trc - RFP, blue light sensor, blue light repressor

THURSDAY, 8/29/2019

here the assembly of three parts is described: pSB1A3 - trc - RFP , pSB1A3 blue light sensor , pSB1C3 - FixK2 - tetR

assemblies

	A	B	C	D	E	F	G	H
1	assembly	pSB1A3 - trc - RFP	pSB1A3 blue light sensor	pSB1C3 - FixK2 - tetR				
2								
3	backbone	pSB1K3	pSB1K3	pSB1A3				
4	insert1	modified: trc - RFP	modified: strong promoter and YF1	modified: FixK2 blue inducible promoter				
5	insert2		modified: RBS - FixJ - half of the other	BBa_S0107				
6								
7								
8								
9								
10								
11								
12								
13								
14								

the parts will be assembled using digestion ligation

quick overview: PCR to modify ends, PCR purification, digestion, ligation, transformation

the ends of inserts trc-RFP, YF1, FixJ & FixK2 are modified by **PCR** to fit together in the desired way

PCR conditions:

Denaturation: 94 degrees celcius, 3 min and 30 sec. Same for all inserts

Annealing (repeated 28 to 30 times):

- FixK2: 58 degrees celcius for 30 sec.
- trc-RFG: 40 degrees celcius for 30 sec.
- YF1: 40 degrees celcius for 30 sec
- FixJ: 40 degrees celcius for 30 sec
- FixJ *: 38 degrees for 30 sec

Extension: All 72 degrees celcius

- FixK2: 20 sec + 5 min
- trc-RFG: 1 min + 5 min
- YF1: 1 min and 15 sec + 5 min
- FixJ: 1 min + 5 min
- FixJ *: 1 min + 5 min

Storage: at 4 degrees celcius in the PCR machine or in the fridge.

FRIDAY, 8/30/2019

PCR Purification

PCR products are purified to remove salts, enzymes etc....

Concentrations:

- FixK2: 54,00
- trc-RFG: 26,65

SUNDAY, 9/1/2019

digestion of the parts

quantities for digestion

	A	B	C	D	E	F	G	H	I	J	K
1	part	pSB1K3	pSB1A3	modified: trc - RFP	modified: strong promoter and YF1	modified: RBS - FixJ - half of the other	modified: FixK2 blue inducible promoter	BBa_S0107			
2	assembly	pSB1A3 - trc - RFP		pSB1A3 - trc - RFP							
3	assembly	pSB1A3 blue light sensor			pSB1A3 blue light sensor	pSB1A3 blue light sensor					
4	assembly		pSB1C3 - FixK2 - tetR				pSB1C3 - FixK2 - tetR	pSB1C3 - FixK2 - tetR			
5											
6	quantities [µl]										
7	DNA	5	5	5	5	5	5	5			
8	buffer 2.1	2.5	2.5	2.5	2.5	2.5	2.5	2.5			
9	BsaI	0	0	0.5	0	0	0.5	0			
10	EcoRI	0.5	0	0	0.5	0	0	0.5			
11	SpeI	0.5	0.5	0	0	0.5	0	0.5			
12	XbaI	0	0.5	0	0	0	0	0			
13	SlaI	0	0	0	2.5	2.5	0	0			
14	water	16.5	16.5	17	14.5	14.5	17	16.5			
15											
16											
17											
18											
19											
20											
21											
22											

parts are digested according to the table above. conditions for digestion:

1. 80 min, 37 °C
2. 20 min, 80 °C

assembly and **ligation** of the parts

quantities for ligation

	A	B	C	D	E	F	G	H
1	assembly	pSB1A3 - trc - RFP	pSB1A3 blue light sensor	pSB1C3 - FixK2 - tetR				
2	quantities [µl]							
3	pSB1K3	2	2	0				
4	pSB1A3	0	0	2				
5	modified: trc - RFP	2	0	0				
6	modified: strong promoter and YF1	0	2	0				
7	modified: RBS - FixJ - half of the other	0	2	0				
8	modified: FixK2 blue inducible promoter	0	0	2				
9	BBa_S0107	0	0	2				
10	ligation buffer	2	2	2				
11	ligase	1	1	1				
12	water	13	11	11				
13								
14								

parts are ligated according to the table above. conditions for ligation:

1. o/n, 4 °C
2. 20 min, 80 °C

MONDAY, 9/2/2019

transformation

transform chemically competent cells with heat shock protocol with the assembled plasmids

1. thaw an aliqot of competent cells on ice (10 min)
2. prepare tubes with 4 µl of ligation product
3. add 60 µl of thawed cells (usually 50, but had only 3 rxn. 20 µl that are leftover in the tube were also used for transformation to test efficiency with T BL) and flick the tube
4. incubate on ice for 30 min
5. heatshock at 42 °C for 30 sec
6. incubate on ice for 5 min
7. add 950 µl SOC (roomtemperature) to the cells
8. outgrow at 37 °C, 250 rpm for 60 min
9. plate on warm plates with the selection antibiotic (overnight)

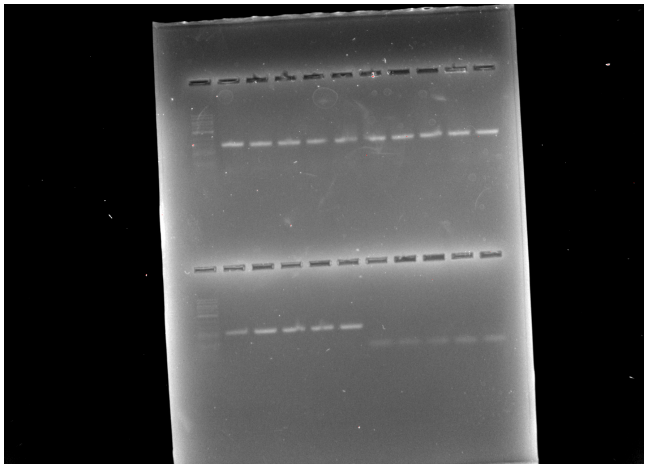
TUESDAY, 9/3/2019

colony PCR to confirm clones

the colony PCR showed all the fragments are too short (Primers VF2, VR)

jkn

BioRad 2019-09-03 20hr 20min.png

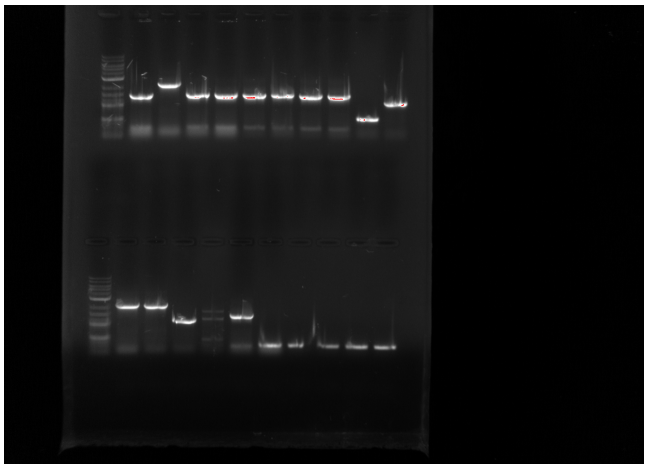


WEDNESDAY, 9/4/2019

colony PCR overnight with different clones (smaller, in case of trc RFP also red coloured ones)

THURSDAY, 9/5/2019

BioRad 2019-09-05 14hr 07min cPCR tbl1tbl2trcRFPfixK2.png



FRIDAY, 9/6/2019

reculture the colonies with correct length of insert in correct antibiotics
TB1,2 ; RFP 3 & RFP5 were recultured in medium with Kanamycin

SATURDAY, 9/7/2019

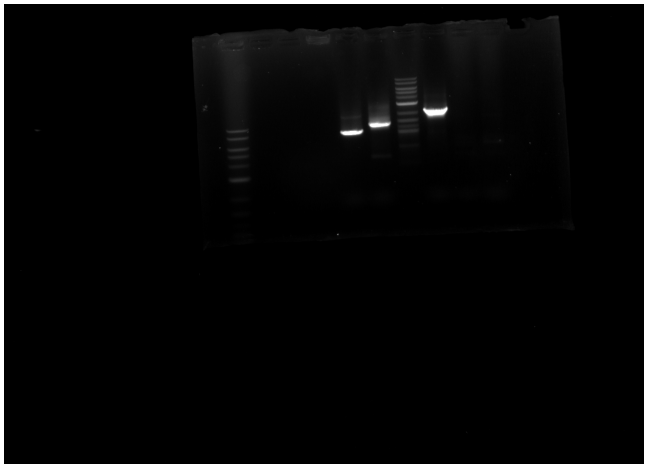
Plasmid purification and overnight PCR with those plasmids for TB12 and RFP 3 & 5

SUNDAY, 9/8/2019

gel of PCR & glycerol stock

glycerol stocks were made by adding 200 µl of glycerol to 600 µl of E. coli culture (TBL, RFP 3 and RFP 5)

BioRad 2019-09-08 14hr 54minrfp5rfp3laddertb12pls23pls24.png



PCR with VF2 and VR primer to confirm the inserts (from left to right: RFP5, RFP3, ladder, TB12)

MONDAY, 9/9/2019

colony PCR of more FixK2 and TetR transformants