

ViTEST

Toehold
switch

7/26-8/12

Flavesence
Dorée and
Boir noir
Toehold
switch
design

Introduction:

From our first experiment we have an idea about how a functional toehold looks like from the sequence map of the toehold plasmid (Banana sensor) we order form BioBits. As Banana sensor is not designed exactly according to Green et al., 2014 paper, it leads us to 2 alternatives of designing the toehold:

- a. Design based on Green et al., 2014
- b. Design based on Banana sensor

Hence, we generated two libraries of toehold sensor, marked as version 1 and 2.

Friday, 26 July 2019

Hairpin loop design: the part which is identical for all toeholds

Version 1:

1. According to the paper (Green et al., 2014), our prototype design has a loop of 15nts at the head of the hairpin. Based on iGEM RBS registry, we chose the RBS from the community collection, which is the same used in the biobits paper. Also suggested by Vimberg et al., 2007 (a A/U RICH regions in the pre-RBS will enhance the the rate of translation). So in our design we have the following sequence as the pre-RBS which has a A/U content of 57%:

gacaugu

2. According to iGEM registry, the optimal distance between the starting codon and RBS is around 6-7 nts, in our first design we choose 6 nts(also A/U enriched):

cuuuaa

3. For starting codon it will always be "aug", refering to Green et al., 2014, it is sequestered by 3 non-complementary nucleotides:

cca

4. Therefore, we have the upper part of our hairpin sequence and structure:

cca cuuuaa gacauguagaggaga uuuaag aug

(NED:8.8%) normalized ensemble defect using NUPACK

Saturday, 27 July 2019

Version 2:

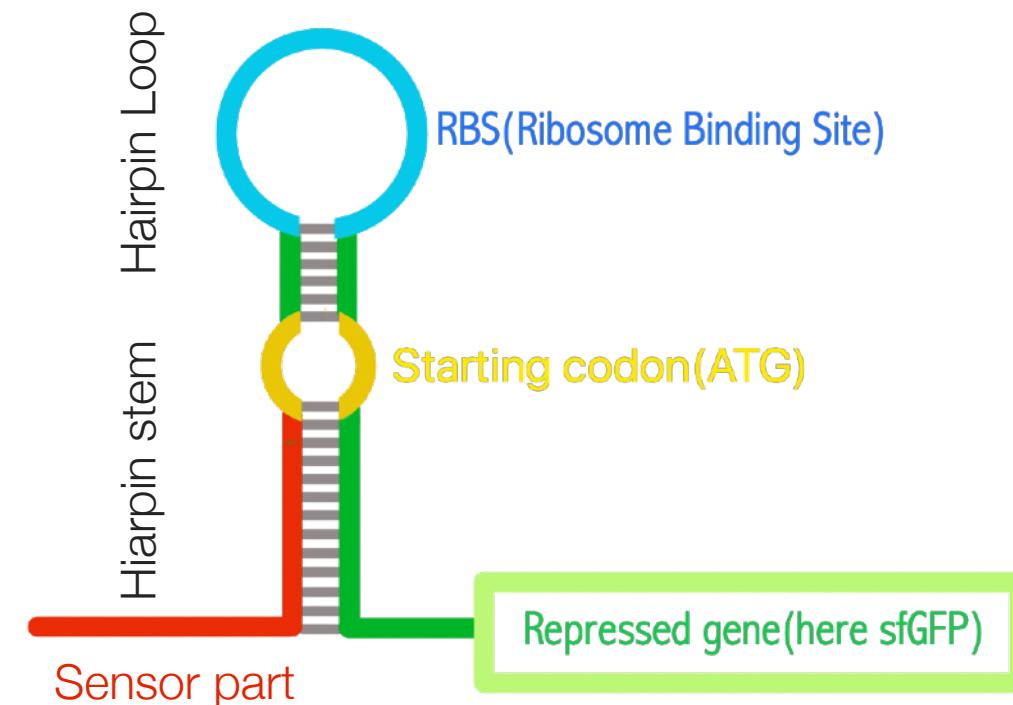
1. Based on the Biobits paper (pCOLA banana sensor sfGFP). The common hairpin sequence is:

gga ctta gaac agaggaga taaag atg
pre-RBS RBS start codon

2. Therefore, we have the upper part of our hairpin sequence and structure:

cca cuuuuaa gaacagaggaga uuuaag aug

(NED: 11.8%) normalized ensemble defect using NUPACK



Hairpin stem design: the part which varies with the sensor part

1. Trigger RNA/DNA:

From the RPA group, we've received the sequence of trigger RNA/DNA. Means the following sequence will appear in the RPA product. For toehold we only need 36 nts of complementary bases from trigger to from the sensor part of toehold.

BN trigger:

AAGCAGGUUUAGCGAUGGUUGUUUUUCCUGAAGGUGGUUUAAAAGAUUCGAAAU
GAUGAAGCAACGGUACCA

FD trigger:

GGGGATAACGTCAAATCATCATGCCCTTATGATCTGGGCTACAAACGTGATACAAT
GGCTATTACAAAGAGTA

EC trigger:

AATCCTGTTTCCGAAAACAACCAAGGGTTCAGAAAACGATAATAAAAAAAAGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAAAACAAATGGAGTTGACT

Monday, 29 July 2019

Using the equation from Green et al. 2014, our toehold switch is sorted by its Design score from lowest to highest. From their function and way of normalising, the lower the score the better.

$$\phi(i) = \beta_1 \bar{l}_{mRNA} + \beta_2 \bar{l}_{toehold} + \beta_3 \bar{n}_{sensor}$$

“Where $\Phi(i)$ is the design score for the sensor at location i of the mRNA, \bar{l}_{mRNA} is the local single-strandedness of the mRNA at the sensor binding site, $\bar{l}_{toehold}$ is the local single-strandedness of the toehold of the sensor, and \bar{n}_{sensor} is the normalised ensemble defect of the sensor. The score ‘-’ above certain variables indicates they were normalised as shown in this equation: $x = x / \max(x)$. the score weight factors used were $\beta_1 = 5$, $\beta_2 = 4$ and $\beta_3 = 3$.”

—Green et al., 2014

Top 4 BN toeholds version 1 and 2:

	T7 promotor	Toehold region	Hairpin region	Refolding	Linker
Toehold_BN_1.1	taatacgact cactataggg	GCGATGGTGTTC CTGAAGGT	GGTATTAAAGATCCACTTTAAGACATGTAGAGG AGATTAAAGATGATCTTTATACC	ggtatta	TGGCGGCAGC GCAAAAG
Toehold_BN_1.2	taatacgact cactataggg	TTCCTGAAGGTGGTAT TAAAGATC	GAAATGATGAAGCCACTTTAAGACATGTAGAGG AGATTAAAGATGCTTCATCATTC	gaaatgc	TGGCGGCAGC GCAAAAG
Toehold_BN_1.3	taatacgact cactataggg	GTTGTTTCCTGAAG GTGGTATT	AAAGATCGAAATCCACTTTAAGACATGTAGAGG AGATTAAAGATGATTCGATCTT	aaagatc	TGGCGGCAGC GCAAAAG
Toehold_BN_1.4	taatacgact cactataggg	AAGGTGGTATTAAAGA TCGAAATG	ATGAAGCAACGCCACTTTAAGACATGTAGAGG AGATTAAAGATGCCGTTGCTTCAT	atgcagc	TGGCGGCAGC GCAAAAG
Toehold_BN_2.1	taatacgactca ctataggg	GCGATGGTGTTC CTGAAGGT	GGTATTAAAGATCCACTTTAAGAACAGAGGGAGA TTAAAGATGATCTTTATACC	ggtatta	TGGCGGCAGC GCAAAAG
Toehold_BN_2.2	taatacgactca ctataggg	TTCCTGAAGGTGGTAT TAAAGATC	GAAATGATGAAGCCACTTTAAGAACAGAGGGAGA TTAAAGATGCTTCATCATTC	gaaatgc	TGGCGGCAGC GCAAAAG
Toehold_BN_2.3	taatacgactca ctataggg	GTTGTTTCCTGAAG GTGGTATT	AAAGATCGAAATCCACTTTAAGAACAGAGGGAGA TTAAAGATGATTCGATCTT	aaagatc	TGGCGGCAGC GCAAAAG
Toehold_BN_2.4	taatacgactca ctataggg	AAGGTGGTATTAAAGA TCGAAATG	ATGAAGCAACGCCACTTTAAGAACAGAGGGAGA TTAAAGATGCCGTTGCTTCAT	atgcagc	TGGCGGCAGC GCAAAAG

*The full library is enclosed in appendix 1.1

Top 4 FD toeholds version 1 and 2:

	T7 promotor	Toehold region	Hairpin region	Refolding	Linker
Toehold_FD_1.1	taatacgactcact ataggg	TAACGTCAAATCATCA TGCCCCTT	ATGATCTGGGCTCCACTTTAAGACATGTAGAGG AGATTAAAGATGAGCCCAGATCAT	acgatct	TGGCGGCAGC GCAAAAG
Toehold_FD_1.2	taatacgactcact ataggg	TATGATCTGGGCTACA AACGTGAT	ACAATGGCTATTCCACTTTAAGACATGTAGAGG AGATTAAAGATGAATTGCCATTGT	acaatgg	TGGCGGCAGC GCAAAAG
Toehold_FD_1.3	taatacgactcact ataggg	CCCCTATGATCTGGG CTACAAAC	GTGATACAATGGCCACTTTAAGACATGTAGAGG AGATTAAAGATGCCATTGTATCAC	gtcatac	TGGCGGCAGC GCAAAAG
Toehold_FD_1.4	taatacgactcact ataggg	GGGGATAACGTCAAAT CATCATGC	CCCTTATGATCTCCACTTTAAGACATGTAGAGG AGATTAAAGATGAGATCATATGGG	cccatat	TGGCGGCAGC GCAAAAG
Toehold_FD_2.1	taatacgactcact ataggg	TAACGTCAAATCATCA TGCCCCTT	ATGATCTGGGCTCCACTTTAAGAACAGAGGAGA TTAAAGATGAGCCCAGATCAT	acgatct	TGGCGGCAGC GCAAAAG
Toehold_FD_2.2	taatacgactcact ataggg	TATGATCTGGGCTACA AACGTGAT	ACAATGGCTATTCCACTTTAAGAACAGAGGAGA TTAAAGATGAATTGCCATTGT	acaatgg	TGGCGGCAGC GCAAAAG
Toehold_FD_2.3	taatacgactcact ataggg	CCCCTATGATCTGGG CTACAAAC	GTGATACAATGGCCACTTTAAGAACAGAGGAGA TTAAAGATGCCATTGTATCAC	gtcatac	TGGCGGCAGC GCAAAAG
Toehold_FD_2.4	taatacgactcact ataggg	GGGGATAACGTCAAAT CATCATGC	CCCTTATGATCTCCACTTTAAGAACAGAGGAGA TTAAAGATGAGATCATATGGG	cccatat	TGGCGGCAGC GCAAAAG

*The full library is enclosed in appendix 1.2

Top 4 EC (endogenous control) toehold version 1 and 2:

	T7 promotor	Toehold region	Hairpin region	Refolding	Linker
Toehold_EC_1.1	taatacgactcact ataggg	CAGAAAACGATAATAA AAAAAGGA	TAGGTGCAGAGACCCTTTAAGACATGTAGAG GAGATTAAAGATGTCTCTGCACCTA	caggtgc	TGGCGGCAGC GCAAAAG
Toehold_EC_1.2	taatacgactcact ataggg	ATAAAAAAAAGGATAGG TGCAGAGA	CTCAATGGAAGCCCCTTTAAGACATGTAGAG GAGATTAAAGATGGCTTCATTGCG	cgcaatg	TGGCGGCAGC GCAAAAG
Toehold_EC_1.3	taatacgactcact ataggg	AAACGATAATAAAAAA AGGATAGG	TGCAGAGACTCACCCTTTAAGACATGTAGAG GAGATTAAAGATGTGCGTCTCTGCA	tgcagag	TGGCGGCAGC GCAAAAG
Toehold_EC_1.4	taatacgactcact ataggg	CAAGGGTTCAGAAAAC GATAATAA	AAAAAGGATAGGCCACTTTAAGACATGTAGAG GAGATTAAAGATGCCTATCCTTTT	caaaagg	TGGCGGCAGC GCAAAAG
Toehold_EC_2.1	taatacgactcact ataggg	CAGAAAACGATAATAA AAAAAGGA	TAGGTGCAGAGACCCTTTAAGAACAGAGGAG ATTAAAGATGTCTCTGCACCTA	caggtgc	TGGCGGCAGC GCAAAAG
Toehold_EC_2.2	taatacgactcact ataggg	ATAAAAAAAAGGATAGG TGCAGAGA	CTCAATGGAAGCCCCTTTAAGAACAGAGGAG ATTAAAGATGGCTTCATTGCG	cgcaatg	TGGCGGCAGC GCAAAAG
Toehold_EC_2.3	taatacgactcact ataggg	AAACGATAATAAAAAA AGGATAGG	TGCAGAGACTCACCCTTTAAGAACAGAGGAG ATTAAAGATGTGCGTCTCTGCA	tgcagag	TGGCGGCAGC GCAAAAG
Toehold_EC_2.4	taatacgactcact ataggg	CAAGGGTTCAGAAAAC GATAATAA	AAAAAGGATAGGCCACTTTAAGAACAGAGGAG ATTAAAGATGCCTATCCTTTT	caaaagg	TGGCGGCAGC GCAAAAG

*The full library is enclosed in appendix 1.3

Conclusion/comment:

Both version should be tested to see the functionality in two perspectives:

- High expression at ON state
- Low leakage at OFF state

But in order to economise the material we will perform only test on BN toeholds first, to see the general difference between version 1 and version 2.