**SNIPR design software**

**Overview**

To facilitate researchers to use SNIPR for gene regulation or molecular probes for SNP detections with cell free system, we provide the design software here to allow people design SNIPR easily. NUPACK3.2 is required to be installed to run the code, which can be downloaded from webserver (<http://www.nupack.org/downloads>). The software need to be run on macOS or Linux. The source code contain four major functions and a number of other supporting functions.

**The usage**

1. **NUPACK installation**

The SNIPR software is build up based on the NUPACK, the users need to download the NUPACK can compile it on your computer. Please follow the the NUPACK manual to install NUPACK.

After the NUPACK installation, we need to add NUPACK to system’s environmental variables to make the usage more conveniently.

Firstly, open the environment variable.

open ~/.bash\_profile

In the text edit environment, add the NUPACK directory as the following example.

export NUPACKHOME=/home/Documents/nupack3.2.0

export PATH=$PATH":${NUPACKHOME}/bin"

After setting the environment variables, Matlab need to be opened from terminal to use NUPACK based functions.

1. **SNIPR sequence input**

In the input folder, fill the target\_input.csv file with the design name, wide type target sequence, mutation type target sequence, and the first 29 bases of protein gene sequence. For the target sequence input, the mutation position should be at 15 bases away from the beginning.

The detailed information about the parameter input can be found in the comments in the source code.

1. **Running the SNIPR**

Before running the main code SNIPR.m，there are couple of parameters need to be specified by the users in the main function.

The size of library that the code will generate for each designs. For each target, there will be 9 conformation designs with different combination of forward and reverse toehold length, the total number of designs generated by the code will be the multiplication of library size and 9. We recommend to set the library number at least 10 to generate optimal designs.

library\_num = 10;

If your computer have multiple core, it’s better to take advantage of it to speed up the design process.

IS\_PARALLEL = 1;

This parameters is used to specify how many designs you want to try experimentally. The SNIPR design is challenging since it targets a very tiny difference. We recommend to try at least 5 designs to get successfully worked sensors.

select\_num = 9;

Sometimes, you may want to differentiate your WT target to SNP target, you can just specify it to be 0.

SNP\_TARGET = 1;

After finish the setting of the parameters, you can just run the main function SNIPR.m to generated the designs. The design may take some time to finish.

1. **Checking out the designs**

After the running is finished, the selected designs will be appeared in the output folder. The SNIPR sequence then will be ready to order to test.