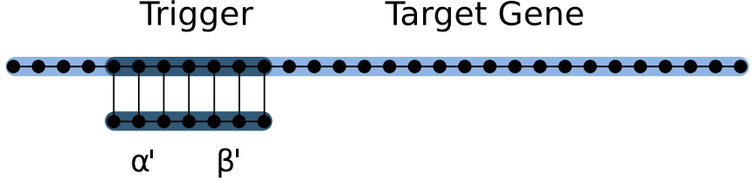
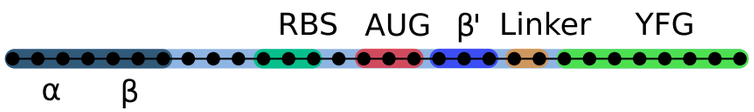
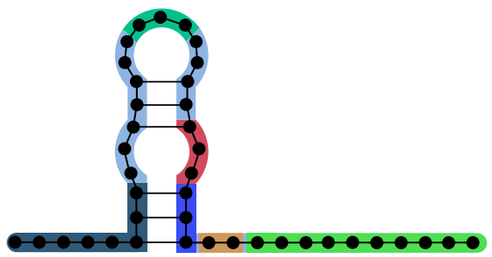


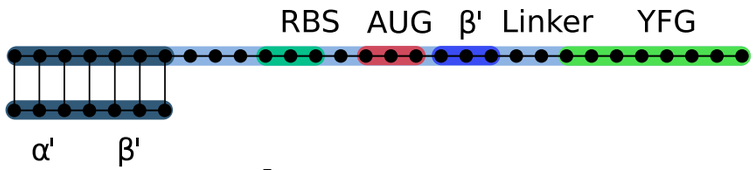
1- Build a toehold for a candidate trigger.



2- Test the secondary structure of the toehold switch.



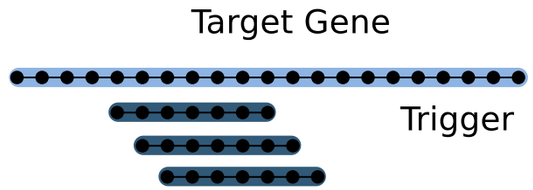
3- Test its binding to the trigger.



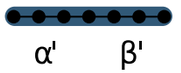
4- Submit it to a toehold library.

5.- Repeat with next trigger

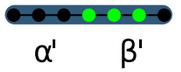
1.1- Scan target gene and select candidate trigger.



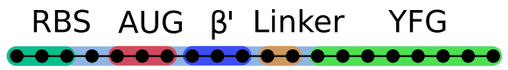
1.2- Generate complement (α' and β').



1.3- Ensure compliance with sequence constraints.

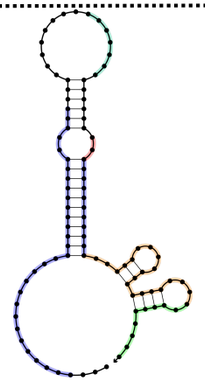


1.4- Add rest of components (RBS, AUG, linker, YFG).



2.1- Check toehold folding using NUPACK.

2.2- Calculate the free energy of the switch when not bound to the target ($\Delta G_{unbound/OFF}$).



3.1- Detect stop codons. If present in frame, toehold is discarded.

3.2- Ensure cannonical base pairing between the toehold and the trigger sequence.

3.3- Calculate free energy of the switch when bound to the target ($\Delta G_{bound/ON}$).