

Table of Contents

Chapter 1 ■ Read Me First! 1

New and Improved Software	2
Just What Is a Phylogenetic Tree?	2
Estimating Phylogenetic Trees: The Basics	4
Beyond the Basics	5
Learn More about the Principles	7
About Appendix VI: F.A.Q.	7
Computer Programs and Where to Obtain Them	8
<i>MEGA</i>	7 8
<i>BEAST</i>	8
<i>FigTree</i>	8
<i>codeml</i>	9
<i>SplitsTree and Dendroscope</i>	9
<i>Graphviz</i>	9
<i>Utility Programs</i>	9
<i>Text Editors</i>	9
Acknowledging Computer Programs	9
The <i>Phylogenetic Trees Made Easy</i> Website	10

Chapter 2 ■ Tutorial: Estimate a Tree 11

Why Create Phylogenetic Trees?	11
About this Tutorial	12
<i>Macintosh and Linux users</i>	12
<i>A word about screen shots</i>	12

Search for Sequences Related to Your Sequence	13
Decide Which Related Sequences to Include on Your Tree	18
<i>Establishing homology</i>	19
<i>To include or not to include, that is the question</i>	20
Download the Sequences	22
Align the Sequences	27
Make a Neighbor Joining Tree	28
Summary	32

Chapter 3 ■ Acquiring the Sequences 33

Background	33
<i>Problems arising from the vast size of the sequence databases</i>	33
<i>The query sequences</i>	34
Hunting Homologs: What Sequences Can Be Included on a Single Tree?	34
Becoming More Familiar with BLAST	35
<i>BLAST help</i>	36
Using the Nucleotide BLAST Page	36
Using BLAST to Search for Related Protein Sequences	39
Finalizing Selected Sequences for a Tree	43
<i>Problems adding coding sequences of protein homologs to the Alignment Explorer</i>	43
Adding Sequences to and Removing Sequences from the Alignment Explorer	47
<i>Add a sequence</i>	47
<i>Import a file of sequences</i>	48
<i>Delete a sequence</i>	48
Other Ways to Find Sequences of Interest (Beware! The Risks Are High)	48

Chapter 4 ■ Aligning the Sequences 53

Aligning Sequences with MUSCLE	53
Examine and Possibly Manually Adjust the Alignment	57
<i>Trim excess sequence</i>	57
<i>Eliminate duplicate sequences</i>	58
Check Average Identity to Estimate Reliability of the Alignment	60
<i>Codons: Pairwise amino acid identity</i>	60
<i>Non-coding DNA sequences</i>	61
Increasing Alignment Speed by Adjusting MUSCLE's Parameter Settings	62
<i>How MUSCLE works</i>	62
<i>Adjusting parameters to increase alignment speed</i>	63

- Aligning Sequences with ClustalW 64
- Aligning Sequences with GUIDANCE2 65
 - Viewing the results* 67
 - Eliminate unreliable parts of the alignment* 68
 - Saving the GUIDANCE2 alignment* 70

Chapter 5 ■ Major Methods for Estimating Phylogenetic Trees 71

- LEARN MORE ABOUT TREE-SEARCHING METHODS 72
- Distance versus Character-Based Methods 74
- LEARN MORE ABOUT DISTANCE METHODS 74

Chapter 6 ■ Neighbor Joining Trees 77

- Using MEGA7 to Estimate a Neighbor Joining Tree 77
- LEARN MORE ABOUT PHYLOGENETIC TREES 78
 - Determine the suitability of the data for a Neighbor Joining tree* 80
 - Estimate the tree* 81
- LEARN MORE ABOUT EVOLUTIONARY MODELS 83
- Unrooted and Rooted Trees 88
- Estimating the Reliability of a Tree 91
- LEARN MORE ABOUT ESTIMATING THE RELIABILITY OF PHYLOGENETIC TREES 92
- What about Protein Sequences? 99

Chapter 7 ■ Drawing Phylogenetic Trees 101

- Changing the Appearance of a Tree 101
 - The Options dialog* 104
 - Branch styles* 108
 - Fine-tuning the appearance of a tree* 109
- Rooting a Tree 112
 - Finding an outgroup* 112
- Subtrees 114
- Saving Trees 118
 - Saving a tree description* 118
 - Saving a tree image* 119
- Captions 119

Chapter 8 ■ Parsimony 121

- LEARN MORE ABOUT PARSIMONY 121

MP Search Methods	123
Using SeaView for Parsimony	125
<i>Estimating a bootstrap tree in SeaView</i>	133
<i>Using MEGA to draw the tree estimated by SeaView</i>	136

Chapter 9 ■ Maximum Likelihood 141

LEARN MORE ABOUT MAXIMUM LIKELIHOOD	141
ML Analysis Using MEGA	143
<i>Test alternative models</i>	144
Estimating the Reliability of an ML Tree by Bootstrapping	148
What about Protein Sequences?	149

Chapter 10 ■ Bayesian Inference of Trees Using BEAST 151

BEAST: An Overview	151
Installing BEAST	152
Prepare the Input Alignment File	152
Run BEAUti	153
LEARN MORE ABOUT BAYESIAN INFERENCE	158
Running BEAST	162
Run Tracer	165
<i>Burnin</i>	166
Run TreeAnnotator	168
What about Protein Sequences?	169
Visualizing the BEAST Tree	170
<i>The icons above the tree</i>	172

Chapter 11 ■ Which Method Should You Use? 175

Criteria to Consider	175
<i>Accuracy</i>	176
<i>Ease of interpretation</i>	176
<i>Time and convenience</i>	176
Results of the Major Methods	178

Chapter 12 ■ Working with Various Computer Platforms 183

Command-line Programs	183
MEGA on the Macintosh Platform	184
<i>Navigating among folders on the Mac</i>	184
<i>Printing trees and text from MEGA</i>	188

The Line Endings Issue 188
Running the Utility Programs 189

Chapter 13 ■ Phylogenetic Networks 191

Why Trees Are Not Always Sufficient 191
Unrooted and Rooted Phylogenetic Networks 192
LEARN MORE ABOUT PHYLOGENETIC NETWORKS 193
Using SplitsTree to Estimate Unrooted Phylogenetic Networks 198
 Estimating networks from alignments 198
 Rooting an unrooted network 204
 Estimating networks from trees 205
 Consensus networks 206
 Supernetworks 210
Using Dendroscope to Estimate Rooted Networks from Rooted Trees 211

Chapter 14 ■ Minimum Spanning Trees 215

Minimum Spanning Trees Are *Not* Phylogenetic Trees! 215
Why Use Minimum Spanning Trees? 215
Origin of MSTs and the Issue of Reliability 216
 What is a minimum spanning tree? 216
Using MSTgold to Estimate MSTs 217
 The MSTgold input files 218
 Two ways for MSTgold to calculate the initial distance matrix 219
 Running MSTgold with the ebgC data 219
 The MSTgold output 220
Bootstrapping MSTgold 223
Exporting MSTs from Graphviz 225
An Alternative Data Set to Illustrate Some Additional
 Features of MSTgold 226
An Alternative to Graphviz: Hypercube 229

Chapter 15 ■ Time Trees 231

Preparations to Estimate a Time Tree 231
Estimating a Time Tree 234
Viewing the Relative Time Tree 238
An Absolute Time Tree 240
 Effect of more calibration points on absolute time trees 244
Postscript 244

Chapter 16 ■ Reconstructing Ancestral Sequences 245

Using MEGA to Estimate Ancestral Sequences by Maximum Likelihood 246

Create the alignment 246

Construct the phylogeny 246

Examine the ancestral states at each site in the alignment 247

Estimate the ancestral sequence 250

Calculating the ancestral protein sequence and amino acid probabilities 256

How Accurate Are the Estimated Ancestral Sequences? 257

Chapter 17 ■ Detecting Adaptive Evolution 259

Effect of Alignment Accuracy on Detecting Adaptive Evolution 261

Using MEGA to Detect Adaptive Evolution 261

Why ebgC is an interesting example for considering adaptive evolution 261

Detecting overall selection 261

Detecting selection between pairs 263

Finding the region of the gene that has been subject to positive selection 264

Using codeml to Detect Adaptive Evolution 266

Installation 267

Run codeml 269

Questions that underlie the models 273

A closer look at ebgC_1.out 275

Summary 278

Postscript 278

Compiling PAML yourself and running codeml without PAMLX 278

Chapter 18 ■ Estimating Phylogenetic Trees from Whole Genome Sequences 281

The Pan-Genome Problem 282

kSNP: An Alternative to Genome Alignment 282

kSNP 2.0 284

kChooser 284

FCK 285

Tree accuracy 286

Always run kChooser before running kSNP 286

Using kSNP3 286

The steps in estimating phylogenetic trees from WGS using kSNP3 287

Chapter 19 ■ Some Final Advice: Learn to Program 297

Appendix I ■ File Formats and Their Interconversion 299

Format Descriptions 299

The MEGA format 299

The FASTA format 300

The Nexus format 301

The PHYLIP format 304

Interconverting Formats 305

FastaConvert, MEGA, and SeaView 305

Appendix II ■ Text Editors 307

Mac OS X: TextWrangler 307

Windows: Notepad++ 308

Linux: Gedit 308

Appendix III ■ The Command-line Environment 311

Introduction and History 311

Terminal and Command Prompt: The Apps for

Accessing the Command-line Environment 312

The current directory 313

Entering Commands 313

Navigating in Terminal 314

The Magic Trick 315

What Is in the CWD? The **ls** Command (§ **dir**) 316

The -l option 317

The -a option 317

The permissions column 318

*The **chmod** command* 318

§The **dir** Command in Command Prompt 319

Some Other Important Commands 320

Copy a file 320

Move a file 321

Rename a file 321

Make a directory 321

Remove a directory 321

Remove a file 321

Print the contents of a file to the screen 321

Clear the screen 321

**Appendix IV ■ Installing and Running
Command-line Programs 323****Installing Command-line Programs 323***Mac OS X/Linux 323**Windows 324***Running Command-line Programs 329***An example 330**The line endings issue 330**The example command line 330**The output file(s) 330**Error checking 330***Appendix V ■ Additional Programs 331****Appendix VI ■ Frequently Asked Questions 335****Literature Cited 339****Index to Major Program Discussions 341****Subject Index 345**