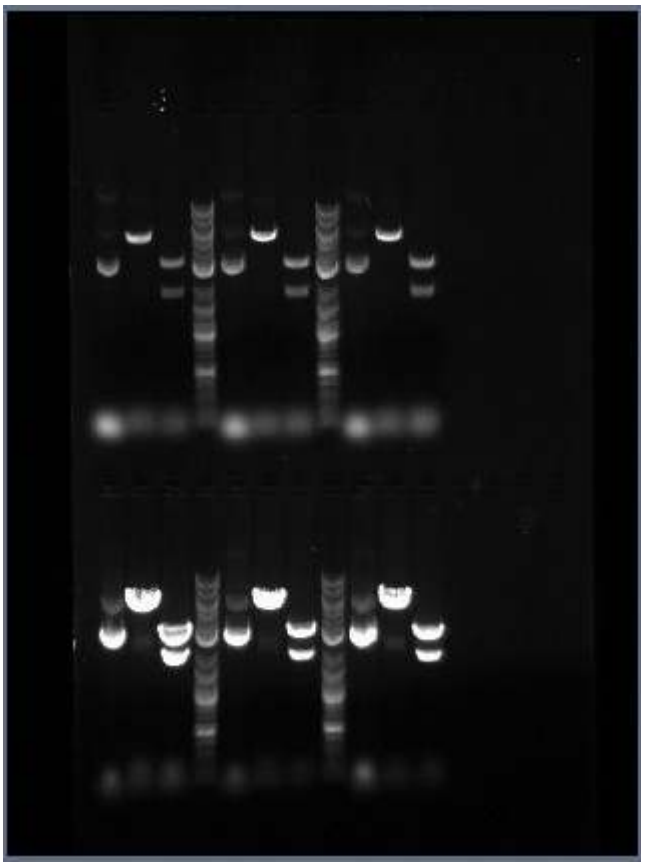
User:Pantalone

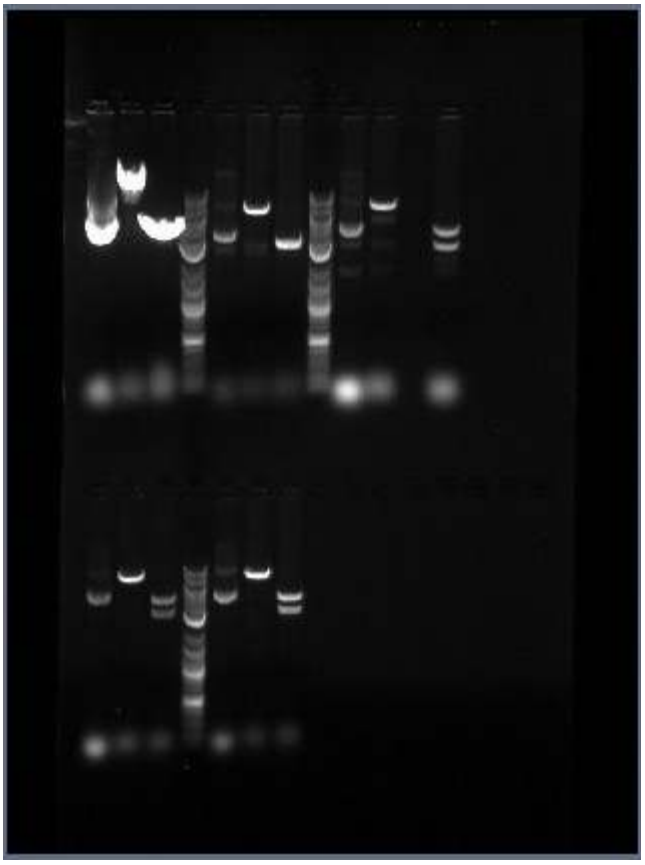
From IGEM WIKI

Nathaniel Pantalone

Notebook 8/25/10

GELs - 30min @ 100V





- 1,5,C, and D display the expected banding patterns for CTC250FP in 3T5, CTC250FP in 4C5, the Key Cass in 4C5, and the Key Cass in 3T5 respectively.

Notebook 8/24/10

only 2 colonies on all four controls. growth on K19+CTC250s but none on Keys. will let grow further.

Updated freezer stocks and made new Amp100 plates.

Finished Justin's multimer cloning. (6uL MultiFWD primer to quench, 2h benchtop ligation, PCR purification, DNA spec, final ligation 3h @ 16C)

Notebook 8/23/10

DIGEST - 2h @ 37C

K19+CTC250- 10uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

Key Cass - 10uL

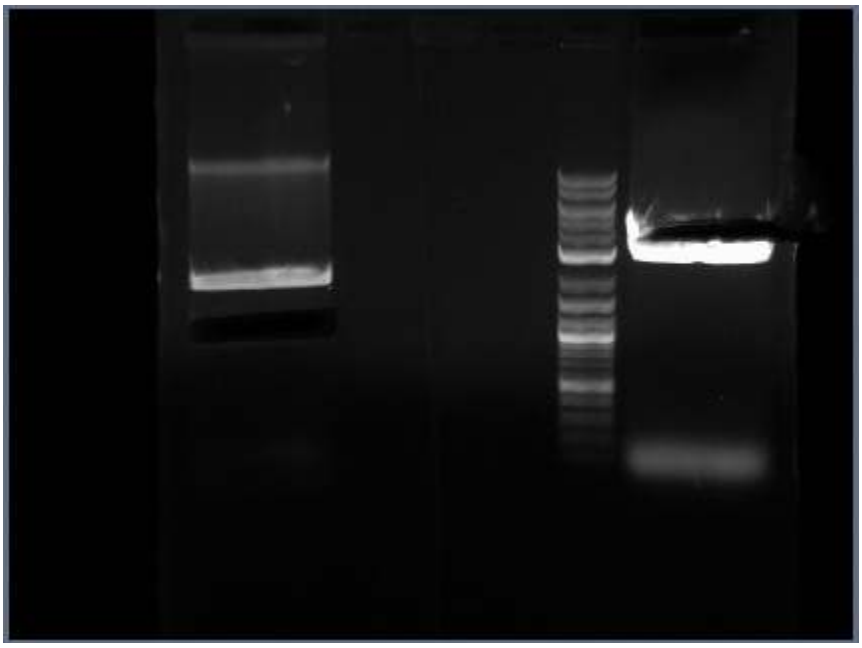
EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 33uL

GEL 35min @ 90V



L to R: ctc, ladder, key

DIGEST - 2h @ 37C

E0240 T - 10uL

EcoRIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

E0240 C - 10uL

EcoRIHF - 1uL

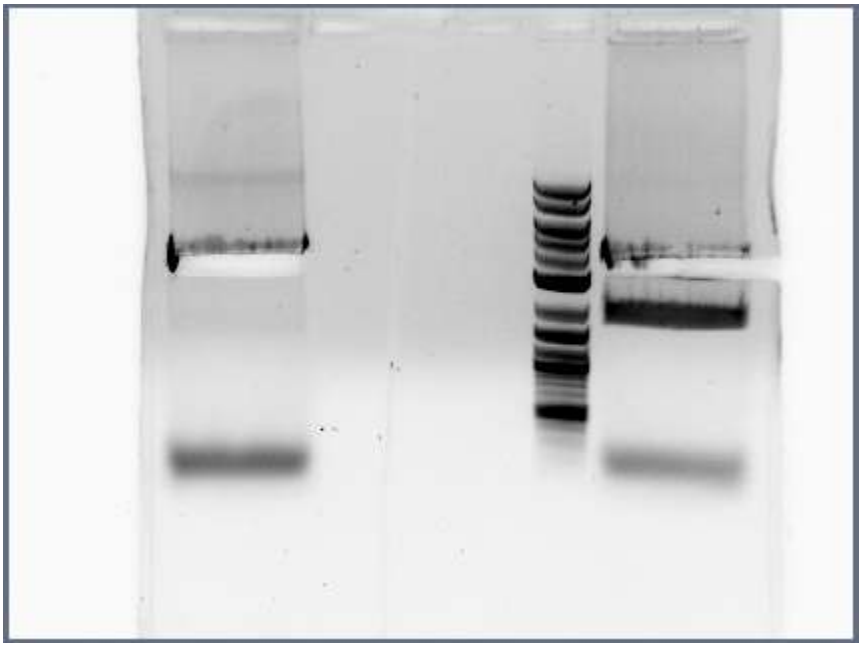
XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

GEL 35min @ 90V



L to R: E02 T, ladder, E02 C

DIGEST - 2h @ 37C

3T5 - 20uL

EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 23uL

4C5 - 10uL

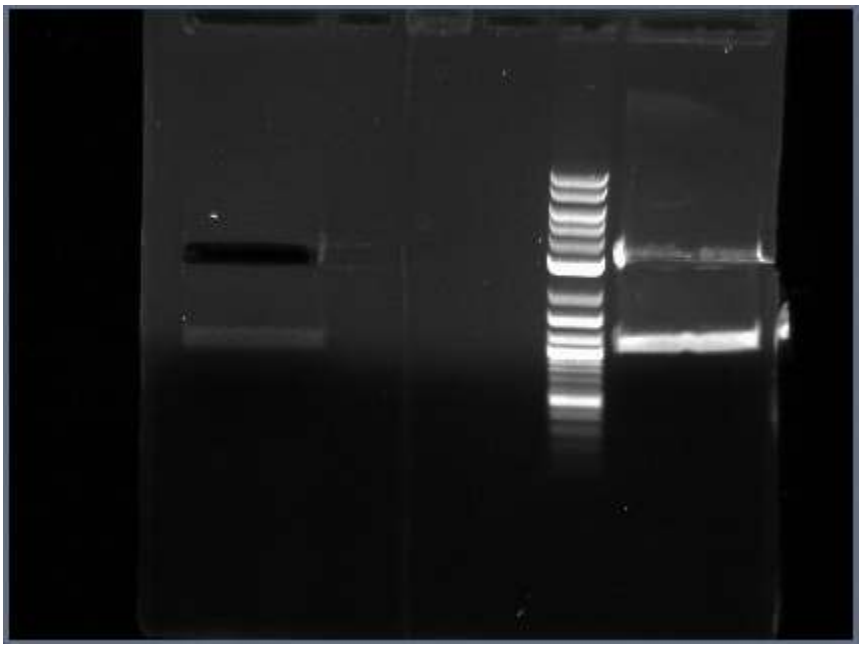
EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 33uL

GEL - 35min @ 90V



L to R: 3T5, ladder, 4C5

- ALL slices were gel extracted using the Qiagen kit

LIGATION - 1h benchtop

4uL 4C5 or 3T5

4uL water or Key

1uL ligase

1uL ligase buffer

3uL E02 T or E02 C

5uL water or CTC

1uL ligase

1uL ligase buffer

TRANSFORMATION - DH10B (1uL lig product)

All between 4.6 and 5.1ms

- shaken for 1h @ 37C before plating

Notebook 8/22/10

Alkaline lysis prep of E0240 in 4C5 and 3T5

Notebook 8/21/10

Alkaline lysis prep of screens 1-3,5,6 (all pellets pink, 5&6 very pink)

DIGEST - 2h @ 37C

Screens 1-3,5,6 - 2uL

PstIHF - 0.5uL

Buffer 4 - 1uL

Sterile water - 6.5uL

Screens 1-3,5,6 - 2uL

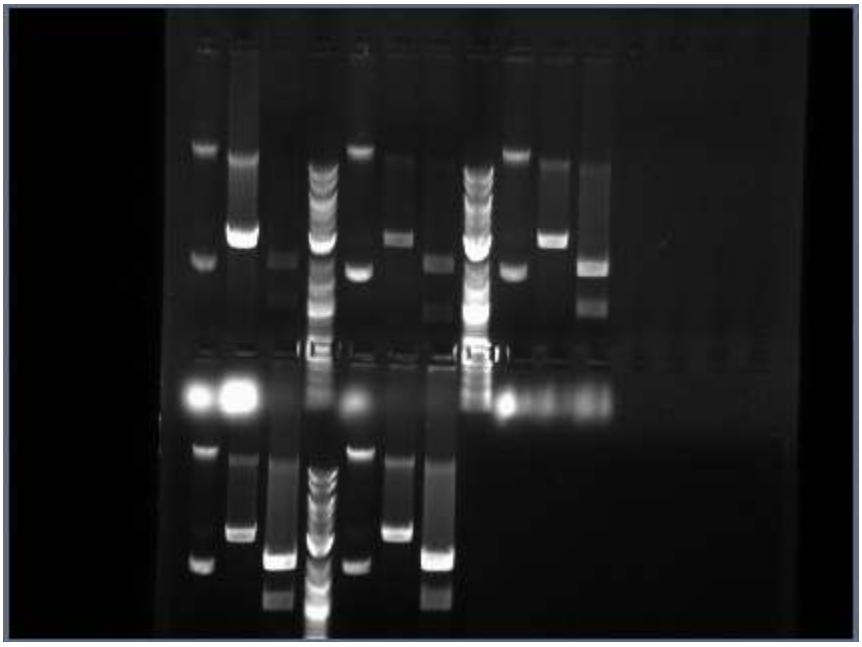
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1uL

Sterile water - 6uL

GEL - 35min @ 90V



1. 1 uncut

2. 1 S

3. 1 D

4. ladder

5. 2 uncut

6. 2 S

7. 2 D

8. ladder

9. 3 uncut

10. 3 S

11. 3 D

12-15. -

16. 5 uncut

17. 5 S

18. 5 D

19. ladder

20. 6 uncut

21. 6 S

22. 6 D

23-30. -

- 3 and 5 show the expected banding patterns for K19+CTC250 and K19+LC respectively.

Notebook 8/20/10

12 colonies on CTC250 control, 10 on LC control; ~125 on K19+CTC250, ~40 on K19+LC; picked 3 colonies of each (K19+CTC250 labeled 1-3, K19+LC labeled 4-6)

DIGEST - 2h @ 37C

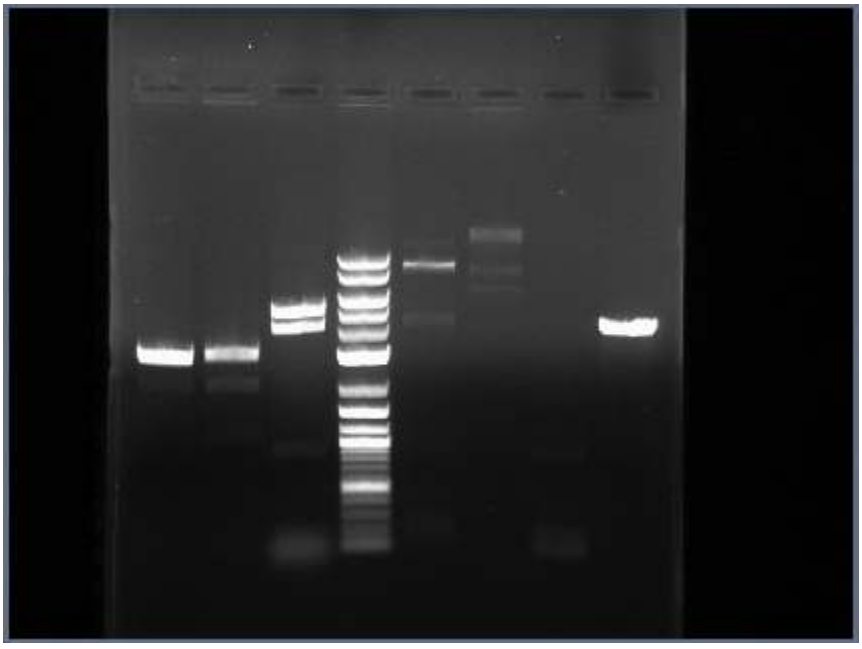
PTC in 3T5 - 2uL

NdeI - 0.5uL

Buffer 4 - 1uL

Sterile water - 6.5uL

GEL 35min @ 90V



1. Justins

2. Justins

3. NdeI digest

4. ladder

5. Marys

6. Marys

7. Marys

8. Marys

- NdeI is either contaminated or DNa is weird. It could be NcoI contamination, yielding a ~890bp piece and a ~70bp piece.

MULTIMER 95C ramp to 25C

27uL MultiBWD

25uL MultiFWD

then

6.5uL ligase buffer

6uL kinase (5:1 kinase buffer:kinase dilution from stock)

Notebook 8/19/10

Alkaline lysis prep for Mary (pGK13)

LIGATION - 1h benchtop

2uL CTC250

6uL water or K19

1uL ligase

1uL ligase buffer

4uL LC

4uL water or K19

1uL ligase

1uL ligase buffer

TRANSFORMATION - DH10B (1uL lig product)

- shaken for 1h @ 37C before plating

Notebook 8/18/10

Alkaline lysis prep of CTC250, LC (2x), Key Cass, and I13501

DIGEST - 2h @ 37C

K199021 - 16uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 26.5uL

LC and CTC250 - 8uL

EcoRIHF - 1uL

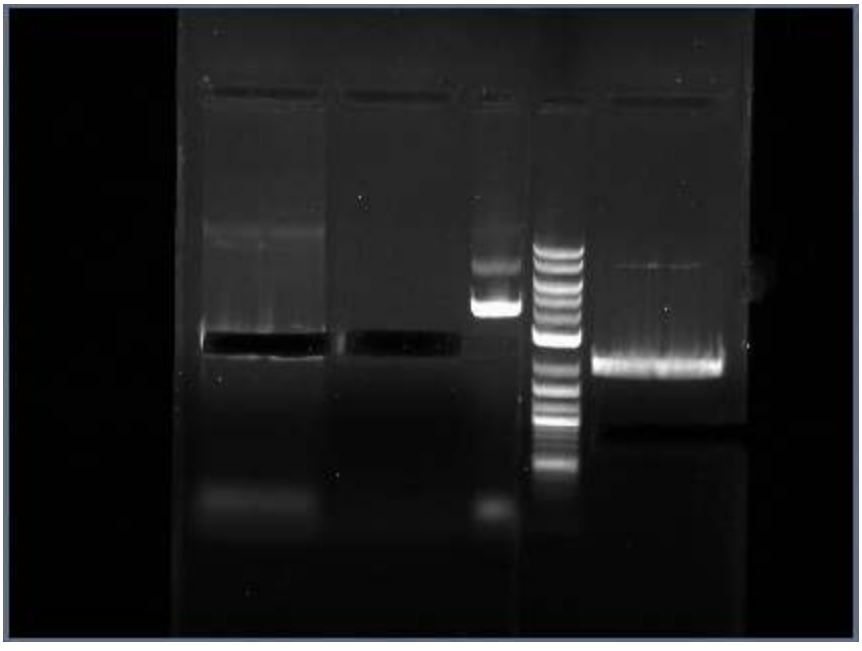
XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 34.5uL

GEL - 35min @ 90V



L to R: CTC250, LC, KeyCass uncut, ladder, K19

- slices were gel extracted with qiagen kit but ligations will be done tomorrow (no ligase)
- the key cass ran as expected

Notebook 8/17/10

Cleaned autoclave with CLR. Made new antibiotics (Amp100 and Kan25) and LB.

P22 screens 1&5 are correct according to sequencing.

Made new Cm25 plates.

Inoculated Key Cass and Lock Cass cultures for tomorrow.

Gel extracted Cre, Hin, and K19 for Justin.

Notebook 8/16/10

DIGEST - 2h @ 37C

P22 ind test 3 and PTC control - 2uL

EcoRIHF - 0.5uL

Buffer 4 - 1uL

Sterile water - 6.5uL

P22 ind test 3 and PTC control - 2uL

EcoRIHF - 0.5uL

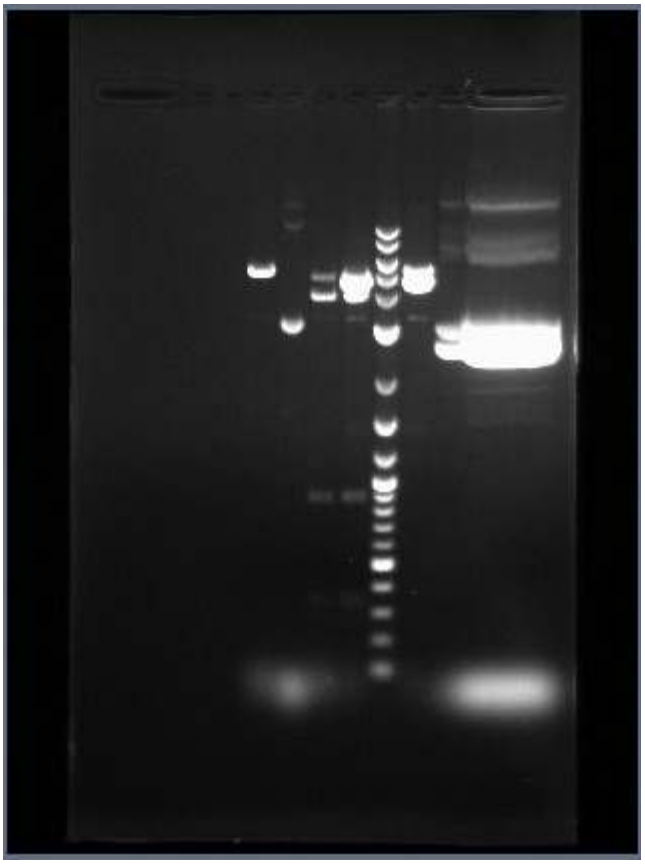
NdeI - 0.5uL

Buffer 4 - 1uL

BSA (10x) - 1uL

Sterile water - 5uL

GEL - 140min @ 50V (1% gel)



L to R: 4-11

4. PTC single

5. PTC uncut

6. PTC double

7. P22 ind test double

8. ladder

9. P22 ind test single

10. - (leakage from 11)

11. P22 ind test uncut

- The ind test was unsuccessful. Both the control and the test show the 900bp band as well as the ~360bp band.

Notebook 8/15/10

Alkaline lysis prep of Key screens 1-4.

DIGEST - 2h @ 37C

Screens 1-4 - 3uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

Screens 1-4 - 3uL

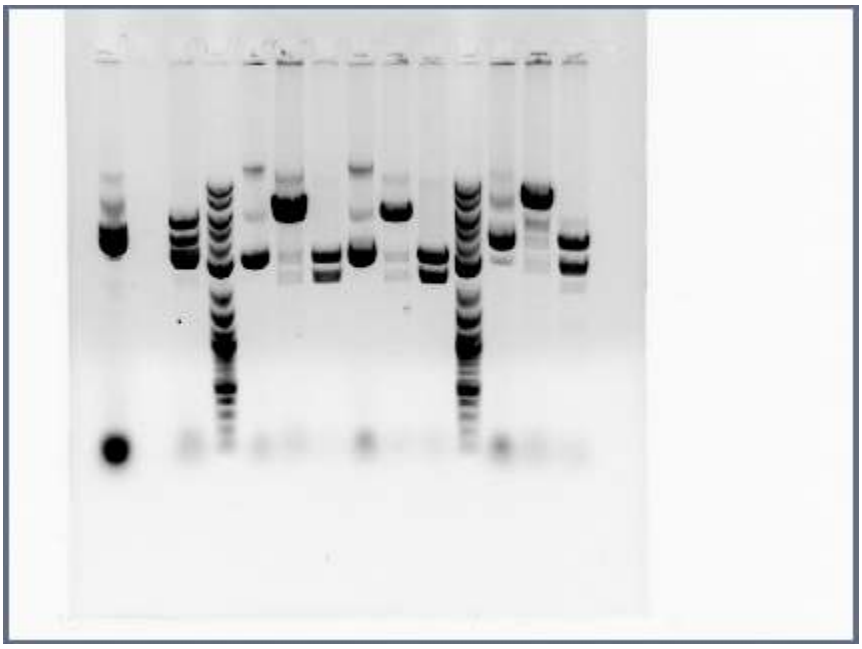
EcoRIHF - 0.5uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GEL - 30min @ 100V



L to R: 1-15

1. 1 uncut

2. 1 S

3. 1 D

4. ladder

5. 2 uncut

6. 2 S

7. 2 D

8. 3 uncut

9. 3 S

10. 3 D

11. ladder

12. 4 uncut

13. 4 S

14. 4 D

15. -

- Screen 4 displays the expected banding pattern.

Notebook 8/14/10

No colonies on Hin control, ~40 on reg Key Cass plate, ~150 on Key pellet plate.

Inoculated 4 LC's of the Key Cass clone for screening.

Checked IC+PTC cultures. No change & no RFP visible. Will grow overnight b/c apparently FIS is expressed during stationary phase.

Notebook 8/13/10

Alkaline lysis prep of Cre Cass and 3 induction tests. (Hin wasnt to density)

Finished making C-Media (made in biohood, floating lint in it >:(but no contamination after a few days NP)

- Made 10mL of MgSO4 (1000x) (in 4C)

Made freezer stocks of fixed Hin Generator

Alkaline lysis prep of Hin Generator.

DIGEST - 2h @ 37C

Cre Cassette - 8uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 34.5uL

Hin Generator - 8uL

EcoRIHF - 1uL

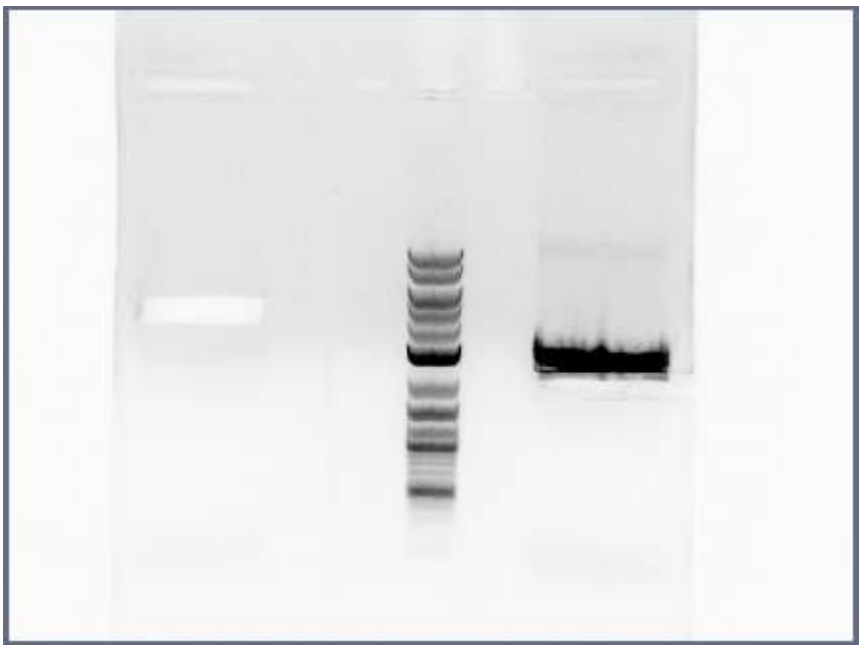
XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 34.5uL

GEL - 35min @ 100V



L to R: Hin Gen, ladder, Cre Cass

DIGEST - 2h @ 37C

P22 ind test 1-3 - 3uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

P22 ind test 1-3 - 3uL

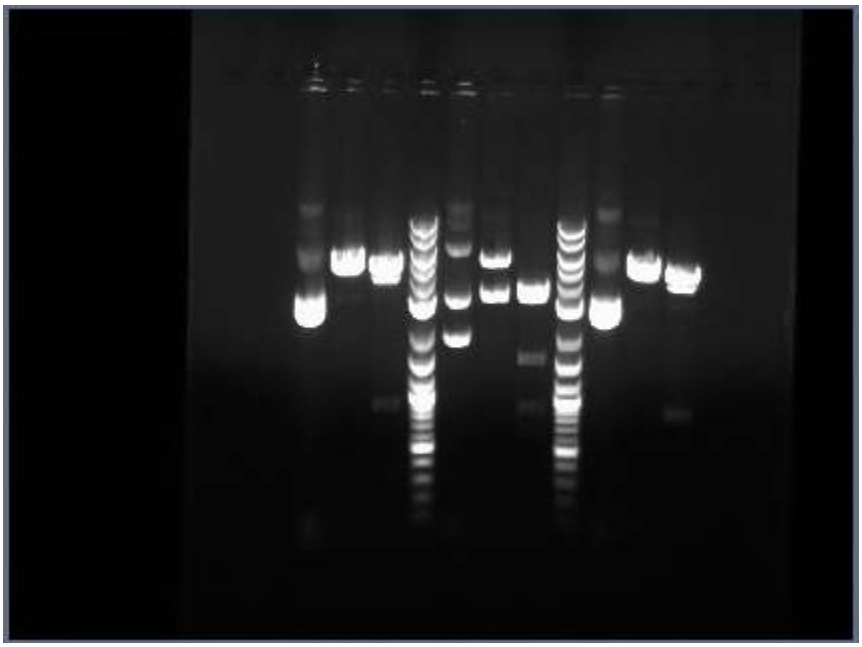
EcoRIHF - 0.5uL

NdeI - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GEL - 35min @ 90V



L to R: 1-15

1. -

2. -

3. 1 uncut

4. 1 S

5. 1 D

6. ladder

7. 2 uncut

8. 2 S

9. 2 D

10. ladder

11. 3 uncut

12. 3 S

13. 3 D

14. -

15. -

- The bands are odd. PTC and IC should be 5.1 and 4.6 respectively. 1 & 3 show this, but 2 has weird banding. The dd of the recombined should show ~1.5kb but neither 1 nor 3 show that. They show ~900 but no bands ~360 for non-recombinants. Its possible that the bands around 360 arent high enough in concentration to be seen.

LIGATION 1h benchtop

5uL Hin

3uL water or Cre

1uL ligase

1uL ligase buffer

Finished Justins gel. Didnt look good. Emailed it to him.

TRANSFORMATION - DH10B (1uL lig product)

Control: 1.8ms | Key: 1.8ms

- shaken for 1h @ 37C before plating

Induced IC+PTC #3 with 100mM IPTG and put Sarah's cultures in shaker.

Notebook 8/12/10

Made C-Media components (see actual notebook for measurments) with Mike Schwartz
begin_of_the_skype_highlighting end_of_the_skype_highlighting including:

- 50mL SS1 amino acids (100x) (in -20C)
- 50mL SS2 amino acids (100x) (in -20C)
- 25mL each of Tyrosine (in 0.08M KOH) and Cysteine (100x each)
- 500mL C-Media buffer (10x) (on shelf)
- 50mL Solution J from RL's 10^6 stock (1000x) (in 4C)

Inoculated new PTC+IC cultures from overnights with 50uL overnight (1,2,3); 10mM,100mM,100mM IPTG respectively; with AKT in 5mL LB medium

Inoculated Hin Gen and Cre Cass cultures for tomorrow

The tyrosine was causing problems by not dissolving so a lot of KOH was added from the initial 0.01M added.

Notebook 8/11/10

Alkaline lysis prep of the four key cass screens.

Inoculated Int Cass + PTC liquid cultures for growth induction tomorrow.

DIGEST - 1.5h @ 37C

Screens 1-4 - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

Screens 1-4 - 3uL

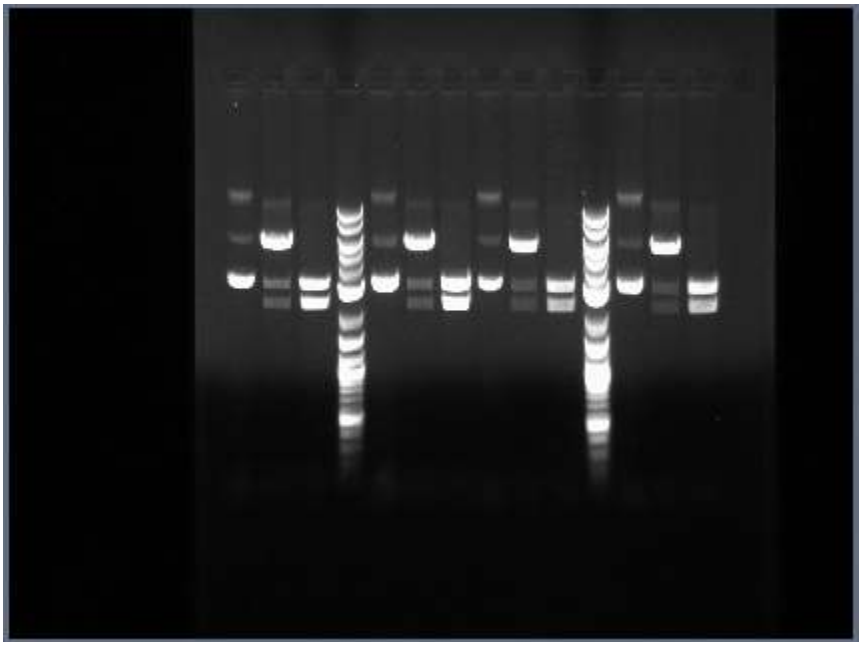
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GEL - 30min @ 100V



1. 1 uncut
2. 1 S
3. 1 D
4. ladder
5. 2 uncut
6. 2 S
7. 2 D
8. 3 uncut
9. 3 S
10. 3 D
11. ladder
12. 4 uncut
13. 4 S
14. 4 D
15. -

- the banding pattern matches the cre cass banding pattern. need to screen more then. massive col pcr to screen?

Notebook 8/10/10

Colony growth on Key Cass. transformants. None on control. Four colonies were picked for screening.

TRANSFORMATION - DH10B

Int Cass and PTC - 5.4ms

- shaken for 70min @ 37C and plated on AKT plate. (AK plate +25uL Tet)

Notebook 8/9/10

Had inoculated cultures Sunday for Justin and for stocks.

DIGEST - 2h @ 37C

Cre Cassette - 20uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 22.5uL

Hin Generator (fixed, B) - 7uL

XbaI - 1uL

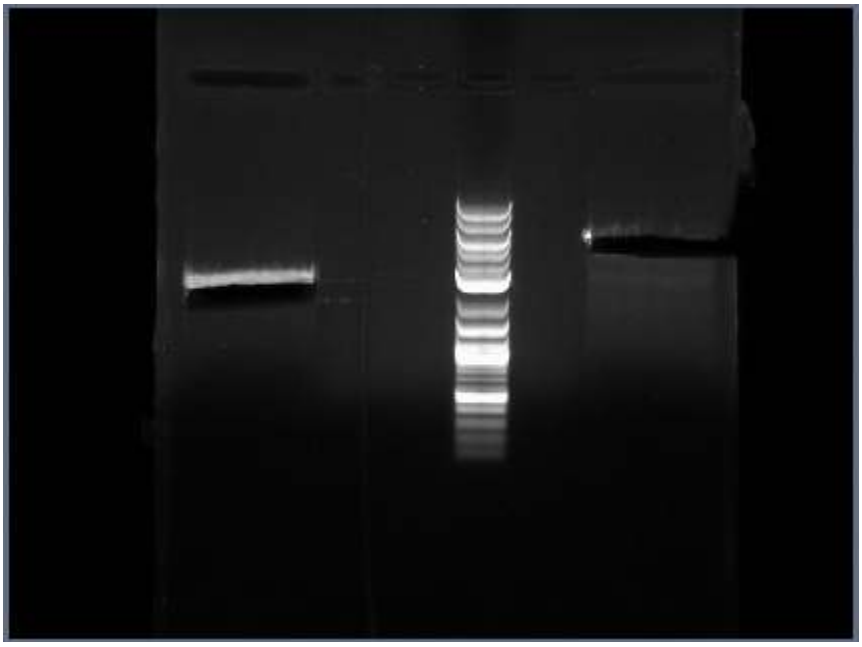
EcoRIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 35.5uL

GEL - 35min @ 90V



From L to R: Cre C, ladder, Hin G

- gel slices were gel extracted using a qiagen kit

LIGATION - 1h benchtop

2uL Hin

6uL Cre or water (for control)

1uL ligase buffer

1uL ligase

TRANSFORMATION - DH10B

Key (Cre+Hin): 4.8ms

Control: 4.8ms

- shaken for 1h @ 37C then plated on AK plates

Notebook 8/6/10

Alkaline lysis prep of screens 1-8

DIGEST - 2h @ 37C

Screens 1-8 - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

Screens 1-8 - 3uL

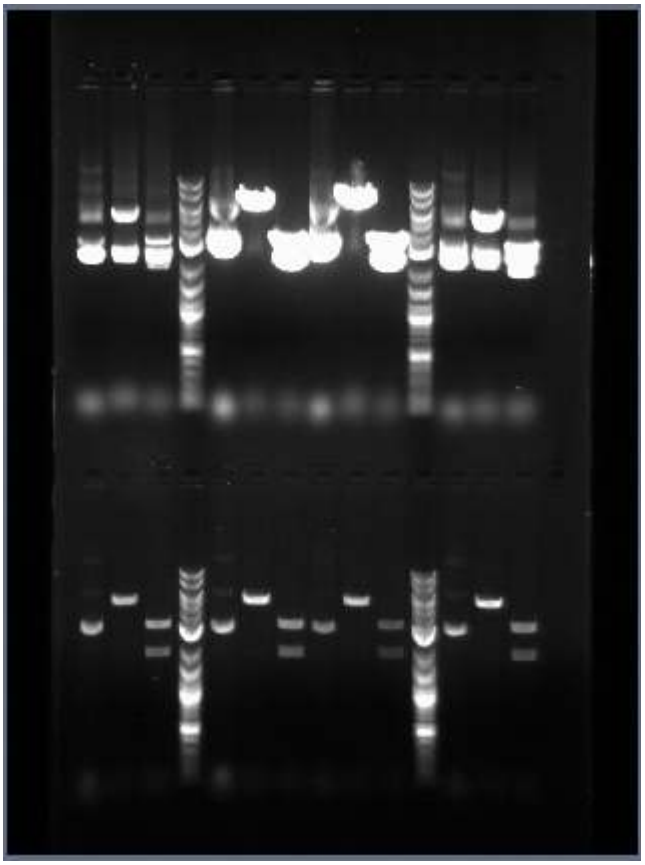
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GEL 30min @ 100V



1. 1 uncut

2. 1 S

3. 1 D

4. ladder

5. 2 uncut

6. 2 S

7. 2 D

8. 3 uncut

9. 3 S

10. 3 D

11. ladder

12. 4 uncut

13. 4 S

14. 4 D

15. -

16. 5 uncut

17. 5 S

18. 5 D

19. ladder

20. 6 uncut

21. 6 S

22. 6 D

23. 7 uncut

24. 7 S

25. 7 D

26. ladder

27. 8 uncut

28. 8 S

29. 8 D

30. -

- DNA concentrations of 1-4 were EXTREMELY high. Screens 5-8 display the expected pattern. 1-4 display a similar pattern to the expected pattern but the concentrations were so large that both 1 and 2 will be sequenced. 5 will be used as well.

- I suspect that 4C5 is not a low copy vector as it is listed in the registry.

Notebook 8/5/10

Colonies appeared on PTC transformations. Still none on the Int Gen clones.

Inoculated culture for screening tomorrow: 4C5 1-4, 3T5 5-8.

Notebook 8/4/10

No growth on plates early...let sit @ 37C for a few more hours.

Still no colonies at noon. will reattempt transformation.

TRANSFORMATION - DH10B

redo of 8/2 clones

Correction: 1 colony grew on PTC in pSB3T5. will pick for screening tomorrow.

Notebook 8/3/10

Discover incubator at 60C! turned to 37C.

Helped Peter with DH10B comp cells.

Transformed ligation from 8/2 into fresh comp cells

Helped Justin with his multimer cloning.

Notebook 8/2/10

DIGEST - 2h @ 37C

pSB3T5 - 10uL

EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 33uL

pSB4C5 - 2uL

EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 41uL

P22 Test Cassette (PTC) - 4uL

EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

Sterile water - 39uL

Int Cassette - 10uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

LacI Cassette - 10uL

XbaI - 1uL

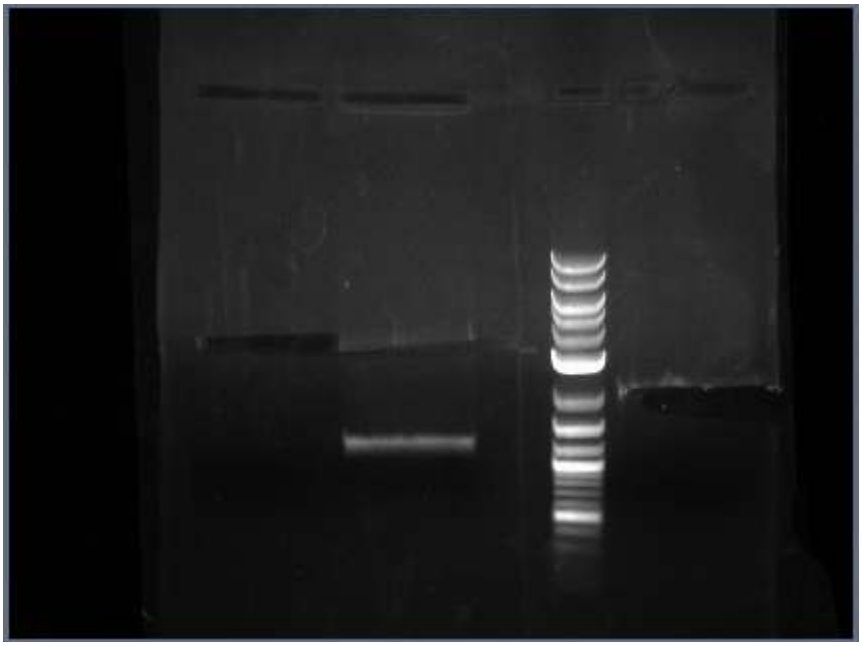
PstIHF - 1uL

Buffer 4 - 5uL

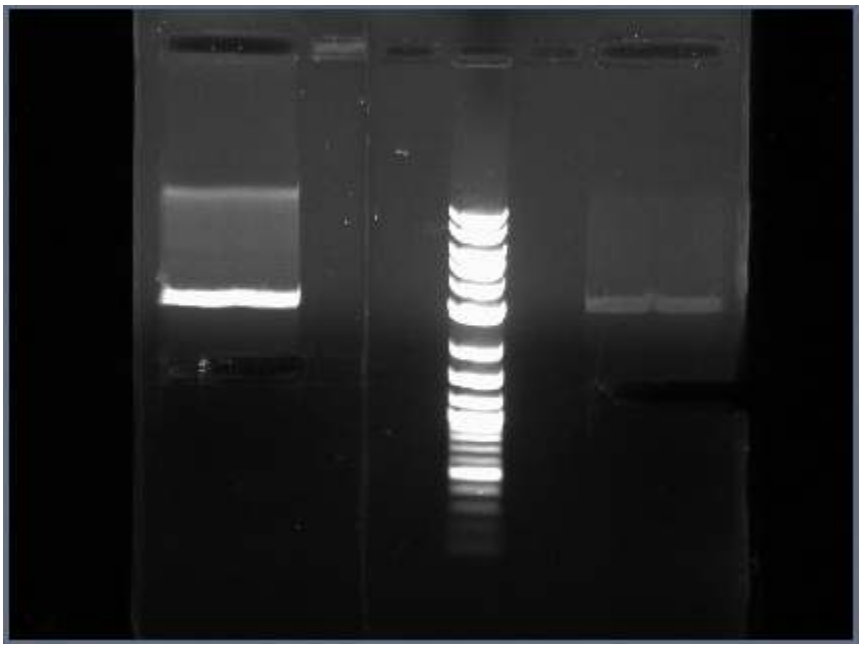
BSA - 0.5uL

Sterile water - 32.5uL

GELS - 50min @ 70V



L to R: 3T5, 4C5, ladder, P22



L to R: Int, ladder, lacI

- gel slices were gel extracted but extremely hard to see

LIGATION - benchtop 1h

out of comp cells... :'(

Notebook 7/30/10

Miniprep of pSB3T5

More colonies on HTC control than other again...

Notebook 7/29/10

One of the 15 colonies grew. It is likely that the plates had less antibiotic and the antibiotic was consumed allowing for contaminate growth.

Made new Amp100 plates and Mike made new 60% glycerol.

Alkaline lysis prep of colony 4 (the one that grew) (HTC1500)

Mike miniprepped the 8 cultures that grew from 7/28. 3T5 didnt grow.

DIGEST - 2h @ 37C

HTC1500 #4 - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

HTC1500 #4 - 3uL

PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

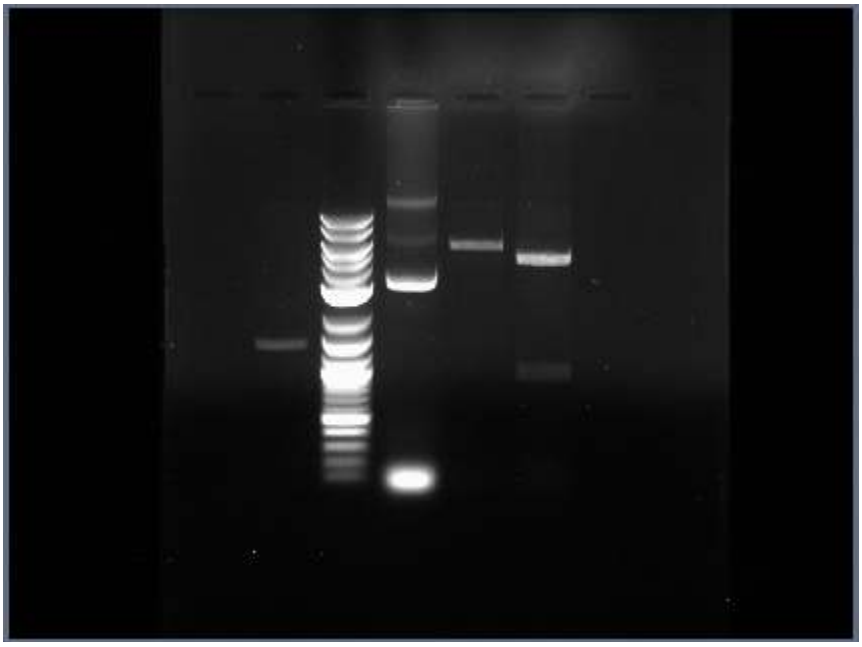
Sterile water - 12.2uL

Justin redid the transformation of the HTC plus junk.

Mike made freezer stocks

Inoculated 3T5 LC's again.

GEL - 35min @ 80V



1. -

2. pSB1A2 HTC

3. ladder

4. HTC1500 #4 uncut

5. HTC1500 #4 single

6. HTC1500 #4 double

7. -

8. -

- The banding pattern is totally incorrect. The cloning must be redone.

Notebook 7/28/10

Made electrocomp cells with Mary. (DH10B and MG1655del(araBAD)del(araE))

Made LC's of pSB3T5, pSB4C5, E0240, k199021, HTC, CTC, LC, and Cre Cass

Picked 15 colonies from HTC clones. HTC1500 1-5, HTC1000 6-10, HTC500 11-15.

Notebook 7/27/10

DIGEST - 1h @ 37C

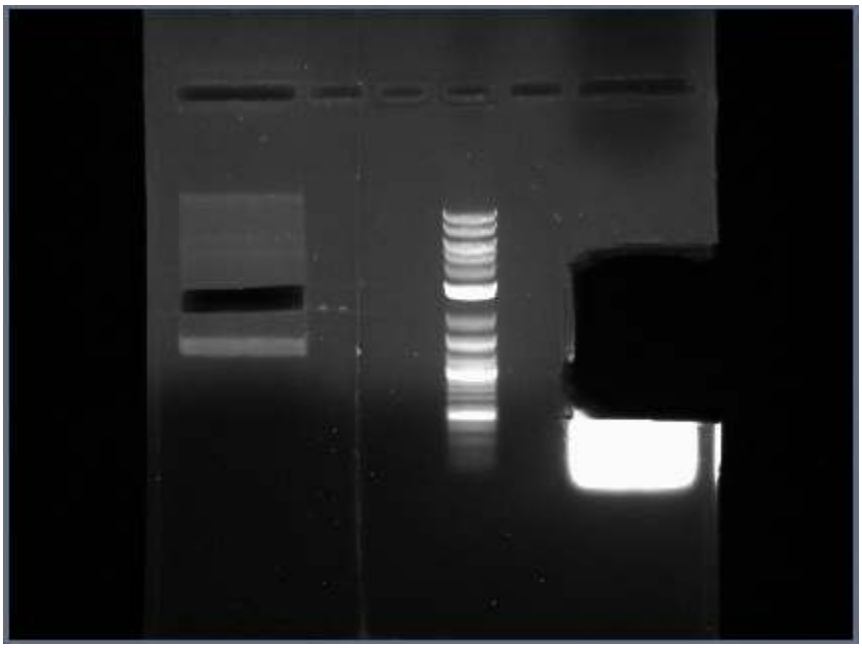
HTC - 10uL

SapI - 1uL

Buffer 4 - 5uL

Sterile water - 34uL

GEL 50min @ 70V



L to R: HTC, ladder, multimer

cut 500, 1000, 1500, and 4000 slices from gel.

- gel extracted with kit. 4000 was too low in []

DEPHOSPHORYLATION 1h @ 37C

12uL HTC

1.4uL Antarctic buffer

1uL Antarctic SAP

- heat inactivated 20min @ 65C afterward

LIGATION 1h benchtop

- HTC 500: 5uL HTC, 3uL 500, 1uL lig buff, 1uL lig
- HTC 1000: 5uL HTC, 3uL 1000, 1uL lig buff, 1uL lig
- HTC 1500: 5uL 1500, 3uL HTC, 1uL lig buff, 1uL lig
- HTC control: 5uL water, 3uL HTC, 1uL lig buff, 1uL lig

Notebook 7/26/10

Made media for various purposes.

Struck plates of Wes's del(araBAD), cre cass, hin cass, e0240, and k199021. also adjusted justins freezer stocks.

MULTIMER REACTION

25uL MultiFWD and 25uL MultiBWD

Thermocycler: 95C 20s then ramp to 25C @ 0.1C/s

Kinase: 5uL kinase and 6uL ligase buffer @ 37C for 30min

Ligation: 5uL ligase for 2.5h

Added 10uL of 50mM EDTA and put in 4C overnight

Finished Justins ligation->transformation

Vacation 7/17-7/25

Notebook 7/16/10

DH10b cells didnt grow for comp cell prep :(

Alkaline lysis prep of screens 1-9

NOTE: Screens 1-3 are Cre Test Cassette, 4-6 are Hin Test Cassette, 7-9 are Lock Cassette.

DIGEST 2h @ 37C

DNA - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

DNA - 3uL

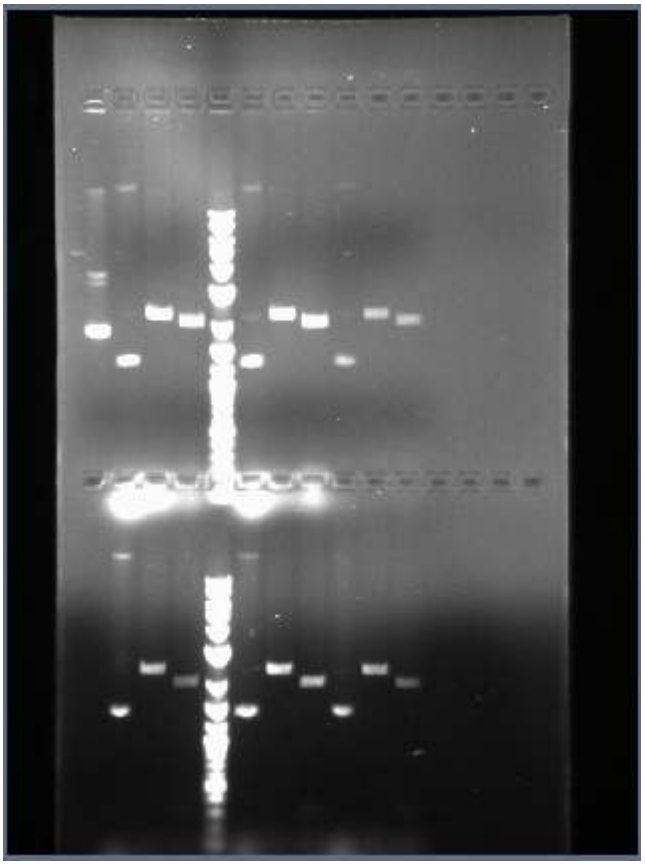
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GELS 85 min @ 50V



1. pSB1A2+1000bp uncut

2. 1 uncut

3. 1 single (S)

4. 1 double (D)

5. ladder

6. 2 uncut

7. 2 S

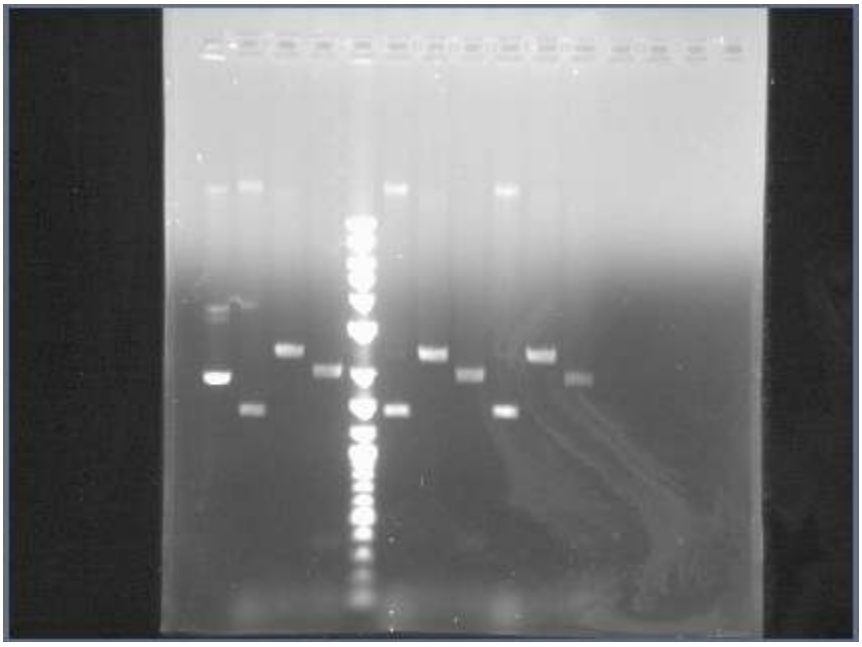
8. 2 D

9. 3 uncut

10. 3 S

11. 3 D

12. -
13. -
14. -
15. -
16. pSB1A2+1000bp uncut
17. 4 uncut
18. 4 S
19. 4 D
20. ladder
21. 5 uncut
22. 5 S
23. 5 D
24. 6 uncut
25. 6 S
26. 6 D
27. -
28. -
29. -
30. -



1. pSB1A2+1000bp uncut

2. 7 uncut

3. 7 single (S)

4. 7 double (D)

5. ladder

6. 8 uncut

7. 8 S

8. 2 D

9. 9 uncut

10. 9 S

11. 9 D

12. -

13. -

14. -

15. -

- Ran the gels too long. The Cre bands (1-3) cannot be seen in the dd. However the single cut is the expected size. For good science, the screening gel should be rerun.
- The Hin (4-6) ran ok. Screen 5 will be used for further experiments.
- The Lock (7-9) ran fast. The band in the dd is expected to be ~430bp and it ran ~370bp. However, it ran

fast during the gel extraction too. Screen 7 will be sequenced and if correct, used for further experiments.

Notebook 7/15/10

Made freezer stocks of pSB3T5 and pSB4C5. Updated freezer stock file and added new boxes (4 and 5).

Transformants look great. Control had 4 colonies early in the day and 6+ later. Lock, Hin, and Cre each had over 100 (probably >200).

Kit miniprep of pSB3T5, pSB4C5, and J31000.

Made LC's of screens labeled 1-9 (1-3 Cre, 4-6 Hin, 7-9 Lock) and LC's of shipment received on 7/14 by Mary.

Notebook 7/14/10

Corrected freezer stock file (pLac Hin parts DO NOT contain the RBS as needed, seemly heterogeneous DNA from iGEM lead to some parts w/o RBS). Updated the file and uploaded it to the wiki. Mike S made freezer stocks and put them in the boxes.

Qiaquick miniprep of Test Cassettes and Lock Cassette. Concentrations were ~750 and ~270 ng/uL respectively.

DIGEST 3h @ 37C

K142003 - 13uL

PstIHF - 1uL

EcoRIHF - 1uL

Buffer 4 - 5uL

Sterile water - 30uL

Test Cass - 4uL

PstIHF - 1uL

EcoRIHF - 1uL

Buffer 4 - 5uL

Sterile water - 39uL

Lock Cass - 10uL

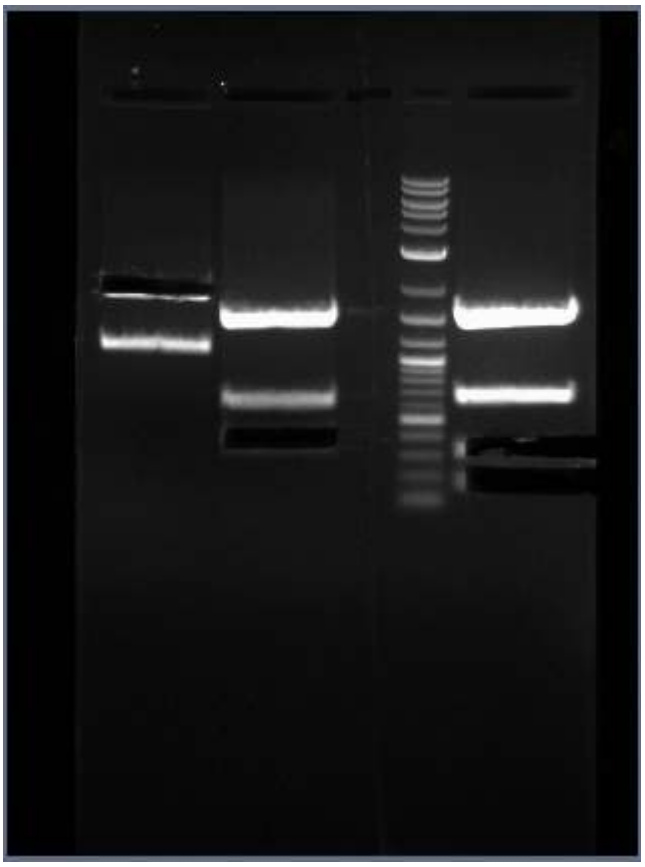
PstIHF - 1uL

EcoRIHF - 1uL

Buffer 4 - 5uL

Sterile water - 33uL

GEL 2h @ 40V



L to R: K142003/pSB1A2, Lock Cassette, ladder, Test Cassettes

- gel slices were extracted using the qiaquick gel extraction kit.

LIGATION 1h benchtop

5uL pSB1A2

3uL insert | water for control

1uL buffer

1uL ligase

TRANSFORMATION DH10B

pSB1A2 control - 5.5ms

Hin Test Cass - 4.3ms

Cre Test Cass - 4.2ms

Lock Cass - 4.9ms

- Put in 37C to shake and Peter plated on Amp100 after ~1h.

Notebook 7/13/10

Lock and Test Cassettes grew in a lawn. Picked some colonies around the edges.

Made LC's of Lock Cass, Test Cass, Hin Gen, Int Cass, and P22 Test Cass.

Transformed pSB4C5 and pSB3T5 from registry (for TetR put 25uL of Tet on normal LB plate)

Struck a plate with J31000 from freezer stocks.

Added BBa_K318010-015, 020-030 to the iGEM Parts Registry with Justin. These new parts have (except for the pLac parts w/o RBS) been completed and are ready for submission to the registry.

Notebook 7/12/10

Received Lock cassette and Test cassettes from GeneArt today. Transformed DH10B cells with them and plated on Kan10 plates.

DIGEST 2h @ 37C

DNA - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

DNA - 3uL

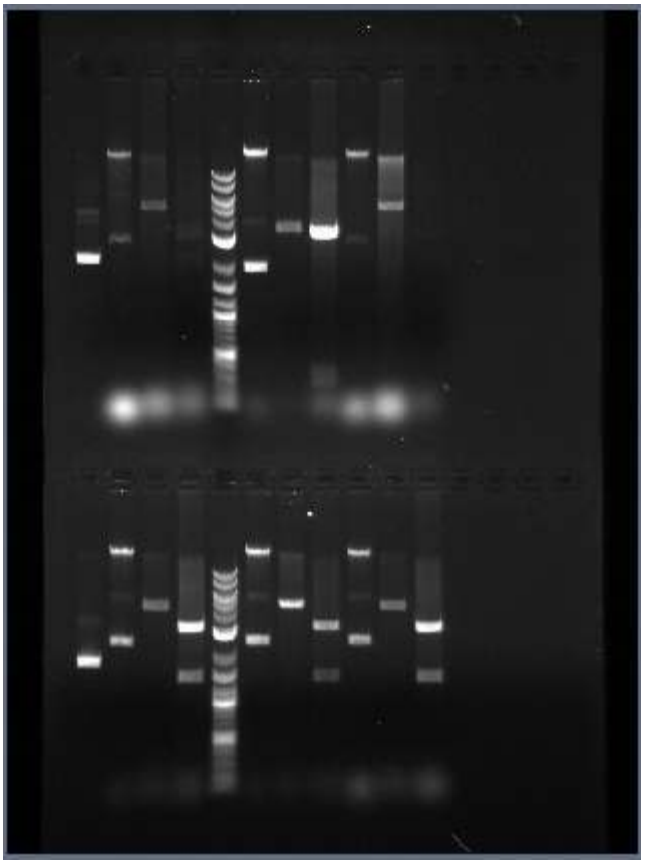
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GELS 50min @ 70V



1. Hin cass uncut

2. 1 uncut

3. 1 single (S)

4. 1 double (D)

5. ladder

6. 2 uncut

7. 2 S

8. 2 D

9. 3 uncut

10. 3 S

11. 3 D

12. -

13. -

14. -

15. -

16. B1 uncut

17. 4 uncut

18. 4 S

19. 4 D

20. ladder

21. 5 uncut

22. 5 S

23. 5 D

24. 6 uncut

25. 6 S

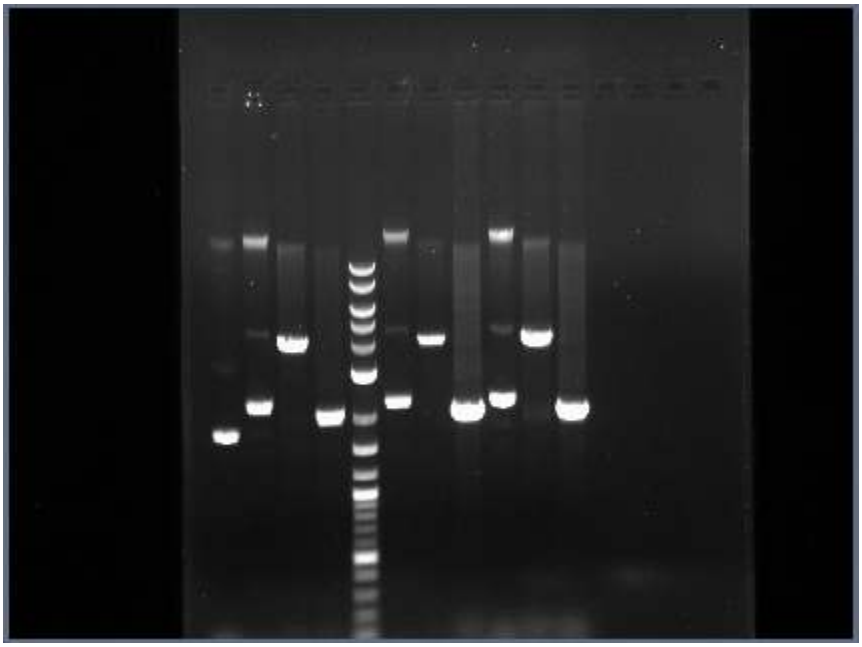
26. 6 D

27. -

28. -

29. -

30. -



(gel run for an additional 20min because resolution at 2kb was not obtained, still not enough resolution to see 2 bands)

1. J23+I32+E02 uncut

2. 7 uncut

3. 7 S

4. 7 D

5. ladder

6. 8 uncut

7. 8 S

8. 8 D

9. 9 uncut

10. 9 S

11. 9 D

- Hin generator was successful. #1 will be saved because Justin has already completed this clone.
- Int cassette was successful. #4 will be used for further experiments. see Int cassette
- P22 test cassette was successful. #8 will be used for further experiments. see P22 test cassette

Notebook 7/10/10

Alkaline lysis prep of screens 1-9

Notebook 7/9/10

plates needed more growth before picking colonies: left in incubator until 3pm

- ~15 on J23 control ~200 on P22 test
- 0 on B1 control ~100 on Int cass
- 1 on Hin control 3 on Hin gen 11 on hin pellet

Inoculated LCs for screening:

- Hin generator labeled 1-3
- Int cassette labeled 4-6
- P22 test cassette labeled 7-9

Notebook 7/8/10

Qiagen miniprep of Hin and LacI cassettes. LacI cassette still yields low concentration likely due to cons pro.

Mike S made freezer stocks. Updated wiki file.

DIGEST - 2h @ 37C

B1 - 6uL

PstIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 36.5uL

K20+I30+B1 - 10uL

PstIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

DIGEST - 2h @ 37C

J23+I32+E02 - 5uL

PstIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 37.5uL

K19+I33 - 4uL

PstIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.5uL

DIGEST - 2h @ 37C

Hin Cass - 10uL

PstIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 32.5uL

LacI Cass - 12.5uL

PstIHF - 1uL

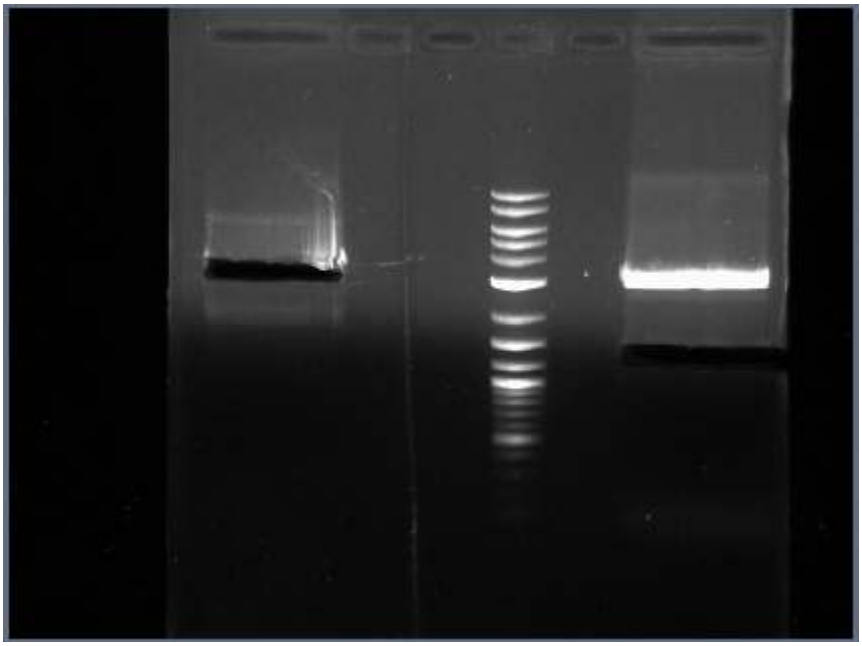
XbaI - 1uL

Buffer 4 - 5uL

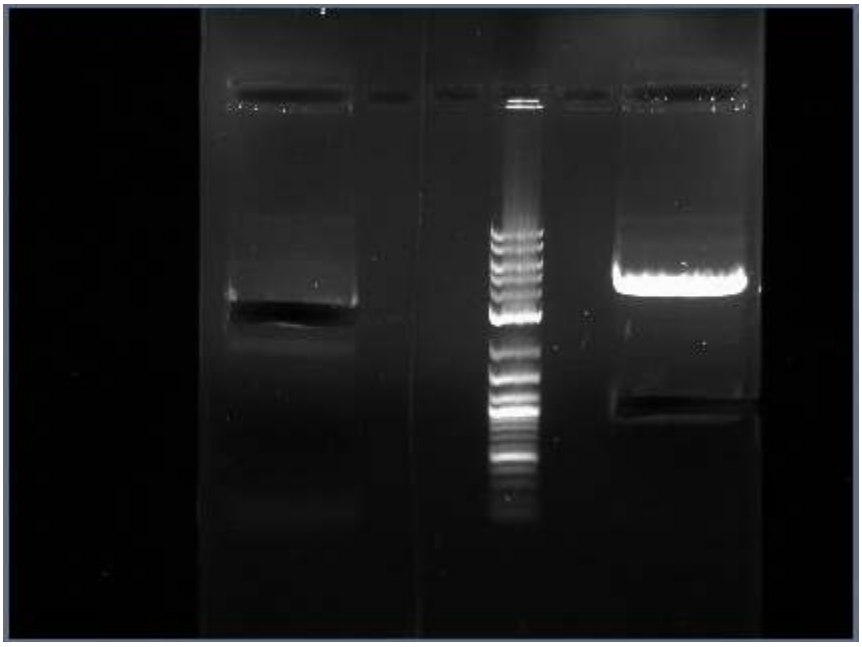
BSA - 0.5uL

Sterile water - 30uL

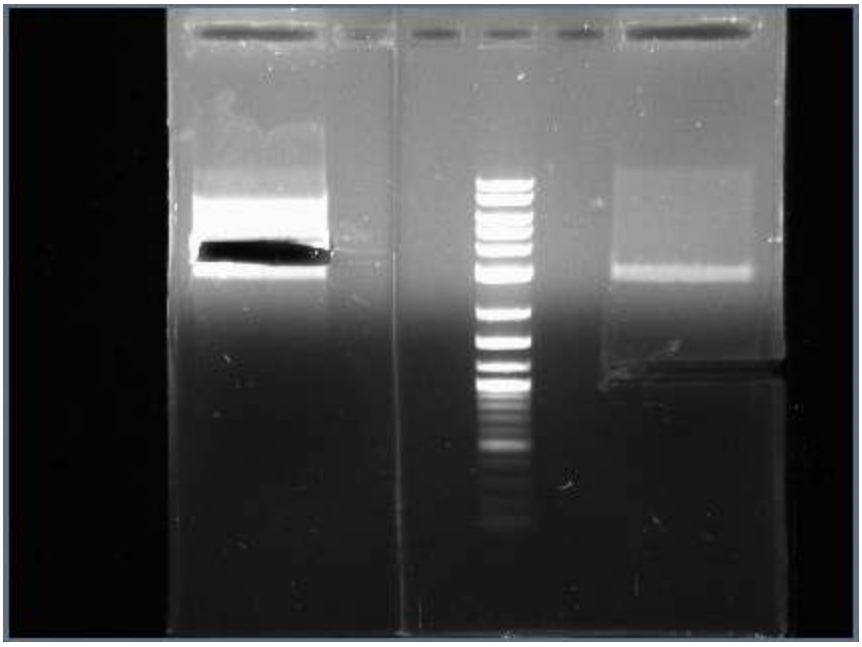
GELS - 50min @ 70V



L to R: B1, ladder, K20+I30+B1



L to R: J23+I32+E02, ladder, K19+I33



L to R: Hin Cass, ladder, LacI Cass

- gel slices were extracted with the Qiaquick gel extraction kit

LIGATION 1h benchtop

Concentrations worked out so that the following was used for all clones:

5uL backbone

3uL insert | water for control

1uL ligase buffer

1uL ligase

TRANSFORMATION

Time constants between 4.5 and 5.5ms

Shaken for 1h @ 37C and then plated on appropriate media

Notebook 7/7/10

Created wiki pages of completed biobricks: I32+E02 , J23+I32+E02 , K20+I30 , K20+I30+B1

Discussed plate experiments with Justin. (4 LB controls, 4 inducers: none, glucose, arabinose, iptg)

Made liquid cultures for freezer stocks and for miniprep

Planned cloning for 7/8

Notebook 7/6/10

Alkaline lysis prep of screens, J23+I32+E02 labeled 1-7 and K20+I30+B1 labeled A-C. (Justin inoculated the LC's for the prep)

DIGEST 2h @ 37C

DNA - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

DNA - 3uL

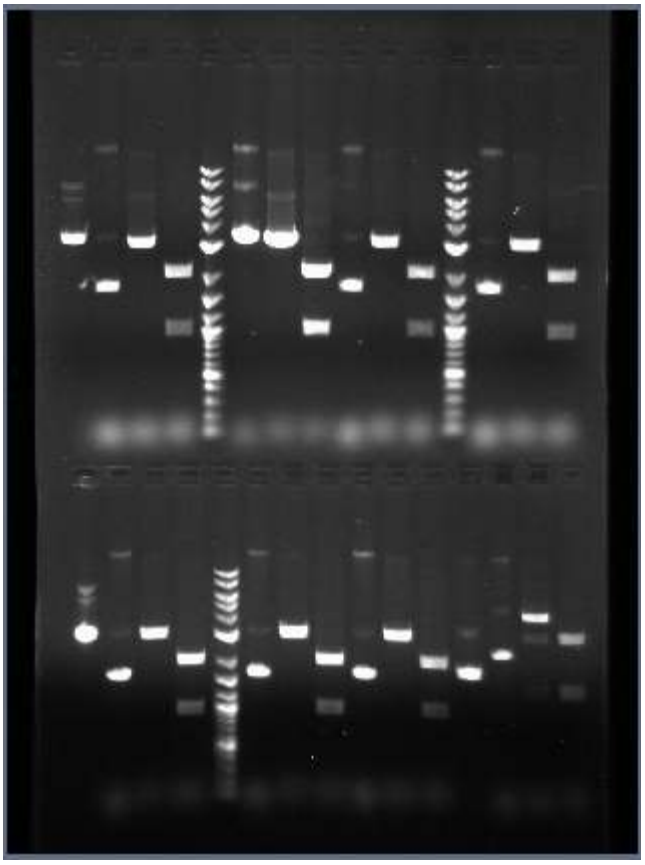
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

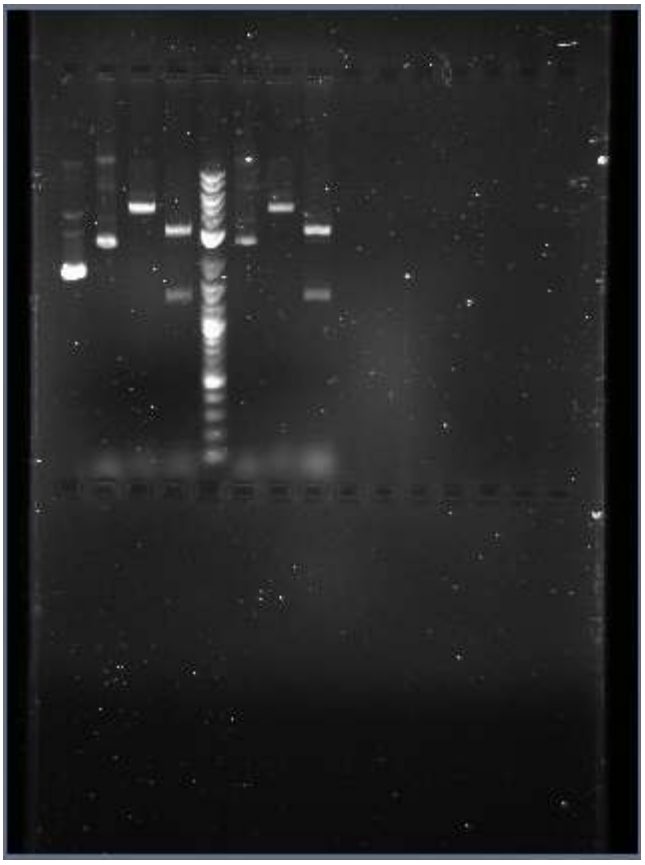
Sterile water - 12.2uL

GELS 50min @ 70V



1. J23 uncut
2. 1 uncut
3. 1 single (S)
4. 1 double (D)
5. ladder
6. 2 uncut
7. 2 S
8. 2 D
9. 3 uncut
10. 3 S
11. 3 D
12. ladder
13. 4 uncut
14. 4 S

15. 4 D
16. J23 uncut
17. 5 uncut
18. 5 S
19. 5 D
20. ladder
21. 6 uncut
22. 6 S
23. 6 D
24. 7 uncut
25. 7 S
26. 7 D
27. B1 uncut
28. A uncut
29. A S
30. A D



1. B1 uncut
2. B uncut
3. B S
4. B D
5. ladder
6. C uncut
7. C S
8. C D

- Screens displayed expected pattern except for "2 uncut." Screens 1 and B will be used for further steps. (Plates were streaked of the LC's to make new LC's for freezer stocks)

Notebook 7/2/10

DIGEST 2h @ 37C

K20+I30 - 20uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 22.5uL

B1006 (B1) - 6uL

EcoRIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 36.5uL

DIGEST 2h @ 37C

I32+E02 - 4uL

PstIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.5uL

J23100 (J23) - 5uL

PstIHF - 1uL

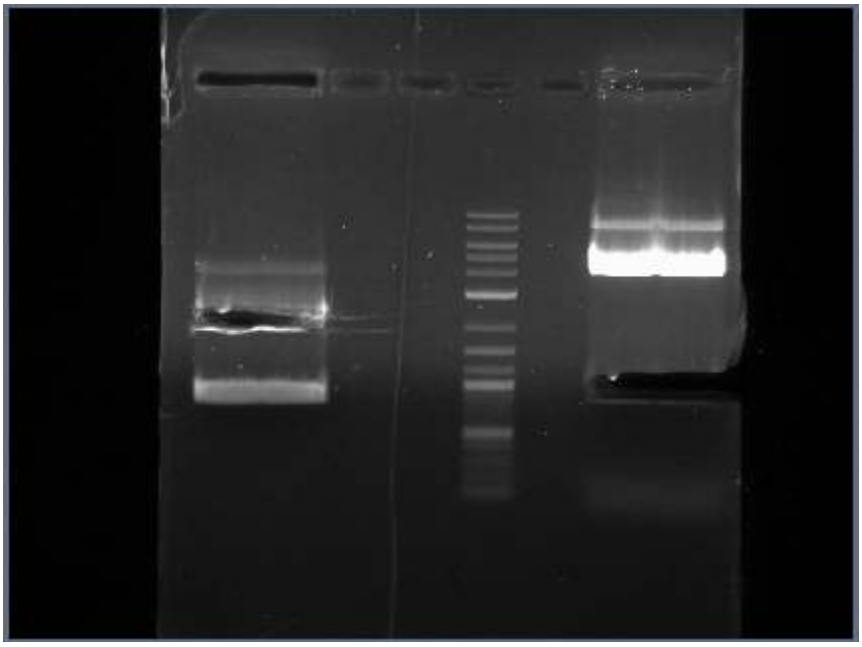
SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

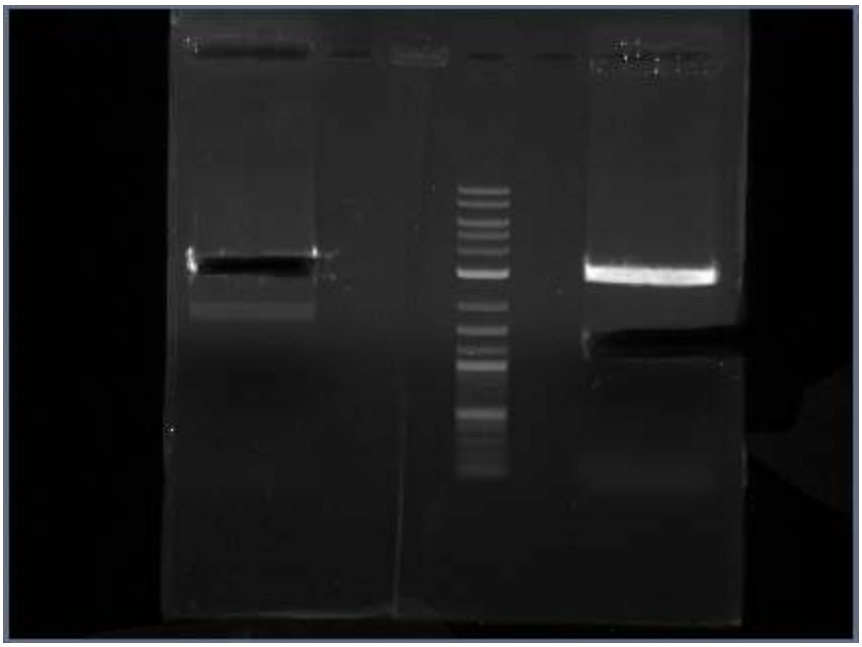
Sterile water - 37.5uL

GEL 50min @ 70V



L to R: J23, ladder, I32+E02

GEL 50min @ 70V



L to R: B1, ladder, K20+I30

- All gel slices were gel extracted using the Qiaquick gel extraction kit

LIGATION benchtop for 1h

3uL backbone

5uL insert or water (for control)

1uL buffer

1uL ligase

Cells (DH10B) were transformed with the ligation products, shaken for 1h @ 37C and plated on appropriate media.

Notebook 7/1/10

Alkaline lysis miniprep of screens 1-12.

DIGEST 2h @ 37C

Screens 1-12 - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

Screens 1-12 - 3uL

PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

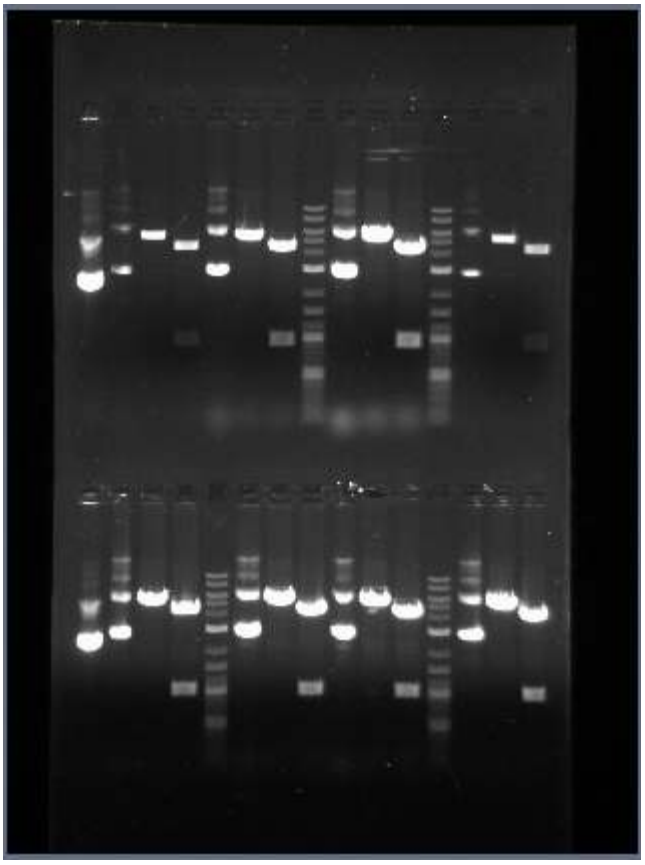
Bands expected:

I32+E02 (#s 1-4) single~5.3 double~0.9

K19+I33 (#s 5-8) single~5.4 double~0.9

K20+I30 (#s 9-12) single~4.4 double~1.3

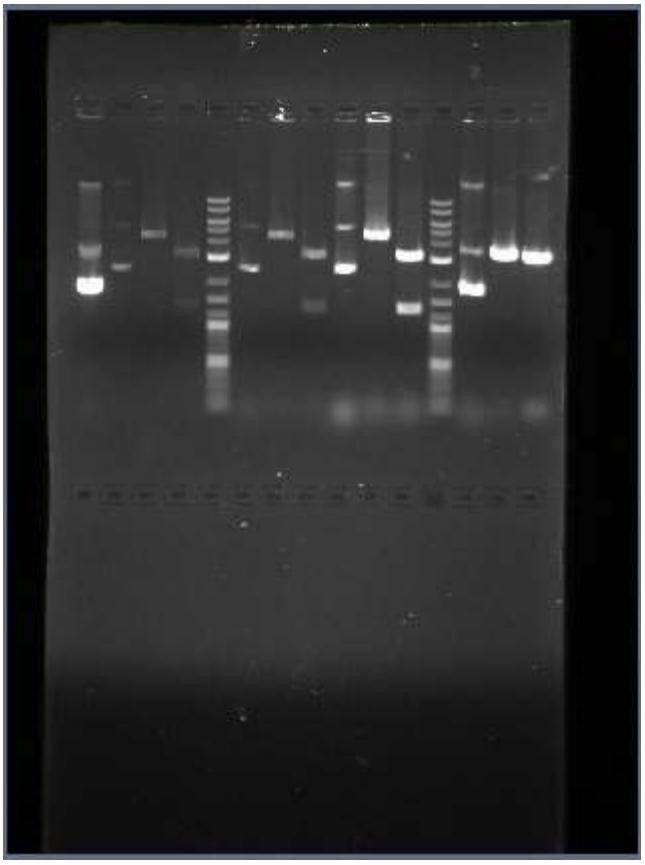
GEL 50min @ 70V



1. I32 uncut
2. 1 uncut
3. 1 S
4. 1 D
5. 2 uncut
6. 2 S
7. 2 D
8. ladder
9. 3 uncut
10. 3 S
11. 3 D
12. ladder
13. 4 uncut
14. 4 S

15. 4 D
16. I33 uncut
17. 5 uncut
18. 5 S
19. 5 D
20. ladder
21. 6 uncut
22. 6 S
23. 6 D
24. 7 uncut
25. 7 S
26. 7 D
27. ladder
28. 8 uncut
29. 8 S
30. 8 D

GEL 50min @ 70V



1. K20 uncut
2. 9 uncut
3. 9 S
4. 9 D
5. ladder
6. 10 uncut
7. 10 S
8. 10 D
9. 11 uncut
10. 11 S
11. 11 D
12. ladder
13. 12 uncut
14. 12 S

15. 12 D

(16-30 empty)

All screens except 12 were successful. 3, 6, and 10 were picked for stocks of I32+E02, K19+I33, and K20+I30 respectively.

I32+E02 and K20+I30 will be used for further cloning on 7/2

Notebook 6/30/10

Transformations were successful, including the K20+I30 that arced.

- There were ~100 colonies on the K19+I33 plate (1 on the I33 control)
- There were ~80 colonies on the I32+E02 plate (3 on the I32 control)
- There were ~40 colonies on the K20+I30 plate (0 on the K20 control)

Liquid cultures were made for screening tomorrow. Plates were also streaked of each screen. Four colonies were picked from each clone. Each labeled 1-12. Numbers 1-4 correspond to I32+E02. Numbers 5-8 correspond to K19+I33. Numbers 9-12 correspond to K20+I30.

Sources for presentation:

File:Cre hetero lox.pdf

File:Cre properties.pdf

File:Hin dna bending.pdf

File:Hin double inversion.pdf

File:Hin hixC function.pdf

File:Hin looping.pdf

File:P22 att site.pdf

File:P22 integrase family.pdf

File:P22 no ihf.pdf

File:P22 site specific rec.pdf

Notebook 6/29/10

DIGEST - 2h @ 37C

I11032 - 4uL

PstIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.5uL

E0240 - 5uL

PstIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 37.5uL

DIGEST 2h @ 37C

K199021 - 8uL

EcoRIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 34.5uL

I11033 - 4uL

EcoRIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.5uL

DIGEST 2h @ 37C

K200021 - 4.5uL

PstIHF - 1uL

SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38uL

I11030 - 8uL

PstIHF - 1uL

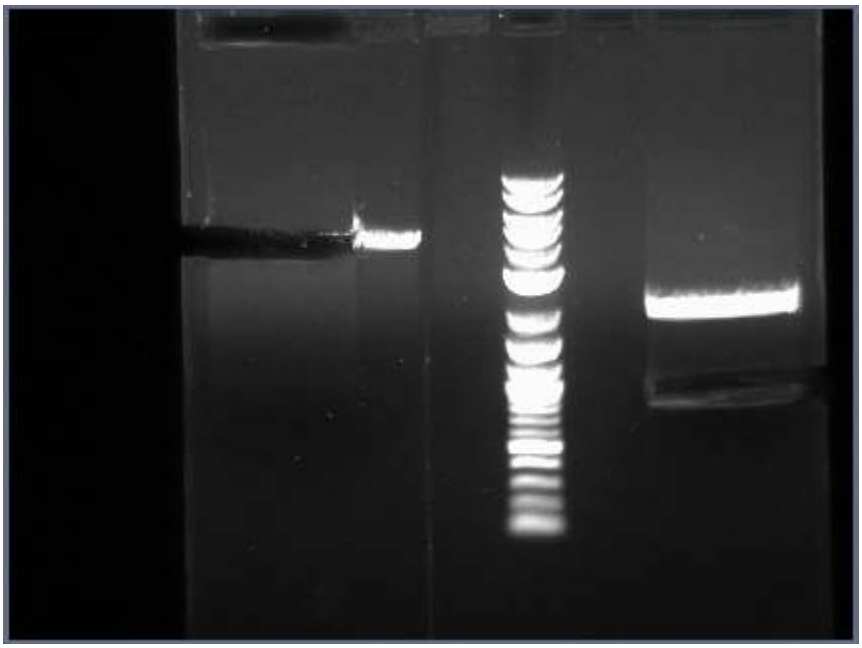
XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 34.5uL

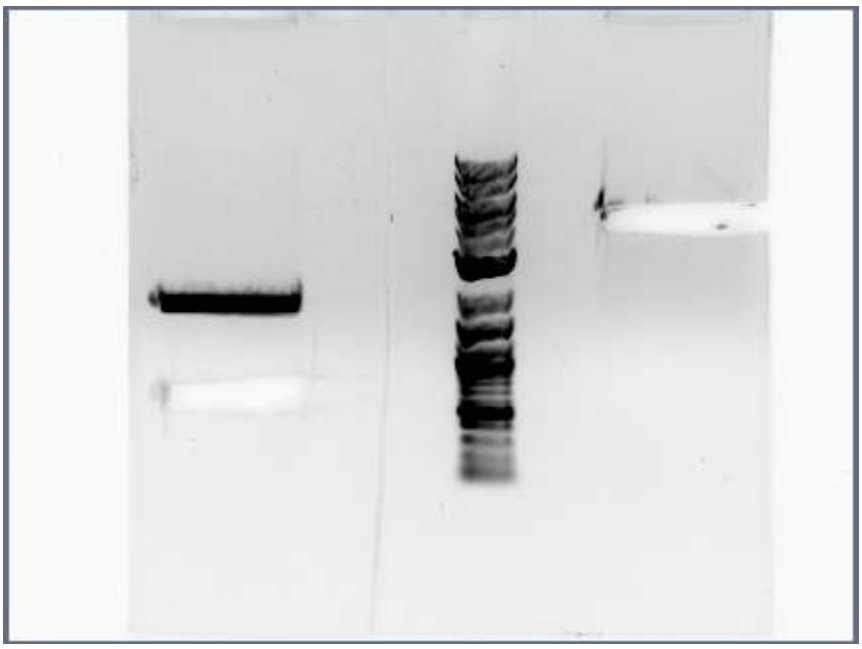
GEL 60min @ 60V



L to R: I32, ladder, E02

expected ~4.5kb and ~0.9kb for extraction

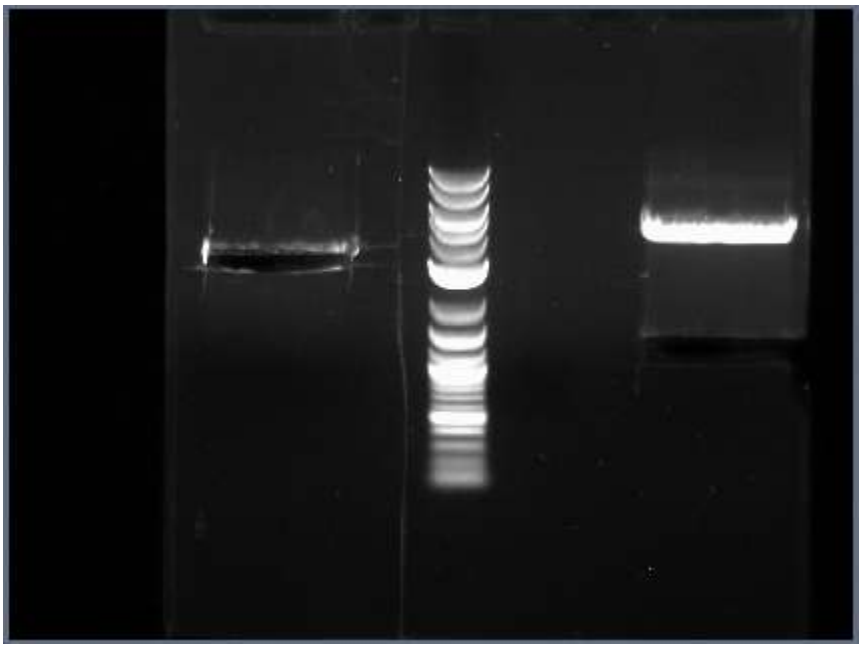
GEL 60 min @ 60V



L to R: K19, ladder, I33

expected ~0.7kb and ~4.6kb for extraction

GEL 60min @ 60V



L to R: K20, ladder, I30

expected ~3.2kb and ~1.2kb for extraction

- All gel slices were processed using the Qiaquick gel extraction kit.

LIGATION 1h benchtop

5uL insert (or sterile water for control)

3uL backbone

1uL buffer

1uL ligase

TRANSFORMATION E. coli DH10b

K20+I30 transformant arced but was plated anyway. All others had times between 4.20 and 4.70ms.

Shook at 37C for 1h before plating on appropriate antibiotics.

Notebook 6/28/10

Miniprepped I11030 for cloning tomorrow. Designed parts to be cloned tomorrow and later this week for the P22 BOB-POP recombination tests. Updated the wiki with Mike S's freezer stocks that he did today (includes the shipment from 6/25). With Justin, made a list of testable variables for encryption constructs. Inoculated Hin and LacI cassettes for miniprep on 6/30.

Vacation 6/19 - 6/26

Notebook 6/18/10

DIGEST 2h @ 37C

K20+J31+B1 - 4uL

PstIHF - 1uL

XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.5uL

B1 - 7uL

PstIHF - 1uL

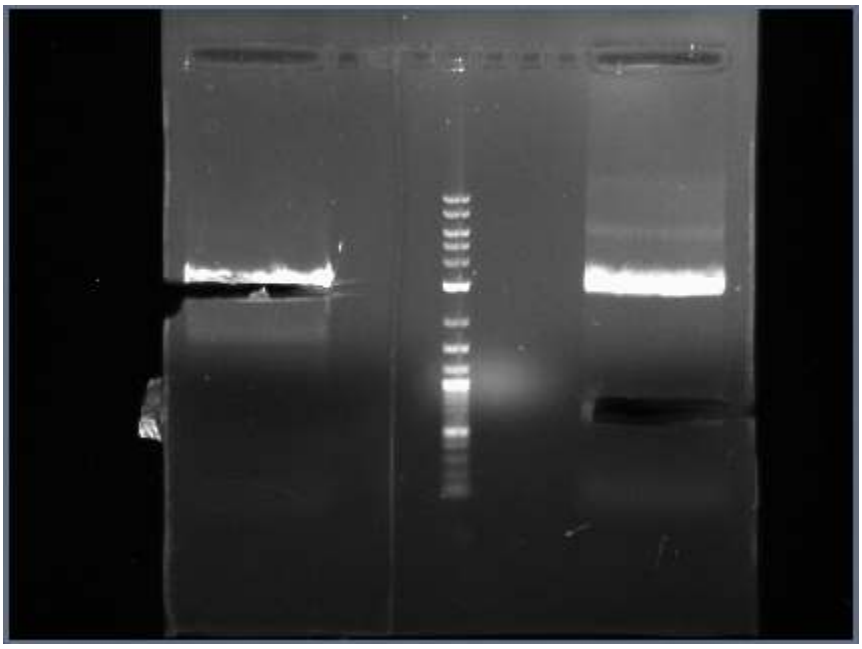
SpeI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 35.5uL

GEL 60min @ 60V



L to R: B1, 2-log ladder, K20+J31+B1

Gel slices were gel extracted using the Qiagen gel extraction kit.

LIGATION 1h benchtop

3uL B1

5uL K20+J31+B1 | water (control)

1uL ligase buffer

1uL ligase

TRANSFORMATION DH10B

B1 control - 4.60ms

B1+K20+J31+B1 (Hin Cassette) - 4.80ms

Plated on AmpKan, left overnight. Justin to take them out tomorrow.

Notebook 6/17/10

Alkaline lysis prepped K20+J31+B1 screens, labeled A-F, and Mary's YgiV in pBad33*BB, labeled 1-2.

DIGEST 2h @ 37C (for A-F)

K20+J31+B1 - 3uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

K20+J31+B1 - 3uL

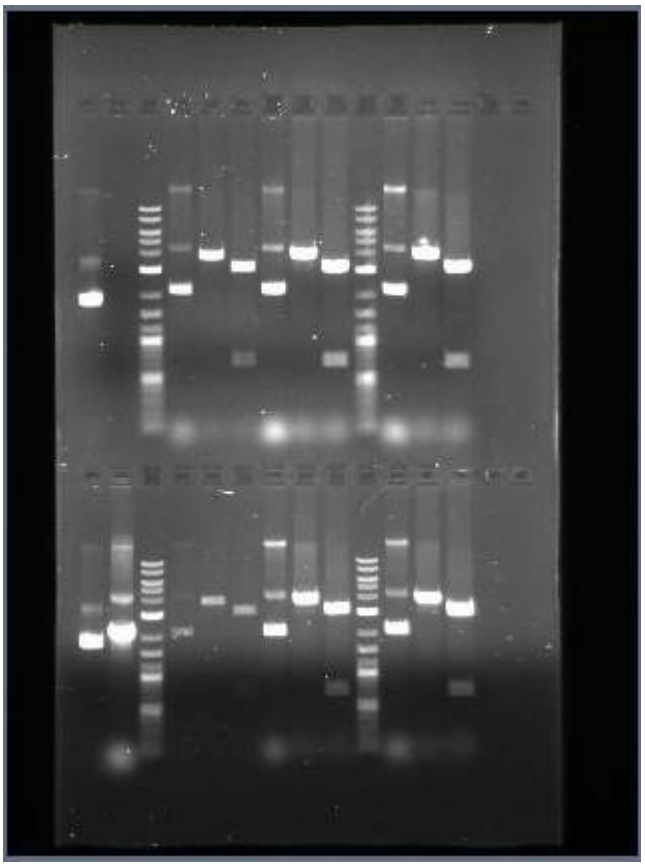
PstIHF - 0.5uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

GEL 50min @ 70V



Top: from L to R 1-15. Bottom: from L to R 16-30

1. uncut B1
2. uncut K20+J31 (missing DNA)
3. 2-log ladder
4. uncut K20+J31+B1 A
5. PstIHF K20+J31+B1 A
6. PstIHF EcoRIHF K20+J31+B1 A
7. uncut K20+J31+B1 B
8. PstIHF K20+J31+B1 B
9. PstIHF EcoRIHF K20+J31+B1 B
10. 2-log ladder
11. uncut K20+J31+B1 C
12. PstIHF K20+J31+B1 C
13. PstIHF EcoRIHF K20+J31+B1 C
14. -
15. -
16. uncut B1
17. uncut K20+J31
18. 2-log ladder
19. uncut K20+J31+B1 D
20. PstIHF K20+J31+B1 D
21. PstIHF EcoRIHF K20+J31+B1 D
22. uncut K20+J31+B1 E
23. PstIHF K20+J31+B1 E
24. PstIHF EcoRIHF K20+J31+B1 E
25. 2-log ladder
26. uncut K20+J31+B1 F
27. PstIHF K20+J31+B1 F

28. PstIHF EcoRIHF K20+J31+B1 F

29. -

30. -

Screens display the correct banding pattern. Screen A will be used for further cloning and freezer stocks. A new part page, K20+J31+B1 composite part, was created.

Sequence files were received and at first glance seem successful. Cre Cassette will be sequenced again because the reverse sequence was weak.

Notebook 6/16/10

K20+J31+B1 grew 2 colonies on the control, ~150 on the pellet, and ~40 on the regular plate. Six colonies, labeled A-F, were picked and used for restriction digest screening.

Miniprepped S03520, I11032, and I11033 for Justin.

DIGEST 2h @ 37C

BBa_J23100 - 5uL

SpeI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 37.5uL

BBa_S03520 - 20uL

XbaI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 22.5uL

Justin continued the above digest through cloning. He also took B1+I7, Cre Cassette, and K20+J31 to be sequenced.

Notebook 6/15/10

Made freezer stocks of L. acidophilus ATCC 4356.

DIGEST - 2h @ 37C

K20+J31 - 3uL

EcoRIHF - 1uL

SpeI - 1uL

BSA - 0.5uL

Buffer 4 - 5uL

Sterile water - 39.5uL

DIGEST - 2h @ 37C

B1006 - 7uL

EcoRIHF - 1uL

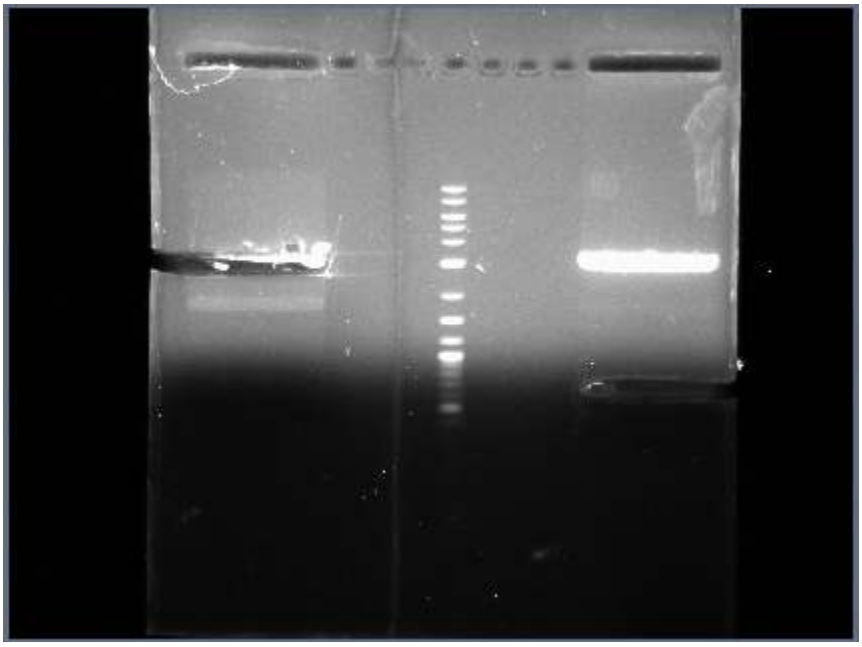
XbaI - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 35.5uL

GEL 60min @ 60V



L to R: B1, 2-log ladder, K20+J31

The gel slices were removed and extracted using the Qiagen gel extraction kit.

B1 (9.4 ng/uL)

K20+J31 (9.8 ng/uL)

LIGATION

3uL water | K20+J31

5uL B1

1uL ligase buffer

1uL ligase

benchtop for 1h then used to transform DH10B cells. (the control contained water instead of the insert)

The cells were shaken for 1h @ 37C and then plated on ampRkanR plates.

Created the Completed Parts section of the wiki to accomidate new composite parts and other parts to be submitted to the iGEM registry in the fall.

Notebook 6/14/10

Two biobrick parts were compiled/deemed successful (minus sequencing) today:

Cre Cassette

K20+J31 composite part

Freezer stocks of liquid cultures from 6/13 were made. The wiki file was updated.

An alkaline lysis miniprep was done on the K20+J31 screen. Three parts from the registry were transformed into DH10B, including a new RBS+lacIq.

DIGEST - 2h @ 37C

K20+J31 - 3uL

EcoRIHF - 1uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

DIGEST - 2h @ 37C

K20+J31 - 3uL

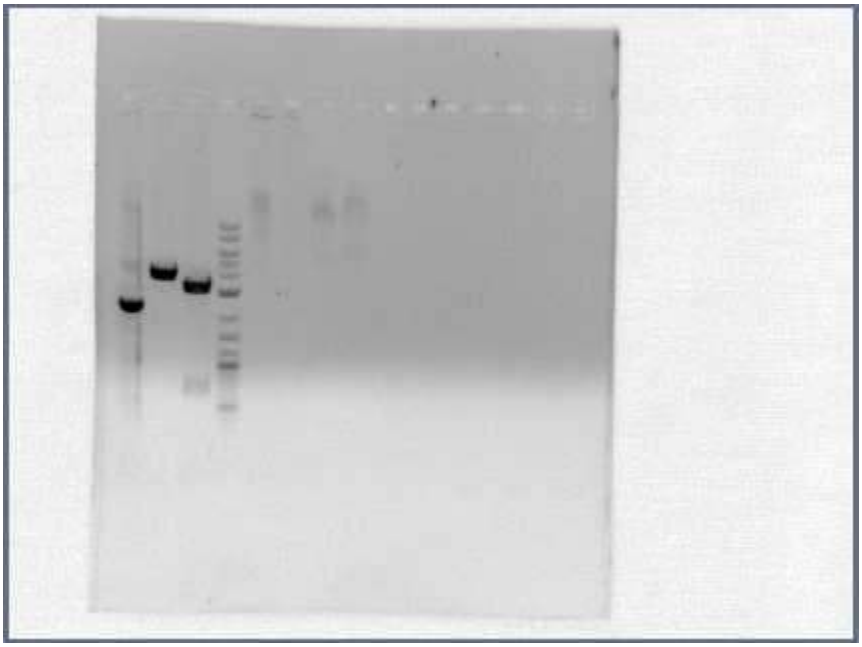
EcoRIHF - 1uL

PstIHF - 1uL

Buffer 4 - 1.8uL

Sterile water - 11.2uL

GEL - 40min @ 80V



From L to R:

1. uncut K20+J31

2. EcoRI K20+J31

3. EcoRI PstI K20+J31

4. 2-log ladder

5. BOB' (11022?)

6. POP'

7. Mary's ligation control

8. Mary's ligation

The K20+J31 displays the correct banding pattern. The clone appears successful.

Notebook 6/11/10

Alkaline lysis of the 12 liquid cultures, Cre Cassette 1-7 and J23+K29 A-E.

DIGEST - 2h @ 37C

Cre Cassette (1-7) - 3uL

EcoRIHF - 0.5uL

PstIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.2uL

Cre Cassette (1-7) - 3uL

EcoRIHF - 0.5uL

Buffer 4 - 1.8uL

Sterile water - 12.7uL

DIGEST - 2h @ 37C

J23+K29 (A-E) - 3uL

BglI - 0.5uL

BglII - 0.5uL

Buffer 3 - 1.8uL

Sterile water - 12.2uL

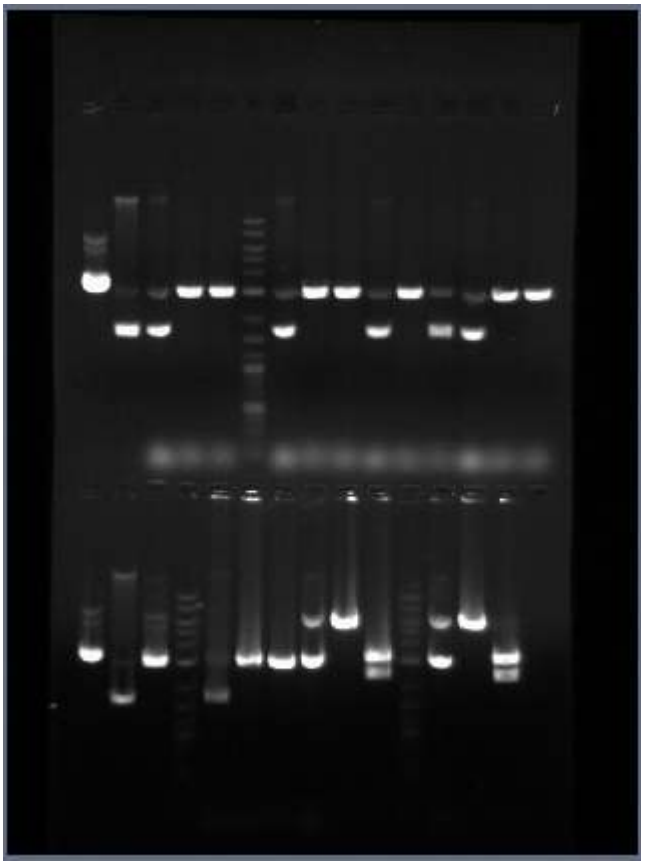
J23+K29 (A-E) - 3uL

BglI - 0.5uL

Buffer 3 - 1.8uL

Sterile water - 12.7uL

GELS - 50min @ 70V



TOP from L to R, 1-15

BOTTOM from L to R, 16-30

1. uncut J23

2. uncut K29

3. uncut J23+K29 A

4. BglI J23+K29 A

5. BglI BglII J23+K29 A

6. 2-log ladder

7. uncut J23+K29 B

8. BglI J23+K29 B

9. BglI BglII J23+K29 B

10. uncut J23+K29 C

11. BglI J23+K29 C

12. BglI BglII J23+K29 C

13. uncut J23+K29 D

14. BglI J23+K29 D

15. BglI BglII J23+K29 D

16. uncut J23

17. uncut K29

18. uncut B1+I7

19. 2-log ladder

20. uncut J23+K29 E

21. BglI J23+K29 E

22. BglI BglII J23+K29 E

23. uncut Cre Cassette 1

24. EcoRIHF Cre Cassette 1

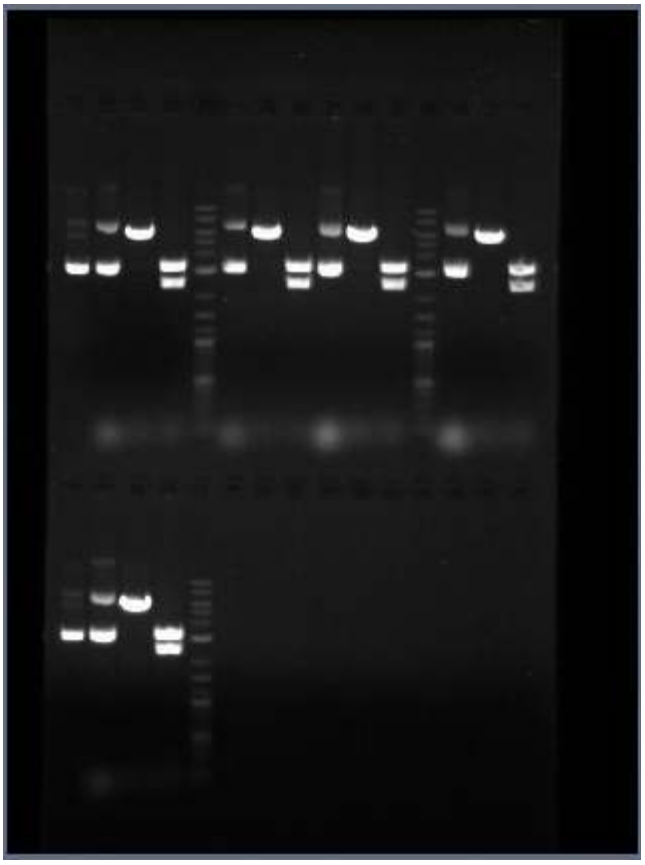
25. EcoRIHF PstIHF Cre Cassette 1

26. 2-log ladder

27. uncut Cre Cassette 2

28. EcoRIHF Cre Cassette 2

29. EcoRIHF PstIHF Cre Cassette 2



TOP from L to R, 1-15

BOTTOM from L to R, 16-30

1. uncut B1+I7

2. uncut Cre Cassette 3

3. EcoRIHF Cre Cassette 3

4. EcoRIHF PstIHF Cre Cassette 3

5. 2-log ladder

6. uncut Cre Cassette 4

7. EcoRIHF Cre Cassette 4

8. EcoRIHF PstIHF Cre Cassette 4

9. uncut Cre Cassette 5

10. EcoRIHF Cre Cassette 5

11. EcoRIHF PstIHF Cre Cassette 5

12. 2-log ladder

13. uncut Cre Cassette 6

14. EcoRIHF Cre Cassette 6

15. EcoRIHF PstIHF Cre Cassette 6

16. uncut B1+I7

17. uncut Cre Cassette 7

18. EcoRIHF Cre Cassette 7

19. EcoRIHF PstIHF Cre Cassette 7

The screens of Cre Cassette 1-7 all have the expected banding pattern (~5.5kb and ~3.2kb & 2.3kb). Screen 3 will be picked and used for further experiments.

The screens of J23+K29 do not have the correct banding pattern. However, screen C deserves further inspection.

Reattempted transformation of K20+J31 clone with same ligation. K20+J31 4.8ms K20 control 5.8ms

Received MG1655 del(araBAD) del(araE) from Wes Marner

Notebook 6/10/10

Antoher colony PCR of 7 colonies from the Cre Cassette clone as well as a B1+I7 control. Used pSB1AK3 FWD and REV primers.

CPCR Protocol:

95C - 0:05:00

95C - 0:00:30

50C - 0:00:30

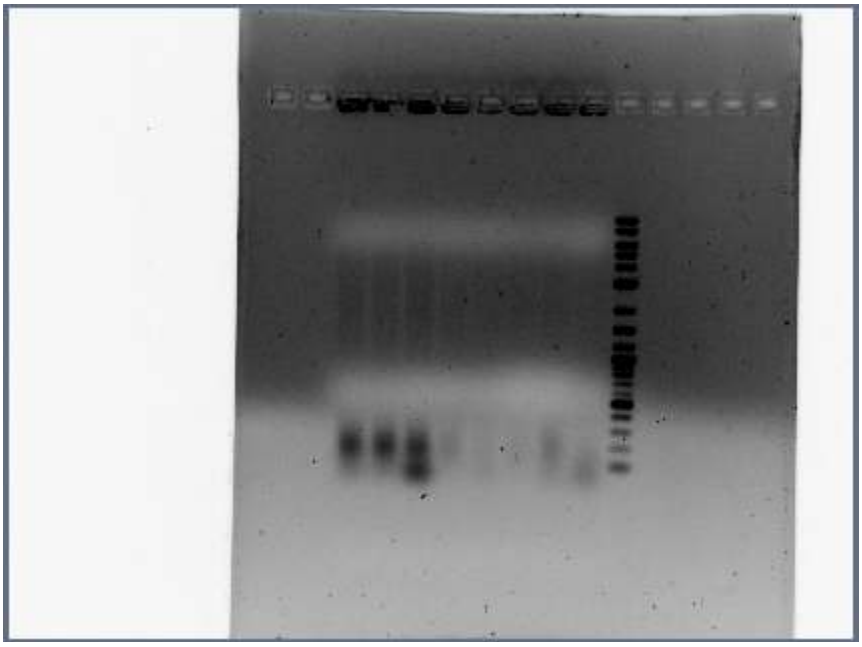
72C - 0:02:20

repeat steps 2-4 25x

72C - 0:05:00

4C - inf

5uL of CPCR product was used for the gel. This CPCR was reattempted because the first was unsuccessful yesterday. A control was used to test the viability of the protocol.



Notebook 6/9/10

Colony PCR of 7 colonies from the Cre Cassette clone. Used pSB1AK3 FWD and REV primers.

CPCR Protocol:

95C - 0:05:00

95C - 0:00:30

50C - 0:00:30

72C - 0:02:20

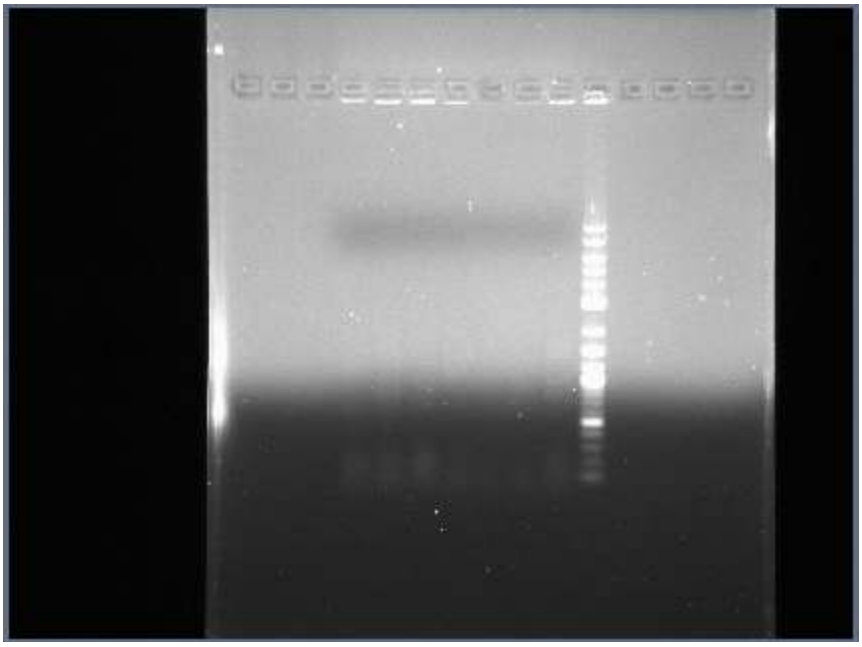
repeat steps 2-4 25x

72C - 0:05:00

4C - inf

5uL of CPCR product was used for the gel

GEL - 40 mins @ 80V



From L to R 1-15, starting in lane 4; screen 1-7,ladder

Nothing appeared. Possibly scewed because of inital PCR protocol fail.

DIGEST

(clone K20+J31, 2h @ 37C)

BBa_K200021 - 4.27uL

SpeI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.23uL

BBa_J31000 - 22.35uL

XbaI - 1uL

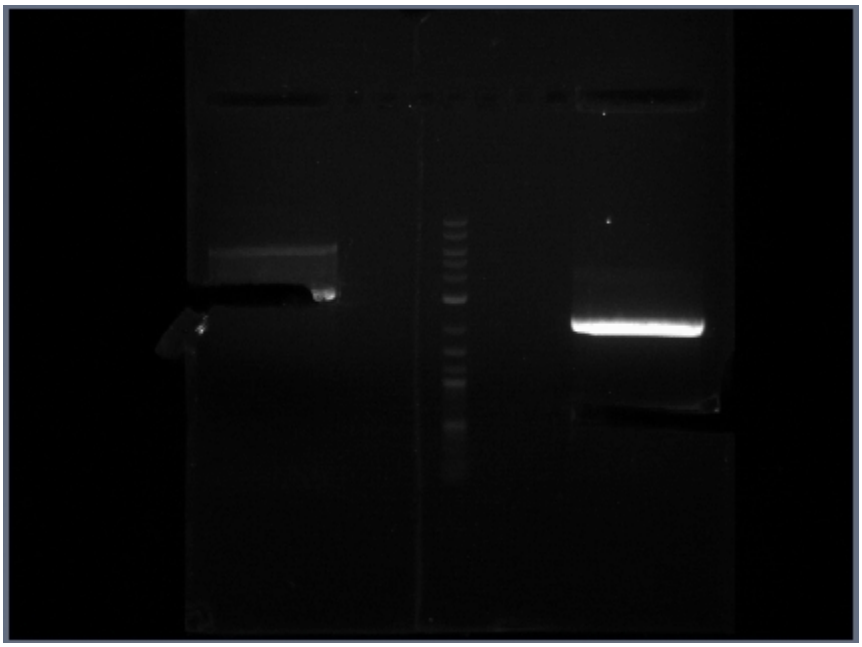
PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 20.15uL

GEL - 60 mins @ 60V



From L to R: K20, ladder, J31

A successful gel. The products were gel extracted and eventually ligated.

K20 (14.9 ng/uL)

J31 (3.7 ng/uL)

DIGEST

(clone K20+I11, 2h @ 37C)

BBa_K200021 - 4.27uL

SpeI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 38.23uL

BBa_I11020 - 13.49uL

XbaI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 29.01uL

GEL - 60 mins @ 70V

File:K20+I11 double digest.jpg

From L to R: K20, ladder, I11.

The K20 band was correct. No band was displayed in the 1.1kb region for I11 as expected. A gel slice was removed anyway because of the fast nature of cloning. The gel slices were not used for further cloning. The banding pattern of I11 was expected to be ~4.4kb and ~1kb. Instead two bands of ~3kb and ~2.5 kb are seen.

DIGEST

(clone J23+K29, 2h @ 37C)

BBa_J23100 - 5.04uL

SpeI - 1uL

PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 37.46uL

BBa_K292006 - 6.34uL

XbaI - 1uL

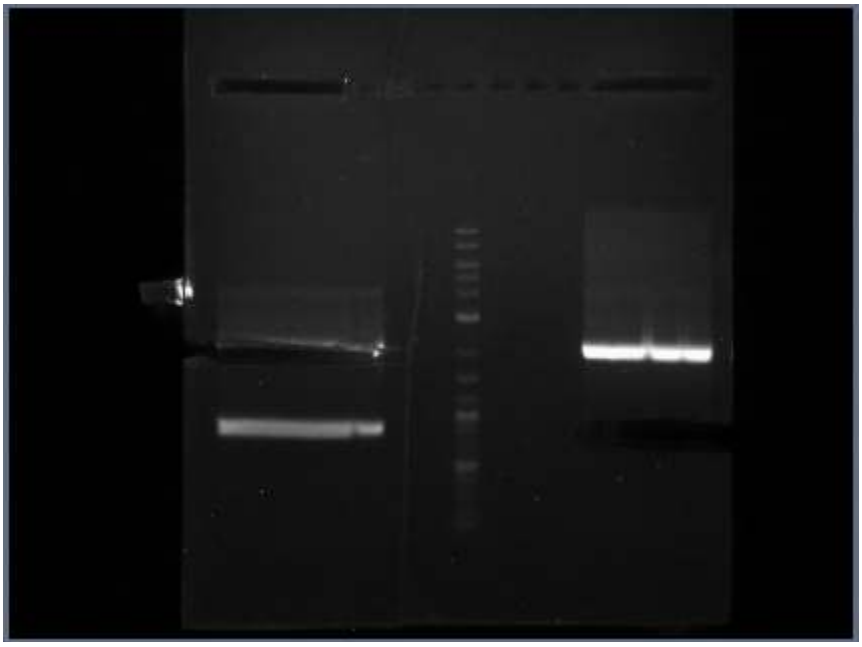
PstIHF - 1uL

Buffer 4 - 5uL

BSA - 0.5uL

Sterile water - 36.16uL

GEL - 60 mins @ 70V



From L to R: J23, ladder, K29.

Another successful gel. The plasmid used in J23 has odd biobrick sites. The small band on the gel in lane J23 is an RFP. The gel slices were gel extracted and used for further cloning. NOTE: the K29 band on the gel was lower than expected. However, the top band was the correct length, so cloning was continued

J23 (15.8 ng/uL)

K29 (4.1 ng/uL)

LIGATION

Ligations of 2/3 clones were prepared. The same procedure was used for both, as the DNA concentrations were similar.

3uL J23 | K20

5uL K29 | J31

1uL ligase buffer

1uL ligase

benchtop for 1h then -20C until Sarah arrived.

Sarah prepared the transformation and further cloning steps.

Notebook 6/8/10

Made freezer stocks of 8 parts received on 6/4

Planned tomorrow's 5 clones with Justin. Made 4 gels in preparation. Yue miniprepped DNA.

Did research with Justin on mechanism of phage recombination to design potential construct.

Notebook 6/7/10

Again, screened 3 colonies from the B1+I7+B1 (a.k.a. Cre cassette) transformation, labeled A, B, and C, with Friday's DNA.

DIGEST (for 2h @ 37C)

BBa_B1006 - 3 uL

BglI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12 uL

DIGEST (for 2h @ 37C)

BBa_B1006+BBa_I718008 - 3 uL

BglI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008+BBa_B1006 - 7 uL

BglI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 8 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008+BBa_B1006 - 7 uL

BamHI - 1 uL

BglI - 1 uL

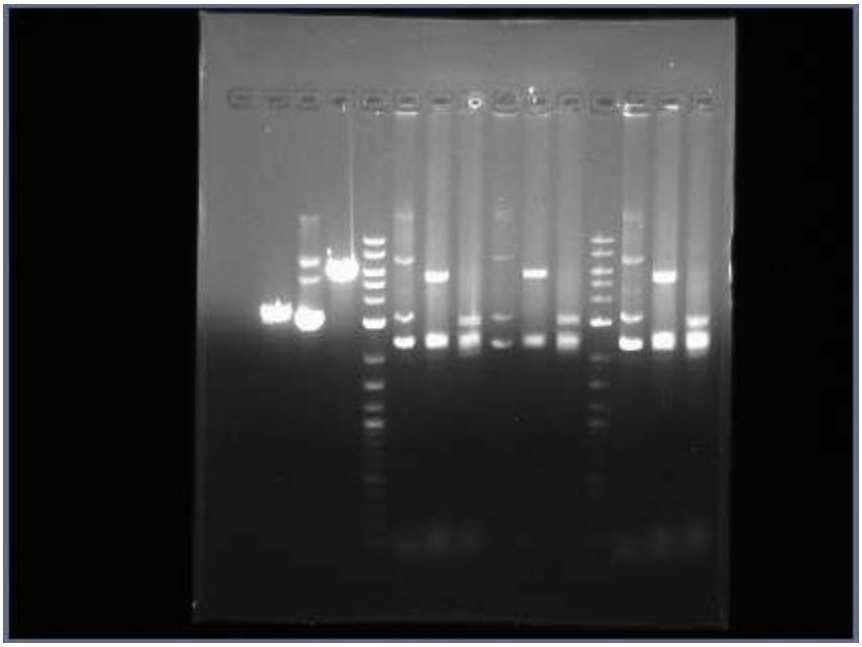
BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 7 uL

GEL (60 min @ 70V)

against 2-log ladder. From L to R; 1-15.



1. uncut B1

2. BglI B1 (expect ~3.2kb)

3. uncut B1+I7

4. BglI B1+I7 (expect ~5.4kb)

5. Ladder

6. uncut Cre cassette A

7. BglI Cre cassette A (expect ~5.5kb)

8. BamHI BglI Cre cassette A (expect ~2.9kb, ~2.1kb, and ~0.5kb)

9. uncut Cre cassette B

10. BglI Cre cassette B (expect ~5.5kb)

11. BamHI BglI Cre cassette B (expect ~2.9kb, ~2.1kb, and ~0.5kb)

12. Ladder

13. uncut Cre cassette C

14. BglI Cre cassette C (expect ~5.5kb)

15. BamHI BglI Cre cassette C (expect ~2.9kb, ~2.1kb, and ~0.5kb)

NOTE: The same band at ~2.5kb is present across all screens. Likely this is unwanted DNA of some kind. Two more colonies were picked and liquid cultures were prepared for additional screening.

Liquid cultures

BBa_K200021 - pSB1AK3

BBa_K112218 - BBa_K112956 (cmR kanR)

BBa_K112224 - BBa_K112952 (ampR cmR)

BBa_K199021 - pSB1A2

BBa_K292006 - pSB1A2

BBa_I11023 - pSB2K3

BBa_I11022 - pSB2K3

BBa_I11020 - pSB2K3

BBa_B1006 - pSB1AK3

BBa_E0240 - pSB1A2

Notebook 6/4/10

Screened 3 colonies from the B1+I7+B1 (a.k.a. Cre cassette) transformation, labeled A, B, and C. Preformed an alkaline lysis miniprep of plasmid DNA.

DIGEST (for 2h @ 37C)

BBa_B1006 - 3 uL

BglI - 0.5 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12.5 uL

DIGEST (for 2h @ 37C)

BBa_B1006+BBa_I718008 - 3 uL

BglI - 0.5 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12.5 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008+BBa_B1006 - 3 uL

BamHI - 0.5 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12.5 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008+BBa_B1006 - 3 uL

BamHI - 0.5 uL

BglI - 0.5 uL

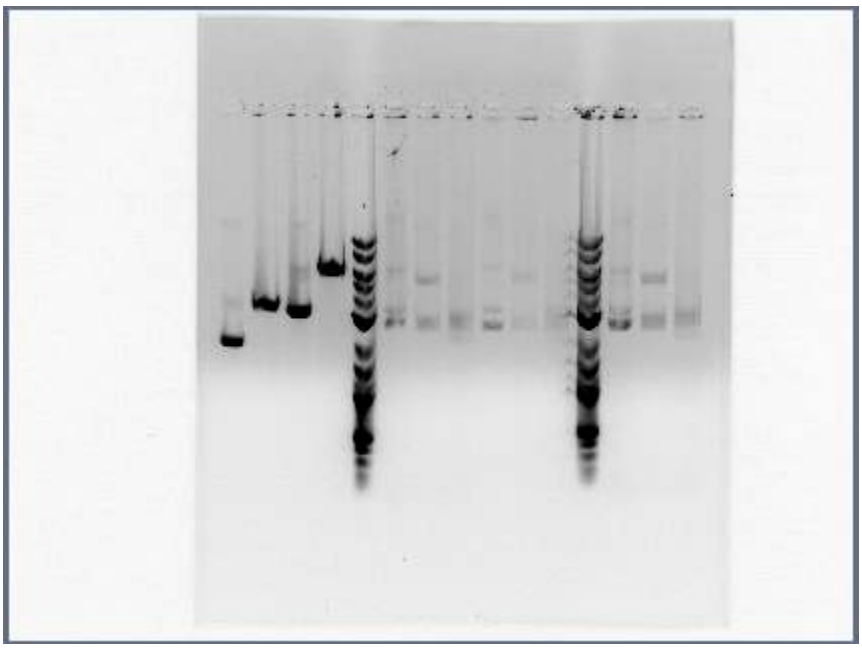
BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12 uL

GEL (35 min @ 90V)

against 2-log ladder. From L to R; 1-15.



1. uncut B1

2. BglI B1 (expect ~3.2kb)

3. uncut B1+I7

4. BglI B1+I7 (expect ~5.4kb)

5. Ladder

6. uncut Cre cassette A

7. BamHI Cre cassette A (expect ~5kb and ~0.5kb)

8. BamHI BglI Cre cassette A (expect ~2.9kb, ~2.1kb, and ~0.5kb)

9. uncut Cre cassette B

10. BamHI Cre cassette B (expect ~5kb and ~0.5kb)

11. BamHI BglI Cre cassette B (expect ~2.9kb, ~2.1kb, and ~0.5kb)

12. Ladder

13. uncut Cre cassette C

14. BamHI Cre cassette C (expect ~5kb and ~0.5kb)

15. BamHI BglI Cre cassette C (expect ~2.9kb, ~2.1kb, and ~0.5kb)

NOTE: The banding pattern of A, B, and C suggest that the BamHI did not cut as well in the 0.5uL concentration. The bands of the screened clones are very faint but they display the expected pattern taking the incomplete BamHI digestion into account. Cre cassette A was streaked on a plate for further cloning and stocks. Further digestion protocols could be attempted for more solid proof of the existence of a good clone. However,

the insert is so large that the shear size change of the plasmid from 3.2 to 5.4 kb suggests that the cloning was successful.

PARTS RECEIVED

A set of 9 biobrick parts were received and struck on plates.

BBa_K200021 - pSB1AK3

BBa_K112218 - BBa_K112956 (cmR kanR)

BBa_K112224 - BBa_K112952 (ampR cmR)

BBa_K199021 - pSB1A2

BBa_K292006 - pSB1A2

BBa_J31001 - pSB1A2

BBa_I11023 - pSB2K3

BBa_I11022 - pSB2K3

BBa_I11020 - pSB2K3

Notebook 6/3/10

There were 7 colonies on the transformation plate of B1+I7+B1 (from here on called Cre Cassette). There were none on the control. Liquid cultures were inoculated of three colonies labeled A, B, and C for screening tomorrow.

Notebook 6/2/10

DIGEST (for 2h @ 37C)

BBa_B1006 (276.6 ng/uL) - 5.4 uL

EcoRIHF - 1 uL

XbaI - 1 uL

BSA - 0.5 uL

NEB Buffer 4 - 5 uL

Sterile water - 37.1 uL

DIGEST

B1+I7 (235.7 ng/uL) - 6.4 uL

EcoRIHF - 1 uL

SpeI - 1 uL

BSA - 0.5 uL

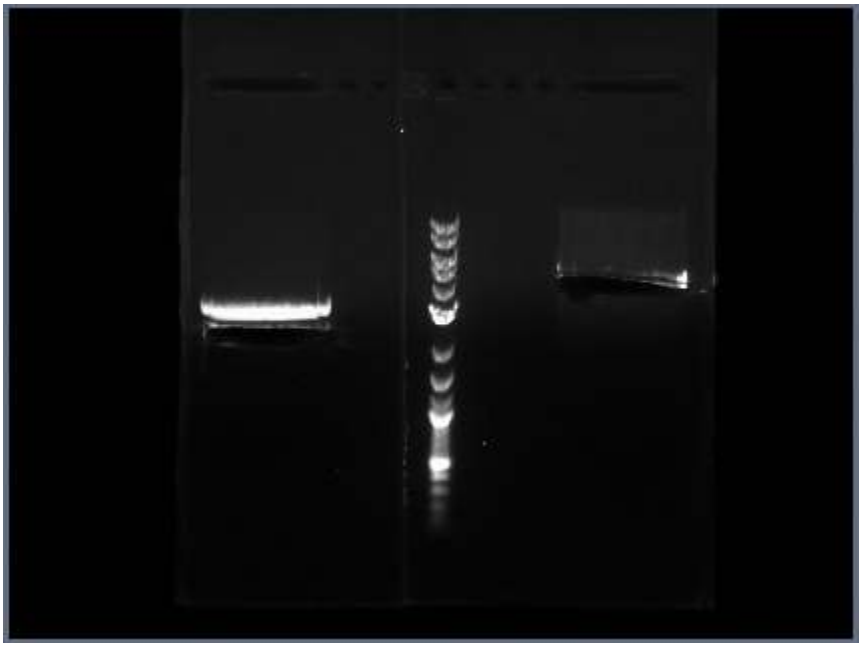
NEB Buffer 4 - 5 uL

Sterile water - 36.1 uL

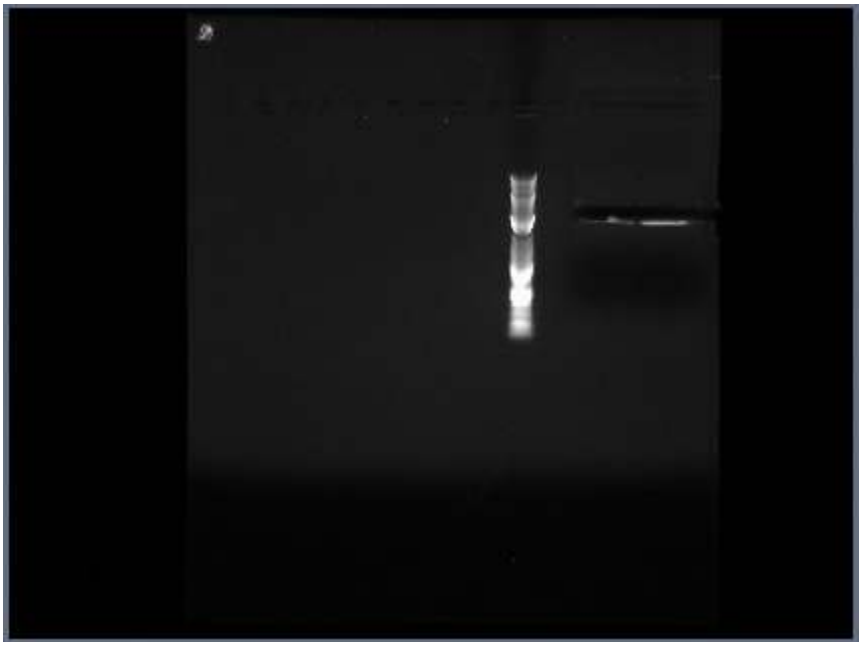
GEL

The first digestion of B1+I7 was correct. However, the digestion of "B1" was actually found to be a digestion of I7, so the B1 digestion was redone and is displayed on the second gel.

(60 mins @ 70V. from L to R; B1+I7, ladder, I7 fail)



(30 mins @ 70V. from L to R; ladder, B1)



The contents were gel extracted using a Qiagen kit. The concentration of B1 was 73.1 ng/uL (which doesnt make sense based upon the inital concentration of the digest) and the concentration of B1+I7 was 11.2 ng/uL

LIGATION

1.36 uL B1

6.64 uL B1+I7

1 uL ligase buffer

1 uL ligase

(benchtop for 1h, then 1h in 4C. the control replaced B1+I7 with sterile water)

TRANSFORMATION

1 uL of each control and B1+I7+B1 was used to transform DH10B cells. The times were both 4.90 ms. Shaken @ 37C for 1h then plated (amp and kan) and left overnight @ 37C.

Notebook 6/1/10

Gel fail. (gel dock setup incorrectly so see 6/2 for digest etc). But miniprep of B1+I7 was successful (235.7 ng/uL). Will do cloning tomorrow.

Notebook 5/31/10

A liquid culture of B1+I7 colony A and 6 plate transformants were inoculated for further cloning and miniprep

Notebook 5/28/10

Screened 3 colonies from the B1+I7 transformation, labeled A, B, and C. With Sarah, preformed an alkaline lysis miniprep of plasmid DNA.

DIGEST (for 2h @ 37C)

BBa_B1006 - 3 uL

BglI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12 uL

DIGEST (for 2h @ 37C)

BBa_I718008 - 3 uL

BamHI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 11 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008 - 3 uL

BamHI - 1 uL

BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 12 uL

DIGEST (for 2h @ 37C) (for each A, B, C)

BBa_B1006+BBa_I718008 - 3 uL

BamHI - 1 uL

BglI - 1 uL

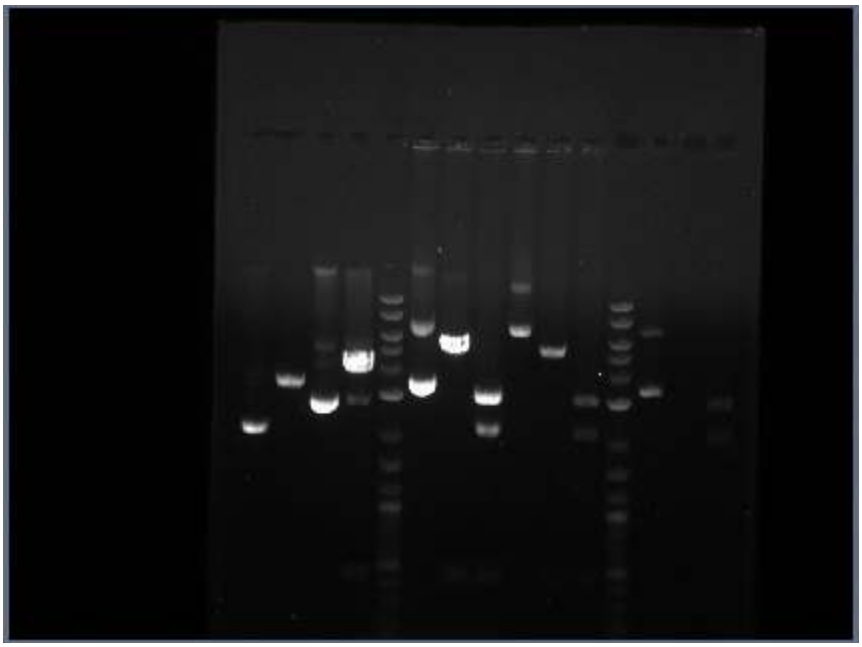
BSA - 0.18 uL

NEB Buffer 3 - 1.8 uL

Sterile water - 11 uL

GEL (60 min @ 70V)

against 2-log ladder. From L to R; 1-15.



1. uncut B1

2. BglI B1 (expect ~3.2kb)

3. uncut I7

4. BamHI I7 (expect ~3.8kb and ~0.5kb)

5. Ladder

6. uncut B1+I7 A

7. BamHI B1+I7 A (expect ~5kb and ~0.5kb)

8. BamHI BglI B1+I7 A (expect ~2.9kb, ~2.1kb, and ~0.5kb)

9. uncut B1+I7 B

10. BamHI B1+I7 B (expect ~5kb and ~0.5kb)

11. BamHI BglI B1+I7 B (expect ~2.9kb, ~2.1kb, and ~0.5kb)

12. Ladder

13. uncut B1+I7 C

14. BamHI B1+I7 C (expect ~5kb and ~0.5kb)

15. BamHI BglI B1+I7 C (expect ~2.9kb, ~2.1kb, and ~0.5kb)

See the page for the new composite part here: B1+I7 composite part

Notebook 5/26/10

Made freezer stocks of transformed cells from 5/24 and DH10B. Yue miniprepped the DNA and the concentration was listed on the vial.

DIGEST (for 2h @ 37C)

BBa_I718008 (418.2 ng/uL) - 3.6 uL

XbaI - 1 uL

PstIHF - 1 uL

BSA - 0.5 uL

NEB Buffer 4 - 5 uL

Sterile water - 38.9 uL

BBa_B1006 (276.6 ng/uL) - 5.4 uL

SpeI - 1 uL

PstIHF - 1 uL

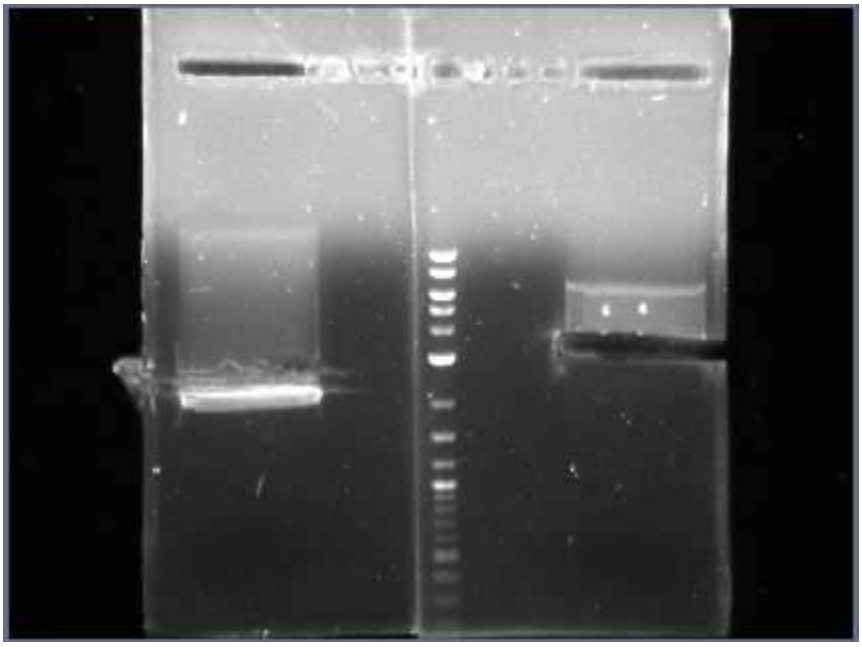
BSA - 0.5 uL

NEB Buffer 4 - 5 uL

Sterile water - 37.1 uL

GEL (70 min @ 70V)

against a 2-log ladder. From L to R; BBa_I718008, 2-log ladder, BBa_B1006.



Concentrations after the gel extraction were: BBa_I718008 (7.6 ng/uL), BBa_B1006 (16.2 ng/uL).

LIGATION

3.2 uL BBa_B1006

4.8 uL BBa_I718008

1 uL ligase buffer

1 uL T4 ligase

(a control with 4.8 uL sterile water instead of I7 was ligated as well)

TRANSFORMATION

40 uL of electrocompetent DH10B cells were transformed with 1 uL of each ligation and plated after 1h of growth on A+K plates. The plates were left overnight.

B1+I7 (3.90ms)

B1 control (4.20ms)

Notebook 5/25/10

Made freezer stocks of 9 requested parts. Transformations from 5/24 were made into liquid cultures for future miniprep and freezer stocks

Notebook 5/24/10

Made stocks of Cm34 in 95% EtOH, re-plated failed gel stab of HinLVA biobrick, compiled additional supply list, compiled additional parts request list, transformed biobricks (BBa_K098995, BBa_K118004, BBa_I718008, BBa_B1006, pSB3C5) from the 2009 and 2010 plate distributions.

Notebook 4/8/10

Digested 18uL pTrc-acs1 (miniprepped Qiagen) with 1uL HindIII for 1h in NEB buffer 2 + BSA (total vol 49uL) then added 1uL XbaI (t.v. 50uL) and let digest 1h Digested 18uL pBad33-BTE (miniprepped Qiagen) with 1uL HindIII for 1h in NEB buffer 2 + BSA (total vol 49uL) then added 1uL NheI (t.v. 50uL) and let digest 1h

ran digests plus 8uL loading dye in .7% agarose gel at 70V for 1h (see gel image: Media:IGEM 2010-04-08 nate-bteacs1-2.jpg) (post extraction: Media:IGEM_2010-04-08_nate-bteacs1-3.jpg) used Qiagen gel extraction kit for each digest product (pTrc ~0.28g;BTE ~0.20g) with optional step and 40uL H2O elution. Ran another gel for concentration estimates (pTrc ~35ng/uL; BTE ~15ng/uL). (concentration gel: Media:IGEM_2010-04-08_nate_concentration.jpg)

did 10uL benchtop (2h run) ligation with 1uL ligase, 1uL ligase buffer and either 5uL BTE and 3uL pTrc or 5uL water and 3uL pTrc. Transformed 0.4uL ligation reaction with 40uL electrocompetent DH10b cells. 2 arcs, then 2 worked. Incubated for 1h and plated 100uL plus pellet.

NOTE: need colony PCR primers!!! in future elute with 30-40uL warm EB and skip optional QG step in gel extraction