

# The RAxML v8.2.X Manual

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## I. About RAxML

RAxML (Randomized Axelerated Maximum Likelihood) is a program for sequential and parallel Maximum Likelihood based inference of large phylogenetic trees. It can also be used for post-analyses of sets of phylogenetic trees, analyses of alignments and, evolutionary placement of short reads.

It has originally been derived from fastDNAmI which in turn was derived from Joe Felsenstein's dnaml which is part of the PHYLIP package.

**When using RAxML please cite:**

A. Stamatakis: "RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies". In *Bioinformatics*, 2014, open access link: <http://bioinformatics.oxfordjournals.org/content/early/2014/01/21/bioinformatics.btu033.abstract?keytype=ref&ijkey=VTEqgUJYCDcf0kP>

## II. Getting help

RAxML support is provided via the RAxML Google group at: <https://groups.google.com/forum?hl=de#!forum/raxml> Note that, Google groups have a search function!

Thus, before posting to the RAxML google group:

1. Search the group to see if your issue has not already been discussed!
2. If you don't want to get a rude reply, read this manual first!
3. Read a standard textbook about phylogenetics such as Ziheng Yang's excellent *Computational Molecular Evolution*. If you haven't read one you should rather not be using RAxML.

**Do never send emails with RAxML questions to A. Stamatakis directly!**

A step by step tutorial with some basic commands is available at: [http://sco.h-its.org/exelixis/web/software/raxml/hands\\_on.html](http://sco.h-its.org/exelixis/web/software/raxml/hands_on.html)

Additional help, links, little helper scripts and documentation is available at the RAxML software page: <http://sco.h-its.org/exelixis/web/software/raxml/index.html>

### III. RAxML web-servers and Graphical User Interfaces

While there exist several web-servers that allow you to run RAxML, I am directly involved in running three of them.

1. The Cipres Portal web server: [http://www.phylo.org/sub\\_sections/portal/](http://www.phylo.org/sub_sections/portal/)
2. The web-server at vital IT in Switzerland: <http://embnet.vital-it.ch/raxml-bb/>
3. A dedicated server for the Evolutionary Placement Algorithm: <http://epa.h-its.org/raxml>

There is no official graphical user interface supported by me, but a GUI has been developed by researchers at the research museum in Frankfurt, which is available here: <http://sourceforge.net/projects/raxmlgui/>

Note that, I will not provide any sort of support for the GUI, you need to contact the original authors for this.

### IV. Downloading RAxML

RAxML is open source under GNU GPL. It is distributed via Alexis github repository: <https://github.com/stamatak/standard-RAxML> where you can always download the most up to date version. Make sure to **watch** the github repository to remain up to date regarding code changes.

We do not provide any support whatsoever for previous versions of the code!

Version numbers follow the notation **x.y.z** where **x** changes with major code reorganizations, **y** changes when new features are added and **z** changes with bug fixes.

### V. Compiling RAxML

RAxML comes in a lot of different flavors.

It can run sequentially, in parallel using either MPI (message passing interface) or PThreads for multi-core shared memory systems.

It also has a hybrid/combined PThreads ↔ MPI parallelization that uses MPI to distribute bootstrap replicates or independent tree searches to different shared memory nodes in a cluster while it uses PThreads to parallelize the likelihood calculations of single tree searches. We call this coarse grain (MPI) and fine-grain (PThreads) parallelism.

Thus, before compiling you need to know on what kind of system you intend to execute RAxML. Also note that, the MPI version only implements/offers a subset of the RAxML functionality, it can only distribute distinct tree searches to processors!

Another important thing to consider prior to compiling is what your target processor architecture is! Modern x86 processors are very powerful because they have so-called vector instructions!

Depending on how new your processor is it will support SSE3 vector instructions, or, if newer also the faster AVX or the even faster AVX2 vector instructions. These instructions are used by RAxML to substantially accelerate the likelihood and parsimony computations it conducts.

Thus, you should always try to compile the code in a way that best exploits the capabilities of your CPU(s). Note that, even most modern laptops have more than 1 CPU/core, hence you will probably always want to compile the PThreads version of RAxML.

Now let's have a look at the RAxML source code, when you download it it will be in a file called:

```
standard-RAxML-8.0.0.tar.gz
```

uncompress it by typing:

```
gunzip standard-RAxML-8.0.0.tar.gz
tar xf standard-RAxML-8.0.0.tar
```

and change into the directory that contains the source files and list the contents:

```
cd standard-RAxML-8.0.0/
ls
```

There is a subdirectory called `usefulScripts` that contains a couple of perl scripts for various little RAxML tasks.

Next, let's list the Makefiles:

```
ls Makefile.*
```

which will generate a listing looking like this:

Makefiles for sequential version, hybrid MPI/Pthreads version, sequential version for MACs using the clang compiler, MPI version, PThreads version, PThreads version for MACs using clang that all rely on the most recent AVX2 vector instructions:

```
Makefile.AVX2.gcc
Makefile.AVX2.HYBRID.gcc
Makefile.AVX2.mac
Makefile.AVX2.MPI.gcc
Makefile.AVX2.PTHREADS.gcc
Makefile.AVX2.PTHREADS.mac
```

Corresponding Makefiles using AVX instructions:

```
Makefile.AVX.gcc
Makefile.AVX.HYBRID.gcc
Makefile.AVX.mac
Makefile.AVX.MPI.gcc
Makefile.AVX.PTHREADS.gcc
Makefile.AVX.PTHREADS.mac
```

Corresponding Makefiles not using any vector instructions (only required when your CPU is more than 5-6 years old!). The file called: `Makefile.QuartetMPI.gcc` is a dedicate Makefile that implements a MPI parallelization of the quartet evaluation functionality, for details see the section describing the command line arguments!

```
Makefile.gcc
Makefile.HYBRID.gcc
Makefile.MPI.gcc
Makefile.PTHREADS.gcc
Makefile.PTHREADS.mac
Makefile.QuartetMPI.gcc
```

Corresponding Makefiles using SSE3 instructions:

```
Makefile.SSE3.gcc
Makefile.SSE3.HYBRID.gcc
Makefile.SSE3.mac
Makefile.SSE3.MPI.gcc
Makefile.SSE3.PTHREADS.gcc
Makefile.SSE3.PTHREADS.mac
Makefile.SSE3.QuartetMPI.gcc
```

Now assume that your computer supports AVX instructions, to compile the sequential version type:

```
make -f Makefile.AVX.gcc
```

this will generate a RAXML executable called:

```
raxmlHPC-AVX
```

Now, to also compile the PThreads version, first remove all object files generated by compiling the sequential version by typing:

```
rm *.o
```

and then type:

```
make -f Makefile.AVX.PTHREADS.gcc
```

which will generate an executable called

```
raxmlHPC-PTHREADS-AVX
```

Compiling all other program flavors is analogous, with the only difficulty that the MPI versions need mpicc, the MPI compiler. A common MPI compiler distribution is provided by OpenMPI and is easy to install on Ubuntu Linux, for instance.

### *When to use which Version?*

The use of the sequential version is intended for small to medium datasets and for initial experiments to determine appropriate search parameters.

The PThreads version will work well for very long alignments, but performance is extremely hardware-dependent!

However, even for relatively short alignments (1,900 taxa, 1,200bp, DNA data) we observed speedups of around factor 6.5 on an 8-core node.

For a long alignment (125 taxa, 20,000 base-pairs, DNA) we observed significant super-linear speedups of around 10-11 on an 8-core system.

**Warning:** Make sure to specify the exact number of CPUs available on your system via the `-t` option. If you start more threads than you have cores available, there will be a significant

performance decrease!

The MPI version is for executing really large production runs (i.e. 100 or 1,000 bootstraps) on a Linux cluster. You can also perform multiple inferences on larger datasets in parallel to find a best-known ML tree for your dataset.

Finally, the rapid BS algorithm and the associated ML search have also been parallelized with MPI.

**Warning:** Reduced functionality of MPI version! The current MPI version only works properly if you specify the `-#` or `-N` option in the command line, since it has been designed to do multiple inferences or rapid/standard BS (bootstrap) searches in parallel!

For all remaining options, the usage of this type of coarse-grained parallelism does not make much sense!

### *Processor Affinity and Thread Pinning with the PThreads Version*

An important aspect if you want to use the PThreads version of the program is to find out how your operating system/platform handles processor affinity of threads. Within the shared-memory or multi-core context processor affinity means that if you run, for instance, 4 threads on a 4-way CPU or 4 cores each individual thread should always run on the same CPU, that is, `thread0` on CPU0, `thread1` on CPU1 etc.

This is important for efficiency, since cache entries can be continuously re-used if a thread, which works on the same part of the shared memory space, remains on the same CPU. If threads are moved around, for instance, `thread0` is initially executed on CPU0 but then on CPU4 etc. the cache memory of the CPU will have to be re-filled every time a thread is moved. With processor affinity enabled, performance improvements of 5% have been measured on sufficiently large and thus memory-intensive datasets.

There is a function that will automatically pin threads to CPUs, that is, enforce thread affinity, under LINUX/UNIX. Because this function might occasionally cause some error messages during compilation due to portability issues it is disabled by default. To enable it, you will need to comment out this flag:

```
#define _PORTABLE_PTHREADS
```

in the `axml.c` source file.

### *How many Threads shall I use?*

It is important to know that the parallel efficiency of the PThreads version of RAxML depends on the alignment length. Normally, you would expect a parallel program to become faster as you increase the number of cores/processors you are using. This is however not generally true, because the more processors you use, the more accumulated time they spend waiting for the input to be parsed and communicating with each other. In computer science this phenomenon is known as Amdahl's law (see [http://en.wikipedia.org/wiki/Amdahl's\\_law](http://en.wikipedia.org/wiki/Amdahl's_law)).

Thus, if you run RAxML with 32 instead of 1 thread this does not mean that it will automatically become 32 times faster, it may actually even become slower. As I already mentioned, the parallel efficiency, that is, with how many threads/cores you can still execute it efficiently in parallel depends on the alignment length, or to be more precise on the number of distinct patterns in your alignment. This number is printed by RAxML to the terminal and into the `RAxML_info.runID` file and look like this:

```
Alignment has 70 distinct alignment patterns
```

As a rule of thumb I'd use one core/thread per 500 DNA site patterns, i.e., if you have less, than it's probably better to just use the sequential version. Single-gene DNA alignments with around 1000 sites can be analyzed with 2 or at most 4 threads. Thus, the more patterns your alignment has, the

more threads/cores you can use efficiently.

Also note that, efficiency varies depending on the type of data, or more precisely, the number of states in your data (e.g., 4 in DNA, 20 for proteins). The more states you have, the fewer site patterns you need per thread/core for RAxML to execute efficiently in parallel. This is because there is more computational work (more mathematical operations) to be done per site pattern as the number of states increases. With protein data you thus require less sites per thread for RAxML to run efficiently. Thus, an MSA with 1000 protein site patterns may still run efficiently when using 16 cores/threads.

Finally, parallel efficiency also depends on the rate heterogeneity model. With the GAMMA model that entails more computations you can thus typically use more threads than with the CAT model that only executes approximately  $\frac{1}{4}$  of the computations the GAMMA model requires.

Note that, these are just very rough rules of thumb, you need to test what the optimal setting is for your dataset!

## VI. RAxML Likelihood Values & Idiosyncrasies

It is very important to note that the likelihood values produced by RAxML can not be directly compared to likelihood values of other ML programs. However, the likelihood values of the current version are very similar to those obtained by other programs with respect to previous releases of RAxML (usually between  $\pm 1.0$  log likelihood units of those obtained e.g. by PHYML or GARLI).

The above of course refers to evaluating the likelihood on identical trees, in terms of tree searches the programs will yield different tree topologies and hence different likelihoods in most cases anyway.

Note, that the deviations between PHYML/RAxML and GARLI likelihood values can sometimes be larger because GARLI uses a slightly different procedure to compute empirical base frequencies (Derrick Zwickl, personal communication, many years ago) while the method in RAxML is exactly the same as implemented in PHYML.

These deviations between RAxML/PHYML on the one side and GARLI on the other side appear to be larger on long multi-gene alignments. Also note that, even likelihood values obtained by different RAxML versions, especially should not be directly compared with each other either.

This is due to frequent code and data structure changes in the likelihood function implementation and model parameter optimization procedures!

Thus, if you want to compare topologies obtained by distinct ML programs with respect to their likelihood, make sure that you optimize branch lengths and model parameters of final topologies with one and the same program.

This can be done by either using the respective RAxML option (`-f e`) or, e.g., the corresponding option in PHYML (see <http://www.atgc-montpellier.fr/phyml/>).

### *Differences in Likelihood scores*

In theory all ML programs implement the same mathematical function and should thus yield the same likelihood score for a fixed model and a given tree topology.

However, if we try to implement a numerical function on a finite machine we will unavoidably obtain rounding errors. Even if we change the sequence (or if it is changed by the compiler, which it usually is) of some operations applied to floating point or double precision arithmetics in our computer we will probably get different results

In my experiments I have observed differences among final likelihood values between GARLI, IQPNNI, PHYML, RAxML (every program showed a different value).

You can also experiment with this by removing the gcc optimization flag `-O2` in one of the RAxML Makefiles. This will yield much slower code, that is in theory mathematically equivalent to the optimized code, but will yield slightly different likelihood scores, due to re-ordered floating point operations.

My personal opinion is that the topological search (number of topologies analyzed) is much more important than exact likelihood scores to obtain *good* final ML trees.

Especially on large trees with more than 1,000 sequences the differences in likelihood scores induced by the topology are usually so large, that a very rough parameter optimization with an epsilon (RAxML `-e` option) of 1 log likelihood unit, i.e., if the difference epsilon between two successive model parameter optimization iterations is smaller than 1.0 we stop the optimization, will already clearly show the differences.

Note that, if you perform a bootstrap analysis you don't need to worry too much about likelihood values anyway, since usually you are only interested in the bootstrapped topologies.

### *The CAT model of rate heterogeneity*

The name of this model has caused a lot of confusion because there is a CAT model also implemented in PhyloBayes (see <http://megasun.bch.umontreal.ca/People/lartillot/www/index.htm>)

Unfortunately, I was not aware of this when I introduced the CAT model in RAxML, the CAT model in RAxML is something completely different! However, I decided not to change the name for backward compatibility such that new RAxML version keep working with old wrapper scripts.

**Warning:** It is not a good idea to use the CAT approximation of rate heterogeneity on datasets with less than 50 taxa. In general there will not be enough data per alignment column available to reliably estimate the per-site rate parameters.

CAT has been designed to accelerate the computations on large datasets with many taxa! Please read the respective paper

[http://ieeexplore.ieee.org/xpl/login.jsp?tp=&arnumber=1639535&url=http%3A%2F%2Fieeexplore.ieee.org%2Fexpls%2Fabs\\_all.jsp%3Farnumber%3D1639535](http://ieeexplore.ieee.org/xpl/login.jsp?tp=&arnumber=1639535&url=http%3A%2F%2Fieeexplore.ieee.org%2Fexpls%2Fabs_all.jsp%3Farnumber%3D1639535)

to understand how CAT works, what the rate categories are (they are conceptually different from the discrete rate categories of the GAMMA model), and what the limitations of this method are.

The *GTRCAT* approximation is a computational work-around for the widely used General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity. CAT serves the analogous purpose, that is, to accommodate searches that incorporate rate heterogeneity.

The aforementioned paper describes what *GTRCAT* is and why I don't like *GTRGAMMA* despite the fact that it is a beautiful Greek letter.

The main idea behind *GTRCAT* is to allow for integration of rate heterogeneity into phylogenetic analyses at a significantly lower computational cost (about 4 times faster) and memory consumption (4 times lower).

Essentially, *GTRCAT* represents a rather un-mathematical *quick & dirty* approach to rapidly navigate into portions of the tree space, where the trees score well under *GTRGAMMA*.

However, due to the way individual rates are optimized and assigned to rate categories in *GTRCAT*, the likelihood values computed under *GTRCAT* are completely meaningless.

**Warning:** never compare alternative tree topologies using their CAT-based likelihood scores!

You will probably obtain a biased assessment of trees. This is the reason why *GTRCAT* is called

approximation instead of model. The same applies to the CAT approximation when used with AA data or any other data type that RAxML supports.

In general, the CAT approximation of rate heterogeneity works very well on datasets with more than 50 taxa for conducting tree searches under a model that accommodates rate heterogeneity among sites. In other words, if you score the trees obtained under CAT using GAMMA you will usually obtain equally good trees as with full searches under GAMMA at a substantially lower computational cost. Also, GAMMA may not work for numerical reasons on very large trees with more than 10,000 taxa (see <http://www.biomedcentral.com/1471-2105/12/470>).

Another detailed study of a CAT-like model is conducted in the FastTree-2 paper, see <http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0009490>

## VII. Alignment input File Formats

### *Alignment & Tree Input Formats*

**Alignments:** The input alignment format of RAxML is relaxed interleaved or sequential PHYLIP or FASTA. *Relaxed* means that sequence names can be of variable length between 1 up to 256 characters.

If you need longer taxon names you can adapt the constant `#define nmlngth 256` in the source file `axml.h` appropriately.

Moreover, RAxML is less sensitive with respect to the PHYLIP formatting (tabs, insets, etc) of interleaved PHYLIP files.

**Trees:** The input tree format is Newick, the RAxML input trees must not always be comprehensive, i.e., need not contain all taxa of the alignment.

See <http://evolution.genetics.washington.edu/phylip/newicktree.html> for details on the Newick format.

### *Alignment Error Checking*

Many alignments need to be checked for the following errors/insufficiencies before running an analysis with RAxML or any other phylogenetic inference program.

RAxML automatically analyzes the alignment and checks for the following errors:

1. Identical Sequence name(s) appearing multiple times in an alignment, this can easily happen when you export a standard PHYLIP file from some tool which truncates the sequence names to 8 or 10 characters.
2. Identical Sequence(s) that have different names but are exactly identical. This mostly happens when you excluded some hard-to-align alignment regions from your alignment and does not make sense to use.
3. Undetermined Column(s) that contain only ambiguous characters that will be treated as missing data, i.e. columns that entirely consist of `x, ?, *, -` for AA data and `n, o, x, ?, -` for DNA data (analogous for other data types)
4. Undetermined Sequence(s) that contain only ambiguous characters (see above) that will be treated as missing data.

Prohibited Character(s) in taxon names are names that contain any form of whitespace character, like blanks, tabulators, and carriage returns, as well as one of the following prohibited characters: `:` or `()` or `[]`.



In case that RAxML detects identical sequences and/or undetermined columns and was executed, e.g., with `-n alignmentName` it will automatically write an alignment file called `alignmentName.reduced` with identical sequences and/or undetermined columns removed.

If this is detected for a partitioned model analysis a respective model file `modelFileName.reduced` will also be written. In case RAxML encounters identical sequence names or undetermined sequences or illegal characters in taxon names it will exit with an error and you will have to fix your alignment.

## VIII. The RAxML options

The single by far most important command is the RAxML help option that displays all options. I also frequently use it because I can not remember them all. In the following I will assume that we are just using the sequential unvectorized code, that is, the `raxmlHPC` executable.

I will discuss each option and provide a simple usage example for it.

Thus, to get on-line help type:

```
raxmlHPC -h
```

and you will get the following, very long listing, that will be discussed at length below:

```
raxmlHPC  -s sequenceFileName -n outputFileName -m substitutionModel
           [-a weightFileName] [-A secondaryStructureSubstModel]
           [-b bootstrapRandomNumberSeed] [-B wcCriterionThreshold]
           [-c numberOfCategories] [-C] [-d] [-D]
           [-e likelihoodEpsilon] [-E excludeFileName]
           [-f a|A|b|B|c|C|d|D|e|E|F|g|G|h|H|i|I|j|J|k|m|n|N|o|p|q|r|R|s|S|t|T|u|
           v|V|w|W|x|y]
           [-F]
           [-g groupingFileName] [-G placementThreshold] [-h] [-H]
           [-i initialRearrangementSetting]
           [-I autoFC|autoMR|autoMRE|autoMRE_IGN]
           [-j] [-J MR|MR_DROP|MRE|STRICT|STRICT_DROP|T_<PERCENT>] [-k] [-K]
           [-L MR|MRE|T_<PERCENT>] [-M]
           [-o outGroupName1[,outGroupName2[,...]]] [-O]
           [-p parsimonyRandomSeed] [-P proteinModel]
           [-q multipleModelFileName] [-r binaryConstraintTree]
           [-R binaryModelParamFile] [-S secondaryStructureFile]
           [-t userStartingTree]
           [-T numberOfThreads] [-u] [-U] [-v] [-V] [-w outputDirectory]
           [-W slidingWindowSize]
           [-x rapidBootstrapRandomNumberSeed] [-X] [-y]
           [-Y quartetGroupingFileName|ancestralSequenceCandidatesFileName]
           [-z multipleTreesFile]
           [-#|-N numberOfRuns|autoFC|autoMR|autoMRE|autoMRE_IGN]
           [--mesquite][--silent][--no-seq-check][--no-bfgs]
           [--asc-corr=stamatakis|felsenstein|lewis]
           [--flag-check]
           [--auto-prot=ml|bic|aic|aicc ]
           [--epa-keep-placements=number]
           [--epa-accumulated-threshold=threshold]
           [--epa-prob-threshold=threshold]
```

```
[--JC69][--K80]
[--set-thread-affinity]
[--bootstop-perms=number]
[--quartets-without-replacement]
[--print-identical-sequences]
```

- a Specify a column weight file name to assign individual weights to each column of the alignment. Those weights must be integers separated by any type and number of white-spaces within a separate file.

In addition, there must of course be as many weights as there are columns in your alignment. The contents of an example weight file could look like this:

```
5 1 1 2 1 1 1 1 1 1 1 2 1 1 3 1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 4 1 1 1 4 1 1
```

**Example:** `raxmlHPC -a wgtFile -s alg.phy -p 12345 -m GTRCAT -n TEST`

- A Specify one of the secondary structure substitution models implemented in RAxML. The same nomenclature as in the PHASE manual is used, available models: S6A, S6B, S6C, S6D, S6E, S7A, S7B, S7C, S7D, S7E, S7F, S16, S16A, S16B

DEFAULT: 16-state GTR model (S16)

Note that, partitioning does not work with secondary structure models. That is a secondary structure can only be superimposed to a single partition. Also note that, you need of course to also specify a file defining the secondary structure via the `-s` option.

**Example:** `raxmlHPC -S secondaryStructureFile -s alg.phy -A S7D -p 12345 -m GTRGAMMA -n TEST`

- b Specify an integer number (random seed) and turn on bootstrapping

DEFAULT: OFF

This option allows you to turn on non-parametric bootstrapping. To allow for reproducibility of runs in the sequential program, you have to specify a random number seed, e.g., `-b 123476`.

Note however, that parallel bootstraps with the parallel version `raxmlHPC-MPI` are not reproducible despite the fact that you specify a random number seed.

**Example:** `raxmlHPC -b 12345 -p 12345 -# 100 -s alg -m GTRCAT -n TEST`

- B specify a floating point number between 0.0 and 1.0 that will be used as cutoff threshold for the MR-based bootstopping criteria. The recommended setting is 0.03.

DEFAULT: 0.03 (recommended empirically determined setting)

This setting allows to specify a threshold for the so-called bootstopping criteria that will automatically determine if you have conducted enough bootstrap replicate searches for obtaining stable support values. Note that, this only has an effect if you use the bootstopping criteria that rely on building majority rule consensus trees for determining convergence. The option will not have an effect when the - not recommended - frequency stopping criterion is being used. Please also read and cite the corresponding paper:

[http://link.springer.com/chapter/10.1007/978-3-642-02008-7\\_13#page-1](http://link.springer.com/chapter/10.1007/978-3-642-02008-7_13#page-1)

**Example:** raxmlHPC -B 0.02 -b 12345 -p 12345 -# AUTOMR -s alg -m GTRCAT -n TEST

- c Specify number of distinct rate categories for RAXML when model of rate heterogeneity is set to CAT. Individual per-site rates are categorized into numberOfCategories rate categories to accelerate computations.

DEFAULT: 25

**Example:** raxmlHPC -c 40 -p 12345 -s alg -m GTRCAT -n TEST

**Warning:** Note that the setting of -c has no effect whatsoever on the number of discrete rate categories that are used to approximate the Gamma distribution of rate heterogeneity! RAXML always uses 4 discrete rate categories to approximate Gamma!

- C Enable verbose output for the "-L" and "-f i" options. This will produce more, as well as more verbose output files

DEFAULT: OFF

The above option will generate verbose output and additional output files when RAXML is used to compute the TC and IC measures as introduced by Salichos and Rokas:

<http://www.nature.com/nature/journal/v497/n7449/full/nature12130.html>

The method is described in more detail in the following paper I wrote with Salichos and Rokas:

<http://mbe.oxfordjournals.org/content/early/2014/02/07/molbev.msu061.abstractkeytype=ref&ijkey=I65FuGNx0HzR2Ow>

**Example:** raxmlHPC -L -m GTRCAT -L MRE -z treeSet -n TEST

- d start ML optimization from random starting tree

DEFAULT: OFF

This option allows you to start the RAXML search with a complete random starting tree instead of the default randomized stepwise addition Maximum Parsimony starting tree. On smaller datasets (around 100-200 taxa) it has been observed that this might sometimes yield topologies of distinct local likelihood maxima which better correspond to empirical expectations.

It has also been observed that, this sometimes yield better - more diverse - starting trees for the analysis of broad phylogenomic alignments that have a very strong phylogenetic signal!

**Example:** raxmlHPC -d -p 12345 -s alg -m GTRGAMMA -n TEST

- D ML search convergence criterion. This will break off ML searches if the relative Robinson-Foulds distance between the trees obtained from two consecutive lazy SPR cycles is smaller or equal to 1%. Usage recommended for very large datasets in terms of taxa.

On trees with more than 500 taxa this will yield execution time improvements of approximately 50% while yielding only slightly worse trees.

DEFAULT: OFF

When enabling this option, RAxML will stop ML and standard, slow bootstrap searches early, when the RF distance between the trees generated by two consecutive cycles of SPR (Subtree Pruning Re-Grafting) moves is smaller than 1%. This leads to substantial speed improvements while the decrease in log likelihood scores is only very small. The option has been tested on several datasets and the results have been included in the following book chapter:

A. Stamatakis: "Phylogenetic Search Algorithms for Maximum Likelihood". In M. Elloumi, A.Y. Zomaya, editors. Algorithms in Computational Biology: techniques, Approaches and Applications, 547-577, John Wiley and Sons, 2011.

**Example:** `raxmlHPC -D -p 12345 -s alg -m GTRCAT -n TEST`

`-e` set model optimization precision in log likelihood units for final optimization of tree topology

DEFAULT: 0.1 for models not using proportion of invariant sites estimate  
0.001 for models using proportion of invariant sites estimate

This allows you to specify up to which likelihood difference, the model parameters will be optimized when RAxML uses the GAMMA model of rate or when you just evaluate a trees with the `-f e` option or a bunch of trees with the `-f n` option or similar options. This has shown to be useful to quickly evaluate the likelihood of a bunch of large final trees of more than 1,000 taxa because it will run much faster.

I typically use e.g. `-e 1.0` or `-e 2.0` in order to rapidly compare distinct final tree topologies based on their likelihood values.

Note that, topology-dependent likelihood-differences are typically far larger than 1.0 or 2.0 log likelihood units. The default setting is 0.1 log likelihood units which proves to be sufficient in most practical cases.

**Example:** `raxmlHPC -f e -e 0.00001 -s alg -t tree -m GTRGAMMA -n TEST`

`-E` specify an exclude file name, that contains a specification of alignment positions you wish to exclude.

Format is similar to Nexus, the file shall contain entries like "100-200 300-400", to exclude a single column write, e.g., "100-100", if you specify a partition file via `"-q"`, an appropriately adapted partition file will also be written.

This option will just make RAxML write a reduced alignment file without the excluded columns that can subsequently be used for the analysis you actually want to conduct.

If you use a partitioned model, an appropriately adapted model file will also be written. Note that, excluding sites with RAxML is a **two-step** procedure. You will first have to invoke RAxML once using the `-E` option to generate a PHYLIP formatted file with the desired sites excluded. Then you will have to invoke RAxML again on the alignment file that was written (called `alg.excludeFile` in the example below) to do an actual tree search, for instance.

**Example:** `raxmlHPC -E excludeFile -s alg -m GTRCAT -q part -n TEST`

In this case the alignment file with columns excluded will be named `alg.excludeFile` and the partition file with the specified columns excluded is called `part.excludeFile`

If you want to exclude sites 100-199 (note that, the borders, that is, sites 100 and 199 are included and will be removed) and sites 200-299 the corresponding exclude file - a plain

text file - would contain the two lines below:

```
100-199
200-299
```

**-f** select algorithm:

The **-f** option is a very powerful option because, in many cases, it allows you to select what kind of algorithm RAXML shall execute. If you don't specify **-f** at all RAXML will execute the standard hill climbing algorithm (**-f d** option)The individual options are listed below.

**-f a** rapid Bootstrap analysis and search for best-scoring ML tree in one program run

Tell RAXML to conduct a rapid Bootstrap analysis and search for the best-scoring ML tree in one single program run.

**Example:** `raxmlHPC -f a -p 12345 -s alg -x 12345 -# 100 -m GTRCAT -n TEST`

**-f A** compute marginal ancestral states on a ROOTED reference tree provided via **-t**

This option allows you to compute marginal ancestral states/sequences on a given, fixed, and rooted reference tree. If you don't know what marginal ancestral states are please read Ziheng Yang's book on Computational Molecular Evolution.

**Example:** `raxmlHPC -f A -t testTree -s testData -m GTRGAMMA -n TEST`

A small phylip-formatted test alignment would look like this:

```
4 4
t1 ACGT
t2 AATT
t3 ATAT
t4 CCGT
```

and a test tree:

```
((t1,t2),(t3,t4));
```

RAXML will then output the rooted binary tree again, but with inner node labels that must be used to associate ancestral sequences with their corresponding positions in the tree. It also writes two files, one containing the marginal probabilities for each inner node label as well as one containing guesses (by taking the maximum probability) for the actual ancestral sequences.

**-f b** draw bipartition information on a tree provided with **-t** (typically the best-known ML tree) based on multiple trees (e.g., from a bootstrap) in a file specified by **-z**

**Example:** `raxmlHPC -f b -t ref -z trees -m GTRCAT -n TEST`

**-f B** optimize br-len scaler and other model parameters (GTR, alpha, etc.) on a tree provided with **-t**. The input tree passed via **-t** needs to contain branch

lengths. The branch lengths will not be optimized, just scaled by a single common value.

This is a slightly idiosyncratic option that was mainly developed to work on conjunction with the PartitionFinder tool (see: <http://www.robertlanfear.com/partitionfinder/>).

The concept of branch length scalers is similar to the one implemented in MrBayes. That is, we assume that all partitions have the same branch lengths proportionally to each other. However, for each partition  $p$  we do a maximum likelihood estimate of a single scaling factor  $s[p]$  by which we multiply the branch lengths. Essentially this represents a parametrization trade-off between estimating a separate set of branch lengths for each partition (heavy increase in the number of parameters → danger of over-parametrization) and doing one common branch length estimate across all partitions (possible under-parametrization). Note that, this works only for evaluating a given, fixed tree with branch lengths and not for tree searches!

**Example:** `raxmlHPC -s alg.phy -t tree -m GTRGAMMA -f B -q part -n TEST`

`-f c` check if the alignment can be properly read by RAxML

**Example:** `raxmlHPC -f c -m GTRCAT -s alg -n TEST`

`-f C` ancestral sequence test for my PhD student Jiajie Zhang, users will also need to provide a list of taxon names via `-Y` separated by white-spaces

This is an algorithm we tried to implement for post-analyzing viral phylogenies. The idea was to build a test that can determine if certain sequences in the viral tree are truly ancestral, that is, if they should essentially be located at or right next to an inner node of the tree.

For this, we built the following test/algorithm:

Given a fixed phylogenetic tree (usually the best-known ML tree) for a set of sequences and, given a list of candidate ancestral sequences via `-Y`, for each of these candidate sequences we compare the likelihood of the fixed tree and the given (optimal) branch length for that putative ancestral sequence with the likelihood of the fixed tree and an essentially zero branch length for the ancestral candidate.

In fact, we apply the test to all three branches the candidate ancestral sequence is attached to, that is, the branch it is directly attached to, as well as the two branches to the right and left of the node to which the candidate ancestral sequence is attached to.

The likelihoods of the different branch length configurations (the three almost-zero branch lengths) are compared using the Shimodaira-Hasegawa test to the original (optimal) likelihood. The Shimodaira-Hasegawa test might not be the most modern test available but was sufficient for conducting initial tests. It turned out that the test worked very well on simulated data, but not at all for real data. Thus, we decided to abandon further testing, but nonetheless keep the option in RAxML.

**Example:** `raxmlHPC -f C -Y candidateTaxonNames -m GTRGAMMA -t tree -s alg -n TEST`

`-f d` new rapid hill-climbing

DEFAULT: ON

This is the default RAxML tree search algorithm and is substantially faster than the original

search algorithm. It takes some shortcuts, but yields trees that are almost as good as the ones obtained from the full search algorithm. The algorithm is described and assessed in this paper here: <http://link.springer.com/article/10.1007/s11265-007-0067-4#page-1>

**Example:** raxmlHPC -f d -m GTRCAT -p 12345 -s alg -n TEST

-f D execute one or more rapid hill-climbing searches that will also generate RELL bootstraps

This is an implementation of the techniques introduced in the following paper: <http://www.ncbi.nlm.nih.gov/pubmed/23418397> Some initial tests have shown that the RAXML implementation yields results that are well correlated with standard bootstrap values. Using this option is recommended when you have very large trees and don't have the resources/time for computing bootstrap replicates.

**Example:** raxmlHPC -f D -m GTRCAT -p 12345 -s alg -n TEST

-f e optimize model parameters+branch lengths for given input tree

You need to provide an input tree via the -t option.

**Example:** raxmlHPC -f e -t ref -m GTRGAMMA -s alg -n TEST

-f E execute very fast experimental tree search, at present only for testing

This option will execute a very fast tree search algorithm that will not try as hard to optimize the likelihood. It is intended for very large trees and follows a similar logic as the FastTree program: <http://meta.microbesonline.org/fasttree/>

**Example:** raxmlHPC -f E -m GTRCAT -p 12345 -s alg -n TEST

-f F execute fast experimental tree search, at present only for testing

This option will execute a fast tree search algorithm that will not try as hard to optimize the likelihood. It is intended for very large trees and follows a similar logic as the FastTree program: <http://meta.microbesonline.org/fasttree/>

**Example:** raxmlHPC -f F -m GTRCAT -p 12345 -s alg -n TEST

-f g compute per site log Likelihoods for one ore more trees passed via -z and write them to a file in treepuzzle format that can be read by CONSEL  
**The model parameters will be estimated on the first tree only!**

**Example:** raxmlHPC -f g -s alg -m GTRGAMMA -z trees -n TEST

-f G compute per site log Likelihoods for one ore more trees passed via -z and write them to a file in treepuzzle format that can be read by CONSEL.  
**The model parameters will be re-estimated for each tree!**

**Example:** raxmlHPC -f G -s alg -m GTRGAMMA -z trees -n TEST

-f h compute log likelihood test (SH-test) between best tree passed via -t and a bunch of other trees passed via -z  
**The model parameters will be estimated on the first tree only!**

**Example:** `raxmlHPC -f h -t ref -z trees -s alg -m GTRGAMMA -n TEST`

`-f H` compute log likelihood test (SH-test) between best tree passed via `-t` and a bunch of other trees passed via `-z`  
The model parameters will be re-estimated for each tree !

**Example:** `raxmlHPC -f H -t ref -z trees -s alg -m GTRGAMMA -n TEST`

`-f i` calculate IC and TC scores (Salichos and Rokas 2013 <http://www.nature.com/nature/journal/v497/n7449/full/nature12130.html>) on a reference tree provided with `-t` based on multiple trees (e.g., from a bootstrap) in a file specified by `-z`

The method is described in more detail in the following paper I wrote with Salichos and Rokas:

<http://mbe.oxfordjournals.org/content/early/2014/02/07/molbev.msu061.abstractkeytype=ref&ijkey=I65FuGNx0HzR2Ow>

The method is then extended here <http://dx.doi.org/10.1101/022053> for partial gene trees.

**Warning:** The default TC/IC calculations are not done exactly as described in our MBE paper. To have RAXML do exactly what is described in the paper, please edit file `bipartitionList.c` by commenting out or removing the line  
`#define BIP_FILTER`

**Example:** `raxmlHPC -f i -m GTRCAT -t referenceTree -z bootstrapTrees -n TEST`

`-f I` a simple tree rooting algorithm for unrooted trees. It roots the tree by rooting it at the branch that best balances the subtree lengths (sum over branches in the subtrees) of the left and right subtree. A branch with an optimal balance does not always exist! You need to specify the tree you want to root via `-t`.

**Example:** `raxmlHPC -f I -m GTRCAT -t unrootedTree -n TEST`

`-f j` generate a bunch of bootstrapped alignment files from an original alignment file. You need to specify a seed with `-b` and the number of replicates with `-#`

**Example:** `raxmlHPC -f j -b 12345 -# 100 -s alg -m GTRCAT -n TEST`

**Warning:** Note that, if you are generating those BS replicate alignments for a dataset for which you subsequently intend to conduct a partitioned analysis you will also need to specify the partition file here via `-q` because RAXML re-samples sites on a per-partition basis!

`-f J` Compute SH-like support values on a given tree passed via `-t`.



This option will compute sh-like support values as described here <http://www.ncbi.nlm.nih.gov/pubmed/20525638> on a given tree. The input tree is typically the best-known ML tree found by a RAxML analysis. Keep in mind that for applying the SH-like test the tree needs to be NNI (Nearest Neighbor Interchange) optimal. Thus, RAxML will initially try to apply NNI moves to further improve the tree and then compute the SH test for each inner branch of the tree.

**Example:** `raxmlHPC -f J -p 12345 -m GTRGAMMA -s alg -t tree -n TEST`

`-f k` Fix long branch lengths in partitioned data sets with missing data using the branch length stealing algorithm.  
This option only works in conjunction with "-t", "-M", and "-q".  
It will print out a tree with shorter branch lengths, but having the same likelihood score.

This option tries to fix the issue of very long branch lengths in partitioned datasets with missing data. For each partition and each branch of a given tree it checks if there is data available for that partition on both sides of the tree as defined by the branch. If this is not the case this means that there is only missing data on one side. In such a case we usually have a very long branch length. The algorithm fixes this issue by stealing branch lengths from those partitions that have data on both sides of the branch under consideration. If there are several other partitions that have data it computes a weighted average for the stolen branch length based on the site counts in each partition from which it stole a branch length. The nice property of this algorithm is that by changing the branch lengths in this way, the likelihood of the tree is not changed.

**Example:** `raxmlHPC -f k -m GTRGAMMA -s alg -q part -M -t tree -n TEST`

A Newick tree file containing a shorter tree with stolen branch lengths will be written to a file called: `RaxML_stolenBranchLengths.TEST`

`-f m` compare bipartitions between two bunches of trees passed via "-t" and "-z" respectively. This will return the Pearson correlation between all bipartitions found in the two tree files. A file called `RaxML_bipartitionFrequencies.outputFileName` will be printed that contains the pair-wise bipartition frequencies of the two sets

**Example:** `raxmlHPC -f m -t trees1 -z trees2 -s alg -m GTRCAT -n TEST`

`-f n` compute the log likelihood score of all trees contained in a tree file provided by -z

**The model parameters will be estimated on the first tree only!**

**Example:** `raxmlHPC -f n -z trees -s alg -m GTRGAMMA -n TEST`

`-f N` compute the log likelihood score of all trees contained in a tree file provided by -z

**The model parameters will be re-estimated for each tree!**

**Example:** `raxmlHPC -f N -z trees -s alg -m GTRGAMMA -n TEST`

`-f o` old and slower rapid hill-climbing without the heuristic cutoff described in <http://link.springer.com/article/10.1007/s11265-007-0067-4#page-1>

If you use this option you will typically get slightly better likelihood scores while the run times are expected to increase by factor 2 to 3.

**Example:** `raxmlHPC -f o -p 12345 -m GTRCAT -s alg -n TEST`

`-f p` perform pure stepwise MP addition of new sequences to an incomplete starting tree and exit

**Example:** `raxmlHPC -f p -t ref -p 12345 -s alg -m GTRCAT -n TEST`

`-f q` fast quartet calculator

This option will calculate the likelihood scores of all quartets for a given input alignment. Initially RAXML will construct a randomized stepwise addition order parsimony starting tree or you can also pass a given tree (e.g., the best-known ML tree) to RAXML via `-t`. This tree is used to estimate model parameters that will then remain fixed during the quartet calculations. For each quartet that is evaluated, only the branch lengths will be optimized.

The algorithm itself has three flavors: (i) it can randomly evaluate a specific number of quartets, (ii) it can evaluate all quartets, or (iii) it can evaluate quartets from a pre-specified quartet constraint tree file that must contain exactly 4 monophyletic and multi-furcating groups.

For details on the input format for the quartet constraint file, see the `-Y` option!

To not evaluate all possible quartets (keep in mind that this number grows quickly as a function of the taxa in the alignment/tree) you have to use either `-#` or `-N` to specify how many quartets you want to randomly evaluate.

**Example 1:** `raxmlHPC -m GTRGAMMA -t tree -s alg -f q -p 12345 -n TEST`

The above example will evaluate all possible quartets.

**Example 2:** `raxmlHPC -m GTRGAMMA -t tree -s alg -f q -p 12345 -N 100 -n TEST`

The above example will evaluate 100 randomly chosen quartets.

The output is written to a file called: `RaxML_quartets.TEST`

It looks like this:

Taxon names and indexes:

```
Cow 1
Carp 2
Chicken 3
Human 4
Loach 5
Mouse 6
Rat 7
Seal 8
Whale 9
Frog 10
```

```

1 2 | 3 4: -2640.417355
1 3 | 2 4: -2640.810748
1 4 | 2 3: -2638.359608
1 2 | 3 5: -2552.181311
1 3 | 2 5: -2543.674965
...

```

First, each taxon name is assigned a number, then the quartets are represented as bipartitions, for instance, 1 2 | 3 4: -2640.417355 is the quartet ((Cow,Carp), (Chicken, Human)) and has a likelihood of -2640.417355 .

The MPI version for this specific option that can be compiled with the corresponding Makefiles will distribute quartet evaluations across processors with MPI.

`-f r` compute pairwise Robinson-Foulds (RF) distances between all pairs of trees in a tree file passed via `-z`. If the trees have node labels represented as integer support values the program will also compute two flavors of the weighted Robinson-Foulds (WRF) distance

**Example:** `raxmlHPC -m GTRCAT -z trees -f r -n TEST`

The two flavors of the Weighted RF distance that are compute are:

1. just add the support of those bipartitions contained in one tree, but not the other
2. add the support of those bipartitions contained in one tree, but not the other and also add the difference in support for each shared bipartition

The output looks as follows (not including weighted RF distances):

```
0 1: 8 0.125000
```

where 0 and 1 denote that this is the distance between tree 0 and 1 in the tree file (the first and second tree in there), where 8 is the plain RF distance and 0.12500 is the normalized RF distance corresponding to 12.5%.

The normalized/relative RF distance is calculated by dividing the plain RF by  $2(n-3)$ , where  $n$  is the number of taxa.

The value of the normalized RF distance ranges between 0.0 and 1.0 and is interpreted as the percentage of splits (bipartitions) that are unique to one of the two trees. In the above example, 12.5% of the splits are unique to either tree 0 or tree 1.

`-f R` compute all pairwise Robinson-Foulds (RF) distances between a large reference tree passed via `-t` and many smaller trees (that must have a subset of the taxa of the large tree) passed via `-z`. This option is intended for checking the plausibility of very large phylogenies that can not be inspected visually any more.

**Example:** `raxmlHPC -f R -m GTRCAT -t hugeTree -z manySmallTrees`

`-f s` split up a multi-gene partitioned alignment into the respective subalignments

**Example:** `raxmlHPC -f s -q part -s alg -m GTRCAT -n TEST`

**Warning:** Note that the sub-alignments will be named by the names you have given to

the individual partitions. If your partition file contains two partitions called part1 and part2, this command will generate two alignment files called part1.phy and part2.phy

-f S compute site-specific placement bias using a leave one out test inspired by the evolutionary placement algorithm

The leave-one-out approach for assessing site-specific congruence/incongruence with the underlying tree is based on the evolutionary placement algorithm, see <http://sysbio.oxfordjournals.org/content/60/3/291.short>

Using a given reference tree (typically the best-known ML tree) a leave-one-out test is conducted by pruning a single taxon  $t$  at a time, carrying out site-specific computations for  $t$ , and subsequently re-inserting it again into its original position. For each taxon  $t$  we use a sliding window of size  $w$  (in the default case  $w:=100$ ) of consecutive sites in the alignment and compute the maximum likelihood placement in the tree with respect to those  $w$  sites only.

This is done repeatedly for each alignment site by moving the window over the alignment on a site by site basis.

Once we have obtained the best placements for each sliding window starting position for a taxon  $t$ , we compute a score  $s[i][t]$  for each site.

The score  $s[i][t]$  is the mean distance (in terms of number of nodes in the tree) between the respective best placements for all sliding windows that comprise site  $i$  and the original placement of taxon  $t$ .

Finally, we calculate a global  $S[i]$  score for each site by computing the mean of the  $s[i][t]$  computed for each taxon  $t$ .

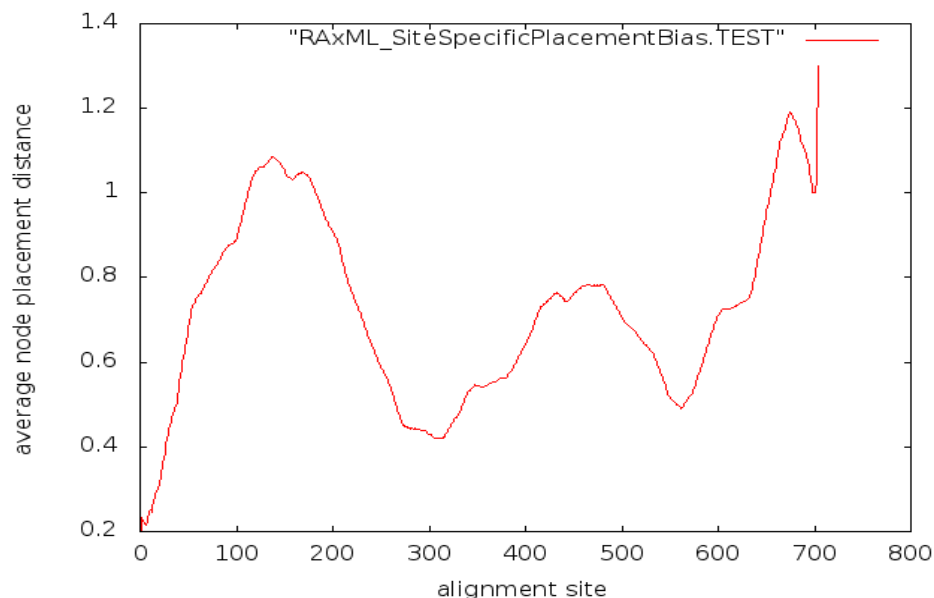
Thus, RAxML will return an list of  $n$   $S[i]$  average node distances, where  $n$  is the number of sites in the alignment.

**Example:** `raxmlHPC -f S -s alg -t tree -m GTRGAMMA -n TEST`

The main idea behind this algorithm is to provide a means for assessing the phylogenetic variability of different areas/parts of a gene in order to decide which part of the gene (e.g., a 16S gene) to amplify and sequence using NGS.

The option above will generate an output file called:

`RaxML_SiteSpecificPlacementBias.TEST` which you can then plot using a tool like gnuplot, to obtain the following plot:



The plot indicates that the alignment site between position 200 and 600 will yield the most congruent signal with the tree calculated on the entire alignment. Overall, the mean node placement distances are relatively low, meaning that this alignment has a relatively stable phylogenetic signal over its entire length.

`-f t` do randomized tree searches on one fixed starting tree

This command will execute a specified number (by `-N` or `-#`) of randomized tree searches. The difference to the standard search algorithm is, that, given a starting tree it will apply SPR (subtree pruning re-grafting) moves in a randomized order and thus may find better trees.

**Example:** `raxmlHPC -f t -t -m GTRCAT -s alg -p 12345 -n TEST`  
`-f T` do final thorough optimization of ML tree from rapid bootstrap search in stand-alone mode

This option allows to do a more thorough tree search that uses less lazy, i.e., more exhaustive SPR moves, in stand-alone mode. This algorithm is typically executed in the very end of a search done via `-f a`.

**Example:** `raxmlHPC -p 12345 -m GTRGAMMA -s alg -f T -t tree -n TEST`

`-f u` execute morphological weight calibration using maximum likelihood, this will return a weight vector. you need to provide a morphological alignment and a reference tree via `-t`

This option will determine to which degree the sites of a morphological alignment are congruent with a given molecular reference tree. As output it will generate a RAxML weight file that reflects the degree of congruence and that can be subsequently read via the `-a` option to conduct a more informed evolutionary placement of fossils using the evolutionary placement algorithm (`-f v` option). For more details, see the corresponding papers:  
Evolutionary placement <http://sysbio.oxfordjournals.org/content/60/3/291.short>  
Fossil calibration [http://ieeexplore.ieee.org/xpl/login.jsp?tp=&arnumber=5586939&url=http%3A%2Fieeexplore.ieee.org%2Fxppls%2Fabs\\_all.jsp%3Farnumber%3D5586939](http://ieeexplore.ieee.org/xpl/login.jsp?tp=&arnumber=5586939&url=http%3A%2Fieeexplore.ieee.org%2Fxppls%2Fabs_all.jsp%3Farnumber%3D5586939)

**Example:** `raxmlHPC -f u -m BINGAMMA -t molecularTree -s morphologicalAlignment`

`-f v` classify a bunch of environmental sequences into a reference tree using thorough read insertions you will need to start RAxML with a non-comprehensive reference tree and an alignment containing all sequences (reference + query)

For details on the design and performance of this algorithm, see: <http://sysbio.oxfordjournals.org/content/60/3/291.short>

RAxML will produce a couple of idiosyncratic output files for the placements, but also an output file according to the common file standard defined by Erick Matsen for his `pplacer` (see <http://matsen.fhcrc.org/pplacer/>) program that is similar to the EPA (Evolutionary Placement Algorithm) and myself.

The common file format is described in this paper here:

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0031009>

**Example:** `raxmlHPC -f v -s alg -t referenceTree -m GTRCAT -n TEST`

If you frequently want to insert different sequence into the same reference tree, there is a shortcut to make this more efficient, because the model parameters and branch lengths are estimated from scratch for the reference tree each time when you invoke the program. If you want to avoid this you proceed as follows.

Generate a binary file containing the model parameters for the reference tree

**Example 1:** `raxmlHPC -f e -m GTRGAMMA -s referenceAlignment -t referenceTree -n PARAMS`

This will generate a file called: `RAxML_binaryModelParameters.PARAMS` which can then be read by the EPA to avoid re-optimizing parameters:

**Example 2:** `raxmlHPC -f v -R RAxML_binaryModelParameters.PARAMS -t RAxML_result.PARAMS -s alg -m GTRGAMMA -n TEST2`

**Warning:** Note that, when using binary model files: The models of rate heterogeneity in examples 1 & 2 (e.g., `GTRGAMMA` or `GTRCAT`) must be the same! Secondly, when using `GTRCAT` you must disable pattern compression via the `-H` flag in both runs! Thirdly, the reference tree read in via `-t` in example 2 must have branch lengths according to the model specified via `-m`. The branch lengths of the reference tree are not re-optimized by the call in example 2. It is very easy to make a mistake and read in a tree whose branch lengths were estimated under `GTRGAMMA` while you are using `GTRCAT`. All of the above only applies when using binary model files through the `-R` option.

`-f V` classify a bunch of environmental sequences into a reference tree using thorough read insertions you will need to start RAxML with a non-comprehensive reference tree and an alignment containing all sequences (reference + query)

This is an experimental extension of the EPA for placing short reads in multi-gene datasets. It uses some computational shortcuts to make the placement faster. It has been used for the following paper: <http://www.nature.com/nmeth/journal/v10/n12/full/nmeth.2693.html>

**Example:** `raxmlHPC -f V -q part -s alg -t referenceTree -m GTRCAT -n TEST`

**Warning:** this is a test implementation for more efficient handling of multi-gene & whole-genome datasets!

-f w compute ELW test on a bunch of trees passed via -z  
**The model parameters will be estimated on the first tree only!**

Computes the Extended Likelihood Weights test by Strimmer and Rambaut, see <http://www.ncbi.nlm.nih.gov/pubmed/11798428>, on a set of trees.

**Example:** raxmlHPC -f w -m GTRGAMMA -s alg -z trees -n TEST

-f W compute ELW test on a bunch of trees passed via -z  
**The model parameters will be re-estimated for each tree**

**Example:** raxmlHPC -f W -m GTRGAMMA -s alg -z trees -n TEST

-f x compute pair-wise ML distances, ML model parameters will be estimated on an MP starting tree or a user-defined tree passed via -t, only allowed for GAMMA-based models of rate heterogeneity

This option will just compute the pair-wise distances (branch lengths) between the sequences of an alignment using a maximum likelihood estimate. To obtain a model parameter estimate (GTR, alpha shape parameter etc.) it will initially either read in a user specified tree (e.g., the best-known ML tree) or generate a randomized stepwise addition parsimony starting tree if no input tree is provided. It then optimizes model parameters on this tree and then computes all pair-wises distances.

**Example:** raxmlHPC -f x -p 12345 -s alg -m GTRGAMMA -n TEST

-f y classify a bunch of environmental sequences into a reference tree using parsimony you will need to start RAXML with a non-comprehensive reference tree and an alignment containing all sequences (reference + query)

This command is analogous to the -f v command with the only difference that it uses parsimony for placing reads into the reference tree. Because it uses parsimony it is orders of magnitude faster than the likelihood-based placement. You can use it for placing millions of reads into extremely large reference trees.

**Example:** raxmlHPC -f y -m GTRCAT -s alg -t referenceTree -n TEST

**Warning:** This algorithm is based on parsimony. Thus, a read may be placed into more than one, equally parsimonious position/branch of the reference tree. Be careful to take all equally parsimonious placements into account when analyzing the results!

-F enable ML tree searches under CAT model for very large trees without switching to GAMMA in the end (saves memory). This option can also be used with the GAMMA models in order to avoid the thorough optimization of the best-scoring ML tree in the end.

DEFAULT: OFF

When conducting analyses under the CAT model of rate heterogeneity in RAXML, the program will in most cases try to evaluate the GAMMA-based score and do some additional optimization of the tree under GAMMA in the very end of the search. If you want to avoid this because of time and/or memory constraints (GAMMA needs four times more memory than CAT) you can use this option.

**Example:** `raxmlHPC -F -m GTRCAT -p 12345 -s alg -N 10 -n TEST`

`-g` specify the file name of a multifurcating constraint tree this tree does not need to be comprehensive, i.e. must not contain all taxa

This option frequently causes confusion among users for two main reasons: (i) if the constraint tree you provide is not comprehensive, that is, does not contain all taxa in the alignment, the taxa not included in the constraint are free, that is, they are allowed to fall into any part of the tree! (ii) users often state that the RAxML tree does not correspond to their constraint tree. This is mostly due to the tree visualization tools that are used, depending at which top level trifurcation (remember that maximum likelihood trees are always unrooted!) the tree is rooted it may look as if it does not comply with the constraint. Please double-check twice and thrice before posting to the RAxML google group!

Finally also note that, any multi-furcations in the input tree will be resolved via a maximum likelihood search.

**Example:** `raxmlHPC -g constraintTree -m GTRGAMMA -p 12345 -s alg -n TEST`

`-G` enable the ML-based evolutionary placement algorithm heuristics by specifying a threshold value (fraction of insertion branches to be evaluated using thorough insertions under ML).

This option can be used in conjunction with the `-f v` and `-f V` options to speed up evolutionary placements of short reads. A setting of 0.1 (10% of branches considered for thorough insertions) yields a good accuracy/speed trade-off. For more details you may actually consider to read the paper: <http://sysbio.oxfordjournals.org/content/60/3/291.short>

**Example:** `raxmlHPC -f v -G 0.1 -s alg -t referenceTree -m GTRCAT -n TEST`

`-h` Display this help message.

**Example:** `raxmlHPC -h`

`-H` Disable pattern compression.

DEFAULT: ON

This option allows to disable alignment site compression in the input alignment. If alignments contain identical sites they can be compressed to become shorter and thus speed up the likelihood calculations. This option has been developed mostly for internal use in my lab.

**Example:** `raxmlHPC -H -s alg -p 12345 -m GTRCAT -n TEST`

**Warning:** using this option may considerably slow down RAxML

`-i` Initial rearrangement setting for the subsequent application of topological changes phase

This option allows to specify the radius (number of nodes away from the original pruning position) up to which pruned subtrees will be re-inserted in the course of SPR (subtree



pruning re-grafting moves) during the tree search. By default this setting will be determined automatically by RAxML. The example below specifies a radius of 10, that is, subtrees will be inserted into all branches that are between 1 and 10 nodes away from their pruning position.

**Example:** `raxmlHPC -i 10 -s alg -m GTRCAT -p 12345 -n TEST`

**-I** a posteriori bootstopping analysis. Use:

`-I autoFC` for the frequency-based criterion  
`-I autoMR` for the majority-rule consensus tree criterion  
`-I autoMRE` for the extended majority-rule consensus tree criterion  
`-I autoMRE_IGN` for metrics similar to MRE, but include bipartitions under the threshold whether they are compatible or not. This emulates MRE but is faster to compute.

You also need to pass a tree file containing several bootstrap replicates via `-z`

This option allows to carry out the bootstrap convergence test, that is, the test that determines if you have computed sufficient BS replicates for getting stable support values, a posteriori, that is after you have already computed, for instance, 100 bootstrap replicates. This option is particularly useful when you are doing large-scale tree searches on supercomputers. Here you would initially run 50 replicates and check if they are sufficient. Then you'd run another 50 and asses if the 100 trees you have now are sufficient, etc.

My favorite flavors in terms of convergence criteria are: `autoMRE` and if the tree is very large in terms of number of taxa (more than 1000) `autoMRE_IGN` to save time. Before using this option you should read the corresponding paper: [http://link.springer.com/chapter/10.1007/978-3-642-02008-7\\_13#page-1](http://link.springer.com/chapter/10.1007/978-3-642-02008-7_13#page-1)

**Example:** `raxmlHPC -m GTRCAT -z RAXML_bootstrap.BS -I autoMRE -n TEST`

Note that, if you have several files containing bootstrap trees they can easily be concatenated by using the Linux `cat` command.

**Example:** `cat RAXML_bootstrap.BS_1 RAXML_bootstrap.BS_2 > allBootstraps`  
**-j** Specifies that intermediate tree files shall be written to file during the standard ML and BS tree searches.

DEFAULT: OFF

This will simply print out a couple of intermediate trees during the tree search and not the final tree only. The intermediate trees are written to files called: `RAXML_checkpoint.TEST.0`, `RAXML_checkpoint.TEST.1`, etc.

**Example:** `raxmlHPC -j -m GTRGAMMA -s alg -p 12345 -n TEST`

**-J** Compute majority rule consensus tree with `"-J MR"` or extended majority rule consensus tree with `"-J MRE"` or strict consensus tree with `"-J STRICT"`. For a custom consensus threshold  $\geq 50\%$ , specify `T_<NUM>`, where  $100 \geq \text{NUM} \geq 50$ .

Options `"-J STRICT_DROP"` and `"-J MR_DROP"` will execute an algorithm that identifies dropsets which contain rogue taxa as proposed by Pattengale et al. in the paper "Uncovering hidden phylogenetic consensus". You will also need to provide a tree file containing several UNROOTED trees

via "-z"

This option actually triggers two different sets of algorithms, one set for building consensus trees and one set for finding rogue taxa. Also note that, these algorithms have been parallelized, that is, if you use the PThreads version they will run faster!

You should read and cite the following papers when using these options!

For consensus trees:

<http://www.sciencedirect.com/science/article/pii/S1877750310000086>

For identifying rogue taxa:

[http://ieeexplore.ieee.org/xpl/login.jsptp=&arnumber=5710874&url=http%3A%2F%2Fieeexplore.ieee.org%2Fxppls%2Fabs\\_all.jsp%3Farnumber%3D5710874](http://ieeexplore.ieee.org/xpl/login.jsptp=&arnumber=5710874&url=http%3A%2F%2Fieeexplore.ieee.org%2Fxppls%2Fabs_all.jsp%3Farnumber%3D5710874)

The two examples below compute an extended majority rule consensus tree and a 75% majority rule consensus tree.

**Example:** `raxmlHPC -J MRE -z trees -m GTRCAT -n TEST`

**Example:** `raxmlHPC -J T_75 -z trees -m GTRCAT -n TEST`

The example below identifies rogues using the aforementioned dropset method and using a majority rule threshold. For details, please read the paper.

**Example:** `raxmlHPC -J MR_DROP -z trees -m GTRCAT -n TEST`

**-k** Specifies that bootstrapped trees should be printed with branch lengths. The bootstraps will run a bit longer, because model parameters will be optimized at the end of each replicate under GAMMA or GAMMA+P-Invar respectively.

DEFAULT: OFF

**Example:** `raxmlHPC -b 12345 -p 12345 -# 100 -k -s alg -m GTRGAMMA -n TEST`

**-K** Specify one of the multi-state substitution models (max 32 states) implemented in RAxML. Available models are: ORDERED, MK, GTR

DEFAULT: GTR model

Evidently, for this option to have an effect you need to have an alignment containing multi-state characters. If you have several partitions that consist of multi-state characters the model specified via -K will be applied to all models. Thus, it is not possible to assign different models to distinct multi-state partitions!

**Example:** `raxmlHPC -p 12345 -m MULTIGAMMA -s multiStateAlignment -n TEST`

**-L** Compute consensus trees labeled by IC supports and the overall TC value as proposed in Salichos and Rokas 2013. Compute a majority rule consensus tree with -L MR or an extended majority rule consensus tree with -L MRE.

For a custom consensus threshold  $\geq 50\%$ , specify -L T\_<NUM>, where 100  $\geq$

NUM >= 50.

You will of course also need to provide a tree file containing several UNROOTED trees via `-z!`

This option allows to compute the TC and IC metrics on a consensus tree, as described by Salichos and Rokas in this paper here:

<http://www.nature.com/nature/journal/v497/n7449/full/nature12130.html>

The method is described in more detail in the following paper I wrote with Salichos and Rokas:

<http://mbe.oxfordjournals.org/content/early/2014/02/07/molbev.msu061.abstractkeytype=ref&ijkey=I65FuGNx0HzR2Ow>

**Warning:** The default TC/IC calculations are not done exactly as described in our MBE paper. To have RAxML do exactly what is described in the paper, please edit file `bipartitionList.c` by commenting out or removing the line  
`#define BIP_FILTER`

There is a dedicated section further down in this manual with detailed instructions.

**Example:** `raxmlHPC -m GTRCAT -L MRE -z trees -n TEST`

`-m` Model of Binary (Morphological), Nucleotide, Multi-State, or Amino Acid Substitution:

This is probably the most complex and confusing command line option because it can be used to specify a lot of things. A very important thing to be aware of is that, in case you use a partitioned alignment the data type in the partitions and the specific models of substitution to be used are actually specified in the partition file. Thus, if you use the `-q` option the only information that will be extracted from this string here that is passed via `-m` is which model of rate heterogeneity is going to be used. Note that, the rate heterogeneity model is always applied to all data partitions, hence you can not have one partition be analyzed under `CAT` and another one be analyzed under `GAMMA`.

In general, the term `CAT` in the strings always indicates that you want to use the `CAT` model of rate heterogeneity. The string `CATI` indicates that after a tree search under `CAT`, you want to evaluate the final trees under a `GAMMA` plus proportion of invariable sites estimate instead of the default pure `GAMMA`.

By appending `x` to the model strings you indicate that you want to use a maximum likelihood estimate for the base frequencies.

By prepending the string `asc_` you indicate that you want to apply an ascertainment bias correction to the likelihood calculations. This is useful for binary/morphological datasets that only contain variable sites (the identical morphological features are usually not included in the alignments, hence you need to correct for this, see, e.g., <http://sysbio.oxfordjournals.org/content/50/6/913.short>).

For DNA data this option might be useful when you analyze alignments of SNPs that also don't contain constant sites. Note that, for mathematical and numerical reasons you can not apply an ascertainment bias correction to datasets or partitions that contain constant sites. In this case, RAxML will exit with an error.

Also note how ambiguous sites are defined in RAxML. If you have a site looking like this:

AAAA---MR

this site is still considered to be an invariable site since - are considered (as in most likelihood-based codes) as completely undetermined characters and thus -, M as well as R could be A. In other words, RAxML will not allow you to conduct an analysis with an ascertainment bias correction if it encounters such a site in your MSA.

We also observed that some SNP datasets do not require a model of rate heterogeneity according to the standard model tests. When this is the case you can use, for instance, -m ASC\_GTRCAT in combination with the -v option. This will make RAxML execute an inference under a plain GTR model without any correction for rate heterogeneity.

Also note that, while I do offer the per-site rate model of rate heterogeneity (the CAT model) with an ascertainment bias correction, I recommend that you either use CAT in combination with -v (no rate heterogeneity) or the corresponding Gamma model of rate heterogeneity with ascertainment bias correction (-m ASC\_GTRGAMMA etc).

Finally, note that, as of version 8.1.7 of RAxML you will need to specify the type of ascertainment bias correction you want to use, we know offer three distinct correction types. For details, please refer to the --asc-corr option.

#### **BINARY:**

- m BINCAT[X] Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under BINGAMMA, depending on the tree search option.  
With the optional "X" appendix you can specify a ML estimate of base frequencies.
  
- m BINCATI[X] Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under BINGAMMAI, depending on the tree search option.  
With the optional "X" appendix you can specify a ML estimate of base frequencies.
  
- m ASC\_BINCAT[X] Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under BINGAMMA, depending on the tree search option.  
With the optional "X" appendix you can specify a ML estimate of base frequencies.  
The ASC prefix will correct the likelihood for ascertainment bias. You will also need to specify the correction type via --asc-corr!
  
- m BINGAMMA[X] GAMMA model of rate heterogeneity (alpha parameter will be estimated). With the optional "X" appendix you can specify a ML estimate of base frequencies.
  
- m ASC\_BINGAMMA[X] GAMMA model of rate heterogeneity (alpha parameter will be

estimated). The ASC prefix will correct the likelihood for ascertainment bias.

With the optional "X" appendix you can specify a ML estimate of base frequencies. You will also need to specify the correction type via --asc-corr!

-m BINGAMMAI[X] Same as BINGAMMA, but with estimate of proportion of invariable sites. With the optional "X" appendix you can specify a ML estimate of base frequencies.

#### **NUCLEOTIDES:**

-m GTRCAT[X] GTR + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated under GTRGAMMA, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies.

-m GTRCATI[X] GTR + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated under GTRGAMMAI, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies.

-m ASC\_GTRCAT[X] GTR + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated under GTRGAMMA, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies. The ASC prefix will correct the likelihood for ascertainment bias. You will also need to specify the correction type via --asc-corr!

-m GTRGAMMA[X] GTR + Optimization of substitution rates + GAMMA model of rate heterogeneity (alpha parameter will be estimated). With the optional "X" appendix you can specify a ML estimate of base frequencies.

-m ASC\_GTRGAMMA[X] GTR + Optimization of substitution rates + GAMMA model of rate heterogeneity (alpha parameter will be estimated). The ASC prefix will correct the likelihood for ascertainment bias. With the optional "X" appendix you can specify a ML estimate of base frequencies. You will also need to specify the correction type via --asc-corr!

-m GTRGAMMAI[X] Same as GTRGAMMA, but with estimate of proportion of invariable sites. With the optional "X" appendix you can specify a ML estimate of base frequencies.

#### **MULTI-STATE:**

- m MULTICAT[X]** Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under MULTIGAMMA, depending on the tree search option.
- With the optional "X" appendix you can specify a ML estimate of base frequencies.
- m MULTICATI[X]** Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under MULTIGAMMAI, depending on the tree search option.
- With the optional "X" appendix you can specify a ML estimate of base frequencies.
- m ASC\_MULTICAT[X]** Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under MULTIGAMMA, depending on the tree search option.
- With the optional "X" appendix you can specify a ML estimate of base frequencies. The ASC prefix will correct the likelihood for ascertainment bias. You will also need to specify the correction type via --asc-corr!
- m MULTIGAMMA[X]** GAMMA model of rate heterogeneity (alpha parameter will be estimated). With the optional "X" appendix you can specify a ML estimate of base frequencies.
- m ASC\_MULTIGAMMA[X]** GAMMA model of rate heterogeneity (alpha parameter will be estimated). The ASC prefix willll correct the likelihood for ascertainment bias. With the optional "X" appendix you can specify a ML estimate of base frequencies. You will also need to specify the correction type via --asc-corr!
- m MULTIGAMMAI[X]** Same as MULTIGAMMA, but with estimate of proportion of invariable sites. With the optional "X" appendix you can specify a ML estimate of base frequencies.
- You can use up to 32 distinct character states to encode multi-state regions, they must be used in the following order:  
 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V  
 i.e., if you have 6 distinct character states you would use 0, 1, 2, 3, 4, 5 to encode these.
- The substitution model for the multi-state regions can be selected via the "-K" option

#### **AMINO ACIDS:**

- m PROTCATmatrixName[F|X]** specified AA matrix + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into numberOfCategories distinct rate

categories for greater computational efficiency. Final tree might be evaluated automatically under `PROTGAMMAmatrixName[F|X]`, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies.

`-m PROTCATmatrixName[F|X]` specified AA matrix + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into `numberOfCategories` distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under `PROTGAMMAmatrixName[F|X]`, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies.

`-m ASC_PROTCATmatrixName[F|X]` specified AA matrix + Optimization of substitution rates + Optimization of site-specific evolutionary rates which are categorized into `numberOfCategories` distinct rate categories for greater computational efficiency. Final tree might be evaluated automatically under `PROTGAMMAmatrixName[F|X]`, depending on the tree search option. With the optional "X" appendix you can specify a ML estimate of base frequencies. The ASC prefix will correct the likelihood for ascertainment bias. You will also need to specify the correction type via `--asc-corr!`

`-m PROTGAMMAmatrixName[F|X]` specified AA matrix + Optimization of substitution rates + GAMMA model of rate heterogeneity (alpha parameter will be estimated). With the optional "X" appendix you can specify a ML estimate of base frequencies.

`-m ASC_PROTGAMMAmatrixName[F|X]` specified AA matrix + Optimization of substitution rates + GAMMA model of rate heterogeneity (alpha parameter will be estimated). The ASC prefix will correct the likelihood for ascertainment bias. With the optional "X" appendix you can specify a ML estimate of base frequencies. You will also need to specify the correction type via `--asc-corr!`

`-m PROTGAMMAmatrixName[F|X]` Same as `PROTGAMMAmatrixName[F|X]`, but with estimate of proportion of invariable sites. With the optional "X" appendix you can specify a ML estimate of base frequencies.

**Available AA substitution models:**

DAYHOFF, DCMUT, JTT, MTREV, WAG, RTREV, CPREV, VT, BLOSUM62, MTMAM, LG, MTART, MTZOA, PMB, HIVB, HIVW, JTTDCMUT, FLU, STMTREV, DUMMY, DUMMY2, AUTO, LG4M, LG4X, PROT\_FILE, GTR\_UNLINKED, GTR

With the optional "F" appendix you can specify if you want to use empirical base frequencies. AUTO and AUTOX are not supported any more, if you specify AUTO it will test prot subst. models with and without empirical

base frequencies now!

Please note that for partitioned models you can in addition specify the per-gene AA model in the partition file. Also note that if you estimate AA GTR parameters on a partitioned dataset, they will be linked (estimated jointly) across all partitions to avoid over-parametrization

**Warning:** When using the **LG4X** protein substitution model, please keep in mind that, this model actually has more free parameters than a normal, fixed protein substitution model. Thus, for each partition that evolves under LG4X there are 6 additional free parameters (3 for the weights and 3 for the rates). Note that it is only 3 weight and 3 rate parameters because the weights need to sum to 1.0 (the 4th parameter is thus determined by the other 3) and the sum of the product of each weight with the respective rate also needs to be 1.0 (see page 2924 in the respective paper: <http://mbe.oxfordjournals.org/content/29/10/2921.full.pdf>). Also note that, in RAxML, LG4X can be used with empirical base frequencies, drawn from the data (LG4XF, LG4MF) and with a ML estimate of base frequencies (LG4XX, LG4MX). Unlike in the published LG4X model, the base frequencies are the same (**shared/linked**) for all 4 substitution matrices of the LG4 models and there are 19 additional parameters in these models. When using this modified form of LG4X please make sure to state it explicitly when you publish results and keep in mind that they have more parameters when you do AIC & BIC test etc. Also, these options have been implemented for convenience in RAxML, but do not correspond to the spirit of the above paper where each of the 4 Q matrices has its own set of base frequencies.  
*Olivier Gascuel asked me to explicitly state that he does not like the following models: LG4XF, LG4MF, LG4XX, LG4MX to be used and I think he has a point!*

The example below will execute a simple search under the CAT approximation of rate heterogeneity on a DNA dataset and evaluate the final tree under GAMMA.

**Example:** `raxmlHPC -m GTRCAT -s alg -p 12345 -n TEST`

The example below will execute a simple search under the CAT approximation of rate heterogeneity on a DNA dataset, but evaluate the final tree under GAMMA+P-Invar.

**Example:** `raxmlHPC -m GTRCATI -s alg -p 12345 -n TEST`

The example below will execute a simple search under the CAT approximation of rate heterogeneity on a DNA dataset and do a maximum likelihood estimate of the base frequencies, instead of using empirical frequencies.

**Example:** `raxmlHPC -m GTRCATX -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity on a DNA dataset.

**Example:** `raxmlHPC -m GTRGAMMA -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity and correct for ascertainment bias on a DNA dataset.

**Example:** `raxmlHPC -m ASC_GTRGAMMA -s alg -p 12345 -n TEST`



The example below will execute a simple search under a plain GTR model (no rate heterogeneity) and correct for ascertainment bias on a DNA dataset.

**Example:** `raxmlHPC -m ASC_GTRCAT -V -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity on a binary dataset.

**Example:** `raxmlHPC -m BINGAMMA -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity on a protein dataset using the substitution matrix and the base frequencies that come with the WAG model.

**Example:** `raxmlHPC -m PROTGAMMAWAG -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity on a protein dataset using empirical base frequencies and the LG substitution model.

**Example:** `raxmlHPC -m PROTGAMMALGF -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA+P-Invar model of rate heterogeneity on a protein dataset using a maximum likelihood estimate of the base frequencies and the LG substitution model.

**Example:** `raxmlHPC -m PROTGAMMAILGX -s alg -p 12345 -n TEST`

The example below will execute a simple search under the GAMMA model of rate heterogeneity on a protein dataset using empirical base frequencies and estimating a GTR model of amino acid substitution.

**Warning:** This may be very slow and you might over-parametrize the model since we need to do a maximum likelihood estimate of 189 rate parameters in the GTR matrix.

**Example:** `raxmlHPC -m PROTGTRGAMMA -s alg -p 12345 -n TEST`

The example below will automatically determine which is the best (the one with the highest likelihood score on the parsimony starting tree) protein substitution model for your dataset using the base frequencies that come with the models. It will chose among the following models: DAYHOFF, DCMUT, JTT, MTREV, WAG, RTREV, CPREV, VT, BLOSUM62, MTMAM, LG, MTART, MTZOA, PMB, HIVB, HIVW, JTTDCMUT, FLU, DUMMY, DUMMY2. These models will not be considered: LG4M, LG4X, PROT\_FILE,GTR\_UNLINKED, GTR!

RAXML will now also automatically test models with and without empirical base frequencies. The criterion for making this choice can now be selected via the `-auto-prot=ml|bic|aic|aicc` option.

**Example:** `raxmlHPC -m PROTGAMMAAUTO -s alg -p 12345 -n TEST`

**-M** Switch on estimation of individual per-partition branch lengths. Only has effect when used in combination with `"-q"`  
Branch lengths for individual partitions will be printed to separate

files

A weighted average of the branch lengths is computed by using the respective partition lengths

DEFAULT: OFF

This will enable an independent per-partition estimate of branch lengths. Note that this increases dramatically the number of free parameters in your model. An unrooted binary tree has  $2n-3$  branch lengths where  $n$  is the number of taxa. If you use this option, instead of  $2n-3$  parameters you will get  $p(2n-3)$  parameters where  $p$  is the number of partitions. RAXML will also run notably slower if you use this option.

Apart from the normal output tree, RAXML will also generate output files for each partition individually that allow to recover the per-partition branch lengths if needed.

**Example:** `raxmlHPC -p 12345 -M -m GTRGAMMA -s alg -q part -n TEST`

-n Specifies the name of the output file.

This option has to be always specified. The arbitrary name passed via `-n` will be appended to all RAXML output files such that you know which files have been generated by which invocation.

If you intend to do two runs that write files into the same directory with the same name specified by `-n` the program will exit with an error to prevent you over-writing output files from a previous run.

**Example:** `raxmlHPC -p 12345 -m GTRGAMMA -s alg  
-n MySuperDuperRAXMLOutputFileName`

-o Specify the name of a single outgroup or a comma-separated list of outgroups, e.g., `-o Rat` or `-o Rat,Mouse`, in case that multiple outgroups are not monophyletic the first name in the list will be selected as outgroup, don't leave spaces between taxon names!

If there is more than one outgroup a check for monophyly of the outgroup in the respective output/final tree(s) will be performed. If the outgroup is not monophyletic the tree will be rooted at the first outgroup in the list and a respective warning will be printed.

Keep in mind that outgroups are just a tree drawing option, the trees remain, essentially unrooted trees.

**Example:** `raxmlHPC -s alg -p 12345 -m GTRGAMMA -o Rat,Mouse -n TEST`

### **A comment on using outgroups: How I would do it.**

In general I'd avoid using outgroups in the initial ML and bootstrap analyses. I'd proceed as follows and provide the rationale for this below

1. Build a tree (ML+BS search) only on the ingroup
2. Then, use EPA (see: <http://sysbio.oxfordjournals.org/content/60/3/291.short>) to place the outgroup(s) onto the tree a posteriori.

This has several advantages:

1. You can use/test different outgroups
2. You don't have to re-run the entire analysis if the outgroup somehow perturbed the

analysis of the ingroup. There have been papers on this issue.

3. You avoid the outgroup affecting the branch lengths of the ingroup because of the way the evolutionary placement algorithm has been built.

4. You get placement probabilities (likelihood weights) for each outgroup, e.g., how likely the outgroup is to fall into one branch or another. This also tells you if your outgroup is good (high probability for being placed into one specific branch) or if it is bad (scatters across the tree)

- O Disable check for completely undetermined sequence in alignment. The program will not exit with an error message when "-O" is specified.

DEFAULT: check enabled

In general it does not make sense to analyze alignments where one or more sequences consist of completely undetermined characters (e.g., -,N,?,X etc.) because you don't have any information about these sequences and they will simply randomly scatter throughout the tree. Normally RAxML will exit with an error message when it detects such sequences. This option here can disable this behavior, but be warned that you really need to know what you are doing when using this option.

**Example:** `raxmlHPC -s alg -p 12345 -m GTRGAMMA -O -n TEST`

- p Specify a random number seed for the parsimony inferences. This allows you to reproduce your results and will help me debug the program.

For all options/algorithms in RAxML that require some sort of randomization, this option must be specified. Make sure to pass different random number seeds to RAxML and not only 12345 as I have done in the examples. When not specifying -p when it is required by RAxML, the program will exit with a respective error message.

In the example below (a simple tree search for the ML tree) the random number seed is required for randomized stepwise addition order parsimony starting tree that is computed prior to the actual ML optimization.

**Example:** `raxmlHPC -s alg -p 2352890 -m GTRGAMMA -n TEST`

- P Specify the file name of a user-defined AA (Protein) substitution model. This file must contain 420 entries, the first 400 being the AA substitution rates (this must be a symmetric matrix) and the last 20 are the empirical base frequencies

Note that, the substitution model you specify via `-m PROTGAMMAWAG` in the example below will be ignored here. RAxML will only extract the information that this is a protein data alignment and that you want to use the GAMMA model of rate heterogeneity from the string and will ignore WAG.

If you want to use your own protein substitution models for partitioned datasets, the substitution model files that have the same format as described here need to be specified in the partition file.

**Example:** `raxmlHPC -s alg -p 12345 -P myProteinModel -m PROTGAMMAWAG -n TEST`

- q Specify the file name which contains the assignment of models to alignment partitions for multiple models of substitution. For the syntax of this file please consult the manual.

This option allows you to specify the regions of your alignment for which an individual model of nucleotide substitution should be estimated. They can also contain different types of data.

This will typically be useful to infer trees for long (in terms of base-pairs) multi-gene alignments. If, e.g., `-m GTRGAMMA` is used, individual alpha-shape parameters, GTR rates, and empirical base frequencies will be estimated and optimized for each partition.

Since RAXML can handle alignments consisting of different data types (binary data, DNA data, protein data etc.) you must specify the type of data for each partition in the partition file, before the partition name.

For DNA data this just means that you have to add `DNA` to each line in the partition file, for AA data this is done by specifying the respective AA substitution matrix (e.g., `WAG` or `LG`) you want to use for a partition. For binary data you'd specify `BIN` and for multi-state data `MULTI`.

Now, if you want to analyze a specific partition using an ascertainment bias correction you need to prepend `ASC_` to the data type, e.g., `ASC_WAG` or `ASC_DNA`.

If for a specific partition you want to use a maximum likelihood estimate for the base frequencies, you'd append `x` to the data type name, e.g., `DNAX` or `LGX`.

If you want to use empirical base frequencies (instead of the default pre-defined ones that ship with the models) you'd write, e.g., `WAGF` or `JTTF`.

If you want to use a protein substitution model of your own for a specific partition, it must be in the same format as specified for the `-P` option. Instead of specifying the data type with `WAG` for instance, you will need to specify the protein substitution model file name in square brackets, e.g., `[myProtenSubstitutionModelFileName]`.

If you want to do a partitioned analysis of concatenated AA and DNA partitions you can either specify `-m GTRGAMMA` or, e.g., `-m PROTGAMMAWAG`. The only thing that will be extracted from the string passed via `-m` is the model of rate heterogeneity you want to use.

If you have a pure DNA alignment with 1,000bp from two genes `gene1` (positions 1-500) and `gene2`(positions 501-1,000) the information in the partition file should look as follows:

```
DNA, gene1 = 1-500
DNA, gene2 = 501-1000
```

If you want an ML estimate of frequencies for the first partition it will look like this:

```
DNAX, gene1 = 1-500
DNA, gene2 = 501-1000
```

To analyze the first partition with ascertainment bias correction you need to write:

```
ASC_DNA, gene1 = 1-500
DNA, gene2 = 501-1000
```

If `gene1` is scattered through the alignment, e.g. positions 1-200, and 800-1,000 you specify this with:

```
DNA, gene1 = 1-200, 800-1,000
DNA, gene2 = 201-799
```

You can also assign distinct models to the codon positions, i.e. if you want a distinct model to be estimated for each codon position in `gene1` you can specify:

```
DNA, gene1codon1 = 1-500\3
DNA, gene1codon2 = 2-500\3
DNA, gene1codon3 = 3-500\3
DNA, gene2 = 501-1000
```

If you only need a distinct model for the 3rd codon position you can write:

```
DNA, gene1codon1andcodon2 = 1-500\3, 2-500\3
DNA, gene1codon3 = 3-500\3
DNA, gene2 = 501-1000
```

As already mentioned, for AA data you must specify the transition matrices for each partition:

```
JTT, gene1 = 1-500
WAGF, gene2 = 501-800
WAG, gene3 = 801-1000
```

The AA substitution model must be the first entry in each line and must be separated by a comma from the gene/partition name, just like the DNA token above. You can not assign different models of rate heterogeneity to different partitions, i.e., it will be either CAT , GAMMA , GAMMAI etc. for all partitions, as specified with `-m` .

If you want to use a protein model of your own, for partition one, you'd write:

```
[prot~myProtenSubstitutionModelFileName], gene1 = 1-500
WAGF, gene2 = 501-800
WAG, gene3 = 801-1000
```

To use a maximum likelihood estimate of the base frequencies for all partitions you'd write:

```
JTTX, gene1 = 1-500
WAGX, gene2 = 501-800
WAGX, gene3 = 801-1000
```

Finally, if you have a concatenated DNA and AA alignment, with DNA data at positions 1-500 and AA data at 501-1,000 with the WAG model the partition file should look as follows:

```
DNA, gene1 = 1-500
WAG, gene2 = 501-1000
```

A partition file for binary data would look like this:

```
BIN, gene1 = 1-500
BIN, gene2 = 501-1000
```

and for multi-state data

```
MULTI, gene1 = 1-500
MULTI, gene2 = 501-1000
```

**Example:** `raxmlHPC -s alg -m GTRGAMMA -q part -p 12345 -n TEST`

**-r** Specify the file name of a binary constraint tree.  
This tree does not need to be comprehensive, i.e. must not contain all taxa

This option allows you to pass a binary/bifurcating constraint/backbone tree in NEWICK format to RAxML.

Note, that using this option only makes sense if this tree contains less taxa than the input alignment. The remaining taxa will initially be added by using parsimony criterion. Once a comprehensive tree with all taxa has been obtained it will be optimized under ML respecting the restrictions of the binary constraint tree.

Thus, option will not change the structure of the binary backbone tree you provide as input at all. What it will do is to just add the taxa not contained in the binary backbone tree to the best positions based on their likelihood. The result is a fully resolved binary tree.

**Example:** `raxmlHPC -s alg -m GTRGAMMA -r constr -p 12345 -n TEST`

**-R** Specify the file name of a binary model parameter file that has previously been generated with RAxML using the `-f e` tree evaluation option. The file name should be: `RAxML_binaryModelParameters.runID`

This option can be used to save time for some other RAxML options, by not re-estimating the model parameters of a fixed tree again, but only doing this once. The binary model parameter input file is always automatically generated by the `-f e` tree evaluation function.

It is useful with the `-f v` option when you want to place different reads into the same reference tree and with the quartet evaluation function `-f q`, in particular the parallel version of it!

**Example:** `raxmlHPC -f v -R RAxML_binaryModelParameters.PARAMS  
-t RAxML_result.PARAMS -s alg -m GTRCAT -n TEST2`

**-s** Specify the name of the alignment data file in PHYLIP or FASTA format

Specify the name of the alignment data file which can be in relaxed PHYLIP format. Relaxed means that you don't have to worry if the sequence file is interleaved or sequential and that the taxon names are too long. What you do need to worry about though is that there always needs to be a space between the taxon name and the sequence.

Sequence names can be of variable length between 1 up to 256 characters. If you need longer taxon names you can adapt the constant `#define nmlength 256` in file `axml.h` appropriately. Moreover, RAxML is not sensitive with respect to the formatting (tabs, insets, etc) of interleaved PHYLIP files.

RAxML can now also parse FASTA format. If RAxML notices that it can't parse a phylip format it will try to parse the alignment file as FASTA format. So far it has been able to parse all FASTA files.

A plethora of small example input files for different data types can be found in the step-by-step on-line tutorial: [http://sco.h-its.org/exelixis/web/software/raxml/hands\\_on.html](http://sco.h-its.org/exelixis/web/software/raxml/hands_on.html)

**Example:** `raxmlHPC -s alignment.fasta -m GTRGAMMA -p 12345 -n TEST`

**-S** Specify the name of a secondary structure file. The file can contain "." for alignment columns that do not form part of a stem and characters "`()<>[]{}"` to define stem regions and pseudoknots

Specifying secondary structure models for an RNA alignment works slightly differently than passing other data-types to RAxML because we read in a plain RNA alignment and then need to tell RAxML by an additional text file that is passed via `-s` which RNA alignment sites need to be grouped/evolve together.

We do this in a standard bracket notation written into a plain text file, e.g., our DNA test alignment has 60 sites, thus our secondary structure file needs to contain a string of 60 characters like this one:

```
.....((.....)).....(.....).....
```

The '.' symbol indicates that this is just a normal RNA site while the brackets indicate stems. Evidently, the number of opening and closing brackets must match. In addition, it is also possible to specify pseudo knots with additional symbols: `<>[]{}` for instance:

```
.....((.....)).....{.....(.....)}.....
```

In terms of models there are 6-state, 7-state and 16-state models for accommodating secondary structure that are specified via `-A`.

Available models are: S6A, S6B, S6C, S6D, S6E, S7A, S7B, S7C, S7D, S7E, S7F, S16, S16A, S16B. The default is the GTR 16-state model (`-A S16`). In RAxML the same nomenclature as in PHASE is used, so please consult the phase manual at <http://intranet.cs.man.ac.uk/ai/Software/phase/phase-2.0-manual.pdf> for a nice and detailed description of these models.

**Example:** `raxmlHPC -m GTRGAMMA -p 12345 -S secondaryStructure.txt  
-s rna.phy -n TEST`

A common question is whether secondary structure models can also be partitioned. This is presently not possible. However, you can partition the underlying RNA data, e.g., use two partitions for our DNA dataset as before. What RAxML will do internally though is to generate a third partition for secondary structure that does not take into account that distinct secondary structure site pairs may be part of different partitions of the alignment.

`-t` Specify a user starting tree file name in Newick format

Specifies a user starting tree file name which must be in Newick format. The branch lengths of that tree will generally be ignored for most options and re-estimated instead by RAxML. Note, that you can also specify a non-comprehensive (not containing all taxa in the alignment) starting tree. This might be useful if newly aligned/sequenced taxa have been added to your alignment and you want to extend the current tree.

Initially, taxa will be added to the tree using parsimony. The comprehensive tree will then be optimized under ML.

**Example:** `raxmlHPC -s alg -m GTRGAMMA -t tree -p 12345 -n TEST`

`-T` PTHREADS VERSION ONLY! Specify the number of threads you want to run. Make sure to set `"-T"` to at most the number of CPUs you have on your machine, otherwise, there will be a huge performance decrease!

T is set to 0 by default, the PThreads version will produce an error if you do not set `-T` to at least 2.

**Example:** `raxmlHPC-PTHREADS -T 4 -s alg -m GTRGAMMA -p 12345 -n TEST`

-u use the median for the discrete approximation of the GAMMA model of rate heterogeneity

DEFAULT: OFF

If you specify this option, RAXML will use the median instead of the mean for the discrete Gamma model of rate heterogeneity. Typically, using the median will yield slightly better likelihood scores.

**Example:** raxmlHPC -p 12345 -u -s alg -m GTRGAMMA -n TEST

-U Try to save memory by using SEV-based implementation for gap columns on large gappy alignments. The technique is described here: <http://www.biomedcentral.com/1471-2105/12/470>

**This will only work for DNA and/or PROTEIN data and only with the SSE3 or AVX-vextorized version of the code.**

This option can help you to save memory and potentially also time on large gappy multi-gene alignments with missing data, in particular in cases where you have a typical gappy partitioned alignment (data for certain taxa missing for certain genes/partitions). The amount of saved memory is roughly proportional to the proportion of missing data.

**Example:** raxmlHPC -p 12345 -U -s alg -m GTRGAMMA -n TEST

-v Display version information

Displays the RAXML version you are using.

**Example:** raxmlHPC -v

-V Disable rate heterogeneity among sites model and use one without rate heterogeneity instead. Only works if you specify the CAT model of rate heterogeneity.

DEFAULT: use rate heterogeneity

This option can be used if your dataset better fits a model without rate heterogeneity. This is rare, but such datasets do exist.

**Example:** raxmlHPC -m GTRCAT -s alg -p 12345 -V -n TEST

-w FULL (!) path to the directory into which RAXML shall write its output files

DEFAULT: current directory

Name of the working directory where RAXML shall write its output files to. Note that you need to specify the full path and not the relative path!

**Example:** raxmlHPC -m GTRCAT -p 12345 -s alg  
-w /home/stamatak/Desktop/myAnalysys -n TEST

-W Sliding window size for leave-one-out site-specific placement bias algorithm only effective when used in combination with -f S



DEFAULT: 100 sites

Defines the sliding window size for the -f S option, 100 usually yields relatively smooth plots.

**Example:** `raxmlHPC -f S -s alg -t tree -m GTRGAMMA -N 100 -W 200 -n TEST`

-x Specify an integer number (random seed) and turn on rapid bootstrapping  
CAUTION: unlike in previous versions of RAxML will conduct rapid BS replicates under the model of rate heterogeneity you specified via -m and not by default under CAT

This will invoke the rapid bootstrapping algorithm described in <http://sysbio.oxfordjournals.org/content/57/5/758.short>

**Example:** `raxmlHPC -x 12345 -p 12345 -# 100 -m GTRCAT -s alg -n TEST`

You can also combine this with the bootstopping option (bootstrap convergence criterion), e.g.:

**Example:** `raxmlHPC -x 12345 -p 12345 -# AUTO_MRE -m GTRCAT -s alg -n TEST`

or also have RAxML search for the best-scoring ML tree after the bootstrap searches using the -f a option:

**Example:** `raxmlHPC -x 12345 -p 12345 -# AUTO_MRE -m GTRCAT -s alg -f a -n TEST`

-X Same as the "-y" option below, however the parsimony search is more superficial.  
RAxML will only do a randomized stepwise addition order parsimony tree reconstruction without performing any additional SPRs.  
This may be helpful for very broad whole-genome datasets, since this can generate topologically more different starting trees.

DEFAULT: OFF

**Example:** `raxmlHPC -X -m GTRCAT -s alg -p 12345 -n TEST`

-y If you want to only compute a parsimony starting tree with RAxML specify -y, the program will exit after computation of the starting tree

DEFAULT: OFF

If you want to only compute a randomized parsimony starting tree with RAxML and not execute an ML analysis of the tree specify -y. The program will exit after computation of the starting tree.

**Example:** `raxmlHPC -y -m GTRCAT -s alg -p 12345 -n TEST`

-Y Pass a quartet grouping file name defining four groups from which to draw quartets  
The file input format must contain 4 groups in the following form:  
(Chicken, Human, Loach), (Cow, Carp), (Mouse, Rat, Seal), (Whale, Frog);

**Only works in combination with -f q !**

The example below, will randomly draw 100 quartets from the predefined quartet grouping above and evaluate their likelihood.

**Example:** `raxmlHPC -f q -m GTRGAMMA -t tree -Y quartetFile -n 100 -n TEST`

**-z** Specify the file name of a file containing multiple trees e.g. from a bootstrap that shall be used to draw bipartition values onto a tree provided with `-t`.  
It can also be used, for instance to compute per site log likelihoods in combination with `-f g` and to read a bunch of trees for a couple of other options (`-f h`, `-f m`, `-f n`).

This option is required in combination with a lot of other RAXML options, in essence every time you need to read in a bunch of trees. Below is an example where you use the option to draw BS support values on a best-known ML tree.

**Example:** `raxmlHPC -f b -m GTRCAT -t mlTree -z bootstrapTrees -n TEST`

**-#|-N** Specify the number of alternative runs on distinct starting trees  
In combination with the `"-b"` option, this will invoke a multiple bootstrap analysis  
Note that `"-N"` has been added as an alternative since `-#` sometimes caused problems with certain MPI job submission systems, since `-#` is often used to start comments.  
If you want to use the bootstopping criteria specify `-# autoMR` or `-# autoMRE` or `-# autoMRE_IGN` for the majority-rule tree based criteria (see `-I` option) or `-# autoFC` for the frequency-based criterion.  
Bootstopping will only work in combination with `-x` or `-b`

DEFAULT: 1 single analysis

Specifies the number of alternative runs on distinct starting trees, e.g., if `-# 10` or `-N 10` is specified, RAXML will compute 10 distinct ML trees starting from 10 distinct randomized maximum parsimony starting trees.

In combination with the `-b` option, this will invoke a multiple bootstrap analysis. In combination with `-x` this will invoke a rapid BS analysis and combined with `-f a -x a` a rapid BS search and thereafter a thorough ML search on the original alignment.

We introduced `-N` as an alternative to `-#` since the special character `#` seems to sometimes cause problems with certain batch job submission systems.

In combination with `-f j` this will generate `numberOfRuns` bootstrapped alignment files.

Note that, several other program options such as the quartet evaluation algorithm (`-f q` option) or the site-specific placement bias algorithm (`-f s` option) require this option to be specified as well. If you forgot to specify this option, RAXML will tell you that you need to do so.

The example below will do 20 independent ML tree searches on 20 randomized stepwise addition order parsimony tree.

**Example:** `raxmlHPC -p 12345 -s alg -n TEST -m GTRGAMMA -# 20`

`--mesquite` Print output files that can be parsed by Mesquite.

DEFAULT: Off

**Example:** raxmlHPC -p 12345 -s alg --mesquite -n TEST -m GTRGAMMA

**--silent** Disables printout of warnings related to identical sequences and entirely undetermined sites in the alignment . The program might run faster when this is enabled.

DEFAULT: Off

**Example:** raxmlHPC -p 12345 --silent -s alg -n TEST -m GTRGAMMA

**--no-seq-check** Disables checking the input MSA for identical sequences and entirely undetermined sites. Enabling this option may save time, in particular for large phylogenomic alignments. Before using this, make sure to check the alignment using the -f c option!

DEFAULT: Off

**Example:** raxmlHPC -p 12345 -s alg --no-seq-check -n TEST -m GTRGAMMA

**--no-bfgs** Disables automatic usage of BFGS method to optimize GTR rates on unpartitioned DNA datasets . Using BFGS can improve speeds for model optimization by up to 30%. It's enabled by default when analyzing single-partition DNA datasets.

DEFAULT: BFGS on

**Example:** raxmlHPC -p 12345 -s alg --no-bfgs -n TEST -m GTRGAMMA

**--asc-corr** Allows to specify the type of ascertainment bias correction you wish to use. There are three types available:

**--asc-corr=lewis:** the standard correction by Paul Lewis

**--asc-corr=felsenstein:** a correction introduced by Joe Felsenstein that allows to explicitly specify the number of invariable sites (if known) one wants to correct for.

**--asc-corr=stamatakis:** a correction introduced by myself that allows to explicitly specify the number of invariable sites for each character (if known) one wants to correct for.

Let's start with the Lewis correction that is easy to handle:

**Example:** raxmlHPC -p 12345 -s alg -n TEST -m ASC\_GTRGAMMA --asc-corr=lewis

The above will run a standard tree search with the ascertainment bias correction by Paul O. Lewis.

The two other options are a bit more tricky to use. They have been designed for cases where the exact number of invariable sites for the dataset is actually known (felsestein option) or the exact frequencies of each invariable character state is known (stamatakis option). Note that, in such cases the number of invariable sites can be larger than the

number of variable sites in the alignment. This is not the case for the Lewis correction, because the maths break down.

For those two corrections we thus need to tell RAxML what the number of invariable sites for each partition (`felsenstein`) is and what the numbers of invariable sites per state for each partition is (`stamatakis`). This is best handled via the partition file, hence even if you only have one partition, you nonetheless need to specify a partition file via `-q` to pass this information to RAxML.

**Example 2:** `raxmlHPC -p 12345 -s alg -n TEST -m ASC_GTRGAMMA --asc-corr=stamatakis -q part`

**Example 3:** `raxmlHPC -p 12345 -s alg -n TEST -m ASC_GTRGAMMA --asc-corr=felsenstein -q part`

Below, we give an example of such a partition file:

```
[asc~p1.txt], ASC_DNA, p4=1-1000  
[asc~p2.txt], ASC_DNA, p5=1001-1965
```

Here, the first entries in each line, provide the name of the file containing the invariable site counts or frequencies for each partition that are stored in plain text files called `p1.txt` and `p2.txt`. Note that, the respective file names must be preceded by the `asc` keyword and the `~` separator symbol!

Thus, each partition is associated with a file containing these counts.

For the Felsenstein correction, these files could look like this:

```
p1.txt:  
1000
```

```
p2.txt:  
2000
```

With that we tell RAxML, that the likelihood first partition shall be corrected for 1000 invariable sites, while the likelihood of the second partition shall be corrected for 2000 invariable sites. Note that, each file (`p1.txt` and `p2.txt`) must only contain a single line comprising integer values!

For my correction, the files could look as follows for DNA data:

```
p1.txt:  
250 300 400 100
```

```
p2.txt:  
500 300 200 200
```

With that we tell RAxML that the likelihood of the first partition shall be corrected for 250 sites consisting of As, 300 sites consisting of Cs, 400 sites of Gs and 100 sites of Ts. For the second partition the likelihood is corrected for 500 invariable sites of As, 300 Cs, 200 Gs and 200 Ts.

The difference among the two corrections is that with Joe Felsenstein's correction we know how many invariable sites there are, but not their composition, so we correct for the absence of 1000 invariable sites in the first partition that could consists of As or Cs or Gs or

Ts. My correction can be used when we do know the exact frequencies of invariable site patterns.

**WARNING:** When generating these ascertainment bias correction files (e.g., p1.txt and p2.txt) for my correction pay particular attention to the order of states that corresponds to the order of frequencies of invariable sites per state:

```
BINARY:      0,1
DNA:         A, C, G, T
PROTEIN:     A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V
```

**--flag-check** When using this option, RAxML will only check if all command line flags specified are available and then exit with a message listing all invalid command line flags or with a message stating that all flags are valid.

This option is intended for 3<sup>rd</sup> party wrapper software that invokes RAxML. It allows for checking beforehand if certain options are available in the RAxML version being used or not. The option will just analyze the command line for valid/invalid flags and then exit with an appropriate message.

**Example 1:** `raxmlHPC --flag-check -f a -s alg -p 12345 -N 20 -x 12345 -m GTRGAMMA -n T1`

**Output:** All options supported

**Example 2:** `raxmlHPC --flag-check -f a -s alg -p 12345 -N 20 -x 12345 -m GTRGAMMA --nonsense-option -n T1`

**Output:** Option `--nonsense-option` not supported

**--auto-prot=ml|bic|aic|aicc** When using automatic protein model selection you can chose the criterion for selecting these models. RAxML will test all available prot subst. models except for LG4M, LG4X, and GTR-based models with and without empirical base frequencies. You can chose between ML score based selection and the BIC, AIC, and AICc criteria.

DEFAULT: ml

**Example:** `raxmlHPC -s alg -p 12345 -m PROTGAMMAAUTO --auto-prot=bic -n T1`

The above invocation will select between protein models using the Bayesian Information Criterion.

For understanding the following three options that all refer to the standard placement output format (.jplace output file of the EPA) we have defined, it will be very helpful if you read the corresponding paper:

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0031009>

**--epa-keep-placements=number** specify the number of potential placements you want to keep for each read in the EPA algorithm .

Note that, the actual values printed will also depend on the settings for

`--epa-prob-threshold=threshold !`

DEFAULT: 7

**Example:** `raxmlHPC -f v --epa-keep-placements=100 -t tree -m GTRCAT -s alg  
-n T1`

Here, RAxML will print at most 100 placements to the `.jplace` placement file, depending on the setting of `--epa-prob-threshold`.

`--epa-prob-threshold=threshold` specify a percent threshold for including potential placements of a read depending on the maximum placement weight for this read. If you set this value to 0.01 placements that have a placement weight of 1 per cent of the maximum placement will still be printed to file if the setting of `--epa-keep-placements` allows for it

DEFAULT: 0.01

**Example:** `raxmlHPC -f v --epa-prob-threshold=0.05 -t tree -m GTRCAT -s alg  
-n T1`

Here, RAxML will print those placements to the `.jplace` placement file that have a likelihood weight of at least 5% of that of the maximum placement weight for the specific read and if the number of placements to print as specified by `-epa-keep-placements` has not been exceeded.

`--epa-accumulated-threshold=threshold` specify an accumulated likelihood weight threshold for which different placements of read are printed to file. Placements for a read will be printed until the sum of their placement weights has reached the threshold value. Note that, this option can neither be used in combination with `--epa-prob-threshold` nor with `--epa-keep-placements`!

**Example:** `raxmlHPC -f v --epa-accumulated-threshold=0.95 -t tree -m GTRCAT -s  
alg -n T1`

Here, RAxML will print those placements of a read to the `.jplace` placement file until their accumulated likelihood weights has reached a value of 0.95 (out of a total of 1.0).

`--JC69` specify that all DNA partitions will evolve under the Jukes-Cantor model, this overrides all other model specifications for DNA partitions.

DEFAULT: Off

**Example:** `raxmlHPC -p 12345 -m GTRGAMMA -s alg --JC69 -n T1`

`--K80` specify that all DNA partitions will evolve under the K80 model, this overrides all other model specifications for DNA partitions.

DEFAULT: Off

Note that, the output of the program might look a bit weird, since unlike in the definition of the model, RAxML actually estimates the rates from  $A \leftrightarrow G$  and  $C \leftrightarrow T$  while all other rates are set to 1.0. Note that, this does not matter, since the rates in the rate matrix are relative

rates; the results (likelihoods) will be the same.

**Example:** `raxmlHPC -p 12345 -m GTRGAMMA -s alg --K80 -n T1`

`--HKY85` specify that all DNA partitions will evolve under the HKY85 model, this overrides all other model specifications for DNA partitions.

DEFAULT: Off

Note that, the output of the program might look a bit weird, since unlike in the definition of the model, RAxML actually estimates the rates from  $A \leftrightarrow G$  and  $C \leftrightarrow T$  while all other rates are set to 1.0. Note that, this does not matter, since the rates in the rate matrix are relative rates; the results (likelihoods) will be the same.

**Example:** `raxmlHPC -p 12345 -m GTRGAMMA -s alg --HKY85 -n T1`

`--set-thread-affinity` specify that thread-to-core affinity shall be set by RAxML for the hybrid MPI-PThreads version

DEFAULT: Off

You might have to use this option for the hybrid MPI-PThreads version of RAxML on some cluster installations such that the code interacts properly with the scheduling system. On other systems however, enabling this option may yield problems! It's unfortunately a matter of trial and error that is due to how different schedulers handle hybrid MPI-PThreads codes!

**Example:** `raxmlHPC-HYBRID -m GAMMA -s alg -p 12345 -N 20 --set-thread-affinity -n T1`

`--bootstop-perms=number` specify the number of permutations to be conducted for the bootstopping/bootstrap convergence test. The allowed minimum number is 100!

DEFAULT: 100

You might want to set this to 1000 or higher to reduce the variation in the number of bootstrap trees you calculate on noisy/difficult datasets. The variance of the number of bootstrap trees that bootstrap runs with different random number seeds will generate will decrease by increasing the number of permutations.

**Example:** `raxmlHPC -B 0.02 --bootstop-perms=1000 -b 12345 -p 12345 -# AUTOMR -s alg -m GTRCAT -n TEST`

`--quartets-without-replacement` specify that quartets are randomly subsampled, but **without** replacement.

DEFAULT: random sampling **with** replacements

**Example:** `raxmlHPC -s alg -p 12345 -m GTRGAMMA -f q -N 50 --quartets-without-replacement -n TEST`

`--print-identical-sequences` specify that RAxML shall automatically generate a .reduced alignment with all undetermined columns removed, **but without** removing exactly identical sequences

DEFAULT: identical sequences will also be removed in the .reduced file

**Example:** `raxmlHPC -s alg -p 12345 -m GTRGAMMA --print-identical-sequences -n TEST`

## IX. Output Files

Depending on the search parameter settings RAxML will write a number of output files. The most important files, a run named `-n exampleRun` will write, are listed below. Since the number and names of output files vary depending on the RAxML options you used, the easiest way to list all output files of the run is to type the following Linux command:

```
ls *exampleRun*
```

`RaxML_info.exampleRun`: contains information about the model and algorithm used and how RAxML was called. The final GAMMA-based likelihood(s) as well as the alpha shape parameter(s) are printed to this file. In addition, if the rearrangement setting was determined automatically (`-i` has not been used) the rearrangement setting found by the program will be indicated.

**This is the most important output file** because it tells you what RAxML did and is always written irrespective of the command line option. In addition it contains information about all other output files that were written by your run.

`RAxML_log.exampleRun`: A file that prints out the time, likelihood value of the current tree and number of the checkpoint file (if the use of checkpoints has been specified) after each iteration of the search algorithm. In the last line it also contains the final likelihood value of the final tree topology. This file is not written if multiple bootstraps are executed, i.e. `-#` and `-b` have been specified. In case of a multiple inference on the original alignment (`-#` option) the Log-Files are numbered accordingly.

`RAxML_result.exampleRun`: Contains the final tree topology of the current run. This file is also written after each iteration of the search algorithm, such that you can restart your run with `-t` in case your computer crashed. This file is not written if multiple bootstraps are executed, i.e. `-#` and `-b` have been specified.

`RAxML_parsimonyTree.exampleRun`: contains the randomized parsimony starting tree if the program has not been provided a starting tree by `-t`. However, this file will not be written if a multiple bootstrap is executed using the `-#` and `-b` options.

`RAxML_randomTree.exampleRun`: contains the completely random starting tree if the program was executed with `-d`.

`RAxML_checkpoint.exampleRun.checkpointNumber`: Printed if you specified by `-j` that checkpoints shall be written. Checkpoints are numbered from 0 to n where n is the number of iterations of the search algorithm. Moreover, the checkpoint files are additionally numbered if a multiple inference on the original alignment has been specified using `-#`. Writing of checkpoint files is disabled when a multiple bootstrap is executed.

`RAxML_bootstrap.exampleRun`: If a multiple bootstrap is executed by `-#` and `-b` or `-x` all final



bootstrapped trees will be written to this one, single file.

`RAXML_bipartitions.exampleRun`: If you used the `-f b` option, this file will contain the input tree with confidence values from 0 to 100 drawn on its nodes! It is also printed when `-f a -x` have been specified, at the end of the analysis the program will draw the BS support values on the best tree found during the ML search.

`RAXML_bipartitionsBranchLabels.exampleRun`: Contains the same information as the file above, but support values are correctly displayed as Newick branch labels and not node labels! **Support values always refer to branches/splits of trees and never to nodes of the tree.** Note that, some tree viewers have problems displaying branch labels, they are however part of the standard Newick format.

`RAXML_bipartitionFrequencies.exampleRun`: Contains the pair-wise bipartition frequencies of all trees contained in files passed via `-t` and `-z` when the `-f m` option has been used.

`RAXML_perSiteLLs.exampleRun`: Contains the per-site log likelihood scores in Treepuzzle format for usage with CONSEL (<http://www.is.titech.ac.jp/~shimo/prog/consel/>). This file is only printed when `-f g` is specified.

`RAXML_bestTree.exampleRun`: Contains the best-scoring ML tree of a thorough ML analysis.

`RAXML_distances.exampleRun`: Contains the pair-wise ML-based distances between all taxon-pairs in the alignment. This file is only printed when the `-f x` option is used.

## X. Computing TC and IC values

In the following I provide some more detailed examples for computing the IC and TC metrics from Salichos and Rokas 2013 <http://www.ncbi.nlm.nih.gov/pubmed/23657258>

The method is described in more detail in the following paper I wrote with Salichos and Rokas: <http://mbe.oxfordjournals.org/content/early/2014/02/07/molbev.msu061.abstractkeytype=ref&ijkey=I65FuGNx0HzR2Ow>

Also note that, as of version 8.2.0 We can conduct TC/IC calculations on collections of partial gene trees. The corrections we apply for partial gene trees are described in this paper here: <http://dx.doi.org/10.1101/022053>

**Warning:** The default TC/IC calculations are not done exactly as described in our MBE paper. To have RAXML do exactly what is described in the paper, please edit file `bipartitionList.c` by commenting out or removing the line `#define BIP_FILTER`

The way it is implemented in RAXML by default and where it differs from the version described in the paper regards the calculation of the ICA and TCA scores. When there are several conflicting bipartitions, the standard method takes all of them into account for computing the ICA score, as long as their frequency exceeds a frequency threshold of 5%. It does however not account for the fact that some of these conflicting bipartitions, with respect to the reference bipartition do not conflict with each other, but are in fact compatible with each other. Thus, by default RAXML only uses those bipartitions that conflict with the reference bipartition that are also mutually incompatible with each other to calculate the ICA score. Thus, less bipartitions will typically be used to calculate the ICA and consequently the TCA scores. My personal opinion is that this is more reasonable, since we are not counting conflicts several times in case these conflicts emerge from compatible bipartitions.

Given a set of gene trees, RAxML can directly calculate a majority rule consensus (MR in RAxML terminology) as well as an extended majority rule consensus tree (MRE in RAxML terminology) on this set that has every internode (that is, internal branch) annotated by their respective IC and ICA scores.

For instance, to compute the IC, ICA, TC, and TCA scores for a given set of gene trees on a MRC tree you would type:

```
raxmlHPC -L MR -z 1070_yeast_genetrees.tre -m GTRCAT -n T1
```

where

- L MR specifies that the scores will be displayed on a MR tree that is computed by RAxML
- z 1070\_yeast\_genetrees.tre specifies the filename that contains the set of gene trees (which are the maximum likelihood trees from the 1,070 yeast genes analyzed by Salichos, and Rokas 2013, and which are provided as supplementary data to this manuscript)
- m GTRCAT is an arbitrary substitution model (this will have no effect whatsoever, but is required as input to RAxML)
- n T1 is the run ID that is appended to output files.

RAxML will automatically build the MR tree, annotate it with the IC and ICA scores, and report both in an output file named `RAxML_MajorityRuleConsensusTree_IC.T1`, which will look like this:

```
(Scer,Spar,(Smik,(Skud,(Sbay,(Scas,(Cgla,(Kpol,(Zrou,((Clus,((Psti,((Ctro,
(Calb,Cdub):1.0[0.95,0.95]):1.0[0.77,0.77],
(Cpar,Lelo):1.0[0.76,0.76]):1.0[0.75,0.75]):1.0[0.11,0.11],
(Cgui,Dhan):1.0[0.02,0.07]):1.0[0.02,0.08]):1.0[0.97,0.97],((Sklu,
(Kwal,Kthe):1.0[0.97,0.97]):1.0[0.32,0.23],
(Agos,Klac):1.0[0.08,0.08]):1.0[0.04,0.10]):1.0[0.59,0.47]):1.0[0.02,0.02]):1.0[0.1
1,0.11]):1.0[0.02,0.02]):1.0[0.97,0.97]):1.0[0.05,0.14]):1.0[0.30,0.27]):1.0[0.54,0
.54]);
```

For each internode or internal branch of the constructed MR tree, RAxML will assign an `length[x,y]` branch label, where `length` corresponds to the branch length (because this is a MRE tree, all internal branch lengths have been arbitrarily set to 1.0 by default), `x` corresponds to the IC score and `y` to the ICA score.

RAxML will also calculate the TC and TCA scores for the MR tree, as well as the relative TC and TCA scores that are normalized by the maximum possible TC and TCA scores for a fully bifurcating tree from the same number of taxa.

The scores are displayed in the terminal output and in the `RAxML_info.runID` standard output file associated with the run (in this case `RAxML_info.T1`) and will look like this:

```
Tree certainty for this tree: 7.642240
Relative tree certainty for this tree: 0.382112
```

```
Tree certainty including all conflicting bipartitions (TCA) for this tree: 7.580023
Relative tree certainty including all conflicting bipartitions (TCA) for this tree: 0.379001
```

Given a set of gene trees, RAxML can also directly calculate an extended MR tree on this set that has every internode (that is, internal branch) annotated by their respective IC and ICA scores. The particularly compute-intensive inference of extended MRC trees (finding the optimal extended MRC tree is, in fact, NP-hard; Phillips, and Warnow 1996) relies on RAxML's fast parallel implementation. Thus if you use the PThreads version of RAxML, this part will run in parallel.

To compute IC, ICA, TC, and TCA scores on an extended MR tree you would type:

```
raxmlHPC -L MRE -z 1070_yeast_genetrees.tre -m GTRCAT -n T2
```

RAxML can compute MR and extended MR trees, using both fully bifurcating and partially resolved/multifurcating trees as an input. RAxML can also compute stricter MR trees with arbitrary threshold settings that range between 51 and 100%. For instance, by typing

```
raxmlHPC -L T_75 -z 1070_yeast_genetrees.tre -m GTRCAT -n T3
```

RAxML will display IC, ICA, TC and TCA scores on a MR tree that only includes those bipartitions that have  $\geq 75\%$  support.

We have also implemented an option (`-f i`) that allows the user to calculate and display IC, ICA, TC, and TCA scores onto a given, strictly bifurcating reference tree (for example, the best-known ML tree).

This is analogous to the standard `-f b` option in RAxML that draws bootstrap support values from a set of bootstrap trees onto a reference phylogeny. The option can be invoked by typing

```
raxmlHPC -f i -t yeast_concatenationtree.tre -z 1070_yeast_genetrees.tre -m GTRCAT -n T4
```

Note that, the tree contained in file `yeast_concatenationtree.tre` needs to be strictly bifurcating and contain branch lengths. In this example, the `yeast_concatenationtree.tre` file is the best-known maximum likelihood tree recovered by concatenation analysis of the 1,070 yeast genes from the paper.

Using this command, RAxML will annotate the tree in `yeast_concatenationtree.tre` with the IC and ICA scores, and report both in an output file named `RAxML_IC_Score_BranchLabels.T4`, which will look like this:

```
(((((Clus:0.47168135428609103688(((Lelo:0.30356174702769450624,Cpar:0.2549087423
9480920682):0.13023178275857649755[0.76,0.76]),
(Ctro:0.18383414558272206940(Calb:0.04124660275465741321,Cdub:0.0429080158839683228
9):0.14526604486383792869[0.95,0.95]):0.12355825028654655873[0.77,0.77]):0.17335821
030783615804[0.75,0.75],Psti:0.42255112174261910685):0.07862882822310976461[0.11,0.
11],
(Cgui:0.45961028886034632768,Dhan:0.28259245937168109286):0.05586015476156453580[0.
02,0.07]):0.08116340505230199009[0.02,0.08]):1.03598510402913923656[0.97,0.97],
((Agos:0.53332956655591512440,Klac:0.47072785596320687596):0.08132006357704427146[0
.08,0.08],
((Kthe:0.17123899487739652203,Kwal:0.17320923240031221857):0.25620117495110567019[0
.97,0.97],Sklu:0.24833228915799765435):0.05646992617871094550[0.32,0.23]):0.0523630
6187235122145[0.04,0.10]):0.10686517691208799463[0.59,0.47],Zrou:0.4130783368556378
2877):0.03792570537296727218[0.02,0.02],Kpol:0.43287284049576529865):0.045603416931
36910068[0.11,0.11],Cgla:0.49584136365135367264):0.04363310339731014259[0.02,0.02],
Scas:0.37212829744050218705):0.29362133996280515014[0.97,0.97],
(Skud:0.06926467973344750673,(Smik:0.06535810850036427588,
(Scer:0.04285848856634000975,Spar:0.03030513540244994877):0.02506719066056842596[0.
54,0.54]):0.02459323291555862850[0.30,0.27]):0.02524223867026276907[0.05,0.14],Sbay
:0.06506923220637816918);
```

For each internode or internal branch of this output tree RAxML will assign a `length[x,y]` branch label, where `length` corresponds to the original branch length in the input tree passed via `-t`, `x` corresponds to the IC score and `y` to the ICA score. RAxML will also display the TC and TCA scores of this tree both in the terminal output and in the `RAxML_info.T4` output file associated with the run.

It should further be noted that the IC and ICA scores are represented as branch labels, since, as is the case for bootstrap support values, information associated to internodes/splits/bipartitions of a tree always refers to branches and not nodes.

Each tree viewer (e.g., Dendroscope <http://ab.inf.uni-tuebingen.de/software/dendroscope/>) that can properly parse the Newick tree format is able to display these branch labels.

The rationale for not providing IC and ICA scores as node labels is that, some viewers may not properly rotate the node labels when the tree is re-rooted by the user, which will lead to an erroneous branch-to-IC and branch-to-ICA score association.

When calculating IC and ICA scores on extended MR trees or when drawing IC and ICA scores onto a given reference tree it may occur that the bipartition that has been included in the tree has lower support than one or more conflicting bipartitions. In this case, RAxML will report IC and ICA scores on the inferred tree with negative signs.

Finally, we have implemented a verbose output option that allows users to further scrutinize particularly interesting conflicting bipartitions.

Verbose mode is activated by adding the `-c` command line switch to any of the above examples. In verbose mode RAxML will generate two additional types of output files: One set of files containing one included bipartition and the corresponding conflicting bipartitions in Newick format (called `RAxML_verboseIC.runID.0 ... RAxML_verboseIC.runID.N-1`, where `N` is the number of bipartitions in the tree) and an output file that lists all bipartitions (included and conflicting) in a PHYLIP-like format (called `RAxML_verboseSplits.runID`).

For example, by adding `-c` to the previous command

```
raxmlHPC -f i -t yeast_concatenationtree.tre -z 1070_yeast_genetrees.tre -m GTRCAT -n T5 -C
```

will produce 20 files (one for each of the 20 bipartitions present in the `yeast_concatenationtree.tre`) named `RAxML_verboseIC.T5.0`, `RAxML_verboseIC.T5.1`, ..., `RAxML_verboseIC.T5.19`

For example, the `RAxML_verboseIC.T5.0` file will look like this:

```
((Cpar, Lelo),(Scer, Smik, Skud, Cgla, Kpol, Zrou, Kwal, Kthe, Agos, Klac, Clus, Cgui, Psti, Ctro, Calb, Cdub, Dhan, Sklu, Scas, Sbay, Spar));  
((Cpar, Ctro, Calb, Cdub),(Scer, Smik, Skud, Cgla, Kpol, Zrou, Kwal, Kthe, Agos, Klac, Clus, Cgui, Psti, Lelo, Dhan, Sklu, Scas, Sbay, Spar));
```

where the first Newick string represents the bipartition that was included in the `yeast_concatenationtree.tre` and all following Newick strings represent the corresponding conflicting bipartitions in descending order of their frequency of occurrence. In the case of the `RAxML_verboseIC.T5.0` file the first bipartition, which is included in the `yeast_concatenationtree.tre` conflicts with only one other bipartition, which is listed as the second bipartition.

Analogously, the output file that lists all bipartitions (included and conflicting) in a PHYLIP-like format (`RAxML_verboseSplits.T5`), looks like this:

1. Scer
2. Smik
3. Skud
4. Cgla
5. Kpol
6. Zrou

7. Kwal
8. Kthe
9. Agos
10. Klac
11. Clus
12. Cgui
13. Psti
14. Cpar
15. Lelo
16. Ctro
17. Calb
18. Cdub
19. Dhan
20. Sklu
21. Scas
22. Sbay
23. Spar

```
partition:
----- ----- ----** ----- ---      956/89.345794/0.761406
----- ----- ----*-  ***--- ---      39/3.644860/0.761406
```

```
partition:
----- ----- ----- -**--- ---      1051/98.224299/0.949483
----- ----- ----- **--- ---      6/0.560748/0.949483
```

.  
.  
.

```
partition:
--***  *****  *****  *****  **_      641/59.906542/0.303620
--*---  -----  -----  -----  -*_      148/13.831776/0.303620
-_*--**  *****  *****  *****  *--      114/10.654206/0.303620
```

```
partition:
-****  *****  *****  *****  **_      825/77.102804/0.545775
--*---  -----  -----  -----  -**      87/8.130841/0.545775
```

Here each block that starts with the partition keyword contains a specific bipartition and all corresponding conflicting bipartitions in descending order. The  $x/y/z$  scores correspond to the frequency of the bipartition ( $x$ ), the support percentage (also known as gene support frequency;  $y$ ), and the IC score ( $z$ ).

## XI. Simple RAxML Analyses

This is a how-to, which describes how RAxML should best be used for a simple real-world biological analysis, given an example alignment named `ex_a1`.

### *The Easy & Fast Way*

The easy and fast way to infer trees with RAxML and to analyze really large datasets (several genes or more than 1,000 taxa) or to conduct a large number of BS replicates is to use the novel rapid BS algorithm and combine it with an ML search.

RAxML will then conduct a full ML analysis, i.e., a certain number of BS replicates and a search for a

best-scoring ML tree on the original alignment.

To just do a BS search you would type:

```
raxmlHPC -x 12345 -p 12345 -# 100 -m GTRGAMMA -s ex_al -n TEST  
or using the bootstrap convergence criterion:
```

```
raxmlHPC -x 12345 -p 12345 -# autoMRE -m GTRGAMMA -s ex_al -n TEST
```

This will conduct rapid bootstrapping and an ML search under the GAMMA model of rate heterogeneity.

Now, if you want to run a full analysis, i.e., BS and ML search type:

```
raxmlHPC -f a -x 12345 -p 12345 -# 100 -m GTRGAMMA -s ex_al -n TEST
```

This will first conduct a BS search and once that is done a search for the best-scoring ML tree. Such a program run will return the bootstrapped trees (RAXML\_bootstrap.TEST), the best scoring ML tree (RAXML\_bestTree.TEST) and the BS support values drawn on the best-scoring tree as node labels (RAXML\_bipartitions.TEST) as well as, more correctly since support values refer to branches as branch labels (RAXML\_bipartitionsBranchLabels.TEST).

Finally, note that, by increasing the number of BS replicates via `-#` you will also make the ML search more thorough, since for ML optimization every 5th BS tree is used as a starting point to search for ML trees.

When `-# autoMRE` is specified RAXML will execute a maximum of 1000 BS replicate searches, but it may, of course converge earlier.

From what I have observed so far, this new ML search algorithm yielded better trees than what is obtained via 20 standard ML searches on distinct starting trees for all datasets with  $\leq 1,000$  sequences.

For larger datasets it might be worthwhile to conduct an additional ML search as described below, just to be sure.

**Warning:** note that the rapid BS search will currently ignore commands associated to user tree files passed via `-t` or `-z`.

However, the constraint and backbone tree options (`-g` and `-r`) do work with rapid BS.

### *The Hard & Slow Way*

Despite the observation that the default parameters and the rapid BS and ML algorithm described above work well in most practical cases, a good thing to do is to adapt the program parameters to your alignment.

This refers to a *good* setting for the rate categories of `-m GTRCAT` and the initial rearrangement setting.

If you use partitioned models you should add `-q partitionFileName` to all of the following commands.

### *Getting the Initial Rearrangement Setting right*

If you don't specify an initial rearrangement setting with the `-i` option the program will automatically determine a good setting based upon the randomized MP starting tree. It will take the starting tree and apply lazy subtree rearrangements with a rearrangement setting of 5, 10, 15, 20, 25. The minimum setting that yields the best likelihood improvement on the starting trees will be used as initial rearrangement setting.

This procedure can have two disadvantages:

Firstly, the initial setting might be very high (e.g. 20 or 25) and the program will slow down considerably.

Secondly, a rearrangement setting that yields a high improvement of likelihood scores on the starting tree might let the program get stuck earlier in some local maximum (this behavior could already be observed on a real dataset with about 1,900 taxa).

Therefore, you should run RAxML a couple of times (the more the better) with the automatic determination of the rearrangement setting and with a pre-defined value of 10 which proved to be sufficiently large and efficient in many practical cases.

In the example below we will do this based on 5 fixed starting trees. So let's first generate a couple of randomized MP starting trees.

Note that in RAxML you also always have to specify a substitution model, regardless of whether you only want to compute an MP starting tree with the `-y` option. Note that, we have to pass different random number seeds via `-p` to obtain distinct starting trees here!

```
raxmlHPC -y -p 12345 -s ex_al -m GTRCAT -n ST0
...
raxmlHPC -y -p 34556 -s ex_al -m GTRCAT -n ST4
```

Then, infer the ML trees for those starting trees using a fixed setting `-i 10`

```
raxmlHPC -f d -i 10 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST0 -n FI0
...
raxmlHPC -f d -i 10 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST4 -n FI4
```

and then using the automatically determined setting on the same starting trees:

```
raxmlHPC -f d -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST0 -n AI0
...
raxmlHPC -f d -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST4 -n AI4
```

The setting that yields the best final likelihood scores as automatically computed under the GAMMA model of rate heterogeneity should then be used for subsequent analyses.

### *Getting the Number of Categories right*

Another issue is to get the number of rate categories right. Due to the reduced memory footprint and significantly reduced inference times the recommended model to use with RAxML on large dataset is GTRCAT if you are doing runs to find the best-known ML tree on the original alignment and for bootstrapping.

Thus, you should experiment with a couple of `-c` settings and then look which gives you the best Gamma-based likelihood value.

Suppose that in the previous section you found that automatically determining the rearrangement setting works best for your alignment.

You should then re-run the analyses with distinct `-c` settings by increments of e.g. 15 rate categories

```
raxmlHPC -f d -c 10 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST0 -n C10_0
...
raxmlHPC -f d -c 10 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST4 -n C10_4
```

You don't need to run it with the default setting of `-c 25` since you already have that data, such that you can continue with ...

```
raxmlHPC -f d -c 40 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST0 -n C40_0
...
raxmlHPC -f d -c 40 -m GTRCAT -s ex_al -t RAxML_parsimonyTree.ST4 -n C40_4
```

and so on and so forth.

Since the GTRCAT approximation is still a new concept little is known about the appropriate setting for `-c 25`.

However, empirically `-c 25` worked best on 19 real-world alignments. So testing up to `-c 55` should usually be sufficient, except if you notice a tendency for final GTRGAMMA likelihood values to further improve with increasing rate category number.

Thus, the assessment of the *good* `-c` setting should once again be based on the final GTRGAMMA likelihood values.

If you don't have the time or computational power to determine both *good* `-c` and `-i` settings you should rather stick to determining `-i` since it has shown to have a greater impact on the final results.

Also note, that increasing the number of distinct rate categories has a negative impact on execution times. Finally, if the runs with the automatic determination of the rearrangement settings from the previous Section have yielded the best results you should then use exactly the same rearrangement settings for each series of experiments to determine a *good* `-c` setting.

The automatically determined rearrangement settings can be retrieved from files `RAxML_info.AI_0` ... `RAxML_info.AI_4`

### *Finding the Best-Known Likelihood tree (BKL)*

As already mentioned RAxML uses randomized stepwise addition order parsimony starting trees on which it then initiates an ML-based optimization. Those trees are obtained by using a randomized stepwise addition sequence to insert one taxon after the other into the tree. When all sequences have been inserted a couple of subtree rearrangements (also called subtree pruning re-grafting) with a fixed rearrangement distance of 20 are executed to further improve the MP score.

The concept to use randomized MP starting trees in contrast to the NJ (Neighbor Joining) starting trees many other ML programs use, is regarded as an advantage of RAxML. This allows the program to start ML optimizations of the topology from a distinct starting point in the immense topological search space each time.

Therefore, RAxML is more likely to find good ML trees if executed several times.



This also allows you to build a consensus tree out of the final tree topologies obtained from each individual run on the original alignment. By this and by comparing the final likelihoods you can get a feeling on how stable (prone to get caught in local maxima) the search algorithm is on the original alignment.

You can also use the `-f r` option to compute pairwise topological Robinson-Foulds distances between the ML trees you have found.

Thus, if you have sufficient computing resources available, in addition to bootstrapping, you should do multiple inferences (I executed 200 inferences in some recent real-world analyses with Biologists) with RAxML on the original alignment.

On smaller datasets or large phylogenomic datasets it will also be worthwhile to use the `-d` option for a couple of runs to see how the program behaves on completely random starting trees. This is where the `-#` option as well as the parallel MPI version `raxmlHPC-MPI` come into play.

So, to execute a multiple inference on the original alignment on a single processor just type:

```
raxmlHPC -f d -p 12345 -m GTRCAT -s ex_al -# 10 -n MultipleOriginal
```

If you have a cluster available you would specify:

```
raxmlHPC-MPI -f d -m GTRCAT -p 12345 -s ex_al -# 100 -n MultipleOriginal
```

preceded by the respective MPI run-time commands, e.g. `mpiexec` or `mpirun` depending on your local installation (please check with your local computer scientist).

### *Bootstrapping with RAxML*

To carry out a multiple non-parametric bootstrap with the sequential version of RAxML just type:

```
raxmlHPC -f d -m GTRCAT -s ex_al -p 12345 -# 100 -b 12345 -n MultipleBootstrap
```

You have to specify a random number seed after `-b` for the random number generator of the bootstraps and `-p` for the random number generator of the parsimony starting trees. This will allow you to generate reproducible results.

To do a parallel bootstrap type:

```
raxmlHPC-MPI -f d -m GTRCAT -s ex_al -# 100 -p 12345 -b 12345 -n MultipleBootstrap
```

once again preceded by the appropriate MPI execution command.

### *Obtaining Confidence Values*

Suppose that you have executed 200 inferences on the original alignment and 1,000 bootstrap runs. You can now use the RAxML `-f b` option to draw the information from the 1,000 bootstrapped topologies onto some tree and obtain a topology with support values. From my point of view the most reasonable thing to do is to draw them on the best-scoring ML tree from those 200 runs. Suppose, that the best-scoring tree was found in run number 99 and the respective treefile is called `RAxML_result.MultipleOriginal.RUN.99`.

If you have executed more than one bootstrap runs with the sequential version of RAxML on distinct computers, i.e. 10 runs with 100 bootstraps on 10 machines you will first have to concatenate the boot-strap files. If your bootstrap result files are called e.g. `RAxML_bootstrap.MultipleBootstrap.0...RAxML_bootstrap.MultipleBootstrap.9` you can

easily concatenate them by using the LINUX/UNIX `cat` command, e.g.

```
cat RAxML_bootstrap.MultipleBootstrap.* > RAxML_bootstrap.All
```

In order to get a tree with bootstrap values on it just execute RAxML as indicated below:

```
raxmlHPC -f b -m GTRCAT -z RaxML_bootstrap.All -t RAxML_result.MultipleOriginal.RUN.99 -n BS_TREE
```

This will return the tree passed via `-t` annotated by support values either as branch labels or as node labels. Some tree viewers have problems with displaying trees with node labels, in particular if they are used to re-root the tree, hence you should use the branch-labeled tree, if possible.

You can now also compute a majority rule consensus tree out of the bootstrap replicates:

```
raxmlHPC -J MR -m GTRCAT -z RaxML_bootstrap.All -n MR_CONS
```

an extended majority rule consensus tree:

```
raxmlHPC -J MRE -m GTRCAT -z RaxML_bootstrap.All -n MRE_CONS
```

or a strict one:

```
raxmlHPC -J STRICT -m GTRCAT -z RaxML_bootstrap.All -n STRICT_CONS
```

For finding rogue taxa you can use the `-J STRICT_DROP` or `-J MR_DROP` options. Alternatively you can use the web-server implemented by my lab: <http://rnr.h-its.org/rnr>

## XII. A Simple Heterotachous Model

We implemented a simple heterotachous model in RAxML by assigning one GTR model to terminal branches of the tree and another distinct GTR model to the inner branches of the tree. The idea was to better capture early rapid radiations with this model, but it didn't work well.

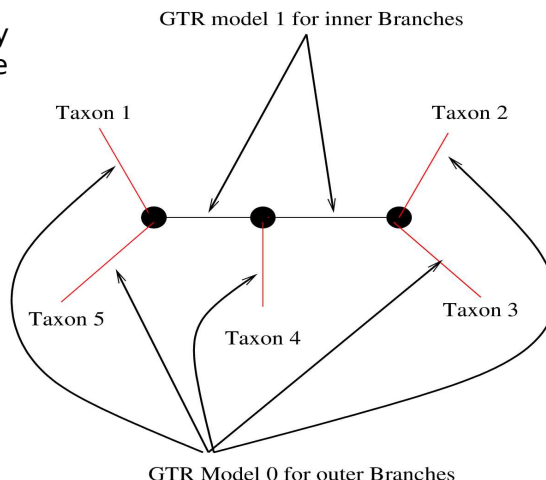
All other parameters such as the alpha shape parameter of the Gamma model of rate heterogeneity and the base frequencies are shared across the entire tree. The model parameters of the two GTR models on the tree are optimized with respect to the overall likelihood of the tree using standard numerical optimization procedures.

This heterotachous model is currently only available in the sequential version of RAxML and only for analyzing DNA sequence data under the Gamma model of rate heterogeneity!

There is no command line switch for enabling this model. Instead, you will need to recompile RAxML by adding `-D_HET` to the line starting by `CFLAGS =` in the respective Makefile. Then, when you specify `-m GTRGAMMA` the search and all other likelihood calculations will be conducted under this heterotachous model.

Keep in mind that, when doing model tests etc., having 2 GTR matrices per partition induces a total of 10 free parameters for the substitution matrix instead of 5 for a single GTR matrix.

Our simple heterotachy model is outlined in the figure below.



### XIII. Frequently Asked Questions

- Q:** When performing a bootstrap search using a partitioned model, does RAxML perform a conserved- bootstrap re-sampling, i.e., does it re-sample within genes so that partitions are sustained?
- A:** That is the case. When performing Bootstraps on partitioned data sets, bootstrapped alignments will be sampled from within partitions, i.e., bootstrapped partitions are sustained and contain exactly the same number of alignment columns as the original partition.
- Q:** Can I use NEXUS-style input files for analyses with RAxML?
- A:** Not directly, but my colleague Frank Kauff (fkauff@rhrk.uni-kl.de) at the University of Kaiserslautern has written a cool biopython wrapper called PYRAXML2. This is a script that reads nexus data files and prepares the necessary input files and command-line options for RAxML. You can download it at <http://www.lutzonilab.net/downloads/>
- Q:** Why don't you like the proportion of Invariable (P-Invar) Sites estimate, despite the fact that you implemented it?
- A:** I only implemented P-Invar in RAxML to make some users happy, but I still strongly disagree with its usage.

*Personal opinion:* It is unquestionable that one needs to incorporate rate heterogeneity in order to obtain *publishable* results. Put aside the *publish-or-perish* argument, there is also strong biological evidence for rate heterogeneity among sites. The rationale for being skeptical about P-Invar in RAxML is that all three alternatives, GTRGAMMA, GTRCAT, and P-Invar represent distinct approaches to incorporate rate heterogeneity. Thus, in principle they account for the same phenomenon by different mathematical means. Also some unpublished concerns have been raised that the usage of P-Invar in combination with Gamma can lead to a *ping-pong* effect since a change of P-Invar leads to a change in Gamma and vice versa. This essentially means that those two parameters, i.e., alpha and P-Invar can not be optimized independently from each other, and might cause significant trouble and problems during the model parameter (everything except tree topology) optimization process. In fact, I already observed this when I was implementing P-Invar in RAxML on a very small AA dataset.

Although this has never been properly documented, several well-known researchers in phylogenetics share this opinion. I'll quote Ziheng Yang from an email in 2008 regarding this part of the RAxML manual:

*I entirely agree with your criticism of the Pinv+Gamma model, even though as you said, it is very commonly used.*

Ziheng also addresses the issue in his book on Computational Molecular Evolution (Oxford University Press, 2006); quote from pages 113-114:

*The model is known as I+G and has been widely used. This model is somewhat pathological as the gamma distribution with alpha already allows for sites with very low rates; as a result, adding a proportion of invariable sites creates a strong correlation between  $p_0$  and alpha, making it impossible to estimate both parameters reliably.*

In any case, I have so far not encountered any difficulties with reviews for the few real phylogenetic analyses I published with colleagues from Biology, when we used the GTRGAMMA model instead of the more widely spread GTR+ $\Gamma$ +I model.

**Q:** Why does RAxML only implement GTR-based models of nucleotide substitution?

**A:** For each distinct model of nucleotide substitution RAxML uses a separate, highly optimized set of likelihood functions. The idea behind this is that GTR is the most common and general model for real-world DNA analysis. Thus, it is better to efficiently implement and optimize this model instead of offering a plethora of distinct models which are only special cases of GTR but are programmed in a generic and thus inefficient way.

*Personal opinion:* My personal view is that using a simpler model than GTR only makes sense with respect to the computational cost, i.e. it is less expensive to compute. Programs such as `Modeltest` propose the usage of a simpler model for a specific alignment if the likelihood of a fixed topology under that simpler model is not significantly worse than that obtained by GTR based on a likelihood ratio test. My experience is that GTR always yields a slightly better likelihood than alternative simpler models. In addition, since RAxML has been designed for the inference of large datasets the danger of over-parameterizing such an analysis is comparatively low. Provided these arguments the design decision was taken to rather implement the most general model efficiently than to provide many inefficient generic implementations of models that are just special cases of GTR.

Finally, the design philosophy of RAxML is based upon the observation that a more thorough topological search has a greater impact on final tree quality than modeling details.

Thus, the efficient implementation of a rapid search mechanisms is considered to be more important than model details.

**Q:** Why has the performance of RAxML mainly been assessed using real-world data?

**A:** *Personal opinion:* Despite the unquestionable need for simulated data and trees to verify and test the performance of current ML algorithms the current methods available for generation of simulated alignments are not very realistic.

For example, only few methods exist that incorporate the generation of gaps in simulated alignments. Since the model according to which the sequences are generated on the true tree is pre-defined we are actually assuming that ML exactly models the true evolutionary process, while in reality we simply don't know how sequences evolved.

The above simplifications lead to *perfect* alignment data without gaps, that evolved exactly according to a pre-defined model and thus exhibits a very strong phylogenetic signal in contrast to real data.

In addition, the given true tree, must not necessarily be the Maximum Likelihood tree. This difference manifests itself in substantially different behaviors of search algorithms on real and simulated data. Typically, search algorithms execute significantly less (factor 5– 10) topological moves on simulated data until convergence as opposed to real data, i.e. the number of successful Nearest Neighbor Interchanges (NNIs) or subtree rearrangements is lower. Moreover, in several cases the likelihood of trees found by RAxML on simulated data was better than that of the true tree.

Another important observation is that program performance can be inverted by simulated data. Thus, a program that yields *good* topological Robinson-Foulds distances on simulated data can in fact perform much worse on real data than a program that does not perform

well on simulated data.

If one is willing to really accept ML as inference criterion on real data one must also be willing to assume that the tree with the best likelihood score is the tree that is closest to the true tree.

My personal conclusion is that there is a strong need to improve simulated data generation and methodology. In addition, the perhaps best way to assess the validity of our tree inference methods consists in an empirical evaluation of new results and insights obtained by real phylogenetic analysis.

This should be based on the prior knowledge of Biologists about the data and the medical and scientific benefits attained by the computation of phylogenies.

**Q:** Why am I getting weird error messages from the MPI version?

**A:** You probably forgot to specify the `-#` or `-N` option in the command-line which must be used for the MPI version to work properly.

**Q:** When using partitioned models, can I link the model parameters of distinct partitions to be estimated jointly, in a similar as way MrBayes does it?

**A:** Currently not, but the implementation of such an option is planned. However, we are still lacking good criteria and methods that tell us how and why to link/unlink certain parameters across certain partitions.

**Q:** How does RAxML handle gaps

**A:** Like most other likelihood-based phylogeny programs it handles them as undetermined characters, that is gaps are treated as `ns`. If this is new to you you may want to read Joe Felsenstein's textbook *Inferring Phylogenies* where he explains why this is a reasonable way to model gaps.

**Q:** Why am I getting long branch lengths on my phylogenomic datasets with missing data

**A:** This is normal and due to the way missing data is modeled, the missing data parts of the sequences about which we don't know become very distant for exactly this reason.