Glucose Transporter Lab Notebook

Project: Glucose Transporter Inhibition experiment

Authors: Lim Cheng Kai

THURSDAY, 5/16/2019

- Transformed SgrS from iGEM team 2011 Peking into 10B cells
- Transformed SgrS + ptsG tagged with GFP reporter into 10B cells

FRIDAY, 5/17/2019

-miniprep to extract Sgrs plamid

TUESDAY, 5/21/2019

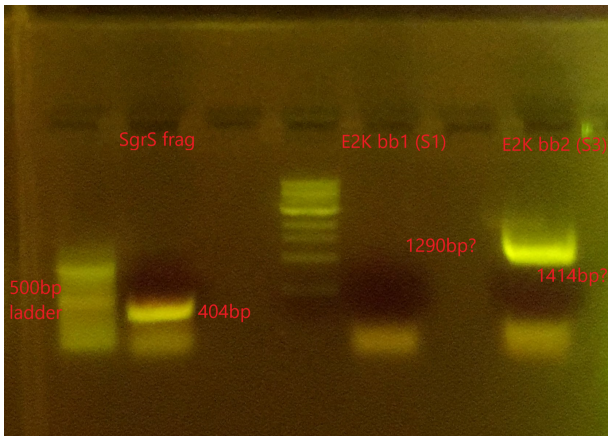
1. Inoculated 10B containing pB backbone into 50mL tube of LB + chloramphenicol overnight @ 37 deg cel.

Purpose: Preparation for Gibson assembly with gene-of-interest (SgrS)

THURSDAY, 6/13/2019

1. PCR-ed to clone SgrS gene into E2K backbone (w/ pTet)

SgrS frag (S2), E2K bb1 (S1), E2K bb2 (S3) gel res ult



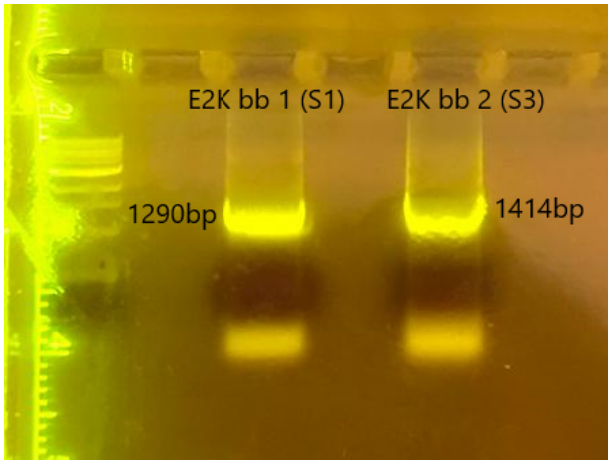
Correct size for SgrS frag, absence of band for E2K bb1 (S1) at 1290bp; <1kbp band for E2K bb2 (S3) unsure whether faint band above is a band or smear

2. Inoculated E2K-RFP plasid in 10b (FROM DAVID's -80degC stock) overnight in LB+K media -> tomorrow miniprep, send for seq, (E2K use as backbone for rerunning of PCR for SgrS bb)

FRIDAY, 6/14/2019

1. Miniprepped overnight E2K-RFP culture (given by David from his -80degC stock) --> sent for sequencing.
2. Leftover of E2K-RFP plasmid obtained, some is used to re-run PCR to get E2K bb1 and bb2 for cloning of SgrS into E2K bb -> ran gel -> gel extracted -> nanodrop (S1: 37.1ng/uL, S2 (frag): 38.4ng/uL, S3: 51.3ng/uL) -> gibson added 2.5ul, 2.5ul, 2ul and 3ul of S1, S2, S3 and dH2O respective -> transformed E2K-SgrS plasmid into TOP10 and 10B (left on bench over weekend)

E2K bb1 and bb2 gel result



Correct band size obtained from David's -80degC E2K cells

MONDAY, 6/17/2019

1. Checked transformed plates from friday:
   Top10 - E2K-SgrS: many colonies BUT RED, picked three for seq
   10B - E2K-SgrS: four colonies, not red; picked three for inoculation/sequencing
   WEIRD to have RFP for most colonies, could be Top10 strain problem since same E2K-SgrS plasmid transformed into two different competent cells (Top10 and 10b) show different color (10b not red)

TUESDAY, 6/18/2019

1. TOP10 competent cells CONTAMINATED --> All inoculated cultures thrown away/ABORTED
2. Miniprepped 10B E2K-SgrS c1, c2 and c3 -> send for seq along
3. Transformed cells
   a. 10B E2K-SgrS c1 plasmid (miniprepped today) **into newly made MG1655 -> RESULT: MANY COLONIES; SEQ OK for 10B**
   b. 10B E2K-SgrS c2 plasmid (miniprepped today) i**nto newly made MG1655 -> RESULT: MANY COLONIES; SEQ OK for 10B**
   c. 10B E2K-SgrS c3 plasmid (miniprepped today) i**nto newly made MG1655 -> RESULT: MANY COLONIES; SEQ OK for 10B**

WEDNESDAY, 6/19/2019

1. Seq result for all E2K-SgrS c1, c2, c3 **in 10b** are SUCCESSFUL!
2. Inoculated one colony from E2K-SgrS MG1655 c2 and c3 (back up) overnight, tomorrow characterize SgrS activity

THURSDAY, 6/20/2019

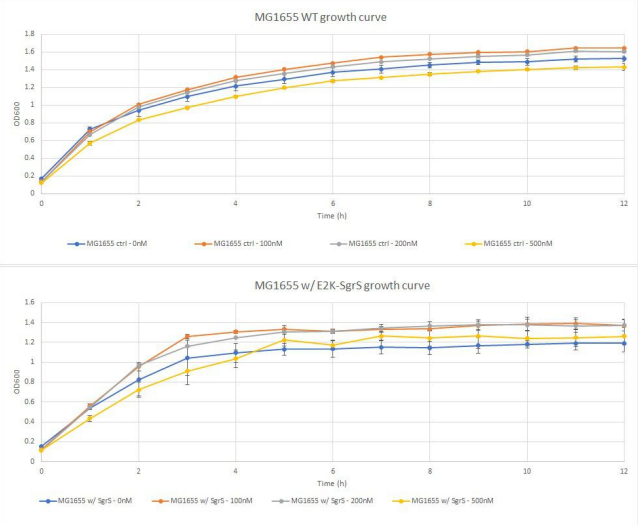
1. Characterized E2K-SgrS in MG1655, induced different ATc conc: 100nM, 200nM and 500nM in WT MG1655 (w/o plasmid), MG1655 w/ E2K plasmid, MG1655 w/ SgrS plasmid; induced at OD600=0.1

Plate layout for FIRST E2k-SgrS characterization

	1	2	3	4	5	6	7	8	9	10	11	12
A	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655
B	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid
C	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2
D												
E			0nM ATC	100nM ATC	200nM ATC	500nM ATC						
F												
G												
H	LB blank	LB blank	LB blank									

Results:

200619 12h read for WT MG1655 and E2K-SgrS characterization, 0, 100nM, 200nM, 500nM Atc.jpg



200619 12h read results for E2K-SgrS characterization, 0, 100nM, 200nM, 500nM Atc.xlsx

200619 E2K-SgrS characterization, 0, 100nM, 200nM, 500nM Atc.xlsx

WEDNESDAY, 6/26/2019

1. Transformed E2K-SgrS c2 (sequenced ok, produced in 10B) into MG1655 -> check colony size to confirm existing MG1655 E2K-SgrS c2 culture in -80degC is indeed MG1655 and not 10B

2. Inoculated WT MG1655 (from toxin characterization culture today), MG1655 E2K, MG1655 E2K-SgrS c2 (unconfirmed identity) for SgrS characterization tomorrow

3. Also inoculated one extra tube of MG1655 E2K-SgrS c2 (unconfirmed identity) -> to miniprep -> seq & check if seq ok

THURSDAY, 6/27/2019

1. Miniprepped MG1655 E2K-SgrS C2 (from -80degC) -> sent for sequencing

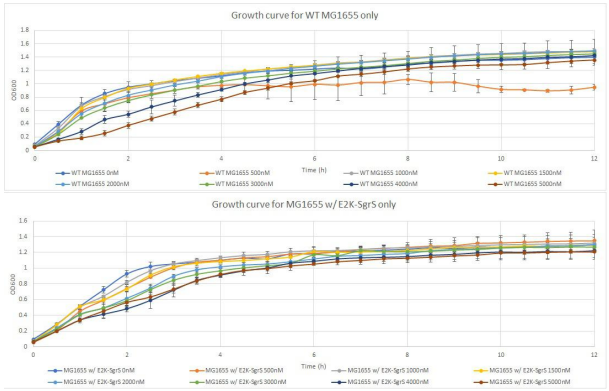
2. SgrS characterization
    - OD600 = 0.05 induction
    - 0nM, 500nM, 1000nM, 1500nM, 2000nM, 3000nM, 4000nM, 5000nM Atc
    - 100uL cells per well
    - 12h continuous read
    - Seq results: MG1655 E2K-SgrS c2 has good seq result

Round two SgrS characterization (trying more Atc inducer conc)

	1	2	3	4	5	6	7	8	9	10	11	12
A	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655
B	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid
C	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2
D	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655	New WT MG1655 ctrl	New WT MG1655	New WT MG1655
E	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid	MG1655 w/ E2K plasmid
F	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2	MG1655 w/ E2K-SgrS c2
G										LB blank	LB blank	LB blank
H		0nM ATC	500nM ATC	1000nM ATC	1500nM ATC	2000nM ATC	3000nM ATC	4000nM ATC	5000nM ATC			

Results:

270619 12h results for WT MG1655 and E2K-SgrS characterization 0, 500nM, 1000nM, 1500nM, 2000nM, 3000nM, 4000nM, 5000nM Atc AT OD=0.05.jpg



270619 E2K-SgrS characterization in MG1655 ctrl, w E2K and E2K-SgrS c2 0, 500nM, 1000nM, 1500nM, 2000nM, 3000nM, 4000nM, 5000nM Atc AT OD=0.05.xlsx

270619 12h results for E2K-SgrS characterization in MG1655 ctrl, w E2K and E2K-SgrS c2 0, 500nM, 1000nM, 1500nM, 2000nM, 3000nM, 4000nM, 5000nM Atc AT OD=0.05.xlsx

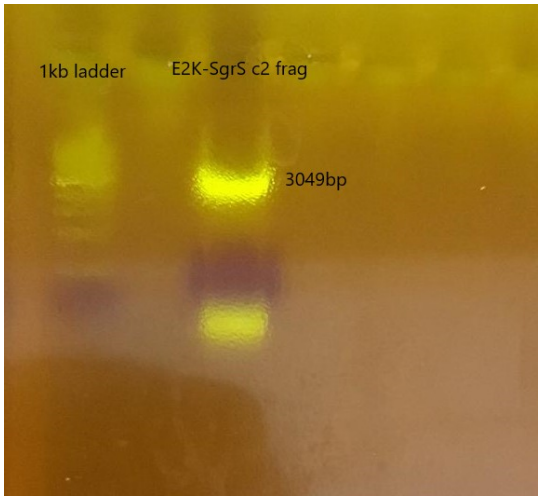
TUESDAY, 7/2/2019

1. Overnight PCR run to get E2K-SgrS c2 frag (for SgrS-GFP cloning work) - used same primer pair as toxin-GFP cloning purposes

WEDNESDAY, 7/3/2019

1. Continued E2K-SgrS-GFP cloning work
    - correct band size: 3049bp
    - gel extraction: 56.8ng/uL

PCR gel result for E2K-SgrS c2 frag



- Gibson with 88ng/uL GFP fragment
- Transform into 10B

THURSDAY, 7/4/2019

1. Inoculated three fluorescing E2K-SgrS c2-GFP : c1, c2, c3 in LB+K overnight

FRIDAY, 7/5/2019

1. Stored 4degC, **miniprep and seq**:
    - 10B E2K-SgrS c2-GFP c1
    - 10B E2K-SgrS c2-GFP c2
    - 10B E2K-SgrS c2-GFP c3

MONDAY, 7/8/2019

1. Transformed seq ok E2K-SgrS-GFP C1 into MG1655 (k plate)

TUESDAY, 7/9/2019

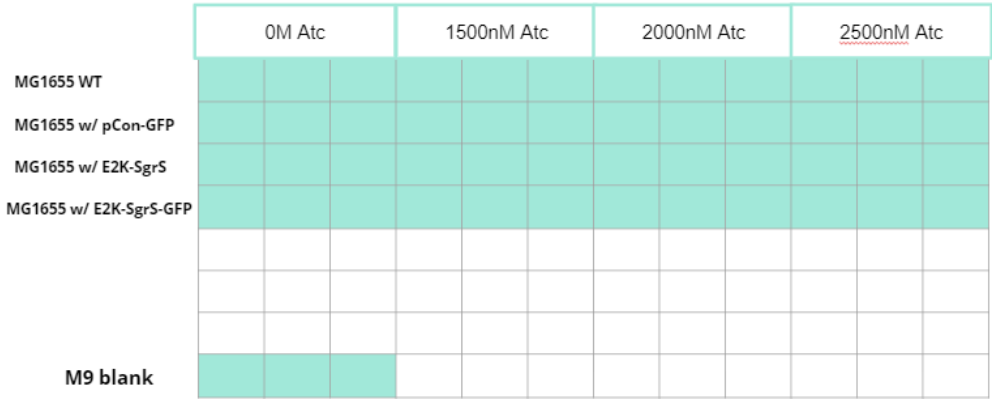
1. Inoculated one colony for MG1655 E2K-SgrS-GFP C1
2. Inoculated from -80degC:
    - MG1655 WT
    - MG1655 w/ pCon-GFP
    - MG1655 E2K-SgrS c2

WEDNESDAY, 7/10/2019

1. Miniprepped MG1655 E2K-SgrS-GFP c1 -> stored in 4degC + sent for sequencing
2. SgrS and SgrS-GFP characterization in **M9 media (0.2% glucose)**
    - OD600 = 0.1 induction
    - 0nM, 1500nM, 2000nM, 2500nM Atc

- 100uL cells per well
- 12h continuous read
- MG1655 E2K-SgrS c2 and MG1655 E2K-SgrS-GFP c1 with **GOOD seq result**

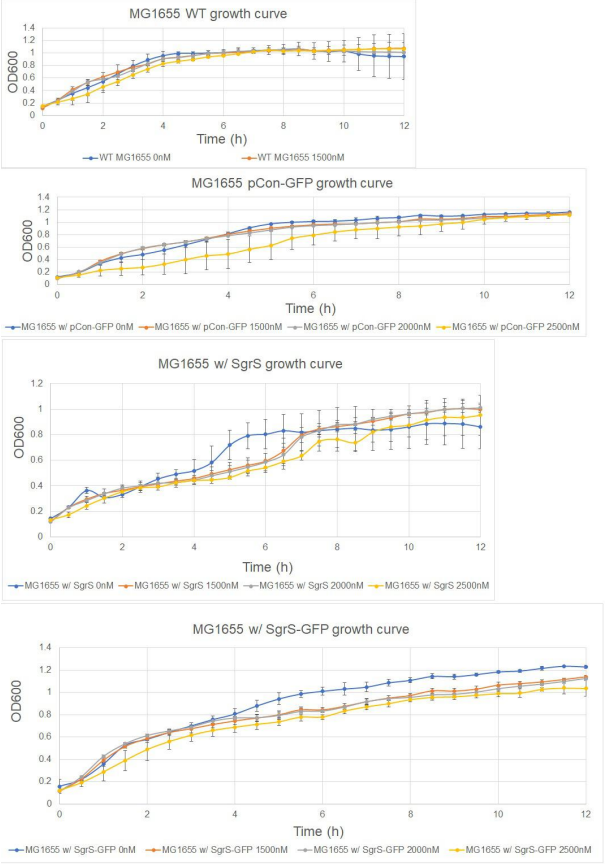
Plate layout



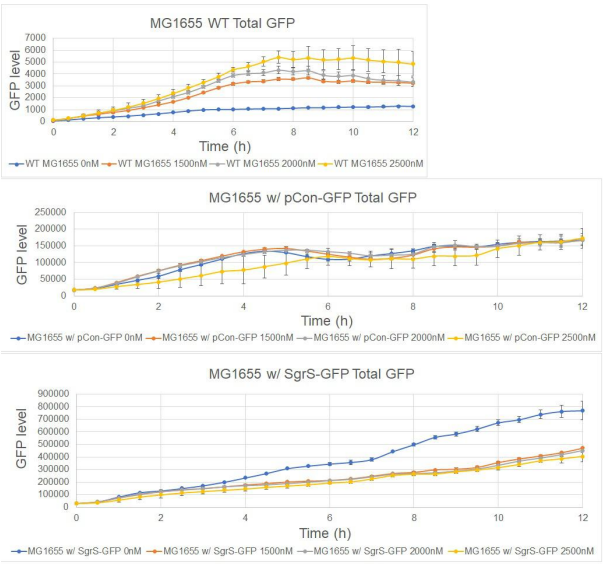
Results:

071019 12h results for New WT MG1655, MG1655 pConGFP, MG1655 E2K-SgrS, MG1655 E2K-SgrS-GFP, 1500nm, 2000nM, 2500nM, 0.1 OD, 12h read.xlsx

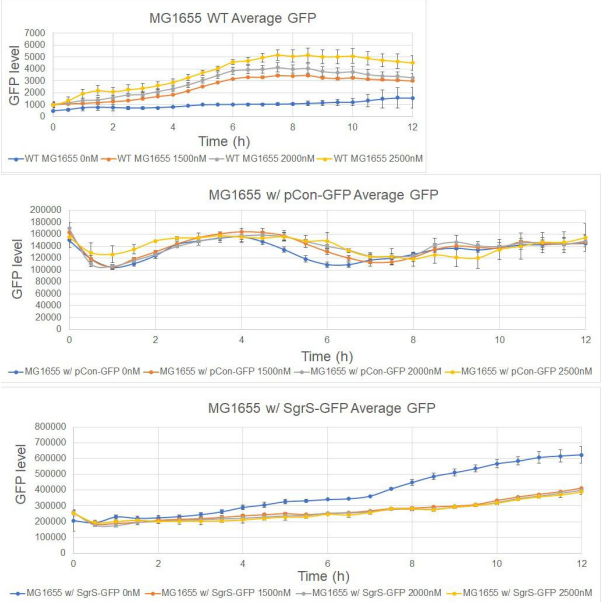
071019 12h cell growth results for New WT MG1655, MG1655 pConGFP, MG1655 E2K-SgrS, MG1655 E2K-SgrS-GFP, 1500nm, 2000nM, 2500nM, 0.1 OD.jpg



071019 12h total GFP results for New WT MG1655, MG1655 pConGFP, MG1655 E2K-SgrS, MG1655 E2K-SgrS-GFP, 1500nm, 2000nM, 2500nM, 0.1 OD.jpg



071019 12h average GFP results for New WT MG1655, MG1655 pConGFP, MG1655 E2K-SgrS, MG1655 E2K-SgrS-GFP, 1500nm, 2000nM, 2500nM, 0.1 OD.jpg



WEDNESDAY, 7/17/2019

1. REPEAT SgrS and SgrS-GFP characterization in **M9 media (0.2% glucose + KANAMYCIN)**
   - OD600 = 0.1 induction
   - 0nM, 1500nM, 2000nM, 2500nM Atc
   - 100uL cells per well
   - 12h continuous read
   - MG1655 E2K-SgrS c2 and MG1655 E2K-SgrS-GFP c1 with **GOOD seq result**
   - **Ran by Chun Yang**

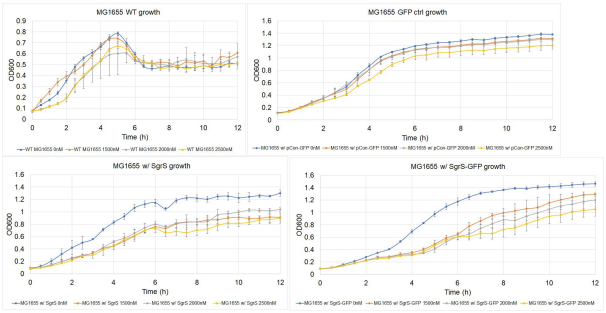
Plate layout

	0M Atc			1500nM Atc			2000nM Atc			2500nM Atc		
MG1655 WT												
MG1655 w/ pCon-GFP												
MG1655 w/ E2K-SgrS												
MG1655 w/ E2K-SgrS-GFP												
M9 blank												

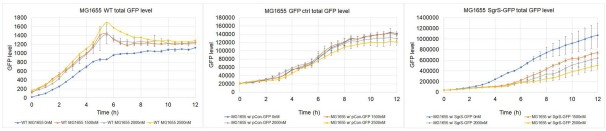
Results:

071719 New wt mg1655, MG1655 gfp ctrl, sgrs, sgrs+Gfp, 0.1 OD, 1500nM 2000nM 2500nM Atc, 12h cont REPEAT of 10Jul.xlsx

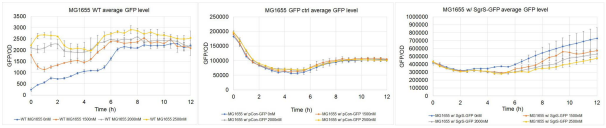
071719 12h OD600 results for New wt mg1655, MG1655 gfp ctrl, sgrs, sgrs+Gfp, 0.1 OD, 1500nM 2000nM 2500nM Atc, 12h.jpg



071719 12h total GFP results for New wt mg1655, MG1655 gfp ctrl, sgrs, sgrs+Gfp, 0.1 OD, 1500nM 2000nM 2500nM Atc, 12h.jpg



071719 12h average GFP results for New wt mg1655, MG1655 gfp ctrl, sgrs, sgrs+Gfp, 0.1 OD, 1500nM 2000nM 2500nM Atc, 12h.jpg



THURSDAY, 8/1/2019

1. Inoculated MG1655 SgrS-GFP c2 and c3 to miniprep and send plasmid for sequencing *tomorrow* (P.S Ck is characterizing the SgrS effect on growth in CELS today, both colonies obtained from transformed plate from a while ago).

FRIDAY, 8/2/2019

1. Miniprepped 8 samples:
    I. MG1655 SgrS-GFP C2 (characterization at CelS)
    II. MG1655 SgrS-GFP C3 (characterization at CelS)

FRIDAY, 9/6/2019

1. Ck repeated SgrS-GFP c2 and new pcon-GFP p2c1 run in E6

MONDAY, 9/9/2019

1. Co-transform Jingyun's pA6A plasmid and 10B E2K SgrS **only c1** plasmid into newly made MG1655 (for long-term sgrs demo)

MONDAY, 9/16/2019

1. Re-streak MG1655 w/ SgrS-GFP c2 and MG1655 w/ pcon-GFP p2c1 (from -80degC) on K plate

TUESDAY, 9/17/2019

1. Inoculate one colony for MG1655 w/ SgrS-GFP c2 and MG1655 w/ pcon-GFP p2c1 (from -80degC)

WEDNESDAY, 9/18/2019

1. Experiment 1: Characterize SgrS-GFP and GFP control in celS (by ck)
2. Experiment 2: 2% Refreshment of MG1655 w/ SgrS GFP

THURSDAY, 9/19/2019

1. Experiment 2 cont: 2% Refreshment; add 1000nM Atc; incubate 24h in 37degC incubator (induced vs uninduced) in 0.2% M9 media (w/o casamino)

FRIDAY, 9/20/2019

1. Experiment 2 cont: single read to read OD and GFP between uninduced and induced SgrS-GFP

MONDAY, 9/23/2019

1. Inoculated MG1655 w/ SgrS_A6A from glycerol stock into LB broth in CeLs

TUESDAY, 9/24/2019

1. Experiment 1: MG1655w/ SgrS_A6A characterization in **M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)**
    - OD600 = 0.1 induction
    - 0nM, 10nM, 100nM, 1000nM Atc
    - 150uM IPTG
    - 190uL cells per well
    - 12h continuous read
    - **Ran by Joanne**

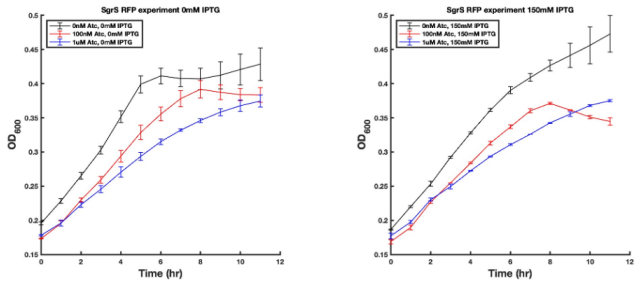
Capture4.PNG

24 Sep (Joanne)

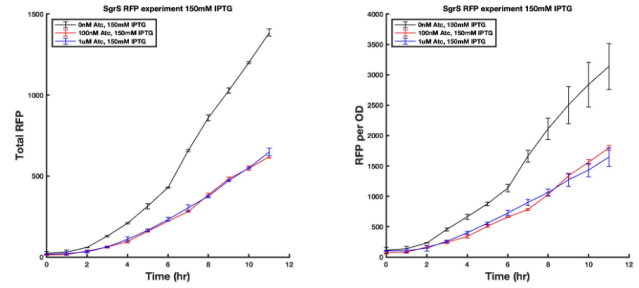
ATC induced at time = 0h
IPTG induced at time = 1h

SgrS-A6A RFP	0nM ATC, 0mM IPTG	100nM ATC, 0 mM IPTG	1uM ATC, 0 mM IPTG
SgrS-A6A RFP	0 nM ATC, 150mM IPTG	100nM ATC, 150 mM IPTG	1uM ATC, 150 mM IPTG
	M9 blank		

SgrS system_24092019_1.PNG



SgrS system_24092019_2.PNG



WEDNESDAY, 9/25/2019

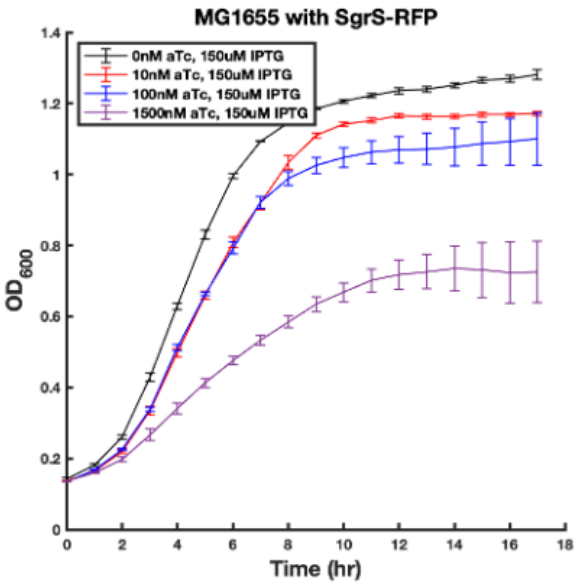
1. Experiment 2 cont: MG1655w/ SgrS_A6A characterization in **M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)**
   - OD600 = 0.1 induction
   - 0nM, 10nM, 100nM, 1500nM Atc; 0hr induction
   - 150uM IPTG; 1hr induction
   - 190uL cells per well
   - 20h continuous read
   - **Ran by Joanne**

SgrS layout.PNG

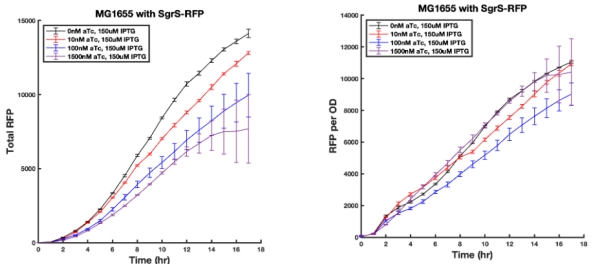
ATC induced at time = 0h
IPTG induced at time = 1h

SgrS-A6A RFP	100nM ATC, 0mM IPTG	10nM ATC, 0mM IPTG	1.5uM ATC, 0mM IPTG
SgrS-A6A RFP	100nM ATC, 150uM IPTG	10nM ATC, 150uM IPTG	1.5uM ATC, 150uM IPTG
SgrS-A6A RFP	0nM ATC, 0mM IPTG	0nM ATC, 150uM IPTG	M9 blank

SgrS system_26092019_1.PNG



SgrS system_26092019_2.PNG



2. D0 of glucose glo assay

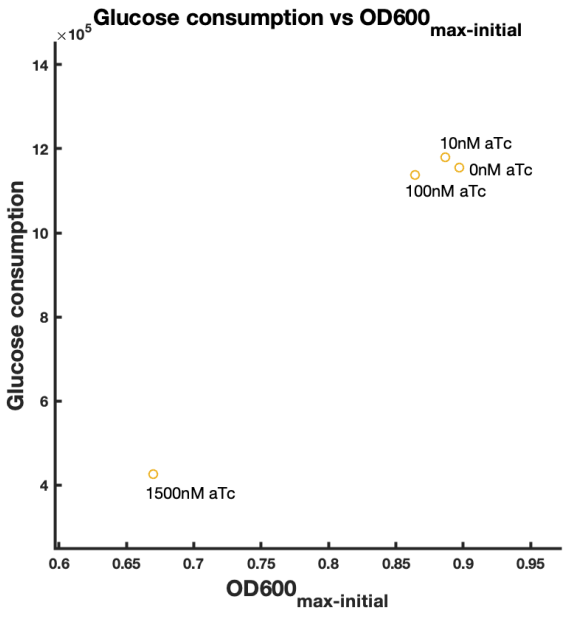
20190926SgrS_GlucoseGloD0.xlsx

THURSDAY, 9/26/2019

1. D1 of Glucose glo assay

20190926SgrS_GlucoseGloD1.xlsx

Glucose consumption vs OD growth.png



WEDNESDAY, 10/2/2019

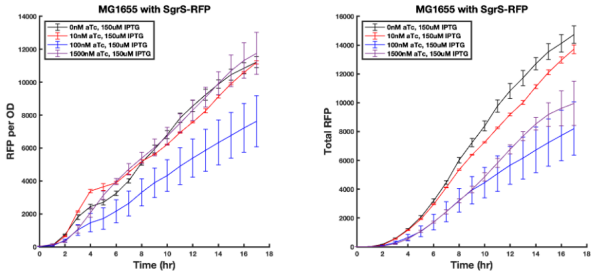
1. Experiment 2 repeat: MG1655w/ SgrS_A6A characterization in **M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)**
   - OD600 = 0.1 induction
   - 0nM, 10nM, 100nM, 1500nM Atc; 0h induction
   - 150uM IPTG; 1hr induction
   - 190uL cells per well
   - 20h continuous read
   - **Ran by Joanne**

SgrS layout.PNG

ATC induced at time = 0h
IPTG induced at time = 1h

SgrS-A6A RFP	100nM ATC, 0mM IPTG	10nM ATC, 0mM IPTG	1.5uM ATC, 0mM IPTG
SgrS-A6A RFP	100nM ATC, 150uM IPTG	10nM ATC, 150uM IPTG	1.5uM ATC, 150uM IPTG
SgrS-A6A RFP	0nM ATC, 0mM IPTG	0nM ATC, 150uM IPTG	M9 blank

SgrS system_02102019_2.PNG



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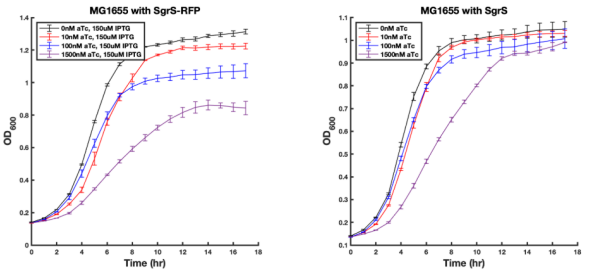


THURSDAY, 10/3/2019

1. Experiment 3 : MG1655w/ SgrS_A6A characterization in **M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)**
   - OD600 = 0.1 induction
   - 0nM, 10nM, 100nM, 1500nM Atc; 0hr induction
   - 150uM IPTG; 1hr and 2hrs induction
   - 190uL cells per well
   - 20h continuous read
   - **Ran by Joanne**

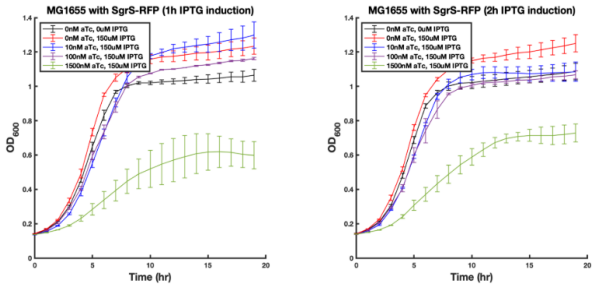
SgrS layout_03102019.PNG

ATC induced at time = 0h

IPTG induced at time = 1h, 2h

SgrS-A6A RFP (1h)	10nM ATC, 150uM IPTG	100nM ATC, 150uM IPTG	1.5uM ATC, 150uM IPTG
SgrS-A6A RFP (1h)	0nM ATC, 150uM IPTG	M9 blank	0nM ATC, 0mM IPTG
SgrS-A6A RFP (4h)	10nM ATC, 150uM IPTG	100nM ATC, 150uM IPTG	1.5uM ATC, 150uM IPTG
SgrS-A6A RFP (4h)	0nM ATC, 150uM IPTG	M9 blank	0nM ATC, 0mM IPTG

SgrS system_03102019_1.PNG



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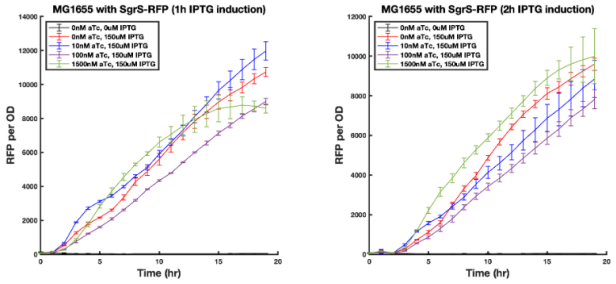
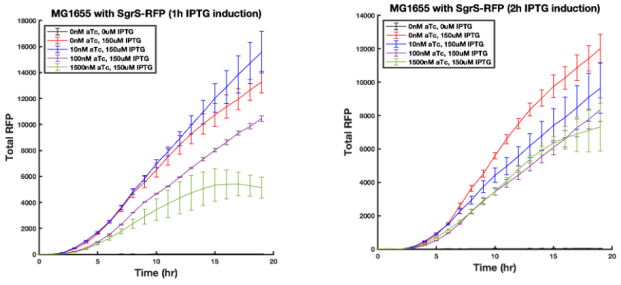


SgrS system_03102019_3.PNG



THURSDAY, 10/10/2019

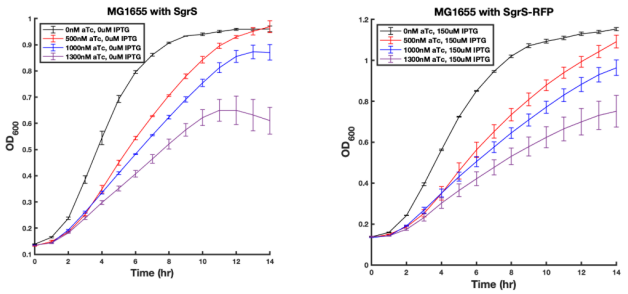
1. Experiment 4 (Final) : MG1655w/ SgrS_A6A characterization in **M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)**
    - OD600 = 0.1 induction
    - 0nM, 500nM, 1000nM, 1300nM Atc; 0hr induction
    - 150uM IPTG; 1hr
    - 190uL cells per well
    - 20h continuous read
    - **Ran by Joanne**

SgrS layout_10102019.PNG

ATC induced at time = 0h
IPTG induced at time = 1h

SgrS-A6A RFP		500nM ATC, 0mM IPTG			1uM ATC, 0mM IPTG			1.3uM ATC, 0mM IPTG				
SgrS-A6A RFP		500nM ATC, 150uM IPTG			1uM ATC, 150uM IPTG			1.3uM ATC, 150uM IPTG				
SgrS-A6A RFP		0nM ATC, 0mM IPTG			0nM ATC, 150uM IPTG			M9 blank				

SgrS system_10102019_1.PNG



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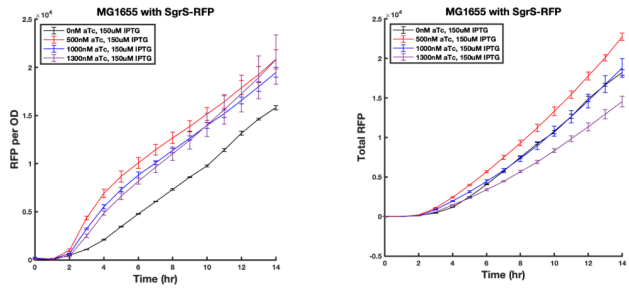

Single-temperature Double Digest

Introduction

This is the Double Digest Protocol with Standard Restriction Enzymes, using a common reaction and same incubation temperature for both enzymes.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

Procedure

Single Temperature DD Reaction

1. Set up the following reaction (total reaction volume 50 µl).

Table2

	A	B
1		**Reagent Volumes (µl)**
2	**Buffer (10x)**	5
3	**DNA ***	Input Volume for ng
4	**Restriction Enzyme #1 ****	1
5	**Restriction Enzyme #2 ****	1
6	**Deionized Water (µl)**	48
7	**Total Volume (µl)**	50

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.
** Restriction Enzymes should be added to the mixture last.

- [x] 2. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.
- [x] 3. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- [x] 4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

01:00:00

▶

Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.