A.C.Camargo Cancer Center
Centro Integrado de Diagnóstico, Tratamento, Ensino e Pesquisa

Immunomics and cancer

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Translational Immuno-oncology Lab.
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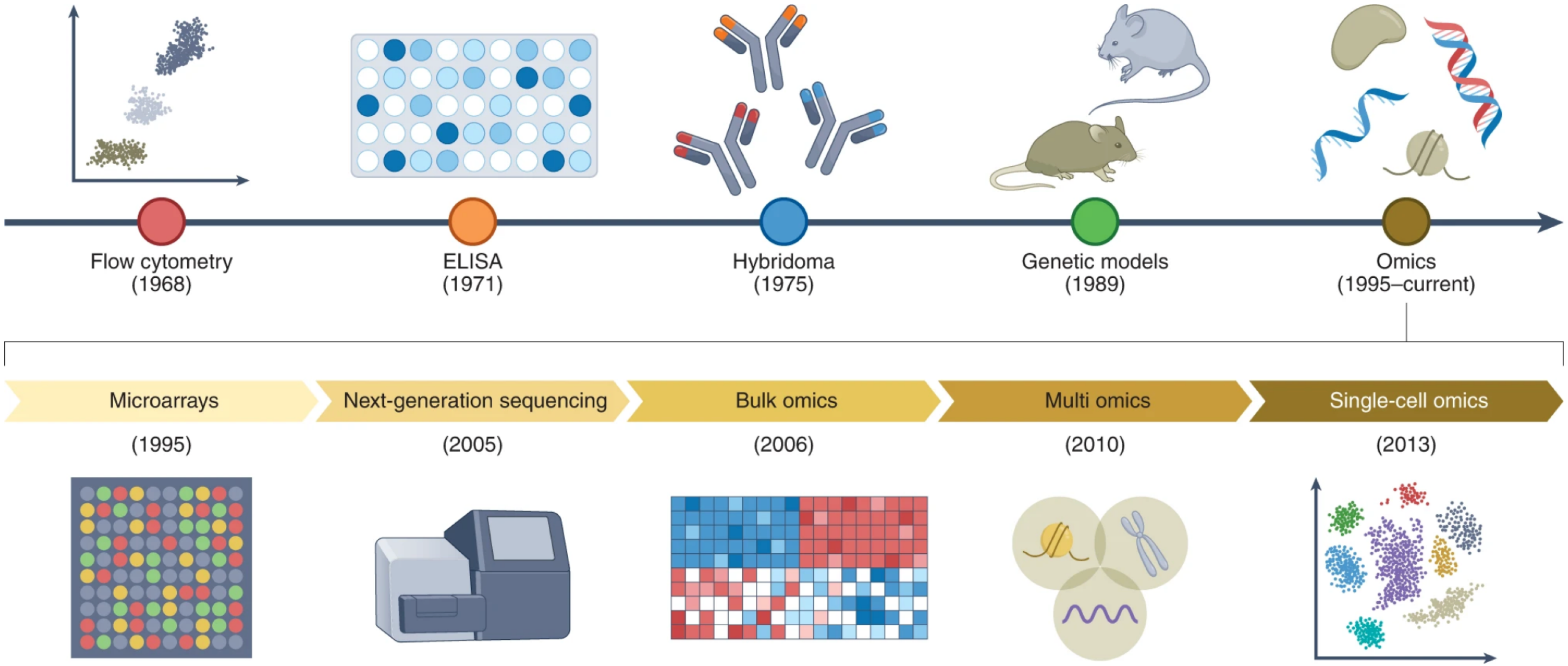
Timeline of methodologies used in immunology research



Bonaguro et al., 2022. Nat Immun.

Summary

Part 1

Introduction to main omics methodologies and data analysis tools.

- Conventional RNA-seq.
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- RNA-seq alternative: hybridization assays.
- scRNA-seq e scTCR-seq/scBCR-seq.
- Spatial transcriptomics.

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Examples of how to use these methodologies in your research project.

- Identifying subgroups of tumor-infiltrating T and B cells using scRNA-seq.
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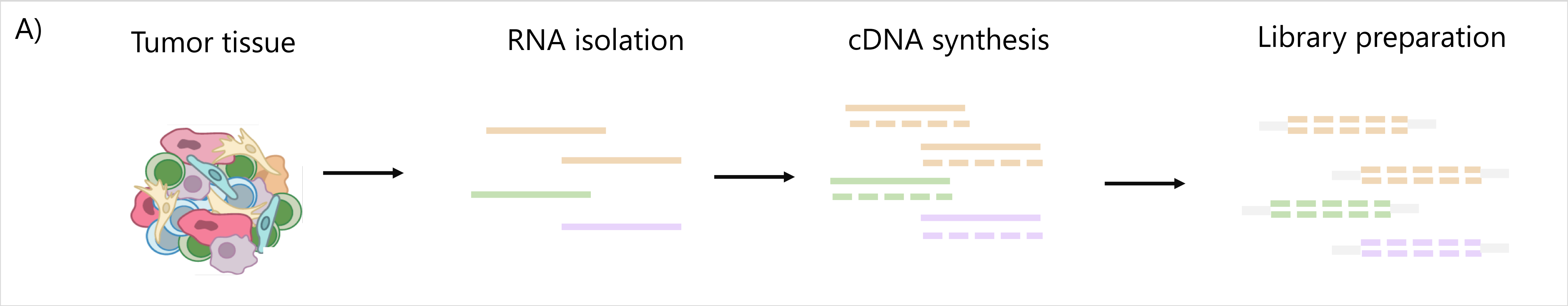
Conventional RNA-seq ("bulk" RNA-seq)

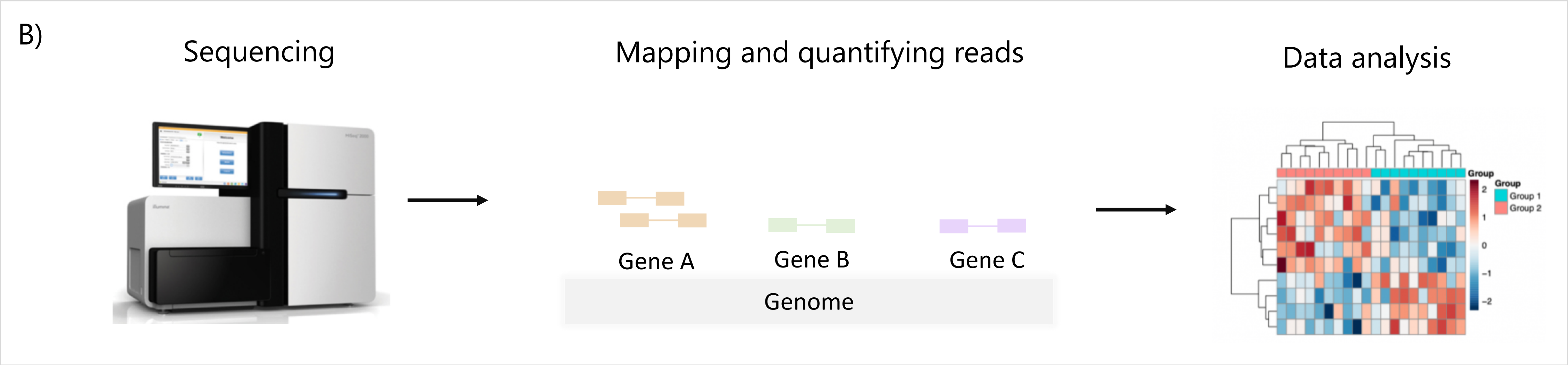

A)

Tumor tissue

RNA isolation

cDNA synthesis

Library preparation


B)

Sequencing

Mapping and quantifying reads

Gene A

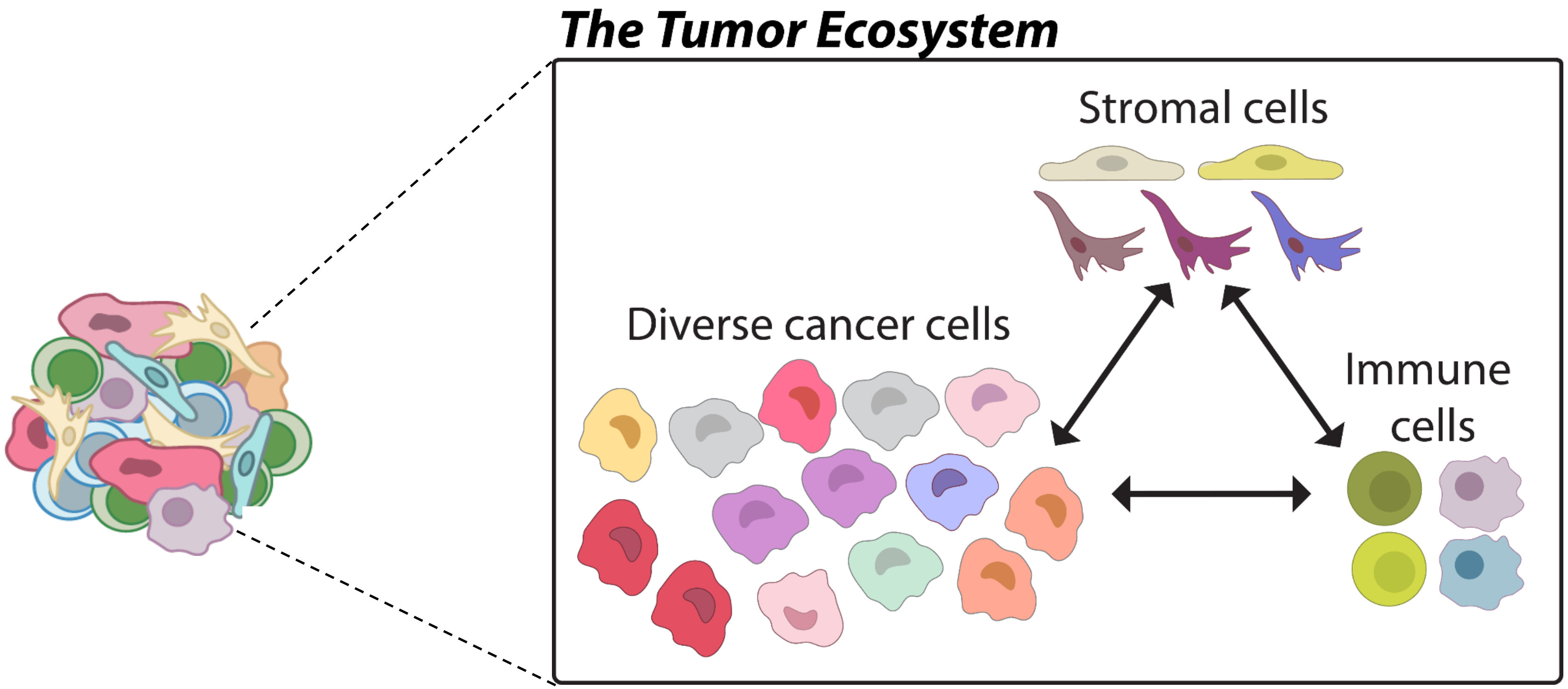
Gene B

Gene C

Genome

Data analysis

Conventional RNA-seq ("bulk" RNA-seq)



Bulk RNA-seq

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Tools to quantify the abundance of immune cells in a tissue profiled by RNA-seq

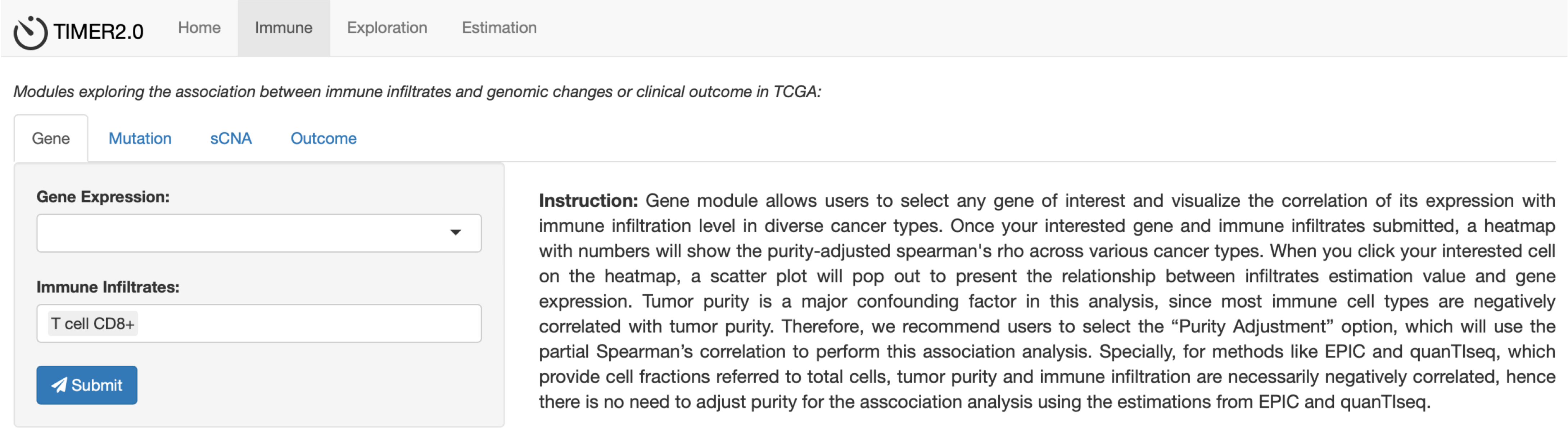
Gene signature-based approaches

- For each immune cell type of interest, there is a list of marker genes.
- The abundance of each immune cell type is estimated separately, based on the expression of marker genes.
- Algorithm options for calculating the abundance scores:
  - **MCPcounter**: mean expression of marker genes.
  - **xCell:** single sample GSEA.
- Compare the abundance of each immune cell type between samples.

Deconvolution-based approaches

- First, define a mixture of immune cell types which will be jointly evaluated. For each cell type, there is a list of marker genes.
- Describe the gene expression of a sample as the weighted sum of the expression profiles of the admixed cell types.
- Algorithm options for calculating the abundance scores:
  - **TIMER:** Linear least square regression.
  - **EPIC:** Constrained least square regression.
  - **CIBERSORT:** v-Support Vector Regression.
- Compare the abundance immune population between samples and also within a sample.

TIMER 2.0

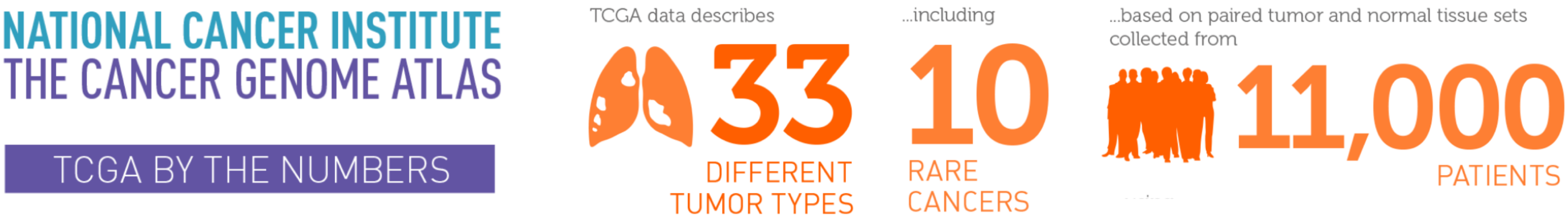


Modules exploring the association between immune infiltrates and genomic changes or clinical outcome in TCGA:

Gene Expression:

Immune Infiltrates: T cell CD8+

Instruction: Gene module allows users to select any gene of interest and visualize the correlation of its expression with immune infiltration level in diverse cancer types. Once your interested gene and immune infiltrates submitted, a heatmap with numbers will show the purity-adjusted spearman's rho across various cancer types. When you click your interested cell on the heatmap, a scatter plot will pop out to present the relationship between infiltrates estimation value and gene expression. Tumor purity is a major confounding factor in this analysis, since most immune cell types are negatively correlated with tumor purity. Therefore, we recommend users to select the “Purity Adjustment” option, which will use the partial Spearman’s correlation to perform this association analysis. Specially, for methods like EPIC and quanTIseq, which provide cell fractions referred to total cells, tumor purity and immune infiltration are necessarily negatively correlated, hence there is no need to adjust purity for the asscociation analysis using the estimations from EPIC and quanTIseq.



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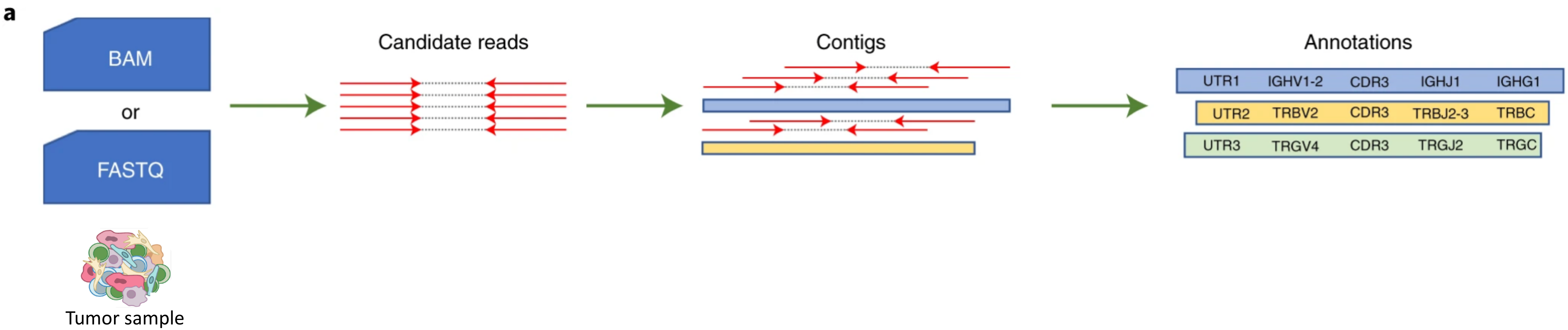
Evaluate the repertoire of T and B cell clones in a tissue profiled by RNA-seq: TRUST 4.0

Select reads that have significant overlap with V, J, C genes

Assemble TCRs and BCRs based from selected reads by creating contigs (series of reads with overlapping sequences)

Align the contig to sequences in the ImmunoGeneTics (IMGT) database for annotating the V, J and C genes.



Song et al., 2021. Nat Met

Evaluate the repertoire of T and B cell clones in a tissue profiled by RNA-seq

Outputs

TCR e BCR

- Receptor diversity
- Rate of clonal expansion
- Shared clonotypes
- Clone reactivity to common viral antigens (VDJdb).

BCR

- Rate of somatic hypermutation
- Rate of Ig class switch recombitaion
- Direction of Ig class switch

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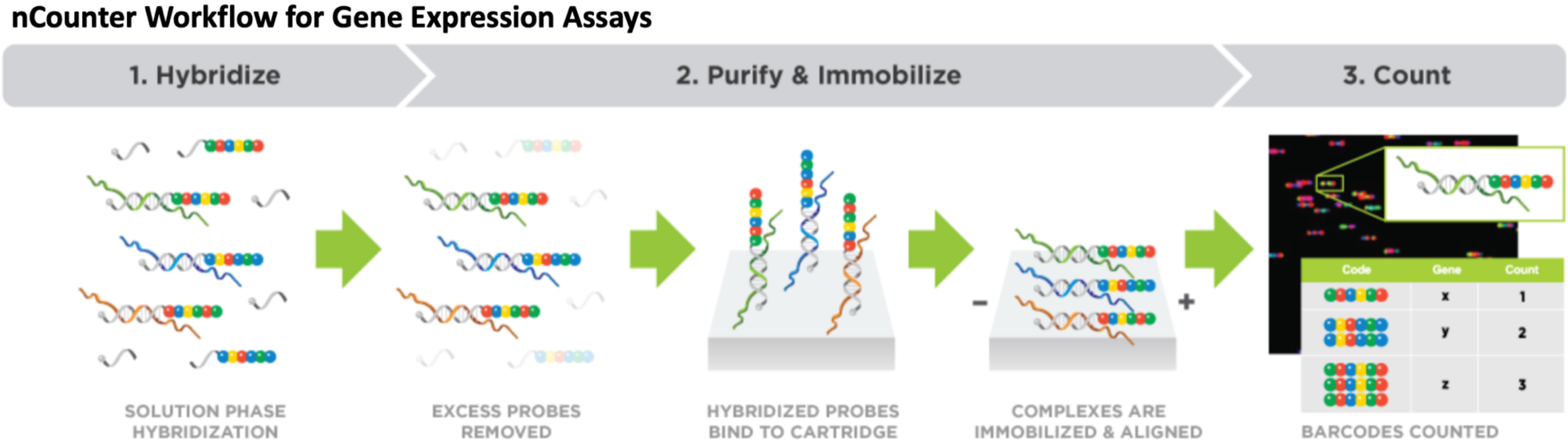
Hybridization assay: a RNA-seq alternative that works well with FFPE samples



Nanostring Resource Documentation

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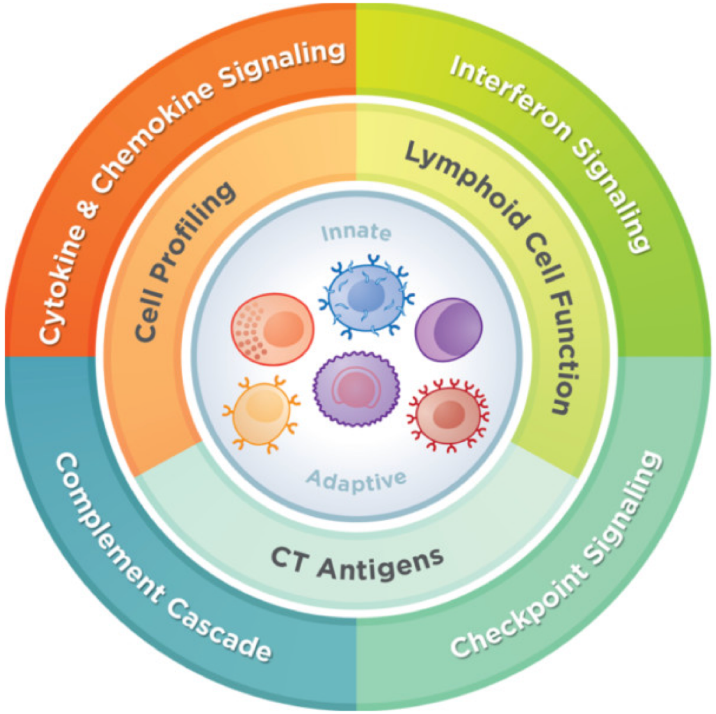

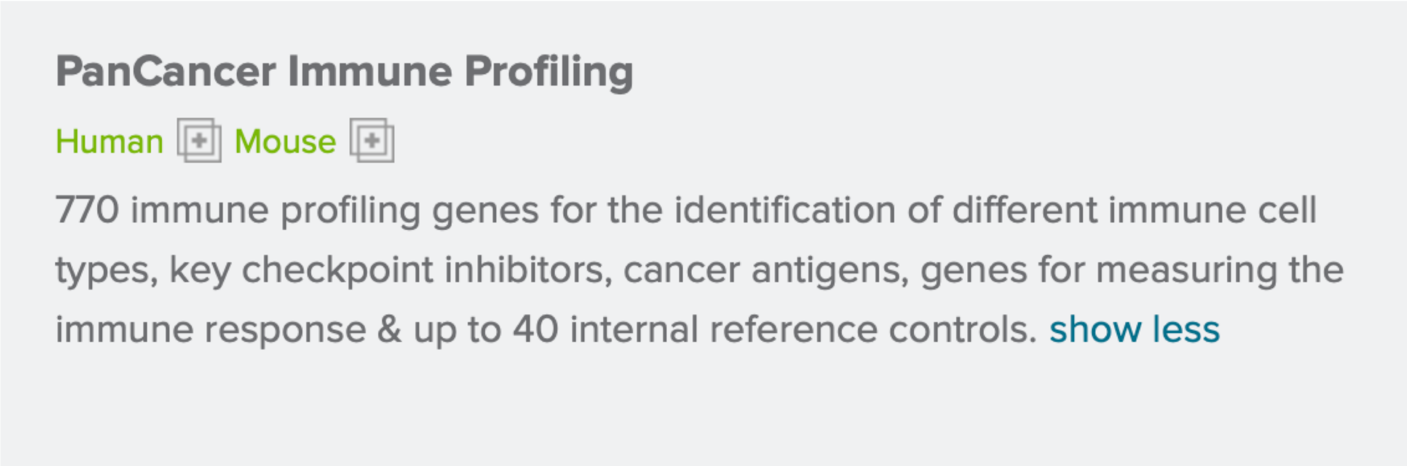

PanCancer Immune Profiling

Human ⊞ Mouse ⊞

770 immune profiling genes for the identification of different immune cell types, key checkpoint inhibitors, cancer antigens, genes for measuring the immune response & up to 40 internal reference controls. show less

Nanostring Resource Documentation

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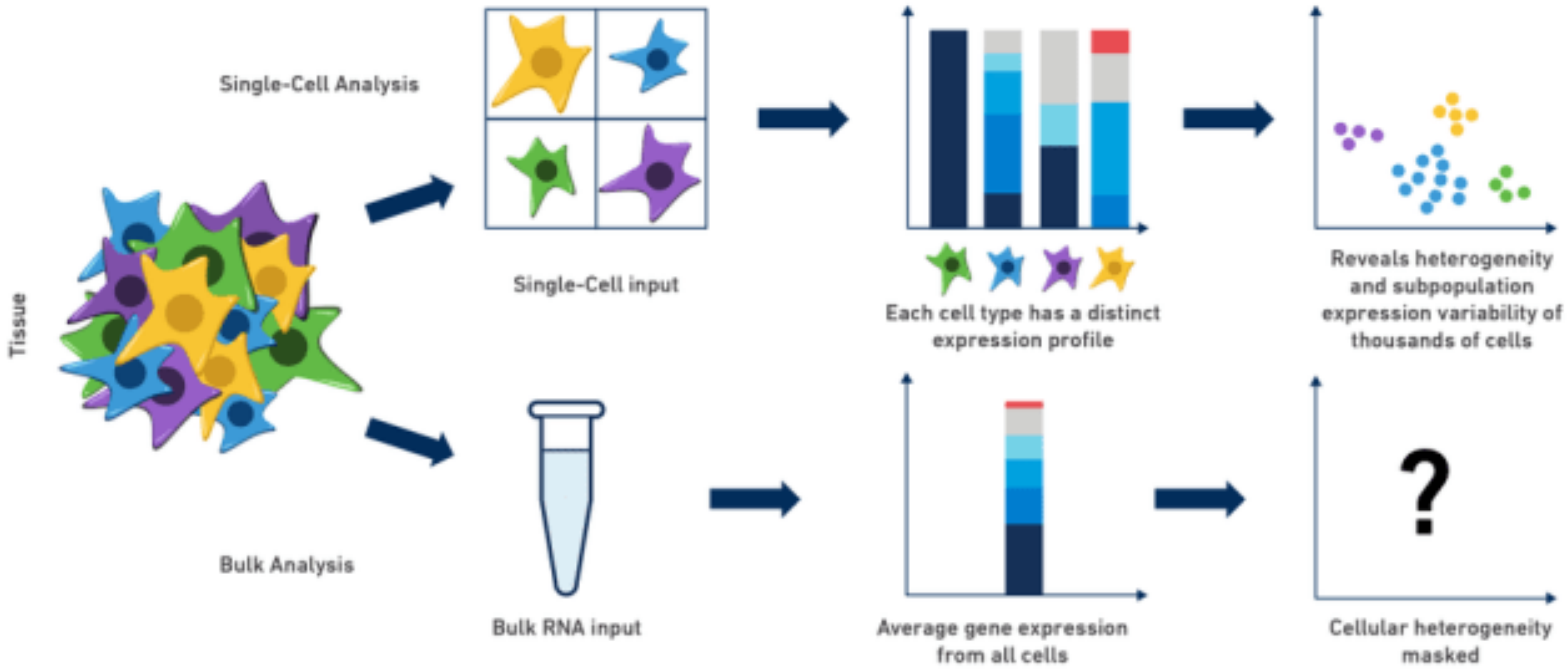
scRNA-seq vs. Bulk RNA-seq



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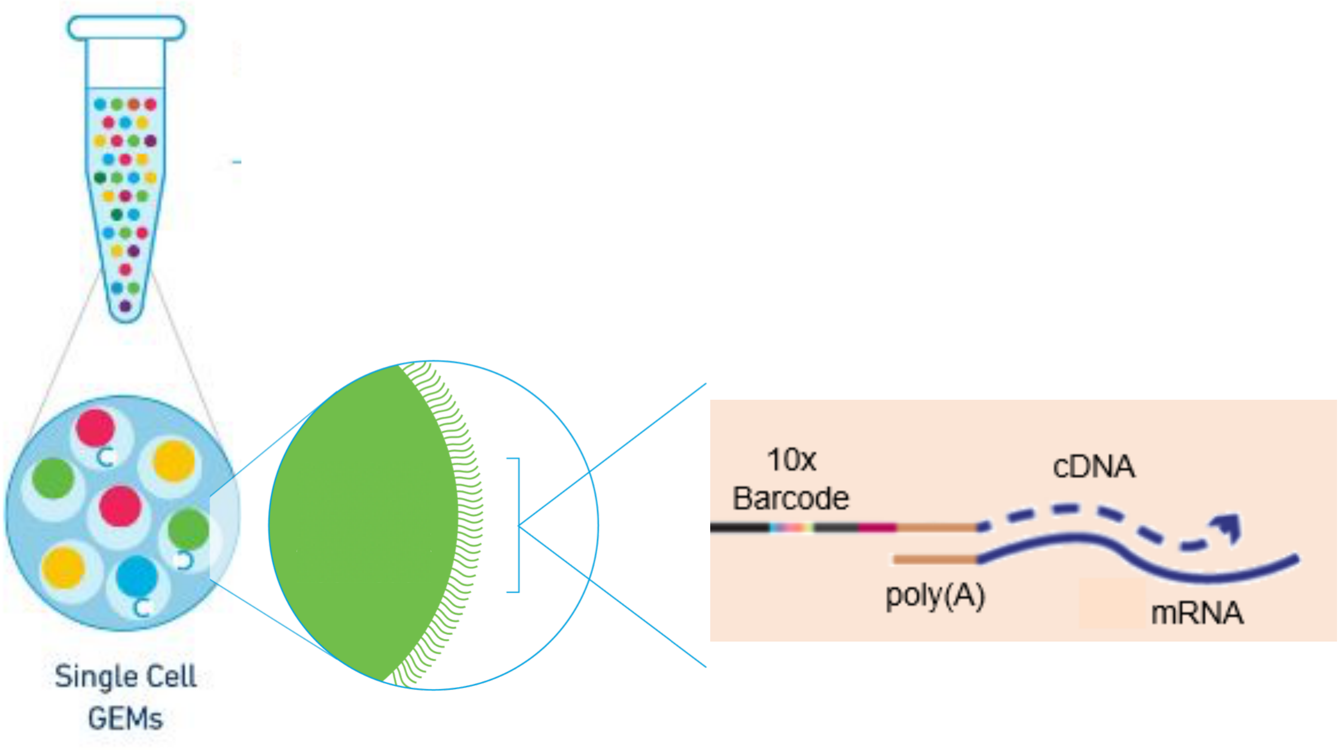
scRNA-seq workflow



→ Amplify cDNA → Construct library → Sequence

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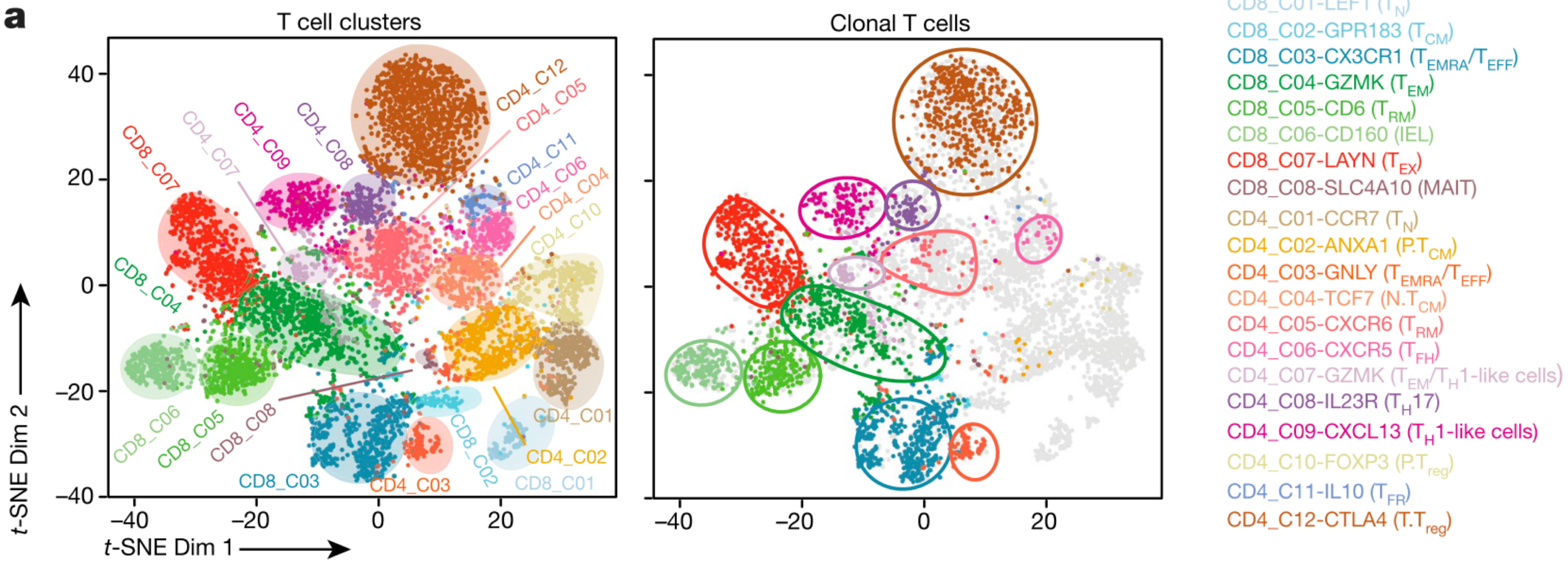
scRNA-seq coupled to scTCR-seq/scBCR-seq



Zhang et al., 2018. Nat Letter

scRNA-seq coupled to scTCR-seq/scBCR-seq

Examples of analyses using tumor samples

- Characterize the expression profile of tumor reactive clones (i.e. clonally expanded).
- Infer trajectories of cellular differentiation using algorithms such as pseudotime.
- Determine whether expanded clonotypes in the tumor tissue could also be detected in the blood.
- Evaluated expanded clones in the tumor microenvironment before and after treatment.
- Detection of bystander tumor-infiltrating lymphocytes that recognize common viral antigens.
- Evaluate the antibody affinity maturation of B cells (i.e. somatic hypermutation and Ig class switch recombination)

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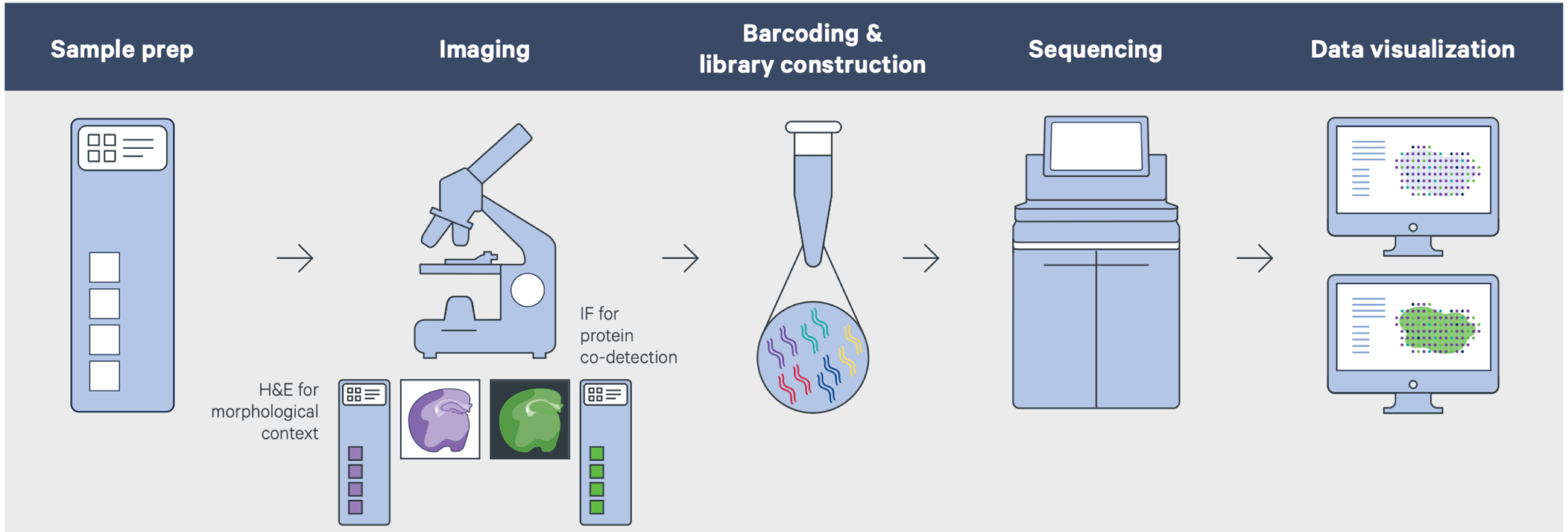
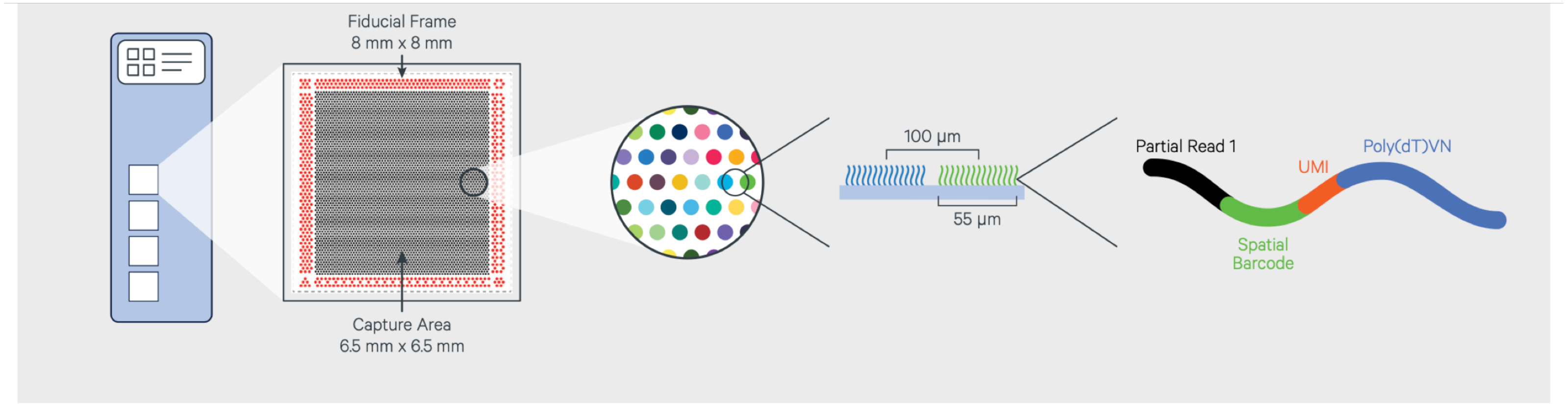
Spatial transcriptomics workflow





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BMJ Journals

Gut

Mature tertiary lymphoid structures are key niches of tumour-specific immune responses in pancreatic ductal adenocarcinomas

Su

Gabriela Sarti Kinker[1], Glauco Akelinghton Freire Vitiello[1], Ariane Barros Diniz[2], Mariela Pires Cabral-Piccin[1], Pedro Henrique Barbosa Pereira[1], Maria Letícia Rodrigues Carvalho[1], Wallax Augusto Silva Ferreira[1,3], Alexandre Silva Chaves[1], Amanda Rondinelli[1], Arianne Fagotti Gusmão[1], Alexandre Defelicibus[1], Gabriel Oliveira dos Santos[4], Warley Abreu Nunes[4], Laura Carolina López Claro[5], Talita Magalhães Bernardo[5], Ricardo Tadashi Nishio[5], Adhemar Monteiro Pacheco[5], Ana Carolina Laus[6], Lidia Maria Rebolho Batista Arantes[6], Julia Lima Fleck[7], Victor Hugo Fonseca de Jesus[8], André de Moricz[5], Ricardo Weinlich[2], Felipe José Fernandez Coimbra[9], Vladmir Cláudio Cordeiro de Lima[8], Tiago da Silva Medina[1,10]

Correspondence to Dr Tiago da Silva Medina, International Research Center, A.C.Camargo Cancer Center, São Paulo, 01508-010, Brazil;
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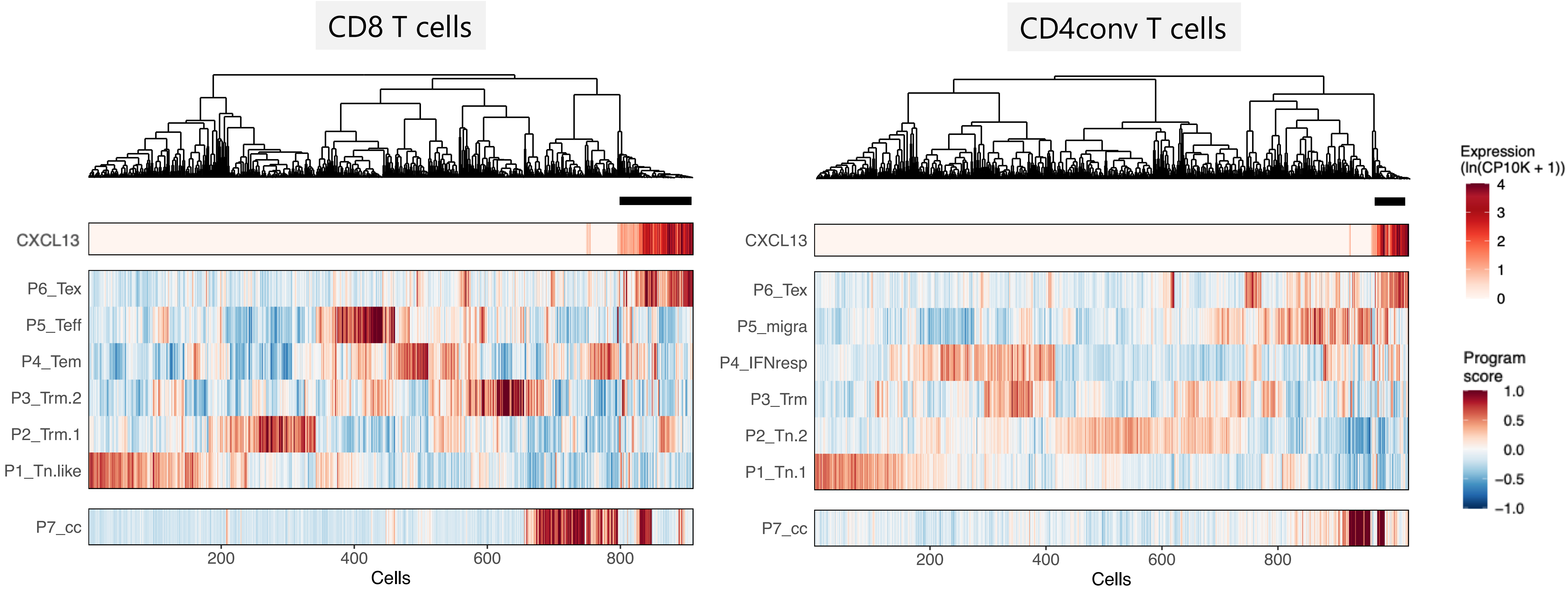
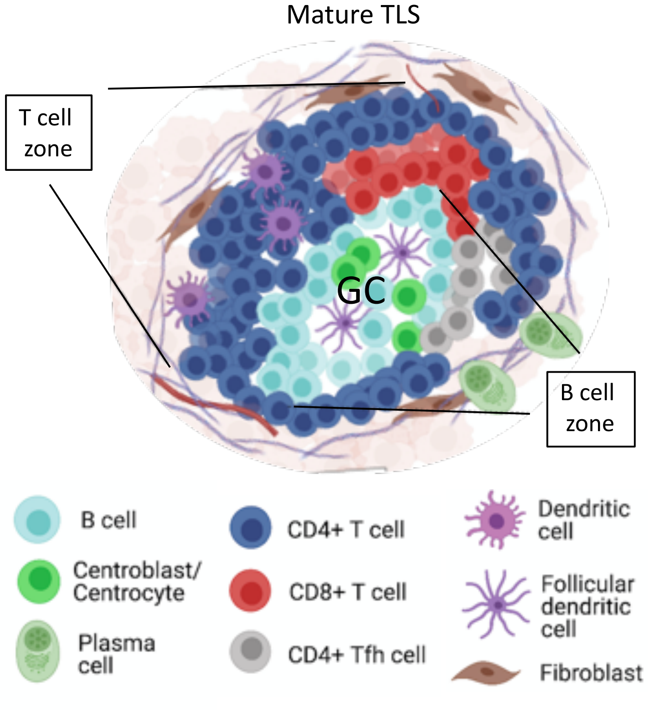
Chronically activated tumor-infiltrating T cells produce de B cell chemoattractant CXCL13

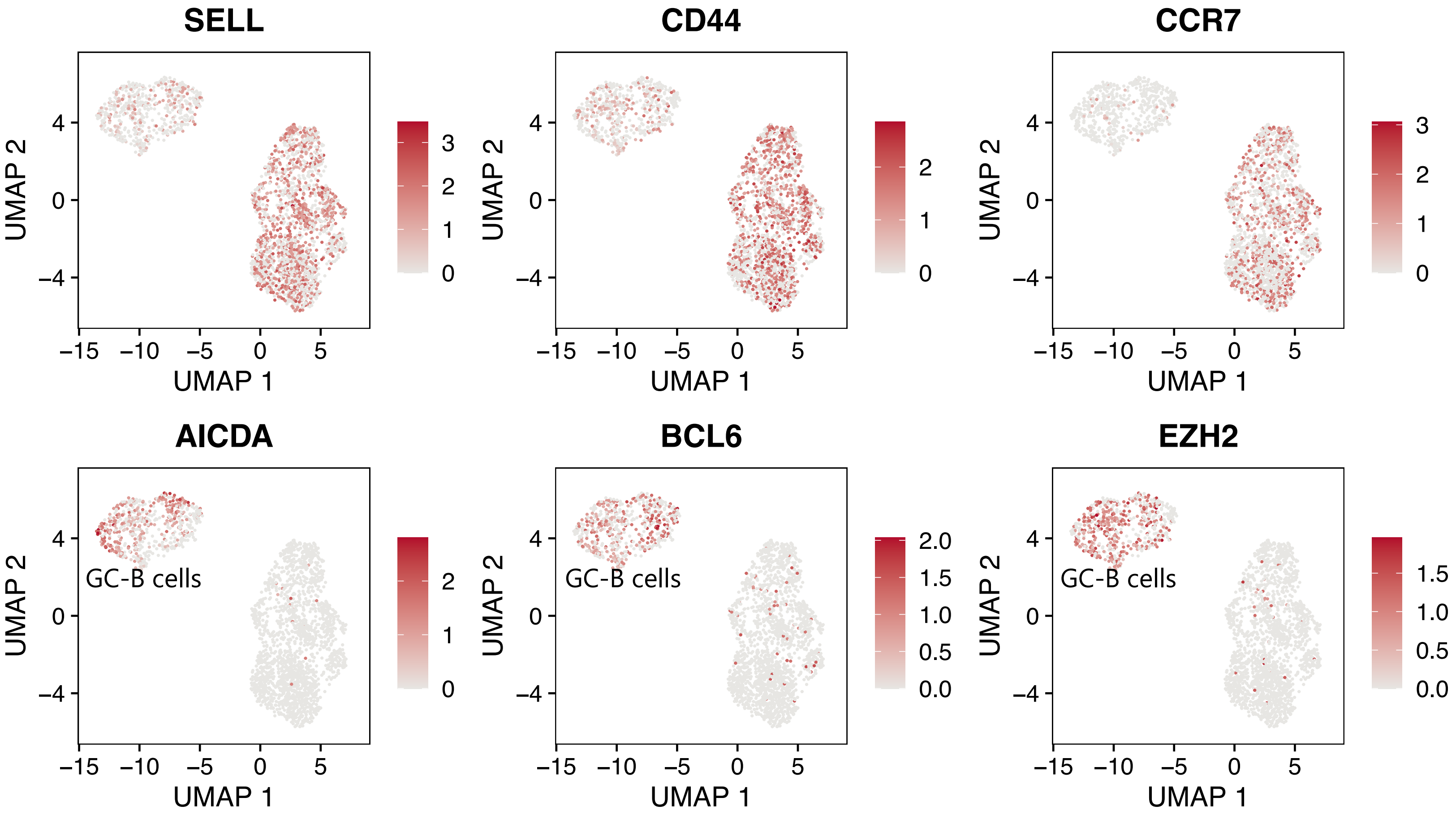


scRNA-seq

CXCL13 orchestrate the formation of tertiaty lymphoid structures (TLSs) in the tumor tissue



B cells



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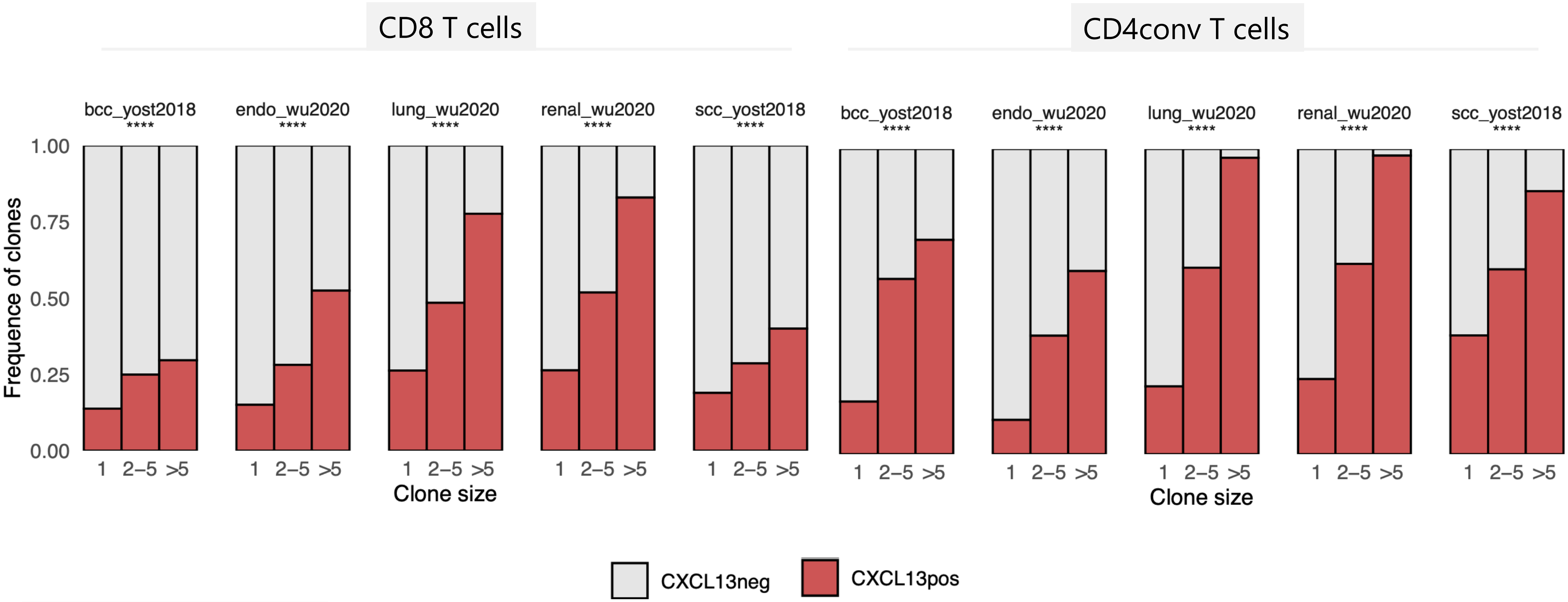
CXCL13-producing T cells are tumor-reactive



scRNA-seq + scTCR-seq

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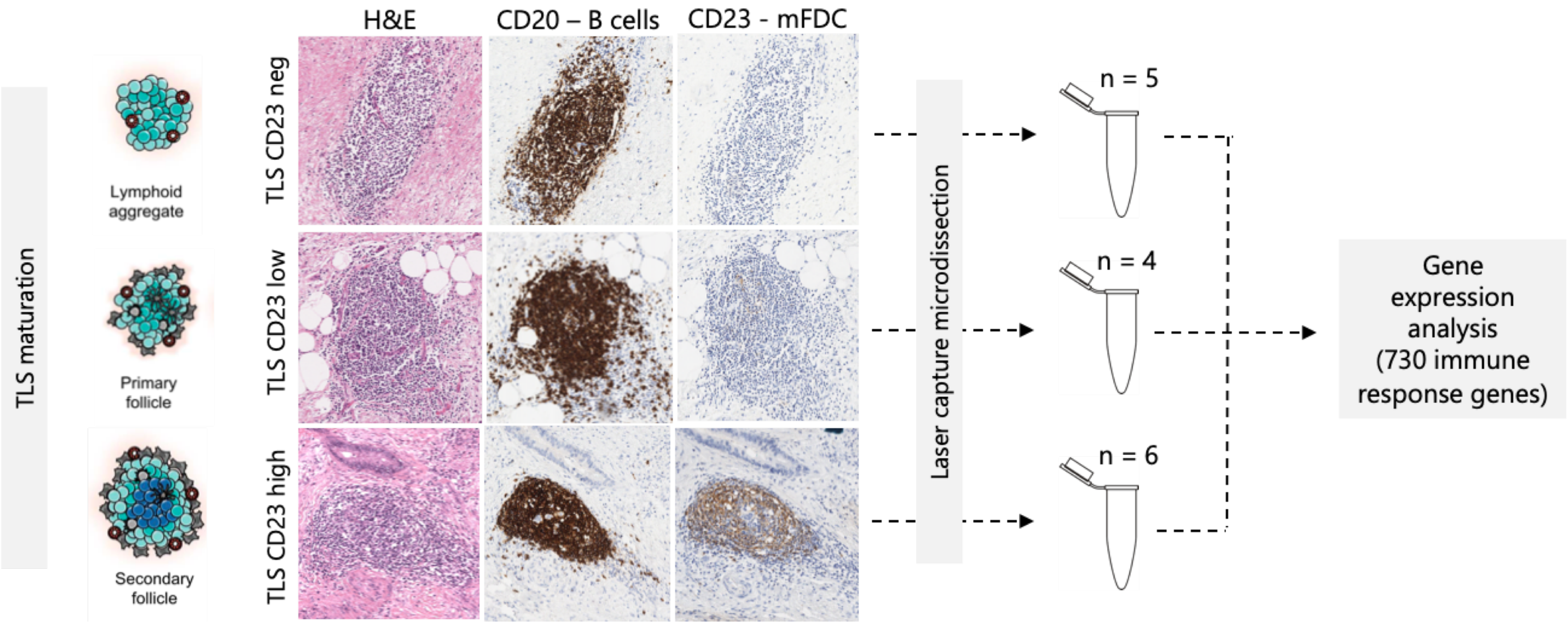
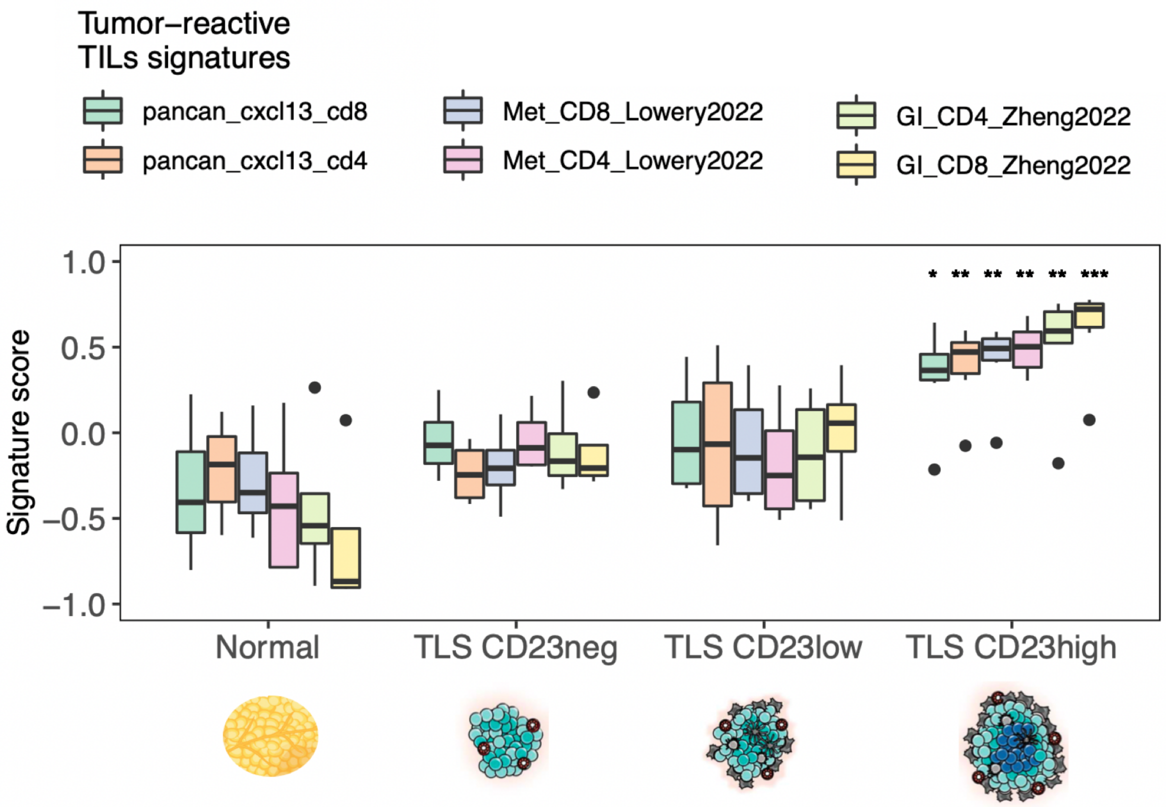
Expression profile of TLSs in different maturation stages

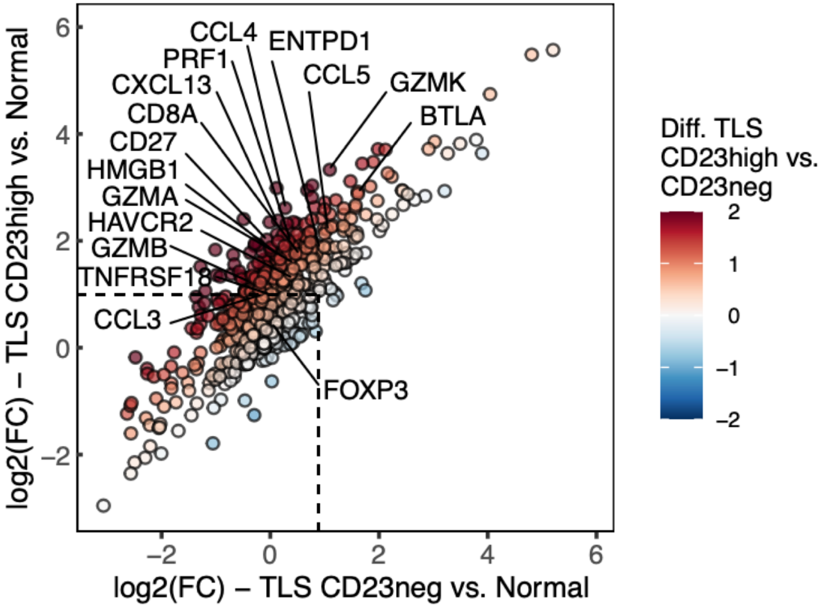


N = 41 treatment-naïve PDACs

Hybridization assay

Mature TLSs are enriched with tumor-reactive T cells





Hybridization assay

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- Identifying subgroups of tumor-infiltrating T and B cells using scRNA-seq.
- Evaluate the dynamics of clonal expansion of tumor-reactive T cells using scTCR-seq.
- Investigate the expression profile of microdissected TLSs using hybridization assays.
- Infer the abundance of different tumor-infiltrating lymphocytes using RNA-seq.
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Summary

Part 1

Introduction to main omics methodologies and data analysis tools.

- Conventional RNA-seq.
  - Infer the abundance of different immune cell types.
  - Evaluate the repertoire of T and B cell receptors.
- RNA-seq alternative: hybridization assays.
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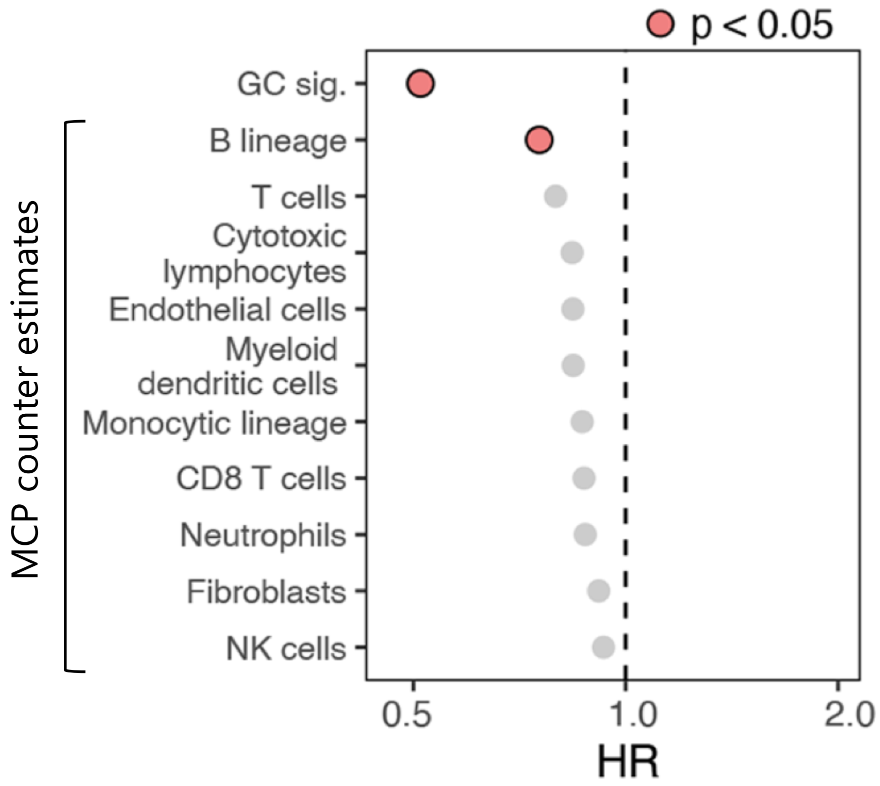
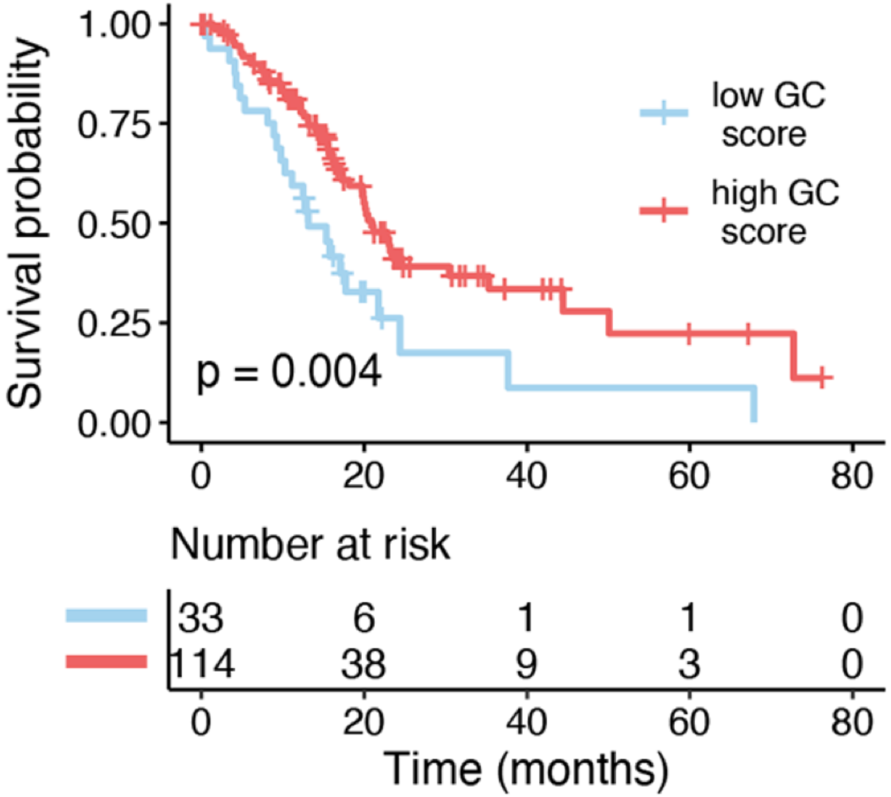
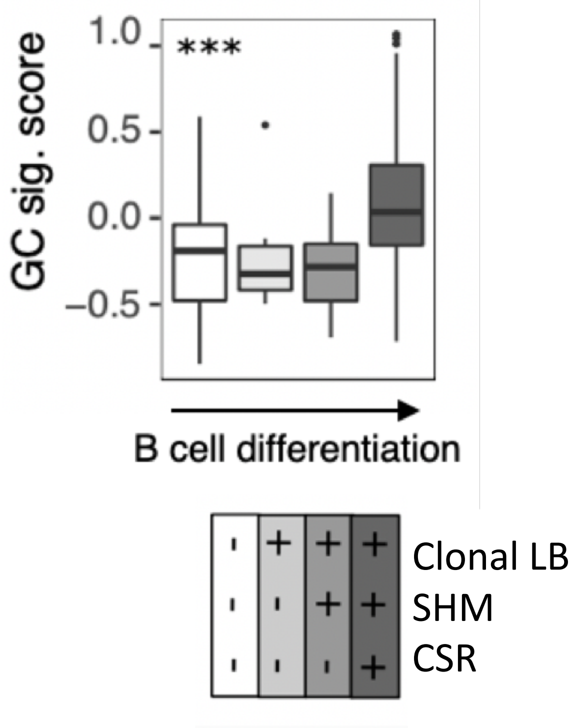
The GC signature is associated with improved prognosis

TCGA bulk RNA-seq







RNA-seq + MCPcounter + TRUST4

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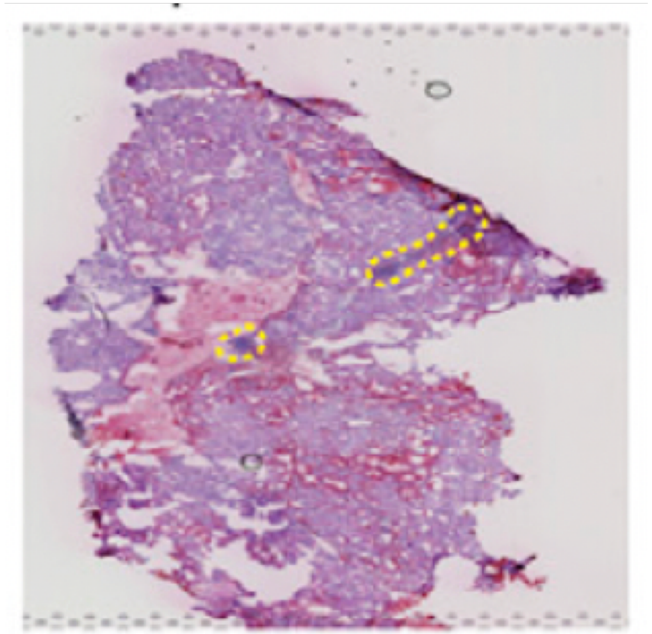
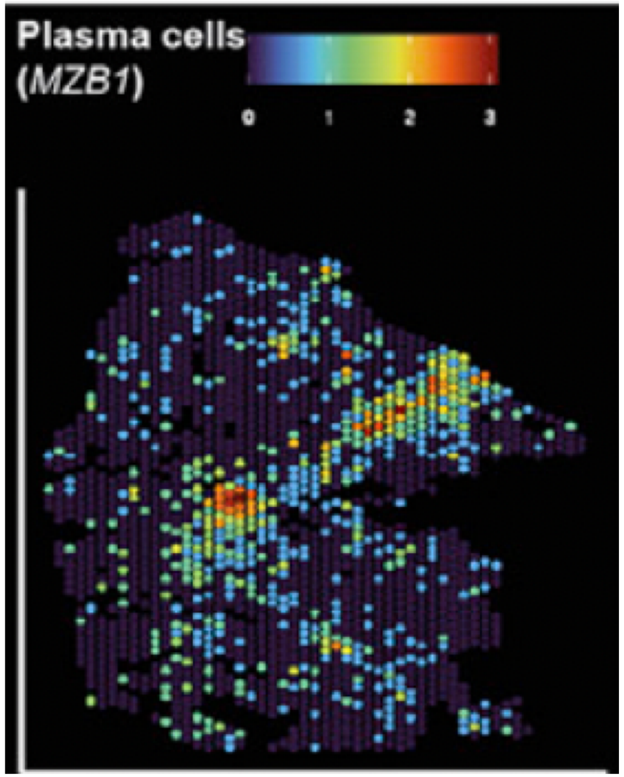
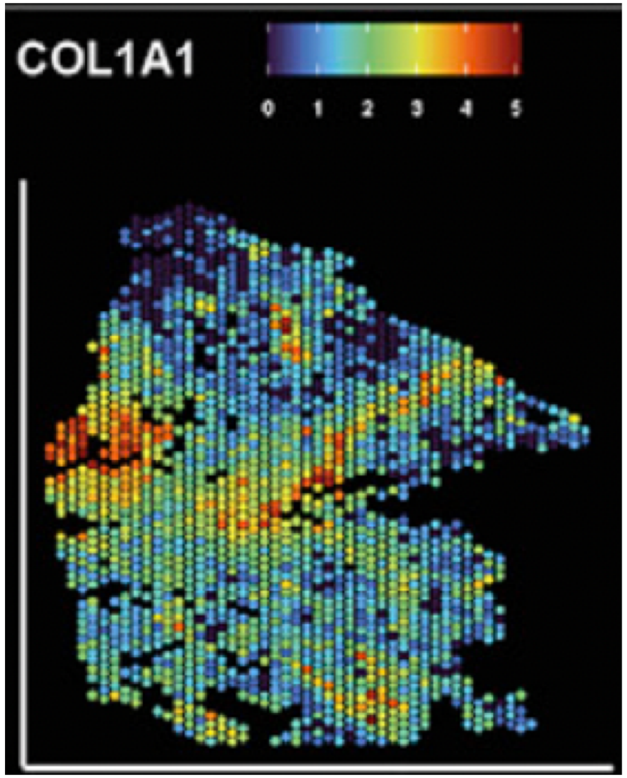
Coming soon ☺

Renal clear cell carcinoma







Maylan et al., 2022. Immunity

⚠️ Reuse and Recycle ⚠️

There is a lot o public omics data out there.

Explore

Be creative

Have fun