

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

Pathway Level:

Differentially expressed markers detected in tumor samples compared against a control are mapped to the KEGG set of metabolic pathways to investigate gene-set enrichment. Incorporated bulk data is used to refine the resultant enriched/suppressed pathways, a crucial step when analyzing expression in heterogenous tissue.

Constraint-Based:

Two methods, Flux-Based (FBA) and Genome-Scale Metabolic Model (GEM)-informed Deep Neural Networks (DNNs) aim to accurately profile the metabolic states of cells and quantify metabolic flux. The neural networks are trained using public metabolic atlas GEM data, to avoid the costly procedure of metabolomic sequencing. However, these steady-state models assume a constant concentration of metabolites, and calculate metabolic flux under these assumptions. Both of the methods take slightly different approaches to modelling the flux distributions using transcriptomic data, so by intersecting the results of the two methods, one can discern the most significant impacts to cellular metabolism, and overlay these results directly to an integrative single-cell analysis.

Kinetic Modelling:

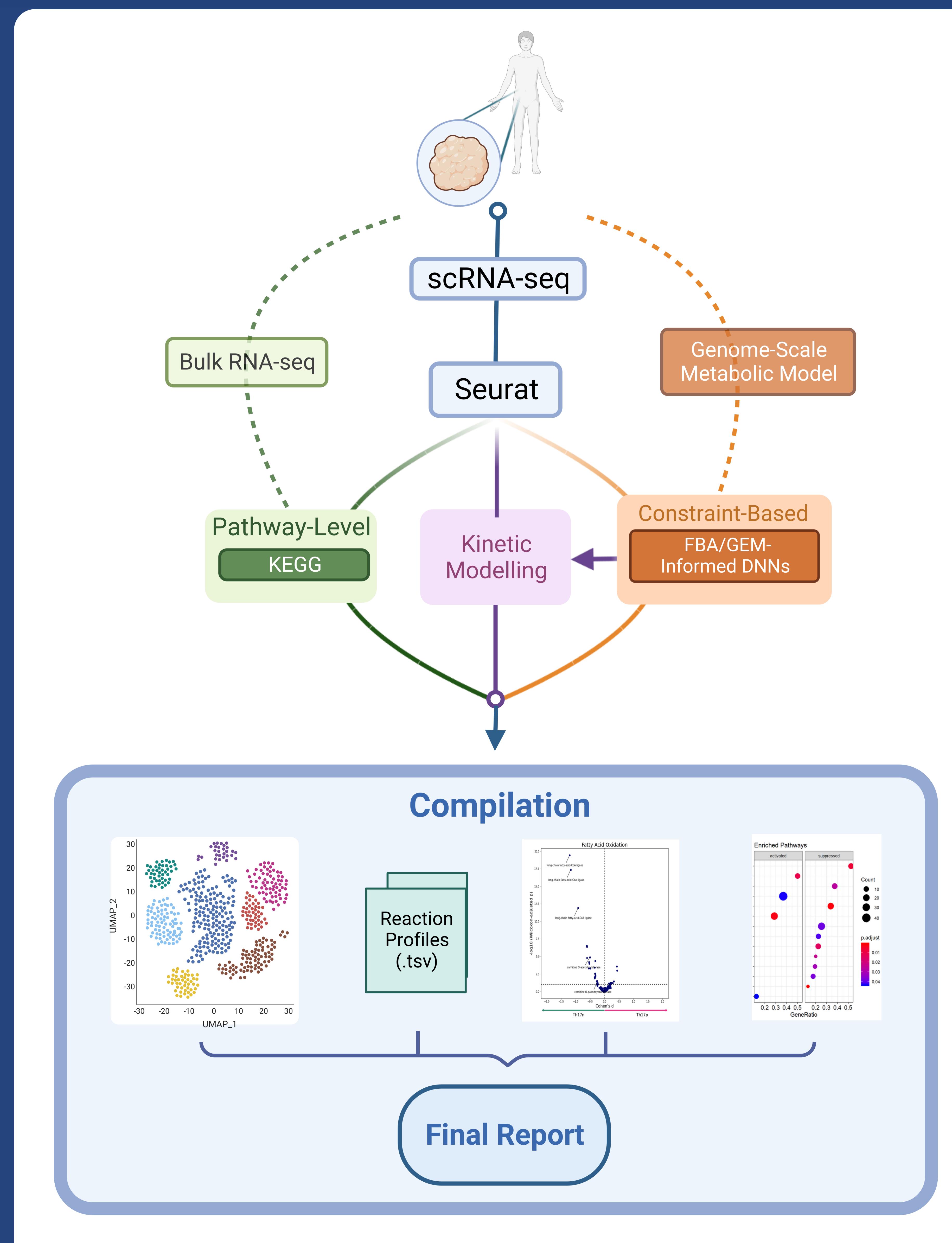
Using the inferred metabolite concentrations generated by the flux-based DNN models, the kinetic model infers metabolic activity using mixture models and serves as an alternative to constraint-based analyses, as it doesn't assume steady-state conditions¹. In the absence of metabolomic data, this approach enables researchers to build off of a more generalized constraint-based model that assumes equivalent metabolic exchange, and allows for a more granular perspective into the condition-specific metabolic changes that one would expect to observe in tumor samples.



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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 publicly 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!

ADVANTAGES

Comparison:

At its core, this pipeline approaches the complex problem of profiling TME metabolism using the "swiss-cheese" approach. By cross-referencing and integrating multiple analytical methods and -omics data, the final refined results can provide an inductive and insightful profile from which researchers can draw meaningful hypotheses. Each of these analyses provides a unique insight into the TME, and become a powerful resource when compiled together.

Flexibility:

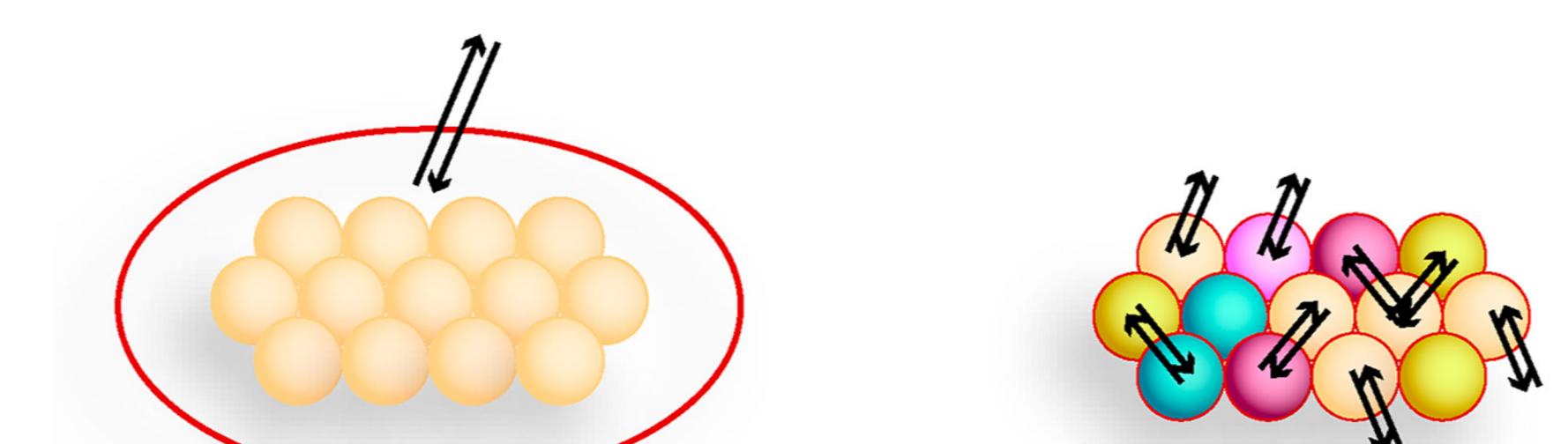
Users have the option to include Bulk RNA-seq data for pathway-level analysis, as well as a custom Genome-Scale Metabolic Model (GEM) rather than opting to use the default models.

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.

Bulk system

Single-cell system



RESULTS

- Each aspect of this pipeline provides unique yet complementary results, leveraging the strengths of each to bolster the weaknesses of the others, with the goal of most accurately profiling the complex metabolic rewiring of tumor tissue.
- Final reports from TMEprofileR are quite comprehensive, from raw reaction profiles to heat maps comparing metabolic activity against public datasets.
- All of the separate analyses are integrated into a broader scRNA-seq analysis, associating pathway expression and reaction profiles to specific cell types and correlating metabolic changes with gene expression

FRAMEWORK

nextflow

slurm
workload manager

R
S

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.