NUPACK Design Parameter Settings

Document by Drylab iGEM UGM 2023

Sofyan Maulana (Biology UGM 2020)

Kayla Queenazima Santoso (Biomedical Engineering UGM 2020)

- Sequence Settings
  Set domains, strands, complexes, and target structures like below:
  a.   Set Domain LIRA Toehold with 3 Segment of Sub-Sequences

ABS-miR21bottomseal1	
ABS	N5
Complementstart	CAU
ABS_	N2
Loop	N2
ATS	N9
Toeholdmir21 - miR92topseal	
toeholdmir21	TCAACATCAGTCTGATAAGCTA
miR21topseal	N8
miR21bottomseal2	N13
miR92bottomseal1	N13
toeholdmiR92	ACAGGCCGGGACAAGTGCAATA
miR92topseal	N8
miR92bottomseal2 - end	
miR92bottomseal2	N13
RBS	TAGAGGAGATG
loopCom	N2
_DBS	N2
StartCodon	AUG
DBSCom	GCTTC

b. Domain miRNA settings

miR21	UAGCUUAUCAGACUGAUGUUGA
miR92a	UAUUGCACUUGUCCCGGCCUGU

c. Strands settings

LIRA	ABS-Complementstart-ABS_-Loop-ATS-loopRBS-miR21bottomseal1-toeholdmir21-miR21topseal-miR21bottomseal2-miR92bottomseal1-toeholdmiR92-miR92topseal-miR92bottomseal2-RBS-loopCom-_DBS-StartCodon-DBSCom
miRNA-21	miR21
miRNA-92a	miR92a

d. Complex and Target Structure settings

- LiRA
  : (((((((((((..(((((((((..((((((((((((((.(((...................-----.))).-)))))))))))(((((((((((.(((...................-----.))).-)))))))
  )))))..-)))))))))).-)))))))))))

- LiRA-miR92a:
  .......................(((((((((((.(((...............-----.))).-)))))))))))..............(((((((((((((((((((...........................-.................+)))))))))))))))))))

- LiRA-miR21:

  ............................(((((((((((((((((........((((((((.(((.....))).-))))))))(((...................-----.)))...........----...........----............ +)))))))))))))))))))

- Constraints settings

1.   GC contents: set with similarity in the hard constraints menu with Reference Sequence 'S30' and a limit of 0.2 to 0.6.

2. banned sequences are set to domain, separate strands, and separate complexes and then set on the off-complex menu. Banned sequences follow the illegal sites rules on the iGEM web:

Ecori: GAATTC GAAUUC
Xbai: TCTAGA UCUAGA
Spei: ACTAGT ACUAGU
Psti: CTGCAG CUGCAG
noti: GCGGCCGC

- Additional Settings
Material: RNA
temperature: 37 Celcius
Trials: 10
model options: ensemble all stacking
must include complexes: LiRA; LiRA-miR92a-miR21; LiRA-miR92a; LiRA-miR21; LiRA-miR21-miR92a
Target concentrations: 2.0 uM