

RNA logic gates

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1 Method

1.1 Design

1.2 Transcription

The oligos from IDT was dissolved in TE buffer [PROTOCOL] to an approximate concentration of $120 \mu\text{M}$, based on the quantity of substance written on the tubes. The final concentration desired was $100 \mu\text{M}$, but due to a risk of inaccurate substance quantities, the dissolved concentration was chosen to be slightly above. The oligos could then be further diluted after measuring their absorbance on the Nanodrop.

After dissolving the oligos, the absorbance of each sample was measured on the Nanodrop in triplets. A program was written which can take the .csv output of the Nanodrop, and calculate the concentration based on each strands extinction coefficient [1].

The measured concentrations (figure 7) was used to dilute the samples further, to a concentration of $100 \mu\text{M}$.

To anneal the templates to the promoter, each of the template strands was mixed with equal amounts of promoter strand in annealing buffer [PROTOCOL], to a final concentration of $1 \mu\text{M}$. The mixed samples were then heated to 90°C for 5 minutes, and left to cool down to room temperature.

To check if samples annealed properly, they were run on a 20% native PAGE gel for 3 hours. Each lane was loaded with $50 \mu\text{l}$ sample, and $10 \mu\text{l}$ native loading buffer [PROTOCOL]. Afterwards the gel was stained in SYBR Gold,

Name	Short	Sequence	Length
T7 promoter	0	GGTAATACGACTCACTATAG	20
Short 1	1	CCTCAAGGAGCTTCAGTCTAGCCCTATAGTGAGTCGTATTACC	43
Short 2	2	CTCCTTGAGGCACATAACTCCCCTATAGTGAGTCGTATTACC	42
Short 3	3	CACATAACTCTACTAAATCTCCCTATAGTGAGTCGTATTACC	42
Short 4	4	GAGTTATGTGCCCTAAGGAGGCCCTATAGTGAGTCGTATTACC	42
Short 5	5	AGATTTAGTAGAGTTATGTGCCCTATAGTGAGTCGTATTACC	42
Long 1	6	GTCATTGCCCTAAGGAGCTTCAGCTAGCCCTATAGTGAGTCGTATTACC	52
Long 2	7	GCTCCCTGAGGCATAATTGACCCATCTTCATTCTACTCCCTACCCCTATAGTGAGTCGTATTACC	62
Long 3	8	CCATCTTCAATTCTACTCTTACCTCAATCCCCATAGTGAGTCGTATTACC	52
Long 4	9	TAGGAGTAGAATGAAGATGGGTCATTGCCCTAAGGAGCCCTATAGTGAGTCGTATTACC	62
Long 5	10	GATTGAGGTATAGGAGTAGAATGAAGATGGCCCTATAGTGAGTCGTATTACC	52

Table 1: Sequences and names of the DNA strands used for transcription.

Name	Short	Sequence	Length
Short 1	1	GCUAGACUGAAGCUCCUUGAGG	23
Short 2	2	GGGAGUUUAUGUGCCUCAAGGAG	22
Short 3	3	GGAGAUUUAGUAGAGUUUAUGUG	22
Short 4	4	GCUCCUUGAGGCACAUACUC	22
Short 5	5	GGCACAUACUCUACUAAAUCU	22
Long 1	6	GCUAGACUGAAGCUCCUUGAGGCCAAUUGAC	32
Long 2	7	GGUAGGAGUAGAAUGAAGAUGGGGUAAUUCGCCUCAAGGAGC	42
Long 3	8	GGGAUUGAGGUAAUAGGAGUAGAAUAGAAGAUGG	32
Long 4	9	GGGCUCCUUGAGGCAGAUUGACCCAUCUUCAUUCUACUCCUA	42
Long 5	10	GGCCAUCUUCAUUCUACUCCUAUACCUAACUAUC	32

Table 2: Sequences and names of the transcribed RNA strands.

and visualised on the Typhoon scanner [PROTOCOL]. The result of the scan can be seen in figure 1.

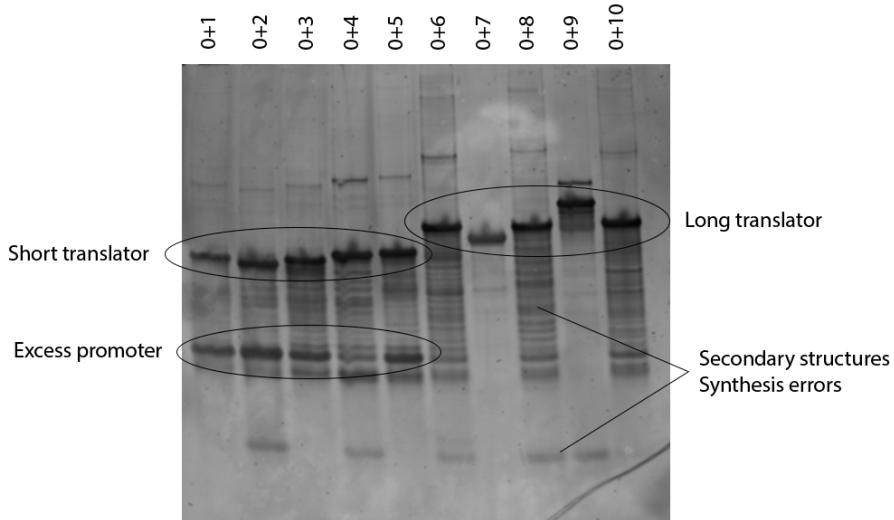


Figure 1: Typhoon scan of SYBR Gold stained native PAGE gel, with the annealed templates and promoter strands. The lanes are labelled by which strands are annealed (see table 1).

As can be seen in figure 1, the darkest bands is the annealed samples. The samples for the short translator runs as about the same size, while there is bigger variation in the long translator samples, as expected based on table 1. The exact positions of the long translator samples does not match with the sequence length, though this can be explained by secondary structures of the single-stranded part of the sample. The shorter bands visible below, is probably excess promoter, other secondary structures, and shorter sequences from synthesis errors. No lane with ladder was run, so the exact position of the bands can't be commented on.

To get the desired RNA sequences, a transcription reaction was run with

	Initial conc.	Final conc.	Volume
Transcription buffer	10X	1X	10 μ L
DTT	100 mM	10 mM	10 μ L
NTP mix	25 mM	2.5 mM	10 μ L
Template	500 nM	50 nM	10 μ L
T7 RNA polymerase			1 μ L
Nuclease-free water			59 μ L
Total			100 μ L

Table 3: Mixing of compounds for the first transcription done on the templates for both the short and long translater.

each of the annealed DNA templates, according to table 3.

The samples were left overnight at 37° C. The day after, 1 μ L of RNase free DNase was added to the samples, and heated for 37° C for an hour. Afterwards, 100 ul of denaturing loading buffer was added to each sample, and 10 ul of the DNA 0 and DNA 5 strands was mixed with 10 ul of denaturing loading buffer, and heated for 5 minutes at 90° C.

To purify the RNA, the transcribed sequences and controls were run on a 20% denaturing PAGE gel, for 4 hours at 20 W.

The RNA product should be visible in UV shadowing, but no product was visible. The gel was then stained with SYBR Gold and scanned on the Typhoon [PROTOCOL]. The result can be seen in figure 2.

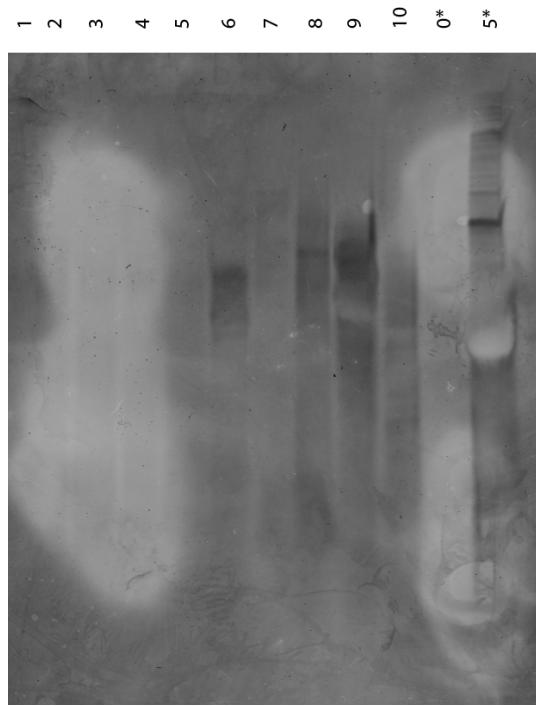


Figure 2: Typhoon scan of SYBR Gold stained native PAGE gel, with the transcribed RNA strands in lanes 1-10, and controls in 11 and 12. The lanes are labelled by which strands are annealed (see Table 2). The asterix refers to the DNA strands in Table 1.

As can be seen in Figure 2, the gel wasn't stained long enough, so after restaining it in SYBR Gold, it was scanned again.

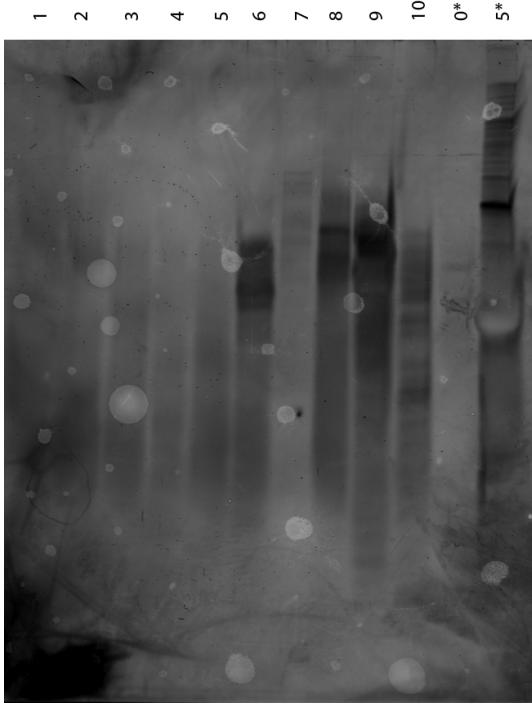


Figure 3: Typhoon scan of SYBR Gold stained native PAGE gel, with the transcribed RNA strands in lanes 1-10, and controls in 11 and 12. The lanes are labelled by which strands are annealed (see table 2). The asterix refers to the DNA strands in table 1.

The lanes 1-10 in Figure 3 does not show distinct bands. There seems to be more product from the long translator transcriptions (lanes 6-10), than in the short ones (lanes 1-5). Even the controls which were loaded in equal amounts does not show up in equal strength. Since the gel from the DNA annealing was run without any controls, it was difficult to see if there was any errors in the annealing. Another 20% native PAGE gel was run with the annealed DNA, using the single stranded oligos as control. To simplify the experiment while trying to the find the error, only the short translator sequences were used.

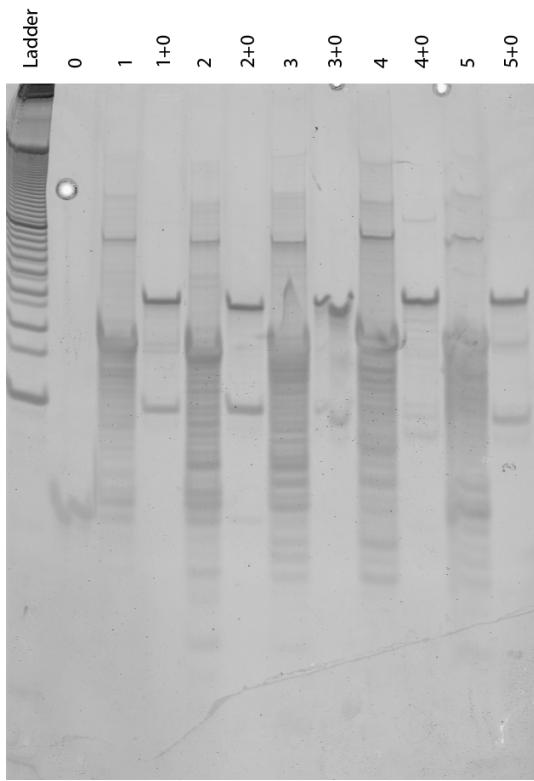


Figure 4: The annealed oligos together with controls and a 10 nt ladder. The lanes are labelled with the oligo names given in Table 1. The plus symbol denotes which strands are annealed.

The results of Figure 4 still shows that the templates have annealed with the promoter, and runs as about 40-50 bp. The expected size is around 60 bp (sum of promoter and template), but it is difficult to say how a partly annealed structure will run on a native gel. The new gel does however show that the bands in the annealed lanes below the assumed product, might not be excess promoter. The promoter is seen in the second lane, and lies below the bands in the annealed structure thought to be excess promoter. The bands below the product might be due to secondary structures of each of the template strands, but comparing with Figure 9, the band in the 5+0 lane would be expected to be less visible, as strand 5 has no secondary structure.

Despite the unexplained bands from the annealing, a new transcription was run on the short template strands to see if better results could be attained. Another transcription was done using the previous protocol (Table 3), and run on a gel. The UV shadowing still didn't show any visible product, so the gel was scanned to check for bands.

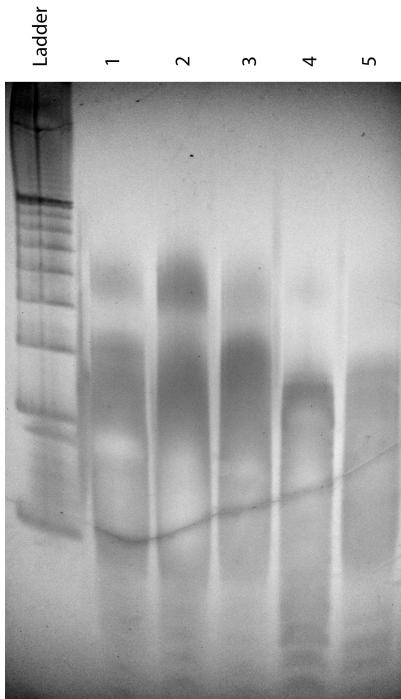


Figure 5: The transcribed short translator sequences and a 10 nt ladder. The lanes are labelled with the oligo names given in Table 2.

[MAYBE MENTION THE OLIGOS RUN AS DYE BASED ON IDT]

As seen in Figure 5, still no clear bands with RNA product was visible. After checking all buffers and materials, it was learned that the T7 polymerase that was used was from 2013, and might have been too old to still function properly. A fresh T7 polymerase was used for a new transcription, same as the previous protocol. The UV shadowing did show some distinct bands this time, so the bands were cut out for purification. The purification was done using the precipitation protocol [PROTOCOL]. After purification the pellets were dissolved in 100 μ L TE buffer, their absorbance measured on the Nanodrop, and their concentration calculated using their extinction coefficients [GET TABLE WITH EXTINCTION COEFF].

To check that the purification worked, 2 μ L of each sample with 1 μ L of denaturing loading buffer was run on a 20% denaturing gel, and visualised on the Typhoon.



Figure 6: The purified short translator RNA oligos.

Figure 6 shows that the RNA has been isolated, but is not very pure. It is possible that when cutting out the RNA from the gel before purification, too large an area was taken. The target RNA sequences was still expected to be in the purified samples (albeit not very pure), and it has previously been shown that strand displacement reactions can work with unpurified components [REFERENCE PLEASE], so the experiment continued with the current RNA.

Based on the concentrations in Figure 8, the samples were further diluted to 1 μ L in TE buffer.

2 Appendix

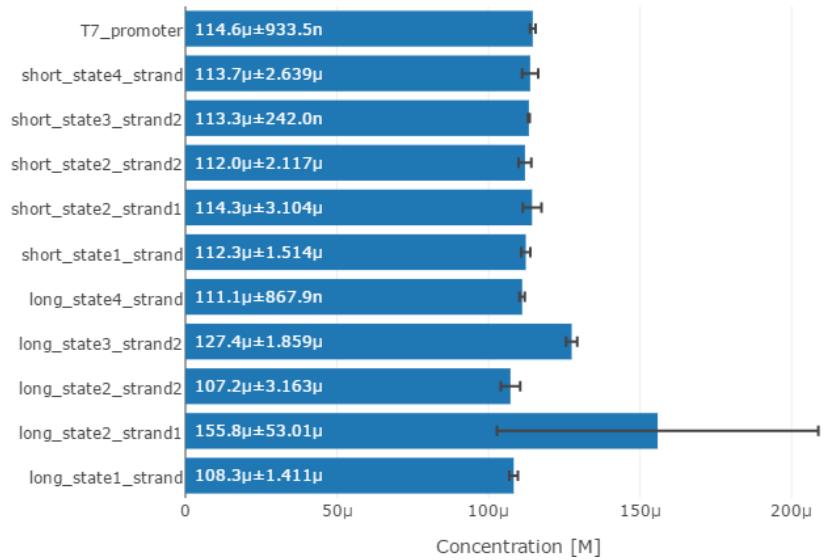


Figure 7: Concentrations of the dissolved DNA oligos ordered from IDT.

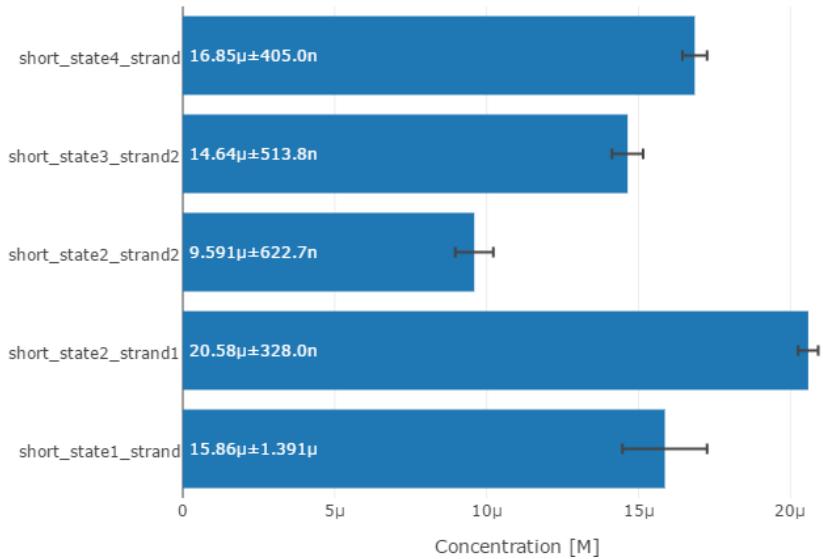


Figure 8: Concentrations of the short translator RNA sequences.

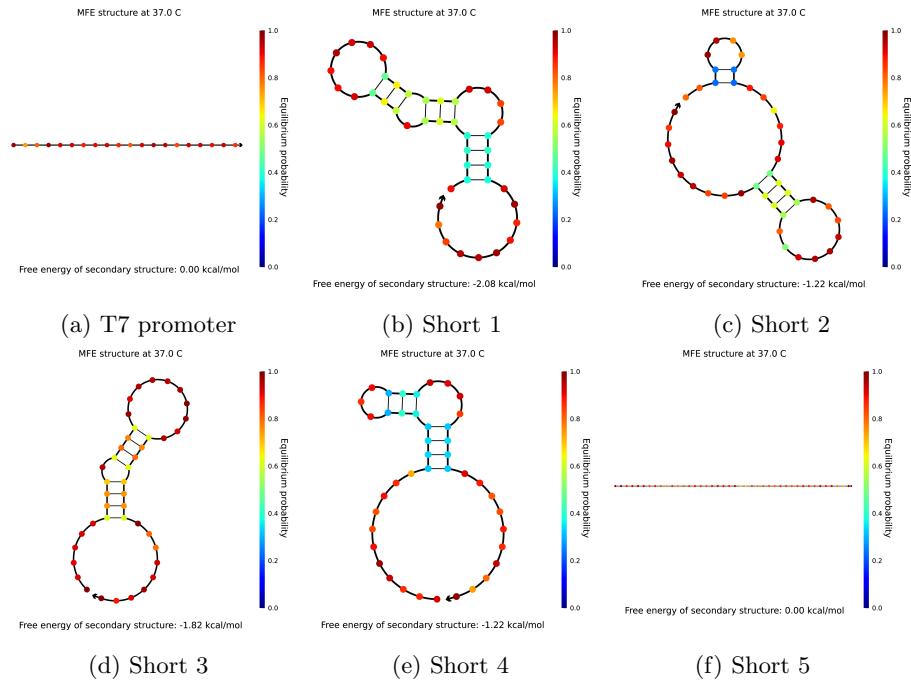


Figure 9: Secondary structures of the DNA sequences for the short translator and the T7 promoter sequence.

References

- [1] <http://nanodropimport.herokuapp.com/>.