12 October 2017 : Amplification of T7 Aptamer Trigger

1. Aim:

Show that the signal of T7 aptamer trigger can be successfully amplified.

2. Materials:

- Nuclease-free water
- LacZalpha 27B toehold
- T7 aptamer trigger 27B
- Aptamer trigger 27B (no T7)
- M15 T7 lysate
- Top10-GamS lysate
- Buffer A
- Energy solution
- Substrate (15 mg/mL)
- Plate reader
- Phusion polymerase
- dNTPs
- T7 RNA Polymerase
- Primer forwardb

3. Procedure:

The following setups will be performed :

1. a) Incubate T7 aptamer trigger with Phusion polymerase and primer for 1 hour prior to Transcription reaction to anneal the primer at the annealing temperature (70°C)
   b) after discussion with Barbara Grisoni, the following procedure was performed : 10 minutes at 70°C , then put on ice for 10 minutes, then continued with step 1.

ANNEALING	
ss T7 aptamer trigger, 100uM	0.7
primer forwardb, 100uM	10
Nuclease-free water	9.3
Total Volume	20

TX control	
ss T7 aptamer trigger, 100uM	0.34
primer forwardb, 100uM	0
Nuclease-free water	9.66
Total Volume	10

2. Continue incubation for two hours for transcription :

    a. for transcription in vitro :

TRANSCRIPTION IN VITRO	
Nuclease-free water	0
NTP Buffer Mix	10
ds T7 aptamer mix	10
T7 RNA Polymerase Mix	2
Total reaction volume	22

    b. for transcription in lysate :

TRANSCRIPTION IN LYSATE	
ds T7 aptamer trigger	3
M15 T7 lysate	1.5
Top10-GamS lysate	1.5
Energy Solution	3
Buffer A	3

After incubation:
Nano-drop for RNA concentration, write that down.

Test:

- Sample from Step2.a) in M15 T7 lysate (usual reaction)
- Sample from Step2.b) in M15 T7 lysate (usual reaction)

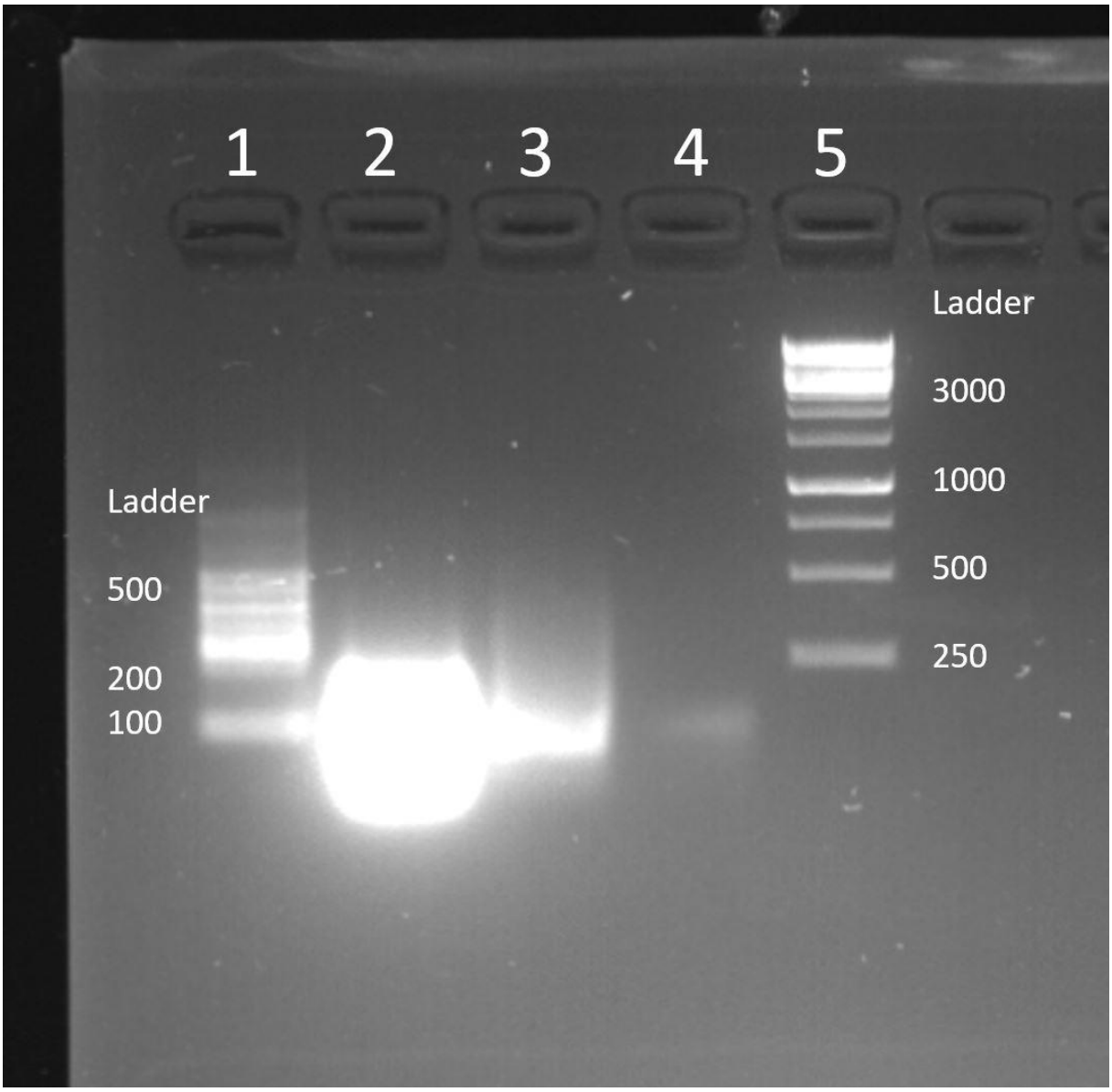
- M15 T7 lysate with T7 aptamer trigger (no prior incubation, same initial concentration, usual reaction)
- M15 T7 lysate with aptamer trigger (old construct without T7 promoter, no prior incubation, same initial concentration, usual reaction)
- No aptamer control (usual control)

Initial aptamer concentration will be at 3uM.

Let the product of in vitro transcription run on a 2% agarose gel, loading :

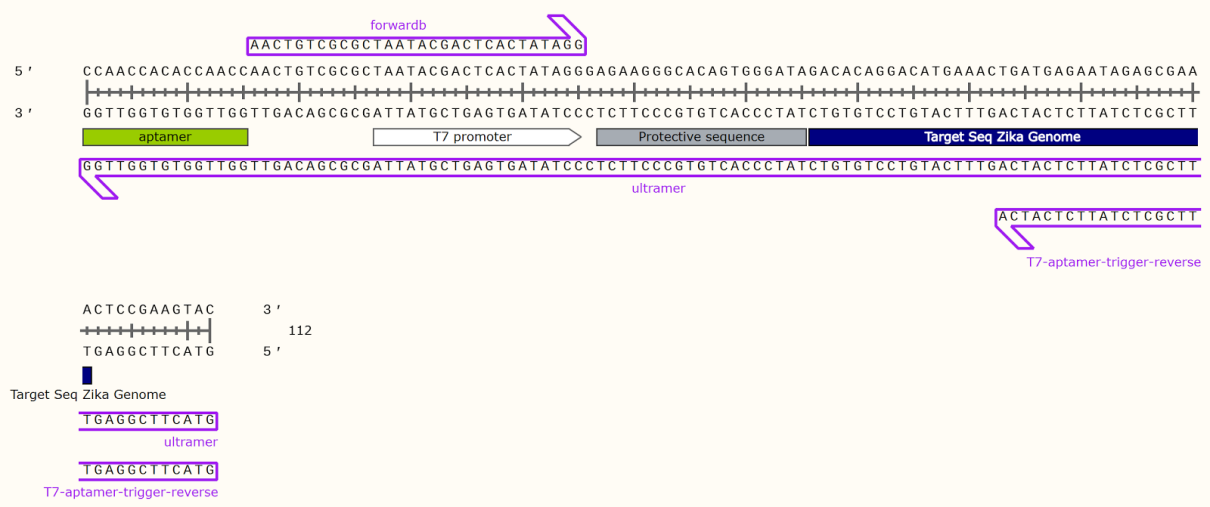
- RNA low range ladder
- the in vitro transcription product
- the in vitro transcription control
- ultramer at the same concentration as was initially added to TX reaction
- DNA 1kb ladder

Gel



PCR approach

Amplify the T7 aptamer trigger ultramer with primers forwardb and T7-aptamer trigger reverse



PCR product : 97 bp
Annealing temperature (Phusion) : 72°C

Run PCR for 20 cycles, 25 uL PCR reactions
Prepare tubes with the following initial DNA concentrations :

- 5nM
- 1nM
- 0.5nM
- 0.1 nM

T7 aptamer concentration	final concentration in PCR reaction	
nM	nM	uL to add to 25 uL PCR reaction
50	5	2.5
50	1	0.5
5	0.5	2.5
5	0.1	0.5

Check the reactions on a 1% agarose gel for bands at ~100bp.
Then continue with in vitro transcription by T7 RNA polymerase

- 2 hours
- 37°C

TRANSCRIPTION IN VITRO

Nuclease-free water	0
NTP Buffer Mix	10
ds T7 aptamer trigger PCR reaction	10
T7 RNA Polymerase Mix	2
Total reaction volume	22

Measure RNA concentration on Nano-Drop.

4. Results:

At the first try, the samples was left 60 minutes at 70°C incubation and then 120 minutes to incubate at 37°C.

This approach did not yield any results on the plate reader as the controls too changed colour.
The procedure was repeated twice more, with the same results each time. We had to conclude that the toehold generated for this experiment somehow was leaky. The toehold was er-extracted and the protocol was changed to incubate at the annealing temperature for 10 minutes, then putting the samples on ice, then leaving them to incubate for two hours at 37°C.

This approach again did not yield any results on the plate reader, with no expression visible for the amplified construct.

We changed our strategy to first test whether we actually amplified the construct in vitro, by letting the

5. Conclusion: