# Week 12

## August 30, 2016

**Digested pgRNA vector for Gibson Assembly**

14 ul pgRNA Humanized
5ul xHo1
2ul BstX1
2ul 3.1 Buffer
26 ul Water

**Neg. Control**

14ul pgRNA Humanized
36ul Water

**Gibson Assembly into pSB1C3 vector**

Adar 1-1x:

2.66 pSB1C3 vector
10 ul NEBuilder
4.23 insert
3.11 water

Adar 1-2x:

2.66 pSB1C3 vector
10 ul NEBuilder
2.67 insert
4.67 water

Adar 1-3x:

2.66 pSB1C3 vector
10 ul NEBuilder
3.29 insert
4.05 water

Adar 2-1x:

2.66 pSB1C3 vector
10 ul NEBuilder
3.49 insert
3.85 water

Adar 2-2x:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 4.19 insert
- 3.15 water

Adar 2-3x:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 2.78 insert
- 4.56 water

Apobec 1x:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 3.11 insert
- 4.23 water

Apobec 2x:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 2.97 insert
- 4.37 water

Apobec 3x:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 3.11 insert
- 4.23 water

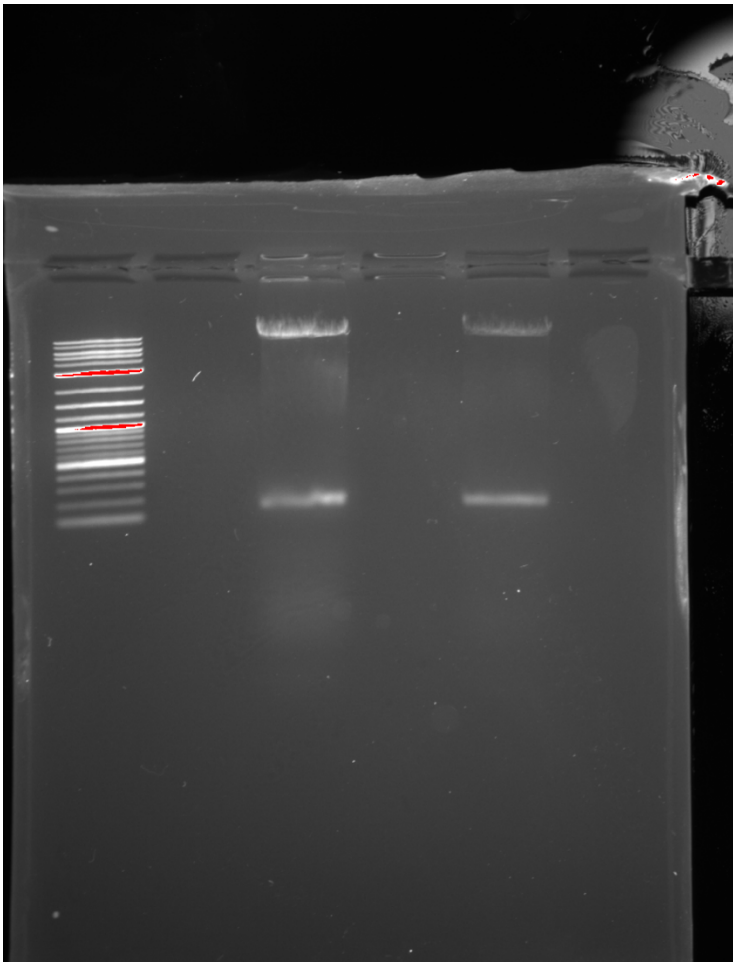
Negative Control:
- 2.66 pSB1C3 vector
- 10 ul NEBuilder
- 7.24 water

The mixtures were set up on ice and then run in a PCR machine for 30 minutes at 50˚C

10µl from each tube was transformed with 50µl dh5a cells and left on ice for 30 minutes
The tubes containing the 60 µl mixture were then put in the 42˚C water bath for 30 seconds, then put on ice for 2 minutes.
950 µl of SOC was added to each tube and gently mixed
The tubes were put in the shakubator for an hour
100 µl of each tube was plated in triplicate on CAM plates and left overnight in the incubator ~7:30pm

# August 31, 2016

**Gel Purified Vector for Gibson Assembly**



Lane 1 – Ladder
Lane 2 – Empty
Lane 3 – PgRNA
Lane 4 – Empty
Lane 5 - pgRNA

**Gibson Cloning**

**Globin pgRNA**

2ul Insert
10ul Gibson mix
9ul Vector

**Neg Control**

2ul vector
10ul Gibson mix
8ul Water

Plates grew very nicely (~30 colonies per plate), but the negative control plates had upwards of 50 colonies as well

Made more CAM plates

1L to 1mL of CAM

Made more CAM stock

.33 g to 10MmL 99.5% EtOH

Picked 8 colonies from Apobec 2-2x plate

Grew Liquid Cultures

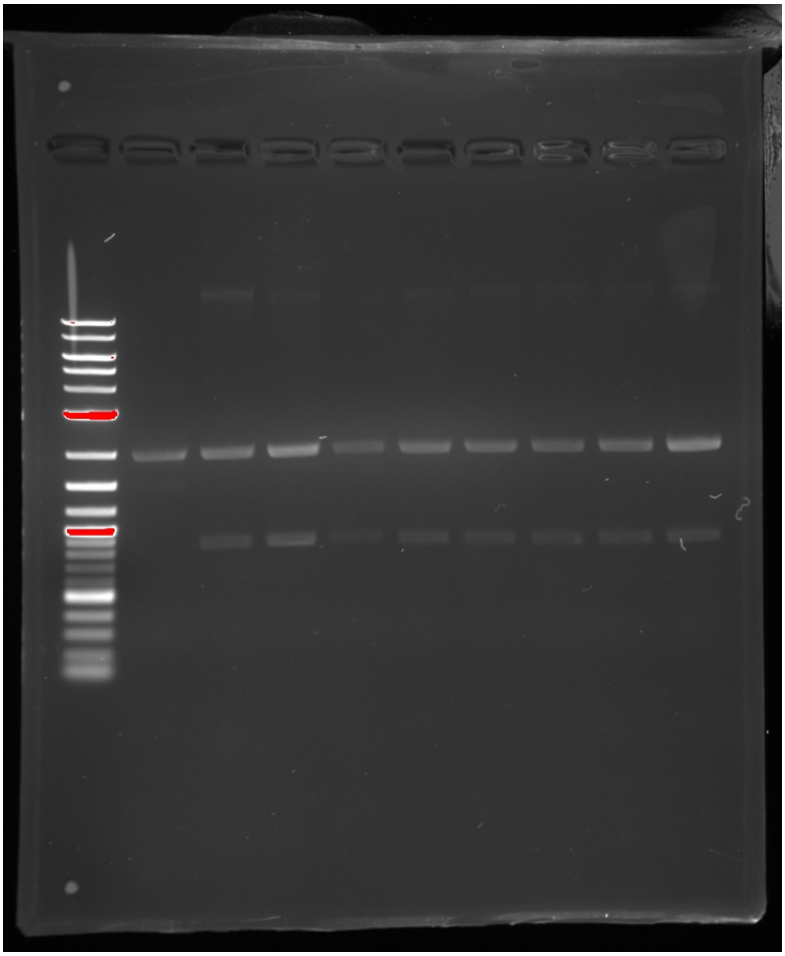
# September 1, 2016

Made glycerol stocks of pSB1C3 Apobec 2x-2 (1-8)

Mini-prepped pSB1C3 Apobec 2x-2 (1-8)
Digested Eco/xba and spe/psd or one digest with not1
XX ul psb1c3 apobec 2x 2-1

**Neg. Control**
Digested mini-preps using protocol:
1ug (volume to be determined) plasmid
2uL cutsmart buffer
2uL Not1
Adjust volume to 20uL

Ran on gel
Added 3µl dye



Lane 1- Ladder
Lane 2- Negative Control
Lane 3- pSB1C3 with Apobec 2x-2 #1
Lane 4- pSB1C3 with Apobec 2x-2 #2
Lane 5- pSB1C3 with Apobec 2x-2 #3
Lane 6- pSB1C3 with Apobec 2x-2 #4
Lane 7- pSB1C3 with Apobec 2x-2 #5
Lane 8- pSB1C3 with Apobec 2x-2 #6
Lane 9- pSB1C3 with Apobec 2x-2 #7
Lane 10- pSB1C3 with Apobec 2x-2 #8

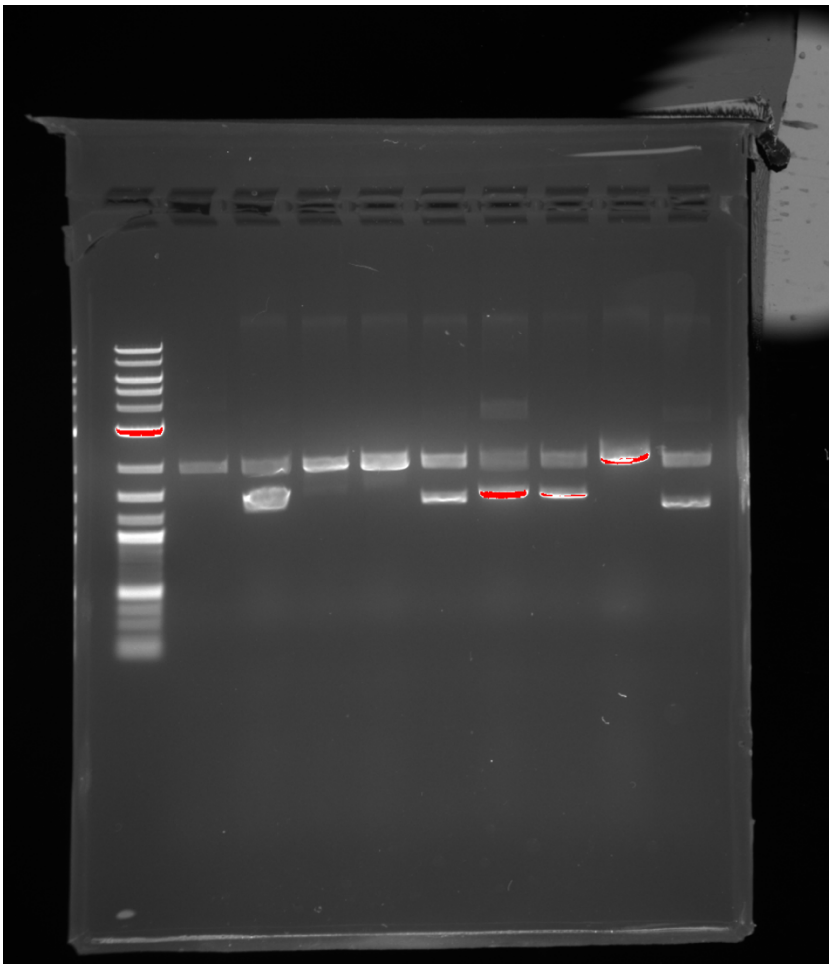
"The most beautiful gel I have ever seen" - Natalie 9/2/16

# September 2, 2016

Globin pgRNA did not grow
Made liquid cultures of PSB1C3 clones

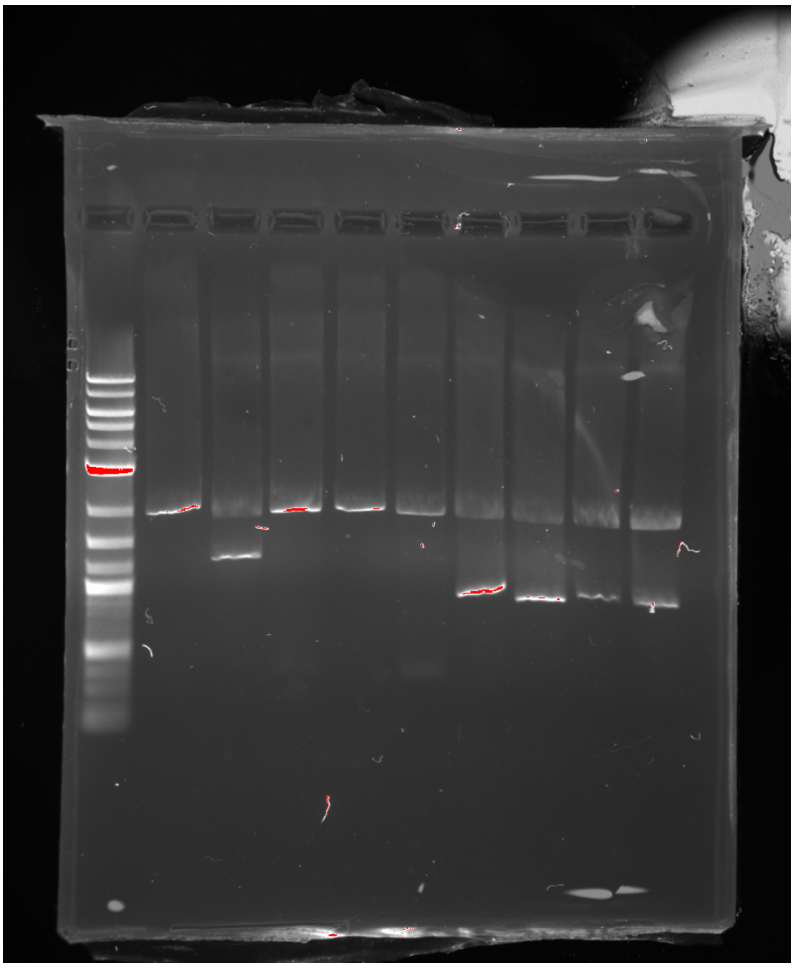
September 3, 2016

Mini-prepped PSB1C3 clones
Digested with Not1
Ran on Gel



Gel 1 – PSB1C3 Clones
Lane 1 – Ladder
Lane 2 – Neg Control
Lane 3 – Adar 1-1x #1
Lane 4 – Adar 1-1x #2
Lane 5 – Adar 1-2x #1
Lane 6 – Adar 1-2x #2
Lane 7 – Adar 1-3x #1
Lane 8 – Adar 1-3x #2
Lane 9 – Adar 2-1x #1
Lane 10 – Adar 2-1x #2

Analysis
The negative control ran correctly. The correct clones are Adar 1-1x #1, Adar 1-2x #2, Adar 1-3x #1 and #2, Adar 2-1x #2.



Gel 2 – PSB1C3 Clones
Lane 1 – Ladder
Lane 2 – Neg Control
Lane 3 – Adar 2-2x #1
Lane 4 – Adar 2-2x #2
Lane 5 – Adar 2-3x #1
Lane 6 – Adar 2-3x #2
Lane 7 – Apobec 1 #1
Lane 8 – Apobec 1 #2
Lane 9 – Apobec 3 #1
Lane 10 – Apobec 3 #2

Analysis
The negative control ran correctly. The correct clones are Adar 2-2x #1, Apobec 1 #1 and #2, and Apobec 3 #1 and #2.