How to use ExCaliBAR

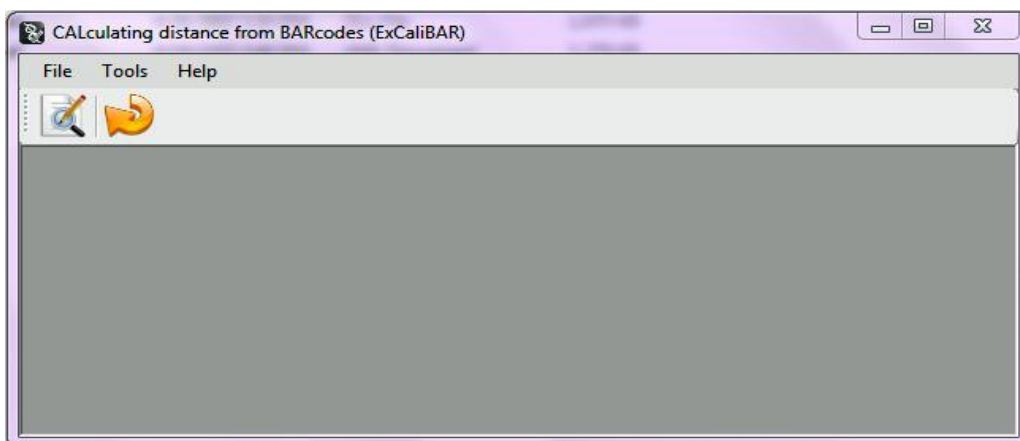**System requirements**

The ExCaliBAR software runs under Microsoft Windows, with Microsoft Net Framework version 2 or higher which is pre-installed in Microsoft Windows 7. Otherwise it can be downloaded and installed in the .NET Framework 4 from Microsoft website. The .NET Framework needs up to 500 MB of free disk space. The software runs only under Windows. It has been tested with Windows XP and Windows Vista, but presumably it should work also with Windows 2000 and Windows 7. Please report any problem with other Windows versions. To work with this program you only need to unzip the ExCaliBAR.zip file in your preferred folder. No further installation is required.

Environment

ExCaliBAR software has a simple environment with three options: "File, Tools and Help". In addition, there are two shortcut icons under the titles menu bar, "start Edit" and "Format Convertor" that can be used to open input Fasta file and distances matrix. (Fig. 1).
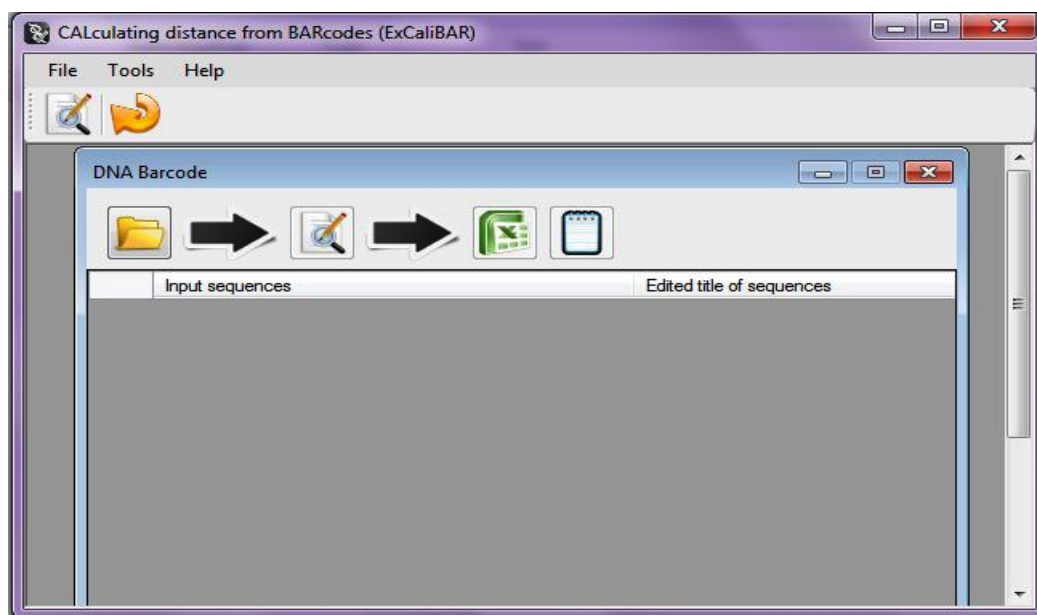
Fig. 1

How to load and edit data

1. Edit the sequence names

- To edit sequences names, select editor option under "Tools", or use "Start Edit" icon, to open the ExCaliBAR window. By browsing the input Fasta file from a given address, the original sequences of Fasta file will appear in the first column or "Input sequences" section of the ExCaliBAR window. The output file, after applying the editing button, will appear in the second column or "Edited title of sequences". The number of read sequences will appear in the menu bar below. The edited file can be saved either in text (Fasta) or excel format. At present some unconventional titles in GenBank or BOLD reside, and the software might not be able to recognize and edit the name of those sequences. In most of these cases, the title of the sequences will be marked with different colours in the 'Edited Genes' column. They can be edited manually (Fig 2).

Fig. 2

2. Format Convertor

- Before starting with this part you need a computed pairwise distance matrix for your sequences. The matrix of general pairwise distances can be calculated with the "Compute pairwise distances" option of MEGA 4 or MEGA 5. Keep in mind that assumed decimals for calculated distances are 4 digits and that the distance matrix should be saved in the lower left format. The text file of distances saved under MEGA (in simple text format) is then used as input file for the Format Convertor.

- Open Format Convertor under "Tools", or click on "Format Convertor" icon in the tool bar menu, to open the ExCaliBAR window

- By clicking on the file icons, browse the computed pairwise distances text file as input file.

- There are two options in the right and left side of the file icons for calculating intra- and inter-specific pairwise molecular distances (Fig.3). The output of inter- and intraspecific distances could be saved as text or excel file. Excel 2007 is restricted to 1,048,576 rows, and if your comparison is above this figure, it is advised to save the output file, particularly for interspecific distances, in text format.

- You can see the progress of computing, and also the sequence counting on the lower part of the menu bar. Depending on the data size this computing takes different amounts of time.

Fig. 3