

Reproducible scRNA-seq Workflow (PBMC)

Technical Summary

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1 Project Objective

This project implements a fully containerized, reproducible single-cell RNA-seq analysis pipeline using Snakemake and Docker. The workflow is demonstrated on publicly available 10x Genomics PBMC datasets (Donors 1–4).

Primary goal: demonstrate production-grade workflow engineering rather than novel biological discovery.

Key design principles:

- Deterministic execution (pinned container + pinned statistical environment)
- Version-locked dependencies (digest-pinned Docker image; `renv.lock`)
- Restart-safe modular execution (`*.done` sentinels; atomic completion markers)
- Donor-aware statistical modeling (pseudobulk DESeq2 with explicit TOST equivalence testing)
- Cross-donor validation (consensus co-expression networks)

2 Architecture

Pipeline layers:

1. Python CLI wrapper (section-based execution)
2. Snakemake DAG (authoritative workflow logic)
3. Docker container (environment isolation)
4. `renv`-locked R environment (statistical layer)

Execution flow:

```
FASTQ → QC → (optional trimming) → STARsolo →  
Seurat QC/Clustering → Pseudobulk DE →  
Equivalence testing (TOST) → Pathway enrichment →  
Consensus co-expression networks
```

Each stage produces atomic `*.done` sentinel files to ensure restart safety and auditability.

3 Reproducibility Strategy

Containerization

- Base image: `rocker/r-ver:4.3.3`
- Digest-pinned release image published to GHCR
- All execution occurs inside Docker (host-independent runtime)

R Environment Control

- `renv.lock` version-locked
- CRAN snapshot pinned (2024-01-01)
- Bioconductor 3.18 pinned
- Seurat / SeuratObject vendored locally (installed from local tarballs)

This eliminates upstream dependency drift and reduces long-term reproducibility risk.

4 Upstream Processing

Data

10x Genomics GEM-X 3' v4 PBMC libraries (Donors 1–4).

Quality Control

FastQC + MultiQC.

Findings:

- High base quality across donors
- No pervasive adapter contamination
- Trimming unnecessary (optional branch retained for pathological runs)

Alignment

Aligner: STARsolo v2.7.11b

Reference: GRCh38 + GENCODE v45

Chemistry: 16 bp cell barcode + 12 bp UMI

Alignment summary:

Metrics are consistent with high-quality 10x 3' scRNA-seq data.

Donor	Unique (%)	Multi (%)
Donor1	71.9	20.2
Donor2	72.4	19.3
Donor3	72.4	19.8
Donor4	68.8	21.7

5 Downstream Analysis

Seurat Processing

- `LogNormalize` (scale factor 10,000)
- 2000 HVGs (VST)
- PCA (30 PCs)
- Louvain clustering (resolution 0.3)
- Marker-based annotation via module scores

Major PBMC lineages are recovered consistently across donors.

Pseudobulk Differential Expression (DESeq2)

Counts are aggregated per donor \times immune group (donor treated as the experimental unit).

Model:

$$\sim \text{donor} + \text{group}$$

Tool: DESeq2 v1.42.1

Strict marker definition:

$$\text{padj} < 10^{-10}, \quad |\log_2 \text{FC}| > 3$$

Contrasts:

- T-like vs B-like
- T-like vs Mono-like
- B-like vs Mono-like

Equivalence Testing (TOST)

Margin:

$$\delta = 0.75$$

Conserved genes:

$$\text{padj}_{\text{equiv}} < 0.05 \quad \text{and} \quad |\log_2 \text{FC}| < \delta$$

This separates true small-effect similarity from statistical non-significance, yielding a contrast-specific marker set alongside a shared “core” program.

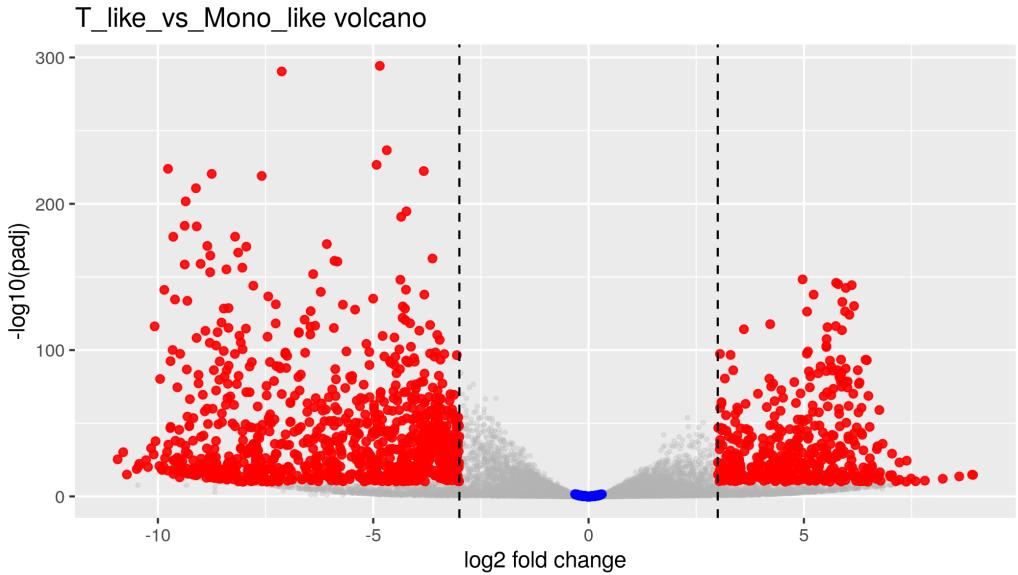


Figure 1: Representative pseudobulk DE contrast (T-like vs Mono-like). Strict markers occupy the distribution tails while near-zero effects concentrate around the origin.

6 Pathway Enrichment

- GSEA (`fgsea`)
- ORA (`clusterProfiler`)
- MSigDB Hallmark + C7

Markers are enriched for inflammatory and immune activation programs (e.g. TNF α /NF κ B and interferon signaling), while conserved genes emphasize shared metabolic and proliferative programs (e.g. oxidative phosphorylation, MYC targets, cell cycle).

7 Consensus Co-expression Networks

Constructed separately for:

- CD4 T cells
- B cells
- CD14 monocytes

Method:

- Metacell aggregation (size = 20)
- Spearman correlation across metacells
- Top- k sparsification ($k = 50$) with $|\rho| \geq 0.25$ (positive edges)
- Consensus retention if edge observed in ≥ 2 donors

Leiden clustering is performed on the consensus graph.

8 What This Demonstrates

- Production-grade Snakemake DAG design with restart-safe sentinel outputs
- Containerized statistical reproducibility (`renv` + digest-pinned Docker)
- Dependency pinning and vendoring discipline (Seurat sources)
- Donor-aware modeling (pseudobulk DESeq2) rather than naive per-cell DE
- Explicit equivalence testing (TOST) to distinguish true small-effect equivalence from statistical non-significance
- Cross-donor consensus network strategy for reproducible co-expression structure

9 Conclusion

This workflow provides a complete, auditable, and reproducible scRNA-seq analysis system from raw FASTQs to donor-aware inference and consensus network construction.

The primary contribution is engineering rigor: reproducibility, modular execution, statistical transparency, and cross-donor validation are treated as first-class design constraints.

Full technical documentation and extended results are available in the accompanying report and repository.

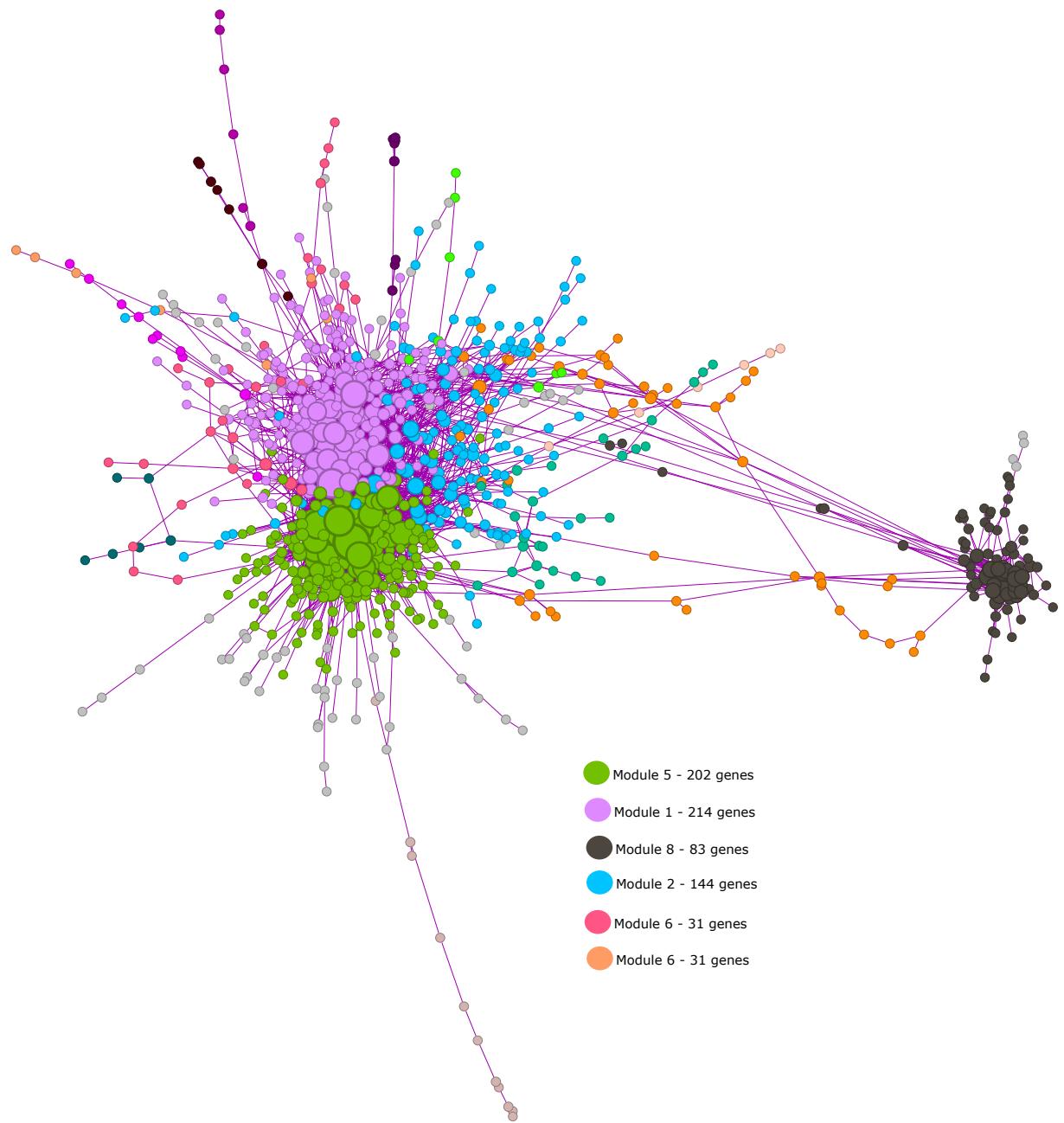


Figure 2: Example consensus network (CD4 T cells) colored by Leiden module. Cross-donor support filtering yields stable modules with clear compartmentalization of inflammatory, interferon, and proliferative programs.