Cloning Journal

TUESDAY, 7/2/2019

quantified the purified Plasmid see table

quantification of Plasmid purification

	A	B	C
1	batch	name	conc (ng/ul)
2	1	1	19.7
3	1	2	68.7
4	1	3	81.0
5	1	4	44.6
6	1	5	48.4
7	1	6	44.4
8	1	7	38.6
9	1	8	47.2
10	1	9	59.0
11	1	10	59.2
12			
13	2	1	45.9
14	2	2	31.2
15	2	3	22.3
16	2	4	28.0
17	2	5	29.0
18	2	6	50.8
19	2	7	5.2
20	2	8	170.8
21	2	9	45.5
22	2	10	28.4
23	2	11	31.5
24	2	12	4.7
25	2	13	31.9
26	2	14	66.1
27	2	15	57.3
28	2	16	45.0
29	2	17	21.6
30			
31			
32			
33			
34			
35			
36			

37			

- Design of Constructs (BioBricks)

Construct I:

(LacI regulated promoter + rbs) + Lysis gene + RFP coding device psB1K3.

Procedure:

3A assembly of the BioBricks cloned in psB1K3 RFP coding device.

i. Restriction digestion
a. Upstream
LacI regulated promoter+rbs

Table1

	A	B	C
1	Reagents	Volume (uL)	
2	EcoRI	0.5	
3	SpeI	0.5	
4	NEB Buffer 2.1 (10x)	2.5	
5	Milli Q	16	
6	LacI plasmid	5.5 (250ng)	
7	TOTAL	25uL	

b. Downstream
Lysis gene

Table2

	A	B
1	Reagents	Volume (uL)
2	EcoRI	0.5
3	SpeI	0.5
4	NEB Buffe 2.1 (10x)	2.5
5	Milli Q	17.3
6	Lysis gene	4.2 (250ng)
7	TOTAL	25uL

c. destination plasmid RFP coding device psB1K3 (kanamycin resistance)

Table3

	A	B
1	Reagents	Volume (uL)
2	EcoRI	0.5
3	SpeI	0.5
4	NEB Buffe 2.1 (10x)	2.5
5	Milli Q	10.3
6	LacI plasmid	11.2 (250ng)
7	TOTAL	25

Ligation of the 2 fragments with the Destination plasmid:

Table4

	A	B
1	Components	Volume (20uL rxn)
2	Upstream part digested	2 uL
3	Downstream part digested	2 uL
4	Destination plasmid digested	2 uL
5	T4 DNA ligase buffer (10x)	2 uL
6	T4 DNA Ligase	1 uL
7	MilliQ	11 uL
8	TOTAL	20 uL

Transformation of the construct with BL21 E.coli competent cells via Heat shock method:

Prepare LB-agar plates with the required selective antibiotic; end concentrations:

- - Ampicillin: 150 µg/ml
- - Kanamycin: 50 µg/ml
- - Chloramphenicol: 10 µg/ml
- - Tetracyclin: 12.5 µg/ml
- ☐ Add ligation mixture or plasmid DNA to the cup with competent cells
  - - Use 40-50 µl competent cells
  - - the volume of DNA should not be higher than 1/10 the volume of the cells
- ☐ 30 min on ice
- ☐ Heat shock 5 min at 37°C water bath (or 1 min at 42°C)
- ☐ Add 850 µl LB
- ☐ Incubate for 60 min at 37°C; 220 rpm to allow expression of antibiotic marker
- ☐ Make dilutions of the transformed cells; plate 100 µl per plate; approx. 3 plates;
  f.e. undiluted + 10x and 100x diluted (this will depend on the expectations of success; transforming ligation mix will give much less transformants than purified plasmid)
- ☐ Add some sterile glycerol to the leftover transformation-cells to an end-conc. of 10% and keep at -20°C
- ☐ Incubate the plates overnight at 37°C

Kanamycin: 250uL to 100mL LB/ LB+agar

Colonies bearing RFP and w/o RFP appeared on the plates with LB+Km

Colony PCR using primers VF2 and VR was carried out:

Picking colonies:

- - Pick the colonies with a toothpick (sterile) [we picked the red colonies, which was false-positive]
- - Make a smear of the cells at the bottom of a PCR tube
- - Then use the toothpick to inoculate 1 ml of LB medium (+ antibiotics) for an

overnightculture (a sterile 1.5 ml Eppendorf tube can be used)

☐ Prepare one PCR master mix for all your samples (+1 extra)

Total volume per reaction = 20 µl

- - DreamTaq buffer, containing MgCl2 (10x)
- - dNTPs (2.5 mM)
- - Forward primer (25 mM)
- - Reverse primer (25 mM)
- - pfuX7
- - mQ

2 µl 2µl 0.5 µl 0.5 µl 0.1 µl 14.9 µl

☐ add 20 µl mastermix to each tube containing colony-cells

Gel electrophoresis to check the construct was carried out.

5uL of loading dye was added to 20uL samples and only 10uL of samples were loaded on the gel.

SATURDAY, 8/3/2019

researched on transformation of Vibrio - naturally competent?-> LBv2 prepared for tomorrow (https://pubs.acs.org/doi/suppl/10.1021/acssynbio.7b00116/suppl_file/sb7b00116_si_001.pdf)

plates poured from 100 ml of LBv2 with Amp (60 ul of 50 mg/ml stock to achieve 30 ug/ml), Tet (50 ul of 5 mg/ml stock to achieve 2.5 ug/ml), Kan (1000 ul of 20 mg/ml stock to achieve 200 ug/ml), Cam (300 ul of 10 mg/ml stock to achieve 15 ug/ml)

Colony PCR using primers VF2 and VR was carried out:

Picking colonies:

- - Pick the colonies with a toothpick (sterile) [white colonies]
- - Make a smear of the cells at the bottom of a PCR tube
- - Then use the toothpick to inoculate 1 ml of LB medium (+ antibiotics) for an overnightculture (a sterile 1.5 ml Eppendorf tube can be used)

☐ Prepare one PCR master mix for all your samples (+1 extra)

Total volume per reaction = 20 µl

- - DreamTaq buffer, containing MgCl2 (10x)
- - dNTPs (2.5 mM)
- - Forward primer (25 mM)
- - Reverse primer (25 mM)
- - pfuX7
- - mQ

2 µl 2µl 0.5 µl 0.5 µl 0.1 µl 14.9 µl

☐ add 20 µl mastermix to each tube containing colony-cells

Gel electrophoresis to check the construct was carried out.

5uL of loading dye was added to 20uL samples and only 10uL of samples were loaded on the gel.

MONDAY, 8/5/2019

Plan: talk to Amanda and finalize gRNA design, figure out new transformation protocol

Confirm construct 1

Culture from Sat was added by 2 ml LB + kanamycin (50 ug/ml) and incubated for another 2 hours

Plasmid was isolation for colony 1,2,3,4,6,7,8,10

Plasmid was digested with PstI and EcoRI for colony 3 & 6

Restriction reaction. Reaction was incubated at 37C overnight

Table5

	A	B
1	Reagents	Volumes
2	EcoRI	1 ul
3	PstI	1 ul
4	NEB Buffe 2.1 (10x)	5 ul
5	Milli Q	35.5 ul
6	Plasmid	7.5 ul
7	TOTAL	50 ul
8		
9		