

Supplementary Figure 1. Distribution of mutations in the experimental phylogeny. Leaf and node numbers are provided at each tip and bifurcation point on the tree, respectively. Nonsynonymous mutations are listed to the right of each branch and synonymous mutations to the left. Nonsynonymous mutations also list the amino acid replacement. Each filled circle represents one round of random-mutagenesis PCR and the color of the circle represents the color-class phenotype of the FP protein at that location in the tree.



Supplementary Figure 2. Diversity of phenotypes displayed by the leaf ('modern') sequences of the phylogeny. Purified proteins from sequences 1-19 are arranged by color-class phenotype and are shown in visible light (top image) and 365 nm UV light (bottom image).



Supplementary Figure 3. Evolution of color emission in the experimental phylogeny. Cladogram drawn with bacteria growing on an agar plate expressing either a node or leaf fluorescent protein and visualized under 365 nm ultraviolet light. Topology is a representation of the true topology shown in the phylogram (Supplementary Fig. 1).



| TrueLCA PAML all 19 PAML Sub1 PAML Sub2 | MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDI MASSEDVIKEFMRFKV <mark>S</mark> MEGSVNGHEFEIEGEGEGRPYEGTQ <mark>S</mark> AKLKVTKGGPLPFAWDI MASSEDVIKEFMRFKV <mark>S</mark> MEGSVNGHEFEIEGEGEGRPYEGTQ MASSEDVIKEFMRFKV <mark>S</mark> MEGSVNGHEFEIEGEGEGRPYEGTQ **************** |
|--|--|
| TrueLCA PAML all 19 PAML Sub1 PAML Sub2 | LSPQFQYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIY LSPQFQYGSKAYVKHPADIPDYMKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGVFIY LSPQFQYGSKAYVKHPADIPDYMKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIY LSPQFQYGSKAYVKHPADIPDYMKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGVFIY ************************************ |
| TrueLCA PAML all 19 PAML Sub1 PAML Sub2 | KVKLRGTNFPSDGPVMQKKTMGWEASTERMYPEDGALKGEVKMRLKLKDGDHYDAEVNTT KVKLGTNFPSDGPVMQKKTMGWEASTERMYPEDGALKGEVKMRLKLKDGCHYEAEVKTT KVKLGTNFPSDGPVMQKKTMGWEASTERMYPEDGALKGEVKMRLKLKDGCHYEAEVKTT KVKLGMNFPSDGPVMQKKTMGWEASTERMYPEDGALKGEVKMRLKLKDGSHYDAEVKTT ****.* |
| TrueLCA PAML all 19 PAML Sub1 PAML Sub2 | YMAKKPVQLPGAYKTDVKLDITSHNEDYTIVEQYERAEGRHSTGA YMAKKPVQLPGAYTTDIKLDITSHNEDYTIVEQYERAEGRHSTGA YMAKKPVQLPGAYTTDIKLDITSHNEDYTIVEQYERAEGRHSTGA YMAKKPVQLPGAYTTDIKLDITSHNEDYTIVEQYERAEGRHSTGA |

Supplementary Figure 4. The effects of taxon sampling on the inference of the last common ancestral sequence. (a) Full phylogeny from Fig. 1 in the main article. (b) Subsample of sequences across the entire spectrum of the full phylogeny used to infer the last common ancestor (LCA). (c) Subsample of sequences composed of clusters from the full phylogeny used to infer the LCA. Scale bars represents amino acid replacements per site per unit evolutionary time. Color corresponds to Fig.1 from the main article. (d) Multiple sequence alignment of LCAs from the different analyses; true ancestor, PAML inferred using all of the 19 leaf sequences, PAML using sequences from b (Subsample1), PAML using sequences from c (Subsample2). Inferred residues that differ from the true ancestral residues are highlighted in magenta. The sites of the highlighted positions are 17, 43, 94, 117, 125, 127, 171, 174, 178, 194 and 197.







b

Supplementary Figure 5. Absorbance spectra for all 70 proteins characterized in the study.

(a) Absorbance spectra for true ancestors and leaves of phylogeny. Color of spectral line represents color-class of encoded protein. (b) Absorbance spectra for true ancestors (solid lines) and incorrectly inferred ancestors (dotted lines).

Supplementary Table 1. Summary statistics for the experimental phylogeny. (a) Total mutations and types of mutations accumulated in the phylogeny. (b) A list of specific nucleotide substitution types and occurrences in the phylogeny. Abbreviations ts and tv represent transitions and transversions, repectively. (c) Types of nucleotide substitutions resulting in either a synonymous (syn) or nonsynonymous (nonsyn) mutation along each individual branch of the phylogeny. Predicted ratio of nonsynonymous to synonymous mutations does not match the observed ratio because of reversion mutations.

| a | | | | | |
|---|-----|--|--|--|--|
| Experimental Phylogeny Mutation Summary | | | | | |
| Total variants in phylogeny | 349 | | | | |
| Nodes | 17 | | | | |
| Leaves | 19 | | | | |
| Total Mutations | 833 | | | | |
| Transitions (ts) | 535 | | | | |
| Transversions (tv) | 298 | | | | |
| Total synonymous (syn) mutations | 461 | | | | |
| Synonymous reversions | 14 | | | | |
| Observed synonymous mutations | 447 | | | | |
| Total nonsynonymous (nonsyn) mutations | 372 | | | | |
| Nonsynonymous reversions | 19 | | | | |
| Observed nonsynonymous mutations | 353 | | | | |

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| Nucleotide Substitution | | | | | | |
|-------------------------|------|------------|-------------------|--|--|--|
| Substitution | Туре | Occurrence | Percentage (%) | | | |
| A> T | tv | 74 | 8.88 | | | |
| A> C | tv | 16 | 1.92 | | | |
| A> G | ts | 117 | 14.05 | | | |
| T> A | tv | 72 | 8.64 | | | |
| T> C | ts | 106 | 12.73 | | | |
| T> G | tv | 19 | 2.28 | | | |
| C> T | ts | 167 | 20.05 | | | |
| C> G | tv | 17 | 2.04 | | | |
| C> A | tv | 33 | 3.96 | | | |
| G> C | tv | 21 | 2.52 | | | |
| G> T | tv | 46 | 5.52 | | | |
| G> A | ts | 145 | 17.41 | | | |

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|---|---|---|
| 1 | L | |
| 1 | ٠ | - |

| Branch number | Syn mutations | Syn reversions | Observed syn mutations | Nonsyn mutations | Nonsyn reversions | Observed nonsyn mutations | Observed nonsyn/syn | Predicted nonsyn/syn |
|------------------|------------------|-------------------|------------------------------|---------------------|----------------------|---------------------------------|------------------------|----------------------|
| 1 | 21 | 0 | 21 | 12 | 1 | 11 | 0.5714 | 0.5238 |
| 2 | 24 | 1 | 23 | 13 | 2 | 11 | 0.5417 | 0.4783 |
| 3 | 16 | 2 | 14 | 17 | 1 | 16 | 1.0625 | 1.1429 |
| 4 | 25 | 0 | 25 | 9 | 0 | 9 | 0.3600 | 0.3600 |
| 5 | 19 | 1 | 18 | 13 | 1 | 12 | 0.6842 | 0.6667 |
| 6 | 21 | 0 | 21 | 11 | 0 | 11 | 0.5238 | 0.5238 |
| 7 | 16 | 0 | 16 | 14 | 1 | 13 | 0.8750 | 0.8125 |
| 8 | 21 | 1 | 20 | 12 | 0 | 12 | 0.5714 | 0.6000 |
| 9 | 23 | 2 | 21 | 16 | 1 | 15 | 0.6957 | 0.7143 |
| 10 | 18 | 1 | 17 | 14 | 1 | 13 | 0.7778 | 0.7647 |
| 11 | 19 | 0 | 19 | 13 | 0 | 13 | 0.6842 | 0.6842 |
| 12 | 17 | 0 | 17 | 13 | 2 | 11 | 0.7647 | 0.6471 |
| 13 | 15 | 0 | 15 | 14 | 1 | 13 | 0.9333 | 0.8667 |
| 14 | 19 | 2 | 17 | 17 | 0 | 17 | 0.8947 | 1.0000 |
| 15 | 23 | 0 | 23 | 13 | 1 | 12 | 0.5652 | 0.5217 |
| 16 | 16 | 1 | 15 | 16 | 0 | 16 | 1.0000 | 1.0667 |
| 17 | 22 | 1 | 21 | 11 | 0 | 11 | 0.5000 | 0.5238 |
| 18 | 21 | 1 | 20 | 17 | 1 | 16 | 0.8095 | 0.8000 |
| 19 | 19 | 1 | 18 | 14 | 1 | 13 | 0.7368 | 0.7222 |
| 22 | 7 | 0 | 7 | 20 | 1 | 19 | 2.8571 | 2.7143 |
| 23 | 4 | 0 | 4 | 4 | 0 | 4 | 1.0000 | 1.0000 |
| 24 | 7 | 0 | 7 | 8 | 1 | 7 | 1.1429 | 1.0000 |
| 25 | 4 | 0 | 4 | 10 | 0 | 10 | 2.5000 | 2.5000 |
| 26 | 1 | 0 | 1 | 1 | 0 | 1 | 1.0000 | 1.0000 |
| 27 | 4 | 0 | 4 | 1 | 0 | 1 | 0.2500 | 0.2500 |
| 28 | 4 | 0 | 4 | 5 | 1 | 4 | 1.2500 | 1.0000 |
| 29 | 1 | 0 | 1 | 4 | 0 | 4 | 4.0000 | 4.0000 |
| 30 | 8 | 0 | 8 | 7 | 0 | 7 | 0.8750 | 0.8750 |
| 31 | 8 | 0 | 8 | 9 | 0 | 9 | 1.1250 | 1.1250 |
| 32 | 2 | 0 | 2 | 2 | 0 | 2 | 1.0000 | 1.0000 |
| 33 | 8 | 0 | 8 | 9 | 1 | 8 | 1.1250 | 1.0000 |
| 34 | 4 | 0 | 4 | 9 | 0 | 9 | 2.2500 | 2.2500 |
| 35 | 2 | 0 | 2 | 2 | 0 | 2 | 1.0000 | 1.0000 |
| 36 | 5 | 0 | 5 | 4 | 0 | 4 | 0.8000 | 0.8000 |
| 37 | 17 | 0 | 17 | 18 | 1 | 17 | 1.0588 | 1.0000 |
| | • | - | • | • | - | Average | 1.0510 | 1.0267 |

Supplementary Table 2. Percentage of sites correctly inferred for the five tested ASR procedures. Procedures follow the color-code scheme (Fig. 2 in main article).

| Method | Correctly Inferred Sites | Incorrectly Inferred Sites | Total Sites |
|-----------------|--------------------------------|----------------------------------|----------------|
| $PAML_{\Gamma}$ | 98.14% | 71 | 3825 |
| FASTML_Γ | 98.17% | 70 | 3825 |
| PAML | 98.12% | 72 | 3825 |
| PHYLO_Γ | 97.88% | 81 | 3825 |
| MP | 98.07% | 74 | 3825 |

Supplementary Table 3. (a) Phenotypes of true ancestors and incorrectly inferred ancestors. Extinction coefficient (ϵ), quantum yield (Φ), brightness ($\epsilon \ge \Phi$), excitation maxima (λ_{ex}), and emission maxima (λ_{em}). The percent error equals zero when the inferred sequence is identical to the ancestral sequence. (b) Phenotypes for all 19 leaf proteins. (c) Summary of phenotypic error for each ASR procedure. Note, nodes 34 and 35 are not included because they outweigh all other error (but their errors are the same for the five procedures so the overall effect is negligible. Procedures follow the color-code scheme (Supplementary Table 2a, c, Fig. 2).

| a | | | | | | | | | | |
|----------|--|------------|--------|------------|-------------------------|--------------------------|-------------------------|--------------------------|------------|---------------------|
| Node | ∑ (M ⁻¹ cm ⁻¹) | Σ Error | 4 | √ Error | λ _{ex} (nm) | λ _{ex} Error | λ _{em} (nm) | λ _{em} Error | Brightness | Brightness Error |
| 21 | 19,003 | - | 0.1213 | - | 586 | - | 608 | - | 2,306 | - |
| PAML Γ | 36,317 | 91.11% | 0.2567 | 111.53% | 585 | 0.17% | 604 | 0.66% | 9,322 | 304.25% |
| FASTML Γ | 36,317 | 91.11% | 0.2567 | 111.53% | 585 | 0.17% | 604 | 0.66% | 9,322 | 304.25% |
| PAML | 36,317 | 91.11% | 0.2567 | 111.53% | 585 | 0.17% | 604 | 0.66% | 9,322 | 304.25% |
| ΡΗΥΙΟ Γ | 36,317 | 91.11% | 0.2567 | 111.53% | 585 | 0.17% | 604 | 0.66% | 9,322 | 304.25% |
| MP | 46,312 | 143.71% | 0.2532 | 108.62% | 585 | 0.17% | 606 | 0.33% | 11,724 | 408.43% |
| 22 | 7.307 | - | 0.0710 | - | 486 | - | 549 | - | 519 | - |
| ΡΑΜΙ Γ | 18.524 | 153.51% | 0.0659 | 7.22% | 486 | 0.00% | 546 | 0.55% | 1.221 | 135.21% |
| FASTML C | 18.524 | 153.51% | 0.0659 | 7.22% | 486 | 0.00% | 546 | 0.55% | 1.221 | 135.21% |
| PAMI | 19.352 | 164.84% | 0.0768 | 8.09% | 486 | 0.00% | 546 | 0.55% | 1,486 | 186.26% |
| PHYLOF | 19,352 | 164 84% | 0.0768 | 8.09% | 486 | 0.00% | 546 | 0.55% | 1 486 | 186 26% |
| MP | 18,276 | 150.12% | 0.0656 | 7.60% | 486 | 0.00% | 546 | 0.55% | 1,199 | 131.11% |
| 23 | 16 329 | | 0.0803 | | 486 | 0.0070 | 546 | 0.0070 | 1 312 | - |
| ΡΔΜΙ Γ | 16 443 | 0 70% | 0.0000 | 4 18% | 486 | 0.00% | 546 | 0.00% | 1,312 | 3 51% |
| FASTML F | 16 443 | 0.70% | 0.0770 | 4.18% | 486 | 0.00% | 546 | 0.00% | 1,200 | 3 51% |
| PAMI | 20 125 | 23 25% | 0.0809 | 0.74% | 486 | 0.00% | 546 | 0.00% | 1,200 | 24 16% |
| | 20,125 | 23.25% | 0.0000 | 0.74% | 486 | 0.00% | 546 | 0.00% | 1,628 | 24.10% |
| MP | 18 353 | 12 40% | 0.0000 | 3 71% | 486 | 0.00% | 546 | 0.00% | 1 419 | 8.23% |
| 24 | 24 473 | 12.4070 | 0.0713 | 0.7170 | 512 | 0.0070 | 560 | 0.0070 | 1,745 | 0.2070 |
| | 24,473 | 7 80% | 0.0713 | 16 / 0% | 511 | 0.20% | 560 | 0.00% | 2 103 | 25 60% |
| | 26,404 | 7 80% | 0.0001 | 16 / 0% | 511 | 0.20% | 560 | 0.00% | 2,195 | 25.09% |
| | 26,404 | 7.89% | 0.0001 | 16.49% | 511 | 0.20% | 560 | 0.00% | 2,103 | 25.60% |
| | 25,605 | 1 99% | 0.0001 | 12.87% | 512 | 0.20% | 561 | 0.00% | 2,135 | 18 50% |
| | 25,035 | 4.3376 | 0.0000 | 7 03% | 512 | 0.00% | 560 | 0.10% | 1,000 | 12 /0% |
| 25 | 17 957 | 4.1470 | 0.0703 | 1.3370 | 272 | 0.0078 | 452 | 0.0078 | 1,501 | 12.4070 |
| | 11,007 | 20 200/ | 0.0000 | 10.20% | 200 | 6.07% | 452 | 0.000/ | 1,550 | 26.2.40/ |
| | 11,010 | 20.3070 | 0.1030 | 10.20% | 200 | 0.97 /0 6 070/ | 451 | 0.22/0 | 1,142 | 20.34 /0 |
| | 24.957 | 30.30% | 0.1030 | 13.00% | 400 | 0.97% | 401 | 0.22% | 2.459 | 20.34% |
| | 24,007 | 30.20% | 0.0303 | 12.00% | 400 | 7.2470 | 451 | 0.22/0 | 2,450 | 58 55% |
| | 11 019 | 28 20% | 0.0909 | 10.20% | 200 | 6.07% | 451 | 0.22 /0 | 2,430 | 26.3.1% |
| 26 | 25 502 | 30.3078 | 0.1030 | 19.3970 | 510 | 0.3770 | 560 | 0.2270 | 1,142 | 20.3470 |
| | 25,592 | 0.00% | 0.0720 | 0.00% | 512 | 0.00% | 560 | 0.00% | 1,042 | 0.00% |
| | 25,592 | 0.00% | 0.0720 | 0.00% | 512 | 0.00% | 560 | 0.00% | 1,042 | 0.00% |
| | 22,002 | 10.00% | 0.0720 | 5.08% | 511 | 0.00% | 560 | 0.00% | 1,042 | 6 30% |
| | 26,150 | 2 /20/ | 0.0708 | 1 64% | 512 | 0.2076 | 560 | 0.00% | 1,725 | 1 74% |
| | 25,486 | 0.41% | 0.0700 | 6.89% | 512 | 0.00% | 560 | 0.00% | 1,074 | 6.45% |
| 27 | 19,410 | 0.170 | 0.0769 | 0.0070 | 496 | 0.0070 | 500 | 0.0070 | 1,301 | 0.4370 |
| | 20 125 | 0.26% | 0.0756 | 6 700/ | 400 | 0.00% | 540 | 0.00% | 1,390 | 16 670/ |
| | 20,125 | 9.20% | 0.0609 | 6 79% | 400 | 0.00% | 540 | 0.00% | 1,020 | 16.67% |
| | 20,125 | 9.20% | 0.0809 | 6 78% | 400 | 0.00% | 546 | 0.00% | 1,020 | 16.67% |
| | 20,125 | 9.20% | 0.0009 | 6 79% | 400 | 0.00% | 540 | 0.00% | 1,020 | 16.67% |
| MP | 16,030 | 8.04% | 0.0009 | 1 75% | 486 | 0.00% | 546 | 0.00% | 1 261 | 9.64% |
| 20 | 17 200 | 0.0470 | 0.0760 | 1.7570 | 496 | - 0.00 70 | 540 | - 0.00 % | 1,201 | 3.0470 |
| | 18 524 | 7 15% | 0.0709 | 1/ 28% | 400 | 0.00% | 545 | 0 1.8% | 1,329 | 8 15% |
| | 18 524 | 7 15% | 0.0059 | 14.20% | 400 | 0.00% | 546 | 0.10% | 1 221 | 8 15% |
| | 18 524 | 7.15% | 0.0659 | 14.20% | 486 | 0.00% | 546 | 0.18% | 1 221 | 8 15% |
| | 15 052 | 7 72% | 0.0053 | 2 01% | 486 | 0.00% | 5/6 | 0.10% | 1 202 | 9.58% |
| FILLO_I | 15,855 | 1.12/0 | 0.0755 | 2.01/0 | 400 | 0.00 /0 | 540 | 0.1070 | 1,202 | 9.00% |

| MP | 18,276 | 5.71% | 0.0656 | 14.63% | 486 | 0.00% | 546 | 0.18% | 1,199 | 9.75% |
|----------------|----------------|----------------|--------|-----------------|-----|---------------|-----|---------------|--------|-----------|
| 29 | 18,034 | - | 0.0803 | - | 487 | - | 547 | - | 1,448 | - |
| PAML_Γ | 17,102 | 5.17% | 0.0775 | 3.54% | 487 | 0.00% | 547 | 0.00% | 1,325 | 8.53% |
| FASTML_Γ | 17,102 | 5.17% | 0.0775 | 3.54% | 487 | 0.00% | 547 | 0.00% | 1,325 | 8.53% |
| PAML | 17,102 | 5.17% | 0.0775 | 3.54% | 487 | 0.00% | 547 | 0.00% | 1,325 | 8.53% |
| PHYLO_Γ | 19,237 | 6.67% | 0.0815 | 1.43% | 486 | 0.21% | 547 | 0.00% | 1,567 | 8.20% |
| MP | 18,034 | 0.00% | 0.0803 | 0.00% | 487 | 0.00% | 547 | 0.00% | 1,448 | 0.00% |
| 30 | 26,360 | - | 0.2398 | - | 586 | - | 606 | - | 6,322 | - |
| ΡΑΜL Γ | 32,151 | 21.97% | 0.2486 | 3.67% | 586 | 0.00% | 604 | 0.33% | 7,993 | 26.44% |
| FASTML Γ | 32,151 | 21.97% | 0.2486 | 3.67% | 586 | 0.00% | 604 | 0.33% | 7,993 | 26.44% |
| PAML | 32,151 | 21.97% | 0.2486 | 3.67% | 586 | 0.00% | 604 | 0.33% | 7,993 | 26.44% |
| PHYLO_Γ | 37,090 | 40.71% | 0.2489 | 3.77% | 585 | 0.17% | 605 | 0.17% | 9,231 | 46.01% |
| MP | 39,835 | 51.12% | 0.2547 | 6.19% | 586 | 0.00% | 605 | 0.17% | 10,145 | 60.48% |
| 31 | 36,941 | - | 0.2528 | - | 584 | - | 602 | - | 9,339 | - |
| PAML Γ | 33,535 | 9.22% | 0.2514 | 0.54% | 584 | 0.00% | 604 | 0.33% | 8,431 | 9.71% |
| FASTML Γ | 33,535 | 9.22% | 0.2514 | 0.54% | 584 | 0.00% | 604 | 0.33% | 8,431 | 9.71% |
| PAML | 45,841 | 24.09% | 0.2437 | 3.60% | 585 | 0.17% | 605 | 0.50% | 11,171 | 19.62% |
| ρηλίο ι | 45,841 | 24.09% | 0.2437 | 3.60% | 585 | 0.17% | 605 | 0.50% | 11,171 | 19.62% |
| MP | 49,196 | 33.17% | 0.2527 | 0.03% | 585 | 0.17% | 605 | 0.50% | 12,433 | 33.14% |
| 32 | 38.038 | - | 0.2527 | - | 586 | - | 608 | - | 9.614 | - |
| PAML Γ | 31.981 | 15.92% | 0.2483 | 1.76% | 585 | 0.17% | 604 | 0.66% | 7.941 | 17.40% |
| FASTML Г | 31,981 | 15.92% | 0.2483 | 1.76% | 585 | 0.17% | 604 | 0.66% | 7,941 | 17.40% |
| PAML | 31.981 | 15.92% | 0.2483 | 1.76% | 585 | 0.17% | 604 | 0.66% | 7.941 | 17.40% |
| ΡΗΥΙΟ Γ | 28.842 | 24.18% | 0.2616 | 3.52% | 585 | 0.17% | 605 | 0.49% | 7.546 | 21.51% |
| MP | 31.981 | 15.92% | 0.2483 | 1.76% | 585 | 0.17% | 604 | 0.66% | 7,941 | 17.40% |
| 33 | 30.069 | - | 0.2572 | - | 584 | - | 604 | - | 7.733 | - |
| ΡΑΜΙ Γ | 30.873 | 2.67% | 0.2178 | 15.31% | 583 | 0.17% | 604 | 0.00% | 6.724 | 13.05% |
| FASTML T | 30.873 | 2.67% | 0.2178 | 15.31% | 583 | 0.17% | 604 | 0.00% | 6.724 | 13.05% |
| PAML | 30.873 | 2.67% | 0.2178 | 15.31% | 583 | 0.17% | 604 | 0.00% | 6.724 | 13.05% |
| ρηλίο ι | 30,873 | 2.67% | 0.2178 | 15.31% | 583 | 0.17% | 604 | 0.00% | 6,724 | 13.05% |
| MP | 30.873 | 2.67% | 0.2178 | 15.31% | 583 | 0.17% | 604 | 0.00% | 6,724 | 13.05% |
| 34 | 26,491 | - | 0.0019 | - | 402 | - | 512 | - | 51 | - |
| PAML Γ | 30.213 | 14.05% | 0.2326 | 11927.88% | 584 | 45.27% | 604 | 17.97% | 7.028 | 13617.80% |
| FASTML T | 30.213 | 14.05% | 0.2326 | 11927.88% | 584 | 45.27% | 604 | 17.97% | 7.028 | 13617.80% |
| PAML | 30,213 | 14.05% | 0.2326 | 11927.88% | 584 | 45.27% | 604 | 17.97% | 7,028 | 13617.80% |
| ρηλίο ι | 30,213 | 14.05% | 0.2326 | 11927.88% | 584 | 45.27% | 604 | 17.97% | 7,028 | 13617.80% |
| MP | 30,213 | 14.05% | 0.2326 | 11927.88% | 584 | 45.27% | 604 | 17.97% | 7,028 | 13617.80% |
| 35 | 8.629 | - | 0.0069 | - | 585 | - | 605 | - | 60 | - |
| PAML Γ | 30.213 | 250.13% | 0.2326 | 3270.90% | 584 | 0.17% | 604 | 0.17% | 7.028 | 11702.63% |
| FASTML Г | 30.213 | 250.13% | 0.2326 | 3270.90% | 584 | 0.17% | 604 | 0.17% | 7.028 | 11702.63% |
| PAML | 30,213 | 250.13% | 0.2326 | 3270.90% | 584 | 0.17% | 604 | 0.17% | 7,028 | 11702.63% |
| ΡΗΥLΟ Γ | 31,380 | 263.66% | 0.2481 | 3496.06% | 583 | 0.34% | 603 | 0.33% | 7,787 | 12977.33% |
| MP | 30,213 | 250.13% | 0.2326 | 3270.90% | 584 | 0.17% | 604 | 0.17% | 7,028 | 11702.63% |
| 36 | 24.031 | - | 0.0010 | - | 406 | - | 511 | - | 23 | - |
| ΡΑΜL Γ | 25.263 | 5.13% | 0.0010 | 1.54% | 400 | 1.48% | 511 | 0.00% | 25 | 6.75% |
| FASTML Г | 27,819 | 15.76% | 0.0011 | 12.79% | 400 | 1.48% | 511 | 0.00% | 30 | 30.57% |
| PAML | 27,819 | 15.76% | 0.0011 | 12.79% | 400 | 1.48% | 511 | 0.00% | 30 | 30.57% |
| ΡΗΥΙΟ Γ | 27,819 | 15.76% | 0.0011 | 12.79% | 400 | 1.48% | 511 | 0.00% | 30 | 30.57% |
| MP | 27,266 | 13.46% | 0.0021 | 119. <u>23%</u> | 400 | 1.4 <u>8%</u> | 511 | 0.00% | 57 | 148.74% |
| 37 | 39,333 | - | 0.2905 | - | 584 | - | 604 | - | 11,425 | - |
| PAML Г | 49,835 | 26.70% | 0.2904 | 0.04% | 584 | 0.00% | 603 | 0.1 <u>7%</u> | 14,470 | 26.65% |
| FASTML C | 49.835 | 26.70% | 0.2904 | 0.04% | 584 | 0.00% | 603 | 0.17% | 14,470 | 26.65% |
| PAML | 49,835 | 26.70% | 0.2904 | 0.04% | 584 | 0.00% | 603 | 0.17% | 14,470 | 26.65% |
| ΡΗΥΙΟ Γ | 49,835 | 26.70% | 0.2904 | 0.04% | 584 | 0.00% | 603 | 0.17% | 14,470 | 26.65% |
| MP | 49,83 <u>5</u> | 26.70 <u>%</u> | 0.2904 | 0.04% | 584 | 0.00% | 603 | 0.17% | 14,470 | 26.65% |

| b | | | | | |
|------|------------|--------|--------------------|--------------------|------------|
| Leaf | ε(M⁻¹cm⁻¹) | Φ | $\lambda_{ex}(nm)$ | $\lambda_{em}(nm)$ | Brightness |
| 1 | 43,663 | 0.2819 | 585 | 604 | 12,309 |
| 2 | 50,293 | 0.2837 | 584 | 602 | 14,270 |
| 3 | 26,750 | 0.0036 | 398 | 511 | 96 |
| 4 | 26,759 | 0.0052 | 425 | 513 | 140 |
| 5 | 61,727 | 0.2717 | 584 | 605 | 16,773 |
| 6 | 38,793 | 0.2541 | 584 | 605 | 9,858 |
| 7 | 30,084 | 8000.0 | 497 | 511 | 24 |
| 8 | 19,474 | 0.0747 | 580 | 604 | 1,455 |
| 9 | 8,572 | 0.2321 | 589 | 609 | 1,990 |
| 10 | 46,478 | 0.2412 | 584 | 603 | 11,208 |
| 11 | 27,193 | 0.0544 | 509 | 557 | 1,480 |
| 12 | 17,339 | 0.1335 | 486 | 532 | 2,314 |
| 13 | 11,352 | 0.0716 | 486 | 548 | 813 |
| 14 | 17,366 | 0.0951 | 486 | 543 | 1,651 |
| 15 | 13,477 | 0.0939 | 486 | 533 | 1,265 |
| 16 | 23,978 | 0.0061 | 510 | 565 | 145 |
| 17 | 25,406 | 0.1648 | 512 | 556 | 4,186 |
| 18 | 29,356 | 0.1348 | 398 | 451 | 3,956 |
| 19 | 10,572 | 0.1038 | 399 | 450 | 1,097 |

С

| Method | د Average Error | Φ Average Error | Brightness Average Error |
|-------------------|--------------------|----------------------|--------------------------------|
| $PAML_{\Gamma}$ | 26.31% | 13.75% | 41.89% |
| $FASTML_{\Gamma}$ | 27.02% | 14.50% | 43.48% |
| PAML | 31.06% | 14.51% | 51.49% |
| ΡΗΥLΟ_Γ | 32.31% | 13.20% | 52.36% |
| MP | 33.73% | 20.87% | 60.79% |

Nodes 34 and 35 not included due to being outliers

Supplementary Note 1

Amino acid sequences for the 37 proteins at the leaves and nodes in the experimental phylogeny.

>01

MASSEDVIKEFMRFRVSMEGSINGHEFEIEGEGEGRPYEGTQTAKLRVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYLKLSFPKGFKWERVMNFEDGGVVTVTQDSTLQDGVLIYKVKLHGINFP SDGPVMQKKTRGWEASTERMYPEDGVLKGEIKMRLKLKDGSHYEAVVKTSYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAVGRHSTGA

>02

MASSEDVIKEFMRFKVSMEGSVNGHEFEIEGDGEGRPYEGTQSAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSTLRDGILIYKVKLHGTNFP SDGPVMQKKTRGWEASTERMYPEDGVLKGEIKMRLKLINGSHYKAEVKTTYIAKKPVQLPGAYKT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>03

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGLPYEGTQVAKLRVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFRWDRIMNFEDGGVVTVIQDTSLRDGEFICKVKLRGTDFP SEGPVMQKQTMGWEASTERMYPDGGMLRGEDNMRLRLKDGGHYYAYVRTTYMAKKPVQLPDAYTI DIKLDVTSHNEDYTIVEQYERAEGRHSTGA

>04

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGRPYEGTQVAKLRVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMKFEDGGVVTVTQDTSLQNGEFICKVKLRGTGFP SEGPVMQKQTMGWEASTERMYPEDGALKGEDTMCLRLKDGGHYDAYIKTTYMAKKPVQLPGAYIV DIKLDVTSHNEDYTIVEQYERAEGRHSTGA

>05

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGRPYEGTQVAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFRWERVMKFEDGGVVTVTQDTSLQDGEFIYKVKLHGTDFP SEGPVVQKQTMGWEASTERMYPEDGALKGEIKMRLRLKGGGQYEADVKTTYMAKKPVQLPGAYIT DIKLDITYHNEDYTIVEQYERAEGRHSTGA

>06

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGVGEGRPYEGTQVAKLKVTKGGPLPFSWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDDGVVIVSQDTSLQNGEFIYKVKLRGIDFP SEGPVMQKQTMGWEASAERMYPEDGALKGEVKMRLRLKDGGHYEADVKTTYMAKKPVLLPGAYIT DIKLDIISHNEDYTIVEQYERAEGRHSTGA

>07

MASSEDVIKEFMRFKVYMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGCPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGLKWDRVMKFEDGGIVTVTQDSSLQDGVFIHKVKVRGTDFP SDGPVMRKQTMGWEASIDRMYPEDGLLKGEAKMRLKLKNGGHYDAEVKTTYMAKKQVQLPGAYII DIKLDTTSHNDDYTIVEQYERAEGRHSTGA

>08

MASSEDVIKEFMRFKVYMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPSDIPDYMKLSFPEGFKWGRVMKFEDGGVVTVTQDSSLQDGVFIYKVKLHGMNFP SDGPVMQKQTMGWEASIERMYPEDGSLKGEAKMRLKLKNGGNYDAEVKTTYMAKKSVQLPGAYII DTKLDITSHNEDYTNVEQYERAEGRHSTGA

MASSEDVIKEFMRFKVCMKGSVNGHVFEIEGEGEGRPYEGTQSVKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPANIPDYVKLSFPEGLKWVRIMNFEDGGVVTVTHDSSLQDGEFIYKVRLVGIDFP SDGPVMQKRTMGWEASTERMYPEDGALKGSVKMRLKLKDGGHYNAEVNTTYMAKKPVQLPGAYIT DIKLDITSHNDDYTVVEQYERAEGRHSTGA

>10

MASSEDVIKEFMRFKVSMEGSVNGHEFEIEGVGEGRPYEGTQSAKLKVTKGGPLPFAWDILSPQF QYGSKVYVKHPADIPDYLKLSFPEGFKWIRIMNFEDGGVVTVTQDSSLQDGVFIYKVKLHGIDFP SDGPVMQKQTMGWEASTERMYPEDGALQGAVKMRLKLKDGGRYGAEVKTTYMAKKHVQLPGAYLT DIKLDITSHNDDYTIVEQYERAEGRHSTGA

>11

MASSEDVIKDFMRFRVRMEGSVNGHEFEIEGEGEGHPYEGTQSAKLKVTKGGPLPFAWDILSPQF MWGSKAYVKHPVDIPDYMKLSFPEGFKWERVMNFEDGGVVIVTQDSSLQDGEFVYEVRLCGTNFP SDGPVMQKKTMGCAAFSERIYSEDGALKGEVKMRLRLKDGDHYEAEVNTTYKAKKAVQLPDAYII YGKLDIISHNEDYTIVEQYERAEGRHSTGA

>12

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGAGRPYEGTQAAKLKVIKGGPLPFAWDILSPQL MWGSKAYVKHPADIPDYMKLSFPEGFKWERVMIFEDGGVLTVTQDSSLQDGEFIYKVRLCGTNFP SDGPVMQKRTMGCAAISERTYPEDGALKGEVKMRLRLKDGGHYEAEVKTTYMAKKTVQLPDAYII DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>13

MASSEDVIKEFMRFKVRMVGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFRWERVMIFEDGGVVTVTQDSSLLDGEFIYEVKLCGTNFP SDGPVMQKKTMGCAAISERIYPKDGALKGEVVMRLRLKDGDHYVAEVKTTYTAKQAVPLPDAYII DGKLDIISYNEDYTIVEQYERAEGRHSTGA

>14

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWDRVMIFEDGGVVTVTQDSSLQDGVLIYKVKVRGTNFP SDGPVMQKKTMGCAAISERVYPEDGALKGRVKMRLRLKGDDYYDAEVSTTYMAKKLVQLPDAYNI DGKLDIISHNKDYTIVEQYERAEARHSTGV

>15

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVIVTHDSSLQDGVFIYKVKLCGTNFP SVGPVMQKRTMGCAAISERIYPEDGALKGEVKMRLRLKDGGHYGAEVNTTYMAKKTVQLPGAYEI DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>16

MASSEDVIKEFMRYKVRMVGSVNGHEFEIKGEGEGRPYEGTQTAILKVTKGGPLPFSWDILSPQF MWGSKAYVKHSADIPDYMKLSFPEGFKWYRVMNFEDGGVITVTHDSSLHDGEFINEVKLRGTNFP SDGPVMQKKTKGCAAFSEHIYPEDGALKGDVIMRLRLEDGDHYVAEVSTAYIAKKRVQLPDAYKI DGKLDIISHNEDYTIVEQYERAEGRHSIGA

>17

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MASSEDVIKEFMRYKVRMEGSVNGHEFEIEGVGEGRPYEGTQTAKLSVTKGGPLPFSWDILSPQF MWGSKTYVKHPPDIPDYMKQSFPEGFRWYRVMNFEDGGVITVTQDSSLQDGKFTYEVKLHGTNFP SHGPVMQKKTNGYAAFSERIYPVDGALKGDVIMRLRLKDGNHYDAQVSTIYMAKKTVQLPDEYKI NGKLDITSHNEDYTIVEQYERAEGRHSTGA

>19

MASSEDVIKEFMRYKVRMEGSINGHEFEIEGVGEGRPYEGTQTAKLRVTRGGPLPFSWDILSPQF MWGSKTYVKHPSDIPDYMKLSFPEGFRWYRVMNFEDGGVITVTQDSSLKDGEFIYEVKLHGTNFP SYGPVMQKKTIGYAAFSERMYPVDGALKGDVIMRLRLKDGSHYDAEVRTTYMAKKTVQLPEEYKI DGKLDITSHNEDYTIVEQYERAEGRHSTGA

>21

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFP SDGPVMQKKTMGWEASTERMYPEDGALKGEVKMRLKLKDGDHYDAEVNTTYMAKKPVQLPGAYKT DVKLDITSHNEDYTIVEQYERAEGRHSTGA

>22

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIYEVKLRGTNFP SDGPVMQKKTMGCAAISERIYSEDGALKGEVKMRLRLKDGDHYDAEVNTTYMAKKTVQLPDAYKI DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>23

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIYEVKLRGTNFP SDGPVMQKKTMGCAAISERIYPEDGALKGEVKMRLRLKDGDHYDAEVSTTYMAKKTVQLPDAYKI DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>24

MASSEDVIKEFMRYKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWYRVMNFEDGGVVTVTQDSSLQDGEFIYEVKLRGTNFP SDGPVMQKKTMGCAAFSERIYPEDGALKGDVIMRLRLKDGDHYDAEVSTTYMAKKTVQLPDAYKI DGKLDITSHNEDYTIVEQYERAEGRHSTGA

>25

MASSEDVIKEFMRYKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLRVTKGGPLPFSWDILSPQF MWGSKTYVKHPADIPDYMKLSFPEGFRWYRVMNFEDGGVITVTQDSSLQDGKFIYEVKLRGTNFP SYGPVMQKKTIGYAAFSERMYPEDGALKGDVIMRLRLKDGDHYDAEVSTTYMAKKTVQLPDEYKI DGKLDITSHNEDYTIVEQYERAEGRHSTGA

>26

MASSEDVIKEFMRYKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWYRVMNFEDGGVVTVTQDSSLQDGEFIYEVKLRGTNFP SDGPVMQKKTMGCAAFSERIYPEDGALKGDVIMRLRLKDGDHYDAEVSTTYMAKKTVQLPDAYKI DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>27

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGVFIYEVKLRGTNFP SDGPVMQKKTMGCAAISERIYPEDGALKGEVKMRLRLKDGDHYDAEVSTTYMAKKTVQLPDAYKI DGKLDIISHNEDYTIVEQYERAEGRHSTGA

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYEVKLCGTNFP SDGPVMQKKTMGCAAISERIYSEDGALKGEVKMRLRLKDGDHYDAEVNTTYMAKKTVQLPDAYII DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>29

MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF MWGSKAYVKHPADIPDYMKLSFPEGFKWERVMIFEDGGVVTVTQDSSLQDGEFIYEVKLCGTNFP SDGPVMQKKTMGCAAISERIYPEDGALKGEVKMRLRLKDGDHYEAEVKTTYMAKKTVQLPDAYII DGKLDIISHNEDYTIVEQYERAEGRHSTGA

>30

MASSEDVIKEFMRFKVCMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTDFP SDGPVMQKQTMGWEASTERMYPEDGALKGGVKMRLKLKDGGHYDAEVNTTYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>31

MASSEDVIKEFMRFKVCMEGSVNGHEFEIEGEGEGRPYEGTQSAKLKVTKGGPLPFAWDILSPQF QYGSKVYVKHPADIPDYVKLSFPEGFKWVRIMNFEDGGVVTVTQDSSLQDGEFIYKVKLHGIDFP SDGPVMQKQTMGWEASTERMYPEDGALKGAVKMRLKLKDGGHYDAEVNTTYMAKKPVQLPGAYIT DIKLDITSHNDDYTIVEQYERAEGRHSTGA

>32

MASSEDVIKEFMRFKVCMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTDFP SDGPVMQKQTMGWEASTERMYPEDGALKGEVKMRLKLKDGGHYDAEVKTTYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>33

MASSEDVIKEFMRFKVCMEGSVNGHEFEIEGEGEGRPYEGTQAAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMKFEDGGVVTVTQDSSLQDGVFIYKVKLRGTDFP SDGPVMQKQTMGWEASIERMYPEDGLLKGEAKMRLKLKNGGHYDAEVKTTYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>34

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGRPYEGTQVAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDTSLQDGEFICKVKLRGTDFP SEGPVMQKQTMGWEASTERMYPEDGALKGEDKMRLRLKDGGHYDADVKTTYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>35

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGRPYEGTQVAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDTSLQDGEFIYKVKLRGTDFP SEGPVMQKQTMGWEASTERMYPEDGALKGEDKMRLRLKDGGHYEADVKTTYMAKKPVQLPGAYIT DIKLDITSHNEDYTIVEQYERAEGRHSTGA

>36

MASSEDVIKEFMRFKVYLEGSVNGHEFEIEGEGEGRPYEGTQVAKLRVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYMKLSFPEGFKWDRVMNFEDGGVVTVTQDTSLQDGEFICKVKLRGTDFP SEGPVMQKQTMGWEASTERMYPEDGVLKGEDKMRLRLKDGGHYDAYVKTTYMAKKPVQLPGAYIT DIKLDVTSHNEDYTIVEQYERAEGRHSTGA

MASSEDVIKEFMRFKVSMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQF QYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSTLQDGVLIYKVKLRGTNFP SDGPVMQKKTRGWEASTERMYPEDGVLKGEIKMRLKLKDGSHYEAEVKTTYMAKKPVQLPGAYKT DIKLDITSHNEDYTIVEQYERAEGRHSTGA