUX demo D1**
   Four tubes (2 uninduced control vs 2 induced with **1mM IPTG**)
   2% refresh followed by addition of **2% arabinose** to **DAY 1 tubes** 1h later, incubated at 29degC -> post 4h induction...
   (a) take picture of entire bottle e
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   Results:

4. **Chemical system characterization**: Studying **REFRESHED HicAB c1 and 3** effect on growth and protein expression when induced with 1mM IPTG and ara conc of 0.125%, 0.2% and 0.4%
   18h cont run
   OD600 = 0.1 at 0h
   500uM IPTG added at 1h
   Ara added at 3.5h
   THIS TIME use restreaked + isolated colony of HicAB c1 and c3

image.png



Results:

MONDAY, 02/09/2019

1. Re-streaked HicAB c1 and c3 on CK plate (to characterize in LB vs M9 media on wed)

2. Miniprepped plasmid retention samples and send for seq

TUESDAY, 03/09/2019

1. Requested for new batch of villu HicAB ko competent cells; agar slab thrown away (all contaminated)

2. Inoculate one colony of HicAB c1 and c3 on CK plate (to characterize in LB vs M9 media on wed)

3. Overnight PCR run to clone RES fragment and HicA bb

4. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 4**
   Eight tubes (4 uninduced control vs 4 induced with **1mM IPTG**)
   2% arabinose was added to **DAY 5 tubes** at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   Characterize both maintained with IPTG vs unmaintained until D5 set

5. **NEW LB BATCH 3 (with refresh) SLEEP-WAKE + LUX demo D4**
   Four tubes (2 uninduced control vs 2 induced with **1mM IPTG**)
   2% refresh followed by addition of **2% arabinose** to **DAY 5 tubes** 1h later, incubated at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

   Did both LUX experiments one day in advance

WEDNESDAY, 04/09/2019

1. pH measurement of spent M9 vs spent LB media for day 10 samples
2. Run gel, gel extraction and gibson RES frag with HicA bb to produce RES only plasmid; transform into 10B
3. Run gel for the GJ c6 and c8 plasmid (which was miniprepped on Monday) to try to separate 3.6kbp plasmid from 3.4kbp plasmid --> failed to separate

THURSDAY, 05/09/2019

1. Inoculate 10B transformed with RES-only

2. Inoculate 10B (from David) to make competent cells tomorrow

3. Inoculate MG1655 SgrS-GFP c3 from -80degC (on ~5 Aug) and pCon-GFP p2c1 for ck to repeat SgrS characterization tomorrow

4. Add IPTG to D10 repeat induced tube (II) for maintenance

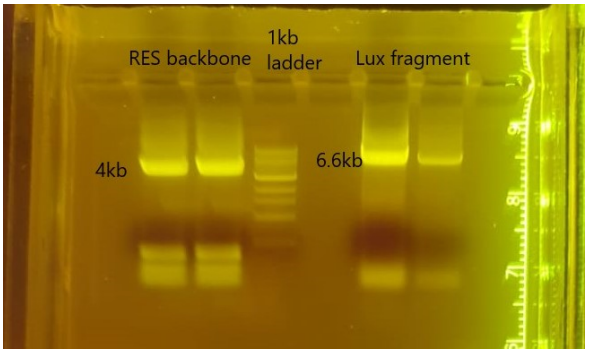
5. SLICE GFP-sok-Axe & J23100-hok-txe --> co-transform into 10B (ck plate) --> no colonies, to re-run PCR for J23100 and GFP fragments

6. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 6**
   Eight tubes (4 uninduced control vs 4 induced with **1mM IPTG**)
   2% arabinose was added to **DAY 5 tubes** at 29degC -> post 4h induction...
   (a) take picture of entire bottle
   (b) read luminescence using microplate (gain= 118) 20h cont
   Microplate reading volume: 200uL
   VERY NICE LUM GRAPH!!!!

FRIDAY, 06/09/2019

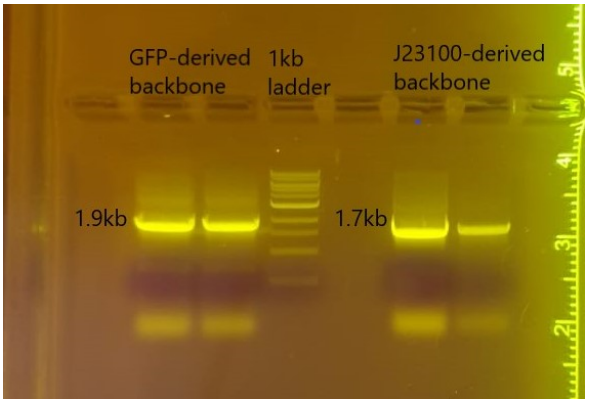
1. Miniprep 10B w/ RES c1, c2 and c3 - stop here (not continuing because focusing on RES-Lux cloning)
2. Make 10B competent cells; spread on c and k plate to test
3. Ran PCR for RES-Lux --> gel extraction -> gibson both fragments together -> transform into 10B (k plate)

image.png



4. Ran PCR for 3frag GFP fragment and J23100 fragment --> SLICE GFP pcr fragment with Sok-Axe gblock and J23100 pcr fragment with Hok-Txe gblock --> co-transform into 10b (CK plate)

image.png



5. Transform GJ P2C8 into MG1655, plate on K plate (see if mostly green)
6. Perform CFU for D6 uninduced and induced tubes (~24h post ara induction)
7. Inoculate inducible RFP cells for russell (to check arabinose effect on constitutive RFP production tomorrow)

SATURDAY, 07/09/2019

1. Check MG1655 GJ c8 k plate - few small colonies but all not glowing
2. RES-Lux and newly co-transformed GJ plates - no colonies at all
3. 10B competent cells made yesterday did not grow on c and k plate - safe for use
4. Re-gibson RES frag and Lux frag together; gibson (instead of SLICE) G frag with sok-txe, J frag with hok-axe again; transform and co-transform both into newly made 10B, K and CK plate respectively
5. Existing WT MG1655 comp cells (previously prepared by chun yang) contaminated with C plasmid, all thrown away
6. Brodersen's HicAB ko WT cells ok - uncontaminated
7. Inoculated (a) Brodersen hicAB ko cells and (b) WT MG1655 (previously checked for Joanne, growth ok in C and K media) over weekend to make comp on Monday

MONDAY, 09/09/2019

1. Make new Brodersen hicAB ko competent cells - inoculate cells in c and k media to check -> **results: C tube is contaminated**
2. Make new WT MG1655 competent cells - inoculate cells in c and k media to check -> **results: both ok**
3. Re-cotransform RES-Lux into newly made WT MG1655 **(no colonies)**
4. Re-co-transform G and J gibson product into newly made WT MG1655 **(no colonies)**
5. Co-transform Jingyun's pA6A plasmid and SgrS **only** plasmid into newly made MG1655 (for long-term sgrs demo) **(only have ONE colony)**
6. Co-transform AHL HicA and HicB into newly made Brodersen hicAB ko comp cells **(no colonies)**
7. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 10**
   DAY 10 tubes (uninduced, induced once, induced thrice) was aliquoted into microplate, triplicates
   Treated with 0% Ara, 0.2% Ara, 1% Ara and 2% Ara
   Ran at 29degC for 20h cont
   Read luminescence using microplate (gain= 118)
   Microplate reading volume: 200uL

TUESDAY, 10/09/2019

1. Inoculated MG1655 SgrS-A6A c1 (only colony) in LB+AK (amp media given by david)
2. Received THREE MG1655 comp cells from David to re-transform:
   a. New GJ (gibson product)
   b. RES-Lux (gibson product) and A8c-Xre plasmid
   c. 10B E2K-SgrS c1 and Jy's pA6A (1) plasmids
3. Obtained a 10B comp cell from David, to transform AHL-HicA c2 (seq ok) to make more plasmid (running out)
4. Re-inoculated Brodersen's hicAB ko stock cells in LB to make comp cells again tomorrow, LB+K and LB+C for testing
5. Re-inoculated WT MG1655 stock cells in LB to make electro-comp cells tomorrow, LB+K and LB+C for testing

WEDNESDAY, 11/09/2019

1. Store cells and keep at 4degC, miniprep and send for seq via biobasic: MG1655 SgrS-A6A c1
2. Add 1mM IPTG into Batch 2 Day 10 II tube for D12 maintenance (previously aliquoted cells out and induced in microplate so tube is still uninduced with ara)
3. Brodersen comp cells turned out fine - not growing in c or k media
4. Transformed plates, inoculated:
   a. David's MG1655 GJ - no colonies
   b. David's MG1655 RES-Lux and Xre - 3 colonies (non-fluorescing) in ck media
   c. David's MG1655 SgrS-A6A (re-transform as backup) - 3 colonies (non-fluorescing) in k+a media
   d. David's 10B HicA-AHL c2 - 1 colony (non-fluorescing) in k media (stock up)
5. Re-SLICE G and J with sok and hok gblocks again because previous product ran out -> transformed into David's 10B in CK plate
6. Co-transformed HicA-Lux and HicB c1 (finished plasmid tube) into Brodersen's hicAB ko comp cells
7. Inoculated v hicAB ko MG1655 w/ HicAB Lux in ck media overnight - label 24h IPTG

THURSDAY, 12/09/2019

1. Store 4degC and miniprep and send for seq (BIOBASIC):
    a. MG1655 RES-Lux and A8c-Xre c1
    b. MG1655 RES-Lux and A8c-Xre c2
    c. MG1655 RES-Lux and A8c-Xre c3
    d. MG1655 SgrS-A6A c1 (on hold, send tomorrow if p1c1 SgrS-A6A is not functional)
    e. MG1655 SgrS-A6A c2 (on hold, send tomorrow if p1c1 SgrS-A6A is not functional)
    f. MG1655 SgrS-A6A c3 (on hold, send tomorrow if p1c1 SgrS-A6A is not functional)
    g. 10B HicA-AHL c2 -> miniprep all (no need to store)
2. Co-transform newly miniprep HicA-AHL c2 w/ HicB-AHL c1 into brodersen hicAB ko MG1655 comp cells -> CK plate
3. Seq ok --> SgrS-A6A cells store in -80degC (two tubes, one for Joanne to bring to Cels, one backup keep in E6)
    a. Also inoculated a tube of MG1655 w/ SgrS-A6A add added 1mM IPTG to test whether inducible RFP is working
4. Inoculate newly co-transformed HicA/B Lux colonies c1 and c2 in LB+CK (15mL)
5. Inoculate RES-Lux and A8c-Xre c1, c2, c3 in LB+CK (10mL)
6. Chun Yang to make chemically comp cells for WT MG1655
7. Inoculated v hicAB ko MG1655 w/ HicAB Lux in ck media overnight - label late log uninduced
8. Measure OD to 0.1 and add 1mM IPTG to v hicAB ko MG1655 w/ HicAB Lux (labeled 24h IPTG) at 2pm

FRIDAY, 13/09/2019

Seq ok:

1. **LB BATCH 3 for HicAB // BATCH 1 for RES/Xre D0 (to maintain IPTG every 3 days)**
   Refresh:
    a. MG1655 w/ RES/Xre lux c2 (seq ok)
    b. b hicAB ko MG1655 w/ HicA/HicB lux c1 (poor seq, no HicA gene detected) and c2 (seq ok)
   to OD600 = 0.1 at 0h (11am)
   1h -> add 1mM iptg to I and II tubes (12pm)
   3.5h -> aliquot cells into microplate wells & add 2% ara at 2.30pm
   Measure for 20h continuously (gain = 118)
2. **Leftover** b hicAB ko MG1655 w/ HicAB Lux c1 and c2 to be **stored at 4degC + miniprepped --> send for seq**
3. If p1c1 for SgrS-A6A is red after adding IPTG, yesterday's miniprepped p2c1 - c3 NO NEED SEND for sequencing
4. Brodersen hicAB ko MG1655 w/ HicAB-AHL **- no colonies :(**
5. Late log vs 24 post IPTG demo:
   (a) Refresh v hicAB ko MG1655 w/ HicAB Lux (labeled late log uninduced) to OD=0.4 at 1.15pm in falcon tube, aliquot and induce 2% ara at 2.30pm
   (b) Aliquot v hicAB ko MG1655 w/ HicAB Lux (labeled 24h IPTG) and induce 2% ara at 2.30pm
6. Inoculated three new colonies from recent transformed 10B with AHL-HicA c2(1), c2(2), c2(3)
7. EL222 cloning: overnight pcr

SATURDAY, 14/09/2019

1. Add 1mM IPTG into Batch 2 Day 10 II tube for D15 maintenance (previously aliquoted cells out and induced in microplate so tube is still uninduced with ara)
2. Miniprep newly re-transformed 10B w/ HicA-AHL c2(1), c2(2), c2(3)
3. EL222 cloning cont:
   Samples:
    a. Ara HicB Brepin plasmid
    b. rrnBP EL222 Brepin plasmid
   gel electrophoresis -> slice -> transform into 10B AND ALSO co-transform with HicA-GFP plasmid into b ko MG1655
4. Co-transform HicA-GFP c2 and A8c-HicB into b ko MG1655 (CK plate)

SUNDAY, 15/09/2019

1. Inoculate transformed 3 colonies for:
    a. b hicAB ko MG1655 w/ HicA-GFP and A8C-HicB
2. EL222 cloning in 10B and b ko MG1655 have no colonies
3. Repeated EL222 cloning

MONDAY, 16/09/2019

1. Send newly re-transformed 10B w/ HicA-AHL c2(1), c2(2), c2(3) for sequencing
2. Send Brodersen hicAB ko MG1655 w/ HicA-GFP and A8C-HicB for sequencing
3. Characterize brodersen ko WT and brodersen ko w/ HicA-GFP and A8c-HicB at 1mM IPTG, 0.2% and 0.4% ara (see if there's difference in OD graph to see if we can keep previous characterized data using villu's ko strain)
4. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D3 maintenance
5. Inoculate seq ok:
    a. Broderden ko MG1655 w/ HicAB Lux c2
    b. MG1655 w/ RESXRE Lux c2

    For batch 2 Brodersen HicAB lux and batch 2 RES/Xre lux demo

TUESDAY, 17/09/2019

1. Co-transform seq ok HicA AHL c**3** w/ HicB AHL c1 into brodersen ko MG1655 (ck plate)
2. Add 1mM IPTG into Batch 2 Day 10 II tube for D18 maintenance (previously aliquoted cells out and induced in microplate so tube is still uninduced with ara)
3. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D0 (to maintain IPTG every 3 days)**

    Refresh:
    a. MG1655 w/ RES/Xre lux c2 (seq ok)
    b. b hicAB ko MG1655 w/ HicA/HicB lux c2 (seq ok)

    to OD600 = 0.1 at 0h (11.18am)

    1h -> add 1mM iptg to I and II tubes (12.18pm)

    3.5h -> aliquot cells into microplate wells & add 2% ara at 2.48pm

    Measure for 20h continuously (gain = 118)
4. Re-cotransform HicA GFP c1 and A8c HicB c4 into Brodersen ko MG1655 (ck plate)

WEDNESDAY, 18/09/2019

1. **LB BATCH 3 for HicAB // BATCH 1 for RES/Xre D5 (to maintain IPTG every 3 days)**

    Samples:

    MG1655 w/ RES/Xre lux c2 (seq ok)

    b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)

    Aliquot U, I, II cells into microplate wells & add 2% ara

    Measure for 20h continuously (gain = 118)
2. Inoculate brodersen ko MG1655 w/ HicAB AHL c1 (one and only colony)
3. Inoculate brodersen ko MG1655 w/ HicA-GFP and A8c HicB c1, c2, c3 (all green)
4. Inoculated one colony for 10B rrnBP1-BREPin hicB cell (I think was left in the incubator for a few days since weekend)

THURSDAY, 19/09/2019

1. **NEW LB BATCH 2 ("REPEAT") SLEEP-WAKE + LUX demo DAY 20**

    Eight tubes (4 uninduced control vs 4 induced with **1mM IPTG**)

    DAY 10 tubes aliquoted into microplate

2% arabinose was added to wells and incubated at 29degC (gain= 118) for 20h cont
Microplate reading volume: 200uL

2. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D6 maintenance
3. Store 4degC and miniprep + send for seq:
    a. brodersen ko MG1655 w/ HicAB AHL c1 (only colony)
    b. brodersen ko MG1655 w/ HicA-GFP and A8c HicB c1, c2, c3

FRIDAY, 20/09/2019

1. Store in -80degC:
    a. 10B EL222 BrepIn HicB c1 (one and only colony; seq ok)
    b. brodersen ko MG1655 w/ HicAB-AHL (all seq ok except hicA gene wasn't covered by primer)
2. Add 1mM IPTG into Batch **4 HicAB** and Batch **2 RES/Xre** II tube for D3 maintenance
3. Co-transform into brodersen ko MG1655:
    a. EL222 BrepIn HicB c1 and HicA-GFP c1
    b. AHL HicA c3 and AHL HicB c1 (repeat to try to get more clones in case existing on cannot arrest growth)
4.

SATURDAY, 21/09/2019

1. Inoculate colonies for brodersen ko MG1655 w/:
    a. EL222 BrepIn HicB c1 and HicA-GFP c1
    b. AHL HicA c3 and AHL HicB c1 (repeat to try to get more clones in case existing on cannot arrest growth)

SUNDAY, 22/09/2019

1. Store 4degC brodersen ko MG1655 w/:
    a. HicAB AHL p2c1 (shino re-transformed on 20th sep)
    b. HicA-GFP Brep-hicB c1, c2, c3

   Refreshed 2% media

2. P1C1 and P2C1 Brodersen ko MG1655 w/ HicAB AHL is added with 100nM AHL during refreshment -> RESULTS: Both turned RED when AHL is added - **FUNCTION OK!**

3. Inoculated in c and k media to test:
    a. Black marker labeled MG1655 comp cells - **OK, not contaminated**
    b. SgrS-GFP in -80degC (labeled c2, since c3 is contaminated with c) - **DAMN, contaminated; thrown away (no SgrS stock in -80degC for now)**

4. Picked one orange colony from P2C8 GJ k plate; inoculated in ck media

5. Co-transformed Jy's PAE-RFP (1) plasmid and David's pBR322 GFP plasmid into MG1655 - have colonies but not green (....yet)

6. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D9 maintenance

7. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D5 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)

MONDAY, 23/09/2019

1. Add 1mM IPTG into Batch **4 HicAB** and Batch **2 RES/Xre** II tube for D6 maintenance

2. **LB BATCH 3 for HicAB // BATCH 1 for RES/Xre D10 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)

   Also Batch **4 HicAB D6** (since D5 missed the wells for reading)

3. Inoculate in CK media brodersen ko MG1655 w/ HicAB AHL to kick start AHL demo tomorrow!
   a. P1C1
   b. P2C1
   (Both tested to be red/functional)

4. EL222 cell testing (in 96well plate)
   Samples: Brodersen ko MG1655 w/ HicA-GFP and EL222-rrnBP1-HicB c1, c2, c3
   Refreshed all to OD=0.1, split into three treatments: 1mM IPTG (in the dark), blue light only, 1mM IPTG + blue light
   Added 1mM IPTG to respective tubes at od=0.1 then immediately put under blue light/shake (same temp)
   Next day read GFP level (post IPTG expression)

5. Stored MG1655 GJ p2c8 (1) in -80degC (orange cells)

6. Miniprepped brodersen ko MG1655 w/:
   I. HicAB AHL p2c1 (shino re-transformed on 20th sep)
   II. HicA-GFP Brep-hicB c1, c2, c3
   III. MG1655 w/ GJ p2c8 (1)
   Sent only p2c1 AHL and GJ p2c8 (1) for seq (biobasic)

7. Inoculated one colony from MG1655 w/ RES-GFP and JY RFP plasmid (ctrl for plasmid retention) p.s plate is 2-day old, plated on sat

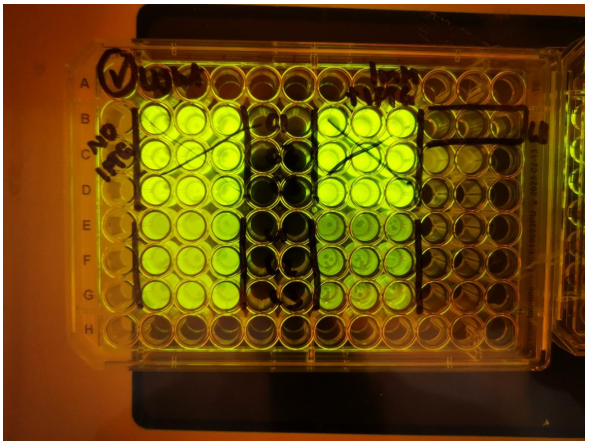
TUESDAY, 24/09/2019

1. **BATCH 1 AHL DEMO D0 (to maintain every 3 days):**
   Refresh:
   b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1
   to OD600 = 0.1 (11am)
   1h later -> add iptg to I and II tubes (12pm)
   3.5h -> aliquot cells into microplate wells & add 100nM AHL

2. Inoculate brodersen ko HicAB GFP c1, c2, c3 (21 sept) in two batch of CK media - one without anything, the other tube in 1mM IPTG (see if with IPTG the GFP is dimmer)

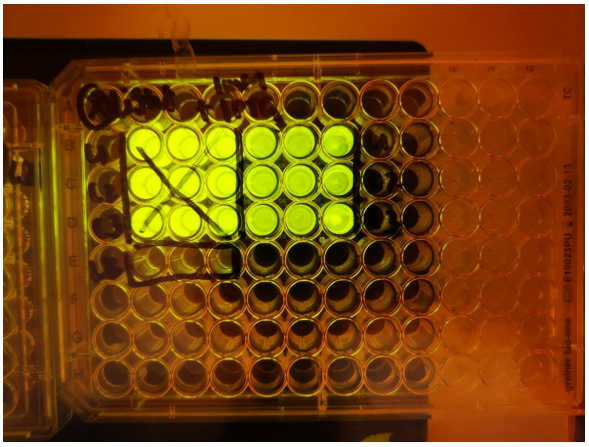
3. brodersen ko MG1655 w/ HicA-GFP and rrnBP-EL222-HicB c1 to c3 all seems ok **-> stored all 3 colonies in -80degC**
   Results: shine blue light + IPTG = lower GFP than IPTG in dark (with AT induced)

image.png



EFG row 2-4: Light only
EFG row 6-8: Light + 1mM IPTG = only toxin present (test)

image.png



BCD row 5-7: No light + 1mM IPTG = toxin and antitoxin present (control)

4. Streaked villu ko MG1655 w/ HicAB-GFP c1 and c3 on CK plate (to characterize on thurs)
5. Sent samples for HPLC:
    a. AHL p2c1 uninduced D0
    b. RES/Xre Batch 2 uninduced D7
    c. RES/Xre Batch 2 induced D7
    d. RES/Xre Batch 2 induced (2) D7
    e. HicAB Batch 4 uninduced D7
    f. HicAB Batch 4 induced D7
    g. HicAB Batch 4 induced (2) D7

WEDNESDAY, 25/09/2019

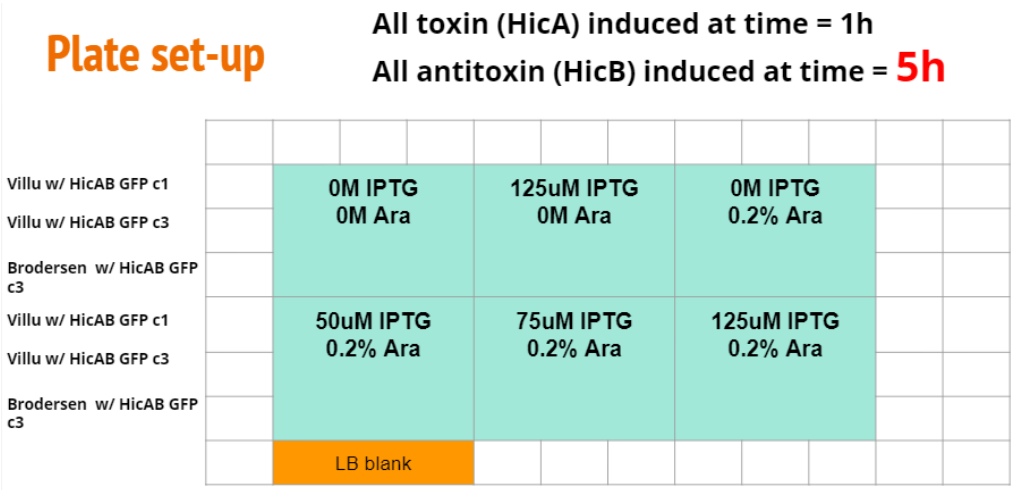
1. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D12 maintenance (suspect batch 2 added IPTG to all tubes)
2. Start **plasmid retention D0** (control & GJ c8(1)) ready (left control culture to grow for 2 days to become orange)
   To passage every 2 days then read RFP and GFP level
3. Visualize brodersen ko MG1655 w/ HicA-GFP and A8c-HicB c1, c2, c3 GFP color (with and without IPTG added) to compare GFP intensity - just to test HicA function since HicB seq ok --> only c3 is cloudy for IPTG treated batch, not sure why c1 and c2 got no cells (clear); nonetheless, od diff between + and - IPTG seems to be apparent so c3 is inoculated and stored in -80degC
4. Inoculate (function ok) brodersen ko HicAB GFP c3 overnight -> characterize tomorrow
5. Inoculate one colony for villu ko MG1655 w/ HicAB-GFP c1 and c3
6. Rerun gel using pcr products from before to get gel photo for wiki:

a. HicA-Lux frag and bb - from 6 Aug
b. E2K-SgrS-GFP - from 2 July

THURSDAY, 26/09/2019

1. Add 1mM IPTG into Batch **4 HicAB** and Batch **2 RES/Xre** II tube for D9 maintenance (HicAB should be left with 10ml - 1.8ml - 1ml (for HPLC); RES/Xre should be left with 10ml - 1.2ml - 1ml (for HPLC)
2. Inoculate fresh HicAB-Lux and RES/Xre culture overnight for new heat-stress experiment (villu hicAB Lux, brodersen hicAB Lux and MG1655 RES/Xre Lux)
3. **Chemical system characterization (using model prediction)**: Repeating **villu HicAB c1 and 3 and Brodersen HicAB c3** effect on growth and protein expression when induced with 50uM, 75uM and 125uM IPTG and 0.2% ara conc
   18h cont run
   OD600 = 0.1 at 0h
   respective IPTG added at 1h
   0.2% Ara added at **5h**
   Non-restreaked culture for brodersen and re-streaked culture for villu used

Plate layout



Results:

FRIDAY, 27/09/2019

1. HEAT STRESS EXPERIMENT:
   Samples: villu HicAB-Lux, brodersen HicAB-Lux and WT MG1655 RES/Xre
   Overnight culture refreshed to OD600=0.1
   Two tubes per sample - with and without 1mM IPTG
   Incubate overnight at 37degC

2. Add 1mM IPTG into AHL hicAB p1c1 and p2c1 for D3 maintenance

3. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D10 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)

4. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D10 CFU!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!**

To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
Samples:
a. HicA/HicB uninduced
b. HicA/HicB induced (1)
c. HicA/HicB induced (2)
d. RES/Xre uninduced
e. RES/Xre induced (1)
f. RES/Xre induced (2)

5. Cloning for work Indonesia team (collab)

6. Inoculate samples for BLUE LIGHT characterization tomorrow
   a. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c1
   b. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c2
   c. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c3

SATURDAY, 28/09/2019

1. Treat HicAB-Lux and RES/Xre overnight culture with heat (50degC) for 1h then measure lux over 20h (**heat stress experiment**)
2. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D15 maintenance
3. **LB BATCH 3 for HicAB // BATCH 1 for RES/Xre D15 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)
4. Refresh overnight cultures to OD=0.1 then induce 1mM IPTG --> shine blue light overnight
   I. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c1
   II. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c2
   III. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c3
5. Continue CFU based on microplate for batch 4 HicA-HicB and batch 2 RES/Xre
6. Continue cloning for indonesia team - inoculate colonies in C plate

SUNDAY, 29/09/2019

1. FIRST BLUE LIGHT CHARACTERIZATION:
   a. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c1
   b. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c2
   c. brodersen ko MG1655 w/ HicA-GFP and rrnBP1-EL222-HicB c3
   Immediately read OD of cells for 3h (in dark) -> then shine light for 3h -> then put back microplate reader for remaining measurement

2. **BATCH 1 AHL DEMO D5 (to maintain every 3 days): READ TOGETHER WITH BLUE LIGHT**
   Samples:
   b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1
   Aliquot U, I, II cells into microplate wells & add 100nM AHL
   Measure for **RFP** 20h continuously

3. **BATCH 1 AHL DEMO D5 CFU BEF AHL INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6 AND 10^7 dilution**
   To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
   Samples:
   a. HicA/HicB uninduced
   b. HicA/HicB induced (1)
   c. HicA/HicB induced (2)

For P1C1 and P2C1

4. **BATCH 1 AHL DEMO D5 ATP MEASUREMENT BEF ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!**
   To perform cfu BEF induction (from microplate, choose consistent data/well)

5. Add 1mM IPTG into Batch **4 HicAB** and Batch **2 RES/Xre** II tube for D12 maintenance (HicAB should be left with 10ml - 2.4ml - 1ml (for HPLC); RES/Xre should be left with 10ml - 1.8ml - 1ml (for HPLC)

6. Miniprep indonesia team plasmids and inoculate gel-ok samples in agar slab for them.

MONDAY, 30/09/2019

1. Add 1mM IPTG into AHL hicAB p1c1 and p2c1 for D6 maintenance

2. Add 1mM IPTG to maintain hicAB heat stress samples (D4); **BATCH 5 LB HicAB Lux // BATCH 3 RES/Xre Lux**

3. **BATCH 1 AHL DEMO D6 (to maintain every 3 days):**
   Samples:
   b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1
   Aliquot U, I, II cells into microplate wells & add **200nM** AHL
   Measure for **RFP** 20h continuously
   For P1C1 and P2C1

4.

TUESDAY, 01/10/2019

1. Add 1mM IPTG into Batch **3 HicAB** and Batch **1 RES/Xre** II tube for D18 maintenance

2. **LB BATCH 3 for HicAB // BATCH 1 for RES/Xre D18 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)

3. **LB BATCH 5 for HicAB // BATCH 3 for RES/Xre D5 CFU BEF ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^7 AND 10^8 dilution**
   To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
   Samples:
   villu HicA-HicB Lux
   Brodersen HicA-HicB Lux
   MG1655 RES-Xre Lux

4. **LB BATCH 5 for HicAB // BATCH 3 for RES/Xre D5 ATP MEASUREMENT BEF ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!**
   To perform cfu BEF induction (from microplate, choose consistent data/well)

5. **LB BATCH 5 for HicAB // BATCH 3 for RES/Xre D5 (to maintain IPTG every 3 days) READ TOGETHER WITH Batch 3 D18**
   Samples:
   Villu hicAB ko MG1655 w/ HicA/HicB lux
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   MG1655 w/ RES/Xre
   Aliquot U, I, II cells into microplate wells & add 2% ara

Measure for 20h continuously (gain = 118)

6. **BATCH 1 AHL DEMO D6 CFU AFT AHL INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^7 only**
   To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
   Samples:
   a. HicA/HicB uninduced
   b. HicA/HicB induced (1)
   c. HicA/HicB induced (2)
   For P1C1 and P2C1

WEDNESDAY, 02/10/2019

1. Add 1mM IPTG into Batch **4 HicAB** and Batch **2 RES/Xre** II tube for D15 maintenance (HicAB should be left with 10ml - 2.4ml - 1ml (for HPLC); RES/Xre should be left with 10ml - 1.8ml - 1ml (for HPLC)

2. Add 1mM IPTG into Batch **5 HicAB** and Batch **3 RES/Xre** II tube for D6 maintenance

3. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D15 (to maintain IPTG every 3 days)**
   Samples:
   MG1655 w/ RES/Xre lux c2 (seq ok)
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)

4. **BATCH 1 AHL DEMO D8 (to maintain every 3 days):**
   Samples:
   b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1
   Aliquot U, I, II cells into microplate wells & add **1000nM** AHL
   Measure for **RFP** 20h continuously
   For P1C1 and P2C1

5. **LB BATCH 5 for HicAB // BATCH 3 for RES/Xre D5 CFU AFT ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6 AND 10^7 dilution**
   To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
   Samples:
   villu HicA-HicB Lux
   Brodersen HicA-HicB Lux
   MG1655 RES-Xre Lux

6. Inoculated brodersen ko MG1655 w/ HicAB Lux c2
7. Refreshed Batch 4 D15 u, i and ii into new tubes (start new batch using this maybe?)

THURSDAY, 03/10/2019

1. Add 1mM IPTG into AHL hicAB p1c1 and p2c1 for D9 maintenance (both left with 5ml - 2.4ml)

2. **BATCH 1 AHL DEMO D8 CFU AFT AHL INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6 only**
   To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
   Samples:
   a. HicA/HicB uninduced
   b. HicA/HicB induced (1)
   c. HicA/HicB induced (2)
   For P1C1 and P2C1

3. New batch of Brod HicAB Lux c2 (from -80) **D0 batch A, B, C ********
    a. Batch A
    b. Batch B
    c. Batch C

    Made up of U, I, II
    Induce 1mM IPTG for I and II, maintain II every 3 days
    to OD600 = 0.1 at 0h (12.30pm)
    1h -> add 1mM iptg to I and II tubes (1.30pm)
    3.5h -> aliquot cells into microplate wells & add 2% ara at 4pm
    Measure for 20h continuously (gain = 118)

4. **LB BATCH A, B, C for HicAB D0 ATP!!!!! FOR MODEL CONSTRUCTION**

5. Streak brodersen HicA-GFP and A8c-HicB c3 in ck plate

6. **LB BATCH 4 for HicAB // BATCH 2 for RES/Xre D15 CFU AFT ARA INDUCTION !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6 only**
    Samples:
        a. HicA/HicB uninduced
        b. HicA/HicB induced (1)
        c. HicA/HicB induced (2)

FRIDAY, 04/10/2019

1. Inoculate brodersen HicA-GFP and A8c-HicB c3 for characterization tomorrow!!!

SATURDAY, 05/10/2019

1. ~~Add 1mM IPTG into Batch **5 HicAB** and Batch **3 RES/Xre** II tube for D9 maintenance~~ **throw away all batch 5 (heat stress) tubes, focusing on batch A B C**

2. Inoculate blue light cells from -80degC C1 - C3 in CK media overnight!!!

3. **BATCH 1 AHL DEMO D11 CFU BEF ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6**
    To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
    Samples:
        a. HicA/HicB uninduced
        b. HicA/HicB induced (1)
        c. HicA/HicB induced (2)
        For P1C1 and P2C1

4. **BATCH 1 AHL DEMO D11 ATP MEASUREMENT BEF ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!**
    To perform cfu BEF induction (from microplate, choose consistent data/well)

5. **BATCH 1 AHL DEMO D11 (to maintain every 3 days): SHARE WITH OD/GFP CHARACTERIZATION RUN**
    Samples:
    b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1
    Aliquot U, I, II cells into microplate wells & add 100nM AHL
    Measure for **RFP** 20h continuously
    For P1C1 and P2C1

CREATE PROTOCOL TO READ OD + RFP + GFP @ 37DEGC

6. **Chemical system characterization (model building with Brod cells)**: Repeating **Brod HicAB c3** effect on growth and protein expression when induced with:
    a. 1mM IPTG and 0.125%, 0.2% and 0.4% ara conc
    b. 10uM, 25uM, 50uM, 75uM IPTG only

    18h cont run
    OD600 = 0.1 at 0h
    respective IPTG added at 1h
    0.2% Ara added at **3.5h**
    Restreaked culture for brodersen used

SUNDAY, 06/10/2019

1. Add 1mM IPTG into AHL hicAB p1c1 and p2c1 for D12 maintenance (both left with 10ml - 3ml)

2. Add 1mM IPTG into Batch **A, B, C HicAB** II tube for D3 maintenance

3. **LB BATCH A, B, C for HicAB D3 ATP!!!!! FOR MODEL CONSTRUCTION**

4. **BATCH 1 AHL DEMO D11 CFU AFT ARA INDUCTION!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! 10^6**
    To perform cfu BEF induction and cfu AFT plate reading (from microplate, choose consistent data/well)
    Samples:
        a. HicA/HicB uninduced
        b. HicA/HicB induced (1)
        c. HicA/HicB induced (2)
        For P1C1 and P2C1

5. Induce appropriate blue light cells set-up with appropriate IPTG/light - leave overnight

MONDAY, 07/10/2019

1. Blue light experiment - excluded IPTG only samples
    Read light only and light + iptg in microplate for 3h
    Shine light again for 3h
    Read plate throughout till end of measurement

TUESDAY, 08/10/2019

1. **LB BATCH A, B, C for HicAB D5 (to maintain IPTG every 3 days)**
    Samples:
    b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
    Aliquot U, I, II cells into microplate wells & add 2% ara
    Measure for 20h continuously (gain = 118)

2. **LB BATCH A, B, C for HicAB D5 CFU 10^6 AND ATP!!!!! (BEFORE ARA INDUCTION)**
3.

WEDNESDAY, 09/10/2019

1. **BATCH 1 AHL DEMO D15 (to maintain every 3 days):**
    Samples:
    b hicAB ko MG1655 w/ HicAB AHL p1c1 and p2c1

Aliquot U, I, II cells into microplate wells & add 100nM AHL
Measure for **RFP** 20h continuously

2. **LB BATCH C for HicAB D5 CFU 10^6 (AFTER ARA INDUCTION) - only C performed due to better lum graph for D5**
3. Add 1mM IPTG into Batch **A, B, C and ref HicAB** II tube for D6 maintenance
4. Inoculate brodersen HicAB Lux c2 (in case need to start a new batch)
5. Inoculated brodersen HicAB-EL222 c1 c2 c3

THURSDAY, 10/10/2019

1. Refresh cells to OD600=0.1 (at 4pm) and induce 1mM IPTG in light+iptg wells leave overnight (refreshed at 11.30am so should be fine to induce directly)
2. ~~Batch Y and Z HicAB LUX D0 (induce 1h after 0.1 OD with 1mM IPTG), 3.5h (3.30pm) induce with 2% ara~~ **didn't do**
3. Added CK antibiotics into **LB BATCH A, B, C for HicAB D7**

FRIDAY, 11/10/2019

1. **LB BATCH A, B, C for HicAB D8 ATP!!!!! FOR MODEL CONSTRUCTION**
2. Blue light characterization: read plate continuously for 20h

SATURDAY, 12/10/2019

1. Add 1mM IPTG into Batch **A, B, C HicAB** II tube for D9 maintenance

SUNDAY, 13/10/2019

1. **LB BATCH A, B, C for HicAB D10 (to maintain IPTG every 3 days)**
   Samples:
   b hicAB ko MG1655 w/ HicA/HicB lux **C2 only** (seq ok)
   Aliquot U, I, II cells into microplate wells & add 2% ara
   Measure for 20h continuously (gain = 118)
2. **LB BATCH A, B, C for HicAB D10 CFU 10^6 AND ATP!!!!! (BEFORE ARA INDUCTION)**
3. Add 1mM IPTG into Batch **Y and Z HicAB** II tube for D3 maintenance
4. Inoculate brodersen HicA-GFP and HicB cells (c3) for characterization based on surface plot data tomorrow.

MONDAY, 14/10/2019

1. **LB BATCH A, B, C for HicAB D10 CFU 10^6 (AFTER ARA INDUCTION) - only for A and Ref with better lum results**
2. Characterization of brodersen HicA-GFP and HicB cells based on:
   Condition 1:
   - Fixed IPTG and varying ara -
     - 1mM IPTG, 0.4% ara
     - 0.5mM IPTG, 0.4% ara
     - 0.1mM IPTG, 0.4% ara

- 0.5mM IPTG, 0.1% ara
- 0.25mM IPTG, 0.05% ara

Condition 2:

- IPTG only - conc of:
  - 0.06mM
  - 0.04mM

Induce IPTG at 1h, Ara at 5h

TUESDAY, 15/10/2019

1. Batch Y and Z HicAB LUX **D5** CYTATION5 READING + ATP + CFU
2. Inoculate blue light brodersen cells c1, c2, c3

WEDNESDAY, 16/10/2019

1. Refresh to OD600 = 0.1 and add IPTG to selected wells (include a IPTG+blue light without putting in dark all the way as control showing that without putting in dark it continue to produce lower level of GFP)
2. Batch Y and Z HicAB LUX **D5** maintenance of tube II
3. **RE-RUN** characterization of brodersen HicA-GFP and HicB cells based on:

   Condition 1:

   - Fixed IPTG and varying ara -
     - 1mM IPTG, 0.4% ara
     - 0.5mM IPTG, 0.4% ara
     - 0.1mM IPTG, 0.4% ara
     - 0.5mM IPTG, 0.1% ara
     - 0.25mM IPTG, 0.05% ara

   Condition 2:

   - IPTG only - conc of:
     - 0.06mM --> change to 0.025mM
     - 0.04mM

   Induce IPTG at 1h, Ara at 3.5h

THURSDAY, 17/10/2019

1. 12.30pm transfer cells into DIFF plate and diff condition.

FRIDAY, 18/10/2019

1. Batch Y and Z HicAB LUX **D8** CYTATION5 READING + ATP + CFU