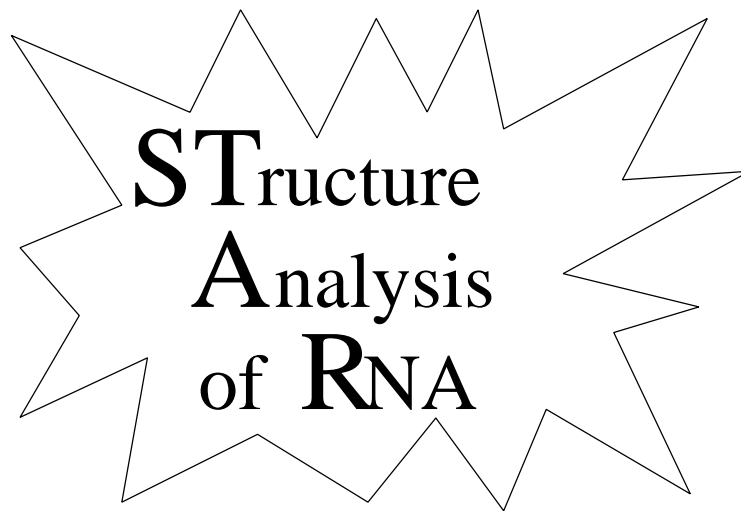


A short introduction to...



(version 5)

Read me please.

This leaflet tells you how to install *STAR* and it gives a short introduction into its use.

If you only have a demo-version of *STAR* this is your only documentation. Otherwise you also have a tutorial.

This leaflet has four sections:

- The first one tells you how to start.
- The second section explains how to install *STAR* on your computer.
- The third section teaches you how to use *STAR*.
- The last section suggests how to proceed further.

1. What to do?

First of all, install *STAR*. Follow the instructions in section 2 of this leaflet.

Next, learn the basic steps to run *STAR*. We wrote section 3 to introduce the basics of the program to you. It takes you approximately half an hour.

Finally, decide how to proceed further. Section 4 offers some guidelines.

2. Install *STAR* on your computer

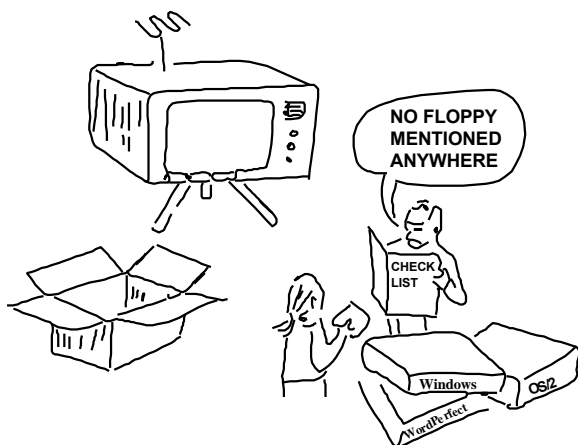
2.1 Contents of the package.

Before you install *STAR*, we suggest that you first check if your package is complete. You should have:

1. this leaflet.
2. a tutorial (not included with the demo).
3. a CD with software

If anything is missing, please let us know.

Tell us your name, address, serial number on the CD (if the CD is not missing, of course) and what the problem is.



2.2 Install procedure for Macintosh

The recipe for Mac OSX installation is simple.

First copy the content of the CD folder Fonts to your personal user folder ~\Library\Fonts^❶.

Next copy the CD folder STAR to your personal folder ~\Applications

To start the program, double click on program *STAR5* (within folder *STAR* within Applications).

To terminate the program, click on [File/Quit], and when *STAR* asks if you really want to quit, click on [OK].

If anything fails, please report this to us so we can help you and, when necessary, adjust the documentation and/or the software.

2.2 Install procedure for Windows

Windows installation is also simple. Just double click on InstallSTAR5.exe (or InstallSTAR5.exe).

This will start an installation dialog. If you like you can install this update in your old folder *STAR* ^❷. To finish the installation properly you have to reboot your computer first.

^❶ This will add the required font APLXMac to the fonts-folder in your Library.

^❷ This will not destroy your old program *STAR* because the new program is called *STAR5*; only a few added sequence files will be updated.

To start the program, click on the *STAR5* icon on your desktop or the *STAR5* in your START menu.

To terminate the program, click on [File/Quit], and when *STAR* asks if you really want to quit, click on [OK].

If anything fails, please report this to us so we can help you and, when necessary, adjust the documentation and/or the software.

3. Using *STAR*

The best way to learn *STAR* is to exercise a bit. The purpose of this section is to help you with this. It takes you through the main procedure of *STAR* using a demo file *STAR.1* of nucleotides of an imaginary RNA. You will predict the secondary structure of that RNA molecule.

If you don't like extensive documentation, read this to get a birds-eye view of *STAR*. After that, stop reading. For others, read this before reading the rest of the documentation.

Step 0: *STAR* procedure

The basic procedure of *STAR* is

1. start *STAR*
2. get a RNA sequence
3. compute structure
4. show structure
5. if unsatisfied, modify parameters and repeat this procedure

Step 1: start *STAR*

After you started *STAR*, it shows you an empty window with new menu-titles: File, Edit, View, Calculate and Help.

Exercise: click [Help] and observe that each of the menu titles have an explanation here. Read some of them.

Step 2: get sequence.

There are two ways to get a sequence: type it or read a file. *STAR* uses uppercase characters ACGU. Lowercase characters are permitted, but these nucleotides remain single stranded!

To type a sequence, use menu-item [File/New]. Type the sequence in uppercase characters. Use blanks and returns where that

feels appropriate. You can use comments whenever you need by writing an asterisk. Text after such an * will be interpreted as comment and ignored in the computations. Terminate with [File/Exit&Save]

Alternatively you could read a file using menu-item [File/Import/Primary]. *STAR* can read many file-types, but if *STAR* ever chokes on a particular file, read that file with a normal text editor, remove all characters except ACGU and save as an ASCII, TEXT file (without special word-processing characters!!).

Exercise: use [File/Import/Primary] and read file STAR.1. Look at it with [Edit/Primary] and observe the comment (after "*").

Step 3: compute structure.

Menu-item [Calculate/Compute/...] computes a structure. You get options: Greedy algorithm, Stochastic algorithm, and Genetic Algorithm.

The Greedy algorithm is the fastest algorithm, the Genetic algorithm is the slowest but the most reliable one; the Stochastic algorithm is in between.

Exercise: use [Calculate/Compute/...] and execute [Greedy algorithm].

Step 4: show structure.

Menu-item [View/Secondary/...] shows the structure on screen and [File/Print/Secondary] yields output on paper and also to file.

STAR has several ways to display output and describing them is less effective than observing yourself. Try them all.

Exercise: activate [View/Secondary/...] and look at each of the alternatives. If you want to study the results quietly, use [File/Print/Secondary/...] for paper output.

Sometimes printing does not work properly. In those cases you can send output to file using [File/Export/Secondary/...]. This file can be printed or read by your favourite text processor and processed for a publication.

Exercise: create a file using [File/Export/Secondary/Table]. Read this file

in your favourite text-processor.

Step 5: modify parameters; iterate.

For some RNA's it is easier to predict a correct secondary structure than for others. So each prediction should be studied critically. Contrary to the minimal energy algorithm, each of the *STAR* algorithms follows a stepwise procedure that can be coaxed to a certain extent. This is done in menu option [Calculate/Parameters].

Some of the various options are:

1. if you want to analyse a part of the structure only, or if some of the predicted stems are not correct you can exclude some nucleotides from stacking (recipe: choose [Calculate/Parameters] and specify after "Single From-To" the range of nucleotides to be excluded).
2. *STAR* uses energy rules at 37 ° C by default. If you want to know how your RNA folds at another temperature choose [Calculate/Parameters] and set the temperature to the one you are interested in. Observe the difference with your previous prediction.
3. folding during simulated growth can improve the quality of stochastic folding (recipe: choose [Calculate/Parameters] and set initial length to a small number like 25).
4. increase of population size can (often, but not always) improve the quality of stochastic folding or of the Genetic Algorithm (choose [Calculate/Parameters] and increase Population size).

After such changes you could do a new prediction with [Calculate/Compute/...] and compare it with a previous prediction.

Exercise: exclude nucleotides 10-15 from calculation using [Calculate/Parameters]; observe the change in the sequence using [View/Primary]. Do a new prediction using [Calculate/Compute/Greedy algorithm] and observe the change in the prediction.

Exercise: do a prediction using Genetic Algorithm and observe the forming *and* removal of intermediate stems.

4. What next?

In the previous section you learned the basic way to use *STAR*. Next you have several alternatives. What you choose depends on your personal style of learning.

If you feel rather confident, you can experiment a bit with the menus. Observe that every menu has a short description below the "Help"-menu. Use your own RNA.

Remember however, that the exercises here use small (sometimes artificial) sequences that are quickly analysed. As computer time roughly increases with a square of sequence length, a long RNA may take many hours. This makes the exercises quite boring.

If you feel a bit unsure we have written a tutorial to help you with exercises and increase your knowledge gradually. Here too, using your own RNA makes the exercises more interesting.

The next step is of course to use your own data. Remember that *STAR* reaches a "final" prediction by following a stochastic folding pathway. Some RNA's have a very stable final result, others may end in a dynamic equilibrium between some states; the latter will be reflected in *STAR* predictions that will vary from one run to another. So we recommend doing several simulations and comparing their results.

Another point to mention is that some RNA's have metastable states. This is reflected in the simulated folding pathway of *STAR* (consult [View/Secondary/Pathway] after a simulation which shows you the addition and removal of stems during the folding process) where you will see that at some point the simulation takes many iterations before the intermediate structure changes. This is an indication for a metastable state.

Finally remember that the predictions of *STAR* try to mimic the real folding process to a certain extent using energy rules as driving mechanism. If the algorithm is too simple, or other processes are involved (such as sheparones), the real secondary structure may be different from the prediction. So the

prediction should not be taken for granted, but checked critically against other (experimental) evidence.

We hope you will find *STAR* useful, please let us know any problems or suggestions you may think of.

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