Group 3 Notebook: July

WEDNESDAY, 11/07/2018

PCR Amplification of XylR gene from Top10 gDNA

Table1

	A	B	C	D	E	F	G	H
1		Volume added (ul)						
2	Top10 gDNA	2.0			Initial denaturation	98°C	30s	
3	10 uM XylR_FP	2.5		34X	Denaturation	98°C	10s	
4	10 uM XylR_RP	2.5			Annealing	**68°C**	30s	
5	10 mM dNTP	1.0			Elongation	72°C	**35s**	1179bp
6	Q5 reaction buffer	5.0			Final elongation	72°C	120s	
7	Q5 polymerase	0.5				12°C	∞	
8	Nuclease-free water	36.5						
9	Total	50.0						

PCR Purification

Concentration of XylR : 90.3 ng/ul in 40 ul

Gel Electrophoresis

<image>

1+ kb band observed.

Restriction Digestion of pQE80L and XylR

Table2

	A	B	C
1	Reaction	pQE80L (289.6 ng/ul)	XylR (90.3 ng/ul)
2		Volume added (ul)	
3	DNA	3.5 (~ 1 ug)	2.2 (~ 0.2 ug)
4	10X FastDigest Green Buffer	2.0	3.0
5	FastDigest BamHI	1.0	1.0
6	FastDigest HindIII	1.0	1.0
7	Nuclease-free water	12.5	22.8
8	Total	20	30

1. Add and mix the above.
2. Incubate at 37°C for 50 min.

PCR Purification

Concentration of pQE80L : 10.1 ng/ul in 40 ul
Concentration of XylR : 2.5 ng/ul in 40 ul

Synchronized degradation tag characterization

1. Transfer 200 µl of overnight culture into fresh 5 mL of LB+K (done the previous day).
2. Incubate at 37°C, 225 rpm for 1 hour (done the previous day).
3. Measure cell density at $OD_{600.}$
4. Transfer 1ml of cell culture final OD_{600} = 0.1, diluted using LB+K, into a 12-well plate. LB+K is added as the blank.
5. Repeat step 6 for triplicates.
6. Measure OD_{600} and fluorescence intensity (535/600 nm) in a microplate reader using the following settings:
   Temperature: Set-point 37°C
   Plate In
   Shake: Orbital for 10s
   Read: (A) 600
   Read: (F) 535,600
   Excitation: 535
   Emission: 600
   Gain: 75
   Read height: 6 mm
   Plate Out
7. Cover the 12-well plate with a dark cloth.
8. Incubate at 25°C without shaking.
9. Repeat steps 8-10 every 1 hour over a period of 8 hours.

Experiment failed due to wrong incubation temperature and rpm used.

THURSDAY, 12/07/2018

Ligation

Table3

	A	B	C	D	E
1	Reaction	Reaction 1: using previously cut pQE80L (52.4 ng/ul)	Reaction 2: using cut pQE80L from above (10.1 ng/ul)		
2		Volume added (ul)			
3	pQE80L	1.0	5.0	~ 37.5 ng	3:1 ratio, using NEBioCalculator for ligation
4	XylR	15.0	15.0	~ 50 ng	
5	T4 DNA ligase	1.0	1.0		
6	T4 DNA ligase buffer	2.0	2.5		
7	Nuclease-free water	1.0	1.5		
8	Total	20.0	25.0		

Incubation at 25°C for 35 min.

Transformation into DH5α

Transform 10 ul from reaction 1 and 15 ul from reaction 2 separately to competent DH5α.
Spread onto AmpR plates: DH5α-pQE80L-XylR-1 and DH5α-pQE80L-XylR-2.

FRIDAY, 13/07/2018

Colony patching

Satellite colonies observed.
Patch 10 colonies from both DH5α-pQE80L-XylR-1 and DH5α-pQE80L-XylR-2 plates onto new agar plates.
Incubate at 37°C.

Colony PCR

Table4

	A	B	C	D	E	F	G	H
1		Volume added (ul)						
2	10 uM XylR_FP	1.0			Initial denaturation	95°C	10s	
3	10 uM XylR_RP	1.0		34X	Denaturation	95°C	10s	
4	10 mM dNTP	1.0			Annealing	**55°C**	30s	
5	10X Std Taq Buffer	5.0			Elongation	72°C	**72s**	1179bp
6	Taq polymerase	0.5			Final elongation	72°C	300s	
7	Nuclease-free water	41.5				12°C	∞	
8	Total	50.0						

1. Prepare two of the above reaction.
2. Mix and transfer 10 ul into four other tubes.
3. Pick colonies 1 to 5 from DH5α-pQE80L-XylR-1 and DH5α-pQE80L-XylR-2 respectively and transfer to respective tubes.

Gel Electrophoresis

1kb band observed for all lanes except lane 10 (DH5α-pQE80L-XylR-2 colony 5).
Intensity of band highest for lane 3 (DH5α-pQE80L-XylR-1 colony 3), followed by lane 5 (DH5α-pQE80L-XylR-1 colony 5).

Quadrant Streak DH5α-pQE80L-XylR-1 colony 3 onto new AmpR agar plate and incubate at 37°C overnight.

Synchronized degradation tag characterization

1. Transfer 200 µl of overnight culture into fresh 5 mL of LB+K (done the previous day).
2. Incubate at 37°C, 225 rpm for 1 hour (done the previous day).
3. Measure cell density at $OD_{600.}$
4. Transfer 1ml of cell culture final OD_{600} = 0.1, diluted using LB+K, into a 12-well plate. LB+K is added as the blank.
5. Repeat step 6 for triplicates.
6. Measure OD_{600} and fluorescence intensity (535/600 nm) in a microplate reader using the following settings:
   Temperature: Set-point 37°C
   Plate In
   Shake: Orbital for 10s
   Read: (A) 600
   Read: (F) 535,600
   Excitation: 535
   Emission: 600
   Gain: 75

Read height: 6 mm
Plate Out

7. Cover the 12-well plate with a dark cloth.
8. Incubate at 37°C, 120 rpm.
9. Repeat steps 8-10 every 1 hour over a period of 8 hours.

MONDAY, 16/07/2018

Inoculation of DH5α-pQE80L-XylR into 10 ml of LB broth and 10 ul of ampicillin

TUESDAY, 17/07/2018

Preparation of DH5α-pQE80L-XylR glycerol stock

Plasmid Extraction

Concentration of pQE80L-XylR : 132.7 ng/ul in 40 ul
To be sent for sequencing.

Synchronized degradation tag characterization (re-run)

1. Transfer 200 µl of overnight culture into fresh 5 mL of LB+K (done the previous day).
2. Incubate at 37°C, 225 rpm for 1 hour (done the previous day).
3. Measure cell density at $OD_{600.}$
4. Transfer 1ml of cell culture final OD_{600} = 0.1, diluted using LB+K, into a 12-well plate. LB+K is added as the blank.
5. Repeat step 6 for triplicates.
6. Measure OD_{600} and fluorescence intensity (535/600 nm) in a microplate reader using the following settings:
   Temperature: Set-point 37°C
   Plate In
   Shake: Orbital for 10s
   Read: (A) 600
   Read: (F) 535,600
   Excitation: 535
   Emission: 600
   Gain: 75
   Read height: 6 mm
   Plate Out
7. Cover the 12-well plate with a dark cloth.
8. Incubate at 25°C without shaking.
9. Repeat steps 8-10 every 1 hour over a period of 8 hours.

THURSDAY, 19/07/2018

XylR sequence confirmed.

PCR Mutagenesis

Table5

	A	B	C	D	E	F	G	H	I	J
1		Reaction 1 (R121C)	Reaction 2 (P363S)	Reaction 3 (R121C, P363S)						
2		Volume added (ul)								
3	pQE80L-XylR (132.7 ng/ul)	0.5	0.5	0.5			Initial denaturation	95°C	120s	
4	10 uM R121C_FP	1.0	0	1.0		30X	Denaturation	95°C	20s	
5	10 uM R121C_RP	1.0	0	1.0			Annealing	**58°C**	20s	
6	10 uM P363S_FP	0	1.0	1.0			Elongation	72°C	**180s**	~6kb
7	10 uM P363S_RP	0	1.0	1.0			Final elongation	72°C	300s	
8	2.5 mM dNTP	4.0	4.0	4.0				12°C	∞	
9	5X TranStart Fast Pfu Buffer	10.0	10.0	10.0						
10	TranStart Fast Pfu DNA polymerase	1.0	1.0	1.0						
11	Nuclease-free water	32.5	32.5	30.5						
12	Total	50.0	50.0	50.0						

DpnI Digestion

Table7

	A	B
1		Volume added (ul)
2	DNA from PCR mutagenesis	30.0
3	10X CutSmart Buffer	3.5
4	DpnI	1.0
5	Nuclease-free water	0.5
6	Total	35.0

The above was conducted for all three reactions. Incubate at 37°C for 1 hr.

TUESDAY, 24/07/2018

PCR Purification

Concentration of DH5α-pQE80L-XylR*-1 (R121C) : 112.6 ng/ul in 40 ul
Concentration of DH5α-pQE80L-XylR*-2 (P363S) : 57.1 ng/ul in 40 ul
Concentration of DH5α-pQE80L-XylR*-3 (R121C, P363S) : 100.8 ng/ul in 40 ul

Transformation into DH5α

2 ul of DNA added to competent DH5α.
DH5α-pQE80L-XylR*-1 (R121C)
DH5α-pQE80L-XylR*-2 (P363S)
DH5α-pQE80L-XylR*-3 (R121C, P363S)

WEDNESDAY, 25/07/2018

Colony Patching and Inoculation

DH5α-pQE80L-XylR*-1 (R121C) : many colonies
DH5α-pQE80L-XylR*-2 (P363S) : no colony
DH5α-pQE80L-XylR*-3 (R121C, P363S) : 7 colonies

Patch 7 colonies from DH5α-pQE80L-XylR*-1 and -3 onto new plate.
Incubate at 37°C.
Inoculate colony 1 from both DH5α-pQE80L-XylR*-1 and -3 in 10 ml of LB broth with 10 ul of ampicillin.

THURSDAY, 26/07/2018

Plasmid Extraction

DH5α-pQE80L-XylR*-1 colony 1 : 118.1 ng/ul in 40 ul
DH5α-pQE80L-XylR*-3 colony 1 : 85.3 ng/ul in 40 ul
To be sent for sequencing

Inoculation

Inoculate colonies 6 and 7 from both DH5α-pQE80L-XylR*-1, and colonies 4 and 6 from DH5α-pQE80L-XylR*-3 in 10 ml of LB broth with 10 ul of ampicillin.

FRIDAY, 27/07/2018

Plasmid Extraction

DH5α-pQE80L-XylR*-1 colony 6 : 102.8 ng/ul in 40ul
DH5α-pQE80L-XylR*-1 colony 7 : 97.8 ng/ul in 40 ul
DH5α-pQE80L-XylR*-3 colony 4 : 119.4 ng/ul in 40 ul
DH5α-pQE80L-XylR*-3 colony 6 : 77.4 ng/ul in 40 ul
To be sent for sequencing.

TUESDAY, 31/07/2018

P363S Mutagenesis

Table6

	A	B	C	D	E	F	G	H	I
1		Reaction 1 (R121C)	Reaction 2 (P363S)						
2		Volume added (ul)							
3	pQE80L-XylR*-1 colony 1 (118.1 ng/ul)	0.5	0.5						
4	10 uM P363S_FP	1.0	10.0			Initial denaturation	95°C	120s	
5	10 uM P363S_RP	1.0	10.0		30X	Denaturation	95°C	20s	
6	2.5 mM dNTP	4.0	4.0			Annealing	**55°C**	20s	
7	5X TranStart Fast Pfu Buffer	10.0	10.0			Elongation	72°C	**180s**	~6kb
8	TranStart Fast Pfu DNA polymerase	1.0	1.0			Final elongation	72°C	300s	
9	Nuclease-free water	32.5	14.5				12°C	∞	
10	Total	50.0	50.0						

Mutagenesis for P363S re-attempted using higher primer concentrations (Reaction 2) and lower Tm (55°C).