XinXin Lin start 8/01/11

From AndersonLab wiki

Contents

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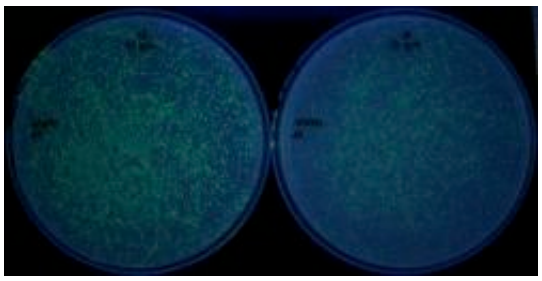
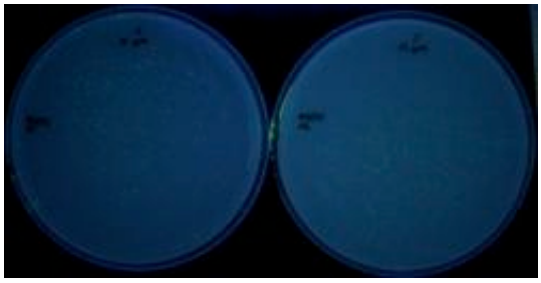
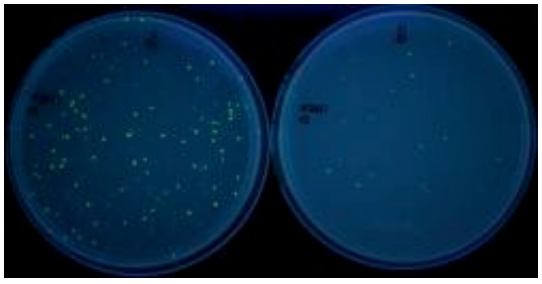
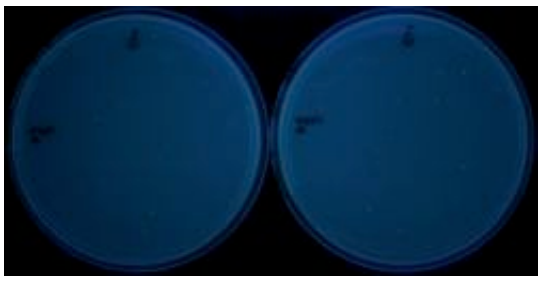
Xin Xin Lin 12:46, 21 September 2011 (PDT)

- Check & Image Selection Plates

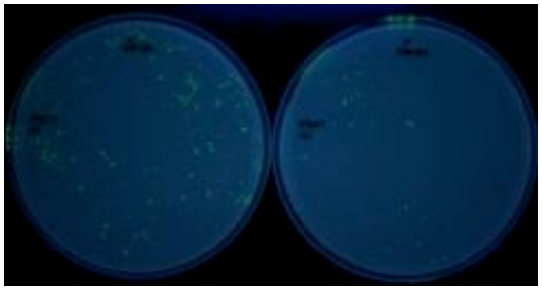
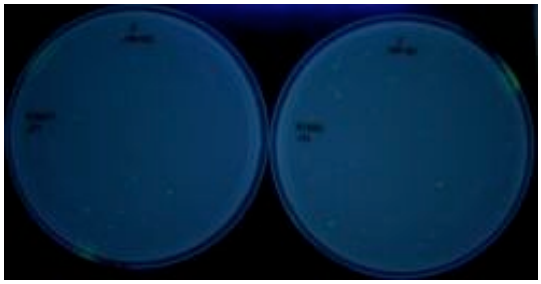
```
No growth on 1e-3 dilution Negative Selection, only growth w/ undiluted
No growth on Negative Control Cam & Spec Plates
```

Negative Selection- Mostly green colonies, very small colonies, dense growth (undiluted)

Positive Selection- Some green colonies w/ white

Non-Selection- 50:50 mix of green & white

Xin Xin Lin 15:01, 20 September 2011 (PDT)

- Negative Selection

```
Added 4uL dP stock to 20mL LB+AS in 50mL Falcon Tube & Mixed
Aliquoted 5mL/Culture Tube- =1uL dP/5mL instead of 0.5uL
Inoculated 1uL Mixed Saturated Cultures
Shake 3hr. @ 37 degree C, 2:28-5:28PM
Media completely clear after 3 hr.- Plated 50uL 1e-3 dilution & 50uL undiluted
```

- Negative Control

```
Spotted -80 Comp Cells pir G-J w/o 2993 (Blue Stripe) on Cam & Spec Plates
```

- Positive Selection

```
Microwaved remaining 300mL of Positive Selection Agar
Added 50mL to 50mL Falcon Tube- Added 50uL Spec & 50uL Amp Antibiotics
-Added 100uL 5FdU, Thymidine, & Uridine- Mixed & Vortexed
Pipetted 12uL agar/plate & dried
Plated 50uL 1e-6 dilution mixed cultures
```

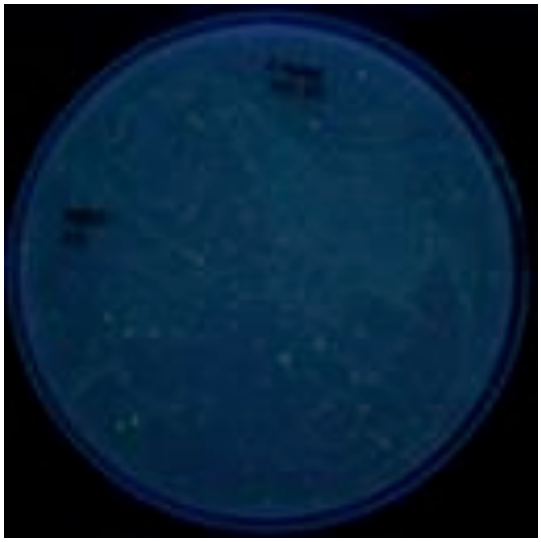
- Non-selection

```
Plated 50uL 1e-6 dilution mixed cultures
```

Xin Xin Lin 15:49, 19 September 2011 (PDT)

- Image pir Strain I Mixed Cultures on Non-Selective Media

```
Redo of Non-Selection- Plated 50uL mixed cultures on Amp/Spec
Very few green colonies
```



- Pick Colonies & Inoculate Overnight Cultures

```
Picked 2 colonies/strain into 3mL LB+AS in 24-well block
-G1-J2 w/ 1832 or ffGFP
Shake overnight @ 37 degree C
```

- To Do

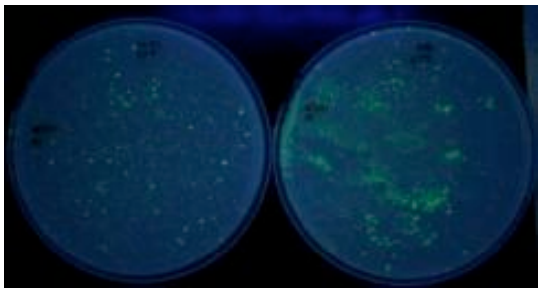
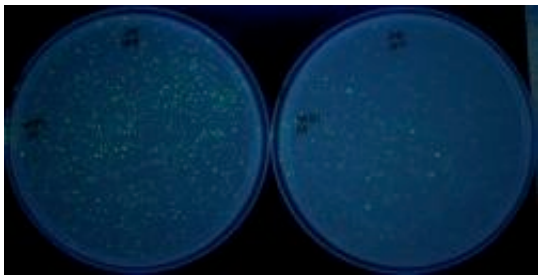
```
Negative Control- 2993 Comp Cells on Spec & Cam Plates
Pour Positive Selection Plates
Mix Cultures 50:50 & Plate on Positive & Non-Selective Media
Seed in Negative Selection Media & Shake 3 hr.- Plate
```

Xin Xin Lin 13:59, 18 September 2011 (PDT)

- Check Transformed Plates & Image under UV

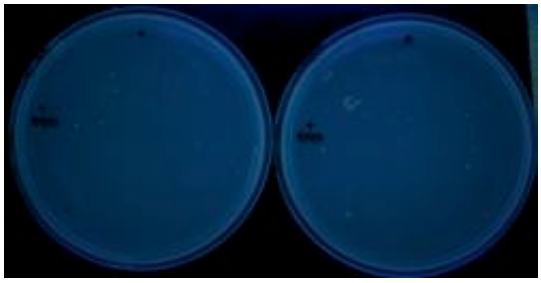
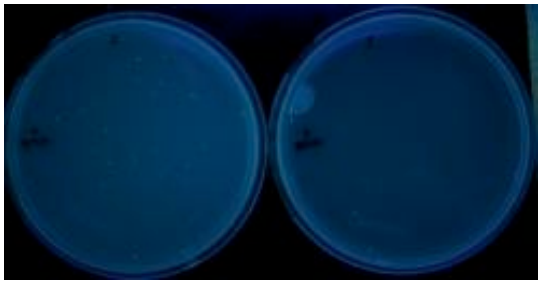
```
Positive Selection- Should be all white colonies
Negative Selection- Should be all green colonies
Non-Selection- Should be 50:50 Green:White Ratio

No growth on Negative Selection 1e-6 Dilutions
```

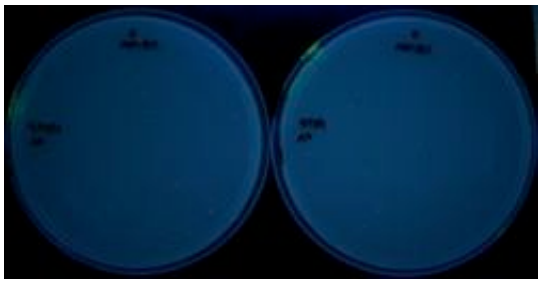
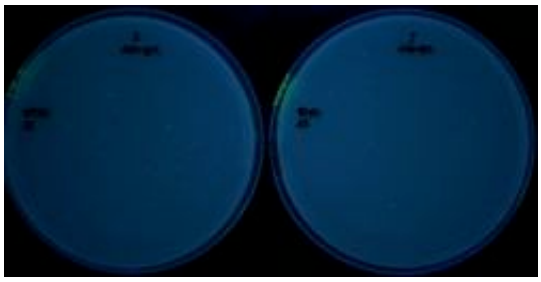
Negative Selection 1e-4 Dilution- G&H, I&J

```
Many white colonies mixed w/ green colonies- Not all killed off?
```

Positive Selection 1e-6 Dilution- G&H, I&J

```
All white colonies
```

Non-Selection 1e-6 Dilution G&H, I&J

```
Very few colonies, all white w/o green
```

- Transform pBca9525-1832 (PrffGH.toxR-mukF) & pBca1256-jtk2828 (ffGFP) into pir G-J Comp Cells w/ 2993

```
Rescue 1hr. @ 37 degree C- 2:10-3:10PM
Plate 50uL on Amp/Spec- Incubate overnight @ 37 degree C
```

- Test Non-Selection Plate

```
Plate 50uL 1:1000 Dilution Mixed Cultures on Amp/Spec
Incubate overnight @ 37 degree C
```

- To Do

```
Pick transformed 1832/GFP Colonies & Inoculate
Check Mixed Cultures Non-Selection Plate- Strain I
```

Xin Xin Lin 11:32, 17 September 2011 (PDT)

- Sequencing Analysis for xl024-xl027 (2993-9007)

```
Pctx & hsvTK parts present
Redo of xl025 w/ G00101
Short read of xl027 w/ ca998
```

- Competent Cell Preparation of pir G-J w/ 2993

```
Inoculate 5mL LB+AK w/ 200uL Saturated Overnight Culture
Shake 2hr. @ 37 degree C (2:30-4:30PM)
-Put rotor in centrifuge & cool to 4 degree C
-Label PCR Strips- Letter G-J on Cap
-Place TSS, 4 50mL Falcon Tube, & PCR strips on ice
Cool down Culture Tubes in ice to below room temp.
Spin down Falcon Tubes for 5min. @ 6500rpm- Pour out supernatant (Visible pellet)
Resuspend in 4mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex to mix
Pour into tray boat & pipet w/ multichannel
Aliquot 150uL/PCR tube on metal block in Ice- 3 PCR Strip/Strain
Label w/ Letters G-J on Cap- Store @ -80 degrees C (In SRS Box, Bottom Shelf of Freezer)
```

- Positive & Negative Selection

```
Mix GFP & 1832 Cultures 50:50- Strain G w/ G-J w/ J
-1mL GFP+1mL 1832 in 2mL Eppendorf Tube
Negative Selection: Reseed Mixed Cultures in Negative Selection Media for 3 hours- Plate 10e-4 &
-Add 0.5mL 1mM dP Stock to 5mL LB+AS
-Inoculate 200uL (1:25 Dilution) Mixed Culture & Shake 3hr. @ 37 degree C (3:00-6:00PM)
-Add 1uL Mixed Culture to 999uL LB+AS (1:1000 Dilution), Add 1uL 1:1000 Dilution to 999uL LB+AS (
-Plate 50uL 1:1000 Dilution & 50uL 1:1e6 Dilution
Positive Selection: Plate Mixed Cultures in 10e-6 dilution onto Positive Selection Plates
-Forgot to add Amp/Spec Antibiotics to Agar- Add 50uL LB+AS/Plate
-Add 1uL Mixed Culture to 999uL LB+AS (1:1000 Dilution), Add 1uL 1:1000 Dilution to 999uL LB+AS (
-Plate 50uL & Spread w/ Sterile Beads
Non-Selection: Plate 50uL Diluted Mixed Cultures 10e-6 on Amp/Spec
Incubate overnight @ 37 degree C
```

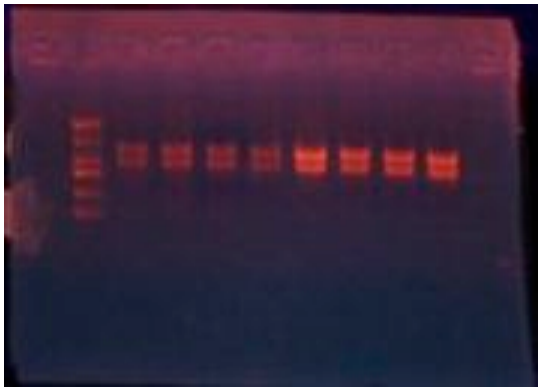
- To Do

```
Transform 1832 & GFP into 2993 Comp Cells
Check Plates
```

Xin Xin Lin 12:16, 16 September 2011 (PDT)

- EcoRI/BamHI Mapping of 2993 Minipreps from pir G-J

```
Digest 1hr. @ 37 degree C- 12:00-1:00PM
Run on Gel 8min. @ 150V- 1:13-1:21PM
Map & Sequence
```



- Inoculate pir G-J w/ 2993/GFP into 24-Well Block

```
3mL LB+AK for 2993, LB+AS for GFP
Incubate overnight @ 37 degree C
Comp Cell Prep tomorrow
```

- Inoculate Transformed pir G-J w/ 2993 & 1832

```
Even lawn of colonies on Amp/Spec Plate
Pick 2 Colonies/Plate & Inoculate 5mL LB+Amp/Spec
```

- Prepare Positive & Negative Selection Media

```
Positive Selection Plates
400mL Bottle of Positive Selection Agar- Microwave
-Pour 40mL into 50mL Falcon Tube
-Add 80uL 0.01g/mL 5FdU, 0.005g/mL Uridine, & 0.005g/mL Thymidine (dT)- Mix
-Pipet/Pour 10mL agar/Plate- Labelled w/ White Marker
Negative Selection Media
-Add 0.5uL of 1mM dP Stock in 5mL LB+Amp/Spec
Non-selective media=LB Agar+Amp/Spec
```

- To Do

```
Need to do sequencing analysis of 2 sequence results
Comp Cell Prep of pir G-J w/ 2993
```

Xin Xin Lin 18:22, 15 September 2011 (PDT)

- Miniprep Transformed pir Strains G-J w/ 2993 (Redo)

```
Miniprep
Map & Sequence next day
```

- Reseed pir Strains G-J w/ 2993 into 24-Well Block

```
1uL Saturated Overnight Culture in 3mL LB+Amp/Kan
Make Comp Cells of G-J w/ 2993 (Redo) next day
```

- Transform 9525-1832 into pir Strains G-J w/ 2993

```
Rescue 1hr., 7:08-8:08PM
Plate 50uL on Amp/Spec
```

- Reseed pir Strains G-J w/ GFP into 24-Well Block

```
1uL Saturated Overnight Culture in 3mL LB+Amp/Spec
```

- To Do

Friday

```
Map & Sequence Minipreps of G-J w/ 2993 Redo
Make Comp Cells of G-J w/ 2993 Redo
Pick colonies of G-J w/ 1832
Reseed G-J w/ GFP
```

Xin Xin Lin 18:01, 14 September 2011 (PDT)

- Pick Colony & Inoculate Overnight Cultures

```
Pick 2 colonies/plate- Grow in 5mL LB+Antibiotics
G-J w/ 2993 on AK- Lawn of colonies
G-J w/ 2993&ffGFP on AS- Lawn of green colonies
G-J w/ 2993&ffGFP on AK- Complete coverage w/ white colonies (background)
Shake overnight @ 37 degree C
```

- Sequencing

```
Submit more sample of xl020 & xl021 for G00101 Sequencing- 8uL each
```

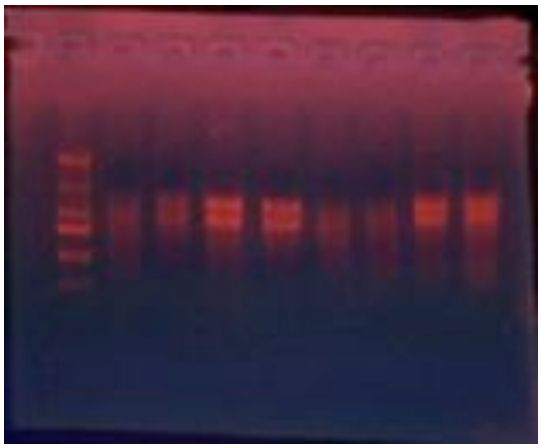
Xin Xin Lin 09:47, 13 September 2011 (PDT)

- Check Negative Control Plates

```
No growth on Cam, few background colonies on all strains on Spec
```

- Map Overnight Digest of Minipreps

```
Load on gel w/ 2uL Loading Dye
Run 10min. @ 180V- 9:45-9:55AM
Visualize under UV- Look for 1.3kb band (Part)
```



Lane 1=DNA Ladder, Lane 2-5=G1-J1, Lane 6-9=G2-J2

```
G1, G2, & H2=Very faint, H1=faint but visible, I&J have bright bands
Sequence G1-J1- 8uL xl20-xl23
```

- Competent Cell Preparation of pir Strains

```
 Inoculate 5mL LB+Kan w/ 200uL Overnight Culture, 2:22-4:22PM
 -Add 1:25 Dilution (200uL) of 5mL pir hsvTK w/ 2993 Plasmid Cultures to 5mL LB+Kan Media
Grow for 2hr. in 37 degree C Shaker (2:23-4:23PM)
-Put rotor in centrifuge & cool to 4 degree C
-Label PCR Strips- Letter G-J on Cap
-Place TSS, 4 50mL Falcon Tube, & PCR strips on ice
Cool down Culture Tubes in ice to below room temp.
Spin down Falcon Tubes for 5min. @ 6500rpm- Pour out supernatant (Visible pellet)
Resuspend in 4mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex to mix
Pour into tray boat & pipet w/ multichannel
Aliquot 150uL/PCR tube on metal block in Ice- 3 PCR Strip/Strain
Label w/ Letters G-J on Cap- Store @ -80 degrees C (In SRS Box, Bottom Shelf of Freezer)
```

```
-80 Stocks- Add 750uL 50% Glycerol to 750uL Saturated Overnight Culture
Label w/ G-J Redo+Date- Store in Personal Box on Bottom Shelf -80 Freezer
```

- Transform pBca1256-jtk2828 into pir Strains G-J w/ 2993 Plasmid

```
Rescue 3:17-4:17PM
Plate 50uL on Amp/Spec
```

- Transform 2993 Plasmid into Competent pir Strains G-J

Transform 2993 Plasmid into Competent pir Strains G-J

```
Plate w/o Rescue 50uL on Amp/Kan
```

Xin Xin Lin 16:35, 12 September 2011 (PDT)

- Chemically Competent Cell Preparation- pir hsvTK Strains G-J

```
Inoculate 120uL Saturated Overnight Culture G-J into fresh LB+Kan
Grow overnight- Start fresh culture
```

- Digest Mapping

```
4:32PM- Digest overnight
Map & Sequence next day
```

- To Do

Tuesday

```
Map Minipreps & Sequence
```

```
Make Comp Cells & Transform 2993-Pctx.rbs.hsvTK (9007)
```

```
Transform 9525-1832 & 1256-2828 into pir Strains w/ 2993-9007- Plate Spec/Amp
```

```
Check Negative Controls on Cam/Spec
```

Xin Xin Lin 12:13, 11 September 2011 (PDT)

- Sequencing Analysis

```
Short reads for ca998 xl018/19 & G00101 xl018, failed read on G00101 xl019- Redo
Pctx promoter present in all reads, hsvTK parts appear complete
```

- Miniprep, Map, & Sequence Transformed pir Strains w/ 2993 Plasmid

```
Map & Sequence later
```

- Re-inoculate pir Strains G-J for Comp Cell Prep

```
Pick 1 colony/strain from streaked plates & inoculate 3mL LB+Kan in 24 well block
Shake overnight @ 37 degree C
```

- Make Chemically Competent Cells of pir Strains w/ 2993 Plasmid & -80 Freezer Stocks

```
Add 1:25 Dilution (200uL) of 5mL pir hsvTK w/ 2993 Plasmid Cultures to 5mL LB+AK Media
Grow for 2hr. in 37 degree C Shaker (3:23-5:23PM)
-Put rotor in centrifuge & cool to 4 degree C
-Label PCR Strips- Letter G-J on Cap
-Place TSS, 4 50mL Falcon Tube, & PCR strips on ice
Cool down Culture Tubes in ice to below room temp.
Spin down Falcon Tubes for 5min. @ 6500rpm- Pour out supernatant (Visible pellet)
Resuspend in 4mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex to mix
Pour into tray boat & pipet w/ multichannel
Aliquot 150uL/PCR tube on metal block in Ice- 3 PCR Strip/Strain
Label w/ Letters G-J on Cap- Store @ -80 degrees C (In SRS Box, Bottom Shelf of Freezer)
```

```
Negative Control- Spot 10uL each strain G-J onto Cam & Spec Plates
Incubate overnight @ 37 degree C
```

```
750uL 50% Glycerol+750uL Cell Culture Strains G-J in 2mL Tube
Label w/ G-J #1/2 & Freeze @ -80 degree C freezer- Located in Personal Box, Bottom Shelf of Freez
```

Xin Xin Lin 21:21, 10 September 2011 (PDT)

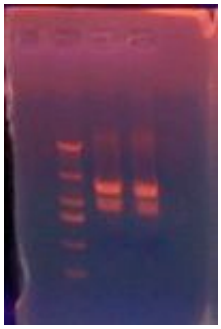
- Inoculate Overnight Culture

```
Mixed phenotypes but growth on all plates G-J
Picked 2 colonies each plate & inoculated 5mL LB+AK
Shake overnight @ 37 degree C
```

Xin Xin Lin 12:56, 9 September 2011 (PDT)

- Miniprep, Map, & Sequence Overnight Cultures

```
EcoRI/BamHI Mapping Digest 12:55-1:55PM
```



Lane 1=DNA Ladder, Lane 2= Miniprep #1, Lane 3=Miniprep #2

```
Part=1.3kb- Visible as smaller band
Both minipreps have part
Submit 8uL both for sequencing
```

- Transform G-J pir hsvTK Strains

```
No Rescue- Plate 50uL on Kan/Amp
Incubate overnight @ 37 degree C
```

Xin Xin Lin 18:14, 8 September 2011 (PDT)

- Pick Transformed Colonies

```
Some growth on Amp plate- Fewer than expected? But no rescue
Some satellite colonies- Uneven spread
Pick 2 colonies into 5mL LB+Amp- Shake overnight @ 37 degree C
```

Xin Xin Lin 11:17, 7 September 2011 (PDT)

- Check Transformed Plates Strains G-J

```
No colonies on any of the AK Plates...
-Comp Cells Issue or Ligation Issue...
Religate & Remake comp cells
```

- Redo EcoRI/BamHI Transfer

```
Digest pBjk2993 & Pctx.rbs.hsvTK EcoRI/BamHI (6:58-7:58PM)
Gel Purify (8:02-8:12PM) & Ligate (8:35-9:05PM)
Transform jtk155- Miniprep & Transform pir hsvTK Strains
No rescue- Plate 50uL on Amp (after adding 200uL 2YT)
```

Xin Xin Lin 15:55, 6 September 2011 (PDT)

- Transform 4 pir Strains G-J w/Pctx.rbs.hsvTK Ligation

```
No rescue- Plate on Kan/Amp
Incubate overnight @ 37 degree C
```

- Check Negative Control Plates

```
No growth on Cam or Amp
Some background on Spec, but only isolated colonies
```

Xin Xin Lin 11:44, 5 September 2011 (PDT)

- Make Chemically Competent pir Strain Stocks+ -80 Freezer Stocks

```
Add 1:25 Dilution (136uL) of 3mL pir hsvTK Cultures to 3.4mL LB Media in 24 well block
Grow for 2hr. in 37 degree C Shaker (1:51-3:51PM)
-Put rotor in centrifuge & cool to 4 degree C
-Label PCR Strips- Color Label=Black Stripe
-Place TSS, Falcon Tube, & PCR strips on ice
Cool down flask in ice to below room temp.
Spin down in block for 5min. @ 5700rpm- Pipet out supernatant (Very tiny pellet- Cells?)
Resuspend in 4mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex (Used only 750uL TSS)
Pour into tray boat & pipet w/ multichannel
Aliquot 100uL/PCR tube on metal block in Ice- 1 PCR Strip/Strain
Label w/ Black Colored Stripe & Letters G-J- Store @ -80 degrees C (In SRS Box, Bottom Shelf of Fr
```

```
Negative Control- Spot 10uL each strain A-J onto Amp, Cam, & Spec Plates
Incubate overnight @ 37 degree C
```

```
750uL 50% Glycerol+750uL Cell Culture Strains A-J
Multichannel 150uL/PCR Tube
Label w/ A-J & Freeze @ -80 degree C freezer- Located in Personal Box, Bottom Shelf of Freezer
```

Xin Xin Lin 13:15, 4 September 2011 (PDT)

- Pick pir Strain Colonies

```
Inoculate 24 Well Block w/ 3mL LB+Kan for 1 Colony/10 strains- A-J
Shake overnight @ 37 degree C
```

Xin Xin Lin 10:18, 3 September 2011 (PDT)

- Sequencing Analysis

```
Mixed peaks w/ ca998- Read not thorough, could not see Pctx.rbs
Good read w/ G00101- Perfect hsvTK Part 2, most of Part 1 visible, Pctx.rbs not in read
Pctx.rbs=Ambiguous

Submit Miniprep #2 for sequencing
```

- EcoRI/BamHI Transfer into pBjk2993 Vector

```
pBjk2993-Bca1144=E9 on JTK Plasmid Stock Plate #19 (AmpR)
Spin down plate & pipet out 4.25uL
```

Digest Pctx.rbs.hsvTK & pBjk2993-Bca1144 EcoRI/BamHI

```
5uL ddH2O              4uL ddH2O
1uL NEB2 Buffer        1uL NEB2 Buffer
3uL pBjk2993-Bca1144 4uL Pctx.rbs.hsvTK Miniprep
0.5uL EcoRI            0.5uL EcoRI
0.5uL BamHI            0.5uL BamHI

Digest 1hr. @ 37 degree C- 1:08-2:08PM
-pBjk2993=2065bp, Bca1144=841bp
Digest products too small to visualize on gel
```

```
Load all of digest into gel w/ 2uL Loading Dye
Run 10-15min. @ 180V (2:17-2:27/32PM)
```

Lane 1=DNA Ladder, Lane 3=Pctx.rbs.hsvTK, & Lane 5=pBjk2993-Bca1144

```
Cut small band for Pctx.rbs.hsvTK- 1.2kb
Cut large band for pBjk2993- 2065bp
```

```
Short Fragment/Regular Zymo Cleanup
Add 1 vol. (10uL) ADB & 500uL EtOH OR 30uL ADB Buffer
Spin 45sec. through Zymo column
Wash w/ 250uL PE Buffer & Spin 30sec.- x2
Dry spin 2min.
Elute w/8.5uL ddH2O
```

Ligate Pctx.rbs.hsvTK Digest into pBjk2993 Digest

```
4.25uL Pctx.rbs.hsvTK Digest
4.25uL pBjk2993 Digest
1uL T4 DNA Ligase Buffer
0.5uL T4 DNA Ligase
Incubate 1/2hr. on benchtop
```

- Streak Out 4 Transduced pir Strains

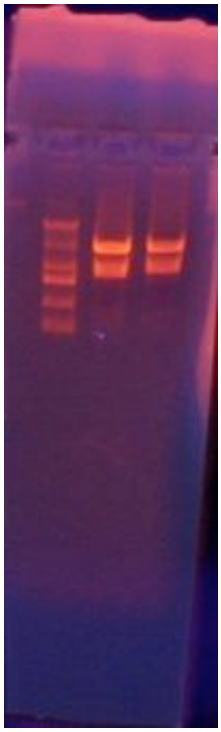
```
-80 Stocks on top of middle pile in bottom shelf of freezer
-Labelled A-J- Correspond to 4,6,8,13,22,23,24
```

Xin Xin Lin 14:46, 2 September 2011 (PDT)

- EcoRI/BamHI Mapping of Pctx.rbs.hsvTK Minipreps

```
5uL ddH2O
1uL NEB2 Buffer
3uL Pctx.rbs.hsvTK #1 & #2 Miniprep
0.5uL EcoRI
0.5uL BamHI
Incubate 1hr. @ 37 degree C- 2:44-3:44PM

Load all of digest w/ 2uL Loading Dye
Run 10min. @ 180V & visualize under UV (3:45-3:55PM)
```



Lane 1=DNA Ladder, Lane 2=Miniprep #1, & Lane 3=Miniprep #2

```
Want ~1.2kb band- Band b/w 1kb & 1.5kb
Submit 8uL Miniprep #1 for Sequencing
```

- Weekly Presentation Meeting

```
Team Name, Logo, Website
Project Track Selection, Abstract
Clotho Bugs- GoogleDoc
Team Uniform- Sherlock Holmes
-Need Prices ($15-30), $7 each for sleeve printing, Sponsor logos on back
-Design do next Wed.
Safety Videos for websites
Content Description for 4 Blocks
-ToxR System, Toxicity & Stress Promoters, ToxR Chimeras, Applications- Biosensors (Estrogen, Ant
-Project Page=Same 4 Images-> Links to subpages
-Start work on Project Page
Websites
-Incorporate Picture Slideshows, About Page
-Team Description of Contributions
Story Writing- Solving Interesting Problems
-Look at previous teams w/ ToxR & Stress Promoter Projects
-Tsinghua 2010 & MIT 2005
9/16- Practice Presentation, 9/28 4PM- BioE 24 Guest Presentation
```

Xin Xin Lin 19:23, 1 September 2011 (PDT)

- Selection Experiment Setup

```
96 Well Block+Overnight Tecan Setup
1mL LB+CAS in all wells of 96-well block- Block divided in half vertically
-Add 1uL Chemical/Column
-Add 2uL Saturated Culture- Experimental Cells 1-4 & Positive/Negative Controls
Transfer 200uL from block to Tecan Plate
-Run overnight 20hr. Tecan measurement
```

```
                  No Chemical  Chemical 1      2       3       4       5       6       7
Experimental 1
2
3
4
Positive Control
Negative Control
```

```
                Chemical 8    9       10      11      12      13      14      15
Experimental 1
2
3
4
Positive Control
Negative Control
```

```
Chemical Key
1- Acetaminophen      8- Nicotine
2- Vanillin           9- 2-Phenethylamine
3- Caffeine           10- Resorcinol
4- Camphor            11- Histamine
5- 1-Butanol          12- Urea
6- Terephthalic Acid  13- Boric Acid
7- Dopamine           14- Dichloropropylketone
```

15- Hexachlorobenzene

- Miniprep Pctx.rbs.hsvTK Overnight Cultures

```
Eluted in 50uL ddH2O- To sequence & map later
```

Xin Xin Lin 11:32, 31 August 2011 (PDT)

- Pick Colony & Inoculate Overnight Culture

```
Plates grew up overnight- Many single colonies
Picked 2 colonies into 5mL LB+Spec
Shake overnight @ 37 degree C
```

Xin Xin Lin 14:56, 30 August 2011 (PDT)

- Assembly of Pctx.rbs.hsvTK

```
Digest & Assemble pBjh1600-Pctx w/ pBjh1600-rbs.hsvTK
```

BglII/XhoI & BamHI/XhoI Digests

```
4uL ddH2O            4uL ddH2O
1uL NEB2 Buffer      1uL NEB3 Buffer
4uL Pctx Miniprep    4uL rbs.hsvTK Miniprep
0.5uL BamHI          0.5uL BglII
0.5uL XhoI           0.5uL XhoI
```

Digest 1hr. @ 37 degree C- 2:42-3:42PM

Used wrong Restriction Enzyme- XhoI gives same sized bands from digest

```
Redo digestions w/ AvrII instead of XhoI
Digest 1.5hr. @ 37 degree C (3:57-5:37PM)
```

Lane 1=DNA Ladder, Lane 3=Pctx, & Lane 5=hsvTK

Gel Purification of Pctx & rbs.hsvTK

```
Load 2uL Dye w/ 10uL Digest into gel
Run 8min. @ 180V
Cut out bands under UV- rbs.hsvTK=75% Large band, Pctx=Small band
Add 700uL ADB Buffer- Heat 5min. @ 55 degree C
Spin 45sec. in Zymo Column
Wash 2x w/ 200uL PE Buffer- Spin 30sec.
Dry spin 2min.
Elute in 8.5uL ddH2O
```

Small Frag Zymo Cleanup of Pctx

```
Add 1 vol.(10uL) ADB Buffer & 500uL EtOH OR 30uL ADB Buffer
Spin 45sec. through Zymo column
Wash w/ 250uL PE Buffer & Spin 30sec.- x2
Dry spin 2min.
Elute w/8.5uL ddH2O
```

Ligation of Pctx & rbs.hsvTK

```
4.25uL Pctx Digest
4.25uL rbs.hsvTK Digest
1uL T4 DNA Ligase Buffer
1uL T4 DNA Ligase
```

Ligate 1/2hr. @ room temp. on benchtop

Transform Assembly into jtk155

```
Add 30uL KCM to -80 Chem. Comp. Cells- Incubate on Ice 1min.
Add 50uL Cell Cocktail to 1uL Ligation DNA
Incubate on ice 10min.
Heat shock 42 degree C for 90sec.
Incubate on ice 1min.
Rescue w/ 200uL 2YT Media- Shake @ 37 degree C for 1hr.
Plate on Spec (pBjh1600) & Incubate overnight @ 37 degree C
```

Xin Xin Lin 15:05, 25 August 2011 (PDT)

Thursday, 2-5PM

- Task List

```
Chemical Stocks
Pour CAS Plates
Enter parts into plate
Rewrite stress promoter abstract
Look up other stress promoter projects
```

Xin Xin Lin 11:09, 24 August 2011 (PDT)

Wednesday, 10AM-4PM

- Task List

```
Autoclave 6 bottles LB Agar Liquid Cycle & Bottles Dry Cycle
Dishes
Clean Bench- Bleach old blocks, toss Tecan plates, store poured plates
1 Paragraph Abstract for Pstress
Cytometry Images using Cyflogic
Research other Stress Promoter Projects- Deliverables
Add QCed parts to Plate
Organize Selection Chemicals
```

- Stress Promoter Abstract

Stress promoters function by regulating expression in response to different types of stress. Based on the microarray results of this paper, the 35 stress promoters of interest were synthesized and coupled to an ffGFP reporter gene. When tested under the different stress conditions of high and low temperatures, high and low pH, and high osmolarity; differential expression could be measured. From the flow cytometry data, it was determined that five of the stress promoters downregulated expression of ffGFP under the cold condition.

Due to the lethal toxicity of toxR on E. coli cells, a stress promoter that downregulated toxR gene expression when the cell was under stress was needed. Stress promoters regulate expression of downstream genes in response to different stresses. Based on the microarray results of the [ ] paper , 35 stress promoters of interest were synthesized, coupled to an ffGFP reporter gene, and tested for downregulation of ffGFP when under stress. From experimental data, four of the stress promoters were shown to have downregulated expression of ffGFP under the cold condition. The stress promoter rffGH, when coupled with toxR, was shown to reduce toxR toxicity and allow cells containing the toxR plasmid to grow.

- Task List

```
Writing abstract/summary of stress promoters
Looking up previous stress promoter projects & determining deliverables
Working on PowerPoint w/ Nikit & Jason
Providing feedback on website versions daily to Jessica
```

Xin Xin Lin 12:50, 18 August 2011 (PDT)

10:30AM-1:30PM

- Pour TetA Selection Plates

```
Microwave 5 bottles LB Agar
Add Amp/Spec to all & 0ug/mL, 10ug/mL, 20ug/mL, 30ug/mL, & 40ug/mL TetA Stock
-400uL, 800uL, 1200uL, & 1600uL
```

Xin Xin Lin 10:24, 11 August 2011 (PDT)

- Obtain Digital Cytometry Data from Cytometry Lab

```
Save Cytometry Data as LMD files from 420 LSA
Schedule 2.5hr. cytometry appointment for next Wed. on book- 10:30AM-1PM
-Training full, only available Monday afternoons
```

- Cytometry Data Analysis

```
Open data to analyze/view images
Download FlowJo from Network Z drive for analysis of cytometry data onto flash drive
-Replace Jar File in Jars folder
-Unable to open LMD files- Requre FCS2.0 or 3.0 format
-Use 30 day trial later on in future
```

Download Cyflogic- Use Dot Plot & Histogram (Density/Contour Plots also available)

```
Tutorial on Basic Usage- http://www.cyflogic.com/files/basic_usage.pdf
Can add quadrant, adjust metrics of graph, distinguish populations, & produce statistics
```

- Lunch & Photos

- To Do

Friday

```
Dishes & Autoclave
Make 6 bottles LB Agar, Positive Selection Plates, & Negative Selection Media
```

Monday

```
Microwave agar & pour plates
```

Tuesday

```
Test selection
```

Xin Xin Lin 10:32, 10 August 2011 (PDT)

- Check Overnight Stress Conditions

```
All cultures grew up over 24hr., 1 ffGFP control in NaOH did not grow to saturation
Tecan 100uL of all samples & analyze data- Cold generates reliable differential expression levels
```

- Serial Dilutions of Saturated Cultures

```
100uL Saturated Culture in 900uL Media for 1:10 Dilution
10uL Saturated Culture in 990uL Media for 1:100 Dilution
Remaining 790uL Saturated Culture for Undiluted
Seal w/ Plastic Cover
```

- Flow Cytometry- Fluorescence-Activated Cell Sorting

```
420 LSA- Cytomics FC5000
-Used 1:100 Dilutions of 10uL Saturated Culture in 1mL 1X PBS
-Bring Negative Control, Gloves, Lab Marker, Pipettes/Multichannel, & Tips
Need Negative Control- White Cells w/o ffGFP Plasmid
Negative Control- Used rffGH in Ctrl & Hot Condition
Positive Control- Used pBgl0002-ffGFP Ctrl 2 in Ctrl Condition
Measure 30,000 Elements- 42 Samples of Pstress in Stress Conditions
Bimodal Distribution of fluorescing & non-fluorescing
Cold Condition results in less fluorescence- Downregulation

Data Analysis w/ Spreadsheet
Record X-Mean All- Average Fluorescence of both bimodal populations
Find B:C Ratio- B=Non-Fluorescent, C=Fluorescent
Find Experiment B:C Ratio:Control B:C Ratio
-If Expt/Ctrl=1       Both change same amount from stress- No significant response to stress
-If Expt/Ctrl>1       B:C of Expt>B:C of Ctrl, Ctrl fluoresced more than Expt- Expt downregulated
-If Expt/Ctrl<1       B:C of Expt<B:C of Ctrl, Ctrl fluoresced less than Expt- Expt upregulated
Compare w/ Tecan Data- Data comparable, cold elicits most response out of stress promoters
```

- Weekly Meeting

- Weekly Meeting

Xin Xin Lin 10:35, 9 August 2011 (PDT)

- Check Overnight Cultures & Inoculate Stress Conditions

```
All cultures in 24 well block grew up- 5 Pstress+2 Controls
Inoculate 3 96 well blocks w/ 1 replicate
-1 Column for Hot & Cold, 4x7 Rows/Columns for Control/HCl/NaOH/NaCl
Shake overnight (~24hr.) @ 37, 25, or 42 degree incubator
```

- Measure OD of Saturated Overnight Culture

```
Saturated Culture (Pstress B2)=1.520OD ~1E9 Cells/mL, Pcon Control=1.471OD
-1:100 Dilution~0.040OD (10uL Saturated Culture in 990uL LB Media), after 1/2hr.~0.030OD (Cells S
Dilute 10x & 100x- Measure OD (~0.0152OD) w/Spectrophotometer
```

```
Blank 2x w/ LB Media, Measure B2 Saturated, Measure Blank, Blank, Measure B2 1:10, Measure Blank,
-Repeat for Pcon Control

OD    Saturated     1:10     1:100
B2    1.510         0.247    0.025
Pcon  1.480         0.265    0.020
```

```
Tecan Data- Blank, B2&Pcon Saturated, 1:10, & 1:100
Compare data- Dilutions not exact
OD values much lower, from cells settling?- Saturated Culture OD very low

Blank   Saturated  1:10     1:100
0.0623  0.4758     0.1150   0.0544       B2 Pstress
        0.5107     0.1149   0.0628       Pcon Control
```

```
Redilute & Remeasure

Blank    Saturated   1:10      1:100
0.0495   0.4611      0.0908    0.0499   B2 Pstress
         0.4779      0.1051    0.0622   Pcon Control
```

- Set Up 2 96 Well Blocks for Serial Dilution- Cytometry Preparation

```
Block 1- Saturated Ctrl/1:10/1:100, HCl/1:10/1:100, NaOH/1:10/1:100, & NaCl/1:10/1:100
Block 2- Saturated Hot/1:10/1:100 & Saturated Cold/1:10/1:100
-No Media for Saturated, 900uL Media for 1:10, & 990uL Media for 1:100
```

- Re-Tecan Overnight Hot Conditions Pstress

```
All grew to saturation but OD still low
Mixed information regarding expression levels of ffGFP
```

- Find & Report Bugs for Clotho

```
Download Java 6 SE & NetBeans v. 6.9
Download iGEM-Clotho / ClothoBiofabEdition as Zip File & Extract to Downloads
Open NetBeans- Open Project- jenhantao-iGEM-Clotho
Right Click- Build/Clean & Build
Build Failed...- Download Java 6 SE instead of Java 7 SE
```

- Task List

```
Autoclave Dry Cycle- 60min. Sterilize & 30min. Dry
```

- To Do

Wednesday

```
Set up 1:10 & 1:100 dilutions of all overnight stress cultures
Flow Cytometry @ 420 LSA- 11AM
```

Xin Xin Lin 10:36, 8 August 2011 (PDT)

- Check Overnight Stress Conditions

```
All cultures grew up after 12hr. shaking, Hot condition appears less saturated than others
Return all 3 blocks to shakers & incubate for additional growth
Tecan 100uL & analyze results
```

- Request EH&S Pickup for Diammonium Phosphate Extinguisher Waste

```
4cu.ft. in 5cu.ft. plastic bag
Print Waste Label
```

- Cytometry Experiment

```
Cytometer Reserved for 11AM-3PM tomorrow- ~10 Samples
-3 Replicates/Condition*5 Conditions*(5 Samples+2 Controls)=105 Samples?
```

```
Reseed 96 well block of LB+KA or Trim from -80 Stock Plate (Pstress-ffGFP)
Shake overnight @ 37 degree C- Inoculate all Pstress into stress conditions Tues.
-36 Pstress Promoters*6 Conditions=216 Samples
FACS (Fluorescence-Activated Cell Sorting) via Flow Cytometer Wed.- Dilute to 1E7 Cells/mL
```

```
Reseed 5 Pstress Promoters & 2 Positive Controls into 24 Well Block- 7 Wells
-3mL LB+KA or LB+Trim, Seed from -80 Stock Plate/Colony from Plate
Shake overnight @ 37 degree C
Cytometry Experiment on 35 samples (7 promoters*5 conditions)
```

- Task List

```
Dishes & Autoclaving
Make & Autoclave Liquid Cycle SOB Media & 10% Glycerol
-25.5g SOB/1L House dI H2O+MgSO4
```

- To Do

Tuesday

```
Inoculate into stress conditions-NaCl early in morning- Allow for 24hr. growth
Measure OD of saturated culture (~1E9/mL)- Dilute 1:100 (~1E7/mL) & Measure OD
Make dilutions of all stress conditions to 1E7- Make 1:10 & 1:100 dilutions
Dishes & Autoclave Dry Cycle (1L Bottles & Blocks)
```

Xin Xin Lin 22:48, 7 August 2011 (PDT)

Sunday, 9:45PM-10:45PM

- Inoculate Overnight Promoters of Interest into Stress Conditions

```
96-Well Block w/ 2 Controls, 3 HCl, 3 NaOH, & 3 NaCl/Pstress; 3 Hot; 3 Cold
-5 Stress Promoters+Pcon (LB+KA) & pBgl0002-ffGFP (LB+Trim) Positive Controls
-Row A=B2, Row B=B4, Row C=C1, Row D=C4, Row E=H1, Row F=Pcon, & Row G=ffGFP
Shake overnight @ 37, 42, & 25 degree C respectively
```

Xin Xin Lin 12:09, 5 August 2011 (PDT)

- Tecan Overnight Stress Conditions

```
All cultures grew up overnight- Controls & Stress Conditions
Tecan 150uL Culture- Control/NaCl, Hot/Cold, & HCl/NaOH
Controls grew up, pBgl0002-ffGFP visibly green & brighter than Pcon.ffGFP Positive Control
-Positive controls brightest in control condition, less bright in stress conditions
-Little growth in hot condition, more growth in cold
```

- Grow up Stress Promoters of Interest

```
Repeat stress conditions w/ more replicates focusing on responsive promoters
Pick from spots on plates & inoculate 3mL LB+KA in 24 Well Block w/ Pcon & pBgl0002-ffGFP Control
fadL, cstC, fadD, cysA, & ffrgh- C1, H1, B2, C4, & B4
```

- Task List

```
Dishes & Autoclave Dry Cycle- Autoclave washed dishes
Make Antibiotic Stocks- Amp, Spec, Kan, & Cam
-Chloramphenicol: 25 mg/mL single copy in H2O- 1.25g in 50mL
-Kanamycin: 25 mg/mL single copy in H2O- 1.25g in 50mL
-Ampicillin: 100 mg/mL- 5g in 50mL
Do not flame sterilize antibiotic stocks- Ethanol as solvent
```

- Lab Cleanup

```
Double bag fire extinguisher waste- Wipe w/ wet paper towels (Wear gloves, goggles, & lab coats)
```

Xin Xin Lin 14:04, 4 August 2011 (PDT)

- Tecan Overnight Stress Conditions

```
All overnight cultures grew up- Transfer to Tecan plates & measure OD/RFU
Combine 5hr. & Overnight Stress into Spreadsheet- Analyze Data w/ Heat Map
Pcon Positive Control=Unreliable- Does not glow under control conditions or stress, glows brightl
-Pcon=Constitutive, should be on in all conditions & control
```

```
Took out spotted plates- Dense spots for all Pstress promoters
-All white, Pcon=green- Parafilm & store in fridge as Pstress stocks
```

```
Spin down Seed Source Overnight Culture & Control- Compare pellet size & fluorescence
Some contamination in Pstress-ffGFP- Pellet=Mixed white & green cells
Reseed stress conditions w/ Pstress-GFP Plate on Kan
-Pick spot w/ P20 Multichannel Pipette Tip & inoculate 96 well block
-Fill Row 5 w/ Green Pcon control, 3 wells Row 6 w/ MC1061 pBgl0002-ffGFP Positive Control (LB+Tr
```

- EH&S Hazardous Waste Disposal Training

```
Take Quiz
Make Templates & Labels for Chemical Waste Bottles- Print Label
-Volume & Chemical Name- List all chemicals for label on bottle (4L Glass Bottle)
```

- Filming for BBC Report on Synthetic Biology
- Task List

```
Dishwashing & Autoclaving Dry Cycle
```

Xin Xin Lin 12:26, 3 August 2011 (PDT)

- Check Overnight Reseeded Cultures

```
All Plain LB & LB+AK Cultures grew up from inoculated -80 stocks
All 3 Stocks=Same-Use Pstress-ffGFP
Inoculate into LB+AK @ 1mL Control, Hot, Cold, 900uL+100uL HCl, NaOH, & NaCl
Shake for 5hr.- Remove 150uL onto Tecan Plate & measure data (12:30PM-5:30PM)
Shake overnight @ 37 degree C to Tecan next day
```

- Task List

```
Autoclave Dry Cycle
Dishes & Autoclave Dry Cycle
Make SOB Media & 10% Glycerol in Autoclaved Bottles
Autoclave Liquid Cycle
```

- Ligation of Pctx.hsvTK rbs Library Digest

```
8.5uL Gel Purified Digest
1uL T4 DNA Ligase Buffer
0.5uL T4 DNA Ligase

Incubate on bench for 30min. (3:15-3:45PM)
```

Zymo Cleanup of Library Material

- Weekly Meeting

```
Team Name/Logo/Costume & Story for Project
Find 3 bugs/comments for BU Clotho Interface
```

Xin Xin Lin 12:53, 2 August 2011 (PDT)

- Check Overnight Cultures

```
Did not grow up- All three seed blocks had no cells in media (Clear)
-Basic Parts=KC (Pstress only), Composite Parts=KA (Pstress-ffGFP)
Spot all 3 stock plates onto LB Agar Plates w/ Pin Tool (BioE140L: Pstress-ffGFP, Fall '10, & Spr
-1mL Plain LB Media in 96-Well Block
-Plain LB Agar Plate
-KC LB Agar Plate
-Cam LB Agar Plate
-Kan LB Agar Plate
Want green in plain LB, white under stress
```

```
Spot on Plain LB Agar Plate, Kan Agar Plate, Plain LB Media, & LB+AK Media
-Let plates dry open under flame & incubate @ 37 degree C overnight
Shake overnight @ 37 degree C
```

- Task List

```
Dishwashing in 4th Floor Equipment Room- ~1hr. (11AM-12PM)
Make 8 bottles LB Media- Autoclave
Autoclave 4 Bottles LB Agar- CS, KS, CK, & Plain (No Carb yet)
Autoclave 8 bottles LB Media
Pour large plates- Bag, tape, label, & store plates in Deli Fridge
```

- To Do:

Come in @ 9AM Wednesday?

```
Dishes & Autoclaving (Liquid/Dry Cycle)- Make LB Agar, 2YT, & LB Media
Make 10% Glycerol
Inoculate under stress conditions w/ Amp/Kan
```

Xin Xin Lin 15:28, 1 August 2011 (PDT)

- Check Overnight Test Cultures

```
No growth from -80 stocks in 5mL LB+KC Media...- Completely clear tubes
-Wrong antibiotic? Or bleach in all containers?
```

- Add Solvent to Chemical Stocks

```
Melamine does not dissolve in DMSO...
```

- Reseed Pstress-ffGFP Cultures from -80 Stocks

```
Load 1mL LB+KC/Well in 3 96-well blocks
-Fall '10
-Spring '11
-No Date
Shake overnight @ 37 degree C
```

- Lab Tasks

```
Clean & Organize Fridge
-Throw out old plates, arrange chemical stocks, parafilm stock plates
Make LB Agar, LB, & 2YT Media
-7 LB Agar (40g/L), 4 2YT (31g/L), & 3 LB (25g/L)
-Autoclave media
Buy Paper Towels from Floor 1 Stock Room
Make 1000X Carb Stocks- 250mg/mL H2O
Pour large agar plates- Spec, Kan/Spec, Cam/Spec, & Carb/Spec (MSD)
```

- Recipe for SOB Media

```
http://www.thelabrat.com/protocols/SOB.shtml
     Measure ~900ml of distilled H2O
     Add 20g Bacto Tryptone
     Add 5g Bacto Yeast Extract
     Add 2ml of 5M NaCl
     Add 2.5ml of 1M KCl
     Add 10ml of 1M MgCl2
     Add 10ml of 1M MgSO4
     Adjust to 1L with distilled H2O
     Sterilize by autoclaving
```

- To Do

Tuesday

```
Dishwashing+Autoclaving 5 Bottles LB Agar- CS, KS, AS (Carb), & CK
Pour plates
Inoculate -80 stock into plain LB in 96-well block
Grow up & spot w/ pin tool on CK plate
Pick colony & grow in LB+CK in block- Use as seed
```