BABAPPA v2.0: A Bash-Based Automated Pipeline with GUI for Codeml-Mediated Selection Analysis with Integrated Alignment Trimming and Recombination Detection

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Abstract:

Detecting positive selection in protein-coding genes is central to understanding adaptive evolution, but conventional codeml (PAML) workflows demand extensive manual configuration and sequential execution, limiting scalability and accessibility. BABAPPA v2.0 (Bash-Based Automated Parallel Positive Selection Analysis) addresses these challenges by providing a fully automated, modular framework that streamlines all stages of codeml analysis from sequence quality control to statistically validated results. In contrast to v1.0, which offered only the basic workflow, v2.0 introduces three flavours: babappa_normal (classic codeml pipeline), babappa_clip (integrating ClipKIT-based alignment trimming with codon integrity checks), and babappa_clipgard (further incorporating recombination detection via HyPhy GARD). These options allow users to balance speed, robustness, and biological realism according to dataset characteristics. In addition, a dedicated graphical user interface (GUI) makes the pipeline accessible to researchers without command-line experience by providing installer-based setup, intuitive workflow selection, and organized results management. Benchmarking on Brassicaceae ortholog sets demonstrated that BABAPPA v2.0 reproduces the exact positive-selection calls of conventional manual workflows while reducing runtime by ~45% (equivalent to an ~80% speedup). By combining reproducibility, efficiency, and usability, BABAPPA v2.0 offers the first practical end-to-end solution for large-scale genome-wide codeml-based selection analyses.

Keywords: Positive Selection, Molecular Evolution, PAML(Codeml), PRANK, IQ-TREE2, Phylogenetics

1. Introduction

The detection of positive (adaptive) selection in protein-coding genes is a cornerstone of molecular evolution studies, as it reveals adaptive changes across lineages[1,2]. Maximum-likelihood models implemented in PAML's codeml (including site, branch, and branch-site models) remain a gold standard for such detection[3]. However, traditional codeml workflows are labor-intensive: researchers must manually edit configuration files, run multiple model comparisons sequentially, and collate likelihood-ratio tests (LRTs) across many genes and branches, applying corrections for multiple testing[4]. These manual steps are prone to error and severely limit throughput when analyzing large gene families or set of orthologs/paralogs. To overcome these bottlenecks, BABAPPA has been developed, a bash-based automated pipeline that orchestrates the entire codeml analysis from raw sequences to statistically validated results. BABAPPA automates sequence quality control, codon-aware alignment, phylogenetic inference, branch annotation, model fitting, and LRT-based selection testing, all with parallel execution to maximize efficiency. It leverages standard bioinformatics tools, such as Biopython[5] for sequence handling, PRANK[6] for codon alignment, IQ-TREE2[7] for phylogenetic inference, and GNU Parallel[8] for parallel processing alongside custom scripting.

In its current version, BABAPPA is available in three complementary flavors. The classic workflow, referred to as babappa_normal, performs codon alignment, tree reconstruction, and codeml-based detection of selection. A second variant, babappa_clip, incorporates ClipKIT[9] for alignment trimming with user-selectable models such as smart-gap or kpic etc., followed by automated quality control to ensure that reading frames are preserved and no premature stop codons are introduced during the trimming process. A third variant, babappa_clipgard, extends babappa_clip by integrating recombination breakpoint detection using HyPhy GARD[10], thereby addressing the well-known confounding effect of recombination on positive selection inference. Together, these extensions enhance both the robustness and reliability of selection analyses, providing users with flexible, reproducible workflows that minimize manual intervention while improving biological interpretability (Figure 1). Here, the design of BABAPPA is described, and its performance is demonstrated against a standard codeml-based pipeline.

2. Materials and Methods

2.1. Validation datasets

To evaluate the accuracy, efficiency, and reproducibility of BABAPPA, three orthologous gene groups from Brassicaceae species has been selected. These gene sets were obtained from the PlantTFDB□, a curated database of plant transcription factors. Each orthogroup comprised five putative orthologs drawn from three closely related Brassicaceae species, representing a realistic yet computationally tractable test case. The use of transcription factor families ensures inclusion of genes typically studied in codeml-based evolutionary analyses, as these genes often exhibit lineage-specific divergence and potential signatures of adaptive evolution. Gene identifiers and sequence details for each orthogroup are provided in Table 1, enabling independent replication of the tests. This dataset design reflects a typical use scenario for BABAPPA: moderate-size orthogroups spanning related species where alignment quality, recombination, and branch-specific signals of selection must all be carefully assessed.

2.2. Pipeline implementation

BABAPPA is implemented as a suite of modular Bash and Python scripts coordinated by a master driver script (`babappa_normal/clip/clipgard.sh`) (Table 2). The pipeline executes an end-to-end workflow that begins with raw nucleotide coding sequences (CDS) and concludes with statistically validated selection results summarized in human-readable formats (Figure 1). The modular design allows users to run individual steps if desired, while the master driver ensures complete automation for batch-scale analyses. In its current release, BABAPPA supports three complementary flavors. The babappa_normal mode reproduces the classic codeml workflow with automated configuration, execution, and aggregation. The babappa_clip mode extends this by incorporating alignment trimming with ClipKIT[9], followed by codon-aware quality control to ensure that no reading frames are disrupted during trimming. Finally, the babappa_clipgard mode builds on babappa_clip by incorporating recombination breakpoint detection using HyPhy GARD[11], addressing the confounding influence of recombination on positive selection inference. This flexible design allows users to choose between speed, robustness, or recombination-aware analysis depending on their research objectives.

2.2.1 Sequence Quality Control

The first step of the pipeline ensures that only biologically meaningful and codon-compatible sequences are retained. A dedicated Python module (`seqQC.py`) applies stringent codon-level filters following best-practice recommendations[1]. Specifically, sequences must begin with an ATG start codon and end with a valid termination codon (TAA, TAG, or TGA). They must be at least >300 base pairs in length to minimize spurious results from very short sequences, contain only the canonical nucleotides A, T, G, and C, and be free of internal in-frame stop codons. In addition, extreme outliers in sequence length (outside 1.5× the interquartile range) are automatically discarded, as such sequences often represent pseudogenes, partial transcripts, or annotation errors. This step ensures that downstream alignment and evolutionary modeling are based on sequences that are structurally and functionally interpretable as protein-coding genes.

2.2.2 Phylogeny-Aware Alignment

High-quality sequences passing initial QC are aligned using PRANK[6] in codon mode (via `script0.sh`). Unlike heuristic aligners, PRANK employs a phylogeny-aware algorithm that considers insertion/deletion events in an evolutionary context. This is particularly important for positive selection analyses, since misplaced indels or misaligned codons can produce false signals of selection. Alignments produced by PRANK therefore preserve codon structure and improve the reliability of downstream likelihood ratio tests. In babappa_normal, these alignments proceed directly to phylogenetic inference, whereas in babappa_clip and babappa_clipgard, they undergo additional refinement through trimming and validation (see below).

2.2.3 Alignment Trimming and Frame Validation (babappa_clip and babappa_clipgard only)

Alignment trimming removes ambiguous or poorly supported regions from multiple sequence alignments. In the babappa_clip and babappa_clipgard workflows, PRANK-generated alignments are processed with ClipKIT[9], which trims poorly aligned or phylogenetically uninformative sites in a codon-aware mode. Users can choose the trimming model most appropriate for their dataset (e.g., smart-gap, kpic), offering flexibility between conservative and aggressive trimming strategies.

To safeguard codon integrity, a custom Python script (`babappa_stopcodon_masker.py`) checks that trimmed alignments remain in-frame and free of errors. This step is critical: even a single frameshift can render an alignment incompatible with codon-based evolutionary models and bias selection inference. Sequences that fail this check are flagged and reported, enabling users to adjust trimming parameters or exclude problematic sequences. Additionally, when trimming introduces spurious internal stop codons, the script masks them to maintain compatibility with downstream analyses.

2.2.4 Recombination Detection (babappa_clipgard only)

Recombination is a major confounding factor in codon-based analyses of positive selection, as recombination breakpoints can mimic or obscure true adaptive signals. To address this, babappa_clipgard integrates the Genetic Algorithm for Recombination Detection (GARD) as implemented in HyPhy[10] though `script0.5.sh`. After trimming and frame validation, alignments are screened for evidence of recombination breakpoints. GARD employs a model selection approach based on genetic algorithms to partition alignments into putative non-recombining segments, reporting both the number and location of detected breakpoints. Alignments with strong evidence of recombination are flagged, and partitioned datasets are optionally provided for downstream codeml analyses. Partitioning is performed by the Python script `split_recombination_blocks.py`, which is integrated into `script0.5.py`.This allows users to explicitly account for recombination or to exclude affected genes from subsequent tests, depending on their study goals.

2.2.5 Phylogenetic Reconstruction

For each codon alignment, BABAPPA infers a maximum-likelihood phylogeny using IQ-TREE2 [7] under an appropriate codon substitution model. By default, we perform 1,000 ultrafast bootstrap replicates [12] and approximate likelihood-ratio tests (aLRT) to assess branch support. This step is executed by `script1.sh`, which collects the resulting tree files.

2.2.6 Foreground Branch Designation

A custom Python script (`4GroundBranchGenerator.py`) processes each inferred tree. It iteratively marks each branch in turn as the "foreground" branch (by appending a #1 tag)

for subsequent branch-site tests [2]. The output is a set of Newick tree files, each with a different branch annotated as foreground.

2.2.7 Codeml Analyses

The codeml models were then fitted to each gene and branch scenario. Control files (`codeml.ctl`) for site, branch, and branch-site models are generated dynamically from a template using embedded `sed` commands, eliminating manual edits. For each gene, `script2.sh` runs branch and branch-site models (alternative and null) in parallel across branches using GNU Parallel[8], while `script3.sh` and `script4.sh` run site models (including M0, M1a, M2a, M3, M7, M8) in parallel. A Python wrapper (`run_codeml.py`) ensures that the correct model versions (alternative vs. null) are run for branch-site comparisons. Throughout, all executions leverage multi-core processing to maximize speed.

2.2.8 Result Aggregation and Statistical Testing

After model fitting, custom scripts (`script5.sh`, `script6.sh`) parse codeml output files to extract log-likelihood (*lnL*) values and parameter counts for each model, writing per-gene summaries in CSV format. A final Python script (`lrt_bh_correction.py`) computes likelihood-ratio statistics ($2\Delta lnL$) between paired models, calculates *p*-values from χ^2 distributions, and applies the Benjamini–Hochberg false-discovery rate (FDR) correction [13] across tests. Raw and adjusted *p*-values, along with significance flags (FDR < 0.05), are output to summary Excel reports in accordance with `script7.sh` and `script8.sh`. All pipeline steps and custom scripts are documented and configurable; source code is available on GitHub.

2.3. Graphical User Interface (GUI)

In addition to the command-line workflows, BABAPPA v2.0 includes a dedicated designed to simplify usage for researchers with little or no experience in scripting. The GUI is distributed as a double-click installer for Windows and automatically sets up all required dependencies. It provides dropdown menus for selecting among the three pipeline flavours (normal, clip, clipgard), tools for input FASTA management and validation, and real-time monitoring of pipeline execution (Figure 2). All outputs, including intermediate alignments, trees, codeml runs, and Excel summary reports, are organized into easily navigable folders with clickable shortcuts. This implementation ensures that the full functionality of

BABAPPA v2.0 is accessible without command-line expertise, while retaining compatibility with the underlying bash-driven framework.

2.4. *Benchmarking and statistical analysis*

BABAPPA was compared to a conventional codeml workflow in which sequence alignments, phylogenies, and codeml runs were performed sequentially with without any parallelization. Both pipelines were executed on a consumer laptop (Intel Core i7 13th Gen CPU, 16 GB RAM, 512 GB SSD) running Ubuntu Linux via Windows Subsystem for Linux. For each of the three orthogroups, both BABAPPA and the standard workflow were run three times to account for run-to-run variability. Wall-clock times were recorded for each complete analysis (in seconds). The distributions of runtimes were compared using a two-sided Wilcoxon rank-sum test (Mann–Whitney U, $\alpha = 0.05$) to assess the statistical significance of speed differences. All tests and data analyses were conducted in R.

3. Results

3.1. *Concordance of positive-selection calls*

BABAPPA's automation preserved the accuracy of codeml's positive-selection detection. Across all Brassicaceae orthogroups, normal flavor reproduced exactly the same results as the manual codeml workflow (FDR < 0.05), demonstrating perfect concordance. The extended flavors supported this finding: clip improved alignment consistency through ClipKIT trimming, while clipgard matched clip mode when no recombination was detected and flagged genes with evidence of recombination for partitioned analysis. Thus, BABAPPA v2.0 maintains codeml's sensitivity and specificity while reducing artifacts from misalignment and recombination and significantly lowers computational time.

3.2. *Computational efficiency*

BABAPPA v2.0 substantially reduced computation time compared to the manual workflow, with mean runtimes of 1,430s for normal flavour versus 2,579s for the sequential approach, a 44.6% reduction and ~80% speed increase (Wilcoxon rank-sum test, $p = 8.2 \times 10^{\square\square}$). The clip flavour added ClipKIT trimming and codon-frame validation without increasing runtime, while clipgard introduced only a modest 12–15% overhead for recombination detection, yet still remained faster than the manual workflow. These gains reflect extensive parallelization and streamlined file handling. The GUI client matched command-line performance while

improving usability through progress indicators, error logs, and organized outputs, enabling non-specialist users to run large-scale codeml analyses efficiently.

4. Discussion

BABAPPA v2.0 streamlines codeml-based selection analysis by unifying quality control, codon-aware alignment, phylogenetic reconstruction, model fitting, and statistical reporting into a fully automated workflow. It eliminates manual configuration and error-prone collation of likelihood-ratio tests while reproducing, without deviation, the positive-selection calls of manual workflows. Its modular design preserves all intermediate files and scripts, ensuring transparency and reproducibility. Three flavours provide flexibility: normal (core workflow with major speedups through parallelization), clip (adds ClipKIT trimming and codon-frame validation to improve alignment reliability), and clipgard (further integrates HyPhy GARD to detect recombination, essential for datasets such as viral or microbial genes etc.). Users can thus choose between maximal speed and enhanced robustness. A dedicated GUI lowers barriers by offering installer-based setup, intuitive flavour selection, and organized outputs, making the pipeline accessible to non-specialists. Unlike other tools, BABAPPA uniquely integrates episodic selection tests within a codeml-centered automated framework. Benchmarking against manual workflows confirms identical inference with significant efficiency gains. Open-source and adaptable, BABAPPA v2.0 allows substitution of aligners, filters, or models, ensuring long-term utility as genomic datasets expand.

BABAPPA v2.0 shares some aims with tools such as EasyCodeML[14], AlexandrusPS [15], and GUIs like pamlX[16] which provide interactive or semi-automated access to codon-based selection analyses. However, unlike these, BABAPPA v2.0 is the first pipeline to integrate branch-site (episodic) selection models fully within an end-to-end automated framework, i.e. pipeline flavours that include alignment trimming (ClipKIT) and recombination detection (HyPhy GARD), along with quality control, phylogenetic inference, codeml model fitting, and FDR-corrected statistical testing. Direct benchmarking against these existing tools is not practicable, because many do not support exactly the same set of models, or do not handle recombination or alignment trimming systematically, or require substantial manual parameter tuning (e.g. labelling of tree branches, control file setup) that BABAPPA automates. For example, EasyCodeML offers GUI presets and custom modes but does not systematically incorporate recombination detection or modular flavours with automatic trimming. What makes BABAPPA unique is its modular design with three flavours (normal, clip, clipgard)

that let users choose between speed, alignment robustness, or recombination awareness; preservation of all intermediate files and full scripting of each step; and the addition of a GUI for users who lack command-line experience. BABAPPA faithfully reproduces manual codeml results while offering major efficiency gains and user accessibility, making it the first practical end-to-end solution for large-scale positive-selection analyses.

5. Conclusion

Detecting positive selection is central to understanding molecular adaptation, but conventional codeml workflows are slow, error-prone, and difficult to scale. BABAPPA v2.0 overcomes these challenges by automating codon-aware filtering, alignment, phylogenetic inference, codeml execution, and FDR-corrected testing in a reproducible framework. On Brassicaceae orthogroups, it reproduced all manual workflow results while reducing runtime by ~80%. Unlike other pipelines, BABAPPA uniquely integrates episodic selection analyses and offers three flavours, normal, clip (with ClipKIT trimming), and clipgard (with HyPhy GARD recombination detection), to balance speed and robustness. A dedicated GUI further lowers barriers, enabling researchers without command-line expertise to run analyses easily. By combining accuracy, efficiency, and accessibility, BABAPPA v2.0 makes large-scale positive-selection analyses broadly feasible.

6. Declarations

6.1. Ethics approval and consent to participate

Not applicable.

6.2. Consent for publication

Nor applicable.

6.3. Availability of data and material

The data are provided within the manuscript, table 1, 2 and/or supplementary information files. Also, the GitHub repository at https://github.com/sinhakrishnendu/babappa.git contains all the supplementary materials in the form of raw and processed data, as well as all necessary scripts to replicate the analyses presented in this manuscript.

6.4. *Competing interests*

The author report that there are no competing interests to declare.

6.5. *Funding*

This research received no funding.

6.6. *Authors' contributions*

K.S. conceived and designed the study, developed and implemented all computational codes, performed data collection, analysis, and interpretation, prepared figures and tables, and wrote and revised the manuscript.

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Group	Species	Gene IDs
Group 1	*Arabidopsis helleri*	Araha.2210s0007.1; Araha.22349s0004.1; Araha.23363s0006.1; Araha.23705s0002.1; Araha.2816s0002.1
	Arabidopsis lyrate	AT1G68520.1; XP_020883914.1; XP_002868216.1; XP_020868537.1; XP_002893503.1
	Arabidopsis thaliana	AT1G25440.1; AT1G28050.1; AT1G49130.1; AT1G49130.2; AT1G68520.1
Group 2	*Arabis alpina*	KFK31035.1; KFK32611.1; KFK36040.1; KFK37540.1; KFK37702.1
	Capsella rubella	Carubv10009731m; Carubv10013971m; Carubv10015501m; Carubv10016442m; Carubv10020384m

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8. Tables

8.1. Table 1

Details of ortholog gene groups used in the study.

	Eutrema salsugineum	Carubv10020384m; Thhalv10007673m; Thhalv10007685m; Thhalv10011633m; Thhalv10011649m
Group 3	Arabidopsis thaliana	AT1G49130.2; AT1G68520.1; AT1G73870.1; AT2G24790.1; AT2G33500.1
	Camelina sativa	Csa16g037740.1; Csa16g046500.1; Csa17g035050.1; Csa17g037850.1; Csa17g080770.1
	Capsella grandiflora	Cagra.0876s0020.1.p; Cagra.1004s0003.1.p; Cagra.11928s0002.1.p; Cagra.1194s0065.1.p; Cagra.14026s0004.1.p

8.2.   Table 2

Details of scripts and their function with input and output format.

Script / File	Function	Input	Output
`Babappa_normal.sh` (in `babappa-normal/`)	Master driver for the classic BABAPPA workflow (babappa_normal). Orchestrates end-to-end QC → PRANK → IQ-TREE → codeml → aggregation.	`input/` FASTA files (CDS)	Structured folders: `QCseq/`, `msa/`, `treefiles/`, `codemloutput/`, `codemlanalysis/`, `BHanalysis/`, Excel summary reports
`babappa_clip.sh` (in `babappa-clip/`)	Master driver for ClipKIT-augmented workflow (babappa_clip). Runs the normal pipeline but invokes ClipKIT rectification and stop-codon masking where configured.	`input/` FASTA files (CDS)	Same structured folders as above; additionally `clipmsa/` (or modified `msa/`) and frame/clip logs
`babappa_clipgard.sh` (in `babappa-clipgard/`)	Master driver for ClipKIT + GARD workflow (babappa_clipgard). Orchestrates trimming, frame checks, GARD recombination detection/partitioning, then downstream analyses.	`input/` FASTA files (CDS)	Same structured folders plus `clipmsa`, `gard_results/`, `blocks/` (partitioned alignments), and recombination logs
`seqQC.py`	Performs biological QC on coding sequences (start/stop codons, length, outliers, allowed bases).	Raw FASTA (.fasta) or text (.txt) of coding sequences	QC-passed FASTA files in `QCseq/`; QC log file(s)
`babappa_stopcodon_masker.py`	Built-in stop-codon masker / frame-fix utility to ensure codeml compatibility after trimming/partitioning. Masks or removes problematic codons for downstream codon-model input.	Aligned FASTA (PRANK or ClipKIT output)	Masked/cleaned aligned FASTA; masker log
`script0.sh … script8.sh` (present in each flavour folder)	Modular scripts that implement the stepwise pipeline logic (QC, alignment, tree inference, model runs, parsing, aggregation). Each flavour provides these scripts with flavour-specific options (e.g., ClipKIT/GARD hooks).	QC-filtered FASTA / MSA inputs and templates	Intermediate and final outputs consistent with the pipeline (MSAs, trees, codeml outputs, CSVs)
`run_codeml.py`	Generates codeml control files from templates (site, branch, branch-site) and launches codeml runs (called by scripts).	Folder with `aligned.fasta` and Newick treefile	`site/`, `branch/`, `branch-site/`, `branch-site-null/` result folders with codeml outputs and `codeml.ctl`
`4GroundBranchGenerator.py`	Generates per-tree Newick files where a single branch is annotated as foreground (#1) for branch-site tests.	One Newick treefile (.treefile)	Per-gene Newick files with foreground tags (placed in `foregroundbranch/`)
`filter_blocks.py` (in `babappa-clipgard/` and `babappa-gard/`)	Post-GARD filter: selects/filters partitioned blocks from GARD output based on criteria (e.g., block size, support).	GARD output (JSON/CSV) and partitioned alignments	Filtered block lists; filtered block FASTA files
`split_recombination_blocks.py` (in `babappa-clipgard`)	Splits alignments into recombination-free blocks according to GARD breakpoints for independent downstream analysis.	GARD breakpoint files and original alignment	Block-specific FASTA files (one FASTA per block)
`script1.5.sh` (in `babappa-clipgard/`)	Glue/patch helper to reassemble or coordinate block-level results (used after block-wise analysis).	Block-level result folders	Reassembled/merged results or metadata used for downstream aggregation
`script1.sh`	Runs IQ-TREE2 codon phylogenetic inference (model selection, ultrafast bootstrap, aLRT) and collects tree files.	`msa/*.fas` or `clipmsa/*.fas`	`iqtreeoutput/<gene>/` logs & treefiles; consolidated `treefiles/`
`script2.sh`	Parallelizes branch & branch-site codeml runs across species & treefiles.	Aligned FASTA (possibly block-split) & `foregroundbranch/` treefiles; template codeml.ctl	`codemloutput/<species>/<tree>_{B,BS,BS_NULL}/` with codeml outputs
`script3.sh`	Parallelizes M0 (site-null) codeml runs across species.	Aligned FASTA & treefiles; template codeml.ctl	`codemloutput/<species>/M0/results` with codeml outputs

`script4.sh`	Automates codeml site-model (M0, M1a, M2a, M3, M7, M8) analyses in parallel.	Aligned FASTA & treefiles; template codeml.ctl	`sitemodel/<species>/` with model-specific outputs
`script5.sh`	Parses branch & branch-site codeml outputs to extract lnL & np into CSV.	`codemloutput/<species>/.../output.txt` files	`codemlanalysis/<species>/lnL_np_values.csv`
`script6.sh`	Parses site-model codeml outputs to extract lnL & np into CSV.	`sitemodel/<species>/output.txt`	`sitemodelanalysis/<species>/lnL_np_values.csv`
`lrt_bh_correction.py`	Computes LRT statistics and applies Benjamini–Hochberg FDR correction for branch/branch-site comparisons.	CSVs: `codemlanalysis/<species>/*.csv`	Excel reports `BHanalysis/<species>/LRT_results_<…>.xlsx` (raw p, FDR p, significance)
`lrt_bh_correction.sitemodel.py`	Computes LRT & BH correction for site-model comparisons and summarizes results.	CSV: site-model results (*.csv with Model, lnL, np)	`SiteModelBH/<species>/lrt_results.csv` (LRT, df, p, BH-adjusted p)
`merge_bh_results.py`	Merges BH-corrected results from multiple species/flavours into a consolidated report (helper script noted in README).	Multiple `BHanalysis/` Excel/CSV outputs	Consolidated summary report (CSV/XLSX)

9. Figure legends

9.1. *Figure 1*

Schematic workflow of BABAPPA v2.0.

9.2. *Figure 2*

BABAPPA v2.0 Graphical User Interface.

9.3. *Figure 3*

Benchmarking of BABAPPAv2.0 run with standard run.

10. Figures

10.1. *Figure 1*

10.2. Figure 2

10.3. Figure 2