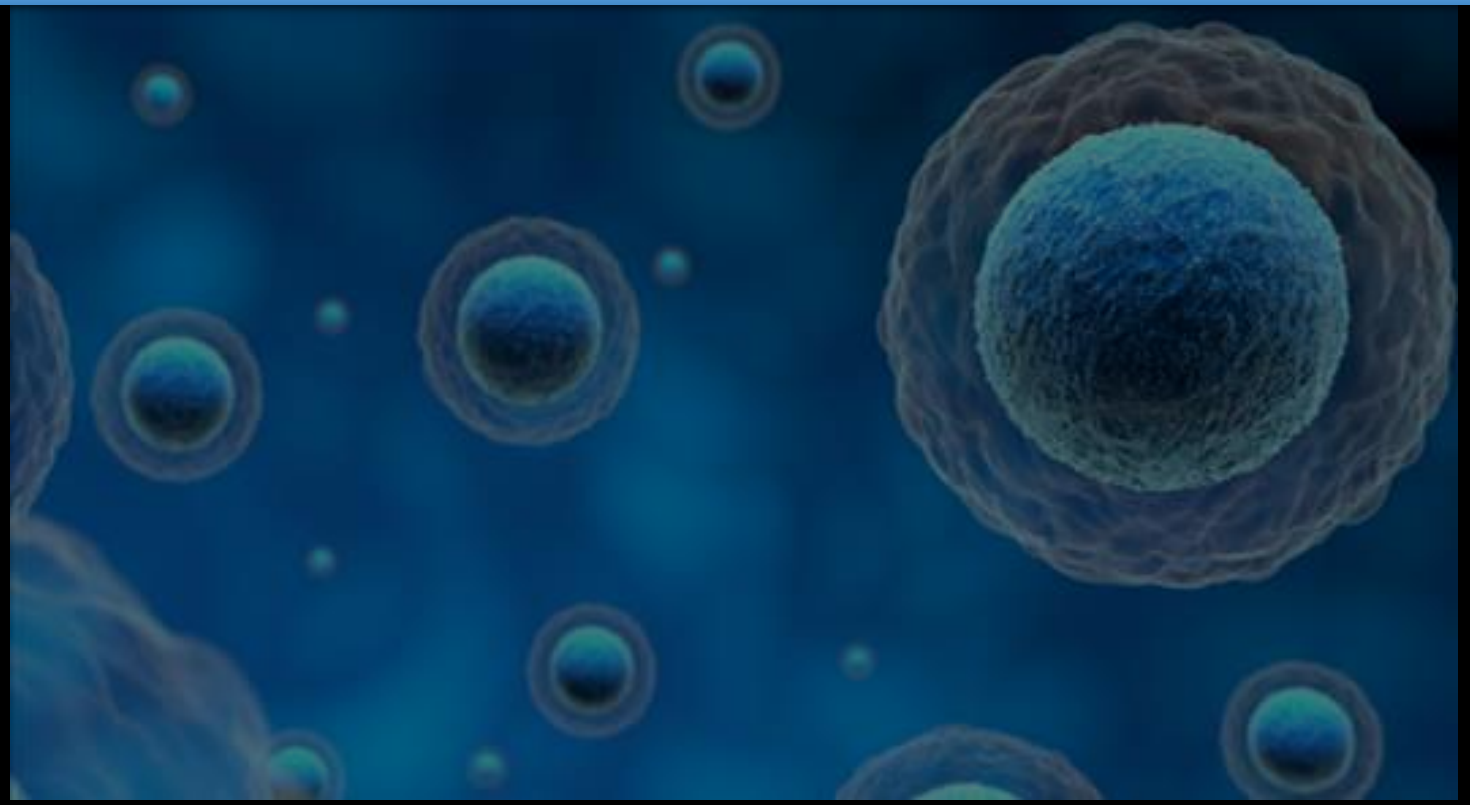


EGY-SEJT FUNKCIONÁLIS GENOMIKA

2018. 11. 29.

AZ ELŐADÁS FELÉPÍTÉSE

I. Egy–sejt funkcionális genomika áttekintése és jelentősége

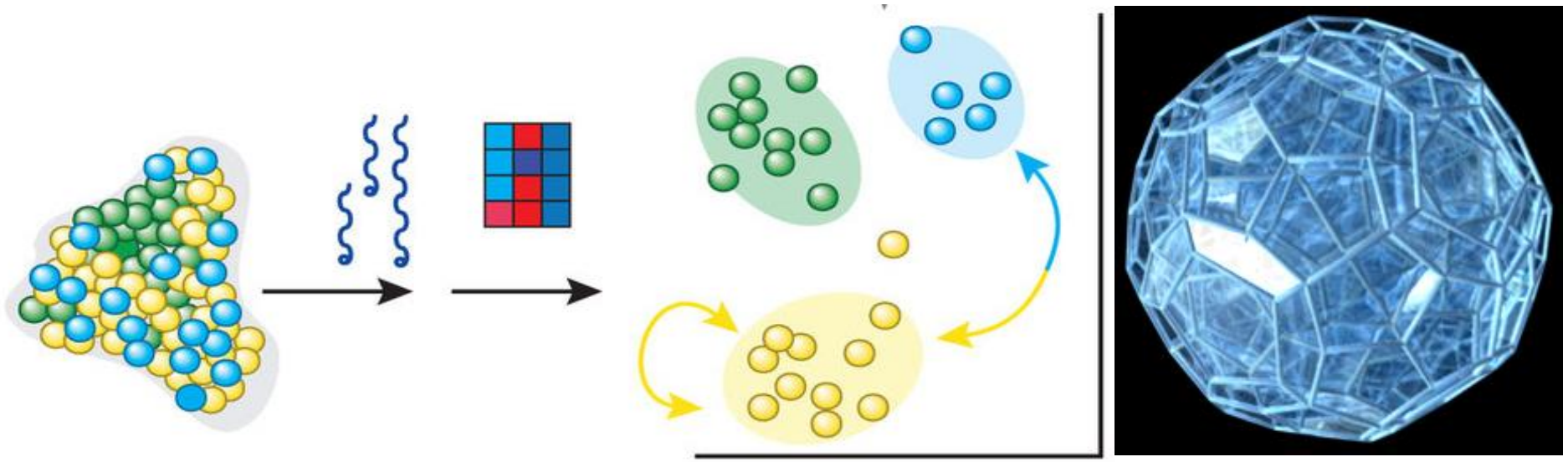
II. Egy-sejt funkcionális genomika alkalmazása

1. Humán Sejt Atlasz (2016 – 2024)
2. Heterogén sejtpopulációk
3. Transzkripciós dinamika
4. Hálózat interferencia

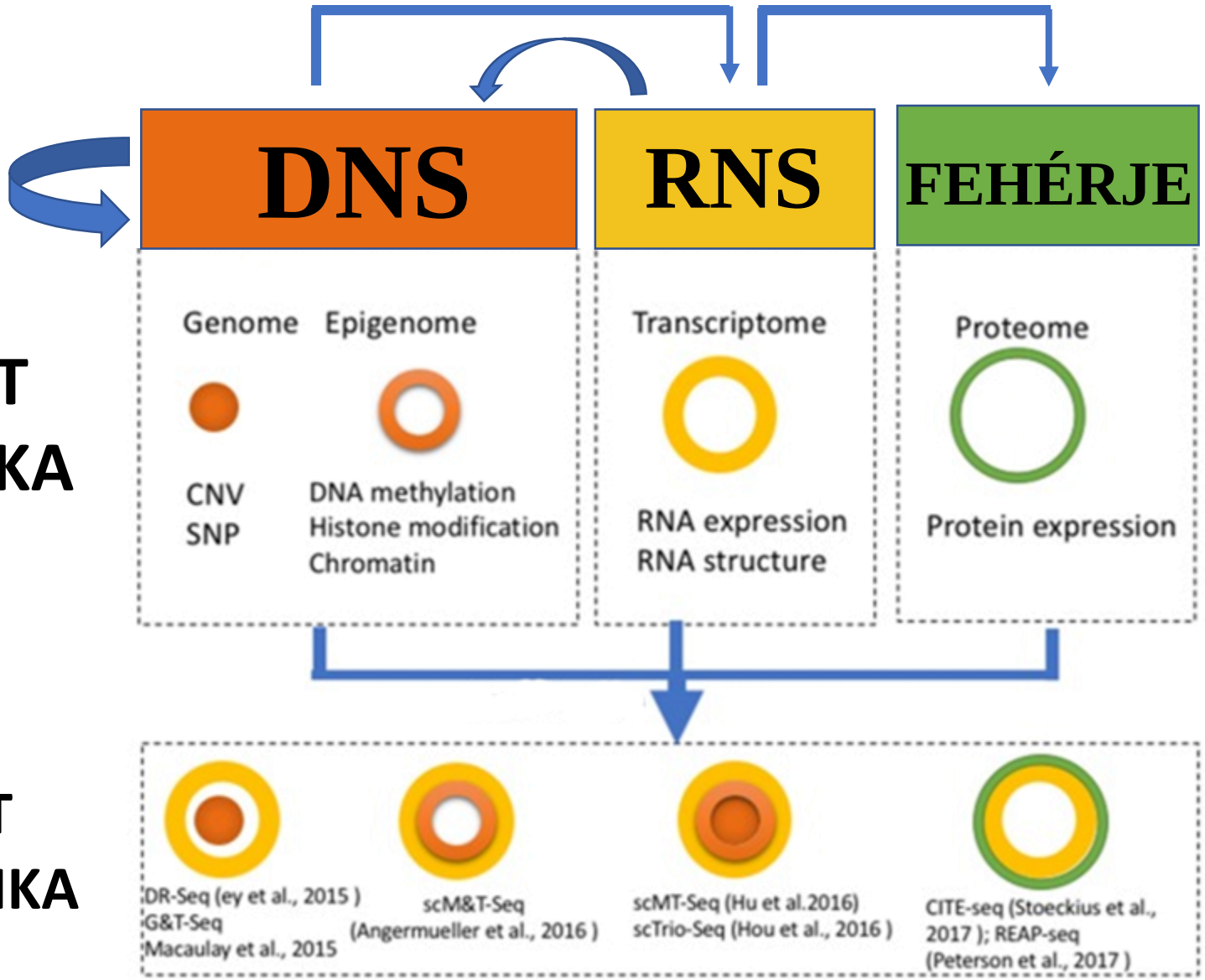
} részletgazdag elemzése

ELŐADÁS CÉLKITŰZÉSE

AZ EGY-SEJT TRANSZKRIPTOMIKAI MÓDSZERTAN ELVÉNEK ISMERTETÉSE, KUTATÁSI ÉS KLINIKAI VETÜLETE

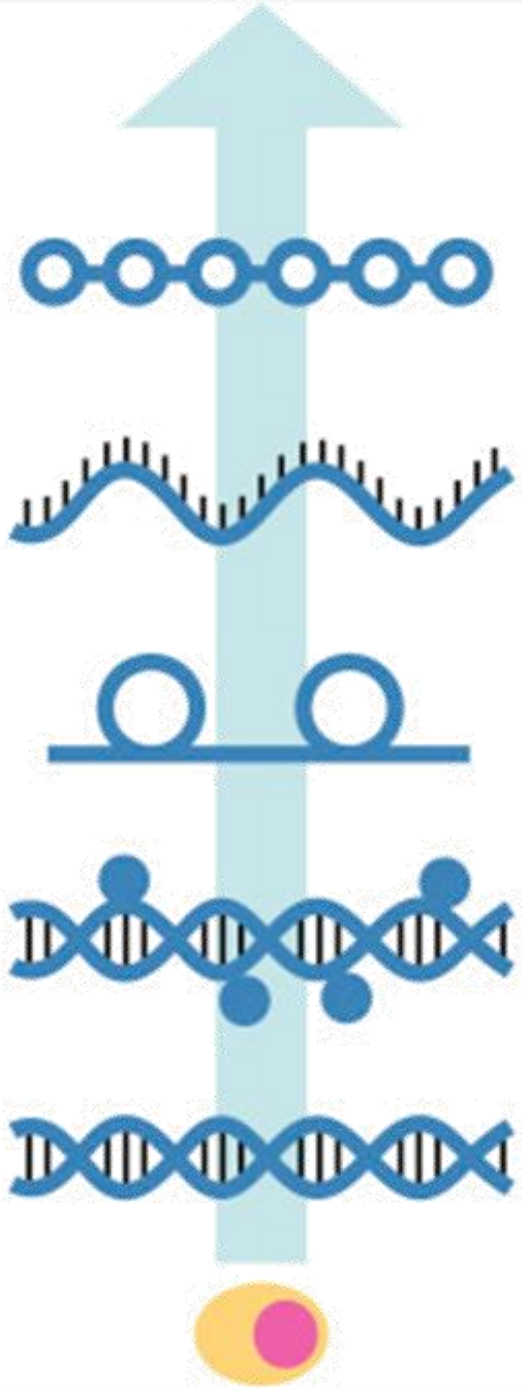


EGY-SEJT GENOMIKA



EGY-SEJT MULTIOMIKA

Hu et al. **Single Cell Multi-Omics Technology: Methodology and Application,** Front Cell Dev Biol. 2018



PROTEOM

TRANSZKRIPTOM

KROMATIN
ARCHITEKTÚRA

METILOM

GENOM

EGY-SEJT

2017: CITE-Seq

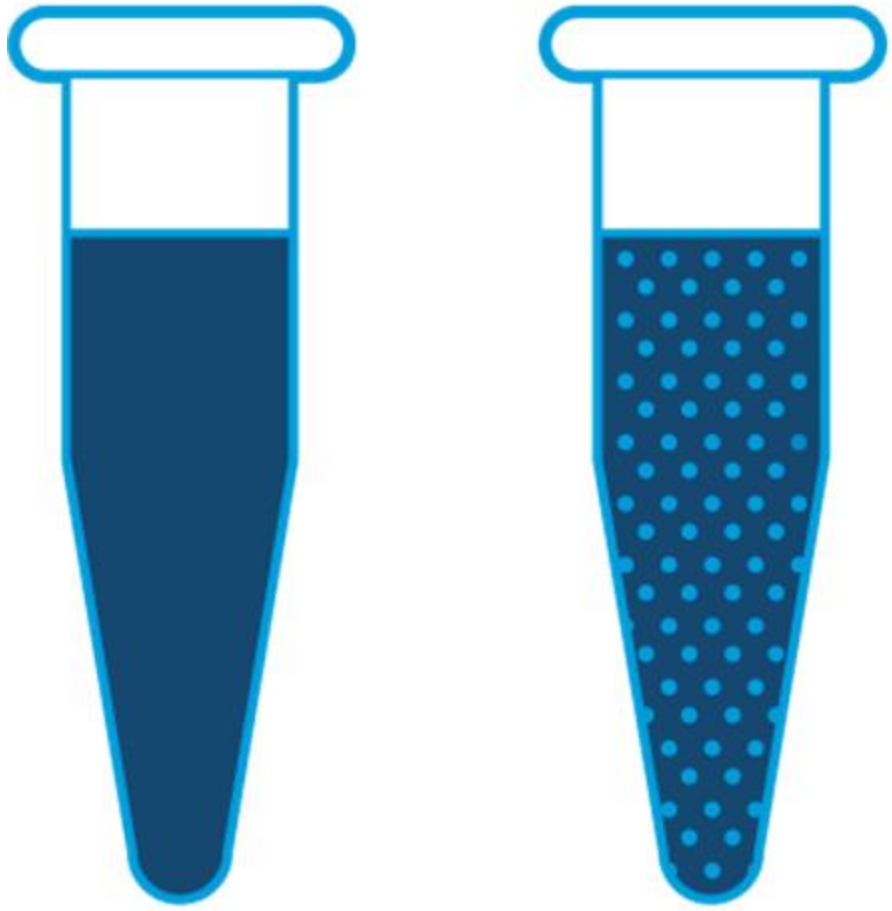
2016: M és G

2015: G és T

SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

HOMOGENIZÁLT MINTA

EGY-SEJT SZUSZPENZIÓ



ÁTLAGOS TRANSZKRIPTOM

SEJTENKÉNTI TRANSZKRIPTOM

EGYEDI SEJTTÍPUS

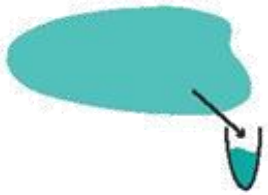
TRANSZKRIPTOM

RITKA SEJT TRANSZKRIPTOM

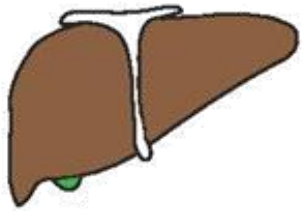
TRANSZKRIPTOM

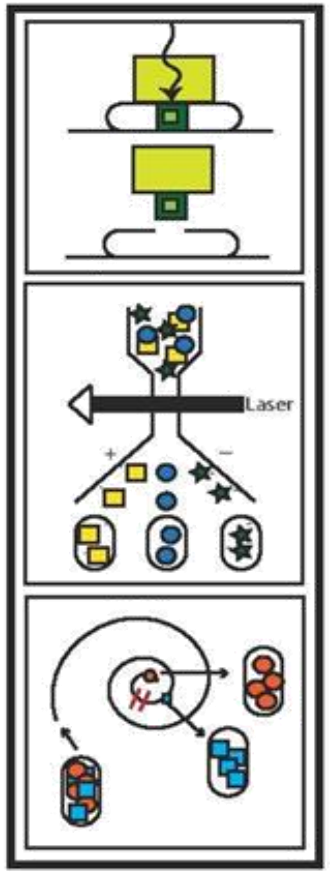
TRANSZKRIPTOM

TRANSZKRIPTOM



BIOLÓGIAI MINTA





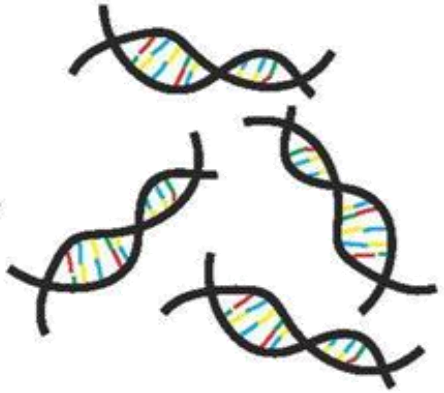
LÉZER MIKRODISSZEKCIÓ

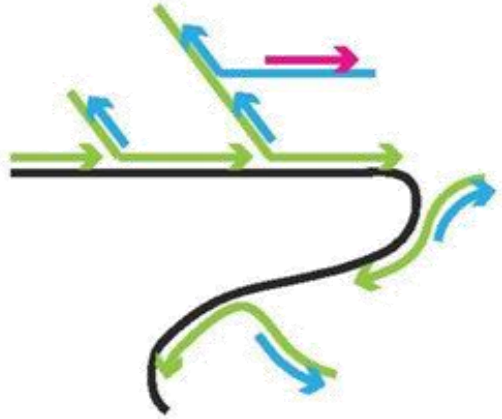
FACS

MIKROFLUIDIKA

EGY-SEJT

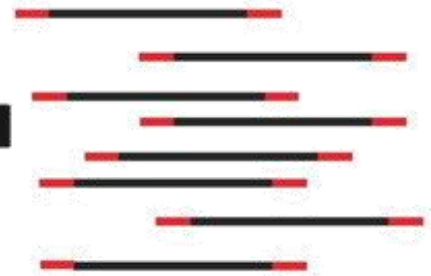
NUKLEINSAV/FEHÉRJE IZOLÁLÁS





REVERZ TRANSZKRIPCIÓ

KÖNVYTÁRKÉSZÍTÉS

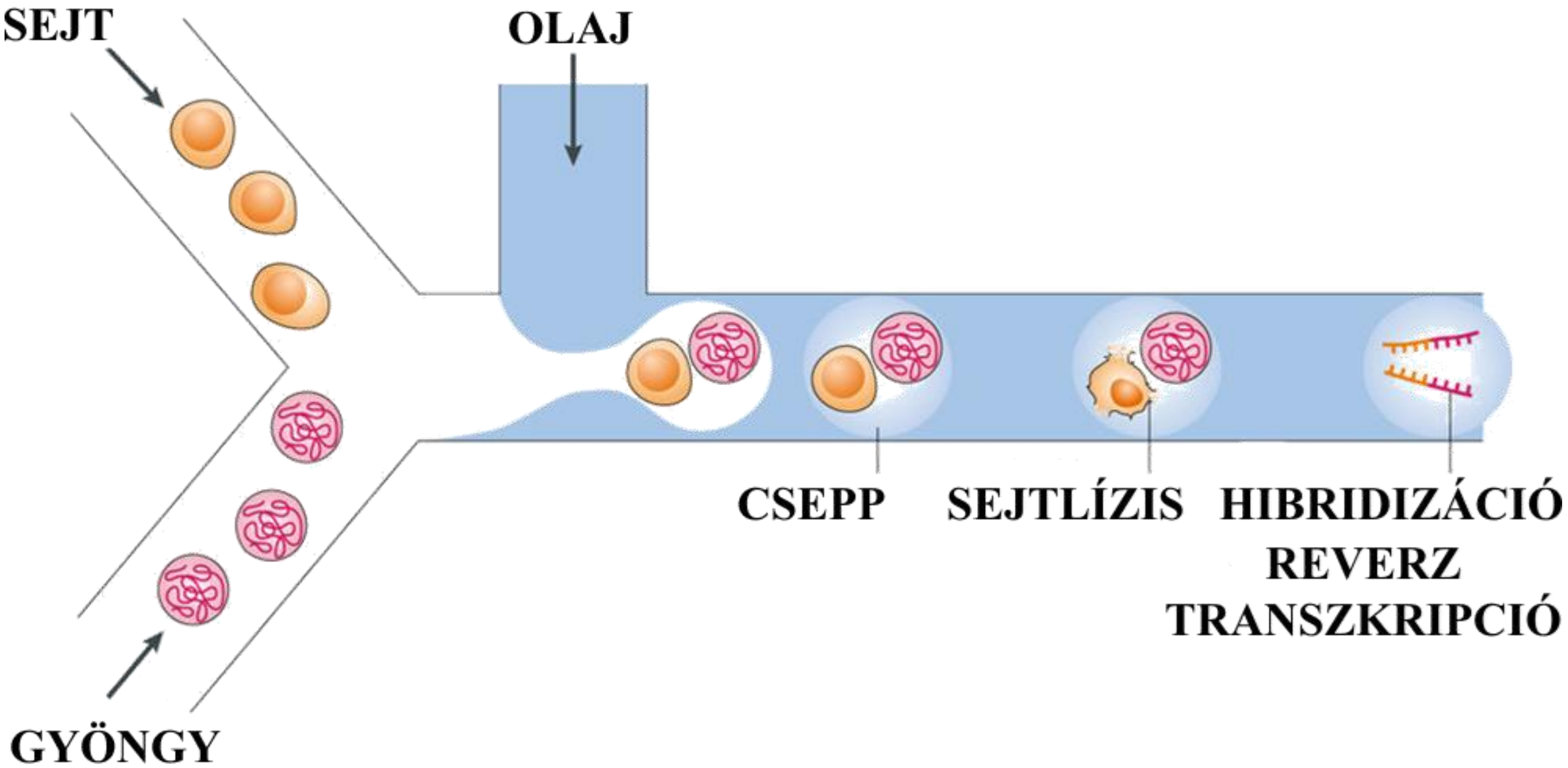


CCTCATGGATAGATTAAT
CCAGGAGTATACAGAAC
GTTGCAAGAGAGTATCA
AGATACAATACAAGGGT
TAAGCCATACCTGAGGG

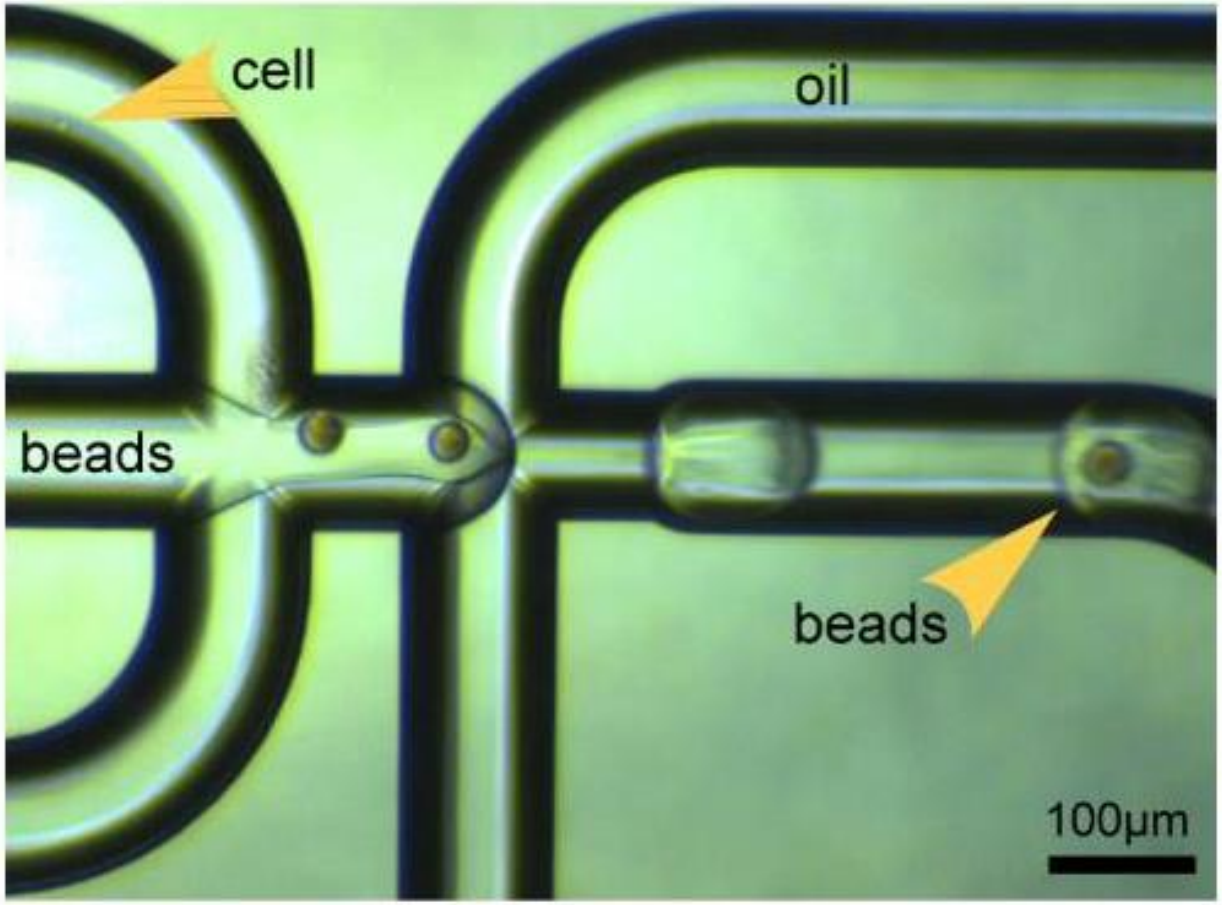
SZEKVENÁLÁS

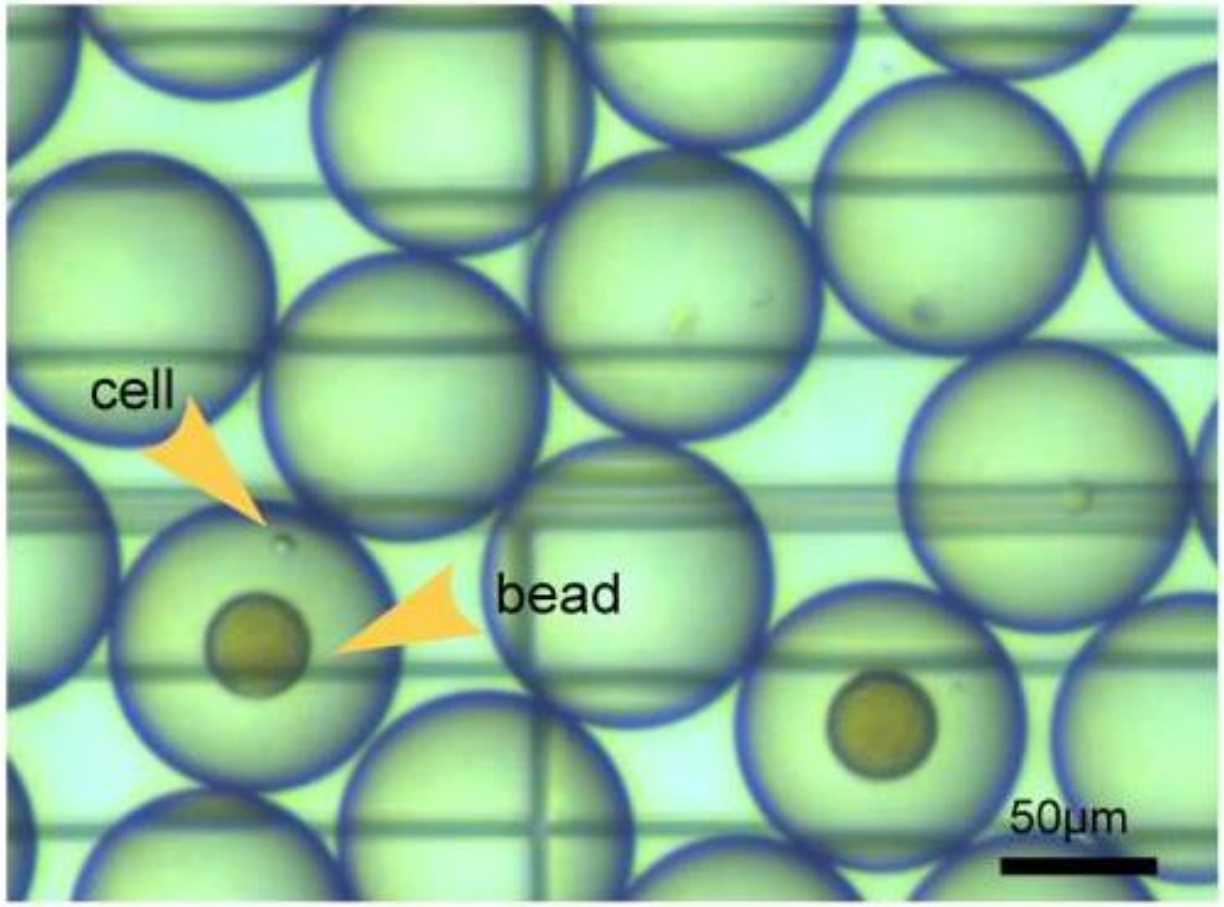
SNP/CNV/
GÉNEXPRESSZIÓ
METILÁCIÓS MINTÁZAT

SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA



SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA





SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

SEJTEK

GYÖNGY

PRIMEREK

SEJTAZONOSÍTÓ

OLAJCSEPP

SEJT

GYÖNGY

REVERZ TRANSZKRIPCIÓ

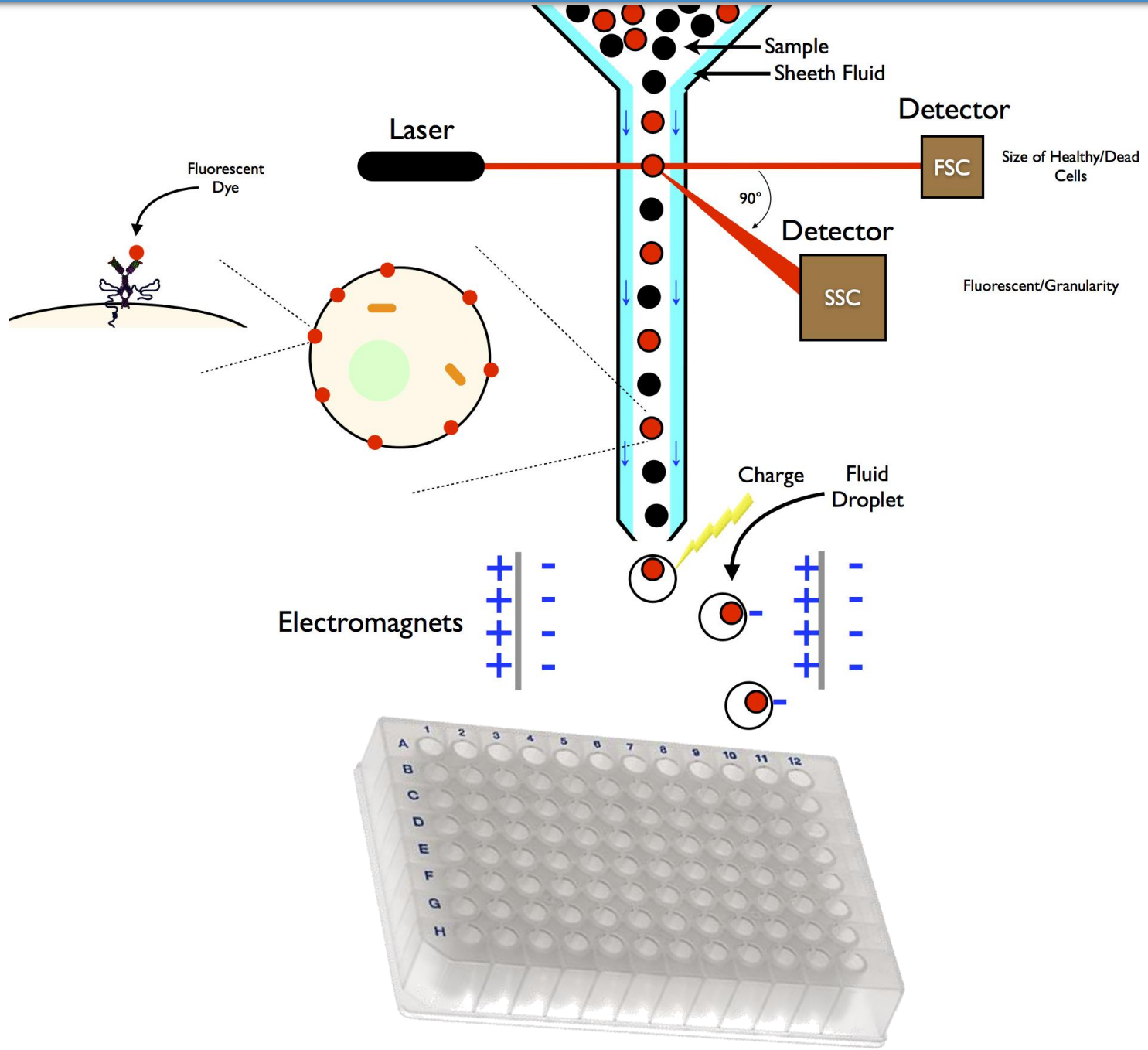
NUKLEINSAV OLAJCSEPPBŐL VALÓ KISZABADÍTÁSA

KÖNYVTÁRKÉSZÍTÉS

SZEKVENÁLÁS ÉS ANALÍZIS

SEJT-
ALPOPULÁCIÓK

EXPRESSZIÓS
KOVARÁCIÓ


Sample
Sheeth Fluid
Detector
Laser
FSC
Size of Healthy/Dead Cells
Fluorescent Dye
90°
Detector
SSC
Fluorescent/Granularity
Charge
Fluid Droplet
Electromagnets
A
B
C
D
E
F
G
H
1
2
3
4
5
6
7
8
9
10
11
12

Heterogén SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

Unravel heterogeneity to decipher dynamic cell state transition

Combinatorial indexing may also facilitate the scalable generation of "joint" single-cell molecular profiles (e.g., RNA-seq and ATAC-seq from each of many single cells).

Multi-omics on single-cells

Single-cell EPIGENOMICS: scATAC-seq, DHS-seq, sc-methylation-seq

Single-cell
active Transcription Factors

Target genes

GRN
Gene Regulatory Networks

Single-cell
Transcribed genes scRNAseq

Egy-sejt transzkriptom

Genome architecture mapping
Hi-C (TAD)

Single-cell

Dual-sequencing
Sc-transcriptogenomics
Sc-G&Tseq
Sc-M&Tseq

Sorting

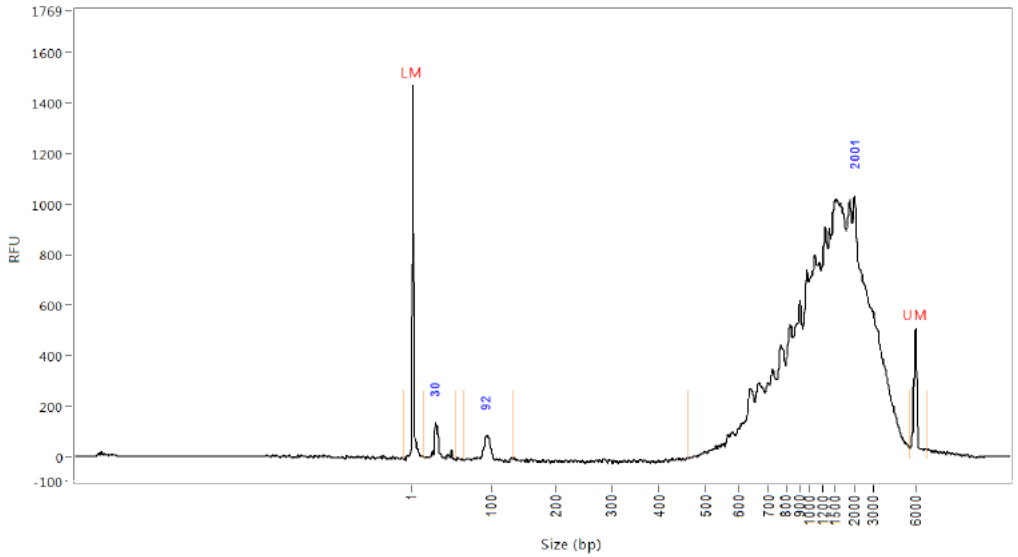
FACS or microfluidics

Proteomics
Mass cytometry, CyTOF

BIOLOGICAL SAMPLE

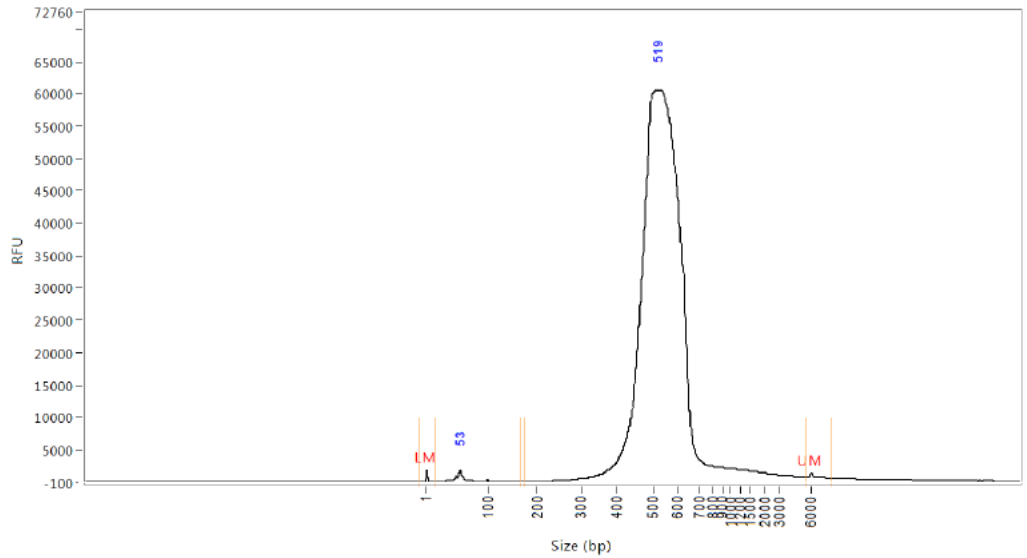
PBMC SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

Preamplification



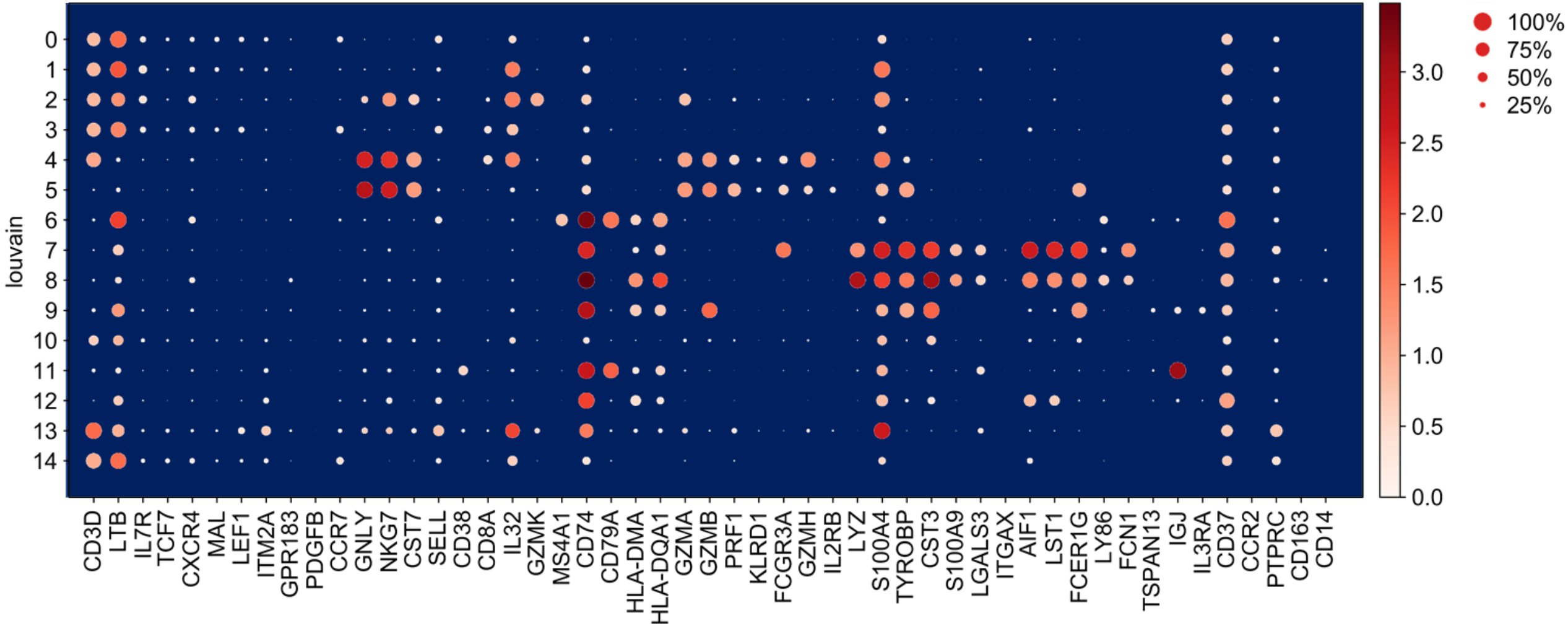
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU
1	1 (LM)	0.0132	0	16	1	156.15	1469
2	30	0.0259	16	56	31	14.28	135
3	92	0.0241	64	133	92	3.01	80
4	2001	2.4261	463	5622	1599	57.72	1033
5	6000 (UM)	0.0042	5622	6886	6033	3.65	506
	TIC:	2.4760	ng/uL				
	TIM:	4.2912	nmole/L				
	Total Conc.:	2.4760	ng/uL				

Postamplification



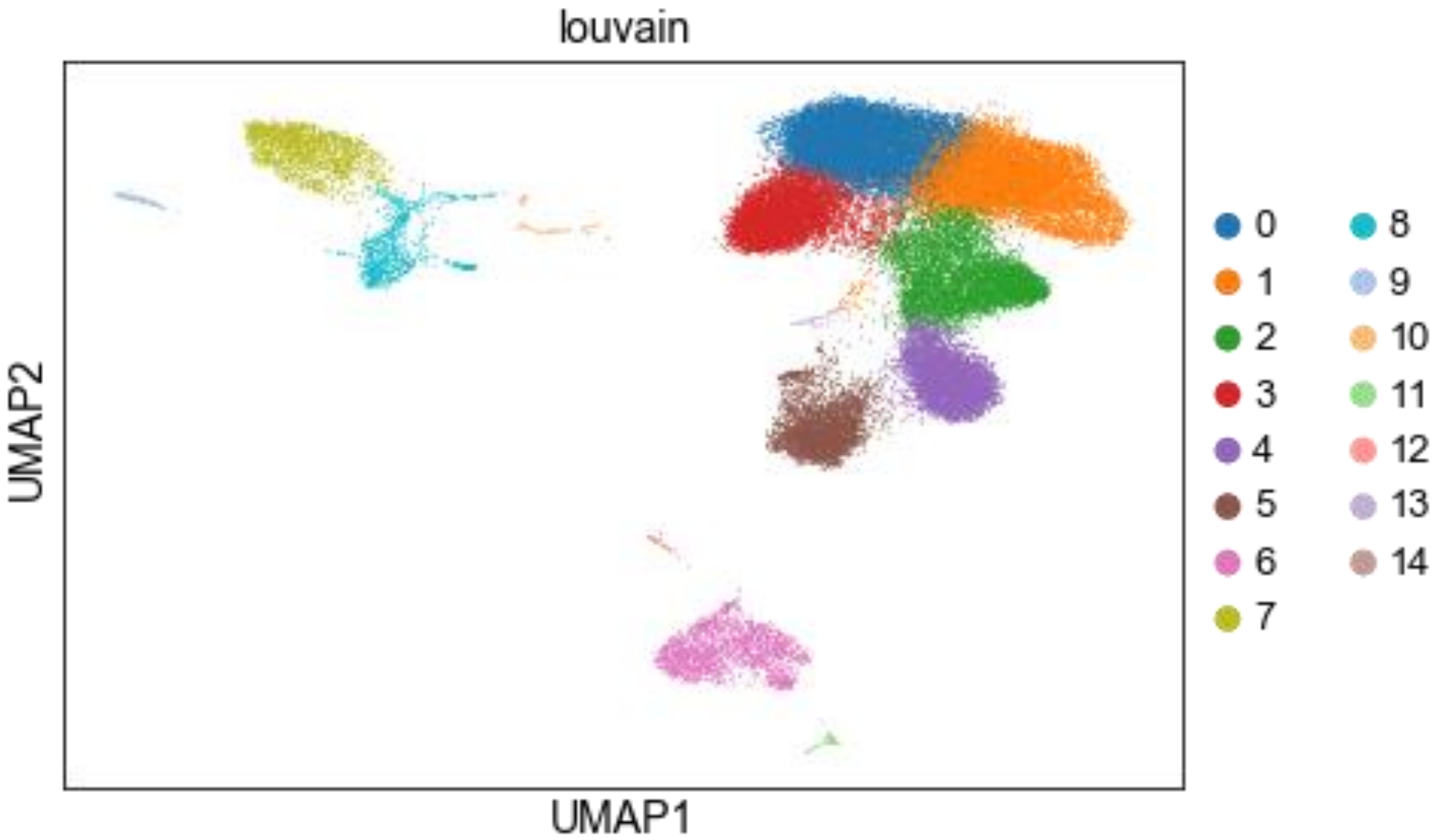
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU
1	1 (LM)	0.0138	0	15	1	155.91	1738
2	53	0.6924	15	164	52	17.96	1755
3	519	105.0950	175	5449	584	59.23	60615
4	6000 (UM)	0.0491	5449	8068	6632	11.27	1400
	TIC:	105.7873	ng/uL				
	TIM:	317.9549	nmole/L				
	Total Conc.:	105.7873	ng/uL				

PBMC SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

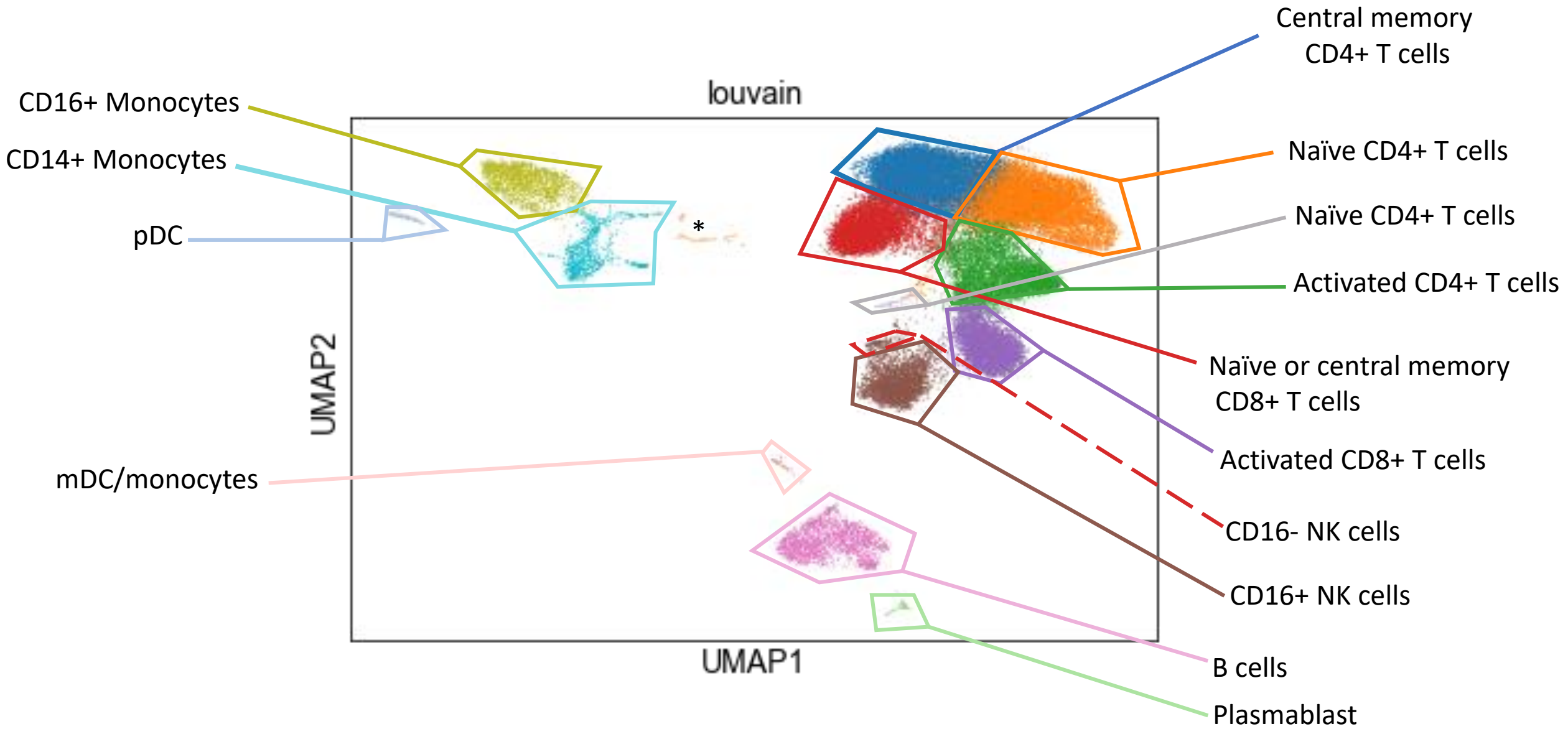


SIZE OF THE BULLETS IS PROPORTIONAL TO THE NUMBER OF CELLS EXPRESSING THE GENE, THE COLOR IS PROPORTIONAL WITH THE LEVEL OF GENE EXPRESSION

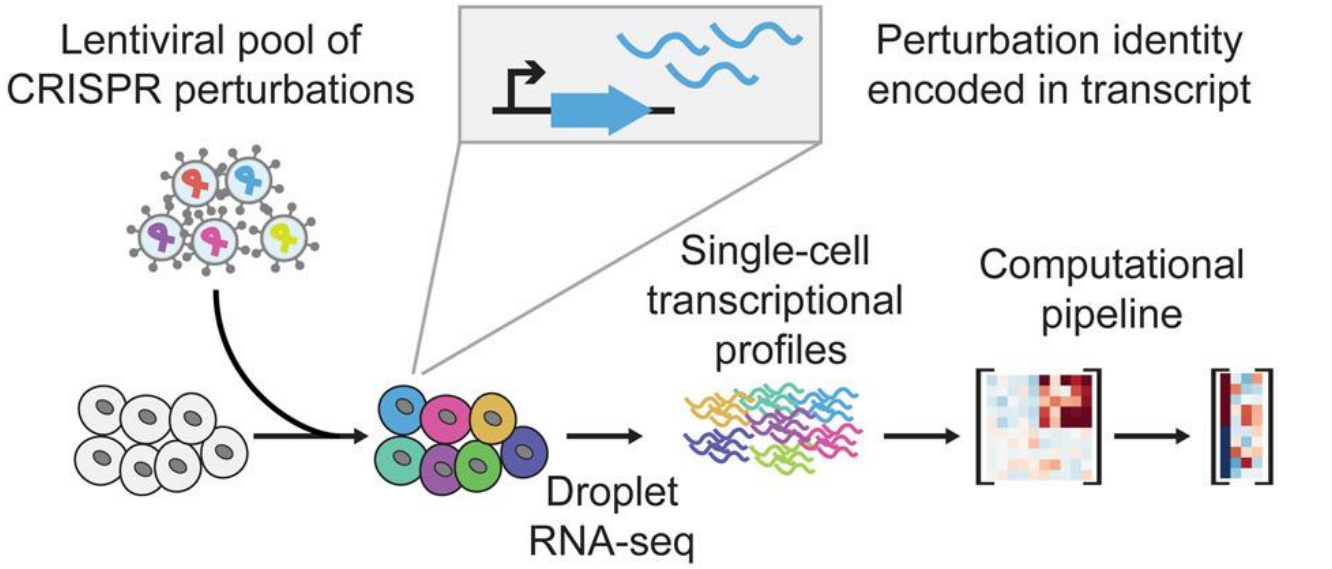
SAMPLE	PLATFORM	SOFTWARE	PROTOCOL	UMAP	tSNE	CELL NUMBER
68K PBMC	10X	SCANPY 1.2.2	SEURAT	YES	YES	68 000

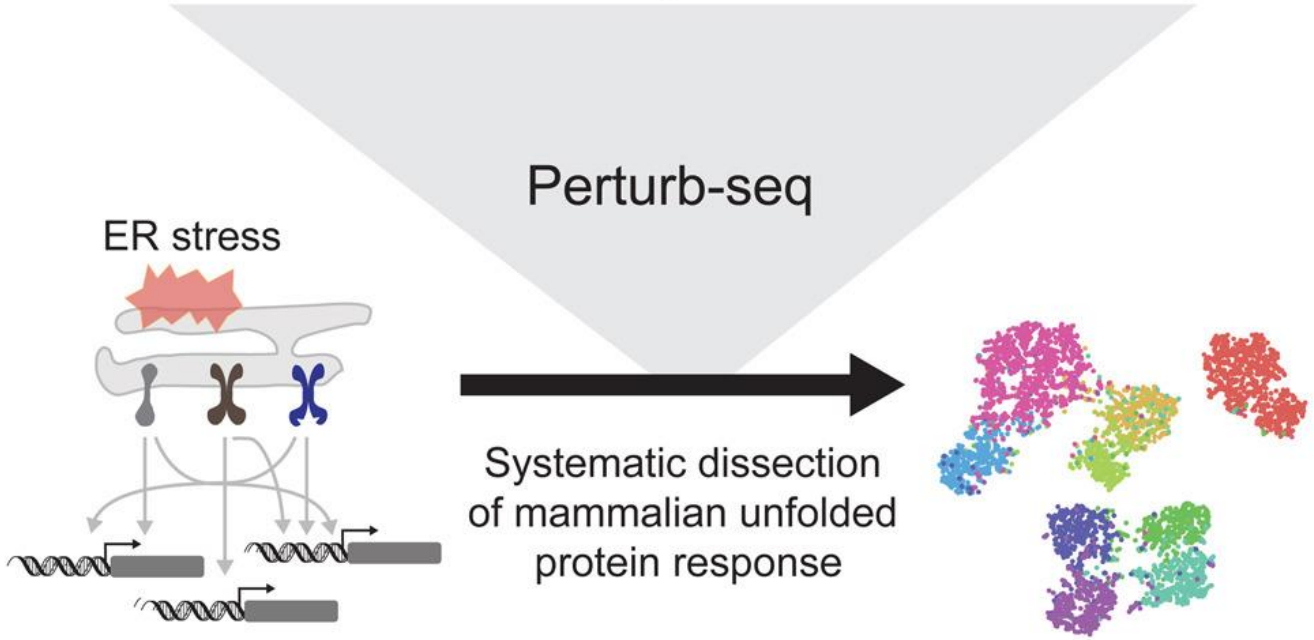


SAMPLE	PLATFORM	SOFTWARE	PROTOCOL	UMAP	tSNE	CELL NUMBER
68K PBMC	10X	SCANPY 1.2.2	SEURAT	YES	YES	68 000

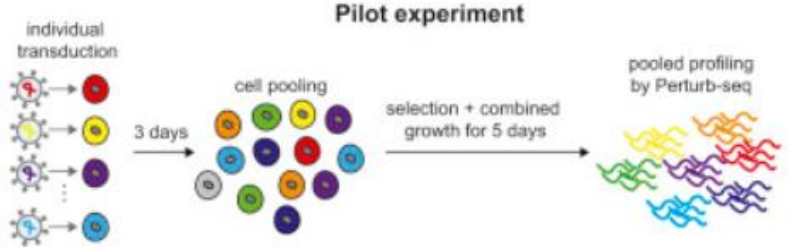


* Putative dublets


Lentiviral pool of
CRISPR perturbations
Perturbation identity
encoded in transcript
Single-cell
transcriptional
profiles
Computational
pipeline
Droplet
RNA-seq


Perturb-seq
ER stress
Systematic dissection
of mammalian unfolded
protein response

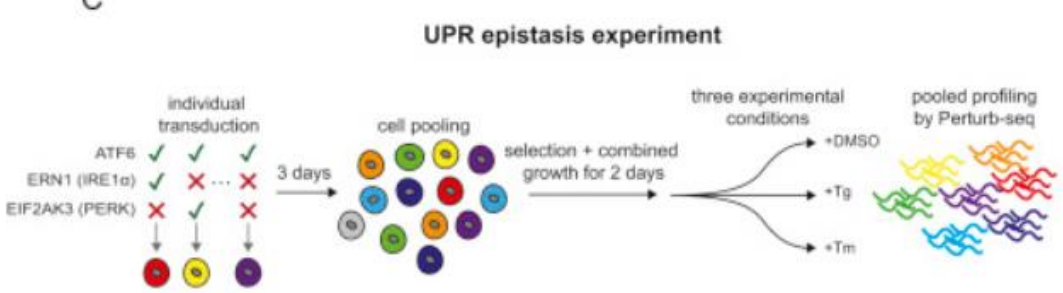
A



B

Perturbation complexity	8 sgRNAs
Cell count	5,768
Unique sgRNA	92%
Multiplet	6%
Unidentifiable	2%
Mean read depth	22,911 per cell
Median UMI depth	9,969 per cell
Median unique genes	2,580

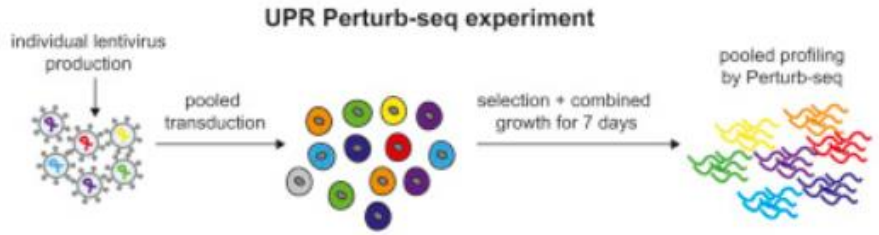
C



D

Perturbation complexity	9 three-guide vectors, 3 conditions
Cell count	15,006
Unique sgRNA	90%
Multiplet	5%
Unidentifiable	5%
Mean read depth	122,670 per cell
Median UMI depth	25,082 per cell
Median unique genes	4,304

E



F

Perturbation complexity	91 sgRNAs, 2 control sgRNAs
Cell count	65,337
Unique sgRNA	77%
Multiplet	13%
Unidentifiable	9%
Mean read depth	66,950 per cell
Median UMI depth	15,355 per cell
Median unique genes	3,690

Adamson et al. 2016 -10.1016/j.cell.2016.11.048

DROPLET-BASED METHODS

e.g. Drop-seq
10X Chromium

+ Extremely high cell throughput (>10^4 cells per experiment)
+ Low cost per cell (< $0.01)
− Smaller cell libraries (~10^4 molecules per cell)

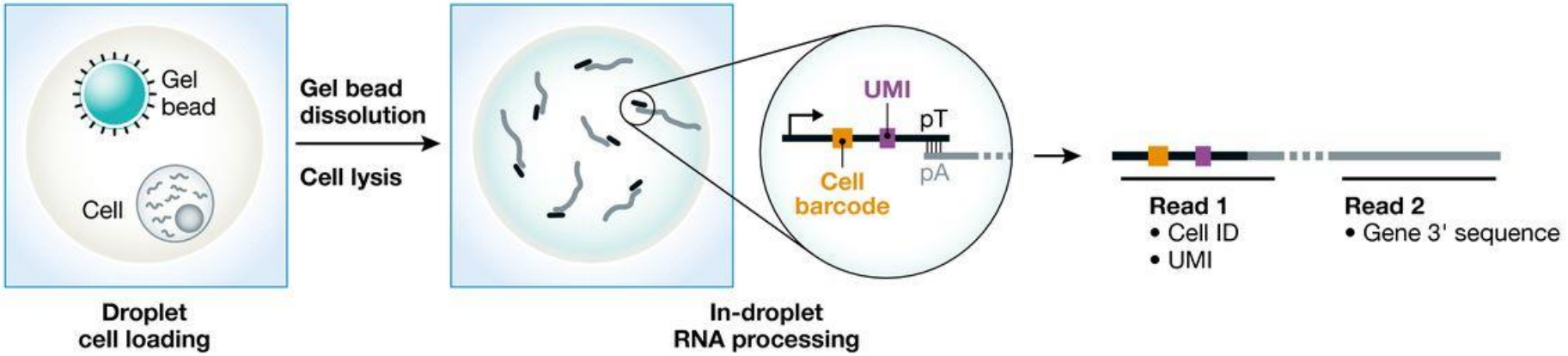
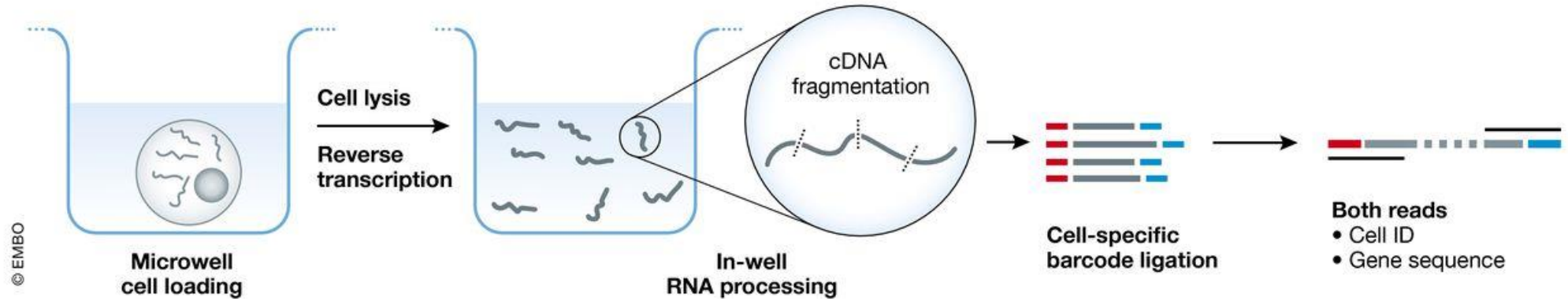


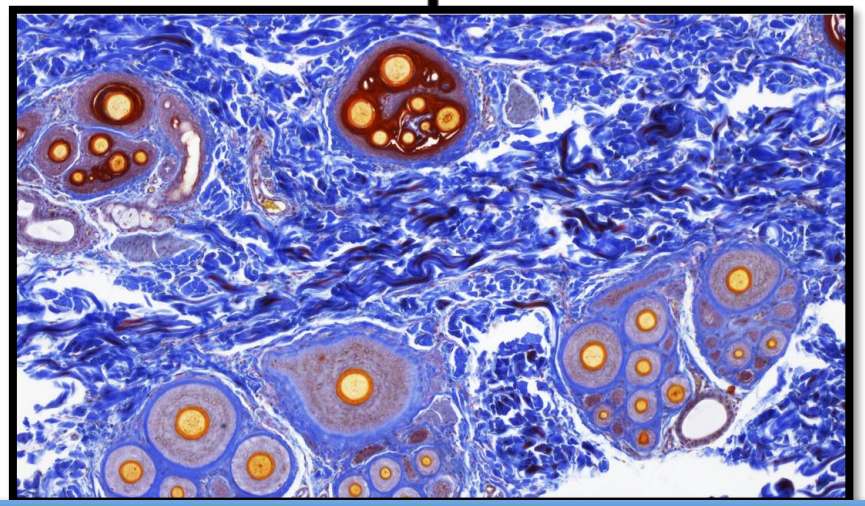
PLATE-BASED METHODS

e.g. Smart-Seq2
MARS-seq

+ High read-depth per cell (>10^6 reads per cell)
+ Reads may be generated across whole transcript length
− Moderate cell throughput (10^2–10^3 cells per experiment)

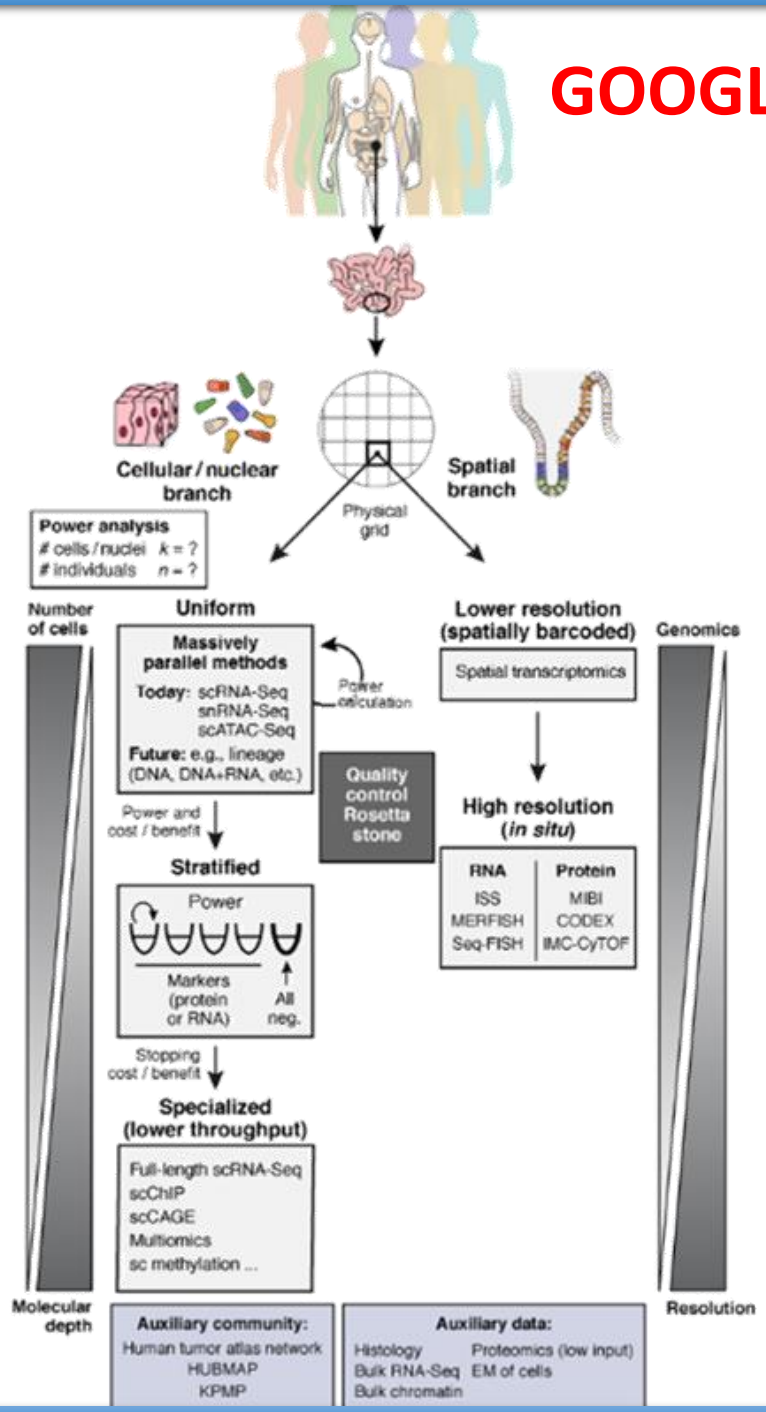


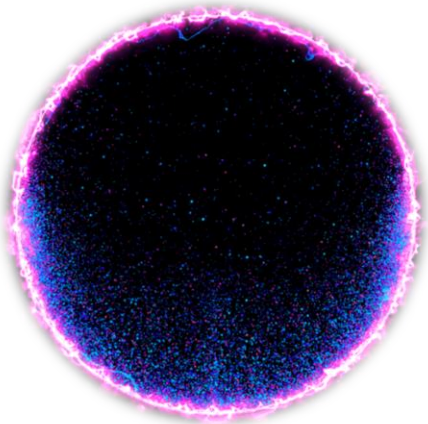
HUMAN CELL ATLAS

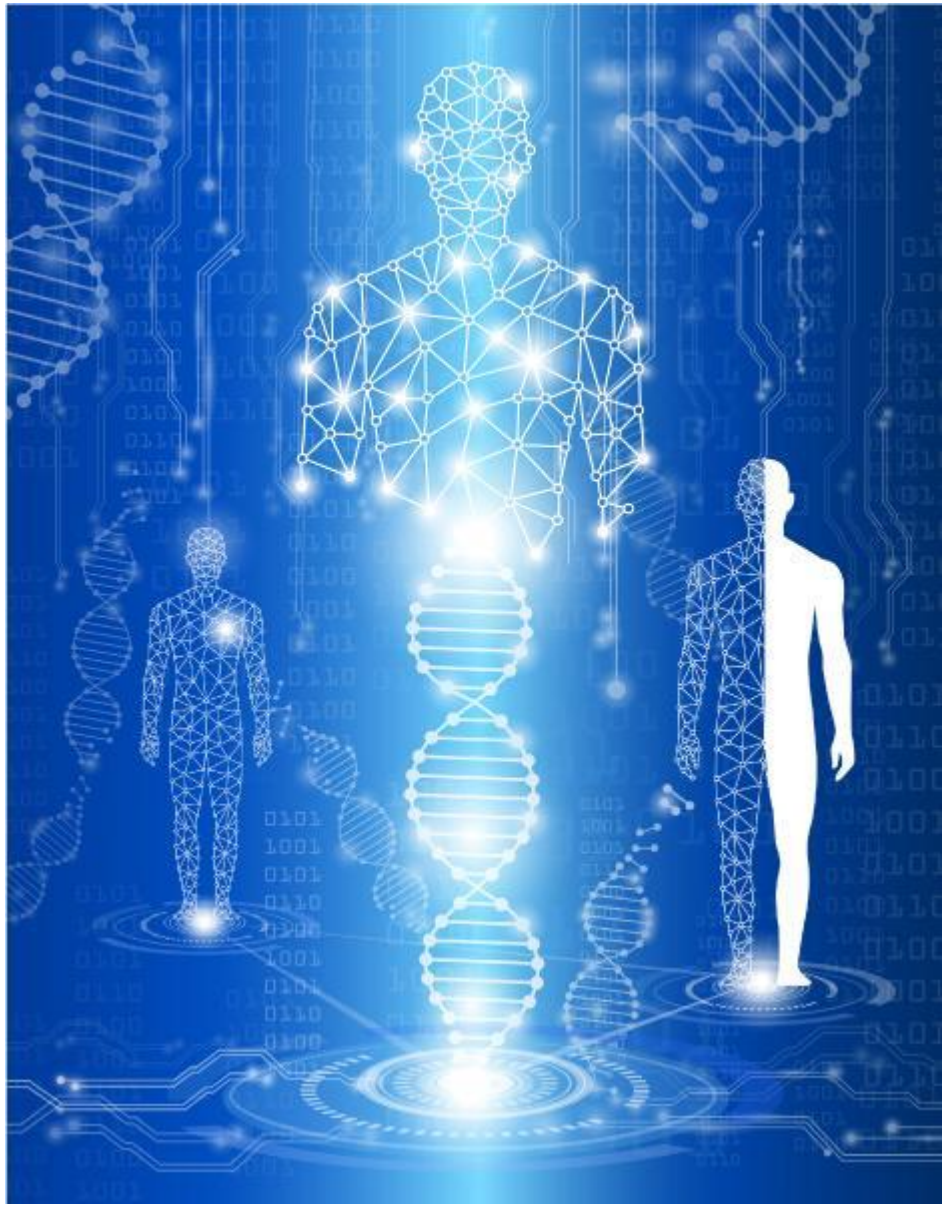


https://www.humancellatlas.org/

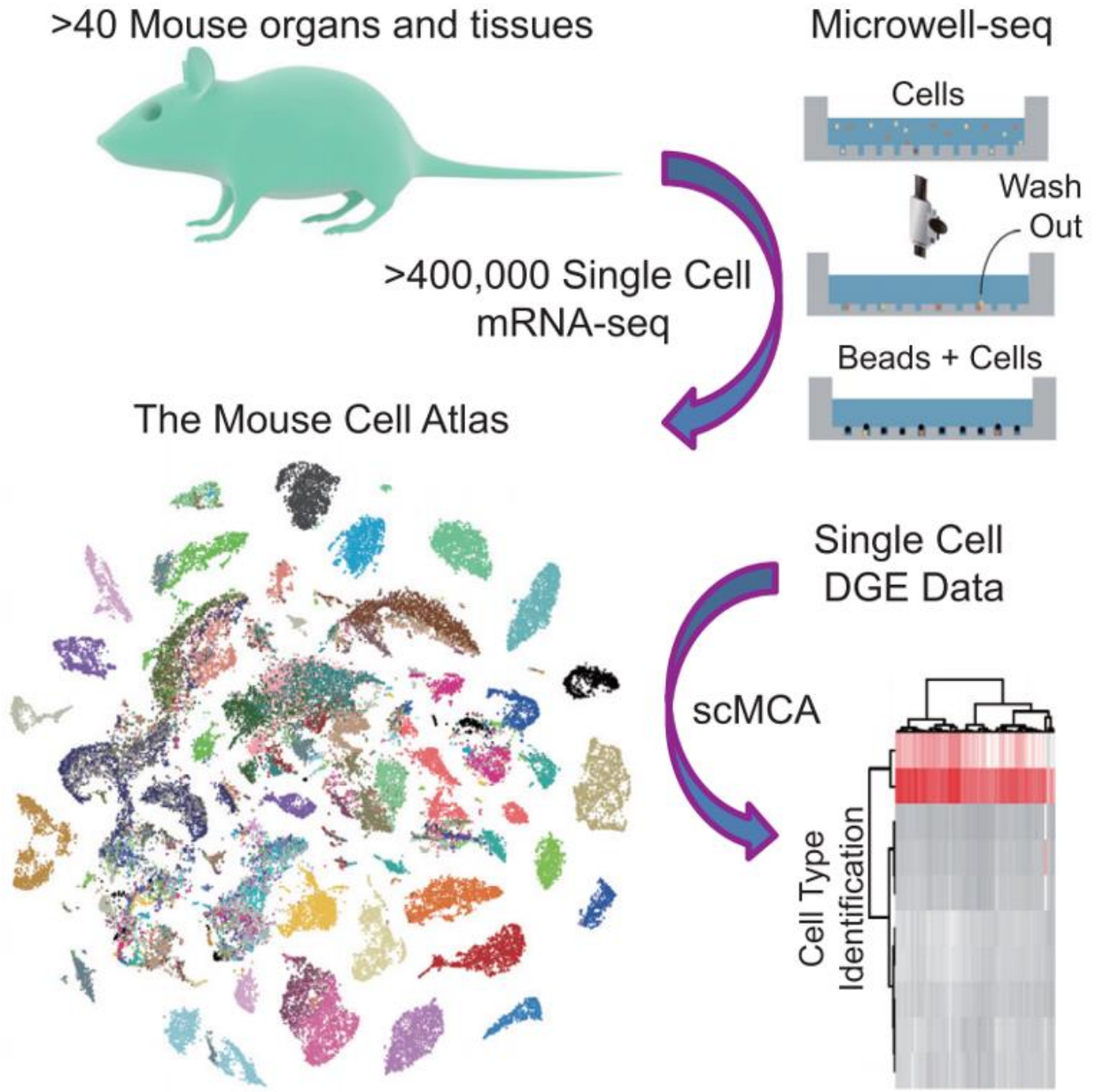
GOOGLE TÉRKÉPE A HUMÁN TESTNEK



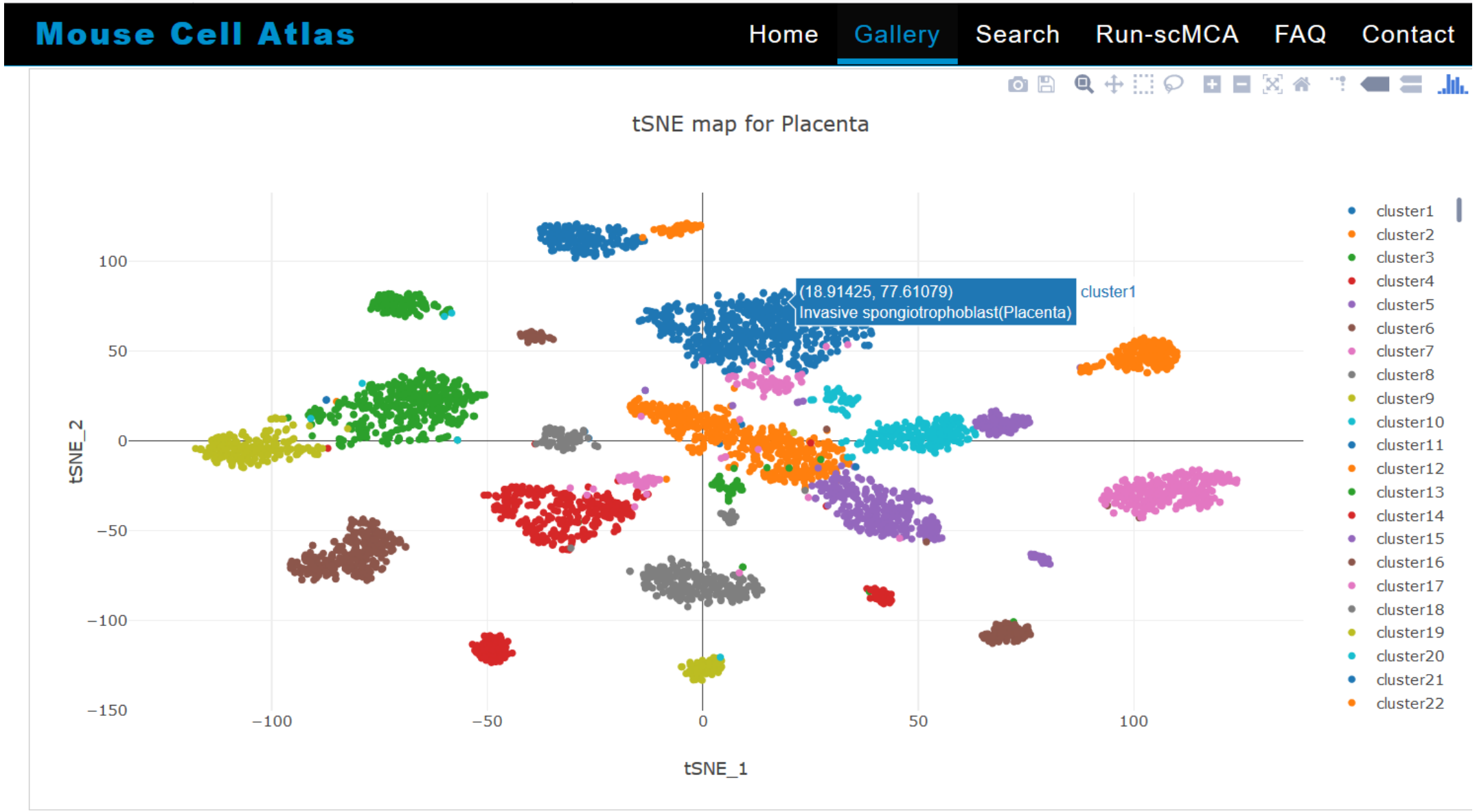




A PROJEKT MEGVALÓSÍTHATÓSÁGA: EGÉR SEJT-ATLASZ LÉTREHOZÁSA



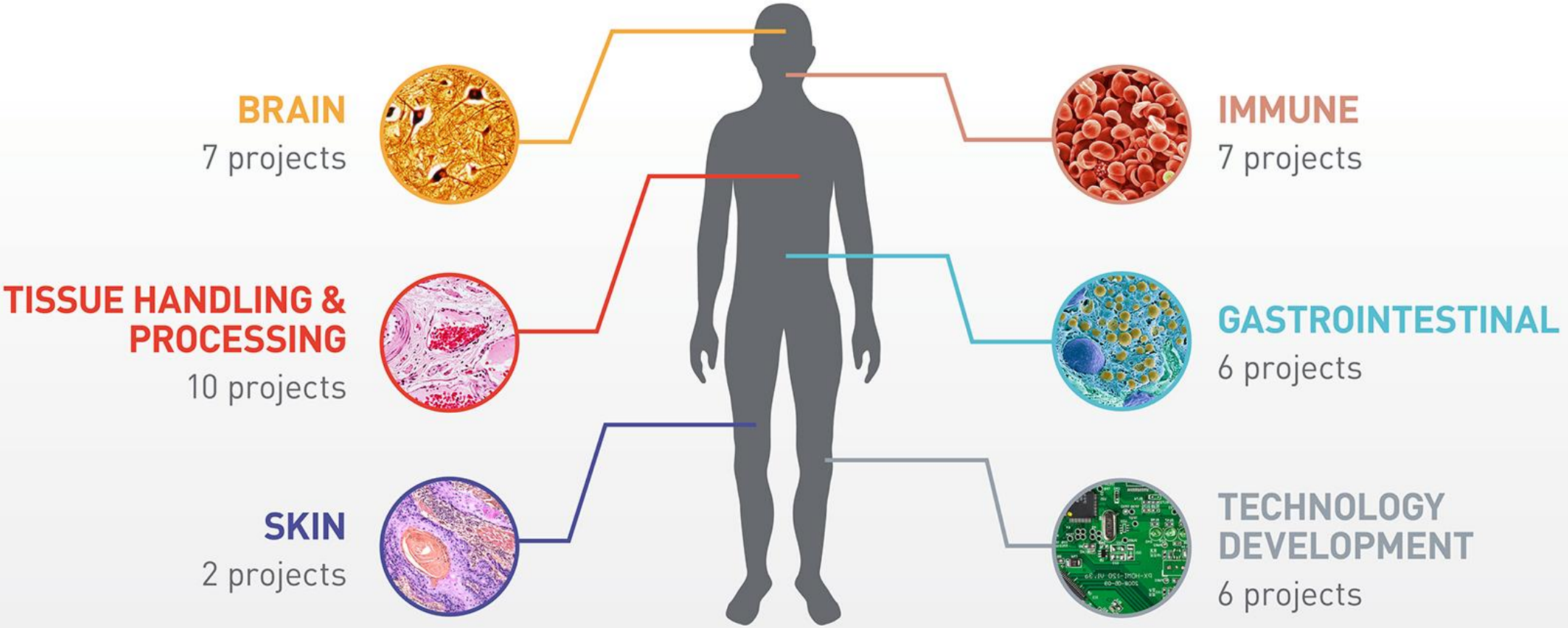
http://bis.zju.edu.cn/MCA/



http://bis.zju.edu.cn/MCA/

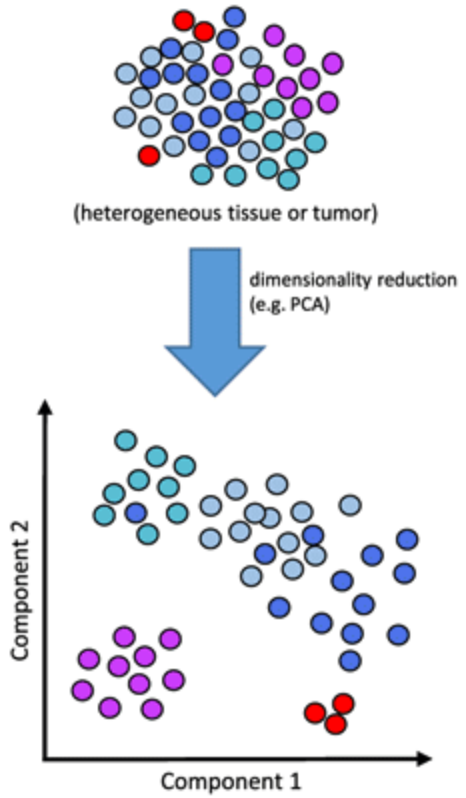
MAPPING THE BASIC UNITS OF LIFE

CZI proudly supports **38 new projects** in these six areas for the **Human Cell Atlas**.

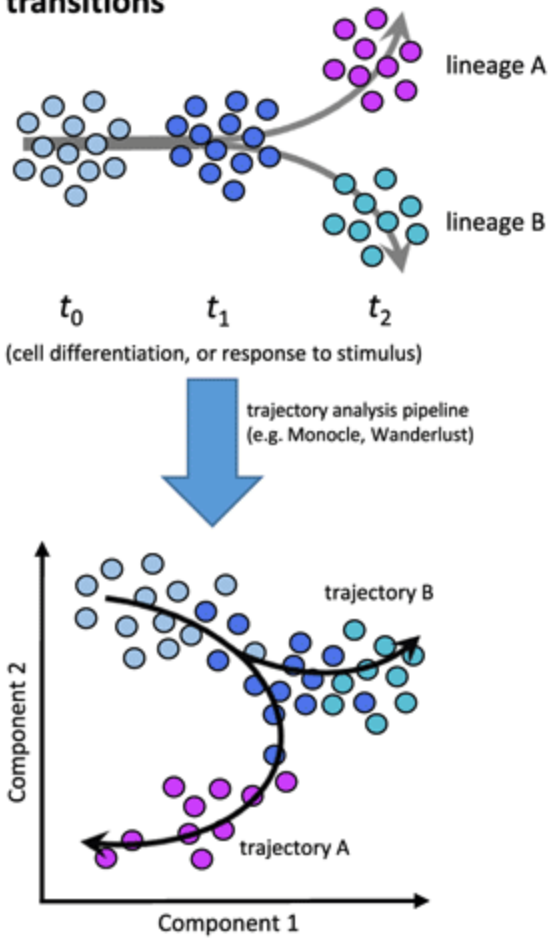


EGY-SEJT TRANSZKRIPTOMIKA ALKALMAZÁSA

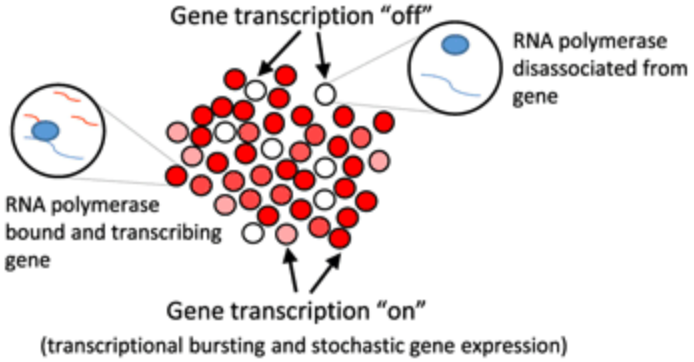
a) Deconvolving heterogeneous cell populations



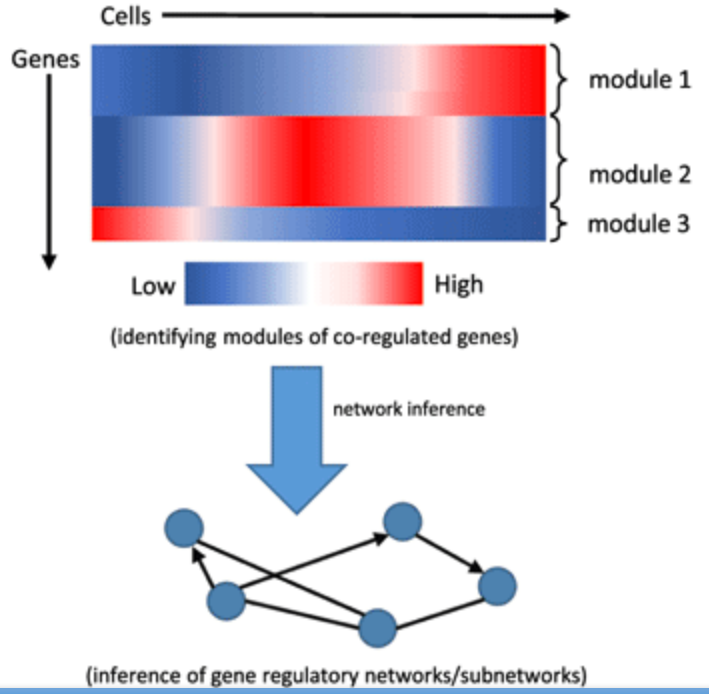
b) Trajectory analysis of cell state transitions

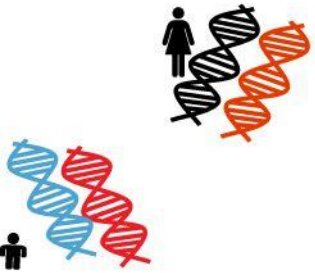


c) Dissecting transcription mechanics

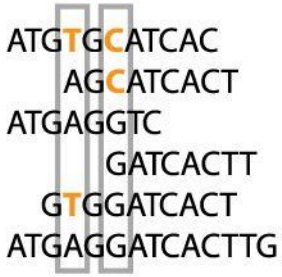


d) Network inference



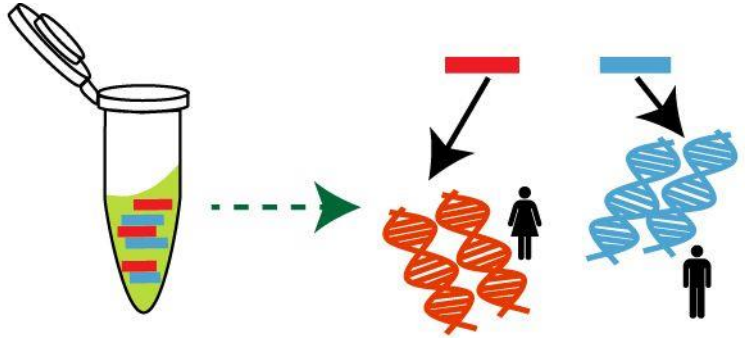


Exome sequencing
of fetus and mother



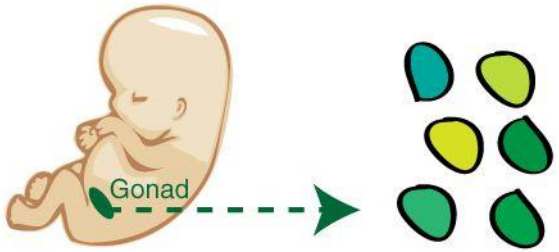
Variant calling from
high coverage
exomic DNA reads

Reconstruction of both,
unique parental haplotypes

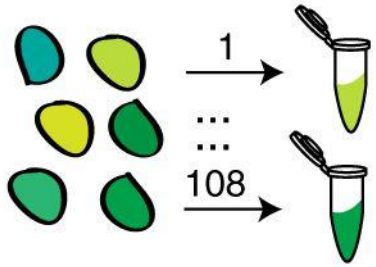


Alignment of RNA reads
to both parental genomes

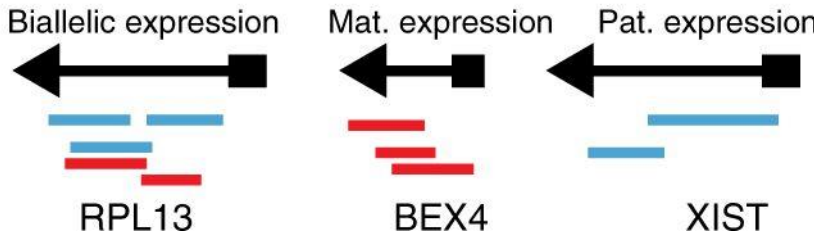
Quantification of parental expression
for all SNPs per haplotype



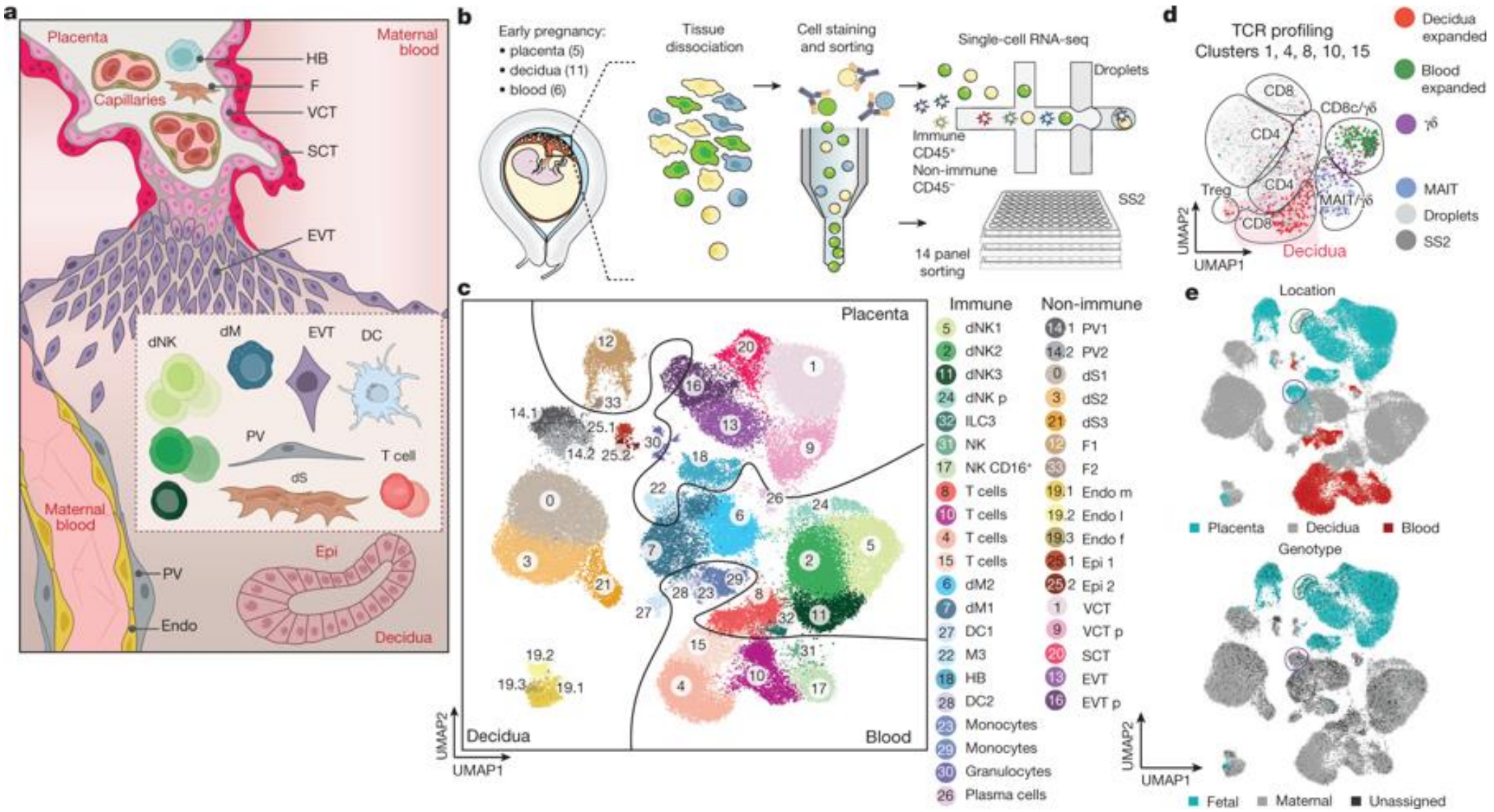
Isolation of single cells
(germ cells and somatic cells)



Single-cell RNA-seq



HUMÁN ANYAI-MAGZATI HATÁRFELÜLET ATLASZA



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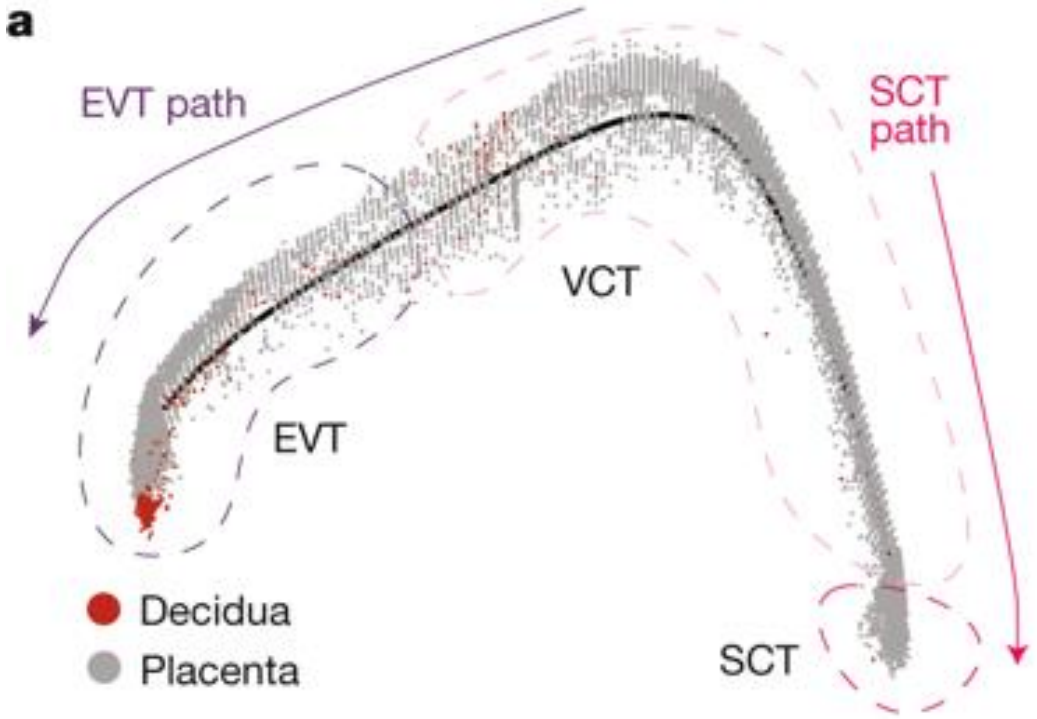
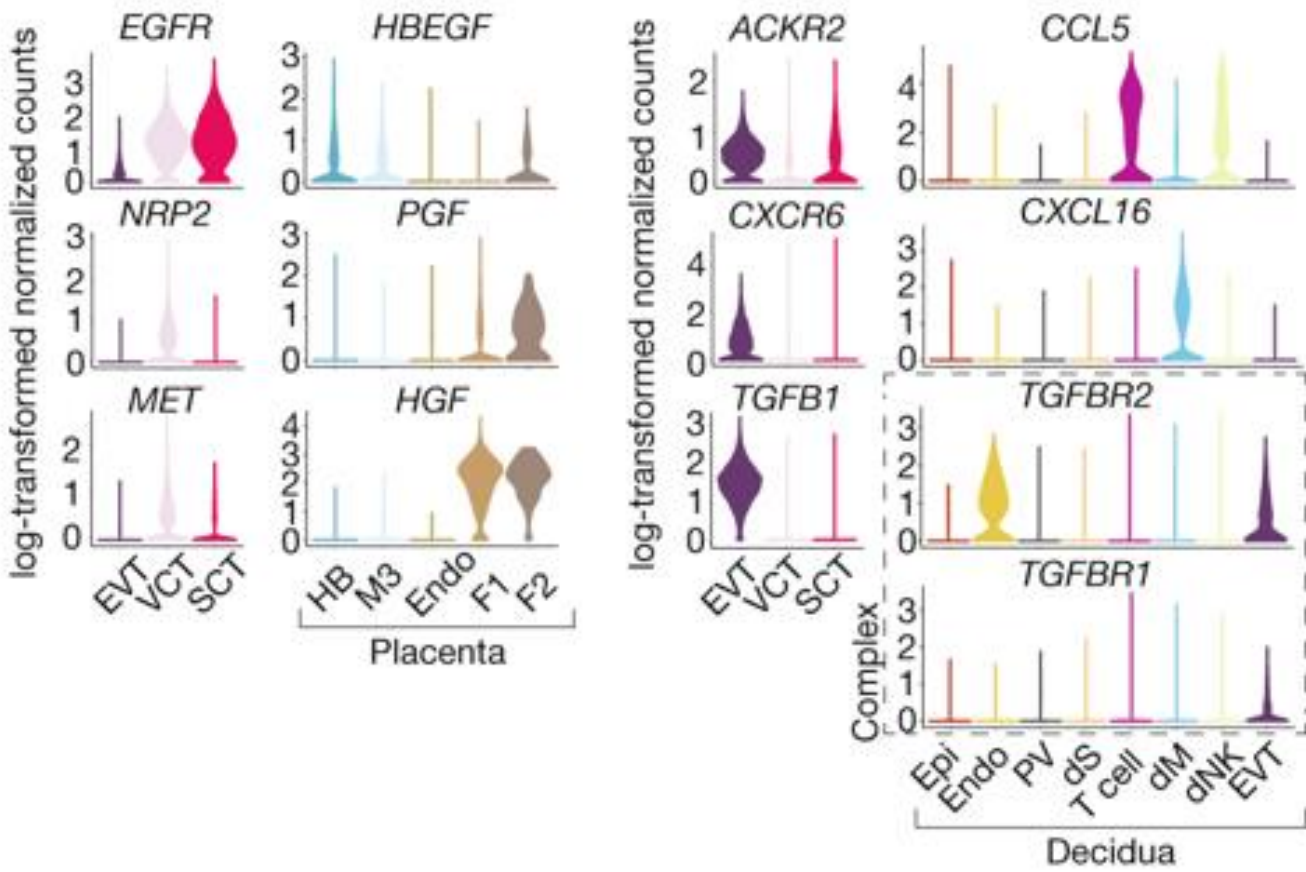
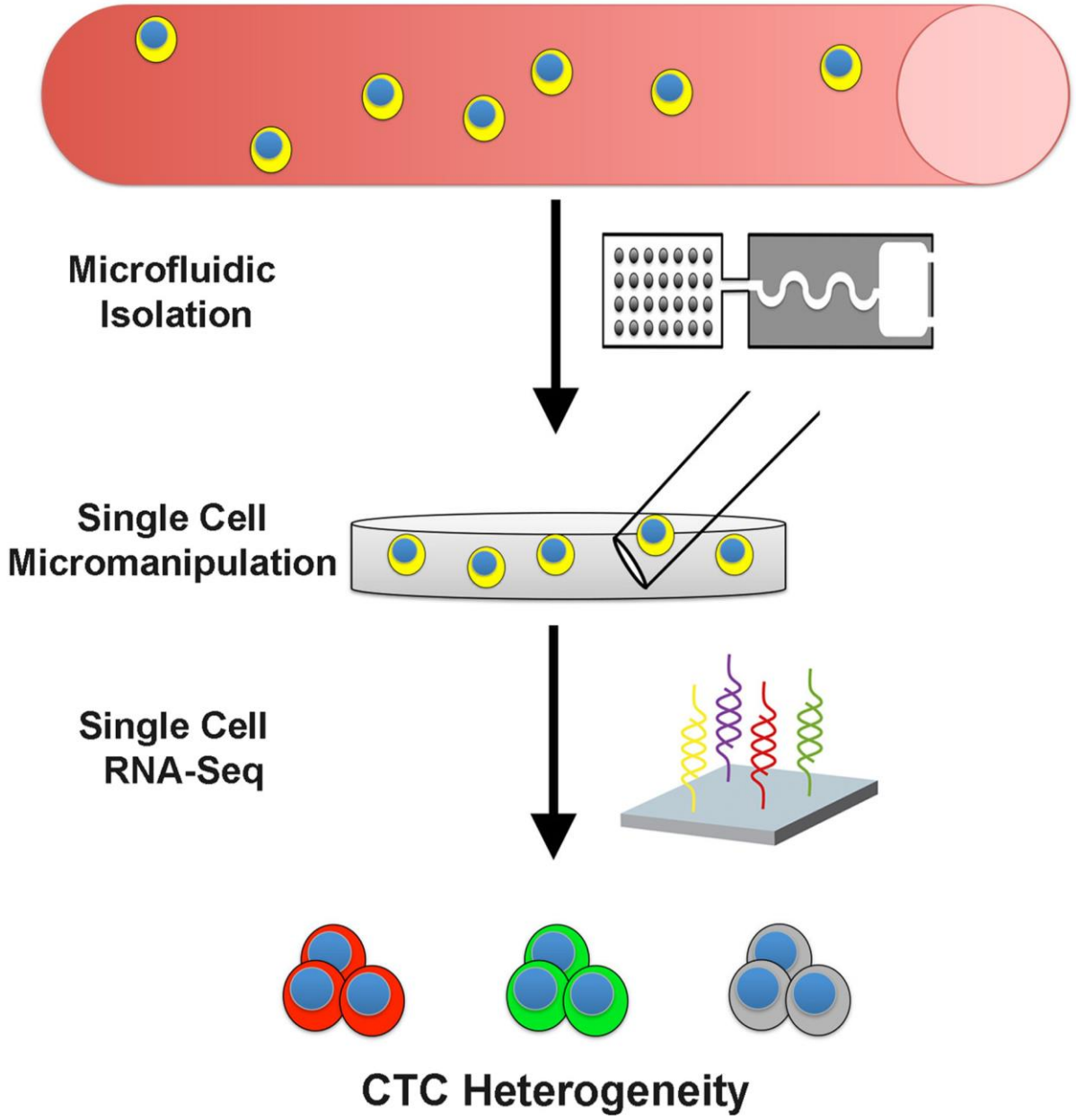
HUMÁN ANYAI-MAGZATI HATÁRFELÜLET ATLASZA





KERINGŐ METASZTATIZÁLÓ TUMORSEJTEK



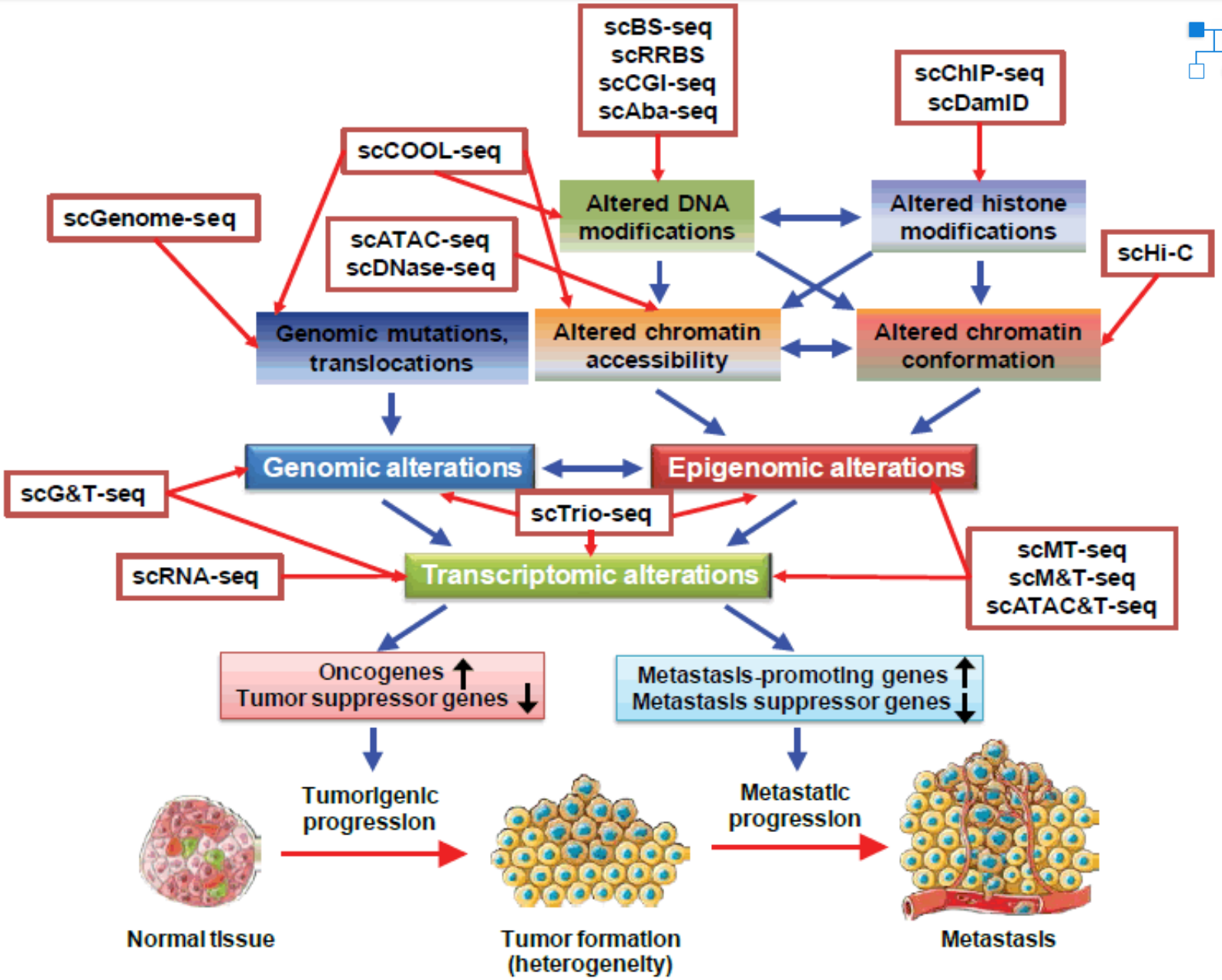
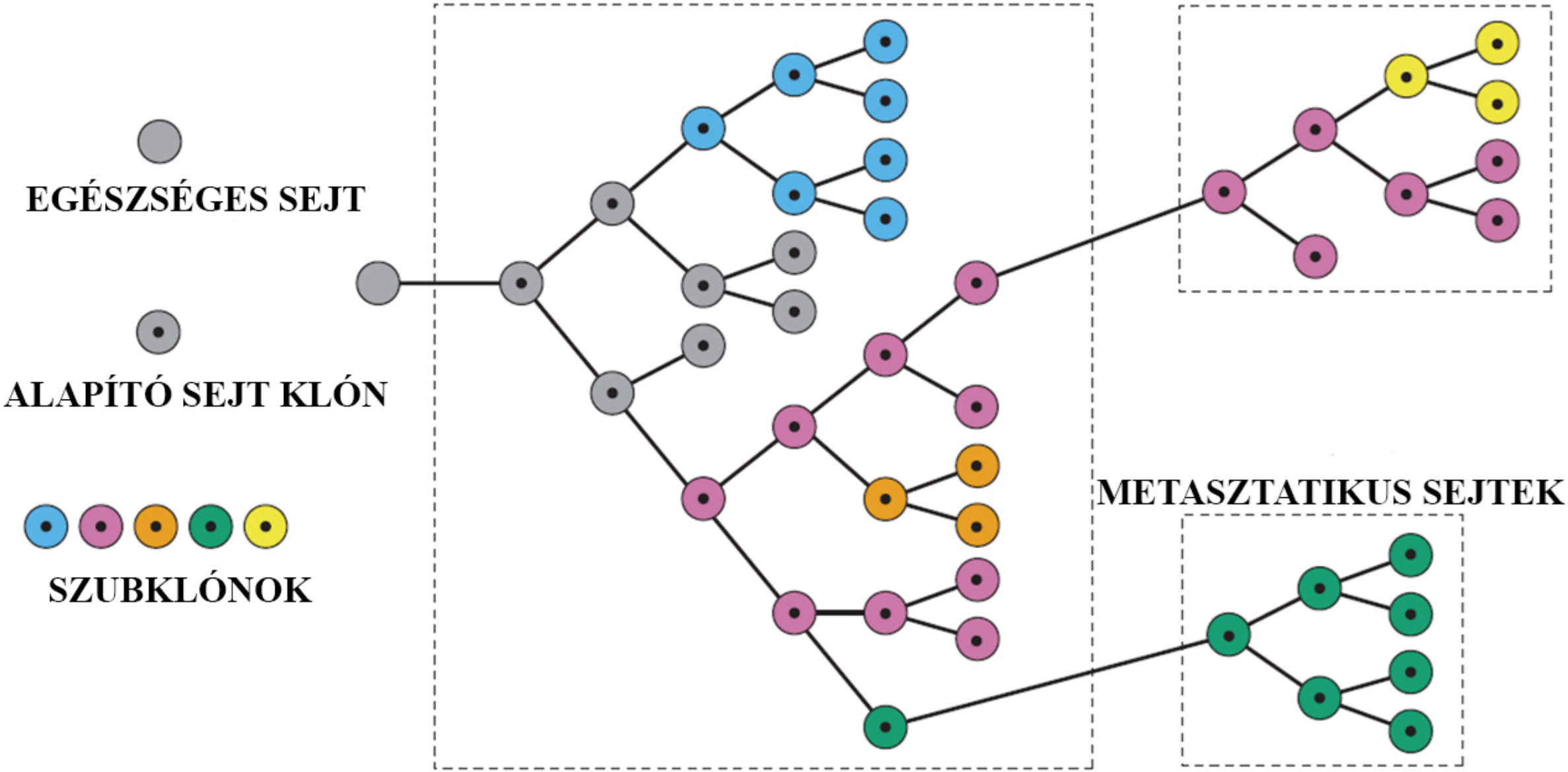


Figure 5: The diagram for illustrating the roles of genomic, epigenomics and transcriptomic alterations in tumorigenesis and metastasis. The current established single-cell genomics, epigenomics, transcriptomics and their derived multi-omics methods are also shown in the diagram to indicate their applications.

SEJTEK MOLEKULÁRIS PORTRÉJÁNAK FESTÉSE (1)



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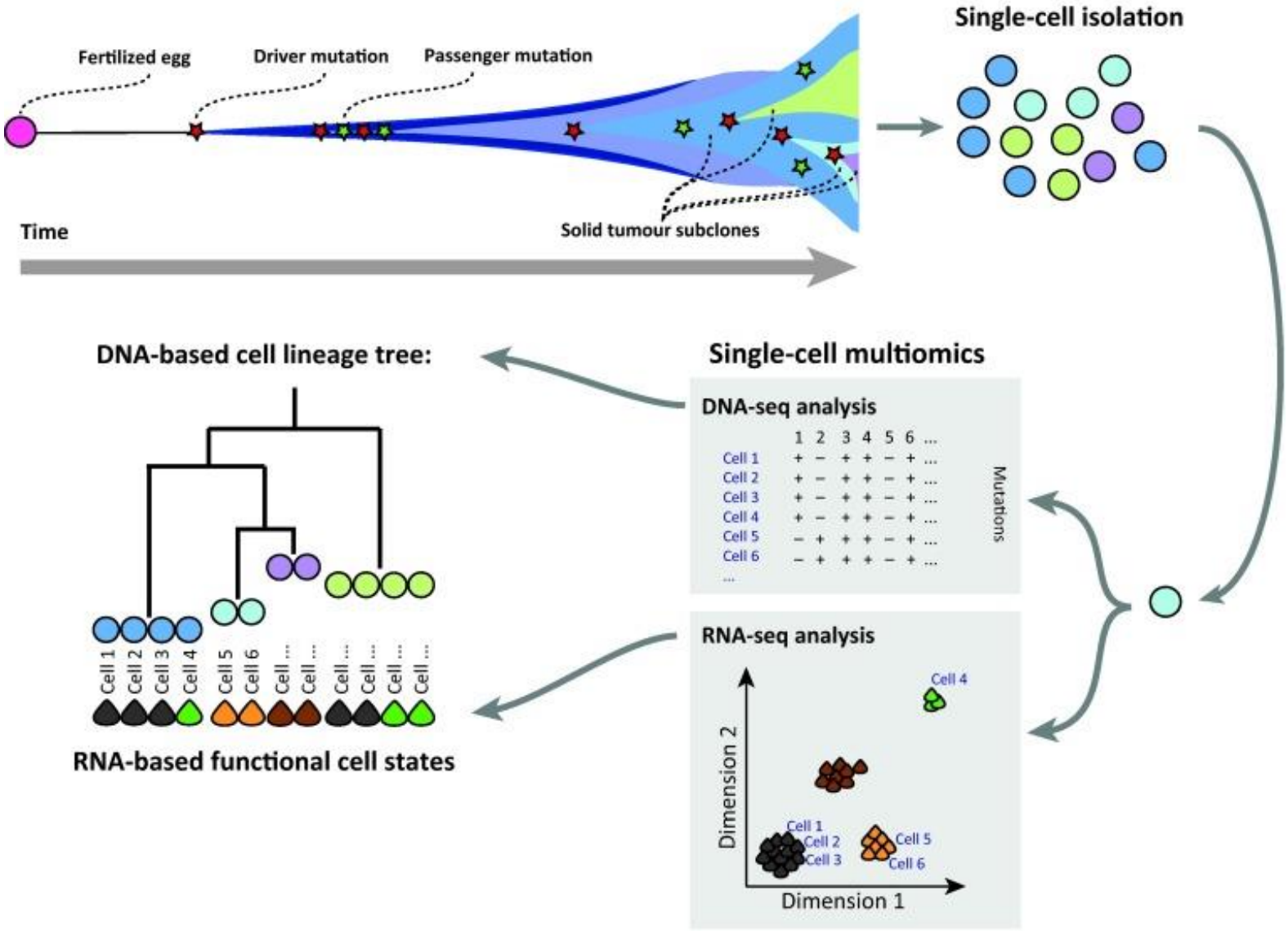

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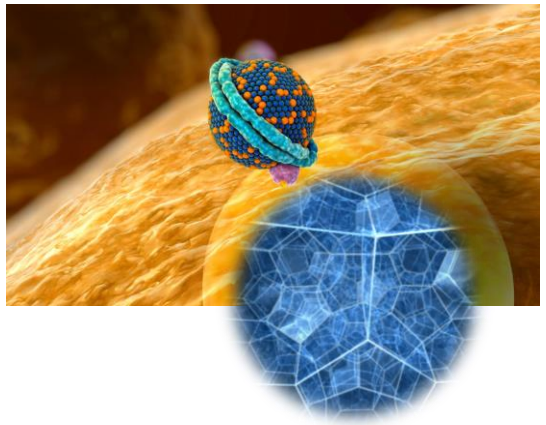
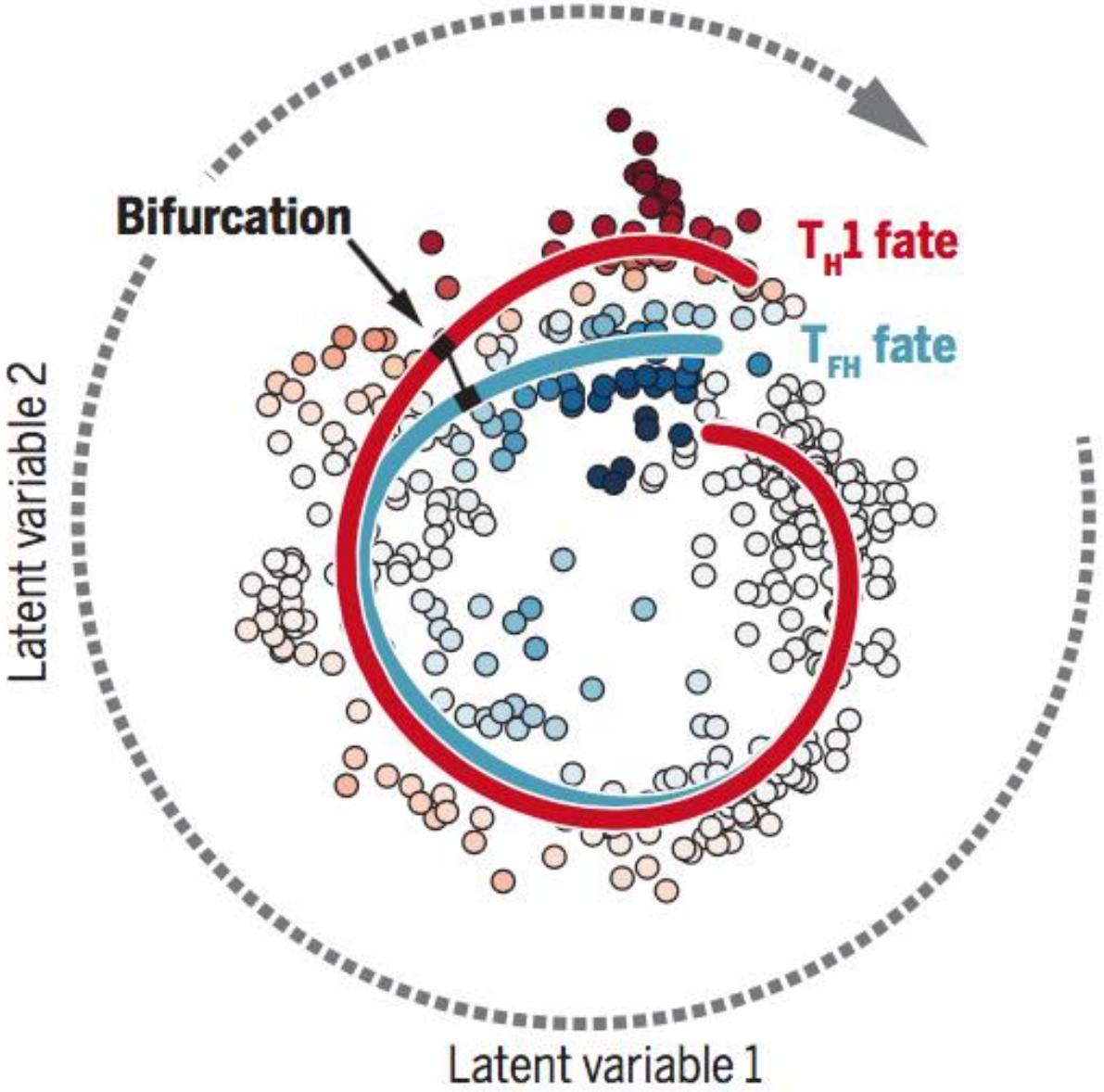
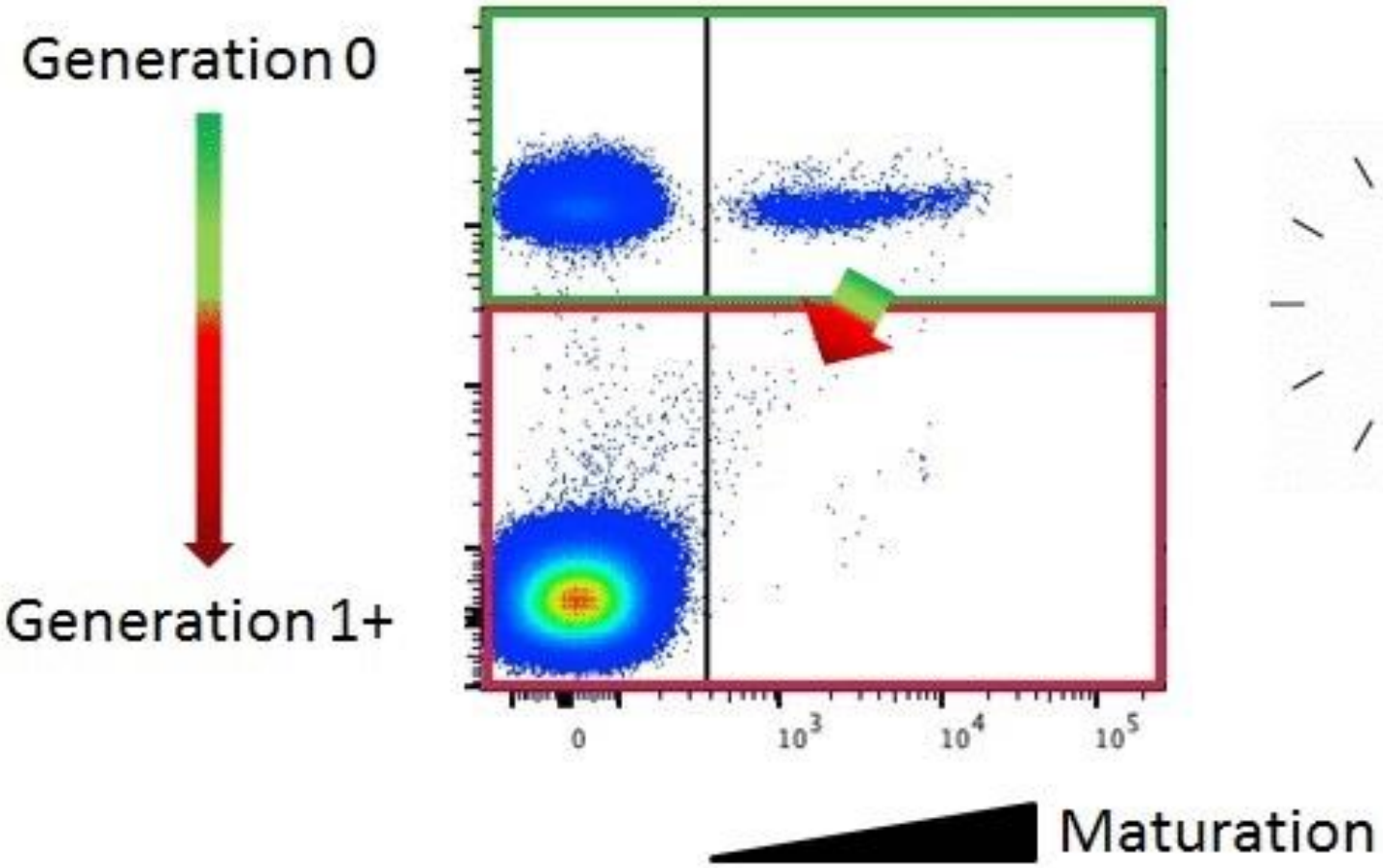


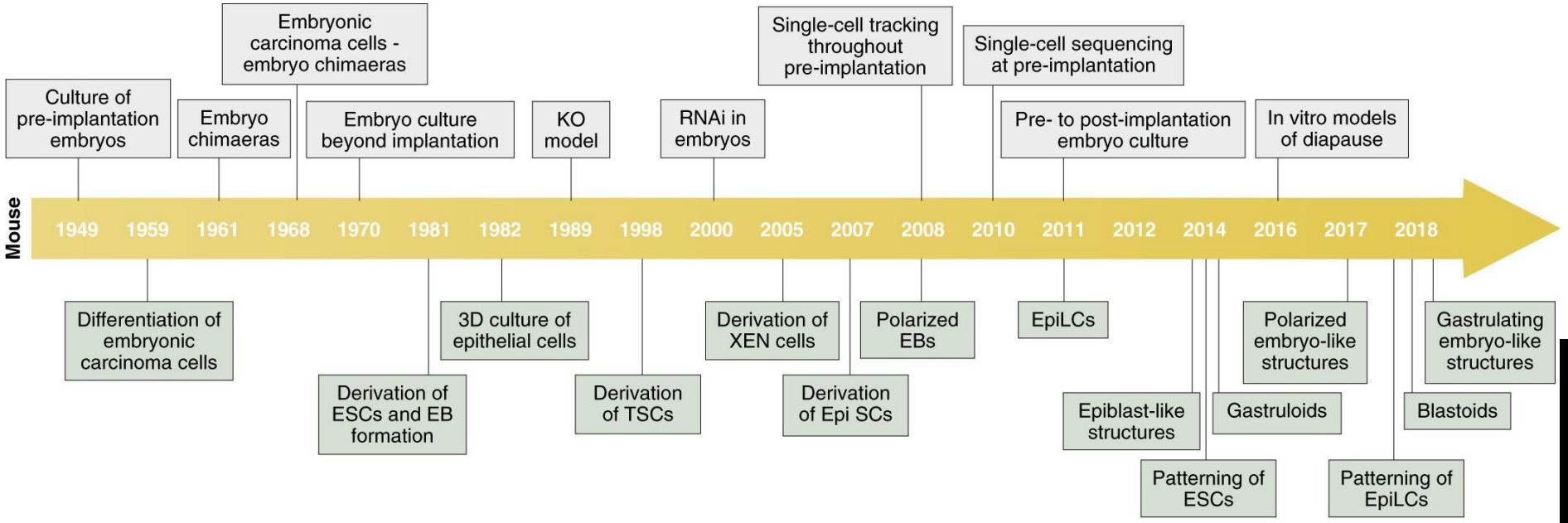


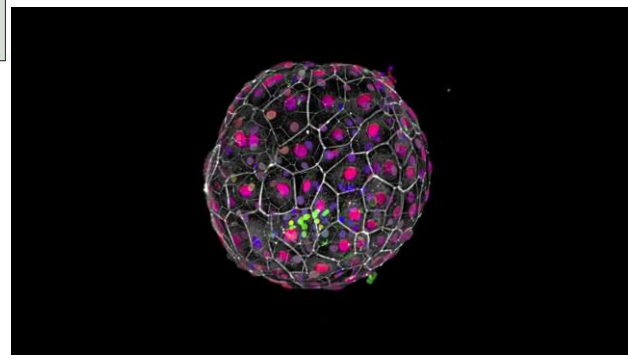
SEJTEK MOLEKULÁRIS PORTRÉJÁNAK FESTÉSE (4)

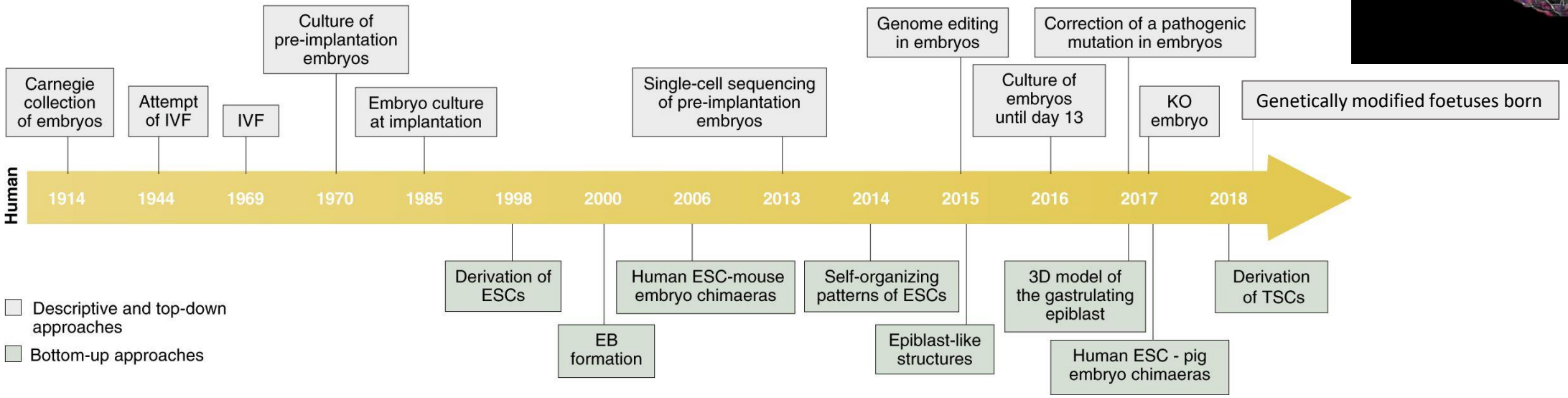


THE MODEL

$$\frac{\partial P}{\partial t}+\lambda\frac{\partial P}{\partial x}=-\boldsymbol{c(x)}P(t,x),\ P(0,x)=P_0 f(x;\mu,\sigma),\ P(t,0)=\boldsymbol{\beta}P(t,x_r)$$

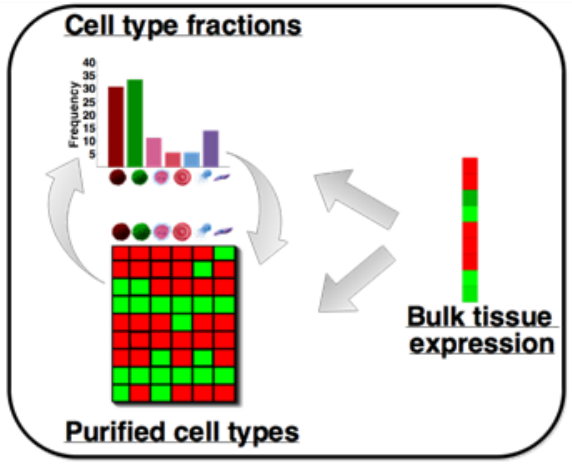


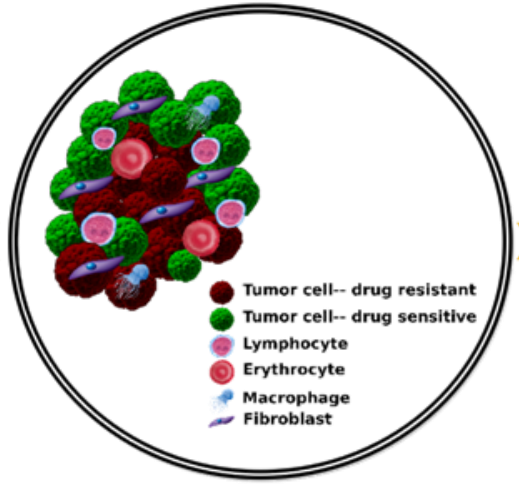


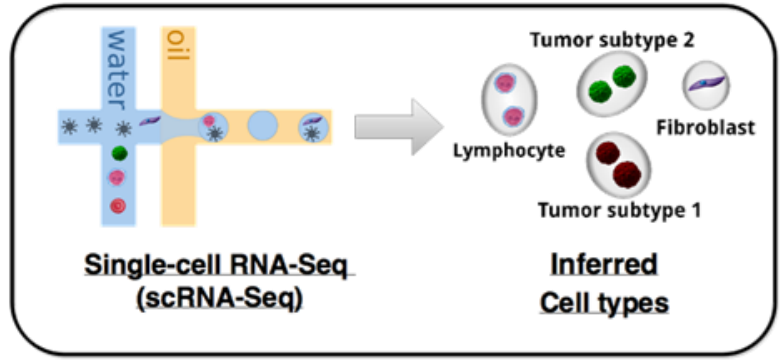


Shahbazi et al 2018 Nature

ELŐADÁS ÜZENETE





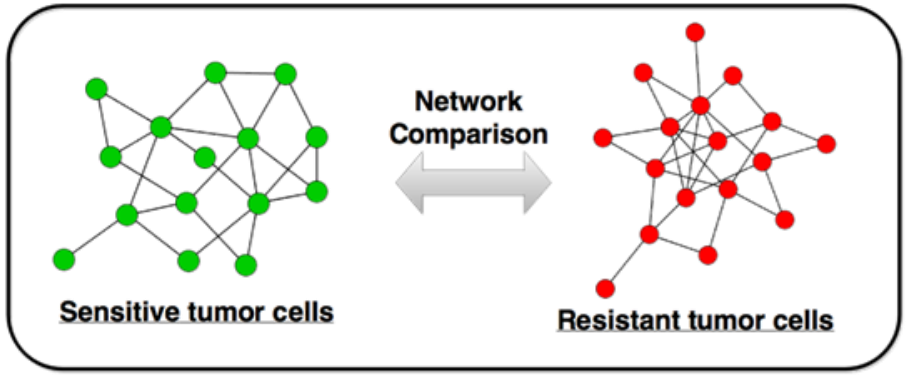


Deconvolving gene expression profiles

A Comprehensive Survey of Deconvolution Methods for Separating Cell types in Complex Tissues, 2016

Identifying cell types from single-cell profiles

Take ACTION to identify high-resolution cell types and associated transcriptional pathways, 2016



Constructing cell type-specific networks

A convex optimization approach for identification of human tissue-specific interactomes, 2016