A Computational Pipeline to Profile the Metabolic Landscape of the Tumor Microenvironment

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BACKGROUND

Tumors are heterogeneous cellular environments with entwined metabolic alterations to support continual growth and evade immune destruction. By profiling the metabolic rewiring of these heterogenous tissues, we can investigate critical components of immune response, proliferation, and distinct metabolic signatures of the tumor microenvironment. A multi-omic approach is crucial to accurately profile these complex interactions.

METHODS

Quality Control:
This pipeline is centered around a Seurat workflow for integrative scRNA-seq analysis, which allows for highly customizable quality-control parameters in the processing of transcriptomic data. This processed data is then provided as the input for all further analysis.

Integration:
Users are required to provide replicates for the single-cell transcriptomic data, which are then processed with Cell Ranger and Seurat, and serve as the basis for the various calculations of gene expression/inferred metabolite concentrations.

Comparison:
The optional (but encouraged) inclusion of bulk RNA-seq data adds an additional layer of granularity to the pathway-level analysis, cross-referencing KEGG enrichment in the scRNA-dataset to elucidate enrichment in heterogenous tissue. Similarly, the inclusion of various analyses represents a "swiss-cheese" approach to most accurately profile the TME.

Flexibility:
Users have the option to include Bulk RNA-seq data for pathway-level analysis, as well as a custom Genome-Scale Metabolic Model rather than opting to use the default.

Reproducibility:
All code dependencies will be wrapped in a Singularity container, and all versions/ parameters will be provided with the final reports of the pipeline. Additionally, all requisite code will be published and made available on GitHub.

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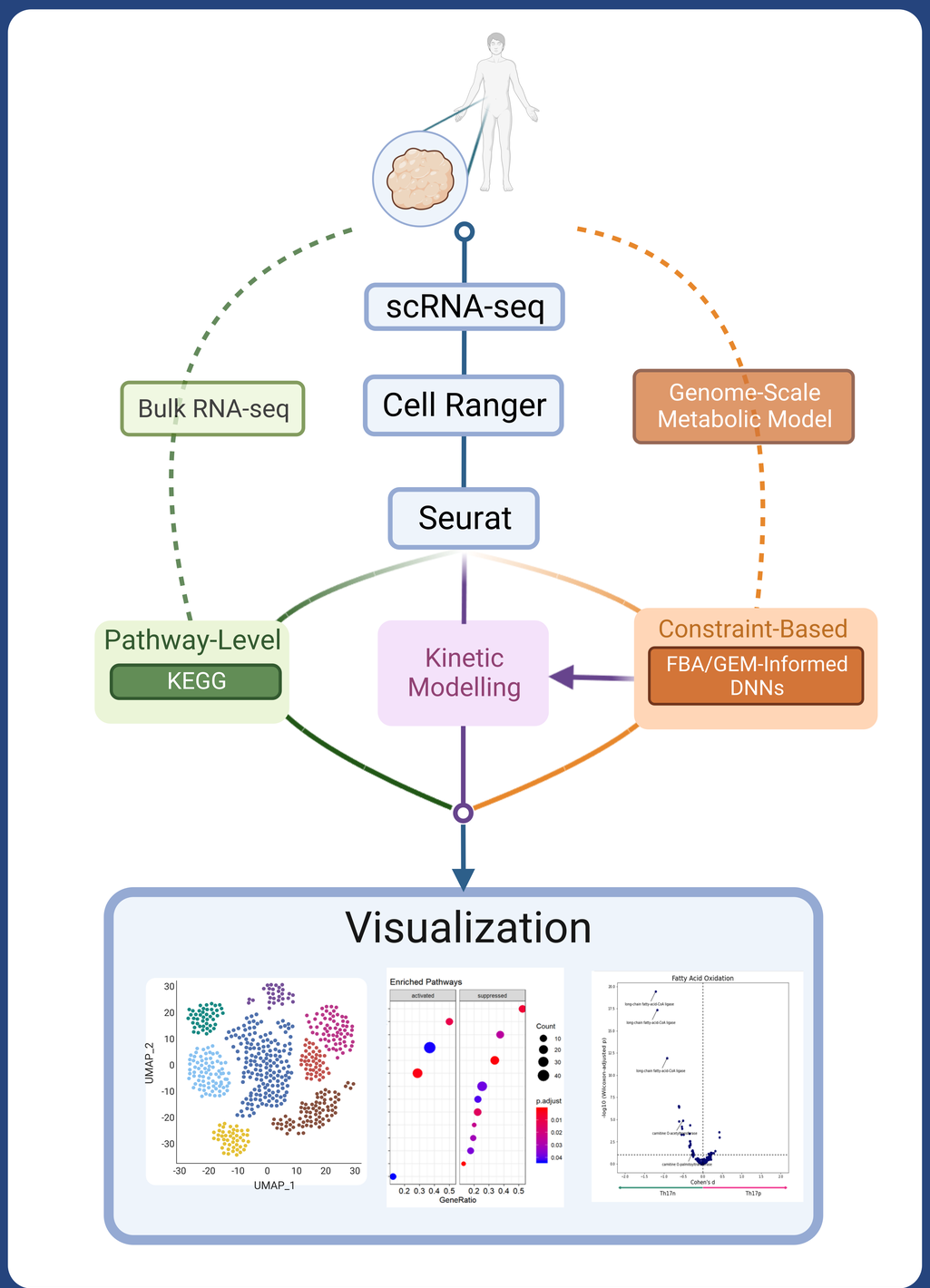
TMEProfileR



The main image shows a graphical abstract of the TMEprofileR analysis pipeline. Some aspects have been simplified or removed for clarity, including the pre-processing and QC of scRNA-seq data, and cell-type annotation. TMEprofileR will be publically available on GitHub, please scan the QR code to access the public directory, where you will find a PDF of this poster and the abstract. Feel free to follow the repository as well!

ANALYSIS

Pathway-Level:
Performed using the ClusterProfiler package in R, this analysis consists of mapping the most differentially-expressed genes between clusters to KEGG pathways through gene-set enrichment analysis. Optionally, these pathways are cross-referenced against bulk RNA-seq enrichment to identify TME-specific pathways.

Constraint-Based:
Comprised of two separate analyses, namely flux-based deep neural network (COMPASS) and GEM-informed graph neural network (scFEA) approaches. These analyses use single-cell transcriptomic contexts to infer metabolite concentrations and model reaction activity as a function of gene expression. The GEM-informed analysis (scFEA) uses a public metabolic atlas as prior-knowledge for quantifying metabolic reactions, and directly interfaces with Seurat.

Kinetic Modelling:
Using the inferred metabolite concentrations from COMPASS (flux-based DNN), the kinetic model infers metabolic activity using mixture models and serves as an alternative to constraint-based analyses as it doesn't assume steady-state conditions[1]

RESULTS

Raw output from the analyses is made available, as well as visualizations showing UMAP clustering of cell types and metabolic flux, ClusterProfiler plots of significant pathways, and various figures highlighting the variance between the provided tumor dataset and public metabolic data.

FRAMEWORK

nextflow

slurm workload manager

REFERENCES

Hrovatin K, Fischer DS, Theis FJ. Toward modeling metabolic state from single-cell transcriptomics. Mol Metab. 2022 Mar;57:101396. doi: 10.1016/j.molmet.2021.101396. Epub 2021 Nov 14. PMID: 34785394; PMCID: PMC8829761.