# Welcome to ABC.13

27. Februar 2025
https://abc.au.dk
abcafe@au.dk
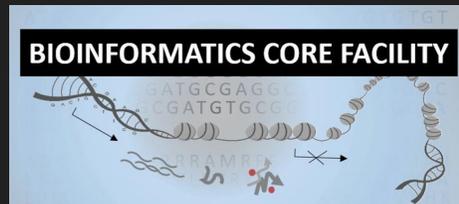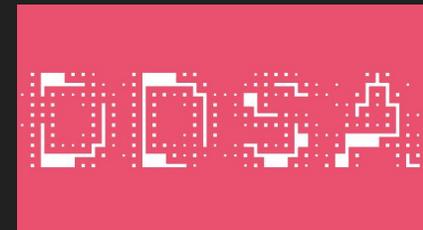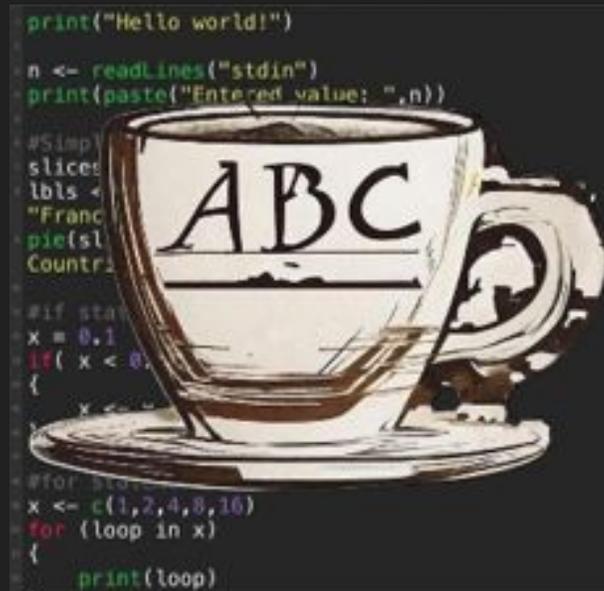
# Agenda

- What's new

- Interesting things from you?

- Topic presentation

- Tutorial and/or open coding

# BulkRNA deconvolution

# BulkRNA deconvolution

Why caring about it?
- Knowledge of tumor microenvironment → Cell types
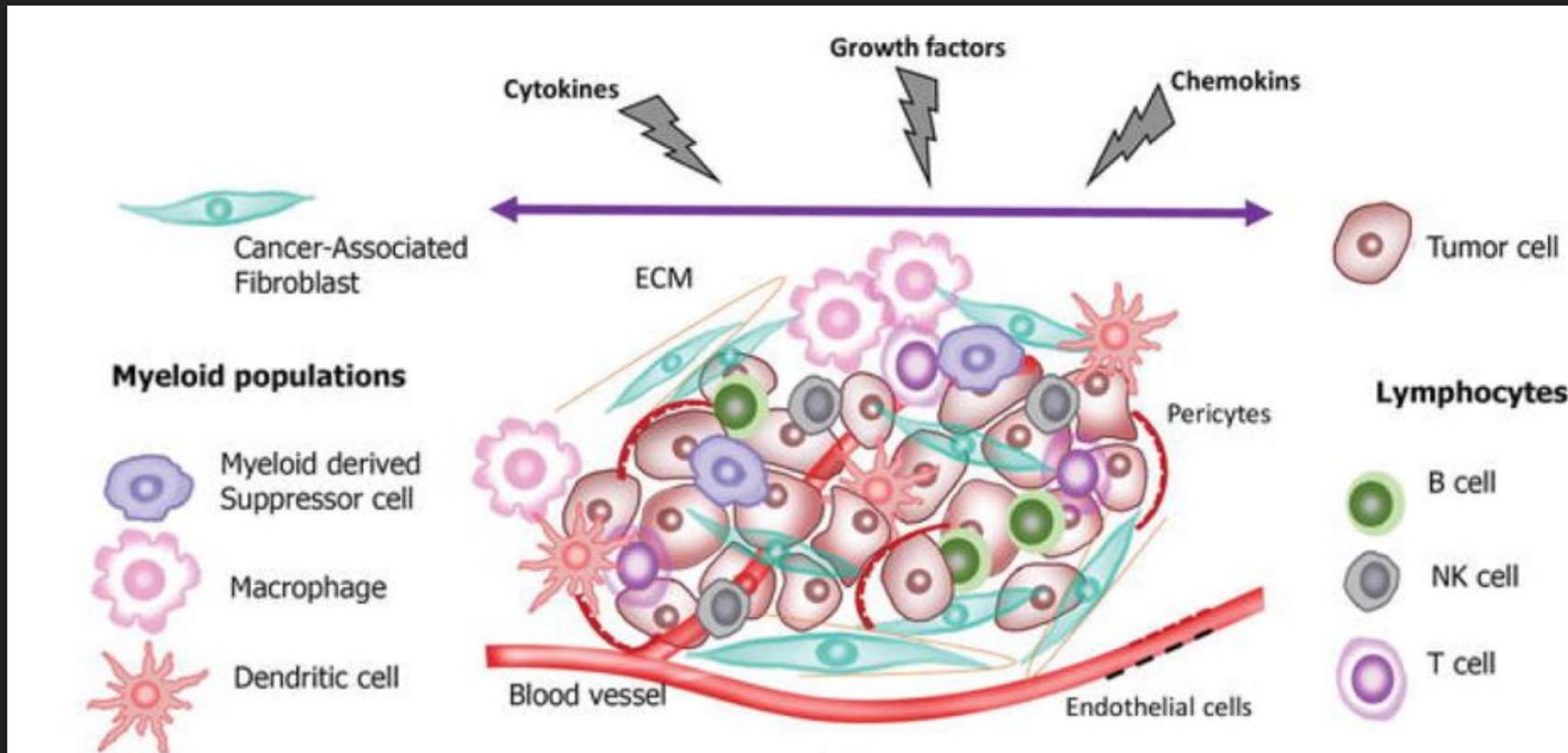
# BulkRNA deconvolution

Bulk RNA-seq = all cells within mixture contribute to final expression levels

Pros
- Can assay entire sample at once
- Can help identify transcription changes in individual cell types
- Huge amount of data out there already
- Cheap(er)

Cons
- Hard to do well

Overabused picture



(Graphic blatantly stolen from the Qiagen website)

$200/sample (Novogene)   $4000-10000/sample

Can we computationally figure out what went into the mixture?

# BulkRNA deconvolution

Before single cell RNA data became common, there have been over 60 methods using

- literature-based marker genes

- microarray data

# BulkRNA deconvolution
# Single-cell methods

- Use scRNA-seq reference with cell types and marker genes

- With optimization find proportions of cell types in bulk data
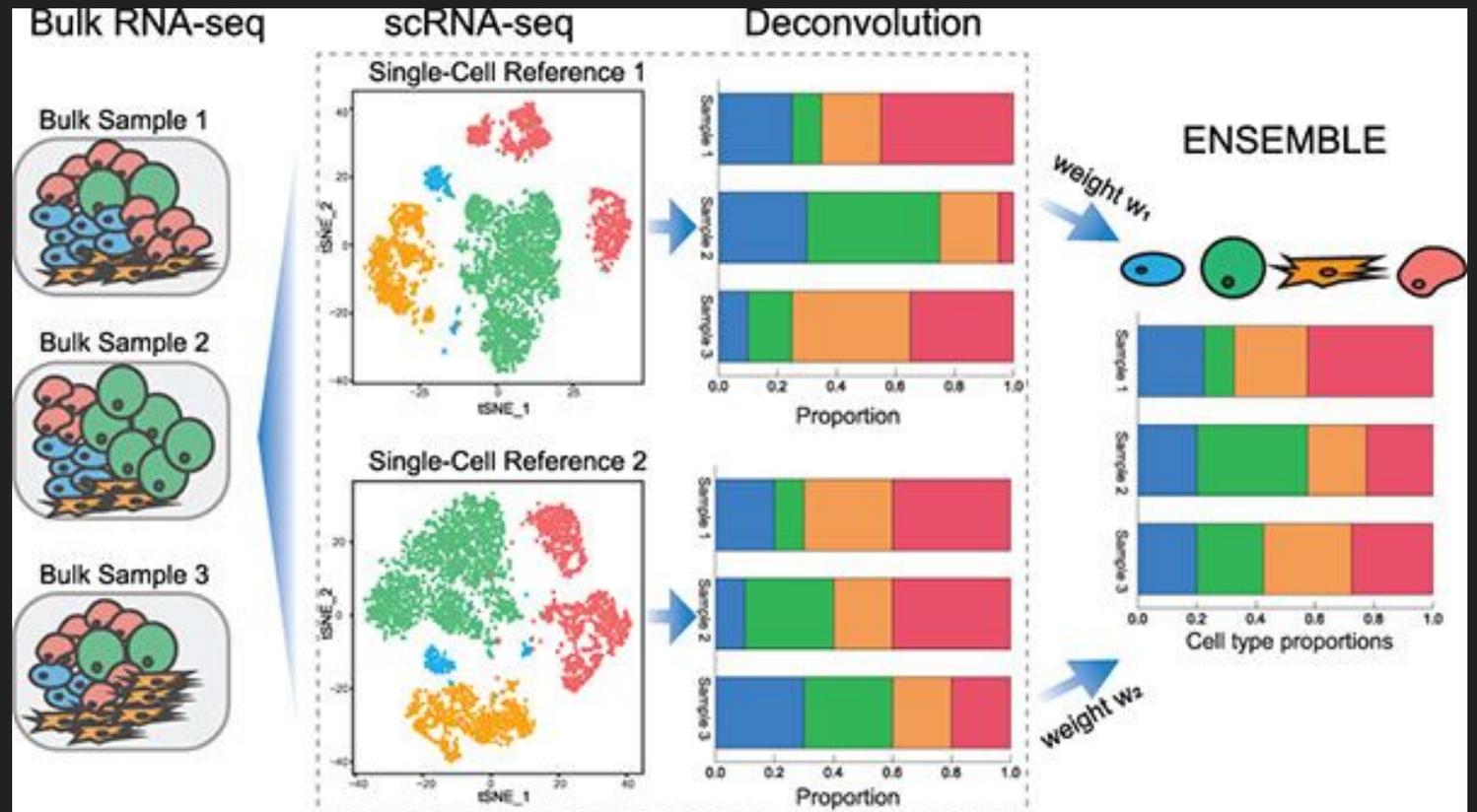
# BulkRNA deconvolution
# Issue - cell size bias

- Cells are not all the same size
- Methods may assume that each cell contributes an equal amount of RNA to total pool
- BUT bigger cells can have more RNA

it is important to NORMALIZE
the data (both bulk and scRNA)
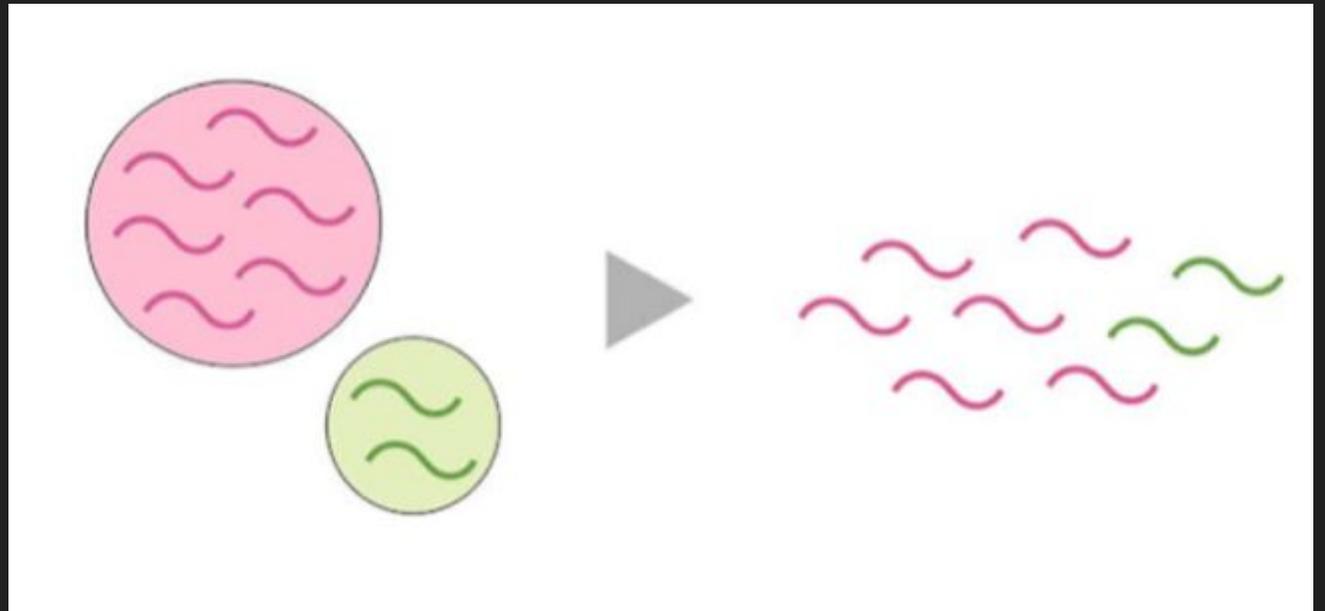
# BulkRNA deconvolution
# Issue - spillover

Closely related cell types have similar cell signatures

Scores that predict enrichment of one cell type may also predict enrichment of another cell type

Other cell type might not even be present



Sturm, G. *et al.* Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. *Bioinformatics* 35, i436–i445 (2019).
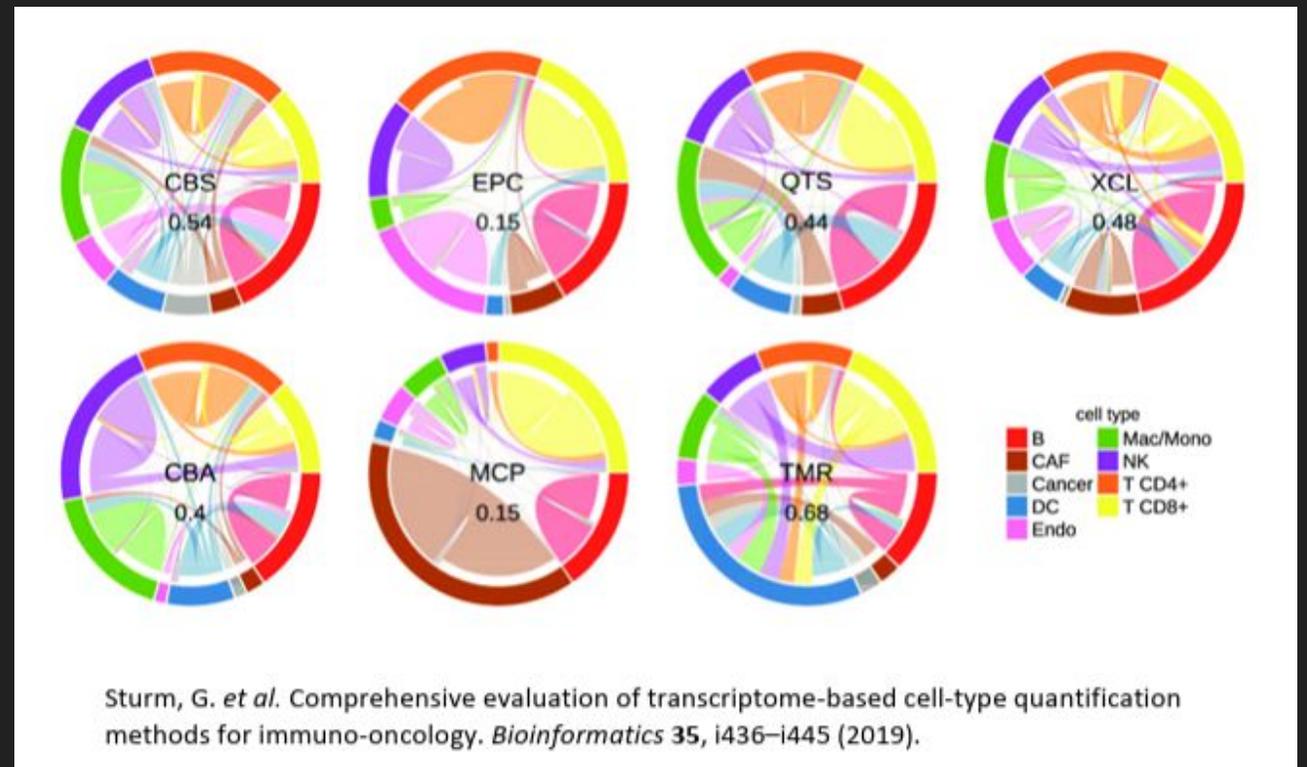
# BulkRNA deconvolution
## Other issues

Microenvironment effect:

Reference sets are often derived from purified non-tumor cells → Do pure cell populations accurately reflect the gene expression patterns of cells in a tumor? → microenvironment affects cell state

You need to always consider which reference you are using

# BulkRNA deconvolution
# Other issues

Many possible preprocessing for bulk and single cell data

- filtering out cells and genes
- samples/studies data integration
- normalization

Sample conservation before scRNA sequencing can also have an effect

There is a large review testing combinations of normalizations, integrations, and preservations (Avila Cobos et al, 2023, Gen.Bio.)

TLDR: scTransform for single cell and logNormalize for bulk data, deconvoluted with DWLS is the best combi. DWLS is best usable with **omnideconv R package**

# BulkRNA deconvolution
# Other issues

From (Avila Cobos et al, 2023, Gen.Bio.)
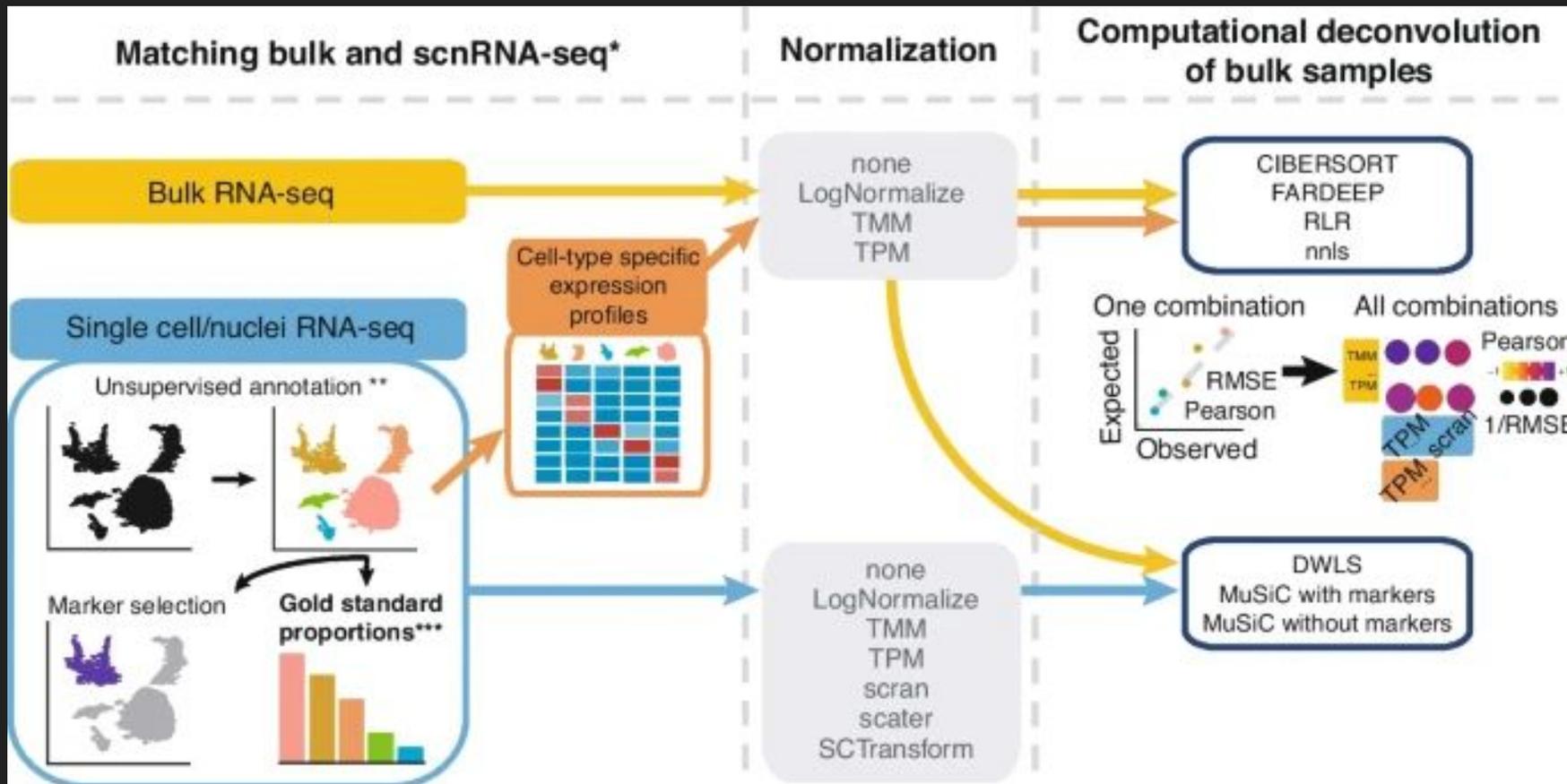
# BulkRNA deconvolution Publications

Some recent papers about technicals of algorithms and improvements

- [Adriana Ivich et al, 2024, Biorxiv](#) Missing cell types in single-cell references impact deconvolution of bulk data but are detectable

- [Shuo Feng et al, 2024, Nat.Com](#). Alleviating batch effects in cell type deconvolution with SCCAF-D

- [Shai Guo, 2024, Gen.Res.](#) A deconvolution framework that uses single-cell sequencing plus a small benchmark data set for accurate analysis of cell type ratios in complex tissue samples

# BulkRNA deconvolution Publications

Some papers with applications

- [Schelker, M. et al.](#) Estimation of immune cell content in tumour tissue using single cell RNA-seq data. Nat.Com. (2017).
- [Wang, X.](#) et al Bulk Tissue Cell Type Deconvolution with Multi-Subject Single-Cell Expression Reference. Nat.Com. (2019)

# BulkRNA deconvolution
# Slide credits

- Harward Chan Bioinformatics Core (slides)

- SCDC – bulk gene expression deconvolution by multiple single-cell RNA sequencing references (paper)

- Unraveling the complexity: understanding the deconvolutions of RNA-seq data (paper)

# Tutorial and open coding

At our home https://abc.au.dk/Documentation you can find

- previous tutorials
- Previous tutorials + 1 conference workshop

Or you can code and ask for help or generic coding/bioinf/data science questions



And have cake