ARB summary

Align sequence(s) to closest relatives using automated aligner

Manually check alignment in sequence editor

Insert aligned sequence(s) into existing tree using parsimony insertion tool (approximate method)

Generate new tree using phylogenetic inference method(s)

Design probes or primers specific for the sequence(s)

Rigorously test resulting topology using:

ARB pros

of interest using probe tools

• easy to import, align and phylogenetically position new sequences

- bootstrap analysis

- using multiple inference methods

- varying outgroup composition

- large aligned reference database which is cumulative
- can expand / collapse tree for easy data handling
- convenient probe design / match function

ARB Getting Started Notes

Getting to the main ARB window

- 1) access ANGIS via Xwindows interface
- 2) goto Unix Command Mode
- 3) type 'arb' at prompt, opens ARB INTRO window
- 4) select database from Existing Files (f) and Directories (D) window, opens database in main ARB window ARB NT

Importing a sequence into ARB

- 1) ftp sequence into ARB directory in ae2, gb, fasta, etc format
- 2) File/Import Sequences and Fields (ARB)
- 3) enter file name
- 4) hit AUTO DETECT (to confirm ARB recognises file format)
- 5) hit GO, opens SEARCH and QUERY window showing imported sequence in HITLIST

Searching sequences in ARB

- 1) Species/Search ... to see SEARCH and QUERY window
- 2) select Search field, type in Search string (* are wildcards)
- 3) hit sequence of interest in HITLIST to see SPECIES INFORMATION window

Aligning sequences in ARB

- 1) Sequence/Edit Marked Sequences using Selected Species and Tree (ARB_EDIT4) ..., opens sequence editor
- 2) Edit/Fast Aligner V1.02 ... [Ctrl-A], opens FastAligner V1.02 window
- 3) type in sequence to be Aligned, and Reference sequence to align against, or let ARB select its own ref sequence using Auto search by pt-server
- 4) hit GO
- 5) manually check alignment; note Align and Insert buttons

Inserting sequence into ARB using the parsimony insertion tool

- 1) Ensure only the sequences you wish to insert are marked
- 2) Tree/Add Species to Existing Tree/Quick Add Marked to a Tree using Parsimony opens SET PARSIMONY OPTIONS window
- 3) in most cases just hit GO

Building a tree in ARB

- 1) MARK (left vertical command bar) sequences in database you wish to build a tree from
- Tree/Build Tree From Sequence Data/Neighbor Joining ..., opens NEIGHBOR JOINING window
- 3) select Filter, Correction and define Name of new Tree
- 4) hit CALCULATE TREE

- 5) goto newly made tree
- 6) select outgroup using S.ROOT (left vertical command bar)
- 7) Tree/Beatify Tree to beautify tree

Making Probes

- 1) MARK sequence(s) in database you wish to design a probe for
- 2) Etc/Probe Functions/Probe Design opens PROBE DESIGN window
- 3) select PT_SERVER (PH updates Snaidr), define parameters
- 4) hit GO, results appear in PD RESULT window, note you will not always get a result
- 5) select probe of interest for further checking
- 6) Etc/Probe Functions/Probe Match opens PROBE MATCH window
- 7) selected probe will automatically be in Target String
- 8) select PT_SERVER, search depth, and other variables in right hand clickable items
- 9) hit MATCH