03/30/15

Objective: Get our feet wet with transformation protocol

Matt Farnitano *et al.*

Transformation from iGEM 2014 kit

- Plate 4 #5H: pSB1A2-BBa_R0011
- Transformed into DH5alpha CCEC
- Plated on LB+amp
- Process
    - Heat shock protocol
- Details/Notes
    - weird pipetting problems at the start--we think some ink got into the DNA
    - heat shock temperature w
    - as not high enough--around 37

Next steps:

- Matt will check the plates for colonies in the morning

05/04/15

Objective: Prepare You Lab lysis genes for plasmid creation

Alvin Han *et al.*

Miniprep for You Lab Samples

- Incubated samples from You lab (PCSaE 500) from ~11:30 AM to _____
- Samples archived @ -80°C, (700 microliters sample, 300 microliters glycerol)
- Process
    - QIAGEN miniprep protocol/kit
- Details/Notes
    - stored miniprepped stuff in the -20

05/11/15

Objective: Prepare the oligonucleotide primers for future use in Golden Gate Assembly

Alvin Han, Sarah Jacobs, Ben Hoover, David Brenes

Reaction/Protocol of elements

- Resuspended dry oligonucleotides in DI water to create 100 µM stocks
- Created 25 µM working stocks; 25 µL stock, 75 µL DI water
- Details/Notes
    - Stored all in -20°C freezer in 2015 iGEM box

05/18/15

Objective: Transformed biobricks (K11700, R0011, K608012) into CCEC

David Brenes, Sarah Jacobs, TJ Ciesla

Transformation of CCEC with biobricks

- Followed Charlie's Cloning Protocols
    - Used Buschler CCEC because we couldn't find any from last year
- Plated the transformed cells and put in incubator at 37◦C

Results (5/19/15): transformation successful!

Next steps:

- Look at plates, prepare an iGEM stock of CCEC
- Miniprep K11700 and R0011
- BioBrick Assembly of K608012 (Induced GFP) - dCas9 -tracrRNA

5/19/15

Objective: Make amp and cm colonies

- Produced 1L of LB+amp and LB+cm

Objective: Design for lac-dcas9 primers

- Ordered

Objective: Grow colonies of K608012, R0011, K117000, DH5alpha

- Picked one colony of each

Results: Low DNA concentration

Next Steps: Grow R0011 on Cm, Grow another colony

5/20/15

Objective: Miniprep K608012, R0011, K117000

- Miniprepped by QIAgen
- Restriction test expected results
    - K608012-pSB1C3: Test (EcoRI/SpeI) ~800 bp and at ~2000 bp
    - R0011-pSB1A2: Test (EcoRI/EcoRV) : Bands at ~900 bp and ~1230 bp
    - K117000-pSB1C3: Test (EcoRI/EcoRV) : ~900 bp and ~ 1300 bp
- **Results:** Everything looks good

Objective: BioBrick Assembly

- Preparatory Digests
    - K117000-pSB1C3 (EcoRI/SpeI): ~ 200 bp
    - R0011-pSB1A2 (EcoRI/XbaI): Collect ~2000 bp
- Gel Purification
    - Used Yellow Box Protocol
- Results: K117000: 12.5 ng/uL
    - R0011: 10.9 ng/uL

Objective: Make competent cells

Protocol

- Used Charlie's protocol
- Stored competent cells in -80°C "Competent Cells" box

Objective: Ligation of K11700 and R0011

David Brenes, Sarah Jacobs, TJ Ciesla, Alvin Han, Jeremy Gonzales, Ben Hoover

Ligation protocol

- Used ligation protocol -> new calculator (http://nebiocalculator.neb.com/#!/ligation)
- 2 µL K117000
- 6 µL R0011

Results(5/21/15):

- Restriction digest of pSB1A2_R0011 using EcoRI/EcoRV was unsuccessful: EcoRV has no restriction site in the pSB1A2 backbone.
-

5/21/15

Objective: Re-Ligation of K11700 and R0011

David Brenes, Sarah Jacobs, TJ Ciesla, Alvin Han, Jeremy Gonzales, Ben Hoover

Ligation protocol

- Used ligation protocol -> new calculator (http://nebiocalculator.neb.com/#!/ligation)
- **6** µL K117000
- **2** µL R0011
- *switched backbone and insert the first time

Objective: Transformed Ligation of K11700 and R0011 into CCEC

David Brenes, Sarah Jacobs, TJ Ciesla, Alvin Han, Jeremy Gonzales

Transformation Protocol

- Followed Charlie's Cloning Protocols
    - used 10 µL of the ligation mix due to low concentration

Objective: Inoculation

5/22/15

Objective: Minipreps

- K11700_1C3
- K608012_1C3
- R0011_1A2
- R0011_1C3
- pdCas9

Objective: Design - Final Construct with Antibiotic

- Antibiotic Attacked: Km

Objective: Design Antimicrobial peptide

- Oligos designed:

Biobrick Assembly

- Part 1: R0011
- Part 2: K117000
- Backbone: pSB1A3
- Results: Success!

5/26/15

Objective: Lysis Gene Experimental Design

[TJ, Ben, Jeremy, Alvin]

Information:

- Measured during log phase (OD = .4)
- 4 time spots (30 min, 1hr, 2hr, 4hr)
- 5 samples
- 70 μL cuvettes
- Induction (mM) = (0/ .01/ .05/ .1/ .25/ .5/ 1/ 2)
    - Total: 3.91mM
- Begin stopwatch when tubes inserted in incubator.

Error Anticipation:

- Keep samples on ice as OD is measured at each time.
- 2 teams, each on a spectrophotometer.
- When measuring, do not test each induction 2 times in a row. Go down the list of assigned inductions.

Steps:

1. Prepare .25L cells.
2. Pipette into tubes
3. Insert IPTG
4. Spectrophotometer samples

05/27/15

Objective: Characterize the effectiveness of the You Lab Lysis gene

Alvin Han, TJ Ciesla, David Brenes, Ben Hoover, Jeremy Gonzales, Sarah Jacobs

Reaction/Protocol of elements

- See final “K_117000 Characterization” protocol
- Details/Notes

Results (if Applicable) (Date): **Significant difference between on and off, but only 3% average difference between no IPTG and max IPTG**

Next steps:

- Add a table of contents/linking abilities to this template

5/28/15

Objective: Anneal GFP1 oligos and RFP1 oligos using Thermocycler

TJ, Jeremy, Sarah, David, Alvin, Ben

Protocol

- “Put 10 μL of RFP1 up with 10μL RFP1 down into PCR tube. Put on thermocycler at 66 °C for 15 min. Let anneal at 50 °C for 45min. Continue annealing at room temperature for 1 hour.” (Done with both GFP1 and RFP1)
- Set Thermocycler to 1 cycle.
- Added 7 μL of the backbone (1C3) to 1μL of the RFP1.

Objective: Insert oligos into rep-seq-rep

- Restriction digest rep-seq-rep with Bsa1
- Ligate GPF1 and RFP1 into rep-seq-rep
- Transform into competent cells
- Standard Protocols
- **Results**: Successful colonies

5/29/15

Objective: Miniprep rep-seq-rep (x2)

- Qiagen kit
- 52.9 and 42.3 ng/uL

Objective: PCR BioBrick prefix and suffix onto the YouLabGene

- Primers: YouLabPrefix, YouLabSuffix
- Anneal temp: 40 C
- Extension Time: 7 seconds
- PCR Cleanup Prepared

Objective: Anneal RNA 2-5 oligos

- Followed protocol using down and up oligos for each gRNA

Objective: Ligate YouLabGene on to pSB1C3

- PCR You Lab Lysis Gene with YouBioBrickPre and YouBioBrickSuf
- Restrict PCRed YouLabGene and pSB1C3 with EcoRI and SpeI
- Ligate parts
- Transform

Objective: Ligate rep-gRNA-rep (RFP 2-5)

- Restrict rep-seq-rep with BsaI
- Ligate in gRNA oligos
    - 1.5 uL of oligos, 5.25 of uL
- Transform

Objective: Gibson pdCas9 into R0011

- PCR of pdCas9
    - Primers: PrefixdCas9Up, dCas9LacDn
    - Anneal temp: 36 C
    - Extension Time: 90 seconds
    - PCR Cleanup Prepared
- PCR of R0011
    - Primers: dCas9LacUp, PrefixdCas9Dn
    - Anneal temp: 36 C
    - Extension Time: 90 seconds
    - PCR Cleanup Prepared
- Gibson Assemble dCas9 into R0011
    - **Not performed, unsuccessful PCR**
- Transform into chemically competent cells

5/30/15

Objective: Store plates in 4C

- Colonies found on all plates, but only 3 on You Lab BioBrick

Objective: Store culture tubes in 4C

5/31/15

Objective: Store culture tubes in 4C

- Store

6/1/15

Objective: Miniprep rep-gRNA-rep (RFP 2-5), You Lab BioBrick (x2)

- Qiagen kit
- RFP 2: 43.4 ng/uL; RFP 3: 71.4 ng/uL; RFP 4: 55.6 ng/uL; RFP 5: 52.1 ng/uL; You Lab Biobrick 1: 36.1 ng/uL; You Lab Biobrick 2: 39.3 ng/uL

Objective: PCR BbsI sites onto the rep-gRNA-rep

- Primers: Bbs1-A-5p and Bbs1-B-3p (RFP 1), Bbs1-B-5p and Bbs1-C-3p (RFP 2), Bbs1-C-5p and Bbs1-D-3p (RFP 3), Bbs1-D-5p and Bbs1-E-3p (RFP 4), Bbs1-E-5p and Bbs1-Z-3p (RFP 5), Bbs1-E-5p and Bbs1-Z-3p (GFP 1), Bbs1-Z-5p and Bbs1-Z-3p (R0011-1A2)
- Anneal temp: 34 C
- Extension Time: 5 seconds
- PCR Cleanup Prepared

Objective: Restrict Bbs1-added rep-gRNA-rep with Bbs1

- Ligation sized restrictions

Objective: Analytic digest You Lab BioBrick

- Cut with EcoRI, EcoRV, SpeI
- Predict bands at 300, 900 and 1150
- **Results: 900 and 1150 bounds found, but 300 band not observed. Further tests necessary**

Objective: Ligate and Transform the R0011-rep-seq-rep-chain-1A2

- Ligate and transform by protocol
- **Results: Successful ligations**

6/2/15

Objective: Prepare You Lab BioBrick and rep-seq-rep for sequencing

- Performed BigDye protocol in Buchler lab
- Delivered samples to BioSci 123

Objective: Inoculate R0011-rep-(RFP 1-4, GFP)-rep-1A2, R0011-rep-(RFP 1-5)-rep-1A2

6/3/15

Objective: Miniprep R0011-rep-(RFP 1-4, GFP)-rep-1A2, R0011-rep-(RFP 1-5)-rep-1A2

- Standard Qiagen protocol

Objective: Gibson pdCas9 into R0011

- PCR of pdCas9
    - Primers: PrefixdCas9Up, dCas9LacDn
    - Anneal temp: 66 C
    - Extension Time: 60 seconds
    - PCR Cleanup Prepared
- PCR of R0011
    - Primers: dCas9LacUp, PrefixdCas9Dn
    - Anneal temp: 66 C
    - Extension Time: 60 seconds
    - PCR Cleanup Prepared
- Gibson protocol

6/4/15

Objective: Begin Assembly of Antibiotic Resistant Plasmid

- PCR of pdCas9
    - Primers: PrefixdCas9Up, dCas9LacDn
    - Anneal temp: 72 C
    - Extension Time: 60 seconds
- PCR of R0011
    - Primers: dCas9LacUp, PrefixdCas9Dn
    - Anneal temp: 72 C
    - Extension Time: 60 seconds

Objective: Biobrick Assembly

Sarah Jacobs and Parth Patel

- Preparatory Digest of pdCas9, R0011, and 1A3 Backbones
    - pdCas9 (EcoR1/Spe1)
    - R0011 (Xba1/Pst1)
    - 1A3 Backbone (EcoR1/Pst1)
- T4 Ligation
    - 4 µL backbone
    - 8.8 µL dCas9
    - 3.2 µL R0011
    - used Charlie's protocol

6/5/15

Objective: Continue Assembly of Antibiotic Resistant Plasmid

- Innoculate pTet (1A2)

6/7/15

Objective: Begin Assembly of Antibiotic Resistant Plasmid

- Innoculate
    - dCas9 + tracr (1C3)
    - Z1 comp cells

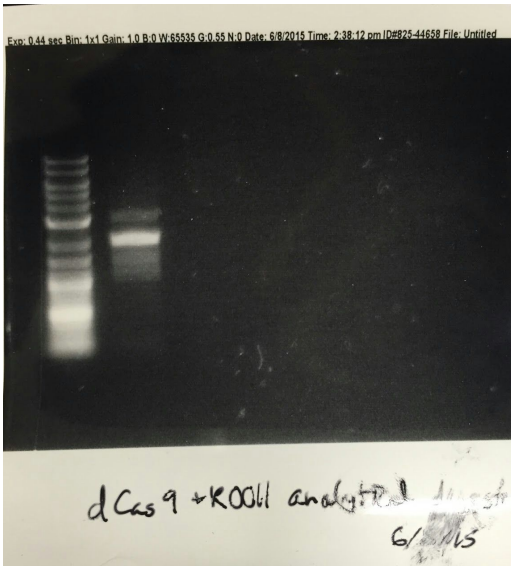
6/8/15

Objective: Lab Maintenance

- Made new batch of Z1 Comp cells

Objective: Begin assembly of GG for antibiotic resistance targeting plasmid

- *Miniprep pTet-1A2, dCas9-R0011-1A3*
    - Qiagen kit
- Assemble GG oligos
    - rehydrate oligos according to recommendations
    - anneal KNR 2-6 up with corresponding KNR 2-6 down
        - melt at 68 C for 15 minutes, anneal at 50 C for 45 min
        - continue annealing at room temp for 1 hour
- dCas 9 + R0011 analytical digest
    - Restriction Enzymes: Ear I and Pst I
    - Gel electrophoresis done



Results: Inconclusive, multiple Ear I binding sites...

- Ligate KNR fragments into rep-seq-rep plasmid
    - rep-seq-rep previously digested with Bsa1

    - 7 µL backbone to 1 µL KNR
    - Charlie's T4 ligation protocol
- Transform comp cells with rep-KNR(2-6)-rep
    - made 10 plates (2 for each insert)
    - standard transformation protocol

6/9/15

Objective: Lab Maintenance

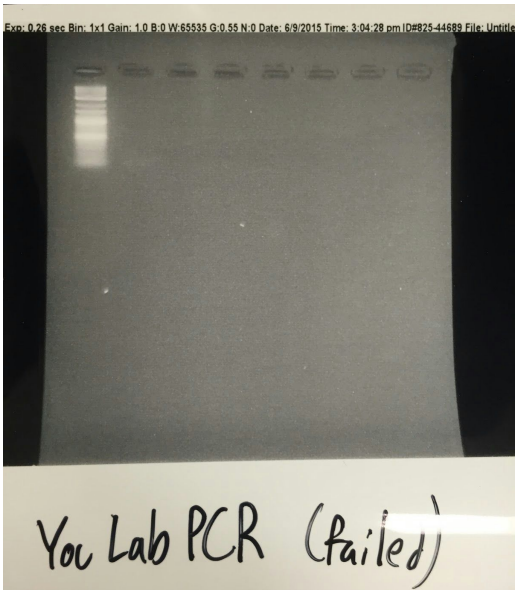
- Made new batch of SOC media

Objective: Inoculate KNR 2-6

- grew overnight in LB-Cm

Objective: Does You Lab Gene Work?

- PCR of You Lab Gene
- Ran Gel Electrophoresis
    - Results Negative for presence of Gene (No Bands)



    - Next Steps: Ordering G-block of Gene

6/10/15

Objective: miniprep KNR 2-6

Parth, Jeremy, Sarah

- QIAGEN Miniprep Kit
    - Standard procedure
    - Changes: Used refrigerated Buffer P1 with RNAse, No Buffer PB added
- Nanodrop Procedure for concentrations of samples

Results:

Sample:	Concentration (ng/μL):	Sample:	Concentration (ng/μL):
Rep-KNR2-Rep-IC3(A)	79.8	Rep-KNR4-Rep-IC3(B)	123.8
Rep-KNR2-Rep-IC3(B)	173.5	Rep-KNR5-Rep-IC3(A)	149.8
Rep-KNR3-Rep-IC3(A)	161.1	Rep-KNR5-Rep-IC3(B)	127.4
Rep-KNR3-Rep-IC3(B)	188.8	Rep-KNR6-Rep-IC3(A)	115.4
Rep-KNR4-Rep-IC3(A)	141.7	Rep-KNR6-Rep-IC3(B)	133.2

Objective: PCR KNR 2-6

Jeremy, TJ

- PCR of KNR 2-6 and R0011&dCAS9 Backbone
    - Sample + Primers:
        - KNR 2 + 5'-BbsI A-Repeat and 3'-BbsI-B-Repeat
        - KNR 3 + 5'-BbsI B-Repeat and 3'-BbsI-C-Repeat
        - KNR 4 + 5'-BbsI C-Repeat and 3'-BbsI-D-Repeat
        - KNR 5 + 5'-BbsI D-Repeat and 3'-BbsI-E-Repeat
        - KNR 6 + 5'-BbsI E-Repeat and 3'-BbsI-Z-Repeat
        - R0011&dCas9 on **1A3** + 5'-BbsI Z-Suffix and 3'-BbsI-A-R0011
    - Anneal temp: 72 C
    - Extension Time: 2 minutes
    - Notes: Used Buchler Lab PCR
- Standard PCR cleanup

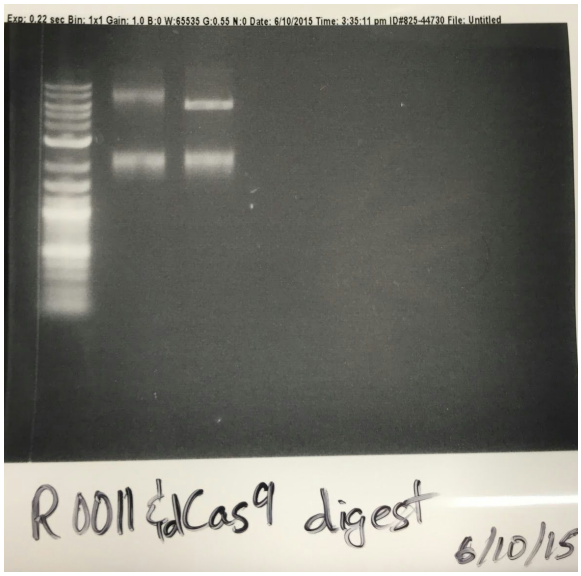
Results:

Sample:	Concentration (ng/μL):	Sample:	Concentration (ng/μL):
KNR2	12.6	KNR5	1.0
KNR3	115.7	KNR6	14.3
KNR4	-7.4	dCas9+R0011+1A3	9.8

Objective: Analytical Digest of R0011&dCas9

- dCas 9&R0011 analytical digest
  Restriction Enzymes:
    - One sample with BamHI
    - One sample with BamHI and XbaI
- Ran gel electrophoresis

Results: Promising, yet ambiguous



Objective: Restriction Digest and then Ligation

- Digested all PCR samples with BbsI
- Ligation of all 6 samples together
    - .5 µL of KNR3 (due to higher concentration), 1.5 µL of all other samples
- Notes: Failed to add backbone to the rest of the samples, Ligation Failed

Next Steps: Religate and continue tomorrow

6/11/15

Objective: Ligation and Transformation of KNR 2-6 and dCas9&R0011&1A3 Backbone

- Ligation of all 6 samples together
    - .5 µL of KNR3 (due to higher concentration), 1.5 µL of all other samples
- Transformation done. 2 plates made

Objective: Discover why PCRs are not working

- *Try new dNTPs*
- *Test BbsI PCR primers of rep-seq-rep*
- *Test BigDye Primers (pSB1C3 backbone)*

Procedure:

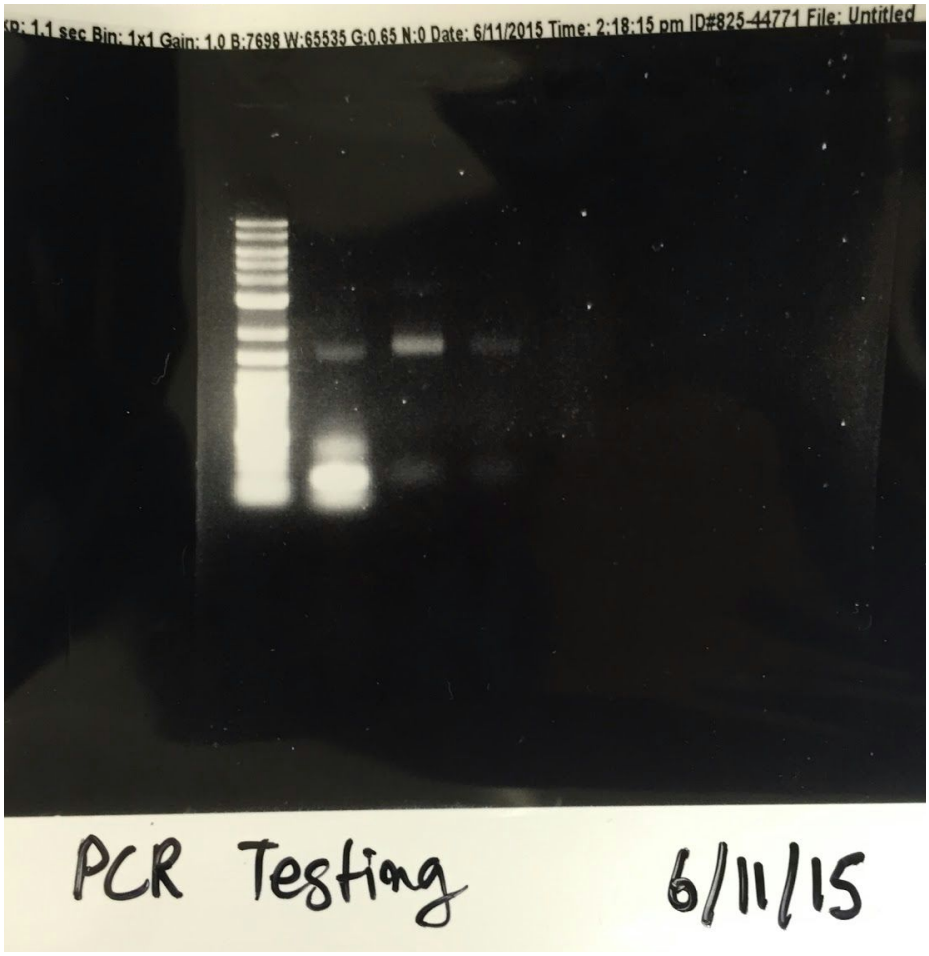
- Ran PCR
    - Samples+Primers:
        - KNR 2B Miniprep + 5'-BbsI-E-repeat and 3'-BbsI-Z-repeat (0.25 µL DNA)
        - KNR 2B Miniprep + 5'-BbsI-E-repeat and 3'-BbsI-Z-repeat (1 µL DNA)
        - Rep-Seq-Rep Plasmid + 5'-BbsI-E-repeat and 3'-BbsI-Z-repeat (0.25 µL DNA)
        - BBa_E2030+ pSB1C3 up and pSB1C3 down (10 µL DNA)

Note: BBa_E2030 was used because it came in a chlor backbone. 10 µL DNA because iGEM notes that there should only be 2-3 ng of DNA in plate wells. New dNTPs were also used for this PCR.

- Annealing Temp: 62 C
- Extension Time: 15 sec
  Note: PCR done using machine here (ie: not Buchler's)

- Ran Gel of PCR

Results: Only the first sample worked.



6/12/15

Objective: Discover why PCRs are not working

- *Test BbsI PCR primers of rep-seq-rep on a temperature gradient*

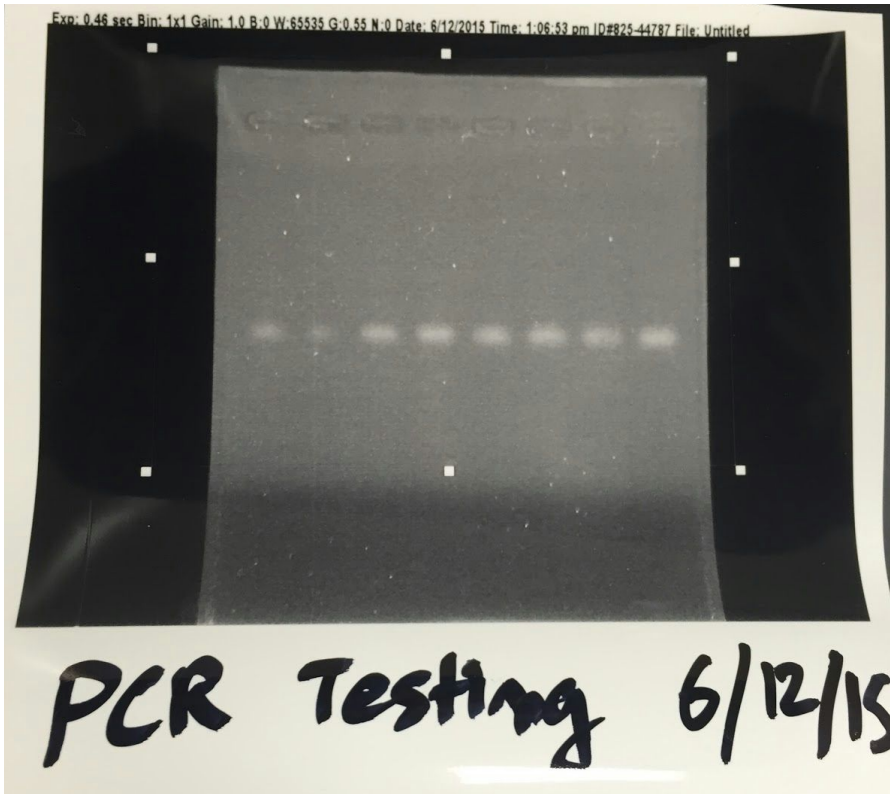
Procedure:

- Ran PCR
  - Samples+Primers:
    - KNR 2A Miniprep + 5'-BbsI-A-repeat and 3'-BbsI-B-repeat (0.25 µL DNA)

  Note: Master Mix of this sample was made and aliquoted to 8 PCR tubes to test the gradient.

- Annealing Temp: 60-72 C
- Extension Time: 15 sec
  Note: PCR done using Buchler Lab's
- Ran Gel of PCR

Results: All wells worked



Objective: Miniprep repeat-sequence-repeat

Jeremy

- Qiagen miniprep kit

Results: rep-seq-rep (77.6 ng/ul)

6/15/15

Objective: Miniprep tracr-dCas9-rep-KNR 2-6 -1A3

Jeremy

- Qiagen miniprep kit

Results:

Sample:	Concentration (ng/ul);
tracr-dCas9-rep-KNR 2-6 -1A3 (A)	65.7
tracr-dCas9-rep-KNR 2-6 -1A3 (A)	66.7

tracr-dCas9-rep-KNR 2-6 -1A3 (B)	66.6
tracr-dCas9-rep-KNR 2-6 -1A3 (B)	112.8

Objective: 3A Assembly of lac/RBS and Biobrick of Tachyplesin

Jeremy, Parth

- Restriction Digest (only 25 ul instead of 50 ul)
    - R0011 (EcoRI and SpeI)
    - B0034 - the RBS (XbaI and PstI)
    - pSB1C3 (EcoRI and PstI)
    - Tachyplesin (EcoRI and PstI)

  Note: RBS sequence was just resuspended for this assembly. It has not yet been transformed
- Ligation
    - pSB1C3 (0.5 µL)
    - R0011 (1.5 µL)
    - B0034 (6 µL)
- Ligation
    - pSB1C3 (5.5 µL)
    - Tachyplesin (2.5 µL)
- Transformation of both samples on to cm plates
  (Used DH5α-Z1 cells)

Results: Transformations failed. Will be religating and retransforming.

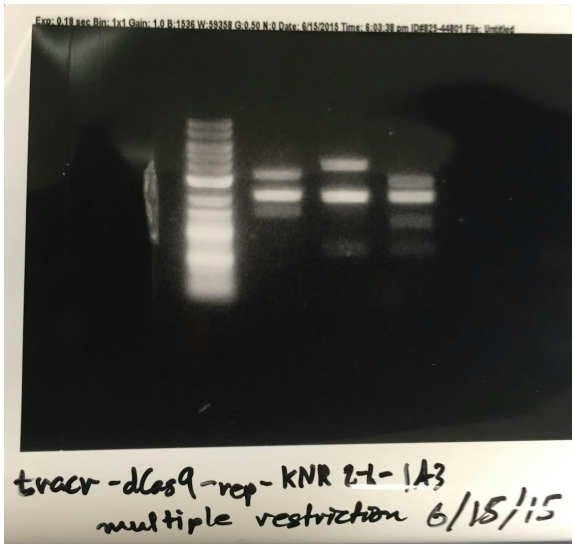
Objective: Analytical digest and gel of tracr-dCas9-rep-KNR 2-6 -1A3 (B)

Jeremy, Parth

- Restriction Digest (3 of the same sample restricted with different enzymes)

1) XbaI and PstI (Predicted bands: 2120 bp, 5110 bp)
2) NheI and PstI (Predicted bands: 3600 bp, 3630 bp)
3) AlwnI and BamHI (Predicted bands: 1920 bp, 5300 bp)

Results: Ambiguous yet promising. All bands add up to the expected ~7000 bp



Objective: Keep the strong RBS sequence on hand

Jeremy

- Transformation of B0034

Results: Transformation failed. Will be retransforming.

6/16/15

Objective: 3A Assembly of lac/RBS and Biobrick of Tachyplesin

Jeremy

- Ligation
  - pSB1C3 (0.5 µL)
  - R0011 (1.5 µL)
  - B0034 (6 µL)
- Ligation
  - pSB1C3 (5.5 µL)
  - Tachyplesin (2.5 µL)
- Transformation of samples and B0034
  (Used DH5α cells)

Objective: Preparation of a lysis gene for use & characterization

Jeremy

- Transformation of K117000 from iGEM 2015 plate 3 well 18G into competent cells

Objective: Grow B0034

Jeremy

- Inoculate 1 colony from plate B0034-1A2 (6/15/15) LB+Amp liquid media

Results: All transformations failed. Now investigating cause.

6/17/15

Objective: Miniprep B0034

Jeremy

- Qiagen miniprep kit & protocol

Results: B0034-1A2 (102.1 ng/ul)

Objective: Attempt 3A assembly of R0011/B0034 construct again, using miniprepped B0034

TJ, Jeremy

- Restriction digest
  - B0034 (XbaI & PstI)
  - re-used previous digests of R0011 (EcoRI & SpeI) and pSB1C3 (EcoRI & PstI)
- Ligation of R0011-B0034-1C3
  - pSB1C3: 0.75 ul
  - B0034: 1.75 ul
  - R0011: 5.50 ul
- Transformation of ligated construct into CCEC (DH5α)

Objective: What's going on with our transformations?

TJ, Jeremy

- Transformation of a kit plate part (K641009)

Objective: Lab Maintenance

- Transformed K117000 from 2014 kit plate (plate 3, well 18G)

Objective: Join iGEM Interlab Study

- Transformation of parts from kit plates
  - J23101 (plate 1, well 20K)
  - J23106 (1, 22A)
  - J23117 (1, 22K)
  - I13504 (4, 21J)

Result: All transformations failed.

6/18/15

Objective: Lab Maintenance

TJ

- Cell culture experiment to find out why transformations are failing
  - plated three samples on six different plates (2 plates per sample)

        - 2 Amp, 2 Cm, 2 LB
    - placed three samples in Buchler lab incubator and another three in BME lab incubator
    - Check both sets of cultures in 6 hours (check at ~4:00)

Results: All plates grew colonies in both incubators. It seems very likely that the problem is the transformation efficiency of our competent cells.

6/19/15

Objective: Lab Maintenance

Sarah, Parth, Ben

- Made Cm plates

Result: failed, accidentally used LB Media instead of Agar. Made ~2x LB Media

Objective: get Gblocks into backbone

Sarah and Ben

- **Digest**
    - tachyplesin already digested with EcoRI and PstI
    - K628000 digest with EcoRI and SpeI
    - You Lab Gene with EcoRI and SpeI
    - dCas9-KNR2-6 with EcoRI and SpeI
    - Binding Site with XbaI and PstI
    - Restrict 1C3 with EcoRI and SpeI (x2)
    - Restrict 1C3 with EcoRI and PstI (x2)
        - Use 10 µL DNA
        - 5 µL buffer
        - 1.25 µL total enzyme
        - water to 25 µL
- **Ligation**
    - Tachyplesin (E/P) (200 bp) + 1C3 (E/P)
        - 1 µL of insert 2.6 µL backbone
    - dCas9-KNR2-6 (E/S) + Binding site (X/P) + 1C3 (E/P)
        - 4 µL backbone 2 µL of binding site, 1 µL of dCas9
    - K628000 (100 bp) (E/S) + 1C3 (E/S)
        - 2 µL insert, 4 µL of backbone
    - You Lab Gene (E/S) (330 bp)+ 1C3 (E/S)
        - 2 µL insert + 6 µL of backbone
    - R0011 B0034
        -
    - ***if dcas9 construct doesn't work - can use Not1 instead of EcoR1
- **Determining transformation efficiency**

  Note: Using iGEM transformation efficiency kit.

Result: All Transformations failed. We now think it was because of our use of LB+Cm instead of SOC Media. Will be remaking or borrowing SOC just in case.

6/22/15

Objective: Lab Maintenance

- Made LB+Cm plates

Note: Used the 35,000 µg/mL Cm instead of the 1000x stock of Cm

Objective: Miniprep Tachyplesin Sample

- Minipreped using QIAGEN Kit
- Nanodrop
    - Concentration: 53.7 ng/µL

Objective: Gel analysis of Tachyplesin DNA

- Restriction Digest of Tachyplesin ()

Note: Used TBE. Gel was not done. Gel samples were accidentally broken up. Will continue tomorrow.

Objective: #TransformationParty

TJ, Sarah, Ben

- Tachyplesin (E/P) (200 bp) + 1C3 (E/P)
- dCas9-KNR2-6 (E/S) + Binding site (X/P) + 1C3 (E/P)
- K628000 (100 bp) (E/S) + 1C3 (E/S)
- You Lab Gene (E/S) (330 bp)+ 1C3 (E/S)
- Positive control - KNR 2B
- negative control

Result: **None of the transformations were successful**

6/23/15

Objective: Lab Maintenance

Parth, TJ

- Biological Waste Management
- Resuspended Tachyplesin and protegrin cytosine removal of G-blocks

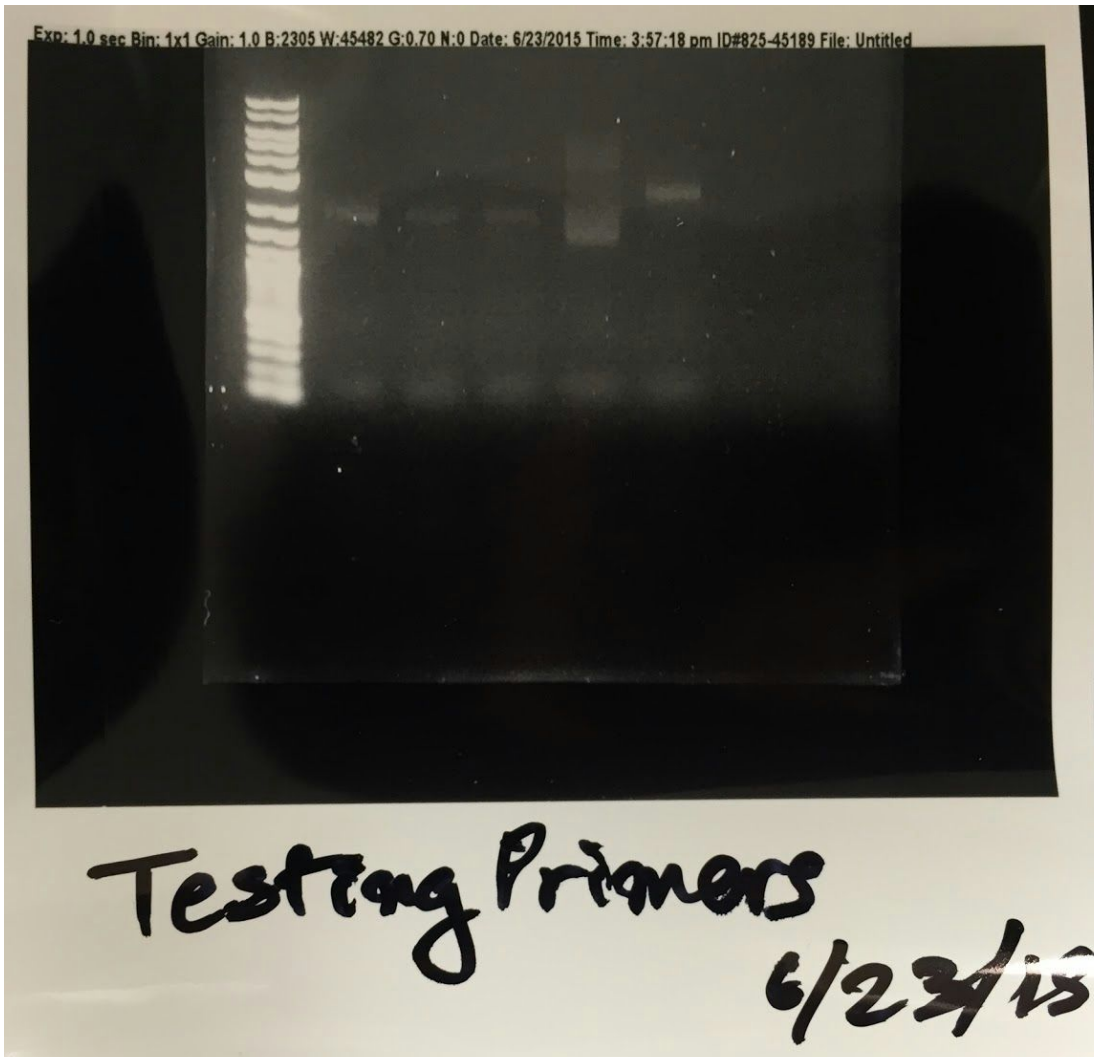
Objective: Testing Primers

Jeremy, Sarah

- PCR using the 5’ prefix and 3’ suffix primers for:
    - Rep-Seq-Rep
    - RFP 1-4 + GFP

    - RFP 1-5
    - dCas9+ KNR 2-6
    - K608012 (Control)
- Annealing Temp: 66°C
- Extension Time: 2 minutes
- Gel analysis

Results: Suspect. Bands seem to match, but there are samples with 200 bp bands that would not be seen properly.



Objective: Transformation Troubleshoot

TJ, Parth

- Conducting experiment with positive and negative controls and on Cm and LB plates
  Transformed the following:
    - dCas9-KNR 2-6 - Binding-1C3 on Cm plate
    - Tachyplesin-1C3 on Cm plate
    - K628000-1C3 on Cm plate

- YouLabGene-1C3 on Cm plate
- KNR 2B (positive control on Cm plate, negative control on LB plate)
- Competent Cells (negative control on Cm plate, background check on LB plate)
- Mert's Plasmid on LB plate

Note: Done in Buchler using their equipment, using Cm plates not made by us
Results: All transformations successful except perhaps: the You lab gene and K628000. The plates do not seem to have growth. We believe that the shaker in the BME lab is to blame for our previous failures now in combination with the incorrect media we were using.

6/24/15

Objective: Move to Buchler Lab

- Started to clear up fridges and tables in preparation for move

Objective: Miniprep dCas9--KNR 2-6--Binding--1C3 growth cultures 1-3

Jeremy

- Used QIAGEN MiniPrep kit and protocol
- Nanodrop procedure

RESULTS:

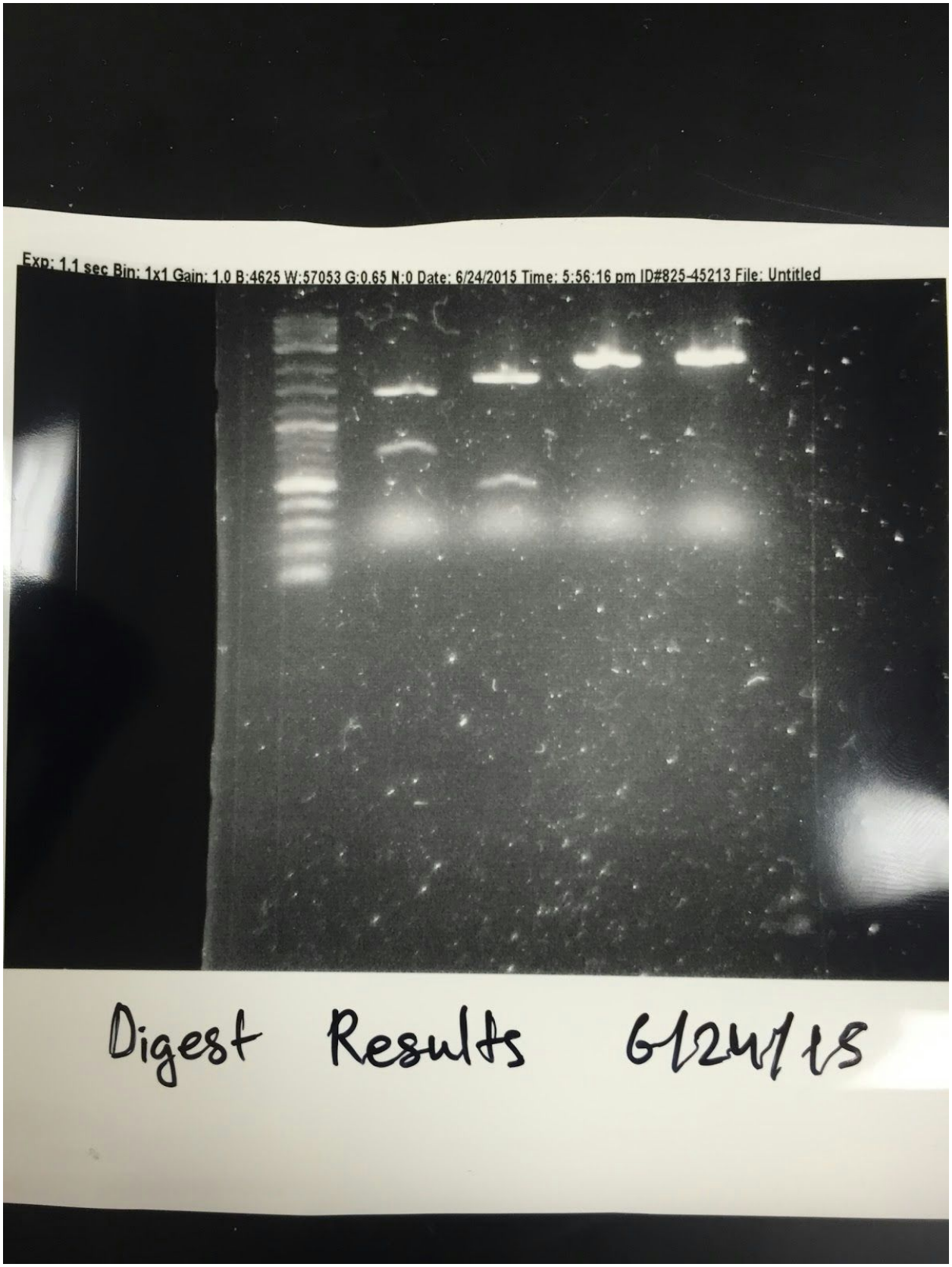
Sample	Concentration (ng/ul)
dCas9--KNR 2-6--Binding--1C3 (#1)	79.4
dCas9--KNR 2-6--Binding--1C3 (#2)	74.1
dCas9--KNR 2-6--Binding--1C3 (#3)	92.7

Objective: Analytical Digest and Gel

Ben

- Digest of:
  - Tachyplesin (EcoRI/AlwNI)
  - Tachyplesin (PvuII/PstI)
  - dCas9--KNR 2-6--Binding--1C3 (EcoRI)
  - dCas9--KNR 2-6--Binding--1C3 (NheI/PstI)

Results:



Digest Results 6/24/15

Objective: PCR

TJ, Jeremy

- Primers: 2014 Primers pSB1C3 up and down
- Samples:
    - RFP 1-4 - GFP
    - RFP 1-5
    - Rep-Seq-Rep
    - dCas - KNR 2-6 - Binding
- Annealing Temp: 64 C
- Extension Time: 60 sec

Objective: 3A Assemble

Parth

- Ligation of:
    - R0011 (4 µL)
    - B0034 (1 µL)
    - 1C3 (2 µL)

Objective: Transformations

- R0011 + B0034 + 1C3
- I13504
- J23101
- J23106
- J23117

Outcome: failure

6/26/15

Objective: Inoculate Transformations

- #2 dCas9-Binding Sites-KNR2-6
- #3 tachyplesin
- #4 YouLab gene
- #5 K628000-IC3
- #6 positive control

Results: all worked except for K628000-1C3

Objective: Transformation

- J23101-I

6/27/15

Objective: miniprep!

- tachyplesin
- YouLab gene
- dCas9-binding

Objective: Make K-resistant media

- 0.5 L of LB Broth mix, autoclaved
- Make 50 mg/L Kan final solution: 2.5 mL of 10 mg/mL solution

Objective: Ligate LacPromoter (R0011) with RBS (B0034)

- 3A Restriction Assembly
- 0.5 uL B0034, 0.5 uL pSB1K3, 5 uL R0011
- Transformed onto K plates

Objective: Transform InterLab Study parts

- J23101-J61002 (Amp)
- J23106-J61002 (Amp)
- J23117-J61002 (Amp)
- K823005-1C3
- K823008-1C3
- K823013-1C3
- I13504-1A2

Objective: Grow K117000 stock

- Transformed K117000 in DH5a

6/29/15

Objective: Miniprep

Jeremy, Ben

- Miniprepped weekend transformations + some:
    - J23101-J61002 (Amp)
    - J23106-J61002 (Amp)
    - J23117-J61002 (Amp)
    - K823005-1C3
    - K823008-1C3
    - K823013-1C3
    - I13504-1A2
    - K117000-1C3
    - Mystery-1C3
    - 2x R0011-B0034-1K3

Objective: Make 1L Amp Plates

Ben

-

Objective: Make Preparatory Digest

Sarah, TJ

- EcoR1/Spe1:
    - K823005
    - K823008
    - K823013
    - J23101
    - J23106
    - J23117
    - Lac-RBS [R0011-B0034] (x3)
- Xba1/Pst1
    - I13504 (x6)
    - You Lab Gene
    - Tachyplesin
    - K117000
    - [dcas9-binding] - just analytic
- EcoR1/Pst1
    - CDT
    - CDP
    - K628000
    - 1A3 (freezer)
    - 1C3 (Ran out, using predigested 1C3)

Objective: Sequencing

Jeremy,TJ

BigDye Protocol

- dCas9-Binding 3 uL
    - forward (A)
    - reverse (G)
- Control: (Miniprep that turned red) J23101 - forward (F) 1 uL
- YouLab gene - forward (B) 3 uL
- Tachyplesin - forward © 3 uL
- rep-seq-rep - forward (D) 3 uL
- K117000 - forward (E) 3 uL

Objective: PCR test

Jeremy, TJ, Sarah

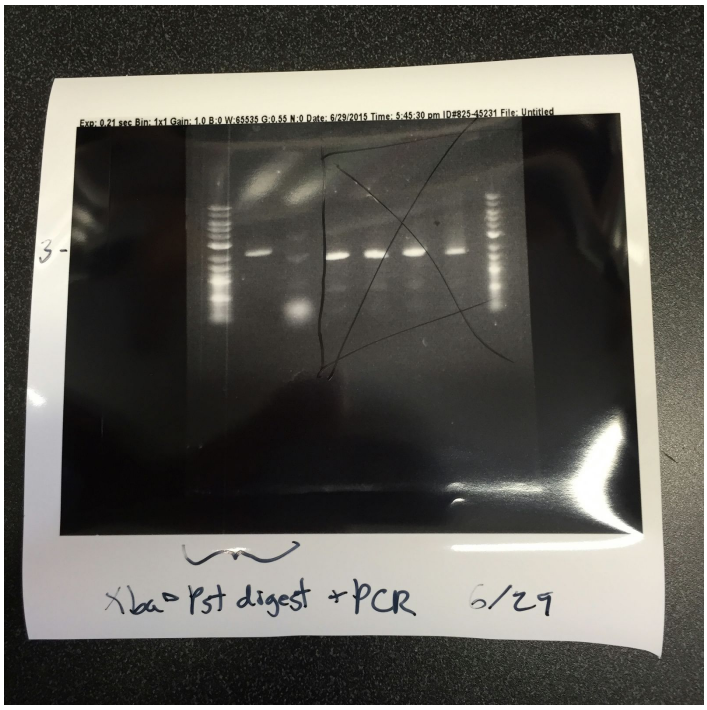
- dCas9-Binding 3 uL
- Control: (Miniprep that turned red) J23101
- YouLab gene - forward

- Tachyplesin - forward
- rep-seq-rep - forward
- K117000 - forward

Objective: Analytical Digest

Jeremy, TJ, Sarah

- dCas9-Binding (on 1C3 backbone)
    - look for location of bands
        - 3400, 2100 means dCas9 chopped in middle
        - 5100, 2100 means perfection
- dCas9-Binding (PCR)
    - 5100 or 3400



Objective: Transformations

- *J23101-I13504-1C3*
- *J23117-I13504-1C3*
- *K823005-I13504-1C3*
- *K823013-I13504-1C3*
- *Lac-RBS-YouLabGene-1A3*
- *Lac-RBS-Tachyplesin-1A3*
- *Lac-RBS-K117000-1A3*
- *R0011-1C3*
- *rep-seq-rep-1C3*

6/30/15

Objective: Restrictions

TJ, Ben, Jeremy

- KNR-5A with EcoR1/Pst1
- dCas9-1C3 with AatII/KpnI
    - failed
- dCas9-binding with AatII/KpnI
    - need to check with analytical digest

Objective: Gel Recovery

Sarah, TJ

- tried to get missing dCas9 segment
    - failed - restriction didn't work

Objective: Lab Maintenance

Sarah, TJ

- made a new plate of gels
- *note for when pouring gels, do so in a 500 mL flask to prevent boiling
- made aliquots for Cm

Objective: Phosphorylate (CIP Protocol)

TJ, Jeremy

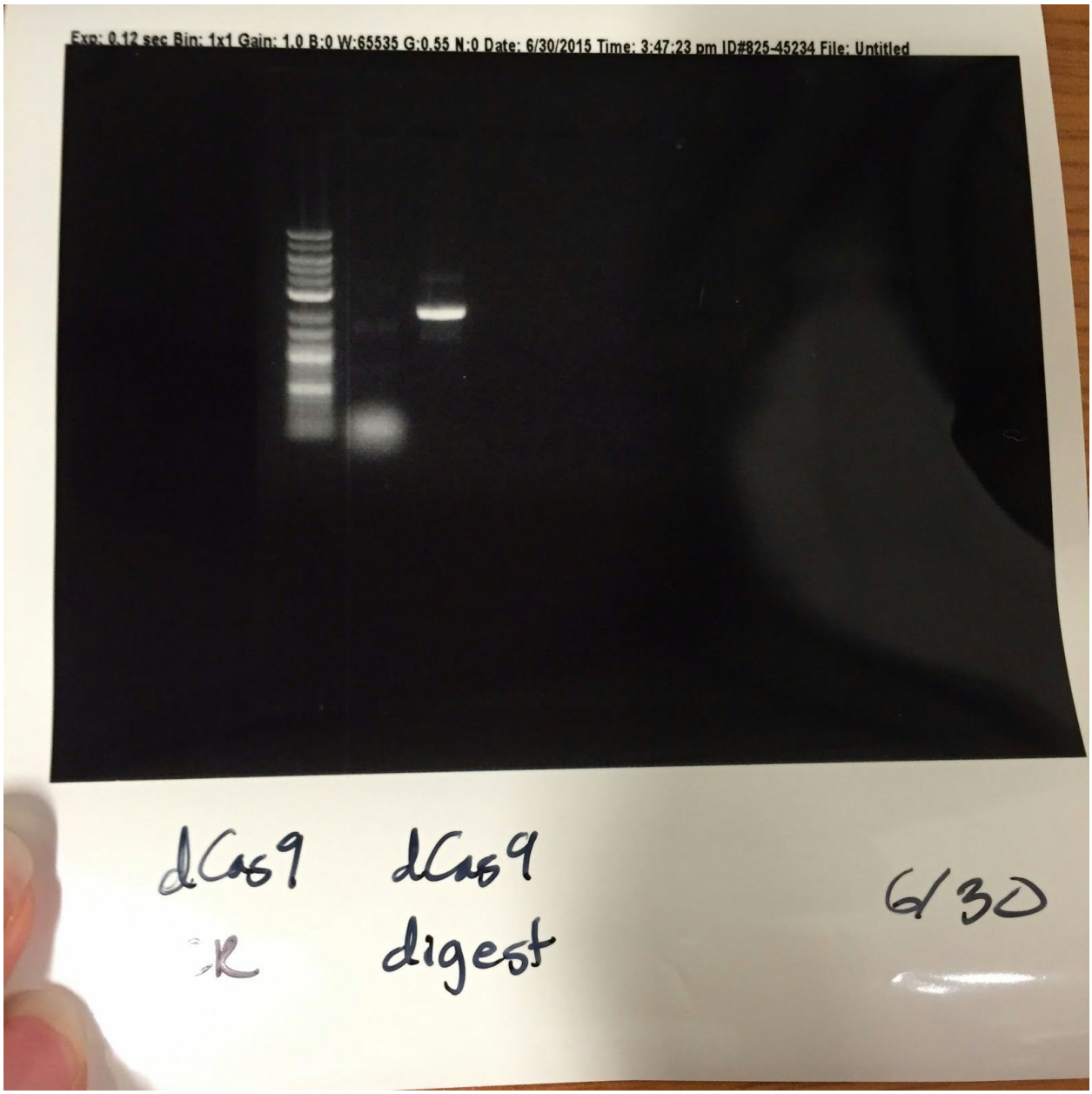
- KNR 5A - 1C3
    - need 1C3 backbone for transformations
- dCas9-binding
- used CIP protocol and proteinase K

Objective: Analytical Digest

Sarah

- dCas9-Binding Digest
    - just to check if this one also failed
- dCas9-Binding PCR

Results: expected bands for 5500 for first, second at 1700 & 5200



Objective: Analytical Digest

Sarah

- see if PCRs from yesterday worked
    - from left to right
    - YouLab (350 bp)
    - Tachyplesin (160 bp)
    - Rep-Sequence-Re (172 bp)
    - K117000 (180 bp)
    - J23101 (control)

Results: Tachyplesin, K117000, J23101 were good! The consistent bands at 2100 are concerning but may be just circularized 1C3 that will go away when we do the gel purification

Objective: Transform programmable cell death genes, InterLab Study constructs and dCas9

- Cysteine-Deleted Protegrin (CDP) - 1C3
- Cysteine-Deleted Tachyplesin (CDT) - 1C3
- K628000 (Protegrin) - 1C3
- J23101 - I13504 - 1C3

- K823008 - I13504 -1C3
- dCas9 - 1C3
- **Results:** K628000-1C3, CDP-1C3 and J23101-I13504-1C3 failed

---

7/1/15

Objective: Miniprep

Jeremy, Ben

- J23106-I13504-1C3
- J23117-I13504-1C3
- K823005-I13504-1C3
- K823013-I13504-1C3
- Lac-RBS-YouLab
- Lac-RBS-Tachyplesin
- Lac-RBS-K11700
- R0011-1C3
- Rep-Seq-Rep - 1C3

Objective: Innoculate for cold stocks

TJ, Jeremy, Sarah

- You Lab Lysis - Spec
- R0011 - rep - RFP 1-5 rep - 1A2
- dCas - rep - KNR 2-6 - rep -1A3
- KNR + Binding - 1C3
- R0011 - B0034 - 1C3
- YouLabGene - 1C3
- Tachyplesin - 1C3
- dCas9 - KNR 2-6 - Binding - 1C3
-

Objective: Innoculate for miniprep

TJ, Sarah, Jeremy

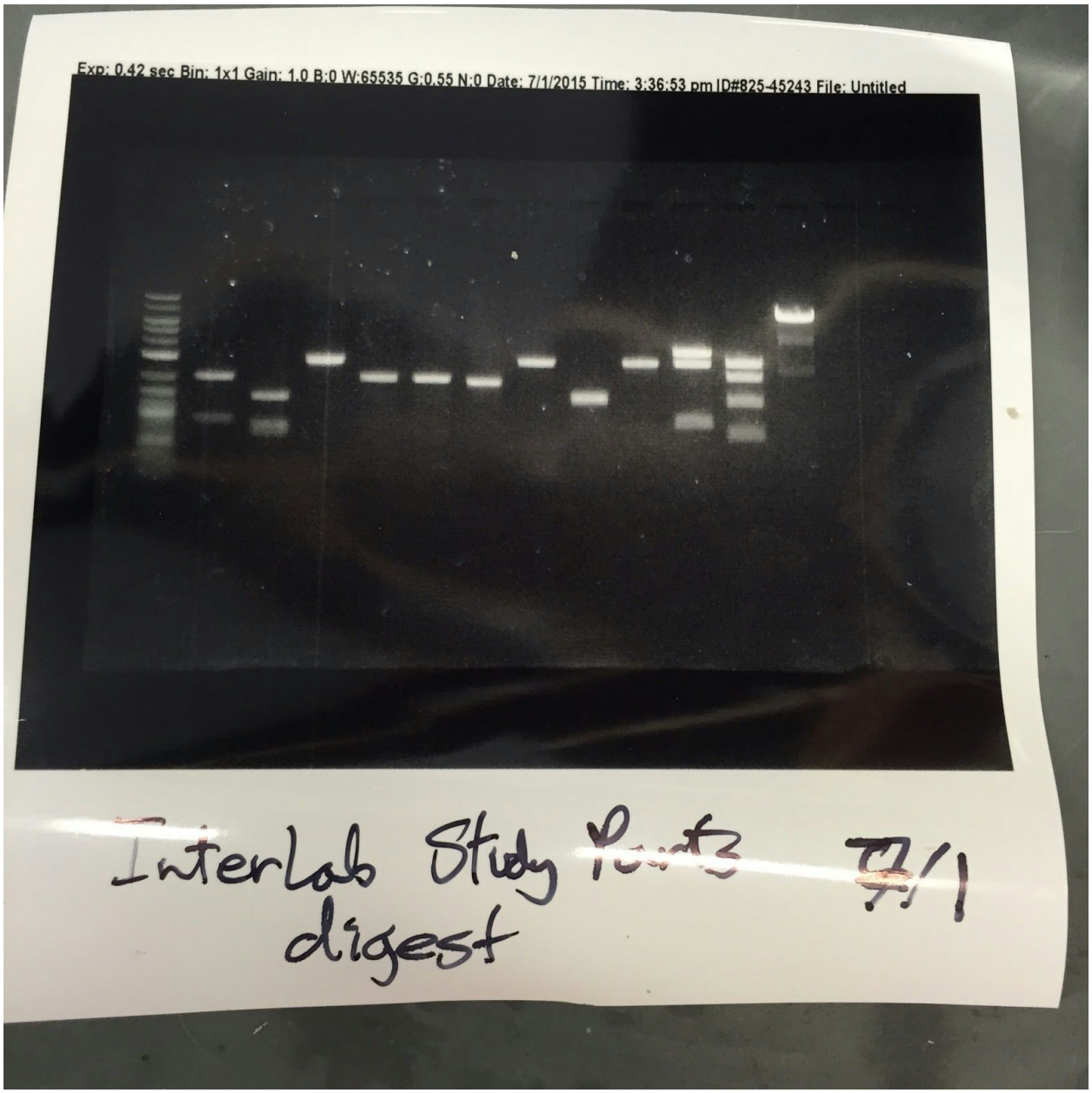
- B0034 - 1A2
- R0011 - 1A2
- R0011 - 1C3 (x2)
- rep-seq-rep - 1C3
- pdCas9
- dCas9 - 1C3
- K823008-I13504 - 1C3
- CDT - 1C3

Objective: Restriction Digest of Interlab Study parts + Gel

TJ, Sarah

- K823005
- K823013
- J23106
- J23117
- all of these with EcoR1/Spe1, AlwN1/XbaI, NheI/BstBI

Results: only K823005 had successful incorporation of promoter + GFP



Objective: Digest Binding Site for ligation

Sarah

- Binding site G-block using Pst1/EcoR1

Objective: ligations

Sarah, TJ

- J23101-I13504-1C3
- CDP-1C3
- K628000-1C3
- Binding site-1C3
- *used 1C3 from phosphorylated KNR 5A

Objective: Transformation

Sarah, TJ, Jeremy

- J23101-I13504-1C3
    - CCEC
- CDP-1C3
    - CCEC
- K62800-1CE
    - CCEC
- Binding site-1C3
    - CCEC
- Lac-RBS-Youlab
    - Z1
- Lac-RBS-Tachyplesin
    - Z1
- Lac-RBS-K117000
    - Z1
- K823005-I13504-1C3
    - CCEC

Results: Youlab, Tachy, K11700 were all successful! CDP, J23101, K823005, binding not so much

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7/2/15

Objective: Miniprep (x19) + Coldstocks (x10?)

TJ, Jeremy, Sarah, Ben

Objective: 3A assembly of Lac-RBS-CDT-1A3

Sarah

- Digest CDT with X/P
- Used precut Lac-RBS, 1A3
- Stopped after ligation
- -> will transform tomorrow

Objective: Redo KNR 2 plasmid

Sarah

- Anneal KNR2 Up/Down
  - 10 uL of each
  - 68 for 15 min, 50 for 45 min
  - 1 hour at room temp
- ligate KNR in rep-seq-rep digested with Bsa1
  - used r-s-r digest found in stocks
- transform into CCEC
  - results: works!

Objective: Learn how to use flow cytometer and test interlab study parts

all

- we learned how to use the machine but all of our samples sucked

Objective: Analytical digest of interlab study parts + cell death parts

Jeremy, TJ

- Lac-RBS-Tachyplesin
  -
- Lac-RBS-K117000
  -
- Lac-RBS-You Lab Lysis Gene
  -
- K823013-I13504-1C3 (A)
  - AlwNI/XbaI
  - NheII/BbstI
  - EcoRI/SpeI
- J23016-I13504-1C3 (A)
  - AlwNI/XbaI
  - NheII/BbstI
  - EcoRI/SpeI
- J23117-I13504-1C3 (A)
  - AlwNI/XbaI
  - NheII/BbstI
  - EcoRI/SpeI

Objective: Analyze sequencing results

7/3/15

Objective: characterize cell death parts

Objective: Ligations and Transformations

Sarah, Jeremy, frands

- K628000
    - 1C3
    - 1K3
    - 1A3
- Binding site
    - 1C3
    - 1K3
    - 1A3

- J23106-I13504

7/4/15

Objective: innoculate

- KNR 2, plates A and B
- K628000-1A3 and -1K3
- Binding Site - 1A3, 1C3, and1K3
- Lac-RBS-CDT-1A3 (x3)

7/5/15

Objective: miniprep

Sarah

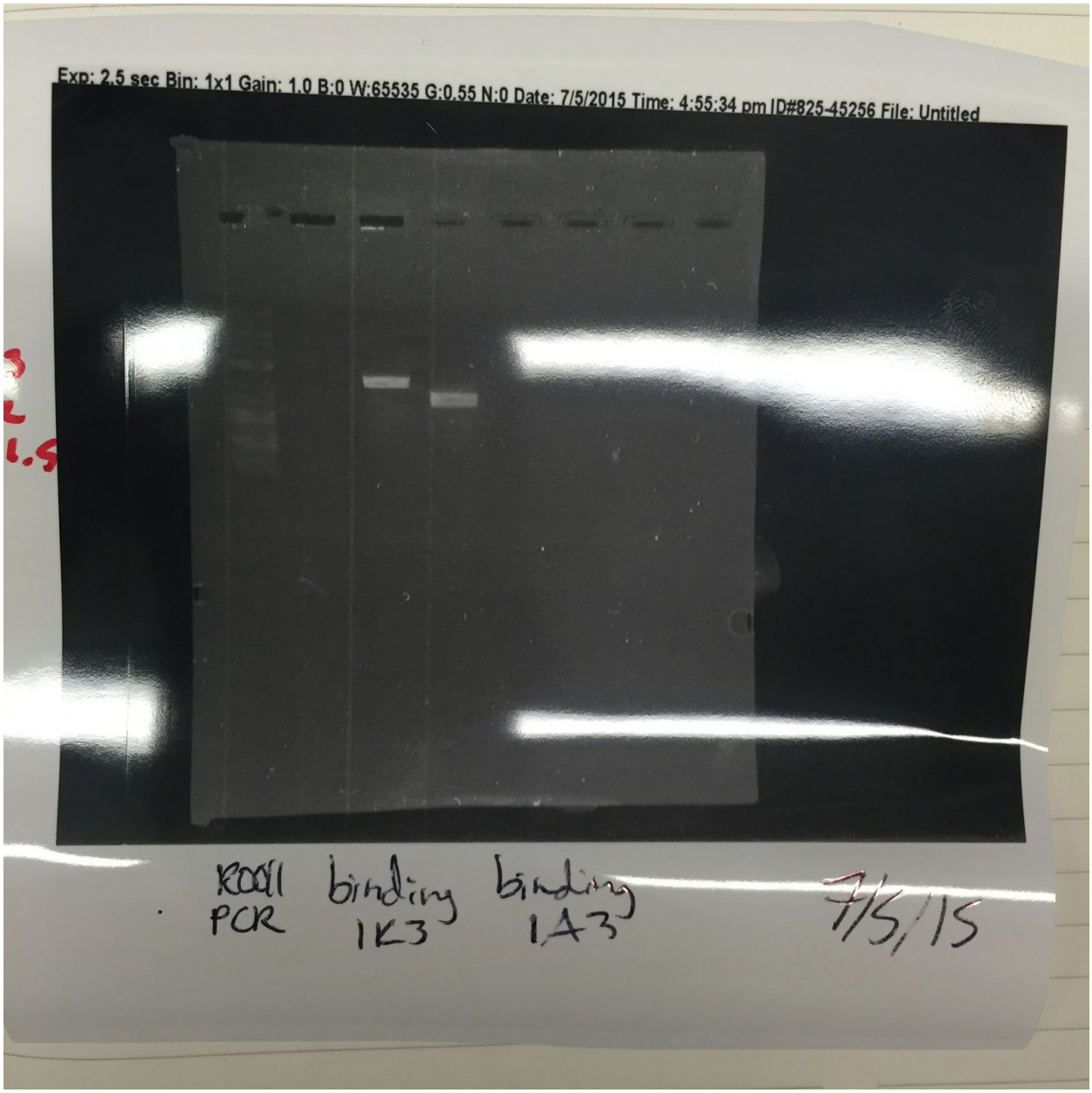
- KNR 2A-1C3
    - 133.9
- KNR 2B-1C3
    - 243.2
- K628000 - 1A3
    - 112.2
- K628000 - 1K3
    - 139.1
- ~~Binding site - 1C3 -~~ no cells present after spin down
- Binding site - 1A3
    - 79.2
- Binding site - 1K3
    - 118.7
- Lac-RBS-CDT-1A3 (A)
    - 110.8
- Lac-RBS-CDT-1A3 (B)
    - 87.7
- Lac-RBS-CDT-1A3 ©
    - 121.6

Objective: Analytical digest of binding site (1A3 and 1K3)

Sarah

- is the binding site really there?

- digest with HindIII
    - on 1K3, expect bands at 970 bp and 130 bp and 1430 bp
    - on 1A3, expect bands at 130 bp and 2350 bp
    - also did R0011 PCR product



Objective: Begin Golden Gate Assembly for KNR 2-6

Sarah

- PCR of KNR 2-6 and R0011 Backbone
    - Sample + Primers:
        - KNR 2 + 5'-BbsI A-Repeat and 3'-BbsI-B-Repeat
        - KNR 3 + 5'-BbsI B-Repeat and 3'-BbsI-C-Repeat
        - KNR 4 + 5'-BbsI C-Repeat and 3'-BbsI-D-Repeat
        - KNR 5 + 5'-BbsI D-Repeat and 3'-BbsI-E-Repeat
        - KNR 6 + 5'-BbsI E-Repeat and 3'-BbsI-Z-Repeat
        - R0011 on **1C3** + 5'-BbsI Z-Suffix and 3'-BbsI-A-R0011

    - Anneal temp: 72 C
    - Extension Time: 2 minutes
- PCR cleanup protocol
    - KNR 2 -> 34.5
    - KNR 3 -> 69.1
    - KNR 4 -> 59.8
    - KNR 5 -> 48.4
    - KNR 6 -> 45.8
    - R0011 1C3 -> 11.4
        - maybe it needed a longer extension time?

Objective: Lab maintenance

Jeremy, Parth

- grow chemically competent E. coli (not Z1 variant)
- prepare LB+Cm plates

7/6/15

Objective: Continue Golden Gate Assembly

Sarah

- Restrict all the parts with Bbs1
    - fun fact: Bbs1 is stored in the -80
    - used CutSmart instead of NEB 1.1 so only 75% transformation
- ligate
- transform

Objective: Is our life a lie? Digest of the binding site to see if it exists

Sarah

- Restrict Binding-1A3, Binding-1K3 with HindIII
    - should see the same results as predicted yesterday

Results: *Binding site not present.*

Objective: Screen J823008-I13504 for possible success

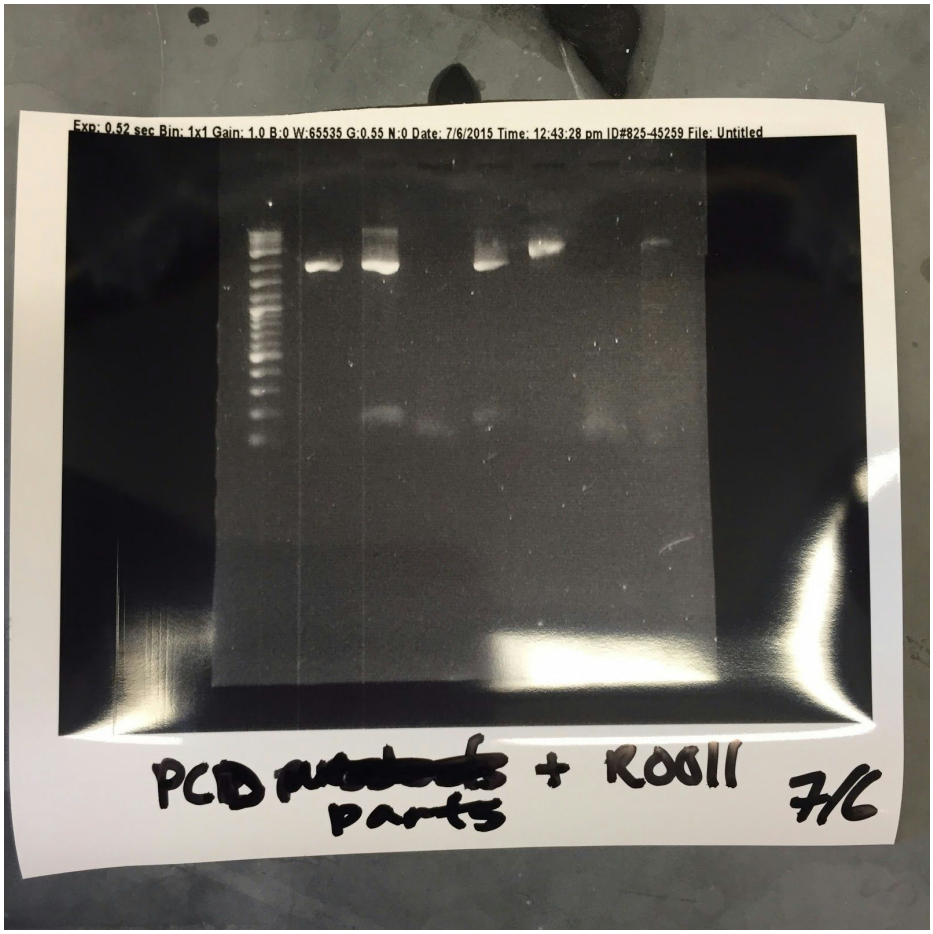
- Flow cytometry revealed no fluorescence
- Restriction digests on samples A-D with the following:
    - EcoRI and SpeI
    - NheI and BstBI
    - AlwN1 and XbaI

Objective: Is anything real? Analytical gel of PCD parts and R0011 PCR product

Sarah

- From left to right: Tachy (X/P), CDT (X/P), CDP (E/P), K117000 (X/P), YouLab (X/P), K628000 (E/S), R0011 PCR Product
- expected band sizes:

Results: tachy isn't there, CDP is a mystery, YouLab isn't there, K628000 is a mystery



Objective: sequence parts

Jeremy

- BigDye protocol
    - all of these done with prefix primer

1) J23117-I13504-1C3
2) K823005-I13504-1C3
3) R0011-B0034-1K3 (2)
4) R0011-B0034-1C3
5) R0011-B0034-1K3 (1)
6) KNR 2B
7) KNR 3B
8) KNR 4B
9) KNR 5A

10) KNR 6B

- Delivered to sequencer

Objective: Transform InterLab Part J823008-I13504

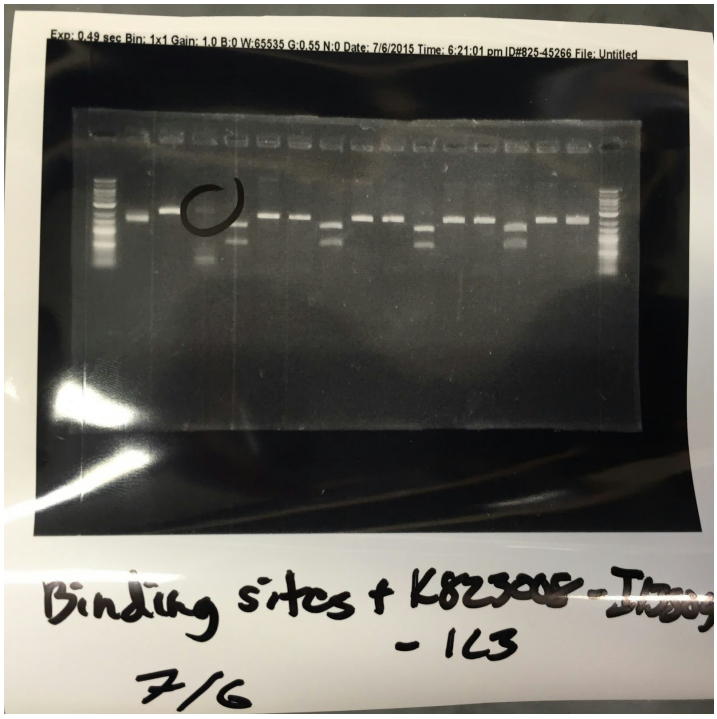
- Transform K823008-I13504 on 1A3 and 1K3
- Transform J23106-I13504 on 1A3 and 1K3

Objective: Transform Programmable Cell Death (PCD) genes

- CDT, CDP, Tachyplesin and YouLabGene onto 1C3, 1A3 and 1K3
- R0011-B0034 onto 1A3 and 1K3

Objective: Analytical Digest and Gel of all the things

- from left to right: Binding site on 1A3, Binding site on 1K3, ligation of KNR + R0011, A (A/X), A (N/B), A (E/S), B (A/X), B (N/B), B (E/S), C (A/X), C (N/B), C (E/S), D (A/X), D (N/B), D (E/S)
    - A=K823005-I13504-1C3
    - B=J23106-I13504-IC3
    - C=J23117-I13504-IC3
    - D=K823008?-I13504-1C3



7/7/2015

Objective: 1A3 assemblies + transformations

Sarah & frands

- K823005-I13504-1K3
- K823005-I13504-1A3
- K823013-I13504-1K3
- K823012-I13504-1A3

7/8/2015

Objective: Miniprep

Jeremy, Sarah

Items

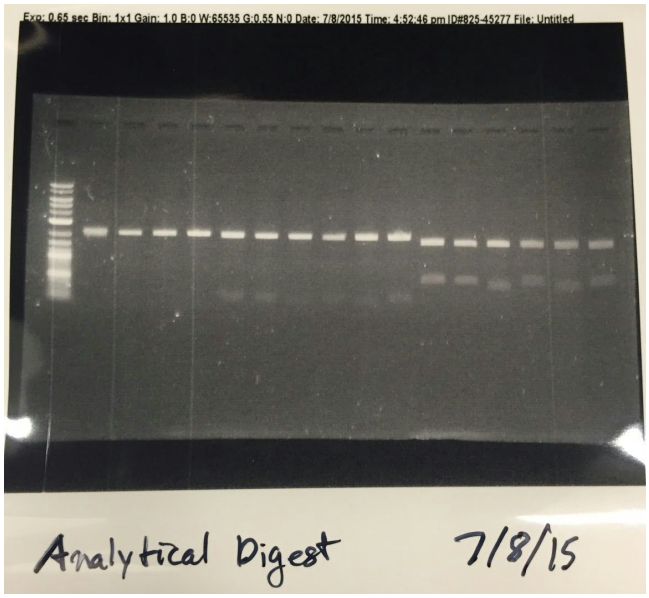
1. K628000-1C3 (2)
2. CDP-1C3 (2)
3. K823008-I-1A3 (3)
4. R0011-KNR2-6-1C3 (A1)
5. J23106-I-1A3
6. Binding-1C3 (1)
7. R0011-KNR2-6-1C3 (B3)
8. CDP-1C3 (3)
9. CDP-1C3 (1)
10. R0011-KNR2-6-1C3 (B2)
11. K823008-I-1A3 (2)
12. K628000-1C3 (1)
13. R0011-KNR2-6-1C3 (A2)
14. K628000-1C3 (3)
15. R0011-KNR2-6-1C3 (A3)
16. Binding-1C3 (3)
17. Binding-1C3 (2)
18. R0011-KNR2-6-1C3 (B1)
19. K823008-I-1A3 (1)

Objective: Analytical Digest

Sarah et al.

- Binding-1C3 with HindIII & EcoRV
- R0011-KNR2-6-1C3 with EcoR1 and Pst1, Aat &Pci1

Result:



Objective: Ligations & Transformations

Adam, Parth

- Controls:
    - Backbone Only (5 µL):
        - 1C3
        - 1A3
        - 1K3
    - Insert Only (2 µL):
        - R0011 (plate on 1C3)
        - R0011 (plate on 1A3)
        - B0034 (1C3)
        - B0034 (1A3)
        - K823005 (1C3)
        - J23101 (1A3)
- Ligations:
    - R0011 - B0034 - (1C3, 1A3, 1K3) (2 µL insert- 2 µL insert- 4 µL backbone)
    - K823005 - I13504 - (1A3, 1K3) (0.6 µL insert- 0.45 µL insert- 4.45 µL backbone)
    - J23101 - I13504 - (1A3, 1K3) (0.6 µL insert- 0.45 µL insert- 4.45 µL backbone)
    - CDT - (1A3, 1K3) (1.35 µL insert- 4.15 µL backbone)
    - Tachy - (1A3, 1K3) (1.35 µL insert- 4.15 µL backbone)
    - You - (1A3, 1K3) (1.35 µL insert- 4.15 µL backbone)
- Transformed

Objective: Inoculations (Tony: if you inoculate 7 and there's a 50% chance of each one being right, you have a 99% chance of at least getting one! TJ: Let's do eight!1!!11!!!)

TJ

- K823013 x8

Objective: Big Dye protocol to sequence things

TJ

- CDP x3
- K628000 x3
- CDT
- Binding-1A3
- K117000

TO SEQUENCE:

- K117000
- CDT
- Binding on 1A3
- K628000? pcr first

7/9/2015

Objective: Test 3A Assembly yield

- 3A doesn't work well with small things, just use two part ligation like everyone but iGEM

Objective: Restriction for ligation

TJ and Jeremy

- K823005
- K823013
- K23008
- B0034 (1A2)
- I13504
- R0011 (1C3)

Objective: Golden Gate 3.0

Sarah et al.

- Anneal KNR2-6 Up/Down
    - 10 uL of each
    - 68 for 15 min, 50 for 45 min
    - 1 hour at room temp
- Ligate into the *real* rep-seq-rep backbone (after gel digest and CIP)
- Tranformations

Objective: Miniprep

Adam and TJ

- idk what they’re doing and no one tells me anything
- K823013 x8

Objective: Gel Purify

Ben, TJ, Jeremy, Sarah

- Ben did a good job doing the cutting of the gel

Objective: CIP all the things - then proteinase K all the things

TJ, Jeremy

- K13 things
- B0034
- rep - seq - rep

Objective: Innoculations

TJ, Jeremy

- Tachy-1A3
- Youlab-1A3
    - 1K3
- CDT-1A3
    - 1K3
- R0011-IC3
    - 1K3
    - K823005-I13504-1K3
- all of these x3

Objective: Ligation all the things

TJ, Jeremy

-

Objective: Analytical Digest

Sarah and frands

- K13-I-1A3 with Nhe1/BstB1
- all eight samples #thanksTJ
    - expected bands: 653, 2312
- **Results:** None

Objective: Transformations

- B0034 - 1A2
- K823005 - 1C3
- K823008 - 1C3
- I13504 - 1C3
- R0011-B0034-1A2
- Rep-Seq-Rep control (backbone only)
- K823005 control (backbone only)
- K823008 control (backbone only)

- K823013 control (backbone only)
- R0011 control (insert only)
- K823005-I13504-1C3
- K823008-I13504-1C3
- K823013-I13504-1C3
- R0011-B0034-1A2
- rep-KNR2-rep-1C3
    - also KNRs 3-6 done individually

Results: None of the ligations worked

7/10/2015

Objective: Miniprep all of the things.

Jeremy

Items:

1. R0011-B0034-1C3 (2)
2. R0011-B0034-1C3 (3)
3. R0011-B0034-1C3 (1)
4. CDT-1K3 (1)
5. CDT-1K3 (2)
6. CDT-1K3 (3)
7. CDT-1A3 (2)
8. CDT-1A3 (1)
9. CDT-1A3 (3)
10. K823005-I13504-1K3 (1)
11. K823005-I13504-1K3 (3)
12. K823005-I13504-1K3 (2)
13. You-1A3 (2)
14. You-1A3 (3)
15. You-1A3 (1)
16. R0011-B0034-1K3 (3)
17. R0011-B0034-1K3 (2)
18. R0011-B0034-1K3 (1)
19. You-1K3 (3)
20. You-1K3 (1)
21. You-1K3 (2)
22. Tachy-1A3 (1)
23. Tachy-1A3 (3)
24. Tachy-1A3 (2)

Objective: Lab maintenance

Ben

- LB+Amp liquid media (500 ml)
- LB+Kan plates (500 ml)
- LB+Cm plates (1000 ml)

Objective: Analytical digest

TJ

- Tachyplesin-1A3
    - EcoRI/PciI -- expected: 293, 1921
- You Lab Lysis Gene-1A3
    - BsaAI/AlwNI -- expected: 644, 1787
- You Lab Lysis Gene-1K3
    - BsaAI/AlwNI -- expected: 644, 1836
- CDT-1A3
    - EcoRI/PciI -- expected: 280, 1921
- CDT-1K3
    - EcoRI/PciI -- expected: 282, 1970
- K823005-I13504-1K3
    - NheI/BstBI -- expected: 853, 2420

Objective: grow colonies of successful transformations

Adam, Jeremy

- B0034 - 1A2 (x3)
- K823005 - 1C3 (x3)
- K823008 - 1C3 (x3)
- I13504 - 1C3 (x3)

Objective: try the ligations again

TJ

- K823005-I13504-1C3
- K823008-I13504-1C3
- K823013-I13504-1C3
- negative controls:
    - K823005-1C3 cut backbone only
    - K823008-1C3 cut backbone only
    - K823013-1C3 cut backbone only

Objective: Transformations

Jeremy

- repeat-sequence-repeat-1C3
- K823005-I13504-1C3
- K823008-I13504-1C3

- K823013-I13504-1C3
- negative controls:
    - K823005-1C3 cut backbone only
    - K823008-1C3 cut backbone only
    - K823013-1C3 cut backbone only

how are we doing more backbone?

7/11/2015

Objective: Miniprep

Jeremy

- B0034-1A2
    a. 119.7 ng/ul
    b. 140.4
    c. 132.3
- K823005-1C3
    a. 245.4
    b. 229.8
    c. 250.0
- K823008-1C3
    a. 153.3
    b. 131.1
    c. 174.6
- I13504
    a. 276.6
    b. 267.6
    c. 280.4

Objective: Move transformed plates from incubator to 4-degree

Jeremy

7/13/2015

Objective: Miniprep

Jeremy

- repeat-sequence-repeat-1C3
    a. Good concentration results for all

Objective: PCR to make more 1C3 (1N3 Oligos)

Jeremy, Ben

- Tubes (hopefully one will work):
    1. r-s-r-1C3 (42.3 ng/uL)
    2. r-s-r-1C3 (42.3 ng/uL)
    3. r-s-r-1C3 (111 ng/uL)
    4. r-s-r-1C3 (111 ng/uL)

- Annealed at 72℃.

Objective: Preparatory restriction digest for ligation:

Jeremy, Ben

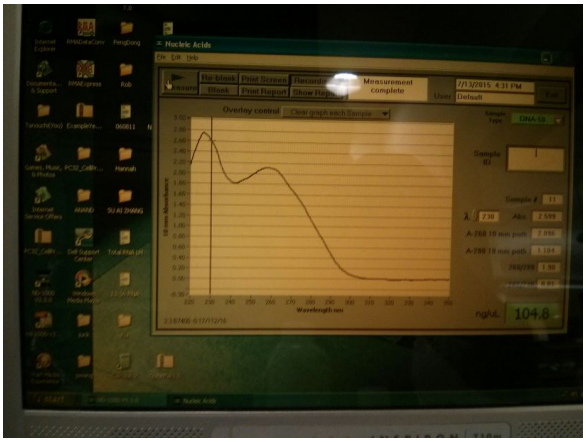
- r-s-r-1C3 (BsaI)
- K823005 (S/P)
- K823008 (S/P)
- K823013 (S/P)
- I13504 (X/P)
- B0034 (E/X)

Results: All is a'ok, boss.

Objective: Gel purify r-s-r-1C3 (Bsa1)

Ben

Results: Concentration = 104.8 ng/uL, but has high agarose contamination at 230 nm.



- Comments: Mert said it should be fine, just run a quick analytical digest to check and see if that is all the contamination (optional). Gersbach's recommendation of a PCR cleanup will waste too much of the 10 uL purified.

Objective: CIP Interlab Study backbones

Jeremy

Objective: Phosphorylate annealed KNRs with PNK from Gersbach's Freezer

Ben

- Used protocol from http://openwetware.org/wiki/PNK_Treatment_of_DNA_Ends

Objective: Ligations:

Jeremy

- K823005-I13504-1C3
- K823008-I13504-1C3
- K823013-I13504-1C3
- R0011-B0034-1A2
- Negative controls
    - K823005 Backbone only
    - K823008 backbone only
    - K823012 backbone only
    - I13504 insert only
    - B0034 insert only

Objective: Transformations

Jeremy

- transformed all ligations plus two things i couldn't find a lot of
    - R0011-1C3
    - K823013-1C3

7/14/2015

Objective: BigDye Sequencing

Jeremy

1) CDT-1A3
2) CDT-1K3
3) K823005-I13504-1K3 (2)
4) K823013-I13504-1A3 D
5) K823013-I13504-1A3 F
6) K823013-I13504-1A3 H

Objective: Resuspend binding site gBlock

Ben

- added 50 ul → 10 ng/ul

Objective: Ligation

Ben, Adam

- repeat-KNR2-repeat
- repeat-KNR3-repeat

- repeat-KNR4-repeat
- repeat-KNR5-repeat
- repeat-KNR6-repeat
- repeat-____-repeat (negative control)

Objective: Transformations

Ben, Adam

- transformed all ligations

Objective: Gibson Assembly

Jeremy

- used 1C3 backbone with conc. of 112.2 ng/ul
- ~~CDT-1C3~~ none left in tube?
- Tachyplesin-1C3
- ~~K628000-1C3~~ couldn't find in freezer?
- ~~CDP-1C3~~ none left in tube?

7/15/2015

Objective: Miniprep

Jeremy

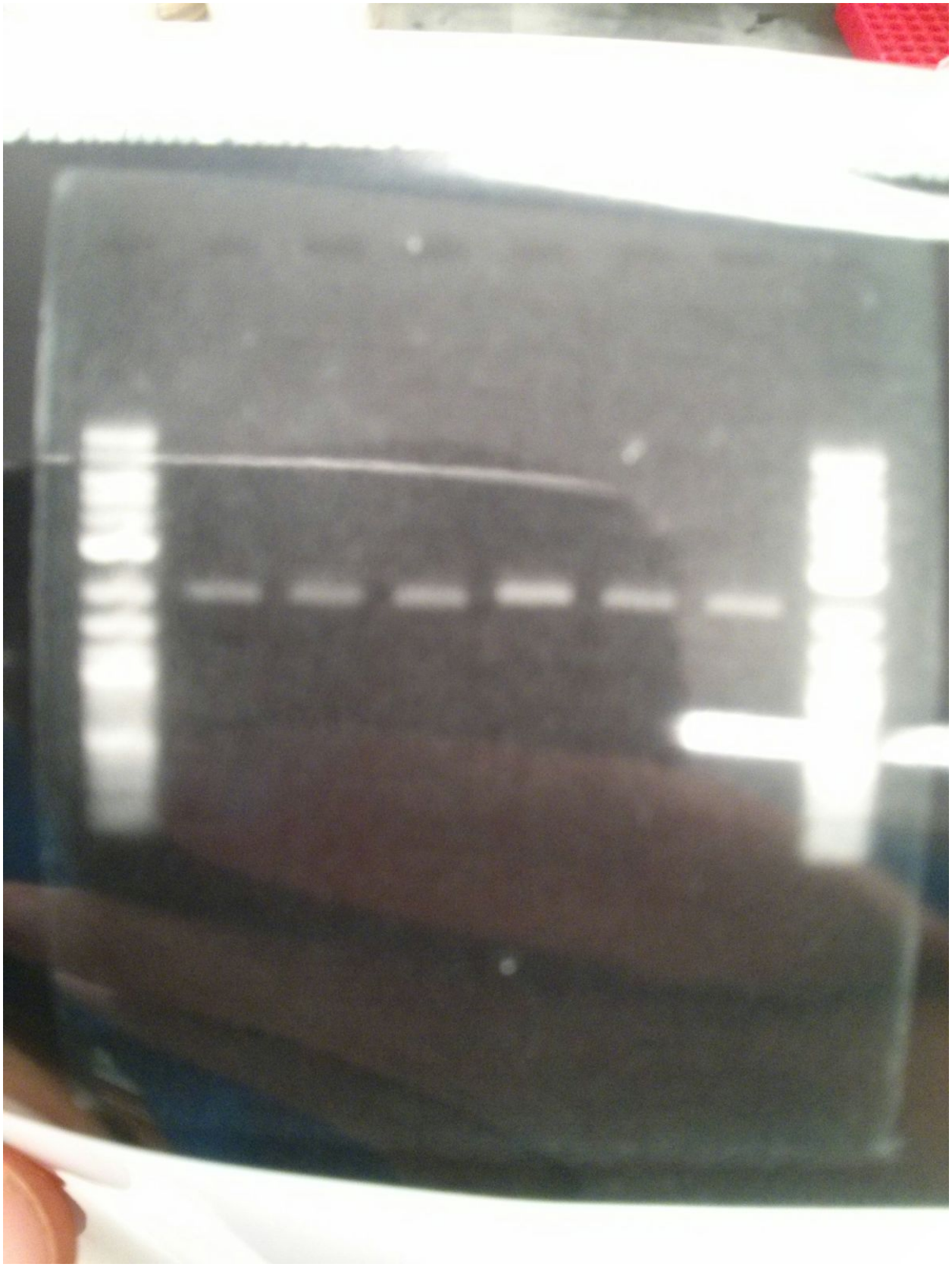
- K823013-1C3 (A, B, C)
    - all good concentrations
- R0011-1C3 (A, B, C)
    - all good concentrations

Objective: Analytical digest

Jeremy, Ben

- R0011-B0034-1C3 (1, 2, 3) -- MfeI/AlwNI
- R0011-B0034-1K3 (1, 2, 3) -- MfeI/AlwNI

  Results: Ugly photo. But all are single uncut bands. No promoter in constructs.



Objective: Send in BigDye sequencing

- CDT-1C3
- CDT-1K3
- K823005-I13504-1K3 (2)
- K823013-I13504-1A3 (D)
- K823013-I13504-1A3 (F)
- K823013-I13504-1A3 (H)

Objective: Prepare repeat-sequence-repeat scaffold for oligos

Objective: Anneal more KNR2-6

Ben, Adam

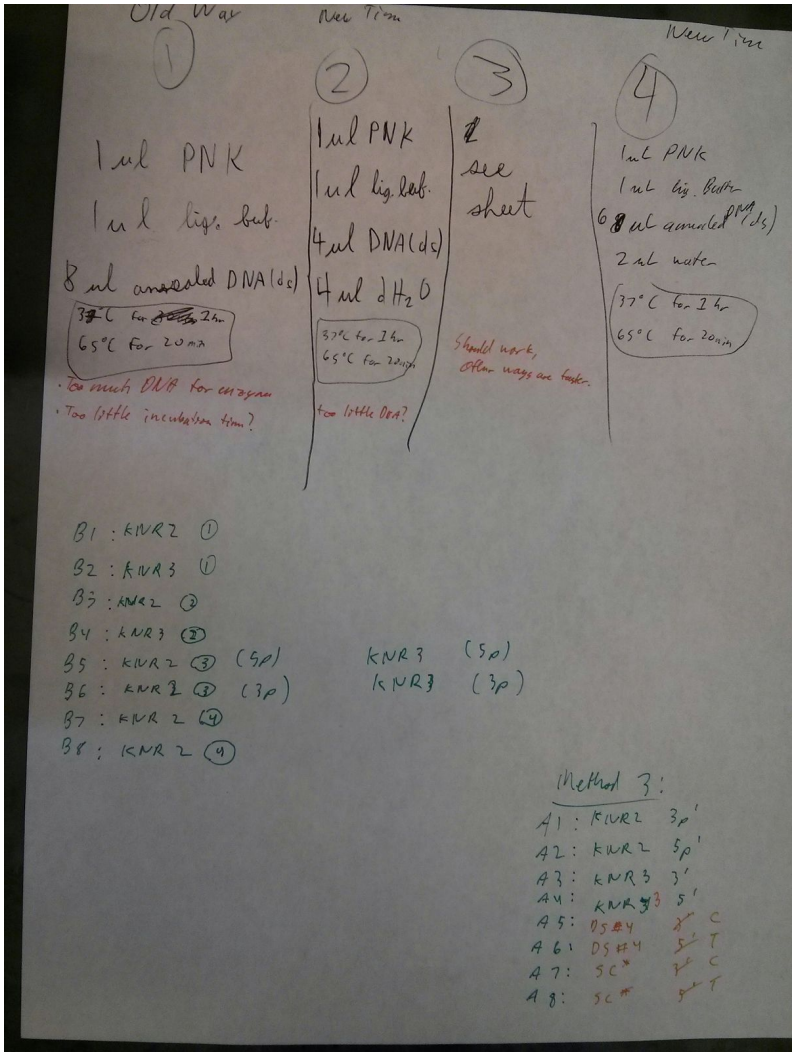
- 2uL each oligo
- 16uL water

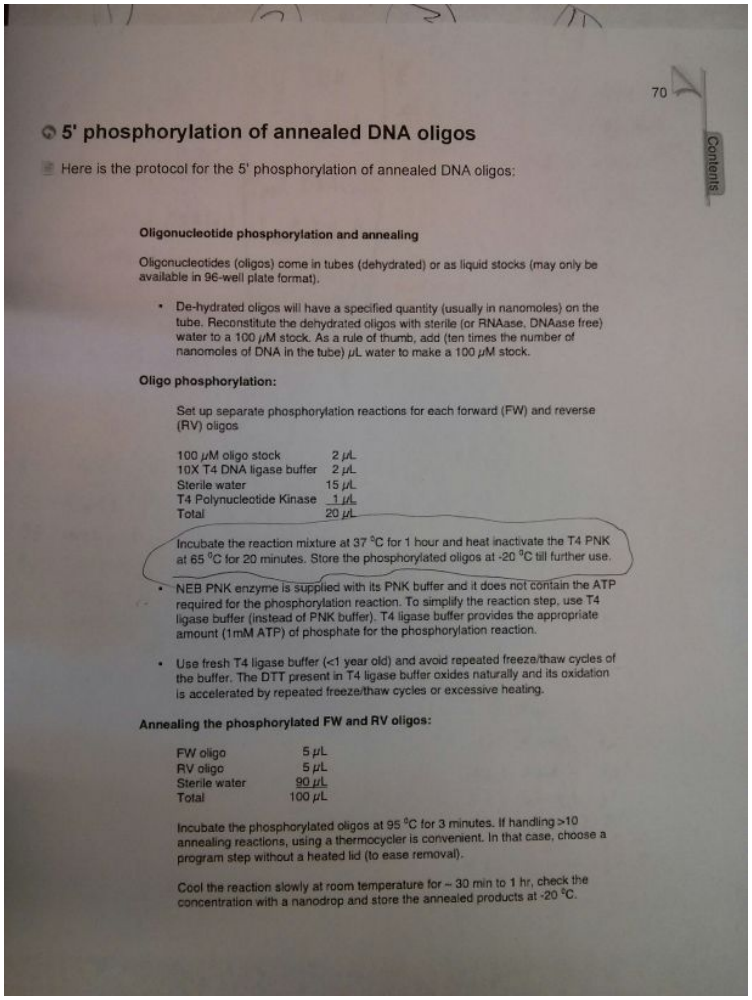
Results: Used to compare different kinase protocols

Objective: Compare different kinase protocols

Ben, Adam

Procedure: Used 3 PCR tube racks. KNR2 and KNR3 with each of the following methods. Method 3 is detailed in the second image below.


Old Way
New Time
New Time
1
2
3
4
1ul PNK
1ul lig. buf.
8 ul annealed DNA(ds)
37°C for 2hr
65°C for 20 min
• Too much DNA for enzyme
• Too little incubation time?
1ul PNK
1ul lig. buf.
4ul DNA(ds)
4ul dH2O
37°C for 1hr
65°C for 20min
too little DNA?
see sheet
Should work, other ways are faster.
1ul PNK
1ul lig. Buffer
6 ul annealed DNA(ds)
2ul water
37°C for 1hr
65°C for 20min
B1 : KNR2 1
B2 : KNR3 1
B3 : KNR2 2
B4 : KNR3 2
B5 : KNR2 3 (5p)
B6 : KNR2 3 (3p)
B7 : KNR2 4
B8 : KNR2 4
KNR3 (5p)
KNR3 (3p)
Method 3:
A1 : KNR2 3p'
A2 : KNR2 5p'
A3 : KNR3 3'
A4 : KNR3 5'
A5 : DS#4 C
A6 : DS#4 T
A7 : SC* C
A8 : SC* T



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Contents

5' phosphorylation of annealed DNA oligos

Here is the protocol for the 5' phosphorylation of annealed DNA oligos:

Oligonucleotide phosphorylation and annealing

Oligonucleotides (oligos) come in tubes (dehydrated) or as liquid stocks (may only be available in 96-well plate format).

- De-hydrated oligos will have a specified quantity (usually in nanomoles) on the tube. Reconstitute the dehydrated oligos with sterile (or RNAase, DNAase free) water to a 100 μM stock. As a rule of thumb, add (ten times the number of nanomoles of DNA in the tube) μL water to make a 100 μM stock.

Oligo phosphorylation:

Set up separate phosphorylation reactions for each forward (FW) and reverse (RV) oligos

100 μM oligo stock	2 μL
10X T4 DNA ligase buffer	2 μL
Sterile water	15 μL
T4 Polynucleotide Kinase	1 μL
Total	20 μL

Incubate the reaction mixture at 37 ^{0}C for 1 hour and heat inactivate the T4 PNK at 65 ^{0}C for 20 minutes. Store the phosphorylated oligos at -20 ^{0}C till further use.

- NEB PNK enzyme is supplied with its PNK buffer and it does not contain the ATP required for the phosphorylation reaction. To simplify the reaction step, use T4 ligase buffer (instead of PNK buffer). T4 ligase buffer provides the appropriate amount (1mM ATP) of phosphate for the phosphorylation reaction.
- Use fresh T4 ligase buffer (<1 year old) and avoid repeated freeze/thaw cycles of the buffer. The DTT present in T4 ligase buffer oxides naturally and its oxidation is accelerated by repeated freeze/thaw cycles or excessive heating.

Annealing the phosphorylated FW and RV oligos:

FW oligo	5 μL
RV oligo	5 μL
Sterile water	90 μL
Total	100 μL

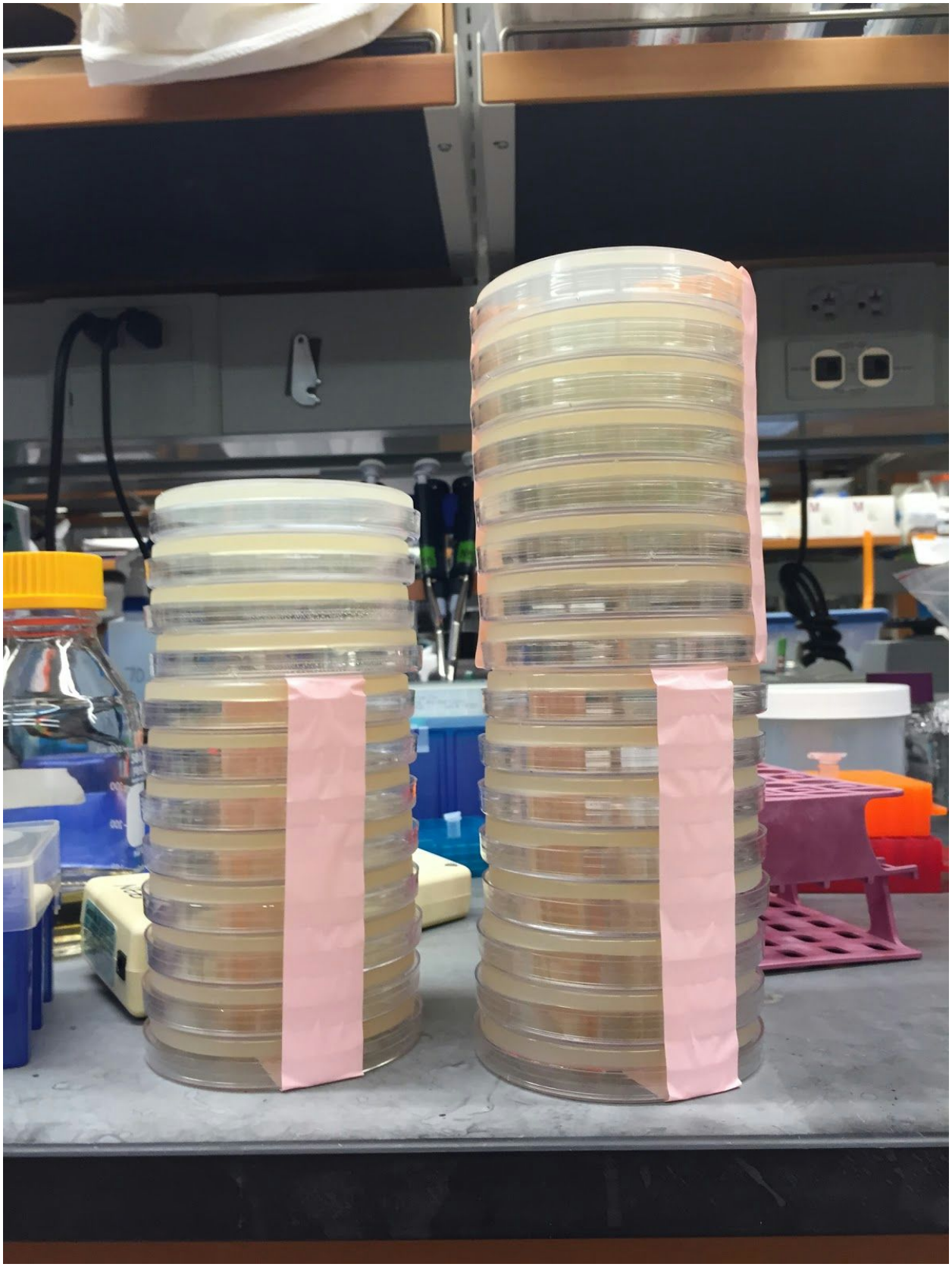
Incubate the phosphorylated oligos at 95 ^{0}C for 3 minutes. If handling >10 annealing reactions, using a thermocycler is convenient. In that case, choose a program step without a heated lid (to ease removal).

Cool the reaction slowly at room temperature for ~ 30 min to 1 hr, check the concentration with a nanodrop and store the annealed products at -20 ^{0}C.

Next Steps: After plating, find out which method works best and perform this method with KNR4-6.

Objective: Transform

- Tachyplesin-1C3 Gibson



Objective: Innoculate InterLab Study Pieces

- K823005-1C3
- K823008-1C3
- K823013-1C3

Objective: MRW Ligations

- **Results:** https://www.youtube.com/watch?v=s_hFTR6qyEo

7/16/2015

Objective: Miniprep with Mert

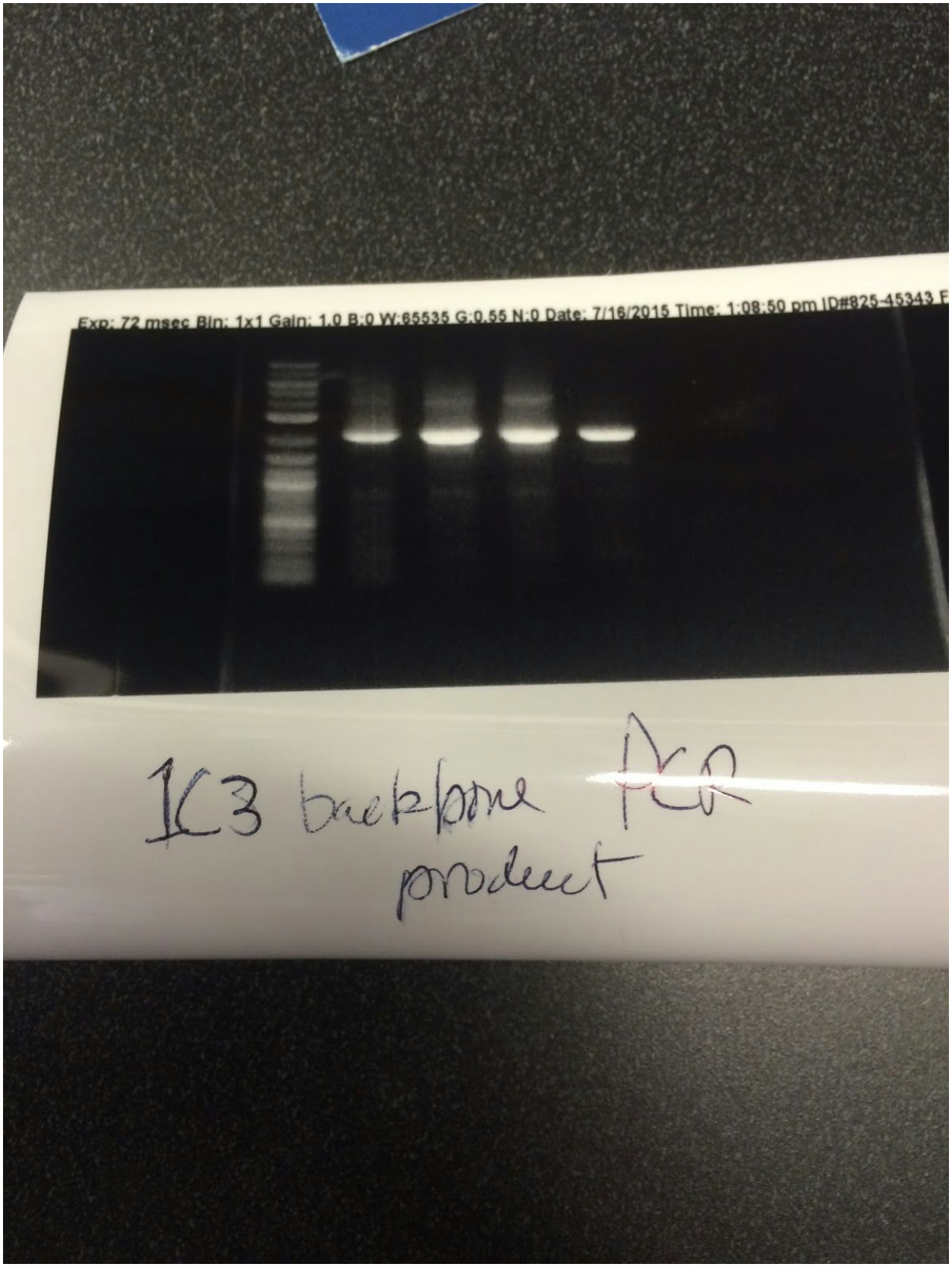
Mert, Jeremy

- K823005
- K823008
- K823013
- I13504
- B0034-1A2
- R0011-1C3

Objective: Run gel on PCR product from 7/13

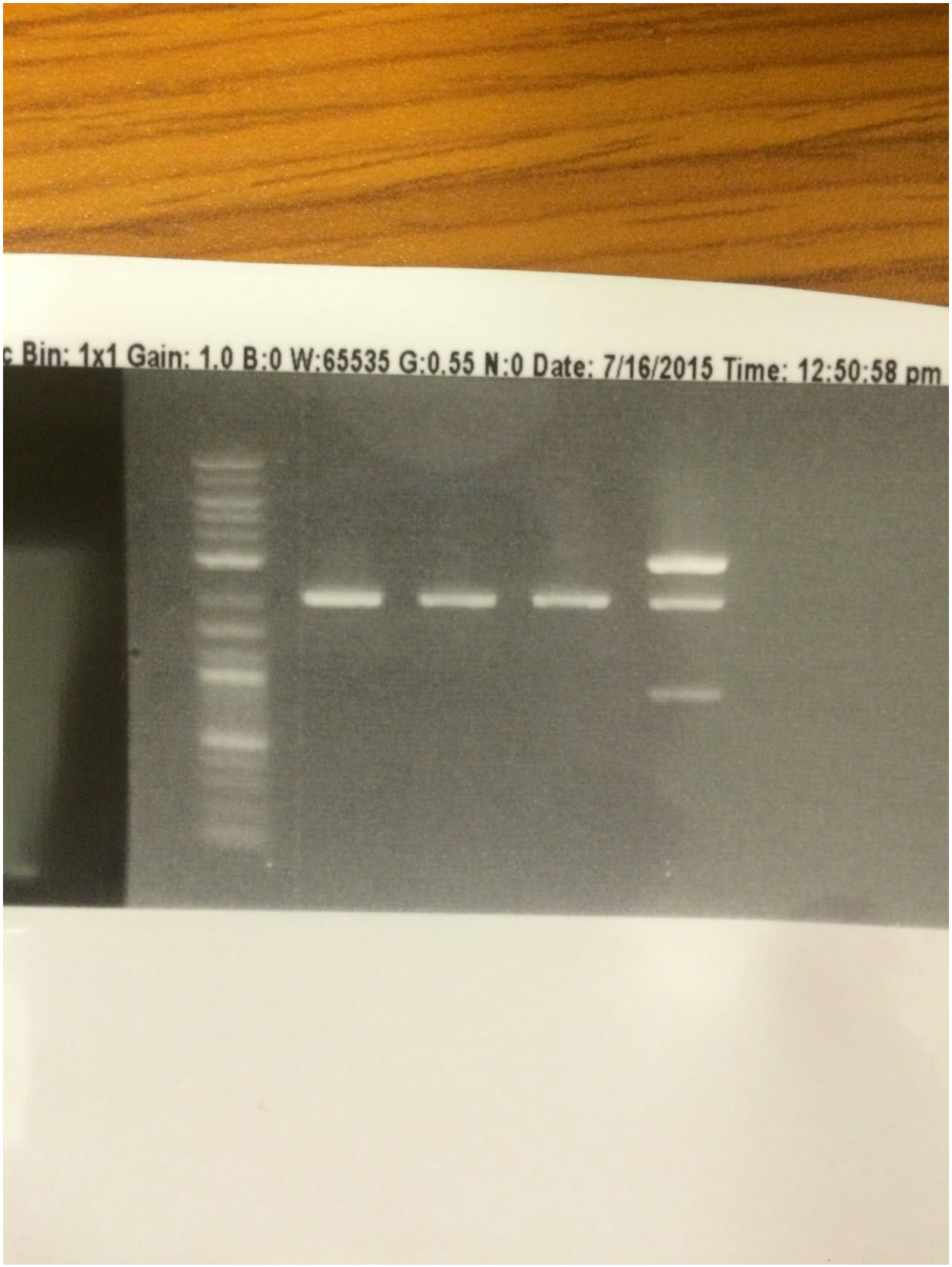
Jeremy

- to verify that we did in fact get the backbone out


Exp: 72 msec Bin: 1x1 Gain: 1.0 B:0 W:65535 G:0.55 N:0 Date: 7/16/2015 Time: 1:08:50 pm ID#825-45343 F
1C3 backbone PCR product

Objective: Preparatory restriction digest

- Check bands to make sure cuts are ok


c Bin: 1x1 Gain: 1.0 B:0 W:65535 G:0.55 N:0 Date: 7/16/2015 Time: 12:50:58 pm

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7/17/2015

Objective: Miniprep

Jeremy

- Tachyplesin-1C3 gibson assembly plate #2 (A, B, C)

Results: all ~170 ng/ul :)

Objective: Analytical Digest of tachyplesin-1C3 A, B, and C

Jeremy

- all cut with EcoRI, EcoRV, and PciI
- run on TAE gel

Results:


Exp: 0.28 sec Bin: 1x1 Gain: 1.0 B:0 W:65535 G:0.55 N:0 Date: 7/17/2015 Time: 2:16:33 pm ID#825-453
A B C
Tachyplesin-HB3 (Gib

Objective: BigDye 1.1 sequencing reaction

Jeremy

1) Tachy-1C3-Gibson2A
2) Tachy-1C3-Gibson2B
3) Tachy-1C3-Gibson2C

Objective: Gibson Assemble gBlocks onto 1C3

- Note: Used cut plasmid, and thus overlap region was small
- Protegrin (K628000) - 1C3
- You Lab Gene (E Lysis gene) - 1C3
- CDP - 1C3
- Backbone Only
- **Results:** Not significantly more colonies on experimental plates than Backbone Only negative control

Objective: Insert gRNA oligos into repeat-sequence-repeat scaffold

- Digested r-s-r with Bsa1 for 2 hours
- Treat r-s-r with CIP
    - 2 uL CIP in 50 uL reaction for an hour
- Gel purify r-s-r, recover with Qiagen Gel Recovery Kit
- Treat gRNAs with Polynucleotide Kinase for 30 minutes then anneal
- Ligate gRNAs
    - 50 ng r-s-r (cut, CIP treated, purified)
    - 1 uL of 1:100 dilute annealed oligos
    - 1 uL Ligase
    - 1 uL Buffer
    - Fill to 10 uL with Water
    - gRNAs Used: KNR 2 - 6, GFPs: -35, -10, Start Codon, Down Stream 1 - 4

Objective: Transform ligated plasmids

- KNR 2 - 6, GFPs: -35, -10, Start Codon, Down Stream 1 - 4 gRNA scaffolds
- Gibson Backbone Only Control, CDP - 1C3, K623000 - 1C3, YouLabGene - 1C3
- CDT - 1A3, CDT - 1K3

Objective: Innoculate colonies from 7/16 ligations

- K823005-I13504-1C3 A - C
- K823008-I13504-1C3 A - C
- K823013-I13504-1C3 A - F

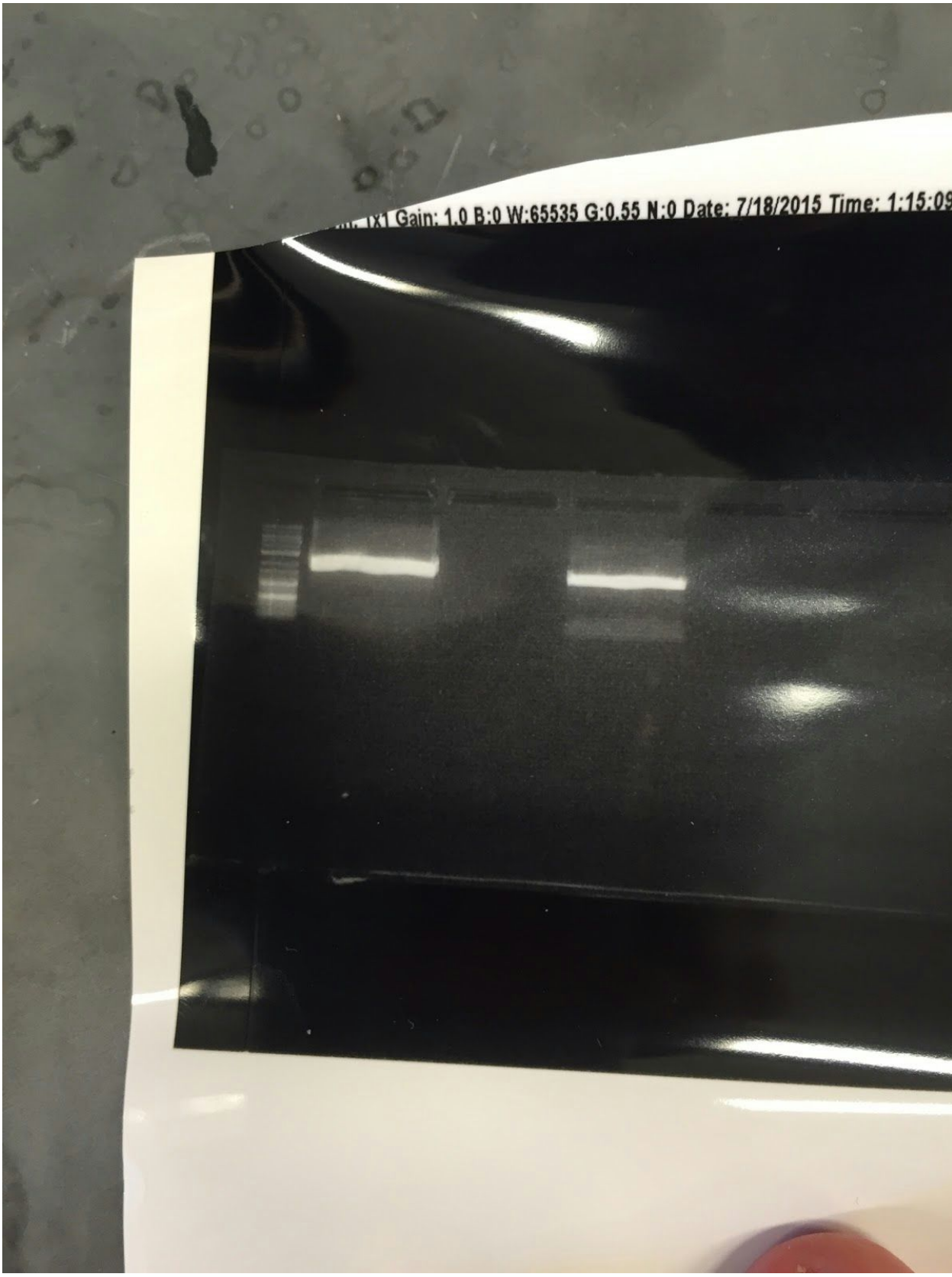
7/18/2015

Objective: Miniprep and Analytical Digest of InterLab Study K8230##-I13504-1C3

- Qiagen Miniprep Kit
- Restrict each plasmid with EcoRI and MfeI
    - Predicted bands: 642, 2346
    - 
    - Left to Right: K...5: A B C; K...8: A B C; K...13: A B C D E F
    - **Next Step:** Sequence K823005-I13504-1C3 and K823008-I13504-1C3 samples

Objective: Attempt R0011-B0034-1A2 Ligation

- Restrict R0011-1C3 with AatII and XbaI
- Restrict B0011-1A2 with AatII and SpeI
- Gel Purify B0011 - 1A2 (2200 band) and R0011 - 1C3 (200 band)
    - R0011 band was present in gel, but was found to be 0.3 ng/uL after clean up

- 

- Ligate

    - 1 uL Backbone at 30.2 ng/uL
    - 7 uL of Insert at 0.3 ng/uL
- Transform over 100 uL of Chemically competent cells
- **Results:** Two colonies, disproven

Objective: Attempt K823013 - I13504 ligation with CIP treated K823013

- 0.5 uL of CIP added to 15 uL of cut, gel purified K823013 in 50 uL total volume
- Treat with 0.5 uL Proteinase K for 15 minutes at room temperature
- Ligate with I13504 from 7/16
- Transform over 100 uL of Chemically competent cells
- **Results:** Nothing

7/19/2015

Objective: Miniprep gRNA constructs and Gibsons

- *Qiagen Miniprep kit r-gRNA-r-1C3 for GFP -35, -10, SC, DS #1-4 and KNR #2-6, CDT-1A3, CDT-1K3, Protegrin-1C3 #1 and 2, CDP-1C3 #1 and 2, YouLabGene - 1C3 #1 and 2*
- *Perform digest:*
    - *YouLabGene: AlwNI and BsaAI: Expect 419, 644, 1283*
    - *CDP and Protegrin: EcoRI/EcoRV/PciI: Expect 280, ~900*


Exp: 0.42 sec Bin: 1x1 Gain: 1.0 B:0 W:65535 G:0.55 N:0 Date: 7/19/2015 Time: 8:02:14 pm ID#825-45369 File: Untitled

- Left to Right: CDT-1K3, Protegrin-1C3 #1 and 2, Ladder, CDP-1C3 #1 and 3, YouLabGene #1 and 2
- **Results:** Very promising but Protegrin #2 strangely high and YouLabGene #1 partially uncut

Objective: Ligate and Transform K823013 - I13504, CDP-1C3, Protegrin-1C3 and YouLabGene-1C3

- *All from previous stocks*
- *K823013 cut with SpeI and PstI, I13504 cut with XbaI and PstI*
- *CDP, Protegrin, YouLabGene, pSB1C3 all cut with EcoRI and PstI*

7/20/2015

Objective: Miniprep and Analytical Digest of R0011-B0034-1A2 colonies

- Qiagen kit
- **Results:** Nah.

Objective: Sequence gRNAs, Tachyplesin, CDP, Protegrin

Objective: Innoculate cells from K823013-I13504-1C3, CDP-1C3, Protegrin-1C3 (K628000-1C3)

- Two colonies pulled from each plate

Objective: Ligate and Transform gRNAs into scaffold

- All 12 gRNAs used

Objective: Gel purify, ligate and transform B0034 into R0011-1C3

- *Used XbaI/SpeI and AlwNI overhangs*
- *Only used 12 uL of B0034-1A2 because that was all that was left*

Objective: Ligate gRNAs into scaffold overnight

Objective: Transform B0034-1A2, K823005-I13504-1C3 (C) and K823008-I13504-1C3 (B)

7/21/2015

Objective: Miniprep

1. Protegrin-1C3 (A)
2. Protegrin-1C3 (B)
3. K823013-I13504-1C3 (A)
4. K823013-I13504-1C3 (B)

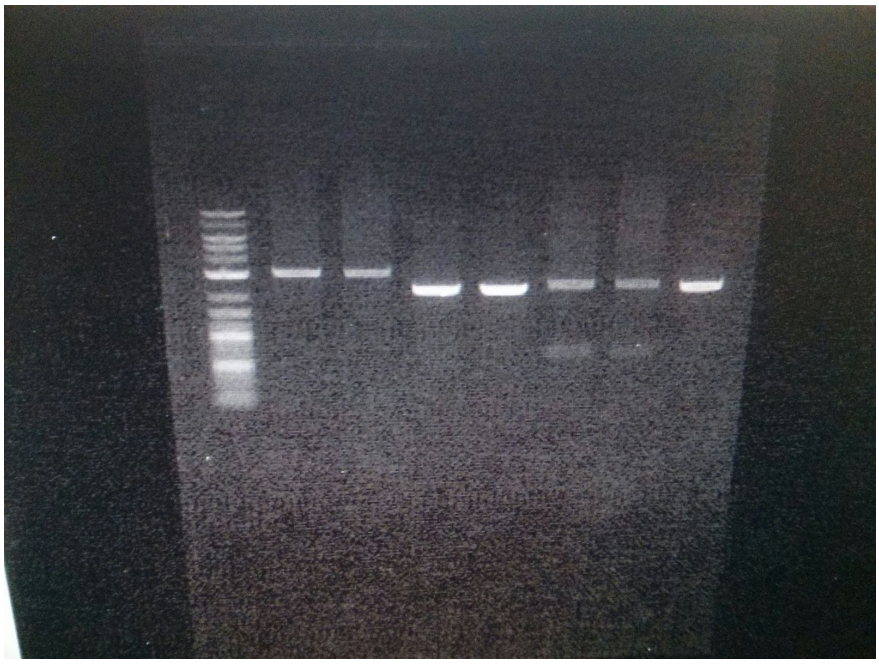
5. CDP-1C3 (A)
6. CDP-1C3 (B)
   Results: 150+ concentrations

Objective: Gel Purification

- r-s-r backbone.
  Results: BsaI did NOT cut all the way. Even after an overnight restriction!!!

Objective: Analyze Efficacy of EcoRV (Previous Objective not yet recorded)

- Mess-Up: Treated Adam's Oligos with EcorR1/Mfe1. Did not yield significant results for testing the presence of his cloned gRNA.
- Results:



  - From left to right:
    - Ladder, K823013-I-1C3 (A) EcoRV, K823013-I-1C3 (B) EcoRV, DS3 (B) EcoRV, DS4 (A) EcoRV, K823013-I-1C3 (A) EcoR1/Mfe1, K823013-I-1C3 (B) EcoR1/Mfe1, DS3 (B) EcoR1/Mfe1

7/22/2015

Objective: Miniprep

Ben

1. KNR6
2. KNR6 (B)
3. DS1
4. DS1 (B)
5. DS3

6. DS3 (B)
7. -10
8. -10 (B)
9. -35
10. -35 (B)
11. R0011-B0034-1C3
12. R0011-B0034-1C3 (B)
13. B0034-1A2

Results: Good

Objective: Analytical Digest with EcoR1 and Pst1

Ben

1. KNR 6 (A & B)
2. -35 (A & B)
3. -10 (A & B)
4. DS1 (A & B)
5. DS3 (A & B)

Notes:

- Ran on TBE, 100V for 25min
- Expect: No more than a 50 basepair difference between bands.

Results:

- Adam's all appear to have worked. KNRs just don't want to ligate.

Objective: Ligate reannealed oligos

Ben

KNR2-6 + Ds2

7/23/2015

Objective: Miniprep

Ben

1. -35 7/21 (1)
2. -35 7/21 (2)
3. SC 7/21 (1)
4. SC 7/21 (2)
5. KNR5 7/21 (2)
6. KNR5 7/21 (1)
7. SC 7/20 (1)
8. SC 7/20 (2)
9. KNR4 7/20 (2)
10. KNR4 7/20 (1)
11. -10 7/21 (1)

12. -35 7/21 (3) ** **[This one or -35 7/21 (2) should be -10 7/21 (2)]**

Objective: Analytical Digest of Morning Minipreps

Notes: All cut with EcoR1 and Pst1

From left to right:

Ladder | SC(1) 7/20 | SC(2) 7/20 | SC(1) 7/21 | SC (2) 7/21 | KNR4 (1) | KNR4(2) | (-) control - DS4 | Ladder | (+) control | KNR5 (1) | KNR5 (2) | -35(1) | -35(2) | -35(?) | -10 (1) | L

Conclusions: None can be made. From this, the only one that appears to not have worked is KNR4 (1). The negative control appears to have worked.

Objective: Re-Ligating

- KNR2-6, DS2

Notes:

- Made two test cases - one where ligations happen overnight and one where ligate for only an hour or two.
- Made 2 backbone only, one for each case
- 10 uL total volume. Make 9uL Master mix. This includes /tube:
    1. 50ng eq of DNA pure r-s-r backbone
    2. 1uL of T4 Ligase Buffer
    3. 1uL of T4 Ligase
    4. Watered to 9uL

Objective: Transformations

1. KNR2-6 + DS2 + K13-I-1C3

Results:

- Yesterday's ligations a failure. Circular backbone was used instead of cut backbone. Discarding all plates.
- 7/24 Inpspection: All inserts have less colonies than Backbone Only. Failure.

Objective: Gel purify Lac and Tet promoter sequences

Ben, TJ

Results:

- Lac - None.
- Tet - None. Really low yield on gel anyways.

7/24/2015

Objective: Digest r-s-r with new Bsa1

Ben

Notes:

- 100 uL total volume:
    - 36uL DNA
    - 10uL Cutsmart
    - 49uL Water
    - 5uL Bsa1
- Plan to run in 37C water bath overnight. Stopwatch is currently running on T1 if want to take out early.
- Run a gel purification afterwards.

Objective: Gibson Assemble Lac-RBS gBlock and TetBinding gBlock into 1C3

- 0.5 uL of 112.2 ng/uL pSB1C3
- 4.5 uL of 10 ng/uL TetBinding
- 2 uL of 10 ng/uL LacRBS
- Fill with water to 5 (0 uL for Tet, 2.5 for LacRBS, 4.5 for BO)
- Gibson to x1

Objective: Innoculate Binding Site Gibson, GFP gRNAs for cold stock

Objective: Interpret Sequencing Results

- K823013-I13504-1C3, YouLab-1C3, r--10-r-1C3, r--35-r-1C3 confirmed

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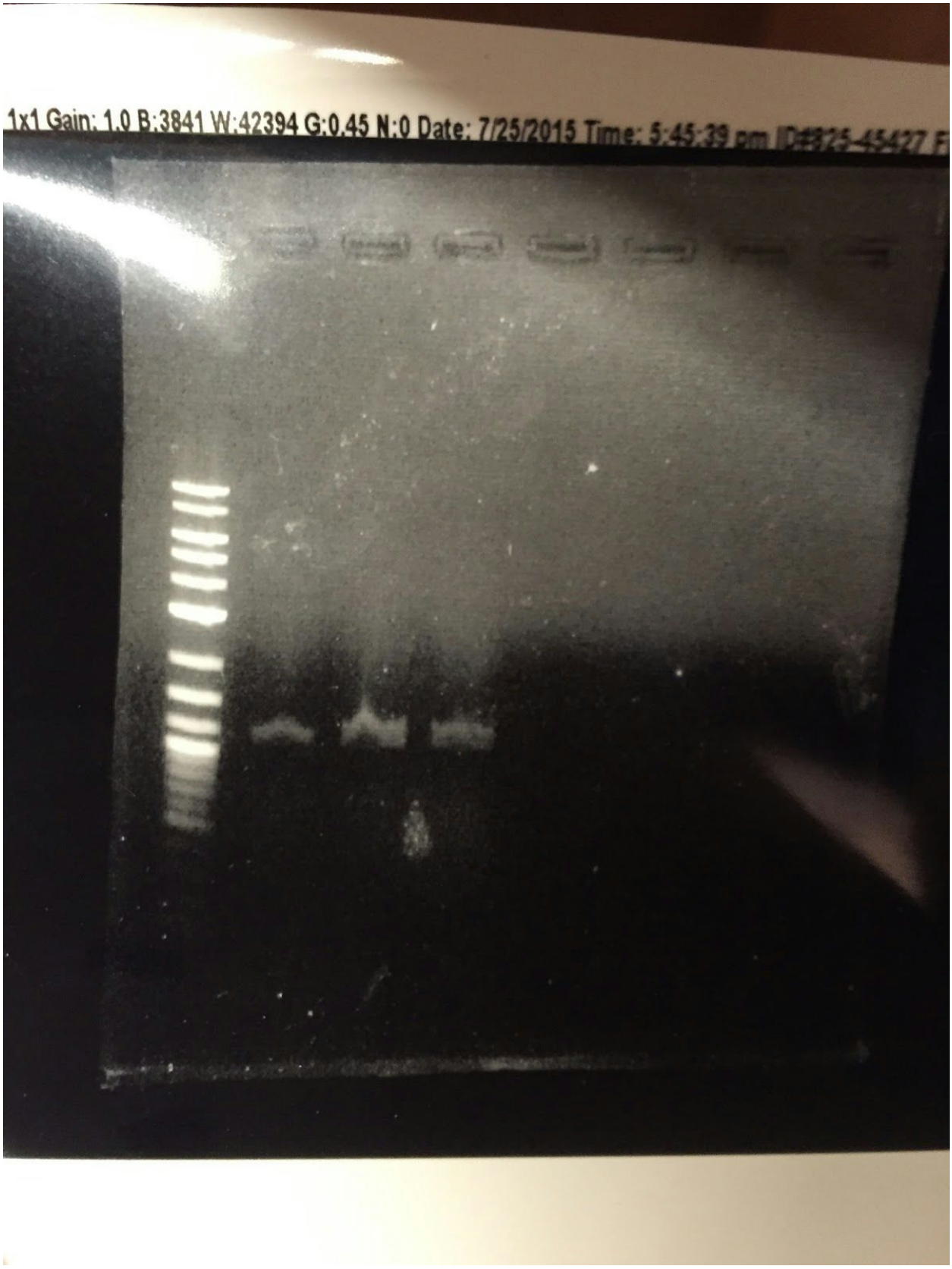
7/25/2015

Objective: Miniprep and analyze GFP Decoy-1C3

- QiaGen Kit
- NdeI/EcoRV: 1200



- **Results:** Looks good, sequence verify?

Objective: Miniprep B1006-1C3

Objective: Gel purify repeat-sequence-repeat and ligate in gRNA

- QiaGen Kit: ~60 ng/uL yield
- Transformed for overnight

Objective: Inoculate Gibson Assemblies of TetBinding-1C3 and LacRBS-1C3

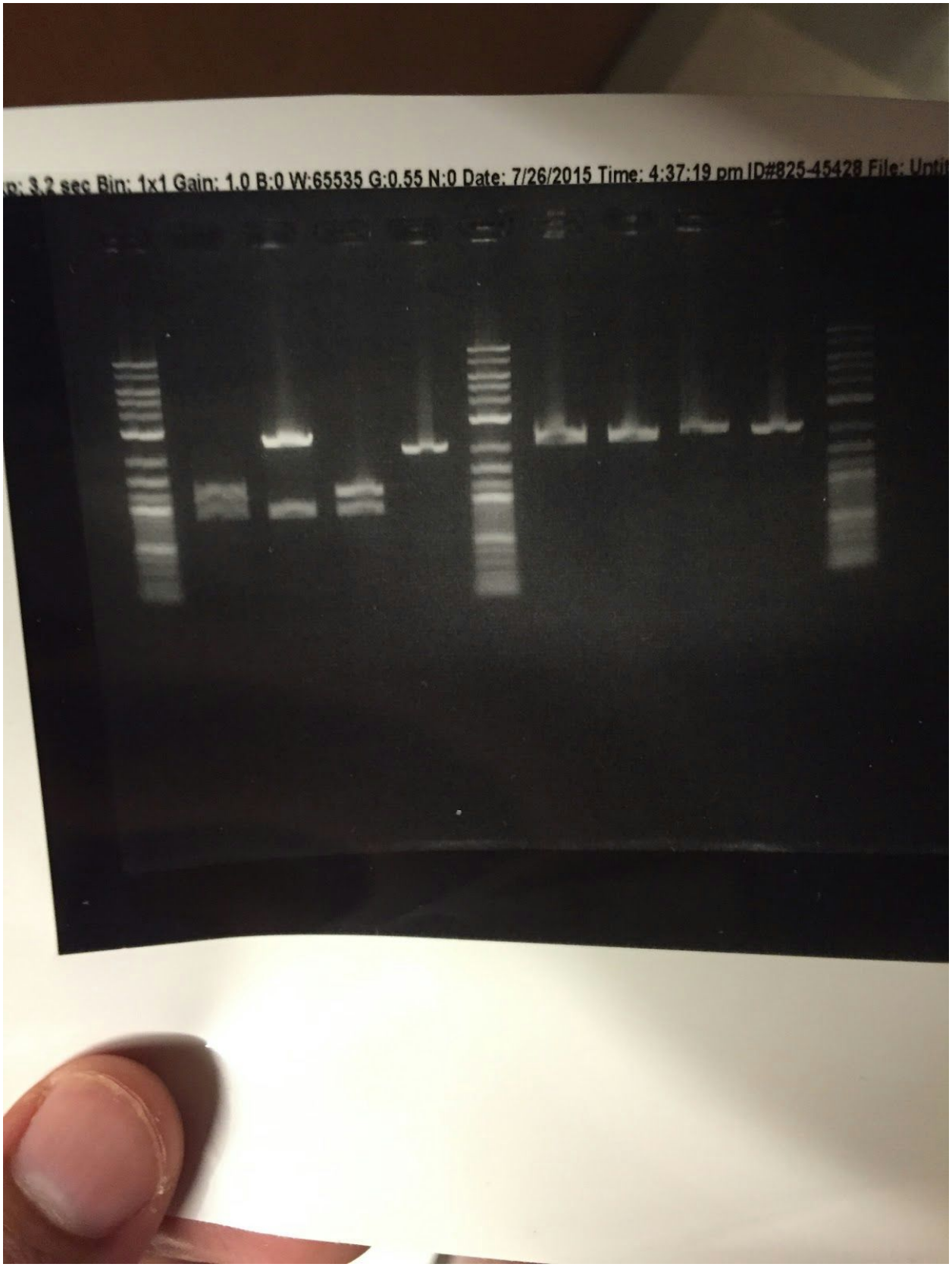
- 4 colonies each
-

7/26/2015

Note: Found error with the TetBinding gBlock that broke the EcoRI site. Could be source of Gibson troubles.

Objective: Miniprep and analyze TetBinding-1C3 and LacRBS-1C3

- Cut TetBinding-1C3 with XmaI/EcoRV (Expect 1170, 1237)
- Cut Lac-RBS-1C3 with MfeI/EcoRV (Expect 934, 1206)

- 

- Left to Right: Lac-RBS-1C3 (A->D), TetBinding-1C3 (A->D)

- **Results:** Lac-RBS-1C3 A and C seemed to work, screen more for TetBinding? Test with different enzyme like HindIII?

Objective: Make LB+Cm plate

- Done by protocol

7/27/2015

Objective: Restriction Digests

TJ

1. LacRBS-1C3:

Objective: Ligate LacRBS onto Cell Death Genes

Objective: PCR CDT out of 1C3 with PrePrefix and PostSuffix

Objective: Gibson CDT onto -1C3 backbone

Objective: Order Cm targeting oligos

Objective: Sequence GFP Decoy, Lac-RBS

Ben

1. DecoyGFP 1
2. DecoyGFP 2
3. DecoyGFP 3
4. LacRBS (A)
5. LacRBS (C)

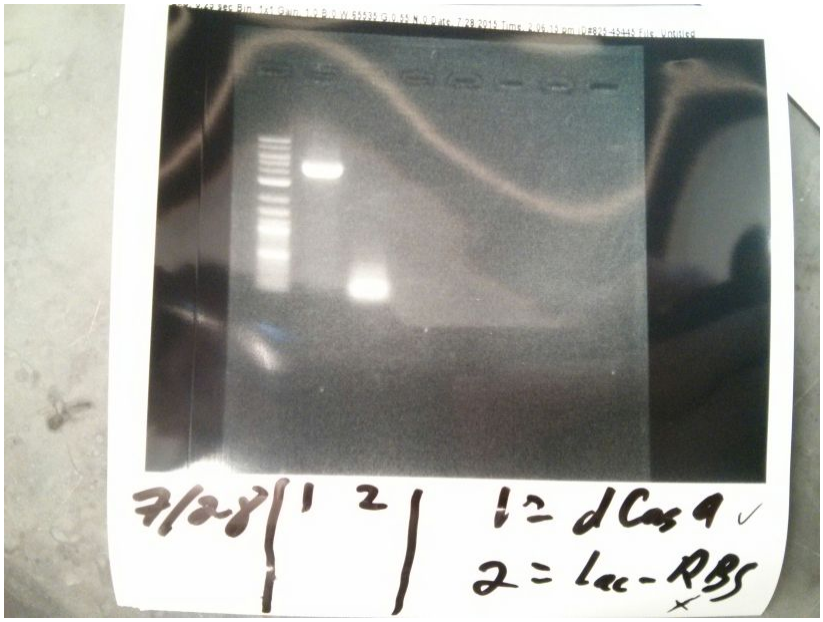
7/28

Objective: Analytical Digest of PCRs

TJ, Ben

1. dCas9 (5'Z)
2. LacRBS (3'A)

Results:

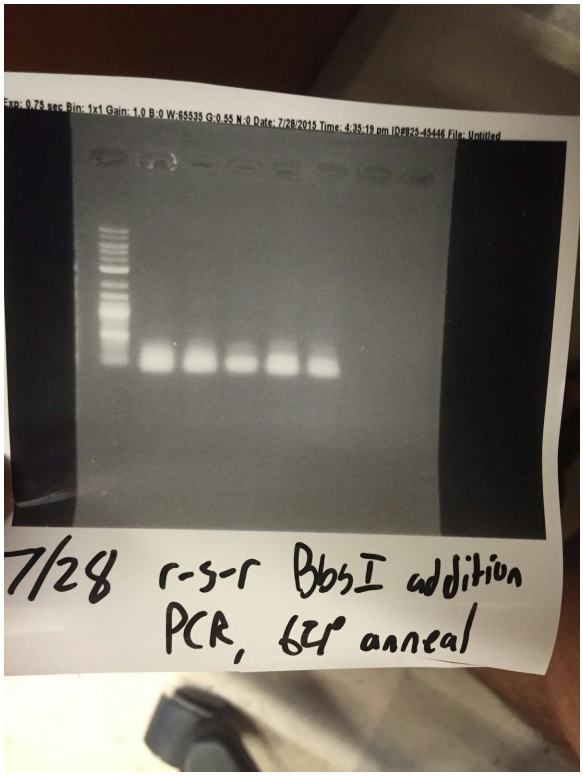


The dCas9 is at 4000 base pairs, as expected. This indicates a successful PCR reaction. The Lac-RBS PCR in column 2 was expected to be 2000 basepairs. Possible problems: The annealing temperature was too low. Solution: set a gradient protocol in the Thermocycler.

Objective: PCR r-s-r with Bbs-1 sites. Initiate Golden Gate Prep

Tj, Ben

1. -10 (5'A, 3'B)
2. SC (5'B, 3'C)
3. DS1 (5'C, 3'D)
4. DS3 (5'D, 3'E)
5. DS4 (5'E, 3'Z)



Results:

Only noise. Try higher temp?

Objective: Restrict BbsI PCR'ed Products

TJ

Objective: Inoculate Possibly Successful Transformations

Ben, TJ

Innoculated Two Samples of:

1. KNR2
2. KNR2 (CIP)
3. KNR3 (CIP)
4. DS2
5. LR-CDT
6. LR-Tachy
7. LR-Proteg
8. LR-CDP
9. LR-K117000

Innoculated for Storage:

1. -35
2. -10
3. SC
4. DS1
5. DS3
6. DS4

8/26/2015

Objective: Prepare fragments for GFP Targeting Golden Gate

- gRNA amplified and primers used
    - -35, BbsI 5' A and BbsI 3' B
    - SC, BbsI 5' B and BbsI 3' C
    - DS1, BbsI 5' C and BbsI 3' D
    - DS3, BbsI 5' D and BbsI 3' E
    - DS4, BbsI 5' E and BbsI 3' Z
    - 72 C annealing, 3 second extension
- Linearize lac-1C3
    - R0011-1C3, BbsI 5' Z-Suffix and BbsI 3' A -Lac
    - 72 C annealing, 60 second extension
    - Run gel to verify success

8/27/2015

Objective: Finish Golden Gate Assembly

- PCR Cleanup lac-1C3
- Restrict PCR Products with BbsI
- Ligate gRNAs onto Lac-1C3
- Transform

Objective: Transform odds and ends for positive control

Objective: Ligate Lac Promoter onto Final Programmable Death Genes

- Restriction
    - LacRBS-1K3, SpeI/AatII
    - CDT-1A3, XbaI/AatII
    - CDP-1C3, XbaI/AatII
    - K117000-1C3, XbaI/AatII

8/28/2015

Objective: Finish Programmable Cell Death Ligations

- Gel Extraction
    - Note: Use TAE, not TBE for easier clean up
    - LacRBS: Collect 200 bp, not 2000
    - CDT: Collect large band
    - CDP: Collect large band
    - K117000: Collect large band

  Ben-"Gel started at 12:20pm. Did not run an analytical first so I hope the restrictions are sufficiently completed. The gel contains (from left to right): Ladder, LacRBS, CDT, CDP, K117000."
- Gel Purification (QiaGen Kit, including isopropanol step)
    -
- Ligations:
    - LacRBS-CDT-1A3
    - CDT-1A3 BO
    - LacRBS-CDP-1C3
    - CDT-1C3 BO
    - LacRBS-K117000-1C3
    - CDT-1C3 BO
- Transform

Objective: Check and innoculate lac-GFPgRNA-1C3 plate

Ben- "Confused on the results of what I think is the BO control and the other two plates in the oven. Looks like contamination. All plates are now sitting on lab bench for further

inspection. I innoculated 3 samples, labeled them with 'i,' and placed the on the left of middle rack in shaker @ 12:00 pm today"

8/29/2015

Objective: Miniprep GFP-gRNA Constructs, CDP, K608012

- Left stock of K608012 for flow cytometry
- CDP complete, other samples were lost

8/30/2015

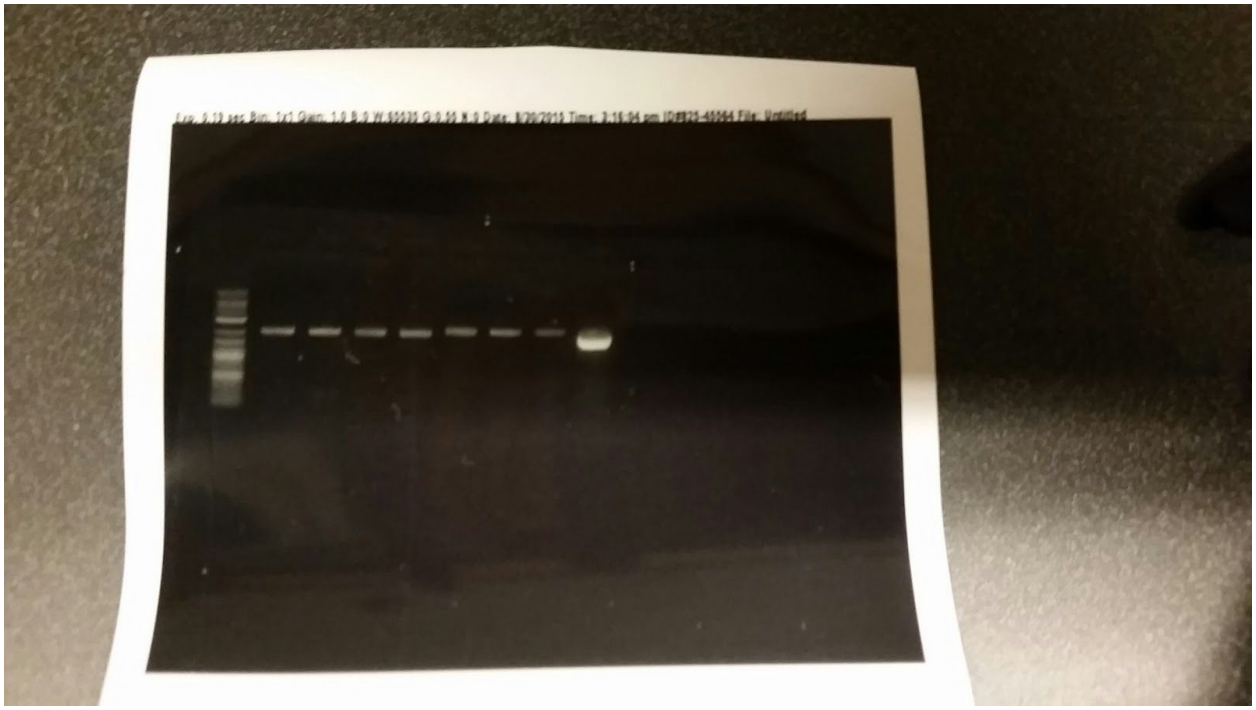
To Do:
Objective: Test Lac-GFPgRNAs-1C3 with Analytic Digest

- Miniprep
    - A - 116.8
    - B - 134.9
    - C - 117.0
    - D - 116.8
- Analytic Digest: EcoRI/PstI

Objective: Test LacRBS-K117000

- Miniprep
    - A - 117.3
    - B - 123.8
    - C - 118.8
- Analytic Digest: MfeI/EcoRV

**backbone only had a concentration of 138, not sure what to digest it with
*For gel below: from left to right, Lac-GFPgRNA-1C3's A, B, C, D; LacRBS-K117000's A, B, C; BO (unrestricted)



8/31/2015

Objective: Gibson Assemble LacRBS-dCas9-1C3

- PCR:
    - LacRBS-1K3 with 5p-1x3 Forward and 3p-dCas9-LacRBS
    - dCas9-1C3 with 5p-LacRBS-dCas9 and Post-Suffix
    - 72 C annealing, 90 second extension
- Gibson

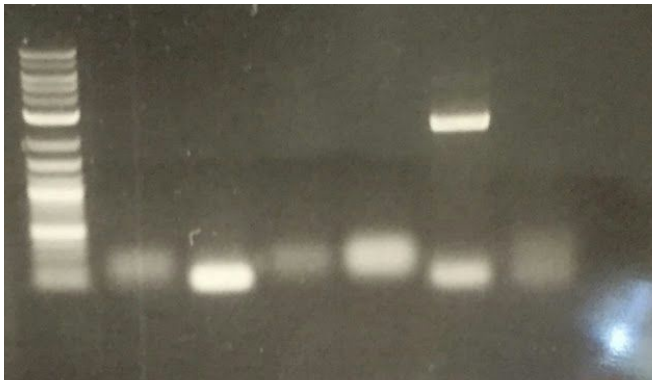
Objective: Gibson Assemble LacRBS-K117000-1K3, LacRBS-CDT-1K3

- PCR:
    - LacRBS-1K3 with 5p-1C3Resist and 3p-K117000LacRBS
    - K117000-1C3 with 5p-LacRBSK117000 and 3p-1C3Resistance
    - LacRBS-1K3 with 5p-1C3Resist and 3p-CDTLacRBS
    - CDT-1C3 with 5p-LacRBS-CDT and 3p-1C3Resistance
    - 72 C anneal, 35 second extension
- Gibson

9/1/2015

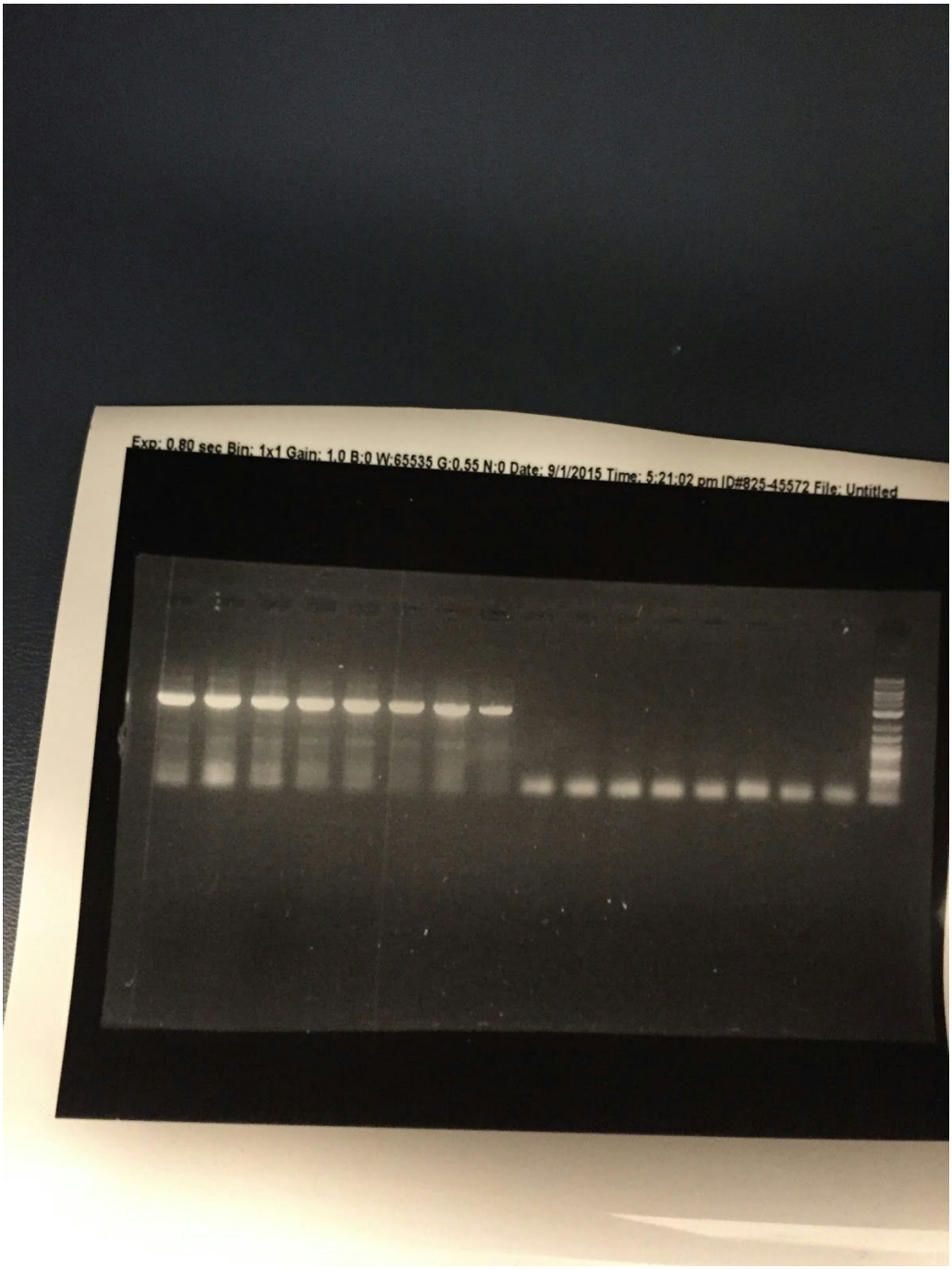
Objective: Grow colony for comp cells

Objective: Confirm PCR products for LacRBS-CDT-1K3, LacRBS-K117000-1K3, LacRBS-dCas9-1K3

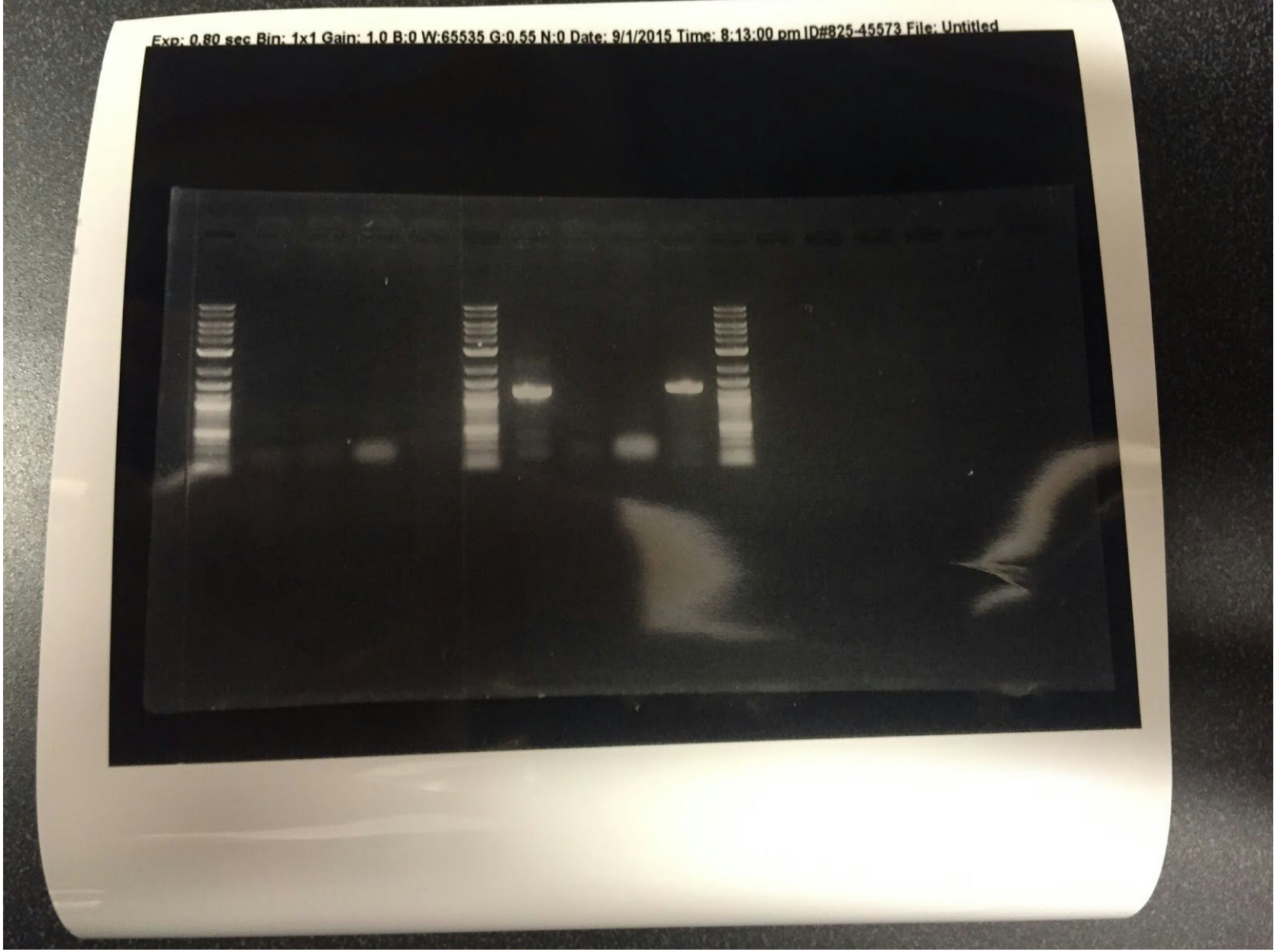
- Ran gel, only dCas9 had a product, but it was the wrong size (2000 bp instead of 4000)
- 
- Next Step: Try at a lower temperature? Use pdCas9 as plasmid source?

Objective: Retry PCR

- PCR:
    - LacRBS-1K3 with 5p-1x3 Forward and 3p-dCas9-LacRBS
    - dCas9-1C3 with 5p-LacRBS-dCas9 and Post-Suffix
    - 60-72 C gradiant annealing, 90 second extension
    - LacRBS worked, not dCas9, design primers for pdCas9?



-
    - Left side was Lac-RBS, right side was dCas9
- PCR:
    - LacRBS-1K3 with 5p-1C3Resist and 3p-K117000LacRBS
    - K117000-1C3 with 5p-LacRBSK117000 and 3p-1C3Resistance
    - LacRBS-1K3 with 5p-1C3Resist and 3p-CDTLacRBS
    - CDT-1C3 with 5p-LacRBS-CDT and 3p-1C3Resistance
    - 60-72 C gradient anneal, 35 second extension
    - K117000 and LacRBS for CDT worked at 60, try even lower?


Exp: 0.80 sec Bin: 1x1 Gain: 1.0 B:0 W:65535 G:0.55 N:0 Date: 9/1/2015 Time: 8:13:00 pm ID#825-45573 File: Untitled

○

    - Left to right: 66 degree: K117000, CDT, LacRBS for K117000, LacRBS for CDT; 60 degree: K117000, CDT, LacRBS for K117000, LacRBS for CDT

9/2/2015

Objective: Prepare comp cells

- LacRBS-1K3 with 5p-1C3Resist and 3p-K117000LacRBS
- CDT-1C3 with 5p-LacRBS-CDT and 3p-1C3Resistance
- 50-65 C gradient anneal, 35 second extension
- Nah.

Objective: Try PCR products at even lower melting temperature for CDT and LacRBS for K117000

Objective: Begin prep for CDT-1C3 Gibson

- PCR:
    - CDT-1K3 with 5p-1C3Resistance and 3p-Post-Suffix
    - Any -1C3 plasmid with 5p-1N3 and 3p-1C3Resistance
    - 55-60 C gradiant annealing, 35 second extension
    - Nah.
- Gibsoned with pre-linearized parts
- Transformed

Objective: Transform GFP-1A2

Objective: Reattempt Golden Gate with new comp cells

9/3/2015

Objective: Innoculate Successful Colonies

9/4/2015

Objective: Redo Lac-1C3 backbone for Golden Gate

- Primers:
    - Bbs1-A-Lac
    - Bbs1-Z-suffix
- *PCR on a temperature Gradient (60-68C)*

- *Extension Time: 60 seconds*

Objective: Perform analytic digest of CDT-1C3 GFP-1A2, Lac-GFPgRNAs

- Miniprep
    - Done. CDT-1C3, GFP-1A2, Lac-GFPgRNAs
- Analytical Digests:
    - DON'T digest CDT-1C3. Will sequence tomorrow
    - Lac-GFPgRNAs: MfeI and PstI (Band should be ~800) **In Unverified**

Objective: Ligate LacRBS-GFP-1K3

- Ligation Sized Restriction
    - LacRBS-1K3 (SpeI/PstI)
    - GFP-1A2 (XbaI/PstI) **In Unverified, next to Lac-GFPgRNAs**
- Gel purification
    - Failed.

Objective: Transform LacRBS-CDT-1K3, LacRBS-K117000-1K3 into DH5alpha Z1

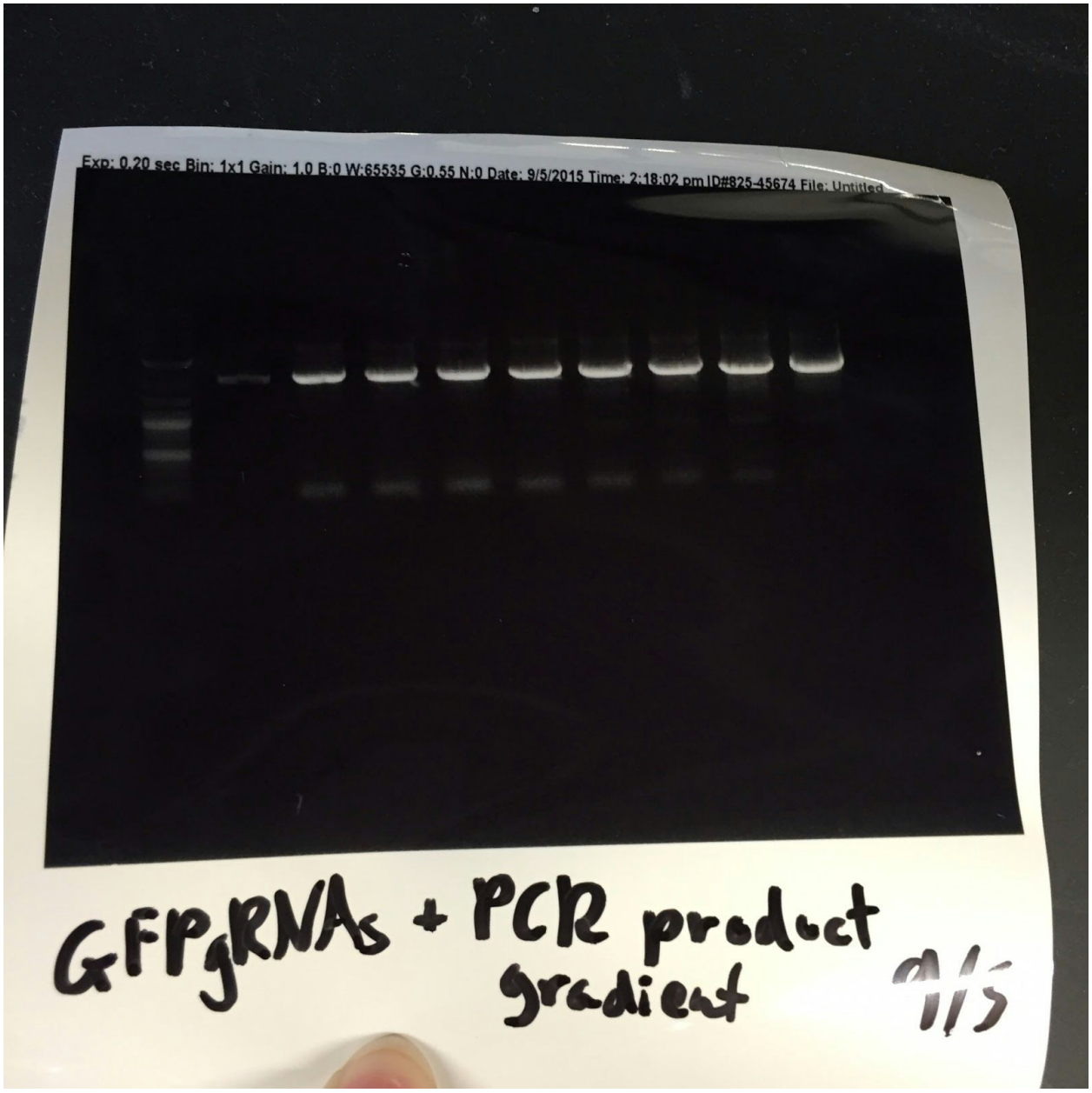
9/5/2015

Objective: Innoculate colonies for the LacRBS-YouLabGene testing tomorrow

- Pick 3 colonies

Objective: Run Lac Golden Gate PCR products and the Lac-GFPgRNAs analytic digest

- PCR products should be ~2000, the analytic should be ~800
- went ladder, GFPgRNAs, PCR products (low to high temp gradient)
- Result:PCR 2kb bands were there, nothing but a 2.1kb band for GFP gRNAs



- *PCR product cleanup: 66.5 (second try)*
    - *placed dead center in oligo box (blue labels)*

Objective: PCR Products for LacRBS-CDT-1K3, LacRBS-K117000-1K3, LacRBS-dCas9-1K3

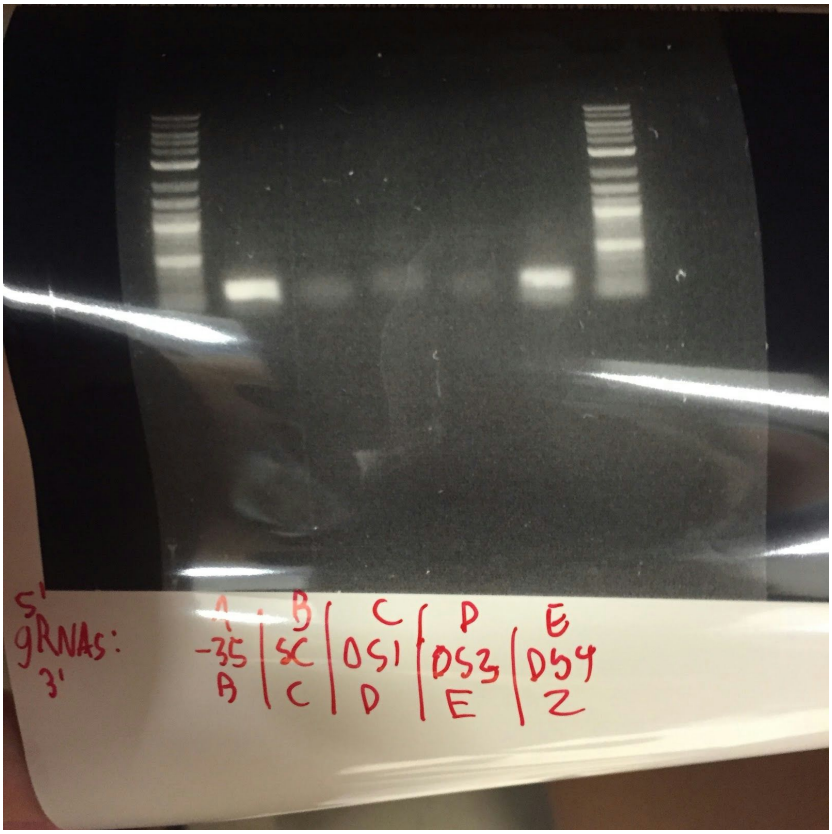
- LacRBS-dCas9-1K3
    - LacRBS: Done
    - dCas9: Try primers5p-LacRBS-dCas9 (In Oligo Box 2) and

9/6/2015

Objective: Retry GFPgRNA with new Lac-1C3

- Restrict Lac-1C3 PCR fragment with BbsI
    - NOTE: Recently we've been using NEB 2 buffer for this, which does not have BSA in it. I used it in this one, but it may behoove us to retry the gRNA restrictions with BSA added as well
    - 

- PCR gRNAs for restriction

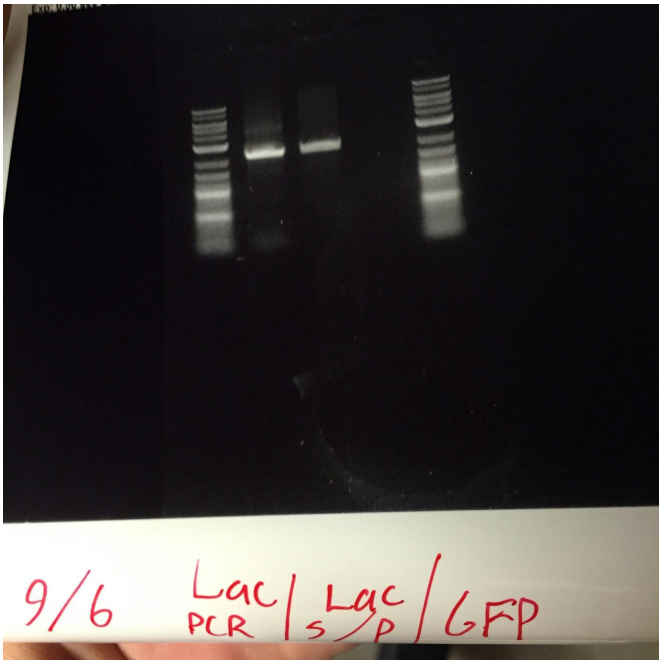
- 

    - -35 and DS4 look promising; DC, DS1 and DS3 questionable
    - Attempt with products but try messing with melting temperature
- Re-Try SC, DS1 and DS3 with lower annealing temperature
- Restrict gRNAs with BbsI
- Ligate
- Transform

Objective: Transform LacRBS-YouLabGene-1C3, LacRBS-Protegrin-1C3, LacRBS-Tachyplesin-1C3 into Z1 cells

Objective: Ligate LacRBS-GFP-1K3

- Restrictions
    - LacRBS-1K3: PstI/SpeI
    - GFP-1A2: PstI/XbaI
    - GFP failed, growing again from colony

- 

- Innoculate GFP-1A2
    - May have contamination

9/7/2015

Objective: Innoculate LacRBS-YouLabGene-1C3, LacRBS-Protegrin-1C3, and LacRBS-Tachyplesin

- Three colonies of each

Objective: Finish LacRBS-GFP-1K3

- Miniprep GFP
    - Pellets very small, can't do ligations. Try to regrow
- Analytic Digest
    - Cut with MfeI and EcoRI (want 560 band and 2300 band)
- Transform from miniprep or from BioBrick Plates

Objective: Troubleshoot failed PCRs for K117000 and LacRBS for CDT

- Try LacRBS with Suffix and 5p-1C3Resist (instead of CDT-LacRBS and 5p-1C3Resist)
- Try K117000 with Prefix and 3p-1C3Resist (instead of LacRBS-K117000 and 3p-1C3Resist)

Objective: Gibson CDP-1C3

- PCR Fragments
    - CDP: Pre-Prefix and 3p-1C3Resist

    - 1C3 Donor: 5p-1C3Resist and 1N3 Reverse
    - Try at 60, 66 and 72 (using gradient under options)
- Run Gel to Verify
- PCR Clean-Up
- Gibson Assembly
- Transform

Objective: Attempt LacRBS-CDT and LacRBS-CDP Ligation

- Restrictions
    - CDT-1A2 (AatI and XbaI)
    - CDP-1C3 (AatI and XbaI)
    - LacRBS-1K3 (AatI and SpeI)
    - Didn't have enough CDP or LacRBS
- Transformation CDP and LacRBS

Objective: Retry gRNAs BbsI PCRs: B-SC-C, C-DS1-D, D-DS3-E

- Try 60, 66 and 72 degrees

Objective: Sequence Experimental Plasmids from Previous results, and CDT-1C3

9/8/2015

The time course gave no results. I checked again with new IPTG, but it still didn't.