Group 3 Notebook: August

WEDNESDAY, 01/08/2018

PCR Purification

Concentration of pQE80L-XylR*-1-1*a (from reaction 1) : 37.9 ng/ul in 40 ul
Concentration of pQE80L-XylR*-1-1*b (from reaction 2) : 111.5 ng/ul in 40 ul

Gel Electrophoresis

Band observed for lane 1 (pQE80L-XylR*-1-1*a).
Primer bands only for lane 2, may account for higher concentration observed for pQE80L-XylR*-1-1*b.

DpnI Digestion (as above)

For pQE80L-XylR*-1-1*a only.

Transformation into DH5α

10 ul added to competent DH5α.

THURSDAY, 02/08/2018

pQE80L-XylR*-1 colony 1 has the mutation (R121C) but contains multiple overlap of fragments

Inoculation

Inoculate colonies 2, 3, 4 from DH5α-pQE80L-XylR*-1, and colonies 1, 2, 3 from DH5α-pQE80L-XylR*-1-1*a in 10 ml of LB broth with 10 ul of ampicillin.

FRIDAY, 03/08/2018

Plasmid Extraction

pQE80L-XylR*-1 colony 2 : 189.5 ng/ul in 40 ul
pQE80L-XylR*-1 colony 3 : 220.7 ng/ul in 40 ul
pQE80L-XylR*-1 colony 4 : 210.2 ng/ul in 40 ul
pQE80L-XylR*-1-1*a colony 1 : 184.6 ng/ul in 40 ul
pQE80L-XylR*-1-1*a colony 2 : 190.2 ng/ul in 40 ul
pQE80L-XylR*-1-1*a colony 3 : 196.4 ng/ul in 40 ul
To be sent for sequencing.

TUESDAY, 07/08/2018

pQE80L-XylR*-1 colony 2 contains the correct sequence.
pQE80L-XylR*-1-1*a colonies have the mutation (P363S) but contain multiple overlap of fragments.

P363S Mutagenesis

Table9

	A	B	C	D	E	F	G	H
1		Volume added (ul)						
2	pQE80L-XylR*-1 colony 2 (189.5 ng/ul)	0.5						
3	10 uM P363S_FP	1.0			Initial denaturation	95°C	120s	
4	10 uM P363S_RP	1.0		30X	Denaturation	95°C	20s	
5	2.5 mM dNTP	4.0			Annealing	**55°C**	20s	
6	5X TranStart Fast Pfu Buffer	10.0			Elongation	72°C	**180s**	~6kb
7	TranStart Fast Pfu DNA polymerase	1.0			Final elongation	72°C	120s	
8	Nuclease-free water	32.5				12°C	∞	
9	Total	50.0						

Mutagenesis for P363S re-attempted using pQE80L-XylR*-1 colony 2 and reduced final elongation time of 2 min, as previous elongation time of 5 min may have contributed to the repeated sequences in pQE80L-XylR*-1 colony 1.

PCR Purification
Concentration of pQE80L-XylR** : 83.3 ng/ul in 40 ul

DpnI Digestion (as above)

Transformation into DH5α
10 ul added to competent DH5α.

WEDNESDAY, 08/08/2018

Colony Patching and Inoculation
pQE80L-XylR** colonies 1 to 9 patched onto new agar plate.
pQE80L-XylR** colonies 2, 4, 9 inoculated in 10 ml of LB broth with 10 ul of ampicillin.

FRIDAY, 10/08/2018

Plasmid Extraction
pQE80L-XylR** colony 2 : 204.1 ng/ul in 40 ul
pQE80L-XylR** colony 4 : 200.0 ng/ul in 40 ul
pQE80L-XylR** colony 9 : 239.0 ng/ul in 40 ul
To be sent for sequencing.

TUESDAY, 14/08/2018

pQE80L-XylR** colony 9 contains the correct sequence.

Inoculation
pQE80L-XylR** colony 9 inoculated in 3 ml of LB broth with 3 ul of ampicillin.

WEDNESDAY, 15/08/2018

Preparation of DH5α-pQE80L-XylR glycerol stock**

THURSDAY, 16/08/2018

Inoculation of DH5α(λpir)-pUC18R6K (suicide plasmid) in 5 ml of LB + gentamycin (15)
Inoculation of DH5α-pTNS2 (helper plasmid) in 5ml of LB + ampicillin (100)

FRIDAY, 17/08/2018

Preparation of DH5α(λpir)-pUC18R6K glycerol stock
Preparation of DH5α-pTNS2 glycerol stock

Plasmid Extraction
pUC18R6K : 202.8 ng/ul in 50 ul
pTNS2 : 217.7 ng/ul in 50 ul

TUESDAY, 28/08/2018

PCR Amplification of gblocks (for stress reporter constructions)

Table1

	A	B	C	D	E	F	G	H	I	J
1		PhtpG1RBS gblock	tSpinach2 gblock	tBroccoli gblock						
2		Volume added (ul)								
3	DNA (10 ng/ul)	1.00	1.00	1.00			Initial denaturation	98°C	180s	
4	10 uM FP*	1.00	1.00	1.00		34X	Denaturation	98°C	10s	
5	10 uM RP*	1.00	1.00	1.00			Annealing**	**50-53°C**	60s	
6	10 mM dNTP	1.00	1.00	1.00			Elongation	72°C	**20s**	<171bp
7	100% DMSO	2.50	2.50	2.50			Final elongation	72°C	300s	
8	10X Std Taq Buffer	5.00	5.00	5.00				12°C	∞	
9	Taq polymerase	0.25	0.25	0.25						
10	Nuclease-free water	38.25	38.25	38.25						
11	Total	50.0	50.0	50.0						

*Respective FP and RP used
**Annealing temperature used for PhtpG1RBS, tSpinach2, and tBroccoli are 50°C. 52°C, and 53°C respectively.

Gel Electrophoresis
50 ul of PCR product mixed with 10 ul of loading dye.

40 ul of mixture, and 7 ul of Quick-Load® 100 bp DNA Ladder loaded.
<image>

20 ul of mixture, 7 ul of primer mixture and 7 ul of Quick-Load® 100 bp DNA Ladder loaded.
<image>

Band is not due to primers.

Gel Extraction
Concentration of PhtpG1 : 35.0 ng/ul
Concentration of tSpinach2 : 18.3 ng/ul
Concentration of tBroccoli : 7.6 ng/ul

WEDNESDAY, 29/08/2018

Restriction Digestion

Table3

	A	B	C	D
1	Reaction	PhtpG1 (35.0 ng/ul)	tSpinach2 (18.3 ng/ul)	tBrocooli (7.6 ng/ul)
2		Volume added (ul)		
3	PCR product (~200 ng)	6.0	11.0	25.0
4	10X FastDigest Green Buffer	3.0	3.0	3.0
5	FastDigest BamHI	1.0	1.0	1.0
6	FastDigest HindIII	1.0	1.0	1.0
7	Nuclease-free water	19.0	14.0	0.0
8	Total	30.0	30.0	30.0

Ligation

Table2

	A	B	C	D	E
1	Reaction	Reaction 1	Reaction 2 (using higher insert volume)		
2		Volume added (ul)			
3	Digested pQE80L (50 ng/ul)	1.0	1.0		
4	Insert: digested PhtpG1 / digested tSpinach2 / digested tBroccoli	1.0	5.0		
5	T4 DNA ligase	1.0	1.0		
6	T4 DNA ligase buffer	2.0	2.0		
7	Nuclease-free water	15.0	11.0		
8	Total	20.0	20.0		

Transformation into Top10

THURSDAY, 30/08/2018

Colony picking

More colonies observed in Reaction 1 using lower volume of insert compared to Reaction 2.
Two colonies from each plate were picked and inoculated in 5 ml of LB broth and 5 ul of ampicillin.