

PSICOLIGOMAKER v1.5

Introduction

PSICOLIGOMAKER is a simple program to identify and design optimal shRNAs. It was created by me (Andrea Ventura) using the AppleScript language and is freely available to the scientific community. It runs exclusively on Apple computers with MacOSX.

The program is based on a set of criteria published by Angela Reynolds et al. (Nature Biotechnology, 2004). Given a DNA sequence the program returns a list of 19mers that have a score equal or higher than a cutoff value chosen by the researcher. 19mers with higher scores have more chances to result in effective shRNA. Usually we select 19mers with a score equal or higher than 6 (the maximum possible score with PSICOLIGOMAKER is 9). The program also converts selected 19mers into sense and antisense oligos ready to be ordered for cloning into pSico, pSicoR or lentilox 3.7

Installation

After downloading the file from the Jacks website (<http://web.mit.edu/ccr/labs/jacks/>), expand it with “stuffit expander”. Double-click on the icon and the program should start. The following window will appear:

TARGETS LIST		
Position	Sequence	Score

How does it work?

1) Finding good 19mers

The program is fairly intuitive. You start by pasting the sequence in the “paste your sequence here” window. The sequence should be “all caps” and contain only legal characters (ACGT). If it does not, click on “clean sequence”: the program will convert it to “all caps” and remove illegal characters.

If you want, you can give a name to your sequence (type it in the “sequence name” box). Select the cutoff score (-2 to 9, default 6) by typing the desired value in the “minimum score” box.

PSico, pSicoR and pLentilox 3.7 require that the 19mer starts with a “G”, other vectors (e.g. pSuper and derivatives) don't. You can tell the program to examine also 19mers that do not start with “G” by un-checking the corresponding checkbox.

When you are comfortable with your choices, click on “Search” and get a coffee, the program will scan the entire sequence (slowly, I must admit) and return a list of 19mers with score equal or higher than the cutoff. The starting position of each 19mer, its sequence and the corresponding score will be shown in the “Targets list” box. While the search is running, a progress indicator will show you the status of the analysis. Take it easy, drink your coffee and wait.

Once the search is done you can scroll the list of targets and copy and paste the list into an Excel or Word file for long term storage. If you find a 19mer that you like, we recommend you to search the Genbank database to be sure that the 19mer is unique to your gene. Click on “Go to BLAST” and the program will open “Netscape” and connect to the BLAST site.

2) Designing the oligos

Given a particular 19mer, designing the correct sense and antisense oligos that have to be ordered is quite tedious and error-prone. PSICOLIGOMAKER takes care of it. Simply paste or type the sequence of the desired 19mer into the “target” box, click on “Make oligos” and PSICOLIGOMAKER will return the forward and reverse oligos in the 5' → 3' orientation, ready to be ordered. By default the oligos are designed so that when annealed they will generate a DNA molecule ready to be cloned in pSico, pSicoR and pLentilox 3.7 (one end blunt, the other sticky compatible with XhoI and a 9 bp loop). You can see and modify the way oligos are designed by clicking on “settings”. This will let you design oligos for RNAi vectors other than ours.

Disclaimer

This should be all you need to know about PSICOLIGOMAKER v1.5. Remember that I am not a programmer, but an MD/PhD with very basic programming skills that I learnt playing with my Mac. I made this little program for fun, because I wanted to learn AppleScript and because I liked the Reynolds' paper. In our hands shRNAs selected using pSICOLIGOMAKER work on average much better than shRNA selected by other means, but of course we can not offer any guarantee that the same will be true for you. If you feel like you need more information, if you have any suggestion/comments or if you just want to tell me that PSICOLIGOMAKER works great also for you, email me at: aventura@mit.edu

Good luck with your experiments!

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