Lab Diary for Gyrase Binding Site

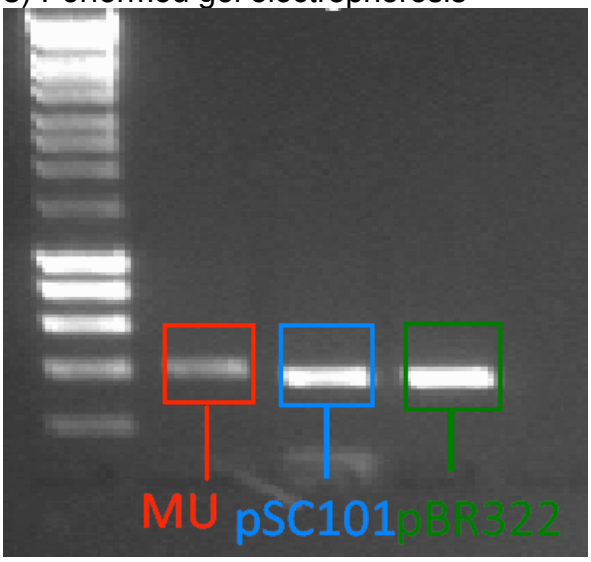
Date: Tuesday, 20 August 2011 11:00
Topic: Re-suspension of primers and PCR reaction for the 3 GBS

1) Carried out re-suspension of primers (forward and reverse primers of mu, pSC101 and pBR322).
2) Conducted a PCR of gene MU, pSC101 and pBR 322.

Materials	Volume (μl)
water	32.5
5X HF buffer	10.0
dNTPs	1.0
Forward primer	2.5
Reverse primer	2.5
Template	1.0
Phusion Enzyme	0.5
Total	50

PCR setting:
Step 1- 1 cycle
98ºC for 30 sec
Step 2 – 30 cycle
98 ºC for 10 sec
65 ºC for 30 sec
72 ºC for 15 sec
Step 3- 1 cycle
72 ºC for 10 min
Step 4
4 ºC – hold

3) Performed gel electrophoresis



Date: Wednessday, 21 August 2011 11:00
Topic: PCR Purification, Digestion, Ligation and Transformation.

1) Carried out PCR purification and recovered 50 μl of PCR product.
2) Set up restriction digestion with PCR purification product.:

Materials	**CM Backbone(μl)**	**pSC101 (μl)**	**pBR322 (μl)**	**MU (μl)**
DNA	20	20	20	20
EcoRI	0.5	0.5	0.5	0.5
PstI	0.5	0.5	0.5	0.5
DpnI	0.5	-	-	-
Buffer 2	5	5	5	5
BSA	0.5	0.5	0.5	0.5
water	23	23.5	23.5	23.5
Total	50	50	50	50

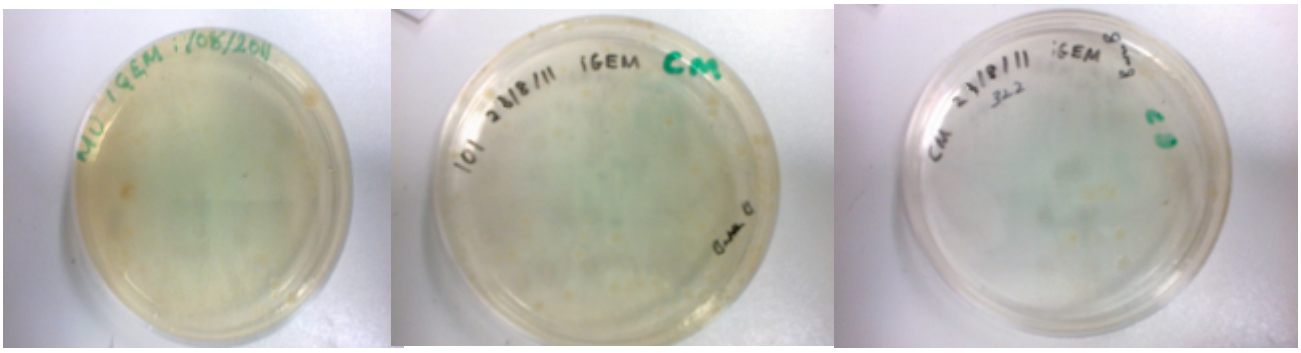
3) Set up the following ligation mixtures:

Material	**A1 (μl)**	**A2 (μl)**	**A3 (μl)**
CM backbone	4.5	4.5	4.5
MU	4.0		
pBR322		4.0	
pSC101			4.0
Ligase	0.5	0.5	0.5
Ligase Buffer	1.0	1.0	1.0
Total	10	10	10

4)Transformation

Organism: TOP 10 *E. Coli*

1) Carried out transformation for Gyrase binding sites MU, pSC101 and pBR322.
2) Plated the transformed cells on the chloramphenicol agar plates.
3) Observed colonies in all the three plates.



Date: Thursday, 22 August 2011 11:00

Topic: Setup Overnight Cultures

1) Set up overnight cultures from a single colony from each of the transformed cell plates in selective LB medium. Left the cultures to grow overnight inside the shaker.

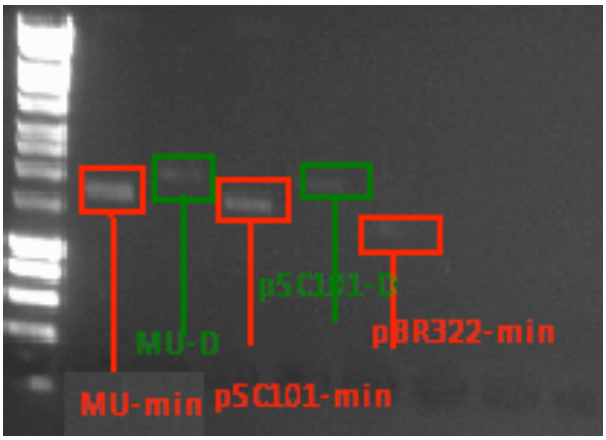
Date: Friday, 23 August 2011 11:00

Topic: Preparing Glycerol stock, Miniprep, Digestion, Gel.

1) Set up glycerol stock from the overnight culture and stored it at -80°C.
2) Mini-prepped the overnight culture into 10 µl volume.
3) Digested 7.5 µl of the mini-prep sample with EcoRI and PstI. Ran the digestion mixture on agarose gel by electrophoreses. The bands confirmed the size of the final construct and plasmid backbone.

Material	**B1 (MU) (µl)**	**B2(pBR322) (µl)**	**B3 (pSB101) (µl)**
Miniprep sample	7.5	7.5	7.5
EcoRI	0.5	0.5	0.5
PstI	0.5	0.5	0.5
Buffer2	1.0	1.0	1.0
BSA	0.5	0.5	0.5
Total	10	10	10

4) Performed gel electrophoresis for Digestion and mini-prep samples.



min- miniprep sample
D- Digestion sample

Date: Saturday, 24 August 2011 11:00
Topic: Sequencing.
1) Sent 10 µl of mini-prep sample to Wolfson Institute for Biomedical Research for sequencing of all three GBS.

Digestion and ligation had failed, the experiment was repeated using new digestion and ligation protocols.
Experiment continued with three GBS (MU, pBR 322, pSC 101) and two backbones (pSB1C3, pSB3T5).

Date: Thursday, 27August 2011 11:00
Topic: Digestion

1) Set up restriction digestion of pSB1C3:

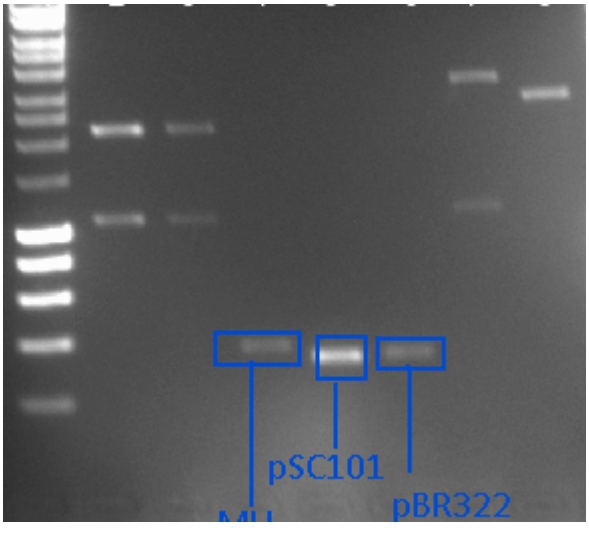
Material	**E13 (µl)**
pSB1C3	4.5
EcoRI	1.0
PstI	1.0
Buffer3	5.0
BSA	0.5

dH2O	38
Total	50

Date: Sunday, 28 August 2011 16:59
Topic: digestion and ligation
1) Set up restriction digestion for MU, pBR322, pSC101 and pSB3T5:

Materials	**E17 (μl)**	**E18(μl)**	**E19(μl)**	**E20(μl)**
Mu	20			
pBR322		19		
pSC101			22	
pSB3T5				3.5
EcoRI	1	1	1	1
PstI	1	1	1	1
Buffer 3	5	5	5	5
BSA	0.5	0.5	0.5	0.5
Water	22.5	23.5	20.5	39.0
Total	50	50	50	50

2) carried out gel electrophoresis of digestion product and all were successful.



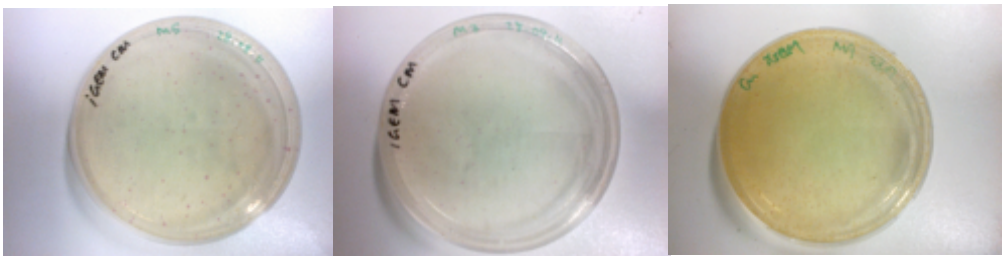
3) Set up the following ligation:

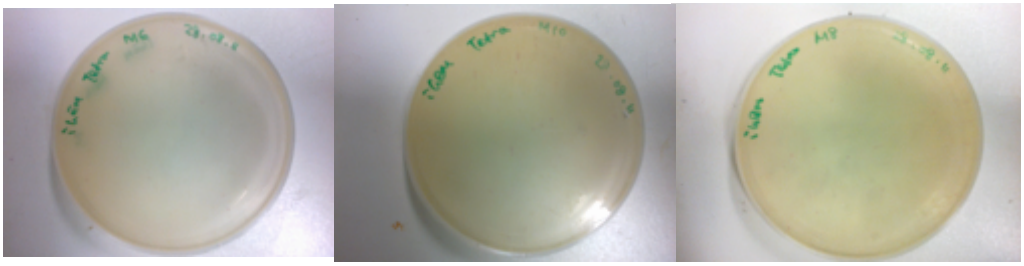
Materials	M5(μl)	M6(μl)	M7(μl)	M8(μl)	M9(μl)	M10(μl)
Mu (E17)	6	6				
pBR322 (E18)			6	6		
pSC101 (E19)					6	6
pSB1C3 (E13)	2		2		2	
pSB3T5 (E20)		2		2		2
Ligase	1	1	1	1	1	1
Ligase buffer	2	2	2	2	2	2
Water	9	9	9	9	9	9
Total	20	20	20	20	20	20

4) Transformation
Transformed a stock of competent E coli with the ligation mixture M5, M6, M7, M8, M9, M10
Plated M5, M7, M9 on chloramphenicol plates
Plated M6, M8 & M10 on tetracycline plates

Date: Monday, 29 August 2011 18:00
Topic: Overnight cultures

1) Observed colonies in all the six plates.





2) Set up overnight cultures of five colonies, from the chloramphenicol plate and tetracycline plates in selective LB medium. Left the culture to grow overnight inside the shaker.

Date: Tuesday, 30 August 2011 11:00
Topic: Mini-preps and competent cells

1) Set up glycerol stock from the chloramphenicol overnight culture and stored it at -80°C, Forgot to set up glycerol stock from the tetracycline overnight cultures.

2) Mini-prepped the overnight culture into 50 µl volume and measured concentration using nano-drop.

Table showing nano-drop readings.

Sample µl	1 nanodrop (ng)	2 nanodrop (ng)	Mean nanodrop (ng)	Volume of DNA for Digestion	Plasmid	BioBrick
M5 mini-prep (1)	3.3	3.2	3.3	154	Mu/ pSB1C3	Mu
M5 mini-prep (2)	46.4	42.7	44.6	11		
M5 mini-prep (3)	30.7	30.7	30.7	16		
M5 mini-prep (4)	25.6	30.7	28.2	18		
M5 mini-prep (5)	80.0	75.4	77.7	6		
M6 mini-prep (1)	45.9	41.8	43.9	11	Mu/ pSB3T5	
M6 mini-prep (2)	70.4	85.4	77.9	6		
M6 mini-prep (3)	94.0	99.7	96.9	5		

M6 mini-prep (4)	83.7	89.6	86.7	6		
M6 mini-prep (5)	72.1	75.5	73.8	7		
M7 mini-prep (1)	36.3	37.2	36.8	14	pBR322/ pSB1C3	pBR322
M7 mini-prep (2)	37.2	37.8	37.5	13		
M7 mini-prep (3)	102.4	96.5	99.5	5		
M7 mini-prep (4)	34.9	36.6	35.8	14		
M7 mini-prep (5)	38.6	34.1	36.4	14		
M8 mini-prep (1)	144.3	157.0	150.7	3	pBR322/ pSB3T5	
M8 mini-prep (2)	75.5	75.8	75.7	7		
M8 mini-prep (3)	209.0	208.9	209.0	2		
M8 mini-prep (4)	85.7	87.4	86.6	6		
M8 mini-prep (5)	105.8	105.3	105.6	5		
M9 mini-prep (1)	168.9	132.9	150.9	3	pSC101/ pSB1C3	pSC101
M9 mini-prep (2)	15.0	16.0	15.5	32		
M9 mini-prep (3)	54.4	56.0	55.2	9		
M9 mini-prep (4)	42.9	44.8	43.9	11		
M9 mini-prep (5)	97.8	97.8	97.8	5		
M10 mini-prep (1)	74.3	96.3	85.3	6	pSC101/ pSB3T5	
M10 mini-prep (2)	71.2	72.5	71.9	7		
M10 mini-prep (3)	84.2	82.5	83.4	6		
M10 mini-prep (4)	nil	nil	76.3	7		
M10 mini-prep (5)	70.1	59.2	64.7	8		

Date: Wednesday, 31 August 2011 12:00
Topic: Digestion

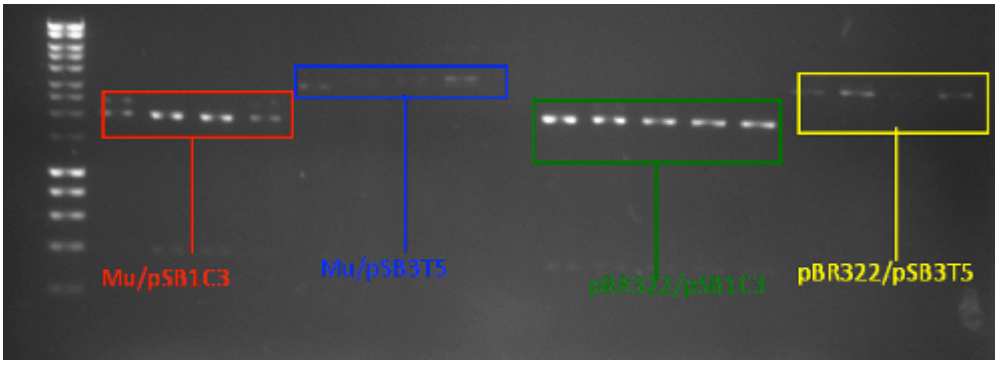
1) Carried out digestion of all the mini-preps and performed gel electrophoresis

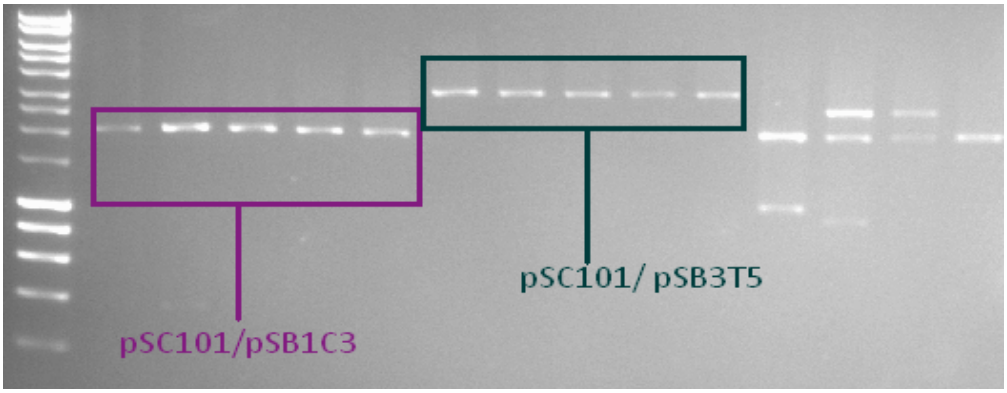
Sample µl	E22	E23	E24	E25	E26	E27	E28	E29	E30	E31	E32	E33	E34	E35	E36
M5 mini-prep (1)															
M5 mini-prep (2)		11													
M5 mini-prep (3)			16												
M5 mini-prep (4)				18											
M5 mini-prep (5)					6										
M6 mini-prep (1)						11									
M6 mini-prep (2)							6								
M6 mini-prep (3)								5							
M6 mini-prep (4)									6						
M6 mini-prep (5)										7					
M7 mini-prep (1)											14				
M7 mini-prep												13			

(2)															
M7 mini-prep (3)													5		
M7 mini-prep (4)														14	
M7 mini-prep (5)															14
EcoRI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PstI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Enzyme Buffer 3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
BSA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water	42.5	31.5	26.5	24.5	36.5	31.5	36.5	37.5	36.5	35.5	28.5	29.5	37.5	28.5	28.5
TOTAL	50	50	10	10	50	50	50	50	50	10	10	50	50	50	50

Sample	E37	E38	E39	E40	E41	E42	E43	E44	E45	E46	E47	E48	E49	E50	E51
M8 mini-prep (1)	3														
M8 mini-prep (2)		7													
M8 mini-prep (3)			2												
M8 mini-prep (4)				6											
M8 mini-prep (5)					5										
M9 mini-prep (1)						3									
M9 mini-prep (2)							32								
M9 mini-prep (3)								9							
M9 mini-prep (4)									11						
M9 mini-prep (5)										5					
M10 mini-prep (1)											6				
M10 mini-prep (2)												7			
M10 mini-prep (3)													6		
M10 mini-prep (4)														7	
M10 mini-prep (5)															8
EcoRI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PstI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Enzyme Buffer 3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
BSA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water	39.5	35.5	40.5	36.5	37.5	39.5	10.5	33.5	31.5	37.5	36.5	35.5	36.5	35.5	34.5
TOTAL	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

2) Performed gel electrophoresis





3) Repeated gel for digested mini-preps for M9, M10.

Date: Thursday, 1 September 2011 11:00
Topic: Sent for sequencing

Sent 10 µl of mini-prep sample to Wolfson Institute for Biomedical Research for sequencing. Sent the following mini-prep sample for sequencing: M5 (3) **Mu/pSB1C3**; M7 (3) **Mu/ pSB3T5**; M9 (2) **Mu/ pSB3T5.**

Sequencing Results:

Mu- success
Mu

TTGTTCAGACGCTGAATAACAGCCGGGAGCGTCAGCTTGCTTTTTCCCTCTGGC
TTAAACGCCTGGCAGGATCACGTCAGACCGGTCATGAAATCCGGGCGCTGGGG
TTTATGACGGGATCCGTGGCTGAAGCCGTATATCAGCGCACCGGGAATATGCCA
GCCCGTCTGCTTGTAATGAACGGGAAAAGTCTGGCGACCACTGCTGATGCCGC
CCTGAAACCGGAGGATTTACAGCGTCTGCCGTCGCTGATGGCAAAACCACAGG
CGGTATTGTGGGACAGGGA

The sequencing data was analysed with 'ApE' software and all the parts for the construct are present.

PBR3222- rev primer wrong.
pSC101- rev primer wrong.

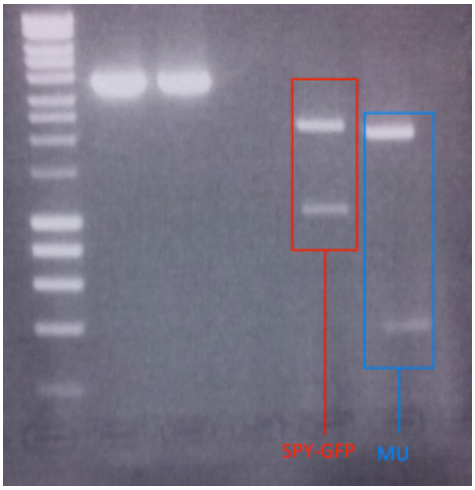
Construct a plasmid with spy device with MU GBS.

Date: Friday, 02/September 2011 11:00
Topic: Digestion and ligation for MU and SPY**.**

1) Set up restriction digestion for Spy and mu
Spy was restricted with XbaI and PstI , MU was digested with ECORI and SpeI

Materials	**E52**	**E53**
SPY-GFP (mini2)	1.5	
Mu		13
ECORI		1
XbaI	1	
PstI	1	
SpeI		1
Buffer	5	5
BSA	0.5	0.5
Water	41.0	29.5
Total	50	50

2) Carried out gel electrophoresis of digestion product and all were successful.



3) Set up the following ligation mixtures

Materials	**M12**
SPY-GFP (E52)	5
Mu(E53)	5
pSB1A3	3
Ligase	1
Ligase Buffer	2
Water	4
Total	20

4) Transformation

Carried out transformation of competent cell stock with Spy/MU miniprep sample.
Plated cells for Spy/MU on ampiciline plates.

Date: Saturday, 03/September 2011 11:00
Topic: Overnight cultures for mu/spy, spy and overnight shake flask cultures of 3 GBS for 1D chloroquine gel.

1) Seed stock: Added 10 ul of spy/GFP glycerol stock from 2009 to10 ml LB medium falcon tubes.
Incubated at 37ºC for 5 hours at 250 rpm
Added 10ul of kanamycin to LB medium and left the cell to grow overnight.

Seed stock: Select 1 colony from the M12 (MU/SPY) plate and set up overnight culture.

Set up overnight shake flask cultures for 3 GBS on pSB1C3 and control of **psB1C3 (spy with RFP insert)**
Added 50 ml LB medium with no antibiotic in each flask
Set to grow at 37 ºC at 175 rpm.

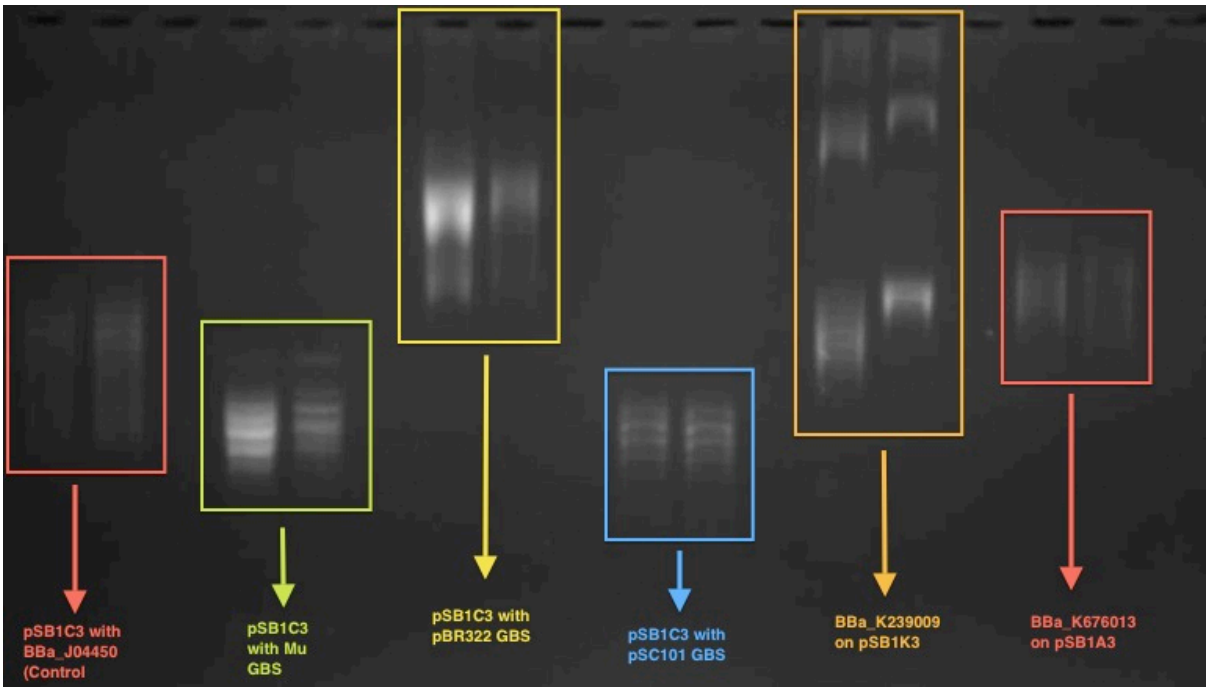
Date: Monday, 05/September 2011 11:00
Topic: Miniprep for the 3 GBS shake flask and setup chloroquine gel

1) Prepared 3 mini-prep samples from each shake flask culture (50ul each)
2) Measured nano drop concentration.
3) Loaded a fixed mass (600ng) of DNA from each sample into a 1D chloroquine gel.

Date: Tuesday, 06/September 2011 11:00
Topic: visualised chloroquine gel for GBS

1) stained, destained and visualised chloroquine gel for 3 GBS

The 1D chloroquine agarose gel electrophoresis



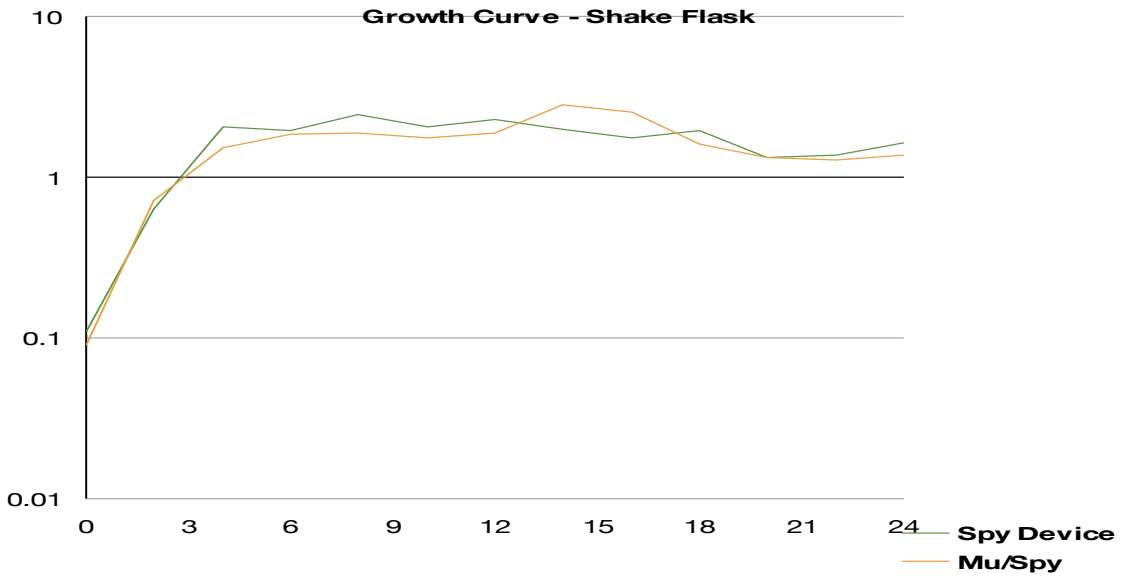
Date: Wednesday, 07/September 2011 11:00
Topic: Shake flask Setup for MU/SPY and SPY and OD reading

1) Setup shake flask (37ºC at 175 rpm for inoculation)
2) Collected 2 ml samples every 2 hours for 24 hours.
3) 1ml will be used to get OD reading and the other 1 ml sample will be centrifuged, pelleted and stored at -20 ºC.

OD Results and Graph

Time (hrs)	OD (S)	OD (MS)
Inoculum	*1.61*	*1.47*
0	0.11	0.09
2	0.63	0.71
4	2.05	1.53
6	1.96	1.85
8	2.45	1.89
10	2.06	1.75

12	2.3	1.87
14	1.98	2.8
16	1.77	2.53
18	1.94	1.62
20	1.32	1.32
22	1.37	1.27
24	1.64	1.38



Date: Thursday, 08/September 2011 11:00
Topic: GFP Scan

1) Re-suspend the shake flask samples in 400 ul of water.
2) Loaded 200ul on to the 96-well plate for GFP Scan.
3) Samples were taken from the shake flask every 2 hours to detect the amount of fluorescent given by the cells.

GFP Scan parameters

SAFIRE II; Serial number: 12904200032; Firmware: V 2.10 12/2007 Safire2; XFLUOR4SAFIREII Version: V 4.62n		
Date:		10/09/2011
Time:		14:27
Measurement mode:		Fluorescence Top
Excitation wavelength:		483 nm
Emission wavelength:		525 nm

Excitation bandwidth:		20 nm
Emission bandwidth:		20 nm
Gain (Manual):		62
Number of reads:		1
FlashMode:		High speed
Integration time:		20 µs
Lag time:		0 µs
Plate definition file:		GRE96ft.pdf
Plate with cover		
Part of the plate:		A1 - E12
Z-Position (Manual):		10200 µm
Target Temperature:		22 °C
Current Temperature:		30.5 °C

Result – GFP SCAN

Time (hours)	2	4	6	8	10	12	14	16	18	20	22	24
Spy	613	1621	2150	2581	2521	2809	2470	3250	3282	3642	2954	3589
Mu/Spy	858	3964	5713	6501	6946	6722	7167	10061	8037	7117	6977	7426
Water	20	18	17	19	17	18	19	19	19	16	17	19
Spy	593	1603	2133	2562	2504	2791	2451	3231	3263	3626	2937	3570
Mu/Spy	838	3946	5696	6482	6929	6704	7148	10042	8018	7101	6960	7407