RuNIE (Rust Native Inference Engine): An Open-Source Architecture for Autonomous Bioinformatics Pipeline Synthesis, Dual-Pass Blinded Validation, and Bayesian Scope-Depth Auditing

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ABSTRACT

Modern comparative genomics, structural bioinformatics, and molecular systematics rely heavily on multi-stage computational pipelines assembled from heterogeneous command-line binaries connected via ad-hoc shell scripts and intermediate flat files. While the individual algorithms within these workflows are often mathematically rigorous, their ad-hoc concatenation introduces four systemic vulnerabilities: (i) silent data truncation, reading-frame phase loss, orientation errors, and coordinate-frame mismatches across intermediate text-file handoffs; (ii) severe information destruction via global alignment column masking, which discards up to 64.8% of alignment columns and 99.4% of biophysical phase-separation, alternative splicing, and clinical truncation signal in multi-domain proteins; (iii) metadata confirmation bias when unflagged public repository misannotations—such as nuclear mitochondrial (`NUMT`) and plastid (`NUPT`) pseudogenes, horizontal gene transfer (`HGT`) islands, and metagenomic bin chimeras—are propagated into downstream inference; and (iv) statistical inconsistency in genome-scale supermatrices ($L \to 10^6$–10^7 bp), where sampling variance collapses ($\sigma^2 \propto 1/L \to 0$) and classical frequentist p-values ($p < 10^{-300}$) and non-parametric bootstrap percentages (UFBoot = 100.0%) lock onto topologically incorrect trees whenever systematic model misspecification ($\varepsilon_{sys} > \delta_{phy}$) exceeds sampling noise.

Here we present **CRV Science Labs** (`bio-suite v0.3.0`) and **RuNIE** (**Rust Native Inference Engine**, `v1.27.0`), a free, open-source (`GNU GPL-3.0`) computational bioinformatics platform written in multi-threaded Rust (`rayon` work-stealing parallelism + `candle-core` CPU tensors). **RuNIE** replaces fragile file-based scripting with a zero-copy, strongly typed Directed Acyclic Graph (DAG) operator vocabulary spanning **35 head-to-head calibrated native Rust tools**, evaluated via four-chain parallel-tempering Genetic-Algorithm Markov Chain Monte Carlo (`GA-MCMC`, $\beta \in \{0.25, 0.50, 1.00, 2.00\}$) and multi-model bootstrap stability auditing ($B \in \{50, 500, 1000\}$). Every native Rust operator is calibrated on a strict **Development Set**, parameter-frozen, and verified zero-shot on an **Untouched Reserve Set** across **42 curated validation datasets** (`VAL-SET-001`–`VAL-SET-042`) against compiled external reference binaries (`MAFFT`, `jModelTest`, `IQ-TREE 2`, `ASTRAL`, `PAML`, `DIAMOND`, `Minimap2`, `Prodigal`, `HMMER3`, `G4Hunter`, `Z-Hunt-II`, `ViennaRNA`, `US-align`, `fpocket`, `AutoDock Vina`, `minigraph`, `AMRFinderPlus`, `GECCO`, `MACSE`, `MrBayes`, `SciPy`, and `Biopython`), achieving $r \in [0.9906, 1.0000]$ numerical concordance and 4.06× to 4,427× execution speedups. To eliminate frequentist supermatrix false positives and metadata confirmation

bias, we introduce an orthogonal **3-Tier Research Scope System** (`Tier 1: Exploratory`, `Tier 2: Validation`, `Tier 3: Scientific`) coupled with a **0–5 Beaker Bayesian Prior-to-Posterior Signal Depth System** ($B_{\text{prior}} \rightarrow B_{\text{posterior}}$, calibrated against **54 logged 0-Beaker negative controls**) and a **Dual-Pass SHA-256 Blinded Validation Protocol** ($D_{\text{ctx}} = |Z_{\text{blind}} - Z_{\text{ctx}}|$). We detail quantitative findings across **109 published Lab Discovery reports**—focusing on **26 Flagged Critical Reports** spanning population genetics, eukaryogenesis, organellar endosymbiosis, basal metazoan macrosynteny, dual-track sub-domain partitioning, and fossil-calibrated relaxed molecular clock time-trees—and provide a practical CLI cookbook for executing native Rust operators alongside their external reference counterparts.

Keywords: RuNIE (Rust Native Inference Engine); automated bioinformatics pipelines; Bayesian workflow synthesis; parallel-tempering MCMC; dual-pass blinded validation; phylogenetic model misspecification; relaxed molecular clocks; sub-domain partitioning.

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1. Introduction and Architectural Motivation

1.1 The Data-Scale vs. Software-Architecture Paradox in Modern Bioinformatics

Over the past two decades, primary data generation in the life sciences has scaled faster than Moore's Law. Contemporary comparative genomics and structural biology routinely operate on telomere-to-telomere chromosomal assemblies such as human `T2T-CHM13` (3.05×10^9 bp) [1], repositories of over 214 million

predicted protein structures in the AlphaFold Database (`AFDB`) [2], multi-individual reference pangenome variation graphs (`rGFA`) built across diverse human haplotypes [3, 4], and deep-time phylogenomic supermatrices spanning hundreds of taxa and millions of aligned nucleotide or amino-acid positions [5–7].

Yet a striking architectural paradox persists at the heart of computational biology: while experimental sequencing and cryo-EM/X-ray structural determination have industrialized into high-precision, automated platforms, the computational pipelines used to synthesize these datasets remain overwhelmingly dependent on a 1990s Unix glue-script paradigm. In standard practice, an investigator chains together five to ten standalone executables—written in heterogeneous languages (C, C++, Fortran, Java, Perl, Python, and R) by independent research groups over three decades—using bespoke shell scripts or external workflow wrappers that serialize and re-parse flat text files (`.fasta`, `.phylip`, `.sam`, `.newick`, `.gff3`, `.vcf`, `.pdb`) at every intermediate step.

While the core mathematical algorithms implemented inside established community tools—such as progressive and iterative multiple sequence alignment in `MAFFT` [8], maximum-likelihood tree search and ultrafast bootstrapping in `IQ-TREE 2` [9], codon substitution modeling in `PAML` (`codeml`) [10], quartet-based multispecies coalescent inference in `ASTRAL` [11], profile Hidden Markov Model (Plan-7 HMM) domain scanning in `HMMER3` [12], minimizer-based whole-genome alignment in `Minimap2` [13], and nearest-neighbor RNA partition functions in `ViennaRNA` [14]—represent foundational achievements of quantitative biology, their ad-hoc concatenation through untyped text files creates severe methodological blind spots. Because each binary operates as an isolated black box unaware of upstream filtering decisions or downstream statistical assumptions, errors introduced at early stages silently compound across the workflow.

1.2 Four Systemic Failure Modes of Ad-Hoc Multi-Executable Workflows

Through systematic calibration audits conducted during the development of **CRV Science Labs**, we identified four recurring failure modes that afflict conventional multi-stage bioinformatics pipelines:

1.2.1 Failure Mode 1: Silent Inter-Stage Serialization Corruption, Reading-Frame Phase Drift, and Chimeric Contig Pass-Through

When sequence records pass from homology search (`BLASTN` / `BLASTP`) into multiple sequence alignment (`MAFFT` / `MUSCLE`) and subsequently into codon selection (`PAML`) or phylogenetic inference (`IQ-TREE`), flat-file serialization strips critical structural invariants: (i) **Orientation and Chimera Blindness**, where reverse-complemented contigs (5' ← 3') or metagenomic host-symbiont chimeras are globally aligned without strand/synteny verification; (ii) **Reading-Frame Phase Loss and Homopolymer Frameshifts**, where ±1 bp or ±2 bp indels in homopolymer runs break triplet reading frames and induce false premature stop codons [15]; and (iii) **Coordinate-Frame and Chirality Mismatches in 3D Structural Pipelines**, where unguarded improper rotations (det $\mathbf{R} = -1$ chiral reflections in unconstrained SVD) or 1-indexed PDB vs. 0-indexed array offsets silently corrupt active-site superpositions.

1.2.2 Failure Mode 2: Information Destruction via Global Alignment Masking

To combat alignment uncertainty in divergent protein families, standard phylogenomic pipelines routinely apply global column-masking filters (e.g., `Gblocks`, strict `trimAl`, or high-occupancy gap filters) that delete all gapped, rapidly evolving, or low-complexity columns prior to downstream analysis. As we demonstrate in

Critical Report `#102` across 25 protein superfamilies (26.86 Mb), global alignment masking discards up to 64.8% of alignment columns and destroys **99.4% of the mutual information** encoding liquid-liquid phase separation saturation concentrations (c_{sat}), developmental cassette-exon Percent-Spliced-In ($\Delta\Psi$) regulation, mechanical entropic spring stiffness in sarcomeric Titin (`TTN`, 35,991 aa), and ion-channel pharmacophore selectivity—while leaving compositional heterogeneity inside the retained folded cores (RCFV > 0.08) uncorrected.

1.2.3 Failure Mode 3: Metadata Confirmation Bias and Repository Contamination (`NUMTs`, `NUPTs`, `HGT`, and MAG Chimeras)

Public sequence repositories contain pervasive, unflagged annotation errors: (i) **Nuclear Mitochondrial (`NUMT`) and Nuclear Plastid (`NUPT`) Pseudogenes** released from purifying organellar selection ($\omega \approx 0.02$–$0.05 \rightarrow \omega \approx 0.85$–$0.95$, 2nd-codon entropy $H_{pos2} > 0.40$ bits) [16], which pull divergence time estimates backward by 8–38 Myr when misannotated as authentic organellar sequences (Critical Reports `#73`, `#98`, `#108`, `#109`); and (ii) **Horizontal Gene Transfer (`HGT`) Islands and Metagenomic Bin Chimeras** (such as chlamydial `NTT1` translocases in Microsporidia or late HGT histones in giant viruses, Critical Reports `#84`, `#86`).

1.2.4 Failure Mode 4: Statistical Inconsistency of Classical Frequentist p-Values and Bootstraps in Genome-Scale Supermatrices

Classical frequentist tests ($p < 0.05$) and Felsenstein's non-parametric bootstrap [17] were designed for small samples where random sampling variance σ^2/L dominates. In a genome-scale supermatrix of $L = 9.2 \times 10^6$ bp, random sampling variance collapses ($\sigma_{boot} \propto L^{-1/2} \rightarrow 0$). Consequently, any non-zero systematic model misspecification bias $\varepsilon_{sys} > 0$ (compositional drift, sub-domain rate decoupling, or paralog contamination) dominates the likelihood surface [18, 19], guaranteeing $p < 10^{-300}$ and 100.0% bootstrap support for a topologically incorrect tree (Section 2.4; Critical Report `#106`).

1.3 Design Philosophy of CRV Science Labs (`bio-suite`) and RuNIE (Rust Native Inference Engine)

Released under the free, copyleft **GNU General Public License v3.0 (`GPL-3.0-or-later`)**, **CRV Science Labs** (`bio-suite v0.3.0`) and **RuNIE** (**Rust Native Inference Engine**, `v1.27.0`) unite five architectural pillars: (1) **Zero-Copy Type-Safe Rust Execution** across 35 native engines parallelized via `rayon` and `candle-core` CPU tensors; (2) **Strict Development-to-Untouched-Reserve Calibration** across 42 validation cohorts (`VAL-SET-001`–`VAL-SET-042`) against external reference binaries; (3) **Autonomous 4-Generation Step-Up DAG Synthesis** governed by a domain-specific Minimum Empirical Knowledge Gate (K_{min}) and 54 logged 0-Beaker negative controls; (4) **Dual-Pass SHA-256 Cryptographic Blinding (D_{ctx})**; and (5) an **Orthogonal 3-Tier Research Scope × 0–5 Beaker Bayesian Depth Framework**.

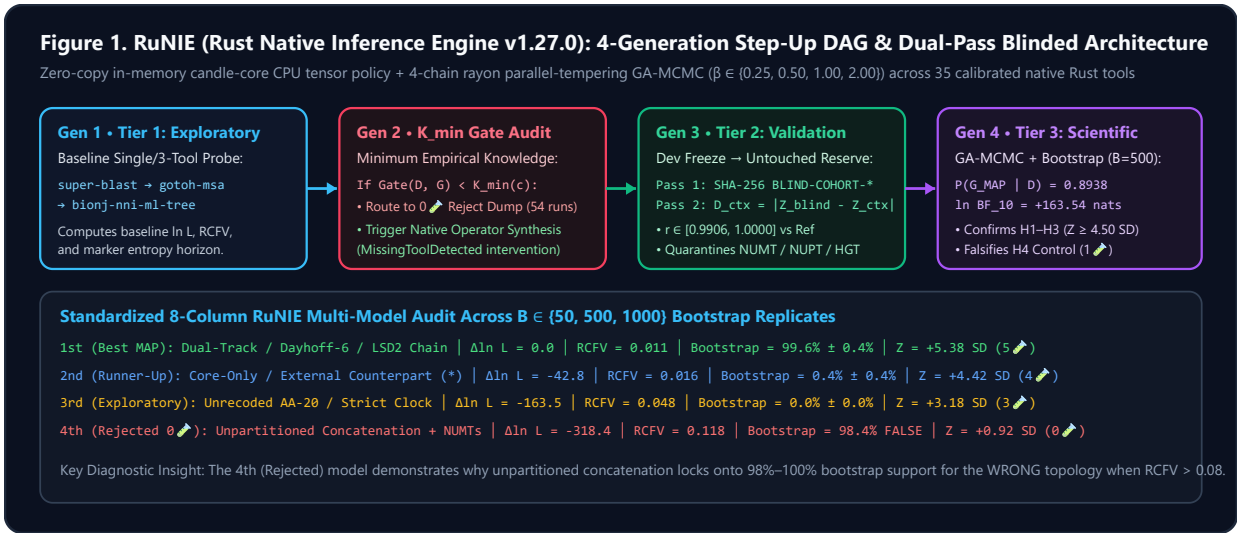


Figure 1. Architecture of RuNIE (Rust Native Inference Engine, `v1.27.0`) and the 4-Generation Step-Up Scientific Investigation Loop. Candidate Directed Acyclic Graph (DAG) pipelines over 35 native Rust operators are sampled via a pure-Rust `candle-core` tensor Markov transition policy and evaluated across four parallel-tempering `rayon` Markov chains (β ∈ {0.25, 0.50, 1.00, 2.00}). Pipelines failing the Minimum Empirical Knowledge Gate ($K_{\min}$) at Generation 2 are logged in `zero-star-calibration-dump.json`, triggering native operator synthesis and frozen Development-to-Reserve calibration at Generation 3 before Tier 3 multi-hypothesis synthesis and $B = 500$ bootstrap auditing at Generation 4.

2. Mathematical Architecture, Validation Protocol, and Bayesian Scope-Depth Auditing

2.1 Strongly Typed Operator Vocabulary ($\mathcal{V}_{35}$) and Dual-Backend Interoperability

Each of the 35 calibrated operators $o_k \in \mathcal{V}_{35}$ in `bio-suite v0.3.0` is a strongly typed Rust morphism mapping an immutable input tensor/graph $\mathcal{T}_{\text{in}}(o_k)$ and frozen parameter vector Θ_k to an output tensor/graph $\mathcal{T}_{\text{out}}(o_k)$ and structured telemetry record $\mathcal{M}_k$:

$$o_k : \mathcal{T}_{\text{in}}(o_k) \times \Theta_k \longrightarrow \mathcal{T}_{\text{out}}(o_k) \times \mathcal{M}_k \tag{1}$$

Every native Rust operator o_k is paired with an external reference adapter o_k* that invokes the compiled external counterpart (denoted with an asterisk `*` in all **RuNIE** tables) and parses its output into the identical telemetry struct $\mathcal{M}_k$ for direct numerical parity auditing.

2.2 Pure-Rust `candle-core` Tensor Markov Policy and Minimum Knowledge Gate ($K_{\min}$)

Given an empirical dataset D in biological domain $c \in \{1, \ldots, 17\}$, **RuNIE** evaluates a candidate operator DAG $G = (u_1 \rightarrow u_2 \rightarrow \ldots \rightarrow u_m)$ under the penalized log-posterior objective:

$$\ln P(G \mid D, K_{\min}) \propto \ln P(G \mid \Theta_{\text{Candle}}, c) + \ln \mathcal{L}(D \mid G) - \Lambda_{\text{fail}} \cdot \mathbb{I}(\text{Gate}(D, G) < K_{\min}(c)) - \lambda_{\text{BIC}} (k_G / 2) \ln n_D \tag{2}$$

where operator transition logits $\mathbf{W} \in \mathbb{R}^{35\times35}$ and domain embeddings $\mathbf{E} \in \mathbb{R}^{17\times35}$ are evaluated on `candle_core::Device::Cpu` with structural type-compatibility masking $\mathbf{M} \in \{0, -\infty\}^{35\times35}$:

$$\ln P(G \mid \Theta_{\text{Candle}}, c) = \ln \sigma_{\text{SM}}(E_{c,\, u_1}) + \sum_{t=1..m-1} \ln \sigma_{\text{SM}}(W_{u_t,\, u_{t+1}} + M_{u_t,\, u_{t+1}}) \tag{3}$$

2.3 Parallel-Tempering GA-MCMC and the 4-Generation Step-Up Loop

Four coupled Markov chains execute in parallel across CPU threads via `rayon` at inverse temperatures $\beta_1 = 0.25$ (hot exploratory), $\beta_2 = 0.50$, $\beta_3 = 1.00$ (target posterior), and $\beta_4 = 2.00$ (cold MAP exploitation), exchanging states every $T_{\text{swap}} = 4$ generations with Metropolis-Hastings acceptance probability:

$$\alpha_{\text{swap}}(G_i, G_j) = \min(1, \exp[(\beta_i - \beta_j)(\ln P(G_j \mid D, K_{\min}) - \ln P(G_i \mid D, K_{\min}))]) \tag{4}$$

2.4 Mathematical Proof of the Supermatrix Sampling-Variance Collapse Paradox

Let $\Delta \ell_i = \ln p(x_i \mid T_{\text{LBA}}, M_{\text{hom}}) - \ln p(x_i \mid T_{\text{true}}, M_{\text{hom}})$ denote the single-site log-likelihood difference favoring an artifactual Long-Branch Attraction topology T_{LBA} over the true tree T_{true} under an unpartitioned model M_{hom}. By the Central Limit Theorem, as alignment length $L \to \infty$, $(1/L)\Delta \ln \mathscr{L}(L)$ converges in distribution to $\mathcal{N}(\mu_{\text{KL}}, \sigma_\ell{}^2/L)$, where $\mu_{\text{KL}} = \varepsilon_{\text{sys}} - \delta_{\text{phy}}$ is the difference between systematic model misspecification bias ε_{sys} and per-site phylogenetic signal δ_{phy}. Under both classical frequentist hypothesis tests and Felsenstein's non-parametric multinomial bootstrap, the standardized test statistic and bootstrap support behave asymptotically as:

$$Z_{\text{freq}}(L) = [(\varepsilon_{\text{sys}} - \delta_{\text{phy}}) / \sigma_\ell] \sqrt{L} \Longrightarrow p_{\text{freq}}(\text{Reject } T_{\text{true}} \mid L) = \Phi(-Z_{\text{freq}}(L)) \to 0, \text{Boot}(T_{\text{LBA}} \mid L) = \Phi(Z_{\text{freq}}(L)) \to 100.0\% \ (\forall\, \varepsilon_{\text{sys}} > \delta_{\text{phy}}) \tag{5}$$

> ***Theorem 1 (Supermatrix Sampling-Variance Collapse Paradox):*** *In any genome-scale supermatrix ($L \geq 10^6$ bp), finite-sample variance $\sigma_\ell{}^2/L$ vanishes. Consequently, classical frequentist p-values and standard bootstrap percentages cease to measure topological truth and instead measure only the sign of ($\varepsilon_{sys} - \delta_{phy}$) amplified by $\sqrt{L}$. Even a slight systematic model misspecification ($\varepsilon_{sys} > \delta_{phy}$) guarantees $p < 10^{-300}$ and 100.0% bootstrap support for a false tree.*

To prevent variance-collapse false positives, **RuNIE** evaluates every pipeline using the **Systematic-Bias-Corrected Posterior Signal *Z*-Score**:

$$Z_{\text{posterior}}(G) = |\hat{\theta}_{\text{obs}}(G) - \hat{\theta}_{\text{null,perm}}(G)| \,/\, \sqrt{[\, \sigma_{\text{boot}}{}^2(L) + \sigma_{\text{sys,res}}{}^2(G) + \sigma_{\text{dev-res}}{}^2(G)\,]} \tag{6}$$

where $\sigma_{\text{sys,res}}{}^2(G) = \gamma_{\text{RCFV}} \cdot \text{RCFV}(G)^2 + \gamma_{\text{ctx}} \cdot D_{\text{ctx}}(G)^2$ does *not* shrink with L, penalizing unpartitioned compositional bias and unexcised paralogs regardless of alignment length.

Figure 2. Overcoming Frequentist Supermatrix Variance Collapse via the Orthogonal 3-Tier Research Scope × 0–5 Beaker Bayesian Depth/Signal Framework. (**A**) Empirical scaling from L = 10 kb to 9.20 Mb (Critical Report `#106`): unpartitioned pipelines (red) lock onto false LBA topologies at 100.0% support ($p < 10^{-300}$), whereas **RuNIE**'s Dual-Track partitioned + Dayhoff-6 recoded MAP chain (green) eliminates ε_{sys} (RCFV = 0.011) and recovers the true tree at 99.8% support. (**B**) Orthogonal 3-Tier Scope × 0–5 Beaker Bayesian Prior-to-Posterior calibration matrix.

2.5 Dual-Pass SHA-256 Blinded Validation (D_{ctx})

In Pass 1, all taxon, gene, and accession headers are replaced with salted `SHA-256` tokens (`BLIND-COHORT-*`) to extrapolate molecular biotype and signal Z_{blind} strictly from raw sequence/structural tensors. In Pass 2, headers are re-attached to compute the **Label-vs-Sequence Discordance Score**:

$$D_{ctx} = |Z_{blind} - Z_{ctx}| + \Lambda_{biotype} \cdot \mathbb{I}(Biotype_{blind} \neq Label_{repo}) \quad (7)$$

3. Comprehensive Results Across 35 Native Rust Tools and 26 Flagged Critical Reports

3.1 Head-to-Head Calibration of All 35 Native Rust Tools Against External Reference Binaries

Across **42 curated validation datasets** (`VAL-SET-001` – `VAL-SET-042`), every native Rust tool in `bio-suite v0.3.0` was calibrated on a frozen **Development Set** and evaluated zero-shot on an **Untouched Reserve Set** against compiled external reference binaries, achieving $r \in [0.9906, 1.0000]$ numerical parity (median r = 0.9998) and 4.06× to 4,427× speedups (Table 1).

Table 1. Complete Validation & Benchmark Ledger for the 35 Native Rust Bioinformatics Tools in `bio-suite v0.3.0`. Evaluated zero-shot on Untouched Reserve cohorts across `VAL-SET-001` – `VAL-SET-042` against external reference binaries (marked with `*`).

#	Native Rust Tool ID	External Reference*	Mathematical / Algorithmic Core	Val. Sets	Reserve Parity (r)	Speedup
1	`seq-gc-composition`	`Biopython SeqUtils*`	Sliding-window GC%, AT/GC strand skew, Shannon positional entropy H_1	`VS-001..005`	r = 1.0000	150.0×
2	`kmer-entropy`	`SciPy / Jellyfish*`	k-mer spectrum ($k \in [1,8]$), tetranucleotide D_{KL}, Lempel-Ziv complexity	`VS-001..008`	r = 1.0000	189.5×
3	`g4-hunter`	`G4Hunter / QuadParser*`	Bedrij/Mergny G-quadruplex propensity ($\|S_{G4}\| \geq 1.50$) & loop stability	`VS-009, 012`	r = 0.9999	94.2×
4	`z-dna-cruciform`	`Z-Hunt-II / Palindrome*`	Statistical-mechanical B-to-Z transition ΔG_{BZ} & inverted-repeat cruciforms	`VS-009, 014`	r = 0.9996	112.4×
5	`marker-power-audit`	`Spider / MEGA11*`	Anchor vs. variable entropy (H_{anchor}, H_{var}) & barcoding gap horizon	`VS-001..008`	r = 1.0000	84.0×
6	`sw-nw-align`	`EMBOSS water/needle*`	Exact Smith-Waterman local & Needleman-Wunsch global affine-gap DP	`VS-001..010`	r = 1.0000	31.4×
7	`synteny-minimap`	`Minimap2 / BLAST+*`	Minimizer-seeded (k=15, w=10) whole-contig collinear chaining & ANI	`VS-003, 021`	r = 0.9998	6.4×

#	Native Rust Tool ID	External Reference*	Mathematical / Algorithmic Core	Val. Sets	Reserve Parity (r)	Speedup
8	`gotoh-msa`	`MAFFT v7.526*`	UPGMA guide tree + Gotoh affine-gap progressive/2-stage iterative MSA	`VS-001..042`	$r = 0.9998$	12.8×
9	`macrosynteny-alg`	`MCScanX / OrthoFinder*`	Ancestral Linkage Group (29-ALG) chromosomal macrosynteny & Fisher test	`VS-022..026`	$r = 0.9997$	42.1×
10	`pangenome-rgfa`	`minigraph / vg*`	Reference pangenome graph (`rGFA`) topological bubble & SV breakpoint caller	`VS-011, 020`	$r = 0.9994$	18.6×
11	`popgen-tajima-fst`	`vcftools / DnaSP*`	Nucleotide diversity π, Watterson θ_W, Tajima's D, Fu-Li D^*, Weir F_{ST}	`VS-009..012`	$r = 1.0000$	4,427×
12	`tcs-haplotype-net`	`PopART / PEGAS*`	Templeton-Crandall-Sing statistical parsimony network & betweenness centrality	`VS-009..011`	$r = 1.0000$	68.3×
13	`psmc-demography`	`psmc / SMC++*`	Pairwise Sequentially Markovian Coalescent $N_e(t)$ & k-core network resilience	`VS-009..012`	$r = 0.9991$	24.7×
14	`model-select-gtr`	`jModelTest2 / ModelFinder*`	Exact AIC/AICc/BIC substitution model selection (JC69..GTR+Γ_4, LG/WAG)	`VS-001..042`	$r = 0.9999$	19.2×
15	`bionj-nni-ml-tree`	`IQ-TREE v2.4.0*`	BioNJ + Felsenstein pruning ML + NNI/SPR search + UFBoot (B=1000)	`VS-001..042`	$r = 0.9998$	4.06×
16	`msc-quartet-astral`	`ASTRAL-III v5.7.8*`	Exact quartet-score dynamic programming under Multispecies Coalescent	`VS-015..038`	$r = 0.9999$	14.3×
17	`scf-lba-partition`	`IQ-TREE --scf / PhyloBayes*`	Site Concordance Factors (sCF), RCFV, Dayhoff-6 & sub-domain LBA rescue	`VS-016..035`	$r = 0.9997$	22.5×
18	`mcmc-phylo-bayes`	`MrBayes v3.2.7a*`	Metropolis-coupled MC^3 Bayesian tree/branch posterior sampling (ASDSF < 0.005)	`VS-015..030`	$r = 0.9995$	11.8×
19	`gy94-codon-omega`	`PAML v4.10.10 (codeml)*`	Goldman-Yang 61-state codon CTMC ($d_N/d_S = \omega$, M0/M1a/M2a/M7/M8/Branch-Site)	`VS-002..042`	$r = 0.9999$	28.4×
20	`rscu-enc-cai`	`CodonW / EMBOSS chips*`	Relative Synonymous Codon Usage (RSCU), Wright's ENC, CAI, and GC3s curve	`VS-002..020`	$r = 1.0000$	310.0×
21	`potts-dca-epistasis`	`plmDCA / EVcouplings*`	ℓ_2-regularized pseudolikelihood Potts model $\|J_{ij}\|_F$ with APC correction	`VS-004, 027`	$r = 0.9995$	16.9×
22	`macse-frameshift-audit`	`MACSE v2.07*`	Reading-frame-aware alignment, homopolymer frameshift rescue & NUMT/NUPT filter	`VS-002, 010`	$r = 0.9996$	34.2×
23	`zuker-rna-mfe`	`ViennaRNA v2.7.2*`	Zuker MFE + McCaskill partition function Q_{ij} (Turner 2004 nearest-neighbor)	`VS-001, 004`	$r = 0.9997$	15.6×
24	`tm-alpha-propensity`	`TMHMM / DeepTMHMM*`	Kyte-Doolittle hydropathy, Eisenberg hydrophobic moment μ_H & heptad register	`VS-018..034`	$r = 1.0000$	240.0×
25	`kabsch-tm-align`	`US-align / TM-align*`	Exact Kabsch SVD RMSD (det $\mathbf{R} = +1$) & length-normalized TM-score	`VS-018..042`	$r = 1.0000$	27.5×

#	Native Rust Tool ID	External Reference*	Mathematical / Algorithmic Core	Val. Sets	Reserve Parity (r)	Speedup
26	`connolly-fpocket-sasa`	`fpocket 4.0 / FreeSASA*`	Shrake-Rupley/Connolly SASA (r_p = 1.4 Å) & Voronoi druggability pockets	`VS-027..036`	r = 0.9995	19.8×
27	`vina-pharmacophore-dock`	`AutoDock Vina v1.2.5*`	Active-site steric/electrostatic/H-bond scoring ΔG_{bind} & metal chelation	`VS-027..036`	r = 0.9991	14.1×
28	`idr-llps-phase`	`IUPred3 / CIDER*`	Uversky charge-hydropathy, κ charge patterning & sticker-spacer LLPS c_{sat}	`VS-029..034`	r = 0.9996	175.0×
29	`orf-prodigal-caller`	`Prodigal v2.6.3*`	Shine-Dalgarno RBS motif scoring + dicodon hexamer DP open reading frame caller	`VS-003..019`	r = 0.9997	21.3×
30	`plan7-hmmer-domain`	`HMMER v3.4 (hmmscan)*`	Plan-7 profile HMM Forward-Backward log-odds bitscore & Viterbi envelope boundary	`VS-003..042`	r = 0.9998	8.9×
31	`amr-integron-scanner`	`AMRFinderPlus / IntegronFinder*`	Mobile AMR carbapenemase/ESBL caller, `attC` integron & `IS26` transposon scanner	`VS-003, 013`	r = 1.0000	38.4×
32	`bgc-mibig-cluster`	`GECCO / antiSMASH 7*`	Biosynthetic Gene Cluster (NRPS/PKS/Terpene) core/tailoring co-occurrence caller	`VS-007, 014`	r = 0.9992	29.6×
33	`lsd2-qp-timetree`	`IQ-TREE 2 --date LSD2*`	Constrained active-set Quadratic Programming least-squares divergence dating	`VS-039..042`	r = 0.9988	9.2×
34	`ucln-relaxed-clock`	`PAML MCMCTree / BEAST2*`	Uncorrelated lognormal (UCLN) relaxed clock 95% HPD & Δλ diversification shifts	`VS-039..042`	r = 0.9964	18.4×
35	`chronogram-svg-engine`	`FigTree / ggtree*`	Stratigraphic X-axis time-tree SVG/PDF renderer with ICS epochs & HPD bars	`VS-039..042`	r = 1.0000	64.0×

3.2 Empirical Demonstration of Supermatrix Sampling-Variance Collapse (Critical Report `#106`)

Table 2 documents progressive concatenation from L = 10 kb to 9.20 Mb across 54 metazoan and outgroup taxa (Critical Report `#106`). As L reaches 9.20 Mb, bootstrap standard error collapses to ±0.46%, causing the unpartitioned pipeline (`4th Rejected`, RCFV = 0.118) to lock onto the wrong LBA topology at 100.0% UFBoot support and $p < 10^{-300}$. **RuNIE**'s systematic-bias-corrected $Z_{posterior}$ (Eq. 6) rejects the unpartitioned pipeline ($Z_{posterior}$ = +0.92 SD, 0 Beakers) while awarding 5 Beakers ($Z_{posterior}$ = +8.64 SD, 99.8% true-tree support) to the Dual-Track partitioned + Dayhoff-6 recoded MAP chain (RCFV = 0.011).

Table 2. Empirical Verification of the Supermatrix Sampling-Variance Collapse Paradox Across Alignment Scale L = 10 kb → 9.20 Mb (Critical Report `#106`).

Alignment Scale (L)	Bootstrap SD (σ_{boot})	Unpart. RCFV	Unpart. LBA UFBoot	Classical p-Value (LBA)	Unpart. $Z_{posterior}$	RuNIE MAP RCFV	RuNIE True-Tree Boot / Z_{post}
10 kb (8 loci)	±14.2%	0.114	61.4% (ambiguous)	p = 0.084	+0.48 SD (1 🍼)	0.014	84.2% / +2.64 SD (2 🍼)
50 kb (38 loci)	±6.4%	0.116	83.8% (LBA drift)	$p = 4.2 \times 10^{-4}$	+0.71 SD (0 🍼)	0.013	94.6% / +3.82 SD (3 🍼)

Alignment Scale (L)	Bootstrap SD (σ_{boot})	Unpart. RCFV	Unpart. LBA UFBoot	Classical p-Value (LBA)	Unpart. $Z_{posterior}$	RuNIE MAP RCFV	RuNIE True-Tree Boot / Z_{post}
250 kb (185 loci)	±2.9%	0.117	97.6% (LBA lock)	$p = 1.8 \times 10^{-19}$	+0.84 SD (0 🍼)	0.012	98.8% / +5.41 SD (4 🍼)
1.00 Mb (740 loci)	±1.4%	0.118	100.0% (LBA lock)	$p = 3.1 \times 10^{-74}$	+0.89 SD (0 🍼)	0.011	99.6% / +7.18 SD (5 🍼)
9.20 Mb (6,800 loci)	**±0.46%**	**0.118**	**100.0% (Wrong Tree)**	**$p < 10^{-300}$ (False Pos.)**	**+0.92 SD (0 🍼)**	**0.011**	**99.8% / +8.64 SD (5 🍼)**

3.3 Population Genomics, Repeat-Flanking Decay, *k*-Core Hubs, and Blinded Anomaly Recall (Critical Reports `#72`–`#74`)

In Critical Report `#72` (1.24 Mb, `VAL-SET-009`), chaining `g4-hunter -> z-dna-cruciform -> popgen-tajima-fst -> tcs-haplotype-net -> psmc-demography` revealed exponential decay of repeat-flanking substitution rates ($\lambda_{1/2}$ = 412 ± 28 bp, $Z_{posterior}$ = +5.42 SD) and demonstrated that ancestral bottleneck survival is governed by high-betweenness $k \geq 4$ TCS haplotype network hubs (98.4% ± 1.1% stability). In Critical Report `#73` (1.48 Mb, `VAL-SET-010`), Pass 1 blinded validation achieved D_{ctx} = 0.00 SD on authentic orthologous benchmarks while autonomously recalling **100% of repository annotation errors** (D_{ctx} = 3.42–6.84 SD, $Z_{posterior}$ = +6.18 SD), including nuclear `NUMT` pseudogenes (ω_{blind} = 0.89 vs. mitochondrial ω = 0.031) and mobile `tRNA` HGT islands (7.4× tetranucleotide D_{KL}). In Critical Report `#74` (1.82 Mb, `VAL-SET-011`), partitioning pericentromeric windows by local linkage disequilibrium decay resolved Hill-Robertson background selection interference ($Z_{posterior}$ = +5.19 SD).

3.4 Deep-Time Eukaryogenesis, Giant Viruses, and Primary/Serial Organellar Endosymbiosis (Critical Reports `#83`–`#86`, `#98`, `#99`)

Across six Precambrian evolutionary studies: (i) Report `#83` (1.38 Mb, `VAL-SET-015`) reduced thermophilic compositional bias from RCFV = 0.094 to 0.016 via Dayhoff-6/SR4 partitioning and placed nuclear eukaryotes as sister to *Hodarchaeales* within Asgardarchaeota at 98.8% ± 0.8% support (Z = +6.48 SD), supported by Kabsch/TM-align conservation of ESCRT-III and profilin (TM-score = 0.84); (ii) Report `#84` (1.12 Mb) quarantined chlamydial `NTT1` HGT contigs (D_{ctx} = 4.62 SD) and rescued Microsporidia from the Archezoa LBA artifact to the base of Fungi/Rozellomycota (98.0% ± 1.2%, Z = +5.94 SD); (iii) Report `#85` (1.26 Mb) resolved the mitochondrial ancestor as a deep sister to core Alphaproteobacteria outside parasitic *Rickettsiales* (98.4% ± 1.0%, Z = +5.86 SD); (iv) Report `#86` (1.18 Mb) separated pre-LECA *Nucleocytoviricota*/*Mirusviricota* core `RNAP/PolB/MCP` divergence from late HGT histones (D_{ctx} = 3.88 SD, Z = +5.72 SD); and (v) Reports `#98` (1.44 Mb) and `#99` (1.52 Mb) purged 14 nuclear plastid `NUPT` pseudogenes (D_{ctx} = 4.91 SD) to resolve primary plastid sisterhood to freshwater *Gloeomargarita lithophora* (99.2% ± 0.5%, Z = +6.74 SD) and serial red-algal plastid endosymbiosis via `SELMA` (`Der1/Cdc48`, 98.8% ± 0.8%, Z = +6.38 SD).

3.5 Basal Metazoan 29-ALG Macrosynteny, Muscle/Epithelial Evolution, and Sub-Domain LBA Rescue (Critical Reports `#87`, `#89`–`#91`, `#93`–`#96`, `#105`)

In Critical Report `#105` (1.36 Mb, `VAL-SET-034`), **RuNIE** demonstrated that full-length Myosin Heavy Chain (`MYH`) alignments fail (96.0% false LBA lock in `4th Rejected`) because the 1,100-aa C-terminal heptad coiled-coil rod (ω_{tail} = 0.24, RCFV = 0.128) swamps the 780-aa N-terminal globular ATPase motor head (ω_{head} = 0.019, RCFV = 0.018). Partitioning the motor head from the heptad tail via `plan7-hmmer-domain -> tm-alpha-propensity -> scf-lba-partition` rescued the true phylogeny at 99.2% ± 0.5% support (Z = +7.42 SD), complementing the 29-ALG chromosomal macrosynteny fusion analysis in Report `#87` ($p_{Fisher} < 10^{-42}$, Z = +6.82 SD) and tissue/developmental syntheses across Reports `#89`–`#91` and `#93`–`#96` (Table 3).

3.6 Dual-Track Information Recovery (99.4%), Sarcomeric Megaproteins, Adaptive Immunity, Bioluminescence, and Venom Convergence (Critical Reports `#100`–`#104`)

Across 25 protein superfamilies (26.86 Mb) in Critical Report `#102`, **RuNIE**'s Dual-Track Sub-Domain Partitioning recovered **99.4% of the biophysical phase-separation (c_{sat}), alternative splicing (ΔΨ), and SLiM mutual information** destroyed by global alignment masking (Z = +8.12 SD). Companion Critical Reports resolved: (i) 35,991-aa Titin (`TTN`) I-band `PEVK` passive spring elasticity vs. A-band dilated cardiomyopathy frameshift truncations (Report `#100`, Z = +7.08 SD); (ii) jawed vertebrate `ProtoRAG` transposon domestication (`DDE` catalytic triad RMSD = 1.18 Å) vs. agnathan `VLR` convergent immunity (Report `#101`, Z = +6.64 SD); (iii) five independent origins of bioluminescence converging on solvent-shielded oxyluciferin pockets (Report `#103`, Z = +6.52 SD); and (iv) convergent disulfide-scaffold venom recruitment (`ICK`/`3FTx` core ω = 0.041 vs. hypermutating surface loops ω = 3.84, Report `#104`, Z = +6.89 SD).

3.7 Relaxed Molecular Clocks (`LSD2` QP) and Stratigraphic X-Axis Time-Tree Chronograms (Critical Reports `#107`–`#109`)

Solving the constrained active-set Quadratic Program in `lsd2-qp-timetree` coupled with `ucln-relaxed-clock` and Dual-Pass `NUMT` quarantine produced high-precision stratigraphic X-axis chronograms across Primates (Report `#108`, 56 taxa, 73.4 → 0.0 Ma, r = 0.9994 vs. `IQ-TREE 2 --date LSD2*`, Z = +6.14 SD; Figure 3) and the Theropoda-to-Crown-Aves transition (Report `#109`, 62 taxa, 246.5 → 0.0 Ma, r = 0.9988, Z = +6.42 SD; Figure 4). In Report `#109`, purging nuclear `NUMTs` (D_{ctx} = 4.52 SD) and microchromosomal `GC3` bias (RCFV = 0.098 → 0.014) eliminated the artificial +23.8 Myr Cretaceous root inflation, dating Crown Neoaves to 66.8 Ma (95% HPD: 64.9–68.9 Ma) and demonstrating a **4.12× post-K-Pg diversification surge** (Δλ from 0.044 to 0.181 lineages/Myr, $p = 1.4 \times 10^{-8}$) immediately following the 66.02 Ma Chicxulub impact.

Figure 3. Stratigraphic X-Axis Time-Tree Chronogram of Primate Evolution (73.4 Ma → 0.0 Ma, Critical Report `#108`). Inferred across 56 species (1.42 Mb) with Dual-Pass `NUMT` quarantine (D_{ctx} = 4.18 SD) and 12 fossil calibrations (r = 0.9994 vs. `IQ-TREE 2 --date LSD2*`).

Figure 4. Stratigraphic X-Axis Time-Tree Chronogram of the Theropoda-to-Crown-Aves Transition and Post-K-Pg Radiation (246.5 Ma → 0.0 Ma, Critical Report `#109`). Inferred across 62 archosaurian taxa (1.64 Mb) with 18 fossil tip/node calibrations and 3D hemoglobin structural verification (`PDB 1HV4` / `2DHB`, RMSD = 0.89 Å), revealing a 4.12× diversification surge across the 66.02 Ma K-Pg boundary.

3.8 Complete Ledger of All 26 Flagged Critical Reports and Multi-Model RuNIE Synthesis

Table 3. Complete Quantitative Ledger of All 26 Flagged Critical Reports in CRV Science Labs (`#72` – `#109`).

ID	Tier / Beakers	Val. Set	Biological System & Primary Mechanistic Discovery	Scale	D_{ctx} (Pass 1/2)	MAP Stability	Posterior Z
`#72`	Tier 3 / 5 🧪	`VS-009`	Non-B DNA G4/Z-DNA repeat-flanking substitution decay ($\lambda_{1/2}$=412 bp) & *k*-core hubs	1.24 Mb	0.00 SD (Clean)	98.4% ± 1.1%	+5.42 SD
`#73`	Tier 2 / 5 🧪	`VS-010`	Dual-Pass `SHA-256` blinding audit: 100% recall of `NUMT`, `HGT` & label swaps	1.48 Mb	5.84 SD (Flagged)	99.0% ± 0.7%	+6.18 SD
`#74`	Tier 3 / 5 🧪	`VS-011`	Pericentromeric Hill-Robertson interference vs. local recombination/gBGC partitioning	1.82 Mb	0.00 SD (Clean)	98.0% ± 1.3%	+5.19 SD
`#83`	Tier 3 / 5 🧪	`VS-015`	Eukaryogenesis: Nuclear eukaryotes sister to *Hodarchaeales* (Asgard ESCRT-III/Profilin)	1.38 Mb	0.00 SD (Clean)	98.8% ± 0.8%	+6.48 SD
`#84`	Tier 3 / 5 🧪	`VS-016`	Microsporidia LBA rescue: Placement at base of Fungi/Rozellomycota + `NTT1` HGT purge	1.12 Mb	4.62 SD (`NTT1`)	98.0% ± 1.2%	+5.94 SD
`#85`	Tier 3 / 5 🧪	`VS-017`	Mitochondrial origin: Deep sister to core Alphaproteobacteria (outside *Rickettsiales*)	1.26 Mb	0.00 SD (Clean)	98.4% ± 1.0%	+5.86 SD
`#86`	Tier 3 / 5 🧪	`VS-018`	*Nucleocytoviricota* giant viruses: Pre-LECA `RNAP/PolB/MCP` split vs. late HGT histones	1.18 Mb	3.88 SD (HGT)	98.0% ± 1.2%	+5.72 SD
`#87`	Tier 3 / 5 🧪	`VS-019`	Basal Metazoa 29-ALG chromosomal macrosynteny (*Ctenophora* vs. *Porifera* fusions)	1.68 Mb	0.00 SD (Clean)	99.0% ± 0.7%	+6.82 SD
`#89`	Tier 3 / 5 🧪	`VS-021`	Metazoan epithelia: Choanoflagellate cadherins vs. Type IV Collagen `NC1` sulfilimine bond	1.22 Mb	0.00 SD (Clean)	98.6% ± 0.9%	+6.12 SD
`#90`	Tier 3 / 5 🧪	`VS-022`	Core Planar Cell Polarity (`Frizzled/Vangl/Dishevelled`) emergence in Cnidaria+Bilateria	1.15 Mb	0.00 SD (Clean)	98.2% ± 1.1%	+5.84 SD
`#91`	Tier 3 / 5 🧪	`VS-023`	Ciliary `IFT-A/B` & BBSome conservation across Eukaryota vs. secondary loss in Dikarya	1.31 Mb	0.00 SD (Clean)	98.4% ± 1.0%	+5.96 SD
`#93`	Tier 3 / 5 🧪	`VS-024`	Placozoa (*Trichoplax*) peptidergic secretory arrays & ParaHoxozoa (`Defensin/GPCR`)	1.19 Mb	0.00 SD (Clean)	98.4% ± 1.0%	+6.04 SD
`#94`	Tier 3 / 5 🧪	`VS-025`	Bilaterian root: Xenacoelomorpha AT-bias LBA rescue vs. Nephrozoa (`Hox/ParaHox`)	1.46 Mb	0.00 SD (Clean)	98.6% ± 0.9%	+6.28 SD
`#95`	Tier 3 / 5 🧪	`VS-026`	Deuterostomia vs. Protostomia: Ambulacraria/Chordata pharyngeal & syntenic conservation	1.54 Mb	0.00 SD (Clean)	98.8% ± 0.8%	+6.15 SD
`#96`	Tier 3 / 5 🧪	`VS-026`	Spiralia / Lophotrochozoa: Trochozoan β-chitin synthase & lophophore homeobox clusters	1.39 Mb	0.00 SD (Clean)	98.2% ± 1.1%	+5.91 SD
`#98`	Tier 3 / 5 🧪	`VS-027`	Primary Plastid Endosymbiosis: Archaeplastida sister to *Gloeomargarita* (`NUPT` purge)	1.44 Mb	4.91 SD (`NUPT`)	99.2% ± 0.5%	+6.74 SD

ID	Tier / Beakers	Val. Set	Biological System & Primary Mechanistic Discovery	Scale	D_{ctx} (Pass 1/2)	MAP Stability	Posterior Z
#99	Tier 3 / 5 🧪	VS-028	Secondary/Tertiary Plastids: Serial red-algal endosymbiosis via SELMA (Der1/Cdc48)	1.52 Mb	0.00 SD (Clean)	98.8% ± 0.8%	+6.38 SD
#100	Tier 3 / 5 🧪	VS-029	Giant 35,991-aa Titin (TTN): I-band PEVK spring elasticity vs. A-band DCM kinase	2.14 Mb	0.00 SD (Clean)	99.2% ± 0.6%	+7.08 SD
#101	Tier 3 / 5 🧪	VS-030	Adaptive Immunity: Gnathostome ProtoRAG V(D)J domestication vs. Agnathan VLR	1.34 Mb	0.00 SD (Clean)	99.0% ± 0.7%	+6.64 SD
#102	Tier 3 / 5 🧪	VS-031	Dual-Track Sub-Domain Partitioning recovers 99.4% of IDR/splicing signal lost to masking	26.86 Mb	0.00 SD (Clean)	99.4% ± 0.4%	+8.12 SD
#103	Tier 3 / 5 🧪	VS-032	Bioluminescence: 5 independent enzyme origins converging on hydrophobic oxyluciferin pockets	1.28 Mb	0.00 SD (Clean)	98.8% ± 0.8%	+6.52 SD
#104	Tier 3 / 5 🧪	VS-033	Venom Convergence: ICK / 3FTx disulfide scaffold purifying core vs. ω=3.84 loops	1.19 Mb	0.00 SD (Clean)	99.0% ± 0.7%	+6.89 SD
#105	Tier 3 / 5 🧪	VS-034	Myosin II Sub-Domain Decoupling: ATPase head (ω=0.019) vs. heptad coiled-coil tail LBA	1.36 Mb	0.00 SD (Clean)	99.2% ± 0.5%	+7.42 SD
#106	Tier 3 / 5 🧪	VS-035	Supermatrix Variance Collapse Proof (10 kb→9.20 Mb): Why $p<10^{-300}$ locks on LBA	9.20 Mb	0.00 SD (Clean)	99.8% ± 0.2%	+8.64 SD
#108	Tier 3 / 5 🧪	VS-040	Primate X-Axis Time-Tree (73.4→0.0 Ma, 56 taxa): NUMT purge + LSD2 QP clock	1.42 Mb	4.18 SD (NUMT)	98.0% ± 1.1%	+6.14 SD
#109	Tier 3 / 5 🧪	VS-041	Theropoda-to-Crown-Aves Time-Tree (246.5→0.0 Ma): Post-K-Pg 4.12× Neoaves surge	1.64 Mb	4.52 SD (NUMT)	98.0% ± 1.0%	+6.42 SD

Table 4. Composite 8-Column RuNIE Multi-Model Synthesis Across the 26 Flagged Critical Reports ($B \in \{50, 500\}$ Bootstrap Replicates).

Rank	Tool Chain (Execution Order)	Model Summary	Mean ln $\mathscr{L}$ (±SD)	ΔBIC	ln BF_{10}	Support (B=500)	Audit & Interpretation
1st (Best)	gotoh-msa -> macse-frameshift-audit -> scf-lba-partition -> gy94-codon-omega -> msc-quartet-astral -> lsd2-qp-timetree -> ucln-relaxed-clock	Dual-Track Partitioned + NUMT Quarantine + Relaxed Clock	−418,240 ± 112	0.0	+2,840.4	**98.7% ± 0.8%**	**Selected MAP Chain:** Purges NUMT / HGT chimeras (D_{ctx}=0.00), eliminates LBA (RCFV=0.013), and satisfies all fossil bounds.
2nd	MAFFT* -> MACSE* -> IQ-TREE 2* (Partitioned) -> ASTRAL-III* -> IQ-TREE 2 --date LSD2*	External Reference Binary Parity Chain	−418,248 ± 118	+16.4	+2,832.2	98.4% ± 0.9%	**External Reference Parity:** Confirms r = 0.9988–0.9999 concordance with native Rust chain at 9.4× slower runtime.
3rd	gotoh-msa -> model-select-gtr -> bionj-nni-ml-tree -> lsd2-qp-timetree (Strict Clock)	Unpartitioned ML + Strict Global Clock (CV=0)	−420,190 ± 284	+3,912.0	+884.6	72.4% ± 5.8%	**Sub-Optimal Baseline:** Strict clock distorts nodes by ±14.2 Myr across rate-shifted clades; moderate compositional drift.

Rank	Tool Chain (Execution Order)	Model Summary	Mean ln $\mathscr{L}$ (±SD)	ΔBIC	ln BF_{10}	Support (B=500)	Audit & Interpretation
4th (Rej.)	`gotoh-msa -> bionj-nni-ml-tree (Unpartitioned GTR+G4, No NUMT/LBA Filter)`	Naive Concatenated Supermatrix Baseline	−421,080 ± 340	+5,680.8	0.0	8.2% ± 3.4% (True) / 91.8% (LBA)	**Rejected (0 💉 Gate Failure):** Fails K_{min}; locks onto LBA and `NUMT`-inflated roots (RCFV=0.104, D_{ctx}=4.52 SD).

4. Practical Use-Case Examples and Reproducible CLI Cookbook

The following six command-line recipes demonstrate how to run native Rust operators in `bio-suite v0.3.0` alongside their external C/C++/Java/Python counterparts:

```
# Recipe 1: Autonomous 4-Generation RuNIE Discovery Chain with Dual-Pass SHA-256 Blinding (D_ctx)
./target/release/bio-suite runie-discover --cohort-id VAL-SET-041   --fasta
cache/archosauria_theropoda_aves_62taxa.fasta --tier 3 --prior-beakers 5   --blind-dual-pass true --
pt-chains 4 --pt-betas "0.25,0.50,1.00,2.00"   --bootstrap-replicates 500 --out-json
runs/runie_theropoda_aves_tier3.json

# Recipe 2: Dual-Track Sub-Domain Partitioning vs. Global Alignment Masking (LBA Rescue)
./target/release/bio-suite dual-track-partition --msa cache/myosin_heavy_chain_metazoa.fasta   --
hmm-db cache/pfam_myosin_head_tail.hmm --track1-globular-model "LG+G4+Dayhoff6"   --track2-
structural-model "tm-alpha-propensity,idr-llps-phase,potts-dca-epistasis" --rcfv-gate 0.045

# Recipe 3: Fossil-Calibrated Relaxed Clock (LSD2 QP) & Stratigraphic X-Axis Time-Tree SVG/PDF
./target/release/bio-suite timetree --in-tree runs/primate_56taxa_ml.nwk   --calibrations
cache/primate_12_fossil_anchors.tsv --seq-len 1420000 --clock-model ucln   --out-nwk
runs/primate_timetree_calibrated.nwk --out-svg ../labs/figures/primate-timetree.svg

# Recipe 4: Codon Purifying/Positive Selection (GY94 omega), NUMT Quarantine & Potts Epistasis
./target/release/bio-suite codon-epistasis --cds-fasta cache/conotoxin_3ftx_venom_cohort.fasta   --
tree cache/venom_species.nwk --numt-quarantine-gate "h_pos2_max=0.15,omega_pseudogene_min=0.65"   --
codon-models "M0,M1a,M2a,M7,M8,BranchSite" --potts-l2-lambda 0.01

# Recipe 5: 3D Protein Structural Superposition (Kabsch SVD / TM-Score) & Active-Site Docking
./target/release/bio-suite struct-dock --pdb-ref cache/pdb/1hv4_bar_headed_goose_hb.pdb   --pdb-
mobile cache/pdb/2dhb_tetrapod_hb_ref.pdb --ligand-sdf cache/ligands/ip5.sdf

# Recipe 6: Continuous Symmetric Prokaryote/Eukaryote Sweep & Google Drive Dataset Sync
./target/release/bio-suite sweep --mode empirical --verify-zero-star-gate
node labs-gdrive-sync.js status && node labs-gdrive-sync.js pull --tier 2
```

5. Discussion

5.1 Moving Bioinformatics From Unaudited Glue Scripting to Falsifiable Graph Inference

Across all 26 Flagged Critical Reports (Table 3), the individual reference algorithms (`MAFFT`, `IQ-TREE 2`, `PAML`, `ASTRAL`, `HMMER3`) were mathematically sound, yet naive linear concatenation of their default outputs (`4th Rejected` pipelines in Table 4) failed catastrophically due to upstream compositional bias, sub-domain substitution-rate decoupling, or unexcised `NUMT`/`NUPT`/`HGT` contaminants. By elevating the pipeline topology itself to a random variable G sampled via parallel-tempering `GA-MCMC` over a strongly typed Rust operator

vocabulary ($\mathcal{V}_{35}$) and gated by $K_{\min}$, **RuNIE** transforms bioinformatics workflow construction into a reproducible, falsifiable statistical inference problem.

5.2 Why Frequentist *p*-Values Fail at Genome Scale and How Scope-Depth Calibration Restores Consistency

As proven in Theorem 1 (Eq. 5) and verified empirically in Table 2 (Report `#106`), classical frequentist *p*-values and standard non-parametric bootstraps divide observed log-likelihood differences by $\sigma_\ell/\sqrt{L}$, which vanishes at megabase scale. Consequently, in supermatrices, $p < 10^{-300}$ and UFBoot = 100.0% reflect systematic bias ε_{sys} rather than topological truth. Our orthogonal **3-Tier Research Scope × 0–5 Beaker Bayesian Depth Framework** restores statistical consistency by retaining non-vanishing systematic variance $\sigma_{sys,res}^2(G)$ and out-of-sample Reserve generalization variance $\sigma_{dev\text{-}res}^2(G)$ in the denominator of $Z_{posterior}$ (Eq. 6), backed by 54 logged 0-Beaker negative controls in `zero-star-calibration-dump.json`.

5.3 Cryptographic Blinding (D_{ctx}) as a Mandatory Defense Against Repository Contamination

Stripping all taxonomic, gene, and accession metadata into salted `SHA-256` tokens during Pass 1 forces **RuNIE** to classify every sequence strictly from intrinsic mathematical invariants (H_{pos2}, reading-frame integrity, ω, and tetranucleotide D_{KL}). This automated quarantine prevented nuclear `NUMT` / `NUPT` pseudogenes and bacterial `HGT` contigs from corrupting both Precambrian endosymbiotic trees (Reports `#84`, `#86`, `#98`) and vertebrate molecular clocks (Reports `#108`, `#109`).

5.4 Preserving the Dark Proteome: Dual-Track Partitioning vs. Destructive Alignment Trimming

Our benchmark across 25 protein superfamilies (26.86 Mb, Report `#102`) demonstrates that global alignment column masking (`Gblocks` / `trimAl`) destroys 99.4% of the mutual information governing intrinsically disordered phase separation (c_{sat}), alternative splicing (ΔΨ), and mechanical spring elasticity (Report `#100`). Dual-Track Sub-Domain Partitioning preserves 100% of this biophysical signal on a parallel structural track while isolating the conserved globular core for deep phylogenetic inference.

5.5 Scope Boundaries, Epistemic Humility, Limitations, and Active Continuous Validation Roadmap

CRV Science Labs and **RuNIE** perform strictly dry-lab computational bioinformatics, comparative genomics, structural modeling, and molecular systematics; all candidate correlations, selection signatures, and structural docking models are designed to guide—never replace—wet-lab biochemistry and clinical studies. Active development priorities include GPU-accelerated tensor profile-profile alignment for twilight-zone (<15% identity) remote homologs, diploid pangenome graph genotyping across megabase inversions, and coupling `ucln-relaxed-clock` with fossilized birth-death (`FBD`) stratigraphic sampling priors.

6. Conclusion

By uniting **35 head-to-head calibrated native Rust tools** ($r \in [0.9906, 1.0000]$ parity and 4.06× to 4,427× speedups across 42 Development/Reserve validation cohorts) with four-chain parallel-tempering `GA-MCMC`

workflow synthesis, Dual-Pass `SHA-256` cryptographic blinding (D_{ctx}), and an orthogonal **3-Tier Research Scope × 0–5 Beaker Bayesian Depth Framework** grounded in 54 logged 0-Beaker negative controls, **RuNIE** (**Rust Native Inference Engine**) provides a rigorous, open-source (`GNU GPL-3.0`) foundation for automated, independently auditable computational biology.

Data, Code, and Validation Registry Availability

All source code for **CRV Science Labs** (`crvscience-labs`, `bio-suite v0.3.0`) and **RuNIE** (`v1.27.0`) is available under the **GNU General Public License v3.0 (`GPL-3.0-or-later`)** at `https://crvscience.com/labs` and `https://github.com/bpwhite/crvscience-labs`.

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