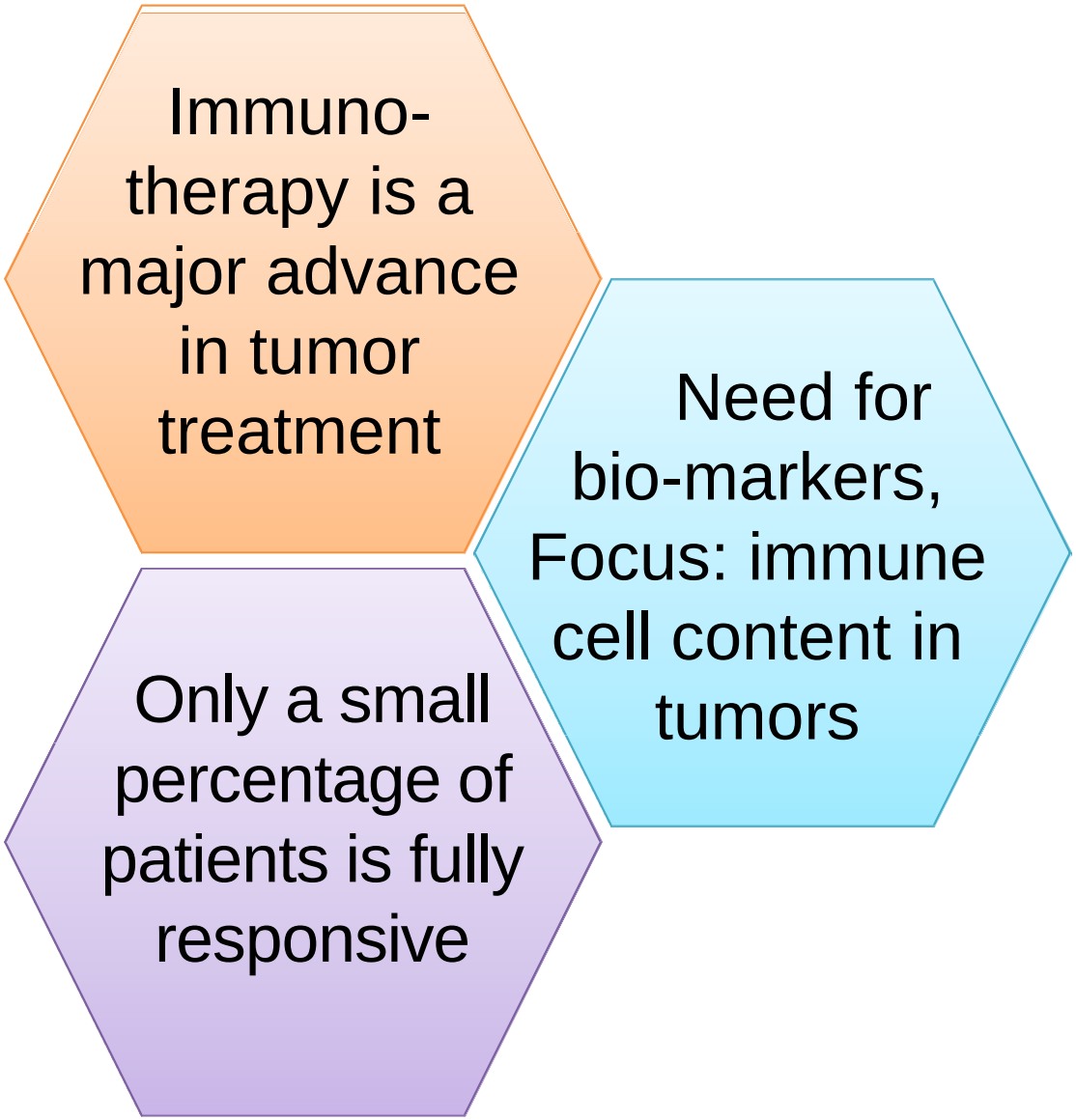
Deconvoluzione computazionale dei dati di trascrittomica per lo studio delle cellule immunitarie infiltranti il tumore

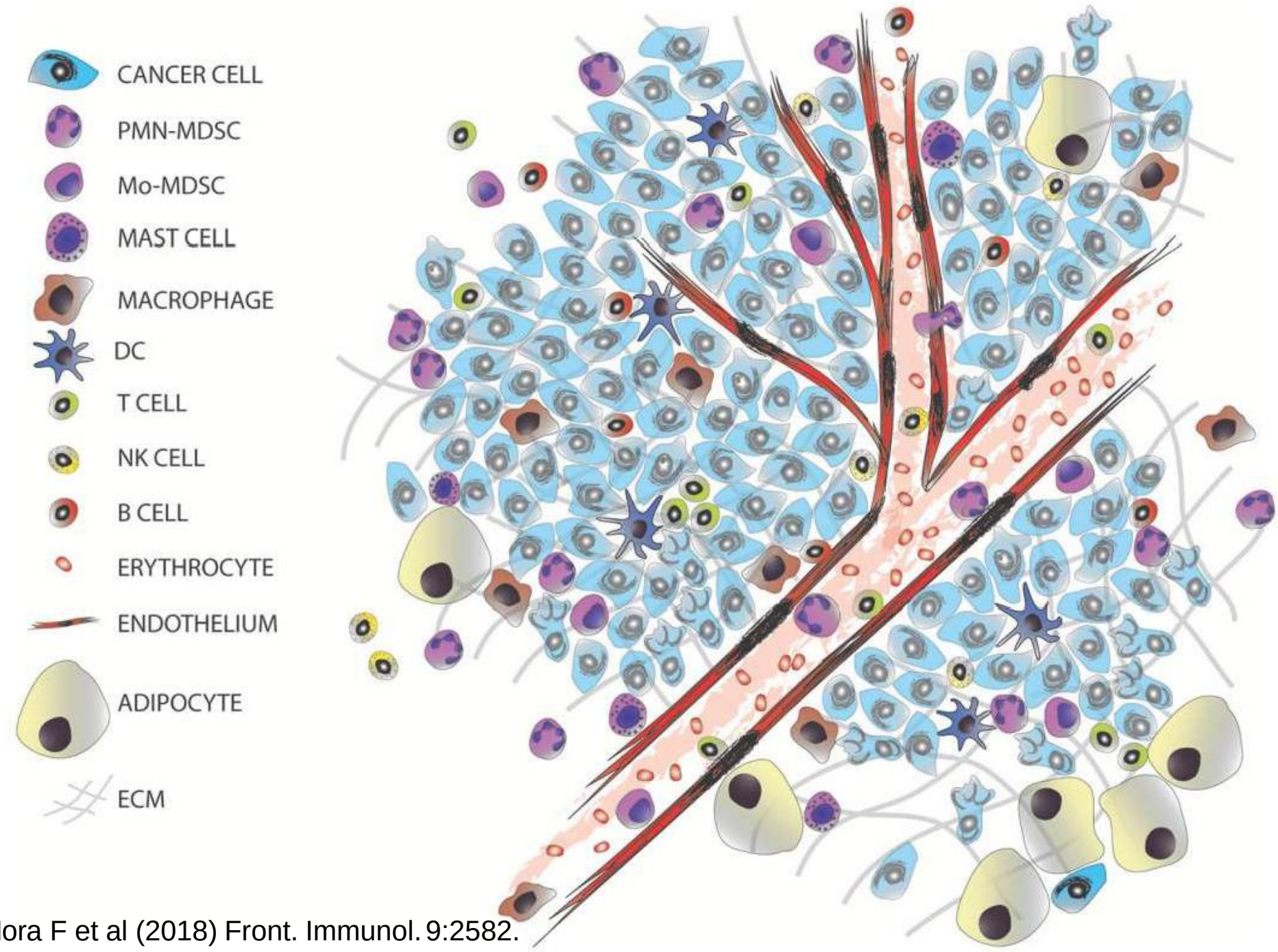
Maddalena Fratelli
Unità di Farmacogenomica

CRO, Aviano, 20 febbraio 2020

BACKGROUND

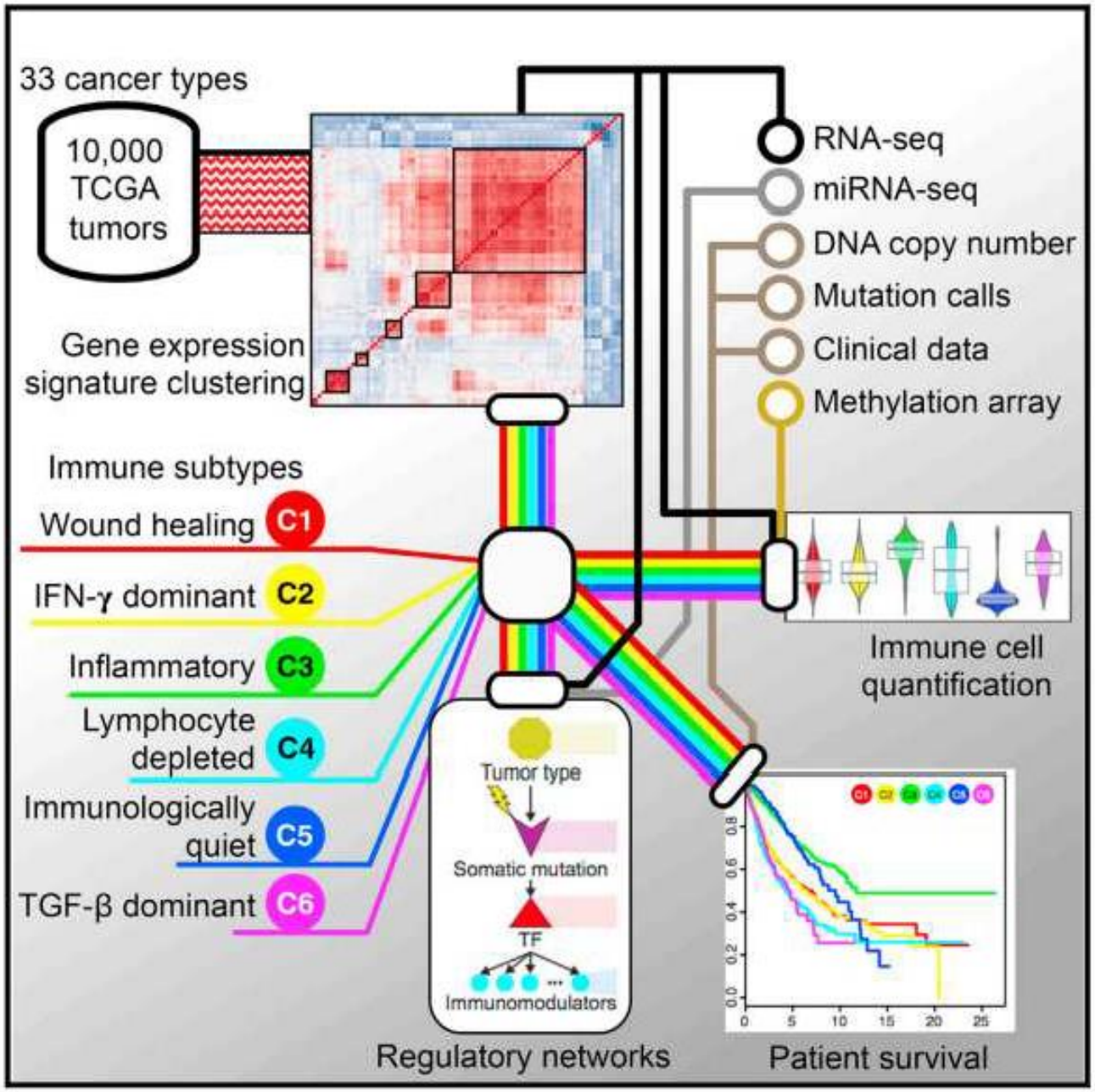

Immuno-therapy is a major advance in tumor treatment
Need for bio-markers, Focus: immune cell content in tumors
Only a small percentage of patients is fully responsive

TUMOR MICROENVIRONMENT



Valdes-Mora F et al (2018) Front. Immunol. 9:2582.

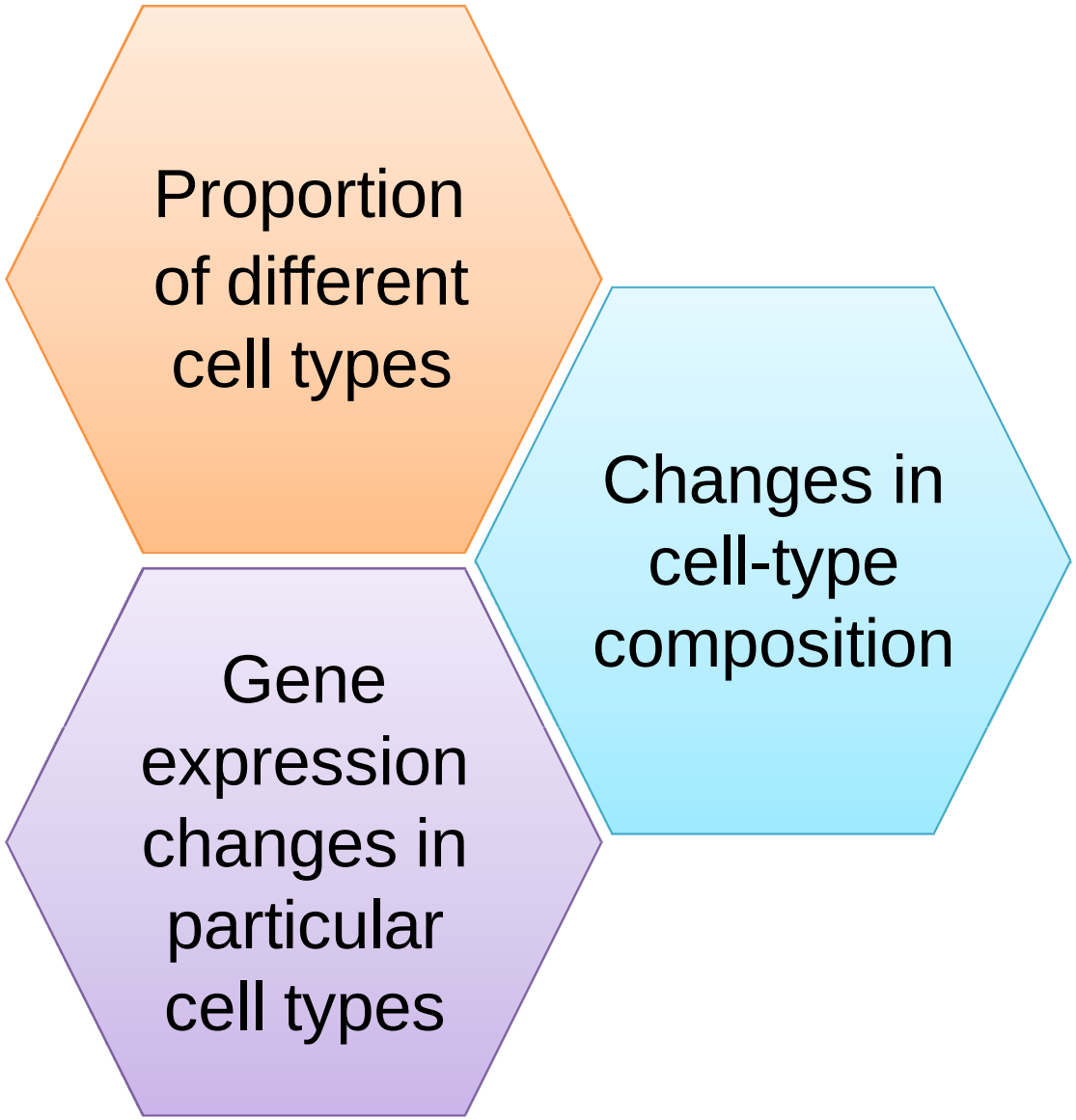
THE IMMUNE LANDSCAPE OF CANCER



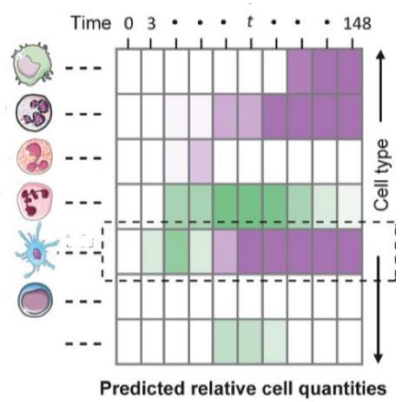
Thorsson V et al.(2018) Immunity 17;48(4):812-830

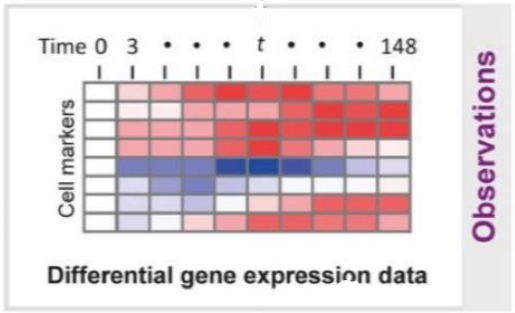
BULK TRANSCRIPTOMICS

SOURCES OF VARIATION:



BULK TRANSCRIPTOMICS DATA DECONVOLUTION

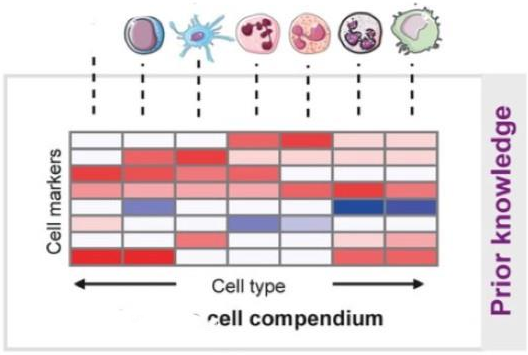




Bulk data

Algorithms

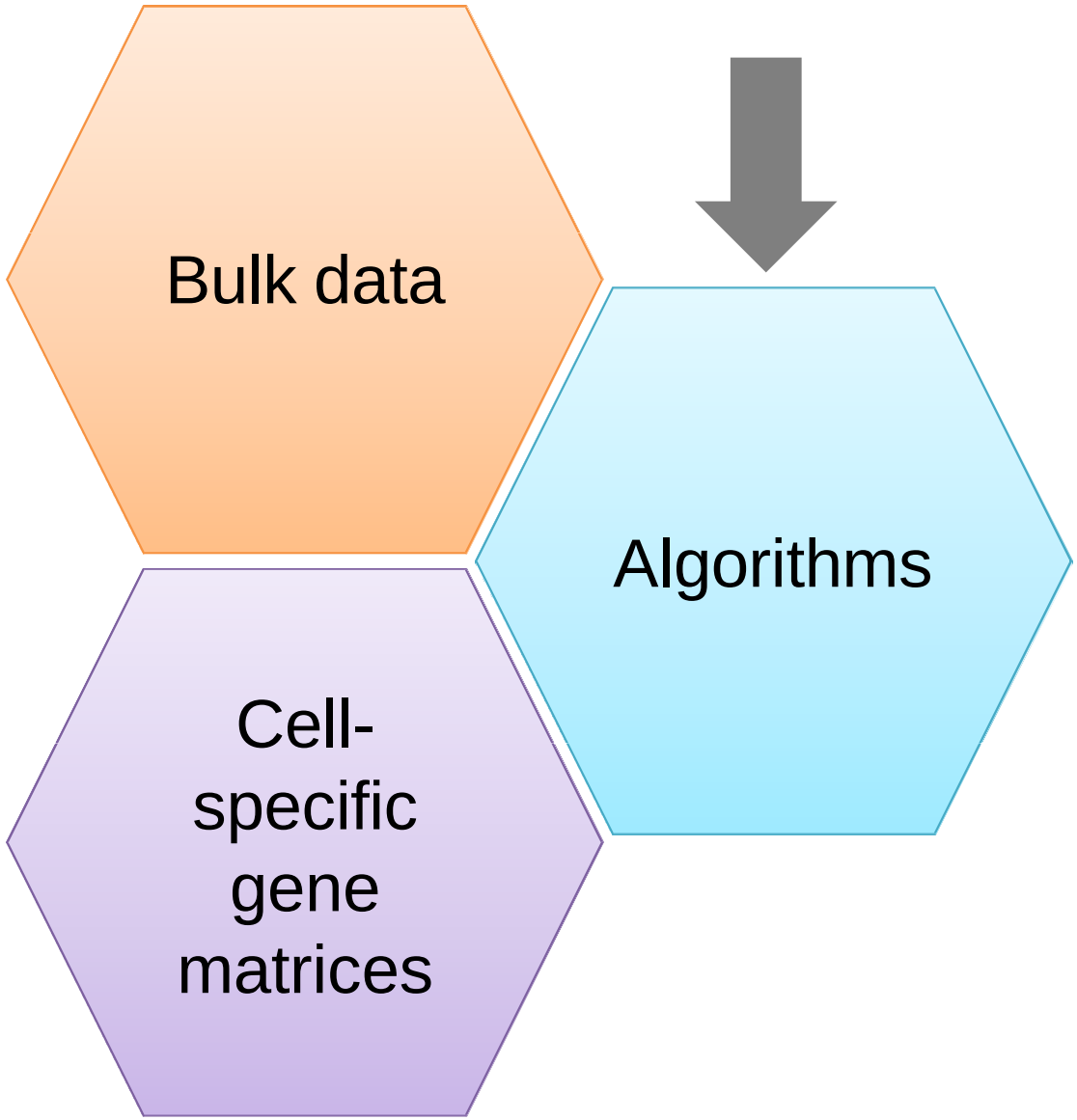
Cell-specific gene matrices



$$Y_{ig}=\pi_{1,i}N_{1,ig}+\pi_{2,i}N_{2,ig}+\pi_{T,i}T_{ig}$$

BULK TRANSCRIPTOMICS DATA DECONVOLUTION

BASED ON:



WIDE VARIETY OF DECONVOLUTION METHODS…

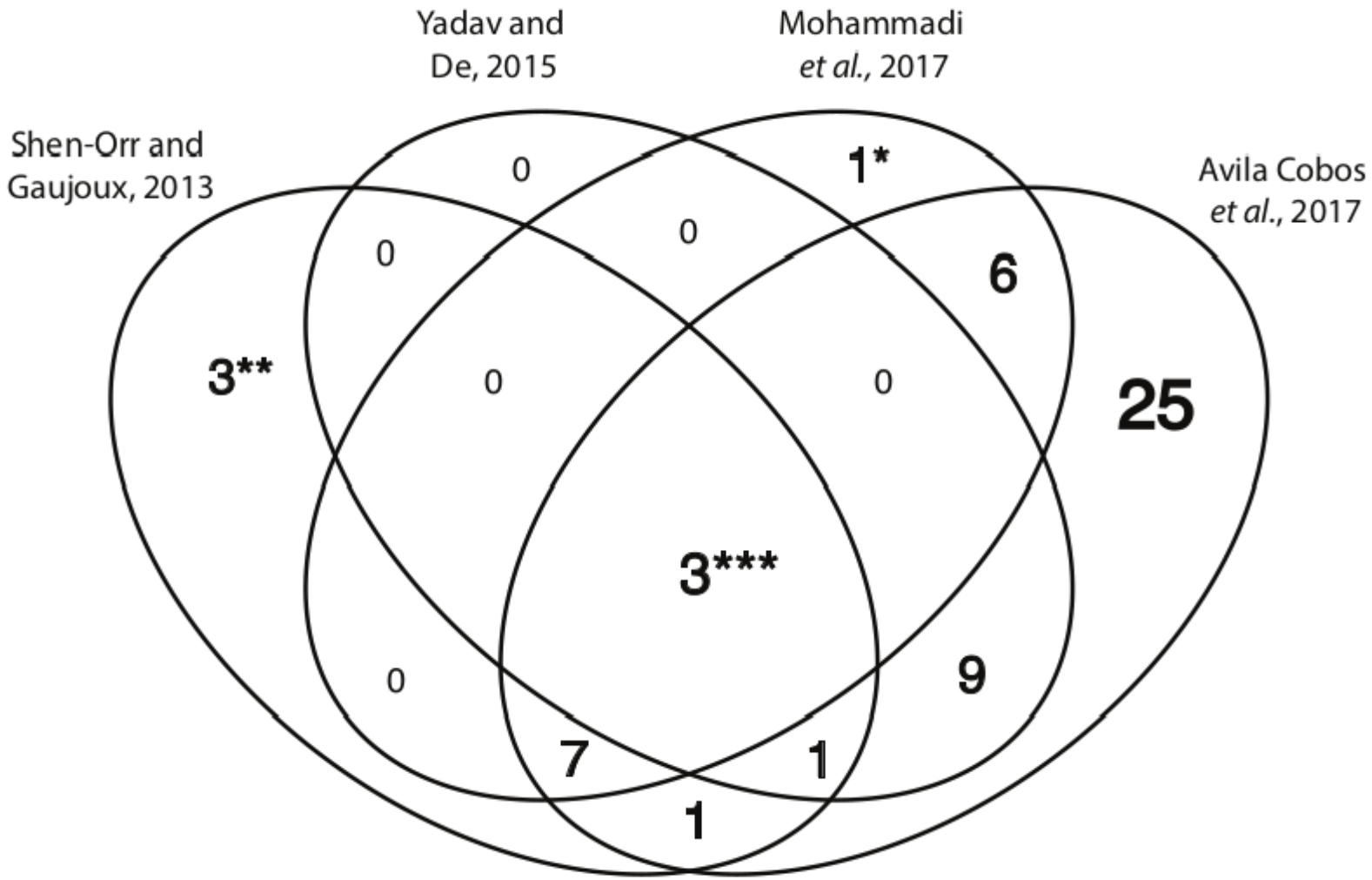
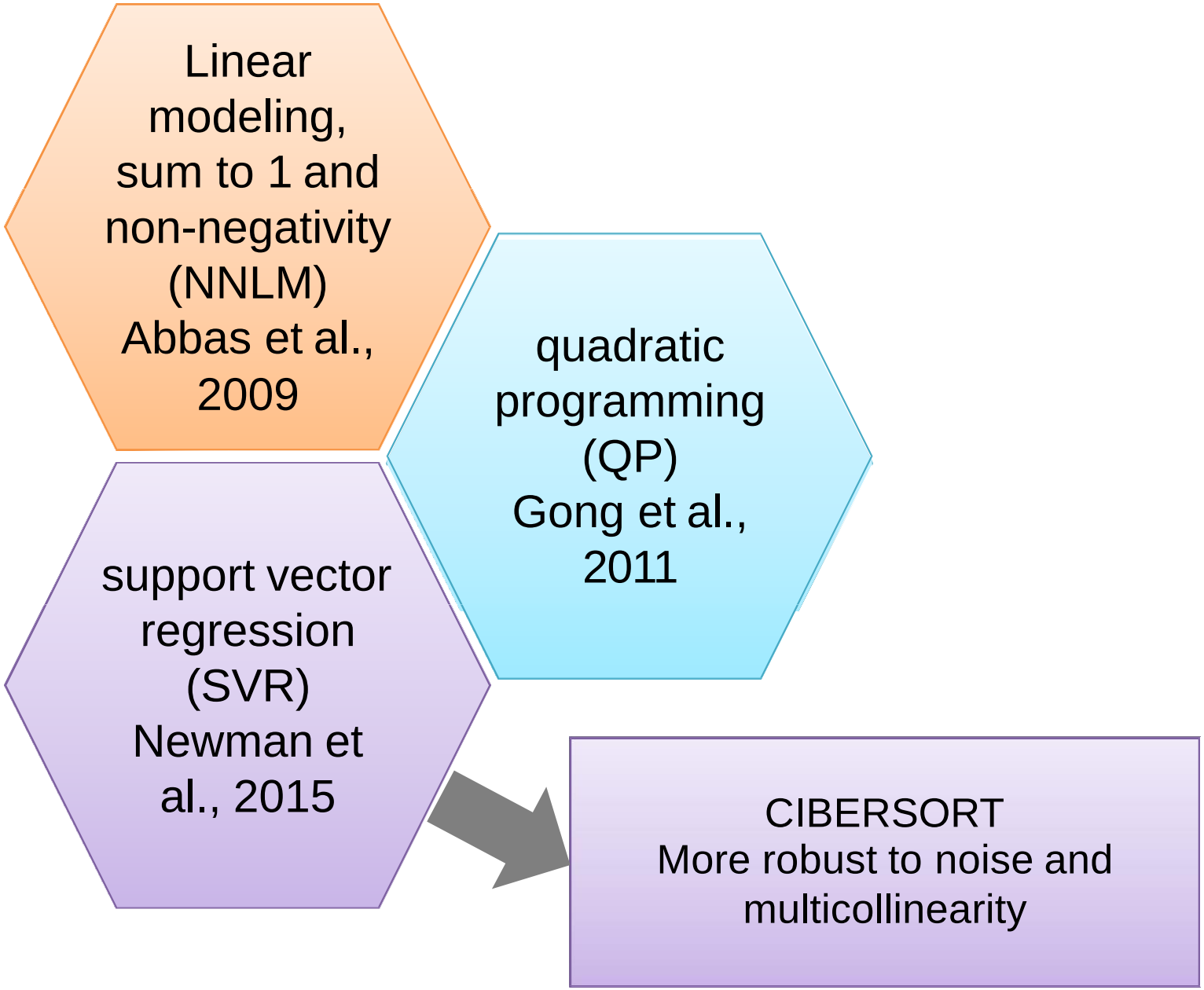


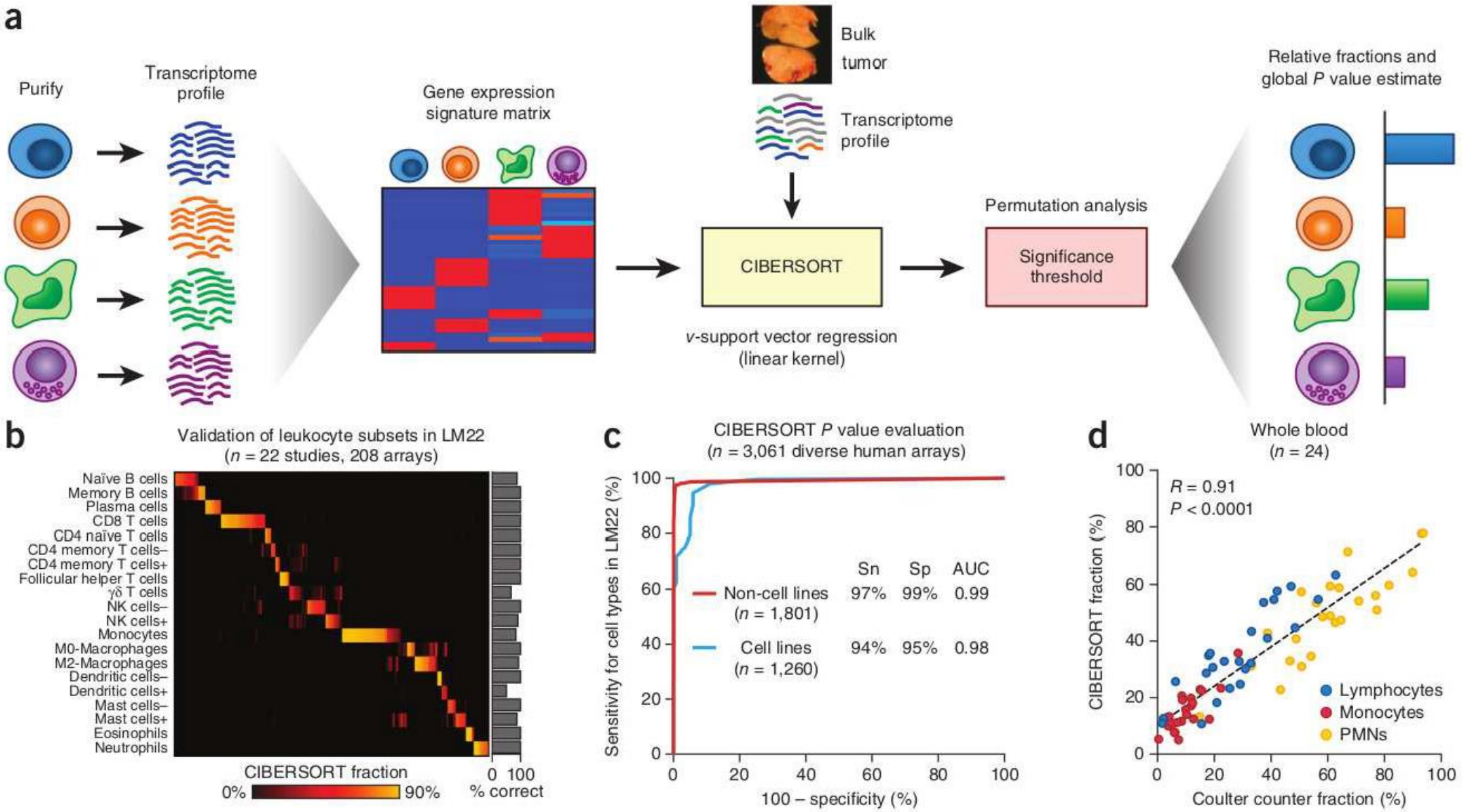
Fig. 1 Venn diagram showing the number of deconvolution methods covered by each review article (using transcriptomics data as input). *(*) We discussed Nano-*

Avila Cobos F et al (2018) Bioinformatics. 34(11):1969-1979.

…BASED ON A VARIETY OF APPROACHES

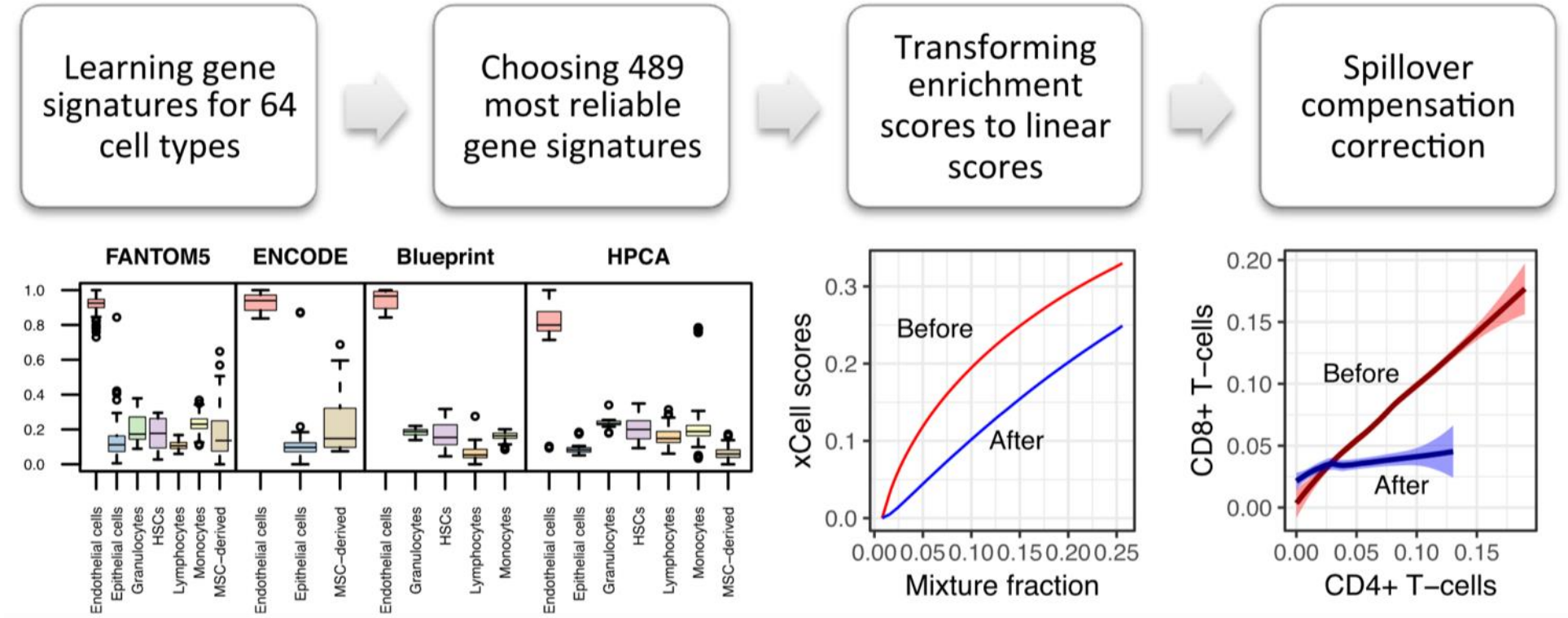


CIBERSORT



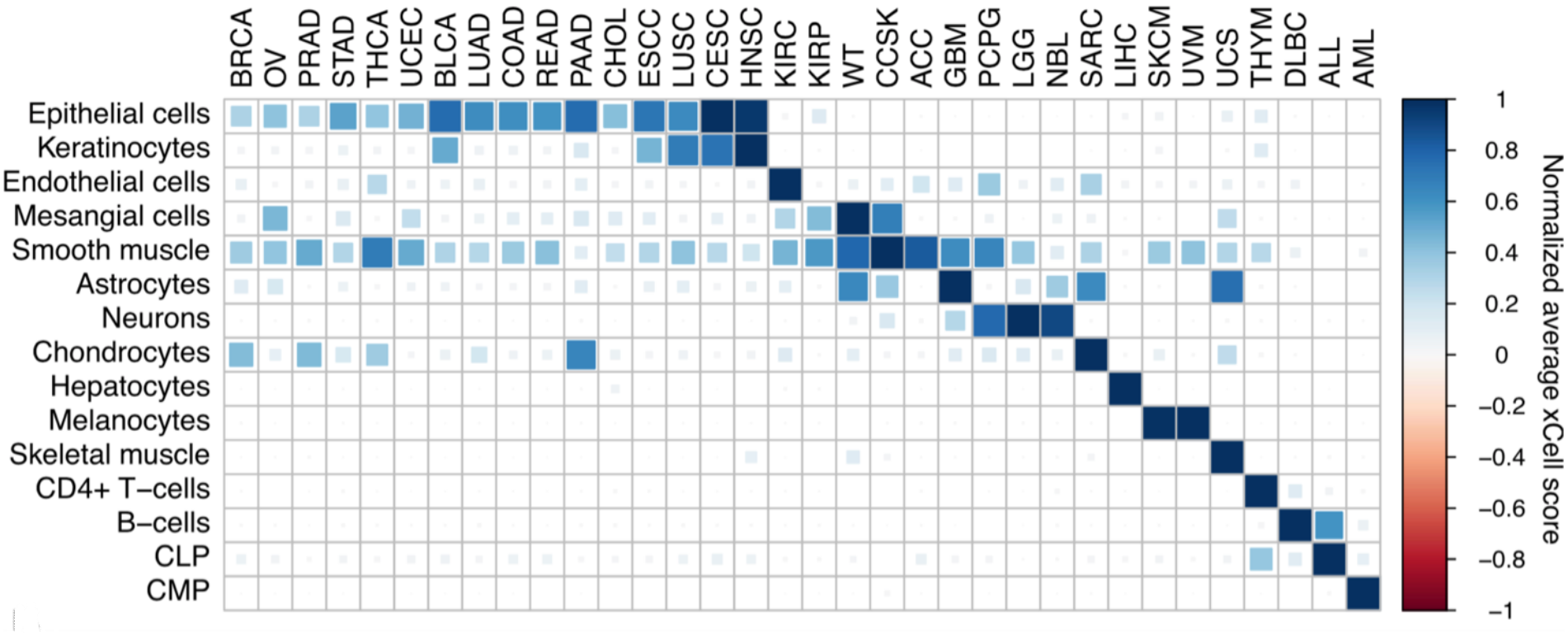
Newman AM et al (2015) Nat Methods. 12(5):453-457

ALTERNATIVE METHOD USING RANK BASED ENRICHMENT ANALYSIS: XCELL



Aran et al. (2017) Genome Biology 18:220

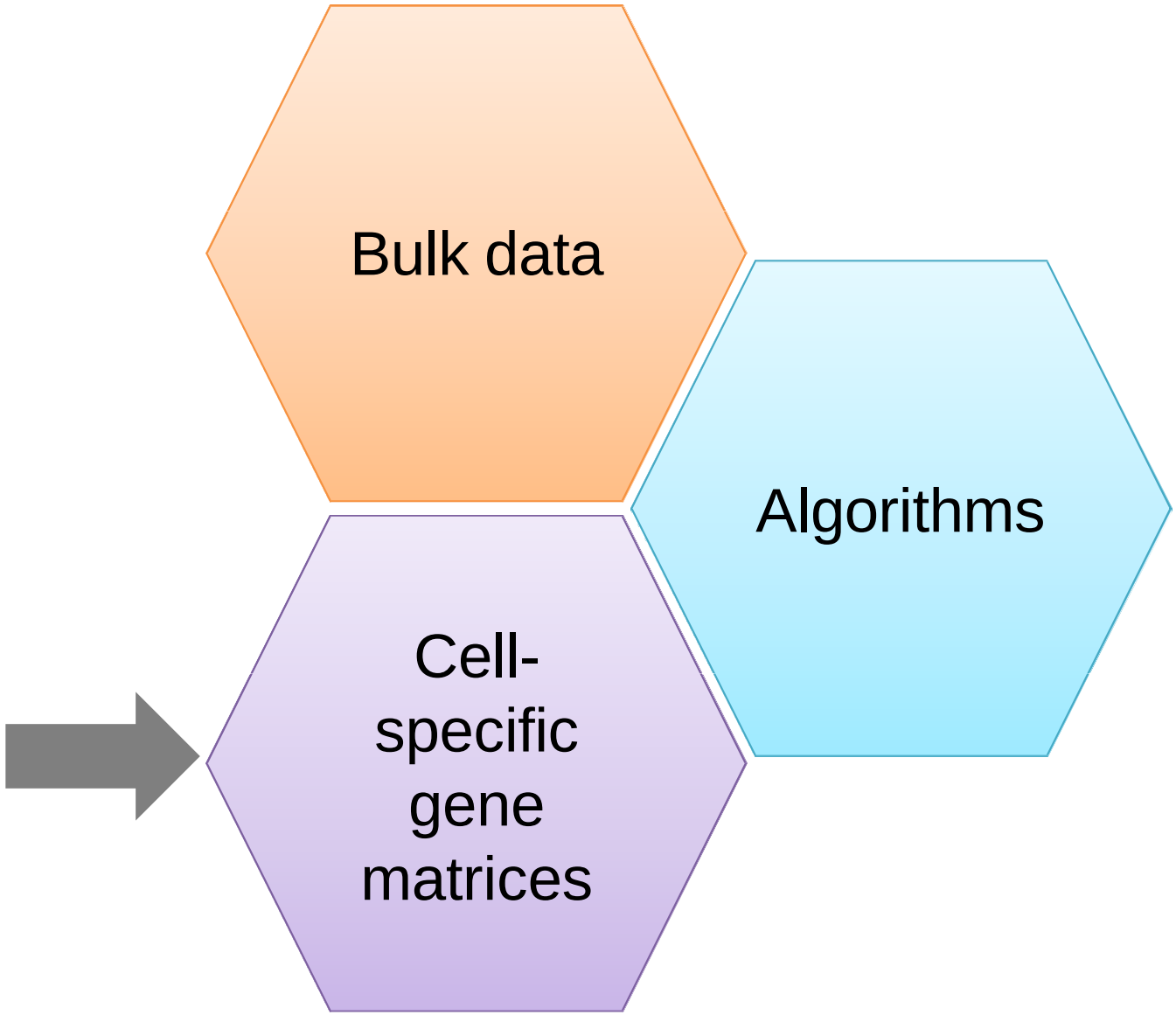
ENRICHMENT OF TUMOR-SPECIFIC CELL TYPES IN TCGA



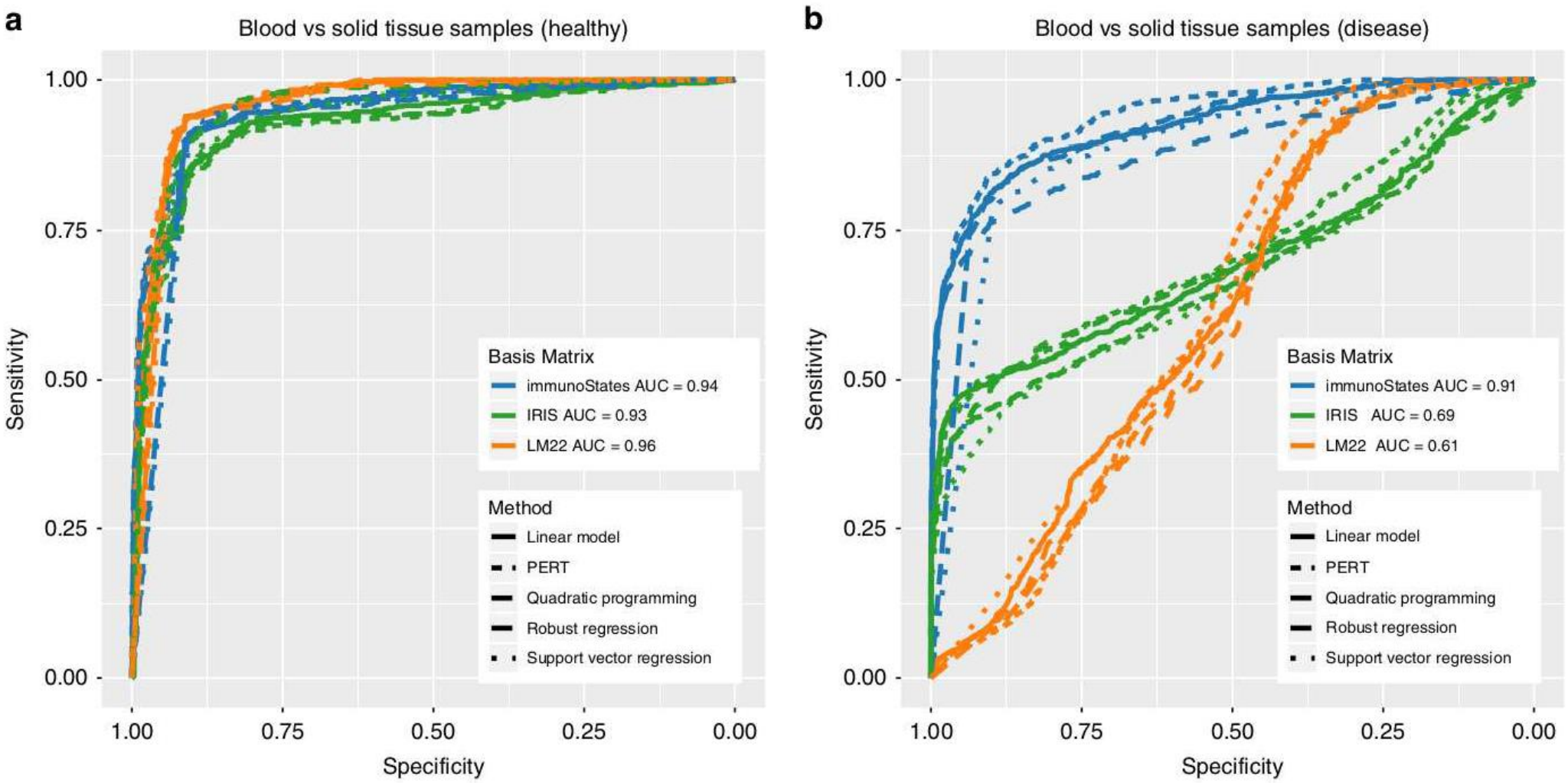
Aran et al. (2017) Genome Biology 18:220

BULK TRANSCRIPTOMICS DATA DECONVOLUTION

BASED ON:



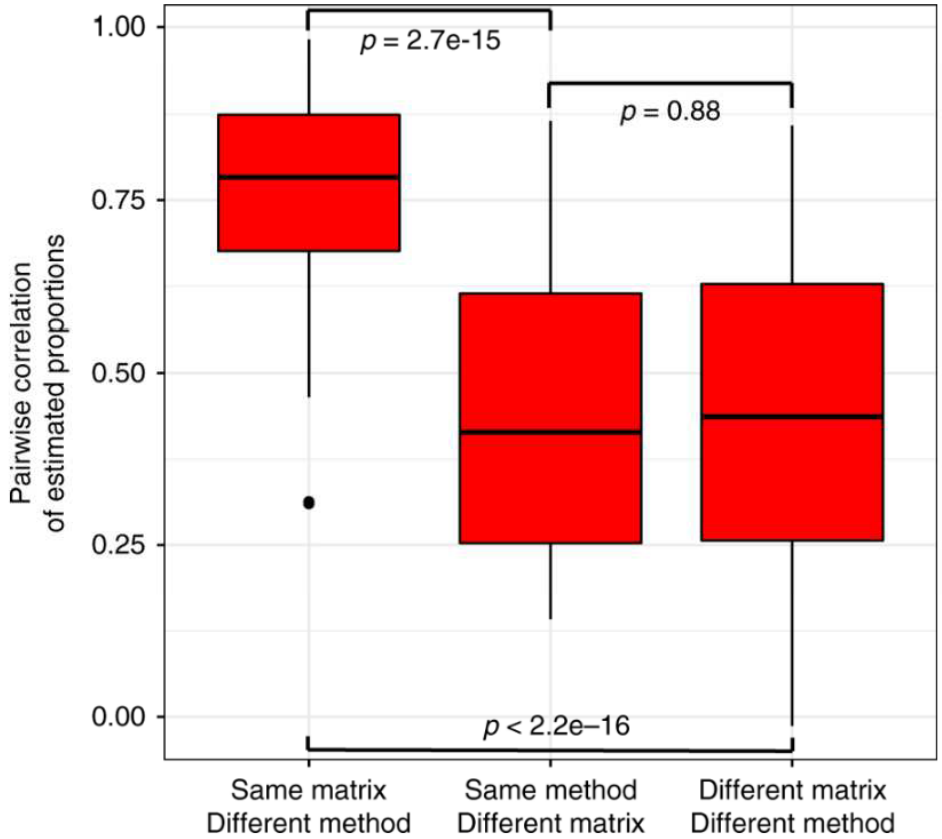
IMMUNOSTATES: A GENE MATRIX LEVERAGING HETEROGENEITY AND DISEASE CONDITIONS



Vallania F et al (2018) Nat Commun 9:4735

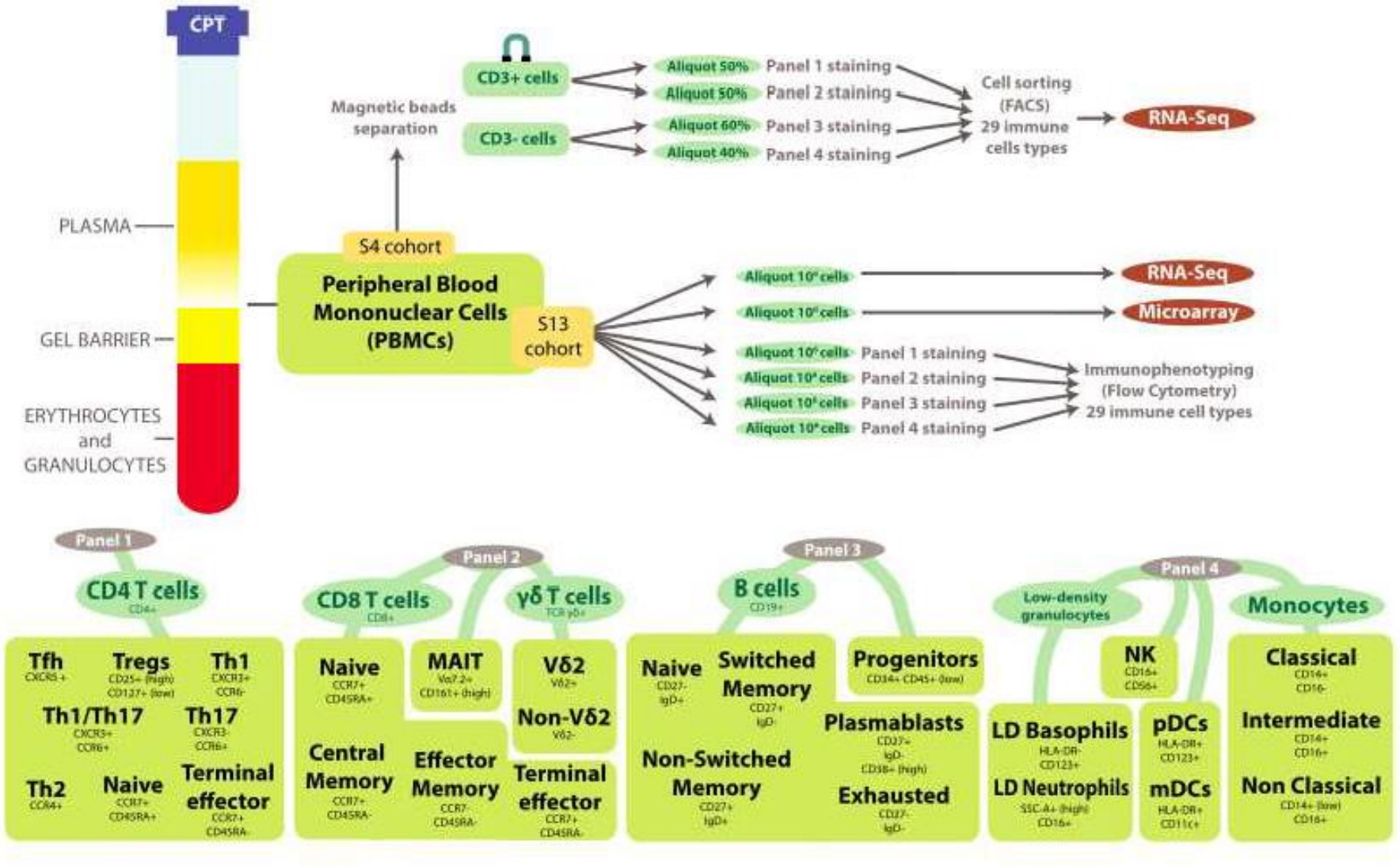
THE QUALITY OF THE RESULTS DEPENDS MORE ON THE GENE MATRICES THAN ON THE ALGORITHMS

DECONVOLUTION CONCORDANCE BY MATRIX AND METHOD:



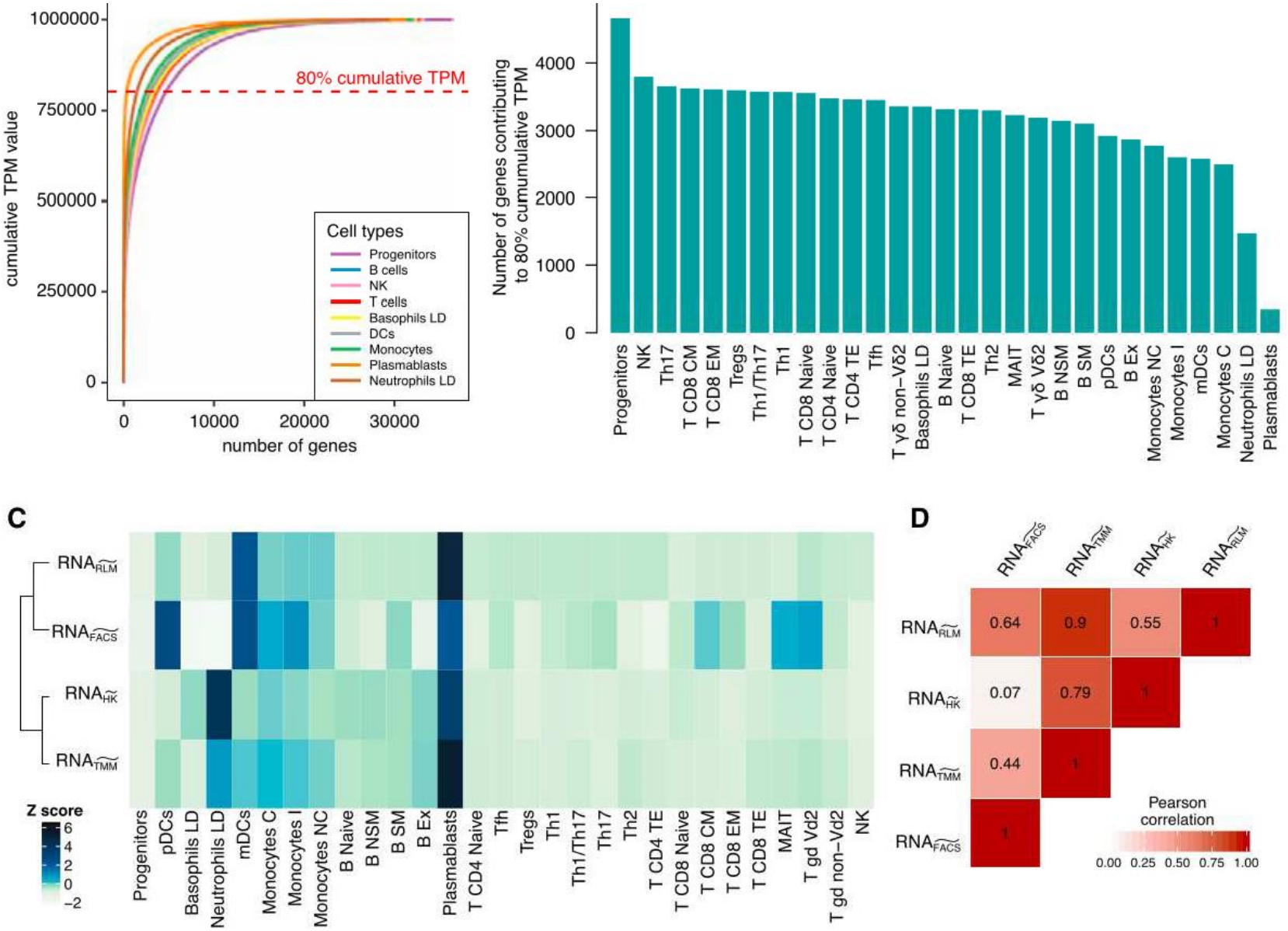
Vallania F et al (2018) Nat Commun. 9(1):4735

CHARACTERIZATION OF 29 HUMAN IMMUNE CELL TYPES BY RNA-SEQ AND FLOW CYTOMETRY



Monaco et al. (2019) Cell Reports 26, 1627–1640

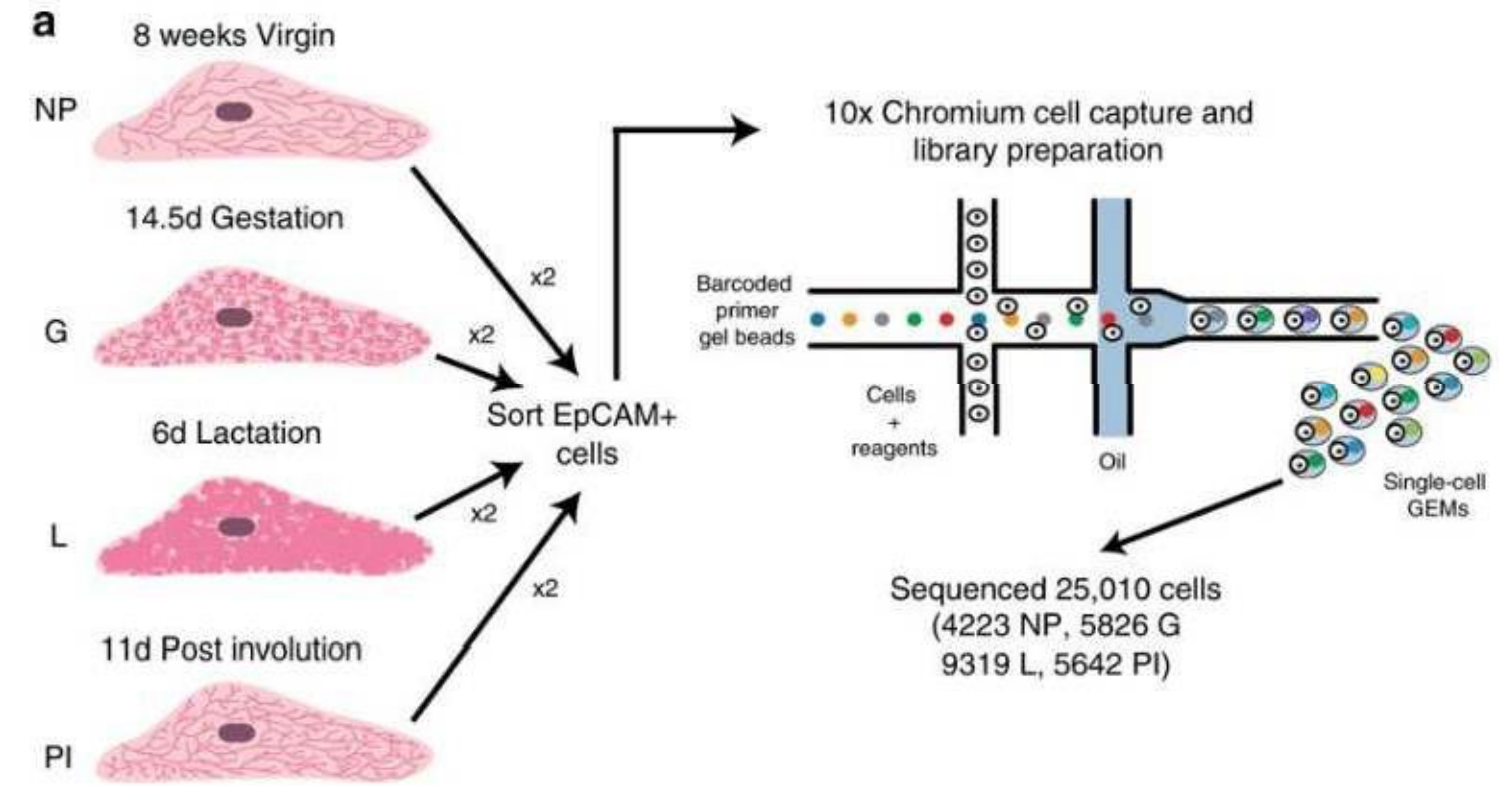
THE ENDLESS PROBLEM OF NORMALIZATION

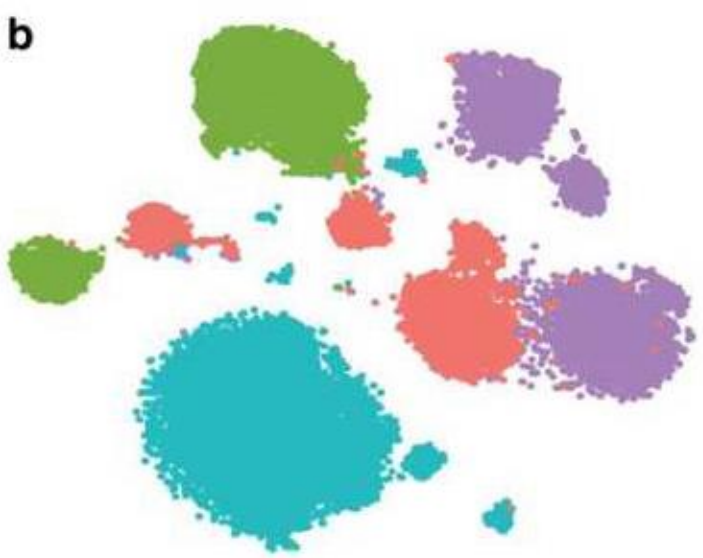


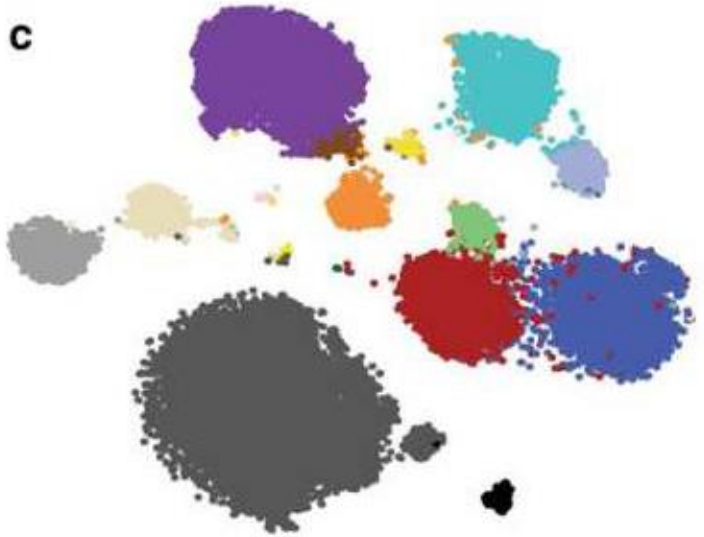
BEST APPROACH: scale the TPM values by a factor that minimizes the error between flow cytometry and deconvolution proportions

Monaco et al. (2019) Cell Reports 26, 1627–1640

SINGLE CELL SEQUENCING

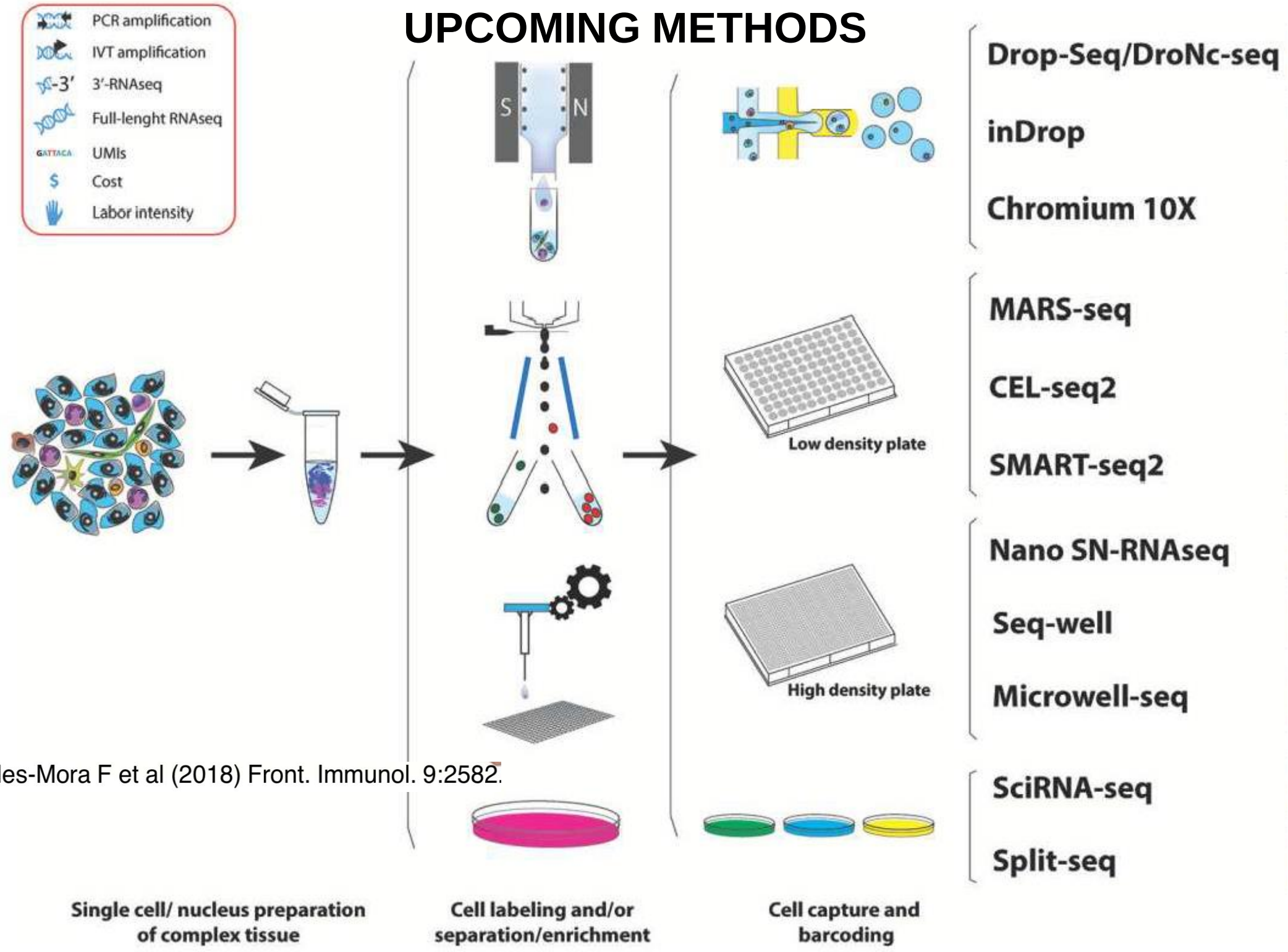






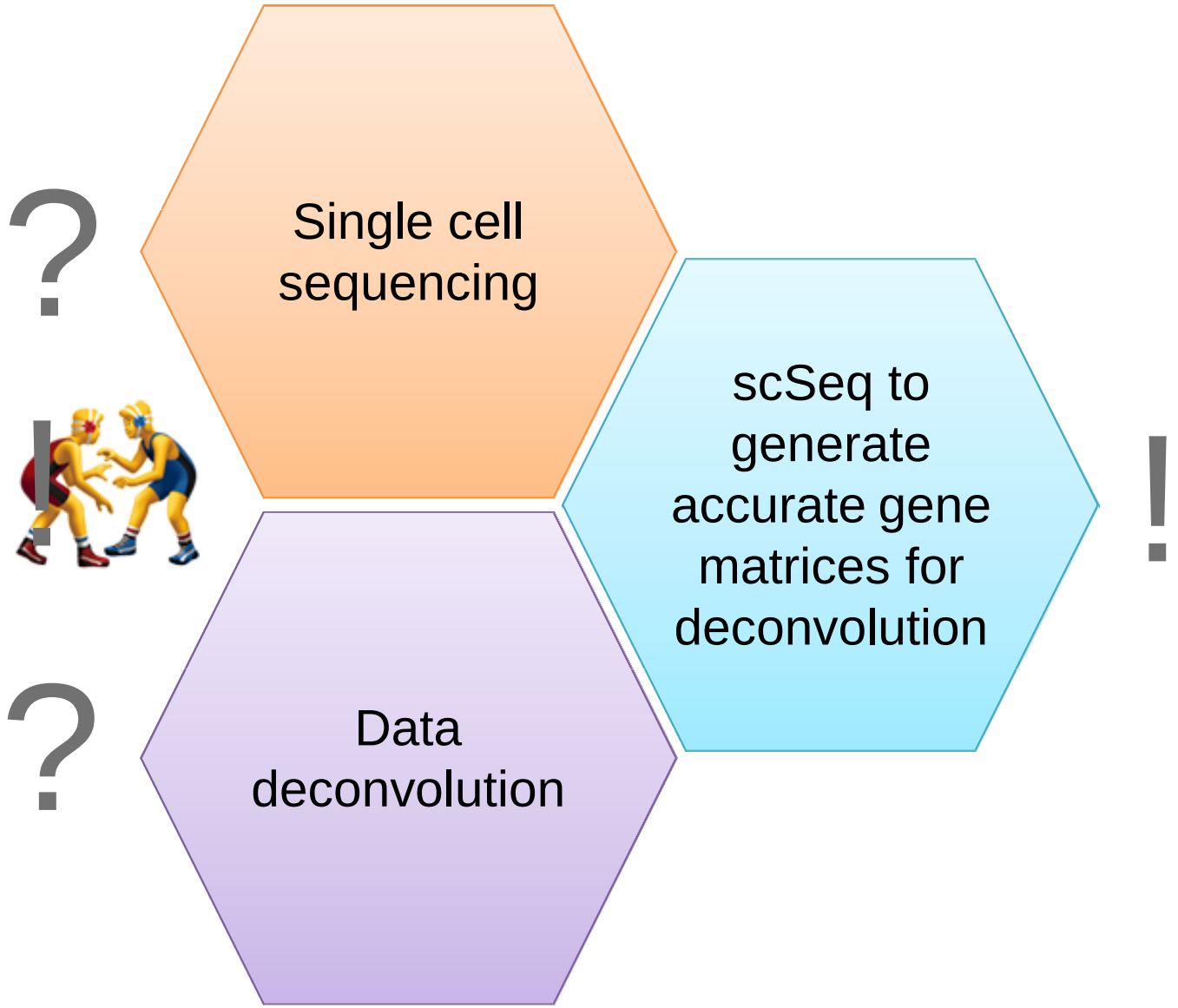
Bach K et al (2017) Nat Commun. 8(1):2128

UPCOMING METHODS



Valdes-Mora F et al (2018) Front. Immunol. 9:2582.

THE FUTURE (last year)



THE FUTURE (now)

TOOLS AND RESOURCES

eLIFE
elifesciences.org

Identifying gene expression programs of cell-type identity and cellular activity with single-cell RNA-Seq

Dylan Kotliar[1,2,3†*], Adrian Veres[1,3,4†], M Aurel Nagy[3,5], Shervin Tabrizi[2], Eran Hodis[3,6], Douglas A Melton[4,7], Pardis C Sabeti[1,2,7]

...tment of Systems Biology, Harvard Medical School, Boston, United States; ...itute of MIT and Harvard, Cambridge, United States; [3]Harvard-MIT ... Health Sciences and Technology, Massachusetts Institute of Technolog... ...ge, United States; [4]Harvard Stem Cell Institute, Harvard University, ...idge, United States; [5]Department of Neurobiology, Harvard Medical Scho... ...on, United States; [6]Biophysics Program, Harvard University, Cambridge, U... ...tes; [7]Howard Hughes Medical Institute, Chevy Chase, United States

OXFORD

Briefings in Bioinformatics, 00(0), 2020, 1–12
doi: 10.1093/bib/bbz166
Article

SCDC: bulk gene expression deconvolution by multiple single-cell RNA sequencing references

Meichen Dong, Aatish Thennavan, Eugene Urrutia, Yun Li, Charles M. Perou, Fei Zou and Yuchao Jiang

Corresponding authors: Fei Zou and Yuchao Jiang, Department of Biostatistics and Department of Genetics, University of North Carolina at Ch... Chapel Hill, NC 27599, USA. feizou@email.unc.edu, yuchaoj@email.unc.edu.

Yu et al. BMC Cancer (2019) 19:715
https://doi.org/10.1186/s12885-019-5927-3

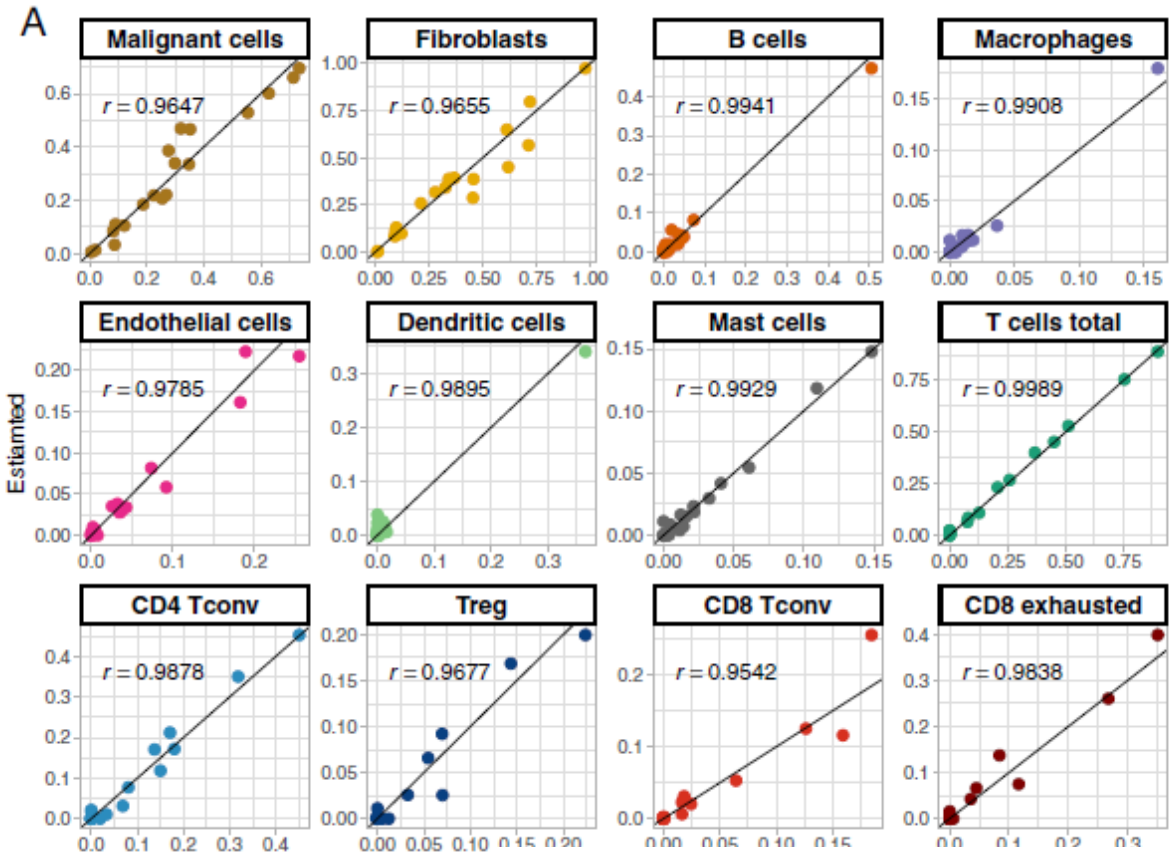
BMC Cancer

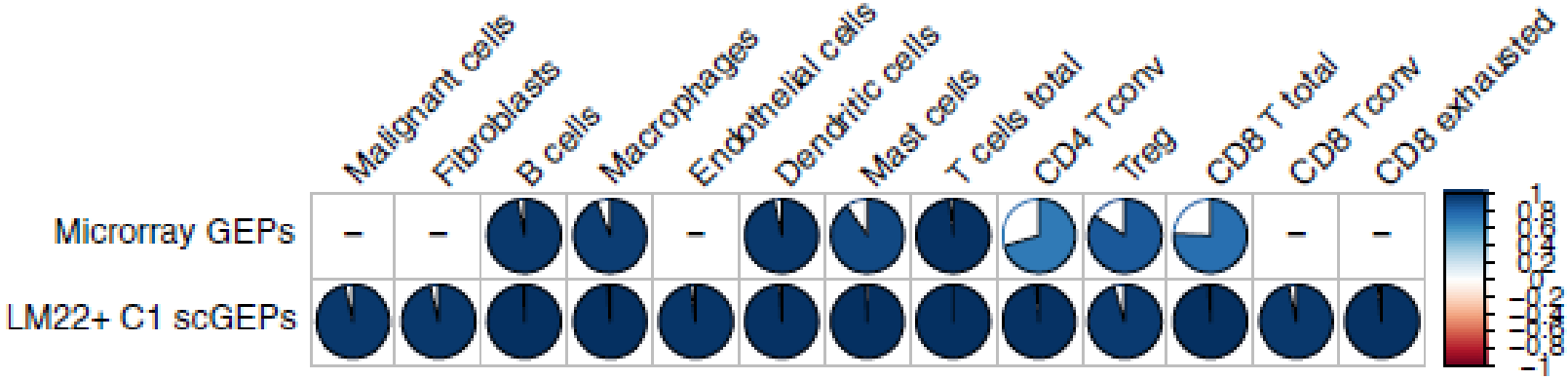
RESEARCH ARTICLE Open Access

Estimation of immune cell content in tumor using single-cell RNA-seq reference data

Xiaoqing Yu[1], Y. Ann Chen[1], Jose R. Conejo-Garcia[2], Christine H. Chung[3] and Xuefeng Wang[1*]

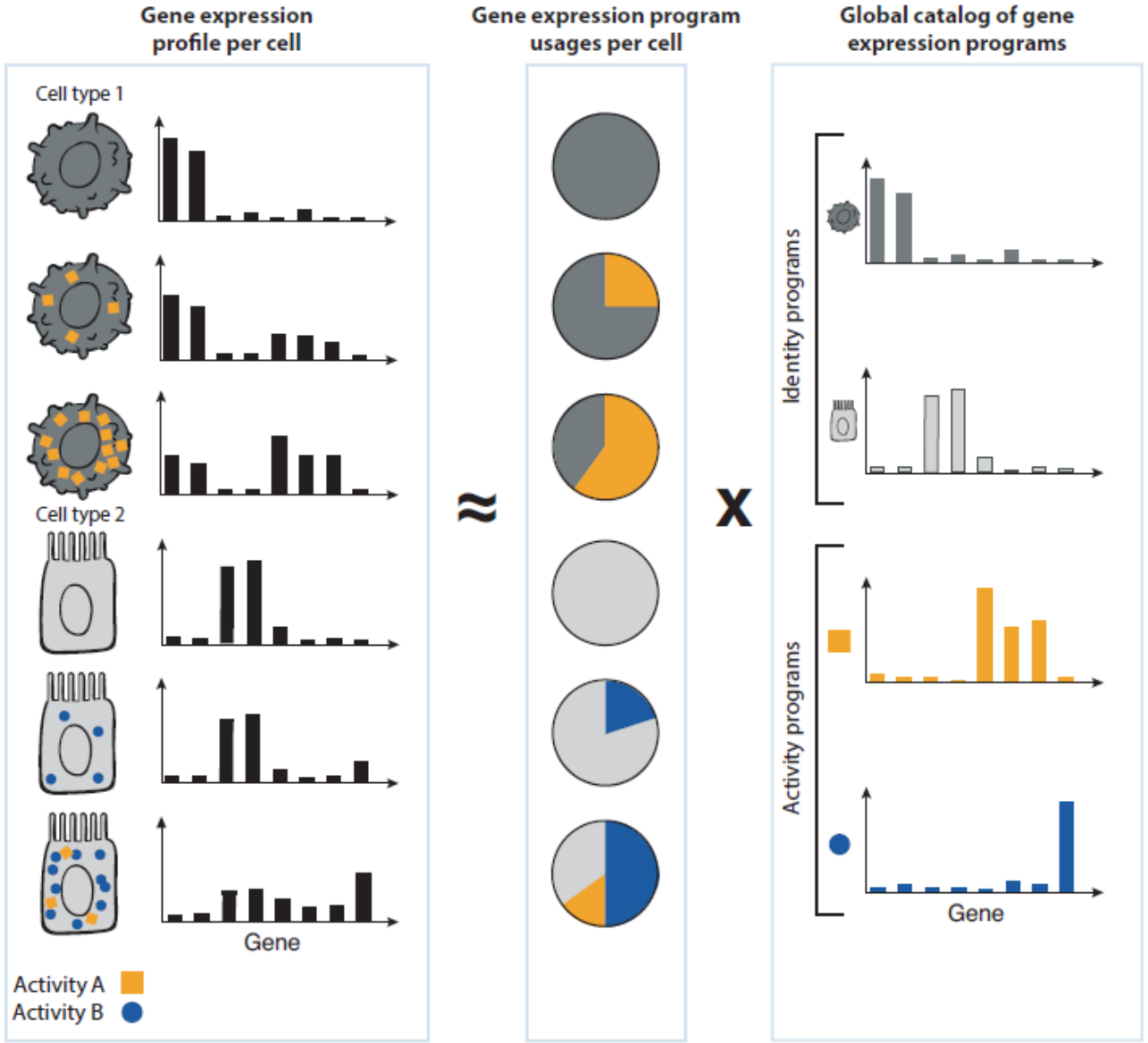
ADVANTAGE OF THE USE OF SINGLE CELL GENE EXPRESSION PROFILES IN SIMULATED BULK TUMOR SAMPLES





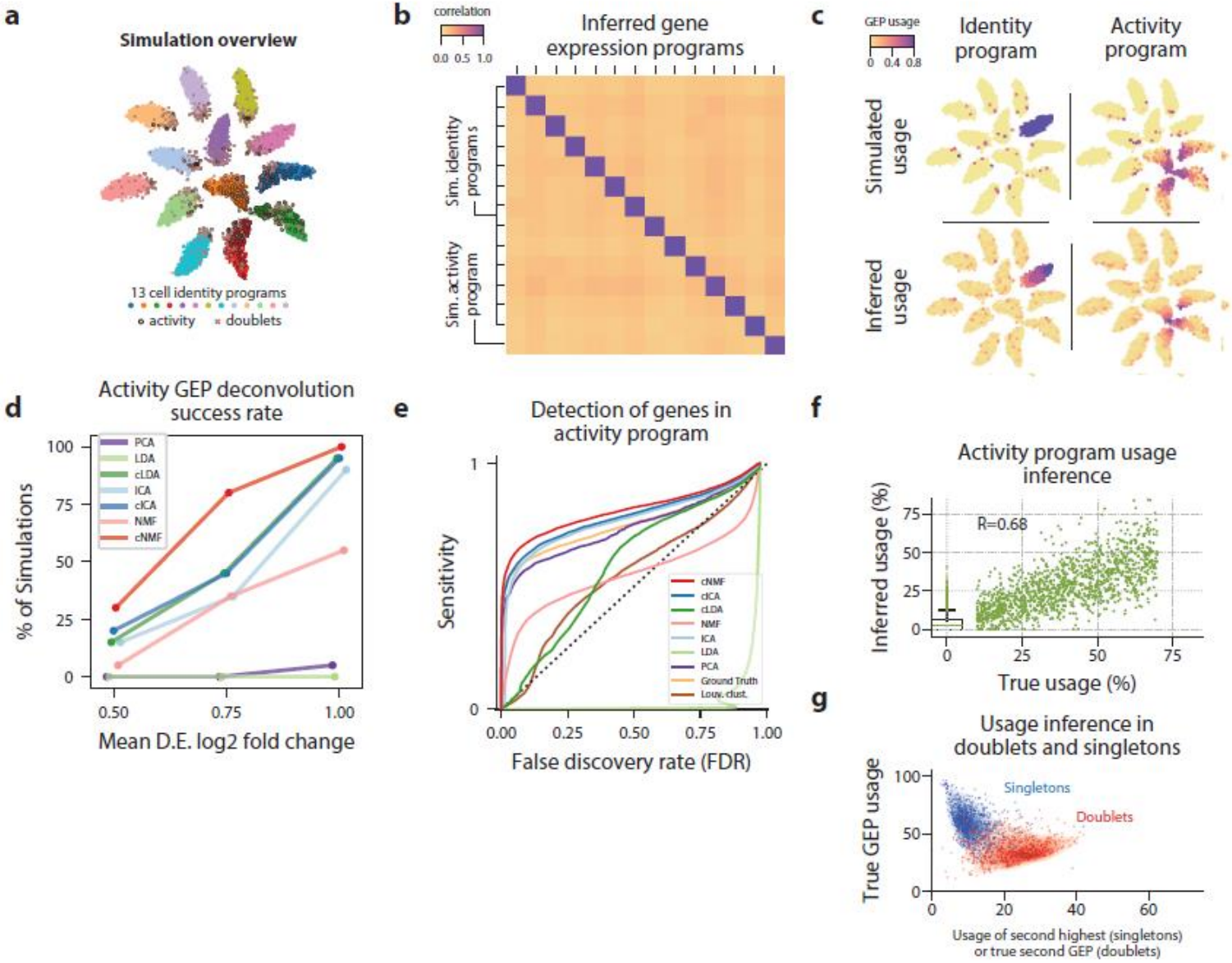
Yu et al. BMC Cancer (2019) 19:715

IDENTIFYING GENE EXPRESSION PROGRAMS UNDERLYING BOTH CELL-TYPE IDENTITY AND CELLULAR ACTIVITIES (E.G. LIFE-CYCLE PROCESSES, RESPONSES TO ENVIRONMENTAL CUES)



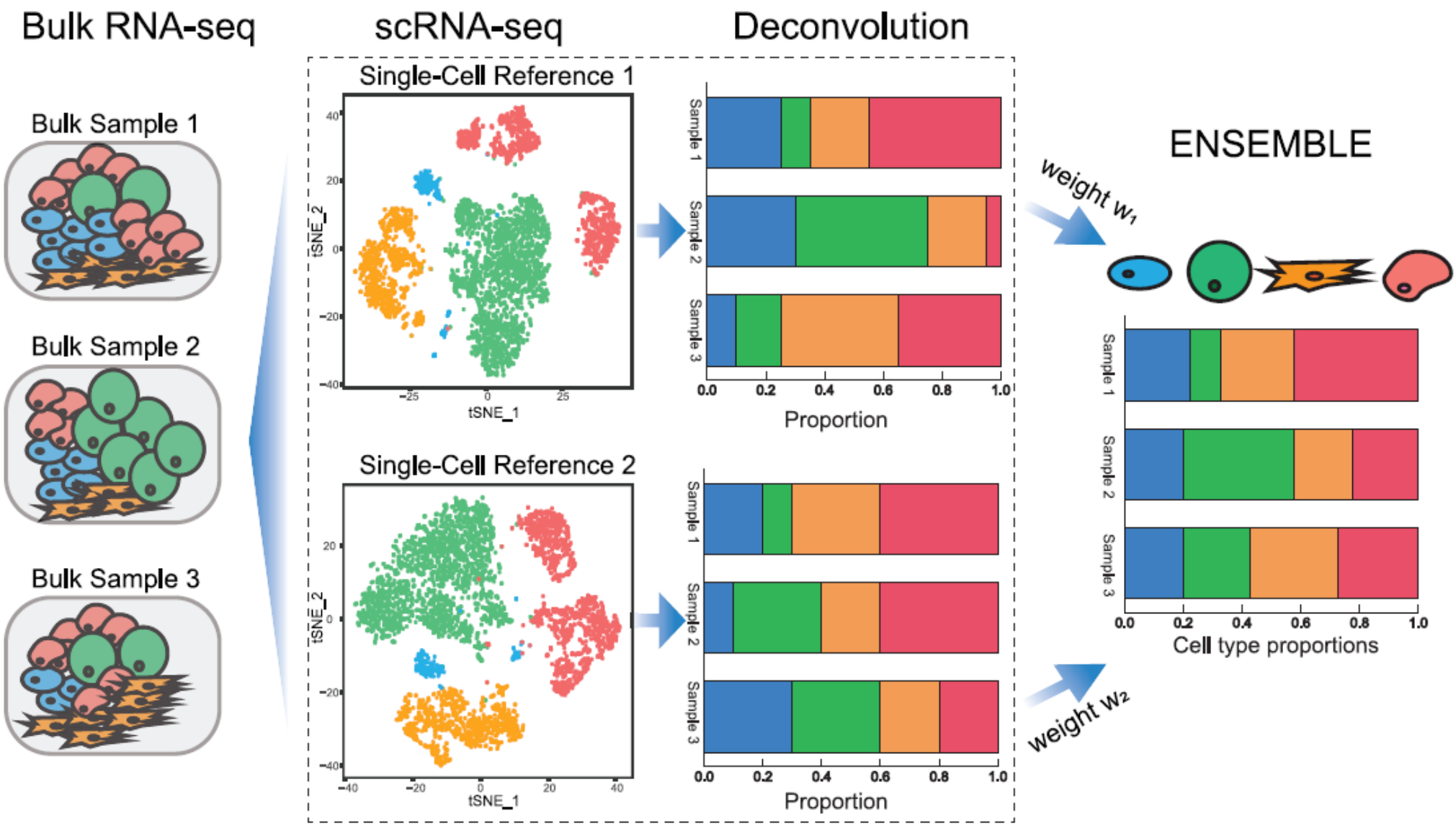
Kotliar et al. eLife 2019;8:e43803

CONSENSUS NON-NEGATIVE MATRIX FACTORIZATION INFERS IDENTITY AND ACTIVITY EXPRESSION PROGRAMS IN SIMULATED DATA



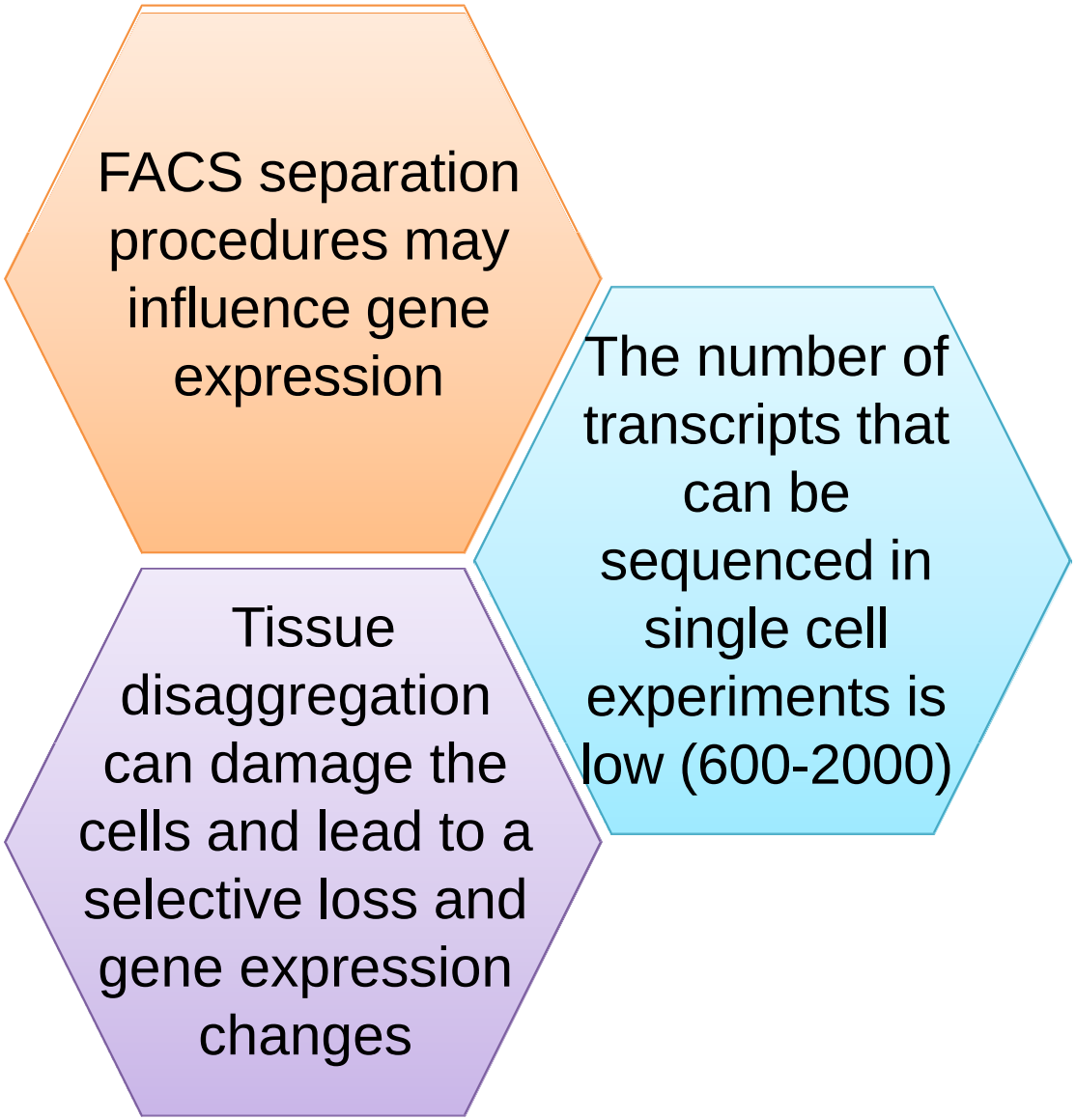
Kotliar et al. eLife 2019;8:e43803

BULK GENE EXPRESSION DECONVOLUTION BY MULTIPLE SINGLE-CELL RNA SEQUENCING REFERENCES



Dong et al., Briefings in Bioinformatics, 00(0), 2020, 1–12

OTHER LIMITS IN THE RELIABILITY OF GENE MATRICES

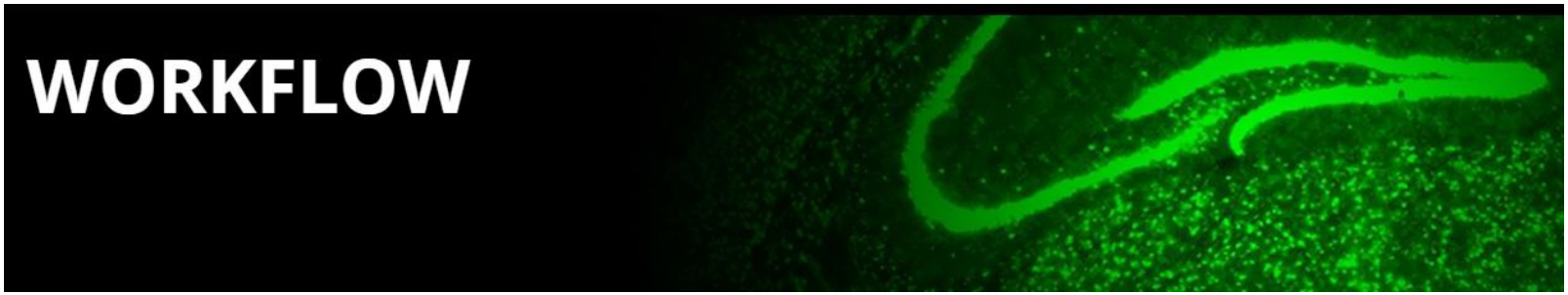


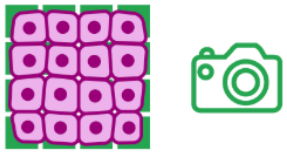
SPATIAL DETECTION OF TRANSCRIPTS IN TISSUE SECTIONS

SPATIAL TRANSCRIPTOMICS

Part of 10x Genomics

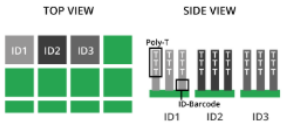
WORKFLOW





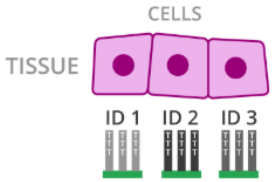
1. Histology

A freshly frozen tissue section is prepared and attached onto our chip. The RNA molecules in each cell contain information about what genes are expressed. The tissue section is imaged in order to retrieve histological information. This allows to see where a cell or a group of cells is located in context of the tis



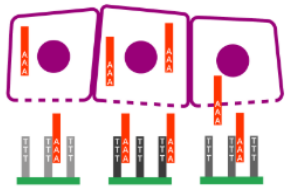
2. The Array

Our chips contain an array of distinguishable capture probes. The Poly-T tails of these capture probes c bind the Poly-A tails of RNA molecules. The arrays are ordered like a chess board where probes with the same ID-Barcode are located in the same square. This allows the determination of where each capture probe, and its bound RNA, originated.



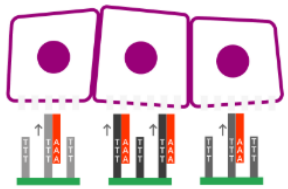
3. Tissue Fixation

The tissue section is fixed. The chip contains a visually detectable frame that is imaged together with th tissue section. This makes it possible to overlay the cell tissue image and the gene expression data in a step.



4. Permeabilisation

The tissue is permeabilised with our Permeabilising Reagent which means that small holes in the cell membrane are created. RNA molecules can exit the cells through these and bind to the adjacent capture probes on the chip. Thus the gene expression information is captured on the chip. The following steps are needed to translate the information stored in the captured RNA molecules as data.



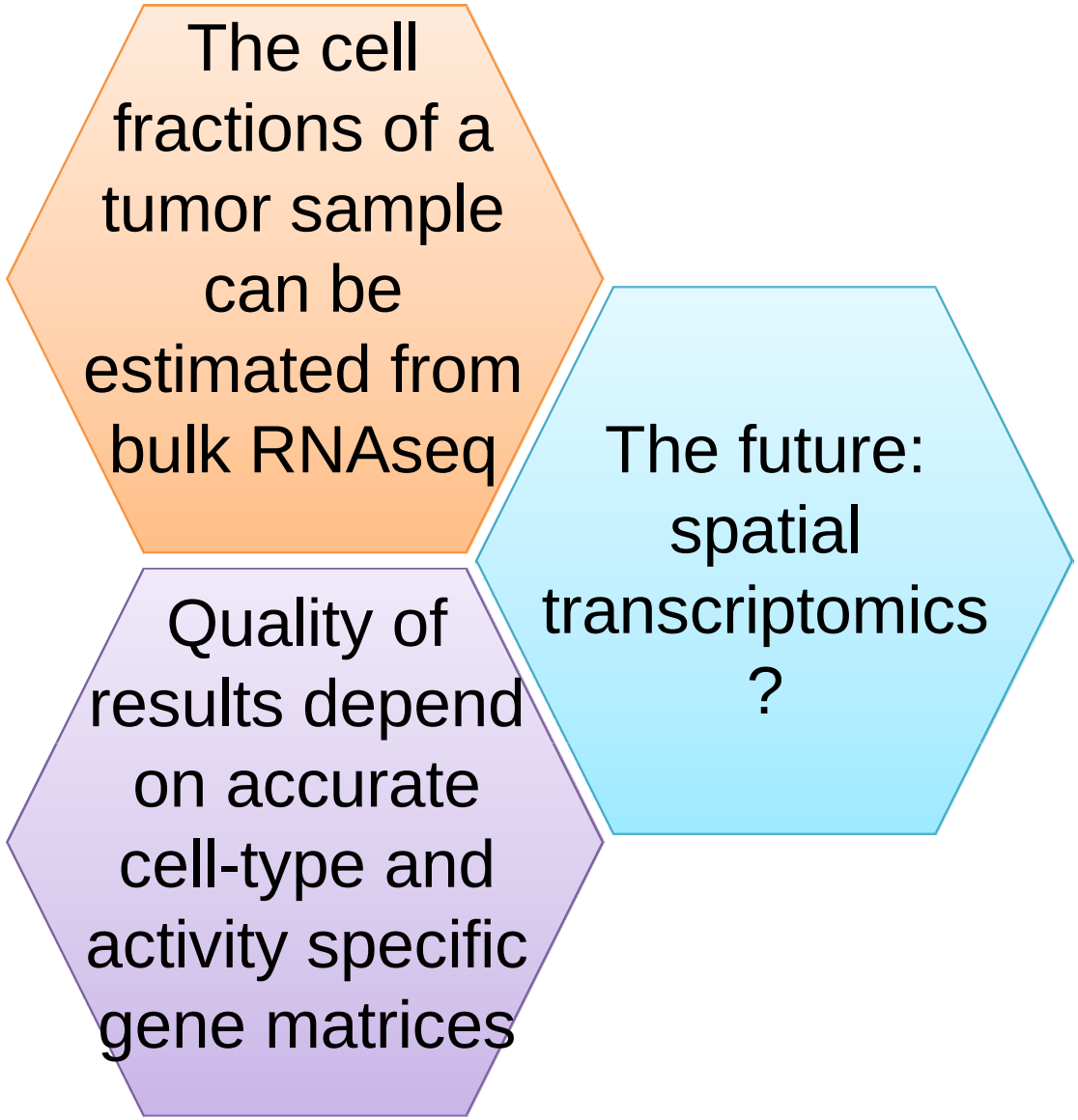
5. cDNA Synthesis

cDNA synthesis is performed to create stable double stranded DNA molecules. This is necessary because cDNA-RNA-hybrids are degraded quickly. Furthermore it is a necessary step before preparation of sequence-able libraries.

6. Library Preparation

The cDNA-RNA-hybrids are cleaved off the chip. Afterwards library preparation is performed with these. This means the molecules are modified in a way to make it possible to read out the information they code for by using a sequencing instrument.

SUMMARY


The cell fractions of a tumor sample can be estimated from bulk RNAseq
The future: spatial transcriptomics ?
Quality of results depend on accurate cell-type and activity specific gene matrices

GRAZIE

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