最佳化分子親緣關係分析平台

- ✓ *Phylogenetic reconstruction by Automatic Likelihood Model selector (PALM)*

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PALM
Phylogenetic reconstruction by Automatic Likelihood Model selector
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Institute of Information Science
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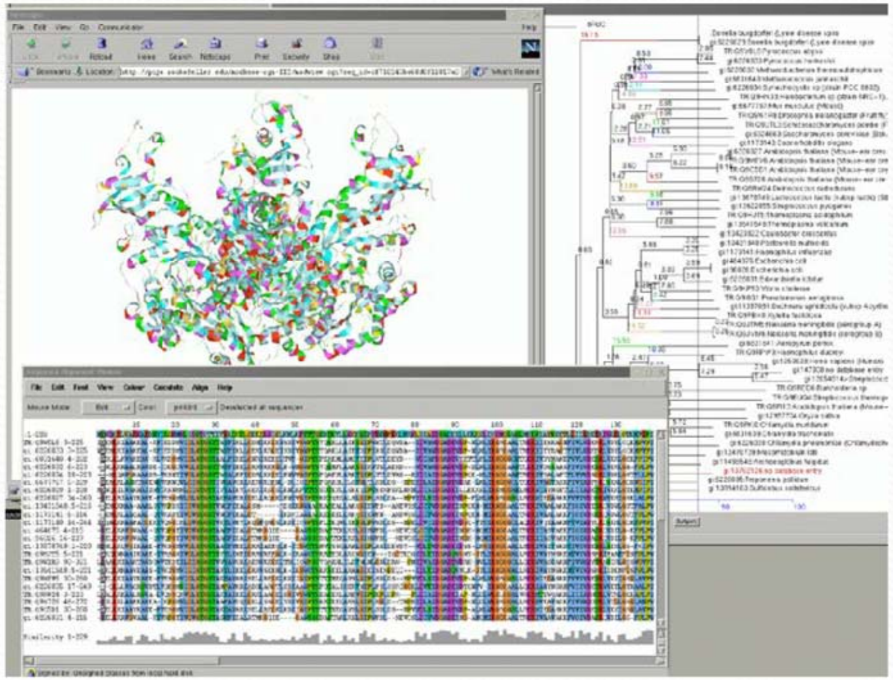
Coding Characters and Defining Homology

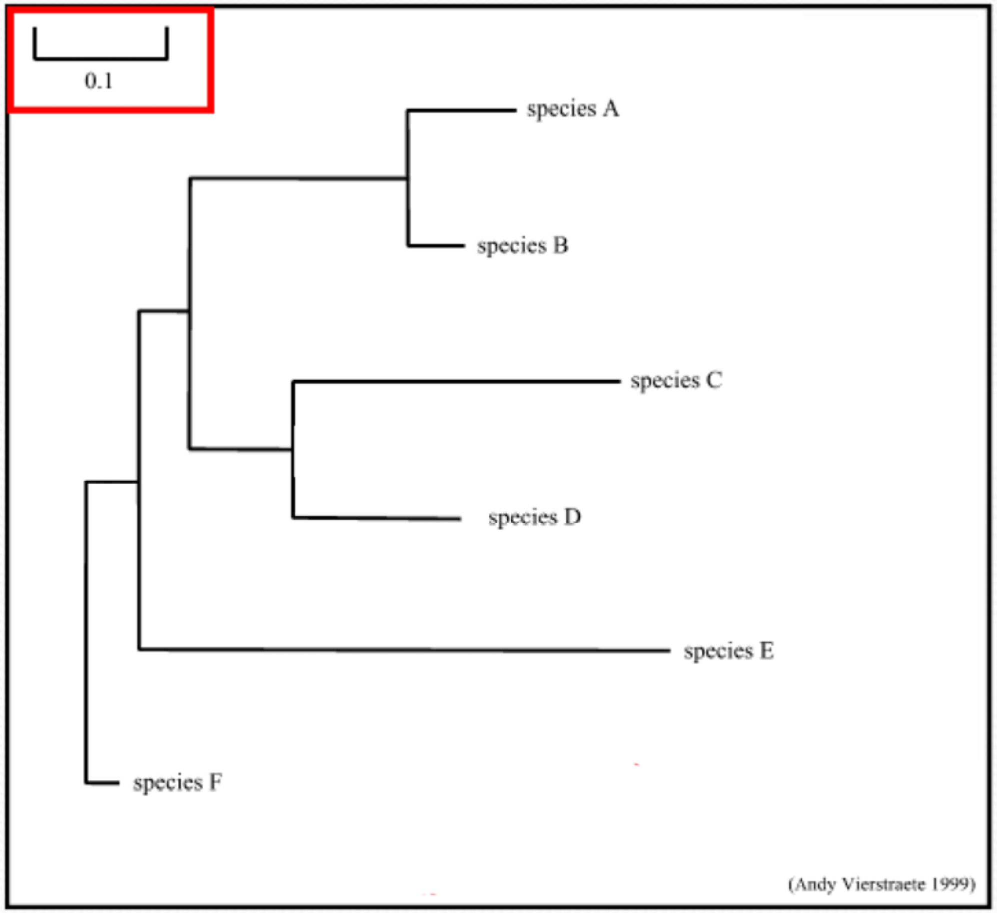


Classical phylogenetic analysis
by Morphology

Molecular phylogenetic analysis
By Bio-Molecules

Phylogenetic Tree

- The tree is composed of nodes connected by branches.

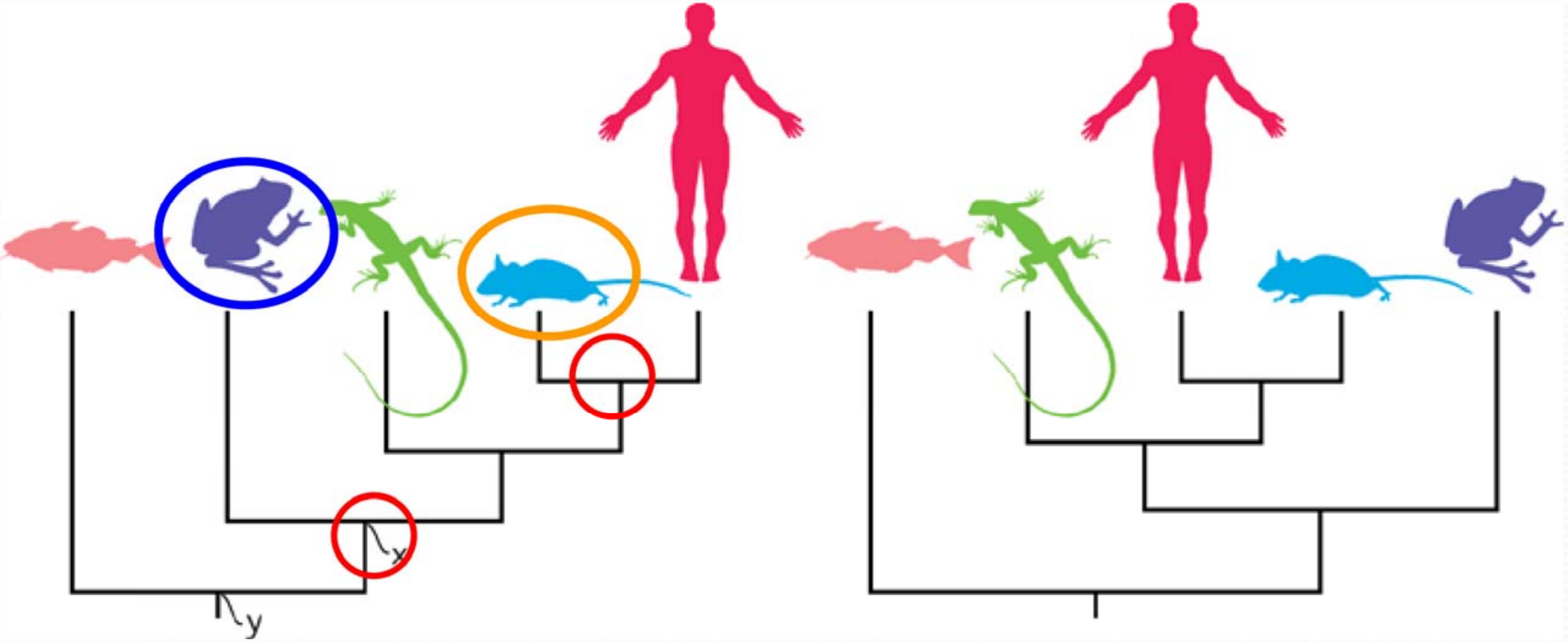


(Andy Vierstraete 1999)

- **node :** a node represents a taxonomic unit.
    - Internal nodes
    - External nodes
- **branch (edge):** defines the relationship between the taxa.
- **branch length :** often represents the number of changes that have occurred in that branch.
- **root :** is the common ancestor of all taxa.
- **distance scale :** scale which represents the number of differences between sequences (e.g. 0.1 means 10 % differences between two sequences)

Trees Only Represent The Order Of Branching

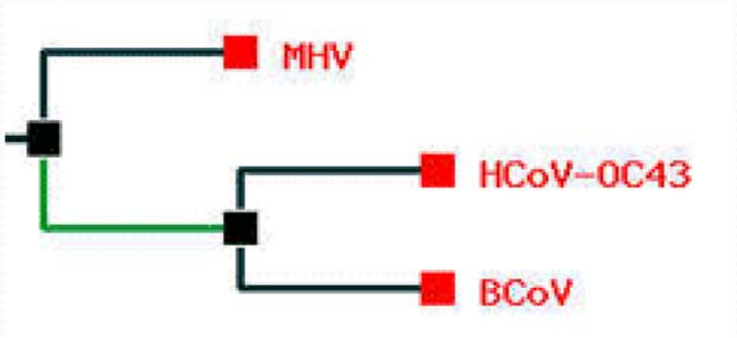
- Same topology in a different style
  - Both trees have identical topologies, with some of the internal nodes rotated.



(David A. Baum et al., Science 11 November 2005:Vol. 310. no. 5750, pp. 979 – 980)

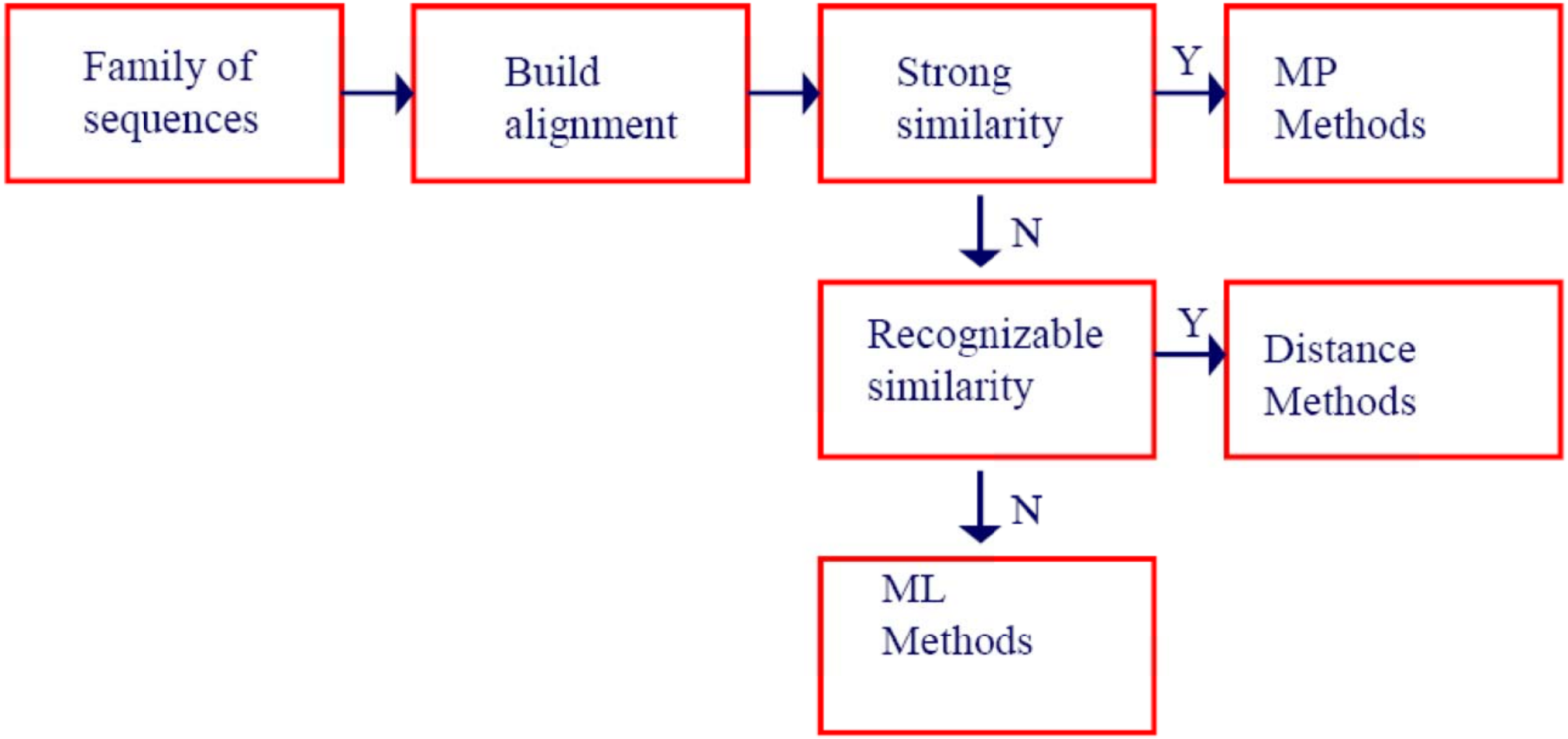
Methods to construct tree

	Optimality search criterion	Clustering
Character state	Maximum parsimony (MP) Maximum likelihood (ML) Bayesian inference	
Distance matrix	Fitch-Margoliash	UPGMA Neighbor-joining (NJ)



(*The phylogenetic handbook 2nd Ed.*)

Flowchart of Analysis



(Mount, *Bioinformatics*)

Distance Method, MP and ML

- Which method should we choose?
- The main disadvantage of distance-matrix methods is their inability to efficiently use information about local high-variation regions that appear across multiple subtrees.
- ML is broadly similar to the maximum-parsimony (MP) method, but maximum likelihood allows additional statistical flexibility by permitting varying rates of evolution across both lineages and sites.
- ML, a better choice?

Maximum Likelihood

- Conditional probability of the data (Aligned sequences) given a hypothesis (a model of substitution with a set of parameter ϴ, and the tree τ, including topology and branch lengths)

$$L(\tau, \Theta\ )=\mathrm{Prob}(\mathrm{Data}|\ \tau, \Theta\ )$$

Or

Prob(Aligned Sequences| tree, model of evolution)

Maximum Likelihood Estimates (MLE)

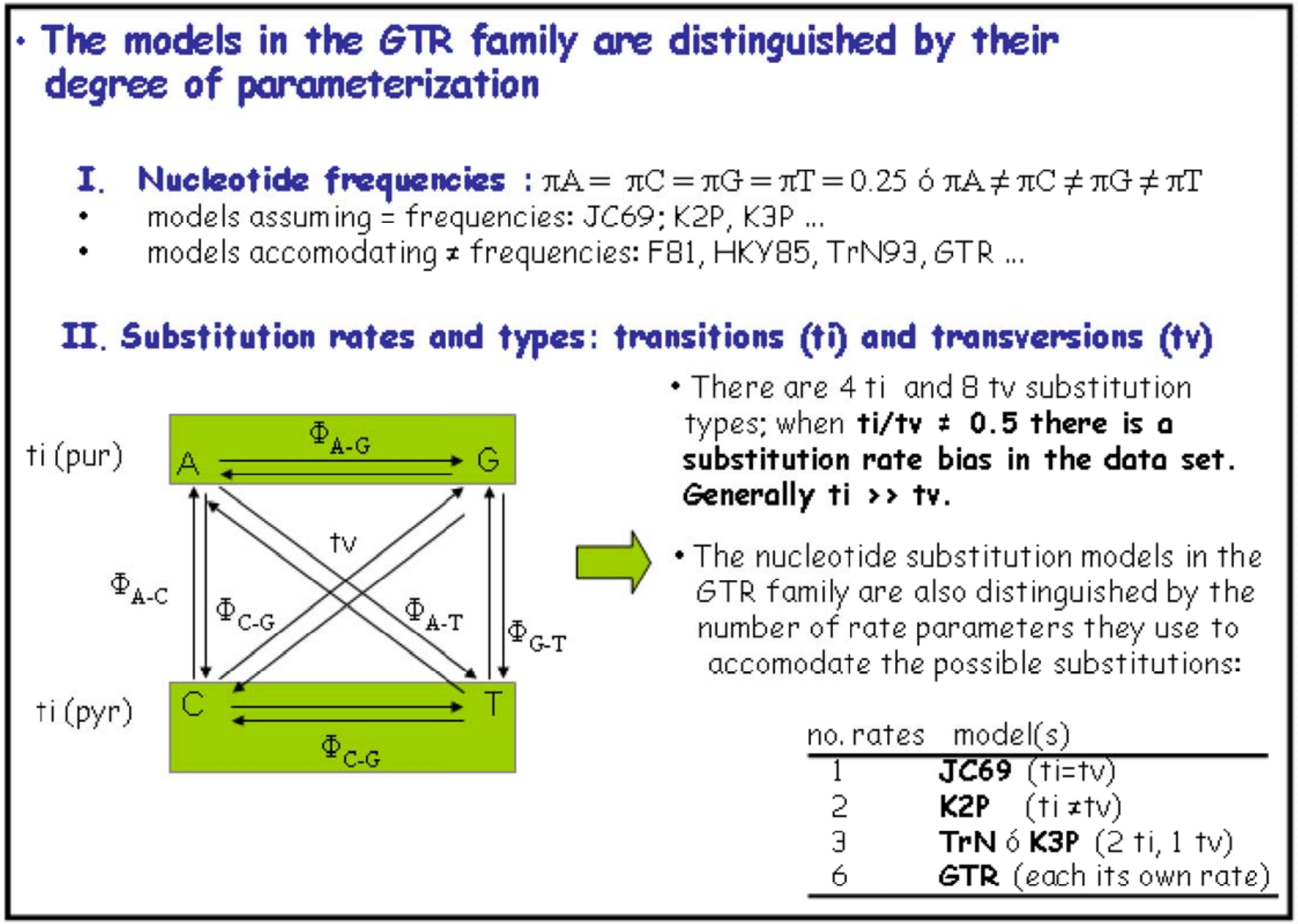
- The maximum likelihood estimates (MLE) of τ, θ are those making the function *L* as large as possible

$$
\begin{aligned}
L(\tau, \theta) &= \Pr(\text{Data} \mid \tau, \theta) \\
&= \Pr(\text{aligned sequence} \mid \text{tree, model of evolution})
\end{aligned}
$$

$$\tau', \theta' = \arg\max L(\tau, \theta)$$

- Hence, what we usually call the likelihood of the tree is **not the likelihood of the tree**, but **the probability of the data given a hypothesis (model of substitution with a set of parameters** θ **and the tree** τ**)**.

Basic Substitution Model



- **The models in the GTR family are distinguished by their degree of parameterization**

I. Nucleotide frequencies : $\pi A = \pi C = \pi G = \pi T = 0.25$ ó $\pi A \neq \pi C \neq \pi G \neq \pi T$

- models assuming = frequencies: JC69; K2P, K3P ...
- models accomodating ≠ frequencies: F81, HKY85, TrN93, GTR ...

II. Substitution rates and types: transitions (ti) and transversions (tv)

ti (pur)
A
Φ_{A-G}
G
tv
Φ_{A-C}
Φ_{C-G}
Φ_{A-T}
Φ_{G-T}
ti (pyr)
C
T
Φ_{C-G}

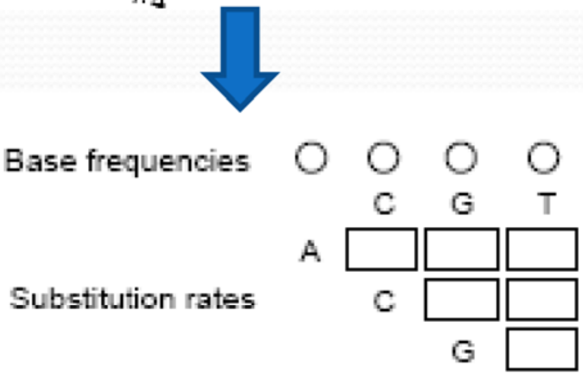
- There are 4 ti and 8 tv substitution types; when **ti/tv ≠ 0.5 there is a substitution rate bias in the data set. Generally ti >> tv.**
- The nucleotide substitution models in the GTR family are also distinguished by the number of rate parameters they use to accomodate the possible substitutions:

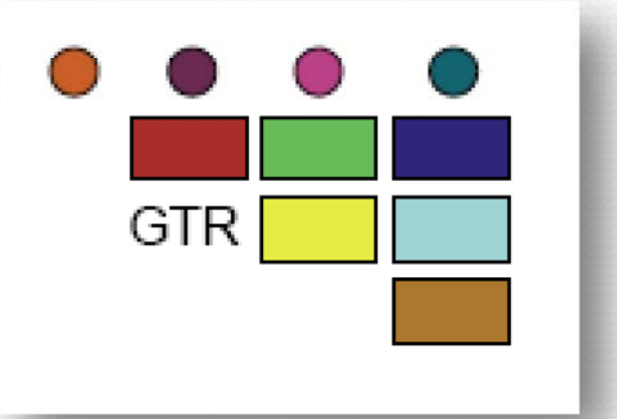
no. rates	model(s)
1	**JC69** (ti=tv)
2	**K2P** (ti ≠tv)
3	**TrN** ó **K3P** (2 ti, 1 tv)
6	**GTR** (each its own rate)

http://www.ccg.unam.mx/~vinuesa/Model_fitting_in_phylogenetics.html

Illustration of DNA substitution Model

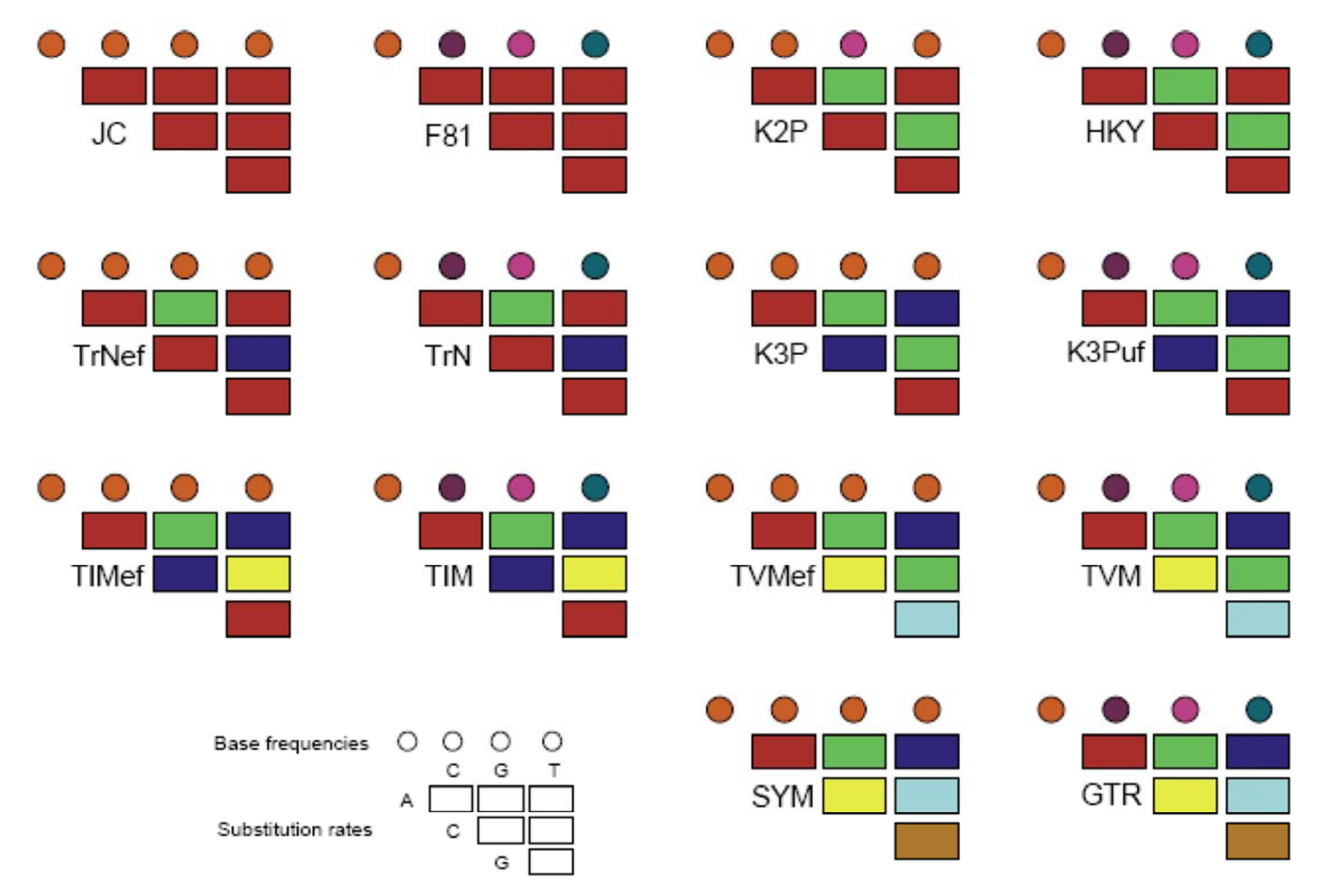
$$Q = \begin{pmatrix} -(x_1+x_2+x_3) & x_1 & x_2 & x_3 \\ \frac{\pi_1 x_1}{\pi_2} & -\left(\frac{\pi_1 x_1}{\pi_2}+x_4+x_5\right) & x_4 & x_5 \\ \frac{\pi_1 x_2}{\pi_3} & \frac{\pi_2 x_4}{\pi_3} & -\left(\frac{\pi_1 x_2}{\pi_3}+\frac{\pi_2 x_4}{\pi_3}+x_6\right) & x_6 \\ \frac{\pi_1 x_3}{\pi_4} & \frac{\pi_2 x_5}{\pi_4} & \frac{\pi_3 x_6}{\pi_4} & -\left(\frac{\pi_1 x_3}{\pi_4}+\frac{\pi_2 x_5}{\pi_4}+\frac{\pi_3 x_6}{\pi_4}\right) \end{pmatrix}$$



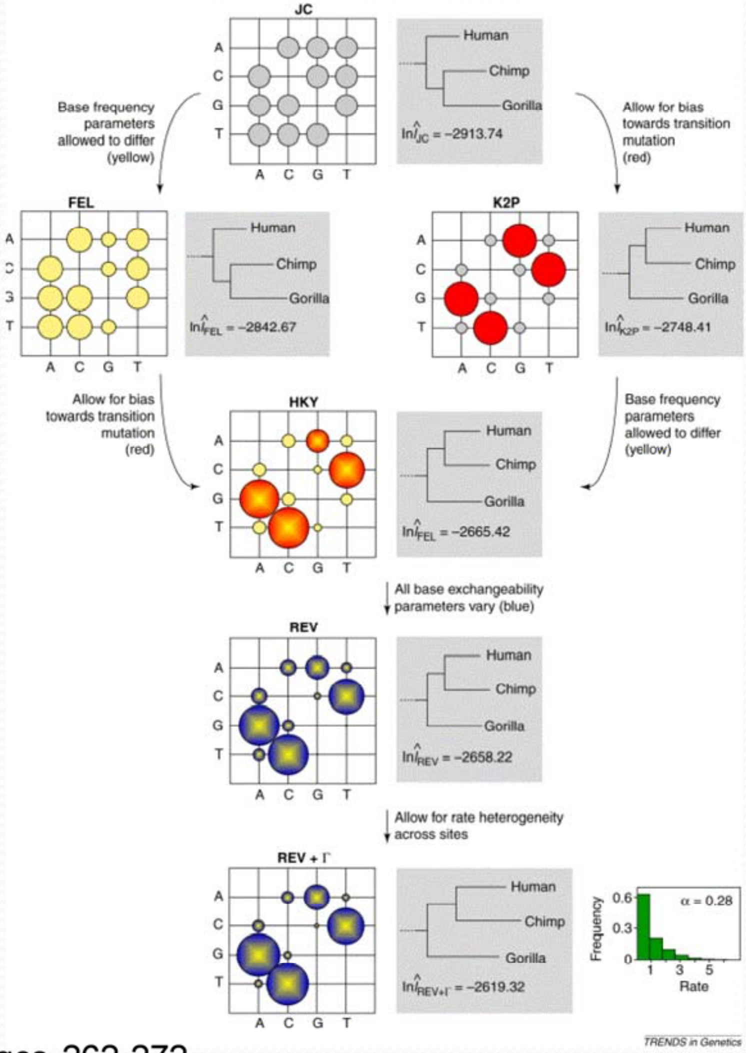


GTR (for four characters, as is often the case in phylogenetics) requires 6 substitution rate parameters (x1~x6), as well as 4 equilibrium base frequency parameters.

Illustration of Models for DNA

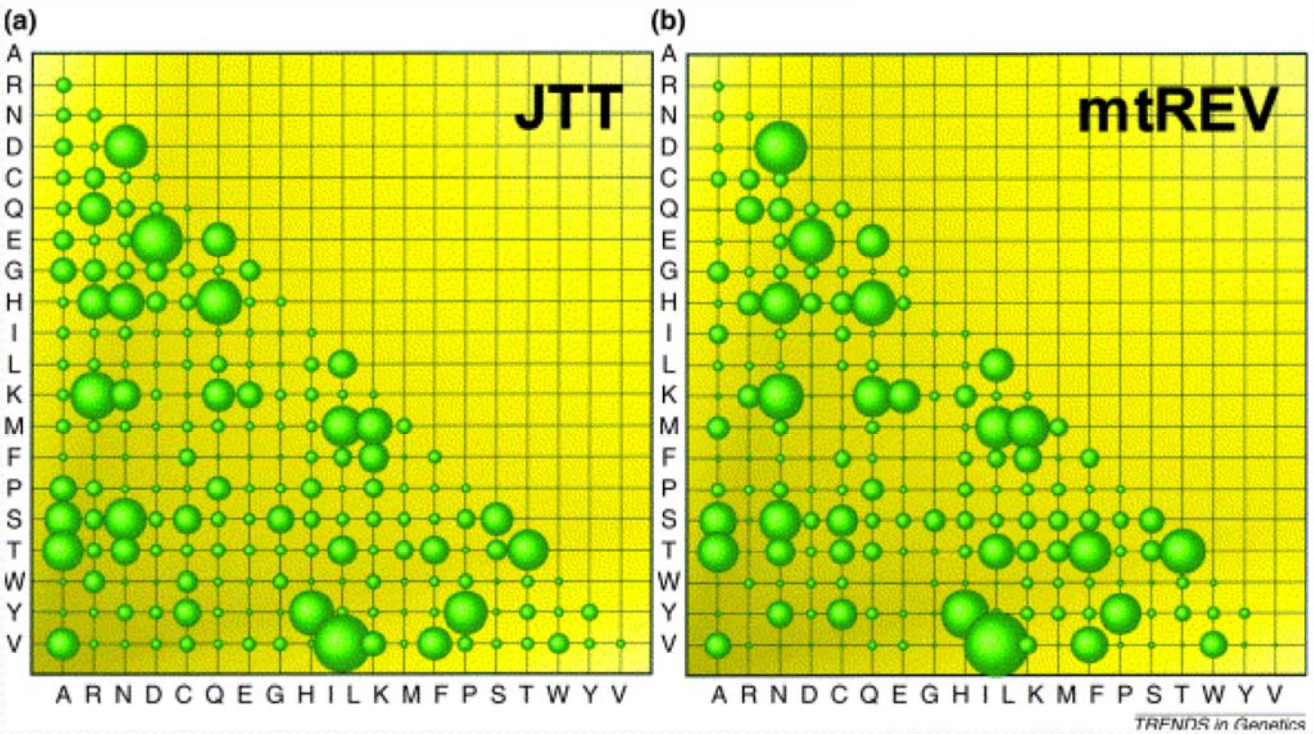


Relationships Among Some Standard Models Of Nucleotide Evolution


JC
A
C
G
T
A C G T
Human
Chimp
Gorilla
$\ln\hat{\ell}_{JC} = -2913.74$
Base frequency parameters allowed to differ (yellow)
Allow for bias towards transition mutation (red)
FEL
Human
Chimp
Gorilla
$\ln\hat{\ell}_{FEL} = -2842.67$
K2P
Human
Chimp
Gorilla
$\ln\hat{\ell}_{K2P} = -2748.41$
Allow for bias towards transition mutation (red)
Base frequency parameters allowed to differ (yellow)
HKY
Human
Chimp
Gorilla
$\ln\hat{\ell}_{FEL} = -2665.42$
All base exchangeability parameters vary (blue)
REV
Human
Chimp
Gorilla
$\ln\hat{\ell}_{REV} = -2658.22$
Allow for rate heterogeneity across sites
REV + Γ
Human
Chimp
Gorilla
$\ln\hat{\ell}_{REV+\Gamma} = -2619.32$
Frequency
0.6
0.3
0
$\alpha = 0.28$
1 3 5
Rate
TRENDS in Genetics

Trends in Genetics
Volume 17, Issue 5, 1 May 2001, Pages 262-272

Models of Amino Acid Replacement



Trends in Genetics
Volume 17, Issue 5, 1 May 2001, Pages 262-272

Models Used in PALM

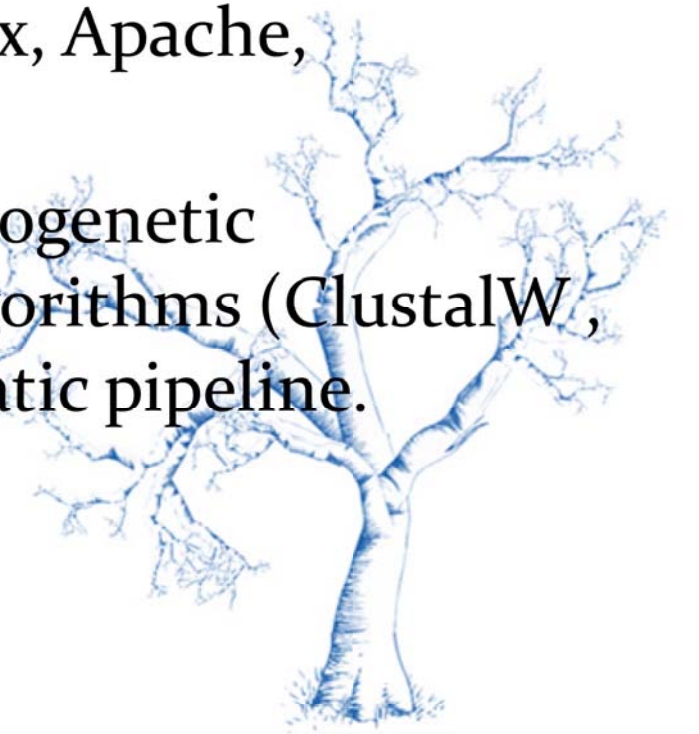
- For DNA (56 models)
  - JC69, K80, F81, HKY, TrN, TrNef, K3P, K3Puf, TIM, TIMef, TVM, TVMef, SYM, GTR
  - +I, +G
- For Protein (112 models)
  - LG, DCMut, JTT, MtREV, MtMam, MtArt, Dayhoff, WAG, RtREV, CpREV, Blosum62, VT, HIVb, HIVw
  - +I, +G, +F

Background for PALM

- Model fitting in phylogenetics has been suggested for many years, yet many authors still arbitrarily choose their models, often using the default models implemented in standard computer programs for phylogenetic estimation.
- Here, we want to show the way that a best-fit model can be readily identified. Consequently, given the relevance of models, model fitting should be routine in any phylogenetic analysis that uses models of evolution.

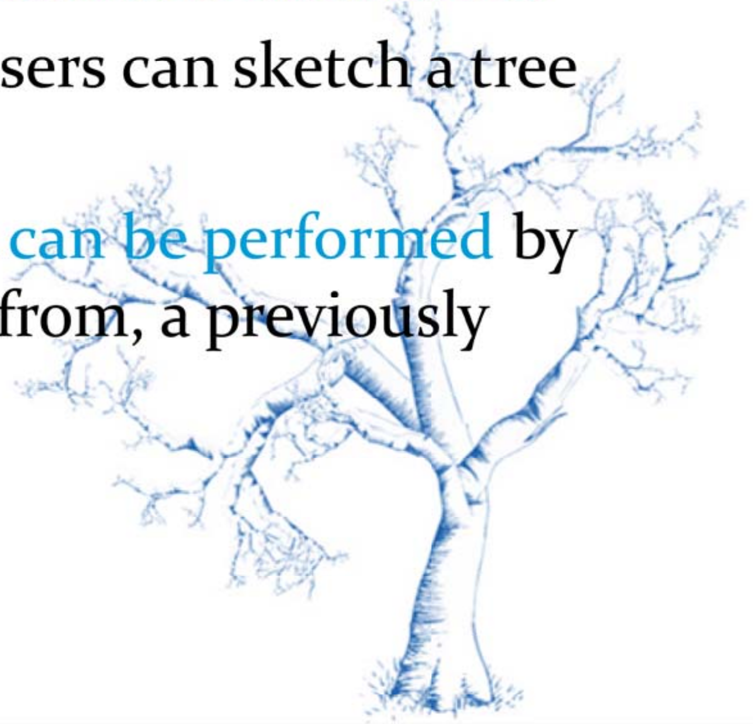
Motivation I

- Provide a seamless way to conduct the complex phylogenetic analysis for Biologists
- An integrated and user-optimized framework for biomolecular phylogenetic analysis
- PALM uses an open-source LAPP (Linux, Apache, PostgreSql, PHP) structure and
- PALM infers genetic distances and phylogenetic relationships using well-established algorithms (ClustalW , PhyML, ProtTest, Modeltest) in automatic pipeline.



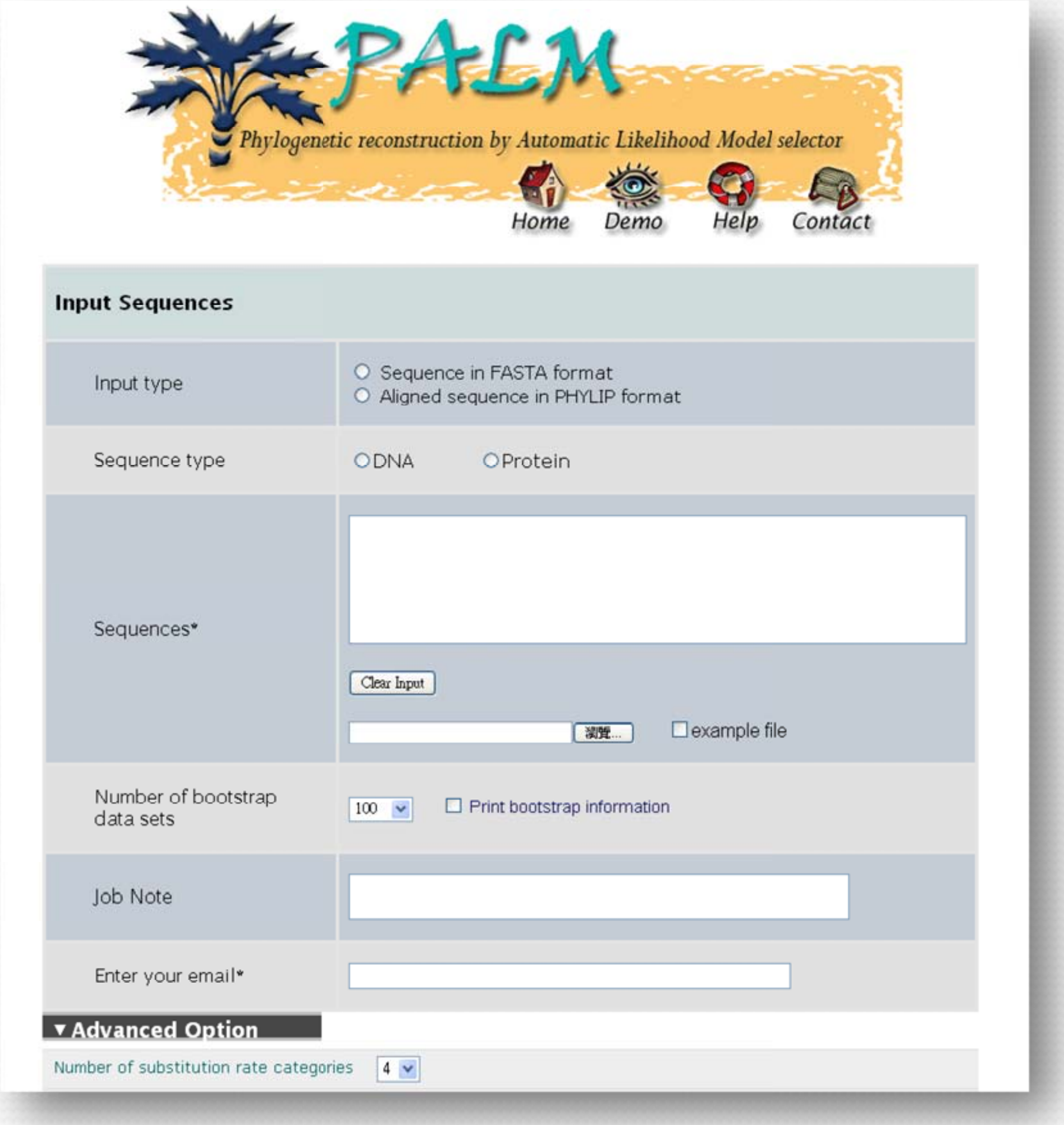
Motivation II

- Model can be selected by following methods including hierarchical likelihood ratio tests (hLRTs), Akaike information criterion (AIC), and Bayesian information criterion (BIC)
- PALM can help user to construct the tree with bootstrap based on best substitution model chosen by maximum likelihood.
- Through a user-friendly web interface, users can sketch a tree effortlessly in multiple steps
- Furthermore, iterative tree construction can be performed by adding sequences to, or removing them from, a previously submitted job



Component Programs of PALM

- PhyML 3.0
- ModelTest 3.7
- ProtTest 2.0
- ClustalW 2.0.8
- Seqret (EMBOSS)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Clear Input
瀏覽...
▾ Advanced Option

Input Sequences

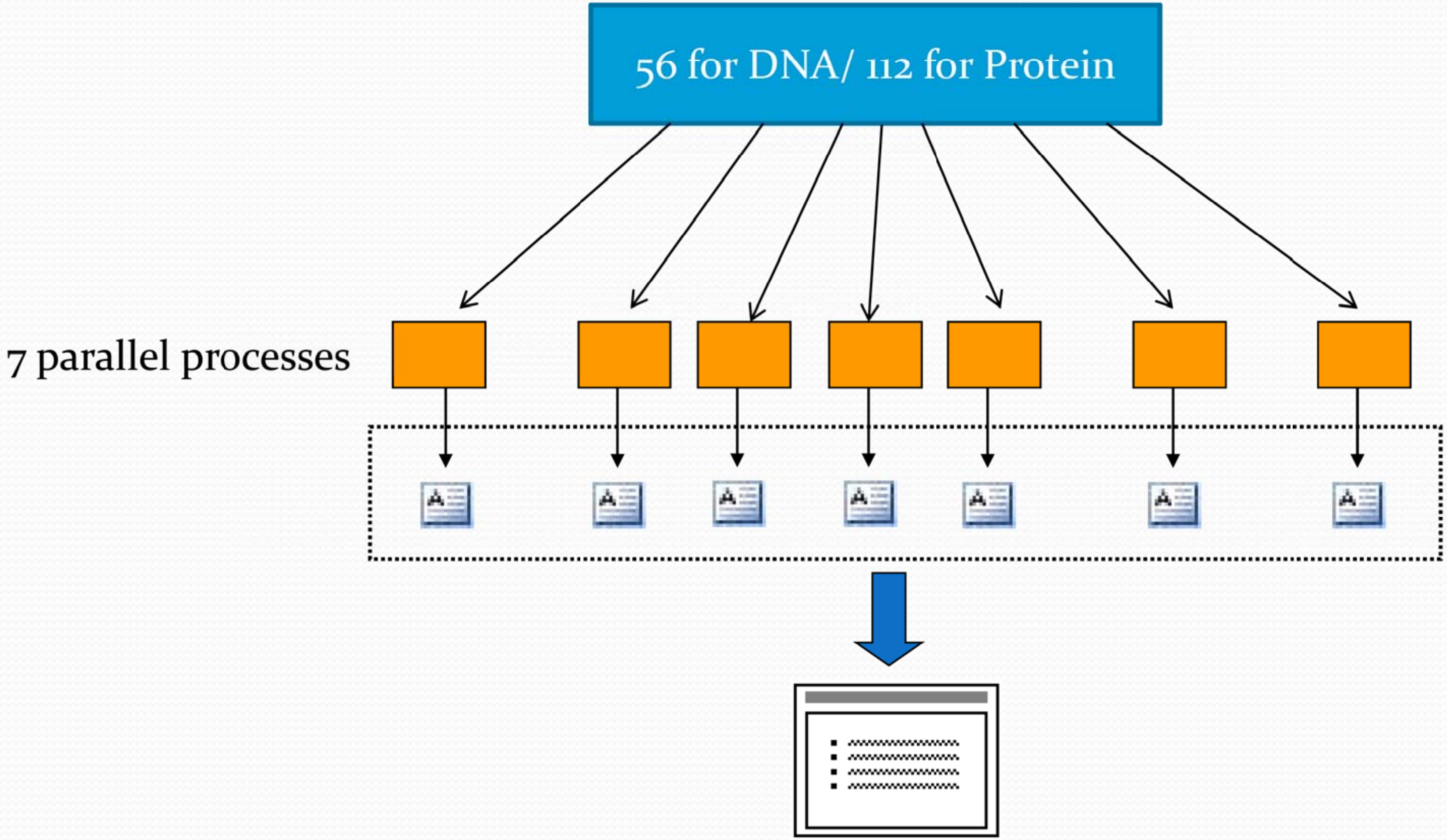
Input type	○ Sequence in FASTA format ○ Aligned sequence in PHYLIP format
Sequence type	○DNA ○Protein
Sequences*	□example file
Number of bootstrap data sets	100 □ Print bootstrap information
Job Note	
Enter your email*	

Number of substitution rate categories 4

Models Used in PALM

- For DNA (56 models)
  - JC69, K80, F81, HKY, TrN, TrNef, K3P, K3Puf, TIM, TIMef, TVM, TVMef, SYM, GTR
  - +I, +G
- For Protein (112 models), **Time consuming**
  - LG, DCMut, JTT, MtREV, MtMam, MtArt, Dayhoff, WAG, RtREV, CpREV, Blosum62, VT, HIVb, HIVw
  - +I, +G, +F

Distribution Computing by PalmMonitor for Each Substitution Model



56 for DNA/ 112 for Protein

7 parallel processes

Decreasing Time by PALMmonitor

- According the algorithm used in PALM, models will take a lot of time to calculate the value of maximum likelihood.

JTT, MtREV	1h:22:21
MtMam, MtArt	1h:51:25
Dayhoff ,WAG	1h:33:51
RtREV, CpREV	1h:33:50
Blosum62, VT	1h:15:58
HIVb, HIVw	1h:35:02
LG, DCMut	1h:38:36

Over 10 Hours

- All Models by PALMmonitor 1h:51:25

Source: 99 sequences with 247 residues for each

Parallel Computing on Bootstrapping

DNA : DNA_Big_24.phy (24 sequences, average 5000 bps substitution model: HKY85- Default)

Bootstrap	100	1000
Runtime	11h:47m:5s	~120 h
Runtime (5 cores/ 8 cores)	2h:53m:2s	17h:52m:15s

1/6 ↓

Protein : Pseq (20 sequences, average 820 a.a., substitution model: LG - Default)

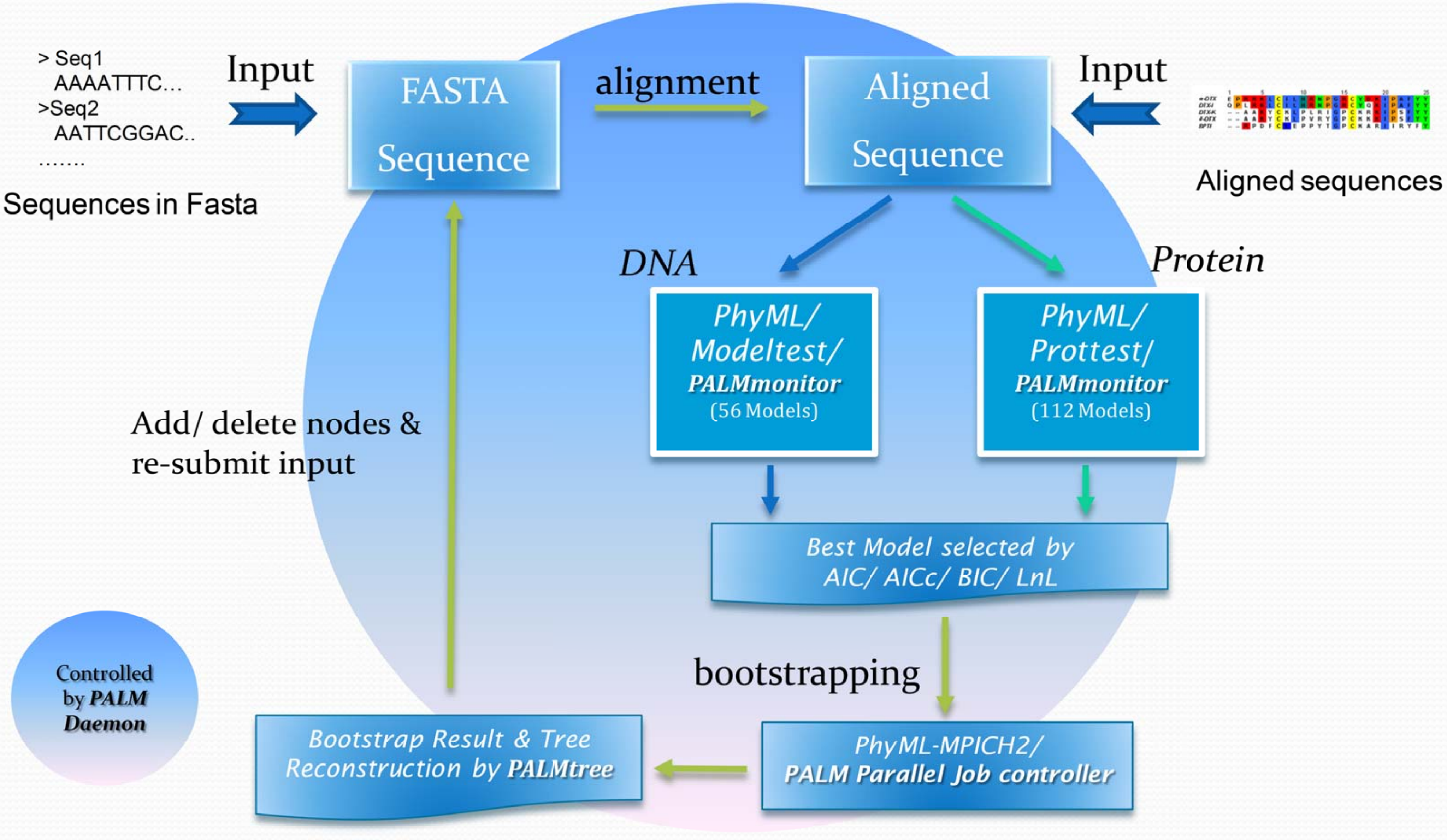
Bootstrap	100	1000
Runtime	17h:31m:33s	~175 h
Runtime (5 cores/ 8 cores)	5h:15m:19s	36h:55m:10s

1/6 ↓

Input and Output of PALM

- Input format (Protein and DNA)
  - Fasta format
  - Phylip format: Aligned Sequences
  - User tree (if submitted and valid)
- Output
  - Tree topology by php and GD library
  - Tree file in Newick format
  - Aligned Sequence in phylip format
  - Best model selected by PALM

Flowchart of PALM



> Seq1
AAAATTTC…
>Seq2
AATTCGGAC..
…….

Sequences in Fasta

Input

FASTA Sequence

alignment

Aligned Sequence

Input

Aligned sequences

DNA

Protein

PhyML/ Modeltest/ ***PALMmonitor*** (56 Models)

PhyML/ Prottest/ ***PALMmonitor*** (112 Models)

Best Model selected by AIC/ AICc/ BIC/ LnL

bootstrapping

PhyML-MPICH2/ ***PALM Parallel Job controller***

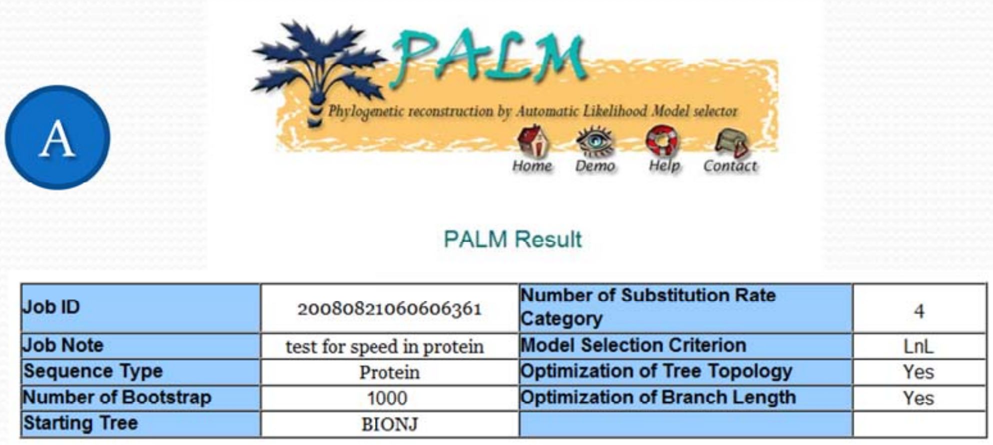
Bootstrap Result & Tree Reconstruction by ***PALMtree***

Add/ delete nodes & re-submit input

Controlled by ***PALM Daemon***

Result of PALM

A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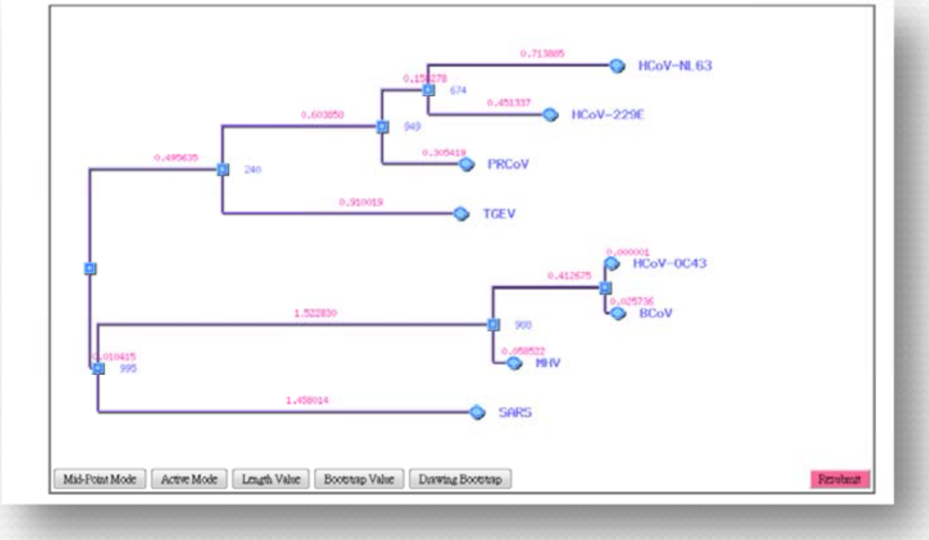
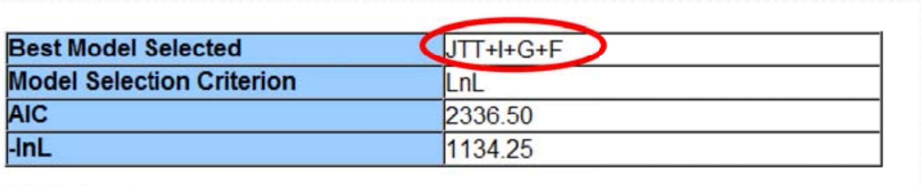
PALM Result

Job ID	20080821060606361	Number of Substitution Rate Category	4
Job Note	test for speed in protein	Model Selection Criterion	LnL
Sequence Type	Protein	Optimization of Tree Topology	Yes
Number of Bootstrap	1000	Optimization of Branch Length	Yes
Starting Tree	BIONJ		

B

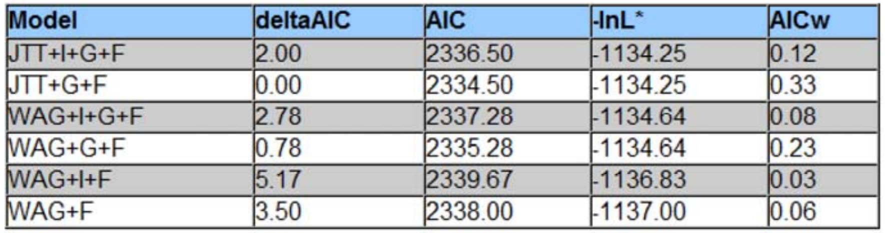


C



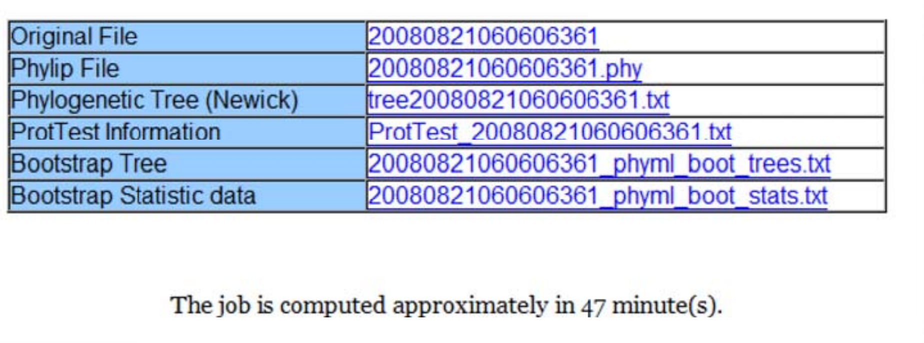
Best Model Selected	JTT+I+G+F
Model Selection Criterion	LnL
AIC	2336.50
-lnL	1134.25

D



Model	deltaAIC	AIC	-lnL*	AICw
JTT+I+G+F	2.00	2336.50	-1134.25	0.12
JTT+G+F	0.00	2334.50	-1134.25	0.33
WAG+I+G+F	2.78	2337.28	-1134.64	0.08
WAG+G+F	0.78	2335.28	-1134.64	0.23
WAG+I+F	5.17	2339.67	-1136.83	0.03
WAG+F	3.50	2338.00	-1137.00	0.06

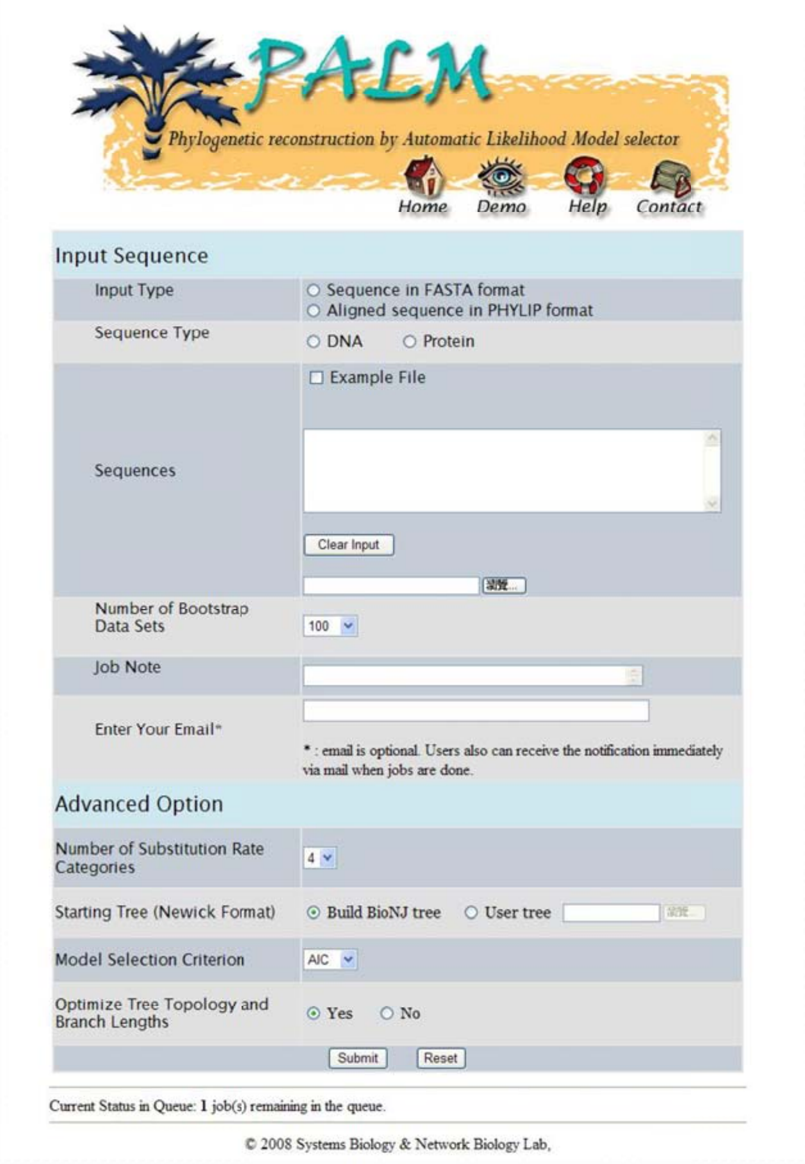
E



Original File	20080821060606361
Phylip File	20080821060606361.phy
Phylogenetic Tree (Newick)	tree20080821060606361.txt
ProtTest Information	ProtTest_20080821060606361.txt
Bootstrap Tree	20080821060606361_phyml_boot_trees.txt
Bootstrap Statistic data	20080821060606361_phyml_boot_stats.txt

The job is computed approximately in 47 minute(s).

Demonstration of PALM



PALM

Phylogenetic reconstruction by Automatic Likelihood Model selector

Input Sequence

Input Type	○ Sequence in FASTA format ○ Aligned sequence in PHYLIP format
Sequence Type	○ DNA ○ Protein
Sequences	□ Example File Clear Input 瀏覽...
Number of Bootstrap Data Sets	100
Job Note	
Enter Your Email*	* : email is optional. Users also can receive the notification immediately via mail when jobs are done.

Advanced Option

Number of Substitution Rate Categories	4
Starting Tree (Newick Format)	● Build BioNJ tree ○ User tree 瀏覽...
Model Selection Criterion	AIC
Optimize Tree Topology and Branch Lengths	● Yes ○ No

Current Status in Queue: 1 job(s) remaining in the queue.

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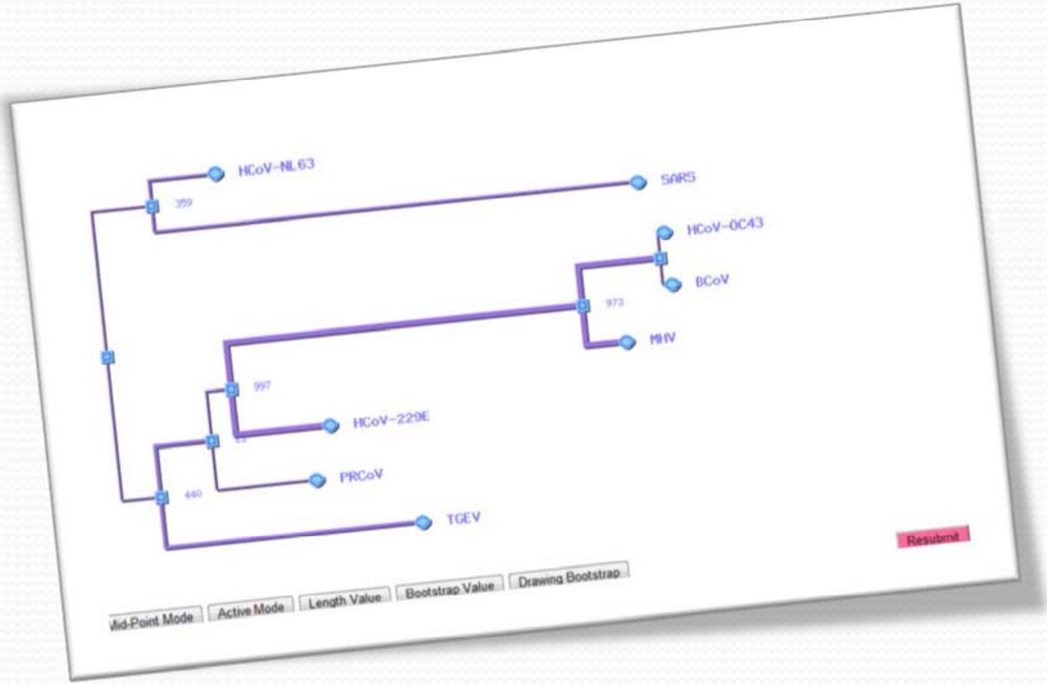
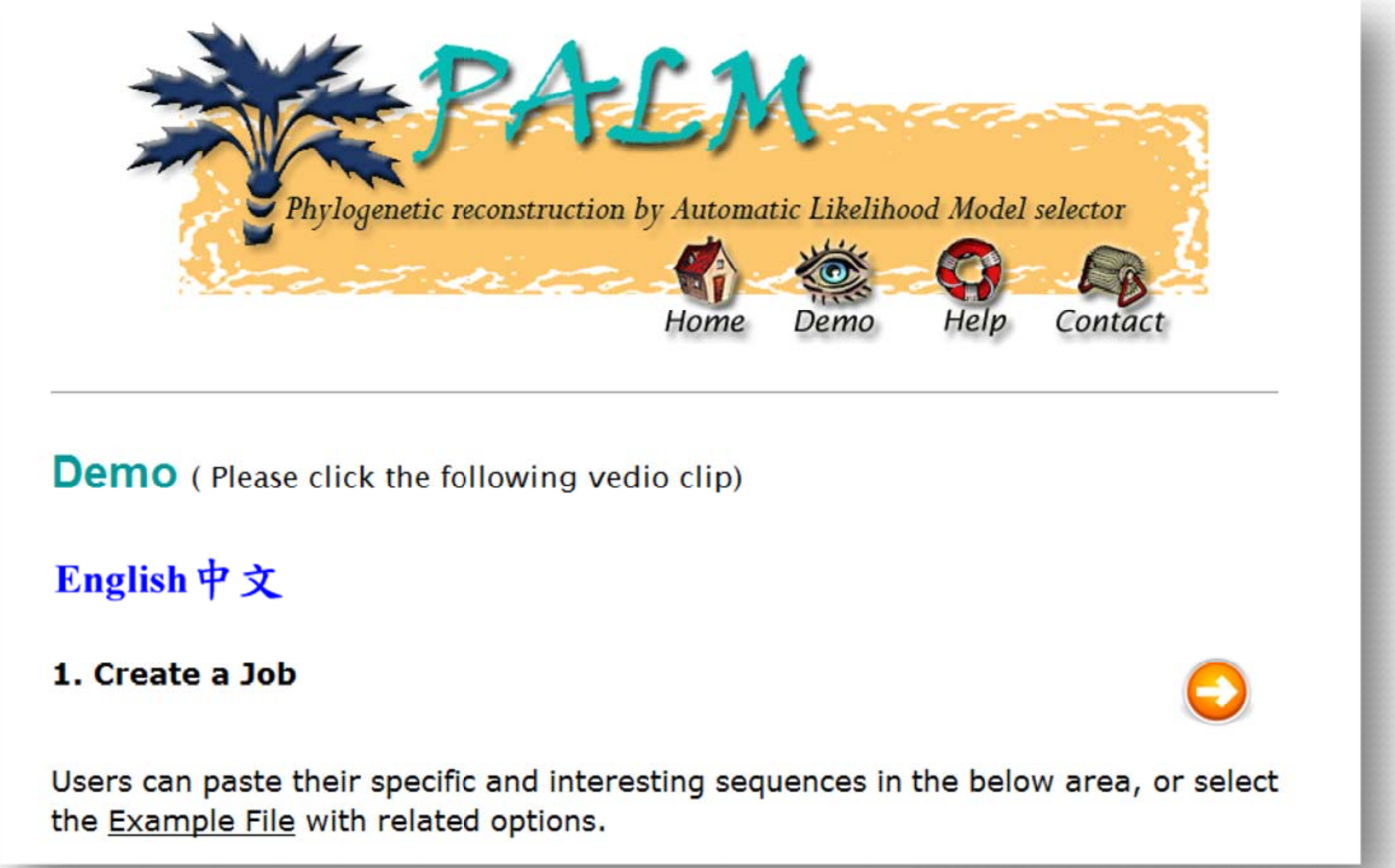


Access : http://palm.iis.sinica.edu.tw

Demo Flash of PALM



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Demo (Please click the following vedio clip)

English 中 文

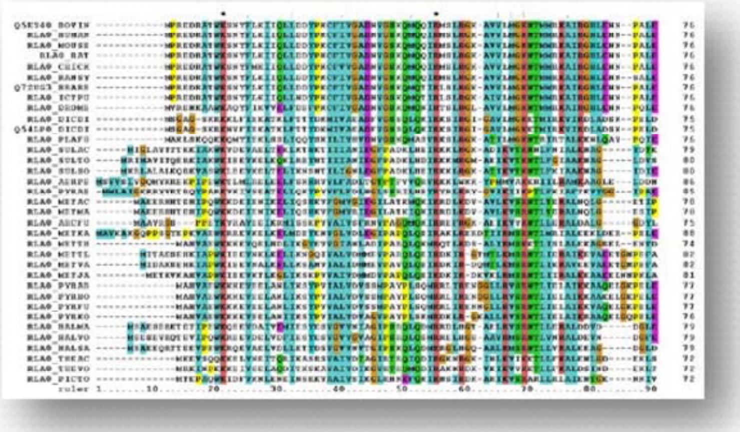
1. Create a Job

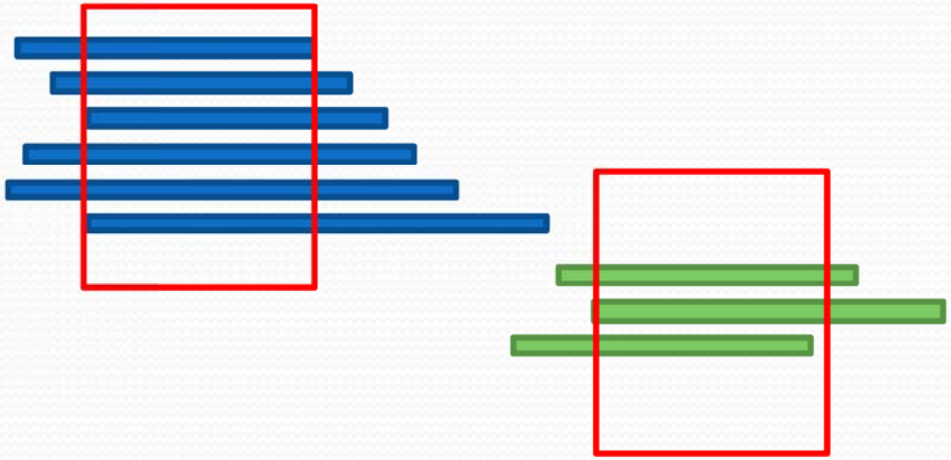
Users can paste their specific and interesting sequences in the below area, or select the Example File with related options.

http://palm.iis.sinica.edu.tw/demo.html

Some Suggestions

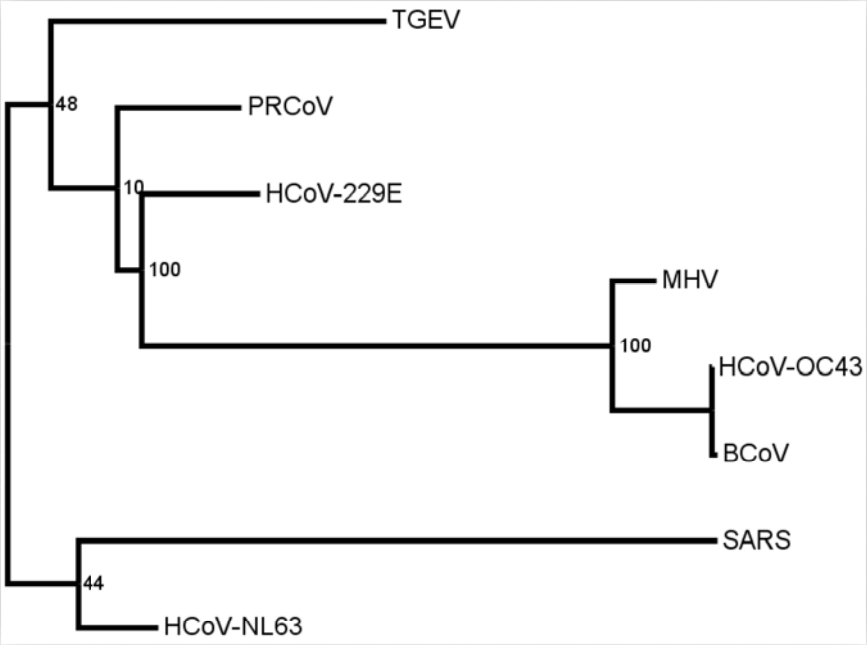
- Please be careful to choose the sequences
- Only well aligned sequences lead to meaningful results.
- RNA editing may introduce bias during analysis.
  - Avoid those regions that may have biases





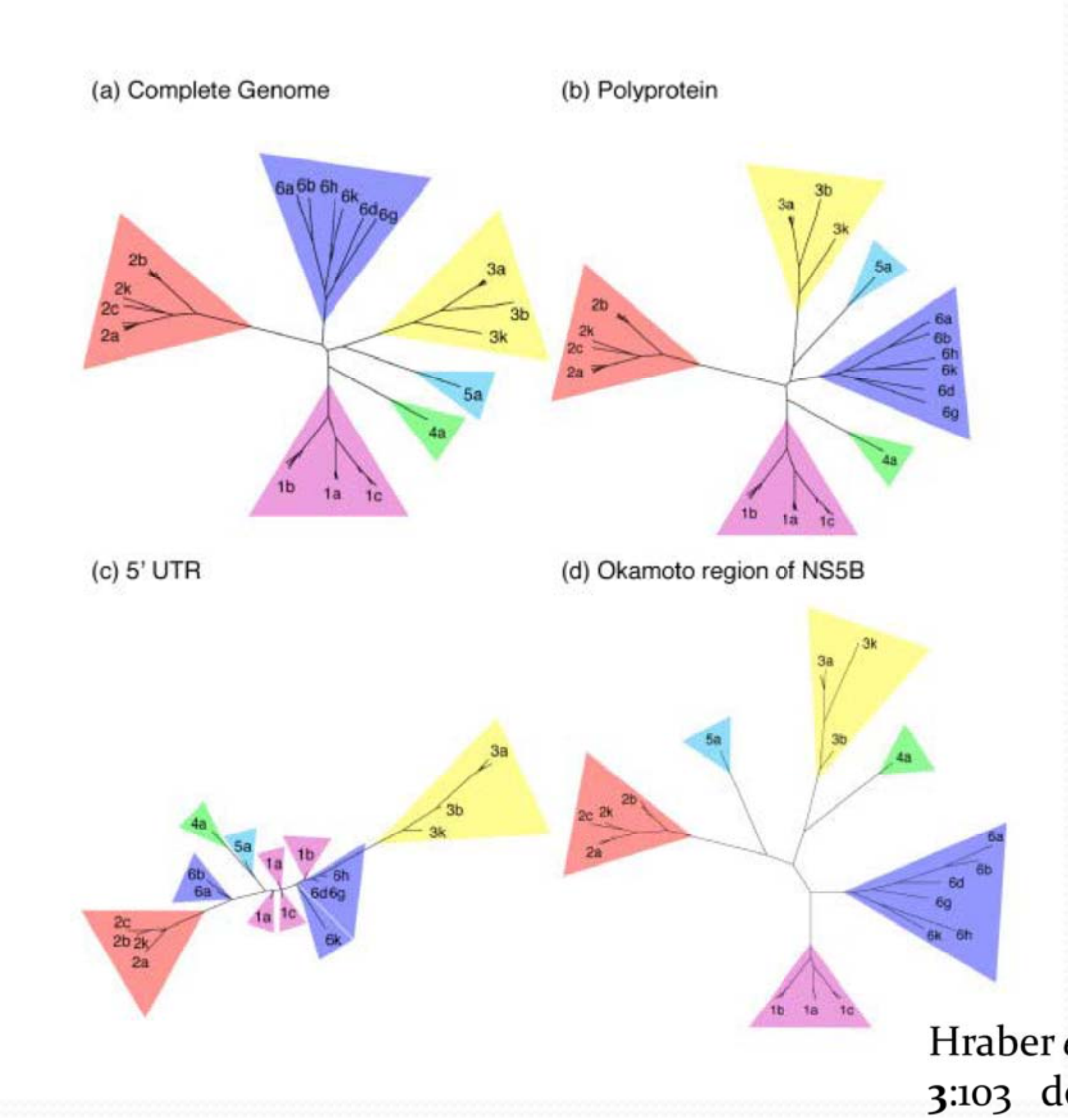
Bootstrap (BS) Analysis

- Bootstrap analysis is the most often used method for statistical evaluation of phylogenies.
- In general:
  - **BS >95%: Often close to 100% confidence in that branch**
  - **BS>75%: Often close to 95% confidence in that branch**
  - BS<75% : Maybe a correct clade due to the original bias cannot be corrected by the re-sampling process.



Input Sequences Make the Tree Different

HIV



Hraber *et al. Virology Journal* 2006 **3**:103 doi:10.1186/1743-422X-3-103

Future Plans for PALM

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- Integrate more substitution models into PALM
- MrBayes will be implemented into this system for Bayesian inference.
- Parallel computing for large scale, ie. 16S RNA tree (near 2000-4000 sequences) reconstruction in metagenomics for revealing microbial community

Acknowledgement

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Tengi Huang
Pao-Han Kuo
Chun-Zen Lo