iGEM Thessaloniki - UParis Collaboration Report

iGEM Thessaloniki and UParis 2021

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igemthessaloniki@gmail.com ***igem.uparis@gmail.com***

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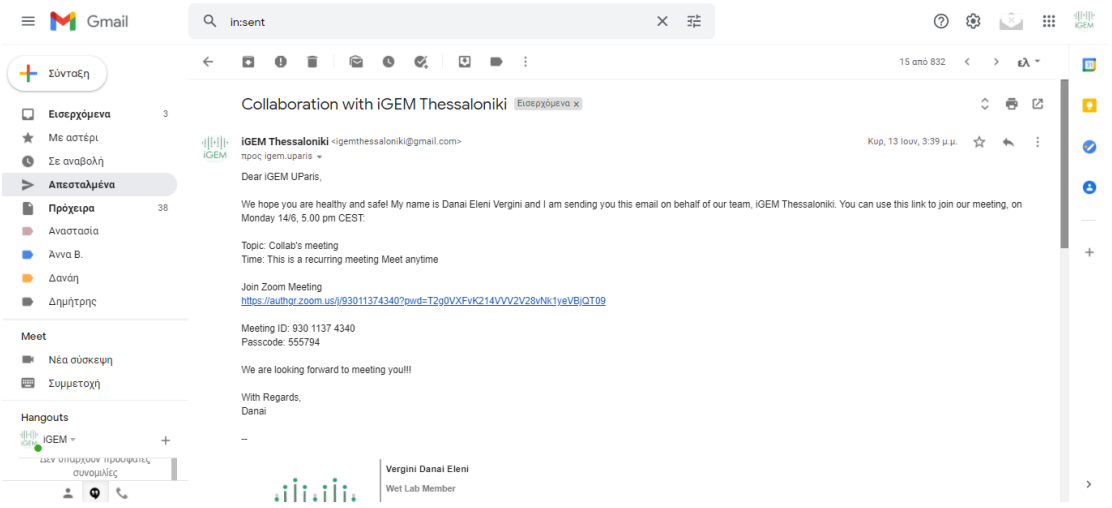
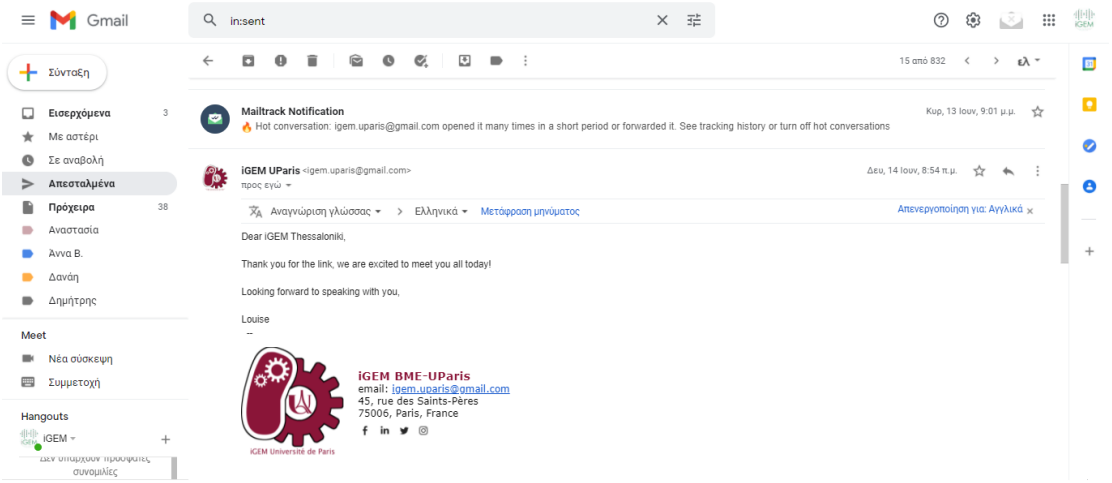
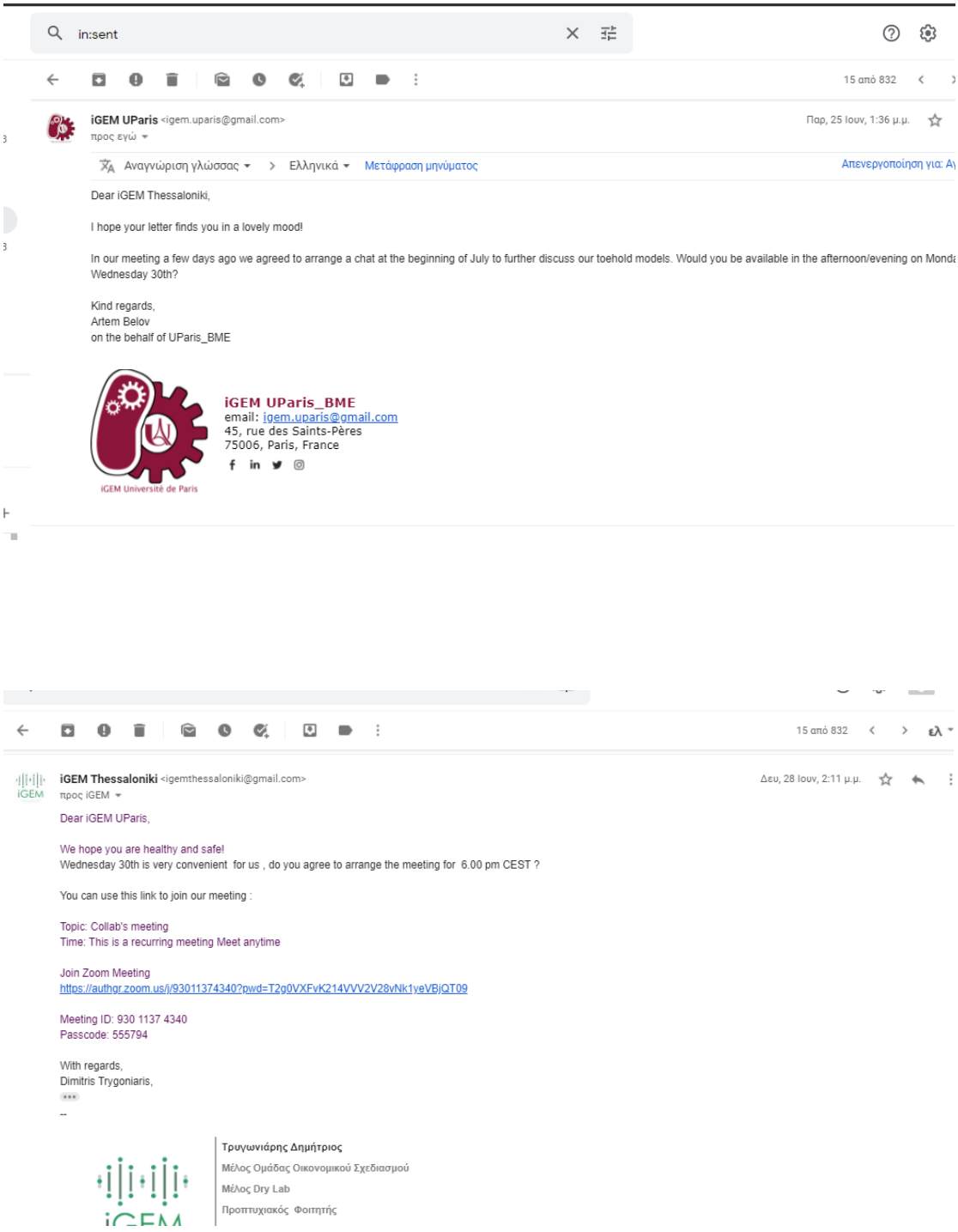
Email Exchange







in:sent

15 από 832

iGEM UParis <igem.uparis@gmail.com>
προς εγώ

Παρ, 25 Ιουν, 1:36 μ.μ.

Αναγνώριση γλώσσας · Ελληνικά · Μετάφραση μηνύματος

Απενεργοποίηση για: Α

Dear iGEM Thessaloniki,

I hope your letter finds you in a lovely mood!

In our meeting a few days ago we agreed to arrange a chat at the beginning of July to further discuss our toehold models. Would you be available in the afternoon/evening on Monda
Wednesday 30th?

Kind regards,
Artem Belov
on the behalf of UParis_BME

iGEM Université de Paris

iGEM UParis_BME
email: igem.uparis@gmail.com
45, rue des Saints-Pères
75006, Paris, France

15 από 832

iGEM Thessaloniki <igemthessaloniki@gmail.com>
προς iGEM

Δευ, 28 Ιουν, 2:11 μ.μ.

Dear iGEM UParis,

We hope you are healthy and safe!
Wednesday 30th is very convenient for us , do you agree to arrange the meeting for 6.00 pm CEST ?

You can use this link to join our meeting :

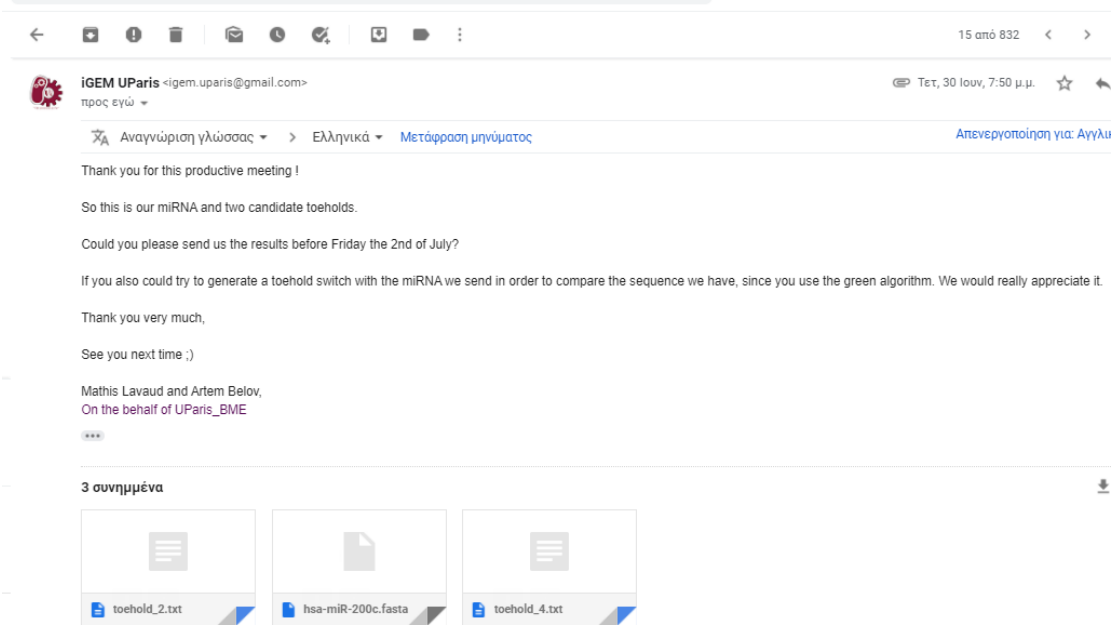
Topic: Collab's meeting
Time: This is a recurring meeting Meet anytime

Join Zoom Meeting
https://authgr.zoom.us/j/93011374340?pwd=T2g0VXFvK214VVV2V28vNk1yeVBjQT09

Meeting ID: 930 1137 4340
Passcode: 555794

With regards,
Dimitris Trygoniaris,
--

Τρυγωνιάρης Δημήτριος
Μέλος Ομάδας Οικονομικού Σχεδιασμού
Μέλος Dry Lab
Προπτυχιακός Φοιτητής



15 από 832

iGEM UParis <igem.uparis@gmail.com>
προς εγώ

Τετ, 30 Ιουν, 7:50 μ.μ.

Αναγνώριση γλώσσας › Ελληνικά Μετάφραση μηνύματος

Απενεργοποίηση για: Αγγλι

Thank you for this productive meeting !

So this is our miRNA and two candidate toeholds.

Could you please send us the results before Friday the 2nd of July?

If you also could try to generate a toehold switch with the miRNA we send in order to compare the sequence we have, since you use the green algorithm. We would really appreciate it.

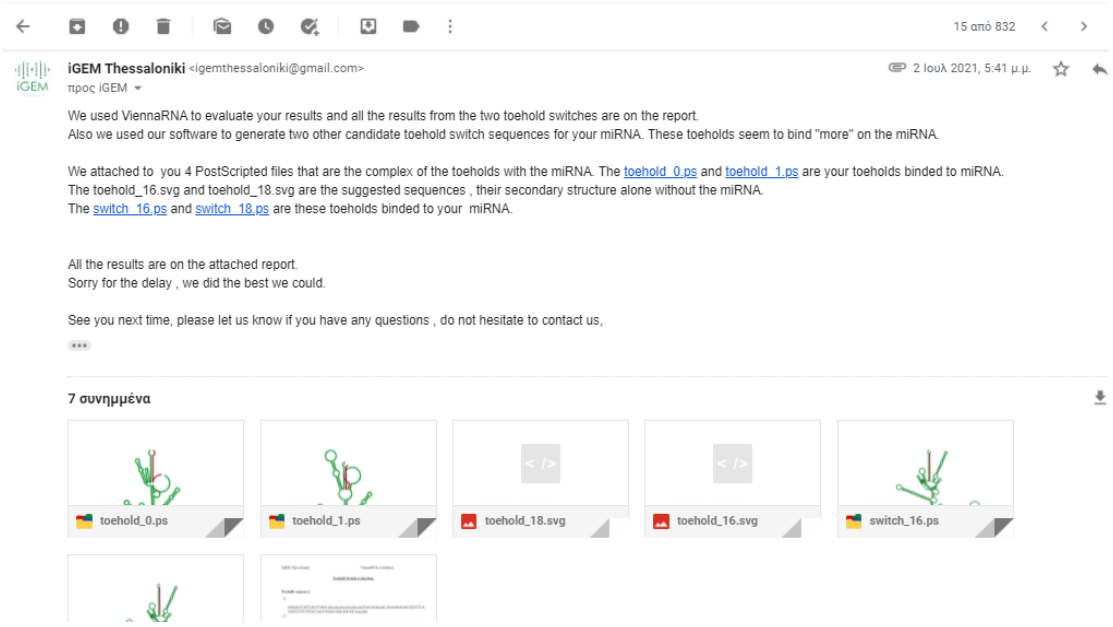
Thank you very much,

See you next time ;)

Mathis Lavaud and Artem Belov,
On the behalf of UParis_BME

3 συνημμένα

toehold_2.txt | hsa-miR-200c.fasta | toehold_4.txt



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iGEM Thessaloniki <igemthessaloniki@gmail.com>
προς iGEM

2 Ιουλ 2021, 5:41 μ.μ.

We used ViennaRNA to evaluate your results and all the results from the two toehold switches are on the report.
Also we used our software to generate two other candidate toehold switch sequences for your miRNA. These toeholds seem to bind "more" on the miRNA.

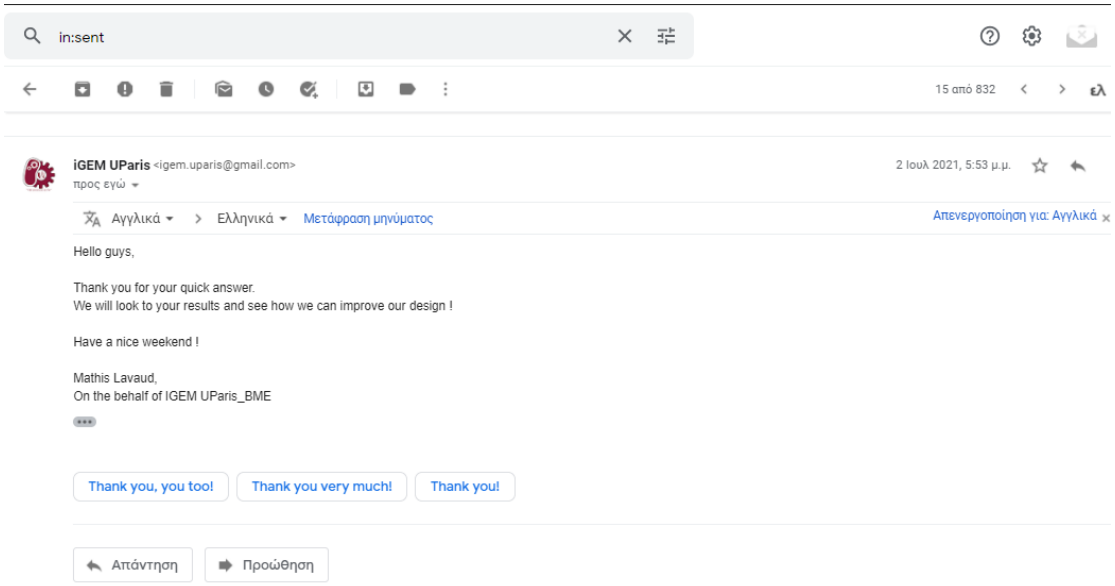
We attached to you 4 PostScripted files that are the complex of the toeholds with the miRNA. The toehold_0.ps and toehold_1.ps are your toeholds binded to miRNA.
The toehold_16.svg and toehold_18.svg are the suggested sequences , their secondary structure alone without the miRNA.
The switch_16.ps and switch_18.ps are these toeholds binded to your miRNA.

All the results are on the attached report.
Sorry for the delay , we did the best we could.

See you next time, please let us know if you have any questions , do not hesitate to contact us,

7 συνημμένα

toehold_0.ps | toehold_1.ps | toehold_18.svg | toehold_16.svg | switch_16.ps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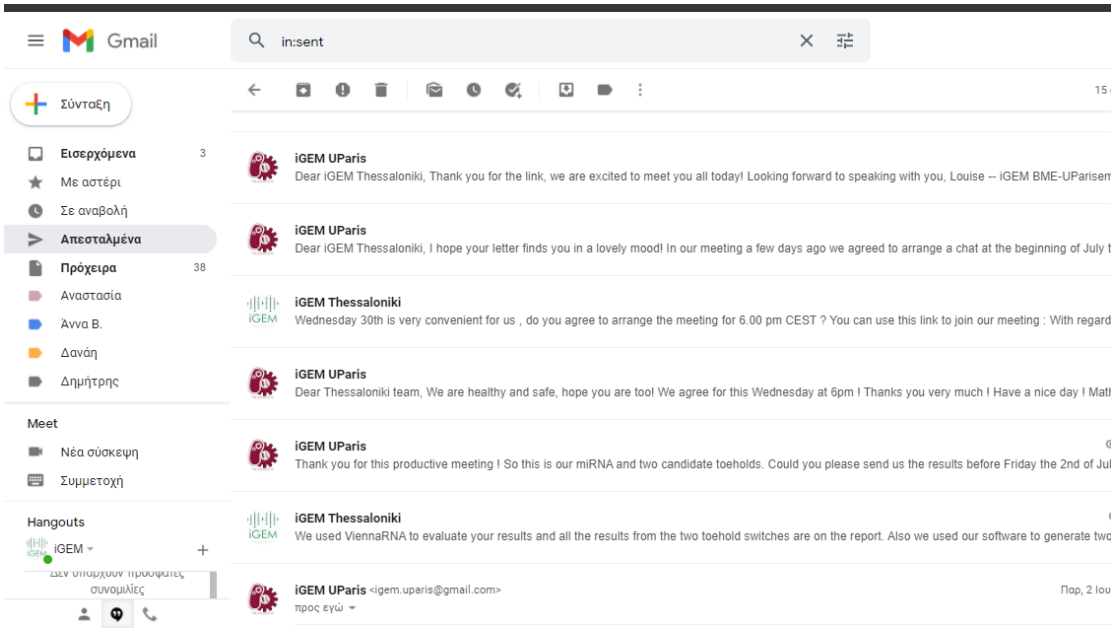
15 από 832
iGEM UParis <igem.uparis@gmail.com>
προς εγώ
2 Ιουλ 2021, 5:53 μ.μ.
Αγγλικά
Ελληνικά
Μετάφραση μηνύματος
Απενεργοποίηση για: Αγγλικά
Hello guys,
Thank you for your quick answer.
We will look to your results and see how we can improve our design !
Have a nice weekend !
Mathis Lavaud,
On the behalf of IGEM UParis_BME
Thank you, you too!
Thank you very much!
Thank you!
Απάντηση
Προώθηση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in:sent
Σύνταξη
Εισερχόμενα 3
Με αστέρι
Σε αναβολή
Απεσταλμένα
Πρόχειρα 38
Αναστασία
Άννα Β.
Δανάη
Δημήτρης
Meet
Νέα σύσκεψη
Συμμετοχή
Hangouts
iGEM
iGEM UParis
Dear iGEM Thessaloniki, Thank you for the link, we are excited to meet you all today! Looking forward to speaking with you, Louise -- iGEM BME-UParisem
iGEM UParis
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iGEM UParis
Dear Thessaloniki team, We are healthy and safe, hope you are too! We agree for this Wednesday at 6pm ! Thanks you very much ! Have a nice day ! Mat
iGEM UParis
Thank you for this productive meeting ! So this is our miRNA and two candidate toeholds. Could you please send us the results before Friday the 2nd of Jul
iGEM Thessaloniki
We used ViennaRNA to evaluate your results and all the results from the two toehold switches are on the report. Also we used our software to generate two
iGEM UParis <igem.uparis@gmail.com>
προς εγώ
Παρ, 2 Ιου

iGEM Thessaloniki's first report

Toehold Switch evaluation:

Toehold sequences :

1. GGGAUCAUUACCCGGCAAAAAAAAAAAAAAUACAGAAAC AGAGGAGAUAUUUUAUGUUUUUUTGCCACCUGGCGGCAG CGCAAAAG
2. GGGCCAUCAUUACCCGGAAAAAAAAAAAAAAUACAGAAA CAGAGGAGAUAUUUUAUGUUUUUUCCGGACCUGGCGGCA GCGCAAAAG

miRNA sequence:

UAAUACUGCCGGGUAAUGAUGGA

GFP sequence:

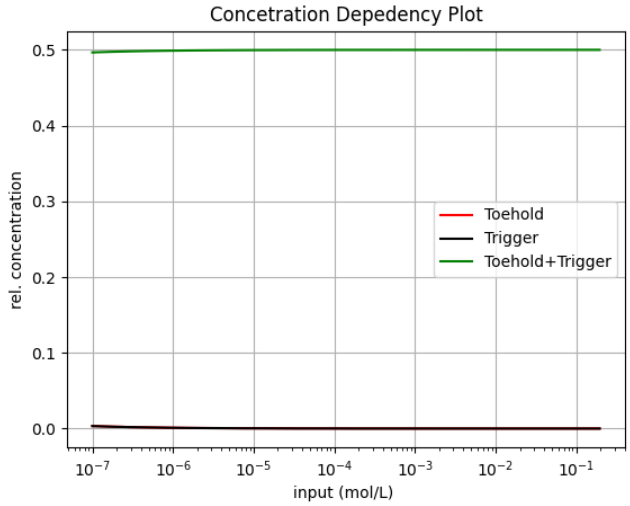
AUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAU UCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUC UGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAA CUUACCCUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUC CGUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAA UGCUUUGCGAGAUACCCAGAUCACAUGAAACAGCAUGACUU UUUCAAGAGUGCCAUGCCCGAAGGUUACGUACAGGAAAGA ACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGC UGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCG AGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUG GACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUAC AUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUU CAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAG CAGACCAUUAUCAACAAAAUACUCCGAUUGGCGAUGGCCC UGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUG CCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGU CCUUCUUGAGUUUGUAACCGCUGCUGGGAUUACACAUGGCA UGGAUGAACUAUACAAA

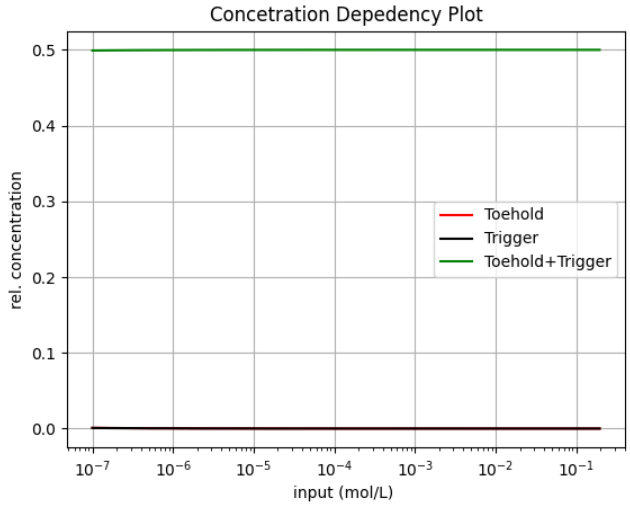
Energy of the binding :

1. Delta G binding : -16.71 kcal/mol , frequency of mfe structure in ensemble 7.95786e-14

2. Delta G binding : -14.63 kcal/mol , frequency of mfe structure in ensemble 2.57708e-14

Concentration Dependency Plots:





Same graphs for both of the toehold switches. The Concentration Dependency Plots generated with the use of **RNAcofold.exe** of **ViennaRNA software.** The binding of the toehold and the miRNA seem to be independent from the input concentration of the two molecules. So the complex that will lead to GFP expression , only depends on initial concentration of toehold and miRNA.

Duplex output:

1. .(((((((((((((.&.)))))))))))))). 3,18 : 6,21
2. .(((((((((((((.&.)))))))))))))). 3,18 : 8,23

The dot-bracket notation refers to the complex that will be formed after the interaction of the two molecules. You can see that not all the miRNA binds upon toehold switch but instead the paired bases that will be formed are 14. The position of the molecules is denoted with the numbers of 3,18 and 6,21 in first case. It means that the paired base of miRNA will be in the position of 4,17 ->14 and from toehold switch 5,20->14 .

Because the binding is continuous without other pairs , the perfect match of the two molecules should be considered upon design.

For the two sequences:

GGAUCAUUACCCGGCAAAAAAAAAAAAAAUACAGAAACAGA
GGAGAUAUUUUAUGUUUUUUTGCCACCUGGCGGCAGCGCAAAAG
(1.0, 14)

GGGCCAUCAUUACCCGGAAAAAAAAAAAAAAUACAGAAACAG
AGGAGAUAUUUUAUGUUUUUUCCGGACCUGGCGGCAGCGCAAAAG
(1.0, 14)

You have 14 paired bases after interaction . The first argument of the tuple (1.0) refers that all the bases of the miRNA that was paired with toehold switch , are continuous and that favors the binding.

The last evaluation is on free energy of the RBS-linker. We need to minimize this energy and the best is to have 0 kcal/mol (linear structure of the RBS-Linker so the ribosome can initiate translation).

CAGAAACAGAGGAGAUAUUUUAUGUUUUUUUGCCACCUGGC
GGCAGCGCAAAAG

(((.....((((((((((...)))))))))).....)))(((....)))..... (-10.30)

.,((((((.{{{(....)))..)))))) | {{{(...{{ | .,.,.}}}.)),,... [-11.68]

..((((((..(((....)))..))))))..... { -3.50 d=11.86}

frequency of mfe structure in ensemble 0.106941; ensemble diversity 16.88

CAGAAACAGAGGAGAUAUUUUAUGUUUUUUCCGGACCUGGC GGCAGCGCAAAAG

(((...(.((((((((((...)))))))))).)...)))(((....))).... (-12.10)

(((...(.((((((((((...)))))))))).)...)))(((....))).... [-12.47]

(((...(.((((((((((...)))))))))).)...)))(((....))).... {-12.10 d=1.01}

frequency of mfe structure in ensemble 0.549266; ensemble diversity 1.79

The first number is the mfe structure and the second number is the centroid structure. The energies are -10.30 kcal/mol and -11.68 kcal/mol for the first case. That means that after binding , the structure does not favor the linearity of RBS-linker .

The sequences that we predict to behave best are :

1. GGGUCCAUCAUUACCCGGCAGUAUUGUAGUGUGUCAGAA ACAGAGGAGAACACACAUGAAUACUGCCAACCUGGCGG CAGCGCAAAAG

1. GGGCCAUCAUUACCCGGCAGUAUUGUAGUGUGUCAGA AACAGAGGAGAACACACAUGAAUACUGCCAACCUGGCG GCAGCGCAAAAG

miRNA sequence:

UAAUACUGCCGGGUAAUGAUGGA

GFP sequence:

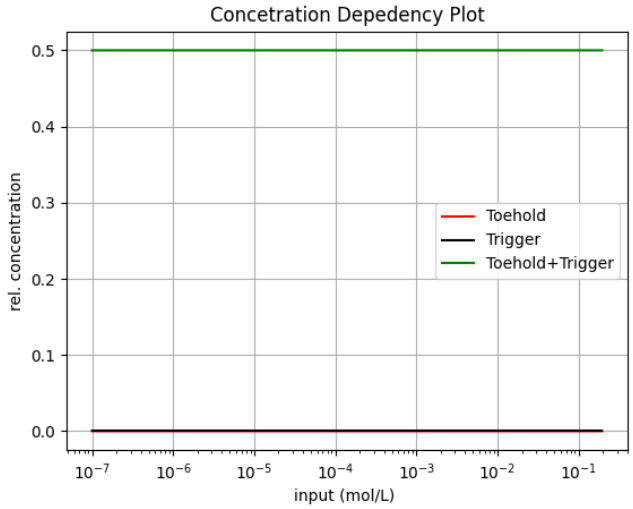
AUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAU UCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUC UGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAA CUUACCCUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUC CGUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAA UGCUUUGCGAGAUACCCAGAUCACAUGAAACAGCAUGACUU

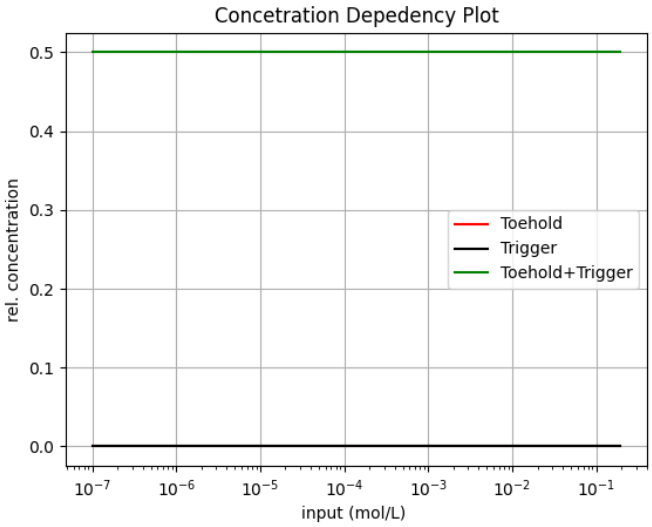
UUUCAAGAGUGCCAUGCCCGAAGGUUACGUACAGGAAAGA
ACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGC
UGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCG
AGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUG
GACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUAC
AUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUU
CAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAG
CAGACCAUUAUCAACAAAAUACUCCGAUUGGCGAUGGCCC
UGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUG
CCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGU
CCUUCUUGAGUUUGUAACCGCUGCUGGGAUUACACAUGGCA
UGGAUGAACUAUACAAA

Energy of the binding :

1. Delta G binding -22.35 kcal/mol , frequency of mfe structure in ensemble 2.12499e-10
2. Delta G binding =-21.48kcal/mol , frequency of mfe structure in ensemble 1.55045e-10

Concentration Dependency Plots:





Duplex output:

1. .(((((((((((((((((((((.&))))))))))))))))))))) 3,27 : 1,23
2. .(((((((((((((((((((((.&))))))))))))))))))))). 3,26 : 1,23

The main difference here is that all the miRNA bind to the toehold switch since the 23 bases are paired upon interaction.

The binding in the second toehold is different. Here the binding involves 22/23 bases of miRNA (small difference)

Perfect matches :

1) -> (1.0, 23)

2) -> (1.0, 22)

The perfect matches depend upon the RNAduplex.exe output.

Delta G RBS-Linker:

1. CAGAAACAGAGGAGAACACACAUGAAUACUGCCAAC CUGGCGGCAGCGCAAAAG

.....(((((.....)))))..... (-8.00)

.....(((((.....,,,))}}}..... [-8.78]

.....(((((.....)))))..... { -8.00 d=6.09}

frequency of mfe structure in ensemble 0.282723; ensemble diversity 7.58

Free energy = -8.00 kcal/mol

2. CAGAAACAGAGGAGAACACACAUGAAUACUGCCAAC CUGGCGGCAGCGCAAAAG

.....(((((.....)))))..... (-8.00)

.....(((((.....,,,))}}}..... [-8.78]

.....(((((.....)))))..... { -8.00 d=6.09}

frequency of mfe structure in ensemble 0.282723; ensemble diversity 7.58

iGEM Thessaloniki's second report

Toehold Switch evaluation:

Toehold Switch sequence – second generation :
GGGCAUCAUUACCCGGCAGUAUAAAAAAAAUACAGAAACAGAGG
AGAUAUUUUAUGUAUACUGCCGACCUGGCGGCAGCGCAAAAG

miRNA-200 c :

UAAUACUGCCGGGUAAUGAUGGA

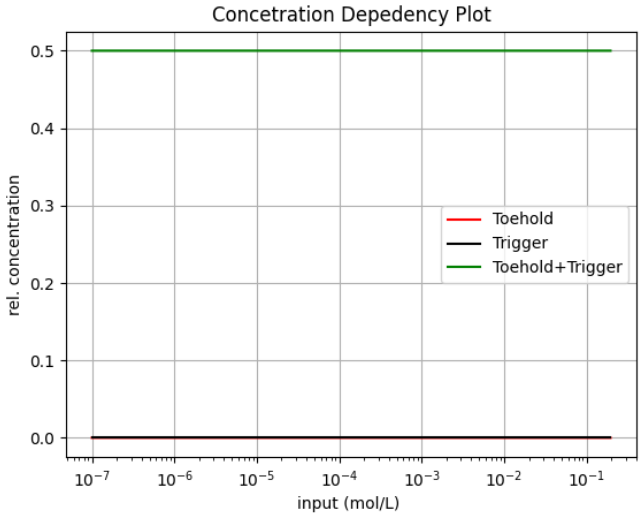
GFP :

AUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUU
GUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUG
GAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUAAAU
UUAUUUGCACUACUGGAAAACUACCUGUUCCGUGGCCAACACUUG
UCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAG
AUCACAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAG
GUUACGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACU
ACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUA
AUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAU
UCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUAC
AUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAA
AUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAU
UAUCAACAAAAUACUCCGAUUGGCGAUGGCCCUGUCCUUUUACCA
GACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUCCC
AACGAAAAGAGAGACCACAUGGUCCUUCUUGAGUUUGUAACCGCU
GCUGGGAUUACACAUGGCAUGGAUGAACUAUACAAA

Energy of the binding :

delta G binding=-20.74 (**better than before -16.71kcal/mol**) , frequency of mfe structure in ensemble 4.26997e-13

Concentration Dependency Plots:



Immediate binding upon interaction and the concentration of the complex is independent from the initial amount of miRNA and toehold switches molecules. The results are the same as the first generation of sequences.

Duplex output:

.((((((((((((((((((.&.))))))))))))))))))). 3,23 : 2,22 (-35.50)

The part of the miRNA that binds to toehold switch starts from 4 position to 22 position. The binding includes the formation of **19 hydrogen bonds.** This result is better than the previous one since the interaction of the first generation of toehold switches had only 14 base pairs upon interaction.

The perfect matches are **19** and the domain on interaction is predicted to be continuous. So the perfect matches for 19 pairs are 100% for this toehold switch sequence.

Energy of RBS-Linker:

CAGAAACAGAGGAGAUAUUUUAUGUAUACUGCCGACCUGGCGGC
AGCGCAAAAG

.....(((.((((....)))).)))...((((((......)))))).... (-11.20)

.....,,,,,,,,,....}}},.|||...((((((......)))))).,,,.... [-12.38]

.....((((((.....))))))..... {-10.30 d=5.43}

frequency of mfe structure in ensemble 0.148513; ensemble diversity 7.39

The Gibbs free energy of the domain of RBS-Linker Structure after binding , is **-11.20 kcal/mol** and this deviate from linear behavior that we would like. The linear behavior is achieved through the 0 kcal/mol free energy value for this domain of the molecule.

To conclude the results of this molecule are predicted to be better with the software of ViennaRNA compared to the first generation of toehold switch sequences.