

RNA toehold switch example

Introduction

The toehold switches are artificial designs which were built to regulate translation of a GFP target gene. A small RNA serves as trigger molecule to activate GFP expression by binding to the 5'UTR region. During the design approach, both sequences were optimized to adopt only one stable secondary structure when on their own. As soon as the sRNA binds the 5'UTR, a stable complex forms and triggers a structural change in the mRNA region. This opens up the hairpin sequestering the RBS and the start codon, thus allowing for ribosomes to bind and initiate translation.

Sequences

The 5'UTR can be found in pAG_TS1_KS001.fa, the sRNA in pAG_TS1_AT001 and both together form pAG_TS1_001.

```
GGGUGAAUGAAUUGUAGGCUUGUUAUAGUUAUG AACAGAGGAG ACAUAAC AUG AACAAG
CCUAACCUGGCGGCAGCGCAAAGAUGCGUAAA
..... ( RBS ) ..... xxx <- start codon
..... coding region ...
```

Constraint folding

structural must change if 5' end is unpaired. Can be checked with hard constraints

```
cat pAG_TS1_KS001.fa pAG_TS1_KS001_bindingsite.fa | RNAfold -C
```

if -C option is removed, then switch will fold into sequestering stem again!

Predict cofolding

cofold with sRNA

```
RNAcofold < pAG_TS1_001.fa
```

To see dot-plot use -p option!

Predict binding site

RNAup to predict binding site with opening and binding energies

```
RNAup -b < pAG_TS1_001.fa
```

Calculate RBS accessibility

use hard constraints to calculate the accessibility of RBS with and without sRNA

Without sRNA binding

```
cat pAG_TS1_KS001.fa | RNAfold -p
cat pAG_TS1_KS001.fa pAG_TS1_KS001_constraint.fa | RNAfold -p -C
```

$$\text{prob}(\text{aptamer}) = \exp((\text{pf} - \text{constraint_energy}) / \text{KT}) \quad \text{KT} = ((\text{temperature} + 273.15) * 1.98717) / 1000.0$$
$$e^{((-27,13+19,33) \div (((37+273,15) \times 1,98717) \div 1000))} = 0,000003189 = 00,00\%$$

states are completely accessible in the full region in and between RBS and AUG.

With sRNA binding

```
cat pAG_TS1_001.fa | RNAcofold -p
cat pAG_TS1_001.fa pAG_TS1_001_constraint.fa | RNAcofold -p -C
```

$$e^{((-79,6+79,28) \div (((37+273,15) \times 1,98717) \div 1000))} = 0.59499031774 = 59,50\%$$

of states are completely accessible in the full region in and between RBS and AUG.

References

Green, Alexander A., Pamela A. Silver, James J. Collins, and Peng Yin. 2014. "Toehold Switches: De-Novo-Designed Regulators of Gene Expression." *Cell* 159 (4): 925-39. doi:10.1016/j.cell.2014.10.002.