

## RF-Net – README

### Software information

RF-Net is a method for estimation of hybridization and reassortment networks given a collection of rooted input trees (e.g., locus trees). RF-Net estimates a phylogenetic network that best embeds all of the given input trees. A major advantage of RF-Net is that it permits errors in the input trees.

The software is implemented in Java 1.8.

### Prerequisites for running RF-Net

- 1) Java 1.8 or higher needs to be installed;
- 2) RF-Net.jar depends on the Robinson-Foulds Supertree (RFS) method by Bansal et al. [1] for construction of a starting tree (i.e., the starting tree for the network search). RFS is available under Windows, Linux, and MacOS platforms.  
Note: last section in this README discusses actions to be taken in case RFS integration is failing.
- 3) For proper work of RF-Net the *dependencies* folder that contains RFS binaries has to be located in the same directory as RF-Net.jar.

### Running RF-Net

The software can be executed as follows. Note that this command has to be executed from the directory where RF-Net.jar is located.

```
java -jar RF-Net.jar [options]
```

*Options:*

- \* *-i, --input*  
*A path to a NEWICK file with input rooted trees*
- \* *-o, --output*  
*A path to a file where to place a resulting network*
- r, --ret*  
*The number of reticulations that a resulting network should contain*  
*Default: 5*
- s, --seed*  
*A path to a starting supertree (level-0 network) in Newick or Nexus format*
- p, --pool*  
*The size of a randomization pool (greater pool size leads to increased running time)*  
*Default: 5*
- a, --anyNetwork*  
*Include this argument if you do not wish to constrain the search space to the tree-child networks only*  
*Default: false (tree-child only)*
- lin32*  
*[32-bit Linux architecture only] specify that option if running on a 32-bit Linux distribution*  
*Default: false*

### Usage example

```
java -jar RF-Net.jar -i sample-dataset/sample-IAVs-8genes.tre -o  
result.newick -r 3
```

The above command would result in RF-Net estimating a phylogenetic network for an IAV dataset (this dataset is distributed along with the software) with three reassortment (reticulation) events.

### Usage example with a given starting tree

```
java -jar RF-Net.jar -i sample-dataset/sample-IAVs-8genes.tre -o  
result.newick -r 3 -s sample-dataset/sample-starting-tree.tre
```

### Network visualization

The output of RF-Net is a network with a specified number of reticulations in the extended Newick format [2]. To visualize such a network we recommend using [Hybroscale](https://hybroscale.com/) or an online tool <https://icytree.org/>. The [Dendroscope](https://dendroscope.org/) package can also be used.

### Possible issues with computing the starting tree via RFS

Since RFS is distributed as a binary file, there could appear difficulties with running it on different architectures/operating systems. Therefore, if you run into an error indicating that RF Supertree method cannot be executed, please, try to do the following:

1) For Linux/MacOS users: try running

```
chmod +x dependencies/RFS.*
```

to make sure that RFS binaries have the execution access permissions.

2) If running on a 32-bit Linux distribution, please, make sure to specify `--lin32` in the option list for RF-Net.jar.

3) If none of these work, please, run RF-Net using the `-s` option (i.e., providing a starting tree) and contact us for assistance. See the above usage example with an `-s` option.

### References

- [1] M.S. Bansal, J.G. Burleigh, O. Eulenstein, and J.G. Fernández-Baca, 2010. Robinson-Foulds Supertrees. *Algorithms for molecular biology*, 5(1).
- [2] G. Cardona, F. Rosselló, and G. Valiente, 2008. Extended Newick: it is time for a standard representation of phylogenetic networks. *BMC bioinformatics*, 9(1).
- [3] A. Markin, T.K. Anderson, V.S.K.T. Vadali, and O. Eulenstein, 2019. Robinson-Foulds Reticulation Networks. *bioRxiv* preprint.