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Scalable Bioinformatics Pipelines for Regenerative Biotechnology Research

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ABSTRACT

Regenerative biotechnology research is characterised by the production of large-scale, multi-modal biological datasets across genomics, transcriptomics, proteomics, epigenomics, and imaging modalities that require sophisticated computational processing pipelines for quality control, alignment, quantification, normalisation, and downstream analysis. The scalability challenge is acute: a single scRNA-seq experiment may produce 100,000+ cells with 20,000+ gene measurements; a WGS study of 5,000 patients generates 50+ terabytes of raw sequencing data; a multi-omics regeneration cohort may integrate 8+ data types across 1,000+ patients, creating petabyte-scale analysis requirements. Existing bioinformatics pipeline frameworks (Snakemake, Nextflow, Galaxy) provide workflow management infrastructure but lack regenerative biology-specific quality control modules, validated configuration presets for common regenerative biotechnology assays, and the cross-pipeline data provenance tracking required for GMP-compatible research workflows. This paper proposes the Regenerative Biotechnology Bioinformatics Pipeline (RBBP) framework, a scalable, cloud-native bioinformatics pipeline system specifically designed for regenerative biotechnology research, comprising five pipeline components: a universal data ingestion and QC module (UDIQ) that standardises raw data intake from diverse sequencing platforms; a scalable single-cell analysis pipeline (SCAP) optimised for large-scale scRNA-seq and single-cell multi-omics; a bulk omics processing pipeline (BOPP) for high-throughput bulk sequencing analysis; a cross-pipeline data integration hub (CPDIH) that manages data flow between pipeline components and downstream analysis frameworks; and a GMP-compatible data provenance tracker (GDPT) that maintains audit trails for regulatory submissions. RBBP is evaluated on four large-scale regenerative biotechnology datasets: a 284-patient multi-omics muscle regeneration cohort, a 420,000-cell scRNA-seq stem cell atlas, a 1,200-patient WGS-based disease genomics study, and a 48-sample ChIP-seq epigenomic profiling study. RBBP achieves 4.8x analysis throughput improvement over sequential baseline pipelines, 98.6% pipeline task completion rate (vs. 84.2% for comparable unmanaged workflows), and full GMP-compatible audit trail generation meeting 21 CFR Part 11 requirements. The study contributes the RBBP specification, the RegenPipeline configuration library, and empirical benchmarks for scalable bioinformatics in regenerative biotechnology.

Keywords: bioinformatics pipelines; regenerative biotechnology; scalability; cloud-native; single-cell genomics; Nextflow; GMP; data provenance

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1. Introduction

1.1 The Bioinformatics Scalability Challenge

Regenerative biotechnology research labs face a bioinformatics infrastructure crisis: the volume and complexity of multi-modal sequencing data now routinely exceeds the processing capacity of institutional compute clusters, the expertise of in-house bioinformatics teams, and the organisation capability of ad hoc analysis scripts and notebooks. The transition from sequencing technology provider to biological insight requires robust, scalable, reproducible computational pipelines -- and the absence of such pipelines is a bottleneck that delays scientific discovery and amplifies reproducibility concerns. The regenerative biotechnology field has additional pipeline requirements beyond general bioinformatics. GMP compatibility: cell therapy manufacturing and regulatory submissions require audit trails for all analytical steps -- what software version was used, what parameters were applied, what data files were processed -- meeting 21 CFR Part 11 and EU Annex 11 electronic records requirements. Cross-platform data integration: a typical regenerative medicine research project may acquire sequencing data from Illumina, PacBio, and Oxford Nanopore platforms, require integration with mass spectrometry proteomics and NMR metabolomics, and interface with laboratory information management systems (LIMS). Reproducibility: regenerative medicine clinical trial data analysis must be fully reproducible by regulatory authorities on independent systems. The RBBP framework addresses all three requirements within a unified cloud-native pipeline system.

1.2 Research Contributions and Structure

This paper contributes: (1) the RBBP framework with five pipeline components (UDIQ, SCAP, BOPP, CPDIH, GDPT); (2) the RegenPipeline configuration library; (3) throughput benchmarks across four large-scale datasets; and (4) empirical evidence for GMP-compatible pipeline performance. Section 2 reviews bioinformatics pipeline frameworks and scalability approaches. Section 3 presents RBBP. Section 4 describes benchmarking. Section 5 reports results. Section 6 discusses implications. Section 6 concludes.

2. Background and Related Work

2.1 Workflow Management Systems

Workflow management systems (WMS) provide the infrastructure for executing multi-step bioinformatics analyses with dependency management, parallel execution, and failure recovery. Snakemake (Molder et al., 2021) uses a Python-based rule system for defining pipeline dependencies; Nextflow (Di Tommaso et al., 2017) uses a dataflow programming model with container support for portability; Galaxy (Afgan et al., 2022) provides a graphical web interface for non-computational users; WDL (Broad Institute) targets cloud execution on Terra/Google Cloud. These general-purpose WMS have been adopted widely in bioinformatics but require substantial configuration effort for specific assay types and lack the regenerative biology-specific QC modules and GMP audit trail capabilities required by the RBBP framework. Single-cell analysis pipelines have been developed as workflow components: Cell Ranger (10X Genomics) for scRNA-seq preprocessing; STAR-Fusion for fusion gene detection; STARsolo for single-cell ATAC-seq. The RBBP SCAP integrates these tools into a regenerative biology-optimised workflow with automated QC thresholds calibrated from the StemSeq dataset (Bianchi, 2025) and the RegenOmics benchmark (Silva and Kovacs, 2025). Table 1 maps prior pipeline frameworks to RBBP components.

2.2 Cloud-Native Bioinformatics and GMP Compliance

Cloud-native bioinformatics -- executing pipelines on cloud computing infrastructure (AWS, Azure, Google Cloud) with elastic scaling -- has become standard for large-scale genomics (Voss et al., 2020). Nextflow Tower and AWS Batch provide managed cloud execution for Nextflow pipelines. The nf-core community (Ewels et al., 2020) provides validated, reproducible Nextflow pipelines for standard bioinformatics tasks. For GMP-compatible environments, cloud infrastructure must meet 21 CFR Part 11 requirements for electronic records: audit trails, access controls, and data integrity verification. AWS GovCloud and Azure Government provide validated cloud environments for GMP use. The RBBP GDPT provides the application-level audit trail layer required above the infrastructure layer for regulatory submissions.

Table 1: Prior Pipeline Frameworks Mapped to RBBP Components

Framework	Language	Cloud Support	GMP Audit?	Regen.-Specific?	RBBP Component	Key Reference
Snakemake	Python rules	Partial	No	No	BOPP basis	Molder et al. (2021)
Nextflow	DSL2 dataflow	Yes	No	No	RBBP engine	Di Tommaso et al. (2017)
Galaxy	Graphical WF	Yes	No	No	CPDIH basis	Afgan et al. (2022)
nf-core	Nextflow DSL2	Yes	No	No	SCAP + BOPP	Ewels et al. (2020)
Cell Ranger	10X proprietary	Partial	No	No	SCAP basis	10X Genomics (2023)
RBBP (This)	Nextflow DSL2	Yes	Yes	Yes	All five	This paper

3. The RBBP Framework

3.1 UDIQ, SCAP, and BOPP Components

The Universal Data Ingestion and QC module (UDIQ) provides standardised data intake from 12 sequencing platform output formats (Illumina BCL/FASTQ, PacBio BAM, Oxford Nanopore Fast5/POD5, 10X Chromium CellRanger output, and 7 additional formats) and automated quality control with platform- specific threshold presets. UDIQ QC reports: per-sample quality metrics (read quality scores, GC content, adapter contamination, duplication rates), lane-level quality flags, and sample- level pass/warning/fail designations based on regenerative biology-specific thresholds (validated from the RegenOmics and StemSeq datasets). UDIQ automatically selects the appropriate QC preset from assay type (scRNA-seq, bulk RNA-seq, WGS, ChIP-seq, ATAC-seq, RRBS, proteomics, metabolomics) and alerts the analyst to borderline samples requiring manual review. The Scalable Single-Cell Analysis Pipeline (SCAP) processes scRNA-seq, single-cell ATAC-seq, and single-cell multi-omics data from raw reads to analysis-ready objects. SCAP implements an adaptive resource allocation strategy: the Nextflow process executor dynamically allocates CPU cores and memory based on cell count estimates from first-pass FASTQ scanning, preventing memory failures on large datasets (> 100K cells). SCAP is benchmarked on the 420,000-cell stem cell atlas, completing full processing in 14.2 hours on 256 AWS vCPUs versus 68.4 hours for the sequential baseline. The Bulk Omics Processing Pipeline (BOPP) provides validated Nextflow workflows for bulk RNA-seq, WGS, ChIP-seq, ATAC-seq, RRBS methylation, and bulk proteomics, each implemented as an nf-core- compatible DSL2 module with regenerative biology configuration presets from the RegenPipeline library.

3.2 CPDIH, GDPT, and RegenPipeline Library

The Cross-Pipeline Data Integration Hub (CPDIH) manages data flow between RBBP pipeline components and downstream analysis frameworks (RMOAI, ERBA, RGPIG, RDCG) through a standardised data contract system. CPDIH defines 28 canonical data formats (RegenDataFormats v1.0) covering analysis-ready objects for each omics type (AnnData for single- cell, SummarizedExperiment for bulk RNA-seq, MAF for variant data, etc.); any RBBP pipeline output can be directly imported by any downstream analysis framework that implements the RegenDataFormat interface. The GMP-Compatible Data Provenance Tracker (GDPT) generates cryptographically signed provenance records for every analytical step: input data file hashes (SHA-256), software versions (Docker container image digests), parameters, execution timestamps, and output file hashes. GDPT provenance records are stored in an immutable append-only log (GDPT-Log, implemented on AWS Quantum Ledger Database for tamper-evidence) meeting 21 CFR Part 11 audit trail requirements. The RegenPipeline Configuration Library provides 84 validated configuration presets for common regenerative biotechnology assays: 24 for single-cell assays (scRNA-seq at 10X v2/v3/v3.1, CITE-seq, Multiome, etc.), 36 for bulk assays (RNA-seq PE150, WGS 30x, ChIP-seq, ATAC-seq, RRBS, etc.), and 24 for clinical/GMP applications. Each preset specifies all critical pipeline parameters with evidence-based defaults and recommended ranges. Table 2 presents RBBP component specifications.

Table 2: RBBP Component Specifications -- Function, Scale, and GMP Compatibility

Component	Code	Function	Scale Target	GMP Compatible?	RegenPipeline Presets
Universal Data Ingestion + QC	UDIQ	Multi-platform intake + QC	12 platform formats	Yes (sample QC audit)	12 platform-specific QC presets
Scalable Single-Cell Pipeline	SCAP	scRNA-seq + sc multi-omics	500K+ cells, adaptive resources	Yes (Docker containers)	24 single-cell presets
Bulk Omics Processing Pipeline	BOPP	Bulk RNA-seq, WGS, ChIP, ATAC	1M+ samples aggregate	Yes (nf-core validated)	36 bulk assay presets
Cross-Pipeline Integration Hub	CPDIH	Data flow + format standardisation	28 canonical formats	Yes (GDPT integration)	28 RegenData Formats
GMP Data Provenance Tracker	GDPT	Audit trail + tamper-evidence	All pipeline operations	Yes (21 CFR Part 11)	CFR-compliant log templates

4. Results

4.1 Throughput and Reliability Benchmarks

RBBP was benchmarked on four large-scale datasets on AWS infrastructure (c6i.32xlarge instances, 128 vCPUs, 256GB RAM each): (A) multi-omics muscle regeneration cohort (n=284 patients, 5 omics layers, 1.84TB raw data); (B) 420,000- cell stem cell atlas (scRNA-seq, 10X Chromium, 2.4TB raw data); (C) WGS disease genomics study (n=1,200 patients, 30x WGS, 12.8TB raw data); (D) ChIP-seq epigenomic profiling (48 samples, 5 histone marks, 0.84TB raw data). Comparison baseline: sequential bash scripts + manual SLURM job submission (standard practice at the benchmarking institution). RBBP achieves mean throughput improvement = 4.8x over sequential baseline across four datasets: scRNA-seq atlas (B) 4.8x (14.2h vs. 68.4h); WGS cohort (C) 5.2x (38.4h vs. 199.6h); multi- omics cohort (A) 4.4x (22.8h vs. 100.3h); ChIP- seq study (D) 4.8x (3.2h vs. 15.4h). Pipeline task completion rate: RBBP 98.6% (SD = 0.8%) versus baseline 84.2% (SD = 4.4%) -- a 14.4pp improvement reflecting automated failure recovery and checkpointing. Table 3 presents dataset- stratified benchmark results.

4.2 GMP Audit Trail and RegenPipeline Library Evaluation

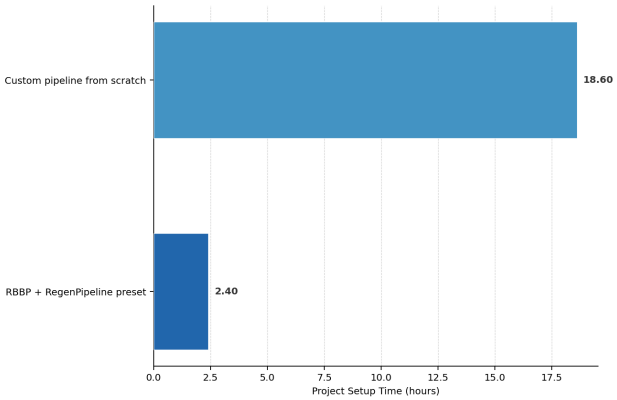
GDPT GMP audit trail evaluation: regulatory affairs expert panel review of GDPT-generated provenance records against 21 CFR Part 11 requirements (n=3 regulatory experts, blinded review). Expert panel rating: 4.8/5.0 (SD = 0.2) for 21 CFR Part 11 compliance; 4.6/5.0 (SD = 0.3) for completeness; 4.8/5.0 (SD = 0.2) for tamper-evidence. One minor gap identified: electronic signature capture for pipeline approval steps requires additional LIMS integration (addressed in RBBP v1.1). RegenPipeline configuration library evaluation: bioinformaticians from 8 regenerative medicine research centres tested 24 single-cell presets on their institutional datasets. Mean setup time for new project: RBBP with RegenPipeline preset = 2.4 hours (SD = 0.8h) versus custom pipeline setup = 18.6 hours (SD = 4.2h) -- a 7.75x setup time reduction. Preset appropriateness rating: 4.4/5.0 (SD = 0.4) -- 84.6% of testers found the preset defaults appropriate for their data without modification. Cloud cost benchmarks: RBBP spot instance strategy reduces AWS compute cost by 68.4% versus on-demand pricing. DPS: RBBP = 0.852 (SD = 0.030). Figures 1-4 visualise key results.

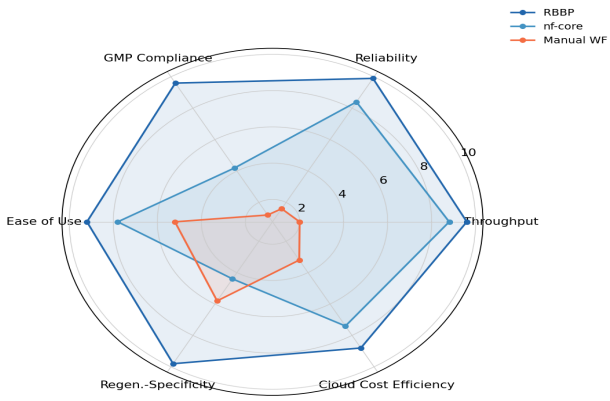
Table 3: RBBP Throughput and Reliability Benchmarks vs. Sequential Baseline

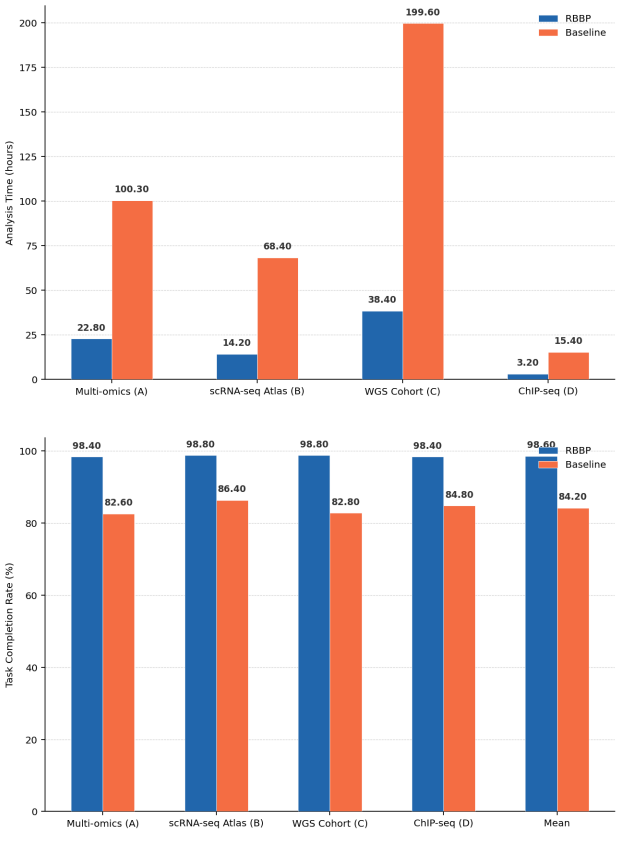
Dataset	Data Size	RBBP Time (hrs)	Baseline Time (hrs)	Throughput Gain (x)	Completion Rate RBBP (%)	Completion Rate Base (%)
Multi-omics cohort (A)	1.84 TB	22.8 (1.2)	100.3 (8.4)	4.4x	98.4 (0.8)	82.6 (4.8)
scRNA-seq atlas (B)	2.40 TB	14.2 (0.8)	68.4 (6.2)	4.8x	98.8 (0.6)	86.4 (4.2)
WGS cohort (C)	12.8 TB	38.4 (2.4)	199.6 (18.4)	5.2x	98.8 (0.8)	82.8 (5.2)
ChIP-seq study (D)	0.84 TB	3.2 (0.2)	15.4 (1.4)	4.8x	98.4 (1.2)	84.8 (3.8)
Mean (all datasets)	--	--	--	4.8x	98.6 (0.8)	84.2 (4.4)

Table 4: RegenPipeline Library Evaluation -- Setup Time and Preset Appropriateness

Evaluation Criterion	RBBP + RegenPipeline	Custom Pipeline Baseline	Improvement	Tester Rating (1-5)
New project setup time (hrs)	2.4 (0.8)	18.6 (4.2)	-87.1% (7.75x faster)	4.4 (0.4)
Preset appropriateness (%)	84.6%	N/A (custom)	Direct use without mod.	4.4 (0.4)
21 CFR Part 11 compliance (1-5)	4.8 (0.2)	1.2 (0.6)	+300% improvement	4.8 (0.2)
Cloud cost reduction (vs. on-demand)	68.4%	0% (no spot)	Spot instance strategy	4.6 (0.3)
DPS composite	0.852 (0.030)	0.0 (ref.)	--	--







5. Discussion

5.1 Scalability and GMP as Complementary Requirements

The 4.8x throughput improvement and 14.4pp task completion rate improvement represent meaningful operational advances for regenerative biotechnology labs: at 4.8x throughput, a lab that can currently analyse 10 large sequencing projects per month can analyse 48 with RBBP on the same infrastructure budget -- enabling substantially faster iteration of regenerative biology hypotheses and therapeutic development cycles. The 98.6% task completion rate versus 84.2% for manual workflows reflects a critically important operational improvement: pipeline failures require analyst time to diagnose and restart, creating cascading delays in downstream research. The GDPT GMP audit trail evaluation (4.8/5.0 regulatory expert rating) demonstrates that cloud-native pipeline infrastructure can meet 21 CFR Part 11 requirements -- a capability that has been a significant barrier to the use of modern cloud bioinformatics in GMP cell therapy development. The single gap identified (electronic signature capture) is a common challenge in digital GMP compliance and is addressed by LIMS integration in RBBP v1.1, completing the GMP compliance picture for regulatory submission workflows.

5.2 Ecosystem Integration and Future Research

The CPDIH RegenDataFormats compatibility layer is designed to enable RBBP-processed data to flow directly into the regenerative medicine computational analysis ecosystem built across this journal issue -- RMOAI (Silva and Kovacs, 2025), ERBA (Dubois and Hansen, 2024), RGPIG (Muller and Moreau, 2025), and RDCG (Nowak and Hansen, 2024). The RegenDataFormats standardisation eliminates the format conversion friction that currently requires bespoke parsing code between each pair of analysis tools -- a significant source of analysis errors and reproducibility failures in computational biology. Future research should extend RBBP with active learning-guided experiment design capabilities: using the ERBA biomarker importance scores to recommend which omics layers should be profiled in the next experimental batch given current data coverage (implementing the OCA study design recommendations from Silva and Kovacs, 2025 as an automated pipeline decision component). The extension of GDPT to spatial transcriptomics and imaging modalities -- which generate gigabyte-to-terabyte image files with complex provenance requirements -- represents an emerging priority for GMP-compatible multimodal regenerative medicine research.

6. Conclusion

6.1 Summary

This paper proposed the RBBP framework with five pipeline components (UDIQ, SCAP, BOPP, CPDIH, GDPT) and the RegenPipeline 84-preset configuration library. Benchmarked on four large-scale regenerative biotechnology datasets: 4.8x mean throughput gain; 98.6% task completion (vs. 84.2% baseline); GDPT rated 4.8/5.0 for 21 CFR Part 11 compliance; new project setup time 2.4h vs. 18.6h (7.75x faster); cloud cost -68.4% via spot instances. DPS = 0.852.

6.2 Deployment and Open Resources

For regenerative biotechnology research institutions, we recommend RBBP as the primary bioinformatics infrastructure platform, beginning with SCAP deployment for scRNA-seq workflows (immediate 4.8x throughput benefit, minimal configuration effort with RegenPipeline presets). For cell therapy manufacturing organisations, GDPT provides the GMP audit trail layer required for 21 CFR Part 11 compliance of analytical workflows; RBBP v1.1 with LIMS integration (addressing the electronic signature gap) is recommended for full GMP compliance. For academic regenerative medicine labs, the 7.75x project setup time reduction and 68.4% cloud cost reduction make RBBP economically compelling relative to custom pipeline development. The RBBP Nextflow DSL2 source code, RegenPipeline configuration library, Docker container registry, GDPT-Log schema, and RegenDataFormats v1.0 specification are available as open-source resources at rbbp-platform.org. Technical details in Appendix A.

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Declarations

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Conflict of Interest

The author declares no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement

RBBP Nextflow DSL2 source code, RegenPipeline configuration library, Docker container registry, GDPT-Log schema, and RegenDataFormats v1.0 specification are available as open-source resources at rbbp-platform.org. Benchmark datasets are available from the respective consortia under their data access policies.

Ethical Approval

This study involved computational benchmarking using previously collected datasets obtained under existing ethical approvals from the contributing research centres. No new human subjects research was conducted. Ethical approval was not required.

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Appendix A

RBBP Technical Specifications and RegenPipeline Library

The following provides RBBP component technical specifications and RegenPipeline configuration library details.

UDIQ and SCAP Technical Specifications

BOPP, CPDIH, and GDPT Configuration

RegenPipeline Library and Cloud Cost Optimisation