XinXin Lin start 7/01/11

From AndersonLab wiki

Contents

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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
```

- EH&S Hazardous Waste Disposal Training

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

- Task List

```
Dishwashing
```

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

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 11:23, 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
```

Xin Xin Lin 12:27, 31 July 2011 (PDT)

Sunday, 11:30AM-12:30PM

- Check Reseeded Pstress Overnight Culture

```
Still no growth- Residual bleach in blocks killing cells?
Inoculate 5mL LB+Kan/Cam in 4 Test Tubes w/ random stress promoters from -80 stock
-#1=H1, #2=H2, #3=H3, & #4=H4
Shake overnight @ 37 degree C
```

- Tecan Lethality Test 2

```
Test conditions w/ chemical mixtures similar to control, but lower OD & higher fluorescence
```

Xin Xin Lin 00:53, 31 July 2011 (PDT)

Saturday, 12:00AM-1:00AM

- Checked Reseeded Pstress Promoters from -80 Stock

```
No growth at all- LB+KC Media still clear!(Wrong media unlikely...)
Made new 50mL LB+Kan/Cam- Inoculated w/ 10uL Old Seed Source into 1mL LB+KC in 96 Well Block
-Remaining wells on previous overnight culture
Shake overnight @ 37 degree C
```

- Checked Lethality Assays -Chemicals

```
All cultures grew up, Control=Green MC1061 w/0002-GFP
Load onto original Lethality Assay Tecan Plate- E1-4
-Control, 100X Mixture, 100X Mixture-Tryptamine/Chloroquine, & 100X Mixture-Try/Chl-Progesterone
Tecan under use?- Save for tomorrow/check Google Calendar...
```

- To Do

Sunday

```
Dishwashing, Autoclaving, & Pouring Plates
Tecan Lethality Assay
Test Stress Conditions
```

Xin Xin Lin 11:57, 29 July 2011 (PDT)

- Tecan Overnight Blocks w/ Stress Conditions

```
Transfer to Tecan Plates & run program
Compare & make heat map
```

```
Cultures did not grow up- Media still clear
-Hot condition had large pellets of material at the bottom of the wells- =Dead Cells
```

```
Reseed LB+Kan/Cam 96 well block from -80 stock plate
Shake overnight @ 37 degree C- Restart Stress Conditions next day
```

- Tecan Chemical Mixture Lethality Assay

```
Transfer to Tecan plate & run program- Single Reading GAL
Heat Map results- 100X mixture=toxic, 1000X mixture not very toxic, Tryptamine/Chloroquine more t
```

```
Acetaminophen | Tryptamine | Dopamine | 1-Butanol | Caffeine | Progesterone | Melatonin | Vanilli
2-Phenethylamine | Fluorescein | Resorcinol | Urea | Boric Acid | Chloroquine | Dichloropropyl Ke
100X Mixture | 1000X Mixture | 1000X 5 Chemicals | 1000X 5 | 1000X 5 | 1000X 5 | 1000X 3 Chemical
1000X 3 Chemicals | 1000X 10 Chemicals | 1000X 10 Chemicals
```

```
Chemicals grouped together in order
```

- Redo Lethality Assay

```
Control, 100X Mixture, 100X Mixture-Tryptamine/Chloroquine, & 100X Mixture-Tryptamine, Chloroquin
Tryptamine darkens LB, Progesterone causes cloudiness, Permethrin creates slight cloudiness/preci
Picked 1 colony into 10uL LB+Trim- Mixed, vortexed, & incubated @ 37 degree C
1mL total in Test Tube- LB+Trim, 10uL Chemical Stock+1uL MC1061 ffGFP
Shake overnight @ 37 degree C
```

- Dissolve 50mL Chemical Stocks in H2O/DMSO Solvent
- 1000X Trim Antibiotic Stocks

```
Trimethoprim: 10 mg/mL (in DMSO) for 1000X- Weigh out 500mg & dissolve in 50mL DMSO
Make 50mL & aliquot 1mL into 50 Eppendorf Tubes- Label w/ Purple Stripe
```

- To Do

```
Dissolve Chemical Stocks, esp. Tyramine, Melamine, + Hexachlorobenzene
Make 10/15mL Stock Solutions?
Test Stress Conditions- Inoculate overnight culture into pH, temperature, & osmolarity stress
Tecan Lethality Assay on same Tecan plate
```

Xin Xin Lin 12:18, 28 July 2011 (PDT)

- Tecan Overnight Stress Condition Results

```
Load 100uL onto Tecan Plate via Multichannel- Future Reference: Combine all measurements to fewer
Label Lids, avoid bubbles
Load onto Tecan- XFluor4Safire, Connect, XFluor4Safire, Multilabelling, GAL, Edit Measurement Par
-Measure OD600 & GFP481 Fluorescence
```

```
Control- No growth on 2 columns, did not inoculate completely...
-OD values comparable to original but slightly higher, fluorescence values different from origina
Original- Use original seed source
-Saturation~0.45OD, Wide range for fluorescence (Very high values)
Low Temp. @ 25 degree C
-Much higher saturation OD ~0.6, variable fluorescence values
High Temp. @ 42 degree C
-Lower OD ~0.3, lower fluorescence values overall
Low pH
-Saturation OD higher than original ~0.5, Variability in fluorescence
High pH- Little growth, pH too basic or seeded incorrectly?
-Very little growth, slightly higher than empty wells but may be due to media, low OD/fluorescenc
High Osmolarity
-Approach saturation @ ~0.43OD, GFP values lower & higher
```

- Redo Stress Condition Experiments

```
Shake pin tool thoroughly, combine 2 conditions per 96 well block
Use 0.01M NaOH stock instead of 0.1M
Reseed from original stock- Not ideal but only available
Control+NaCl, HCl+NaOH, Heat, & Cold
```

- Making Chemical Stock Solutions

```
Make 50mL 100mM Stocks of all Chemicals (23)
Hexachlorobenzene did not dissolve in 1mL or 10mL DMSO- Did not use
DDT & Chlordane=5000ug/mL, Only 1mL->Transfer to Glass Vial (Assume=100X)
```

- Lethality Assay- 1/100 Dilutions & Chemical Mixtures

```
Load 1mL LB+Trim into 96 well block-Add 10uL all 100mM Chemical Stock Solution
-100X Toxicity Test=23 Chemicals+1 Control- 24 Wells
-1000X Mixture all Chemicals (10uL each Chemical=230uL+770uL LB+Trim)- 1 Well
-10,000X Mixture all chemicals (1uL each Chemical=23uL+977uL LB+Trim)- 1 Well
-1000X Mixture of 5 Chemicals- 5 Wells (5,5,5,5,&2)
-1000X Mixture of 3 Chemicals- 8 Wells (3,3,3,3,3,3,3,&2)
-1000X Mixture of 10 Chemicals- 2 Wells (10&12)
Inoculate w/ 1uL MC1061+ffGFP (Trim)
-Picked 1 green colony from plate & inoculated 1mL LB+Trim, vortex & incubate 1-2hr. @ 37 degree
Shake @ 37 degree C overnight
```

```
Observations- Melatonin/Progesterone leads to cloudiness
```

Xin Xin Lin 10:50, 27 July 2011 (PDT)

- Check JW1226 Test Plate

```
Streaked on Spec- Colonies grew up->Spec Resistance
Retreak on Trim & Amp to determine other antibiotic resistances
```

- pH & Osmolarity Standard Curve Results

```
Image 96 well blocks under UV to detect green fluorescence
```

Left=Bottom Lighting, Right=Overhead Lighting

```
Rows 1&2=HCl (1:10 Dilution Series from 0.1M), Rows 4&5=NaOH (1:10 Dilution Series from 0.1M), & 
No growth @ 2 highest HCl & highest NaOH Concentration, Growth @ all Osmolarity
```

Tecan Data

```
Higher numbers on bottom row due to bubbles
```

<> 1 2 3 4 5 6 7 8 9 10 11 12

A 0.4536 0.4304 0.4047 0.4075 0.4757 0.4130 0.4130 0.4175 0.4069 0.4196 0.4194 0.4157

B 0.3994 0.3522 0.3907 0.3913 0.3862 0.3876 0.3862 0.3927 0.4631 0.4013 0.4179 0.4098

C 0.0325 0.0331 0.0332 0.0334 0.0335 0.0333 0.0330 0.0338 0.0340 0.0334 0.0335 0.0327

D 0.0843 0.2902 0.3602 0.3894 0.3879 0.3911 0.4173 0.4011 0.4014 0.3813 0.3960 0.4172

D 0.0345 0.2962 0.3002 0.3694 0.3879 0.3911 0.4175 0.4011 0.4014 0.3815 0.3960 0.4172

E 0.0798 0.3736 0.4094 0.4314 0.5320 0.4458 0.4342 0.4278 0.4384 0.4540 0.4441 0.4234

F 0.0380 0.0386 0.0388 0.0387 0.0393 0.0387 0.0392 0.0396 0.0404 0.0394 0.0393 0.0378

G 0.0507 0.1029 0.3835 0.3752 0.3840 0.4028 0.3532 0.3843 0.3860 0.3983 0.3913 0.6918

H 0.0761 0.3334 0.4304 0.3849 0.3212 1.2187 1.1297 0.3973 0.3265 0.5067 0.9180 0.6746

```
All NaCl Cultures (A&B) grew to saturation, Low OD for 1st NaOH concentration & 1st 2 HCl concent
Forgot controls...- Last Rows should not have HCl/NaOH/NaCl added
```

- Reseed Overnight Pstress Culture into Different Conditions

```
High & Low Temperature- 42 degree C & 25 degree C, High & Low pH Titration Curve, & High Osmolari
Load A5-C5 & D4-H4 w/ 1mL LB+Trim- 35 Stress Promoters, Use pin tool & inoculate (7  Concentratio
```

```
Start w/ 0.1M for NaCl, 0.01M for NaOH, & 0.001M for HCl, 1 Control
1 Control Block @ 37 degree C
1 Block @ 42 degree C
1 Block @ 25 degree C
1 Block @ Low pH, High pH, & High Salt @ 37 degree C (6 Blocks Total)
-0.001M HCl, 0.01M NaOH, & 0.1M NaCl
```

- Redo Expand PCR of bth8189 w/ & w/o DMSO

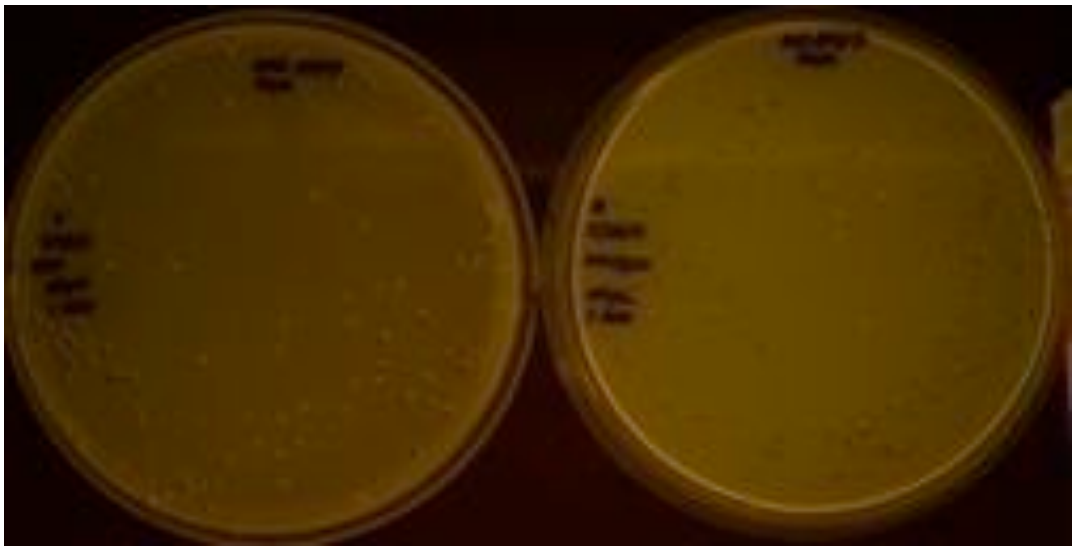
```
Pstress project on hold for now
```

- Weekly Meeting

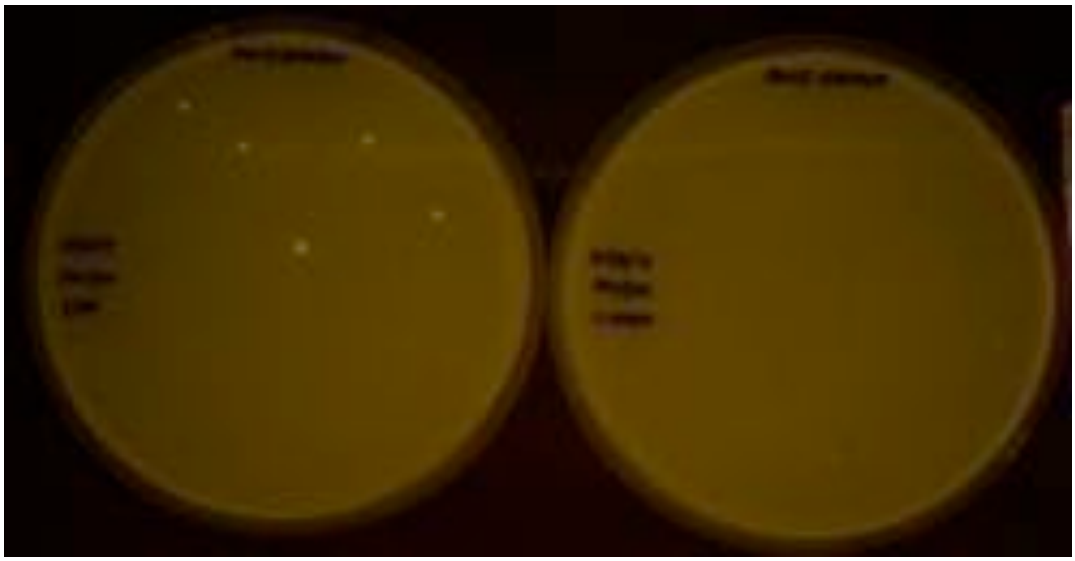
Xin Xin Lin 10:58, 26 July 2011 (PDT)

- Check All Plates

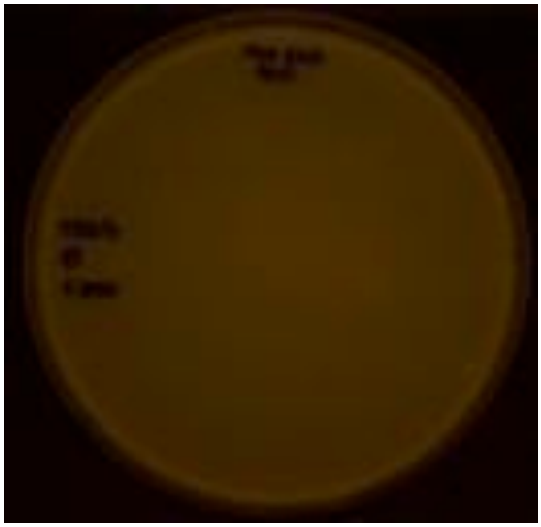
```
Pcon/0002 ffGFP 1:10000 Overnight Culture on Kan- Medium-sized Colonies, Green:White=0:139
Pcon/1600 ffGFP 1:10000 Overnight Culture on Kan/Spec- Small Colonies, Green:White=51:105
-1:100 Dilutions- Lawn of Colonies on both plates, Mix of Green&White on 1600, All White on 0002
-50uL Undiluted Pcon Culture on Kan- Complete lawn all over plate, All White

Pcon/ffGFP 1600 1:10000 Negative Selection Survivors on Kan/Spec- No Colonies
Pcon/ffGFP 1600 1:100 Negative Selection Survivors on Kan/Spec- 5 Green Colonies
Pcon/ffGFP 1600 1:10000 Positive Selection on Positive Selection Plate- No Colonies (Too Dilute..
Image all Plates

Streaked JW1226 Stock Plate- Colonies formed
Pick Colony & Streak Kan Plate/Inoculate LB+Kan Overnight Culture
```



Plated Mixed Overnight Cultures, 1:10000 Dilutions



Negative Selection Survivors from Mixed Overnight Cultures, 1:100 & 1:10000 Dilutions



Positive Selection from Mixed Overnight Cultures, 1:10000 Dilution

- Redo Positive Selection

```
Pour new Positive Selection Plate & Plate New Mixed Overnight Culture
```

- PCR & Assembly of Toxic Gene Plasmids

```
Spin down PCR plates- Balance & spin in large centrifuge, Pulse spin
Take out 1uL from plate well
```

Expand PCR w/ & w/o DMSO

```
Add to Expand Master Mix:
10uM Primer 1- ca998    1uL
10uM Primer 2- G00101   1uL
Template DNA            1uL
Expand Polymerase       0.75uL

Run 4K55 Program ~4hr.- Max PCR Fragment Size=4kb (1:10PM-~4:10PM)
```

- Reseed -80 Stock of Pstress Promoters

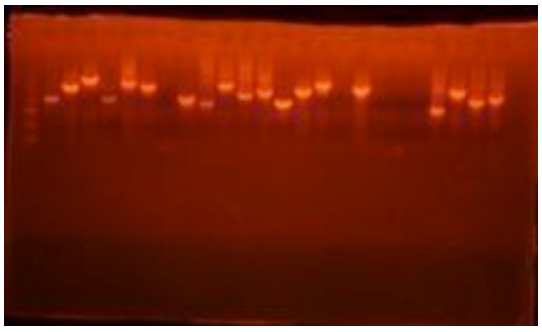
```
Find 96-Well Plate w/ Stock Cells & Reporter Plasmids (4th Shelf, Right Side)
Dip plastic pin into -80 stock & inoculate 96-Well Plate w/ 1mL LB+Kan/Cam
Plasmid Backbone=1601KC KanR/CamR, A5-C5, D4-H4
Shake Overnight @ 37 degree C
```

- pH & Osmolarity Standard Curve

```
Make 1:10 Dilution of Stock 10M HCl & NaOH- 100uL in 900uL ddH2O=1M HCl+NaOH
Make 1M NaCl Stock Solution- 58.44mg/mL ddH2O
Serial Dilution in Duplicate w/ MC1061 & pBgl0002-ffGFP (Trim)
900uL LB+Trim in 96-Well Block- Add 100uL 1M Stock, Take 100uL & add to next well
12-Order Magnitude Dilution- 1/10 Dilution each series
Shake overnight @ 37 degree C
```

- Zymo Cleanup & Analytical Gel of PCR Fragments

```
Load 5uL PCR Product w/ 2uL Dye & 5uL DNA Ladder
Run 20min. @ 180V (5:07-5:17PM) on large gel box
Visualize under UV
```



Lane 1=DNA Ladder, Order=w/DMSO->w/o DMSO

Lane 1=DNA Ladder, Order=w/DMSO > w/o DMSO

```
#7 (bth8189) has no product w/ or w/o DMSO, w/ DMSO yields less PCR Product
```

- To Do

```
Inoculate Overnight Culture block into Variable Conditions- Low/High pH, Low/High Temp. (42&25 de
Grow overnight & Tecan
```

Xin Xin Lin 10:47, 25 July 2011 (PDT)

- Plate Mixed Overnight Cultures

```
Plate 50uL each overnight culture (1uL/2mL) on Kan/Spec- Pcon/1600 ffGFP
Plate 50uL each overnight culture (1uL/2mL) on Kan- Pcon/0002 ffGFP
Redo Plates- 1mL Saturated Overnight Culture=1e9 Cells, 1uL=1e6 Cells->Want 100-1000cfu
-Dilute 1uL 1600 Culture in 99uL LB+Kan/Spec, Dilute 1uL 1:100 Dilution in 99uL LB+Kan/Spec (Plat
Incubate @ 37 degree overnight- Compare ratio Green:White
```

- Sequencing Analysis of pBjh1600-Pcon.rbs.hsvTK

```
Both Samples w/ ca998/G00101- Perfect promoter, rbs, & hsvTK parts, but 2bp (AG) insert b/w promo
-2bp Insert=Extra 2bp added to rbs to make it 8bp before the start codon
```

- Procure JW1226 Plate

```
JW1226 strain from KEIO collection streaked on Kan plate
Incubate @ 37 degree C overnight
Pick colony & streak on Spec plate- Inoculate 5mL LB+Kan Media
Grow up overnight @ 37 degree C
-Check if there is growth on Spec
```

- Inoculate Overnight Culture of Pcon.rbs.hsvTK

```
Inoculate 1uL saturated overnight culture (from 7/23) Pcon.rbs.hsvTK in 5mL LB+Kan/Spec
Shake overnight @ 37 degree C

Inoculate Pcon/ffGFP Positive Control Negative Selection Media
1uL saturated overnight culture (from 7/23) Pcon.rbs.hsvTK in 5mL LB+Kan/Spec+dP
Shake 2hr. @ 37 degree C & Plate Kan/Spec (3:35-5:35PM)- 100uL of 1:100
Incubate overnight @ 37 degree C
Expect no growth- Constitutively ON Promoter->Lethal Mutagenesis (hsvTK+dP)
```

```
Plate on Positive Selection Plate
Pour 1 Positive Selection Plate- Used 25mL Agar
-5FdU Stock- 10mg in 1mL ddH2O- Add 50uL to Agar
-Thymidine Stock- 5mg in 1mL ddH2O- Add 50uL to Agar
-Uridine Stock- 5mg in 1mL ddH2O- Add 50uL to Agar
Forgot to add Kan...
Let dry- Labelled w/ Black Stripe
Plate Pcon.rbs.hsvTK/ffGFP (1600)- Dilute 1uL in 99uL, 1uL 1:100 in 99uL & Plate
```

- Project Updates

```
Spec Contamination- Plated Spec->Kan & Kan->Spec, Growth on both plates
New JW1226 Cells streaked out- Remake -80 degree C competent cells

New Project: Stress Promoter Research
Promoter.hsvTK rbs Library
DNA
```

Have EIPCR rbs Library Product (50uL) & EIPCR rbs Library Digest (20uL)Need to ligate before retransforming

```
Strains
```

Find out Wed. RE: SpecR of JW1226 Make new -80 comp cells

```
Pcon.rbs.hsvTK/ffGFP Competitive Assay
10^9 Cells/mL in saturated culture=10^6 Cells/uL
want 100-1000cfu
Plate mixed culture on Kan/Spec- Dilute 1uL in 99uL, Dilute 1uL in 99uL, Plate 100uL
Negative Selection- 1uL in 5mL Negative Selection Media
```

Dilute 1uL in 99uL, Dilute 1uL in 99uL, Plate 100uL

```
Positive Selection- Dilute 1uL in 99uL, Dilute 1uL in 99uL, Plate 100uL
```

Plate on Positive Selection Plates

```
Photograph plates for green:white ratio
```

```
Stress Promoters
Find Cre, TetA, ToxR, Methyl Transferase, jtk2245, 2544, 2937, 2979, 3106, 2796
PCR w/ ca998/G00101- Zymo cleanup, analytical gel
EcoRI/BamHI digest & drop into 1600 plasmid (p15A Spec)
Make -80 comp cell stock
```

```
Find stress promoter plate
```

ID vector & location (if p15A SpecR, use different plasmid than 1600 for toxic gene)

```
Transform Pstress promoters into -80 comp cells
Colonies by wed.- inoculate culture
Tecan w/ dilutions of Arabinose/cytometry
Record on parts page
```

- To Do

```
Assemble collection of toxic genes
-Email about parts & location
-Summarize vectors
-Find Pstress plate
Digest toxic genes EcoRI/BamHI- Drop into pBjh1600 Digest
PCR toxic genes w/ ca998/G00101
```

```
Cre- KA 9008
TetA- 1600 Plasmid
ToxR- DC006, DC023, & DC038
I716101-jtk2245  {rbs.SSSI}                        Plate 5-E6
pBca9523-jtk2544 {PY54_RepA}                       11-B8
pBjk2741-jtk2937 {Pcon){rbs.HlyE}{b0015}           18-A11
pBca9145-jtk2979 {rbs.recA}{rbs.gam-bet-exo-tL3}   19-D10
pBjk2741-jtk3106 {O7 5/6}                          22-F9
pBca9145-jtk2796 {Pcon){rbs.TetR.term}             14-D12
pBjk2741-Bth8189 (Pcon.AcuImet)                    4-G12
```

Xin Xin Lin 14:09, 24 July 2011 (PDT)

Sunday, 2:00PM-2:45PM

- Check Test Plates

```
Kan->Spec- Growth of assorted tiny colonies
Spec->Kan- Growth of fat colonies
Stored in fridge
```

- Competitive Assay

```
0002 ffGFP greener than 1600 ffGFP Overnight Culture- Higher Copy Plasmid
Mix 50-50 ffGFP & Pcon.rbs.hsvTK cultures
-0002 ffGFP w/ Pcon #1
-0002 ffGFP w/ Pcon #4
-1600 ffGFP w/ Pcon #1
-1600 ffGFP w/ Pcon #4
OR
5mL LB+Kan- 1uL Pcon #1 & 0002 ffGFP
5mL LB+Kan/Spec- 1uL Pcon #1 & 1600 ffGFP
1mL LB+Kan- 2mL Pcon #1 & 0002 ffGFP
1mL LB+Kan/Spec- 2mL Pcon #1 & 1600 ffGFP
Shake overnight @ 37 degree C & plate Kan/Spec or Kan
```

Xin Xin Lin 15:18, 23 July 2011 (PDT)

Saturday, 1:15PM-4:40PM

- Check Transformed Plates

```
JW1226 w/ 0002 ffGFP on Kan/Trim- Green Lawn
JW1226 w/ 1600 ffGFP on Kan/Spec- Green Lawn
JW1226 on Kan- Dense Lawn
JW1226 on Spec- Less Dense Lawn
JW1226 w/ rbs Library on Spec- Less Dense Lawn
JW1226 w/ rbs Library on Kan/Trim- Relatively even spread of medium sized colonies
```

```
SpecR Contamination?
Parafilm rbs Library Plates & store in fridge
```

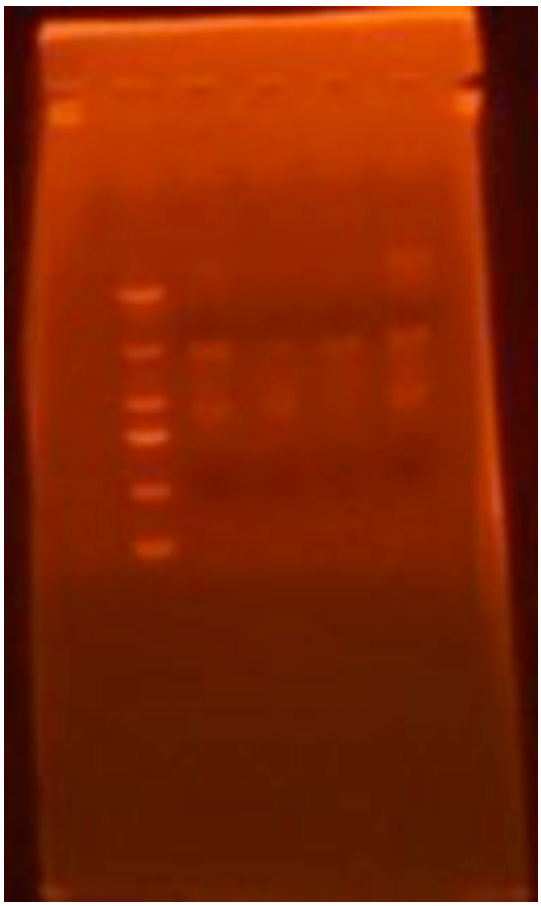
```
Determine if there is mixture of cells
Streak Colony from Kan onto Spec
Streak Colony from Spec onto Kan
```

- Miniprep of Pcon Overnight Cultures

```
4 Overnight Cultures- Pcon.rbs.hsvTK #1-4
```

- Mapping

```
EcoRI/BamHI Digest of 3uL each Miniprep 1hr. @ 37 degree C- 2:33-3:33PM
Run Gel @ 180V for 10min.- 4:00-4:10PM
Visualize under UV- Check for 2.6kb & 1.3kb band
```



Lane 1=MW DNA Ladder (6kb, 3kb, 1.5kb, 1kb, 500bp, & 200bp), Lane 2-5=Pcon.rbs.hsvTK #1-4

```
#1&4=Brightest- Sequence 1&4, All bands=visible
```

Submit for Sequencing

```
xl014 & xl015=#1&#4, ca998/G00101 Pcon.rbs.hsvTK
12uL Miniprep DNA- Very faint bands
```

- Inoculate Overnight Culture of ffGFP, Pcon, & JW1226

```
1uL Pcon Overnight Culture into 5mL Kan/Spec
1 Colony each ffGFP into 5mL Kan/Spec or Kan/Trim
1 Colony JW1226 into Kan
```

- To Do

```
Competitive Assay
Sequencing Analysis
```

Xin Xin Lin 10:52, 22 July 2011 (PDT)

- Sequencing Analysis of Pcon.rbs.hsvTK

```
xl012 ca998- No match to template at all
xl012 G00101- Perfect hsvTK Part 2, Early Sequences cut off
xl013 ca998- No match template at all
xl013 G00101- Majority match to hsvTK Part 2, Early Sequences cut off
Incomplete part sequenced for all- Forward sequence only shows below sequences & do not match
```

- Check Transformed & Test Plates

```
Pc+.hsvTK JW1226 Antibiotic Test- No growth on Amp
Pctx.hsvTK JW1226 Antibiotic Test- Mixed phenotypes on Spec

Pc+.hsvTK JW1226 Control- No growth on Kan/Trim/Amp (Expected)
Pc+.Pbad-ExsA JW1226 Transformed- No growth on Kan/Trim/Amp (Unexpected)
Pctx.hsvTK JW1226 Control- Mixed phenotypes on Kan/Trim/Spec (Unexpected)
Pctx.toxR-mukF JW1226 Transformed- Mixed phenotypes on Kan/Trim/Spec (Unexpected)
```

```
Pcon.rbs.hsvTK #1 JW1226- Even growth of colonies on Kan/Spec (Expected)
#2 Ligation+30min.- No growth on Kan/Trim (Expected)
#3 8uL Ligation- No growth on Kan/Trim (Expected)
```

```
Issue w/ JW1226 Comp Cells w/ Pctx/Pc+- Remake comp cells? Need to remove background
```

- Pcon.rbs.hsvTK Competitive Assay

```
Pick 4 Pcon.rbs.hsvTK colonies & inoculate 5mL LB+Kan/Spec Overnight Cultures
Incubate @ 37 degree shaker overnight

Transform pBjh1600-jtk2828 & pBgl0002-jtk2828 ffGFP into JW1226 Cells
Plate Kan/Spec & Kan/Trim
```

- Miniprep Overnight Cultures in 24-Well Block

```
Spin 7000rpm for 5min. @ 27 degrees C, S5700 Rotor for blocks- Pipet out supernatant
Add 250uL P1 Buffer & 250uL of P2 Buffer- Swirl block gently
Add 350uL N3 Buffer- Swirl then shake vigorously to break membrane b/w P2&N3
Spin 5300rpm for 10min.
Pipet off supernatant into Miniprep column on Pig- Turn on vacuum to remove liquid
Add 500uL Buffer PB & 750uL Buffer PE
Dry spin in centrifuge for 2min.
Elute in Eppendorf w/ 50uL dIH2O
Centrifuge 1min. @ 13.4k rpm
```

- rbs Library Dual Selection

```
Focus on Pctx.hsvTK only
Transform rbs Library into JW1226 Cells
-Control- Plate JW1226 Cells on Spec
Plate Trim/Kan
-Control- Plate transformed JW1226 Cells on Spec
Streak fresh JW1226 on Kan for stock
Rescue for 1hr. @ 37 degree C- 1:42-2:42PM
```

Plate Summary

```
JW1226 on Spec- Control (Plated 100uL of 1:10 Dilution- 10uL of (10uL Cells in 90uL) in 90uL, use
JW1226 on Kan- Stock, JW1226=Kan (Plated 100uL of 1:10 Dilution- 10uL of (10uL Cells in 90uL) in
JW1226 rbs Library on Spec- Control (50uL)
JW1226 rbs Library on Kan/Trim- rbs Library=Trim (50uL)
JW1226 0002 ffGFP on Kan/Trim- 0002=Trim (50uL)
JW1226 1600 ffGFP on Kan/Spec- 1600=Spec (50uL)

Incubate overnight @ 37 degree C
```

- To Do

Saturday

```
Scrape w/ 3-5mL LB+TRIM/Kan- Inoculate 1uL in 5mL liquid Negative Selection Media
Grow 2-3hr.
Plate LB+Kan/TRIM

Miniprep, map, & sequence Pcon.rbs.hsvTK

Make chemical stock solutions
Combined mixture lethality assay
```

Xin Xin Lin 11:30, 21 July 2011 (PDT)

- Check Transformed Plates

```
Pctx & toxR-mukF- Lawn of different phenotypes on both plates (Transformed & Control)
Pc+ & Pbad-ExsA- No colonies on either plates (Transformed & Control)
Did not scrape cells

Test Antibiotic Resistances- Spread Pctx & Pc+ JW1226 Comp Cells on Spec Only & Amp Only Plates
```

```
Redo Transformation
1uL pBca9145-Bss38 in Pc+ JW1226 (Light Green), 1uL pBca9525-Bca1832 in Pctx JW1226 (Light Blue)
Incubate on ice 10min.- 11:10-11:20AM
Rescue w/ 200uL 2YT & shake @ 37 degree C for 1hr.- 11:25AM-12:25PM
Plate 50uL on Kan/Trim/Spec (Pctx) & Kan/Trim/Amp (Pc+)- 2 Controls (Not Transformed) also plated
-Pour Kan/Trim/Amp Plates
```

- Redo Digestion & Ligation of pBjh1600-hsvTK & Pcon.rbs

Digestion

```
4uL ddH2O                   4uL ddH2O
1uL NEB2 Buffer             1uL NEB2 Buffer
4uL Pcon.rbs Wobble PCR     4uL pBjh1600-hsvTK
0.5uL EcoRI                 0.5uL EcoRI
0.5uL BamHI                 0.5uL BglII

Digest 1.5hr. @ 37 degree C
```

Ligation

```
4.25uL pBjh1600-hsvTK Digest
4.25uL Pcon.rbs Wobble Digest
1uL T4 DNA Ligase Buffer
0.5uL T4 DNA Ligase

Ligate on bench for 30min. (10:52-11:22AM)
```

Transform JW1226 Competent Cells

```
1uL Ligation in 50uL JW1226 Cells+KCM- Plate 50uL on Kan/Spec (pBjh1600 Plasmid)
1uL Ligation- After 30min. #1- Plated on Kan/Spec
-Incubate on Ice 10min.- 11:36AM-11:46AM
-Rescue for 1hr. @ 37 degree C- 11:49AM-12:49PM
1uL Ligation- After 1hr. #2- Plated on Kan/Trim- Should have no growth
-Incubate on Ice 10min.- 11:52AM-12:02PM
-Rescue 1hr. @ 37 degree C- 12:06-1:06PM
8uL Ligation- After 1hr. #3- Plated on Kan/Trim- Should have no growth
-Incubate 10min. on Ice- 12:07-12:17PM
-Rescue 1hr. @ 37 degree C- 12:22-1:22PM
Pour Kan/Trim Plates
```

Submit for Sequencing

```
Pcon.rbs.hsvTK Large Colony #1 & Small Colony #2- Bands from Mapping @ approximate locations (Dir
Submit 12uL to sequence w/ ca998/G00101
-xl012=Large Colony #1
-xl013=Small Colony #2
```

- Inoculate Overnight Culture of Cells

```
3mL LB+Amp/Kan in 24 well block
Inoculate w/ 1 colony- 3 colonies/strain=5 strains & 15 total inoculations
Shake @ 37 degree C overnight & Miniprep next day
-A1, A2, A3, E1, E2, E3
-F1, F2, F3, G1, G2, G3
-H1, H2, H3
```

- Miniprep 3 Samples- 1, 10, & 25
- Clean up Empty Bench

```
Salvage extra chemicals & lab supplies
Clean out old dishes
```

- To Do

```
Make Rhodamine Stock Solution
```

Xin Xin Lin 13:29, 20 July 2011 (PDT)

- Miniprep of Pcon.rbs.hsvTK Overnight Cultures

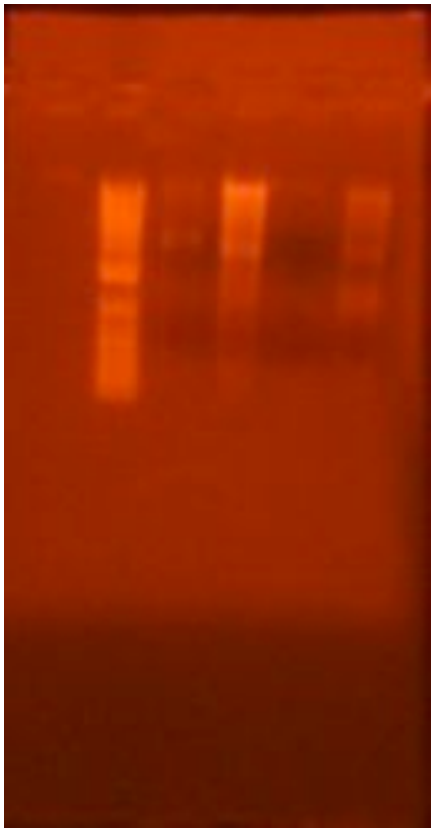
```
Miniprepped 3mL of each saturated overnight culture- Eluted in 50uL ddH2O
Large Colony #1/#2 & Small Colony #1/#2
```

EcoRI/BamHI Digest Mapping

```
5uL Box H2O
1uL NEB2 Buffer
3uL Miniprep
0.5uL EcoRI
0.5uL BamHI

Digest 1hr. @ 37 degree C (1:22PM-2:22PM)
```

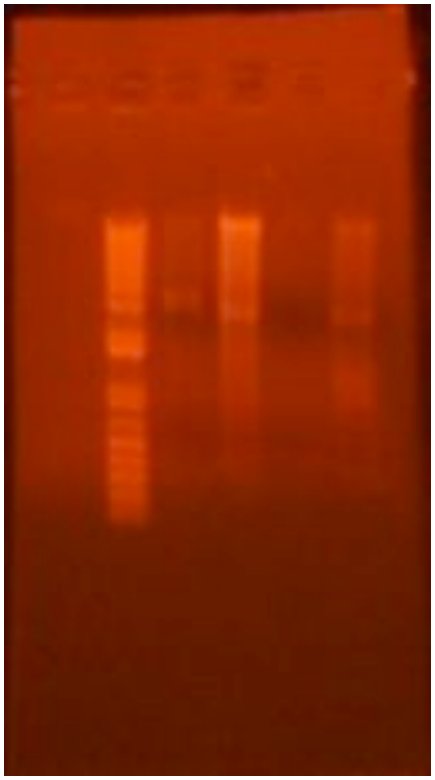
Mapping Analytical Gel

```
Load gel w/2uL Dye in Digests & 5uL DNA Ladder
Run 10min. @ 180V (2:32-2:42PM)
Visualize under UV- Look for 1kb Part & 2.5kb Vector (pBjh1600)
```



Lane 1=DNA Ladder, Lane 2=Large Colony #1, Lane 3=Large Colony #2, Lane 4=Small Colony #1, & Lane 5=Small Colony #2

```
Little separation between ladder bands, smear or no bands visible from digests...
Run additional 5min. (2:46-2:51PM)
```



Still unresolved- Digest fragments not clear

```
Large Colony #1- Band at ~3kb but very faint
Large Colony #2- Bright smear
Small Colony #1- No bands...
Small Colony #2- Band at ~3kb w/ some background
```

Submit for Sequencing?

```
12uL of each of 4 Minipreps
-xl012- Pcon.rbs.hsvTK, Large Colony #1
-xl013- Pcon.rbs.hsvTK, Large Colony #2
-xl014- Pcon.rbs.hsvTK, Small Colony #1
-xl015- Pcon.rbs.hsvTK, Small Colony #2
Sequence w/ca998/G00101

Redo Ligation w/ Cleaned Digests of pBjh1600-hsvTK & Pcon.rbs- Retransform JW1226
```

Make -80 degree C Stocks of Remaining Overnight Culture- Only if mapping works out

```
Pipet out remaining cell culture & add to 2mL 50% Glycerol- 1/2 Cells & 1/2 50% Glycerol
Multichannel 150uL/PCR Tube from tray
Store @ -80 degree C- Pcon.rbs.hsvTK
Streak out Kan/Spec plate for fresh colonies- Incubate 37 degree C overnight
Transform JW1226 w/pBjh1600-jtk2828 & incubate w/ streaked Pcon.rbs.hsvTK

Leave cultures as is- Did not transform correctly...
Did not transform ffGFP into JW1226
```

- Check Negative Selection Survivors Transformed Plates

```
1uL Transformed Survivors=Complete Lawn
1:10 Dilution Transformed Survivors=Lawn of slightly larger & fewer colonies
```

```
Scrape survivors w/ 3mL LB+Trim/Kan- Pctx #1 1uL Undiluted & Pc+ #2 1:10 Dilution
Add 1:100 dilution (50uL) into 5mL LB+Kan/TRIM
Shake & grow for 2hr. @ 37 degree C (12:08PM-2:08PM)
```

Small Scale Chemically Competent Cell Prep of Negative Selection Survivors

```
Put rotor in centrifuge & cool to 4 degree C- A-10 Rotor
Label PCR Strips- Color Label=Pale & Dark Blue Stripes (Pctx.hsvTK) & Mint & Green Stripes (Pc+.hs
-Circled in green- Contaminated w/ Ice Water?
Place TSS, Falcon Tube, & PCR strips on ice
Cool down culture tubes in ice to below room temp.
Spin down in 50mL Falcon Tube for 5min. @ 6500rpm & Remove supernatant
Resuspend in 5mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex
Pour into tray boat & pipet w/ multichannel
Aliquot 150uL/PCR tube on metal block in Ice- 4 Strips Pctx & 4 Strips Pc+
```

Transform pBca9525-Bca1832 into Pctx & pBca9145-bss38 into Pc+- Control=Not Transformed

```
Controls- No plasmid is transformed in
Used freshly made chemically competent cells for transformation
-Added 30uL KCM to 1 Pctx PCR Tube & 1 Pc+ PCR Tube- Marked w/ Blue/Green Dot on Lid
-2 Controls w/o DNA Transformed- Pctx & Pc+ w/o toxR-mukF/Pbad-ExsA
-50uL Cell Cocktail added to 1uL DNA- Incubate on ice 10min. (3:54-4:04PM)
Rescue w/200uL 2YT & shake 1hr. @ 37 degree C- 4:10-5:10PM
Plate 50uL on Kan/TRIM/Spec (toxR-mukF) or Kan/Trim/Amp ({Pc+.rbs.ffGFP}{AraC-Pbad}{rbs.exsA!})
-Pour LB+Kan/Trim/Amp Plates
```

- Weekly Meeting

```
Add Rhodamein to Chemical Stock Selection- Make 100mM Stock Solution
Combine all chemicals & test lethality on culture

Test pBjh1600-Pcon.rbs.hsvTK in JW1226 thru Racing Assay w/ pBjh1600-ffGFP in JW1226
Mix cultures 1:1- Negative & Positive Selection, Characterize colonies
```

- To Do

Thursday

```
Re-ligate Pcon.rbs Wobble PCR & pBjh1600-hsvTK Digests
Transform JW1226 & Plate Kan/Spec
```

```
Scrape transformed survivors & wash w/ PBS- Spin, Discard, Resuspend x3-4
Resuspend in PBS & Plate Kan/TRIM/Spec Positive Selection- Vary dilutions 1e-4 & 1e-6
```

Xin Xin Lin 12:55, 19 July 2011 (PDT)

- Checked All Transformed Plates

Retransformed pBgl0002-Promoter.hsvTK JW1226 rbs Library on Kan/Trim

```
Many small colonies in even lawn- 100uL Spread w/ Beads
Approximately equal numbers for A1 & B1 (Pctx & Pc+)
```

Transformed pBjh1600-Pcon.rbs.hsvTK JW1226 on Kan/Spec

```
Small area of medium-sized colonies due to uneven spreading (Dry Agar->Scratches)- 50uL w/ Spread
Some small colonies- From contaminated 2YT?
```

Transformed pBgl0002-Promoter.rbs.hsvTK JW1226 Negative Selection Survivors w/toxR-mukF on Kan/Trim/Spec

```
Uneven spread of large & small colonies due to very uneven spreading
Used contaminated TSS to make competent cells->Re-Do
```

- Dual Selection

```
Scrape w/ 3mL LB+TRIM/Kan- Inoculate 1uL in 5mL liquid Negative Selection Media
-Negative Selection Media=LB+Kan/Trim+dP
-Tested 2 different 1mM dP Stocks- 1mL Stock (#1) & 10mL Stock (#2)
Grow 2hr. in 37 degree C Shaker (11:55-1:55PM)
-After 2hr., both Pctx cultures are cloudier than Pc+ cultures- All are relatively clear
Plate LB+Kan/TRIM w/ Beads- 2 Dilutions (100uL of 1uL Cells in 99uL LB+Trim/Kan & 100uL 1:100 Dil
-1:10=10uL Cells in 90uL LB+Kan/Trim- Take 10uL & add to 90uL LB+Kan/Trim
Incubate overnight @ 37 degree C
```

```
Make -80 Cell Stocks of rbs Library
Pipet out remaining scraped cell solution & add to 3mL 50% Glycerol- 1/2 Cells & 1/2 50% Glycerol
Multichannel 150uL/PCR Tube from tray
Store @ -80 degree C- Pctx=Purple Stripe, Pc+=Purple & Blue Stripe
```

```
Future: When plating on Kan/Trim/Spec after transforming in toxR-mukF, use untransformed as negat
-Untransformed should die on Spec b/c there is no toxR-mukF SpecR plasmid
```

- Pick Colony & Inoculate Overnight Culture- Pcon.rbs.hsvTK

```
Inoculate 5mL LB+Kan/Spec Overnight Culture w/ 1 Colony
-2 Large Colonies & 2 Small Colonies
Shake overnight @ 37 degree C
```

- Made trash can from 2YT Broth Container
- Purchased Chemicals through RES
- To Do

Wednesday

```
Scrape & Comp Cell Prep of Negative Selection Survivors
Miniprep, map, & sequence Pcon.rbs.hsvTK
Make chemical stock solutions
```

Xin Xin Lin 11:41, 18 July 2011 (PDT)

- Electrocompetent Cell Preparation

```
Filled flask w/ 1L 2YT Media- Removed 2mL into Eppendorfs for blanking Spec
Inoculated w/ 5mL Overnight Culture- Labeled w/ Yellow Tape
Shake in Warm Room for 1.5hr. (10:15-11:45PM)
```

- Transform Constitutive toxR-mukF Plasmid into Competent JW1226 w/Promoter.hsvTK

```
Add 50uL Pctx/Pc+.hsvTK JW1226 (w/ 30uL KCM) to 1uL pBca9525-Bca1832 (toxR-mukF)
Incubate on Ice for 10min. (11:19-11:29AM)
Rescue w/ 200uL 2YT Media & shake 1hr. @ 37 degree C (11:34-12:34PM)
Plate 50uL, 1:10 Dilution, & 1:100 Dilution on Kan/Trim/Spec
```

- Purchase 5 Remaining Selection Chemicals

```
DDT, Hexachlorobenzene, Chlordane, Citronella, & Camphor
Make 1mL 100mM Stock Solutions, w/ Tyramine & Melamine
```

- pBgl0002-Pcon.rbs.hsvTK Assembly

```
Available Parts: Pcon.rbs, pBjh1600-hsvTK, & pBgl0002-ffGFP
```

```
Digest Pcon.rbs & pBgl0002-ffGFP EcoRI/BamHI
Digest pBjh1600-hsvTK & pBgl0002-ffGFP EcoRI/BamHI
Small Frag/Zymo Cleanup, ligate, & Transform jtk155- Plate Trim
Miniprep, map, & sequence

Digest pBgl0002-Pcon.rbs BamHI/XhoI & pBgl0002-hsvTK BglII/XhoI
Gel purify, ligate, & transform jtk155- Plate Trim
Miniprep, map, & sequence

Transform JW1226 w/ pBgl0002-Pcon.rbs.hsvTK- Plate Kan/Trim
Small Scale Chemically Competent Cell Prep
Transform JW1226 (Pcon.rbs.hsvTK) w/ pBca9525-Bca1832 (toxR-mukF)
```

No need to transform in toxR-mukF

Digest Pcon.rbs Wobble PCR Product EcoRI/BamHI & pBjh1600-hsvTK EcoRI/BglII

```
4uL ddH2O                   4uL ddH2O
1uL NEB2 Buffer             1uL NEB2 Buffer
4uL Pcon.rbs Wobble PCR     4uL pBjh1600-hsvTK
0.5uL EcoRI                 0.5uL EcoRI
0.5uL BamHI                 0.5uL BglII

Digest 1.5hr. @ 37 degree C- 1:00-2:30PM
```

Regular & Small Frag Zymo Cleanup

```
Digest products too small to visualize on gel
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
```

Ligation

```
4.25uL pBjh1600-hsvTK Digest
4.25uL Pcon.rbs Wobble Digest
1uL T4 DNA Ligase Buffer
0.5uL T4 DNA Ligase

Ligate on bench for 30min. (2:50-3:20PM)
```

Transform Chemically Competent JW1226

Transform Chemically Competent JW1226

```
1uL Ligation Product in 50uL JW1226/KCM Cell Cocktail
Incubate on ice 10min. (3:38-3:48PM)
Rescue w/ 200uL 2YT- Shake 1hr. @ 37 degree C (3:51-4:51PM)
Plate 50uL on Kan/Spec
```

- Retransform rbs Library

```
TSS (Used for Negative Selection Survivor Comp Cell Prep) was contaminated
Add 50uL JW1226 Chemically Competent Cells (+30uL KCM) to 1uL EIPCR Ligation Product (A1&B1)
Incubate on ice for 10min. (4:09-4:19PM)
Rescue w/ 200uL 2YT & shake for 1hr. @ 37 degree C (4:24-5:24PM)
Plate 100uL on Kan/Trim Plates
```

- To Do

Tuesday

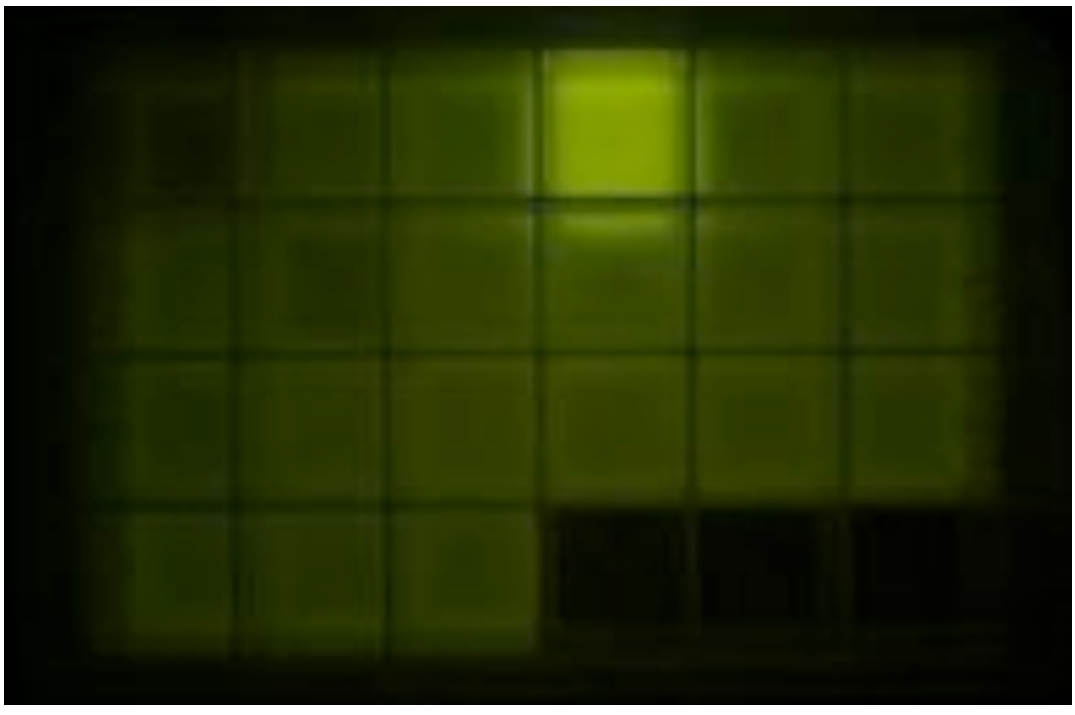
```
Pick Pcon.rbs.hsvTK colony & inoculate overnight LB+Kan/Spec Culture
```

Xin Xin Lin 14:08, 17 July 2011 (PDT)

Sunday, 2PM-7PM

- Lethality Assay Results

```
Check 24-well block after shaking overnight
All green saturated culture & most comparable to both Media Only & DMSO Controls
-Dopamine 1/1 is least green- Media appeared brownish yellow
-Chloroquine 1/2 less green than others
-Fluoroscein especially bright due to fluorescence of chemical
```

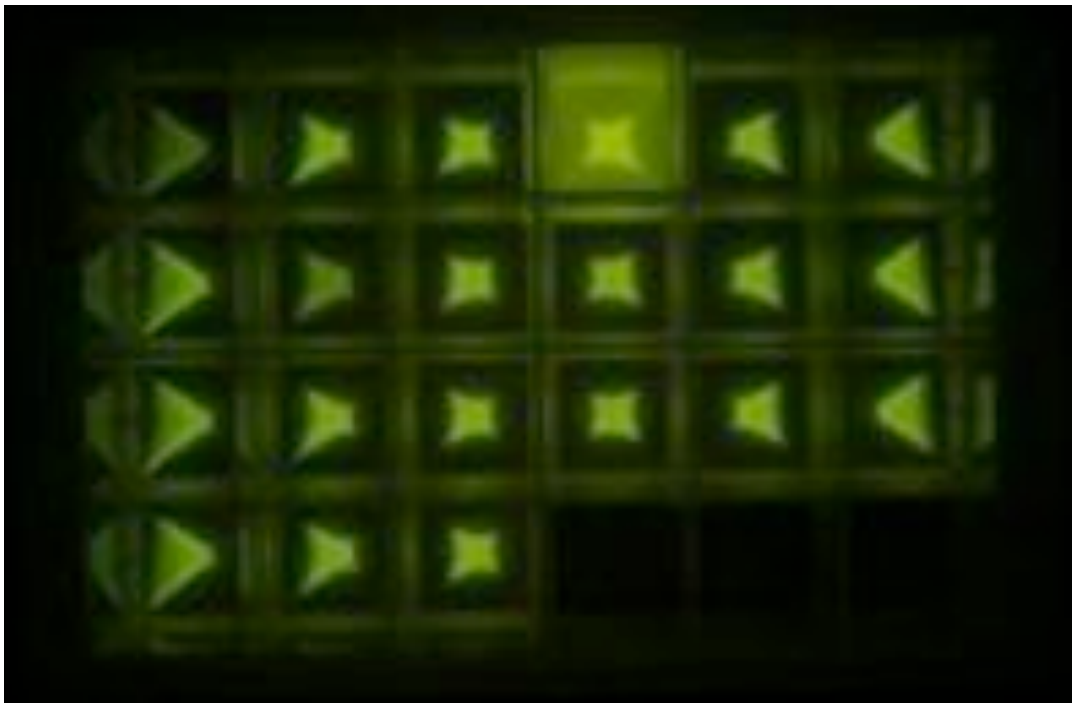


6x4 24 Well Block

Dopamine	Caffeine	Histamine	Fluoroscein	Resorcitol	Urea
Boric Acid	Chloroquine	Acetaminophen	Progesterone	Tryptamine	Melatonin
Vanillin	Terephthalic Acid	Permethrin	2-Phenethylamine	1-Butanol	Dicyclopropyl Ketone
Nicotine	Control MC1061	DMSO Control			

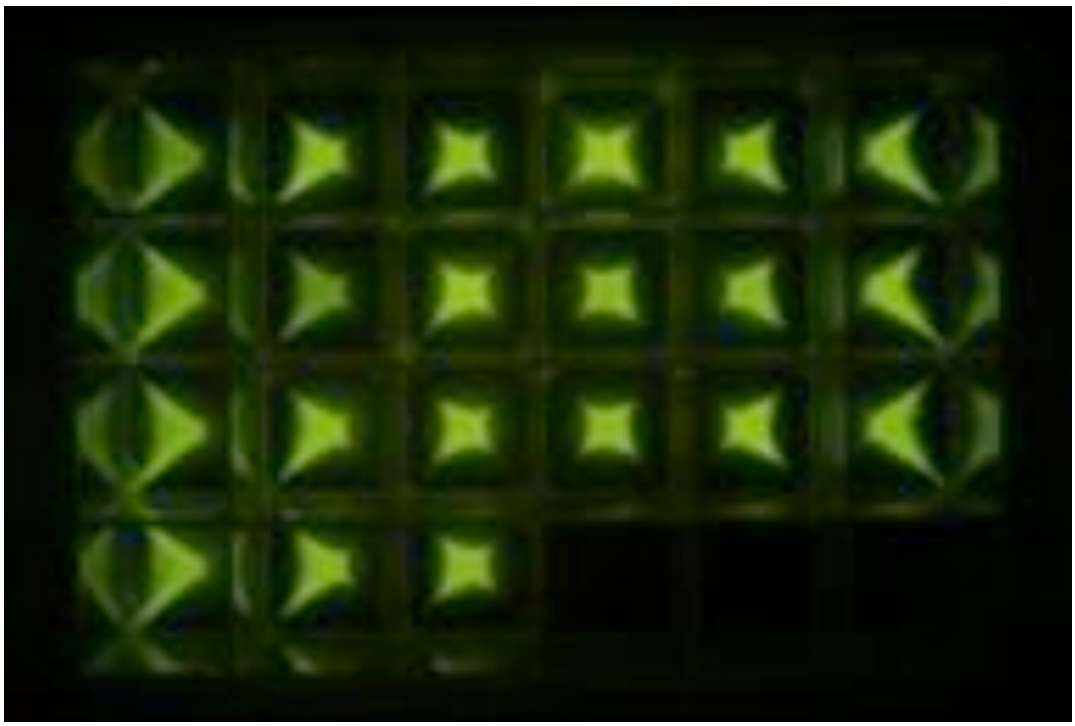
Spin down to pellet in centrifuge

Balance & Spin 4min. @ 5400rpm on S5700 Rotor for Spinning Blocks
Image spun down blocks



Spun Down Pellet w/ Media- Fluorescein in media glows

Pellets approximately similar size- Dopamine pellet slightly fainter



Spun Down Pellet w/o Media- Media removed

```
No pellet is noticeably smaller- Chemical Stocks not lethal @ concentration
```

- Negative Selection Overnight Cultures

```
Both Pctx & Pc+ cultures appear fully saturated
```

- Check Negative Selection Survivor Plates

```
Complete lawn of many tiny colonies on all plates at all dilutions
Scrape survivors of 1:100 dilution plates w/ 2mL LB+Kan/Trim- Most even spread of colonies
-Store unscraped 1uL & 1:10 Dilution plates in fridge
Inoculate LB+Kan/Trim Media w/ 1uL
Grow 2 hours in 37 degree Shaker (3:06-5:06PM)
Culture only slightly cloudy- Not as saturated as when 20uL scraped cells=added
-When spun down, only tiny pellet visible
-Add additional 19uL scraped cells from plates & incubate additional 30min. in shaker (5:25-5:55P
-Pellet by splitting 5mL culture into 3 1.5mL Eppendorf Tubes- 1.5mL each (Small pellet)
-Resuspend in 1.5mL TSS in each Eppendorf & pour into tray for multichanneling into PCR strips
```

Small Scale Chemically Competent Cell Preparation of Negative Selection Survivors

```
Put rotor in centrifuge & cool to 4 degree C- A-10 Rotor
Label PCR Strips- Color Label=Magenta Stripe (Pctx.hsvTK) & Magenta+Blue (Pc+.hsvTK)- No stripes
Place TSS, Falcon Tube, & PCR strips on ice- TSS appears contaminated (Cloudy & foamy/bubbly w/ c
Cool down culture tubes in ice to below room temp.
Spin down in 50mL Falcon Tube for 5min. @ 6500rpm & Remove supernatant
Resuspend in 5mL cold TSS (Vol.=Competent Cell Mix Vol.)- Vortex
Pour into tray boat & pipet w/ multichannel
Aliquot 150uL/PCR tube on metal block in Ice- 3 Strips Pctx & 3 Strips Pc+
Label w/ Colored Stripe & Store @ -80 degrees C (in SRS Box w/Bss52 Cells, Bottom Shelf)
```

- To Do

```
Transform constitutive toxR-mukF plasmid into cells (BOBP)
Plate Kan/TRIM/Spec
```

Xin Xin Lin 16:09, 16 July 2011 (PDT)

Saturday, 1:30PM-5:30PM

- Check Transformed JW1226 rbs Library Plates

```
Many colonies on both plates for Pctx.hsvTK & Pc+.hsvTK in pBgl0002- Expected many more colonies
Scrape w/ 3mL LB+TRIM/Kan- Inoculate 20uL in 5mL liquid Negative Selection Media
Grow 2-3hr. @ 37 degree C shaker (2:49-4:49PM)- Culture slightly cloudy
Plate varying dilutions on LB+Kan/TRIM & incubate overnight @ 37 degree C
-1uL in 49uL 2YT Media (Plate 50uL), 10uL in 90uL 2YT (Plate 10uL 1:10), 10uL in 990uL 2YT (Plate
-Serial Dilutions: 1:10=1uL in 9uL 2YT, 1:100=1uL 1:10 in 9uL 2YT
Return cultures to shake overnight- Check next day for saturation of culture & compare w/ -dP cul
```

Negative Selection Media=500mL LB+Kan/Trim+0.026925mg dP (100nM dP)

```
Make 1mM dP Stock Solution for Immediate Use- 0.269mg dP in 1mL ddH2O
Use 0.5uL of 1mM Stock in 5mL LB+Kan/Trim
-Density=1.8 g/cm3 or 1.8g/mL, MW=269.2539g/mol
```

- Lethality Assay

```
Take out overnight cultures of MC1016 w/ffGFP
Add 24 well block w/ 3mL LB+Trim
-Inoculate w/ 3uL Saturated Overnight Culture
-Add 100uM (1/1000 Dilution) of Chemical Stocks- 3uL 100mM Stock
Shake overnight @ 37 degree C & confirm growth to saturation
-Photograph culture & spun down pellet
Control=No chemicals added
```

- Wobble PCR Reaction of Pcon.rbs Zymo Cleanup

```
No Analytical Gel- Part too short (Only 73bp)
Short Fragment Zymo Cleanup
 Add 1 volume (50uL) ADB Buffer w/ 500 uL EtOH
 Pour onto Zymo spin column
 Spin 15sec. @ 12.5 rcf
 Wash w/ 250uL Wash Buffer 2x & spin 15sec. @ 12.5 rcf
 Full speed 60sec. drying spin
 Elute in 50uL ddH2O
```

- To Do

```
Digest Pcon.rbs & pBjh1600 EcoRI/BamHI
Gel Purify pBjh1600 Vector Digest & Small Fragment Zymo Cleanup Pcon.rbs Digest
Ligate & Transform jtk155- Plate Spec
Pick colony & inoculate overnight culture
Miniprep, map, & sequence
Digest BamHI/XhoI & pBjh1600-hsvTK BglII/XhoI
Gel purify, ligate, & transform jtk155- Plate Spec
Pick colony & inoculate overnight culture
Miniprep, map, & sequence
Digest pBjh1600-Pcon.rbs.hsvTK & pBgl0002 EcoRI/BamHI
Gel purify, ligate, & transform JW1226- Plate Trim
Pick colony & inoculate overnight culture
Miniprep, map, & sequence
Dual Selection w/ Pcon.rbs.hsvTK
```

```
Remake dP Stock Solution?- 10mM Stock=2.6925mg/10mL
Retest Negative Selection

Scrape survivors & grow several hours in 1:50-1:100 dilution in LB+Kan/TRIM
Small scale chem. comp cell prep of survivors
Transform constitutive toxR-mukF plasmid into cells (BOBP)
Plate Kan/TRIM/Spec

Image Lethality Assay results
```

Xin Xin Lin 09:39, 15 July 2011 (PDT)

- Pick MC1061 ffGFP Colonies & Inoculate Overnight Cultures

```
Colonies grew on Trim w/ & w/o Rescue- Same volume (50uL) but w/ Rescue had higher colony density
-Uneven spreading of cells on w/o Rescue plate?- Used plate spinner...
Picked 1 colony from each plate & inoculated 5mL LB+Trim culture- All colonies green
Shake overnight @ 37 degree C
```

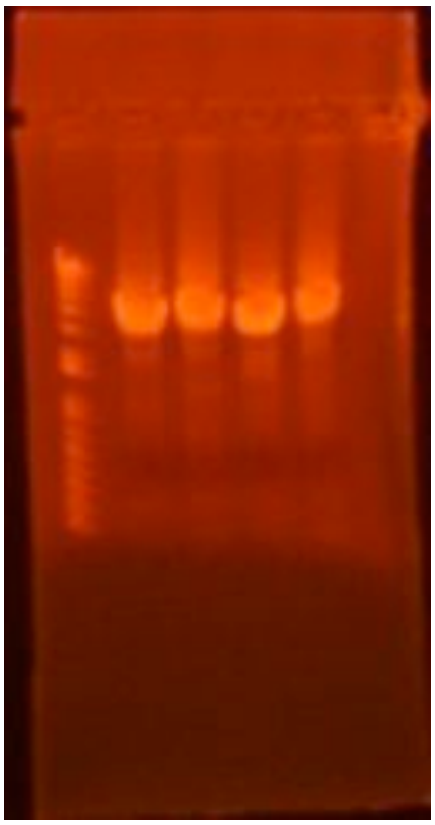
- Sequencing Analysis of pBgl0002-Promoter.hsvTK/ffGFP

```
Long contiguous reads- Samples read well
Complete hsvTK & ffGFP Part, Perfect Pctx Promoter, Same point mutation in Pc+ Promoter
```

- Analytical Gel & Zymo Cleanup on EIPCR rbs Library

```
Load 5uL PCR Product w/ 2uL Dye & 5uL DNA Ladder
Run 10min. @ 180V (9:24-9:34PM)
```

```
Visualize under UV
```



Lane 1=DNA Ladder, Lane 2=A1 (Pctx.hsvTK w/o DMSO), Lane 3=B1 (Pc+.hsvTK w/o DMSO), Lane 4=A2 (w/DMSO), & Lane 5=B2 (w/DMSO)

```
See 3kb band for all reactions-> All worked
-Use A1 & B1
```

Zymo Cleanup

```
Added 150uL ADB Buffer to 50uL volume PCR reaction
Heat @ 55 degree C for 5min.
Spin through Zymo Column 45sec.
Add 200uL Buffer PE 30sec. x2
Dry spin 2min.
Elute w/ 50uL ddH2O (Same Volume as PCR Reaction)
```

- Digestion of EIPCR Product w/ BsaI/DpnI

```
20uL Scale Digestion:
8uL ddH2O
2uL NEB2
8uL Cleaned EIPCR Product
1uL BsaI
1uL DpnI

Digest @ 37 degree C for 1hr. (10:12AM-11:12AM)
Waited extra 30min. for gels to be made- Already added dye
```

- Gel Purification

```
pBgl0002-Bxl9002 Pctx.hsvTK=2921bp
pBgl0002-Bxl9004 Pc+.hsvTK=3146bp
Load all of digest w/ 2uL loading dye & 5uL DNA Ladder
Run 10min. on gel @ 180V (11:44-11:54AM)
Cut out single 3kb band under UV

Dissolve in 700uL ADB Buffer @ 55 degree C for 5min. (12:00-12:05PM)
Spin through Zymo Column 45sec.
Wash w/ 200uL PE Buffer for 30sec. x2
Dry spin 2min.
Elute in 20uL ddH2O for Immediate Ligation
```

- Ligation

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

Incubate on bench for 30min. (12:22-12:52PM)
```

- Transformation

```
Add 50uL JW1226 Chemically Competent Cells (+30uL KCM) to 1uL EIPCR Ligation Product
Incubate on ice for 10min. (1:16-1:26PM)
Rescue w/ 200uL 2YT & shake for 1hr. @ 37 degree C (1:31-2:31PM)
Plate 50uL on Kan/Trim Plates
```

- Wobble Reaction of Pcon.rbs

```
Oligos for Overlap Extension Delivered
Resuspend to 10uM & Make 1:10 Dilution (10uL in 90uL ddH2O)

34uL ddH2O
5uL 10X Expand buffer
5uL 2mM in each dNTP
5uL 100uM Oligos- 2.5uL of each xl045F & xl046R
1uL Taq- Use Expand instead
```

```
PCR Program- Overlap Extension Wobble "Wobb" Program
 Initial Denaturation of 94 degree C for 2 min.
 55 degree C for 30sec.
 72 degree C for 30sec.
 Repeat Steps 2-3 x10
 Hold at 4 degree C Forever

Run PCR Program overnight
```

- SynBERC Picnic

```
Meeting/Activities w/ NorCal iGEM Teams
```

Xin Xin Lin 12:43, 14 July 2011 (PDT)

- Miniprep Overnight Cultures of pBgl0002-Promoter.hsvTK/ffGFP

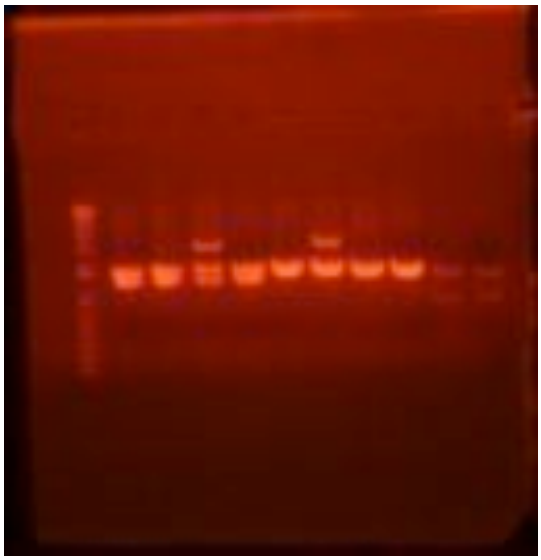
```
10 Cultures=10 Minipreps- A1-4=Pctx.hsvTK, B1-4=Pc+.hsvTK, & C1-2=ffGFP
Elute in 50uL & Map EcoRI/BamHI
```

- EcoRI/BamHI Mapping of Miniprep & Analytical Gel

```
5uL Box H2O
1uL NEB2 Buffer
3uL Miniprep
0.5uL EcoRI
0.5uL BamHI

Vortex well, spin down, & Digest 1hr. @ 37 degree C (12:26-1:26PM)
```

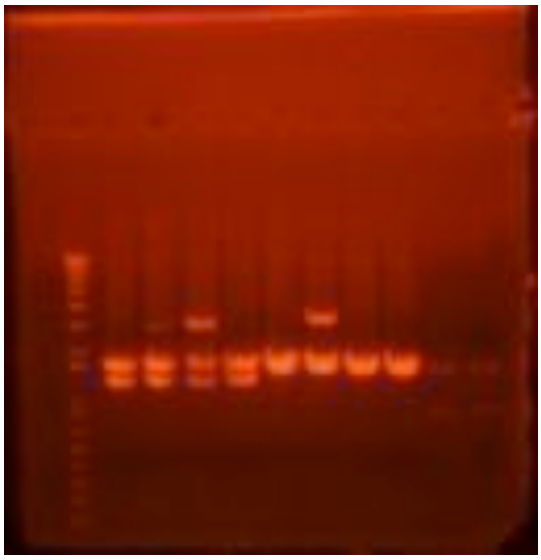
Run Analytical Gel for 10min. @ 180V (1:44PM-1:54PM)

```
Add 2uL Loading Dye to Digests, run w/ 5uL DNA Ladder
Visualize under UV & find Part Bands (Pctx.hsvTK ~1200bp, Pc+.hsvTK ~1500bp, ffGFP ~900bp)
```



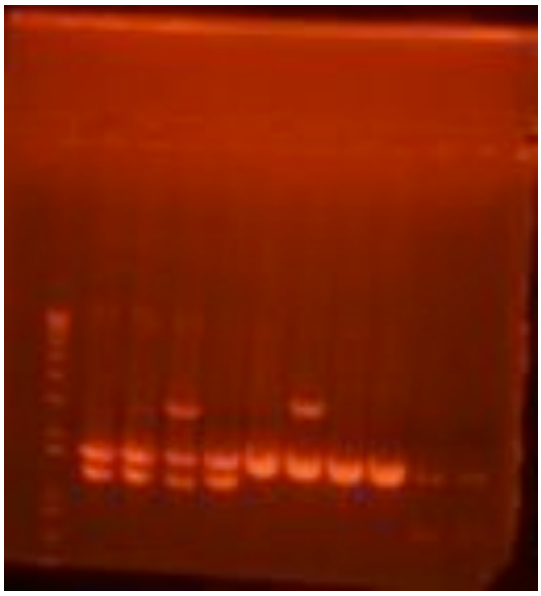
Lane 1=DNA Ladder, Lane 2-5=A1-4, Lane 6-9=B1-4, & Lane 10-11=C1-2

```
Bands much brighter than previous maps- More DNA in Minipreps
Difficult to see separation b/w vector & part for Promoter.hsvTK- Run for additional 5min. (1:59-
ffGFP clearly distinguishable, A3 & B3 have large singly cut band
```



Separation visible for Pctx.hsvTK, Pc+.hsvTK appears undigested?- Only see one band...

```
Bands=very close together/overlap->appear as 1 thick band
Run additional 5min. (2:10-2:15PM)
```



Pc+.hsvTK appears undigested, only 1 singly cut, others unmapped...

```
Sequence A1, B1, & C1- Continue w/ EIPCR on A1 & B1
```

Sequence Minipreps w/ brightest bands

```
xl009, xl010, & xl011- pBgl0002-Pctx.hsvTK, Pc+.hsvTK, & ffGFP
Submit 8uL to sequence w/ ca998/G00101
-Don't need to sequence ffGFP- Green fluorescent phenotype visible
```

- EIPCR rbs Library on Promoter.hsvTK

```
Use Expand Only w/ and w/o DMSO
Use Expand & Phusion Polymerases +/- 2uL DMSO- Phusion said to work better
3 Minipreps/Promoter Construct*2 Polymerases*2 DMSO/No DMSO=24 EIPCR Reactions
-Check Map for best constructs- Use A1 & B1
```

```
Use 50uL Expand & Phusion Master Mix- ddH2O, Buffer, & dNTPs
Pctx.hsvTK                Pc+.hsvTK
1uL xl042F                1uL xl042F
1uL xl043R                1uL xl044R
1uL Pctx.hsvTK Miniprep   1uL Pc+.hsvTK Miniprep
0.5uL Expand              0.5uL Phusion
(2uL 4% DMSO)             (2uL 4% DMSO)

Run 4K55 PCR Program on PCR Block A in Back- Run for ~4hr. (2:55PM-6:55PM)
-Should be 3K55 for both (~3kb Plasmids)
4 EIPCR Reactions- All Expand: A1=pBgl0002-Pctx.hsvTK, B1=pBgl0002-Pc+.hsvTK, A2=Pctx w/ DMSO, B2=

Leave overnight in PCR block- To finish ~7PM
Run analytical gel on EIPCR product- Should have one 3kb band for all reactions
Zymo cleanup & store in freezer
```

- Pcon.rbs Overlap Extension Oligo Design

```
5' Tail-EcoRI-atg-BglII-Pcon.rbs-BamHI-3' Tail
Canonical rbs=Shine-Dalgarno Sequence rbs AGGAGGT for E. coli, 8bp before atg
```

Construction File for pBjh1600-Pcon.rbs

```
Oligos: xl045F Forward CCATAgaattcatgAGATCTttgacaGCTAGCtcagtCCTAGG
        xl046R Reverse CTGATGGATCcACCTCCTGCTAGCattataCCTAGGactgaGCTAGCtgtc
```

```
Wobble xl045F/xl046R          (73bp, EcoRI/BamHI)
Sub into pBjh1600-Bxl9001     (EcoRI/BamHI, 2626/1143bp, L)
Product is pBca9145-Bca9939   {Pcon.rbs}
----
xl045F   Forward construction of Pcon.rbs basic part
CCATAgaattcatgAGATCTttgacaGCTAGCtcagtCCTAGG
xl046R   Reverse construction of Pcon.rbs basic part
CTGATGGATCcACCTCCTGCTAGCattataCCTAGGactgaGCTAGCtgtc
```

- Lethality Assay of Chemical Stocks on E. coli Cells

```
Test whether chemicals kill/prevent growth to saturation
1:1000 overnight culture of MC1061 (SpecR) w/ ffGFP in LB+Spec
-3mL LB+Spec in 24 Well Block w/ 3uL Saturated Culture
-Add 100uM (1/1000 Dilution) of Chemical Stocks
Shake overnight & confirm growth to saturation
-Photograph culture & spun down pellet
Control=No chemicals added
```

```
Transform pBjh1600-jtk2828 (or pBgl0002-jtk2828 ffGFP) into MC1061 chemically competent cells
Add 50uL MC1016+30uL KCM to 0.5uL ffGFP Miniprep
Incubate on ice 10min.- 4:04PM-4:14PM
Rescue in 200uL 2YT Media- Plate 50uL immediately on Trim & shake rest for 1hr. (4:20-5:20PM)
```

```
Plate 50uL on Trim after rescue
```

- To Do

Friday- Come in @ 9AM

```
Run analytical gel on EIPCR product & Zymo Cleanup
-Look for 1 3kb band
Digest BsaI/DpnI, Ligate, & Transform JW1226- Plate Kan/Trim

Create rest of chemical stocks & order additional chemicals
Pick MC1061 colonies & inoculate overnight culture
```

Xin Xin Lin 10:42, 13 July 2011 (PDT)

- Check Transformed Plates & Start Overnight Culture

```
pBgl0002-jtk2828 (ffGFP)- Lawn of tiny green colonies
pBgl0002-Bxl9002 (Pctx.hsvTK)- Many green colonies w/ several white colonies
pBgl0002-Bxl9004 (Pc+.hsvTK)- Many green colonies w/ several white colonies
```

```
Green colonies=singly cut parent plasmids (~1kb difference b/w singly & doubly cut)
-Cut out singly cut band w/ doubly cut band during gel purification
-Ligation for singly cut more efficient->more green colonies
```

```
Inoculate 4 white colonies/Promoter.hsvTK & 2 green colonies for ffGFP in 5mL LB+Trim
Shake overnight @ 37 degree C
-A1-4=Pctx.hsvTK
-B1-4=Pc+.hsvTK
-C1-2=ffGFP
```

- Chemical Stock Solutions

```
Make 10mL 100mM Stocks for liquid chemicals
-1-Butanol, 2-Phenethylamine, Nicotine, Dicyclopropyl Ketone & Tyramine (~1-1.5uL)
Vortex & store in bench fridge
```

- Weekly Meeting

```
Need suggestions/ideas for new safety plan
Need Pcon.hsvTK to test for functionality- Positive Control
-Pc+ not needed- Not using relay system
-Need rbs library for Pcon & Pctx
Weigh chemicals in 1.5mL Eppendorf on balance to prevent loss of material in transfer
-Hygroscopic/Light-Sensitive?- Store in dark
-Add solvent directly to stock bottle if small volumes
```

- To Do

Thursday- Come in @ 9AM

```
Miniprep, Map, & Sequence pBgl0002-Promoter.hsvTK/ffGFP
EIPCR rbs Library onto Assembly, Analytical Gel, & Zymo Cleanup
Digestion w/ BsaI & DpnI- 8uL DNA in 20uL Total
Gel Purification- Elute 20uL
Ligation- Self-Ligate BsaI sites
Transform into -80 degree C chemically competent stock JW1226 cells
Plate Kan/Trim & grow overnight @ 37 degree C

X Pcon.hsvTK Assembly- Digest pBjh1600-Bca1108 BamHI/XhoI & pBjh1600-Bxl9001 BglII/XhoI
X Gel purify, ligate, & transform jtk155 cells- Plate Spec & grow overnight @ 37 degree C
Design Wobble/Klenow Extension Oligos for SOEing & Order- Pcon of varying strengths & Canonical ri
```

Friday- Come in @ 9AM

```
Scrape w/ 3-5mL LB+TRIM/Kan- Inoculate 1uL in 5mL liquid Negative Selection Media
Grow 2-3hr. @ 37 degree C shaker
Plate LB+Kan/TRIM & incubate overnight @ 37 degree C

X Pick colonies & inoculate overnight LB+Spec culture
X Sequencing Analysis of pBgl0002-Promoter.hsvTK Constructs
Wobble Reaction, EcoRI/BamHI Digest, & Zymo Cleanup
Drop into hsvTK Plasmid Digest- BglII/XhoI & BamHI/XhoI

SynBERC/iGEM Picnic/Activities @ 12PM
```

Saturday

```
Scrape survivors & grow several hours in 1:50-1:100 dilution in LB+Kan/TRIM
Small scale chem. comp cell prep of survivors
Transform constitutive toxR-mukF plasmid into cells (BOBP)
Plate Kan/TRIM/Spec & grow overnight

Miniprep, map, & sequence Pcon.hsvTK
Move into pBgl0002 vector- Digest EcoRI/BamHI, gel purify, ligate, & transform jtk155 cells
Plate LB+Trim
```

Sunday

```
Scrape & wash w/ PBS- Spin, Discard, Resuspend x3-4
Resuspend in PBS & Plate Kan/TRIM/Spec- Vary dilutions 1E-4 & 1E-6

Pick colonies & inoculate overnight culture
Sequencing analysis of pBgl0002-Pcon.hsvTK
```

Xin Xin Lin 14:00, 12 July 2011 (PDT)

- Check Transformed Plates- Pctx/Pc+.hsvTK in pBgl0002/ffGFP

```
Colonies on all plates, some a lot smaller

Plate #1, Pctx/gl0002 #1-  Colonies all green
Plate #2, Pctx/GFP #1-     Not green
Plate #3, Pc+/gl0002 #1-   All green w/ smaller colonies in streaks
Plate #4, Pc+/GFP-         No green, some tiny colonies

Plate #5, Pctx/gl0002 #2-  Mostly green, w/ some not green
Plate #6, Pctx/GFP #2-     Not green, all small colonies
Plate #7, Pc+/gl0002 #2-   Mostly green, w/ some not green
Plate #8, Pc+/GFP #2-      No green, all tiny colonies
```

```
ffGFP should be green, pBgl0002 should not be green- Possible switch?!
Plated on wrong antibiotic- Parts moved from SpecR pBjh1600 into Trim/p15A!
-Should have plated on LB+Trim Plates instead of LB+Spec, all current colonies=background
Can't pick colonies & inoculate overnight culture...
Discard plates in Biohazard Waste
```

- Repeat Assembly Experiment of Promoter.hsvTK into pBgl0002/ffGFP

```
pBgl0002-jtk2828 Plasmid Available=ffGFP Reporter Gene (jtk2828) in pBgl0002 Vector
-Transform 0.5uL into jtk155 Cells for more ffGFP Stock
```

EcoRI/BamHI Digestion of Pctx.hsvTK, Pc+.hsvTK, & pBgl0002-jtk2828

```
Want pBgl0002 vector to insert Promoter.hsvTK parts
-ffGFP Part=889bp, pBgl0002 Vector=1637bp Want larger vector
```

```
4uL ddH2O
1uL NEB2 Buffer
4uL Miniprep- Pctx.hsvTK, Pc+.hsvTK, & pBgl0002-jtk2828
0.5uL EcoRI
0.5uL BamHI

Digest 1hr. @ 37 degree C- 11:45AM-12:45PM
```

Gel Purification

```
Add 2uL Loading Dye & run on gel w/ 5uL DNA Ladder for 10min. @ 180V
Visualize under UV Light- Cut out small Promoter.hsvTK bands & large pBgl0002 vector band
```

```
Add 700uL ADB Buffer- Heat 5min. @ 55 degree C
Spin 45sec. in Zymo Column
Add 200uL PE Buffer & Spin 30sec.- x2
Dry spin 2min.
Elute 8.5uL Box H2O for Immediate Ligation
```

Ligation

```
Ligate Pctx.hsvTK/Pc+.hsvTK into pBgl0002 vector

4.25uL Insert- Promoter.hsvTK
4.25uL Vector- pBgl0002
1uL T4 DNA Ligase Buffer
0.5uL T4 DNA Ligase

Ligate on bench @ room temp. for 0.5hr. (1:55PM-2:25PM)
```

Transformation

```
Transform jtk155 chemically competent cells
-1uL pBgl0002-Pctx.hsvTK & pBgl0002-Pc+.hsvTK, 0.5uL pBgl0002-jtk2828 (ffGFP)
Add 50uL Cell Cocktail w/ 30uL KCM
Incubate on ice 10min. (2:40-2:50PM)
Rescue in 200uL 2YT for 1hr. @ 37 degree C (2:55-3:55PM)
Plate on Trim plates & incubate overnight @ 37 degree C
```

- Sequencing Analysis

```
xl008 (Pc+.hsvTK) ca998 Forward- Point mutation in promoter (Substitution of C for T)
-Point mutations towards end of hsvTK Part 1 but poorer read
-hsvTK Part 2 not read well
xl008 G00101 Reverse- Very short read & not of part
```

```
xl007 (Pctx.hsvTK) Forward- Perfect promoter sequence
-Point mutations towards end of hsvTK Part 1 w/ poorer read
-hsvTK Part 2 not read well
xl007 Reverse- Perfect hsvTK Part 2
-Point mutation towards end of hsvTK Part 1- Insertion of C Frameshift
```

- Make LB+Trim Media & Pour Plates

```
Microwave 1L LB Agar for 4min. @ 100%
Inverter Turbo Defrost 6.0lb. for 22min.
Microwave additional 2-3min.
Let cool to touch & add 900uL Trim Antibiotic
```

```
Pour 2 bags of Trim plates- Label w/ purple marker
Let dry on benchtop
```

- Make Chemical Stock Solutions

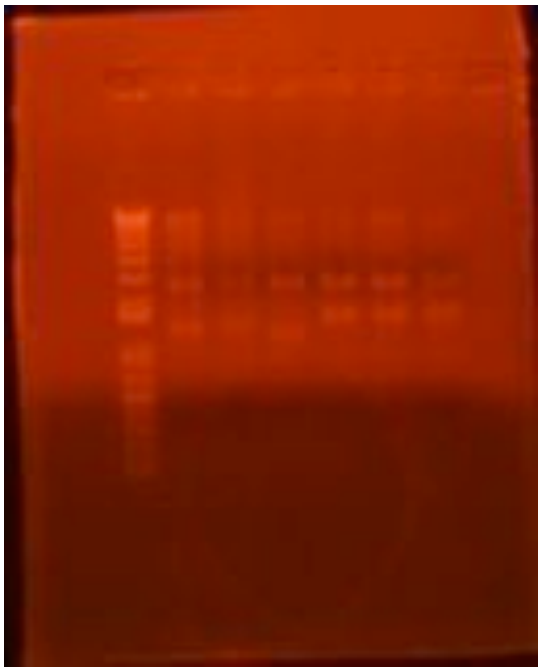
```
Calculate mass/volume needed to make 1mM stock solution
MW(g)/L=1M->0.001MW(g)/L=1mM->0.001MW(mg)/mL=1mM
Dissolve in H2O or DMSO- Gives 1mL of 1mM Stock Solution
```

```
Make 1mL 100mM Chemical Stock instead?- Use 100mM Calculator
https://spreadsheets.google.com/spreadsheet/ccc?key=0ApSMw9eiO5C-dC15eEV2ZmJIelhHd3REemo4T1FJTEE&
-Add 1mL Box H2O/DMSO into 1.5mL Eppendorfs
-Weigh out 0.001MW(mg) (~10-50mg) on Weight Paper in Balance in Fume Hood & add to solvent
-Measurements likely inaccurate due to negative pressure of fume hood- Changes constantly
-Vortex to dissolve

Stored stock solutions & chemicals in fridge (Bench fridge & Equipment Room fridge)
```

Xin Xin Lin 11:23, 11 July 2011 (PDT)

- EcoRI/BamHI Mapping of Promoter.hsvTK Minipreps

```
Map all 6 Minipreps- Find best map (Part ~1200bp. Vector ~2600bp) & Submit for sequencing
5uL ddH2O
1uL NEB2 Buffer
3uL hsvTKI Miniprep
0.5uL EcoRI
0.5uL BamHI

Digest @ 37 degree C for 1hr. (10:38AM-11:38PM)
Run on Gel 10min. @ 180V (11:51AM-12:01PM) & Image on Gel Box- Expect 1293bp Pctx.hsvTK & 1518bp
-5uL Digest+2uL Dye & 5uL Ladder
Identify best of 3 Pctx & Pc+
```



Correct Mapping: Lane 1=DNA Ladder, Lanes 2-4=Pctx.hsvTK, Lanes 5-7=Pc+.hsvTK

```
1000bp<Pctx.hsvTK<Pc+.hsvTK<1650bp, Digested pBjh1600>2000bp
Pctx.hsvTK 1+3 & Pc+.hsvTK 1+2= Brighter- All are visible but fainter than expected
Use Pctx.hsvTK #1 & Pc+.hsvTK #1 for Sequencing & Digestion
```

Sequencing

Sequencing

```
Submit 12uL Pctx.hsvTK #1 & Pc+.hsvTK #1 for sequencing w/ ca998/G00101
xl007=Pctx.hsvTK, xl008=Pc+.hsvTK
```

- Sequencing Analysis of Assembled hsvTK (xl006)

```
xl006 w/ ca998- Good Trace 30-790bp
-Perfect Read of hsvTK Part I & portions of Part II- Insertion of G @ 799bp towards end (Frameshi
xl006 w/ G00101- Bad Read, Not enough sample for reverse read...
-Submit more sample for reverse read? Submit new assembled promoters
```

- Assembly of Promoter.hsvTK into pBgl0002 Trim/p15A Plasmid

Digestion

```
EcoRI/BamHI Digest Pctx.hsvTK & Pc+.hsvTK Minipreps- Pre-Digested pBgl0002 & ffGFP (Control)
4uL ddH2O
1uL NEB2 Buffer
4uL Promoter.hsvTK Miniprep #1
0.5uL EcoRI
0.5uL BamHI

Digest 1hr. @ 37 degree C (1:03-2:03PM)
```

Gel Purification

```
Run on gel for 12min. @ 180V (2:12-2:24PM)
Visualize under UV & cut out smaller band (~1200 & 1500bp)
```

```
Add 700uL Buffer ADB- Heat @ 55 degree C for 5min. & Mix/Shake (2:34-2:39PM)
Run through Zymo column for 45sec.
Wash w/ 200uL Buffer PE & Spin 30sec.- x2
Dry 90sec. & Elute w/ 8.5uL ddH2O
-Forgot to dry before eluting- 1st Elution=#1 (EtOH in Wash Buffer messes up DNA...)
-Dry column again for 2min. & elute 8.5uL- 2nd Elution=#2 (DNA in either Elution 1 or 2)
```

Ligation

```
Ligate Promoter.hsvTK Sets #1 & #2 w/ pBgl0002 & ffGFP
-Pctx.hsvTK/pBgl0002, Pc+.hsvTK/pBgl0002, Pctx.hsvTK/ffGFP, & Pc+.hsvTK/ffGFP x2 (#1/#2)
-Not enough pBgl0002 & ffGFP- Add Box H2O until Vol.=17uL (4.25uL*4 Sets)
```

```
8.5uL DNA- 4.25uL Promoter.hsvTK & 4.25uL Vector
1uL Ligase Buffer [w/ ATP, DTT, MgCl2]
0.5uL T4 DNA Ligase

Ligate 1/2hr. on bench (3:28PM-4:08PM)
```

Transform Chemically Competent jtk155 Cells

```
1uL Ligation DNA + 50uL jtk155 Cells/30uL KCM
Incubate on Ice 10min.- 4:18PM-4:28PM
Labels: 1-pBgl0002-Pctx.hsvTK #1
        2-ffGFP-Pctx.hsvTK #1
        3-pBgl0002-Pc+.hsvTK #1
        4-ffGFP-Pc+.hsvTK #1

        5-pBgl0002-Pctx.hsvTK #2
        6-ffGFP-Pctx.hsvTK #2
        7-pBgl0002-Pc+.hsvTK #2
        8-ffGFP-Pctx.hsvTK #2
Rescue w/ 200uL 2YT Media & Shake 1hr. @ 37 degree C- 4:35PM-5:35PM
```

Pour Spec Plates

```
Microwave 500mL LB Agar 2min. @ 100%
Inverter Turbo Defrost @ 3.0lb.- 12min., stops after 6min.->Press Start again
Microwave additional 1-2min. if still not completely melted
Let cool to touch, Add 500uL Spec Antibiotic, Swirl, & Pour Plates
```

```
Let dry under flame/cool on ice+fridge & plate 50uL Transformants (Can incubate up to 1.5hr.)
-Incubated transformants in rescue media for extra 30min. (To 6:05PM)
Incubate @ 37 degree C overnight
```

- Order Oligos for KpnI/SpeI Split of mukF Interface 6aa Library

```
xl006R & xl007F- xl005F & xl008R already ordered, assumed others were ordered as well but not the
Checked Logged Oligos Orders & Emailed Bioneer
```

- EIPCR rbs Library on Promoter.hsvTK- Start Wednesday... Use Expand Only w/ and w/o DMSO

```
Use Expand & Phusion Polymerases +/- 2uL DMSO- Phusion said to work better
3 Minipreps/Promoter Construct*2 Polymerases*2 DMSO/No DMSO=24 EIPCR Reactions
-Check Map for best constructs
```

```
Use 50uL Expand & Phusion Master Mix- ddH2O, Buffer, & dNTPs
Pctx.hsvTK                Pc+.hsvTK
1uL xl042F                1uL xl042F
1uL xl043R                1uL xl044R
1uL Pctx.hsvTK Miniprep   1uL Pc+.hsvTK Miniprep
0.5uL Expand              0.5uL Phusion
(2uL 4% DMSO)             (2uL 4% DMSO)
```

- To Do

Tuesday

```
Pick 2-3 colonies from all plates & inoculate 5mL LB+Spec overnight culture
Sequencing Analysis of Promoter.hsvTK Constructs
Clarify Protocol for rbs Library Screening
Order Chemicals?
Make 100mM Chemical Stock Solutions
```

Wednesday

```
Miniprep, Map, & Sequence Promoter.hsvTK in pBgl0002/ffGFP
EIPCR rbs Library, 4K55 PCR Program, Zymo Cleanup, & Analytical Gel
Digest w/ BsaI & DpnI, Gel Purify, Self-Ligate, & Transform Chemically Competent JW1226 Cells
-Plate Kan/Trim
```

Xin Xin Lin 19:05, 10 July 2011 (PDT)

Sunday, 6:15PM-7:30PM

- Miniprep Promoter.hsvTK Overnight Culture

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6 Miniprep Products- Pctx.hsvTK 1-3 & Pc+.hsvTK 1-3
Need to EcoRI/BamHI Digest & Map
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Xin Xin Lin 22:37, 9 July 2011 (PDT)

Saturday, 10:00PM-10:32PM

- Inoculate Overnight Cultures

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Plates taken out earlier from 37 degree C incubator
Inoculated 6 overnight cultures w/ 3 colonies/promoter
-Pctx.hsvTK 1-3 & Pc+.hsvTK 1-3
Shake overnight @ 37 degree C
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- Sequencing Analysis

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Got results for assembled hsvTK product w/ ca998/G00101
Not enough DNA for reverse read?- Good forward read
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Xin Xin Lin 11:51, 8 July 2011 (PDT)

- Miniprep of Assembled hsvTKI in jtk155

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Spin 1.5mL overnight culture in 2mL tube for 30sec.- Discard supernatant & repeat
Add 250uL P1- Vortex vigourously to mix well
Add 250uL P2- Swirl & invert gently
Add 350uL N3- Swirl & invert, then shake vigorously
Spin 10min.- Pour off supernatant into Miniprep column
Spin 30sec.- Discard flowthrough
Add 500uL PB- Spin 30sec.
Add 750uL PE- Spin 30sec.
Spin Dry 2min.
Elute in 50uL Box H2O- Let sit 1min. & spin 1min.
```

- EcoRI/BamHI Digest Mapping of hsvTK Miniprep

- EcoRI/BamHI Digest Mapping of hsvTK Miniprep

```
5uL ddH2O
1uL NEB2 Buffer
3uL hsvTKI Miniprep
0.5uL EcoRI
0.5uL BamHI

Digest @ 37 degree C for 1hr. (11:38AM-12:38PM)
Run on Gel 10min. @ 100V (12:43-12:53PM) & Image on Gel Box- Expect 1128bp sized hsvTK product
-5uL Digest+2uL Dye & 5uL Ladder
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2 very faint bands of hsvTK digests- Run 10 more min. to separate ladder bands
Should be 1143bp (Part) & 2626bp (Vector) bands
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Bands still faint, but present- Ladder bands difficult to resolve & compare
Appears ~1kb & 2kb bands- Digestion products
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- Assembly of Promoter & hsvTK Constructs