Vibrio SPL pTet and pBAD

FRIDAY, 9/6/2019

Primer design and order, digestion & ligation

Primers were designed and ordered at IDT

the goal is to optimize pTet and pBAD

primers ordered to make the SPL

	A	B	C
1	SPL fw pBAD	attagcggatcctacctgacgnnnnnnnnnnnnnnnnnntawwatnnnnacatacccgttttttg	
2	SPL rev pBAD	caggtaggatccg	
3	SPL rev pTet	agggactctagaagc	
4	SPL fw pTet	gccgcttctagagnnnnnnnnnnnnnnnnnnnttgacannnnnnnnnnnnnnnnngatactnnnnactactagagaaagag	

procedure:

- [ ] clone pBAD_mCherry (4153 bp) & pTet_mCherry (2998 bp)
- [ ] run PCR with the according Primers
- [ ] circulize Plasmid with BamHI (pBAD_mCherry) & XbaI (pTet_mCherry)
- [ ] screen for non leaky constructs (no fluorescence)
- [ ] induce and screen for strong constructs (high fluorescence)
- [ ] quantify the promoter

Cloning

pSB1K3 , BBa_K808000 , BBa_J06602 , BBa_R0040 were digested according to 3A assembly cloning strategy

digestion

	A	B	C	D	E	F	G	H
1	part	pSB1K3	BBa_K808000	BBa_J06602	BBa_R0040			
2	length (bp)		1209	780				
3	assembly	pBAD_mCherry	pBAD_mCherry	pBAD_mCherry				
4	assembly	pTet_mCherry		pTet_mCherry	pTet_mCherry			
5								
6	quantities [µl]							
7	DNA	5	5	5	5			
8	buffer 2.1	2.5	2.5	2.5	2.5			
9	PstI	0.5	0	0.5	0			
10	EcoRI	0.5	0.5	0	0.5			
11	SpeI	0	0.5	0	0.5			
12	XbaI	0	0	0.5	0			
13								
14	water	16.5	16.5	16.5	16.5			

90 min at 37 °C, 20 min at 80 °C (heat inactivation)

Next, the parts were combined according to the table below

ligation

	A	B	C
1	assembly	pBAD_mCherry	pTet_mCherry
2	quantities [µl]		
3	pSB1K3	2	2
4	BBa_J06602	2	2
5	BBa_K808000	2	0
6	BBa_R0040	0	2
7			
8	ligation buffer	2	2
9	ligase	2	2
10	water	10	10
11			
12			
13			

First, the inserts were incubated in RT for approx an hour, after which the backbone was added and incubated at 4 °C overnight.

SATURDAY, 9/7/2019

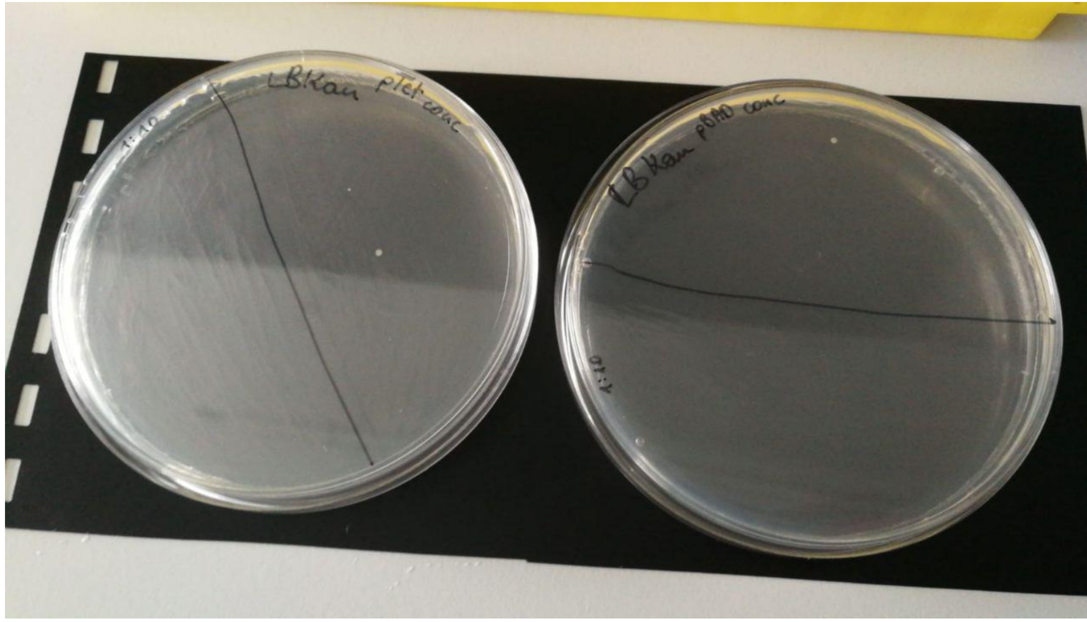
transformation & check of ligation

there is no gel dye, so the transformation is done without confirming the ligation

SUNDAY, 9/8/2019

colony PCR & reculturing of correct clones

photo_2019-09-09_09-44-15.jpg

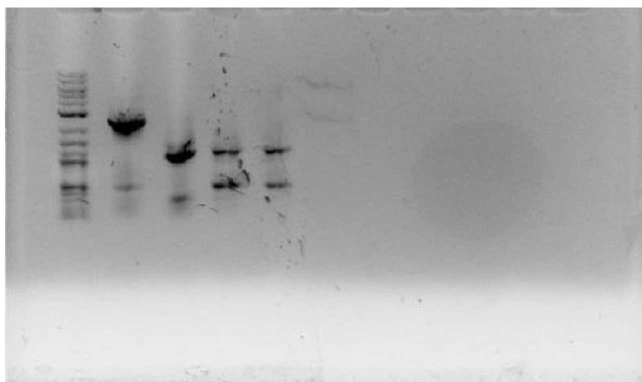


left: pTet_mCherry (3 clones), right: pBad_mCherry (1 clone)

only 4 clones in total have grown

- plated the all of the rest of the transformation (approx. 600 μl; was in the fridge overnight in non selective media)
- colony PCR with all of the grown clones (more clones but not many more)

WhatsApp Image 2019-09-08 at 20.19.44.jpeg



the desired lenghts: pTet_ mCherry (1108 bp) pBAD_mCherry (2263 bp); not desired length of RFP coding device (1367 bp)

TUESDAY, 9/10/2019

Plasmid purification & trial to confirm constructs

The strains were plated on plates with and without inducer (500 ng/ml aTC or 0.2 % w/v arabinose)

- one of the platings (pTet 1) is red without inducer -> RFP coding device is in

WEDNESDAY, 9/11/2019

pTet4, pTet5 and pBAD cultured o/n to make glycerol stocks

THURSDAY, 9/12/2019

SPL PCR & start of Vibrio culture for chemically competent cells

SPL PCR

PCR and check on gel -> if gel purification is needed

circulize Plasmids

digestion & ligation

if transformation doesn't work try gibson ligation (maybe one of the kits from jena included exonuclease)

FRIDAY, 9/13/2019

preparation of competent cells & chemical transformation of Vibrio with SPL plasmids