Week 6

July 11, 2016

Results from Weekend Transformations

5 colonies on 5ul + 15ul insert plate
- 3 single
- 2 double

5 colonies on 5ul + 2ul insert plate
- 5 single

Nothing grew from our ligation

7/8 colonies on each pgRNA transformation

Retry of pgRNA
digested 10ug with Xbst1 and Xho1, will sit overnight

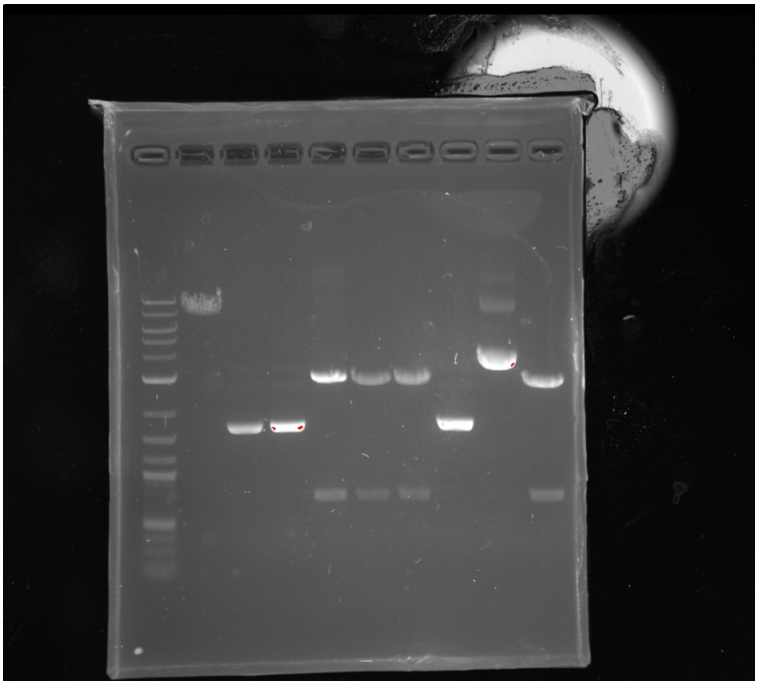
Picked colonies from old ligation method, grew liquid cultures

PCR of ATG-eGFP and ACG-eGFP

- Digest (vector prep)
  - DNA: pgRNA
  - Enzymes: BstXI, XhoI
  - 3.1 buffer, 5 μL
  - 10 μg (12 μL) DNA (0.833 μg/μL)
  - 2 μL each enzyme
  - Total = 50 μL
- PCR
  - GFP – wild type/ACG mutant
- PCR clean up
- Digest (GFP insert)
  - Enzymes: BamHI, XbaI
- Liquid culture
  - pRetro-dCas9 ligation colonies (16 total)

July 12, 2016

Gel 1: Digested DNA

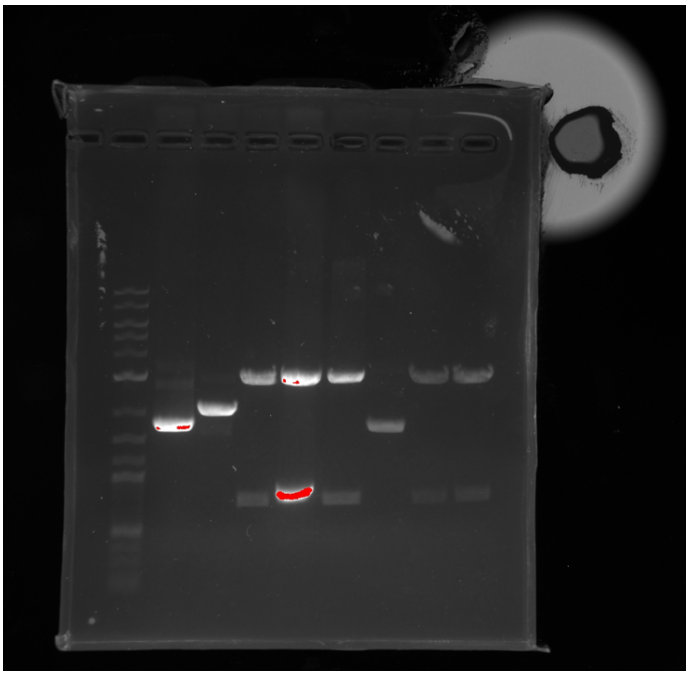


Lane 1 – Ladder
Lane 2 – Neg. Control
Lane 3 – Clone 2-5 #1
Lane 4 – Clone 2-5 #2
Lane 5 – Clone 2-5 #3
Lane 6 – Clone 15-5 #1
Lane 7 – Clone 15-5 #2
Lane 8 – Clone 15-5 #3
Lane 9 – Clone 15-5 #4
Lane 10 – Clone 15-5 #5

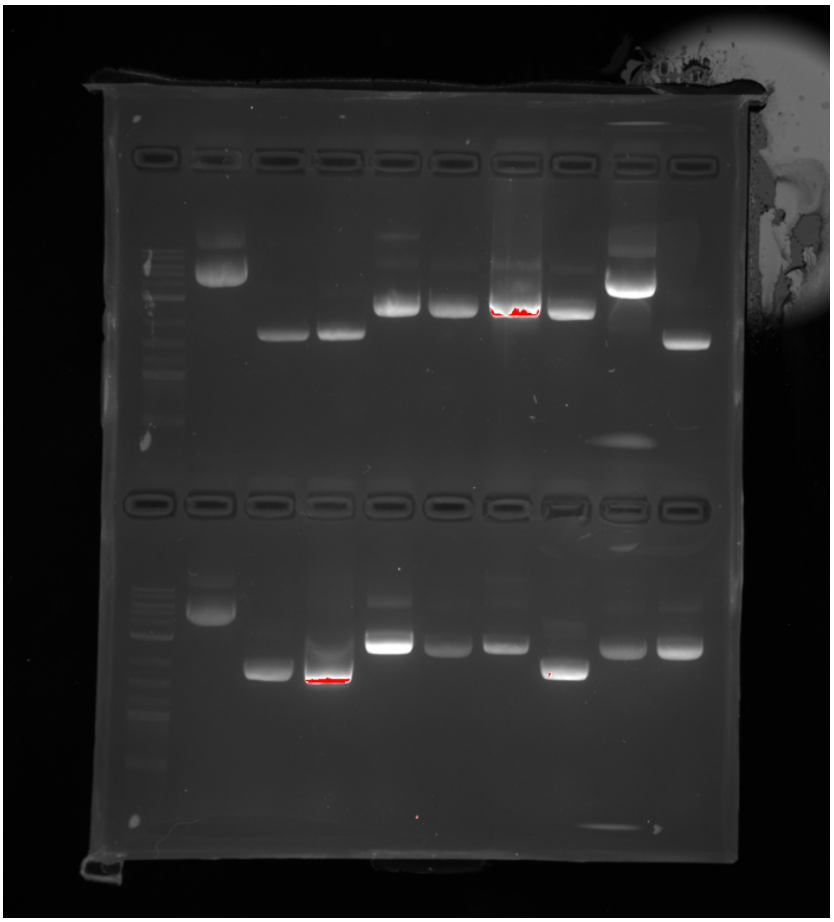
Analysis

The negative control ran a little higher than the 6.8kb mark. Every other clone did not run correctly, some show dCas9 by itself without a vector while others cannot be explained.

Gel 2: Digested DNA



Lane 1 – Ladder
Lane 2 – Clone 8-5 #1
Lane 3 – Clone 8-2 #2
Lane 4 – Clone 8-5 #3
Lane 5 – Clone 8-5 #4
Lane 6 – Clone 8-5 #5
Lane 7 – Clone 8-5 #6
Lane 8 – Clone 8-5 #7
Lane 9 – Clone 8-5 #8



Top
Lane 1 – Ladder
Lane 2 – Neg. Control
Lane 3 – Clone 2-5 #1
Lane 4 – Clone 2-5 #2
Lane 5 – Clone 2-5 #3
Lane 6 – Clone 15-5 #1
Lane 7 – Clone 15-5 #2
Lane 8 – Clone 15-5 #3
Lane 9 – Clone 15-5 #4
Lane 10 – Clone 15-5 #5
Bottom
Lane 1 – Ladder
Lane 2 – Neg. Control
Lane 3 – Clone 8-5 #1
Lane 4 – Clone 8-5 #2
Lane 5 – Clone 8-5 #3
Lane 6 – Clone 8-5 #4
Lane 7 – Clone 8-5 #5
Lane 8 – Clone 8-5 #6
Lane 9 – Clone 8-5 #7
Lane 10 – Clone 8-5 #8

Analysis

Nothing ran correctly, except for the ladder!

WE ARE READY TO TRY SOMETHING NEW!!

- Glycerol stock
  - pRetro-dCas9 ligation liquid cultures (16 total)
- Miniprep
  - 2-5 #2: 197.7 ng
  - 8-5 #8: 399.5 ng
- Digest
  - Miniprep-ed DNA
  - Enzymes: PacI, BamHI
- Gel
  - pgRNA digested with BstXI, XhoI
    - 60 µL total volume
- Gel purification
  - pgRNA (Top band)
    - 0.4 g gel → 800 µL NTI
- Gel
  - pRetro-dCas9 (digested & undigested)

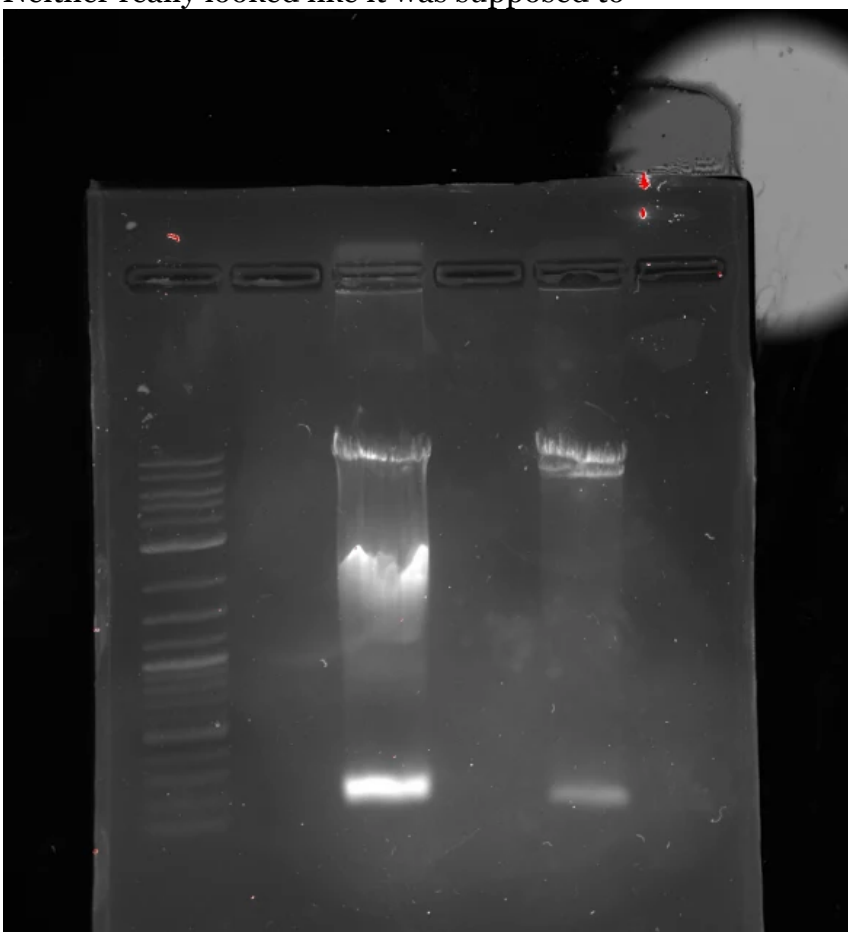
Heat Killed ACG/ATG eGFP inserts
Gel Purified

gel ran pgRNA
purified
.4g DNA, .8mL NT1

miniprepped liquid cultures, most 200-400 ng/uL
Mastermix
18uL Cutsmart
9uL each enzyme
72uL water
4uL DNA

ran gel or uncut and cut with Pac1 and BamH1

Neither really looked like it was supposed to



July 12, 2016

• Primer design

Ligation of eGFP into pcDNA3.1
2uL vector (pcDNA),
0ul/2ul/8ul/15ul eGFP insert
15ul/13ul/7ul/0ul Water
2 uL Ligation Buffer
1 ul T4 Ligase

Incubated at RT for 30 minutes
Transformed into DH5Alpha cells, (5uL/10uL Ligation into 50 uL DH5Alpha Cells)

GM:
Liquid cultures RFP, GFP

July 14, 2016

Interview with Dr. Wen Xue and his grad student Zachary Kennedy
Transformed BE2 given to us by ^

Made 2x Amp plates
40 g of LB AGAR powder in 1 L water
Mix well, autoclave, let cool to touchable temp
Add 1mL of 200 Amp

• Digest (vector prep)
DNA: pRetro
Enzymes: BamHI, PacI
- 10 µg DNA

• Colony PCR
Primers:
- GFP only: #62, #63
- Sequence: 3' BGH, 5'
(12 per set of primers)

DNA:
- WT (ATG) eGFP
- Mutant (ACG) eGFP

Per reaction (15 µL):
- 7.5 µL MasterMix
- 0.5 µL each primer
- 4.5 µL water
- 1 µL template

(Make stock for 25 reactions for each set of primers)
Total 48 reactions

• Gel
48 colony PCRs
- Total volume (~15 µL)
- 3 µL dye

• Liquid culture
Colonies 1-6 (WT & ACG) + 1 negative control each

P-retro digestion and gel purification with NotI and BamHI
11uL p-retro
32uL water
3uL buffer
2uL each enzyme

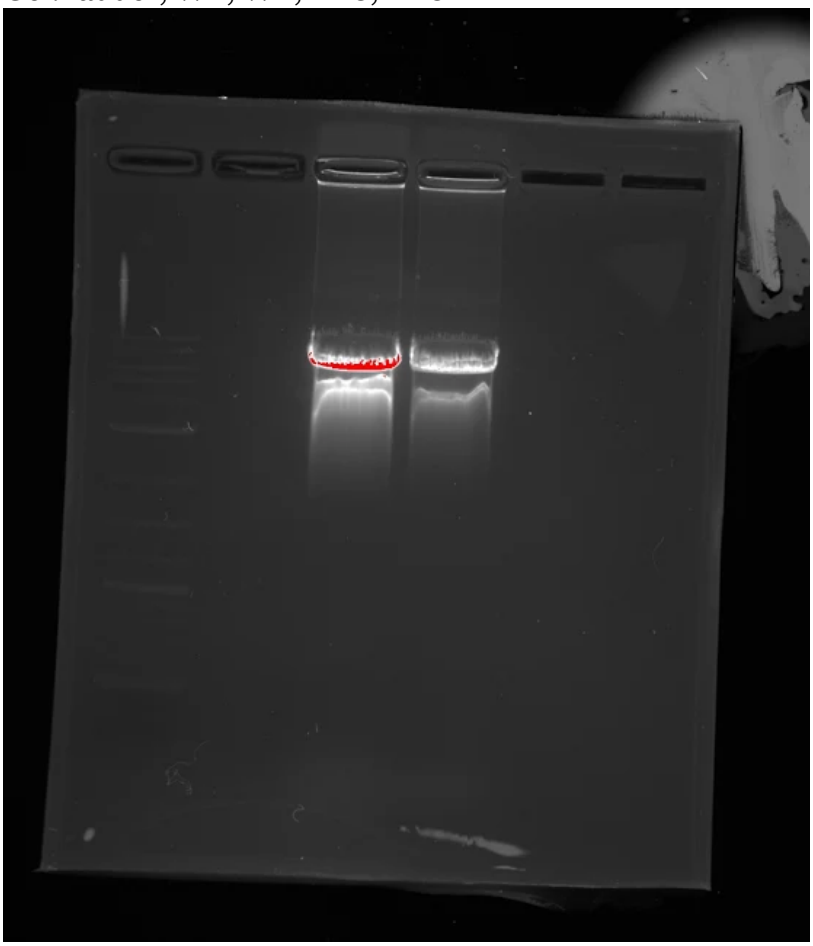
New insert, PTC and WT globin
spin down and add 50uL water
20uL of each to tube
3uL cutsmart
1uL XhoI

1uL BamHI
5uL water

digest 2hours
heat kill 80 degrees 20 mins

Gel purified even though we weren't supposed to

Gel: ladder, WT, WT, PTC, PTC



nanodropped, WT- about 4ng/uL, PTC about 8ng/uL

Colony PCR of ACG-GFP +WT-GFP
-One set using GFP Primers
-One set using Sequencing Primers

Selected first 6 of WT and Mutant for liquid cultures

July 15, 2016

Ligated globin into peGFP
Insert: 0uL, 8uL, 15uL
heated 2 mins, let return to room temp
transformation
plated on Kan plates

Mini prepped eGFP DNA
-Checked on gel
Digested with xbaI and BamHI
Heat killed
Ran on gel

- Miniprep
  12 WT + ACG eGFP (+ 2 negative)
  WT: 637.3, 766.4, 532.8, 815.3, 823.7, 887.1, 678.3
  ACG: 853.8, 633.3, 925.8, 862.3, 908.1, 721.0, 750.5
- Digest
  Miniprep-ed DNA
  Enzymes: BamHI, XbaI
  - 1 µL each enzyme
  - 2 µL CutSmart buffer
  - About 1 µg DNA

  (20 µL total)
- Gel
  Digested WT & ACG eGFP

  - Band at about 5-6 kbp for all (negative control included)