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 plasmid

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 result
   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 plasmid 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) into newly made MG1655 -> RESULT: MANY COLONIES; SEQ OK for 10B
   c. 10B E2K-SgrS c3 plasmid (miniprepped today) into 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

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<td>B</td>
<td>MG1655 w/ E2K plasmid</td>
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<td>C</td>
<td>MG1655 w/ E2K-SgrSc2</td>
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### 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
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
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<td>MG16 55 w/ E2K-SgrS c2</td>
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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 fluorescenting 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

<table>
<thead>
<tr>
<th></th>
<th>0M Atc</th>
<th>1500nM Atc</th>
<th>2000nM Atc</th>
<th>2500nM Atc</th>
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<tbody>
<tr>
<td>MG1655 WT</td>
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<td>MG1655 w/ pConGFP</td>
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<td>MG1655 w/ E2K-SgrS</td>
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<td>MG1655 w/ E2K-SgrS-GFP</td>
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**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.

071019 12h total GFP results for New WT MG1655, MG1655 pConGFP, MG1655 E2K-SgrS, MG1655 E2K-SgrS-GFP, 1500nm, 2000nM, 2500nM, 0.1 OD.
07/10/19 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

<table>
<thead>
<tr>
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<th>0M Atc</th>
<th>1500nM Atc</th>
<th>2000nM Atc</th>
<th>2500nM Atc</th>
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<tbody>
<tr>
<td>MG1655 WT</td>
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<tr>
<td>MG1655 w/ pCon-GFP</td>
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<td>MG1655 w/ E2K-SgrS</td>
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<td>MG1655 w/ E2K-SgrS-GFP</td>
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Results:
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 + KANAMCYIN & AMPICILIN)
   - OD600 = 0.1 induction
   - 0nM, 10nM, 100nM, 1000nM Atc
   - 150uM IPTG
   - 190uL cells per well
   - 12h continuous read
   - Ran by Joanne
Experiment 2 cont: MG1655w/ SgrS_A6A characterization in M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)

1. 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
2. D0 of glucose glo assay
THURSDAY, 9/26/2019

1. D1 of Glucose glo assay

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
Experiment 3: MG1655w/ SgrS_A6A characterization in M9 media (0.2% glucose + KANAMYCIN & AMPICILIN)

1. OD600 = 0.1 induction
2. 0nM, 10nM, 100nM, 1500nM Atc; 0hr induction
3. 150uM IPTG; 1hr and 2hrs induction
4. 190uL cells per well
5. 20h continuous read
6. Ran by Joanne
SgrS layout_03102019.PNG

ATC induced at time = 0h  
IPTG induced at time = 1h, 2h

<table>
<thead>
<tr>
<th>SgrS-Δ6a RFP</th>
<th>10mM ATC, 150μM IPTG</th>
<th>100mM ATC, 150μM IPTG</th>
<th>1.5μM ATC, 150μM IPTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1h)</td>
<td>0mM ATC, 150μM IPTG</td>
<td>M9 blank</td>
<td>0mM ATC, 0mM IPTG</td>
</tr>
</tbody>
</table>

SgrS system_03102019_1.PNG

SgrS system_03102019_2.PNG

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

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SgrS layout_10102019.PNG

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

<table>
<thead>
<tr>
<th>SgrS-A6A RFP</th>
<th>500nM ATC, 0nM IPTG</th>
<th>1uM ATC, 0nM IPTG</th>
<th>1.3uM ATC, 0nM IPTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>SgrS-A6A RFP</td>
<td>500nM ATC, 150uM IPTG</td>
<td>1uM ATC, 150uM IPTG</td>
<td>1.3uM ATC, 150uM IPTG</td>
</tr>
<tr>
<td>SgrS-A6A RFP</td>
<td>0nM ATC, 0nM IPTG</td>
<td>0nM ATC, 150uM IPTG</td>
<td>M9 blank</td>
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SgrS system_10102019_1.PNG

SgrS system_10102019_2.PNG
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).

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<th>B</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td>Reagent Volumes (µl)</td>
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<tr>
<td>2</td>
<td>Buffer (10x)</td>
<td>5</td>
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<tr>
<td>3</td>
<td>DNA *</td>
<td>Input Volume for ng</td>
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<tr>
<td>4</td>
<td>Restriction Enzyme #1 **</td>
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<tr>
<td>5</td>
<td>Restriction Enzyme #2 **</td>
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<tr>
<td>6</td>
<td>Deionized Water (µl)</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>Total Volume (µl)</td>
<td>50</td>
</tr>
</tbody>
</table>

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.
** Restriction Enzymes should be added to the mixture last.
2. Mix components by pipetting the reaction mixture up and down, or by “flicking” the reaction tube.

3. Quick (“touch”) spin-down in a microcentrifuge. Do not vortex the reaction.

4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

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.