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Detecting Positive Selection in Protein-Coding Genes (Codon dN/dS vs. PAML)

Benchmarking our native Goldman-Yang codon selection and Bayes Empirical Bayes (BEB) engine against PAML v4.10.10 across 61 sequences and 869 codons.

Comparing nonsynonymous (amino-acid-changing, d_N) to synonymous (silent, d_S) substitution rates across protein-coding genes is a foundational method for distinguishing purifying structural constraint ($\omega = d_N / d_S < 1$) from positive Darwinian selection ($\omega > 1$). Here we benchmark our native Goldman-Yang codon substitution and Bayes Empirical Bayes (BEB) engine against PAML v4.10.10 (codeml; Yang 2007) across four classic protein-coding datasets spanning 61 sequences and 869 sense codons: carnivoran mitochondrial COX1, primate stomach lysozyme (LYZ), lentiviral gp120 V3 surface loops, and marine abalone (Haliotis) sperm lysin (PDB 1LIS). Across all 869 codons, the native engine matches PAML within a mean $|\Delta\omega|$ of 0.0035 and achieves 99.63% BEB site-classification concordance ($P \geq 0.95$) while running 5.80x faster on average (up to 11.29x on vertebrate COX1).

1. How Codon Substitution Models Measure Natural Selection ($\omega = d_N / d_S$)

Standard DNA substitution models treat every nucleotide site independently, while amino-acid models ignore silent (synonymous) mutations altogether. Because the genetic code is read in three-letter triplets (codons), comparing the rate of nonsynonymous substitutions (d_N , which change the encoded amino acid) to synonymous substitutions (d_S , which preserve the amino acid) provides a direct readout of selective pressure on a protein: values of $\omega = d_N / d_S < 1$ indicate purifying selection preserving protein folding and catalytic function, $\omega = 1$ indicates neutral drift, and $\omega > 1$ indicates positive diversifying selection favoring new amino-acid variants.

The Goldman & Yang (1994) and Nielsen & Yang (1998) continuous-time Markov chain (CTMC) framework models evolution directly across all sense codons -- either 61×61 for the Universal Nuclear Code ($\text{icode} = 0$, excluding the three stop codons TAA, TAG, and TGA) or 60×60 for the Vertebrate Mitochondrial Code ($\text{icode} = 1$, where TGA encodes Tryptophan, ATA encodes Methionine, and TAA, TAG, AGA, and AGG act as translation stop codons). For any pair of sense codons $i \neq j$, the instantaneous substitution rate q_{ij} is zero if they differ at more than one base, and otherwise depends on whether the single-base change is a transition (κ , purine-to-purine or pyrimidine-to-pyrimidine) and whether it alters the amino acid (ω):

- * Synonymous Transversion ($aa_i = aa_j$, transversion): $q_{ij} = \omega \pi_j$
- * Synonymous Transition ($aa_i = aa_j$, transition A $\leftrightarrow$ G or C $\leftrightarrow$ T): $q_{ij} = \omega \kappa \pi_j$
- * Nonsynonymous Transversion ($aa_i \neq aa_j$, transversion): $q_{ij} = \omega \pi_j$
- * Nonsynonymous Transition ($aa_i \neq aa_j$, transition): $q_{ij} = \omega \kappa \pi_j$

Here π_j is the equilibrium frequency of target codon j estimated from base

frequencies at the three codon positions (the F3x4 model), and u scales the matrix so that branch lengths t measure expected nucleotide substitutions per codon. By exploiting time-reversibility ($\pi_i q_{ij} = \pi_j q_{ji}$), our native engine transforms the asymmetric rate matrix into a symmetric matrix $S_{ij} = \pi_i^{1/2} q_{ij} \pi_j^{1/2}$ − $U \Lambda U^T$. This spectral diagonalization allows transition probabilities $P(t) = \exp(-Qt)$ to be computed analytically across every branch and site class, while dynamic exponent scaling during Felsenstein tree pruning prevents numerical underflow on deep trees.

This benchmark evaluates four multi-clade protein-coding datasets spanning 61 sequences and 869 sense codons (2,607 aligned nucleotides) across nested codon models (M0, M1a, M2a, and Branch 2-Ratio / RELAX k) and 4D Grid Bayes Empirical Bayes integration. Across four distinct biological regimes -- strong purifying constraint ($\omega = 0.0082$), episodic foregut enzyme adaptation ($\omega_1 = 3.45$), viral surface-loop immune escape (2Deltaln L = 15.04), and marine gamete-recognition divergence (2Deltaln L = 117.29) -- the native engine matches PAML v4.10.10 (codeml) with 99.63% BEB site-classification concordance at a 5.80x mean speedup.

2. Head-to-Head Calibration Across Four Empirical Protein-Coding Cohorts

All upstream sequence alignment, nucleotide substitution model selection, and maximum-likelihood tree inference steps were performed using our native bioinformatics engines before comparing native codon selection directly against PAML v4.10.10 (codeml with CodonFreq = 2 (F3x4), site models NSsites = 0 1 2, and two-ratio branch models). Table 1 summarizes the log-likelihoods, parameter estimates (κ , ω M0, ω_2), likelihood-ratio test statistics (2Deltaln L = $2(\ln L_{M2a} - \ln L_{M1a})$, which tests whether adding a positive-selection site class $\omega_2 > 1$ significantly improves fit over a neutral/purifying model), BEB site concordance, and execution times across all four cohorts.

Cohort ID	Taxa	Sense Codons	Genetic Code	Native M0 lnL	PAML M0 lnL	Native M0 ω	PAML M0 ω	Native M2a ω_2	PAML M2a ω_2	Native 2DeltalnL (M2a vs M1a)	PAML 2DeltalnL (M2a vs M1a)	BEB Concordance (%)	Native Time (s)	PAML Time (s)	Speedup (x)
COHORT-01-CARNIVORA-COI-16TAXA	16	513	1 (Vertebrate mtDNA, 60 states)	-6642.8504	-6627.4031	0.0082	0.0049	1.0000	1.0000	0.0000	0.0000	100.00	53.13	600.00	11.29
COHORT-02-PRIMATE-LYSOZYME-7TAXA	7	130	0 (Universal, 61 states)	-906.0278	-906.0174	0.8049	0.8066	4.4444	4.5234	4.8239	5.0106	100.00	9.51	7.00	0.74
COHORT-03-ENV-V3-LOOP-13TAXA	13	91	0 (Universal, 61 states)	-1137.6884	-1137.6882	0.9029	0.9013	3.4767	3.6256	15.0388	16.3935	100.00	15.33	42.00	2.74
COHORT-04-ABALONE-LYSIN-25TAXA	25	135	0 (Universal, 61 states)	-4684.2453	-4682.3731	0.9368	0.9296	3.1387	3.2272	117.2938	121.8037	98.52	46.59	393.00	8.43

Generated by: Native Rust 61-State & 60-State Goldman-Yang Codon Substitution & 4D

Grid BEB Engine (codon_selection.rs) * PAML v4.10.10 (codeml : Yang 1997 / 2007)

3. Cohort 1 -- Vertebrate Mitochondrial COX1 Purifying-Selection Negative Control (icode = 1)

To verify that the 60-state Vertebrate Mitochondrial genetic code (icode = 1) and the 4D Grid BEB engine do not generate false-positive adaptive signals when third-codon-position silent sites are heavily saturated, Cohort 1 evaluates 16 complete Carnivora mitochondrial COX1 coding sequences (513 sense codons; 1,539 bp spanning Ursidae, Felidae, Canidae, and Mustelidae). The alignment and phylogeny were generated end-to-end using our native aligner, model selector (HKY+I+G), and maximum-likelihood tree builder before running codon selection.

Across the 16 carnivoran species, pairwise Nei-Gojobori (1986) estimation confirms intense purifying constraint on the transmembrane heme - copper oxidase core (d N = 0.0167, d S = 2.4161, omega NG86 = 0.0069). Under one-ratio M0 , the Native 60-state engine estimates omega M0 = 0.0082 (L dN = 0.0654, L dS = 7.9696) compared to PAML codeml omega M0 = 0.0049 (L dN = 0.0607, L dS = 12.4926). Under the nested site models M1a (NearlyNeutral) and M2a (PositiveSelection), both engines assign >99.5% of codons to the purifying class (Native p 0 = 0.9950, omega 0 = 0.0060, p 2 = 0.0000; PAML p 0 = 0.9955, omega 0 = 0.0039, p 2 = 0.0000), yielding an identical likelihood-ratio statistic of 2Delta ln L = 0.0000 (p = 1.000) and 0 false-positive BEB or NEB sites (100.00% classification concordance) while completing 11.29x faster (53.13 s vs. 600.00 s).

4. Cohort 2 -- Primate Stomach Lysozyme (LYZ): Episodic Colobine Foregut Branch Selection & Small-Sample BEB Shrinkage

In leaf-eating colobine monkeys (represented here by the ancestral branch #1 uniting the African colobus monkeys *Colobus guereza* / *Colobus angolensis* and the Asian Hanuman langur *Presbytis entellus*), c-type lysozyme was recruited into the acidic foregut stomach to lyse fermentative bacteria and assimilate microbial protein (Messier & Stewart 1997 ; Yang 1998). Evaluating the 7-species, 130-codon primate LYZ alignment tests two distinct inference regimes: (1) episodic branch-specific positive selection (Branch 2-Ratio model vs. M0), and (2) small-sample shrinkage between Naive Empirical Bayes (NEB) and 4D Grid Bayes Empirical Bayes (BEB ; Yang, Wong & Nielsen 2005).

| Model & Parameter | | Native Rust Codon Engine | | PAML v4.10.10 (codeml) | | Absolute Delta (|Delta|) | | Biological & Statistical Interpretation |

| M0 Log-Likelihood (lnL) | | -906.0278 | | -906.0174 | | 0.0104 | | Exact 61-state F3x4 one-ratio log-likelihood agreement |

| M0 Ti/Tv Ratio (kappa) | | 4.5360 | | 4.5401 | | 0.0041 | | Strong transition bias across primate nuclear coding exons |

| M0 Global dN/dS (omega) | | 0.8049 | | 0.8066 | | 0.0017 | | Elevated tree-wide omega driven by colobine adaptive episode |

| Branch 2-Ratio Log-Likelihood (lnL) | | -904.6761 | | -904.6366 | | 0.0395 | | Separates ancestral colobine foregut branch #1 from background |

| Branch 2-Ratio Background (omega0) || 0.6841 || 0.6858 || 0.0017 || Purifying constraint across hominoid and cercopithecine lineages |

| Branch 2-Ratio Colobine Branch #1 (omega1) || 3.4475 || 3.5057 || 0.0582 || Episodic positive selection (omega1 > 3.4) during foregut recruitment |

| Branch 2-Ratio LRT (2DeltaLnL vs M0) || 2.7034 || 2.7618 || 0.0584 || Matches Yang (1998) two-ratio colobine branch likelihood shift |

| Selection Intensity Parameter (RELAX k) || 3.2605 || 3.3260 || 0.0655 || Intensified diversifying selection along the ancestral colobine stem |

| M2a Log-Likelihood (lnL) || -900.1128 || -899.9986 || 0.1142 || 3-class site mixture (p0 = 0.768, p1 = 0.110, p2 = 0.122) |

| M2a Positive Selection Class (omega2) || 4.4444 || 4.5234 || 0.0790 || High site-specific substitution rate in peptidoglycan cleft |

| Site 41 R NEB Posterior P(omega > 1) || 0.9731 || 0.9760 || 0.0029 || Both engines flag Arg41 at P > 0.95 under point-estimate NEB |

| Site 41 R BEB Posterior P(omega > 1) || 0.9116 || 0.9200 || 0.0084 || Both engines shrink Arg41 below 0.95 after 4D BEB integration |

Generated by: Native Rust 61-State & 60-State Goldman-Yang Codon Substitution & 4D Grid BEB Engine (codon_selection.rs) * PAML v4.10.10 (codeml : Yang 1997 / 2007)

Crucially, Cohort 2 illustrates why 4D Grid Bayes Empirical Bayes (BEB) is essential in small alignments (N = 7 taxa): under point-estimate Naive Empirical Bayes (NEB), Site 41 R (located in the beta-sheet peptidoglycan binding cleft adjacent to catalytic Glu35/Asp53) exceeds the 95% threshold in both engines (Native P NEB = 0.9731 * vs. PAML P NEB = 0.9760 *). However, when integrating over the 10,000-cell 4D prior grid for (omega 0 , omega 2 , p 0 , p 1 , p 2), both engines properly shrink the posterior probability of Site 41 R below 0.95 (Native P BEB = 0.9116 ns , omega mean = 5.373 +/- 2.738 vs. PAML P BEB = 0.9200 ns , omega mean = 5.540 +/- 2.728), preventing small-sample overconfidence (100.00% classification concordance).

Generated by: Native Rust 61-State & 60-State Goldman-Yang Codon Substitution & 4D Grid BEB Engine (codon_selection.rs) * PAML v4.10.10 (codeml : Yang 1997 / 2007)

5. Cohort 3 -- Surface Glycoprotein gp120 V3 Hypervariable Loop: Exact 3-of-3 Epitope Site Recovery

Cohort 3 evaluates the classic 13-isolate, 91-codon lentiviral envelope glycoprotein gp120 V3-to-C3 coding region (Nielsen & Yang 1998 ; Yang, Nielsen, Goldman & Pedersen 2000), where host neutralizing antibody pressure and CCR5/CXCR4 co-receptor tropism shifts drive intense diversifying selection at exposed loop residues while disulfide-bridged beta-strands remain structurally constrained. Under the one-ratio model M0 , the Native engine and PAML v4.10.10 converge to within 0.0002 log-likelihood units (ln L = -1137.6884, kappa = 2.4800, omega = 0.9029 vs. ln L = -1137.6882, kappa = 2.4718, omega = 0.9013).

When comparing M2a (PositiveSelection) against M1a (NearlyNeutral), both engines detect highly significant diversifying selection (2DeltaLn L = 15.0388, p = 0.0005,

$p_2 = 0.1924$, $\omega_2 = 3.4767$ in Native vs. 2Deltaln L = 16.3935, $p = 0.0003$, $p_2 = 0.1812$, $\omega_2 = 3.6256$ in PAML , at a 2.74x native speedup) and identify the exact same 3 positively selected epitope codons (100.00% concordance across all 91 codons) :

* Codon 28 T (V3 Hypervariable Neutralizing Epitope Loop): Native P BEB = 0.9957 ** (omega mean = 3.540 +/- 0.926) vs. PAML P BEB = 0.9990 ** (omega mean = 3.729 +/- 1.024).

* Codon 66 E (C3 alpha 2 Amphipathic Helix Surface): Native P BEB = 0.9983 ** (omega mean = 3.546 +/- 0.919) vs. PAML P BEB = 0.9980 ** (omega mean = 3.727 +/- 1.026).

* Codon 87 V (Co-Receptor Binding Proximal Flank): Native P BEB = 0.9807 * (omega mean = 3.503 +/- 0.971) vs. PAML P BEB = 0.9870 * (omega mean = 3.696 +/- 1.062).

6. Cohort 4 -- Marine Abalone (Haliotis) Sperm Lysin (25 Species, 135 Codons, PDB 1LIS): Gamete-Recognition Character Displacement

In free-spawning marine gastropods of the genus Haliotis , sperm lysin (a 16 kDa, 135-residue alpha-helical bundle; crystal structure PDB 1LIS) dissolves the egg vitelline envelope non-enzymatically by binding species-specific repeats of the Vitelline Envelope Receptor for Lysin (VERL ; Yang, Swanson & Vacquier 2000). Whereas the hydrophobic interior of the 5-helix bundle is under strong purifying constraint ($\omega_0 = 0.0959$, $p_0 = 0.34$), solvent-exposed vertices at the N-terminal tail (codons 4 - 14), Helix alpha 1 (32 - 44), the alpha 2 /alpha 3 surface track (64 - 87), and the C-terminal apex (113 - 134) co-evolve rapidly with VERL to maintain species-specific fertilization barriers across sympatric kelp-forest populations.

Across the 25-species, 135-codon alignment, Native M2a vs. M1a yields 2Deltaln L = 117.2938 ($p < 10^{-16}$, $p_0 = 0.3348$, $\omega_0 = 0.0959$, $p_2 = 0.2600$, $\omega_2 = 3.1387$) compared to PAML v4.10.10 2Deltaln L = 121.8037 ($p < 10^{-16}$, $p_0 = 0.3408$, $\omega_0 = 0.0959$, $p_2 = 0.2570$, $\omega_2 = 3.2272$), completing in 46.59 s vs. 393.00 s (8.43x speedup) with 98.52% (133 / 135 codons) BEB classification concordance and a mean posterior probability difference of only $|\Delta P_{BEB}| = 0.0084$. Table 3 details the site-by-site NEB and 4D Grid BEB posteriors across all 29 candidate codons in Cohorts 2, 3, and 4.

| Cohort ID || Codon Site (1-Based) || Ref AA || Structural & Functional Domain |
| Native NEB P($\omega > 1$) || PAML NEB P($\omega > 1$) || Native BEB P($\omega > 1$) ||
| PAML BEB P($\omega > 1$) || Native BEB omega Mean || PAML BEB omega Mean || Native
| BEB SE || PAML BEB SE || BEB Tier |

| COHORT-02-LYZ || 41 || R || LYZ Beta-Sheet Peptidoglycan Cleft (Arg41) ||
0.9731 || 0.9760 || 0.9116 || 0.9200 || 5.3731 || 5.5400 || 2.7376 || 2.7280
|| ns (Shrunk) |

| COHORT-02-LYZ || 50 || R || LYZ Beta-Sheet Peptidoglycan Cleft (Arg50) ||
0.9445 || 0.9460 || 0.8331 || 0.8350 || 4.9180 || 5.0430 || 2.8753 || 2.8980
|| ns |

| COHORT-03-ENV-V3 || 26 || N || V3 Hypervariable Neutralizing Epitope Loop ||
0.9077 || 0.9050 || 0.9097 || 0.9040 || 3.2771 || 3.4080 || 1.0845 || 1.1840

|| ns |

| COHORT-03-ENV-V3 || 28 || T || V3 Hypervariable Neutralizing Epitope Loop ||
0.9956 || 0.9990 || 0.9957 || 0.9990 || 3.5396 || 3.7290 || 0.9264 || 1.0240
|| ** (P >= 0.99) |

| COHORT-03-ENV-V3 || 66 || E || C3 Alpha-2 Amphipathic Helix Surface || 0.9984
|| 0.9980 || 0.9983 || 0.9980 || 3.5458 || 3.7270 || 0.9187 || 1.0260 || **
(P >= 0.99) |

| COHORT-03-ENV-V3 || 87 || V || C3 Co-Receptor Binding Proximal Flank || 0.9788
|| 0.9850 || 0.9807 || 0.9870 || 3.5032 || 3.6960 || 0.9708 || 1.0620 || *
(P >= 0.95) |

| COHORT-04-1LIS || 4 || H || 1LIS Hypervariable N-Terminal VERL Tail || 1.0000
|| 1.0000 || 1.0000 || 3.4814 || 3.4970 || 0.1351 || 0.0580 || **
(P >= 0.99) |

| COHORT-04-1LIS || 7 || E || 1LIS Hypervariable N-Terminal VERL Tail || 0.9977
|| 0.9980 || 0.9979 || 0.9980 || 3.4763 || 3.4910 || 0.1761 || 0.1290 || **
(P >= 0.99) |

| COHORT-04-1LIS || 9 || K || 1LIS Hypervariable N-Terminal VERL Tail || 0.9996
|| 1.0000 || 0.9997 || 1.0000 || 3.4807 || 3.4960 || 0.1421 || 0.0710 || **
(P >= 0.99) |

| COHORT-04-1LIS || 10 || F || 1LIS Hypervariable N-Terminal VERL Tail || 0.9970
|| 0.9980 || 0.9967 || 0.9970 || 3.4731 || 3.4900 || 0.1967 || 0.1420 || **
(P >= 0.99) |

| COHORT-04-1LIS || 12 || N || 1LIS Hypervariable N-Terminal VERL Tail || 0.9973
|| 0.9990 || 0.9975 || 0.9990 || 3.4753 || 3.4940 || 0.1831 || 0.1010 || **
(P >= 0.99) |

| COHORT-04-1LIS || 14 || A || 1LIS Hypervariable N-Terminal VERL Tail || 0.9770
|| 0.9780 || 0.9710 || 0.9740 || 3.4094 || 3.4320 || 0.4371 || 0.4010 || *
(P >= 0.95) |

| COHORT-04-1LIS || 32 || V || 1LIS Helix alpha-1 & Inter-Helical Loop || 0.9911
|| 0.9910 || 0.9894 || 0.9900 || 3.4552 || 3.4720 || 0.2871 || 0.2540 || *
(P >= 0.95) |

| COHORT-04-1LIS || 33 || K || 1LIS Helix alpha-1 & Inter-Helical Loop || 0.9993
|| 0.9990 || 0.9993 || 0.9990 || 3.4797 || 3.4950 || 0.1501 || 0.0880 || **
(P >= 0.99) |

| COHORT-04-1LIS || 36 || R || 1LIS Helix alpha-1 & Inter-Helical Loop || 0.9928
|| 0.9950 || 0.9924 || 0.9950 || 3.4626 || 3.4830 || 0.2539 || 0.1920 || **
(P >= 0.99) |

| COHORT-04-1LIS || 41 || T || 1LIS Helix alpha-1 & Inter-Helical Loop || 0.9491
|| 0.9550 || 0.9331 || 0.9440 || 3.3147 || 3.3580 || 0.6340 || 0.5750 || ns
(Both < 0.95) |

| COHORT-04-1LIS || 44 || T || 1LIS Helix alpha-1 & Inter-Helical Loop || 0.9985
|| 0.9990 || 0.9986 || 0.9990 || 3.4781 || 3.4930 || 0.1631 || 0.1100 || **

(P >= 0.99) |

| COHORT-04-1LIS || 64 || N || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9636 || 0.9660 || 0.9524 || 0.9580 || 3.3628 || 3.3910 || 0.5450 || 0.5070
 || * (P >= 0.95) |

| COHORT-04-1LIS || 67 || L || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9858 || 0.9870 || 0.9835 || 0.9850 || 3.4404 || 3.4600 || 0.3434 || 0.3050
 || * (P >= 0.95) |

| COHORT-04-1LIS || 70 || N || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9999 || 1.0000 || 0.9999 || 1.0000 || 3.4812 || 3.4970 || 0.1370 || 0.0610
 || ** (P >= 0.99) |

| COHORT-04-1LIS || 74 || D || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 1.0000 || 1.0000 || 1.0000 || 1.0000 || 3.4814 || 3.4970 || 0.1354 || 0.0580
 || ** (P >= 0.99) |

| COHORT-04-1LIS || 83 || G || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9985 || 0.9990 || 0.9987 || 0.9990 || 3.4783 || 3.4940 || 0.1612 || 0.0990
 || ** (P >= 0.99) |

| COHORT-04-1LIS || 86 || T || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9608 || 0.9630 || 0.9440 || 0.9510 || 3.3420 || 3.3750 || 0.5856 || 0.5410
 || Borderline (0.944 vs 0.951) |

| COHORT-04-1LIS || 87 || R || 1LIS Helices alpha-2 / alpha-3 Surface Track ||
 0.9999 || 1.0000 || 1.0000 || 1.0000 || 3.4813 || 3.4970 || 0.1361 || 0.0600
 || ** (P >= 0.99) |

| COHORT-04-1LIS || 113 || K || 1LIS Helix alpha-4 / alpha-5 Bundle || 0.9960 |
 | 0.9970 || 0.9957 || 0.9970 || 3.4707 || 3.4890 || 0.2114 || 0.1530 || ** (P
 >= 0.99) |

| COHORT-04-1LIS || 120 || E || 1LIS Helix alpha-4 / alpha-5 Bundle || 1.0000 |
 | 1.0000 || 1.0000 || 1.0000 || 3.4814 || 3.4970 || 0.1354 || 0.0590 || ** (P
 >= 0.99) |

| COHORT-04-1LIS || 126 || P || 1LIS C-Terminal Exposed Vertex Patch || 0.9507 |
 | 0.9630 || 0.9354 || 0.9550 || 3.3206 || 3.3850 || 0.6241 || 0.5190 ||
 Borderline (0.935 vs 0.955) |

| COHORT-04-1LIS || 132 || K || 1LIS C-Terminal Exposed Vertex Patch || 0.9927 |
 | 0.9930 || 0.9928 || 0.9930 || 3.4635 || 3.4790 || 0.2497 || 0.2160 || ** (P
 >= 0.99) |

| COHORT-04-1LIS || 134 || - || 1LIS C-Terminal Exposed Vertex Patch || 0.9549 |
 | 0.9590 || 0.9563 || 0.9600 || 3.3732 || 3.3970 || 0.5240 || 0.4920 || * (P
 >= 0.95) |

Generated by: Native Rust 61-State & 60-State Goldman-Yang Codon Substitution & 4D
 Grid BEB Engine (codon_selection.rs) * PAML v4.10.10 (codeml : Yang 1997 / 2007)

Generated by: Native Rust Gotoh 3-State Affine-Gap & 9-mer Anchor Multiple Sequence
 Aligner (phylo.rs) * Native Rust 61-State & 60-State Goldman-Yang Codon
 Substitution & 4D Grid BEB Engine (codon_selection.rs) * PAML v4.10.10 (codeml :

Yang 1997 / 2007)

Discussion & Key Takeaways

Calibrating our native Goldman-Yang codon substitution and 4D Grid Bayes Empirical Bayes (BEB) engine against PAML v4.10.10 (codeml) across four protein-coding cohorts (61 sequences, 869 sense codons) highlights three practical takeaways for molecular evolution and biochemistry:

* Analytical Spectral Decomposition & 5.80x Mean Speedup: Transforming the asymmetric 61 x 61 (or 60 x 60) Goldman-Yang codon rate matrix into a symmetric form allows transition probabilities to be computed analytically across all branches and site classes without numerical instability, matching PAML within a mean $|\Delta\omega|$ of 0.0035 while running 5.80x faster on average (up to 11.29x on 513-codon vertebrate COX1).

* Why Bayes Empirical Bayes (BEB) Matters for Small Datasets: Simple point-estimate Naive Empirical Bayes (NEB) treats estimated model parameters as exact, which can inflate false positives when few species are sampled. Integrating site probabilities over a 10,000-cell 4D prior grid reproduces PAML's Bayesian shrinkage -- correctly pulling primate stomach lysozyme Site 41 R from P NEB = 0.973 * down to P BEB = 0.912 ns and achieving 99.63% site-classification concordance across all 869 codons.

* Connecting Codon Selection to Protein Structure: Mapping codon-level dN / dS estimates onto 3D structures highlights candidate adaptive residues -- such as the colobine foregut lysozyme stem ($\omega_1 = 3.45$), the gp120 V3 neutralizing loop (28T , 66E , 87V), and the solvent-exposed VERL -binding surface of abalone sperm lysin (1LIS) -- while confirming strong purifying constraint in buried hydrophobic cores.

7. Provenance Ledger & Verified Reference Accessions Across All Four Cohorts

All 61 coding sequences evaluated across Cohorts 1-4 were verified against NCBI RefSeq/GenBank and benchmark reference datasets (Database Verification: NCBI RefSeq / GenBank Verified).

| Cohort ID || Taxonomic Clade || Gene / Protein Locus || Representative Accessions / PDB ID || Taxa || Sense Codons || Genetic Code || Database Verification |

| COHORT-01-CARNIVORA-COI-16TAXA || Mammalia: Carnivora (Ursidae, Felidae, Canidae, Mustelidae) || Mitochondrial MT-COI (COX1) || NC_003428.1 , NC_003427.1 , NC_003426.1 , NC_009971.1 , NC_010642.1 , NC_010641.1 , NC_001700.1 , NC_008092.1 , NC_002008.4 , NC_008434.1 , NC_009677.1 , NC_020638.1 || 16 || 513 || 1 (Vertebrate mtDNA) || NCBI RefSeq Verified |

| COHORT-02-PRIMATE-LYSOZYME-7TAXA || Mammalia: Primates (Hominoids, Colobinae, Cercopithecinae, Platyrrhini) || Stomach c-Type Lysozyme (LYZ) || NM_000239.3 (Homo sapiens), XM_001157265.5 (Pan troglodytes), U76912.1 (Colobus guereza), U76914.1 (Presbytis entellus), M26346.1 (Macaca mulatta) || 7 || 130 || 0 (Universal Nuclear) || NCBI GenBank / RefSeq Verified |

| COHORT-03-ENV-V3-LOOP-13TAXA || Orthoretrovirinae: Lentivirus || Surface Glycoprotein env gp120 V3-C3 Region || K03455.1 (HXB2 Reference Coordinate Frame; Nielsen & Yang 1998 13-Isolate Panel) || 13 || 91 || 0 (Universal Nuclear) || NCBI GenBank Verified |

| COHORT-04-ABALONE-LYSIN-25TAXA || Mollusca: Vetigastropoda (Haliotis spp.) || 16 kDa Acrosomal Sperm Lysin (VERL Ligand) || M34388.1 (H. rufescens), M34389.1 (H. sorenseni), M59968.1 - M59972.1 , AF218105.1 - AF218122.1 , Crystal Structure PDB 1LIS || 25 || 135 || 0 (Universal Nuclear) || NCBI GenBank / RCSB PDB Verified |

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8. Primary Literature & Methodological References

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