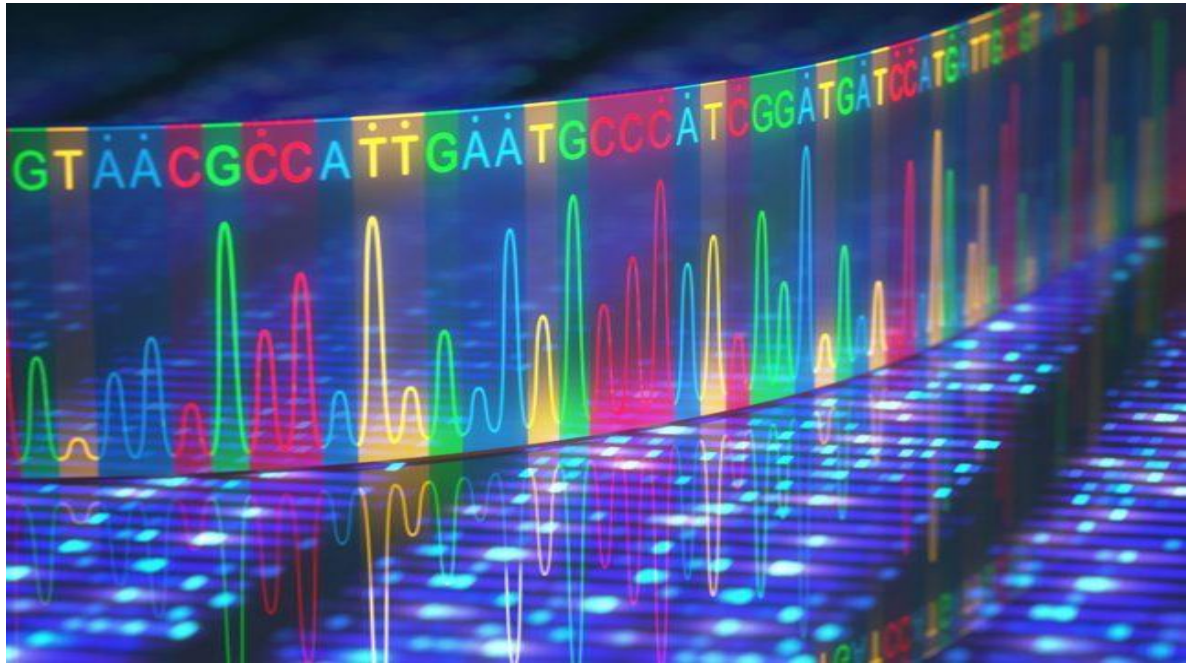
Benchmarking Cell Typing Methods in Spatial Transcriptomics

- Howard Baek
- Eliza Chai
- Alexis Harris
- Ingrid Luo
- Makayla Tang



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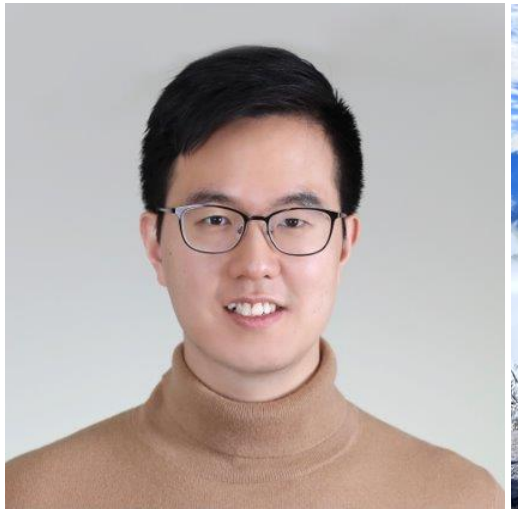
BIOSTATISTICS
SCHOOL OF PUBLIC HEALTH

Meet the Team

Alexis Harris

Eliza Chai

Ingrid Luo



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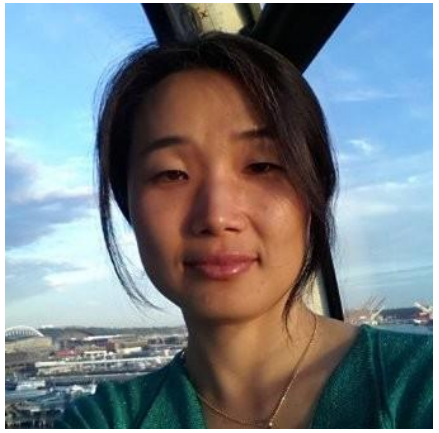
Acknowledgements

Patrick Danaher

Edward Zhao

Ronalyn Vitancol



Sangsoon Woo

Lloyd Mancl

Patrick Heagerty

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Spatial Transcriptomics and Cell Typing

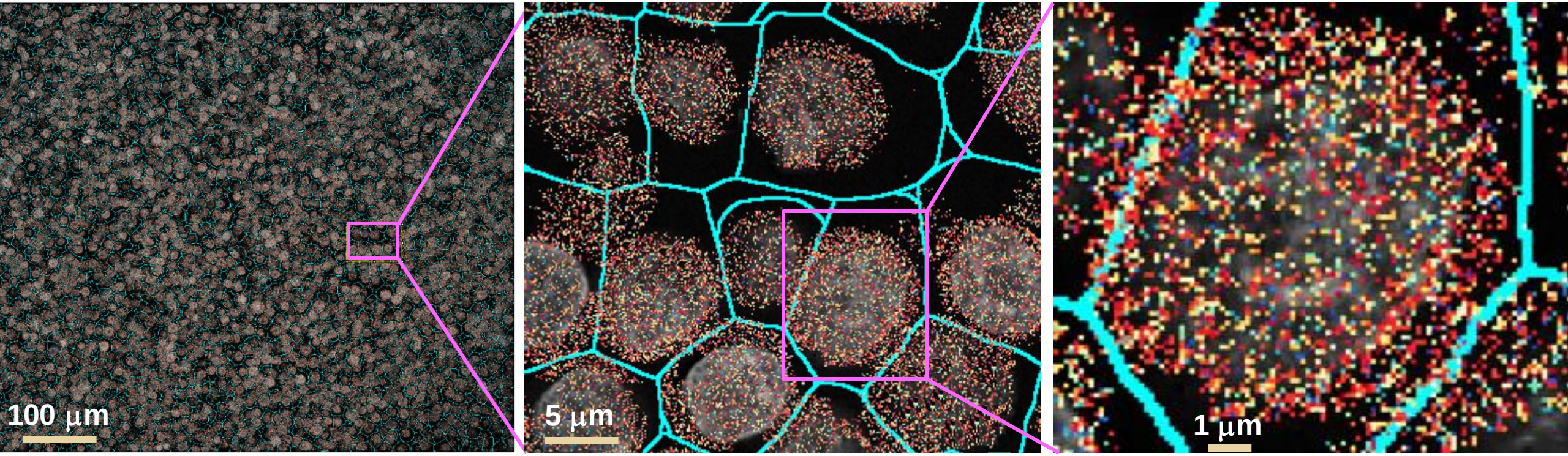
Spatial transcriptomics technologies measure single cell gene expression while recording cells' positions within a tissue

- detect individual RNA molecules where they lie on tissue slides
- produce datasets of single-cell gene expression data while preserving cells' location data

Cell typing is a classification used to identify cells that share features

- supervised and unsupervised methods

Single Cell Spatial Imager Localizes RNA at Subcellular Scale



Thousands of RNA transcripts
(represented as different colored single pixel in the image)
detected per single cell

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InSituType Cell Typing

- *in situ* - "in the original place"
- Likelihood model for spatial transcriptomics data
- Supervised cell typing from reference datasets via a Bayes classifier
- Unsupervised and semi-supervised cell typing via an Expectation Maximization (EM) algorithm

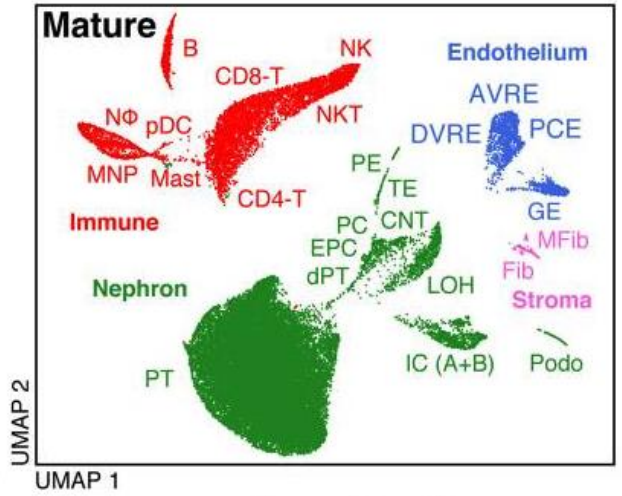
Dataset - Supervised

Training set: Kidney Single Cell Atlas.

- scRNA study that makes an analysis of all the cell types
- 40,268 mature kidney cells, consisting of 33 cell types

Test set: Kidney core biopsy from a lupus nephritis patient profiled using the CosMx™ Spatial Molecular Imager

- scRNA dataset with 61,073 mature kidney cells
- No cell type data
- Have a priori about whether the cells are located within the glomerulus or not



Dataset - Unsupervised

- Unsupervised method can train on any tissue
- Cell pellet array (CPA) profiled using the CosMx™ Spatial Molecular Imager
- Dataset of 52,518 cells
- 13 known clusters
- Benchmarking is performed on the full dataset and two downsampled datasets containing a random sample of 50% of the genes and the 10% most highly variable genes

Objectives

Objective 1 – Supervised Cell Typing

Benchmark **InSituType supervised** against established cell typing methods: SingleR, CHETAH, SeuratV3, SingleCellNet, scPred and SVM

- Evaluate the accuracy of the results using known kidney biology
- Compare running time

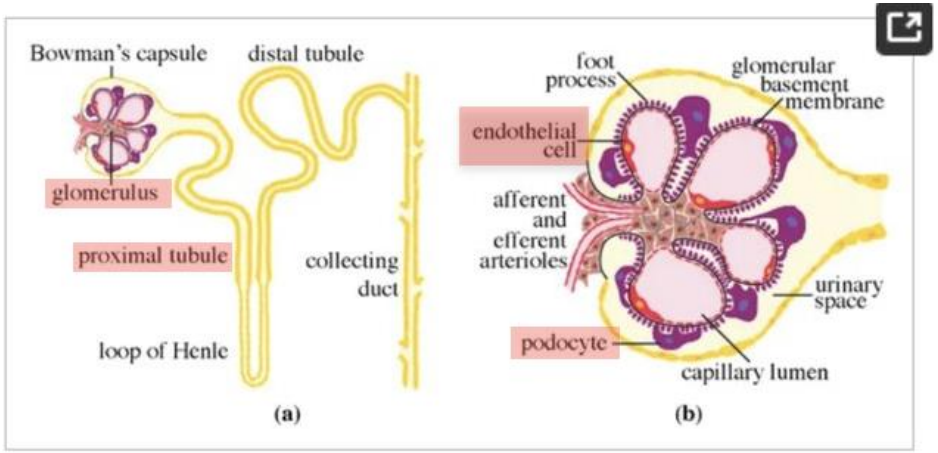
Objective 2 – Unsupervised Cell Typing

Optimize the performance of **InSituType unsupervised** method by tuning parameters

Methods

Supervised Cell Typing

- Score cell typing results based on the distribution of distinct cell types in substructures called "glomeruli"
- Record the intensity of specific marker proteins (PanCK and CD45) that are highly informative of cell types (cytokeratin and immune cells)



Unsupervised Cell Typing

Modify tuning parameters (# of clusters, iterations, Subsample size, number of cohorts)

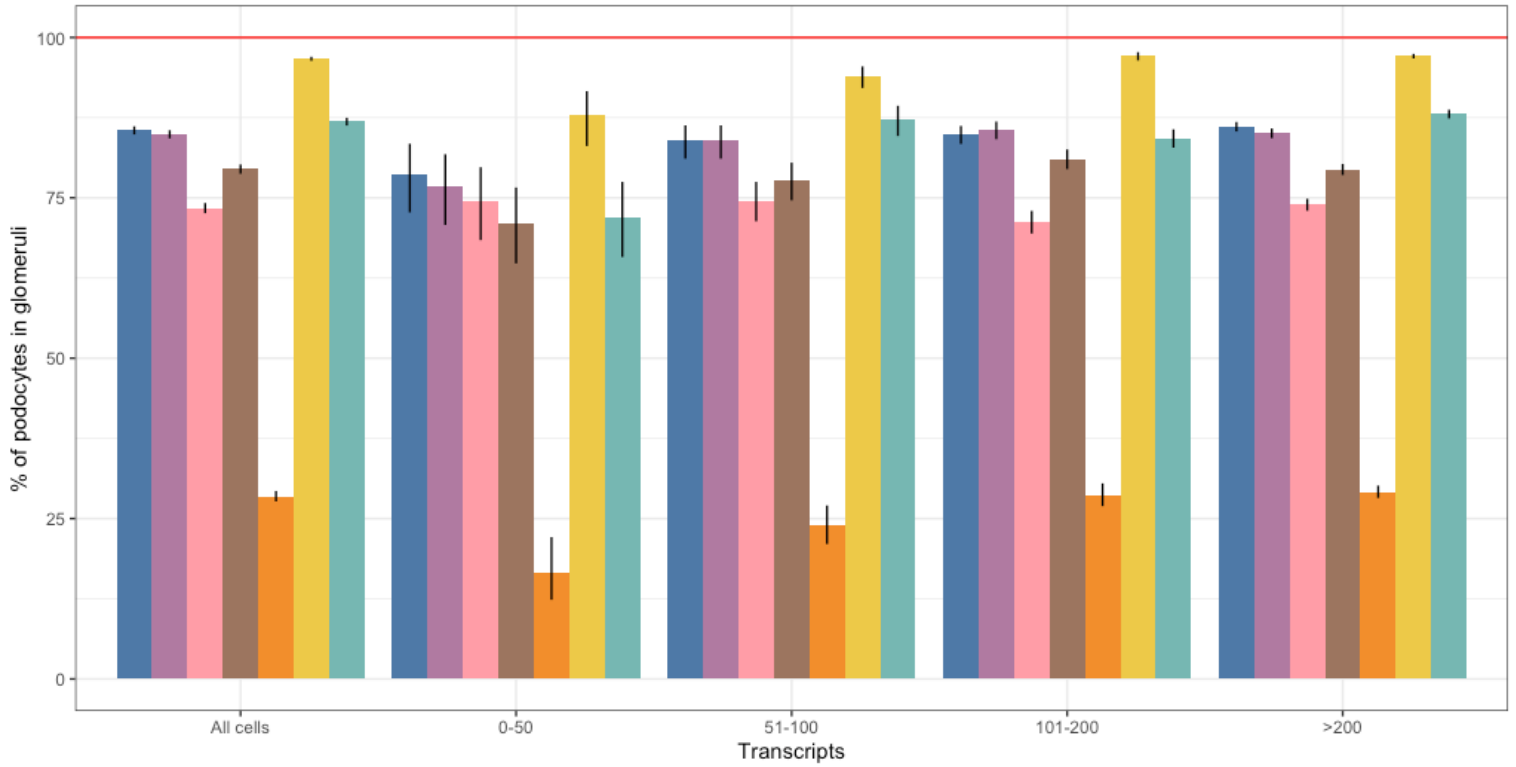
Metrics: Bayesian information criteria (BIC), Adjusted rand index (ARI), Computation time

Supervised

Results – Accuracy of glomerular cell assignments

All genes

Note that scPred has 98% unassigned cells;
CHETAH has 50% unassigned cells



Best: SVM

Worst: CHETAH

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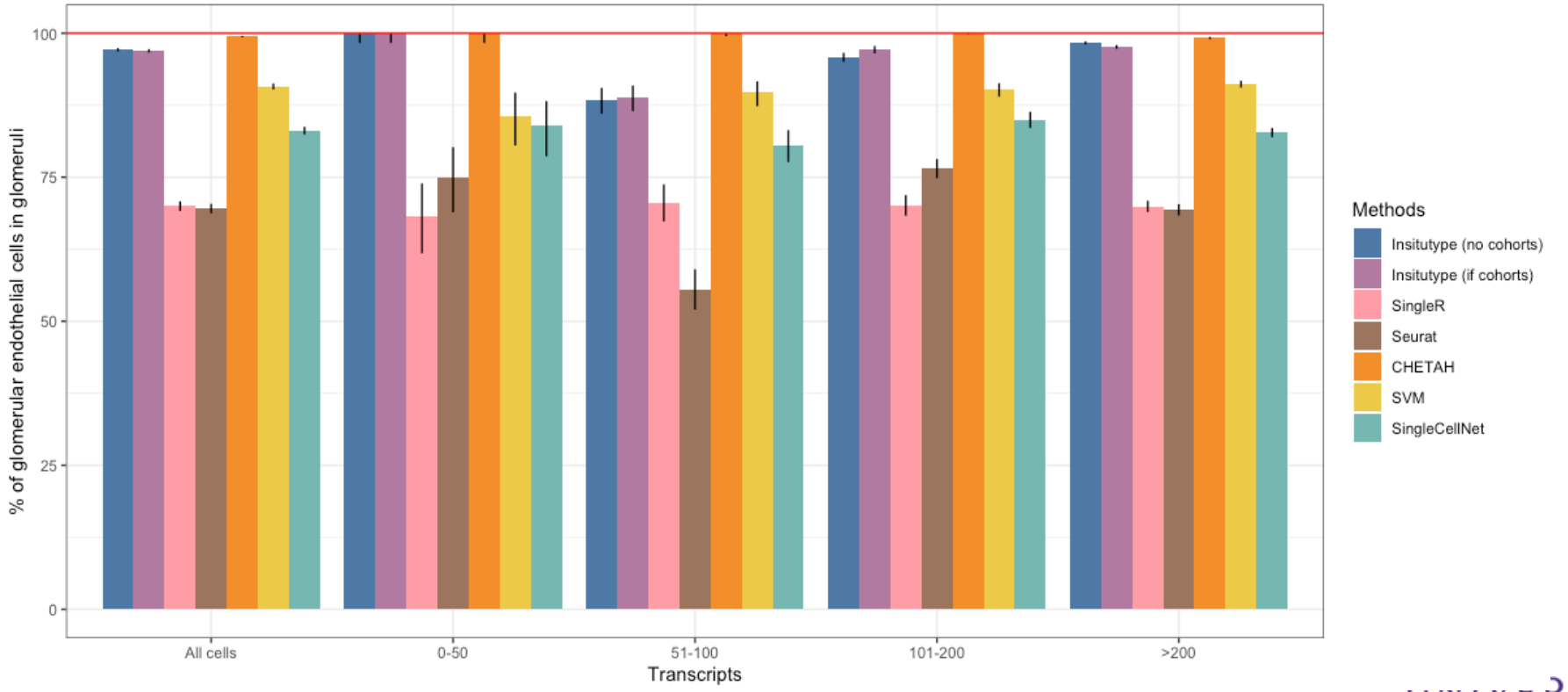
Supervised

Results – Accuracy of glomerular cell assignments

All genes



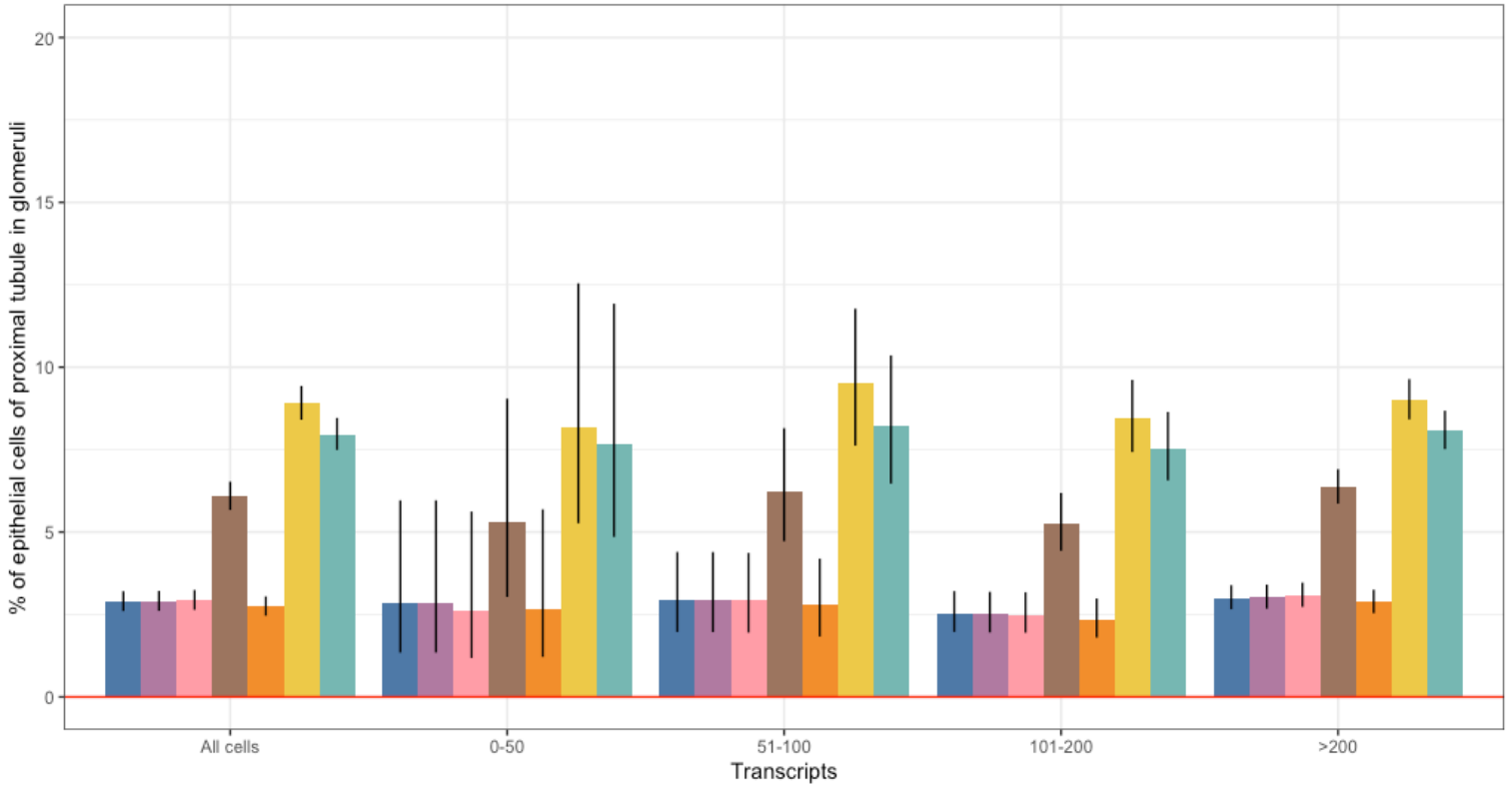
Best: CHETAH

Worst: Seurat

Supervised

Results – Accuracy of glomerular cell assignments

All genes



Best: /

Worst:

SVM

SingleCellNet

Seurat

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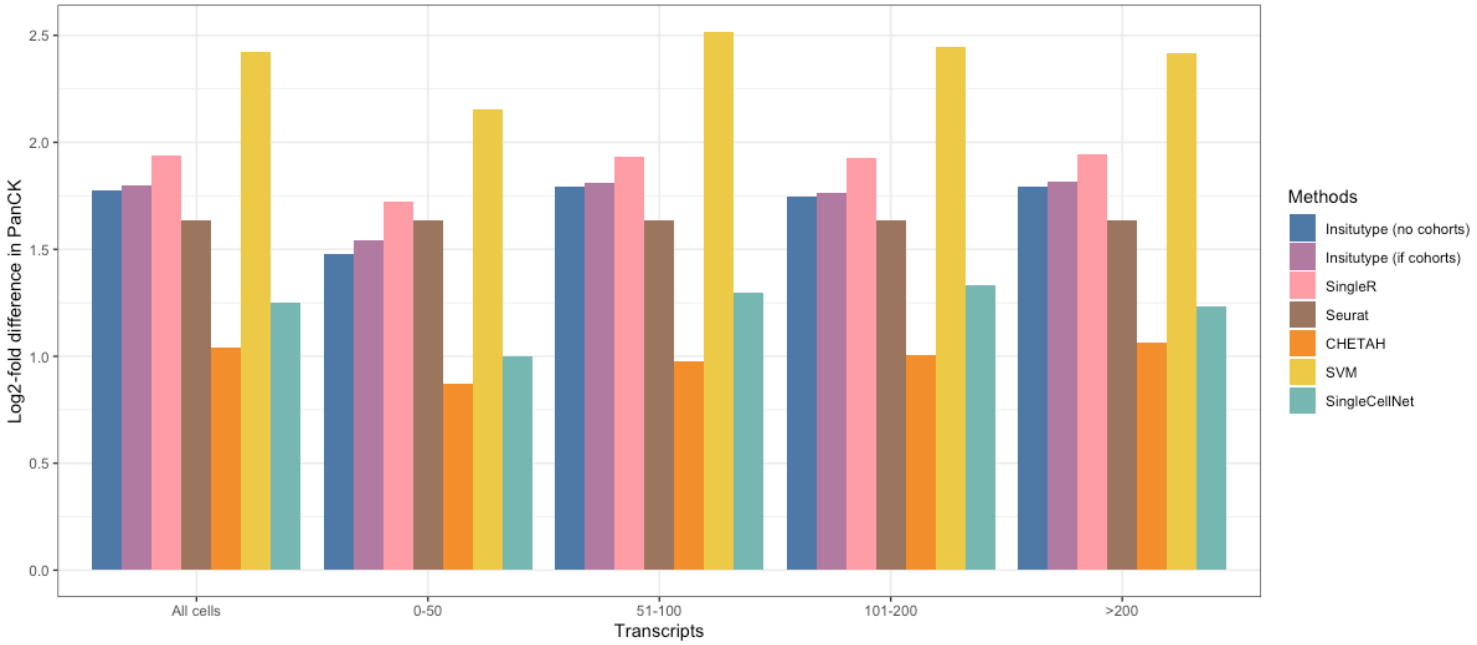
Supervised

Results – Difference in PanCK intensity

- Cytokeratin+ cells show strong PanCK staining; Cytokeratin- cells do not show PanCK staining
- Significant difference in PanCK intensity between cytokeratin+ and cytokeratin-cells

  -> cell typing is accurate

All genes



Best: SVM

Worst: CHETAH

Cytokeratin + cell types:

epithelial cell of proximal tubule,
kidney connecting tubule **epithelial** cell,
kidney loop of Henle thick ascending limb **epithelial** cell,
kidney **epithelial** cell,
renal **alpha-intercalated** cell,
renal **beta-intercalated** cell,
renal **intercalated** cell, renal principal cell

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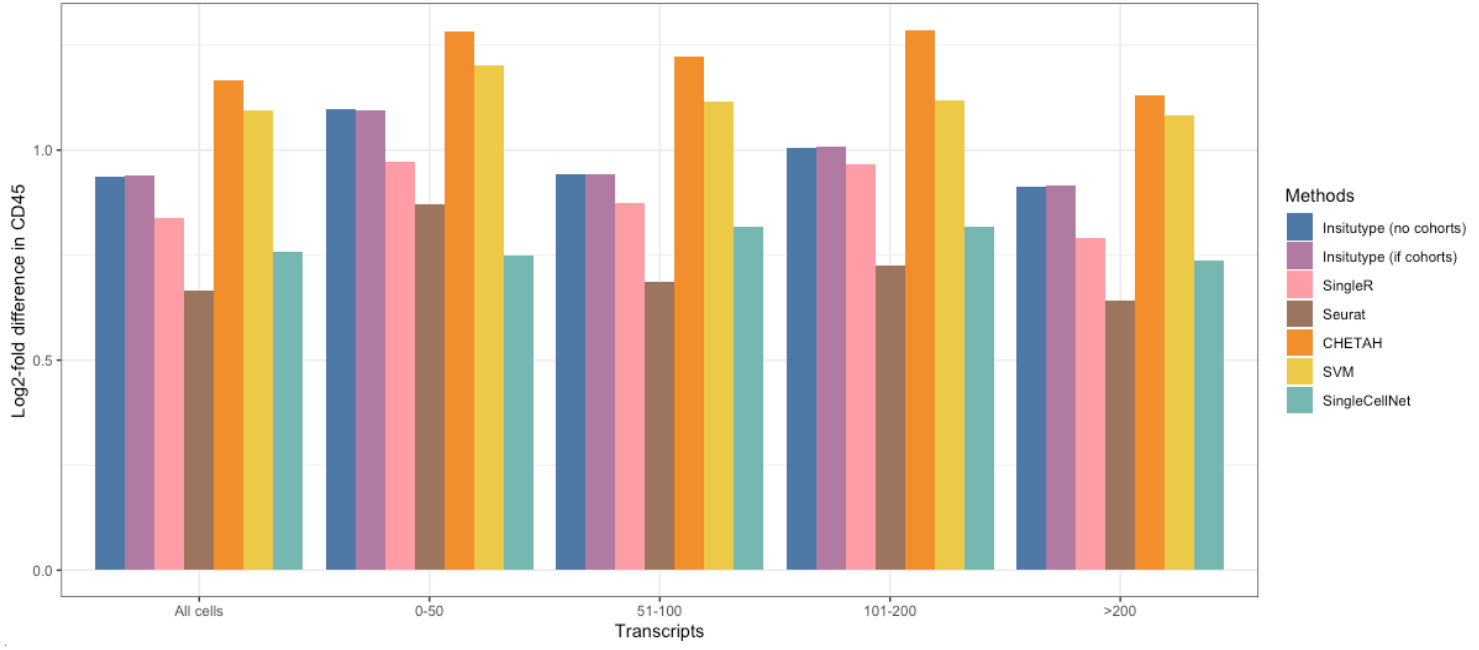
Supervised

Results – Difference in CD45 intensity

- Immune cells express high levels of CD45; Non-immune cells express low levels of CD45
- Significant difference in CD45 intensity between immune and non-immune cells

  -> cell typing is accurate

All genes



Best: **CHETAH**

Worst: **Seurat**

Immune cell types:

B cell, CD4-positive alpha-beta T cell, CD8-positive alpha-beta T cell, classical monocyte, dendritic cell, kidney resident macrophage, mature NK T cell, natural killer cell, neutrophil, non-classical monocyte, plasmacytoid dendritic cell

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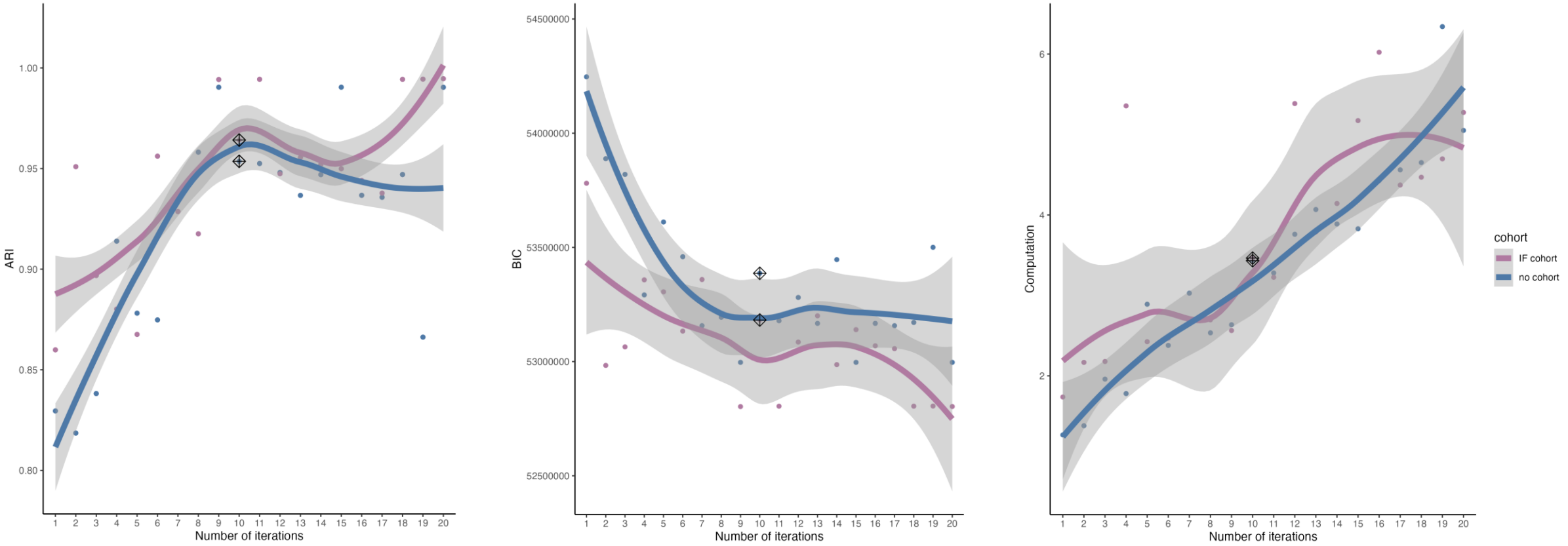
Results – Summary

- No method that outperforms all others in every aspect
- **InSituType** performs relatively well
- Consistent accuracy rates across cells with different transcript numbers
- Inconsistent accuracy rates across all genes, half genes, and 10% highly variable genes (not displayed in the slides, but will be included in the final report)
- Running time: SingleR and InSituType are the fastest (<1 min), SingleCellNet is the slowest (~1.5 hrs)

Unsupervised

Results – Number of iterations

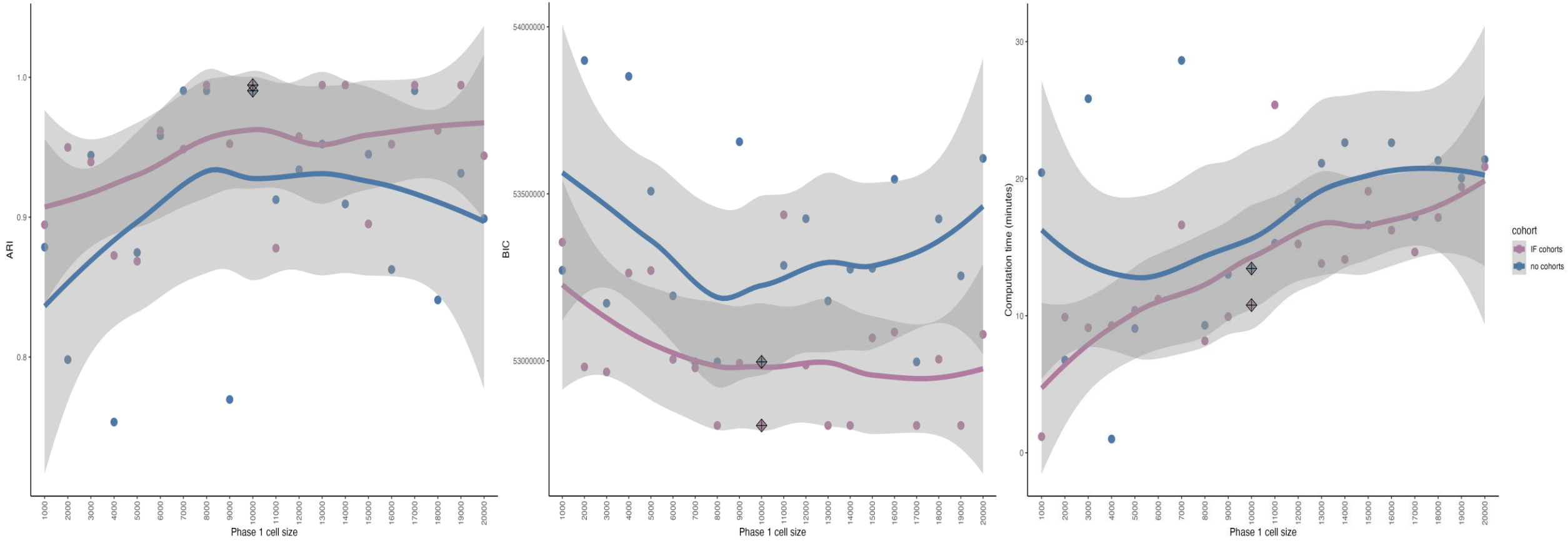
All genes



Unsupervised

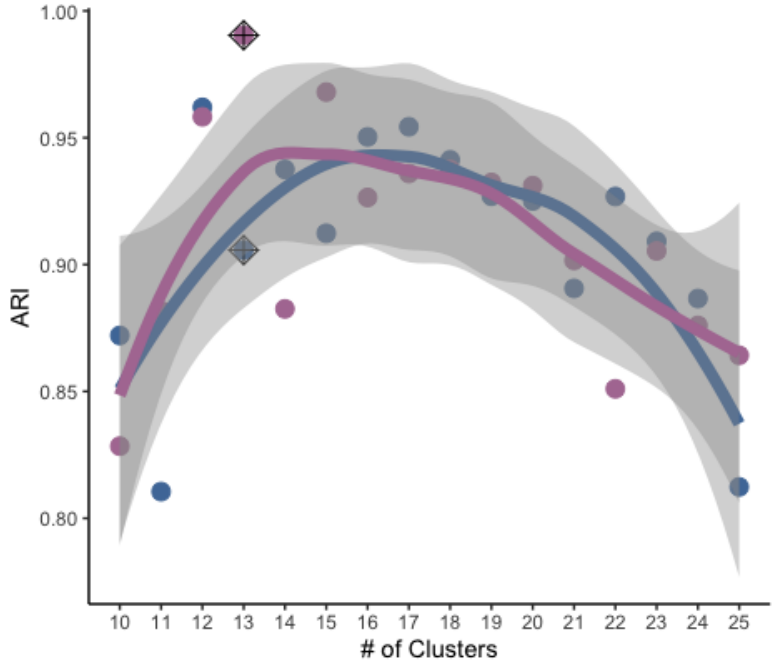
Results – Number of cells in phase 1

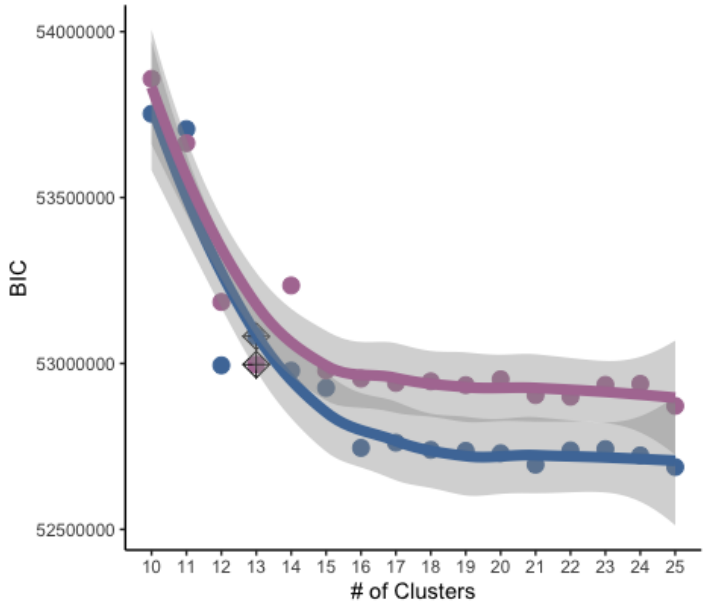
All genes

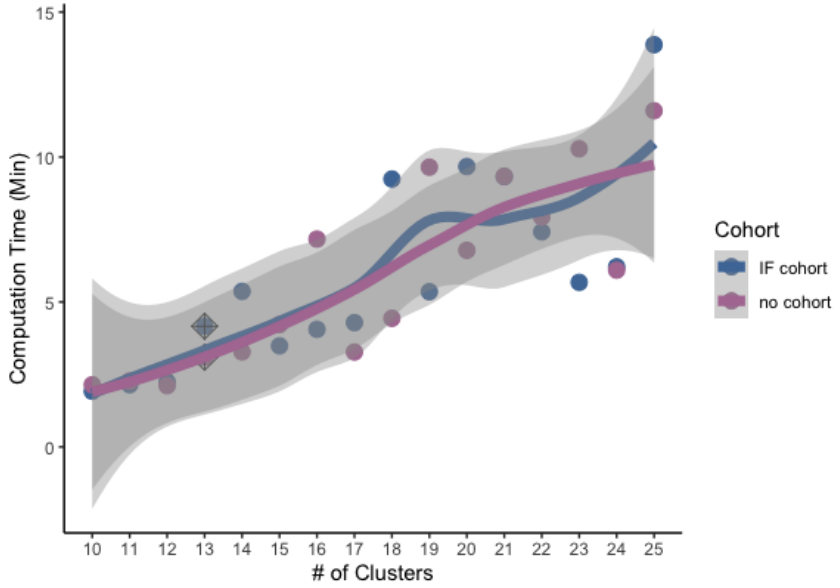


Results – Number of clusters

All genes







Results – Summary

- **Default parameters** perform reasonably well regarding ARI, BIC, and computation time
  - Variability in accuracy for cells in phase 1 parameter
- Computation time increases relatively linear as parameter values increase

Future Work

Supervised Methods

- Experiment with different tuning parameters for SingleCellNet

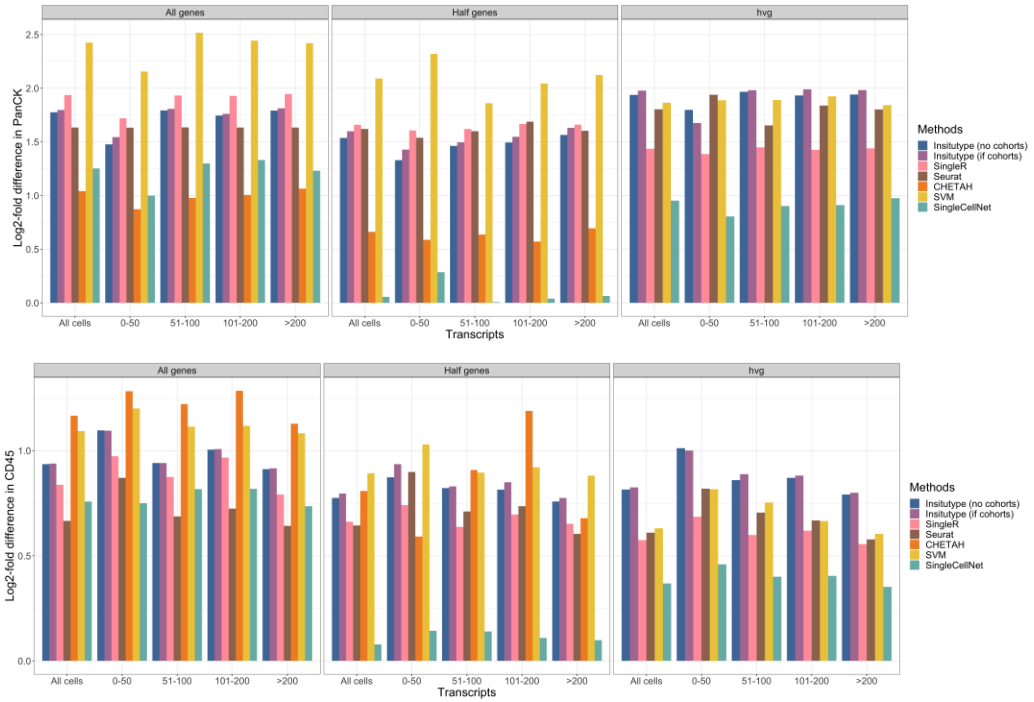
Unsupervised Methods

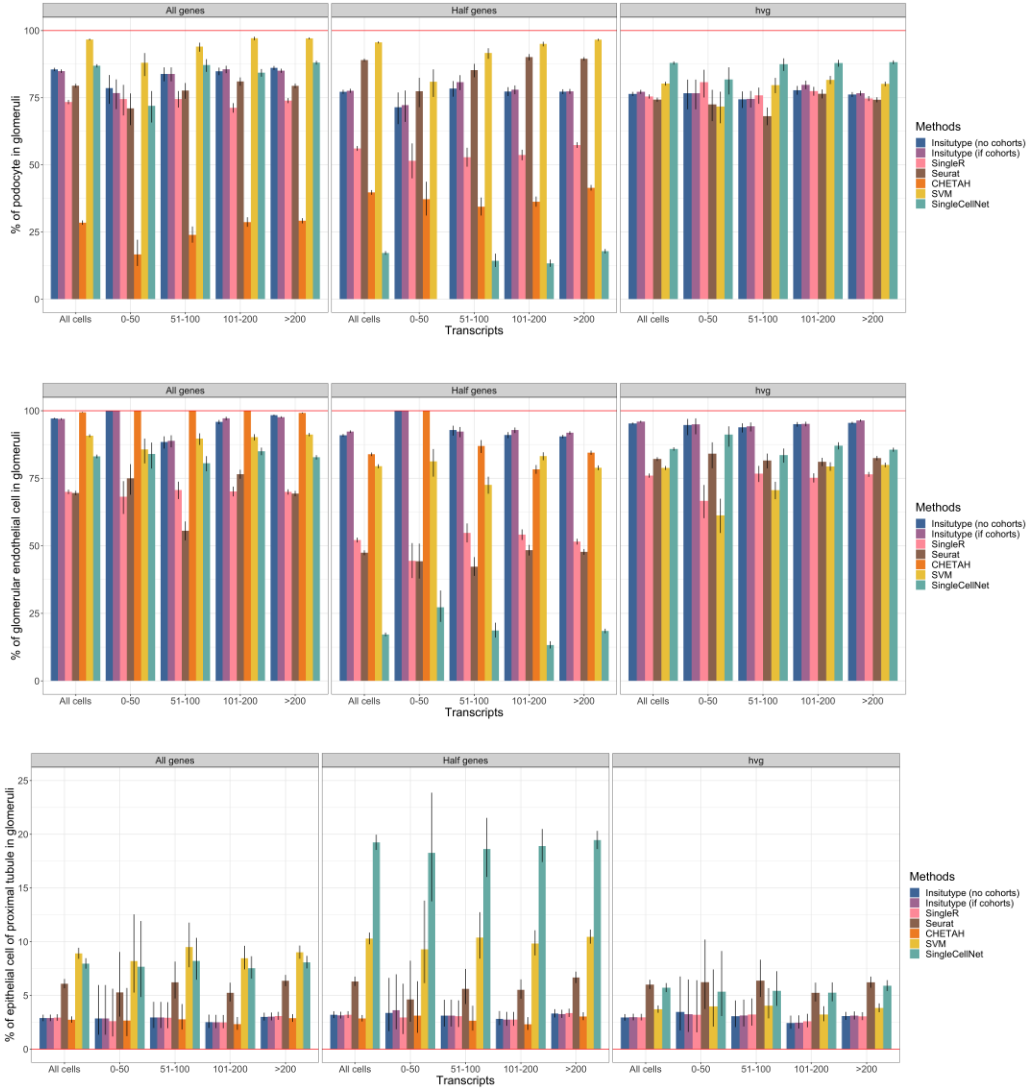
- Benchmark different combinations of parameters
- Utilize parallel computation and run all models on the same computing environment such as Amazon EC2

Thank you!

Any Questions?

Appendix





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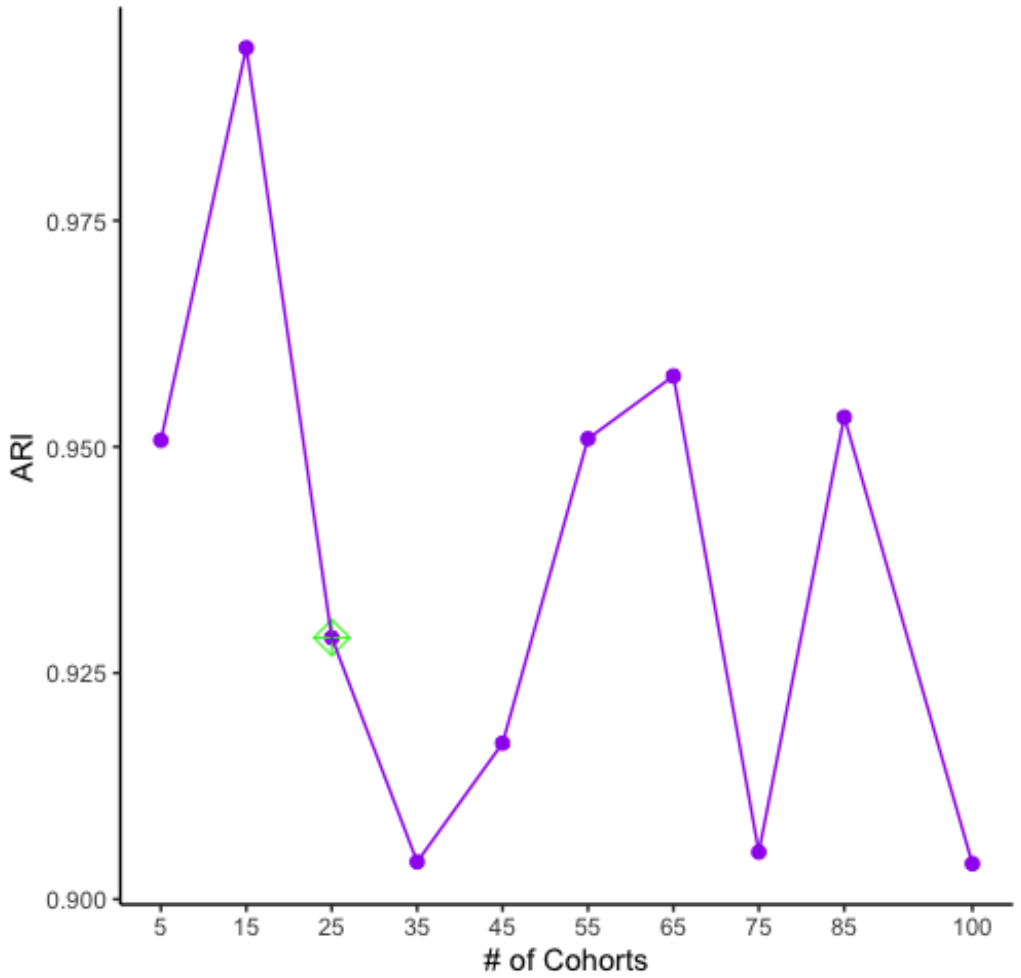
Appendix

	InSituType (no cohort)	InSituType (if cohort)	SingleR	Seurat	CHETAH	SVM	SingleCellNet
All genes	31.25 sec	33.0 sec	1.18 min	2.99 min	26.11 min	6.48 min	~1.5 hr
Half genes	15.65 sec	15.73 sec	44.07 sec	3.99 min	34.27 min	3.55 min	64.13 min
10% hvg	5.24 sec	5.06 sec	3.93 sec	5.93 min	NA	1.79 min	39.58 min

Appendix-

unsupervised cell typing performance (number of cohorts in `fastCohorting()`)

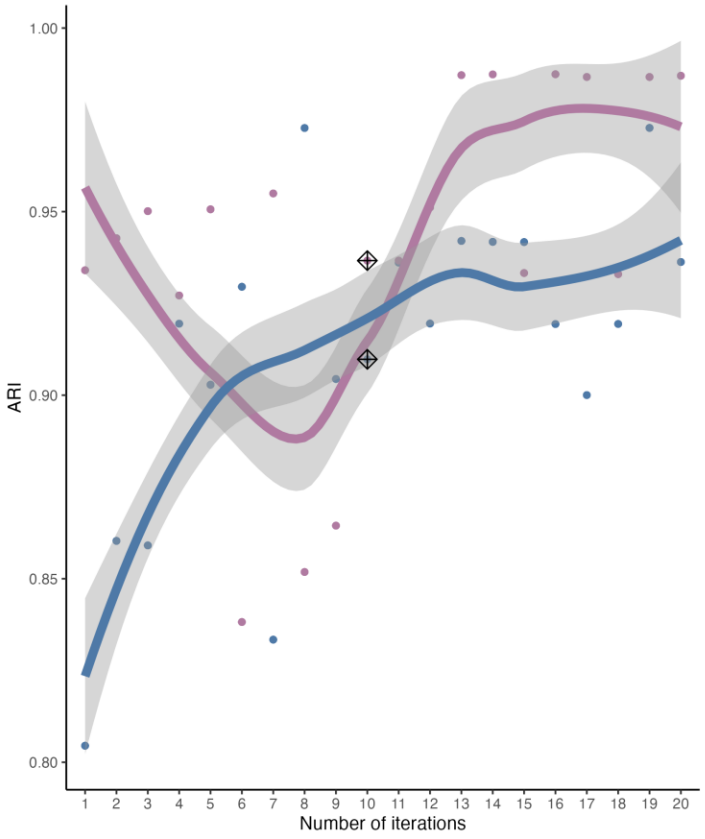
Full gene data set

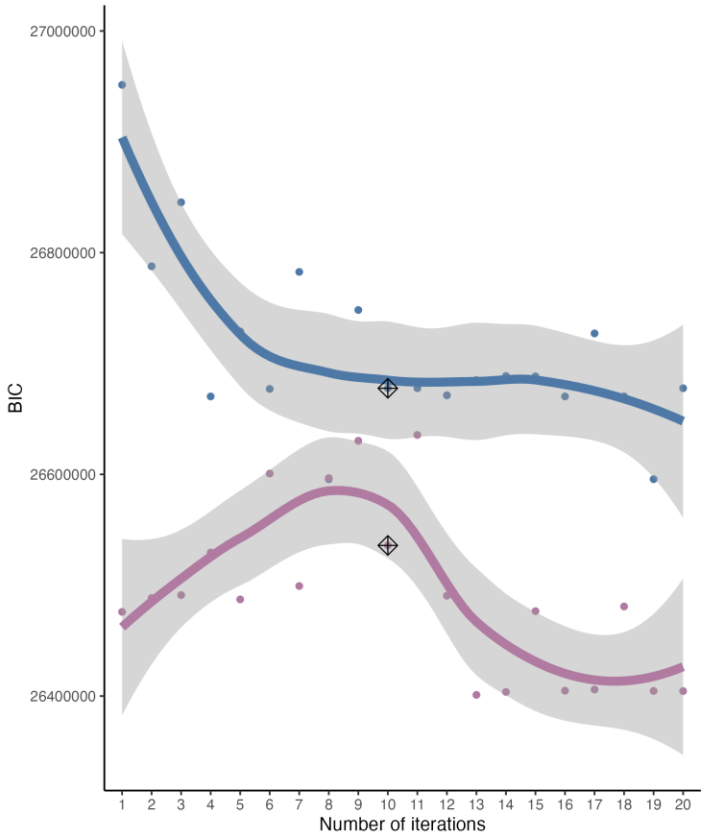


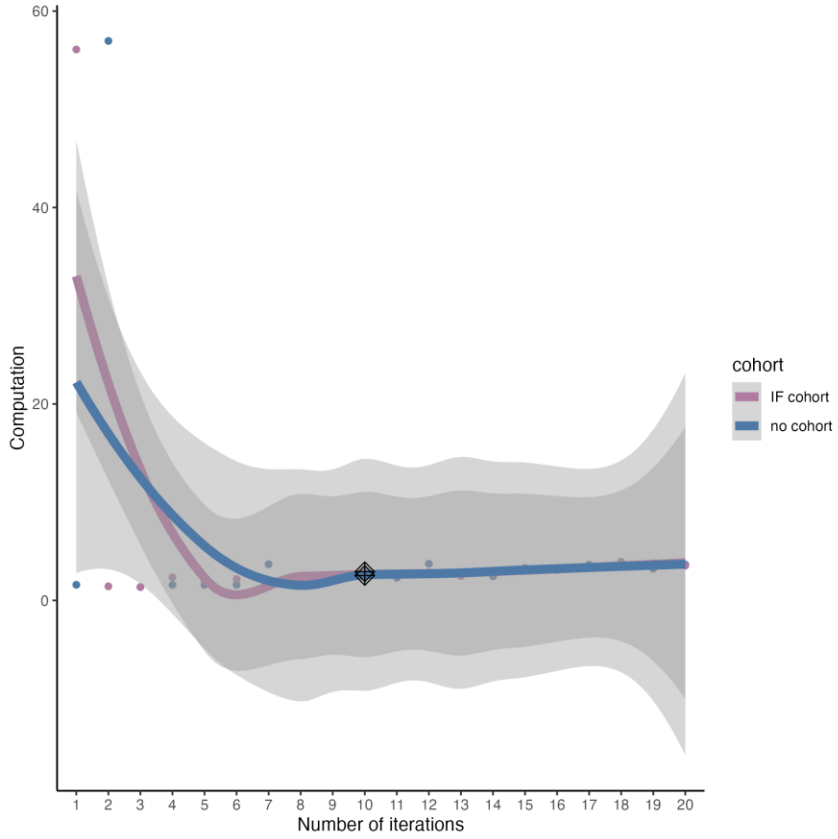
Results –

unsupervised cell typing performance (number of iterations)

Half gene data set



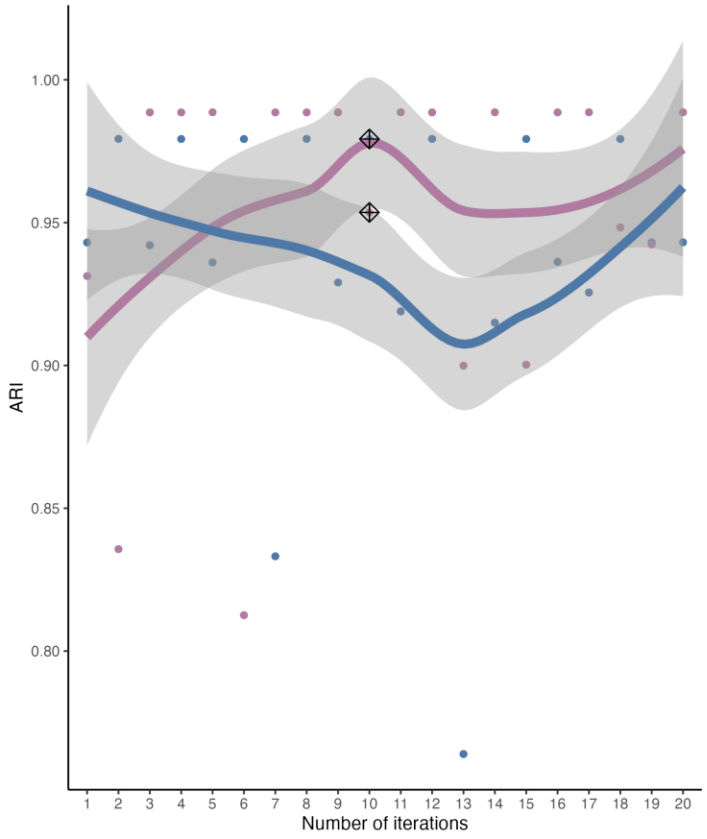


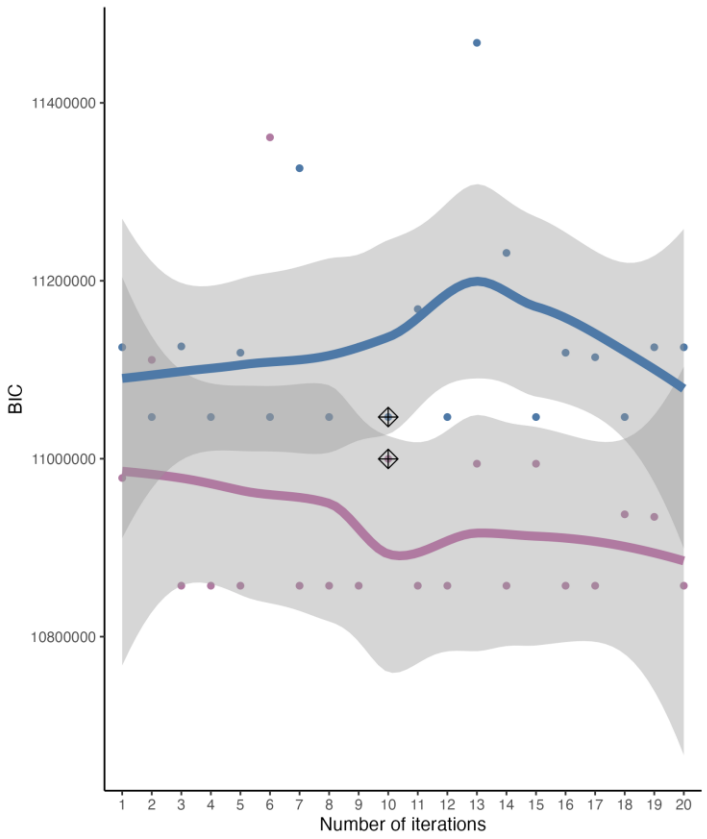


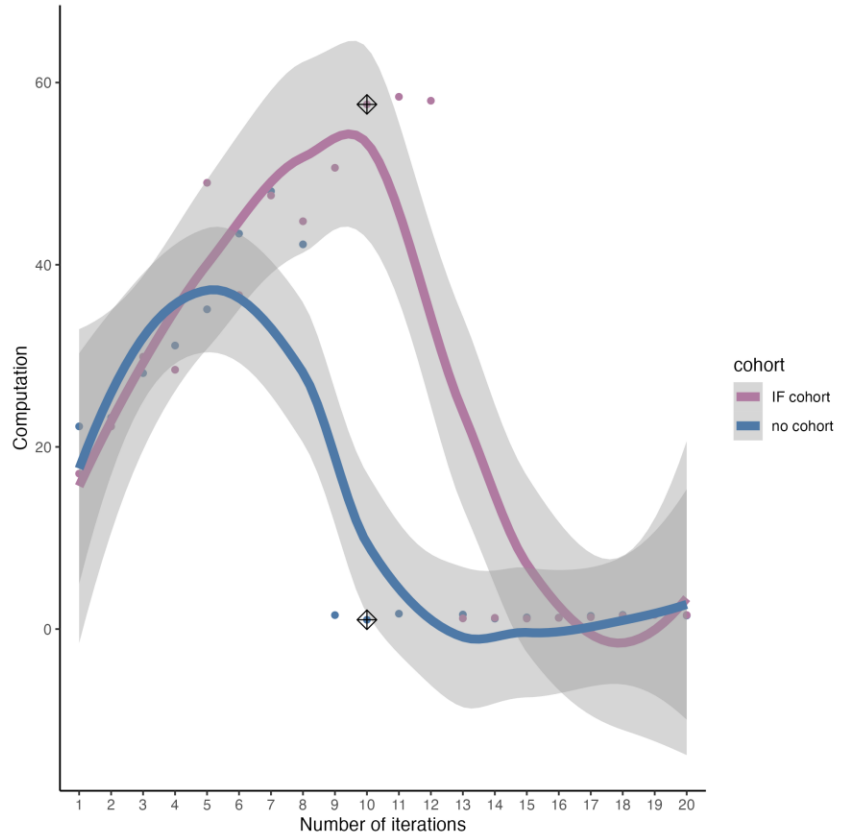
Results –

unsupervised cell typing performance (number of iterations)

10% Highly Variable Gene



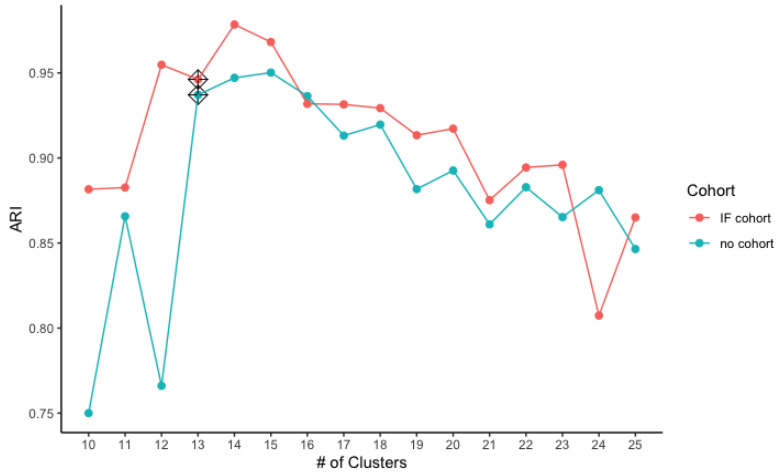


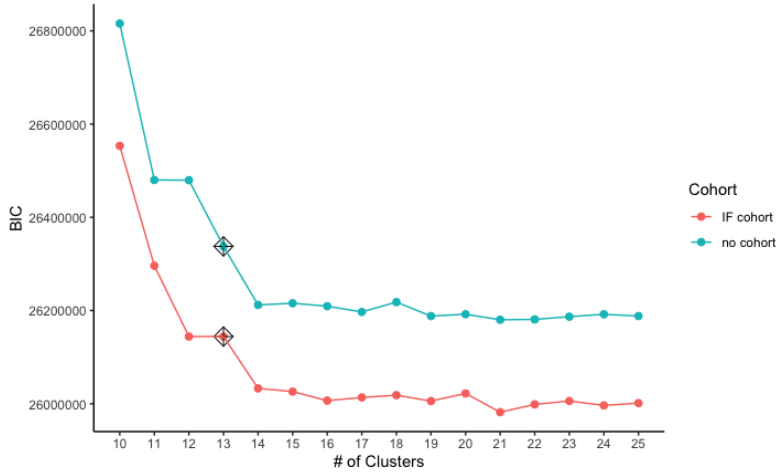


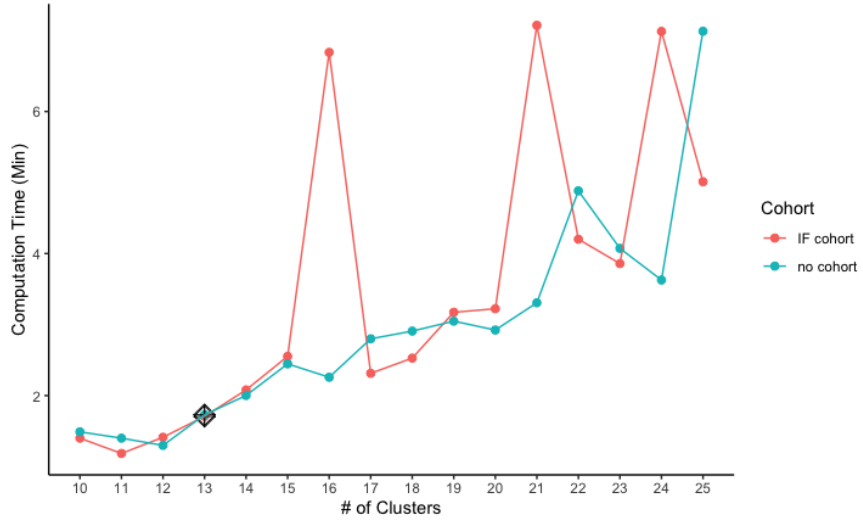
Results –

unsupervised cell typing performance (number of clusters)

Half gene data set







Results –

unsupervised cell typing performance (number of clusters)

10% Highly Variable Gene