2021 Lab Log

Project: iGEM Guelph 2021
Authors: Enzo Baracuhy
Entry Created On: 2021-02-17 04:30:21 PM +0000
Entry Last Modified: 2021-10-19 12:21:54 AM +0000
Export Generated On: 2021-10-19 12:22:34 AM +0000

WEDNESDAY, 2/17/2021

- **Note in the lab log, the word guide may be referred to as "guide" or "g" followed by a number (i.e., g4 for guide 4)**

pfloral Namra Hamid Enzo Baracuhy Kulay Janneh

Golden gate using the NEB kit protocol

Two guide inserts were used in this protocol (SPCH1 and CLE18). Both guide inserts we used in a CRISPRa and a CRISPRi reaction. Both reactions were done in duplicates, for a total of eight reactions.

The reactions were set up as follows:

SPCH Guide 1/CLE18 Guide 1 CRISPRi

	A	B
1	Reagent	Volume (µL)
2	CRISPRi plasmid (undiluted)	1
3	Guide insert (SPCH Guide 1 or CLE18 Guide 1) (undiluted)	1
4	NEB enzyme mix	1
5	NEB buffer mix	2
6	dH2O	15
7	Total	20

SPCH Guide 1/CLE18 Guide 1 CRISPRa

	A	B
1	Reagent	Volume (µL)
2	CRISPRa plasmid (undiluted)	1
3	Guide insert (SPCH Guide 1 or CLE18 Guide 1) (undiluted)	1
4	NEB enzyme mix	1
5	NEB buffer mix	2
6	dH2O	15
7	Total	20

All eight reaction were subjected to the following PCR cycles:
Step 1: 99 cycles

- 2min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 time)
Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees
- infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

THURSDAY, 2/18/2021

Namra Hamid Danielle Halasz Kulay Janneh Enzo Baracuhy

Transformations Using GoldenGate Plasmids

The 8 plasmids from the Golden Gate reactions set up above (2 of SPCH guide 1 into CRISPRi, 2 of SPCH guide 1 into CRISPRa, 2 of CLE18 guide 1 into CRISPRi, 2 of CLE18 guide 1 into CRISPRa) were transformed into *E. coli* DH5α as follows:

1. The entire ligation mix from the Golden Gate reaction (20µL) was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat-shocked at 42°C for 45 seconds. Cells were rested on ice for 5 min.
4. 950µL of LB media were added to cells. Cells were shaken for 1 hr at 37C.
5. Plate 50µL or 950µL on LB plates containing kanamycin. Incubate overnight at 37C.

PCR using the Quiagen Kit protocol

The Quiagen Kit and Quiagen protocol were used to perform PCR on SPCH Guide 1 (CRISRa and CRISPRi patch plates)

1. The primers were diluted 1:10 from the IDT tubes before being added to the master mixes. Today, we did 50µL into 450µL.
    a. Tubes were labelled as follows: A or I (for activation or inhibition); 1, 2, 3, 4, 5 (for plate number); A, B, C, D, E, F (for patch designation)
    b. The following reagents were added to each PCR tube (see table below).
    c. The thermocycler was run as follows:

- 94°C for 3 minutes
- [94°C for 30 seconds
- 54°C for 30 seconds
- 72°C for 2 minutes] x35 cycles
- 72°C for 10 minutes
- Hold at 4°C

d. A total of 10 PCRs were run on experimental colonies plus one positive control and one negative control.
   I. Positive control: CRISPRi CLE18 guide 1
   II. Negative control: empty pHSN6i01 vector

- the following is in µL

Qiagen PCR Protocol

	A	B	C
1	10x Buffer	5	60
2	10mM dNTP (Fisher brand)	1	12
3	Forward primer (dilute)	5	60
4	Reverse primer (dilute)	5	60
5	Taq Polymerase	0.25	3
6	Sterile mQ Water	33.75	405
7	Total volume	50	600

FRIDAY, 2/19/2021

Danielle Halasz

Gels were run of the PCR products prepared on Thursday

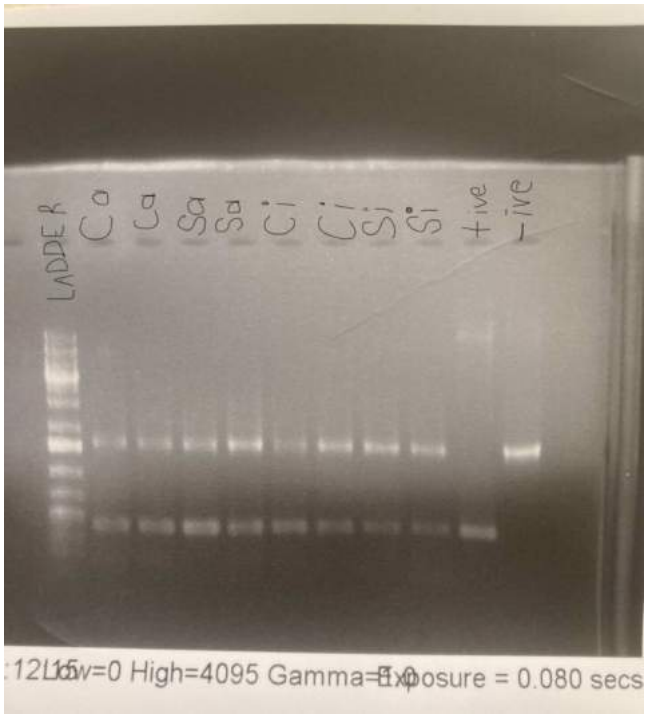
1. A 1% agarose gel was prepared using 100 mL TAE buffer and 1.0 g agarose.
2. 5.0 µL of RedSafe was added to the gel after the mixture cooled to a reasonable temperature.
3. Gel was poured and allowed to solidify.
4. Samples were prepared on parafilm wax: 2µL of loading dye was added to 9µL of Gene Ruler 1kb DNA Ladder (ladder did not already contain dye). 2µL of loading dye was added to 9µL of each sample.
   a. Note: Some of the samples partially dried on the parafilm wax, resulting in some of the loading dye and possibly dye sticking to the parafilm wax and not entering the wells.
5. Samples were loaded as follows:

Ladder, Ca Ca Sa Sa Ci Ci Si Si positive control negative control

a = CRISPRa, i = CRISPRi, C = CLE18, S = SPCH; (i.e. Ca = CLE18 CRISPRa)

6. Gel was run for 40 minutes at 114V

image.png

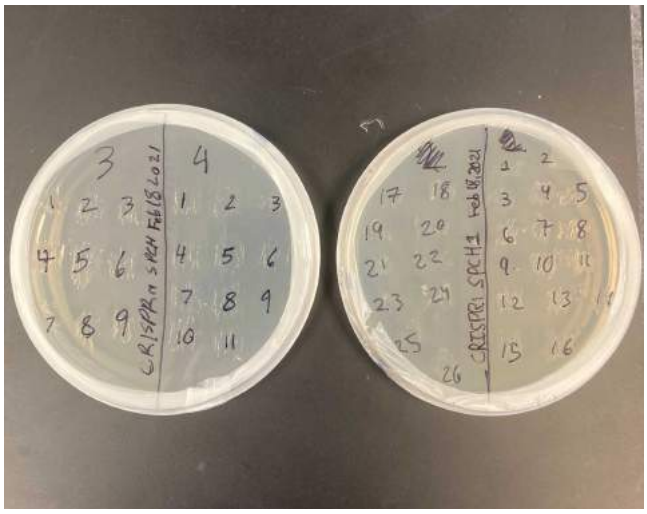


Patch Plate Preparation

Patch plates were prepared for 2 plates containing CLE18 CRISPRi guide, and 2 plates containing DXR CRISPRi guide 1.

1. Colonies were scraped off of the plates listed above using a sterile loop
2. Each colony was spread in a small patch on an LB + Kanamycin plate.
3. patches were made on each plate.

image.png



Protocol for visualizing a gel:

The protocol for vizualizing a gel that was just run is to go to the room with the machine that lets you view gels, go to the computer next to it, and then click: Bio Rad Gel Doc XR, Basic, file, gel dox xr, in that order, and then go over to the machine itself to turn on the UV ray button, then your gel image will appear on the computer screen. Make sure the imager door is completely closed. It might seem like it is, but if you cannot see an image, give it an extra push.

SATURDAY, 2/20/2021

Danielle Halasz Enzo Baracuhy

golden gate using expired BSAI enzyme

- A total of four golden gate reactions were run using two SPCH CRISPRi, and two CLE18 CRISPRi backbones, in order to test expired BSAI restriction enzyme
- one of each CRISPR backbone was run using the usual procedure for golden gate reactions, and the other of each CRISPR backbone was used in a golden gate procedure where the amount of BSAI restriction enzyme was doubled
- Reagents were added in the following order: H_2O, CRISPR plasmid, gRNA, NEB buffer mix, NEB enzyme mix

CRISPR backbones

	A	B
1	CRISPR backbone	abreviation
2	SPCH CRISPRi back bone, 1 µL of BSAI enzyme	Si 1
3	SPCH CRISPRi back bone, 2 µL of BSAI enzyme	Si 2
4	CLE18 CRISPRi back bone, 1 µL of BSAI enzyme	Ci 3
5	SPCH CRISPRi back bone, 2 µL of BSAI enzyme	Ci4

Standard Golden Gate procedure

	A	B
1	**Ingredient**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	BsaI	**1**
5	T4 ligase	1
6	10x CutSmart	2
7	H2O	**12**
8	ATP	2
9	**Total**	**20**

Golden Gate procedure using double the usual BSAI enzyme amounts

	A	B
1	**Ingredient**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	BsaI	**2**
5	T4 ligase	1
6	10x CutSmart Buffer	2
7	H2O	**11**
8	ATP	2
9	**Total**	**20**

Thermocycler cycles set as follows:

Step 1: 99 cycles;

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 times)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees
- Infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

SUNDAY, 2/21/2021

Danielle Halasz Amira Bouchema Namra Hamid

Master mix for PCR

- Followed SOP for PCR outlined on experiments page.
    - GG = Golden Gate

Table1

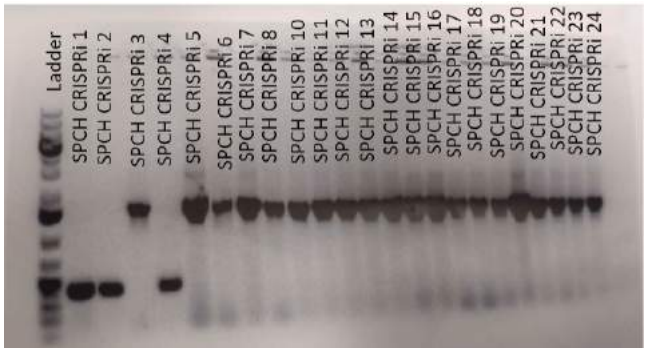
	A	B	C	D	E	F
1	reagent	x1 (cDNA)	x1 (GG)	x48 (cDNA)	x4 (GG)	x17 (cDNA)
2	Buffer	5	5	240 µL	20 µL	85 µL
3	miliQ water		23.75	1476 µL	95 µL	403.75 µL
4	dNTP	1	1	48 µL	4 µL	
5	Forward Primer	5	5	240 µL	20 µL	85 µL
6	Reverse Primer	5	5	240 µL	20 µL	85 µL
7	DNA	10	3	10 µL/well	3/per well	
8	Taq	0.25	0.25	12 µL	1	4.25

Gel 1

Samples were loaded as follows: LADDER, SPCH CRISPRi (1 2 3 4 5 6 7 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24)

- numbers represent their respective patch on its patch plate

image.png

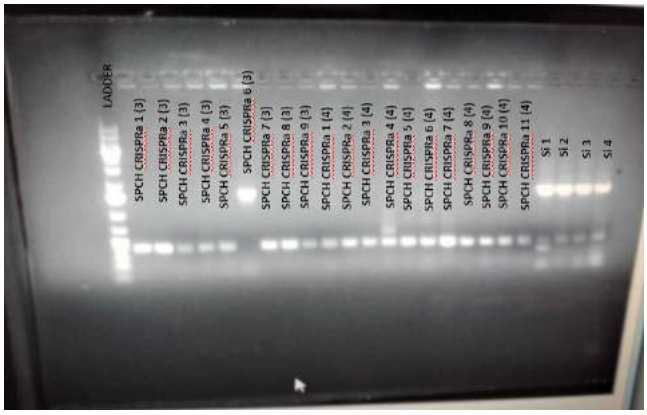


Gel 2

Samples were loaded as follows: LADDER, SPCH CRISPRa 3 (1 2 3 4 5 6 7 8 9), SPCH CRISPRa 4 (1 2 3 4 5 6 7 8 9 10 11) Golden gate (Si 1, Si2, Si 3, Si 4)

- numbers represent their respective patch on its patch plate; Si = SPCH gene CRISPRi

image.png



For Gel 2, colours were inverted for clairity

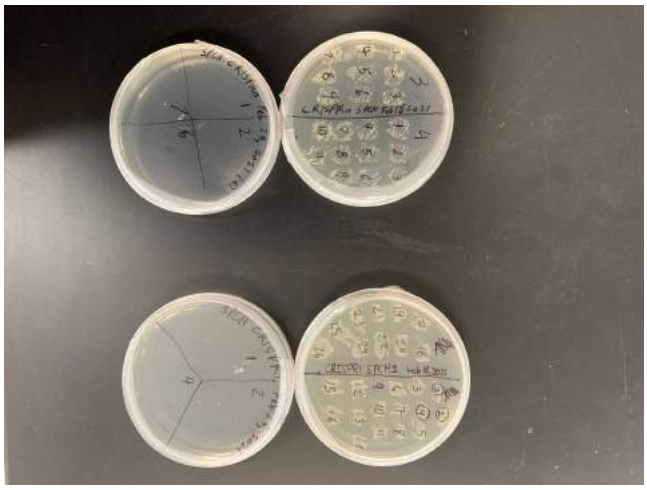
WEDNESDAY, 2/24/2021

Danielle Halasz

Streak plating of patch plates

1. brought 2 Petri dishes from the fridge labelled "LB+KAN" as well the two filled petri dishes containing patch plates of CRISPRa SPCH and CRISPRi SPCH from the 4 Celsius fridge.
2. Sterilized area
3. divided the petri dish for CRISPRa SPCH into quarters, and streaked patches 1, 2, 6, and 7 from section 3 onto the new plate,
    a. Patches 1, 2, 6, and 7 corresponded to the strongest positive band reads present from the gel run on Sunday Feb 21
4. divided the petri dish for CRISPRi SPCH into thirds, and streaked patches 1, 2, and 4 onto the new plate
5. Took off the parafilm from already culture filled petri dish from the 4 Celsius fridge
6. Light up a bunsen burner and sterilized the innoculating loop and let it cool for 10 seconds.
7. scraped from the middle part of the culture filled petri dish with the innoculating loop and closed the culture as soon as possible.
8. Streaked the new petri dish accordingly and closed the new petri dish.
9. Wrapped both petri dishes with para film.
10. settled the new petri dishes in the 37C incubator, and the patch plates were returned to the fridge

image.png



THURSDAY, 2/25/2021

Enzo Baracuhy

The new plates made by Danielle Halasz yesterday were taken from the 37C incubator and placed in the 4C fridge

FRIDAY, 2/26/2021

Amira Bouchema Danielle Halasz Namra Hamid

Preparation of overnights for glycerol stocks

1. 7 sterile glass tubes were labelled "Ci SPCH 1, Ci SPCH 2, Ci SPCH 4, Ca SPCH 1, Ca SPCH 2, Ca SPCH 6, Ca SPCH 7" backbone"
    a. Here Ci = CRISPRi and Ca = CRISPRa
2. A bench area was wiped with ethanol and a bunsen burner was lit
3. 3mL of LB media was put into each tube
4. Kanamycin was taken from the freezer and 3µL was put into each tube
5. An inoculating loop was used to swab a sample from each of the streak plates made on Wednesday labelled "SPCH CRISPRi 1, 2, 4", and SPCH CRISPRa 1, 2, 6, 7" and placed into their corresponding test tubes containing LB and kanamycin
6. Tubes were put to shake in the incubating chamber within the lab space

Golden gate reaction using expired BsaI enzyme

- A total of four golden gate reactions were run using two SPCH CRISPRa, and two CLE18 CRISPRa backbones, in order to test expired BsaI restriction enzyme on CRISPRa plasmids
    - although the expired BSAI restriction enzyme did not successfully ligate inserted into the CRISPRi plasmids (as seen on Sunday, Feb 21), there is a possibility that the enzyme still works and it is an issue with the CRISPRi backbone itself since there were so many other negative results with the CRISPRi plasmid, but nearly all the CRISPRa plasmids used in the gel were positive.
- Reagents were added in the following order: H_2O, CRISPR plasmid, gRNA, NEB buffer mix, NEB enzyme mix

Table2

	A	B
1	**Ingredient**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	BsaI	**1**
5	T4 ligase	1
6	10x CutSmart	2
7	H2O	**12**
8	ATP	2
9	**Total**	**20**

Thermocycler cycles set as follows:

Step 1: 99 cycles;

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 times)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees

- Infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

- temperature is in Celsius

SATURDAY, 2/27/2021

Danielle Halasz Amira Bouchema Namra Hamid

***overenights from previous night were thrown out since they were run at the incorrect temperature

PCR using the Quiagen Kit protocol: Golden Gate products prepared Friday

The Quiagen Kit and Quiagen protocol were used to preform PCR on CRISPRa SPCH Guide 1 and CLE18 Guide 1

1. The primers were diluted 1:10 from the IDT tubes before being added to the master mixes. Today, we did 50µL into 450µL.
    a. **Tubes were labelled as follows: S= SPCH or C= CLE18; A (CRISPR activation) 1, 2 (sample number)**
    b. The following reagents were added to each PCR tube (see table below).
    c. The thermocycler was run as follows:
        - 94°C for 3 minutes
        - [94°C for 30 seconds
        - 54°C for 30 seconds
        - 72°C for 2 minutes] x38 cycles
        - 72°C for 10 minutes
        - Hold at 4°C
    d. A total of 4 PCRs were run on experimental colonies plus one positive control and one negative control.
        I. **Positive control:** CRISPRa CLE18 guide 1
        II. **Negative control**: Empty pHSN6i01 vector

- the following values are in µL
- GG = Golden Gate

Table3

	A	B	C
1	10x CL Buffer	5	40
2	10mM dNTP (Fisher brand)	1	8
3	Forward primer (dilute)	5	40
4	Reverse primer (dilute)	5	40
5	Taq Polymerase	0.25	2
6	GG DNA	2	2/well
7	Sterile mQ Water	31.75	254
8	Total volume	50	386

Gels were run of the PCR products prepared on Saturday

1. A 1% agarose gel was prepared using 50 mL TAE buffer and 0.5 g agarose.
2. 2.5 µL of RedSafe was added to the gel after the mixture cooled to a reasonable temperature.
3. Gel was poured and allowed to solidify.
4. Volume loaded as follows:

5µL of Gene Ruler 1kb DNA Ladder, 10 µL of each sample loaded

5. Samples were loaded as follows:

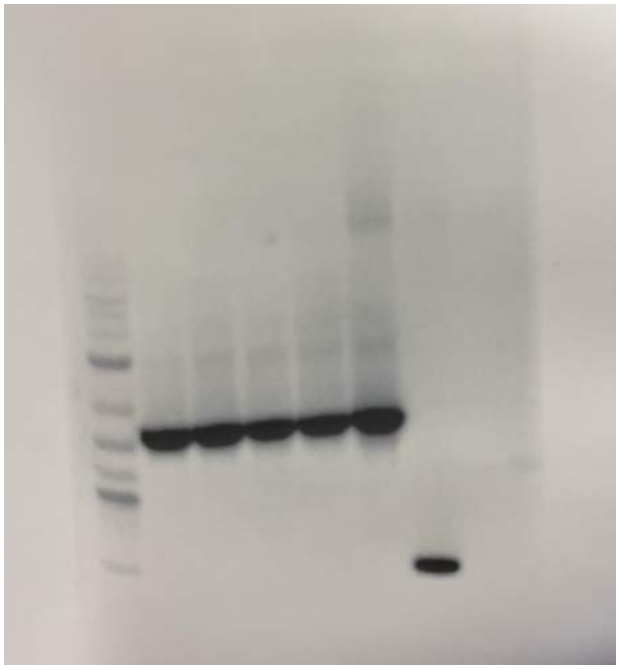
Ladder, Sa1, Sa2, Ca1, Ca2, Negative control, Positive control

S = SPCH
C = CLE18
a = CRISPRa
Negative control= pHSN6i01 vector
Positive control = CRISPRa CLE18 Guide 1

Gel was run for 30 minutes at 109 V

Gel

Gel



Conclusion: Expired BSA1 enzyme has lost activity.

SUNDAY, 2/28/2021

Enzo Baracuhy Danielle Halasz Namra Hamid

Preparation of overnights for glycerol stocks

1. 2 sterile glass tubes were labelled "Ci SPCH 1, and Ca SPCH 1"
    a. Ci = CRISPRi; Ca = CRISPRa
2. A bench area was wiped with ethanol and a bunsen burner was lit
3. 3mL of LB media was put into each tube

4. Kanamycin was taken from the freezer and 3µL was put into each tube
5. An inoculating loop was used to swab a sample from each of the streak plates made on Wednesday labelled "SPCH CRISPRi 1, 2, 4", and SPCH CRISPRa 1, 2, 6, 7" and placed into their corresponding test tubes containing LB and kanamycin
6. Tubes were put to shake in the warm room shaker in room 3202B-C

MONDAY, 3/1/2021

Preparation of archives

Enzo Baracuhy Danielle Halasz Namra Hamid

this procedure must be done with utmost care and sterility. Archives are what we return to when we need to reuse a plasmid, and if they are contaminated then we will have to start from scratch to recreate them

1. The overnights of archives from yesterday were taken from the warm room shaker in room 3202B-C
2. 2 cryotubes were labelled as "Ci SPCH 1" and "Ca SPCH 1"
3. An area was sterilzed and a bunsen burner was lit
4. 950µL of 40% sterile glycerol was put into a labelled cryotube followed by 950µL of the 3mL overnight culture of the corresponding plasmid while working close to the flame for a total volume of 1.9mL per cryotube.
5. cryotubes containing the archive were placed in the -80ºC freezer in room 2202A in the box labelled C.C iGEM on the left-hand side of the top shelf.

TUESDAY, 4/27/2021

Enzo Baracuhy Namra Hamid Kulay Janneh Sarah Cumberland Hannah Vujovic

Watered Apr 14 batch of plants

Golden Gate on DXR guide 4 for CRISPRi and CRISPRa

Reactions were set up using the standard Golden Gate procedure.

Standard Golden Gate Reaction

	A	B
1	**Ingredient**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	BsaI	**1**
5	T4 ligase	1
6	10x CutSmart	2
7	H2O	**12**
8	ATP	2
9	**Total**	**20**

The thermocycler was set to the same parameters (saved under GGOG on the thermocycler).

Thermocycler cycles set as follows:

Step 1: 99 cycles;

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 times)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees
- Infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

ICE2 guides

ICE2 guides were prepared from lyophilized samples.

1. Tubes were centrifuged at 13000 rpm for 2 minutes
2. Amount of duplex buffer used to resuspend was determined by the amount of sample in each tube (described in nmol). Decimal is moved one space to the right i.e 34.1 nmol = 341 µL of duplex buffer
3. Fresh centrifuge tubes were labelled as ICE2 guide ___ duplex
4. 50 µL of forward and reverse primer was added to this tube and then heated at 95C for 2 minutes
5. Tubes were rested at room temperature for 10 minutes and then placed in the freezer

WEDNESDAY, 4/28/2021

Danielle Halasz

Preparation of overnights for miniprep

1. 4 sterile glass tubes were used, 2 were labelled "PHSN6IO1", and 2 labelled "PHSN6AO1"
2. A bench area was wiped with ethanol and a bunsen burner was lit
3. 5mL of LB media was put into each tube
4. Kanamycin was taken from the freezer and 5µL was put into each tube
5. An inoculating loop was used to swab a sample from each of the streak plates made on Tuesday labelled PHSN6IO1 and PHSN6AO1 and placed into their corresponding test tubes containing LB and kanamycin
6. Tubes were put to shake in the warm room shaker in room 3202B-C at 8:15 am

Danielle Halasz Enzo Baracuhy Kulay Janneh Namra Hamid Hannah Vujovic

Miniprep of the 4 CRISPRi and CRISPRa backbone Overnights

1. The 4 of "CRISPRi backbone" and "CRISPRa backbone" overnights prepared earlier in the day were pelleted at 13,000 rpm for 2 minutes in a microcentrifuge tube
2. 250µL of resuspension solution was used and both vortexed and pipetted up and down until no clumps remained
3. 250 µL of lysis solution was added and the tubes were inverted 6 times and incubated for 2 minutes
4. 350µL of neutralization solution was added and both were inverted 6 times and centrifuged for 5 minutes at 13,000 rpm
5. Since some pellets remained in solution, both were centrifuged again at 14,000 rpm for one minute
6. The supernatant was decanted into a GeneJET spin column and pipetted the remaining drops, centrifuged for one minute at 13,000 rpm and discarded flow-through
7. 500µL of Wash Solution was added and centrifuged at 13,000 rpm for one minute and discarded the flow-through
8. Step 7 was repeated once more
9. The GeneJET spin column was moved to a microcentrifuge tube and 50µL of warm elution buffer (65 celsius) was added. The 4 tubes were then incubated at room temperature (22.5 Celcius) for two minutes and centrifuged for two minutes at 13,000 rpm
10. Concentrations were measured on the nanodrop, all were between 200-400ng/µL, and had an A260/280 reading between 1.8 - 1.9

Enzo Baracuhy Namra Hamid

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of DXR into CRISPRi and 2 of DXR into CRISPRa) were transformed into *E. coli* DH5α as follows:

1. The entire ligation mix from the Golden Gate reaction (20µL) was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat shocked at 42°C for 45 seconds. Cells were rested on ice for 5 min.

4. 950µL of LB media were added to cells. Cells were shaken for 1 hr at 37C.
5. Plate 50µL and 950µL on LB plates containing kanamycin. Incubate overnight at 37C.

THURSDAY, 4/29/2021

Danielle Halasz Sarah Cumberland Hannah Vujovic

Golden Gate on DXR guide 4 for CRISPRi and CRISPRa

Reactions were set up using the standard Golden Gate procedure.

Table4

	A	B
1	**Ingredient**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	BsaI	**1**
5	T4 ligase	1
6	10x CutSmart	2
7	H2O	**12**
8	ATP	2
9	**Total**	**20**

The thermocycler was set to the same parameters (saved under GGOG on the thermocycler).

Thermocycler cycles set as follows:

Step 1: 99 cycles;

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 times)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees
- Infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

- temperatures are in Celsius

FRIDAY, 4/30/2021

Sarah Cumberland

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of DXR Guide 4 in CRISPRi and 2 of DXR Guide 4 in CRISPRa) were transformed into *E. coli* DH5α as follows:

1. The entire ligation mix from the Golden Gate reaction (20µL) was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat-shocked at 42°C for 45 seconds. Cells were rested on ice for 5 min.
4. 950µL of LB media were added to cells. Cells were shaken for 1 hr at 37C.
5. Plate 50µL or 950µL on LB plates containing kanamycin. Incubate overnight at 37C.
6. Controls were set up as follows:
    I. Positive cell control: competent cells on LB plate
    II. Positive plasmid control: 20µL of confirmed plasmid (DXR guide 1 CRISPRi @ 539.3ng/µL) on LB + kanamycin

SUNDAY, 5/2/2021

Enzo Baracuhy Danielle Halasz

Golden Gate using NEB Protocol

We used DXR guide 4 on both CRISPRa and CRISPRi using bother the Old and NEB protocol. 2 replicates of each reaction were made. fo a total of 8 reactions

The thermocycler was set to the same parameters (saved under GGOG on the thermocycler).

Thermocycler cycles set as follows:

Step 1: 99 cycles;

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 times)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees
- Infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

- temperatures are in Celsius

MONDAY, 5/3/2021

Sarah Cumberland Brittany Alexander

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20 minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of DXR Guide 4 in CRISPRi old plasmid, 2 of DXR Guide 4 in CRISPRa old plasmid, 2 of DXR Guide 4 in CRISPRi new plasmid, 2 of DXR Guide 4 in CRISPRa new plasmid) were transformed into *E. coli* DH5α as follows:

1. 18µL from the Golden Gate reaction was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat-shocked at 42°C for 45 seconds.
4. Cells were rested on ice for 5 min.
5. 950µL of LB media was added to cells.
6. Cells were shaken for 1 hr at 37C. (Shaker in room 3202.)
7. Plate 50µL or 950µL on LB plates containing kanamycin.
8. Incubate overnight at 37C.

9. Controls were set up as follows:
    I. Positive cell control: competent cells on LB plate
    II. Positive plasmid control: 18µL of confirmed plasmid (CLE18 Guide 1 CRISPRi @ 510.1ng/µL) on LB + kanamycin

Golden Gate Using NEB Kit Protocol

Sarah Cumberland

Golden gate reactions were performed using the following combinations of plasmids/backbone: ICE2 guide 1 in CRISPRi, ICE2 guide 1 in CRISPRa, ICE2 guide 2 in CRISPRi, ICE2 guide 2 in CRISPRa

1. Reactions were set up as follows.
    a. GG = Golden Gate

Table8

	A	B
1	**Reagent**	**Volume (µL)**
2	Plasmid DNA	1
3	sgRNA insert	1
4	T4 ligase buffer	1
5	NEB GG mix BsaI	2
6	dH2O	15
7	**Total**	**20**

2. Thermocycler was run at the following conditions:
    - 2 min @ 37 degrees
    - 5 min @ 16 degrees
    - Repeat 2 steps above 99 times
    - 15 min @ 65 degrees
    - 20 min @ 80 degrees
    - Hold @ 4 degrees
- Above is measured in Celsius

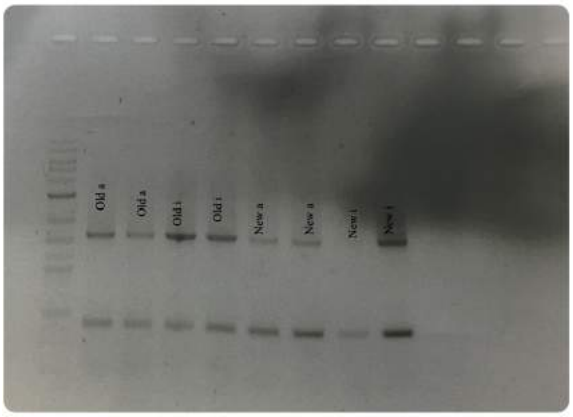
Charlotte Fletcher Kulay Janneh

Gel Electrophoresis on old and new DXR guide 4 CRISPRi and CRISPRa plasmids

No controls were made or added to the gel. Each plasmid was done in replicates (2 of old CRISPRi plasmids, 2 of the old CRISPRa plasmids, 2 of the new CRISPRi plasmids, and 2 of the CRISPRa plasmids)

1. A large 1% gel was cast using 100mL of TAE buffer and 1.0g of Agarose powder, following the Gel Electrophoresis protocol
2. 5 µL of Redsafe was added to the mixture after it has cool to a reasonable temperature
3. Gel was allowed to solidify
4. The following volumes were loaded onto the gel:
    a. 5 µL of ladder
    b. 12 µL of each sample
5. Samples were loaded as following
    a. DXR guide 4 old CRISPRa plasmid (old a),
    b. DXR guide 4 old CRISPRi plasmid (old i).
    c. DXR guide 4 new CRISPRa plasmid (old a).
    d. DXR guide 4 new CRISPRi plasmid (old i).

Screen Shot 2021-05-03 at 10.41.50 PM.png



TUESDAY, 5/4/2021

Namra Hamid Brittany Alexander

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 8 plasmids from the Golden Gate reactions set up above (2 of ICE2 Guide 1 in CRISPRi plasmid and 2 of ICE2 Guide 2 in CRISPIRi plasmid, 2 of ICE2 Guide 1 in CRISPRa plasmid, and 2 of ICE2 Guide 2 in CRISPRa plasmid were transformed into *E. coli* DH5α as follows:

1. 20µL from the Golden Gate reaction was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat-shocked at 42°C for 45 seconds.
4. Cells were rested on ice for 5 min.
5. 950µL of LB media was added to cells.
6. Cells were shaken for 1 hr at 37C. (Shaker in room 3202.)
7. Plate 50µL or 950µL on LB plates containing kanamycin.
8. Incubate overnight at 37C.
9. Controls were set up as follows:
    I. Positive cell control: CLE18 CRISPRi
    II. Negative cell control: *E. coli* cells

Dennis Tran Gerry Koot Namra Hamid

Golden gate using the NEB kit protocol

Two guide inserts were used in this protocol (ICE2 Guide 3 and ICE2 Guide 4). Both guide inserts we used in a CRISPRa and a CRISPRi reaction. Both reactions were done in duplicates, for a total of eight reactions.

The reactions were set up as follows:

- GG = Golden Gate

Table5

	A	B
1	Reagent	Volume (µL)
2	CRISPRi plasmid (Duplex)	1
3	Guide insert (ICE2 Guide 3 or ICE2 Guide 4) (Duplex)	1
4	T4 DNA Ligase Buffer	1
5	NEB GG mix BsaI	2
6	dH2O	15
7	Total	20

- GG = Golden Gate

Table6

	A	B
1	Reagent	Volume (µL)
2	CRISPRa plasmid (Duplex)	1
3	Guide insert (ICE2 Guide 3 or ICE2 Guide 4) (Duplex)	1
4	T4 DNA Ligase Buffer	1
5	NEB GG mix BsaI	2
6	dH2O	15
7	Total	20

All eight reaction were subjected to the following PCR cycles:

Step 1:

- 2 min @ 37 degrees
- 5 min @ 16 degrees

Step 2: Repeat step 1 (99 time)

Step 3: 1 cycle

- 15 min @ 65 degrees
- 20 min @ 80 degrees

- infinite time for 4 degrees

The reaction was preformed for approx. 13 hrs

- Temperatures are in Celsius

NOTE ICE2 Guide 4 CRISPRi duplicate 1 contains less than 2 microlitres of NEB Golden Gate mix BsaI due to running out.

Hannah Vujovic Rebecca S-G Isha Anum Anjum @Samantha Segal

Another round of To Arabidopsis were planted and left to germinate in the phytotron.

WEDNESDAY, 5/5/2021

Hannah Vujovic

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of ICE2 guide 3 in CRISPRi, 2 of ICE2 guide 3 in CRISPRa, 2 of ICE2 Guide 4 in CRISPRi, 2 of ICE2 Guide 4 in CRISPRa) were transformed into *E. coli* DH5α as follows:

1. 18µL from the Golden Gate reaction was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 30min.
3. Cells were heat-shocked at 42°C for 45 seconds.
4. Cells were rested on ice for 5 min.
5. 950µL of LB media was added to cells.
6. Cells were shaken for 1 hr at 37C. (Shaker in room 3202.)
7. Plate 50µL or 950µL on LB plates containing kanamycin.
8. Incubate overnight at 37C.

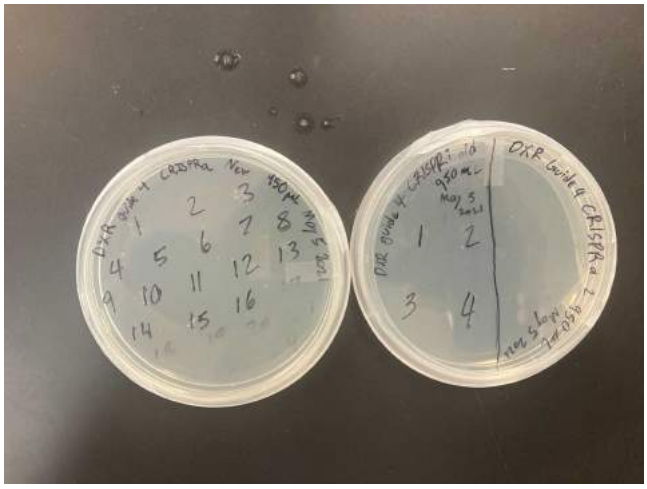
Controls were set up as follows:
Positive cell control: competent cells on LB plate
Positive plasmid control: 18µL of confirmed plasmid (CLE18 Guide 1 CRISPRa) on LB + kanamycin

Danielle Halasz Brittany Alexander @Mike Green

Streak plating of patch plates

1. brought 2 Petri dishes from the fridge labelled "LB+KAN" as well the three filled Petri dishes containing patch plates of DRX guide 4 CRISPRa Old and new (in reference to previously done golden gate) and DXR guide 4 CRISPRi from the 4 Celsius fridge.
2. Sterilized area
3. divided the petri dish for DXR guide 4 CRISPRi and DXR Guide 4 CRISPRa into half, and streaked patches
4. Took petri dish for DXR guide 4 CRISPRa, and streaked patches 1-16 on the new plate
5. Took off the parafilm from already culture filled petri dish from the 4 Celsius fridge
6. Light up a bunsen burner and sterilized the innoculating loop and let it cool for 10 seconds.
7. scraped from the edge part of the culture filled petri dish with the innoculating loop and closed the culture as soon as possible.
8. Streaked the new petri dish accordingly and closed the new petri dish.
9. Wrapped both petri dishes with parafilm.
10. settled the new petri dishes in the 37C incubator, and the patch plates were returned to the fridge

image.png



THURSDAY, 5/6/2021

Harkamal Samra Amira Bouchema

Preparing colony water

1. For each patch on the patch plate, a PCR tube with 100µL of sterile water was filled and labelled
2. Using a sterile P1000 tip under the flame, a small streak of the patch was scooped and placed into its respective PCR tube containing the 100µL of water. Do not use an inoculating loop

as the metal can negatively impact DNA

PCR using the Quiagen Kit protocol

The Quiagen Kit and Quiagen protocol were used to preform PCR on DXR Guide 4 (CRISRa and CRISPRi patch plates)

1. The reagents required for the PCR were thawed at room temperature (except Taq, which was added in at the end and quickly put it back in the freezer).
2. The following reagents were added to each PCR tube (see table below).
3. The thermocycler was run as follows:
    a. 94°C for 3 minutes
    b. [94°C for 30 seconds
    c. 54°C for 30 seconds
    d. 72°C for 2 minutes] x35 cycles
    e. 72°C for 10 minutes
    f. Hold at 4°C
4. A total of 20 PCRs were run on experimental colonies

Table7

	A	B	C
1	Reagents	Volume 1X	Volume 20X
2	10x Buffer (Red)	5 µL	100 µL
3	dNTP mix	1 µL	20 µL
4	Taq polymerase	0.25 µL	5 µL
5	10 uM Forward primer	5 µL	100 µL
6	10 uM Reverse primer	5 µL	100 µL
7	Nuclease Free Water (Sterile Water)	24 µL	480 µL
8	Colony Water	10 µL	200 µL
9	Total Volume	50.25 µL	1005 µL

Danielle Halasz @Mike Green Taylor @Tiffany Arnone

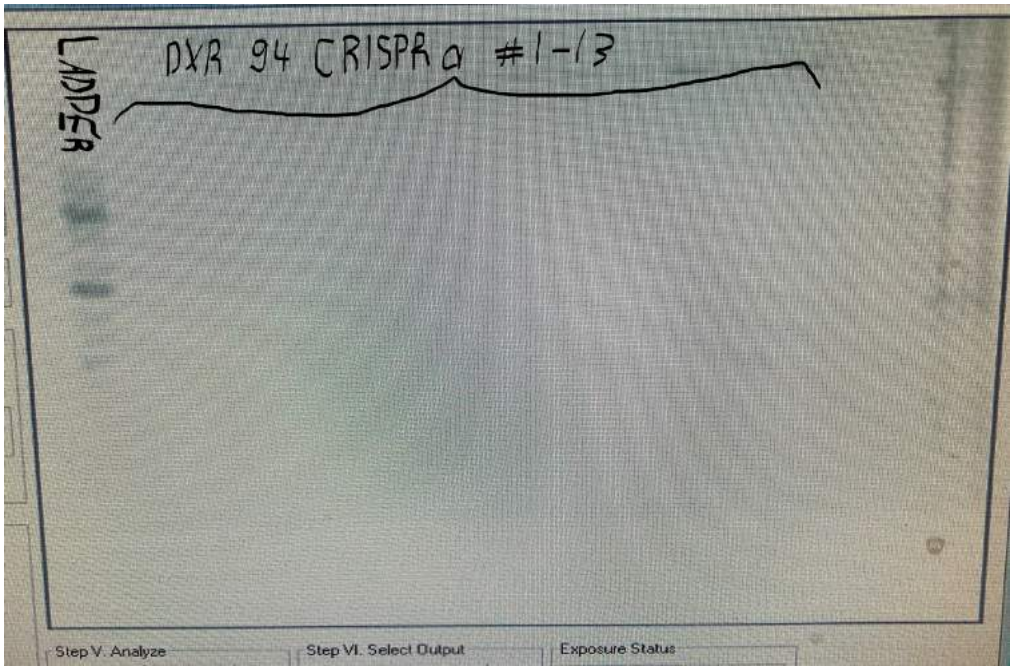
Gels were run of the PCR products prepared earlier this day

1. A 1% agarose gel was prepared using 100 mL TAE buffer and 1.0 g agarose, and a 1% agarose gel was also prepared using 50 mL TAE buffer and 0.5 g agarose
2. 5.0 µLof RedSafe was added to the larger gel, and 2.5 µL of RedSafe was added to the smaller gel after the mixture cooled to a reasonable temperature.
3. Gels were poured and allowed to solidify for 20 minutes
    a. Note: for future referance, gels should be allowed to solidify for longer periods of time in given our lab conditions, approximatly 30-40 minutes
4. Loading dye + ladder was prepared on parafilm wax: 2µL of loading dye was added to 9µL of Gene Ruler 1kb DNA Ladder (ladder did not already contain dye). Samples from PCR reaction already contained loading dye included in the buffer used to run the PCR itself, so it was not required to add loading dye to each sample
5. Samples were loaded as follows into the larger gel: Ladder, DXR guide 4 CRISPRa (from NEB golden gate protocol) (1,2,3,4,5,6,7,8,9,10,11,12,13)
6. Samples were loaded as follows into the smaller gel: Ladder, DXR guide 4 CRISPRa (from NEB golden gate protocole) (14,15,16), DXR guide 4 CRISPRi (from old golden gate protocol) (1,2,3,4)
7. Gel was run for 40 miniutes at 114V

- the numbers refer to their respective patch on the patch plate

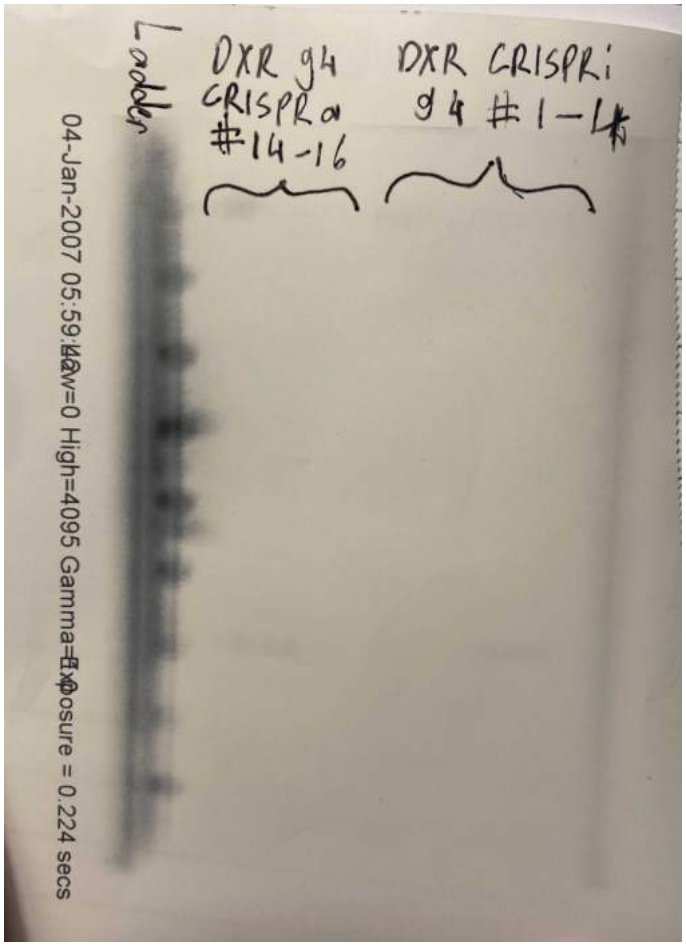
Larger gel:

image.png



Smaller gel:

image.png



FRIDAY, 5/7/2021

Sarah Cumberland Gesjana Rustemi

Preparing Colony Water

Colony water was prepared using:

- 3 patches of ICE2 Guide 4 CRISPRi
- 4 patches of DXR Guide 4 CRISPRa (new backbone) (patches 1-4)
- 4 patches of DXR Guide 4 CRISPRi (old backbone)
- positive control of "CLE18 CRISPRi positive +"

1. For each patch, a PCR tube with 100µL of sterile water was filled and labelled.
2. Using a sterile P1000 tip under the flame, a small streak of the patch was scooped and placed into its respective PCR tube with the 100µL of water. Do not use an inoculating loop as the metal can do bad things to the DNA.

PCR using the Quiagen Kit protocol

The Quiagen Kit and Quiagen protocol were used to preform PCR on:

- 3 patches of ICE2 Guide 4 CRISPRi
- 4 patches of DXR Guide 4 CRISPRa (new backbone) (patches 1-4)
- 4 patches of DXR Guide 4 CRISPRi (old backbone)
- positive control of "CLE18 CRISPRi positive +"

1. PCR reagents (except Taq) were thawed at room temperature.
2. The following reagents were added to each PCR tube (see table below).
3. The thermocycler was run as follows:
   - 94°C for 3 minutes

- [94℃ for 30 seconds
- 54℃ for 30 seconds
- 72℃ for 2 minutes] x35 cycles
- 72℃ for 10 minutes
- Hold at 4℃

4. A total of 11 PCRs were run on experimental colonies + 1 positive control.

Table9

	A	B	C
1	Reagents	Volume 1X	Volume 14X
2	10x Buffer (Red)	5 µL	70 µL
3	dNTP mix	1 µL	14 µL
4	Taq polymerase	0.25 µL	7 µL
5	10 uM Forward primer	5 µL	70 µL
6	10 uM Reverse primer	5 µL	70 µL
7	Nuclease Free Water (Sterile Water)	24 µL	336 µL
8	Colony Water	10 µL	(added per tube)
9	Total Volume	50.25 µL	—

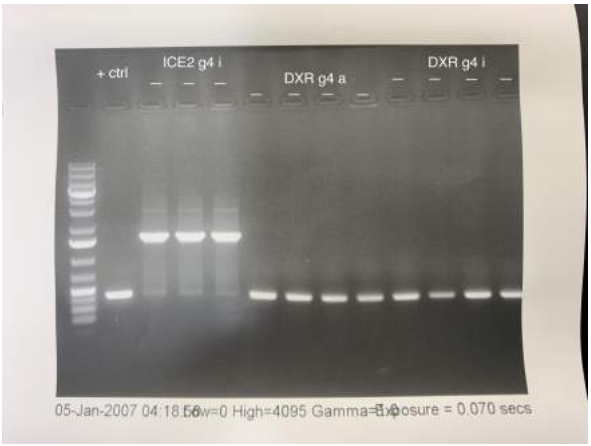
Sarah Cumberland Emma Lee

Gels were run of the PCR products prepared earlier this day

1. A 1% agarose gel was prepared using 100 mL TAE buffer and 1.0 g agarose
2. 5.0 µL of RedSafe was added to the agarose dissolved in TAE buffer mixture after it cooled to a reasonable temperature.
3. Gels were poured and allowed to solidify for 30 minutes.
4. Loading dye + ladder was prepared on parafilm wax: 2µL of loading dye was added to 9µL of Gene Ruler 1kb DNA Ladder (ladder did not already contain dye). Samples from PCR reaction already contained loading dye included in the buffer used to run the PCR itself, so it was not required to add loading dye to each sample
5. Samples were loaded as follows into the gel as follows: Ladder, positive control of "CLE18 CRISPRi positive +", 3 of ICE2 Guide 4 CRISPRi, 4 of DXR Guide 4 CRISPRa (new backbone), 4 of DXR Guide 4 CRISPRi (old backbone)
6. Gel was run for 40 minutes at 114V

Gel:

IMG_3982.JPG



Danielle Halasz

Preparation of overnights for glycerol stocks

1. 2 sterile glass tubes were labelled "DXR guide 4 CRISPRi 1" and "DXR guide 4 CRISPRa 1"
2. A bench area was wiped with ethanol and a bunsen burner was lit
3. 5mL of LB media was put into each tube
4. Kanamycin was taken from the freezer and 5µL was put into each tube
5. An inoculating loop was used to swab a sample from each of the streak plates made on Wednesday labelled "DXR guide 4 CRISPRi 1", and "DXR CRISPRa 1" and placed into their corresponding test tubes containing LB and kanamycin
6. Tubes were put to shake in the warm room shaker in room 3202B-C

SATURDAY, 5/8/2021

Hannah Vujovic

Miniprep of the DXR guide 4 CRISPRi and CRISPRa backbone Overnights

1. The DXR guide 4 of "CRISPRi backbone" and "CRISPRa backbone" overnights prepared yesterday were pelleted at 13,000 rpm for 2 minutes in a microcentrifuge tube
2. 250µL of resuspension solution was used and both vortexed and pipetted up and down until no clumps remained
3. 250 µL of lysis solution was added and the tubes were inverted 6 times and incubated for 2 minutes
4. 350µL of neutralization solution was added and both were inverted 6 times and centrifuged for 5 minutes at 13,000 rpm
5. The supernatant was decanted into a GeneJET spin column and pipetted the remaining drops, centrifuged for one minute at 13,000 rpm and discarded flow-through
6. 500µL of Wash Solution was added and centrifuged at 13,000 rpm for one minute and discarded the flow-through
7. Step 6 was repeated once more
8. The flow-through was decanted and columns were dry spun for 1 minute at 13000rpm
9. The GeneJET spin column was moved to a microcentrifuge tube and 50µL of warm elution buffer (65 Celcius) was added. The 4 tubes were then incubated at room temperature (22.5 Celcius) for two minutes and centrifuged for two minutes at 13,000 rpm

Concentrations were measured on the nanodrop, all were between 480-500ng/µL, and had an A260/280 reading between 1.8 - 1.9

SUNDAY, 5/9/2021

Overnights of glycerol stocks

Enzo Baracuhy

5mL overnight cultures of DXR guide 4 and guide 1 CRISPRi/a, CLE18 guide 1 CRISPRi/a, and SPCH guide 1 CRISPRi/a were prepared and left in room 3202 to shake overnight at 200rpm. 5µL of kanamycin was used as the selectable antibiotic

MONDAY, 5/10/2021

Hannah Vujovic

Miniprep of DXR/SPCH/CLE18 guide 1 CRISPRa/i

Sequence of Procedures (SOP) outlined in the experiments page was followed. Concentrations fell between 300-400ng/µL with acceptable A260/A280 readings.

Hannah Vujovic Rebecca S-G Brittany Alexander

Golden Gate of ICE2 guides 1/2 CRISPRa/i

SOP outlined in the experiments page was followed.
Protocol Changes:

1. CRISPRa/i plasmids were diluted to 200ng/µL
2. Total reaction volume was decreased to 15µL
3. NEB enzyme mixture was used

Enzo Baracuhy Sarah Cumberland Hannah Vujovic Amira Bouchema Danielle Halasz Kulay Janneh

Transformation of Golden Gate Plasmids into Competent *Agrobacterium tumefaciens*

1. Four 1.5mL tubes of competent *Agrobacterium* cells (~100 to 140µL cells per tube) were taken from -80C freezer
2. The following plasmids were taken from the -20C freezer:
    - SPCH guide 1 CRISPRa x2 (1 replicate by Danielle, 1 replicate by Amira)
    - CLE18 guide 1 CRISPRa x2 (1 replicate by Danielle, 1 replicate by Amira)
    - SPCH guide 1 CRISPRi x2 (1 replicate by Danielle, 1 replicate by Amira)
    - CLE18 guide 1 CRISPRi (by Kulay)
    - DXR guide 4 CRISPRa x2 (by Kulay)
    - DXR guide 1 CRISPRa (by Hannah & Sarah)
    - DXR guide 4 CRISPRi (by Hannah & Sarah)
    - DXR guide 1 CRISPRi (by Hannah & Sarah)
3. 1ug of plasmid was pipetted into each tube (one type of plasmid per tube). Note: use c=n/v to find the volume of each plasmid to use.
4. *Agrobacterium* + plasmid mixture was flicked to mix, and then left on ice for 15min.
5. *Agrobacterium* + plasmid mixture was put into a -80C freezer for 2min. (Note: liquid nitrogen for 1 minute also works)
6. *Agrobacterium* + plasmid mixture was put in a 37C hot plate for 3 min.
7. 300µL LB broth was added to the mixture and put into a 28C shaker for 2hr
8. 50µL of the *Agrobacterium*+plasmid+LB mxiture was pipetted onto a LB+Kanamycin+gentamycin plate, and the rest was poured onto another LB+Kanamycin+gentamycin plate.
9. Plates (24 in total) were put in a 28C incubator for 48 hours

TUESDAY, 5/11/2021

Sarah Cumberland Brittany Alexander Gesjana Rustemi

E. coli Transformation

E. coli was transformed with the following plasmids from the Golden Gate reaction yesterday, as per the SOP for *E. coli* Transformation outlined in the experiments page.

- ICE2 guide 1 CRISPRi x2
- ICE2 guide 1 CRISPRa x2

- Note: ran out of BsaI while doing Golden Gate for the two above rxns so 1xreplicate of each of the above has less BsaI than protocol calls for
- ICE2 guide 2 CRISPRi x2
- ICE2 guide 2 CRISPRa x2

The following changes were made to the protocol: 5µL of plasmid DNA were added to each transformation instead of 20µL.

THURSDAY, 5/13/2021

Hannah Vujovic Emma Lee Isha Anum Anjum samantha segal

Thinned plants from May 4th. There was further moss growth on the first two planters that were planted that was scrapped off the topsoil.

FRIDAY, 5/14/2021

Sarah Cumberland Brittany Alexander

Transformation of Golden Gate Plasmids into Competent *Agrobacterium tumefaciens*

1. Four 1.5mL tubes of competent *Agrobacterium* cells (~100 to 140µL cells per tube, 50µL cells per transformation) were taken from -80C freezer.
2. The following plasmids were taken from the -20C freezer:
   - SPCH guide 1 CRISPRa --360.8 ng/µL -- 2.77 µL
   - SPCH guide 1 CRISPRi -- 357.4 ng/µL -- 2.80 µL
   - CLE18 guide 1 CRISPRa -- 305.5 ng/µL --3.27 µL
   - CLE18 guide 1 CRISPRi -- 350.8 ng/µL-- 2.85 µL
   - DXR guide 4 CRISPRa -- 491.4 ng/µL-- 2.04 µL
   - DXR guide 4 CRISPRi -- 463.7 ng/µL -- 2.16 µL
   - DXR guide 1 CRISPRa -- 346.4 ng/µL --2.89 µL
   - DXR guide 1 CRISPRi -- 352.9 ng/µL --2.84 µL
3. 1 ug of plasmid was pipetted into each tube (one type of plasmid per tube). Note: use c=n/v to find the volume of each plasmid to use.
4. *Agrobacterium* + plasmid mixture was flicked to mix, and then left on ice for 15min.
5. *Agrobacterium* + plasmid mixture was cooled in liquid nitrogen for 1 minute.
6. *Agrobacterium* + plasmid mixture was put in a 37C hot plate for 3 min.
7. 300µL LB broth was added to the mixture and put into a 28C shaker for 2hr
8. 50µL of the *Agrobacterium*+plasmid+LB mixture was pipetted onto an LB+Kanamycin+gentamycin plate, and the rest was poured onto another LB+Kanamycin+gentamycin plate. Note: the gentamycin was 1000x too diluted in these plates.
9. Controls were set up as follows:
   - 50µL of DXR guide 4 CRISPRa tranformed into *Agrobacterium* on to a plain LB plate
   - 50µL of DXR guide 4 CRISPRi tranformed into *Agrobacterium* on to a plain LB plate
10. Plates (18 in total) were put in a 28C incubator for 48 hours

Sarah Cumberland

Streak Plates

- Competent *Agrobacterium tumefaciens* cells from someone in Dr. Wang's lab were streaked on to a plain LB plate and on to an LB + gentamycin plate, then placed in 28C incubator to grow.
- Non-competent *Agrobacterium* cells from Dr. Wang were streaked on to a plain LB plate and on to an LB + gentamycin plate, then placed in 28C incubator to grow.

SUNDAY, 5/16/2021

Sarah Cumberland

Patch Plates

Patches were made from the following transformation plates:

- SPCH guide 1 CRISPRi
- SPCH guide 1 CRISPRa
- CLE18 guide 1 CRISPRa
- DXR guide 1 CRISPRa
- DXR guide 4 CRISPRi
- DXR guide 4 CRISPRi

MONDAY, 5/17/2021

Sarah Cumberland Brittany Alexander

Preparing Colony Water

Colony water was prepared using:

- DXR guide 1 CRISPRa -- 5 colonies
- DXR guide 1 CRISPRi -- 1 colony
- DXR guide 4 CRISPRa -- 2 colonies
- DXR guide 4 CRISPRi -- 3 colonies
- CLE18 guide 1 CRISPRa -- 2 colonies
- CLE18 guide 1 CRISPRi -- 1 colony
- SPCH guide 1 CRISPRa -- 1 colony
- SPCH guide 1 CRISPRi -- 1 colony
- positive control of "CLE18 CRISPRi positive +"

1. For each colony, a PCR tube with 100µL of sterile water was filled and labelled.
2. Using a sterile P1000 tip under the flame, a small streak of the patch was scooped and placed into its respective PCR tube with the 100µL of water. Do not use an inoculating loop as the metal can negatively impact DNA.

PCR using the Quiagen Kit protocol

The Quiagen Kit and Quiagen protocol were used to preform PCR on:

- DXR guide 1 CRISPRa -- 5 colonies
- DXR guide 1 CRISPRi -- 1 colony
- DXR guide 4 CRISPRa -- 2 colonies
- DXR guide 4 CRISPRi -- 3 colonies
- CLE18 guide 1 CRISPRa -- 2 colonies
- CLE18 guide 1 CRISPRi -- 1 colony
- SPCH guide 1 CRISPRa -- 1 colony
- SPCH guide 1 CRISPRi -- 1 colony
- positive control of "CLE18 CRISPRi positive +"
- one replicate of CLE18 guide 1 CRISPRi, with the addition of 10µL of CLE18 guide 1 primer mix

1. PCR reagents (except Taq) were thawed at room temperature.
2. The following reagents were added to each PCR tube (see table below).
3. The thermocycler was run as follows:
    - 94°C for 3 minutes
    - [94°C for 30 seconds
    - 54°C for 30 seconds
    - 72°C for 2 minutes] x35 cycles
    - 72°C for 10 minutes
    - Hold at 4°C

A total of 16 PCRs were run on experimental colonies + 1 positive control + one replicate of CLE18 guide 1 CRISPRi using the duplexed CLE18 oligos as PCR primers

Table10

	A	B	C
1	Reagents	Volume 1X	Volume 20X
2	10x Buffer (Red)	5 µL	100 µL
3	dNTP mix	1 µL	20 µL
4	Taq polymerase	0.25 µL	5 µL
5	10 uM Forward primer	5 µL	100 µL
6	10 uM Reverse primer	5 µL	100 µL
7	Nuclease Free Water (Sterile Water)	24 µL	480 µL
8	Colony Water	10 µL	(added per tube)
9	Total Volume	50.25 µL	—

Patch Plates

Patches were made from the following transformation plates:

- 1st Patch Plate:
- DXR guide 1 CRISPRa -- 4 patches
- DXR guide 1 CRISPRi -- 1 patch
- 2nd Patch Plate:
- DXR guide 4 CRISPRa -- 1 patch
- DXR guide 4 CRISPRi -- 2 patches
- 3rd Patch Plate:
- CLE18 guide 1 CRISPRa -- 1 patch
- CLE18 guide 1 CRISPRi -- 1 patch
- SPCH guide 1 CRISPRi -- 1 patch

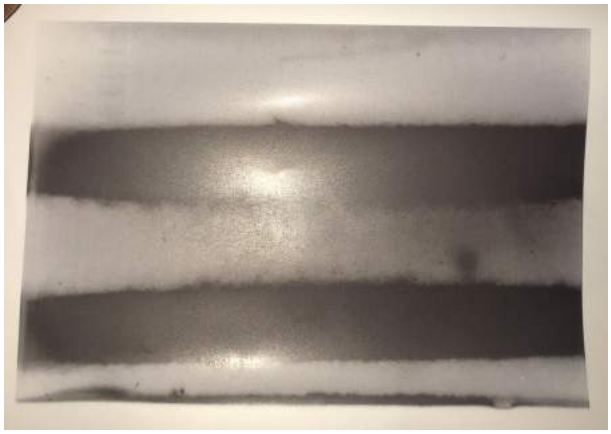
Kulay Janneh Gerry Koot Charlotte Fletcher

Agarose gel on PCR products

Experiment was carried out following the protocol using the gRNAs PCRed above.

- The voltage supplier made abnormal sounds and may have to be inspected.

IMG_3444.JPG



PCR products were put into the fridge to be run tomorrow.

TUESDAY, 5/18/2021

Dennis Tran Kulay Janneh Brittany Alexander

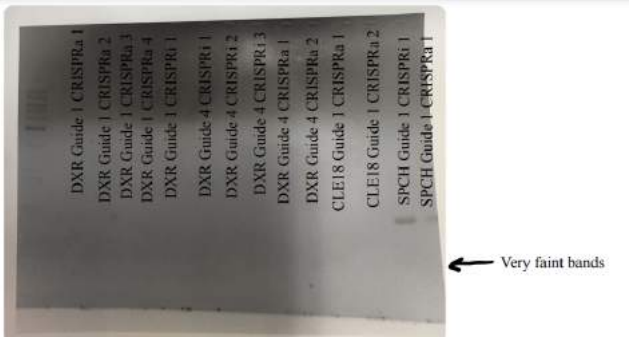
Gels were run of the PCR products prepared on Monday May 18th

Larger Gel

1. A 1% agarose gel was prepared using 100 mL TAE buffer and 1.0 g agarose
2. 5.0 µL of RedSafe was added to the agarose dissolved in TAE buffer mixture after it cooled to a reasonable temperature.
3. Gels were poured and allowed to solidify for 30 minutes.
4. Ladder (Gene Ruler 1kb DNA) and samples from PCR reaction already contained loading dye included in the buffer used to run the PCR itself, so it was not required to add loading dye to each sample;
    a. 5 µL of ladder was added
    b. 12 µL of sample was added
5. Samples were loaded as follows into the gel as follows (brackets indicate replicates):
    a. Ladder
    b. DXR Guide 1 CRISPRa (1)
    c. DXR Guide 1 CRISPRa (2)
    d. DXR Guide 1 CRISPRa (3)
    e. DXR Guide 1 CRISPRa (4)
    f. DXR Guide 1 CRISPRi (1)
    g. DXR Guide 4 CRISPRi (1)
    h. DXR Guide 4 CRISPRi (2)
    i. DXR Guide 4 CRISPRi (3)
    j. DXR Guide 4 CRISPRa (1)
    k. DXR Guide 4 CRISPRa (2)
    l. CLE18 Guide 1 CRISPRa (1)
    m. CLE18 Guide 1 CRISPRa (2)
    n. SPCH Guide 1 CRISPRi (1)
    o. SPCH Guide 1 CRISPRa (2)
6. Gel was run for 20 minutes at 115V. See bolded note.

Gel:

image.png

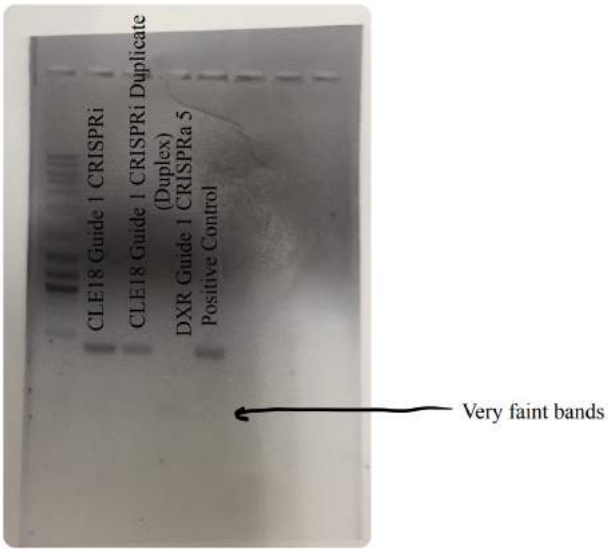


Smaller Gel

1. A 1% agarose gel was prepared using 50 mL TAE buffer and 0.5 g agarose
2. 2.5 µL of RedSafe was added to the agarose dissolved in TAE buffer mixture after it cooled to a reasonable temperature.
3. Gels were poured and allowed to solidify for 30 minutes.
4. Ladder (Gene Ruler 1kb DNA) and samples from PCR reaction already contained loading dye included in the buffer used to run the PCR itself, so it was not required to add loading dye to each sample;
    a. 5 µL of ladder was added
    b. 12 µL of sample was added
5. Samples were loaded as follows into the gel as follows:
    a. Ladder
    b. DXR Guide 1 CRISPRa (5)
    c. Positive Control
    d. CLE18 Guide 1 CRISPRi
    e. CLE18 Guide 1 CRISPRi duplicate (duplex)
6. Gel was run for 20 minutes at 115V. See bolded note below.
    About 20 minutes into the run, the voltage supplier started beeping. It was turned down to 100V for the remaining 20 minutes and the beeping stopped. Supplier beeped again 37 minutes into the run. Each cassette was plugged into its own supplier (111 V supplied to the larger gel and 118V supplied to the smaller gel). After the full 40 minutes, the larger gel ran well. The smaller gel ran to about the middle and was run for an additional 10 minutes.

Gel:

image.png



Enzo Baracuhy Namra Hamid

Media Prepartion

4 bottles of media were made and autoclaved in prepartion for the competent *Agrobacterium* protocol:

- Two 400mL bottles of 20g/L Lysogeny Broth (LB)
- One ~40mL aliquot of 100% glycerol
- One 100mL bottle of 20mM calcium chloride (0.22g of anhydrous calcium chloride added to 100mL of miliQ water)

WEDNESDAY, 5/19/2021

Hannah Vujovic Harkamal Samra Brittany Alexander @Samantha Segal

Agrobacterium tumefaciens PCR

SOP for PCR outlined on the experiments page was followed. Changes to protocol made include the following:

1. The colony water was heated at 95C for 30 minutes before being added to the PCR tubes
2. 5µL of 1:10 diluted duplexed guide were added to each PCR tube

THURSDAY, 5/20/2021

Sarah Cumberland

Golden Gate

Golden gate ligation was performed on the following gRNAs:

- DXR guide 2 CRISPRi
- DXR guide 3 CRISPRi
- DXR guide 5 CRISPRi

A total of 18 Golden Gate reactions were set up as follows:

Golden Gate Scaled Down & Modified as Per Dr. Wang's Suggestions

	Ingredient	Volume	Optimization Rep 1	Optimization Rep 2	Optimization Rep 3
1	Sterile H2O	--			
2	Plasmid DNA	--	100 ng	100 ng	200 ng
3	gRNA Insert DNA	--	100 ng	20 ng	50 ng
4	T4 DNA ligase buffer	1µL			
5	CutSmart Buffer	1µL			
6	BsaI	1µL			
7	T4 DNA ligase	0.5 µL			
8	Total	13 µL			

- **Note:** Dr. Wang recommended 10µL total volume but had to increase it due to plasmid/gRNA volumes.
- **Note:** Volumes were found via V = m/C, ie. V = 100ng/315.8 ng/µL = 0.317µL. We have no pipettes that go down that low so this DNA was diluted 1 in 5 to use 1.6µL.
- Thermocycler was run as indicated in: "SOP - Golden Gate Cloning outlined on the experiments page.

Danielle Halasz Mike Green @Nathaniel Petersen

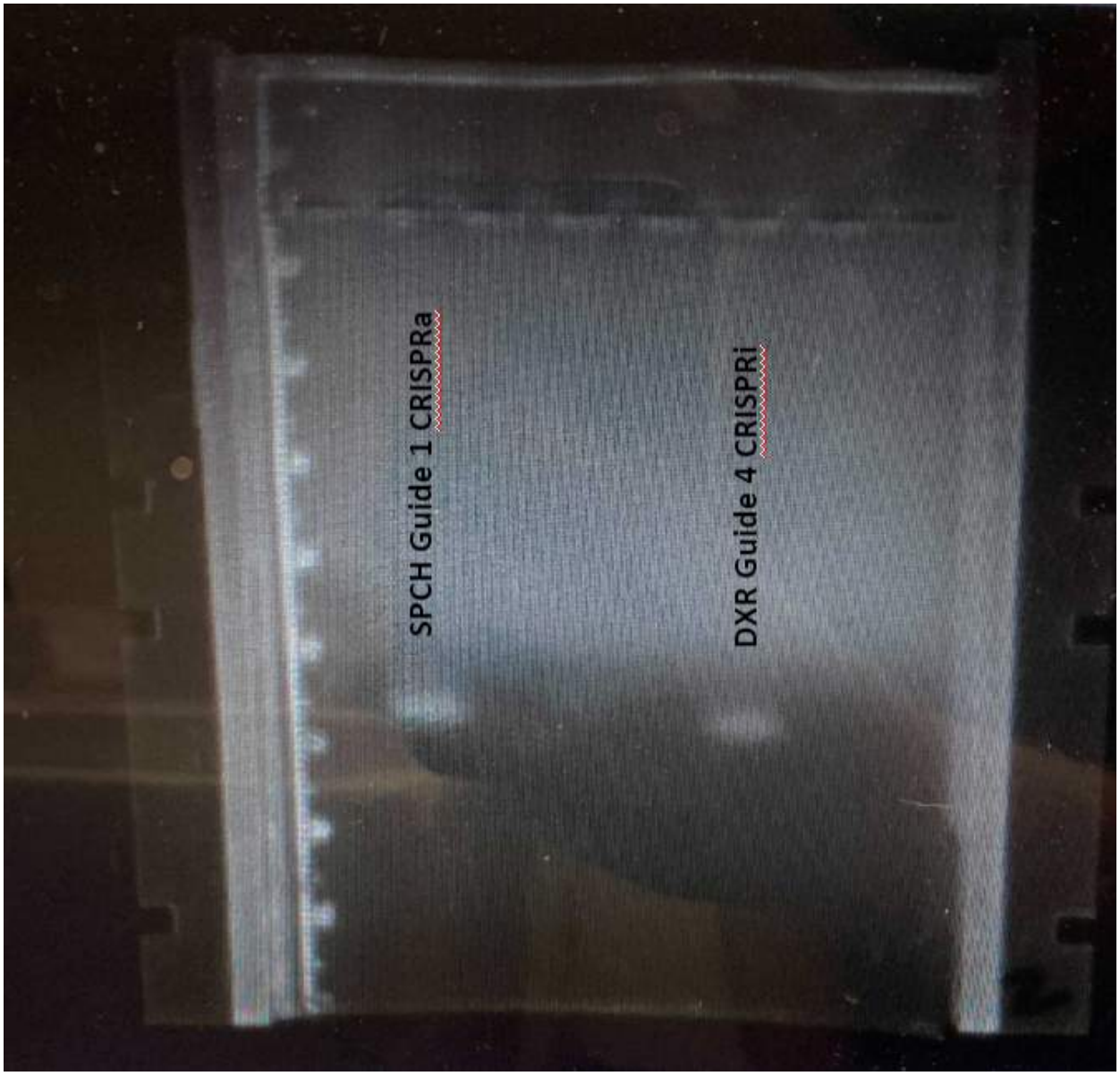
Gels were run of the PCR products prepared on Wednsday May 19th

SOP for Gel Electrophoresis outlined on the experiments page was followed. Changes to protocol or things of notice include the following:

- Two large 100 mL and one small 50 mL gels wre made, at 1% agarose gel concentration (32 samples in total)
- contents of PCR tube labled "c" was taken from the fridge and used as a control
- there was issues with running the gels as the power supply alarm went off that notified that there was high voltage
- the gel reading were largely unsuccessful except for wells with bands correspond to SPCH Guide1 CRISPRa on left, and on the right, DXR Guide 4 CRISPRi

Small gel:

image.png



The two larger gels, where only the control appeared:

image.png

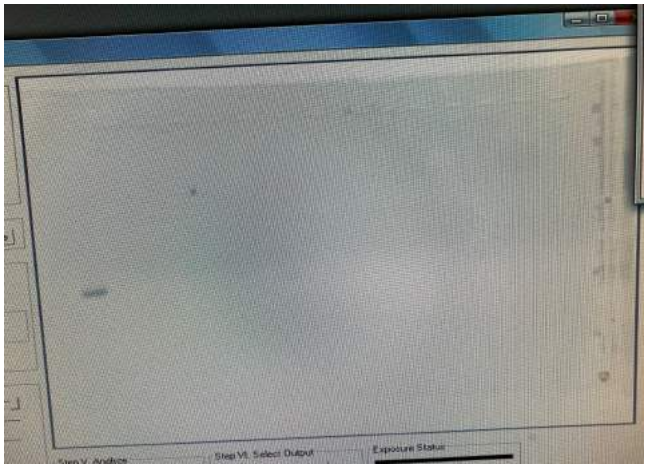


image.png

Sarah Cumberland Dennis Tran @Tiffany Arnone

Competent *Agrobacterium tumefaciens* Preparation: Overnight Cultures

- 2x5mL overnight cultures of *Agrobacterium tumefaciens* MP90 strain were prepared as per SOP outlined on the experiments page.

FRIDAY, 5/21/2021

Sarah Cumberland

Competent *Agrobacterium tumefaciens* Preparation

- *Agrobacterium tumefaciens* MP90 strain was prepared as per "SOP - *A. tumefaciens* Competent Cell Preparation" outlined on the experiments page. OD didn't get high enough so no competent *Agrobacterium* aliquots were produced.

Danielle Halasz Lisa Thuy Duyen Tran Harkamal Samra

Transformation of Golden Gate plasmids from Thursday May 20 into competent *E.coli*

- The "SOP - *E. coli* Transformation iGEM" protocol outlined on the experiments page was followed.

- a total of 36 plates were prepared from the 18 golden gate reactions prepared on Thursday, using both our own competent *E.coli* cells and Dr. Wang's cells. Plates that have "Wang" and "ours" written on them correspond to the competent *E.coli* obtained from Dr. Wang, and our own *E.coli,* respectively

SUNDAY, 5/23/2021

Sarah Cumberland

Patch Plates

Patch plates were prepared from colonies on the following transformation plates:

- Our cells, DXR Guide 2 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 3 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 5 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 2 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 3 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 5 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 5 Rep 3 (200ng plasmid/50ng insert)

MONDAY, 5/24/2021

Sarah Cumberland Brittany Alexander Charlotte Fletcher Artur Karapetov

PCR

PCR was performed as per "SOP - Polymerase Chain Reaction (PCR) - Colony Crack iGEM" outlined on the experiments page. PCR was performed on 2 replicates each of one colony from each of the following transformation plates. (Note: none of the patches had grown yet, hence why PCR was done on transformation plate colonies rather than patches. Each colony was too small to both do colony PCR and make a patch from).

- Our cells, DXR Guide 2 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 3 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 5 Rep 1 (100ng plasmid/100ng insert)
- Our cells, DXR Guide 2 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 3 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 5 Rep 2 (100ng plasmid/20ng insert)
- Our cells, DXR Guide 5 Rep 3 (200ng plasmid/50ng insert)

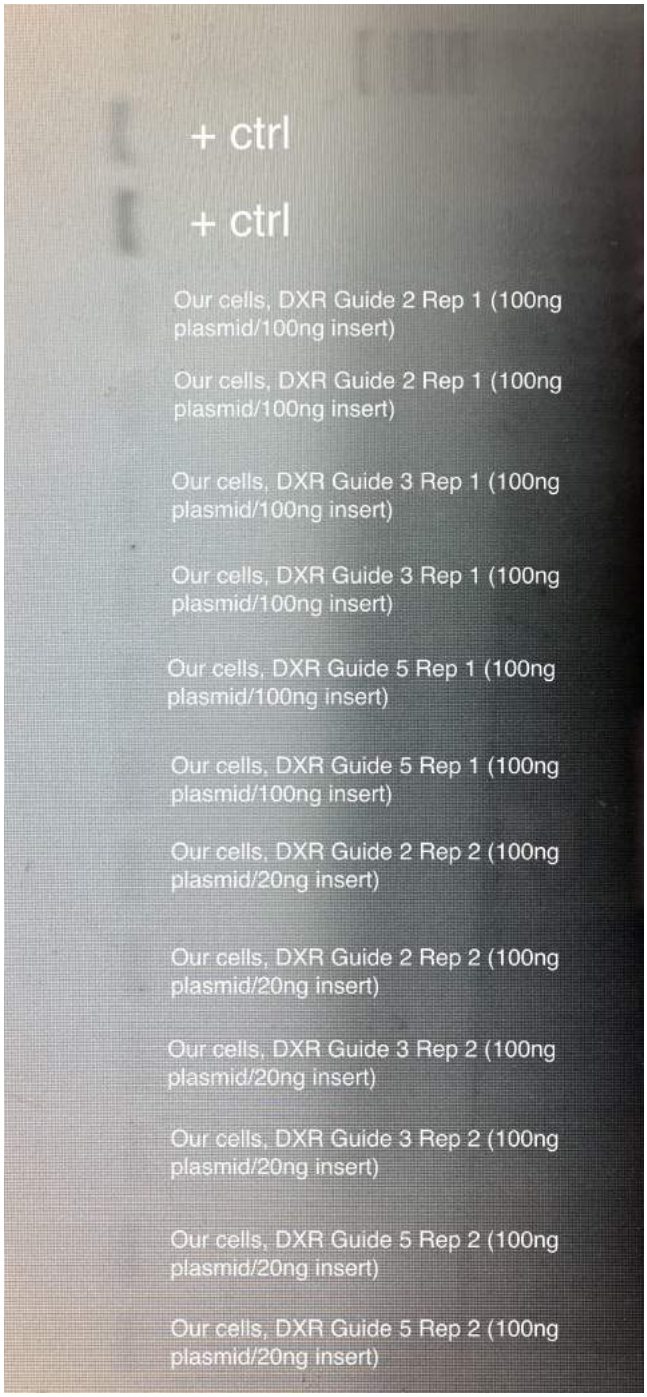
Sarah Cumberland Brittany Alexander Charlotte Fletcher Artur Karapetov

Gel Electrophoresis

Gel Electrophoresis was carried out as per "SOP - Gel Electrophoresis iGEM" outlined on the experiments page. Gel electrophoresis was performed on the sample listed in the previous experiment. One large gel and one small gel were prepared.

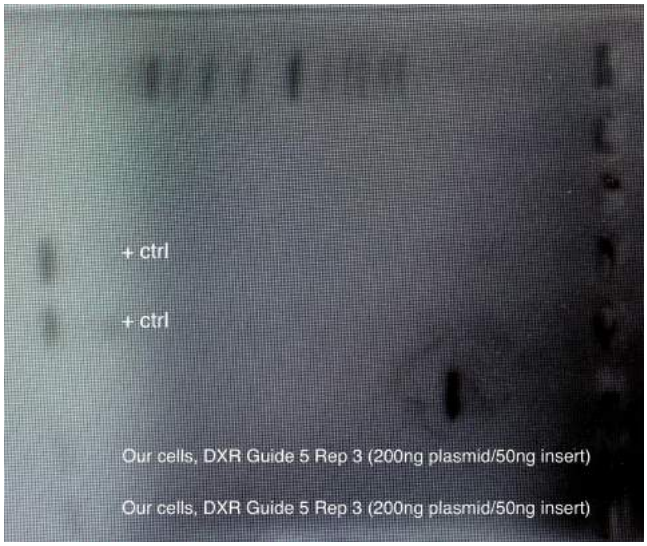
Large gel:

IMG_4225.jpeg



Small gel:

IMG_4222.jpeg



Hannah Vujovic Brittany Alexander

Golden Gate

C=m/V because (ng/µL) = ng/µL
C=m/V -> CV=m -> V=m/C

SOP outlined on the experiments page was followed using CLE18 guides 2-5 CRISPRi.

Table11

	Reagent	Volume (µL)	Mass (ng)	Concentration (ng/µL)
1	Molecular grade H2O (Found in Plant box “H2O”)	4.5		
2	Plasmid DNA	1	100	100
3	Duplexed gRNA insert	1	100	100
4	10x CutSmart Buffer	1		
5	10x T4 Ligase Buffer	1		
6	BsaI	1		
7	T4 ligase	0.5		
8	**Total**	10		

TUESDAY, 5/25/2021

Amira Bouchema Harkamal Samra

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of CLE18 guides 2-5 in CRISPRi) were transformed into *E. coli* DH5α as follows:

1. 20µL from the Golden Gate reaction was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 15min.
3. Cells were heat-shocked at 42°C for 45 seconds.
4. Cells were rested on ice for 5 min.
5. 950µL of LB media was added to cells.
6. Cells were shaken for 1 hr at 37C.
7. Plated 50µL and 950µL on LB plates containing kanamycin.
8. Incubated overnight at 37C.

Controls were set up as follows:
Positive cell control: competent cells on LB plate
Positive plasmid control:

WEDNESDAY, 5/26/2021

Sarah Cumberland

Competent *Agrobacterium tumefaciens* Preparation: Overnight Cultures

- 2x5mL cultures of *Agrobacterium tumefaciens* MP90 strain were prepared as per "SOP - *A. tumefaciens* Competent Cell Preparation iGEM" outlined on the experiments page at 9 am. Another 2x5mL cultures were prepared at 9pm.

Danielle Halasz Mike Green

Golden gate of SPCH Guide 2, 3, 4, 5

- Golden gate was performed as per "SOP - Golden Gate Cloning" outlined on the experiments page.
- Doubles were made for each guide insert duplex
- All guides and the CRISPRi plasmid backbone were diluted to 100ng/µL and excess SPCH guides that were diluted were placed in the freezer in the oligo box (therefore 4.5 microliters of water in final volume)
    - g2 C1 = 1279.2
    - g3 C1 = 1709.0 ng/µL
    - g4 C1 = 1713.9 ng/µL
    - g5 C1 = 2298.7 ng/µL
    - use these concentrations for these SPCH guides so that you don't have to use the nanodrop in the future, and just dilute as nessecary!
- instructions for labled tubes are on a sticky note ontop of the thermocycler

Kulay Janneh

Patch Plate of DXR Guides

- Two patch plates of *E.coli* transformants made on Tuesday were made
- The following was plated on one plate:
    - DXR guide 2 CRISPRi (50 and 950 µL) and the "DXR guide 2 CRISPRi patch plate" labelled plate
- The following was plated on the second plate:
    - DXR guide 5 CRISPRi (50 and 950 µL) and the DXR guide 3 CRISPRi (50 and 950 µL)

Both plates where parafilmed and placed in the 37 degree incubator

Molecular water aliquots

- 2mL of molecular water was pipetted into 2.0 mL Eppendorf (labelled H2O) and placed in front of the blackboard in an orange rack

THURSDAY, 5/27/2021

Sarah Cumberland

Competent *Agrobacterium tumefaciens* Preparation

- *Agrobacterium tumefaciens* MP90 strain was prepared as per "SOP - *A. tumefaciens* Competent Cell Preparation" outlined on the experiments page using the 2x5mL cultures prepared at 9am on the previous day.
- 79x110µL aliquots were produced plus 1x50µL aliquot

PCR on CLE18 and DXR Patches

PCR was performed as per "SOP - Polymerase Chain Reaction (PCR)" outlined on the experiments page on the following patches:

1. CLE18 CRISPRi guide 2 patch #1
2. CLE18 CRISPRi guide 2 patch #2
3. CLE18 CRISPRi guide 2 patch #3
4. CLE18 CRISPRi guide 4 patch #1
5. CLE18 CRISPRi guide 4 patch #2
6. CLE18 CRISPRi guide 5 patch #1
7. DXR CRISPRi Rep 1 guide 2 patch #10 (Wang cells)
8. DXR CRISPRi Rep 1 guide 2 patch #1 (Wang cells)
9. DXR CRISPRi Rep 1 guide 2 patch #3
10. DXR CRISPRi Rep 1 guide 2 patch #5
11. DXR CRISPRi Rep 1 guide 3 patch #5
12. DXR CRISPRi Rep 1 guide 3 patch #4
13. DXR CRISPRi Rep 1 guide 3 patch #3
14. DXR CRISPRi Rep 1 guide 2 patch #2
15. DXR CRISPRi Rep 1 guide 5 patch #4
16. DXR CRISPRi Rep 2 guide 2 patch #1
17. DXR CRISPRi Rep 2 guide 2 patch #5
18. DXR CRISPRi Rep 2 guide 2 patch #6
19. DXR CRISPRi Rep 2 guide 3 patch #2
20. DXR CRISPRi Rep 2 guide 3 patch #5
21. DXR CRISPRi Rep 2 guide 5 patch #2
22. DXR CRISPRi Rep 2 guide 5 patch #4
23. DXR CRISPRi Rep 3 guide 5 patch #1
24. DXR CRISPRi Rep 3 guide 5 patch #2
25. DXR CRISPRi Rep 3 guide 5 patch #4
26. Positive Control (CLE18 Guide 1)
27. CLE18 CRISPRi guide 2 patch #1 (double primer replicate)

Hannah Vujovic Dennis Tran

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformation of SPCH CRISPRi guides 2-5 into *E. Coli*

SOP outlined on the experiments page for *E. Coli* transformations was followed

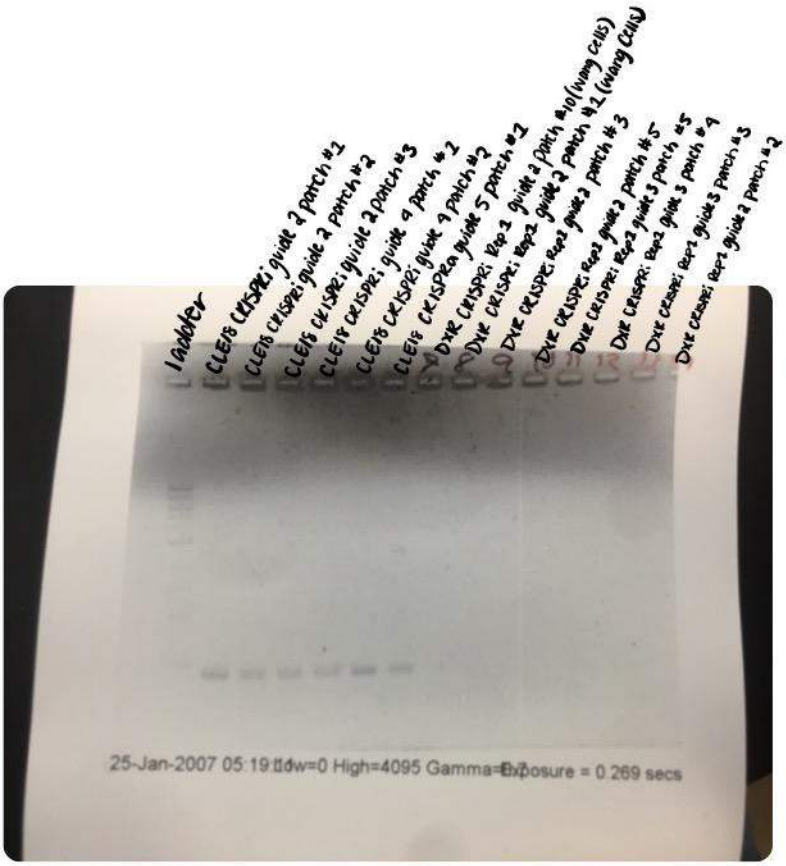
Positive control = CLE18 guide 1 from 2019 plated on LB+Kanamycin
Negative control = empty *E. Coli* plated on LB

Kulay Janneh Dennis Tran Brittany Alexander @Tiffany Arnone

Gel on PCR products

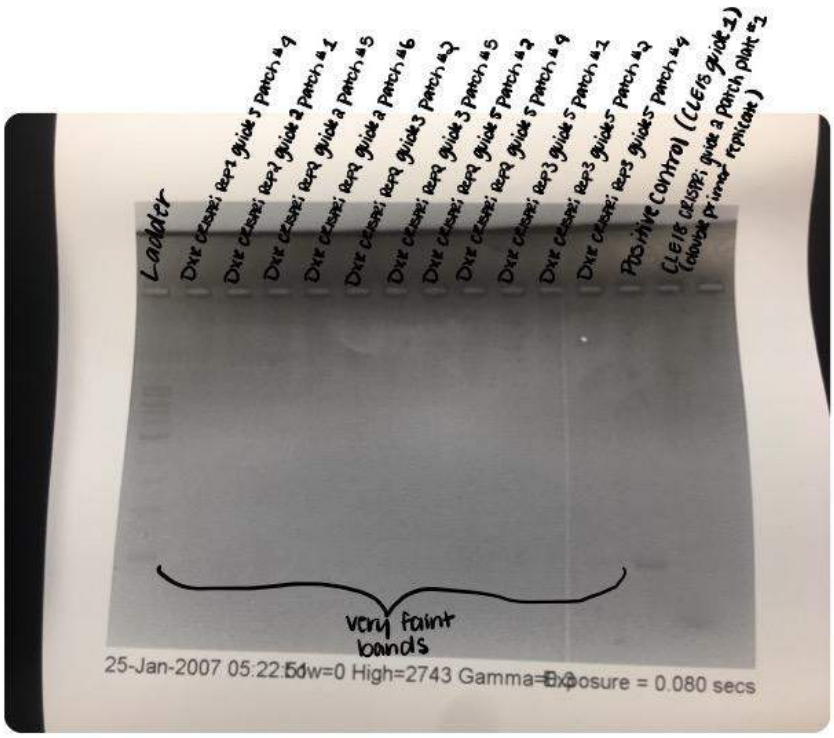
- 2 large gel were made following the "SOP - Gel Electrophoresis iGEM" protocol outlined on the experiments page on the PCR products listed above
- Both gel were run at 114V for 36 minutes
- Voltage supplier started beeping on the 14 well gel and was turned down to 99V for the remaining 4 minutes

Gels from May 27th.jpg



*Note: CLE18 CRISPRa guide 5 patch #1 is actually CRISPRi

Gels from May 27th (2).jpg



Enzo Baracuhy

E. coli Overnight Cultures

6mL overnight *E. coli* cultures were prepared for the following plasmids:

- CLE18 CRISPRi guide 2
- CLE18 CRISPRi guide 4
- CLE18 CRISPRi guide 5
- DXR CRISPRi guide 2
- DXR CRISPRi guide 3
- DXR CRISPRi guide 5

FRIDAY, 5/28/2021

Sarah Cumberland Gesjana Rustemi Mike Green

Miniprep

- A miniprep was done on the following overnight *E. coli* cultures, as per "SOP - Plasmid Preparation (MiniPrep)" outlined on the experiments page.
  - CLE18 CRISPRi guide 2
  - CLE18 CRISPRi guide 4
  - CLE18 CRISPRi guide 5
- Note: the other overnight cultures were not dense enough to miniprep at this time.

- 4 x 50µL samples of each plasmid were obtained, at the following concentrations:
- CLE18 CRISPRi guide 2:
  - 67.0ng/µL
  - 68.5ng/µL
  - 76.3ng/µL

- 78.1ng/µL
- CLE18 CRISPRi guide 4:
  - 245.7ng/µL
  - 254.4ng/µL
  - 197.3ng/µL
  - 194.1ng/µL
- CLE18 CRISPRi guide 5:
  - 77.9ng/µL
  - 79.7ng/µL
  - 79.4ng/µL
  - 73.4ng/µL

Glycerol Stocks

- Glycerol stocks were prepared of the following *E. coli* cultures, as per "SOP - Glycerol Stock Preparation" outlined on the experiments page.
- 2 x replicates of each culture were prepared.
  - CLE18 CRISPRi guide 2
  - CLE18 CRISPRi guide 4
  - CLE18 CRISPRi guide 5

SATURDAY, 5/29/2021

Brittany Alexander Namra Hamid Danielle Halasz Dennis Tran Gerry Koot

Agrobacterium tumefaciens transformation

- following plasmids were taken from CRISPRi and CRISPRa box in -20 freezer:
  - CRISPRi backbone (as negative control)
  - SPCH g1 CRISPRa (NOTE: small amount spilled from epindorph when being transfered to shaking room, and plate with 250 µLwas accidentaly teared)
  - DXR g1 CRISPRa
  - CLE18 g1 CRISPRa
  - DXR g4 CRISPRa
  - CLE18 g1 CRISPRi
  - DXR g4 CRISPRi
  - DXR g1 CRISPRi

1. 1ug of plasmid was pipetted into each tube (one type of plasmid per tube). Note: use c=n/v to find the volume of each plasmid to use.
2. *Agrobacterium* + plasmid mixture was flicked to mix, and then left on ice for 15min.
3. *Agrobacterium* + plasmid mixture was put into a -80C freezer for 2min. (Note: liquid nitrogen for 1 minute also works)
4. *Agrobacterium* + plasmid mixture was put in a 37C hot plate for 3 min.
5. 300µL LB broth was added to the mixture and put into a 28C shaker for 2hr
6. 50µL of the *Agrobacterium*+plasmid+LB mxiture was pipetted onto a LB+Kanamycin+gentamycin plate, and the rest was poured onto another LB+Kanamycin+gentamycin plate.

Plates (16 in total) were put in a 28C incubator for 48 hours

MONDAY, 5/31/2021

Sarah Cumberland

Patch Plates & PCR

PCR was performed as per the SOP outlined on the experiments page. PCR was performed on the following colonies, and patches/patch plates were also made from the following colonies:

- SPCH CRISPRi guide 5 patches 1-4
- SPCH CRISPRi guide 4 patches 1-4

- SPCH CRISPRi guide 3 patches 1-4
- SPCH CRISPRi guide 2 patches 1-4

An additional patch plate was made using the following colonies (these colonies were not PCR'd):

- SPCH CRISPRi guide 5 patches 5-8
- SPCH CRISPRi guide 4 patches 5-8
- SPCH CRISPRi guide 3 patches 5-8
- SPCH CRISPRi guide 2 patches 5-8

An additional "patch pour plate" was prepared using the remaining colony water from the PCRs. The same colonies as were PCR'd were also made into these "pour patches", ie. patches 1-4 of guides 2-5.

Hannah Vujovic @Samantha Segal

Golden Gate on ICE2 guides 1-5 and CLE18 guide 3 CRISPRi

Golden gate was performed as per "SOP - Golden Gate Cloning" outlined on the experiments page.

Kulay Janneh Gerry Koot Charlotte Fletcher

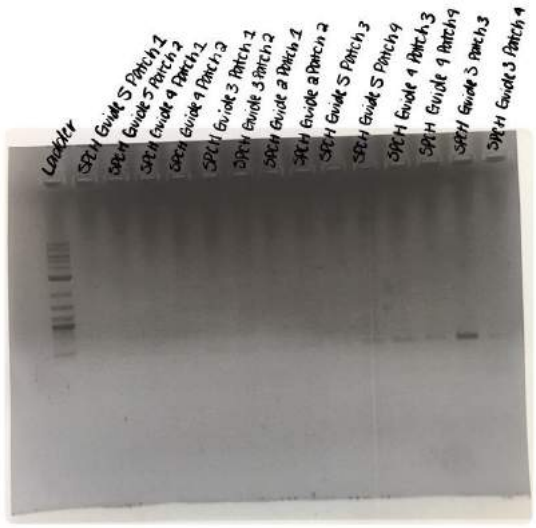
Gel Electrophoresis on SPCH CRISPRi guides 2-5

- Two gel Electrophoresis was carried out as per "SOP - Gel Electrophoresis" outlined on the experiments page.
- 10 µL of each PCR product were loaded into a well

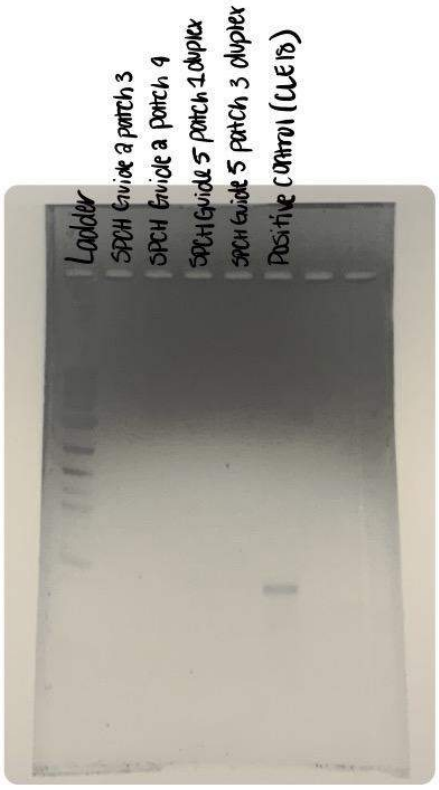
1. SPCH Guide 5 Patch 1
2. SPCH Guide 5 patch 2
3. SPCH Guide 4 patch 1
4. SPCH Guide 4 patch 2
5. SPCH Guide 3 patch 1
6. SPCH Guide 3 patch 2
7. SPCH Guide 2 patch 1
8. SPCH Guide 2 patch 2
9. SPCH Guide 5 patch 3
10. SPCH Guide 5 patch 4
11. SPCH Guide 4 patch 3
12. SPCH Guide 4 patch 4
13. SPCH Guide 3 patch 3
14. SPCH Guide 3 patch 4
15. SPCH Guide 2 patch 3
16. SPCH Guide 2 patch 4
17. SPCH Guide 5 patch 1 duplex
18. SPCH Guide 5 patch 3 duplex
19. Positive control (CLE18)

- The larger gel was run at 101V and the smaller gel was run at 116V for 40 minutes.

Gel.May31(1).jpg



gel.May31.jpg



TUESDAY, 6/1/2021

Amira Bouchema Harkamal Samra Mike Green

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour

3. Heat inactivate the BsaI for 20minutes at 80C
4. Hold at 4C infinitely

Transformations Using GoldenGate Plasmids

The 4 plasmids from the Golden Gate reactions set up above (2 of ICE2 guides 1-5 in CRISPRi and 2 of CLE18 guide 3 in CRISPRi) were transformed into *E. coli* DH5α as follows:

1. 11µL from the Golden Gate reaction was added to 50µL of competent *E. coli* cells.
2. Cells were incubated on ice for 15min.
3. Cells were heat-shocked at 42°C for 45 seconds.
4. Cells were rested on ice for 5 min.
5. 250µL of LB media was added to cells.
6. Cells were shaken for 1 hr at 37C.
7. Plated 50µL and 250µL on LB plates containing kanamycin.
8. Incubated overnight at 37C.

Controls were set up as follows:
Positive control: competent cells on LB plate
Negative control: empty *E. coli* cells on LB plate

WEDNESDAY, 6/2/2021

Hannah Vujovic

Agrobacterium tumefaciens transformation

1. Four 1.5mL tubes of competent *Agrobacterium* cells (~100 to 140µL cells per tube) were taken from -80C freezer
2. The following plasmids were taken from the -20C freezer:
    - CLE18 guide 2 CRISPRi
    - CLE18 guide 4 CRISPRi
    - CLE18 guide 5 CRISPRi
3. 1ug of plasmid was pipetted into each tube (one type of plasmid per tube). Note: use c=n/v to find the volume of each plasmid to use. Duplicates were done of each guide.
4. *Agrobacterium* + plasmid mixture was flicked to mix, and then left on ice for 15min.
5. *Agrobacterium* + plasmid mixture was put into a -80C freezer for 2min. (Note: liquid nitrogen for 1 minute also works)
6. *Agrobacterium* + plasmid mixture was put in a 37C hot plate for 3 min.
7. 300µL LB broth was added to the mixture and put into a 28C incubator (non-shaking) for 2hr
8. 300µL of the *Agrobacterium*+plasmid+LB mxiture was pipetted onto a LB+Kanamycin+gentamycin plate
9. Plates (6 in total) were put in a 28C incubator for 48 hours

Danielle Halasz Dennis Tran Gesjana Rustemi @Kartikay

Patch plate preparation of successful *E.coli* and *Agrobacterium tumefaciens*

5 Patch plates were prepared for *Agrobacterium* and 2 plates for *E.coli*

1. Colonies were scraped off of the plates listed above using a sterile loop
2. Each colony was spread in a small patch on an LB + Kanamycin plate for *E.coli*, and a LB + Kanamycin + gentamycin plate for *Agrobacterium*
3. patches were made on each plate., *Agrobacterium* plates were placed in the 28* incubator, and *E.coli* plates placed in 37* incubator

Danielle Halasz Dennis Tran Brittany Alexander

Golden gate of SPCH Guide 2, 3, 4, 5, DXR guide 2, 3, 5, and CLE18 g3

- Protocol for golden gate was followed as per "SOP - Golden Gate Cloning" outlined on the experiments page.
- doubles were made for each guide insert duplex

- all guides and the CRISPRi plasmid backbone were diluted to 100ng/µL and excess guides that were diluted were placed in the freezer in the oligo box (therefore 4.5 microliters of water in final volume)
- instructions for labled tubes are on a sticky note ontop of the thermocycler

Sarah Cumberland

E. coli Competent Cell Prep: Overnight Cultures

- Followed "SOP - *E. coli* TSS Chemically Competent Cell Preparation" outlined in the experiments page.

2x5mL cultures of *E. coli* were set up to shake overnight at 37C.

THURSDAY, 6/3/2021

Danielle Halasz Sarah Cumberland

E.coli Competent Cell Prep

- Followed "SOP - *E. coli* TSS Chemically Competent Cell Preparation" outlined in the experiments page.
- 5mL of culture was added to 45 mL of LB (x2) for a total of 100mL of culture
- a total of 91 tubes of 100 µL of competent cells were obtained
- tubes were placed in new box in -80 freezer, and each tube was labelled "*E.coli* 3 June 2021"

Kulay Janneh

Pre-Transformation GoldenGate Product Prep

1. Add 1µL of BsaI to the Golden Gate mixture
2. Heat at 37C for 1 hour
3. Heat inactivate BsaI for 20 minutes at 80C
4. Hold at 4C infinitely

Kulay Janneh Dennis Tran @Adrian Monrad

E.coli transformation with Golden Gate products

- Transformations using SPCH guides 2-5, DXR guides 2-5 and CLE18 Guide 3 plasmids were performed following the "SOP-*E.coli* transformation" outlined on the experiments page.
- 320 µL was plated on kanamycin + LB plates and left to dry in the biosafety cabinet

Kulay Janneh Gerry Koot Taylor @TiffanyArnone

PCR on DXR, CLE18 and SPCH guides

- PCR was performed following the "SOP-PCR-Colony crack" protocol outlined on the experiments page.
- PCR was performed on the following patches. Samples 31-30 are from *Agrobacterium* plates and samples 31-35 are from *E. coli* plates.

1. SPCH guide 1 CRISPRa patch 1 (50 µL)
2. SPCH guide 1 CRISPRa patch 2 (50 µL)
3. SPCH guide 1 CRISPRa patch 1 (250 µL)
4. DXR guide 1 CRISPRa patch 1 (50 µL)
5. DXR guide 4 CRISPRa patch 1 (250 µL)
6. DXR guide 4 CRISPRa patch 2 (250 µL)
7. DXR guide 4 CRISPRa patch 3 (250 µL)
8. DXR guide 4 CRISPRa patch 4 (250 µL)
9. DXR guide 4 CRISPRa patch 5 (250 µL)
10. DXR guide 4 CRISPRa patch 6 (250 µL)
11. DXR guide 4 CRISPRa patch 7 (250 µL)
12. DXR guide 4 CRISPRa patch 8 (250 µL)
13. DXR guide 4 CRISPRi patch 1 (250 µL)
14. DXR guide 4 CRISPRi patch 2 (250 µL)

15. DXR guide 4 CRISPRi patch 3 (250 µL)
16. DXR guide 4 CRISPRi patch 4 (250 µL)
17. DXR guide 4 CRISPRi patch 5 (250 µL)
18. DXR guide 4 CRISPRi patch 6 (250 µL)
19. DXR guide 4 CRISPRi patch 7 (250 µL)
20. DXR guide 4 CRISPRi patch 8 (250 µL)
21. CLE18 guide 1 CRISPRi patch 1 (250 µL)
22. CLE18 guide 1 CRISPRi patch 2 (250 µL)
23. CLE18 guide 1 CRISPRa patch 1 (250 µL)
24. CLE18 guide 1 CRISPRa patch 2 (250 µL)
25. CLE18 guide 1 CRISPRa patch 3 (250 µL)
26. CLE18 guide 1 CRISPRa patch 4 (250 µL)
27. DXR guide 1 CRISPRi patch 1 (250 µL)
28. DXR guide 1 CRISPRi patch 2 (250 µL)
29. DXR guide 1 CRISPRi patch 3 (250 µL)
30. DXR guide 1 CRISPRi patch 1 (50 µL)
31. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
32. ICE2 guide 2 CRISPRi patch 2 (*E. coli*)
33. ICE2 guide 1 CRISPRi patch 1 (*E. coli*)
34. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
35. ICE2 guide 4 CRISPRi patch 1 (*E. coli*)

- Legend, similar to this, can be found on the lid of the thermocycler.

FRIDAY, 6/4/2021

Sarah Cumberland Dennis Tran

PCR on ICE2 Guide 2 Patches

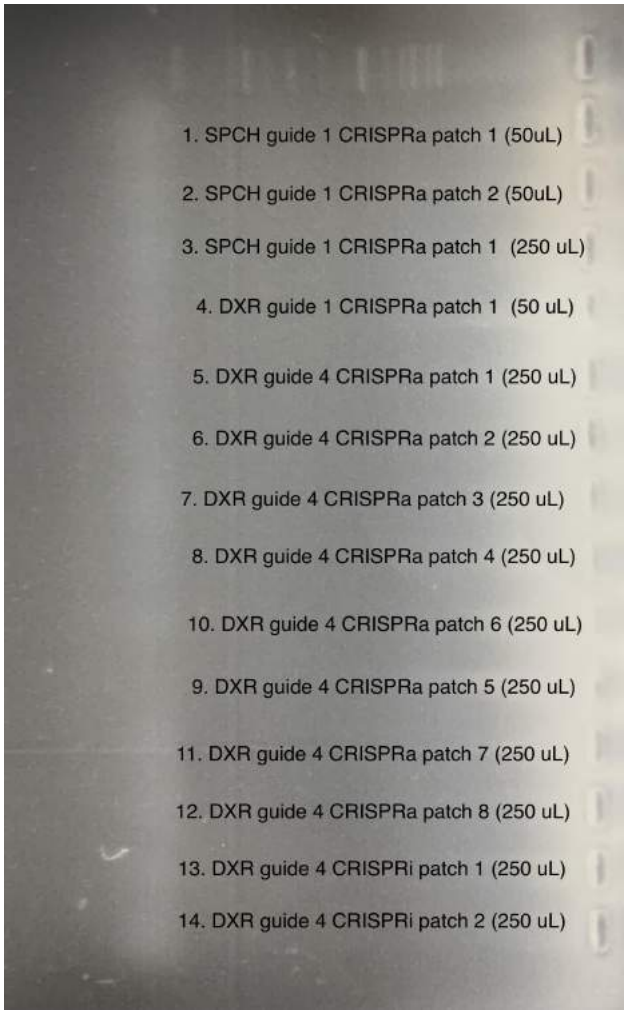
- PCR was performed as per "SOP - Polymerase Chain Reaction (PCR) - Colony Crack iGEM" outlined on the experiments page on the following colonies:
  1. ICE2 guide 2 CRISPRi patch 1
  2. ICE2 guide 2 CRISPRi patch 2
  3. ICE2 guide 2 CRISPRi patch 3

  Positive control: CLE18 guide 1 CRISPRi

Gel Electrophoresis on PCRs from *Agrobacterium tumefaciens* Colonies

- Gel Electrophoresis was performed as per "SOP - Gel Electrophoresis iGEM" outlined on the experiments page on PCRs from the following *Agrobacterium* colonies:
  1. SPCH guide 1 CRISPRa patch 1 (50 µL)
  2. SPCH guide 1 CRISPRa patch 2 (50 µL)
  3. SPCH guide 1 CRISPRa patch 1 (250 µL)
  4. DXR guide 1 CRISPRa patch 1 (50 µL)
  5. DXR guide 4 CRISPRa patch 1 (250 µL)
  6. DXR guide 4 CRISPRa patch 2 (250 µL)
  7. DXR guide 4 CRISPRa patch 3 (250 µL)
  8. DXR guide 4 CRISPRa patch 4 (250 µL)
  9. DXR guide 4 CRISPRa patch 5 (250 µL)
  10. DXR guide 4 CRISPRa patch 6 (250 µL)
  11. DXR guide 4 CRISPRa patch 7 (250 µL)
  12. DXR guide 4 CRISPRa patch 8 (250 µL)
  13. DXR guide 4 CRISPRi patch 1 (250 µL)
  14. DXR guide 4 CRISPRi patch 2 (250 µL)

Gel 1.jpg



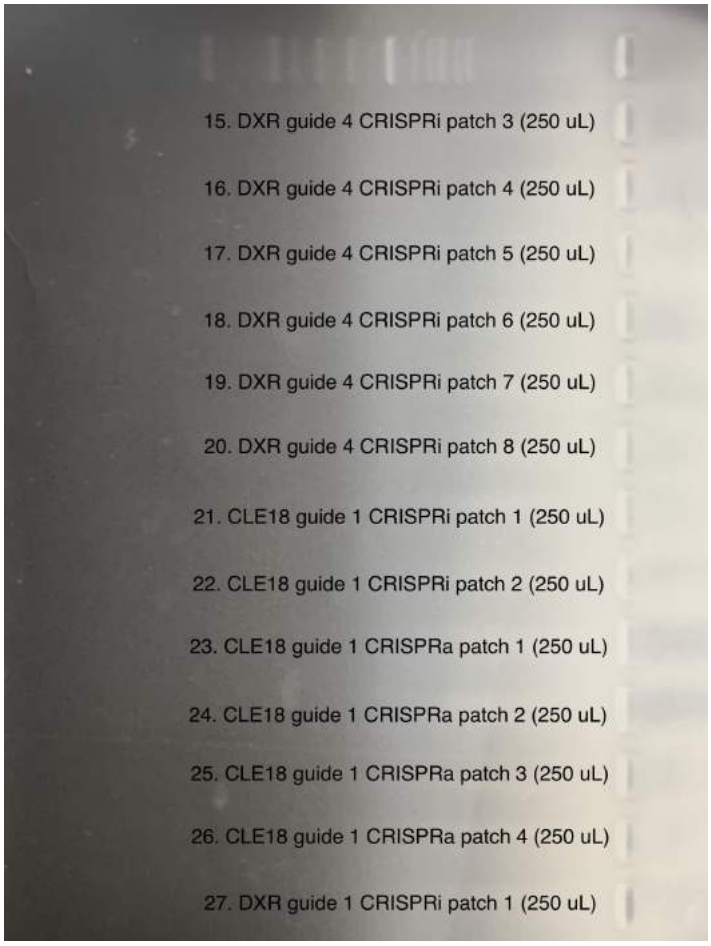
Sarah Cumberland Dennis Tran Brittany Alexander Emma Lee

Gel Electrophoresis on PCRs from *Agrobacterium tumefaciens* & *E. coli* Colonies

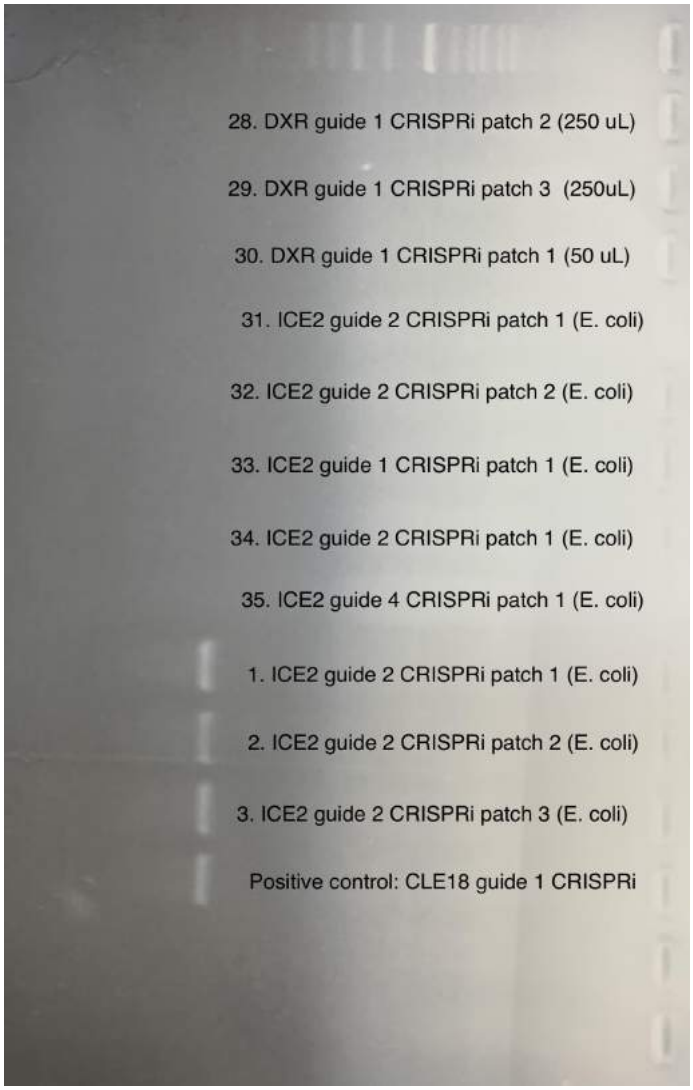
- Gel Electrophoresis was performed as per "SOP - Gel Electrophoresis iGEM" outlined on the experiments page on PCRs from the following *Agrobacterium* colonies:
  15. DXR guide 4 CRISPRi patch 3 (250 µL)
  16. DXR guide 4 CRISPRi patch 4 (250 µL)
  17. DXR guide 4 CRISPRi patch 5 (250 µL)
  18. DXR guide 4 CRISPRi patch 6 (250 µL)
  19. DXR guide 4 CRISPRi patch 7 (250 µL)
  20. DXR guide 4 CRISPRi patch 8 (250 µL)
  21. CLE18 guide 1 CRISPRi patch 1 (250 µL)
  22. CLE18 guide 1 CRISPRi patch 2 (250 µL)
  23. CLE18 guide 1 CRISPRa patch 1 (250 µL)
  24. CLE18 guide 1 CRISPRa patch 2 (250 µL)
  25. CLE18 guide 1 CRISPRa patch 3 (250 µL)
  26. CLE18 guide 1 CRISPRa patch 4 (250 µL)
  27. DXR guide 1 CRISPRi patch 1 (250 µL)
  28. DXR guide 1 CRISPRi patch 2 (250 µL)
  29. DXR guide 1 CRISPRi patch 3 (250 µL)
  30. DXR guide 1 CRISPRi patch 1 (50 µL)

- Gel Electrophoresis was also performed on PCRs from the following *E. coli* colonies:
  31. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
  32. ICE2 guide 2 CRISPRi patch 2 (*E. coli*)
  33. ICE2 guide 1 CRISPRi patch 1 (*E. coli*)
  34. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
  35. ICE2 guide 4 CRISPRi patch 1 (*E. coli*)
  1. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
  2. ICE2 guide 2 CRISPRi patch 2 (*E. coli*)
  3. ICE2 guide 2 CRISPRi patch 3 (*E. coli*)
  Positive control: CLE18 guide 1 CRISPRi

Gel 2.jpg



Gel 3.jpg



SATURDAY, 6/5/2021

Sarah Cumberland Hannah Vujovic Emma Lee

PCR on DXR, CLE18 and SPCH guides

- PCR was performed following the "SOP-PCR-Colony crack" protocol outlined on the experiments page.
- PCR was performed on the following patches. Samples 31-30 are from *Agrobacterium* plates and samples 31-35 are from *E. coli* plates.

1. SPCH guide 1 CRISPRa patch 1 (50 µL)
2. SPCH guide 1 CRISPRa patch 2 (50 µL)
3. SPCH guide 1 CRISPRa patch 1 (250 µL)
4. DXR guide 1 CRISPRa patch 1 (50 µL)
5. DXR guide 4 CRISPRa patch 1 (250 µL)
6. DXR guide 4 CRISPRa patch 2 (250 µL)
7. DXR guide 4 CRISPRa patch 3 (250 µL)
8. DXR guide 4 CRISPRa patch 4 (250 µL)
9. DXR guide 4 CRISPRa patch 5 (250 µL)
10. DXR guide 4 CRISPRa patch 6 (250 µL)

11. DXR guide 4 CRISPRa patch 7 (250 uµL)
12. (no patch left)
13. DXR guide 4 CRISPRi patch 1 (250 µL)
14. DXR guide 4 CRISPRi patch 2 (250 µL)
15. DXR guide 4 CRISPRi patch 3 (250 µL)
16. DXR guide 4 CRISPRi patch 4 (250 µL)
17. DXR guide 4 CRISPRi patch 5 (250 µL)
18. DXR guide 4 CRISPRi patch 6 (250 µL)
19. DXR guide 4 CRISPRi patch 7 (250 µL)
20. (no patch left)
21. CLE18 guide 1 CRISPRi patch 1 (250 µL)
22. CLE18 guide 1 CRISPRi patch 2 (250 µL)
23. CLE18 guide 1 CRISPRa patch 1 (250 µL)
24. CLE18 guide 1 CRISPRa patch 2 (250 µL)
25. CLE18 guide 1 CRISPRa patch 3 (250 µL)
26. CLE18 guide 1 CRISPRa patch 4 (250 µL)
27. DXR guide 1 CRISPRi patch 1 (250 µL)
28. DXR guide 1 CRISPRi patch 2 (250 µL)
29. DXR guide 1 CRISPRi patch 3 (250 µL)
30. DXR guide 1 CRISPRi patch 1 (50 µL)
31. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
32. ICE2 guide 2 CRISPRi patch 2 (*E. coli*)
33. ICE2 guide 1 CRISPRi patch 1 (*E. coli*)
34. ICE2 guide 2 CRISPRi patch 1 (*E. coli*)
35. ICE2 guide 4 CRISPRi patch 1 (*E. coli*)

- Legend, similar to this, can be found on the lid of the thermocycler.

SUNDAY, 6/6/2021

Amira Bouchema

Gel was made for the PCR that was made on Saturday 5/6/2021 using SOP outlined on the experiments page.

No colonies tested positive.

Please follow legend as prescribed on Saturday 5/6/2021

image.png

image.png

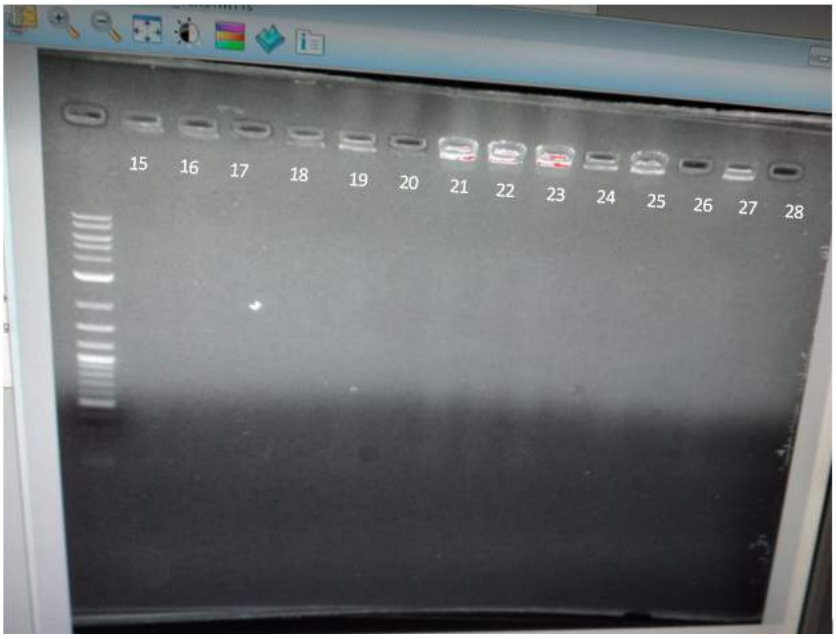


image.png

MONDAY, 6/7/2021

Sarah Cumberland @Nathaniel Petersen

Golden Gate on the Following Guides: CLE18 guide 3, DXR guides 2, 3, 5, SPCH guides 2, 3, 4, 5

Golden gate was performed on CLE18 guide 3, DXR guides 2, 3, 5, SPCH guides 2, 3, 4, 5 as per "SOP - Golden Gate Cloning" outlined on the experiments page.

Table12

	Reagent	Volume (µL)	Mass (ng)	Concentration (ng/µL)	20x reaction volume
1	Molecular grade H2O (Found in Plant box “H2O”)	4.5			90
2	Plasmid DNA	1	100	100	20
3	Duplexed gRNA insert	1	100	100	
4	10x CutSmart Buffer	1			20
5	10x T4 Ligase Buffer	1			20
6	BsaI	1			20
7	T4 ligase	0.5			10
8	**Total**	10			200

TUESDAY, 6/8/2021

Transformation

Transformations using CLE18 guide 3, DXR guides 2, 3, 5, SPCH guides 2, 3, 4, 5 plasmids were performed following the "SOP- *E.coli* transformation" outlined on the experiments page.

PCR Verification

- PCR was performed following the "SOP-PCR-Colony crack" protocol.
- PCR was performed on the following patches. Samples 1-29 are from *Agrobacterium* plates and samples 1-2 are from *E. coli* plates.

WEDNESDAY, 6/9/2021

Namra Hamid Dennis Tran

Gel electrophoresis

See protocol outlined on the experiments page.

- 3 Gels performed on the PCR products done yesterday.

Samples:

1* *E. coli* ICE 2 Guide 1

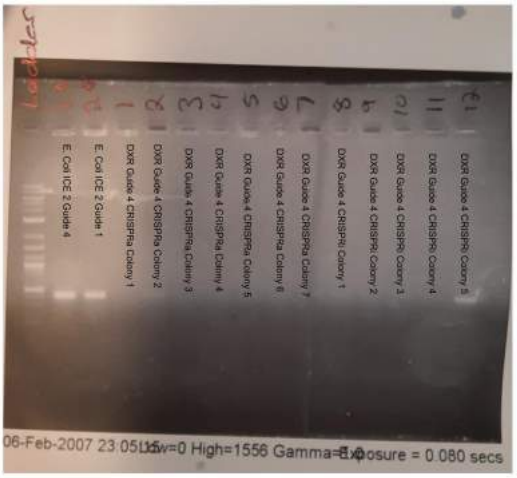
2* *E. Coli* ICE 2 Guide 4

1. DXR Guide 4 CRISPRa Colony 1
2. DXR Guide 4 CRISPRa Colony 2
3. DXR Guide 4 CRISPRa Colony 3
4. DXR Guide 4 CRISPRa Colony 4
5. DXR Guide 4 CRISPRa Colony 5
6. DXR Guide 4 CRISPRa Colony 6
7. DXR Guide 4 CRISPRa Colony 7

8. DXR Guide 4 CRISPRi Colony 1
9. DXR Guide 4 CRISPRi Colony 2
10. DXR Guide 4 CRISPRi Colony 3
11. DXR Guide 4 CRISPRi Colony 4
12. DXR Guide 4 CRISPRi Colony 5
13. DXR Guide 4 CRISPRi Colony 6
14. DXR Guide 4 CRISPRi Colony 7
15. DXR Guide 1 CRISPRa
16. DXR Guide 1 CRISPRi (50µL)
17. DXR Guide 1 CRISPRi (250µL) Colony 1
18. DXR Guide 1 CRISPRi (250µL) Colony 2
19. DXR Guide 1 CRISPRi (250µL) Colony 3
20. SPCH Guide 1 CRISPRa (250µL)
21. SPCH Guide 1 CRISPRa (50µL) Colony 1
22. SPCH Guide 1 CRISPRa (50µL) Colony 2
23. CLE18 Guide 1 CRISPRa Colony 1
24. CLE18 Guide 1 CRISPRa Colony 2
25. CLE18 Guide 1 CRISPRa Colony 3
26. CLE18 Guide 1 CRISPRa Colony 4
27. CLE18 Guide 1 CRISPRi Colony 1
28. CLE18 Guide 1 CRISPRi Colony 1
29. Positive Control

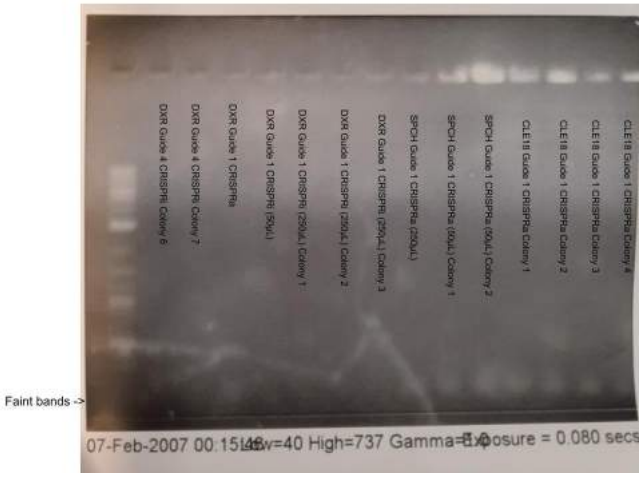
Gel 1:

Gel 1 (1).jpg



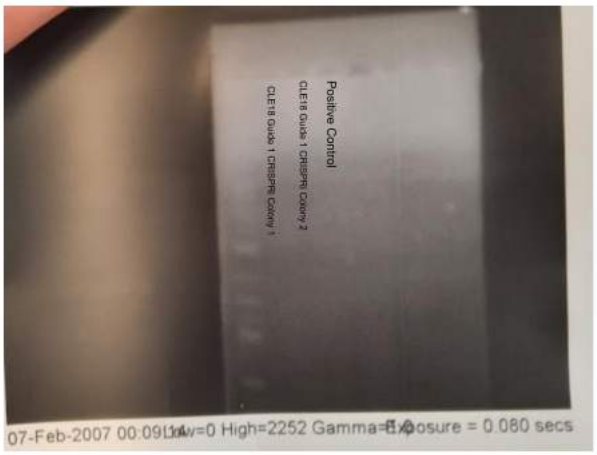
Gel 2:

Gel 2.jpg



Gel 3: No bands found

Gel 3.jpg



Kulay Janneh

Patch Plating

One patch plate of the DXR, CLE18 and SPCH guide 3 *E.coli* transformants were made and incubated in the 37 degree incubator.

THURSDAY, 6/10/2021

Danielle Halasz Mike Green @Adrian Monrad

Golden gate of guide inserts into CRISPRi backbone

Golden gate was run for the following guide inserts into CRISPRi backbone, following the protocol "**SOP - Golden Gate Cloning**" outlined on the experiments page.

Following were made with duplicates:

1. DXR CRISPRi guide 2
2. DXR CRISPRi guide 5
3. SPCH CRISPRi guide 2
4. SPCH CRISPRi guide 4
5. SPCH CRISPRi guide 5

Sarah Cumberland Harkamal Samra @Adrian Monrad

PCR Verification of CRISPRi DXR guide 3, SPCH guide 3, CLE18 guide 3

PCR was run on the following patches, as per "SOP - Polymerase Chain Reaction (PCR) - Colony Crack" outlined on the experiments page.

1. DXR CRISPRi guide 3 patch 1
2. DXR CRISPRi guide 3 patch 2
3. SPCH CRISPRi guide 3 patch 1
4. CLE18 CRISPRi guide 3 patch 1
5. CLE18 CRISPRi guide 3 patch 2
6. CLE18 CRISPRi guide 3 patch 3

- positive control (CLE18 CRISPRi)

Legend can be found on top of thermal cycler

Kulay Janneh Gerry Koot Charlotte Fletcher Taylor @rebecca taylor

Gel on PCR products (CRISPRi DXR guide 3, SPCH guide 3, CLE18 guide 3)

- A small gel was made following the "SOP - Gel electrophoresis iGEM" outlined on the experiments page.
- 10 µL of each PCR product was loaded into a well and run at 104V for 40 minutes
- All colonies used in the PCR that was loaded into this gel were circled with a red marker

GelJun 10, 2021.jpg

Kulay Janneh

Overnight Cultures of *E.coli* and *Agrobacterium tumefaciens*

6mL of LB + 6µL of kanamycin and 6mL LB + 6µL kanamycin + 6µL gentamycin were made for *E.coli* and *Agrobacterium* respectively. The following overnights were made and shaken overnight:

1. ICE2 guide 1 CRISPRi *E.coli*
2. ICE2 guide 2 CRISPRi *E.coli*
3. ICE2 guide 4 CRISPRi *E.coli*
4. SPCH guide 1 CRISPRa *Agrobacterium*
5. DXR guide 1 CRISPRa *Agrobacterium*
6. DXR guide 1 CRISPRi *Agrobacterium*

7. DXR guide 4 CRISPRi *Agrobacterium*
8. DXR guide 4 CRISPRa *Agrobacterium*
9. CLE18 guide 1 CRISPRa *Agrobacterium*
10. CLE18 guide 1 CRISPRi *Agrobacterium*

FRIDAY, 6/11/2021

Danielle Halasz

Transformation of guide inserts from yesterday into competent *E.coli* cells

followed the protocol "SOP - *E. coli* Transformation" outlined on the experiments page, total of 10 plates were made

Overnight of *Agrobacterium tumefaciens* (repeate from yesterday)

5mL LB+5µL Kanamycin + 5µL gentamycin were made for *Agrobacterium*

The following overnights were made and shaken overnight in room 410:

1. SPCH guide 1 CRISPRa *Agrobacterium*
2. DXR guide 1 CRISPRa *Agrobacterium*
3. DXR guide 1 CRISPRi *Agrobacterium*
4. DXR guide 4 CRISPRi *Agrobacterium*
5. DXR guide 4 CRISPRa *Agrobacterium*
6. CLE18 guide 1 CRISPRa *Agrobacterium*
7. CLE18 guide 1 CRISPRi *Agrobacterium*

Hannah Vujovic @adrian

Miniprep and glycerol stocks of ICE2 guides 1, 2, and 4 CRISPRi

SOP outlined on the experiments page was followed for miniprep experiment.

SATURDAY, 6/12/2021

Hannah Vujovic Dennis Tran @samantha segal

Agrobacterium tumefaciens transformations of all miniprepped guides

SOP outlined on the experiments page was followed using CLE18 guides 1/2/4/5, DXR guide 1 and ICE2 guides 1/2/4 all CRISPRi.

Sarah Cumberland

Miniprep

Minprep was performed on the 1 successful overnight culture from the day before. Protocol followed was "SOP - Plasmid Preparation (MiniPrep) iGEM" outlined on the experiments page.

- DXR guide 4 CRISPRi *Agrobacterium*.

MONDAY, 6/14/2021

Colony PCR of *Agrobacterium tumefaciens*

Enzo Baracuhy

The following *Agrobacterium* colonies were colony PCR'd using the protocol "SOP - Polymerase Chain Reaction Colony Crack" outlined on the experiments page. I took big glops of *Agrobacterium* colonies to make the colony water, vortexed it, and used 20µL of colony water instead of 10. I also removed 10µL from the amount of water we added.

- CLE18 g4 CRISPRi
- CLE18 g5 CRISPRi
- CLE18 g2 CRISPRi
- CLE18 g1 CRISPRi
- CLE18 g1 CRISPRa
- SPCH g1 CRISPRa

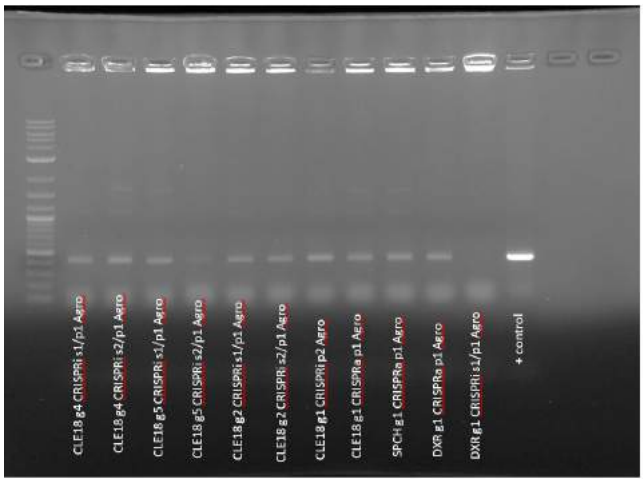
- DXR g1 CRISPRa
- DXR g4 CRISPRi

Agarose Gel

Amira Bouchema

- A gel was made following the "SOP - Gel electrophoresis iGEM" outlined on the experiments page.

image.png



Danielle Halasz @Nathaniel Petersen

Transformation of *Agrobacterium tumefaciens*

- followed **SOP - *A. tumefaciens* transformation protocol outlined on the experiments page,** using the following minipreped plasmids, and made duplicates of each:
  - DXR g1 CRISPRi
  - DXR g4 CRISPRa
  - ICE2 g4 CRISPRi
  - ICE2 g2 CRISPRi NOTE: out of plasmid, concentration was very low (approx. 50 ng/µL, so alot had to be used to reach desired 1 ug required for transformation, therfeore we are out)
  - ICE2 g1 CRISPRi

Danielle Halasz @Nathaniel Peterson

6 mL Overnights of *Agrobacterium tumefaciens* that were sucsessfuly transformed

- duplicates made of each sample, therefore total of 16 overnight tubes made
  - CLE18 g1 CRISPRi
  - CLE18 g2 CRISPRi
  - CLE18 g4 CRISPRi
  - CLE18 g5 CRISPRi
  - CLE18 g1 CRISPRa
  - SPCH g1 CRISPRa
  - DXR g1 CRISPRa
  - DXR g4 CRISPRi

TUESDAY, 6/15/2021

Sarah Cumberland Enzo Baracuhy

5 mL Overnights of *Agrobacterium tumefaciens*

- none of the overnights from the previous day grew, therefore repeat of yesterday's setup
- duplicates made of each sample, therefore total of 16 overnight tubes made
    - CLE18 g1 CRISPRi
    - CLE18 g2 CRISPRi
    - CLE18 g4 CRISPRi
    - CLE18 g5 CRISPRi
    - CLE18 g1 CRISPRa
    - SPCH g1 CRISPRa
    - DXR g1 CRISPRa
    - DXR g4 CRISPRi

WEDNESDAY, 6/16/2021

Sarah Cumberland

- The following overnight cultures grew:
    - CLE18 g2 CRISPRi
    - CLE18 g5 CRISPRi
    - CLE18 g1 CRISPRa
    - SPCH g1 CRISPRa
    - DXR g4 CRISPRi
- The following overnight cultures did not grow:
    - CLE18 g1 CRISPRi
    - CLE18 g4 CRISPRi
    - DXR g1 CRISPRa

Floral Dip: Day 2

- 300mL cultures were prepared for Day 2 of the floral dip protocol, as per "SOP - Floral Dip (*Agrobacterium*-mediated transformation of Arabidopsis) iGEM" outlined on the experiments page.
- 4mL of starting culture (overnight culture) were used instead of 1mL as per Dr. Wang's suggestion (given our shorter than usual time for the 300mL cultures to grow).
- 300mL cultures were prepared from the following *Agrobacterium* cultures:
    - CLE18 g2 CRISPRi
    - CLE18 g5 CRISPRi
    - CLE18 g1 CRISPRa
    - SPCH g1 CRISPRa
    - DXR g4 CRISPRi

Glycerol Stocks

- Glycerol stocks were prepared as per "SOP - Glycerol Stock Preparation iGEM" outlined on the experiments page.
- 1 x glycerol stock was prepared for each of the following *Agrobacterium* cultures:
    - CLE18 g2 CRISPRi
    - CLE18 g5 CRISPRi
    - CLE18 g1 CRISPRa
    - SPCH g1 CRISPRa
    - DXR g4 CRISPRi
- Stocks were placed in the glycerol stock box in the -80 freezer

Kulay Janneh @Adrian Monrad

Patch plates

- Two patch plates were made of the following guides:

1. CLE18 guide 1 CRISPRi

2. CLE18 guide 2 CRISPRi
3. CLE18 guide 4 CRISPRi
4. CLE18 guide 5 CRISPRi
5. ICE2 guide 1 CRISPRi
6. ICE2 guide 2 CRISPRi
7. ICE2 guide 4 CRISPRi
8. DXR guide 1 CRISPRi

- Plates were placed in the 28 degree incubator

Danielle Halasz

Golden Gate

- Golden gate was performed as per 'SOP - Golden Gate Cloning iGEM' outlined on the experiments page.
- 2 replicates were made of each of the following constructs:
  - CLE18 g3 CRISPRi
  - DXR g2 CRISPRi
  - DXR g3 CRISPRi
  - DXR g5 CRISPRi
  - SPCH g2 CRISPRi
  - SPCH g3 CRISPRi
  - SPCH g4 CRISPRi
  - SPCH g5 CRISPRi
  - ICE2 g3 CRISPRi
  - ICE2 g5 CRISPRi

THURSDAY, 6/17/2021

Sarah Cumberland

Glycerol Stocks

- Glycerol stocks were prepared as per "SOP - Glycerol Stock Preparation iGEM" outlined on the experiments page.
- 1 x glycerol stock was prepared for each of the following *Agrobacterium* cultures:
  - CLE18 g2 CRISPRi
  - CLE18 g5 CRISPRi
  - CLE18 g1 CRISPRa
  - SPCH g1 CRISPRa
  - DXR g4 CRISPRi
- Stocks were placed in the *Agrobacterium* Deep archives Glycerol Stock Box in the -80 freezer.

Sarah Cumberland Harkamal Samra @Nathaniel Peterson

Transformation into *E. coli*

- Transformation was performed as per "SOP - *E. coli* Transformation" outlined on the experiments page on 2 replicates each of the following plasmids:
  - CLE18 g3 CRISPRi
  - DXR g2 CRISPRi
  - DXR g3 CRISPRi
  - DXR g5 CRISPRi
  - SPCH g2 CRISPRi
  - SPCH g3 CRISPRi
  - SPCH g4 CRISPRi
  - SPCH g5 CRISPRi
  - ICE2 g3 CRISPRi
  - ICE2 g5 CRISPRi

- A total of 22 plates were prepared
    - 20 samples
    - 1 x positive control (CLE18 CRISPRa g1)
    - 1 x cell control (*E. coli* on plain LB)

Danielle Halasz Mike Green @Kartikay

PCR Verification

- Verification was followed as per "**SOP - Polymerase Chain Reaction (PCR) - Colony Crack iGEM**" outlined on the experiments page on 2 replicates of each of the following inserts:
    - ICE2 G4 CRISPRi (patch 1 +2)
    - ICE2 G1 CRISPRi (patch 1 +2)
    - CLE18 G5 CRISPRi (patch 4 + 3)
    - CLE18 G4 CRISPRi (patch 1 + 6)
    - CLE18 G1 CRISPRi (patch 4 + 1)
    - DXR G1 CRISPRi (patch 1 + 2)

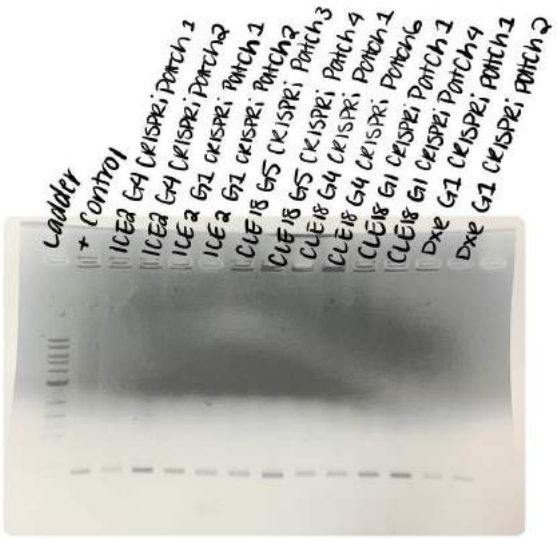
Kulay Janneh Taylor

Agarose gel

- A large gel was prepared following the "SOP- Gel electrophoresis" outlined on the experiments page with the following PCR products:
    - positive control
    - ICE2 G4 CRISPRi patch 1
    - ICE2 G4 CRISPRi patch 2
    - ICE2 G1 CRISPRi patch 1
    - ICE2 G1 CRISPRi patch 2
    - CLE18 G5 CRISPRi patch 3
    - CLE18 G5 CRISPRi patch 4
    - CLE18 G4 CRISPRi patch 1
    - CLE18 G4 CRISPRi patch 6
    - CLE18 G1 CRISPRi patch 1
    - CLE18 G1 CRISPRi patch 4
    - DXR G1 CRISPRi patch 1
    - DXR G1 CRISPRi patch 2

- 10 µL of PCR product was loaded into each well and the gel was run at 103V for 70 minutes

GelJun17.jpg



Kulay Janneh Taylor

Agrobacterium tumefaciens Overnights

- 3mL of LB and kanamycin (3µL) and gentamycin (3µL) were prepared
- The following patches were used to make the overnights
  - ICE2 G4 CRISPRi (duplicates)
  - ICE2 G1 CRISPRi (duplicates)
  - CLE18 G5 CRISPRi (duplicates)
  - CLE18 G4 CRISPRi (duplicates)
  - CLE18 G1 CRISPRi (duplicates)
  - DXR G1 CRISPRi (duplicates)
- Overnights were left shaking in room 4109

FRIDAY, 6/18/2021

Hannah Vujovic Sarah Cumberland

Agrobacterium tumefaciens Glycerol Stocks

- Glycerol stocks of the successful overnight cultures from the previous day were prepared as per "SOP - Glycerol Stock Preparation iGEM" outlined on the experiments page.
- 4 x cryotubes of glycerol stocks were prepared of each of the following *Agrobacterium tumefaciens* cultures:
  - DXR guide 1 CRISPRi
  - ICE2 guide 1 CRISPRi
  - ICE2 guide 4 CRISPRi
  - CLE18 guide 4 CRISPRi
  - CLE18 guide 5 CRISPRi
- 2 x cryotubes of each culture were placed in the *Agrobacterium* Glycerol Stocks Box in the -80 freezer and 2 x cryotubes were placed in the *Agrobacterium* Deep Archives Glycerol Stock Box in the -80 freezer.

Ligation Protocol WITH T4 DNA Ligase (M0202)

Introduction

Please see the NEB website for supporting information on this protocol.

Materials

- 10X T4 DNA Ligase Reaction Buffer
- T4 DNA Ligase
- Vector DNA (4kb)
- Insert DNA (1kb)
- Nuclease-free water

Procedure

Set up the T4 DNA Ligase Reaction

Note: T4 DNA Ligase should be added last. The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. *Use NEB calculator to calculate molar ratios.*

1. Thaw the T4 DNA Ligase Buffer and resuspend at room temperature.

   Tip: Alicuote the 10x buffer less concentrated so when thawing, the DTT gets soluble more easily.

2. Set up the following reaction in a microcentrifuge tube on ice:

Table1

	A	B
1	**Component**	**Volume (µl)**
2	10X T4 DNA Ligase Buffer	2
3	Vector DNA: 50 ng (0.020 pmol)	
4	Insert DNA: 37.5 ng (0.060 pmol)	
5	Nuclease-free water	17
6	T4 DNA Ligase	1
7	Total	20

3. Gently mix the reaction by pipetting up and down and microfuge briefly.

4. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours.

- [x] 5. Heat inactivate at 65 degrees C for 10 minutes.

00:10:00

- [x] 6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

  Use 25 uL DH5α cells, and add 2 uL of reaction mixture

2021 Lab Log (Part 2)

Project: iGEM Guelph 2021
Authors: Sarah Cumberland
Entry Created On: 2021-06-16 09:40:00 PM +0000
Entry Last Modified: 2021-10-18 11:56:05 PM +0000
Export Generated On: 2021-10-19 12:23:19 AM +0000

SUNDAY, 6/20/2021

Namra Hamid

Golden Gate

- Golden gate was performed as per "SOP - Golden Gate Cloning iGEM"
- 2 x replicates of each of the following constructs were prepared:
  - CLE18 CRISPRi guide 3
  - DXR CRISPRi guide 2
  - DXR CRISPRi guide 3
  - DXR CRISPRi guide 5
  - SPCH CRISPRi guide 2
  - SPCH CRISPRi guide 3
  - SPCH CRISPRi guide 4
  - SPCH CRISPRi guide 5
  - ICE2 CRISPRi guide 3
  - ICE2 CRISPRi guide 5

Sarah Cumberland

Overnight Cultures: Agrobacterium & E. coli

- 1 x 5mL culture of each of the following E. coli cultures were set up:
  - CLE18 CRISPRi guide 1
  - DXR CRISPRi guide 1
  - DXR CRISPRi guide 4
  - SPCH CRISPRi guide 1
  - ICE2 CRISPRi guide 1
  - ICE2 CRISPRi guide 2
  - ICE2 CRISPRi guide 4
- Left in incubate in 37C shaking incaubator in room 4224B
- 1 x 5mL culture of each of the following Agrobacterium cultures were set up:
  - CLE18 CRISPRi guide 1
  - ICE2 CRISPRi guide 2
- Left in incubate in 28C shaking incubator in room 4109

Floral Dip 5mL Overnight Cultures

- 1 x 5mL culture of each of the following Agrobacterium cultures were set up to be used for floral dip:
  - SPCH CRISPRa guide 1
  - CLE18 CRISPRa guide 1
  - CLE18 CRISPRi guide 1
  - CLE18 CRISPRi guide 2
  - CLE18 CRISPRi guide 4
  - CLE18 CRISPRi guide 5
  - ICE2 CRISPRi guide 1
  - ICE2 CRISPRi guide 2
  - ICE2 CRISPRi guide 4

    - DXR CRISPRi guide 1
    - DXR CRISPRi guide 4
    - DXR CRISPRa guide 1
    - DXR CRISPRa guide 4
- Left in incubate in 28C shaking incubator in room 4109

MONDAY, 6/21/2021

Hannah Vujovic Amira Bouchema Brittany Alexander @nate

Glycerol Stocks

- Agrobacterium Glycerol stocks were made following "SOP - Glycerol Stock Preparation iGEM" for the following Agrobacterium CRISPRi guides:
    - ICE2 CRISPRi guide 2
    - CLE18 CRISPRi guide 1
- Deep glycerol stocks were made following "SOP - Glycerol Stock Preparation iGEM" for the following E.coli CRISPRi guides:
    - SPCH CRISPRi guide 1
    - CLE18 CRISPRi guide 1
    - DXR CRISPRi guide 1
    - DXR CRISPRi guide 4
    - ICE2 CRISPRi guide 1
    - ICE2 CRISPRi guide 4
    - ICE2 CRISPRi guide 2

Patch Plates

- Patch plates were made from the following plates:
    - DXR CRISPRi guide 2
    - DXR CRISPRi guide 3
    - ICE2 CRISPRi guide 3
    - ICE2 CRISPRi guide 5

E.Coli Transformation

1. Obtained and labelled 11 1.5 mL tubes.
2. Took (insert number here) tubes of competent DH5a cells on ice taken from the -80ºC freezer
3. Pipetted 50 µL of competent cells into the sterile chilled 1.5 mL tubes so each tube has 50µL of competent cells, and Pipetted 11ul (not sure how much it was) of DNA into the competent cells.
4. Incubated cells and DNA in 1.5 mL tubes on ice for 30 minutes.
5. Heat shocked cells at 42ºC in a hot plate or water bath for 45 seconds.
6. Immediately let the cells incubate ice for 5 minutes
7. Added 250 µL LB media into each transformation tube.
8. Incubated cells at 37ºC for 1 hour
9. Warmed up LB kanamycin plates in the 37ºC incubator to reduce condensation and avoid temperature shocking the cells too much.
10. Pipetted all of the reaction mixture onto a single plate for each reaction.
11. Spread the culture using a sterile glass spreader.
12. Incubated transformations overnight at 37ºC agar-side up to avoid condensation.

Samples transformed were:

1. CLE18 CRISPRi G3
2. DXR CRISPRi g2
3. DXR CRISPRi g3
4. DXR CRISPRi g5

5. SPCH CRISPRi g2
6. SPCH CRISPRi g4
7. SPCH CRISPRi g5
8. ICE2 CRISPRi g3
9. ICE2 CRISPRi g5
10. SPCH CRISPRi g3
11. Positive control
12. Negative Control

TUESDAY, 6/22/2021

Hannah Vujovic Sarah Cumberland

Golden Gate

- Golden gate was performed as per "SOP - Golden Gate Cloning iGEM".
- 2 x replicates of each of the following constructs were prepared:
  - CLE18 CRISPRi guide 3
  - DXR CRISPRi guide 3
  - DXR CRISPRi guide 5
  - SPCH CRISPRi guide 2
  - SPCH CRISPRi guide 3
  - SPCH CRISPRi guide 4
  - SPCH CRISPRi guide 5
  - ICE2 CRISPRi guide 3

Patch Plates

- Patch plates were made of the successful tranformations and placed in the 37C incubator:
  - DXR CRISPRi guide 2
  - ICE2 CRISPRi guide 5

WEDNESDAY, 6/23/2021

Amira Bouchema Mike Green Harkamal Samra Kulay Janneh

E.coli Transformation

- Transformation was preformed following the "SOP - E. coli Transformation iGEM" protocol
- The following transformants where plated and left to incubate in the 37 degree incubator:
  - CLE18 CRISPRi guide 3
  - DXR CRISPRi guide 3
  - DXR CRISPRi guide 5
  - SPCH CRISPRi guide 2
  - SPCH CRISPRi guide 3
  - SPCH CRISPRi guide 4 (one replicate was missing)
  - SPCH CRISPRi guide 5
  - ICE2 CRISPRi guide 3
  - Positive control
  - Negative control

Floral dip overnights

- Two 300mL overnights of CLE18 guide 5 CRISPRi and ICE2 guide 1 CRISPRi were made and left shaking in room 4109

gRNA duplex

- Duplexing was preformed for the new guides (CLE18 guide A-E and ICE2 guides A-E).
- Duplexed guides and leftover forward and reverse guides were placed in the old oligo box because the current box is full

THURSDAY, 6/24/2021

Danielle Halasz Brittany Alexander

300mL agrobacterium overnights for floral dip

- Additional 4 mL of culture was added to overnights done on wednesday
- 300mL overnights were made using 5mL of:
  - ICE2 G4 CRISPRi
  - CLE18 G2 CRISPRi
  - ICE2 G2 CRISRPi
  - CLE18 G4 CRISPRi

Danielle Halasz Brittany Alexander Lisa Thuy Duyen Tran

Plant Thinning

- Plants that were planted a week ago were thinned.

FRIDAY, 6/25/2021

Danielle Halasz Dennis Tran Mike Green adrian Gerry Koot

PCR of E.coli

- Followed protocol "Polymerase Chain Reaction (PCR) - Colony Crack iGEM" SOP
  - Positive control: ICE2 G3
  - ICE 2 G3 patch 3 rep 1
  - ICE2 G3 patch 1 rep2
  - ICE2 G5 patch 1
  - ICE2 G5 patch 2
  - DXR G2 rep 1 patch 1
  - DXR G2 rep2 patch 2

Sarah Cumberland Dennis Tran Charlotte Fletcher

Floral Dip

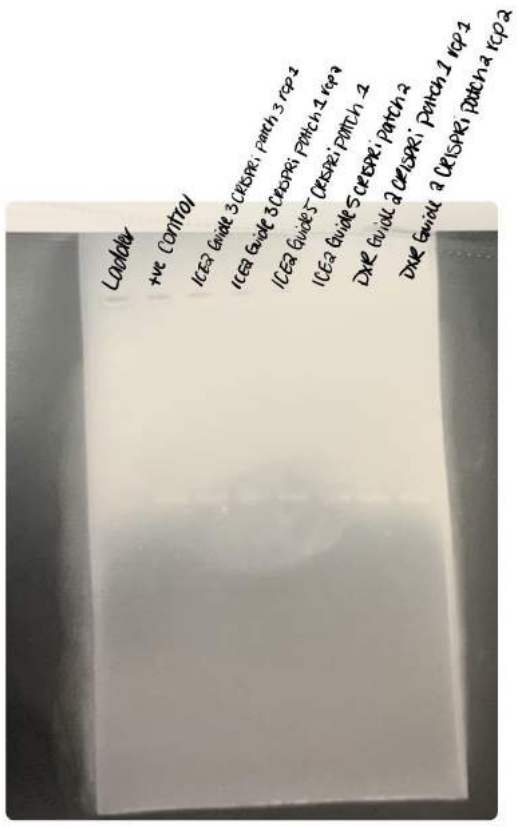
- Day 3 of floral dip protocol was followed as per "Floral Dip (Agrobacterium-mediated transformation of Arabidopsis) iGEM" SOP
  - 2 plants were floral dipped using an Agrobacterium culture of ICE2 guide 1
  - 2 plants were floral dipped using an Agrobacterium culture of CLE18 guide 5
- Plants were left in the darkness under iceboxes for 22 hours at 6:20pm on Friday -> ready to return to phytotron at 4:20pm on Saturday.

Kulay Janneh Dennis Tran Brittany Alexander

Agarose gel on PCR products

- A small gel was made following the "Gel electrophoresis iGEM" SOP
- 10 uL of the following PCR products was added to each gel and run for 103V for 1 hour
  - Positive control
  - ICE2 Guide3 replicate 1 patch 3
  - ICE2 Guide3 replicate 2 patch 1
  - ICE2 Guide 5 patch 1
  - ICE2 Guide 5 patch 2
  - DXR guide 2 replicate 1 patch 1
  - DXR guide 2 replicate 1 patch 2

GelJun 25, 2021.jpg



Kulay Janneh

E.coli overnights

- 7mL overnights (+7uL Kanamycin) of ICE2 CRISPRi guide 3 and 5 as well as DXR CRISRPi guide 2 were prepared and left to incubate in room 3202

SATURDAY, 6/26/2021

Sarah Cumberland

Glycerol Stocks

- Glycerol stocks were prepared as per "SOP - Glycerol Stock Preparation iGEM". 2 x glycerol stocks were prepared of each of the following plasmids in E. coli:
  - ICE2 CRISPRi guide 3
  - ICE2 CRISPRi guide 5
  - DXR CRISPRi guide 2
- 1 x glycerol stock of each sample was stored in the E. coli Glycerol Stocks Box in the -80C freezer and 1 x glycerol stock of each sample was stored in the E. coli Deep Archives box.

Sarah Cumberland Dennis Tran Brittany Alexander

Miniprep

- Miniprep was performed as per "SOP - Plasmid Preparation (MiniPrep) iGEM". The following plasmids were miniprepped:
  - ICE2 CRISPRi guide 3
  - ICE2 CRISPRi guide 5

- DXR CRISPRi guide 2

Namra Hamid Dennis Tran

Agrobacterium Transformation

- Transformation was performed as per "SOP - A. tumefaciens transformation". The following plasmids were transformed into Agrobacterium
  - ICE2 CRISPRi Guide 3
  - ICE2 CRISPRi Guide 5
  - DXR CRISPRi Guide 2

SUNDAY, 6/27/2021

Danielle Halasz

Golden gate

- New guides for CRISPRa backbone were duplexed, concentrations were read and calculated, and dilutions of 100 ng/uL were made for each ICE2 A, B, C, D, E, and CLE18 A, B, C, D, E
- Original duplexes and their dilutions were moved to CRISPRa box, and old CRISPRa guides were moved to past project box
- Followed "SOP - Golden Gate Cloning iGEM protocol", and made doubles of each sample (total of 20 samples)
- A p10 broke after attempting to dispense a pipett tip

MONDAY, 6/28/2021

Danielle Halasz @Samantha Segal Nathaniel Petersen

Transformation of E.coli

- Product of golden gate from yesterday were transformed into E.coli following the "SOP - E. coli Transformation iGEM" protocol
- Total of 20 plates were made

TUESDAY, 6/29/2021

Sarah Cumberland

Golden Gate

- Golden gate was performed on the following constructs as per "SOP - Golden Gate Cloning iGEM protocol"
  - CLE18 CRISPRi g3
  - DXR CRISPRi g3, g5
  - SPCH CRISPRi g2, g3, g4, g5
- 2 replicates were made of each sample

Patch Plates

- Agrobacterium patches were made from the following colonies:
  - ICE2 CRISPRi guide 3
  - ICE2 CRISPRi guide 5
  - DXR CRISPRi guide 2
- E. coli patches were made from the following colonies:
  - ICE2 CRISPRa guide A
  - ICE2 CRISPRa guide B
  - ICE2 CRISPRa guide D
  - ICE2 CRISPRa guide E

5mL Overnight Cultures

- 7 x 5mL overnight cultures were prepared using glycerol stocks of the following plasmids in Agrobacterium:
  - CLE18 CRISPRi guide 1

    - CLE18 CRISPRi guide 2
    - CLE18 CRISPRi guide 4
    - DXR CRISPRi guide 1
    - DXR CRISPRi guide 4
    - ICE2 CRISPRi guide 2
    - ICE2 CRISPRi guide 4
- Cultures were left to incubate at 28C in room 4109.

WEDNESDAY, 6/30/2021

Kulay Janneh Harkamal Samra Nathaniel Petersen

E.coli Transformation

- Pre-transformation prep and transformations were performed following the "SOP-E.coli transformation iGEM" protocol
    - CLE18 CRISPRi guide 3 (Replicates)
    - DXR CRISPRi guide 3 and 5 (Replicates)
    - SPCH CRISPRi guides 2,3,4 and 5 (Replicates)
- A total of 16 plates (14 plus positive and negative controls) were made and left to incubate in the 37° incubator

Kulay Janneh

Agrobacterium Cultures for floral dip

- Three 200mL Agrobacterium cultures were made of the following guides:
    - ICE2 Guide 3 CRISPRi
    - ICE2 Guide 5 CRISPRi
    - CLE18 Guide 5 CRISPRi
- Colonies were taken from Agrobacterium patch plates
- Cultures were left to incubate in room 4109

THURSDAY, 7/1/2021

Kulay Janneh Dennis Tran

Patch plates

- Patch plates where made from the following guides:
    - DXR CRISPRi guide 5
    - SPCH CRISPRi guide 2, 3, 4 and 5
    - CLE18 CRISPRi guide 3

Sarah Cumberland Namra Hamid Dennis Tran

Floral Dip

- 2 plants were floral dipped using each of the following constructs as per "SOP - Floral Dip (Agrobacterium-mediated transformation of Arabidopsis) iGEM".
    - ICE2 Guide 3 CRISPRi
    - ICE2 Guide 5 CRISPRi
    - CLE18 Guide 5 CRISPRi
- Plants were left in darkness at 7pm for 24hrs.

Sarah Cumberland

Agrobacterium Cultures for floral dip

- 3 200mL cultures were made of the following guides:
    - CLE18 Guide 1 CRISPRi
    - CLE18 Guide 2 CRISPRi
    - CLE18 Guide 4 CRISPRi

- Colonies were taken from Agrobacterium patch plates
- Cultures were left to incubate in room 4109

FRIDAY, 7/2/2021

Hannah Vujovic

PCR verification

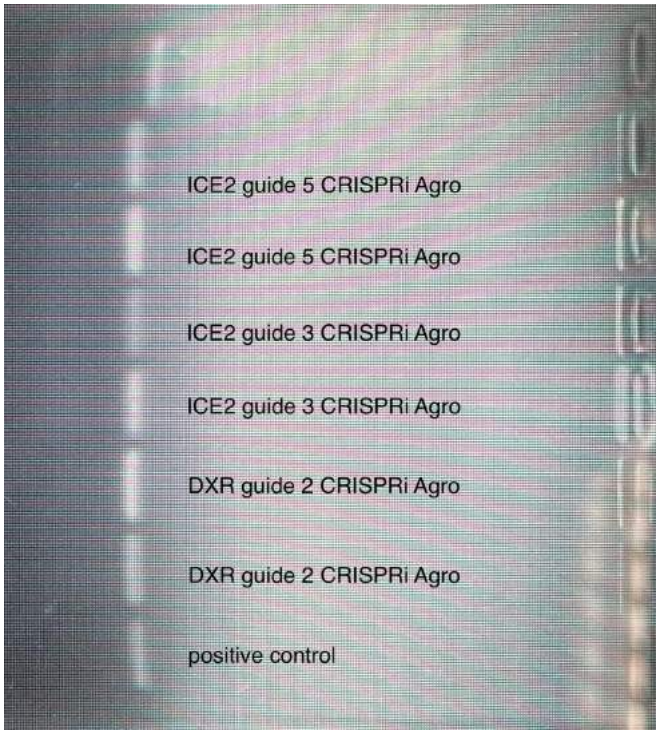
- "Polymerase Chain Reaction (PCR) - Colony Crack iGEM" SOP was followed for the following guides, all were done in duplicates:
- Agrobacterium CRISPRi
  - ICE2 guides 3/5
  - DXR guide 2
- E.coli CRISPRa
  - ICE2 guides A/B/D/E

Sarah Cumberland Hannah Vujovic

Gel Electrophoresis

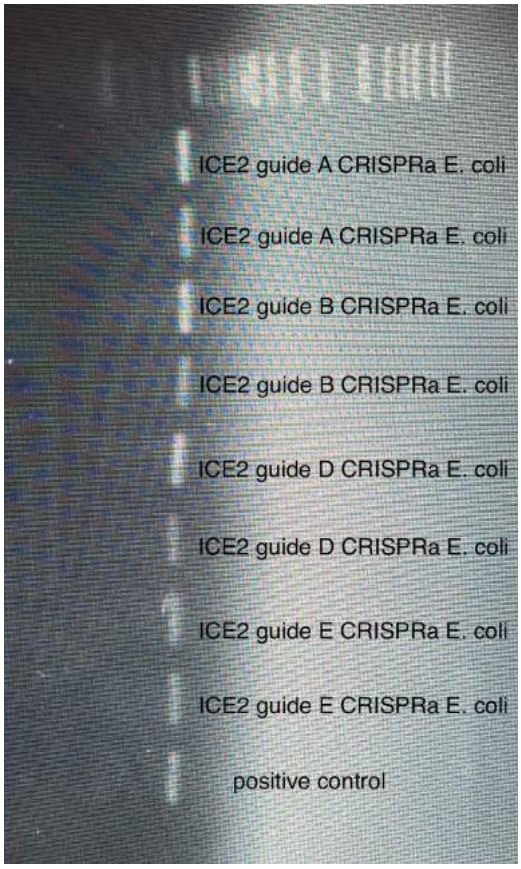
- 2 gels were run as per "SOP - Gel Electrophoresis iGEM".
  - Agrobacterium gel:

IMG_2104.jpg



  - E. coli gel:

e coli.jpg



7mL Overnight Cultures

- 7mL overnight cultures were prepared of the following constructs for glycerol stocks & minipreps:
  - ICE2 guide 5 CRISPRi Agrobacterium
  - ICE2 guide 3 CRISPRi Agrobacterium
  - DXR guide 2 CRISPRi Agrobacterium
  - ICE2 guide A CRISPRa E. coli
  - ICE2 guide B CRISPRa E. coli
  - ICE2 guide D CRISPRa E. coli
  - ICE2 guide E CRISPRa E. coli

SATURDAY, 7/3/2021

Hannah Vujovic Namra Hamid @samantha segal

Miniprep

SOP was followed for the miniprep of ICE2 guides A/B/D/E CRISPRa in e. coli

Glycerol stocks

SOP for creating glycerol stocks was followed. Regular and deep glycerol stocks were made for ICE2 guides A/B/D/E CRISPRa in e. coli.

Kulay Janneh Harkamal Samra

Agrobacterium Transformation

- Duplicate transformations were made for ICE2 guides A,B,D and E following the A.tumefaciens SOP.

SUNDAY, 7/4/2021

Hannah Vujovic Amira Bouchema

Glycerol stocks

- Protocol "SOP - Glycerol Stock Preparation iGEM" was followed. Regular and deep glycerol stocks were made for ICE2 guides 3/5 and DXR guide 2 CRISPRi in agrobacterium.

Golden Gate

- "SOP - Golden Gate iGEM" protocol was followed for reactions on the following CRISPRa guides:
  - ICE2 guide C
  - CLE18 guides A/B/C/D/E

MONDAY, 7/5/2021

Sarah Cumberland Kulay Janneh Mike Green Nathaniel Petersen

E.coli Transformation

- "SOP - E. Coli Transformation iGEM" was followed for the transformation of ICE2 guide C (in duplicate), and CLE18 guides A/B/C/D/E into E. coli

TUESDAY, 7/6/2021

Hannah Vujovic Sarah Cumberland

Golden Gate

"SOP - Golden Gate iGEM" was followed for the following guides:

- CLE18 guide 3 CRISPRi
- DXR guides 3 and 5 CRISPRi
- SPCH guides 2/3/4/5 CRISPRi
- CLE18 guides A/B/C/D/E/Multiplex CRISPRa

Patch plates

- Patch plates were made of Agrobacterium ICE2 guides A/E/D CRISPRa

WEDNESDAY, 7/7/2021

Hannah Vujovic Kulay Janneh Charlotte Fletcher

E.coli Transformation

- "SOP - E. Coli Transformation iGEM" was followed to transform E.coli with the following constructs:
  - CLE18 guide 3 CRISPRi
  - DXR guides 3/5 CRISPRi
  - SPCH guides 2/3/4/5 CRISPRi
  - CLE18 guides A/B/C/D/E/Multiplex CRISPRa
- Duplicates were made for each construct

Hannah Vujovic Kulay Janneh

Patch Plates

- Patch plates of Agrobacterium ICE2 guide B and ICE2 guide C CRISPRa (E.coli) was made

Kulay Janneh

Floral Dip Cultures

- 3 300mL cultures of CLE18 guide 1, 2, and 4 were prepared and left to incubate in room 4109

THURSDAY, 7/8/2021

Hannah Vujovic Anum Anjum Isha adrian

Patch plates

Patch plate was made for CLE18 guide C CRISPRa e. coli

Sarah Cumberland

Floral Dip

- The following plasmids in Agrobacterium were floral dipped into Arabidopsis thaliana as per "SOP - Floral Dip (Agrobacterium-mediated transformation of Arabidopsis) iGEM".
    - ICE2 CRISPRa guide A
    - ICE2 CRISPRa guide D
- 2 plants were dipped into each of the above cultures and left to sit in darkness for 24 hours.

Golden Gate

- Golden gate was performed as per "SOP - Golden Gate Cloning iGEM".
- 2 x replicates of each of the following constructs were prepared:
    - CLE18 CRISPRa guide A
    - CLE18 CRISPRa guide B
    - CLE18 CRISPRa guide D
    - CLE18 CRISPRa guide E
    - CLE18 CRISPRa multiplex
    - ICE2 CRISPRa multiplex

FRIDAY, 7/9/2021

Dennis Tran Mike Green Danielle Halasz

PCR

- The following plasmids were PCR'd for the following Guide RNAs according to the "Polymerase Chain Reaction (PCR) - Colony Crack iGEM" SOP:
    - E. coli:
        - CLE18 Guide C CRISPRa (x3)
        - CLE18 Guide C CRISPRa Rep 2
        - ICE2 Guide C CRISPRa (x3)
    - Agrobacterium:
        - ICE2 Guide B (x2)
        - ICE2 Guide D2 (x2)
        - ICE2 Guide E2 (x2)
        - ICE2 Guide A (x2)

E. coli Transformation

Nathaniel Petersen

- E.coli transformation SOP was followed to transform E.coli with the following constructs:
- Duplicates were made for each construct
    - CLE18 CRISPRa gA (Both using Homemade SOC)
    - CLE18 CRISPRa gB (Both using Homemade SOC)
    - CLE18 CRISPRa gD (Both using Homemade SOC, one with Dr. Seah's competent cells)

- CLE18 CRISPRa gE (One with Homemade SOC and Dr. Seah's competent cells, One with Industry SOC and iGEM's typical cells)
- CLE18 CRISPRa multiplex (Both using Homemade SOC)
- ICE2 CRISPRa multiplex (Both using Homemade SOC, one with Dr. Seah's competent cells)

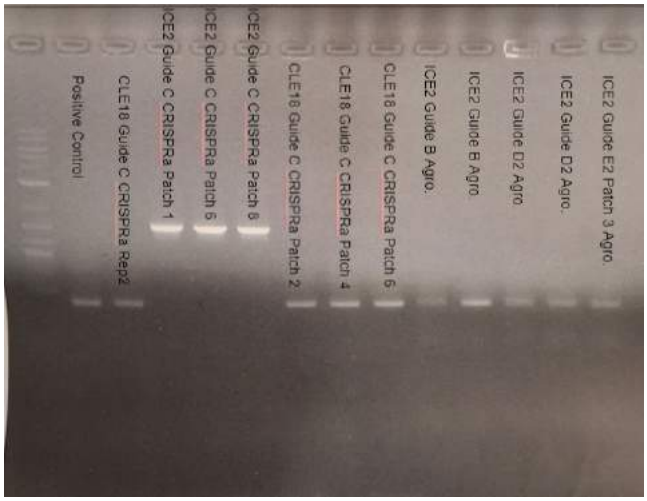
Gel Electrophoresis

Mike Green Amira Bouchema Dennis Tran

- Gel was run according to the SOP on the plasmids above.

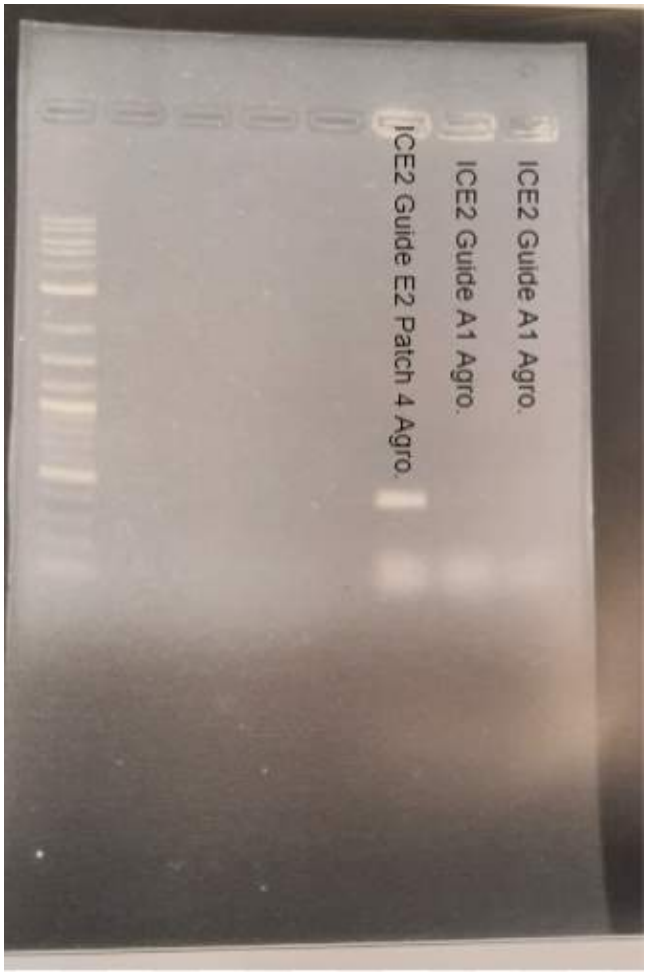
Gel 1: Colonies with ICE2 Guide C CRISPRa failed.

image.png



Gel 2: All Fail.

image.png



SATURDAY, 7/10/2021

Namra Hamid

Glycerol Stocks

Glycerol stocks were prepared as per "SOP - Glycerol Stock Preparation iGEM". 2 x glycerol stocks were prepared of each of the following plasmids in E. coli:

- CLE 18 CRISPR a Guide C

Glycerol stocks were prepared as per "SOP - Glycerol Stock Preparation iGEM". 2 x glycerol stocks were prepared of each of the following plasmids in Agrobacterium:

- ICE 2 CRISPR a Guide B
- ICE 2 CRISPR a Guide D2
- ICE 2 CRISPR a Guide A

Miniprep

SOP was followed for the miniprep of ICE2 guide B CRISPRa in Argo and CLE18 guide C in E.coli.

Namra Hamid Danielle Halasz

Agrobacterium Transformation

Transformations were made for CRISPR a ICE2 guide E and CLE 18 guide C following the A.tumefaciens Transformation SOP.

SUNDAY, 7/11/2021

Danielle Halasz

Golden Gate Cloning

Floral Dipping followed as per SOP - Floral Dip (Agrobacterium-mediated transformation of Arabidopsis) iGEM

- 2 plants were floral dipped, only overnight for ICE2 guide B CRISPRa was used.
    - Overnight was made directly from culture. was allowed to grow for nearly 48 hours.
- Final OD for 200mL culture was 0.46, was pelleted and then resuspended in 115 mLof infiltration media in order to reach final OD of 0.8.

MONDAY, 7/12/2021

Amira Bouchema

E. coli transformation

- E.coli transformation SOP was followed to transform E.coli with the following constructs:
    - CLE18 guide C CRISPRa
    - ICE2 guide C CRISPRa
    - Duplicates were made

TUESDAY, 7/13/2021

Danielle Halasz

- 8 tubes of gentamycin 25 mg/mL made
- 2L LB made, previous one was contaminated
- made three 200 mL overnights of CRISPRa ICE2 guide E for floral dip, used rep 1 patch 1 and 2, and rep 2 patch 1
- planted a tray of arabidopsis

WEDNESDAY, 7/14/2021

Amira Bouchema Kulay Janneh Gerry Koot Dennis Tran

- Overnights did not grow
- Autoclave was done
- Plants were watered

300mL Agrobacterium Overnight

1. CLE18 CRISPRa Guide C (x3)
2. ICE2 CRISPR a Guide E (x3)

PCR

PCR was done following "SOP - PCR iGEM" for the following patches:

1. CLE18 guide C CRISPRa E.coli Rep 2
2. CLE18 guide C CRISPRa E.coli (1)
3. CLE18 guide C CRISPRa E.coli (2)
4. CLE18 guide C CRISPRa E.coli (3)
5. CLE18 guide C CRISPRa E.coli (4)
6. CLE18 guide C CRISPRa E.coli (5)
7. CLE18 guide C CRISPRa E.coli (6)
8. ICE2 guide C CRISPRa E.coli (1)
9. ICE2 guide C CRISPRa E.coli (2)
10. ICE2 guide C CRISPRa E.coli (3)
11. ICE2 guide C CRISPRa E.coli (4)

12. ICE2 guide C CRISPRa E.coli (5)
13. ICE2 guide C CRISPRa E.coli (6)
14. ICE2 guide C CRISPRa E.coli (7)
15. ICE2 guide C CRISPRa E.coli (8)
16. ICE2 guide B CRISPRa Agrobacterium (1)
17. ICE2 guide B CRISPRa Agrobacterium (2)
18. ICE2 guide B CRISPRa Agrobacterium (3)
19. ICE2 guide B CRISPRa Agrobacterium (4)
20. ICE2 guide B CRISPRa Agrobacterium (5)
21. ICE2 guide B CRISPRa Agrobacterium (6)
22. ICE2 guide B CRISPRa Agrobacterium (7)
23. ICE2 guide B CRISPRa Agrobacterium (8)
24. ICE2 guide B CRISPRa Agrobacterium (9)
25. ICE2 guide B CRISPRa Agrobacterium (10)

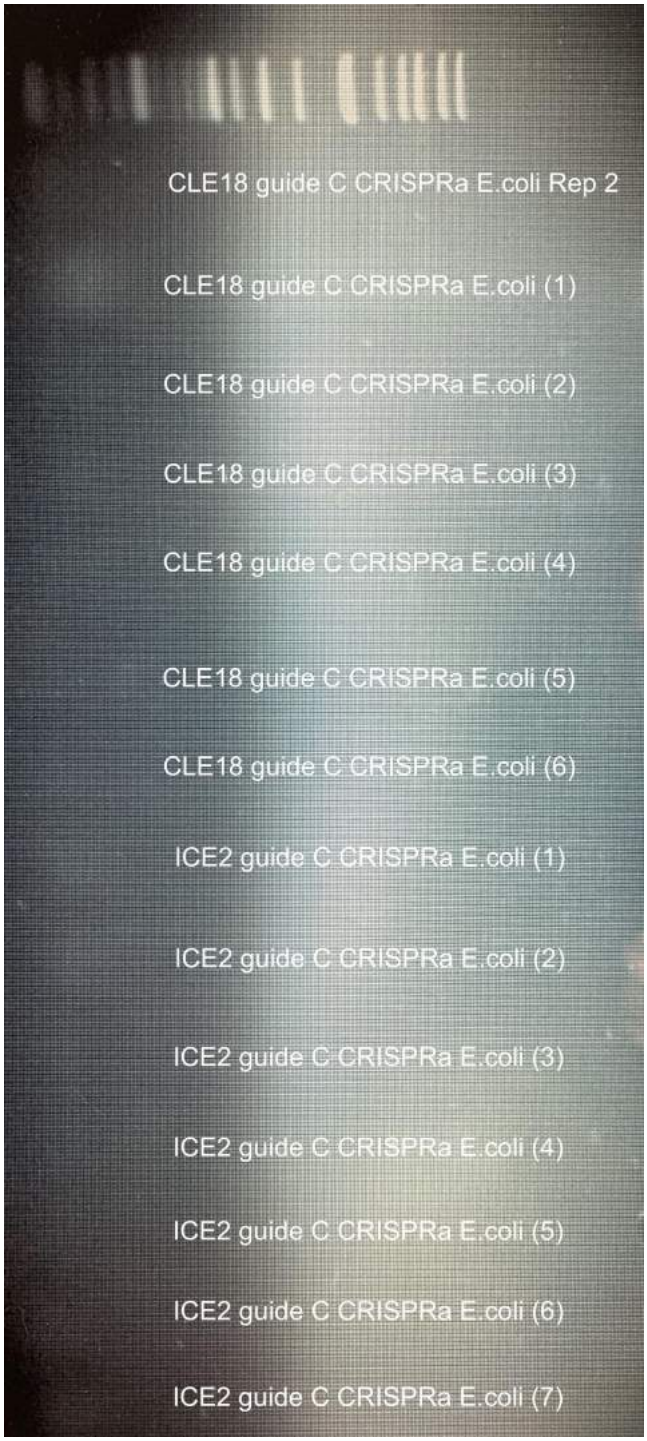
THURSDAY, 7/15/2021

Sarah Cumberland

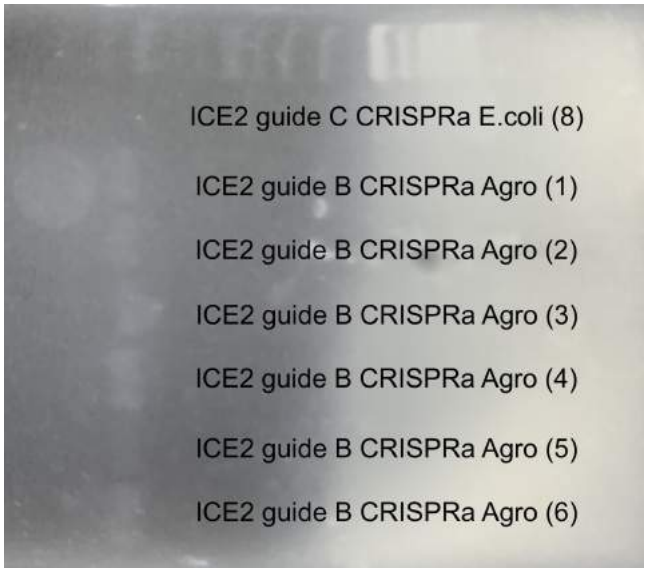
Agarose Gels

- 3 agarose gels were prepared as per the protocol.
- Note: all Agrobacterium colonies showed bands at ~500bp, E. coli showed some faint and smeared bands at a lower size than expected.

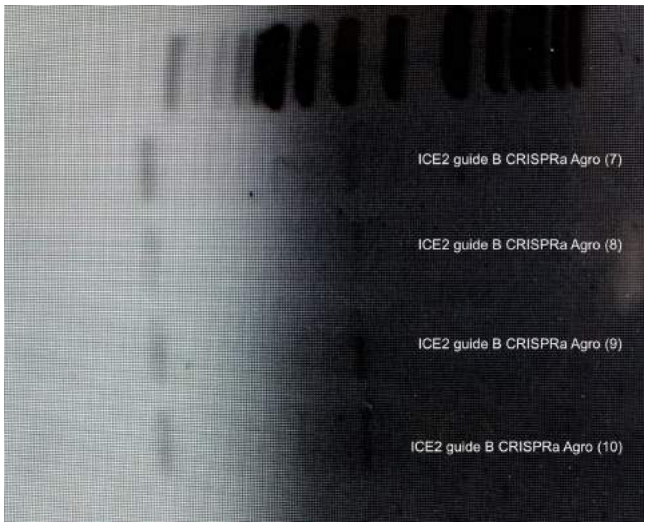
IMG_4687.jpg


CLE18 guide C CRISPRa E.coli Rep 2
CLE18 guide C CRISPRa E.coli (1)
CLE18 guide C CRISPRa E.coli (2)
CLE18 guide C CRISPRa E.coli (3)
CLE18 guide C CRISPRa E.coli (4)
CLE18 guide C CRISPRa E.coli (5)
CLE18 guide C CRISPRa E.coli (6)
ICE2 guide C CRISPRa E.coli (1)
ICE2 guide C CRISPRa E.coli (2)
ICE2 guide C CRISPRa E.coli (3)
ICE2 guide C CRISPRa E.coli (4)
ICE2 guide C CRISPRa E.coli (5)
ICE2 guide C CRISPRa E.coli (6)
ICE2 guide C CRISPRa E.coli (7)

IMG_4689.jpg



IMG_4686.jpg



Sarah Cumberland Hannah Vujovic Brittany Alexander

Floral Dip

- Floral dip was performed into Arabidopsis thaliana as per protocol on the following constructs:
  - CLE18 guide C CRISPRa (agrobacterium)
  - ICE2 guide E CRISPRa (agrobacterium)
- 2 pots were transformed with each plasmid

Overnight Cultures

- Overnight cultures were prepared for the following plasmids:
  - ICE2 guide B CRISPRa (Agrobacterium)
  - ICE2 guide C CRISPRa (E. coli)
  - pHSN6A01 (E. coli)
  - pSiM-24 GUS (E. coli)
- 2 replicates of each culture were prepared

FRIDAY, 7/16/2021

Sarah Cumberland Dennis Tran Sofia Finley Brittany Alexander

Miniprep

- Miniprep was performed on the following plasmids as per protocol:
  - ICE2 guide C CRISPRa (E. coli)
  - pHSN6A01 (E. coli)
  - pSiM-24 GUS (E. coli)

Sarah Cumberland

Glycerol Stocks

- 2 x glycerol stocks were prepared of each of the following plasmids as per protocol:
  - ICE2 guide C CRISPRa (E. coli)
  - pHSN6A01 (E. coli)
  - pSiM-24 GUS (E. coli)

Kulay Janneh Dennis Tran

Agrobacterium Transformation

- Duplicate transformations of ICE2 Guide C CRISPRa, pSiM-24 GUS replicate 1 and replicate 2 was prepared following the A.tumefaciens Transformation SOP
- Plants floral dipped yesterday were put back in the growth chamber, no one was in the phytotron to get a string but they are at the very back on the transformants tray

TUESDAY, 7/20/2021

Sarah Cumberland Enzo Baracuhy

Golden Gate

- Golden Gate NEB Kit Protocol was followed (as per SOP).
- The following changes were made to the protocol:
  - Initial 37C step for 5 minutes was added
  - 37C step was changed to 3 minutes
  - 16C step was kept as is
  - Cycle was repeated 80 times not 99 times
  - 4C hold step was kept as is
- Plasmid was at a concentration of 100ng/uL therefore 100ng were used
- Single guide inserts were at a concentration of 100ng/uL therefore 100ng were used
- Multiplexing inserts were at a concentration of 20ng/uL each therefore 100ng were used
- The following constructs were assembled:
  - CLE18 CRIPSRa guide A
  - CLE18 CRIPSRa guide B
  - CLE18 CRIPSRa guide D
  - CLE18 CRIPSRa guide E
  - ICE2 CRISPRa guide C
  - CLE18 multiplex
  - ICE2 multiplex

WEDNESDAY, 7/21/2021

Sarah Cumberland Hannah Vujovic Brittany Alexander Charlotte Fletcher @samantha

Patch Plates

- Patch plates were made of ICE2 Guide C and pSIM-24 Gus, and left in the 28C incubator

Transformation

- The following constructs were transformed into E. coli as per the protocol:
  - CLE18 CRIPSRa guide A (Invitrogen competent cells)
  - CLE18 CRIPSRa guide B (Invitrogen competent cells)
  - CLE18 CRIPSRa guide D (Invitrogen competent cells)
  - CLE18 CRIPSRa guide E (NEB competent cells)
  - ICE2 CRISPRa guide C (NEB competent cells)
  - CLE18 multiplex (NEB competent cells)
  - ICE2 multiplex (NEB competent cells)

THURSDAY, 7/22/2021

Danielle Halasz

Golden Gate

- Followed old protocol (10uL) and NEB protocal (20uL) as duplicates
- The following constructs were assembled:
  - CLE18 CRIPSRa guide A
  - CLE18 CRIPSRa guide B
  - CLE18 CRIPSRa guide D
  - CLE18 CRIPSRa guide E

Patch plates were made of following:

- ICE2 CRISPRa guide C
- CLE18 multiplex
- ICE2 multiplex

FRIDAY, 7/23/2021

Nathaniel Petersen Dennis Tran Namra Hamid

Transformation

- The following constructs were transformed into E. coli as per the E. Coli Transformation SOP protocol
  - CLE18 CRIPSRa guide A x2 (One old protocol, one NEB)
  - CLE18 CRIPSRa guide B x2 (One old protocol, one NEB)
  - CLE18 CRIPSRa guide D x2 (One old protocol, one NEB)
  - CLE18 CRIPSRa guide E x2 (One old protocol, one NEB)

SUNDAY, 7/25/2021

Sarah Cumberland Hannah Vujovic Dennis Tran Emma Lee

Floral Dip

- ICE2 CRISPRa Guide C (in Agrobacterium) was floral dipped into Arabidopsis thaliana as per the Floral Dip SOP protocol

Kulay Janneh Amira Bouchema

Miniprep

- The following constructs were mini-prepped following the Miniprep SOP protocol
  - CLE18 multiplex replicate 1 E.coli
  - ICE2 Guide C E.coli

    - ICE2 Guide C Agrobacterium
- The following constructs were mini-prepped following the protocol in the NEB kit
    - CLE18 multiplex replicate 2-4 E.coli
    - ICE2 multiplex replicate 1-4 E.coli
- Mini-prepped constructs were placed in plasmid box

Kulay Janneh Amira Bouchema

Glycerol stocks

- Glycerol stocks and deep archives of the following constructs were made following the Glyceral Stock SOP protocol
    - ICE2 Multiplex replicates 1-4 E.coli
    - CLE18 Multiplex replicates 1-4 E.coli
    - ICE2 Guide C E.coli
    - ICE2 Guide C Agrobacterium

Kulay Janneh

Agrobacterium Transformation

- Duplicates of the following constructs were made following the A. tumefaciens protocol
    - CLE18 multiplex
    - ICE2 multiplex
    - ICE2 Guide C from E.coli
    - ICE2 Guide C from Agrobacterium

MONDAY, 7/26/2021

Sarah Cumberland

Restriction Digest

- The following plasmids were digested using the following enzymes:
    - pSIM-24 GUS -> EcoRI & HindIII
    - pHSN6A01 (CRISPRa plasmid) -> SphI & NotI
- Reaction was set up as follows:

Table1

	A	B
1	Plamid	2ug
2	10X CutSmart Buffer	3uL
3	Enzyme 1	1uL
4	Enzyme 2	1uL
5	Molecular Grade H2O	To a total volume of 30uL

- Thermocycler was run was follows:
    - Digest at 37C for 3.5 hours
    - Heat inactivate at 80C for 20 minutes
    - Hold at 4C

PCR Amplification

- The AlcR ethanol inducible promoter (inside a plasmid from IDT) was PCR amplified out of the plasmid from IDT.
- Reaction was set up as follows:

Table2

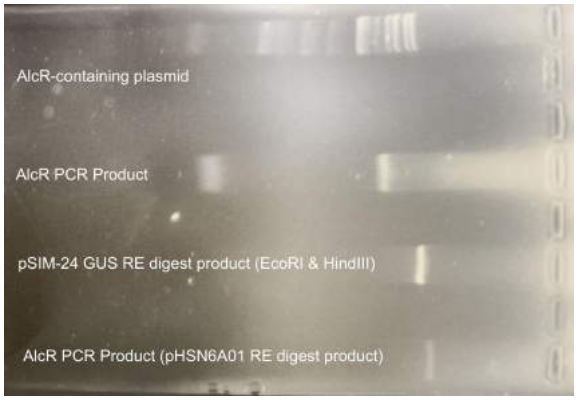
	A	B
1	Q5 High-Fidelity 2X Master Mix	12.5 µl
2	10 µM Forward Primer	1.25 µl
3	10 µM Reverse Primer	1.25 µl
4	Template DNA (AlcR-containing plasmid)	10ng (1uL)
5	Molecular Grade H2O	To a total volume of 25uL (9uL)

- Thermocycler was run as follows:
  - Initial Denaturation at 98°C for 30 seconds
  - Denaturation at 98°C for 10 seconds
  - Annealing at 58C for 30 seconds
  - Elongation at 72°C for 2 minutes (30 seconds/kb)
  - Repeat the previous 3 steps 30 times
  - Final extension at 72°C for 2 minutes
  - Hold at 4°C

Gel Electrophoresis

- A gel was run on the digested pSIM-24 GUS product, the digested pHSN6A01 product, the AlcR plasmid, and the AlcrR PCR product.
- The wells were filled as follows:
  - 1uL (10ng) of AlcR-containing plasmid + 2uL of purple loading dye (6x)
  - 25uL of AlcR PCR Product + 5uL of purple loading dye (6x)
  - 30uL of pSIM-24 GUS RE digest product + 6uL of purple loading dye (6x)
  - 30uL of pHSN6A01 RE digest product + 6uL of purple loading dye (6x)

IMG_4892.jpg



DNA Extraction from Gel

- Gel extraction was perfomed as per the following protocol: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012661_GeneJET_Gel_Extraction_UG.pdf
- Proctol was performed as follows:
  - Gel collection tubes were pre-weighed
  - Gel fragments were excised and the weight of the fragments was determined
  - 1uL of Binding Buffer was added to each tube for every 1mg of agarose gel
  - Mixture was incubated at 60C for 10 minutes until gel was fully dissolved. Tube was inverted every few minutes. Tube was briefely vortex.
  - 1 gel volume's worth of H2O was added to the dissolved pHSN6A01 mixture because the fragment is >10kb long
  - Mixture was added to column and centrifuged for 1 min at 12 000 rpm (repeating this step until all of mixture has been passed through column)
  - Column was washed with 700uL of wash buffer and centrifuged for 1 min
  - Column was centrifuged again (w/no addition of buffer)
  - Elution buffer was heated to 65C
  - 50uL elution buffer was added to each tube and flow through was collected in clean eppendorf tubes
- Yields:
  - AlcR: 14ng/uL
  - GUS: 14.1ng/uL
  - CRISPRa: 16.3ng/uL

TUESDAY, 7/27/2021

Danielle Halasz Lisa Thuy Duyen Tran Sofia Finley

Agrobacterium Transformation

Agrobacterium transformation following A. tumefaciens Transformation SOP protocol for duplicates of:

- ICE2 multiplex
- CLE18 multiplex
- ICE2 guide C

Kulay Janneh

Sterilization of Seeds

- 1 eppendorf of seeds was sterilized according to the Seed Sterilization SOP and yielded a total of 14 plates
- 3 of the 14 plates were MS + Hygromycin
- 11 of the 14 plates were just MS

- Plates were parafilmed and left in the tissue culture room in the phytotron

Collection of seeds

- WT seeds were collected in labelled eppendorfs and placed in the " WT arbadiphosis seeds" box in the fridge

WEDNESDAY, 7/28/2021

Sarah Cumberland

Infusion Reaction

- Infusion reaction was carried out as follows:

Table3

	A	B
1	5X In-Fusion HD Enzyme Premix	2 ul
2	Linearized vector	50ng
3	Purified PCR fragment	50ng
4	H2O	Up to 10uL
5	Total Volume	10 ul

- Thermocycler was set up as follows:
  - Ligation at 50° C for 15 min
  - Hold at 4°C

Restriction Digest

- The following plasmids were digested using the following enzymes:
  - pSIM-24 GUS -> EcoRI & HindIII
  - pHSN6A01 (CRISPRa plasmid) -> SphI & NotI
- Reaction was set up as follows:

Table4

	A	B
1	Plasmid	2ug CRISPRa; 900ug GUS
2	10X CutSmart Buffer	3uL
3	Enzyme 1	1uL
4	Enzyme 2	1uL
5	Molecular Grade H2O	To a total volume of 30uL

- Thermocycler was run was follows:
    - Digest at 37C for 12 hours
    - Heat inactivated at 80C for 20 minutes
    - Hold at 12C

Amira Bouchema Mike Green Emma Lee

PCR Verification

- PCR verification was done as per FroggaBio protocol
- The following guides were PCR'd from E. coli colonies:
    - 1-4: NEB CLE18 GB CRISPRa E.coli
    - 5-8: CLE18 GD CRISPRa E.coli
    - 9-12: NEB CLE18 GE CRISPRa E.coli
    - 13-16: CLE18 GA CRISPRa E.coli
    - 17-20: CLE18 GB CRISPRa E.coli
    - 21-24: CLE18 GE CRISPRa E.coli
    - 25: NEB CLE18 GA CRISPRa E.coli
    - 26: NEB CLE18 GD CRISPRa E.coli
    - 27: ICE2 GC CRISPRa Agrobacterium
    - CLE18 CRISPRa guide A
    - CLE18 CRISPRa guide B
    - CLE18 CRISPRa guide D
    - CLE18 CRISPRa guide E
    - ICE2 CRISPRa guide C

Amira Bouchema Gerry Koot

Gel Electrophoresis

- Gel electrophoresis was run on the samples set up in the PCR above.
- All CLE18 constructs had at least one positive transformant replicate. ICE2 CRISPRa guide C did not have a positive transformant

image.png

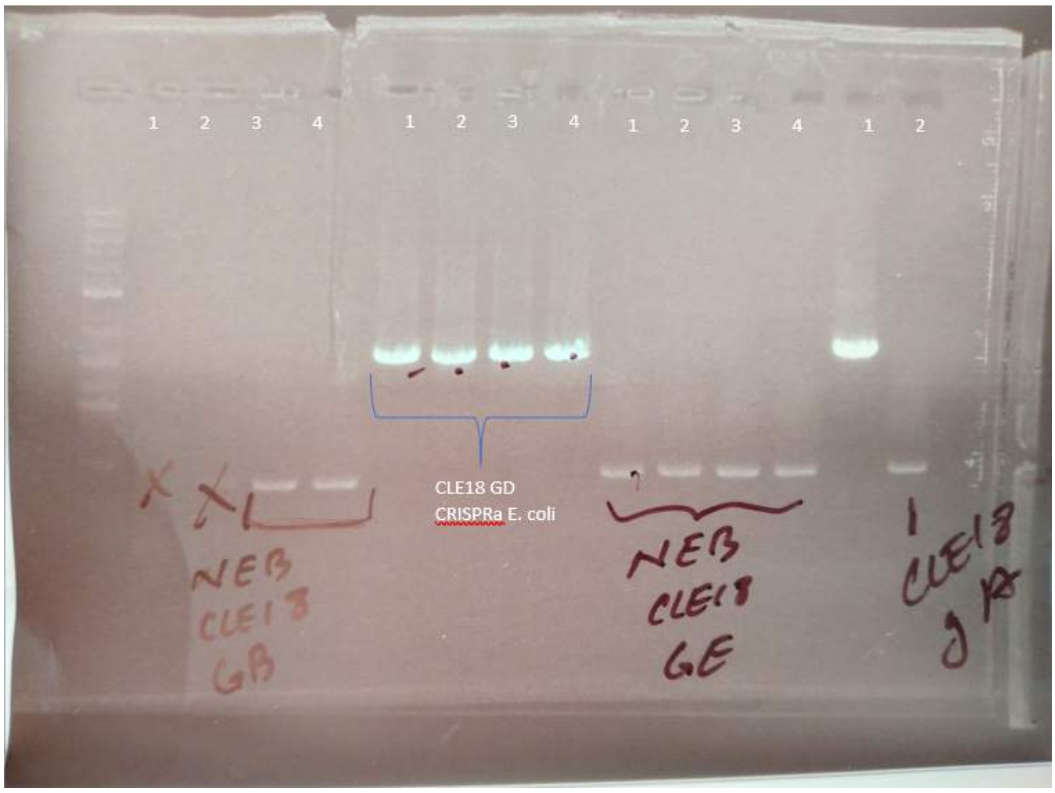
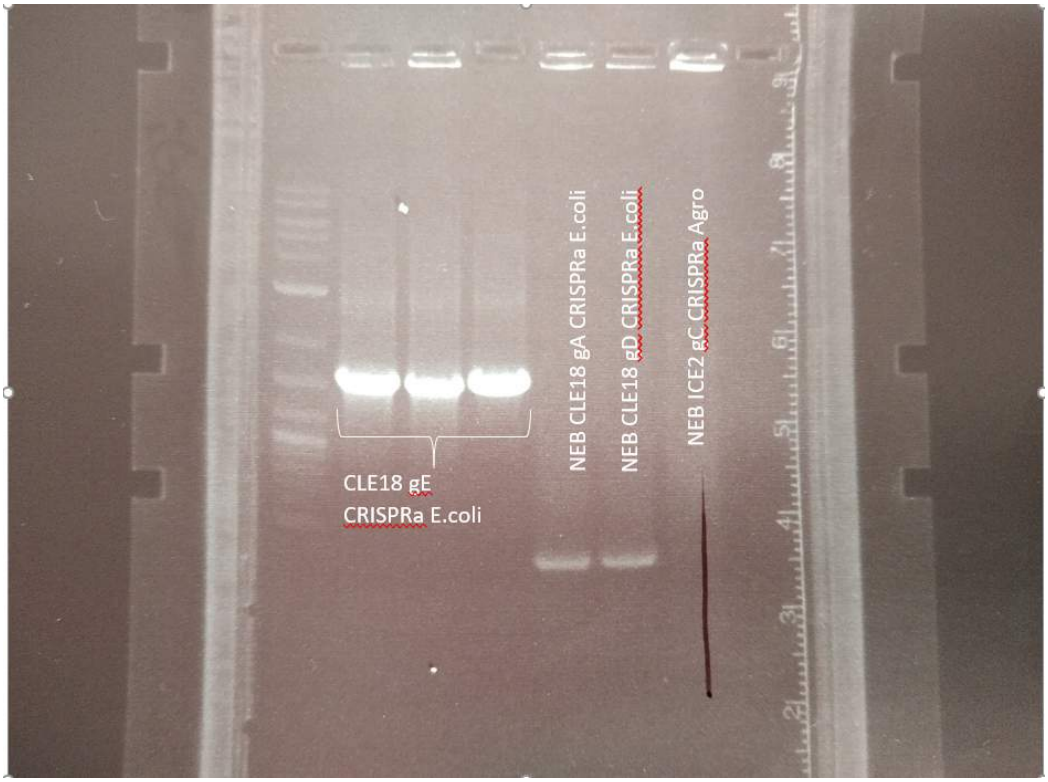

1 2 3 4 1 2 3 4 1 2 3 4 1 2
CLE18 GD
CRISPRa E. coli
NEB
CLE18
GB
NEB
CLE18
GE
1
CLE18
GB

image.png



Overnights

- NEB CLE18 Guide B CRISPRa E.coli Patch 3
- NEB CLE18 Guide E CRISPRa E.coli Patch 11
- CLE18 Guide A CRISPRa E.coli Patch 16
- NEB CLE18 Guide D CRISPRa E.coli Patch 26
- GUS E.coli
- GUS Agrobacterium
- CRISPRa Backbone

THURSDAY, 7/29/2021

Hannah Vujovic Dennis Tran Anum Anjum

Miniprep

- The SOP was followed with adjustments made using the Monarch reagents for CLE18 guides A/B/D/E CRISPRa and the GUS plasmid

Glycerol Stocks

- Made following SOP for CLE18 guides A/B/D/E CRISPRa and the GUS plasmid in E. coli

300mL Overnights

- Made from patch plate colony for the GUS plasmid and ICE2 guides D/E/A CRISPRa

Sarah Cumberland

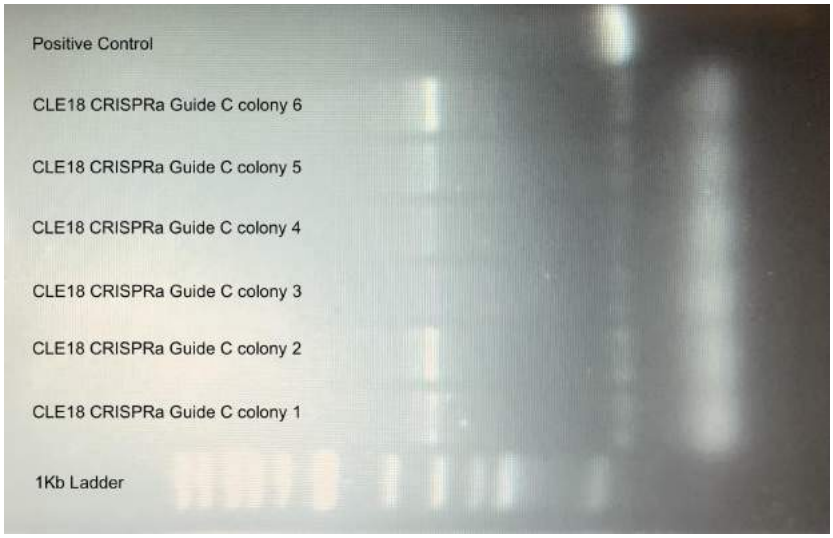
PCR

- PCR was run on 6 additional colonies of ICE2 CRISPRa Guide C as per FroggaBio protocol

Gel Electrophoresis

- Gel was run on PCR products described above as per protocol

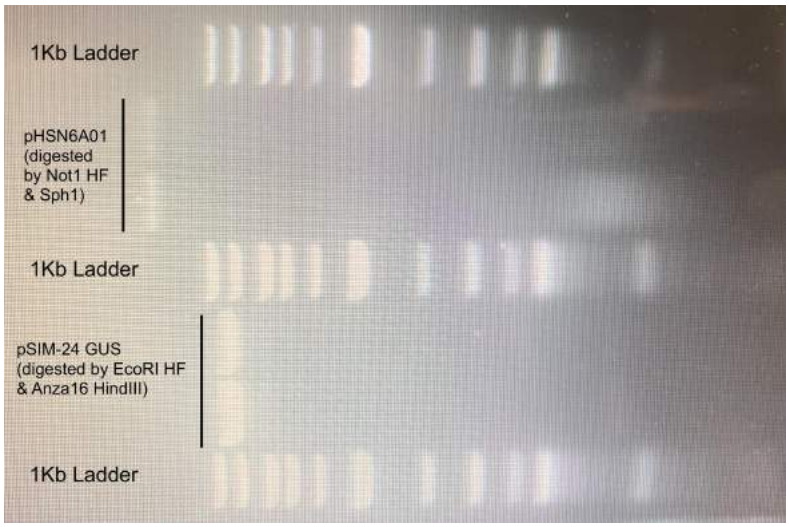
IMG_4944.jpg



Gel Electrophoresis on Digest Products

- Gel was run on the products of the restriction digest done yesterday (GUS plasmid and CRISPRa plasmid) as per protocol

IMG_4940.jpg



Emma Lee Danielle Halasz @Tiffany Mike Green

LB Aliquoting

LB was aliquoted into 50mL aliquots

Pipette Tip Racking

Pipette tips were racked for the p1000 and the p10 tips, and were set for autoclaving

MilliQ Water Aliquoting

MilliQ water was aliquoted into 2mL aliquots

FRIDAY, 7/30/2021

Hannah Vujovic

Agrobacterium Transformation

- SOP for A. tumefaciens transformation was followed for ICE2 mutliplex/guide C and CLE18 multiplex/guides A/B/D/E CRISPRa

Patch Plates

- Patch plate was made for agrobacterium transformations of ICE2 multiplex and guide C as well as for CLE18 multiplex

Sarah Cumberland

DNA Extraction from Gel

- Gel extraction was perfomed as per the following protocol: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012661_GeneJET_Gel_Extraction_UG.pdf
- Proctol was performed as follows:
    - Gel collection tubes were pre-weighed
    - Gel fragments were excised and the weight of the fragments was determined
    - 1uL of Binding Buffer was added to each tube for every 1mg of agarose gel
    - Mixture was incubated at 60C for 10 minutes until gel was fully dissolved. Tube was inverted every few minutes. Tube was briefely vortex.
    - 1 gel volume's worth of H2O was added to the dissolved pHSN6A01 mixture because the fragment is >10kb long
    - Mixture was added to column and centrifuged for 1 min at 12 000 rpm (repeating this step until all of mixture has been passed through column)
    - Column was washed with 700uL of wash buffer and centrifuged for 1 min
    - Column was centrifuged again (w/no addition of buffer)
    - Elution buffer was heated to 65C
    - 50uL elution buffer was added to each tube and flow through was collected in clean eppendorf tubes
- pHSN6A01 (digested by Not1 HF & Sph1) was extracted/eluted from gel as was pSIM-24 GUS (digested by EcoRI & Anza16 HindIII)

Infusion Reaction

- Infusion reactions to put together pHSN6A01 with AlcR, and to put together PSIM-24 GUS with AlcrR, were set up as follows:

Table5

	A	B
1	5X In-Fusion HD Enzyme Premix	2 ul
2	Linearized vector	50ng
3	Purified PCR fragment	50ng
4	H2O	up to 15uL (should be 10 but concentrations were too low)
5	Total Volume	15uL (should be 10 but concentrations were too low)

- Incubated for 15 minutes at 50C, then left to hold at 12C

Hannah Vujovic Sarah Cumberland Nathaniel Petersen

Floral Dip

- The following constructs were floral dipped into Arabidopsis:
  - pSIM-24 GUS
  - ICE2 CRISPRa Guide A

Hannah Vujovic Sarah Cumberland

Overnight Cultures

- 2 replicates were set up of each of the following overnight cultures:
  - GUS in E. coli (5mL for miniprep)
  - CRISPRa backbone in E. coli (5mL for miniprep)
  - GUS in Agrobacterium (3mL for glycerol stocks)

SATURDAY, 7/31/2021

Danielle Halasz

Miniprep

- Miniprepped CRISPRa plasmid, final concentration was 192 ng/uL, used thermofisher miniprep kit
- There was no growth in GUS plasmids for miniprep

Glycerol Stocks

- 2 x 20% glycerol stocks made of GUS plasmid in Agrobacterium, and placed in -80C

Kulay Janneh Dennis Tran Harkamal Samra @Samantha

Seed harvesting

- Seeds were collected from plants floral dipped with the following constructs:
  - CRISPRi ICE2 Guide 1

    - CRISPRi ICE2 Guide 3
    - CRISPRi ICE2 Guide 5
    - CRISPRi CLE18 Guide 5
- Seeds were placed in eppendorfs that were labelled and placed in the "WT Arabidophsis seeds" box in the fridge

SUNDAY, 8/1/2021

Sarah Cumberland

E. coli transformation

- The following E. coli transformations were set up using Invitrogen cells:
    - CRISPRa + AlcR (from Infusion Reaction), plated on Kanamycin
    - CRISPRa (digested by Sph1 & Not1 HF), plated on Kanamycin
    - AlcR (from PCR), plated on Kanamycin
    - GUS + AlcR (from Infusion Reaction), plated on Ampicillin
    - GUS (digested by EcoRI HF & Anza16 HindIII), plated on Ampicillin
    - AlcR (from PCR), plated on Ampicillin
- 2.5uL of Infusion Reaction was added to 50uL cells
- 10ng of digested plasmid was added to 50uL cells
- 15ng of AlcR fragment was added to 50uL cells

Overnight Cultures

- 2 x 5mL overnight cultures were set up of each of the following:
    - pSIM-24 GUS in E. coli
    - CLE18 Guide C CRISPRa (Agrobacterium)
    - ICE2 Guide E CRISPRa (Agrobacterium) (rep 1) (labelled 1, 2)
    - ICE2 Guide E CRISPRa (Agrobacterium) (rep 2) (labelled 3, 4)

Patch Plates Agrobacterium

- No colonies were found on the plates, left to grow for longer.

MONDAY, 8/2/2021

Danielle Halasz

Patch Plates

- Streak plates were made from GUS + ALCR, GUS plasmid digest, and AlcR fragment as there was lawn growth, making it impossible to patch plate. The other three transformations had no growth

Amira Bouchema

PCR

- PCR was done using the FROGGA bio protocol on the following contstructs
    - 1-4: CLE18 multiplex CRISPRa (Agrobacterium)
    - 5-7: ICE2 Guide C CRISPRa (Agrobacterium)
    - 8-9: ICE 2 multiplex (Agrobacterium)

Sarah Cumberland

PCR

- PCR was done using the FROGGA bio protocol on the following constructs:
    - 2 x positive control

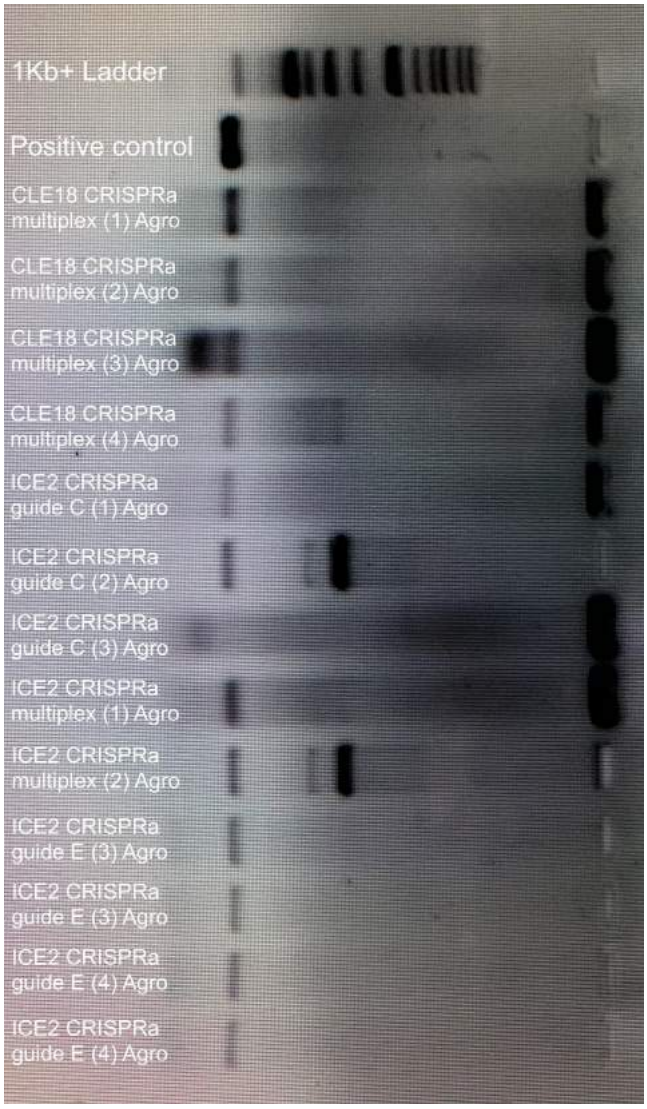
PCR Purification

- PCR purification was done as per Thermo Scientific GeneJET PCR Purification Kit Protocol: http://2017.igem.org/wiki/images/8/81/T--Chalmers-Gothenburg--Thermo_Scientific_GeneJET_PCR_Purification_Kit.pdf
    - A 1:1 volume of binding buffer was added to DNA
    - Binding buffer/DNA was added to a purification column & centrifuged for 1 min at 12k RPM
    - 700uL wash buffer was added & column was centrifuged as above
    - Again, 700uL wash buffer was added & column was centrifuged as above
    - Dry centrifuge spin, conditions as above
    - 50uL of warmed (at 65C) elution buffer was added to column and spun as above to elute DNA (into a fresh eppendorf)
    - Flow through was added back to column and spun as above one more time
- The following constructs were purified:
    - AlcR fragment
    - GUS (digested by EcoRI HF & Anza16 HindIII)
    - CRISPRa (digested by Sph1 & Not1 HF)

Gel Electrophoresis

- Gel was run on the PCR products prepared by Amira & Sarah today (as per protocol):

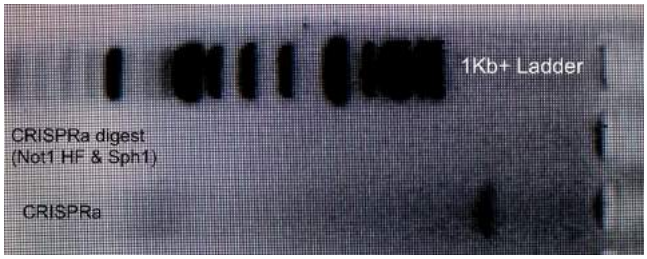
IMG_4961.jpg



- Gel was run on the PCR purified products prepared today (as per protocol):

IMG_4959.jpg

IMG_4960.jpg



Miniprep & Glycerol Stocks

- Glycerol stocks were made as per protocol
- pSIM-24 GUS in E. coli had growth, so was miniprepped
- no growth in CLE18 Guide C CRISPRa (Agrobacterium)
- no growth in ICE2 Guide E CRISPRa (Agrobacterium) (rep 1)
- ICE2 Guide E CRISPRa (Agrobacterium) (rep 2) had growth, so 2 x glycerol stocks were made. One was placed in Agrobacterium Glycerol Stocks box and one was placed in Agrobacterium Deep Archives box.

Overnight Cultures

- 2 x 5mL overnight cultures were set up of the following:
  - CLE18 Guide C CRISPRa (Agrobacterium)
  - ICE2 Guide C CRISPRa (Agrobacterium)
  - CLE18 multiplex CRISPRa (Agrobacterium)
  - ICE2 multiplex CRISPRa (Agrobacterium)

PCR Amplification

- The AlcR ethanol inducible promoter (inside a plasmid from IDT) was PCR amplified out of the plasmid from IDT.
- 2x replicates of the following reaction were set up as follows:

Table6

	A	B
1	Q5 High-Fidelity 2X Master Mix	12.5 µl
2	10 µM Forward Primer	1.25 µl
3	10 µM Reverse Primer	1.25 µl
4	Template DNA (AlcR-containing plasmid)	10ng (1uL)
5	Molecular Grade H2O	To a total volume of 25uL (9uL)

- Thermocycler was run as follows:
  - Initial Denaturation at 98°C for 30 seconds
  - Denaturation at 98°C for 10 seconds
  - Annealing at 58C for 30 seconds
  - Elongation at 72°C for 2 minutes (30 seconds/kb)
  - Repeat the previous 3 steps 30 times
  - Final extension at 72°C for 2 minutes
  - Hold at 12°C

TUESDAY, 8/3/2021

Danielle Halasz Gerry Koot Charlotte Fletcher Mike Green

PCR Verification

- PCR was done following SOP for AlcR Fragment in E.coli (triplicate), Gus + AlcR E.coli Amp (triplicate), and Gus plasmid digest E.coli Amp (triplicate)

PCR Clean-Up

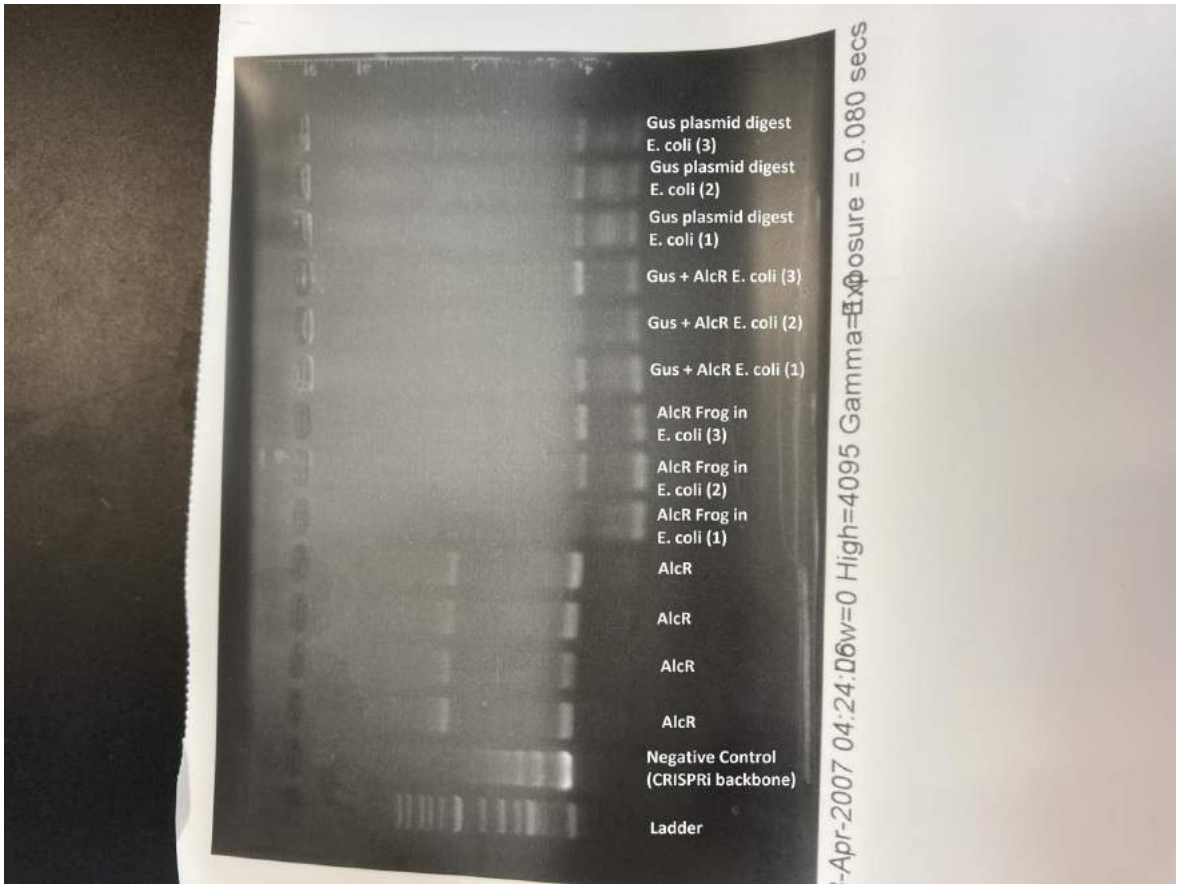
- PCR clean-up was done on AlcR ethanol inducable promoter (inside plasmid from IDT) following SOP

Danielle Halasz @Tiffany Mike Green

Gel Electrophoresis

- Gel electrophoresis was done following SOP

Gel (August 3, 2021).jpg



Overnight Cultures

- 300 mL Overnight cultures were made of:
  - ICE2 guide C (Agrobacterium) (duplicate)
  - CLE18 multiplex (Agrobacterium) (duplicate)
  - ICE2 multiplex (Agrobacterium) (duplicate)
- Excess 2mL was placed in culture fridge

WEDNESDAY, 8/4/2021

Hannah Vujovic Anum Anjum Isha

Overnight Cultures

- 5mL overnight cultures were set up of the following:
  - CLE18 Guide A CRISPRa (Agrobacterium)
  - CLE18 Guide B CRISPRa (Agrobacterium)
  - CLE18 Guide D CRISPRa (Agrobacterium)
  - CLE18 Guide E CRISPRa (Agrobacterium)
  - CLE18 multiplex CRISPRa (Agrobacterium)
  - ICE2 multiplex CRISPRa (Agrobacterium)
  - ICE2 Guide C CRISPRa (Agrobacterium)

Restriction Digest

- The following plasmids were digested using the following enzymes:

- pSIM-24 GUS -> EcoRI & HindIII
- pHSN6A01 (CRISPRa plasmid) -> SphI & NotI

- Reaction was set up as follows:

Table7

	A	B
1	Plasmid	4ug CRISPRa; 4ug GUS
2	10X CutSmart Buffer	3uL
3	Enzyme 1	1uL
4	Enzyme 2	1uL
5	Molecular Grade H2O	To a total volume of 30uL

- Thermocycler was run was follows:
  - Digest at 37C for 12 hours
  - Heat inactivated at 80C for 20 minutes
  - Hold at 12C

Patch Plates

- SOP was followed
  - CLE18 Guide A CRISPRa (Agrobacterium)
  - CLE18 Guide B CRISPRa (Agrobacterium)
  - CLE18 Guide D CRISPRa (Agrobacterium)
  - CLE18 Guide E CRISPRa (Agrobacterium)
  - CLE18 multiplex CRISPRa (Agrobacterium)
  - ICE2 multiplex CRISPRa (Agrobacterium)
  - ICE2 Guide C CRISPRa (Agrobacterium)

Glycerol Stocks

- Glycerol stocks were made following the SOP for the following constructs in Agrobacterium tumefaciens:
  - ICE2 guide C CRISPRa
  - ICE2 multiplex CRISPRa
  - CLE18 multiplex CRISPRa

Kulay Janneh

Sterilization of transformant's seeds

- Seeds for floral dipped plants were sterilized following SOP
- The following 12 MS + hygromycin plates were placed in the tissue culture room on shelf 4.3
  a. CRISPRi ICE2 Guide 1
  b. CRISPRi ICE2 Guide 3 (4 replicates)
  c. CRISPRi ICE2 Guide 5 (4 replicates)
  d. CRISPRi CLE18 Guide 5 (3 replicates)

Sarah Cumberland Taylor

DNA Extraction from Gel

- Gel extraction was perfomed as per the following protocol: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012661_GeneJET_Gel_Extraction_UG.pdf
- Proctol was performed as follows:
  - Gel collection tubes were pre-weighed
  - Gel fragments were excised and the weight of the fragments was determined
  - 1uL of Binding Buffer was added to each tube for every 1mg of agarose gel
  - Mixture was incubated at 60C for 10 minutes until gel was fully dissolved. Tube was inverted every few minutes. Tube was briefely vortex.
  - 1 gel volume's worth of H2O was added to the dissolved pHSN6A01 mixture because the fragment is >10kb long
  - Mixture was added to column and centrifuged for 1 min at 12 000 rpm (repeating this step until all of mixture has been passed through column)
  - Column was washed with 700uL of wash buffer and centrifuged for 1 min
  - Column was centrifuged again (w/no addition of buffer)
  - Elution buffer was heated to 65C
  - 50uL elution buffer was added to each tube and flow through was collected in clean eppendorf tubes
- Note: PCR cleanup should be done as there was high RNA contamination. Note 2: did that.

THURSDAY, 8/5/2021

Sarah Cumberland Harkamal Samra

PCR

- PCR was set up as per Frogga protocol. The following patches were PCR'd:
- Plate 1
  - CLE18 guide E patch 1
  - CLE18 guide E patch 2
  - CLE18 multiplex patch 3
  - CLE18 multiplex patch 4
  - CLE18 guide A patch 5
  - CLE18 guide A patch 6
  - CLE18 guide B patch 7
  - CLE18 guide B patch 8
- Plate 2
  - ICE2 guide C patch 1
  - ICE2 guide C patch 2
  - ICE2 guide C patch 3
  - ICE2 guide C patch 4
  - ICE2 multiplex patch 5
  - ICE2 multiplex patch 6
  - ICE2 multiplex patch 7
  - CLE18 guide D patch 8
  - CLE18 guide D patch 9
  - CLE18 guide D patch 10
  - ICE2 guide C patch 11
  - ICE2 guide C patch 12

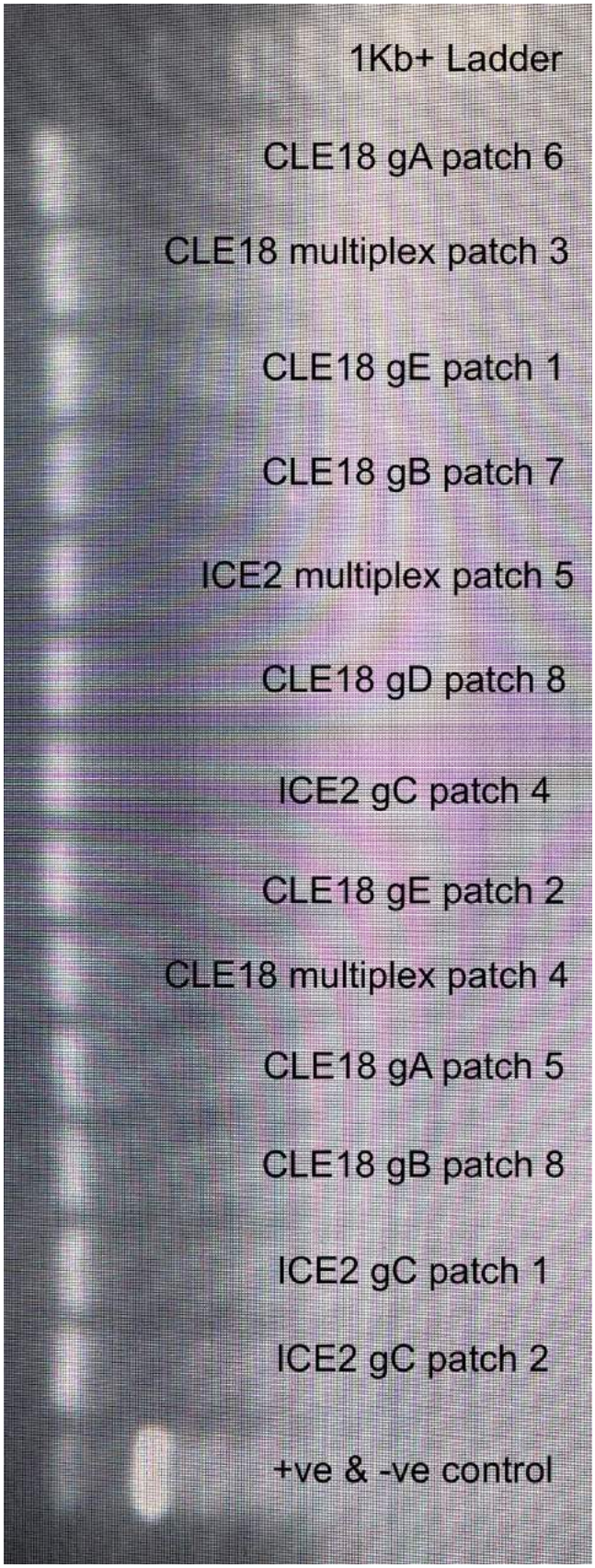
Sarah Cumberland Harkamal Samra Hannah Vujovic

Gel Electrophoresis

- Gel was run on the following PCR products as per the protocol:
- Gel 1
  - Ladder

- CLE18 guide A patch 6
- CLE18 multiplex patch 3
- CLE18 guide E patch 1
- CLE18 guide B patch 7
- ICE2 multiplex patch 5
- CLE18 guide D patch 8
- ICE2 guide C patch 4
- CLE18 guide E patch 2
- CLE18 multiplex patch 4
- CLE18 guide A patch 5
- CLE18 guide B patch 8
- ICE2 guide C patch 1
- ICE2 guide C patch 2
- positive & negative control

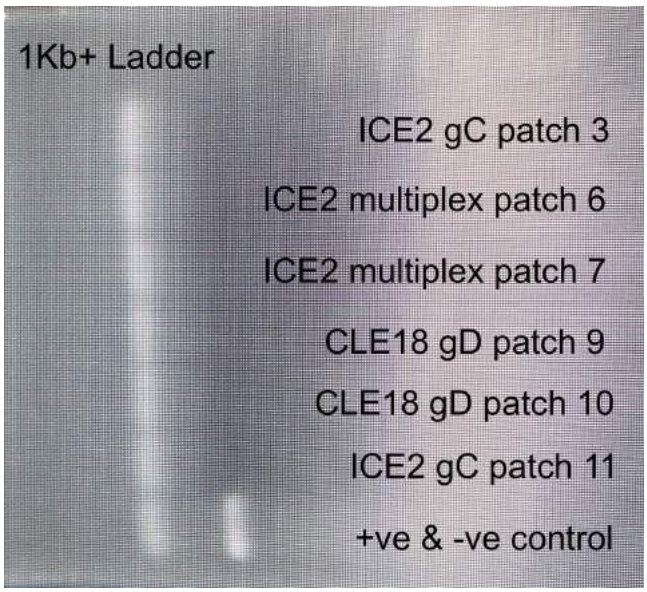
IMG_2345.jpg



- Gel 2
  - Ladder
  - ICE2 guide C patch 3

- ICE2 multiplex patch 6
- ICE2 multiplex patch 7
- CLE18 guide D patch 9
- CLE18 guide D patch 10
- ICE2 guide C patch 11
- Positive & negative control

IMG_2346.jpg



Hannah Vujovic

Glycerol Stocks

- Glycerol stocks were made of the following constructs in Agrobacterium. 2 replicates were made of each and one was placed in the Agrobacterium Glycerol Stocks box and one was placed in the Agrobacterium Deep Archive box in the -80C freezer
  - CLE18 Guide A CRISPRa (Agrobacterium)
  - CLE18 Guide B CRISPRa (Agrobacterium)
  - CLE18 Guide D CRISPRa (Agrobacterium)
  - CLE18 Guide E CRISPRa (Agrobacterium)
  - CLE18 multiplex CRISPRa (Agrobacterium)
  - ICE2 multiplex CRISPRa (Agrobacterium)
  - ICE2 Guide C CRISPRa (Agrobacterium)

Sarah Cumberland

PCR Purification

- PCR purification was done as per Thermo Scientific GeneJET PCR Purification Kit Protocol: http://2017.igem.org/wiki/images/8/81/T--Chalmers-Gothenburg--Thermo_Scientific_GeneJET_PCR_Purification_Kit.pdf
  - A 1:1 volume of binding buffer was added to DNA
  - Binding buffer/DNA was added to a purification column & centrifuged for 1 min at 12k RPM
  - 700uL wash buffer was added & column was centrifuged as above
  - Again, 700uL wash buffer was added & column was centrifuged as above
  - Dry centrifuge spin, conditions as above
  - 50uL of warmed (at 65C) elution buffer was added to column and spun as above to elute DNA (into a fresh eppendorf)
  - Flow through was added back to column and spun as above one more time
- The following constructs were purified:
  - AlcR fragment

- GUS (digested by EcoRI HF & Anza16 HindIII)
- CRISPRa (digested by Sph1 & Not1 HF)

Restriction Digest

- 200ng of AlcR fragment were digested using FD EcoRI & FD HindIII from Dr. Seah
- Reaction was set up as follows:
  - 15.4uL AlcR (13.0ng/uL -> 200ng)
  - 1uL EcoRI
  - 1uL HindIII
  - 2uL FastDigest Enzyme
  - H2O up to 30uL (11.5uL)
  - Total: 30uL
- Reaction was run at 37C for 3 hours then heat inacivated at 80C for 10 min as per heat inactivation temperatures/times for the enzymes

Hannah Vujovic Sarah Cumberland

Floral Dip

- The following constructs were floral dipped into Agrobacterium tumefaciens as per the protocol
  - ICE2 CRISPRa multiplex
  - ICE2 CRISPRa guide C

Overnight Cultures

- The following constructs in Agrobacterium tumefaciens were prepared in 300mL overnight cultures & left to shake at 28C:
  - CLE18 CRISPRa multiplex
  - CLE18 CRISPRa guide A
  - CLE18 CRISPRa guide B
  - CLE18 CRISPRa guide D
  - CLE18 CRISPRa guide E

FRIDAY, 8/6/2021

Sarah Cumberland

Gel Electrophoresis

- Gel was set up as per the protocol
  - Ladder
  - GUS digest
  - GUS digest
  - GUS digest
  - Ladder
  - CRISPRa digest
  - CRISPRa digest
  - CRISPRa digest
  - Ladder
  - AlcR digest
  - AlcR digest
  - Ladder
- Gel was not imaged in order to reduce UV exposure

DNA Extraction from Gel

- Gel extraction was perfomed as per the following protocol: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012661_GeneJET_Gel_Extraction_UG.pdf

Sarah Cumberland Namra Hamid

Floral Dip

- The following constructs were floral dipped into Agrobacterium tumefaciens as per the protocol:
  - CLE18 multiplex CRISPRa
  - CLE18 guide A CRISPRa
  - CLE18 guide D CRISPRa
- 3 pots dipped per sample

SATURDAY, 8/7/2021

Kulay Janneh

Overnights

- Two 5mL cultures of the following constructs were made and left to shake in room 4224B (Labelled iGEM)
  - GUS plasmid
  - CRISPRa plasmid

SUNDAY, 8/8/2021

Hannah Vujovic

Miniprep

- Miniprep of CRISPRa plasmid was done following monarch protocols included with the kit

Overnights

- 300mL overnight cultures were made of CLE18 guides B/E CRISPRa

MONDAY, 8/9/2021

Sarah Cumberland

Ligation

- A ligation between digested GUS and digested AlcR was performed as per the NEB Ligation Protocol with T4 DNA Ligase (M0202): https://international.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202
- Gently mixed the reaction by pipetting up and down and microfuge briefly.
- For cohesive (sticky) ends, incubated at room temperature for 10 minutes. Overnight at 15C. 15C room on 3rd floor of SSC, or thermal cycler. Or put tubes in the vegetable compartment of the lower drawer.
- Heat inactivate at 65°C for 10 minutes.
- Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.
- 2 replicates of this reaction were prepared

Table8

	A	B
1	T4 DNA Ligase Buffer (10X)	3 µl**
2	Vector DNA (GUS) (8.2 kb)	50 ng
3	Insert DNA (AlcR) (4 kb)	73.2 ng*
4	Nuclease-free water	to 20 µl
5	T4 DNA Ligase	1 µl
6	Total	31 µl**

*As calculated using the NEBioCalculator to get a 1:3 molar ratio of vector DNA to insert DNA http://nebiocalculator.neb.com/#!/ligation
**Total should have been 20uL but the DNA concentrations were too low so total was increased to 31uL & buffer was increased to 3uL

Floral Dip

- Cancelled due to cultures not being grown enough, so new 300mL overnight cultures of CLE18 guides B/E CRISPRa were set up to grow overnight

Enzo Baracuhy

In-Fusion of alcR + digested CRISPRa

- 4uL of alcR fragment and 4uL of CRISPRa digest was mixed into a PCR tube with 2uL of InFusion master mix. Put into thermocycler at 50C for 15min
- A transformation was done acroding to the IN-Fusion transformation protocol using the entire 10uL

TUESDAY, 8/10/2021

Hannah Vujovic Isha

300mL Overnight Cultures

- The following 300mL overnight cultures were prepared as per the protocol
  - ICE2 guide C/multiplex
  - CLE18 multiplex

Sarah Cumberland Mike Green Isha

E. coli transformation

- E. coli transformation was performed as per the protocol. 2 replicates of each of the following were prepared.
  - GUS + AlcR -> plated @ 2 dilutions
  - GUS digest
  - GUS plasmid (undigested)
  - AlcR plasmid (undigested)
  - Empty (no plasmid)

Kulay Janneh Dennis Tran Harkamal Samra

Seed collection

- Seeds were collected from T_0 plants floral dipped with the following constructs
  - CLE18 Guide C CRISPRa
  - ICE2 Guide B CRISPRa
  - ICE2 Guide E CRISPRa
- Seeds are in the fridge on an eppendorf tube rack labelled "Non-sterile transformed seeds"

Kulay Janneh Dennis Tran Harkamal Samra

Patch plate + Streak plate

- One patch plate of GUS + AlcR plasmids (50 uL) in E.coli was patch plated
- All other plates except for one formed a lawn (these plates are in the 4°C incubator)
- GUS + AlcR plasmid in E.coli was streak plated and left to incubate in the 37°C incubator

Sarah Cumberland Dennis Tran Sofia Finley

Floral Dip

- Floral dip was performed as per the protocol. The following constructs were dipped:
  - CLE18 CRISPRa multiplex

THURSDAY, 8/12/2021

Sarah Cumberland Nathaniel Petersen

Miniprep

- The following cultures were miniprepped as per the NEB Monarch Protocol (to be used in restriction digest):
  - GUS/AlcR patches (3 replicates)
  - GUS/AlcR from transformation plate
  - GUS (digested) from transformation plate
  - GUS (undigested) from transformation plate
  - AlcR from transformation plate

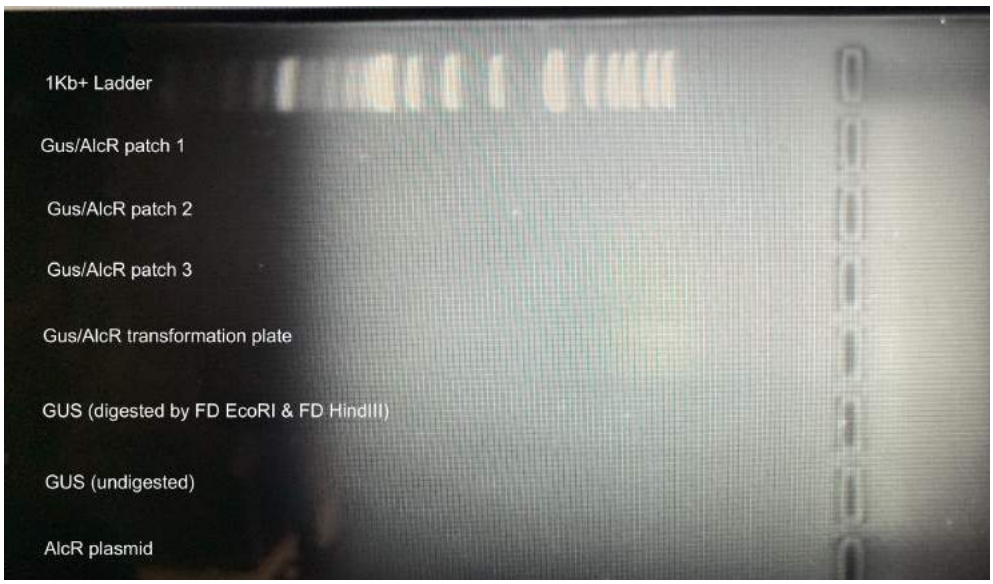
Restriction Digest

- Restriction digest was performed on colonies from the following plates:
  - 4 x GUS/AlcR plasmid
  - GUS (undigested)
  - GUS (digested)
  - AlcR plasmid
- Digest was set up as follows:
  - 200ng DNA
  - 1uL EcoRI
  - 1uL HindIII
  - 2uL FastDigest Buffer
  - H2O up to 30uL
  - Total: 30uL
- Reaction was run at 37C for 3 hours then heat inactivated at 80C for 10 min as per heat inactivation temperatures/times for the enzymes

Gel Electrophresis

- Gel electroporesis was performed as per the protocol

IMG_5042.jpg



Patch Plate

- A patch plate was made of GUS/AlcR on an Amp plate at the proper Amp concentration.

FRIDAY, 8/13/2021

Kulay Janneh

Miniprep

- Miniprep was preformed following protocol in the Monarch miniprep kit on PHSN6A01 plasmid
- DNA was placed in CRISPRa box

300mL Cultures

- Cultures were made using the CLE18 Guide B and E constructs and left in 4109

Namra Hamid

Agrobacterium Transformation

- Transformed CLE18 guide C into agrobacterium as per SOP

SATURDAY, 8/14/2021

Amira Bouchema

Floral Dip

- Floral dip was performed on the following guides:
  - CLE18 guide B CRISPRa
  - CLE18 guide B CRISPRa

SUNDAY, 8/15/2021

Hannah Vujovic

Patch Plates

- Patch plate was made for CLE18 guides B/E CRISPRa.

MONDAY, 8/16/2021

Sarah Cumberland Hannah Vujovic

PCR Verification

- PCR verification was performed on the following guides as per the FroggaTaq protocol:

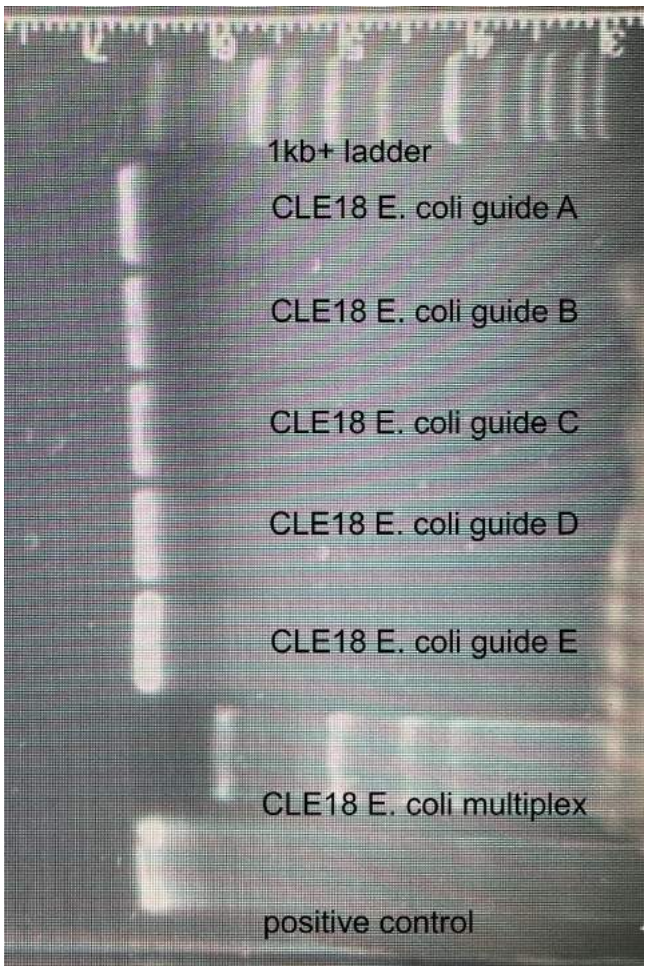
1. CLE18 multiplex (E. coli)
2. CLE18 guide A (E. coli)
3. CLE18 guide B (E. coli)
4. CLE18 guide C (E. coli)
5. CLE18 guide D (E. coli)
6. CLE18 guide E (E. coli)
7. ICE2 multiplex (E. coli)
8. ICE2 multiplex (E. coli)
9. ICE2 guide A (E. coli)
10. ICE2 guide B (E. coli)
11. ICE2 guide C (E. coli)
12. ICE2 guide D (E. coli)
13. ICE2 guide E (E. coli)
14. CLE18 guide A (Agrobacterium)
15. CLE18 guide B (Agrobacterium)
16. CLE18 guide D (Agrobacterium)
17. CLE18 guide E (Agrobacterium)
18. CLE18 multiplex (Agrobacterium)
19. CLE18 multiplex (Agrobacterium)
20. CLE18 multiplex (E. coli)
21. ICE2 multiplex (Agrobacterium)
22. ICE2 multiplex (Agrobacterium)
23. ICE2 guide A (Agrobacterium)
24. ICE2 guide B (Agrobacterium)
25. ICE2 guide C (Agrobacterium)
26. ICE2 guide D (Agrobacterium)
27. ICE2 guide E (Agrobacterium)
28. CRISPRa (E. coli)
29. GUS (E. coli)
30. GUS (Agrobacterium)
31. DXR positive control
32. DXR positive control
33. DXR positive control
34. GUS negative control

- Thermocycler was run as follows:
  - 94°C for 3 minutes
  - [94°C for 30 seconds
  - 59°C for 30 seconds
  - 72°C for 4.5 minutes] x35 cycles
  - 72°C for 10 minutes
  - Hold at 16°C

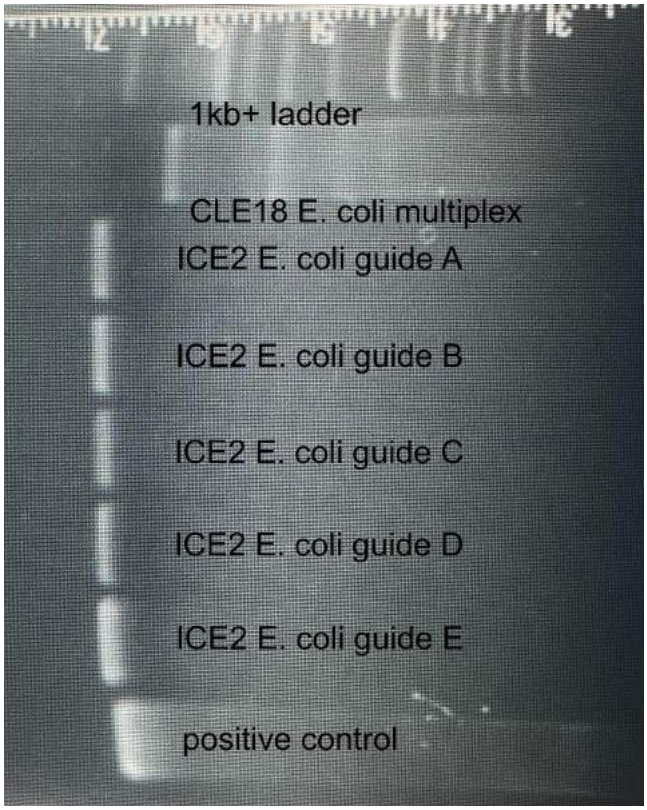
Gel Electrophoresis

- Gel electrophoresis was performed as per the protocol

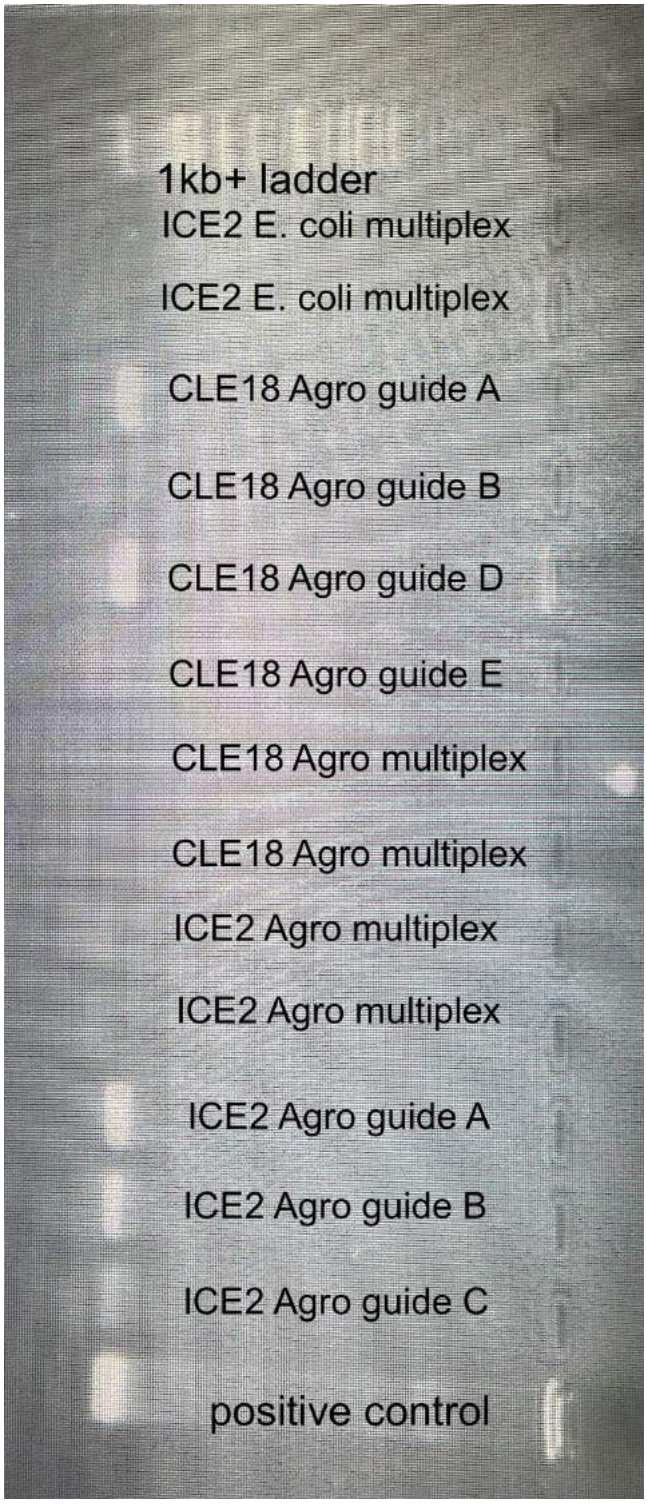
IMG_2367.jpg


1kb+ ladder
CLE18 E. coli guide A
CLE18 E. coli guide B
CLE18 E. coli guide C
CLE18 E. coli guide D
CLE18 E. coli guide E
CLE18 E. coli multiplex
positive control

IMG_2369.jpg


1kb+ ladder
CLE18 E. coli multiplex
ICE2 E. coli guide A
ICE2 E. coli guide B
ICE2 E. coli guide C
ICE2 E. coli guide D
ICE2 E. coli guide E
positive control

IMG_2370.jpg



TUESDAY, 8/17/2021

Danielle Halasz

Agrobacterium transformation

Following the protocol, transformations of the following (with duplicates) were made:

- CLE 18 Guide E CRISPRa (rep 1 and 2)
- CLE18 multiplex (rep 1 and 2)

- ICE2 multiplex (rep1 and 2)

ICE2 Guide B had growth on patch plate made previous day, and was moved to fridge to have 7mL overnights made on thursday

WEDNESDAY, 8/18/2021

Emma Lee Amira Bouchema

Patch Plates

- No growth was found on the plates. Plates were left at 28C to grow more

Double Digest

37C - 1h

80C - 20min

Reaction was carried out as shown here:

Table9

	A	B	C
1	Reagents	AlcR amp EB (Aug 11 - 22ng) - 10ul 15ul molecular h2o. 3ul fd buffer	GUS
2	HindIII	1	1
3	EcoRI	1	1
4	Plasmid	10	20
5	Buffer	3	3
6	H2O	15	5

THURSDAY, 8/19/2021

Amira Bouchema Harkamal Samra Dennis Tran

Agrobacterium Transformation

- Transformation was completed using SOP A. tumefaciens transformation on the following constructs:
  - CLE18 Multiplex
  - ICE2 Multiplex
  - CLE18 Guide E

FRIDAY, 8/20/2021

Danielle Halasz

Gel extraction of GUS plasmid

- There was no growth on agrobacterium plates transformed

SATURDAY, 8/21/2021

Kulay Janneh

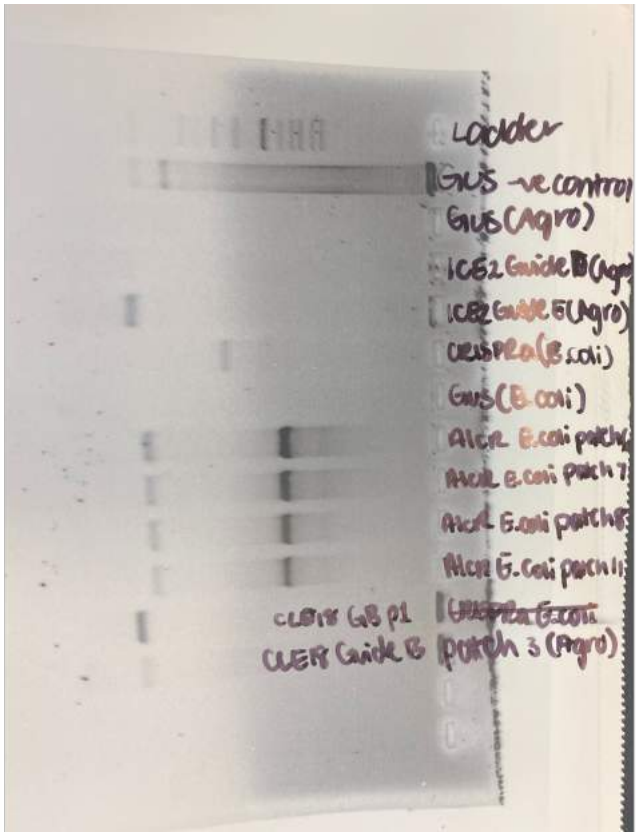
PCR

- PCR was performed following the PCR FroggaBio Protocol from the following patches:
  - AlcR promoter E.coli patch 6
  - AlcR promoter E.coli patch 7
  - AlcR promoter E.coli patch 8
  - AlcR promoter E.coli patch 11
  - CLE18 Guide B CRISPRa Agrobacterium Patch 1 (duplicate 1)
  - CLE18 Guide B CRISPRa Agrobacterium Patch 3 (duplicate 2)

Gel Electrophoresis

- Was carried out following the Gel Electrophoresis protocol using the PCR products from above as well as the following PCR products at 115V:
  - GUS negative control
  - GUS (Agrobacterium)
  - ICE2 guide D (Agrobacterium)
  - ICE2 guide E (Agrobacterium)
  - CRISPRa (E. coli)
  - GUS (E. coli)

GEL.pdf
p. 1



AlcR overnight

- 6 labelled 4 mL overnight of AlcR were made and left to shake in 4224A

SUNDAY, 8/22/2021

Sarah Cumberland

Miniprep

- Overnights from yesterday (AlcR plasmids in E. coli) were miniprepped as per the NEB Monarch nucleic acid purifcation kit protocol.

Restriction Digest

- AlcR plasmid was digested as follows:
  - 27 uL AlcR plasmid (150.3ng/uL)
  - 1 uL EcoRI
  - 1uL Hindiii
  - 5uL 10x FD buffer
  - 16uL mol water
- Thermocycler was run as follows:
  - 37C for 3 hours
  - 80C for 20 min
  - Rest at 12C

Hannah Vujovic

Patch Plates

- Patch plate was made following SOP for CLE18 guide E/multiplex and ICE2 multiplex

Overnights

- 3mL overnight culture was prepared following SOP for CLE18 guide B CRISPRa Agrobacterium

MONDAY, 8/23/2021

Sarah Cumberland Isha Anum Anjum

Agrobacterium transformation

- Agrobacterium transformation was performed as per the SOP to transform the following constructs into Agrobacterium:
  - CLE18 guide B
  - CLE18 guide C
  - CLE18 guide E
  - CLE18 multiplex
  - ICE2 guide D
  - ICE2 multiplex

PCR

- PCR was performed as per the SOP on the following patches:
  - 1. CLE18 guide E CRISPRa patch 1
  - 2. CLE18 guide E CRISPRa patch 2
  - 3. positive control for guide verification
  - 4. AlcR-pHSN6A01 Infusion patch 1
  - 5. AlcR-pHSN6A01 Infusion patch 2
  - 6. AlcR-pHSN6A01 Infusion patch 3
  - 7. AlcR-pHSN6A01 Infusion patch 4
  - 8. AlcR-pHSN6A01 Infusion patch 5
  - 9. AlcR-pHSN6A01 Infusion patch 6
  - 10. AlcR-pHSN6A01 Infusion patch 7
  - 11. AlcR-pHSN6A01 Infusion patch 8
  - 12. AlcR-pHSN6A01 Infusion patch 9
  - 13. AlcR-pHSN6A01 Infusion patch 10
  - 14. negative control pHSN6A01

- Legend was left on the thermocycler

Enzo Baracuhy

Gel Electrophoresis

A gel was run on the above PCR, excluding samples 12 and 13 because room had to be made for the AlcR digest from yesterday

TUESDAY, 8/24/2021

Hannah Vujovic Danielle Halasz Harkamal Samra

E.coli transformation

- SOP was followed with the following changes: Ligation was heat-inactivated at 65C for 10 minutes and 5uL was added to Invitrogen cells. Ampicillin plates were used.

Glycerol Stocks

- SOP was followed for glycerol and deep glycerol stocks for CLE18 guide B CRISPRa agrobacterium.

Floral Dip

- CLE18 guide E had no growth; therefore, only CLE18 guide B was floral dipped following SOP - Floral Dip (Agrobacterium-mediated transformation of Arabidopsis

WEDNESDAY, 8/25/2021

Danielle Halasz Charlotte Fletcher

- Created patch plates of E.coli GUS-alcR digestion
- Performed PCR following SOP on what was left inside the thermocycler
- Made 12 MS + hygromycin plates
- Made 0.2% and 0.3% agar for seeds

THURSDAY, 8/26/2021

Danielle Halasz

Agrobacterium transformation:

- alcR PHSN6A01
- ICE2 Guide D
- CLE18 Guide B
- CLE18 Guide C
- CLE18 Guide E
- CLE18 multiplex
- ICE2 multiplex
- Positive control
- Negative control

E.coli transformation:

- E.coli ligation
- Positive control

FRIDAY, 8/27/2021

Hannah Vujovic Nathaniel Petersen

PCR

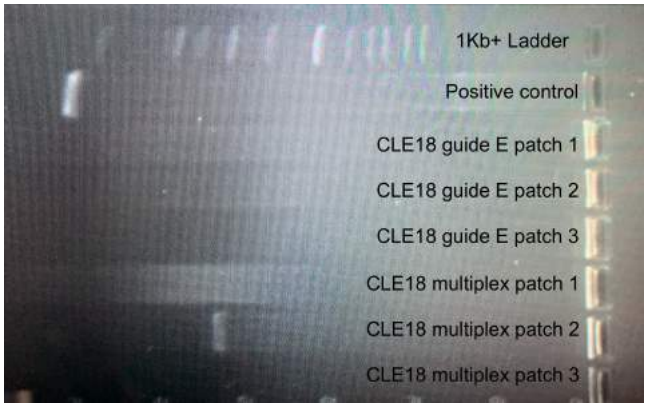
SOP was followed for the PCR of the following samples:

- CLE18 guide E patches 1-3
- CLE18 multiplex patches 1-3
- ICE2 multiplex patches 1-3
- GUS-AlcR (not ligated) patches 1,2
- GUS-AlcR ligation (E. coli) patches 1-15
- Positive controls

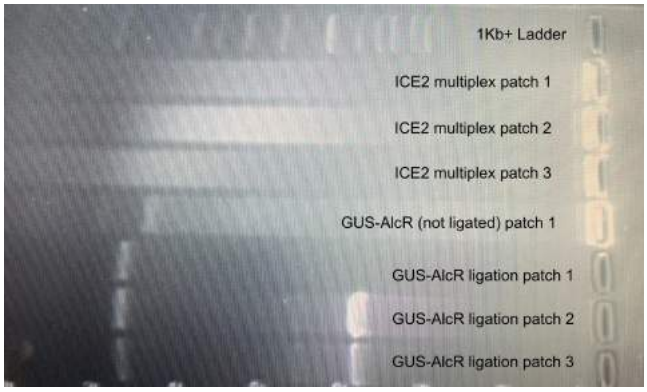
Gel Electrophoresis

- Gel electrophoresis was performed as per the protocol

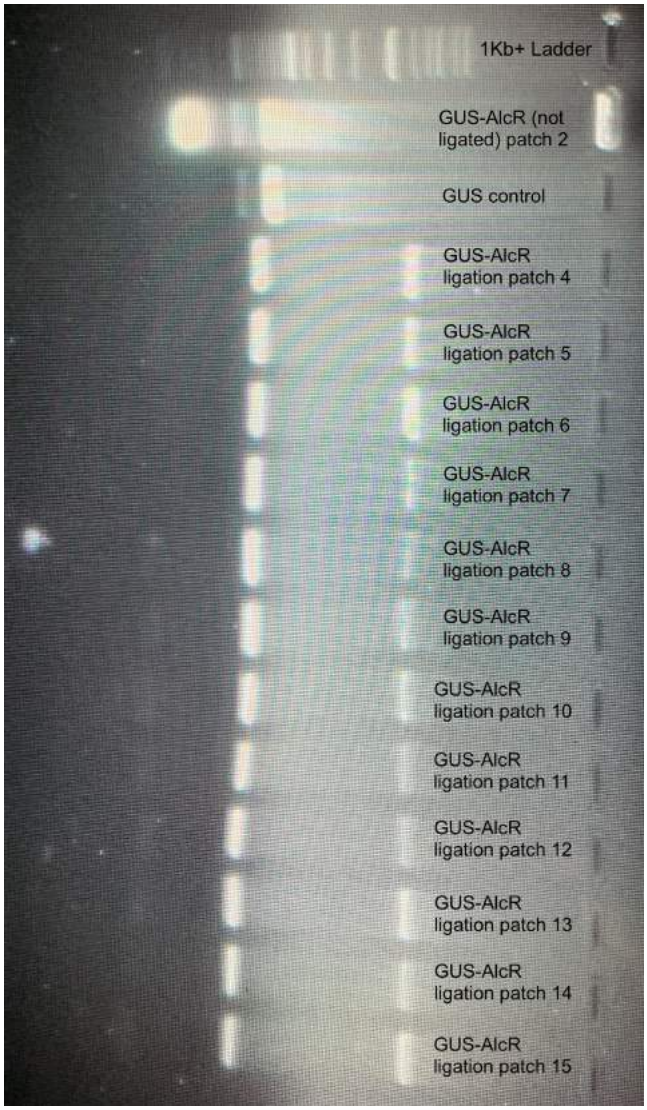
Screen Shot 2021-08-27 at 8.18.32 PM.png



IMG_5416.jpg



IMG_5419.jpg



300mL Overnights

- 300mL overnight cultures were set up for a floral dip following the protocol. Cultures were made from the following patches:
  - CLE18 multiplex patch 2 (Agrobacterium)
  - ICE2 multiplex patch 2 (Agrobacterium)

SATURDAY, 8/28/2021

Kulay Janneh Sarah Cumberland

Seed harvesting

- Seeds from plants dipped in the following constructs we collected:
  - ICE2 Guide A
  - ICE2 Guide C
  - CRISPRa GUS
- Seeds leftover after sterilization were put in Dr. Seah's lab.

Kulay Janneh

Seeds sterilization

- Seeds collected from earlier today were sterilized to yield the following number of plates:
  - 3 x ICE2 Guide A
  - 3 x ICE2 Guide C
  - 4 x CRISPRa GUS
- A positive and negative control were also plated
- All 12 plates were left in Dr.Seah's lab's fridge.

MONDAY, 8/30/2021

Kulay Janneh

Patch plates

- Patch plates of the following colonies were made:
  - ICE2 Guide D (Agrobacterium)
  - ICE2 Multiplex (Agrobacterium)
  - CLE18 Guide B (Agrobacterium)
  - CLE18 Guide C (Agrobacterium)
  - CLE18 Guide E (Agrobacterium)
  - CLE18 Multiplex (Agrobacterium)
  - GUS/AlcR Ligation (E. coli [Room 4224A])

Sarah Cumberland Hannah Vujovic

DNA prepared for sequencing

- 300ng samples of the following plasmids were aliquoted into PCR tubes for sequencing:
  - 2 x AlcR-pHSN6A01
  - 2 x ICE2 multiplex
  - 2 x CLE18 multiplex

Overnight Cultures

- 3x3mL overnights of GUS-AlcR (ligated) were prepared and left to shake in Room 4224A
- 3x3mL overnights & 3x2mL overnights (5mL total) of GUS-AlcR (ligated) were prepared and left to shake in Room 4224A

LB (no plates)

- 3L of LB were prepared & autoclaved
- 500mL of this can be used to make LB + Kan plates if agar is added & re-autoclaved
- 500mL of this can be used to make LB + Gen + Amp plates if agar is added & re-autoclaved

TUESDAY, 8/31/2021

Kulay Janneh

Glycerol stocks

- Were made following the protocol for the following construct:
  - GUS-AlcR

Miniprep

- Was carried out following the monarch miniprep kit protocol for the following constructs
  - GUS-AlcR
  - AlcR- PHSN6A01

Amira Bouchema

PCR

- Was carried out following the Frogga protocol for the following constructs
    - CLE18 Guide E x 12
    - CLE18 Guide C x 8
    - CLE18 Guide B x 7
    - AlcR PHSN6A01 x 11
    - ICE2 Guide D x 5
    - CLE18 Multiplex x 4
    - ICE2 Multiplex x 4

Danielle Halasz Namra Hamid

Golden gate

- Using the NEB protocol on the following constructs:
    - CLE18 multiplex AlcR PHSN60A1
    - CLE18 multiplex AlcR pHSN6A01
    - ICE2 multiplex AlcR PHSN6A01
    - ICE2 multiplex AlcR PHSN6A01

Dropped off the templates and primers for sequencing

Gel Electrophoresis

Ran one large gel, loaded in this order from left to right:

- ladder
- CLE18 guide E patch 8
- CLE18 guide E Patch 9
- CLE18 Guide C patch 3
- CLE18 Guide C patch 6
- CLE 18 Guide B patch 1
- CLE 18 Guide B patch 6
- AlcR PHSN6A01 patch 5
- AlcR PHSN6A0o1 patch 7
- ICE2 Guide D patch 4
- ICE2 Guide D patch 5
- CLE18 multiplex patch 3
- CLE18 multiplex patch 4
- ICE2 multiplex patch 1
- ICE2 multiplex patch 3

Gel image:

image.jpg

Made overnights of one of each duplicate

THURSDAY, 9/2/2021

Danielle Halasz

Agrobacterium Transformation

Transformation with duplicates of agrobacterium:

- AlcR-pHSN6A01 (2 of them)
- GUS-AlcR (2 of them)

Patch Plates

Patch plates of yesterday's sucessful transformation; 12 patches of the following:

- ICE2 multiplex AlcR pHSN60A1 (E. coli)
- CLE18 multiplex AlcR pHSN6A01 (E. coli)

FRIDAY, 9/3/2021

Sarah Cumberland

PCR on Agrobacterium Patches

- PCR verification was performed on the following patches (E. coli) as per the FroggaBio protocol:

1. negative control: empty pHSN6A01
2. positive control sgRNA (DXR)
3. CLE18 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 1
4. CLE18 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 2
5. CLE18 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 3
6. CLE18 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 2 patch 1
7. CLE18 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 2 patch 2
8. negative control: empty pHSN6A01
9. positive control: AlcR-pHSN6A01 (AlcR primers)
10. CLE18 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 1
11. CLE18 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 2
12. CLE18 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 3
13. CLE18 multiplex AlcR-pHSN6A01 (AlcR primers) plate 2 patch 1

14. CLE18 multiplex AlcR-pHSN6A01 (AlcR primers) plate 2 patch 2
15. negative control: empty pHSN6A01
16. positive control sgRNA (DXR)
17. ICE2 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 1
18. ICE2 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 2
19. ICE2 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 1 patch 3
20. ICE2 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 2 patch 1
21. ICE2 multiplex AlcR-pHSN6A01 (sgRNA primers) plate 2 patch 2
22. positive control: AlcR-pHSN6A01 (sgRNA & AlcR primers)
23. positive control: AlcR-pHSN6A01 (AlcR primers)
24. ICE2 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 1
25. ICE2 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 2
26. ICE2 multiplex AlcR-pHSN6A01 (AlcR primers) plate 1 patch 3
27. ICE2 multiplex AlcR-pHSN6A01 (AlcR primers) plate 2 patch 1
28. ICE2 multiplex AlcR-pHSN6A01 (AlcR primers) plate 2 patch 2

possible gel setup:
ladder

1. empty phsn6a01
2. sgnra positive
3. cle18 multiplex (sgrna primers)
4. cle18 multiplex (sgrna primers)
5. cle18 multiplex (sgrna primers)
6. cle18 multiplex (sgrna primers)
7. cle18 multiplex (sgrna primers)

ladder
8. empty phsn6a01
9. AlcR positive
10. cle18 multiplex (AlcR primers)
11. cle18 multiplex (AlcR primers)
12. cle18 multiplex (AlcR primers)
13. cle18 multiplex (AlcR primers)
14. cle18 multiplex (AlcR primers)

ladder
15. empty phsn6a01
16. sgnra positive
17. ice2 multiplex (sgrna primers)
18. ice2 multiplex (sgrna primers)
19. ice2 multiplex (sgrna primers)
20. ice2 multiplex (sgrna primers)
21. ice2 multiplex (sgrna primers)
22. AlcR positive 4 primers
23. AlcR positive
24. ice2 multiplex (AlcR primers)
25. ice2 multiplex (AlcR primers)
26. ice2 multiplex (AlcR primers)
27. ice2 multiplex (AlcR primers)
28. ice2 multiplex (AlcR primers)

SATURDAY, 9/4/2021

Hannah Vujovic

Glycerol Stocks

- 2x glycerol stocks were prepared as per the protocol of each the following constructs in E. coli:
  - CLE18 multiplex AlcR-pHSN6A01
  - ICE2 multiplex AlcR-pHSN6A01
- 2x glycerol stocks were prepared as per the protocol on of the following constructs in Agrobacterium:
  - ICE2 CRISPRa multiplex
  - ICE2 CRISPRa guide D
  - CLE18 CRISPRa multiplex
  - CLE18 CRISPRa guide B
  - CLE18 CRISPRa guide C

Sarah Cumberland Hannah Vujovic

Floral Dip

- Floral dip was performed as per the protocol on the following constructs into Arabidopsis thaliana:
  - ICE2 CRISPRa multiplex
  - ICE2 CRISPRa guide D
  - CLE18 CRISPRa multiplex
  - CLE18 CRISPRa guide B
  - CLE18 CRISPRa guide C

SUNDAY, 9/5/2021

Enzo Baracuhy

Miniprep

The following cultures were minipreped from E. coli according to the NEB miniprep kit protocol and placed in the plant box:

- ICE2 Multiplex AlcR
- CLE18 Multiplex AlcR

Sarah Cumberland

- 2L of LB made
- Plants moved up to phytotron

MONDAY, 9/6/2021

Hannah Vujovic

Agrobacterium transformation

SOP was followed for agrobacterium transformation for:

- ICE2 multiplex AlcR
- CLE18 multiplex AlcR
- GUS-AlcR
- AlcR- pHSN6A01

Sarah Cumberland

Overnight Cultures

300mL overnight cultures were set up of each of the following constructs in Agrobacterium as per the protocol:

- 2 x CLE18 guide E CRISPRa
- 2 x CLE18 multiplex CRISPRa

- 2 x ICE2 multiplex CRISPRa

TUESDAY, 9/7/2021

Nathaniel Petersen Sarah Cumberland

Glycerol Stocks

2 x glycerol stocks were made of each of the following constructs from 300mL overnight Agrobacterium cultures, as per the glycerol stocks SOP:

- CLE18 guide E CRISPRa
- CLE18 multiplex CRISPRa
- ICE2 multiplex CRISPRa

Floral Dip

Floral dip was performed as per the protocol using the following constructs into Arabidopsis thaliana:

- CLE18 guide E CRISPRa
- CLE18 multiplex CRISPRa
- ICE2 multiplex CRISPRa

WEDNESDAY, 9/8/2021

Danielle Halasz

- It was not possible to create patch plates or overnights. The plates were left to grow longer. GUS-AlcR had lawn growth, so a streak plate was made

FRIDAY, 9/10/2021

Hannah Vujovic

PCR

PCR was done following SOP on the following:

- AlcR-pHSN6A01 patches 1/2/3/4/6/7/8
- ICE2 multiplex AlcR patches 1/2/3/4/5/6
- CLE18 multiplex AlcR patches 1/3/4/5/6/7/8/9/10
- GUS-AlcR streak 1
- Positive and negative controls

Patch Plates

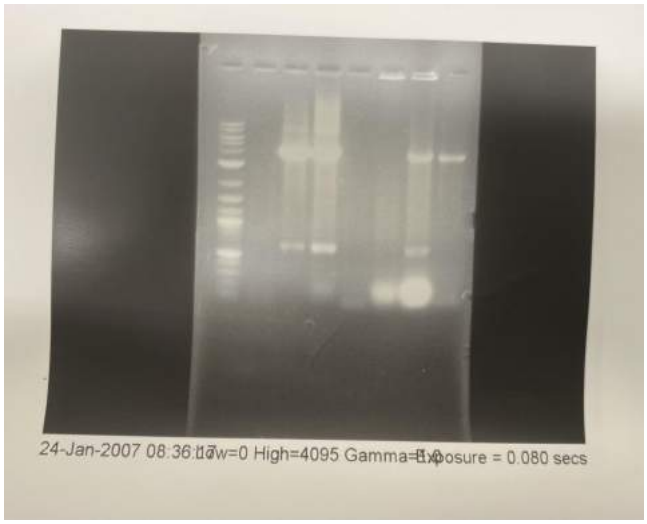
Patch plates were made for the above samples

Namra Hamid

Gel 1

ladder
AlcR pH5N6A01 patch 1
AlcR pH5N6A01 patch 2
AlcR pH5N6A01 patch 3
AlcR pH5N6A01 patch 4
AlcR pH5N6A01 patch 5
AlcR pH5N6A01 patch 6
AlcR pH5N6A01 patch 7
AlcR pH5N6A01 patch 8

65301505357__BB9C639C-4E07-4070-B7FB-5919BE6860BB.jpeg



Gel 2

ladder

ICE2 multiplex AlcR patch 1

ICE2 multiplex AlcR patch 2

ICE2 multiplex AlcR patch 3

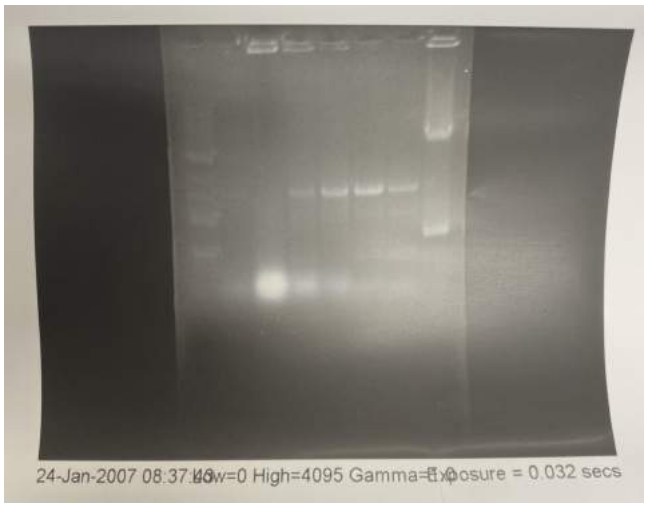
ICE2 multiplex AlcR patch 4

ICE2 multiplex AlcR patch 5

ICE2 multiplex AlcR patch 6

AlcR- pH5N6A01 Control

65301506374__C437395C-AA0E-4396-ACDF-0BB01AFAD425.jpeg



Gel 3

ladder

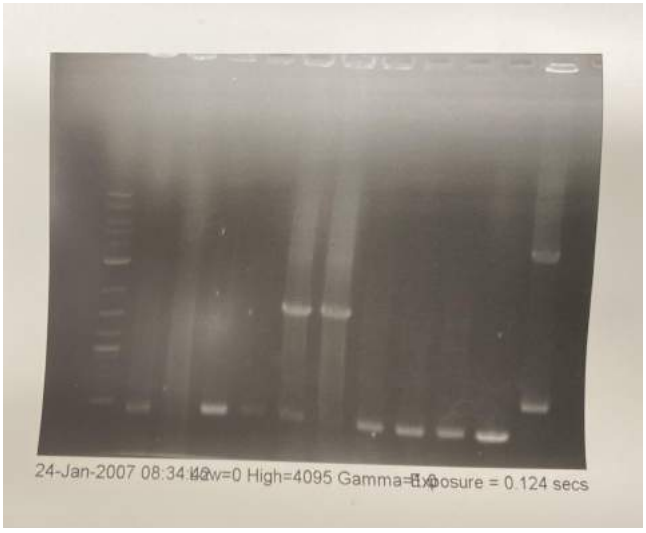
CLE18 multiplex AlcR patch 1

Gus alcR 1

CLE18 multiplex AlcR patch 3

CLE18 multiplex AlcR patch 4
CLE18 multiplex AlcR patch 5
CLE18 multiplex AlcR patch 6
CLE18 multiplex AlcR patch 7
CLE18 multiplex AlcR patch 8
CLE18 multiplex AlcR patch 9
CLE18 multiplex AlcR patch 10
Multiplex +ve control
Gus AlcR control

65301503919__09A8B087-F592-4042-B372-2608CD1B49A6.jpeg



Overnights

300mL overnight cultures were set up of each of the following constructs in Agrobacterium as per the protocol:

- 1 x ICE2 multiplex AlcR patch 3
- 1 x AlcR pH5N6A01 patch 3

SATURDAY, 9/11/2021

Danielle Halasz Amira Bouchema

Glycerol Stocks

- CLE18 multiplex AlcR patch 3
- AlcR pH5N6A01

Floral Dip

- CLE18 multiplex AlcR patch 3
- AlcR pH5N6A01

MONDAY, 9/13/2021

Sarah Cumberland Hannah Vujovic

- Floral dipped plants from Saturday brought up to phytotron

Agrobacterium transformation

- The following constructs were transfrormed into Agrobacterium tumefaciens as per the protocol:

- ICE2 multiplex AlcR
- CLE18 multiplex AlcR
- GUS-AlcR
- AlcR- pHSN6A01

Amira Bouchema

Agrobacterium transformation

- The following constructs were PCR Tested:
  - CLE18 multiplex AlcR - Sept 10 (guide primers)
  - CLE18 multiplex AlcR - Sept 10 (CRISPRa AlcR primers)
  - GUS-AlcR - Sept 10 (GUSAlcR primers)

Danielle Halasz

Gel + overnights

- Gel was run on the following samples, but issues arrose that made it difficult to see any results

image.png

image.png

- A new Gel and PCR will be run tomorrow, but overnights were made regadles of CLE18 multiplex AlcR patch 20 and GUS-AlcR patch 4

TUESDAY, 9/14/2021

Danielle Halasz Sarah Cumberland

Floral dip

- There was growth for CLE18 multiplex AlcR (patch 20), but no growth for GUS-AlcR Agrobacterium
- Floral dip according to protocol was followed only for CLE18 multiplex AlcR.
- Made 1L of LB

THURSDAY, 9/16/2021

Amira Bouchema Dennis Tran

- Made 5L worth of hygromycin/MS plates

FRIDAY, 9/17/2021

Nathaniel Petersen

PCR

PCR was done following SOP on the following:

- AlcR-pHSN6A01 patches 1/2/3/4/5/6/7/8/9 (Tubes 8-16 are patches 1-9 with guide insert primer, tubes 35-43 are patches 1-9 with ALCR primer)
- ICE2 multiplex AlcR patches 1/2/3/4/5/6 (Tubes 17-22 are patches 1-6 with guide insert primer, tubes 44-49 are patches 1-6 with ALCR primer)

- CLE18 multiplex AlcR patches 1/2/3/4/5/6/7 (Tubes 1-7 are patches 1-7 with guide insert primer, tubes 28-34 are patches 1-7 with ALCR primer)
- GUS-AlcR streak 1 (Tube 55)
- Controls (Tubes 23,24 are controls with guide insert primer, tubes 50,51 are controls with ALCR primer)

SATURDAY, 9/18/2021

Sarah Cumberland Harkamal Samra

Seed Sterilizarion & Plating

The following seeds were sterilized and plated as per the protocol:

- CLE18 guide A: 6 plates
- CLE18 guide D: 6 plates
- CLE18 multiplex: 7 plates
- ICE2 multiplex: 5 plates
- ICE2 guide C: 2 plates
- WT seeds on hygromycin: 1 plate
- WT seeds on no antibiotic: 2 plates

Plated Seed Types/Plate Counts

Table10

	Seed Type	# of Plates	# of +ve Transformants
1	ICE 2 g5	1	0
2	CLE18 gC	3	0
3	ICE2 m	1	0
4	ICE2 gB	2	0
5	WT	10	N/A
6	ICE2 g3	1	0
7	ICE2 gE	3	0
8	GUS	4	0
9	ICE2 gA	3	0
10	ICE2 gC	3	0

MONDAY, 9/20/2021

Sarah Cumberland Hannah Vujovic

Gel Electrophoresis

- Gel electrophoresis was performed on the PCR samples from Friday as per the protocol
- All lanes were smeared with no clear bands.

Kulay Janneh Mike Green

PCR

- PCR was preformed on the following patches following the protocol
  - CRISPRa ALcR

    - CLE18 Multiplex AlcR CRISPRa
    - ICE2 Multiplex AlcR CRISPRa
- PCR TUBES 1-4 are CRISPRa ALcR, 5-8 are CLE18 Multiplex AlcR CRISPRa, 9-12 are ICE2 Multiplex AlcR CRISPRa. A legend is on Thermocycler.
- Colony water is in the fridge in case these PCR products don't show anything

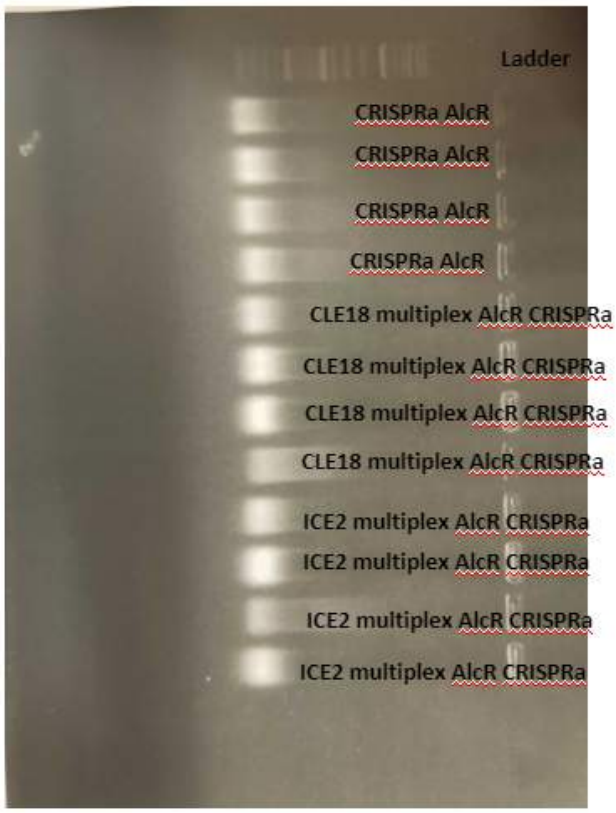
TUESDAY, 9/21/2021

Danielle Halasz Amira Bouchema

Gel Electrophoresis

- Performed on PCR products from yesterday; bands with streaks were still observed.

image.png



SATURDAY, 9/25/2021

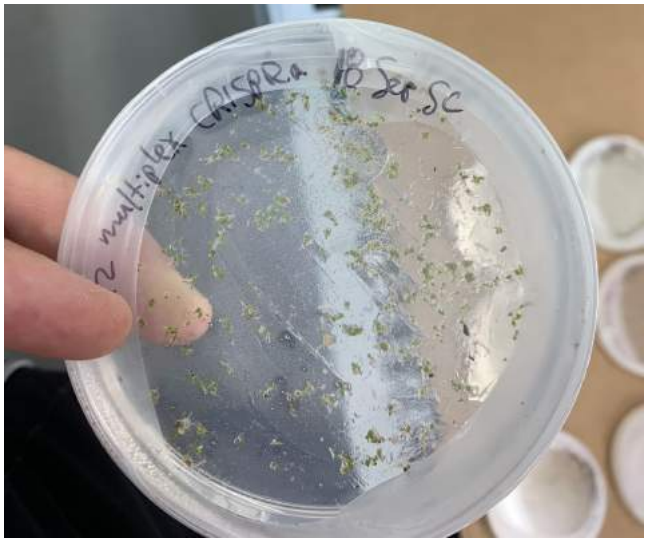
Sarah Cumberland Harkamal Samra Dennis Tran

Seed Plating

Seed plating was performed as per the protocol, with the following changes made:

- Each Eppendorf tube was filled with only 50uL of seeds. Same volumes of ethanol, bleach, and water were used. Seeds were resuspended in same volume of 0.1% glycerol at the end (1mL). Each Eppendorf was split between 2 plates, therefore ~25uL of seeds were plated on each plate. This was done in order to achieve low concentration of seeds on plates. Plates should have a final density similar to the following plate:

IMG_5962.jpeg



The following seeds were plated:

- CLE18 guide B CRISPRa
- CLE18 guide E CRISPRa

MONDAY, 9/27/2021

Sarah Cumberland Harkamal Samra

PCR

PCR was performed on the following patches as per protocol:

- pHSN6A01-AlcR patch 4
- pHSN6A01-AlcR CLE18 multiplex patch 4
- pHSN6A01-AlcR ICE2 multiplex patch 1

Replicates were set up using varying amounts of primers:

- 1:10, 1:50, 1:100

Tubes were labelled as follows:

1. positive control (DXR sgRNA) - guide insert verification primers @ 1:10
2. negative control (pHSN6A01) - guide insert verification primers @ 1:10
3. pHSN6A01-AlcR - guide insert verification primers @ 1:10
4. pHSN6A01-AlcR CLE18 multiplex - guide insert verification primers @ 1:10
5. pHSN6A01-AlcR ICE2 multiplex - guide insert verification primers @ 1:10
6. positive control (DXR sgRNA) - AlcR insert verification primers @ 1:10
7. negative control (pHSN6A01) - AlcR insert verification primers @ 1:10
8. pHSN6A01-AlcR - AlcR insert verification primers @ 1:10
9. pHSN6A01-AlcR CLE18 multiplex - AlcR insert verification primers @ 1:10
10. pHSN6A01-AlcR ICE2 multiplex - AlcR insert verification primers @ 1:10
11. positive control (DXR sgRNA) - guide insert verification primers @ 1:50
12. negative control (pHSN6A01) - guide insert verification primers @ 1:50
13. pHSN6A01-AlcR - guide insert verification primers @ 1:50
14. pHSN6A01-AlcR CLE18 multiplex - guide insert verification primers @ 1:50
15. pHSN6A01-AlcR ICE2 multiplex - guide insert verification primers @ 1:50
16. positive control (DXR sgRNA) - AlcR insert verification primers @ 1:50
17. negative control (pHSN6A01) - AlcR insert verification primers @ 1:50
18. pHSN6A01-AlcR - AlcR insert verification primers @ 1:50

19. pHSN6A01-AlcR CLE18 multiplex - AlcR insert verification primers @ 1:50
20. pHSN6A01-AlcR ICE2 multiplex - AlcR insert verification primers @ 1:50
21. positive control (DXR sgRNA) - guide insert verification primers @ 1:100
22. negative control (pHSN6A01) - guide insert verification primers @ 1:100
23. pHSN6A01-AlcR - guide insert verification primers @ 1:100
24. pHSN6A01-AlcR CLE18 multiplex - guide insert verification primers @ 1:100
25. pHSN6A01-AlcR ICE2 multiplex - guide insert verification primers @ 1:100
26. positive control (DXR sgRNA) - AlcR insert verification primers @ 1:100
27. negative control (pHSN6A01) - AlcR insert verification primers @ 1:100
28. pHSN6A01-AlcR - AlcR insert verification primers @ 1:100
29. pHSN6A01-AlcR CLE18 multiplex - AlcR insert verification primers @ 1:100
30. pHSN6A01-AlcR ICE2 multiplex - AlcR insert verification primers @ 1:100

TUESDAY, 9/28/2021

Hannah Vujovic Dennis Tran

Gel electrophoresis

SOP was followed for the following gels:

Using guide insert primers 1:10

gel1.pdf

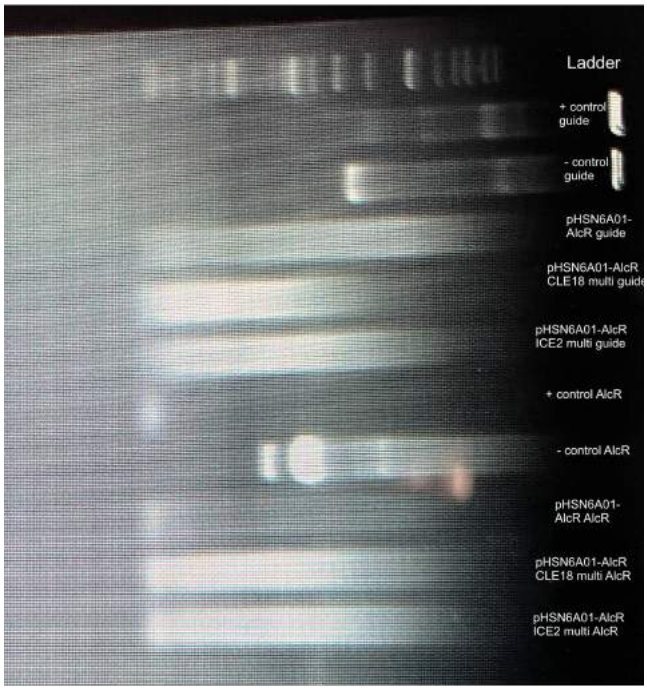
p. 1

Using AlcR primers 1:10

gel2.pdf
p. 1

Using primers 1:50

gel5.pdf
p. 1



Using AlcR primers 1:100

gel 4.pdf
p. 1

WEDNESDAY, 9/29/2021

Kulay Janneh Danielle Halasz

PCR

Frogga protocol was followed to perform PCR on the following:

- 1 ICE2 multi AlcR Patch 1 (alcR primers used)
- 3 ICE2 multi AlcR Patch 3 (alcR primers used)
- 5 CRISPRa AlcR Patch 1 (alcR primers used)
- 9 CLE18 multi AlcR Patch 1 (alcR primers used)
- 11 CLE18 multi AlcR Patch 3 (alcR primers used)
- 2 ICE2 multi AlcR Patch 2 (guide insert primers used)
- 4 ICE2 multi AlcR Patch 4 (guide insert primers used)
- 6 CRISPRa AlcR Patch 2 (guide insert primers used)
- 8 CRISPRa AlcR Patch 4 (guide insert primers used)
- 12 CLE18 multi AlcR Patch 4 (guide insert primers used)

PCR was run for 25 amplification steps instead of 35, colony watrer is in fridge for back up incase this gel doenst work.

THURSDAY, 9/30/2021

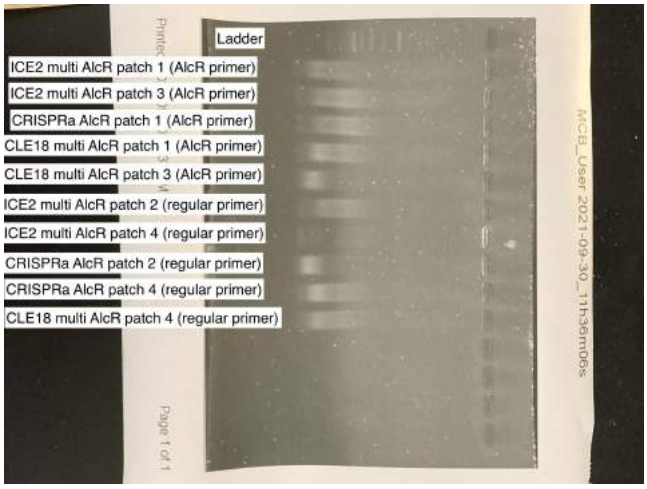
Amira Bouchema Mike Green

Gel Electrophoresis

- Done on the previous day PCR; followed as SOP

- All bands were smeared and unreadable

Gel image 30-09-2021.jpeg



SATURDAY, 10/2/2021

Amira Bouchema Dennis Tran

Seed Collection

Seeds collected are the following:

1. ICE2gD
2. CLE18 Multiplex
3. CRISPRa CLE18 GE
4. CRISPRa CLE18 Multiplex
5. CLE18 guide B

Full Tray was emptied as a result, One tray and a half to go.

TUESDAY, 10/5/2021

Danielle Halasz Mike Green

PCR

PCR following Frogga protocol on the following:

- 1 ICE2 multi AlcR Patch 1 (alcR primers used)
- 2 ICE2 multi AlcR Patch 2 (alcR primers used)
- 3 CRISPRa AlcR Patch 1 (alcR primers used)
- 4 CRISPRa AlcR Patch 2 (alcR primers used)
- 5 CLE18 multi AlcR Patch 2 (alcR primers used)
- 6 CLE18 multi AlcR Patch 1 (alcR primers used)
- 7 ICE2 multi AlcR Patch 3 (guide insert primers used)
- 8 ICE2 multi AlcR Patch 4 (guide insert primers used)
- 9 CRISPRa AlcR Patch 3 (guide insert primers used)
- 10 CRISPRa AlcR Patch 4 (guide insert primers used)
- 11 CLE18 multi AlcR Patch 3 (guide insert primers used)
- 12 CLE18 multi AlcR patch 4 (guide insert primers used)
- 13 + ive control (CLE18 multi AlcR) (guide insert primer used)
- 14 + ive control (ICE2 multi AlcR) (AlcR primers used)

Made new 1:10 dilutions of AlcR as well as guide insert forward and reverse primners

WEDNESDAY, 10/6/2021

Amira Bouchema

Gel Electrophoresis

Legend:

- 1 ICE2 multi AlcR Patch 1 (alcR primers used)
- 2 ICE2 multi AlcR Patch 2 (alcR primers used)
- 3 CRISPRa AlcR Patch 1 (alcR primers used)
- 4 CRISPRa AlcR Patch 2 (alcR primers used)
- 5 CLE18 multi AlcR Patch 2 (alcR primers used)
- 6 CLE18 multi AlcR Patch 1 (alcR primers used)
- 7 ICE2 multi AlcR Patch 3 (guide insert primers used)
- 8 ICE2 multi AlcR Patch 4 (guide insert primers used)
- 9 CRISPRa AlcR Patch 3 (guide insert primers used)
- 10 CRISPRa AlcR Patch 4 (guide insert primers used)
- 11 CLE18 multi AlcR Patch 3 (guide insert primers used)
- 12 CLE18 multi AlcR patch 4 (guide insert primers used)
- 13 + ive control (CLE18 multi AlcR) (guide insert primer used)
- 14 + ive control (ICE2 multi AlcR) (AlcR primers used)

Danielle Halasz Dennis Tran

Seed Sterilization

- CLE18 multi x 3 on hygromycin
- ICE2 guide D x 2 on hygromycin
- CLE18 guide B x4 on hygromycin
- ICE2 multi x 2 on hygromycij
- WT on hygromycin + MS
- WT on plain MS

Placed in the 4C fridge in Dr. Seah's room

MONDAY, 10/18/2021

Sarah Cumberland

RNA Extraction

RNA extraction, DNase I digestion, and RNA cleanup were performed as per the Qiagen RNeasy Plant Mini Kit protocols. The following samples were processed:

- 4 x samples of WT Arabidopsis thaliana (leaves)
- 2 x samples of ICE2 multiplex transformed Arabidopsis thaliana (leaves)
- 4 x samples of WT Arabidopsis thaliana (whole plant including roots)
- 2 x samples of CLE18 guide B transformed Arabidopsis thaliana (whole plant including roots)