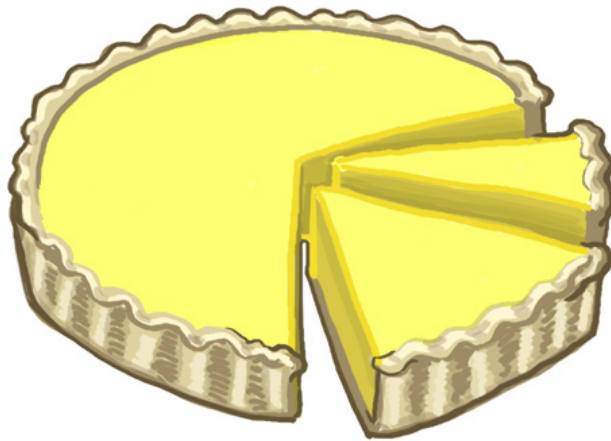


# PartitionFinder2

## Manual

Rob Lanfear, August 2015

Icon © Ainsley Seago. Thanks Ainsley!



Questions, suggestions, problems, bugs? Search or post on the discussion group at:  
<http://groups.google.com/group/partitionfinder>

**Step-by-step tutorial:** <http://www.robertlanfear.com/partitionfinder/tutorial/>

**FAQs:** <http://www.robertlanfear.com/partitionfinder/faq/>

### Citations

PartitionFinder2 incorporates many years of hard work from many people, presented in various different papers. Citations are the only way that we know people are using our methods. So if you use it PartitionFinder2 in your published work please cite the appropriate papers (there will often be more than one). The correct citations are listed in the 'Citations' section, below, and will also included at the bottom of the best\_scheme.txt.

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## Disclaimer

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*This program is free software: you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation, either version 3 of the License, or (at your option) any later version. This program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU General Public License for more details. You should have received a copy of the GNU General Public License along with this program. If not, see <<http://www.gnu.org/licenses/>>. PartitionFinder also includes the PhyML program and the RAxML program, using PartitionFinder implies that you agree with those licences and conditions as well.*

## What PartitionFinder2 is for

PartitionFinder2 is a program for selecting best-fit partitioning schemes and models of molecular evolution for nucleotide and amino acid alignments. The user provides an alignment, and optionally some pre-defined data blocks (e.g. 9 data blocks defining the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> codon positions of 3 protein-coding genes, see Figure 1). The program then finds the best partitioning scheme for this dataset, at the same time as selecting best-fit substitution models for each subset of sites. Here are a few things you can do with the programs:

1. Find the best-fit partitioning scheme for any nucleotide or amino acid dataset
2. Compare any number of user-defined partitioning schemes
3. Find best-fit models of molecular evolution for each subset in any partitioned dataset (much like you might do with ModelTest or ProtTest).

The idea is that finding best-fit partitioning schemes and models of molecular evolution will improve any downstream analyses of your data, like estimating phylogenetic trees or molecular dates. All of those kinds of analyses assume that your model of evolution is correct, and PartitionFinder2 helps make the model as good as it can be.

PartitionFinder2 can be downloaded from [www.robertlanfear.com/partitionfinder](http://www.robertlanfear.com/partitionfinder). It is designed to take the hard work out of comparing partitioning schemes, and to help find a scheme that maximises the fit of the model to your data, without including more parameters than are necessary. PartitionFinder2 implements three information-theoretic measures for comparing models of molecular evolution and partitioning schemes: the Akaike Information Criterion (AIC), the corrected Akaike Information Criterion (AICc), and the Bayesian Information Criterion (BIC). We recommend that you use the AICc, see below.

At the end of a run, you are given output files that tell you the best partitioning scheme, along with the best-fit model of molecular evolution for each subset (sometimes called a 'partition', but that term is a bit misleading) in that scheme. So you can then move straight on to your phylogenetic analyses.

## **Operating systems (Mac, Windows and Linux work)**

Mac OSX and Windows are supported. For Linux, we do our best to help out. Details of what to do to get up and running are provided in the FAQs on the website:

<http://www.robertlanfear.com/partitionfinder/faq/>

## QuickStart – simple use cases

In all of these examples, things in quotes and brackets (i.e. "<>") indicate that you should use full file paths. E.g. "<PartitionFinder.py>" might be /Desktop/partitionfinder/PartitionFinder.py

Also, defining data blocks by gene and codon position refers DNA datasets from protein coding genes. For amino acid datasets, or DNA datasets from non-coding regions, just define data blocks by gene.

### For a small multilocus dataset (e.g. ~10 loci)

For DNA, use a greedy search with PhyML. For amino acids, use a greedy search with RAXML. These options give, respectively, the most models for DNA and amino acids.

1. Define data blocks by gene and codon position
2. In the .cfg file, set the following options:

```
branchlengths = linked;
models = all;
model_selection = aicc;
search=greedy;
```

3. Run PartitionFinder from the commandline as follows for DNA:

```
python "<PartitionFinder.py>" "<InputFoldername>"
```

or as follows for amino acids:

```
python "<PartitionFinderProtein.py>" "<InputFoldername>" --raxml
```

### For a larger dataset (e.g. ~100 loci)

Use a greedy search with RAXML, for both DNA and amino acids. This will usually be fairly quick.

1. Define data blocks by gene and codon position
2. Set the .cfg file options as above.
3. Run PartitionFinder using RAXML, as follows for DNA:

```
python "<PartitionFinder.py>" "<InputFoldername>" --raxml
```

or as follows for amino acids:

```
python "<PartitionFinderProtein.py>" "<InputFoldername>" --raxml
```

If that's too slow for proteins, it's probably because there are a lot of models being compared, so try specifying a smaller list of models in the .cfg file, e.g.:

```
models = LG, LG+G, LG+I+G, LG+I+G+F, LG4X;
```

## For a really big dataset (e.g. ~1000 loci)

For datasets of this size, the greedy algorithm is likely to be too slow. So we can use some faster algorithms that try and guess the best partitioning schemes instead.

1. Define data blocks by gene and codon position
2. Set the .cfg file options as above, except:

```
search=rcluster;
```

3. Run PartitionFinder using RAxML, as follows (for amino acids, change 'PartitionFinder.py' to 'PartitionFinderProtein.py':

```
python "<PartitionFinder.py>" "<InputFoldername>" --raxml
```

This implements the relaxed clustering algorithm described in Lanfear et al 2014 (Selecting optimal partitioning schemes for phylogenomic datasets. *BMC evolutionary biology*, 14(1), 82). If the default behaviour is still too slow on your dataset, reduce rcluster-max from the default of 1000 to e.g. 100 like this (see below for an explanation of what this does):

```
python "<PartitionFinder.py>" "<InputFoldername>" --raxml --rcluster-max 100
```

The rcluster algorithm gives you a lot of control over the balance between speed and accuracy with two parameters: --rcluster-max and --rcluster-percent. Read below for more information on these.

## When you can't define meaningful data blocks

Some datasets just don't lend themselves to defining useful data blocks, like UCE datasets. In this case, we made an algorithm that does its best to group together similar sites, without you having to decide beforehand what those groupings should be. In our experience, this is very quick, even on very very large datasets.

1. Define a single data block with all of your sites.
2. Set the .cfg file options as above, except:

```
search=kmeans;
```

3. Run PartitionFinder using PhyML or RAxML (depending on what models you are interested in, and the size of your dataset), e.g. with RAxML:

```
python "<PartitionFinder.py>" "<InputFoldername>" --raxml
```

This implements the kmeans partitioning algorithm described in Frandsen et al 2015 (Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. *BMC Evolutionary Biology*, 15(1), 13), with one important difference – we use the entropy (instead of TIGER rates) to group similar sites together. This is faster and gives better results, and because of that we no longer support TIGER rates.

## To compare all possible models of evolution

PartitionFinder2 implements lots more models of evolution than PF1. Most of the above examples use `models=all` which implements all of the models most people are interested in most of the time. However, there are some models that are not included in `models=all`, these are mostly models in which state frequencies (e.g. frequencies of A, C, T, and G) are estimated with maximum likelihood instead of just by counting frequencies from the data. These kinds of models are usually just a tiny bit better in terms of their fit to the data, but they can take a lot longer to optimise because you are optimising additional parameters (a lot of them in the case of protein datasets). If you really want a list of models with all possible models (though I wouldn't recommend it, see below for more information) use this setting in the `.cfg` file:

```
models = allx;
```

## Overview

Partitioning involves splitting sites in your alignment into sets that have evolved under similar models. For example, if you have a dataset of 3 protein-coding genes you might suspect that each of the three genes has been evolving differently – perhaps they come from different chromosomes, or have experienced different evolutionary constraints. Furthermore, you might think that each codon position within each gene has been evolving differently – different codon positions tend to evolve at different rates, and experience different substitution processes thanks to the triplet structure of the genetic code. Because of this, you might split your data into 9 sets of sites (we call those data blocks) for this alignment – one for each codon position in each gene. But is this too many different sets? Perhaps it would be better to join together the 1<sup>st</sup> and 2<sup>nd</sup> codon sites of each gene, so defining 6 sets of sites. Or perhaps it would be better to forget the divisions between genes, and define only 2 sets of sites – 1<sup>st</sup> and 2<sup>nd</sup> codon sites versus 3<sup>rd</sup> codon sites. The trouble is that if you start with 9 possible sets of sites, there are a lot of different possible partitioning schemes you might consider, 21147 in fact. This creates a problem – how do we find the best scheme from that many schemes?

Even worse, what if you have a dataset (like one made up of UCE's, for example) that doesn't have convenient features like codon positions? How do you partition that?

PartitionFinder2 (PF2) solves these problems in one of two ways. If you have pre-defined data blocks, PF2 can quickly and efficiently search for the best scheme from the set of all possible schemes, by trying lots of combinations of data blocks (this is what the greedy, rcluster, and hcluster algorithms do). In the example above, all you would need to do is define your 9 possible sets of sites (i.e. the largest number of sets of sites you think is sensible to define) as data blocks, and PF2 will do the rest. If you do not have pre-defined data blocks, you can specify a single set of sites and use the K-means algorithm in PF2, which will attempt to find a good partitioning scheme by splitting your alignment into sets of sites which evolve in similar ways.

At the end of a run you are told the best partitioning scheme that PF2 could find (in the `best_scheme.txt` file), and also which model of molecular evolution you should use for each subset of sites in that scheme (i.e., you don't have to use ModelTest or ProtTest or similar programs on your partitioned dataset, PartitionFinder does all of this model selection for you at the same time as finding a partitioning scheme). You can then go straight on to performing your phylogenetic analysis, without any additional model-testing or comparisons of partitioning schemes.

PF2 is pretty flexible, and should be able to accommodate most of the popular kinds of partitioned analyses people like to do. This includes options for how to treat branch lengths between subsets, which models of molecular evolution to consider, and many other things. This manual describes in detail all of these steps.



## Running PartitionFinder2 on a Mac

### 1. Install python using Anaconda

PartitionFinder needs Python 2.7.10 or higher and some additional libraries to run. By far the simplest way to set this up is to install the Anaconda Python distribution, which is a simple point-and-click installer which can be downloaded from here:

<http://continuum.io/downloads>

Follow the link for the Python 2.7 graphical installer, then open it and follow the prompts. You need to make sure that you have version 2.3.0 or higher of the Anaconda Python distribution.

### 2. Install PartitionFinder2

1. Download the latest version of PartitionFinder2 from [www.robertlanfear.com/partitionfinder](http://www.robertlanfear.com/partitionfinder)
2. Double-click the .zip file, and it will automatically unzip. You will get a folder called something like 'PartitionFinder2.0.0'
3. Move it to wherever you want to store PartitionFinder2 (e.g. in /Applications)

### 3. Run PartitionFinder2

These instructions describe how to run the 'example/nucleotide' analysis using PartitionFinder. This is a DNA alignment with 9 data blocks. To use partitionfinder with amino acid alignments, just follow these instructions but replace 'PartitionFinder' with 'PartitionFinderProtein' in step 2, and 'example/nucleotide' with 'example/aminoacid'.

1. Open Terminal (on most Macs, this is found in Applications/Utilities)
2. In the Terminal, you need to tell the computer where to find Python, PartitionFinder2, and your input files. The easiest way to do this is as follows:
  - a. Type "python" followed by a space
  - b. Drag and drop the "PartitionFinder.py" file (which is in the PartitionFinder folder you just unzipped) onto the command prompt. The path to 'PartitionFinder.py' will be added automatically.
  - c. Type another space
  - d. Drag and drop the blue 'example/nucleotide' folder (in the PartitionFinder folder) onto the command prompt
3. Hit Enter/Return to run PartitionFinder2

That's it!

More generally, you run PartitionFinder2 by typing a command line that looks like this:

```
python "<PartitionFinder.py>" "<InputFoldername>"
```

Where <PartitionFinder.py> is the full path to the PartitionFinder.py (or PartitionFinderProtein.py) file, and <InputFoldername> is the full path to your input folder, which should contain an alignment and a .cfg file. Note that the input folder can be anywhere on your computer, it doesn't have to be in the PartitionFinder folder like the example file.

Once PartitionFinder2 is running, it will keep you updated about its progress. If it hits a problem, it will (hopefully) provide you with a useful error message that will help you correct that problem. Hopefully, you won't have too many problems and your terminal screen will look something like that shown below.

```

Last login: Wed Jul 13 18:36:05 on ttys005
tarquin:~ Rob$ python /Applications/PartitionFinder/PartitionFinder.py /Users/Rob/Desktop/frogs
INFO | 2011-07-13 18:39:01,492 | You appear to have 16 cpus
INFO | 2011-07-13 18:39:01,494 | Using folder: '/Users/Rob/Desktop/frogs'
INFO | 2011-07-13 18:39:01,494 | Loading configuration at '/Users/Rob/Desktop/frogs/partition_finder.cfg'
INFO | 2011-07-13 18:39:01,509 | Setting 'alignment' to 'frogs.phy'
INFO | 2011-07-13 18:39:01,509 | Setting 'branchlengths' to 'linked'
INFO | 2011-07-13 18:39:01,510 | Setting 'models' to 'all'
INFO | 2011-07-13 18:39:01,510 | Setting 'model_selection' to 'bic'
INFO | 2011-07-13 18:39:01,522 | Setting 'search' to 'all'
INFO | 2011-07-13 18:39:01,523 | Beginning Analysis
WARNING | 2011-07-13 18:39:01,567 | Columns defined in partitions range from 29 to 3328, but these columns in the alignment are missing: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 1577, 1578, 1579, 1580, 1581, 1582, 1583, 1584, 1585, 1586, 1587, 1588, 1589, 1590, 1591, 159
2, 1593, 1594, 1595, 1596, 1597, 1598, 1599, 1600, 1601, 1602, 1603, 1604, 1605, 1606, 1607, 1608, 1609, 1610, 1611, 1612, 1613, 1614, 1615, 1616, 1617, 1618, 1619, 16
20, 1621, 1622, 1623, 1624, 1625, 1626, 1627, 1628, 1629, 1630, 1631, 1632, 1633, 1634, 1635, 1636, 1637, 1638, 1639, 1640, 1641, 1642, 1643, 1644, 1645, 1646, 1647, 2
333, 2334
INFO | 2011-07-13 18:39:01,568 | Making BioNJ tree for /Users/Rob/Desktop/frogs/analysis/start_tree/filtered_source.phy
INFO | 2011-07-13 18:39:02,155 | Estimating initial branch lengths on BioNJ tree
INFO | 2011-07-13 18:41:30,134 | Initial tree and branchlength estimation finished
INFO | 2011-07-13 18:41:30,134 | BioNJ tree with GTR+I+G brensi is stored here: /Users/Rob/Desktop/frogs/analysis/start_tree/filtered_source.phy_phym1_tree.txt
INFO | 2011-07-13 18:41:30,135 | Analysing all possible schemes for 9 starting partitions
INFO | 2011-07-13 18:41:30,135 | This will result in 21147 schemes being created
INFO | 2011-07-13 18:41:30,135 | PartitionFinder will have to analyse 511 subsets to complete this analysis
INFO | 2011-07-13 18:41:30,135 | Generating all possible schemes for the partitions...
INFO | 2011-07-13 18:41:31,575 | Analysing scheme 1/21147
INFO | 2011-07-13 18:41:31,575 | Analysing subset 1/511: 0.28% done
INFO | 2011-07-13 18:43:13,098 | Analysing scheme 2/21147
INFO | 2011-07-13 18:43:13,099 | Analysing subset 2/511: 0.39% done

```

## Running PartitionFinder on Windows

### 1. Install python using Anaconda

PartitionFinder needs Python 2.7.10 or higher and some additional libraries to run. By far the simplest way to set this up is to install the Anaconda Python distribution, which is a simple point-and-click installer which can be downloaded from here:

<http://continuum.io/downloads>

Follow the link for the Python 2.7 graphical installer, then open it and follow the prompts. You need to make sure that you have version 2.3.0 or higher of the Anaconda Python distribution.

### 2. Install PartitionFinder2

1. Download the latest version of PartitionFinder2 from [www.robertlanfear.com/partitionfinder](http://www.robertlanfear.com/partitionfinder)
2. Double-click the .zip file, and it will automatically unzip. You will get a folder called something like 'PartitionFinder2.0.0'
3. Move it to wherever you want to store the PartitionFinder2

### 3. Run PartitionFinder2

These instructions describe how to run the 'example/nucleotide' analysis using PartitionFinder. This is a DNA alignment. To use partitionfinder with amino acid alignments, just follow these instructions but replace 'PartitionFinder' with 'PartitionFinderProtein' in step 2, and 'example/nucleotide' with 'example/aminoacid'.

1. Open a command prompt. To do this, click on the Start Menu, then navigate to the command prompt like this: "All Programs" -> "Accessories" -> "Command Prompt". On **Windows 7** you can just type "cmd" into the search box area, and you'll see it.
2. In the command prompt, you need to tell the computer where to find Python, PartitionFinder2, and your input files. The easiest way to do this is as follows:
  - a. Type "python" followed by a space
  - b. Drag and drop the "PartitionFinder.py" file (which is in the PartitionFinder folder you just unzipped) onto the command prompt. The path to 'PartitionFinder.py' will be added automatically.
  - c. Type another space
  - d. Drag and drop the blue 'example/nucleotide' (in the PartitionFinder folder) onto the command prompt
3. Hit Enter/Return to run PartitionFinder

That's it!

More generally, you run PartitionFinder2 by typing a command line that looks like this:

```
python "<PartitionFinder.py>" "<InputFoldernam>"
```

Where <PartitionFinder.py> is the full path to the PartitionFinder.py (or PartitionFinderProtein.py) file, and <InputFoldernam> is the full path to your input folder, which should contain an alignment and a .cfg file. Note that the input folder can

be anywhere on your computer, it doesn't have to be in the PartitionFinder folder like the example file.

Once PartitionFinder is running, it will keep you updated about its progress. If it hits a problem, it will (hopefully) provide you with a useful error message that will help you correct that problem. Hopefully, you won't have too many problems and your terminal screen will look something like that shown below.

```

C:\WINDOWS\system32\cmd.exe - python "c:\Program Files\partitionfinder\PartitionFinder.py" "c:\Documents and Settings\brett\My Documents\frogs"
C:\Documents and Settings\brett>python "c:\Program Files\partitionfinder\PartitionFinder.py" "c:\Documents and Settings\brett\My Documents\frogs"
INFO | 2012-03-30 12:23:25,960 | You appear to have 1 cpus
INFO | 2012-03-30 12:23:25,970 | ----- PartitionFinder v0.9 -----
INFO | 2012-03-30 12:23:25,970 | Setting working folder to: 'c:\Documents and Settings\brett\My Documents\frogs'
INFO | 2012-03-30 12:23:25,970 | ----- BEGINNING NEW RUN -----
INFO | 2012-03-30 12:23:25,970 | Loading configuration at '.\partition_finder.cfg'
INFO | 2012-03-30 12:23:25,990 | Setting 'alignment' to 'test.phy'
INFO | 2012-03-30 12:23:25,990 | Setting 'branchlengths' to 'linked'
INFO | 2012-03-30 12:23:25,990 | Setting 'models' to 'all'
INFO | 2012-03-30 12:23:25,990 | Setting datatype to 'DNA'
INFO | 2012-03-30 12:23:25,990 | Setting 'model_selection' to 'bic'
INFO | 2012-03-30 12:23:26,000 | Setting 'search' to 'greedy'
INFO | 2012-03-30 12:23:26,000 | Beginning Analysis
INFO | 2012-03-30 12:23:26,000 | Removing Schemes in '.\analysis\schemes' (they will be recalculated from existing subset data)
INFO | 2012-03-30 12:23:26,020 | Making BioNJ tree for '.\analysis\start_tree\filtered_source.phy'
INFO | 2012-03-30 12:23:26,040 | Found program phym1.exe at 'c:\Program Files\partitionfinder\programs\phym1.exe'
INFO | 2012-03-30 12:23:26,040 | Estimating GTR+I+G branch lengths on tree
INFO | 2012-03-30 12:23:28,375 | Branchlength estimation finished
INFO | 2012-03-30 12:23:28,375 | Starting tree with branch lengths is here: '.\analysis\start_tree\filtered_source.phy_phym1_tree.txt'
INFO | 2012-03-30 12:23:28,375 | Performing greedy analysis
INFO | 2012-03-30 12:23:28,375 | This will result in a maximum of 121 schemes being created
INFO | 2012-03-30 12:23:28,375 | PartitionFinder will have to analyse a maximum of 73 subsets of sites to complete this analysis
INFO | 2012-03-30 12:23:28,375 | Analysing starting scheme (scheme 1)
INFO | 2012-03-30 12:23:28,375 | Analysing scheme 1/121
INFO | 2012-03-30 12:23:28,375 | Analysing subset 1/73: 1.37% done
INFO | 2012-03-30 12:23:37,687 | Analysing subset 2/73: 2.74% done

```

## Input Files

PartitionFinder2 needs two input files, a Phylip alignment and a configuration file. The best way to get a feel for how this works is to have a look in the examples we've provided in the 'example' folder. There is also an online tutorial at [www.robertlanfear.com/partitionfinder/tutorial](http://www.robertlanfear.com/partitionfinder/tutorial). You can copy and paste these folders onto your desktop (or anywhere) and try running them by following the instructions above. Playing around with the options in the .cfg files give you a good idea of what's possible.

In the rest of this section, we describe in detail exactly what the two input files should look like, and what they do.

### Alignment File in phylip format

**The phylip format:** Your alignment needs to be in Phylip format. We use the same version of Phylip format that PhyML uses, which is described in detail here <http://www.atgc-montpellier.fr/phyml/usersguide.php?type=phylip>. In brief, this format should contain a line at the top with the number of sequences, followed by the number of sites in the alignment. After that, there should be one sequence on each line, where a sequence contains a name, followed by some whitespace (either spaces or tabs) and the sequence. Names can be up to 100 characters long. There should be nothing else on the line other than the name and the sequence – watch out if you use MacClade, which adds some extra things to the end of each line.

**Converting other formats to phylip:** If you have an alignment in some other format and want to convert it into phylip format, the best (free!) tool to use is Geneious. Other alignment editors tend to cut the names short in phylip files (the original definition had a 10 character limit on names), but Geneious doesn't. If you don't have Geneious, it's free and you can download it from <http://www.geneious.com/>. Once you have Geneious, follow these steps to convert your alignment file to phylip:

1. Open up your alignment file in Geneious, and highlight it
2. Go the 'File' menu and click 'Export', then 'Selected documents...'
3. Scroll down the list of options and choose 'Phylip (\*.phy)', and click 'OK'.
4. Now a box of options will come up, choose 'Export full length'.
5. Save the phylip alignment file in the same folder as your .cfg file for PartitionFinder.

**One more thing:** Often, you'll have sites in your alignment that you don't intend to use in your final analysis, or perhaps you have an alignment of mixed data types like DNA, protein, and morphological data. In PartitionFinder2 this is OK. You don't need to make separate alignments of each datatype. You can just ignore the sites you're not interested in by setting the '[data\_blocks]' option to only focus on the data you want PartitionFinder2 to analyse, more instructions below.

## Configuration File

PartitionFinder2 gets most of its information on the analysis you want to do from a configuration file. This file should always be called “partition\_finder.cfg”. The easiest thing to do is to base your own .cfg on one of the examples provided in the “example” folder. An exhaustive list of everything in that file follows. **Note that all lines in the .cfg file except comments and lines with square brackets have to end with semi-colons.**

In the configuration file, white spaces, blank lines and lines beginning with a “#” (comments) don’t matter. You can add or remove these as you wish. All the other lines do matter, and they must all stay in the file in the order they are in below. There is one exception – the user\_tree\_topology option (see below).

The basic configuration file looks like this:

```
# ALIGNMENT FILE #
alignment = test.phy;

# BRANCHLENGTHS #
branchlengths = linked;

# MODELS OF EVOLUTION #
models = all;
model_selection = aicc;

# DATA BLOCKS #
[data_blocks]
Gene1_pos1 = 1-789\3;
Gene1_pos2 = 2-789\3;
Gene1_pos3 = 3-789\3;

# SCHEMES #
[schemes]
search = greedy;

# user schemes (see manual)
```

The options in the file are described below. Where an option has a limited set of possible commands, they are listed on the same line as the option, separated by vertical bars like this “|”.

### alignment

The name of your sequence alignment. This file should be in the same folder as the .cfg file, and must be in the correct phylip format (see above).

### branchlengths: linked | unlinked

This determines how branch lengths will be estimated during your analysis. How you set this will depend to some extent on which program you intend to use for your final phylogenetic analysis. Almost all phylogeny programs support linked branchlengths, but only some support unlinked branchlengths (e.g. MrBayes, BEAST, and RaxML).

**branchlengths = linked;** only one underlying set of branch lengths is estimated. Each subset has its own scaling parameter (i.e. its own subset-specific rate). This allows subsets to evolve at different rates, but doesn’t change the length of any one branch relative to any other. The total number of branch length parameters here is quite small. If there are N species in your dataset, then there are 2N-3 branch lengths in your tree,

and each subset after the first one adds an extra scaling parameter. For instance, if you had a scheme with 10 subsets and a dataset with 50 species, you would have 106 branch length parameters.

**branchlengths = unlinked;** each subset has its own independent set of branch lengths. In this case, branch lengths are estimated independently for each subset, so each subset has its own set of  $2N-3$  branch length parameters. With this setting, the number of branch length parameters can be quite large ( $2NS - 3S$ , where  $S$  is the number of species in your alignment). So, a scheme with 10 subsets and a dataset with 50 species would have 970 branch length parameters.

**models all | allx | beast | mrbayes | gamma | gammai | <list>**

Most people will just want to set models to 'all'. Below is a very long description of what all of the models are, and what the other options do. This is written mostly for those who are confused about, or new to, models of molecular evolution in phylogenetics. If you just want to know what the options do, skip to the description of the options themselves.

The 'models' option sets which models of molecular evolution to consider during model selection. The models that are available for any particular analysis will depend on two things: your data type (i.e. nucleotides or amino acids) and the phylogeny program you are using (i.e. PhyML, which is the default, or RAxML which you can specify using the -raxml command line option). There are many models you can specify, and although lists are provided below, perhaps the best way to understand what is available is to look at the models.csv file, which is located in the /partfinder folder. This lists all available models, as well as details of the models themselves, and whether each model is implemented in PhyML or RAxML.

Whatever you set for 'models', PF2 will print out the list of models it will use at the start of the analysis. You should check this and make sure it is what you want. For every subset of sites that PF2 looks at, it will fit every single model in your list of models, and pick the best one according to your chosen information theoretic score (AIC, AICc, or BIC). Your results therefore tell you not only the best partitioning scheme, but also which model of molecular evolution is most appropriate for each subset in that scheme. This means that you don't need to do any further model selection after PartitionFinder2 is done.

### A very short primer on models in phylogenetics

Over the years I've had a lot of questions about models in phylogenetics. So here's a primer if you're interested. There are two important things to understand: the differences between models, and how the models are named.

The difference between all of the models is in which parameters are fixed a-priori vs estimated from the data (we call these free parameters), and how the free parameters are estimated. In general, all models (both amino acid and nucleotide models) have three components: the frequencies of nucleotides or amino acids (e.g. the proportion of A, C, T, and G in your data), the relative rates at which the different nucleotides or amino acids are replaced by each other (e.g. the rate at which A replaces G in your data), and the distribution of rates of evolution among sites in your dataset. There are many many possible models of evolution for nucleotide and amino acid alignments. All of the models implemented in PF (plus a few that are not possible) are listed in the models.csv

file, which is in the partitionfinder folder, under /partfinder/models.csv. As you read the next paragraph, it might help to open up this file and take a look at the models to familiarise yourself with what's possible.

**Frequencies of nucleotides or amino acids** can be fixed in advance, estimated from the data by simply counting the proportions observed in your alignment, or estimated using maximum likelihood. In the models.csv file, this is described in the base\_frequencies column. There are four possible types of base frequency: **'equal'** is when all frequencies are set to equal (e.g. proportions of 0.25 for A, C, T, and G in the JC model) and is only implemented for nucleotide models in PF2; **'model'** is when frequencies are determined a-priori from some other dataset, which is common for amino acid models (e.g. the JTT model); **'empirical'** is when the frequencies are determined by just counting the proportions of nucleotides or amino acids in your data; **'ML'** is when the frequencies are determined using maximum likelihood. The last two options tend to give very very similar answers (ML is guaranteed to be better, but the difference is usually so marginal that it makes no difference to the phylogeny) but 'empirical' is much faster, and so most people never use ML base frequencies. One more important thing – thanks to historical naming conventions, the way that these models are named differs for nucleotide and amino acid models. For amino acid models, it's easy, we'll use the LG model as an example. If you specify 'LG', then amino acid frequencies will be estimated from the model (the LG model comes with some best-guess amino acid frequencies estimated from a large collection of datasets). If you specify 'LG+F' then the amino acid frequencies are estimated empirically from your data. If you specify 'LG+X' then the amino acid frequencies are estimated with maximum likelihood from your data. For nucleotide models, it's different. Take a look at rows 2-22 of the models.csv file. First, no nucleotide models have a priori base frequencies included in the model (i.e. the 'model' option), so your only options are to have them all equal, estimate them empirically, or estimate them with maximum likelihood. Historically, the first two of these options were given completely different model names, rather than using the '+F' notation that we use for amino acid models. Let's take the JC model as an example. If you specify 'JC', that assumes all base frequencies are equal. But there is no JC+F model, it does exist, but it's just called the F81 model. This is the reason why there are no '+F' models in the list of possible models for DNA sequence alignments. Rows 2-22 spell this out: each model with 'equal' base frequencies is paired with a model with empirical base frequencies. For example, the model in row 1 (JC) matches the model in row 8 (F81), row 2 (K80) matches row 9 (HKY), and so on. To complete the picture, we can specify that we want our base frequencies for nucleotide models estimated with ML (few people use these models though). To do that, we just specify the name of the empirical model with a '+X', e.g. 'F81+X'. Note that most of these kinds of nucleotide models are only implemented in PhyML, not RAxML.

**Relative rates of substitution (this is usually called the rate matrix)** can be fixed in advance, or estimated from the data. For DNA models, there are really just 6 types of relative rate matrix. JC and F81 have all rates equal (no free parameters), other models set certain parameters to be equal (1-4 free parameters, depending on the model), and at the other end of the scale the SYM and GTR models allow all six reversible rate parameters to differ (5 free parameters, since one is set arbitrarily to 1.0). In the amino acid models, almost all models use pre-estimated rate matrices. The matrices tend not to be estimated from the data, because most datasets do not contain enough information to estimate so many parameters (189 free parameters in an amino acid replacement matrix). These matrices have names like WAG, LG, JTT, etc. There is one exception – one can estimate a GTR model for amino acid data – this has a LOT of parameters (189



free parameters), but for very big datasets it might be appropriate, and so might be worth including in your list of possible models (note that only this model is only supported when you are using the '--raxml' cmdline option (see below).

**The distribution of rates across sites** is modelled in one of five ways. The first four work the same for most nucleotide and amino acid models, so I'll just use the HKY model as an example. First, one can assume that all sites evolve at the same rate (not a very good assumption in most cases), in which case the model would be just 'HKY'. Second, we could assume that some sites never change, and so model a proportion of invariant sites. In this case, we estimate one free parameter (the proportion), and the model would be 'HKY+I'. Third, we could assume that the sites evolve according to some distribution of rates, which we can model with a gamma distribution (we use this because it can take lots of different shapes). In this case, we estimate 1 free parameter, which determines the shape of the distribution, and the model would be 'HKY+G'. Fourth and last, we could combine the proportion of invariant sites and the gamma distribution, in this case we estimate two free parameters and the model would be 'HKY+I+G'. Finally, there is a new class of rate distribution models which are often called 'free rate' models. In these models, one specifies some number of categories of rate, and instead of modelling them as coming from a distribution (like the gamma distribution) one estimates the rate of each category. These models have been implemented for nucleotides and amino acids, but because of various technical limitations the only model like this that's implemented in PartitionFinder is the LG4X model (which works only with the '--raxml' cmdline option, see below). The LG4X model has four rate categories, and it also has four separate sets of amino acid frequencies. It's a very neat model, and often fits the data better than other amino acid models. If you use it, you should read and reference this paper: <http://mbe.oxfordjournals.org/content/29/10/2921>.

**models = all;** Chooses the largest sensible set of models possible, depending on the analysis being conducted (i.e. whether you are using DNA or amino acid alignments, and whether you are using PhyML or RAxML). Note that this list does NOT include models in which the base or amino acid frequencies are estimated with maximum likelihood, because these models are very rarely used, and in practice take a lot longer to estimate for extremely marginal gains in performance.

If you are analysing **DNA sequences** with the **default options** in PF2, then **models = all** will compare 56 models of nucleotide evolution for each subset. These 56 models comprise the 14 most commonly used models of molecular evolution (JC, K80, TrNef, K81, TVMef, TIMef, SYM, F81, HKY, TrN, K81uf, TVM, TIM, and GTR), each of which comes in four flavours: on its own, with invariant sites (+I), with gamma distributed rates across sites (+G), or with both gamma distributed rates and invariant sites (+I+G).

If you are analysing **DNA sequences** with the **--raxml cmdline option**, then **models = all** will compare the 3 models of nucleotide evolution available in RAxML: GTR, GTR+G, GTR+I+G.

If you are analysing **amino acid sequences** with the **default options** in PF2, then **models = all** will compare 112 models of evolution, comprising 14 amino acid rate matrices (LG, WAG, MTREV, DAYHOFF, DCMUT, JTT, VT, BLOSUM62, CPREV, RTREV, MTMAM, MTART, HIVB, HIVW), each of which comes in 8 flavours: with or without empirical amino acid frequencies (+F), combined with the four types of distributions of rates across sites described above (i.e. all rates equal, +G, +I, and +I+G). E.g. the 8

flavours of the LG model are: LG, LG+F, LG+G, LG+G+F, LG+I, LG+I+F, LG+I+G, LG+I+G+F.

If you are analysing **amino acid** sequences with the **--raxml commandline option**, then models = all will compare 128 models of evolution. Most of these work as above – there are 21 models (LG, WAG, MTREV, DAYHOFF, DCMUT, JTT, VT, BLOSUM62, CPREV, RTREV, MTMAM, MTART, HIVB, HIVW, MTZOA, PMB, JTTDCMUT, FLU, STMTREV, DUMMY, DUMMY2), each of which comes in 6 flavours: with or without empirical amino acid frequencies (+F), combined with 3 types of rate distribution across sites (all rates equal, +G, and +I+G). Note that RAxML does not estimate models with just +I, and that's why there are 6 not 8 flavours of each model when using the --raxml option. The final two models are the LG4X model (described above), and the LG4M+G model. There is only one flavor of each of these models implemented in RAxML, if you're interested to know why, there is a great description in the RAxML manual and the paper that describes the original models <http://mbe.oxfordjournals.org/content/29/10/2921>.

**models = allx;** this is a list of models that includes every single model listed in the 'models = all' description above, but also includes models in which base or amino acid frequencies are estimated using maximum likelihood (+X) rather than empirically (+F) where possible.

For DNA sequences: with the **default options** this list comprises 84 models (all the 56 models from the 'models = all;' list, plus the following base models in with +X, +I+X, +G+X and +I+G+X: F81, HKY, TrN, K81uf, TVM, TIM, and GTR); with the **--raxml commandline option** it comprises 6 models (GTR, GTR+X, GTR+G, GTR+G+X, GTR+I+G, GTR+I+G+X).

For **amino acid** sequences: with the **default options** this list comprises the same 112 models as in 'models = all;' because PhyML does not allow for amino acid frequencies to be calculated with maximum likelihood (i.e. +X models are not implemented in PhyML, so are not possible to estimate with the default options of PF2); with the **--raxml commandline option** it comprises 195 models. These 195 models include all the models from the 'models = all;' list, plus variants of the 21 rate matrices in RAxML combined with amino acid frequencies estimated from the data (e.g. LG+X, LG+G+X, LG+I+G+X) as well as all 6 variants of the protein GTR model, in which the rate matrix is itself estimated from the data. You should be careful before you use the GTR model, since it can take a very long time to run and is only likely to provide good information theoretic scores on exceptionally large datasets.

**models = mrbayes; models = beast;** tells PartitionFinder to use only the nucleotide models available in MrBayes3.2, or BEAST2 respectively. This can be useful if you intend to use one of these programs for your phylogenetic analysis, as it restricts the models that are compared to only those that are implemented in the particular programs. This is not only the most appropriate thing to do for a Bayesian analysis though, so be careful (see above).

**models = gamma; models = gammai;** tells PartitionFinder to use only a subset of models from the 'models = all;' list. 'gamma' uses only those models from 'models = all;' that have '+G'. 'gammai' uses only those models from models = all that have '+I+G' but not '+G' for their rate distribution.

**models = <list>;** This can be any list of models appropriate for the data type. If you are not sure which models are possible, you can either study the models.csv file (in the /partfinder folder) or just try out a list. If you include a model that won't work, PF2 will tell you which models didn't work an error message before your analysis gets underway. Each model in the list should be separated by a comma. For example, if I was only interested in a few nucleotide models in PartitionFinder, I might do this:

```
models = JC, JC+G, HKY, HKY+G, GTR, GTR+G;
```

Or, for protein models in PartitionFinderProtein I might do this:

```
models = LG, LG+G, LG+G+F, WAG, WAG+G, WAG+G+F;
```

Note that in this list you can specify either nucleotide models, or amino acid models, but not a mixture of both. If you have a mixed dataset (i.e. some data blocks are amino acid, some are nucleotides, you have to run PartitionFinder on the nucleotide data, then PartitionFinderProtein on the amino acid data.

A complete list of all models implemented in PF2 is provided in the models.csv file. This list also includes notes on some of the models, including many models that look like they could be implemented but are not for various reasons.

### **model\_selection: AIC | AICc | BIC**

Sets which metric to use for model selection. It also defines the metric for comparing partitioning schemes if you use search=greedy (see below).

The AIC, AICc, and BIC are similar in spirit – they all reward models that fit the data better, but penalise models that have more parameters. The idea is include parameters that help the model fit the data more than some specified amount, but to avoid including too many parameters (overparameterisation). The BIC penalises extra parameters the most, followed by the AICc, and then the AIC. Which model\_selection approach you use will depend on your preference. There are lots of papers comparing the merits of the different metrics, and my current favourite is the AICc. In general, you should never use the AIC since the AICc is always preferable. However, it's included in PartitionFinder mostly for historical reasons.

### **[data\_blocks]**

On the lines following this statement you define the starting subsets for your analysis (we call these data blocks). Each data block has a name, followed by an "=" and then a description. The description is built up as in most Nexus formats, and tells PartitionFinder which sites of your original alignment correspond to each data block. The best way to understand this it to look at a couple of examples.

Imagine a DNA sequence alignment with 1000bp of protein-coding DNA, followed by 1000bp of intron DNA. Let's imagine that some of the intron was unalignable too, so we don't want that included in our analysis, but we don't want to cut it out of our alignment file. Your data block definitions might look like this:

```
Gene1_codon1 = 1-1000\3;           ❶
Gene1_codon2 = 2-1000\3;           ❷
Gene1_codon3 = 3-1000\3;           ❸
```

```
intron          = 1001-1256 1675-2000;          ④
```

①–③ are typical of how you might separate out codon positions for a protein coding gene. The numbers either side of the dash define the first and last sites in the data block, and the number after the backslash defines the spacing of the sites. Every third site will define a codon position, as long as your alignment stays in the same reading frame throughout that gene.

④ shows how you can include ranges of sites without backslashes, and demonstrates that you can combine more than one range of sites in a single data block. Here, we excluded sites 1257-1674 because they were unalignable.

The total list of data blocks does not have to include all the sites in your original alignment. For instance, you might exclude some sites you're not interested in, or that were unalignable. You'll get a warning from PartitionFinder if all of the sites in the original alignment are not included in the data blocks you've defined. Also, note that data blocks cannot be overlapping. That is, each site in the original alignment can only be included in a single data block.

To help with cutting and pasting from Nexus files (like those used by MrBayes) you can leave "charset" at the beginning of each line. So, the following would be treated exactly the same as the example above:

```
charset Gene1_codon1 = 1-1000\3;
charset Gene1_codon2 = 2-1000\3;
charset Gene1_codon3 = 3-1000\3;
charset intron       = 1001-1256 1675-2000;
```

Note that if you are using the kmeans algorithm (i.e. search = 'kmeans') you should define a single datablock that incorporates all of the sites you want to use in your downstream analyses.

## [ schemes ]

On the lines following this statement, you define how you want to look for good partitioning schemes, and any user schemes you want to define. You only need to define user schemes if you choose search=user.

**search: all | greedy | rcluster | hcluster | kmeans | user**

This option defines which partitioning schemes PartitionFinder will analyse, and how thorough the search will be. In general 'all' is only practical for analyses that start with 12 or fewer data blocks defined (see below). A rough guide is to use 'all' for very small datasets, 'greedy' for datasets of ~10 loci, and 'rcluster' for datasets of 100's of loci. We do not recommend you use 'hcluster', but rather that if 'rcluster' is too slow, you make it quicker using the '--rcluster-max' commandline argument (see below). 'kmeans' can be useful if you do not have data for which you can define useful data blocks.

**search = all;** Tells PartitionFinder to analyse all possible partitioning schemes. That is, every scheme that includes all of your data blocks in any combination at all. Whether you can analyse all schemes will depend on how much time you have, and on what is computationally possible. **If you have any more than 12 data blocks to start with you should not choose 'all'.** This is because the number of possible schemes can be extremely large. For instance, with 13 data blocks there are almost 28 million

possible schemes, and for 16 data blocks the number of possible schemes is over 10 billion. It's just not possible to analyse that many schemes exhaustively. For 12 data blocks, the number of possible schemes is about 4 million, so it might be possible to analyse all schemes if you have time to wait, and a fast computer with lots of processors.

**search = greedy;** Tells PartitionFinder to use a greedy algorithm to search for a good partitioning scheme. This is a lot quicker than using `search=all`, and will often give you the same answer. However, it is not 100% guaranteed to give you the best partitioning scheme. If you use this algorithm, please cite the 2012 PartitionFinder paper (see citations, below, or here: <http://mbe.oxfordjournals.org/content/29/6/1695>) in addition to the PF2 paper.

**search = rcluster;** Tells PartitionFinder to use a relaxed hierarchical clustering algorithm to search for a good partitioning scheme. This option only works with the `--raxml` commandline option (see above). It works by measuring the similarity of different subsets, then looking at schemes that combine the most similar subsets. It usually performs worse than the greedy search option, and always performs better than the `hcluster` option. You can control this algorithm using the `'--rcluster-max'`, `'--rcluster-percent'` and `'--weights'` command line options (see below). The `rcluster` algorithm is a very efficient way to search, and can be used even on large phylogenomic datasets with 1000's of loci. It's designed for use with datasets that are too large to analyse with the greedy algorithm. If you use this algorithm, please cite the 2014 paper in which it is described (see Citations, or here: <http://www.biomedcentral.com/1471-2148/14/82>).

**search = hcluster;** Tells PartitionFinder to use a strict hierarchical clustering algorithm to search for a good partitioning scheme. This option only works with the `--raxml` commandline option. This algorithm is faster than the `rcluster` algorithm, but performs worse, often a great deal worse. In general, I do not recommend using this algorithm under any circumstances. It is better to use the `'rcluster'` algorithm with `'--rcluster-max'` set to some very low number (e.g. 10, see below) instead. The `hcluster` algorithm is almost the same as using the `rcluster` algorithm with `'--rcluster-max'` set to 1. You can control this algorithm using the `'--weights'` command line options (see below). If you use this algorithm, please cite the 2014 paper in which it is described (see Citations, or here: <http://www.biomedcentral.com/1471-2148/14/82>).

**search = kmeans;** Tells PartitionFinder to use a kmeans algorithm to group together sites that have been evolving at similar rates. This is very different to the other search algorithms. All of the other algorithms start with data blocks that you define, and then *group together* similar data blocks. The kmeans algorithm starts with a single data block (which should be all of the sites in your alignment that you want to use), and then *splits up* those sites into ones with similar evolutionary rates. The kmeans algorithm will be particularly useful for datasets in which it is not possible to define useful datablocks. E.g. UCE datasets often comprise bits of intergenic DNA, and we don't know much about their biology. It is therefore difficult to divide a UCE up into sensible datablocks (unlike e.g. a protein coding gene, where we know that the different codon positions are likely to evolve at different rates a-priori, so we can define sensible data blocks). Here's an example of a single data block definition, that could be used as the starting point for the kmeans algorithm:

```
allsites = 1-1000 2500-3000 3017-5196;
```

Before using this algorithm, we recommend that you read the paper that describes it, and as always, please cite the paper if you use the kmeans algorithm in your published work (see Citations, or here: <http://www.biomedcentral.com/1471-2148/15/13>).

**search = user;** Use this option to compare partitioning schemes that you define by hand. User-defined schemes are listed, one-per-line, on the lines following “search=user”. A scheme is defined by a name, followed by an “=” and then a definition. To define a scheme, simply use parentheses to join together data blocks that you would like to combine. Within parentheses, each data block is separated by a comma. Between parentheses, there is no comma. All user schemes must contain all of the data blocks defined in [data\_blocks].

Here’s an example. If I’m working on my one protein-coding gene plus intron alignment above, I might want to try the following schemes: (i) all data blocks analysed together; (ii) intron analysed separately from protein coding gene; (iii) intron separate, 1<sup>st</sup> and 2<sup>nd</sup> codon positions analysed separately from 3<sup>rd</sup> codon positions; (iv) all data blocks analysed separately. I could do this as follows, with one scheme on each line:

```
together      = (Gene1_codon1, Gene1_codon2, Gene1_codon3, intron);
intron_123    = (Gene1_codon1, Gene1_codon2, Gene1_codon3) (intron);
intron_12_3   = (Gene1_codon1, Gene1_codon2) (Gene1_codon3) (intron);
separate      = (Gene1_codon1) (Gene1_codon2) (Gene1_codon3) (intron);
```

### user\_tree\_topology

This is an additional option which can be added into the .cfg file after the ‘alignment’ line. It’s used if you’d like to supply PartitionFinder with a fixed topology, rather than relying on the neighbour joining topology that the program estimates by default. This might be useful if you know ahead of time what the true tree is, for instance when doing simulations. To use the option, just add in an extra line to the .cfg file like this:

```
# ALIGNMENT FILE #
alignment = test.phy;
user_tree_topology = tree.phy;
```

Where “tree.phy” is the name of the file containing a newick formatted tree topology (with or without branch lengths). The file name can be anything – it doesn’t have to be ‘tree.phy’. The tree file must be in the same folder as the alignment and the .cfg file. When you use this option, the topology you supply in the tree file will be fixed throughout the analysis. Branch lengths will be re-estimated using a GTR+I+G model on the whole dataset, as in a standard analysis.

If you don’t want to use this option, you can just leave out the user\_tree\_topology line from the .cfg file.

## Output files

All of the output is contained in a folder called “analysis” which appears in the same file as your alignment. There is a lot of output, but in general you are likely to be interested in four things, maybe this order:

### **best\_schemes.txt**

has information on the best partitioning scheme found, and the settings used to find it. This includes a detailed description of the scheme, as well as the model of molecular evolution that was selected for each subset in the scheme. It also contains a description of the each scheme in RAxML and Nexus formats.

### **subsets folder**

is a folder which contains the results of the model selection on each subset of sites that was analysed. These are .txt files, in which each model you included in your analysis is listed, in order of increasing AICc score (i.e. best model is at the top). The default is to save model selection results of only those subsets that made it into the best partitioning scheme. If you want to save the model selection results from all subsets of sites that were analysed, then you can use the --save-phylofiles commandline option (but beware, this results in writing a lot of files – see info on the option, below). The subsets folder also contains a database (data.db) of information on the subsets of sites that were analysed, so that PartitionFinder can re-run analyses without re-calculating lots of results.

### **schemes folder**

is a folder which contains detailed information on the schemes that were analysed during the analyses, each in a separate .txt file that is very like the best\_scheme.txt file. For the greedy and clustering algorithms, this folder contains only the starting scheme and the best scheme that was found at each step of the algorithm. For the kmeans algorithm, it will just contain the start\_scheme.txt file, because we cannot save schemes along the way during the kmeans algorithm (read the paper to find out why).



## Command line options

There are a number of additional commands you can pass to PartitionFinder from the commandline. These can be used to fine-tune your analyses.

### **--all-states**

Only affects the k-means algorithm. Specifically, this limits the k-means algorithm to only produce subsets that contain all possible states. We allow ambiguous DNA states that code for <4 states, and we count these as representing all possible resolutions of the ambiguity. We do not count '?', 'N', or '-' states as representative of anything. This option is designed to try and ensure that the partitioning schemes produced by the k-means algorithm are easy to analyse in downstream software like RAxML.

### **--force-restart**

This will delete all previous workings (by deleting the 'analysis' folder) before restarting a run. The default is not to do this, so PartitionFinder can use results that it has already calculated.

### **--min-subset-size**

Default: 100 for k-means; ignored for rcluster.

Only affects the k-means and rcluster algorithms. This option sets the minimum size of a subset that the k-means or rcluster algorithms will produce. For the k-means algorithm, a subset will not be split if either of the daughter subsets has fewer than the specified number of sites. For the rcluster algorithm, subsets smaller than the specified size are merged with their 'nearest neighbour' subsets before the main analysis begins. The 'nearest neighbour' is defined by the 'weights' argument, see below.

### **-p N, --processors N**

Default – use all available processors.

N is the number of processors you want PartitionFinder to use. This controls the number independent PhyML or RAxML runs that PartitionFinder will run at any one time. The default is for PartitionFinder to use all of the available processors (look for this message at the start of the run, to see how many it found: "You appear to have N cpus"). However, if you don't want it to use all the processors, control with this option. E.g. -p 5 would tell PartitionFinder to use up to 5 processors at once.

### **--quick, -q**

This option will stop PartitionFinder from writing out unnecessary summaries of partitioning schemes during the analysis. Most people will not need to use this option, but if you are running really big analyses, particularly with the greedy algorithm, it can marginally speed things up.

### **--raxml**

This tells PartitionFinder and PartitionFinderProtein to use RAxML rather than PhyML (the default). You might want to do this because RAxML is faster than PhyML, or because it implements the models you are interested in (NB, RAxML implements fewer nucleotide models, but many more amino acid models, than PhyML). Because of the nature of RAxML, we can't guarantee that the RAxML executables we have provided in the 'programs' folder will work on all Windows and Mac machines. So if you use this



option and RAxML doesn't work, you'll need to download and compile RAxML yourself, on your own computer.

### **--rcluster-max N**

Default: --rcluster-max 1000

See below for a description.

### **--rcluster-percent N**

Default: --rcluster-percent 10

These two options control the thoroughness of the relaxed clustering algorithm together. Setting either of them higher will tend to make the search more thorough and slower. Setting them lower will tend to make the search quicker but less thorough. The rcluster algorithm works by finding the rcluster-max most similar pairs of data blocks, OR the top rcluster-percent of similar datablocks, whichever is smaller. It then calculates the information score (e.g. AICc) of all of these data blocks, and keeps the best one. Setting --rcluster-max to 1000 and --rcluster-percent to 10 (i.e. the default values) is usually sufficient to ensure that PF2 will estimate a robust partitioning scheme, even on very large datasets in which there may be millions of possible pairs of data blocks. Please note that it is better to use rcluster with --rcluster-max set to a very small number (e.g. 10) than to use the hcluster algorithm.

Why do we have two parameters to control the rcluster algorithm? What's important is that the smaller value of --rcluster-max and --rcluster-percent changes as the algorithm progresses. Let's imagine that you have rcluster-max at 1000 and rcluster-percent at 10, and that you have a large dataset with a lot of data blocks, for which the optimal partitioning scheme happens to have a small number of subsets. In the early stages of the algorithm, there are A LOT of potential combinations of subsets, so the algorithm will consider the most similar 1000 subsets (because 10% of A LOT will be bigger than 1000, so the rcluster-max cutoff will be working; and remember that similarity is defined by measuring attributes of subsets like their rate of evolution). But as we get towards the end of the algorithm, we will get to a partitioning scheme with just a few subsets, because we've merged most of the subsets. Specifically, let's imagine that the algorithm is currently working on a partitioning scheme with 100 subsets. If there are 100 subsets then there are  $100 \text{ choose } 2 = 4950$  possible combinations. Now, rcluster-max is 1000, but rcluster-percent is 10, and 10 percent of 4950 is 495. So in this case the rcluster algorithm will consider just the most similar 495 subsets. It will not consider the top 1000 subsets, because 495 (determined by rcluster-percent = 10) is smaller than 1000 (determined by rcluster-max = 1000).

Why is this sensible? There are two answers to this. One is empirical: I've tested this algorithm on a huge range of empirical datasets, and the default settings seem to provide the best balance between speed and accuracy across those datasets. The other answer is theoretical: when you have rcluster-percent set to 100, you sometimes spend a long time at the end of the algorithm analysing really big subsets because at the end of the algorithm you have joined together a lot of the initial data blocks into larger subsets. And here's the important bit – all this searching seems to almost never matter. The improvements in AICc scores we get at the end of the algorithm are very small, and on top of that it seems to be much easier to guess which are the best subsets to merge as the subsets get bigger, probably because we can more accurately guess which subset

combinations will lead to improvements in the AICc score when the subsets are bigger and have more information. So it's much better to analyse fewer subsets at the end of the algorithm - we may lose a tiny bit of the improvement in AICc score (my tests show that we often lose nothing in terms of AICc score though!), but we gain an awful lot in terms of speed. So, because the whole point of the rcluster algorithm is that it's less thorough but a lot quicker than the greedy algorithm I think setting the default for rcluster-percent to 10 is sensible. Of course, you can set these parameters however you like.

### **--save-phylofiles**

This option will make PartitionFinder write a lot of extra files to disk. In general, you will not need to use this. Specifically, it will write the input and output of every single phylogenetic analysis into the /analysis/phylofiles folder (beware, this can be many millions of files, and can take up a lot of disk space). It will also write the results of model selection on every single subset encountered, rather than just the subsets in the best scheme that was estimated. This option can be particularly useful if PartitionFinder (or the programs it uses, like RAxML and PhyML) can't analyse your dataset. It can help track down bugs in the programs, or errors in your input files.

### **--weights "W<sub>rate</sub>, W<sub>base</sub>, W<sub>model</sub>, W<sub>alpha</sub>"**

Default: --weights "1, 0, 0, 0"

A list of weights to use in the clustering algorithms (NB, this only works in combination with the --raxml option and either the hcluster or rcluster search options). This list allows you to assign different weights to the overall rate for a subset, the base/amino acid frequencies, the model parameters, and the alpha parameter (which describes gamma distributed rates across sites). This will affect how subsets are clustered together. For instance:

--weights '1, 1, 1, 0.1'

would weight the subset rate, base frequencies, and the model parameters equally, but the alpha parameter as 10x less important. You can play around with these parameters to try and find the best scheme that you can.

## Citations

Depending on your analysis, you may need to cite up to three papers. One for PartitionFinder2, one for the algorithm you use (if you use the `rcluster`, `hcluster`, or `kmeans` options), and one for either PhyML or RAxML.

### PartitionFinder2

If you are reading this manual, then you are probably using PartitionFinder2. The paper describing PF2 will be out (we hope) in 2015, so please take a quick look here to see if you can see it: [www.robertlanfear.com/publications](http://www.robertlanfear.com/publications). If the paper is not yet out, please cite the PF1 paper:

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular biology and evolution*, 29(6), 1695-1701.

### Using search = 'rcluster' or search = 'hcluster'

These algorithms are described in the following paper, if you use them please cite:

Lanfear, R., Calcott, B., Kainer, D., Mayer, C., & Stamatakis, A. (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC evolutionary biology*, 14(1), 82.

### Using search = 'kmeans'

This algorithm is described in the following paper, if you use it please cite:

Frandsen, P. B., Calcott, B., Mayer, C., & Lanfear, R. (2015). Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. *BMC Evolutionary Biology*, 15(1), 13.

### PhyML

If you use PF2 without the `--raxml` command line option, PF2 relies heavily on PhyML version 3.0, so please cite:

New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. *Systematic Biology*, 59(3):307-21, 2010.

### Using the --raxml command line option

If you use the `--raxml` commandline option, PF2 uses RAxML v8.0 for calculations. If you use it, please cite:

A. Stamatakis, RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models, *Bioinformatics* **22**, 2688–2690 (2006)

