Week 13

RNA thermoswitches for OmpA-His and MstX-OmpA-His constructs

AIM:

To show that MstX improves OmpA-His insertion into the membrane, we have to be sure, that protein expression in the PURE system starts only after the Bam complex insertion into the membrane.

REAGENTS USED:

Table 1. List of primers used in the experiment

Name of primer pair	**Forward sequence**	**Reverse sequence**
AukseRv		CTGCAGCGGCCGCTACTAGTACATATGCCCAATG
Sw11BBpre	GAATTCGCGGCCGCTTCTAGAGGGTGCGGTGTATAAG	
T7	TAA TAC GAC TCA CTA TAG GG	
Sw11Suff		CTGCAGCGGCCGCTACTAGTACCCAATGACTCCTTTC

EXPERIMENT DESCRIPTION:

Sw11 from report about Vienna switches is used to insert in constructs OmpA-His and MstX-OmpA-His. However, the Sw11 switch synthesized previously does not have the correct digestion site, to insert it into the plasmid with constructs of interest. That is why, one more PCR has to be compiled to get the insert with the right restriction enzyme cutting site.

EXPERIMENT PROTOCOL:

1. Run PCR reaction to synthesize Sw11 with right restriction enzyme cutting sites.

2. PCR

Reagent	**Amount**
Sw11 (with bad cutting sites)	5 ng
Primer AukseRv	2.5 µL
Primer Sw11BBpre	2.5 µL
Super Fi MM x2	25 µL
H_2O	Up to 50 µL
Total:	50 µL

3. Purify PCR product using GeneJet PCR Purification Kit (Thermofisher scientific)
4. Cut PCR product and plasmid with construct using XbaI and NdeI restriction enzymes for 30-45 minutes.

Reagent	**Amount**
DNA	200 ng / 1000 ng

FastAP	- / 3 µL
10x Fastdigest buffer	2 / 3 µL
XbaI	0.5 / 3 µL
NdeI	0.5 / 3 µL
H_20	To 20 / 30 µL
Total:	20 / 30 µL

5. Purify cut PCR products using PCR Purification Kit (Thermofisher scientific).
6. Run DNA electrophoresis gel and purify cut plasmid from the gel using GeneJet Gel Extraction Kit (Thermofisher scientific)
7. Ligate PCR product and plasmid for 45 minutes.

Reagent	**Amount**
5x Rapid ligation buffer	4 µL
T4 ligase	1 µL
DNA	100 ng
Insert	X ng (7:1 compared to DNA by molar mass)
H_2O	Up to 20 µL
Total:	20 µL

8. Transform DH5α and BL21 competent cells with the ligation mixture. (10 µL for each)
9. Plate cells on LB+ agar plates with antibiotics. (Amp was used here, because pRESET has resistance to it).
10. Grow in 37˚C for 16 hours.
11. PCR reaction mixture

1 cycle	95°C	2 minutes

35 cycles	95°C	15 seconds
	72˚C	15 seconds
1 cycle	72°C	5 minutes
1 cycle	4°C	indefinite period

12. Run colony PCR to check if bacteria have the thermoswitch.

Reagent	**Amount**
Primer T7 promoter	0.5 µL
Primer Sw11BBsuff	0.5 µL
DreamTaq MM x2	2 µL
H_2O	4 µL
Total:	10 µL

RESULTS:

After PCR reaction was performed, the DNA electrophoresis gel was run. The expected product is about 80 bp. You can see it in Fig. 1. Then the plasmid and PCR products were cut, ligated and the bacterial transformation was done. After 16 hours in 37˚C colony PCR was conducted. You can see the results in Figure 2. Most colonies were positive. It might look, that products are of different length, but it is due to 2% gel that does not form consistent wells.

Fig. 1 PCR reaction of Sw11 synthesis

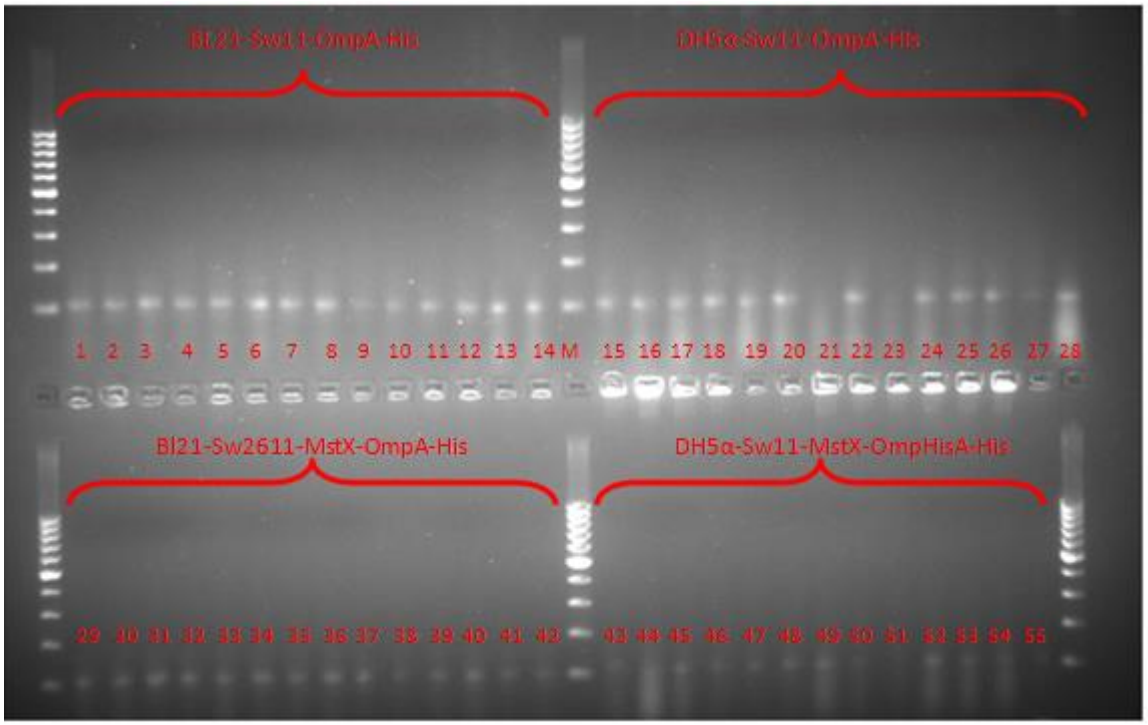

Fig. 2 Colony PCR run on 2% agarose gel.

CONCLUSIONS:

1. PCR, digestion, ligation and transformation were successful.
2. Constructs for protein expression experiment to check if RNA thermoswitches work were made.