Week 14

Vienna RNA thermoswitches

AIM:

Make pre-made RNA thermoswithes for GFP nano (igA) constructs.

REAGENTS USED:

Tab. 1 List of reagents used in the experiment

Name of reagent
100pb Plus DNA ladder (Thermo Scientific)
GeneJet PCR purification Kit
GeneJet Gel Purification Kit
DNA loading dye 6x
Isopropanol
DH5α competent cells
LB media
LB + agar plates
Chloramphenicol
SOC

Tab. 2 List of primers used in the experiment

Name of primer pair	Forward sequence	Reverse sequence
GJ2	GAATTCGCGGCCGCTTCTAGAGGTATCCCTCACTTACTAG	CTGCAGCGGCCGCTACTAGTAGCTAGCGGGTATATCTCCTTCTG
GJ3	GAATTCGCGGCCGCTTCTAGAGGGTCCCTCACTTACT	CTGCAGCGGCCGCTACTAGTAGCTAGCGGGTATATCTCCTTCTG
GJ6	GAATTCGCGGCCGCTTCTAGAGTCTCCTTCACTAGTAA	CTGCAGCGGCCGCTACTAGTAGCTAGCGGGTATATCTCCTTTT
GJ9	GAATTCGCGGCCGCTTCTAGAGCTCCTTACTAGTCTGC	CTGCAGCGGCCGCTACTAGTAGCTAGCGGGTATATCTCCTTCTG
GJ10	GAATTCGCGGCCGCTTCTAGAGTCTCCTTACTAGTCTGC	CTGCAGCGGCCGCTACTAGTAGCTAGCGGGTATATCTCCTTCTG
Sw2Core	AAGAGAAAGCGGTCCGTCTAAGGAGCCTGAGGT	ACCTCAGGCTCCTTAGACGGACCGCTTTCTCTT
Sw2BB	GAATTCGCGGCCGCTTCTAGAGAAGAGAAAGCGGTCCG	CTGCAGCGGCCGCTACTAGTAACCTCAGGCTCCTTAG
Sw3Core	AGTGCGGTGTATTTATAACAAGGAGCGGGATGG	CCATCCCGCTCCTTGTTATAAATACACCGCACT
Sw3BB	GAATTCGCGGCCGCTTCTAGAGAGTGCGGTGTATTTAT	CTGCAGCGGCCGCTACTAGTACCATCCCGCTCCT
Sw6Core	GTGCGGTGCCGTGGTCACTAAGGAGCCCTTGGG	CCCAAGGGCTCCTTAGTGACCACGGCACCGCAC
Sw6BB	GAATTCGCGGCCGCTTCTAGAGGTGCGGTGCCGT	CTGCAGCGGCCGCTACTAGTACCCAAGGGCTCCT
Sw7Core	GATCGCCCCTAGGAGGAACAAGGAGCTATCGTT	AACGATAGCTCCTTGTTCCTCCTAGGGGCGATC

Sw7BB	GAATTCGCGGCCGCTTCTAGAGGATCGCCCCTAGGA	CTGCAGCGGCCGCTACTAGTAAACGATAGCTCCTTGT
Sw9Core	GAGGTTTTATAGTCCTTCTAAGGAGATTGAGCT	AGCTCAATCTCCTTAGAAGGACTATAAAACCTC
Sw9BB	GAATTCGCGGCCGCTTCTAGAGGAGGTTTTATAGTCCTTC	CTGCAGCGGCCGCTACTAGTAAGCTCAATCTCCTTAG
Sw11Core	GGTGCGGTGTATAAGGTGAAAGGAGTCATTGGG	CCCAATGACTCCTTTCACCTTATACACCGCACC
Sw11BB	GAATTCGCGGCCGCTTCTAGAGGGTGCGGTGTATAAG	CTGCAGCGGCCGCTACTAGTACCCAATGACTCCTTTC

EXPERIMENT DESCRIPTION:

Due the lack of time, RNA thermoswitches were synthesized using 4 primers. Core primers make the thermoswitche sequence, and BB primers insert biobrick sites at the beginning and the end of the gene.

EXPERIMENT PROTOCOL:

1. Run PCR using for primers: **BB**forward, **BB**reverse, **Core**forwad and **Core**reverse primers to synthesize RNA thermoswithes and use GJx primers, to insert biobrick sites on the old RNA thermoswitches.

2. PCR reaction

Reagent	**Amount**

Super Fi MM x2	25 µL
Rcore+Fcore / GJx forward	0.5+0.5 µL (2.5 nM each) / 2.5 (10 nM)
BBpre+BBsuff / GJx reverse	2.5+2.5 µL (10 nM each) /2.5 (10 nM)
H_2O	19 / 20 µL
Total:	50 µL

3. Run gel electrophoresis.
4. Purify PCR products using GeneJet PCR Purification Kit (Thermofisher scientific).
5. Digest GJ_x with XbaI and NheI, Sw_x with XbaI and BcuI restriction enzymes and pSB1C3 plasmid with XbaI and BcuI using FastAP. Digest for 1 hour.
6. Digestion reaction

Reagent	**Amount**
DNA	200 ng / 3000 ng
FastAP	- / 5 µL
10x Fast Digest buffer	2 / 5 µL
XbaI	0.5 / 5 µL
BcuI (NheI for Sw_x)	0.5 / 5 µL
H_20	To 20 / 50 µL
Total:	20 / 50 µL

7. Run gel electrophoresis and purify cut plasmid using the GeneJet Gel Purification Kit Thermofisher scientific).
8. Ligate RNA thermoswitches in to cut plasmid for 1 hour.
9. Ligation reaction

Reagent	**Amount**
5x Rapid ligation buffer	4 µL

T4 ligase	1 µL
DNA	60 ng
Insert	Xng (7:1 compared to DNA by molar mas)
H_2O	Up to 20 µL
Total:	20 µL

10. Transform DH5α competent cells with ligation mix.
11. Plate cells on LB+ agar plates with chloramphenicol.
12. Grow in 37˚C for 16 h.
13. Run colony PCR using BBforward and BBreverse primers for Sw_x and for GJx use forward and reverse primers.

Reagent	**Amount**
Primer BBreverse	0.5 µL
Primer BBforward	0.5 µL
DreamTaq MM x2	2 µL
H_2O	4 µL
Total:	10 µL

14. Two step PCR.

1 cycle	95°C	2 minutes
6 cycles	95°C	15 seconds
	50/54/57°C	15 seconds

	72°C	10 seconds
1 cycle	72°C	3 minutes
30 cycle	95°C	15 seconds
	72°C	10 seconds
1 cycle	72°C	5 minutes
1 cycle	4°C	indefinite period

15. Data was analysed by running the electrophoresis gel after PCR reaction, digestion and colony PCR.

RESULTS:

After PCR reaction products of 88 bp- in first line, 87 bp-second line, 102 bp-third line, 81 bp- forth line, 82 bp-fifth line and 76 bp in the rest of lines were expected. You can see them in Figure 1.

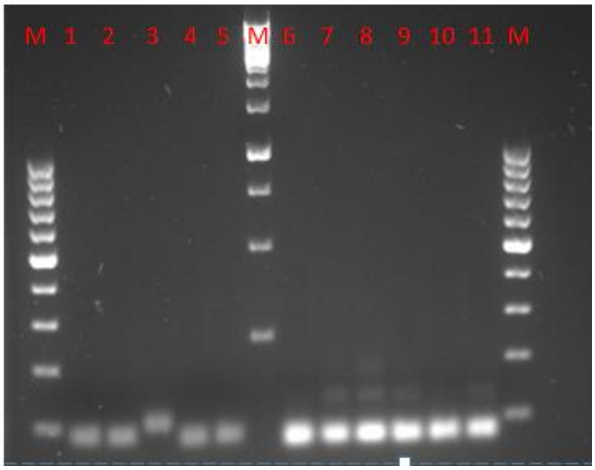

Fig. 1 Electrophoresis gel of PCR. 1- GJ2, 2- GJ3, 3- GJ6, 4- GJ9, 5- GJ10, 6- Sw2, 7- Sw3, 8- Sw6, 9- Sw7, 10- Sw9, 11- Sw11

After PCR, products were cut, purified and ligated to pSB1C3 plasmid. After transformation and 16 h growth in 37˚C, transformed bacteria were checked by running colony PCR. All colonies came out to be positive, expect a few ones with non-specific PCR products (those colonies were not transfer to mini preps for plasmid multiplication). You can see the result in a picture two. PCR product length is the same as in the first PCR.

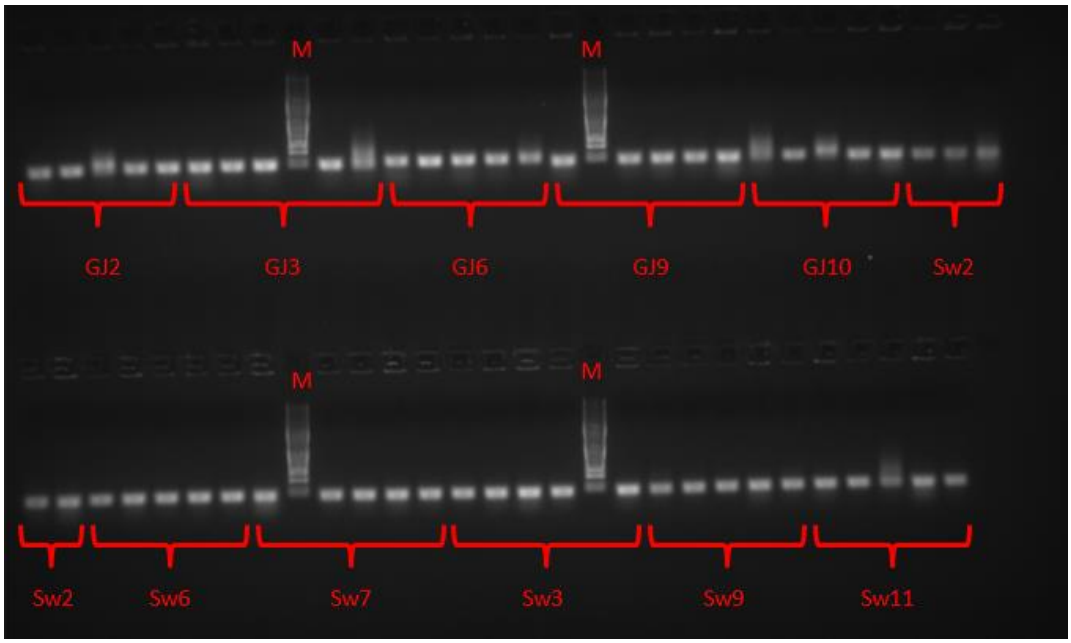

Fig. 2 Colony PCR gel electrophoresis.

CONCLUSIONS:

1. Designed primers were specific for RNA thermoswitches production, as well as for the colony PCR.
2. Both ligation and the bacterial transformation showed the plasmid with genes of interest.