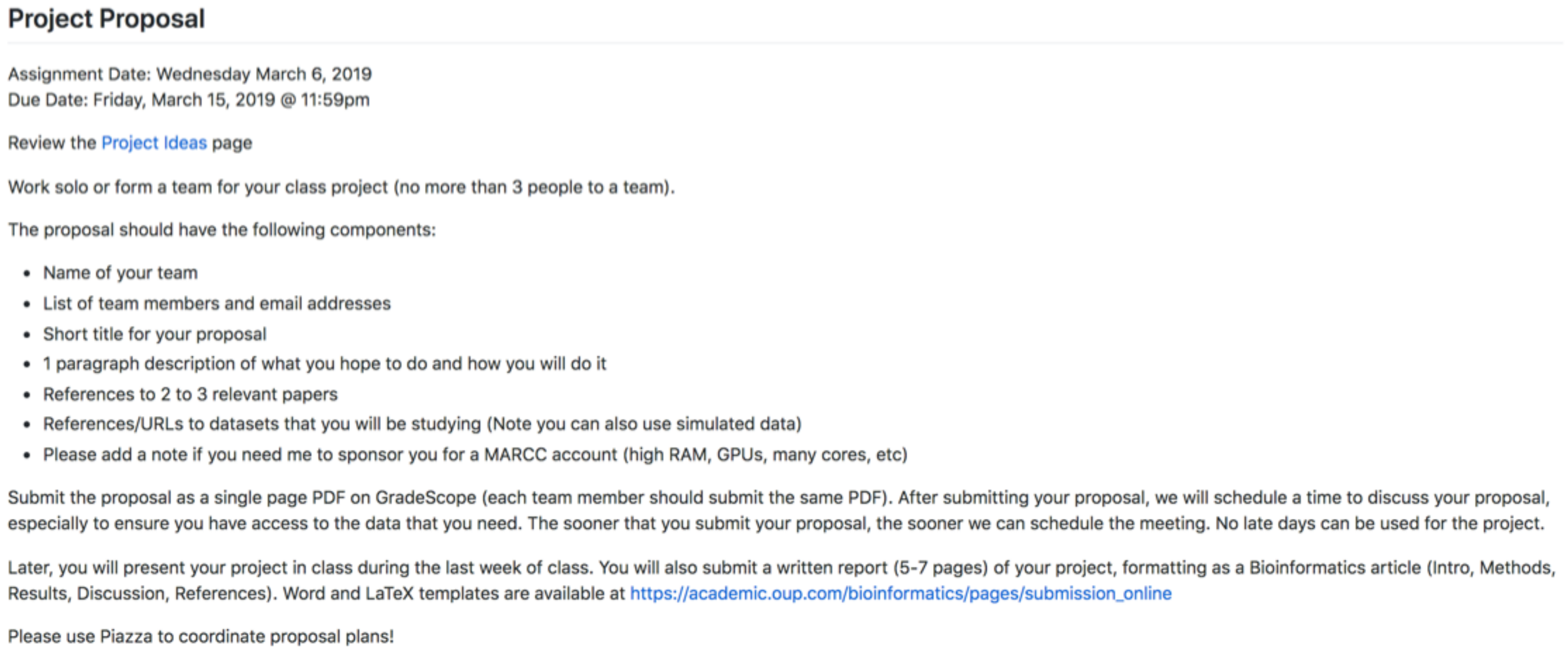
Lecture 15. Single Cell Analysis

Michael Schatz

March 25, 2019

JHU 601.749: Applied Comparative Genomics

Project Proposal!
Due March 15



Project Proposal

Assignment Date: Wednesday March 6, 2019
Due Date: Friday, March 15, 2019 @ 11:59pm

Review the Project Ideas page

Work solo or form a team for your class project (no more than 3 people to a team).

The proposal should have the following components:

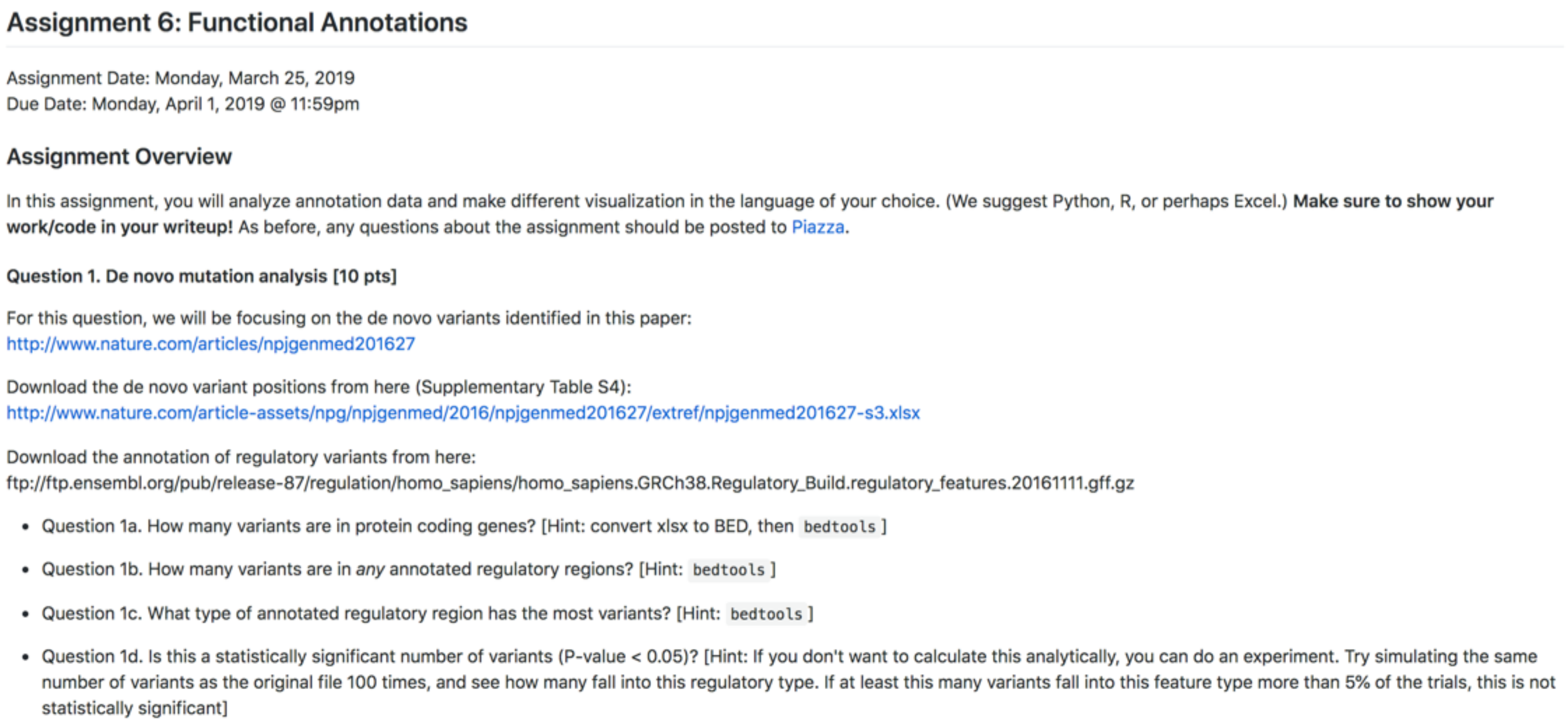
- Name of your team
- List of team members and email addresses
- Short title for your proposal
- 1 paragraph description of what you hope to do and how you will do it
- References to 2 to 3 relevant papers
- References/URLs to datasets that you will be studying (Note you can also use simulated data)
- Please add a note if you need me to sponsor you for a MARCC account (high RAM, GPUs, many cores, etc)

Submit the proposal as a single page PDF on GradeScope (each team member should submit the same PDF). After submitting your proposal, we will schedule a time to discuss your proposal, especially to ensure you have access to the data that you need. The sooner that you submit your proposal, the sooner we can schedule the meeting. No late days can be used for the project.

Later, you will present your project in class during the last week of class. You will also submit a written report (5-7 pages) of your project, formatting as a Bioinformatics article (Intro, Methods, Results, Discussion, References). Word and LaTeX templates are available at https://academic.oup.com/bioinformatics/pages/submission_online

Please use Piazza to coordinate proposal plans!

HW6: Due April 1



Assignment 6: Functional Annotations

Assignment Date: Monday, March 25, 2019
Due Date: Monday, April 1, 2019 @ 11:59pm

Assignment Overview

In this assignment, you will analyze annotation data and make different visualization in the language of your choice. (We suggest Python, R, or perhaps Excel.) **Make sure to show your work/code in your writeup!** As before, any questions about the assignment should be posted to Piazza.

Question 1. De novo mutation analysis [10 pts]

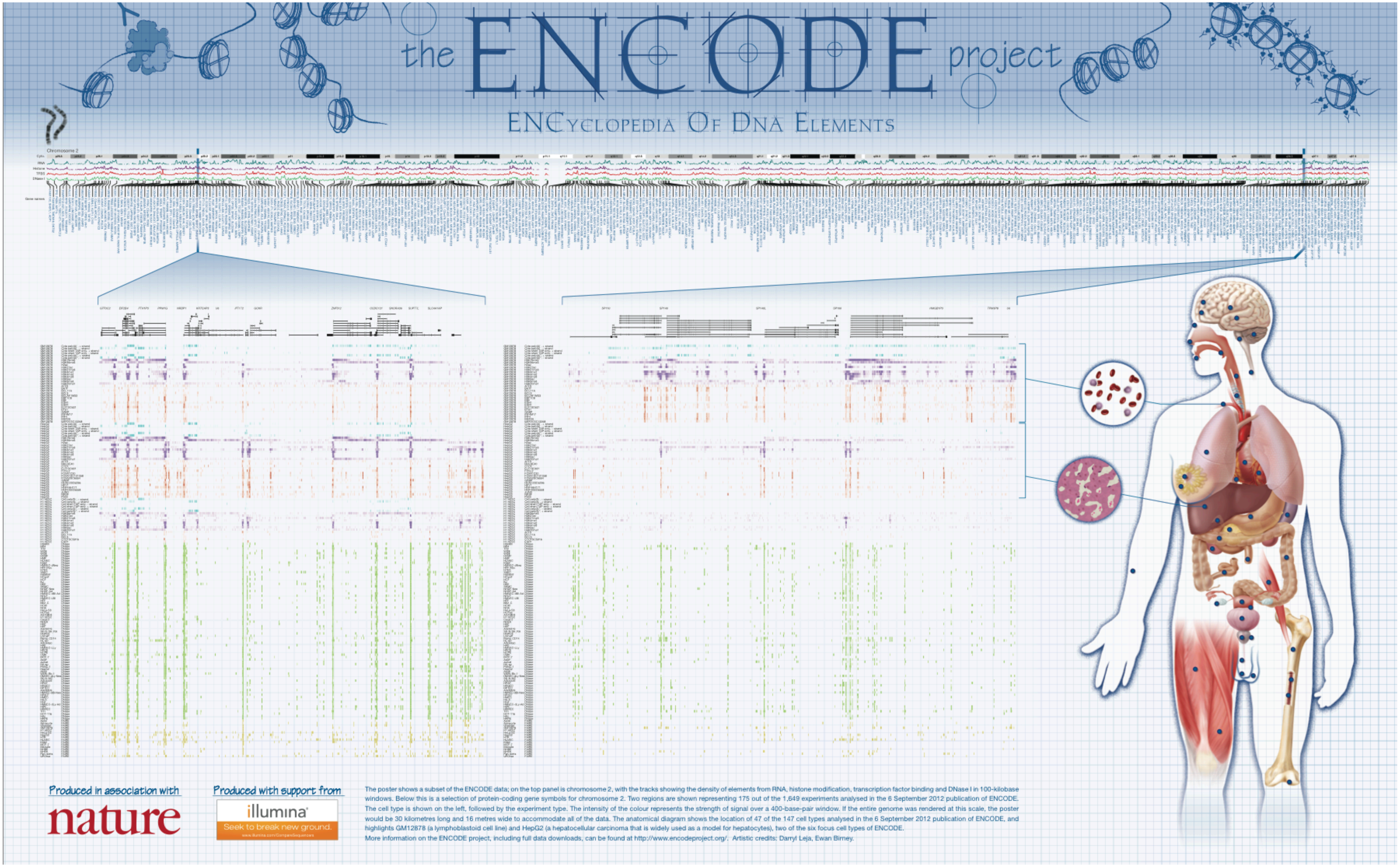
For this question, we will be focusing on the de novo variants identified in this paper:
http://www.nature.com/articles/npjgenmed201627

Download the de novo variant positions from here (Supplementary Table S4):
http://www.nature.com/article-assets/npg/npjgenmed/2016/npjgenmed201627/extref/npjgenmed201627-s3.xlsx

Download the annotation of regulatory variants from here:
ftp://ftp.ensembl.org/pub/release-87/regulation/homo_sapiens/homo_sapiens.GRCh38.Regulatory_Build.regulatory_features.20161111.gff.gz

- Question 1a. How many variants are in protein coding genes? [Hint: convert xlsx to BED, then `bedtools`]
- Question 1b. How many variants are in *any* annotated regulatory regions? [Hint: `bedtools`]
- Question 1c. What type of annotated regulatory region has the most variants? [Hint: `bedtools`]
- Question 1d. Is this a statistically significant number of variants (P-value < 0.05)? [Hint: If you don't want to calculate this analytically, you can do an experiment. Try simulating the same number of variants as the original file 100 times, and see how many fall into this regulatory type. If at least this many variants fall into this feature type more than 5% of the trials, this is not statistically significant]

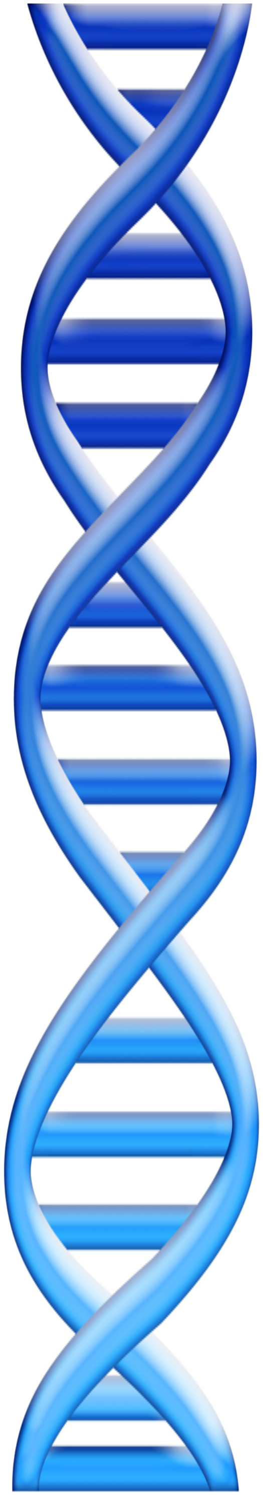
ENCODE Data Sets



The poster shows a subset of the ENCODE data; on the top panel is chromosome 2, with the tracks showing the density of elements from RNA, histone modification, transcription factor binding and DNase I in 100-kilobase windows. Below this is a selection of protein-coding gene symbols for chromosome 2. Two regions are shown representing 175 out of the 1,649 experiments analysed in the 6 September 2012 publication of ENCODE. The cell type is shown on the left, followed by the experiment type. The intensity of the colour represents the strength of signal over a 400-base-pair window. If the entire genome was rendered at this scale, the poster would be 30 kilometres long and 16 metres wide to accommodate all of the data. The anatomical diagram shows the location of 47 of the 147 cell types analysed in the 6 September 2012 publication of ENCODE, and highlights GM12878 (a lymphoblastoid cell line) and HepG2 (a hepatocellular carcinoma that is widely used as a model for hepatocytes), two of the six focus cell types of ENCODE.

More information on the ENCODE project, including full data downloads, can be found at http://www.encodeproject.org/. Artistic credits: Darryl Leja, Ewan Birney.

1,640 data sets total over 147 different cell types



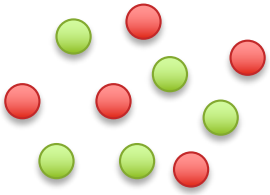
Single Cell Analysis

1. Why single cells?
2. scDNA
3. scRNA and other assays

Population Heterogeneity

Red cells express twice the abundance of “brain” genes compared to green cells

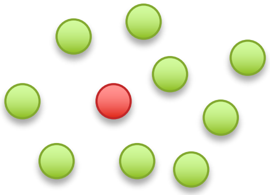
Experiment 1: 50/50



Compared to a control sample of pure green cells, this sample will show:

50% 2x + 50% 1x
= 1.5x over expression of brain genes

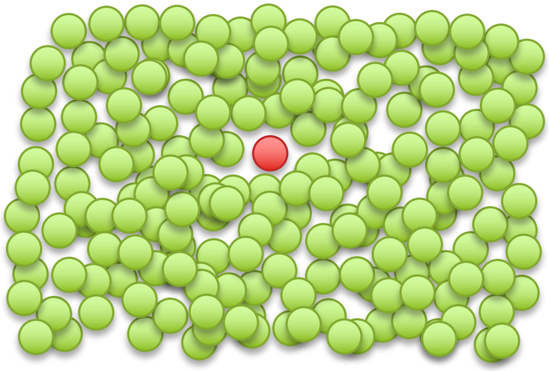
Experiment 2: 1/10



Compared to a control sample of pure green cells, this sample will show:

10% 2x + 90% 1x
= 1.1x over expression of brain genes

Experiment 3: 1/1000



Compared to a control sample of pure green cells, this sample will show:

0.1% 2x + 99.1% 1x
= 1.001x over expression of brain genes

The limitations of averages

	Drug A	Drug B
Overall Response	78% (273/350)	**83% (289/350)**

The limitations of averages

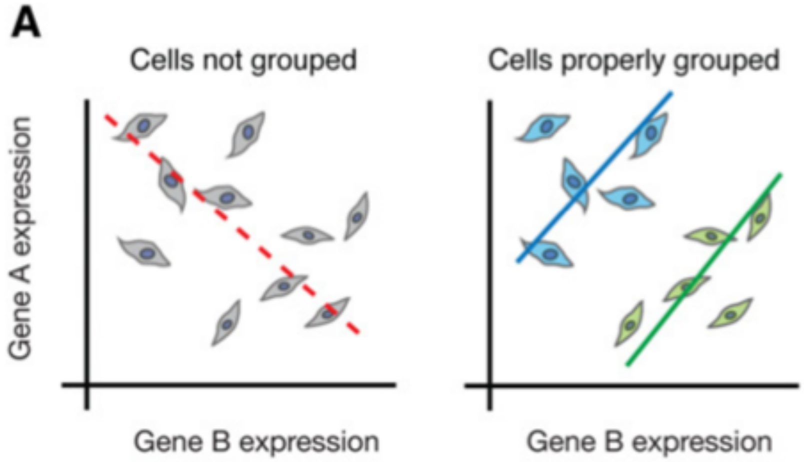
	Drug A	Drug B
Overall Response	78% (273/350)	**83% (289/350)**
Male Response	**93% (81/87)**	87% (234/270)
Female Response	**73% (192/263)**	69% (55/80)

What??? How can the better performing drug depend on if you examine the overall response or separately examine by gender

Example of Simpson's paradox:
Trend of the overall average may reverse the trends of each constituent group

In this example, the "lurking" variable (or confounding variable) is the severity of the case (represented by the doctors' treatment decision trend of favoring B for less severe cases), which was not previously known to be important until its effects were included. (Based on real analysis of kidney stone treatments)

The paradox of averages



What??? How can the better performing drug depend on if you examine the overall response or separately examine by gender

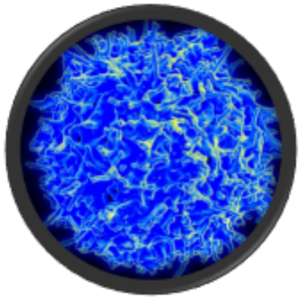
Example of Simpson’s paradox:
Trend of the overall average may reverse the trends of each constituent group

In this example, the "lurking" variable (or confounding variable) is the severity of the case (represented by the doctors' treatment decision trend of favoring B for less severe cases), which was not previously known to be important until its effects were included. (Based on real analysis of kidney stone treatments)

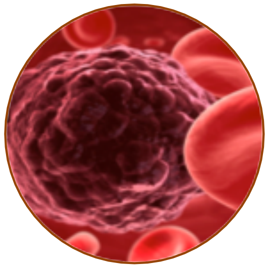
(Trapnell, 2015, Genome Research)

Sources of (Genomic) Heterogeneity

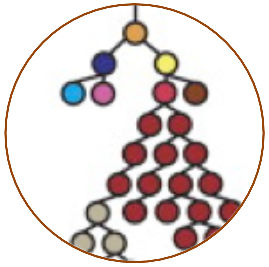
Neuronal mosaicism



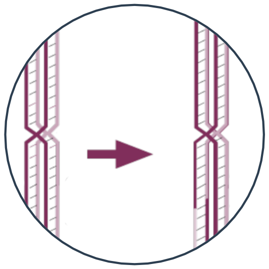
Immune Cells



Circulating tumor cells

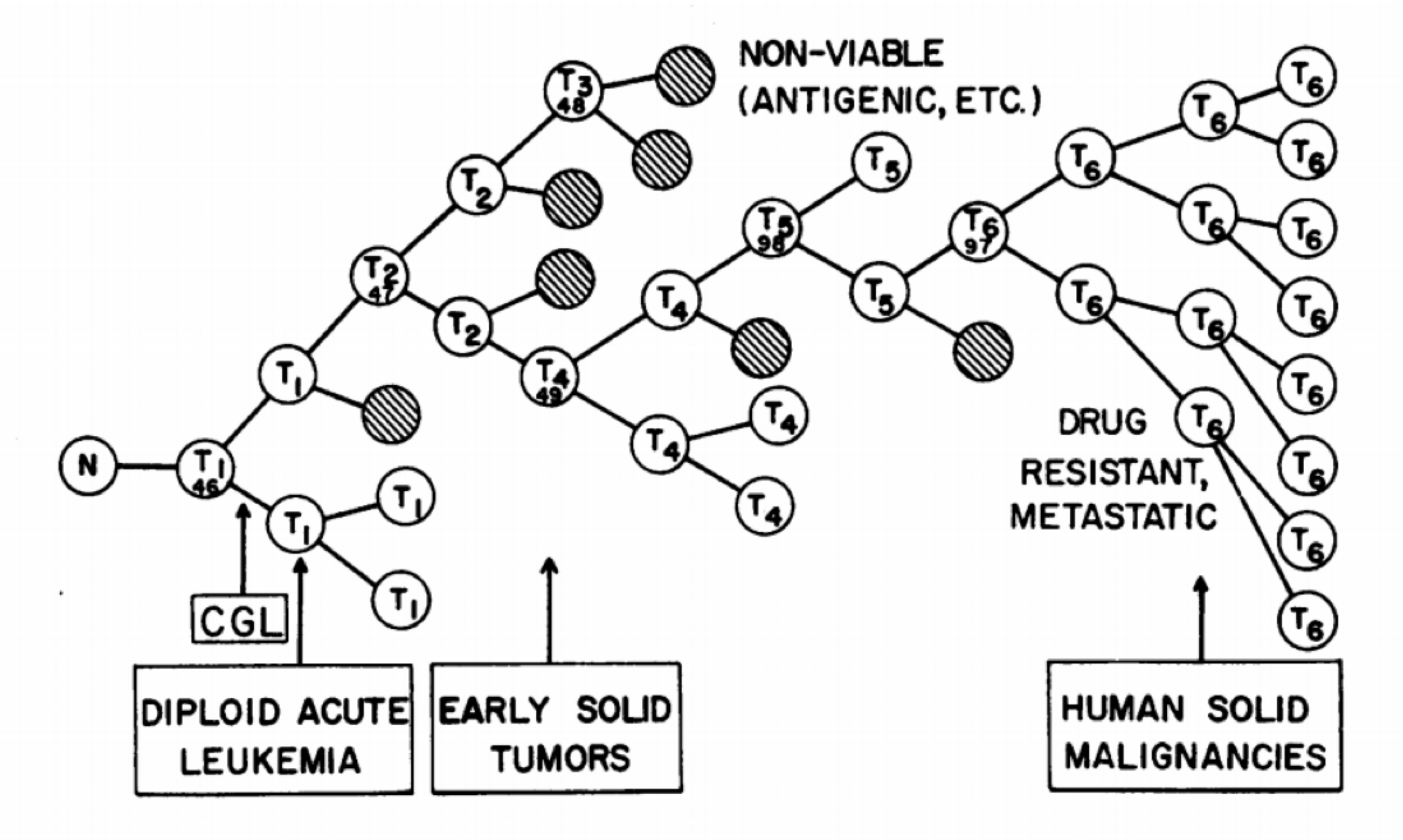


Tumor evolution



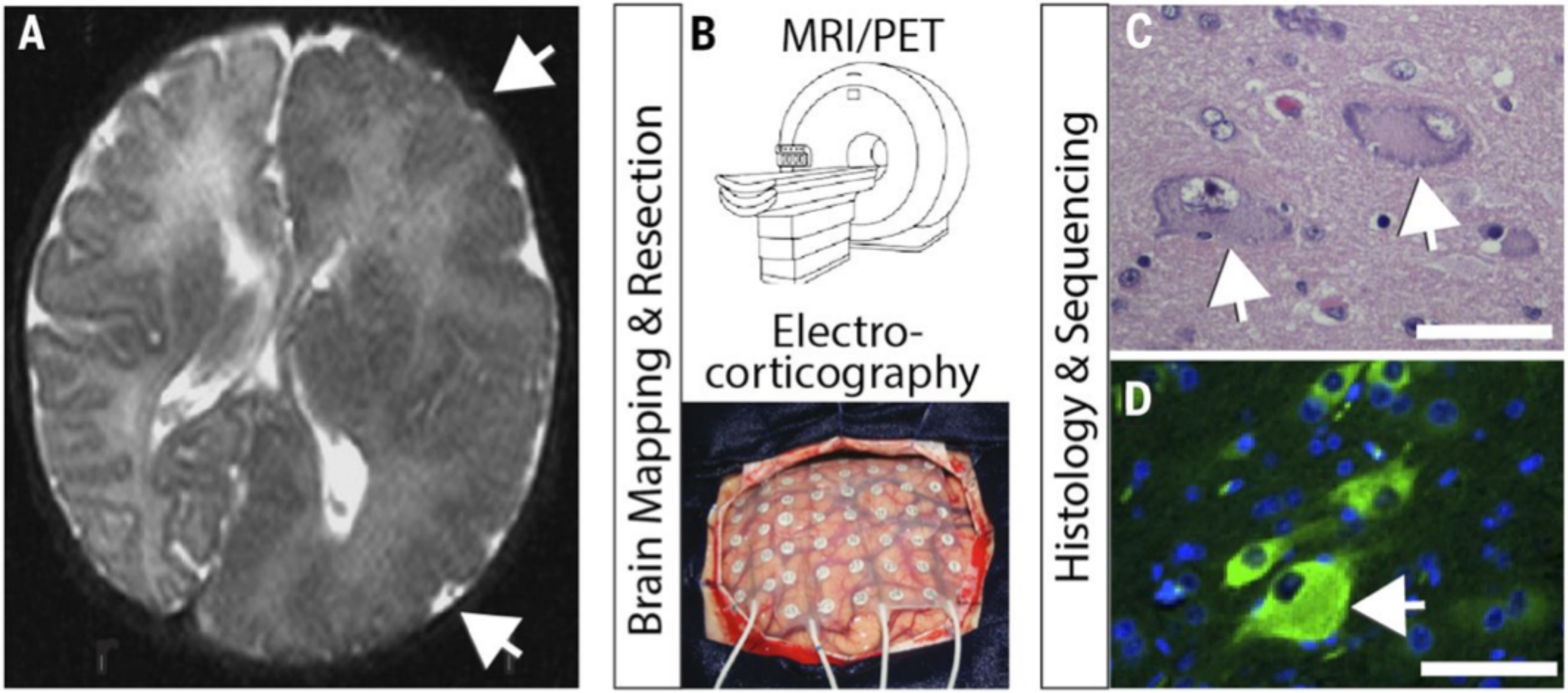
Recombination/crossover in germ cells

Tumor Evolution



The Clonal Evolution of Tumor Cell Populations

Peter C. Nowell (1976) *Science*. 194(4260):23-28 DOI: 10.1126/science.959840

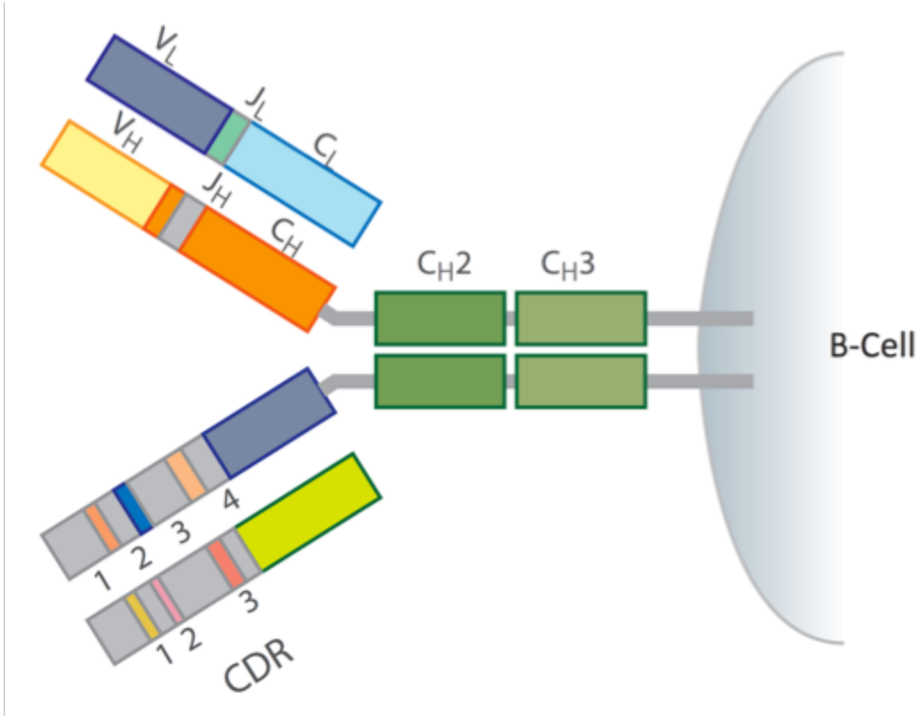


An example of brain somatic mosaicism that leads to a focal overgrowth condition.

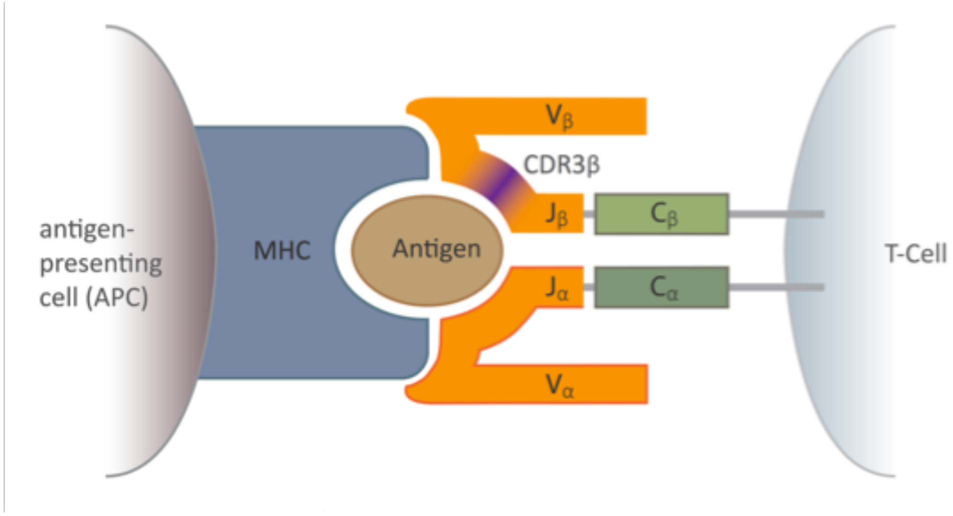
(**A**) Axial brain MRI of focal overgrowth from a 2-month-old child with intractable epilepsy and intellectual disability. (**B**) Brain mapping using high-resolution MRI is followed by surgical resection of diseased brain tissue. (**C**) Histological analysis with hematoxylin/eosin showing characteristic balloon cells consisting of large nuclei, distinct nucleoli, and glassy eosinophilic cytoplasm. (**D**) After surgery, the patient showed clinical improvement.

Intersection of diverse neuronal genomes and neuropsychiatric disease: The Brain Somatic Mosaicism Network. McConnell et al (2017) Science. doi: 10.1126/science.aal1641

Immunology



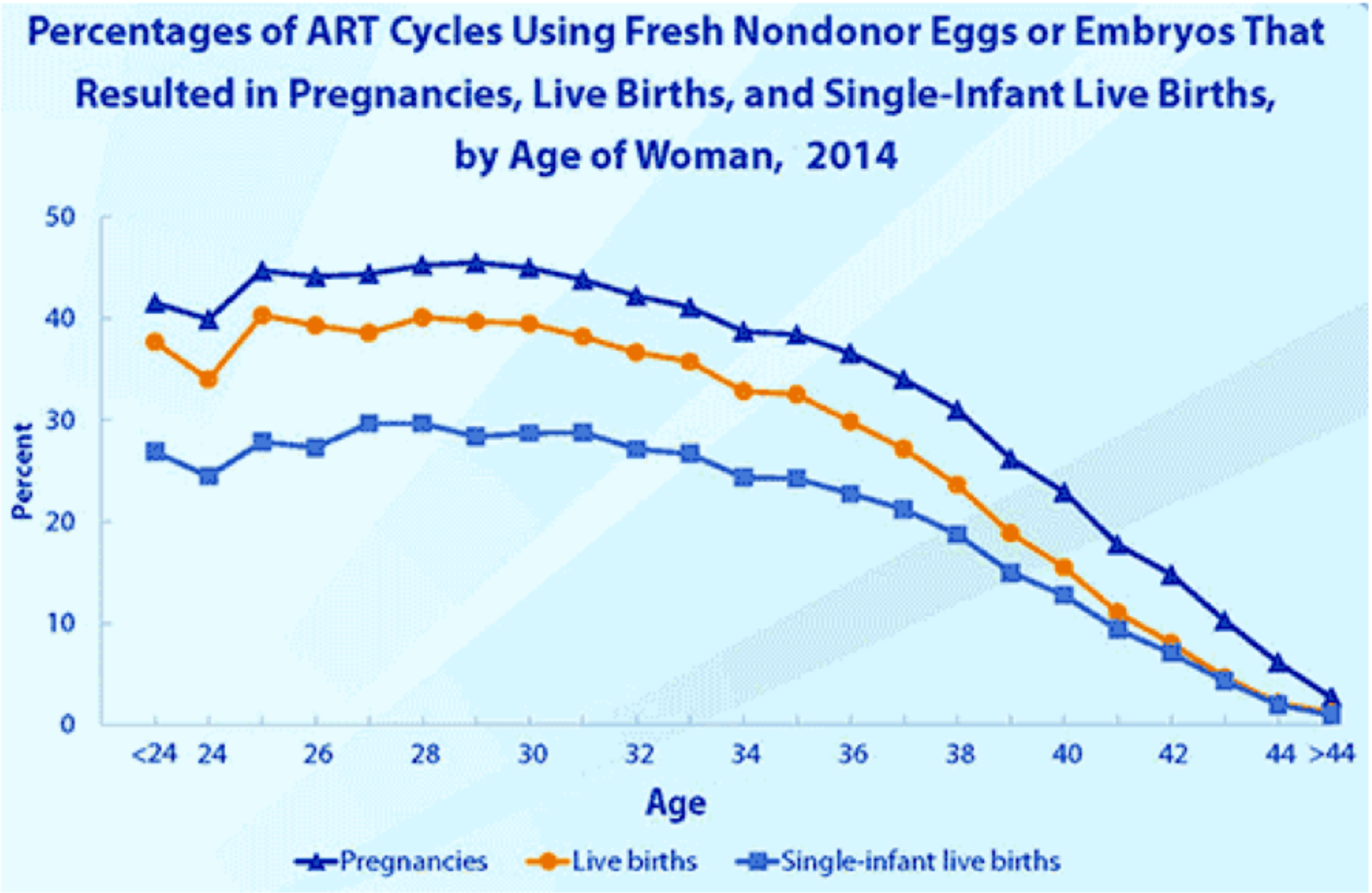
- Massive diversity rivaled only by germ cells
- Somatic recombination



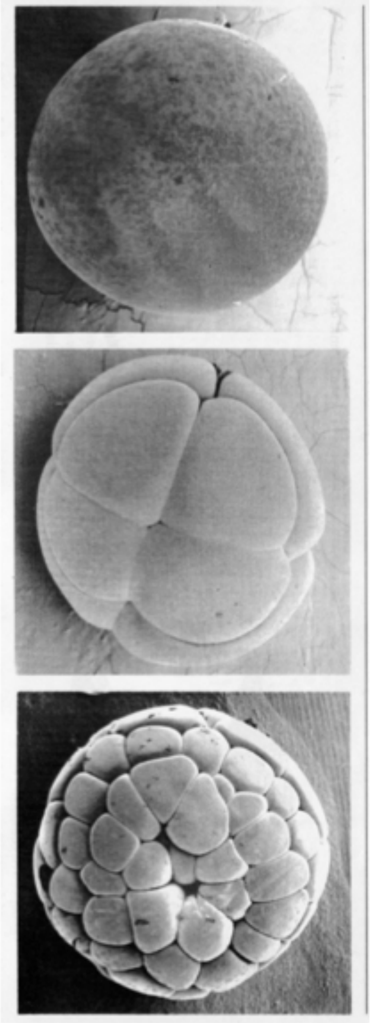
- B cells – antibody generation
- T cells – antigen response

Single cell research. Illumina.

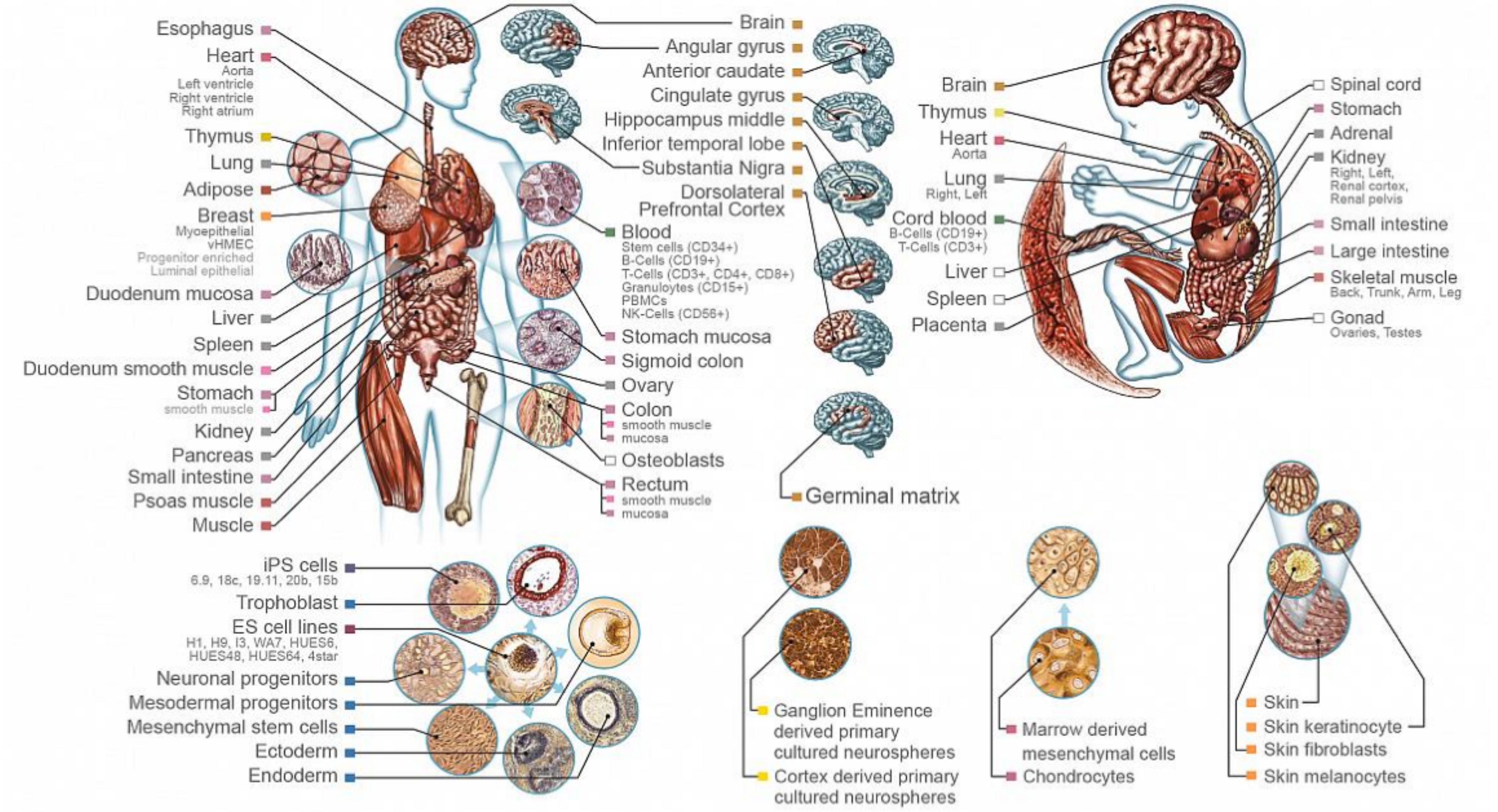
In-vitro Fertilization



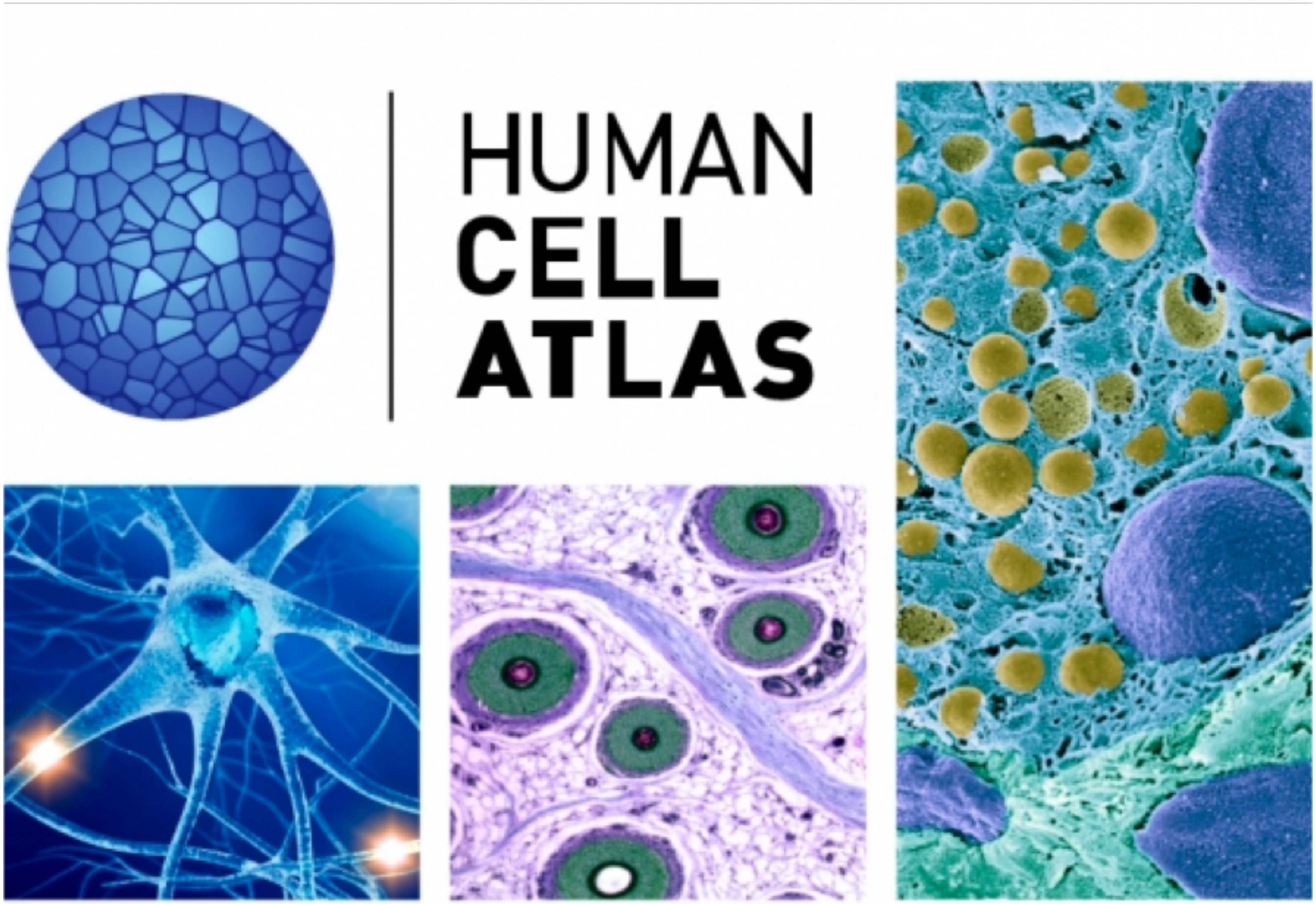
Percentages of ART Cycles Using Fresh Nondonor Eggs or Embryos That Resulted in Pregnancies, Live Births, and Single-Infant Live Births, by Age of Woman, 2014



Sources of (Cellular) Heterogeneity



Roadmap Epigenomics Consortium



https://www.humancellatlas.org/

Clustering Refresher

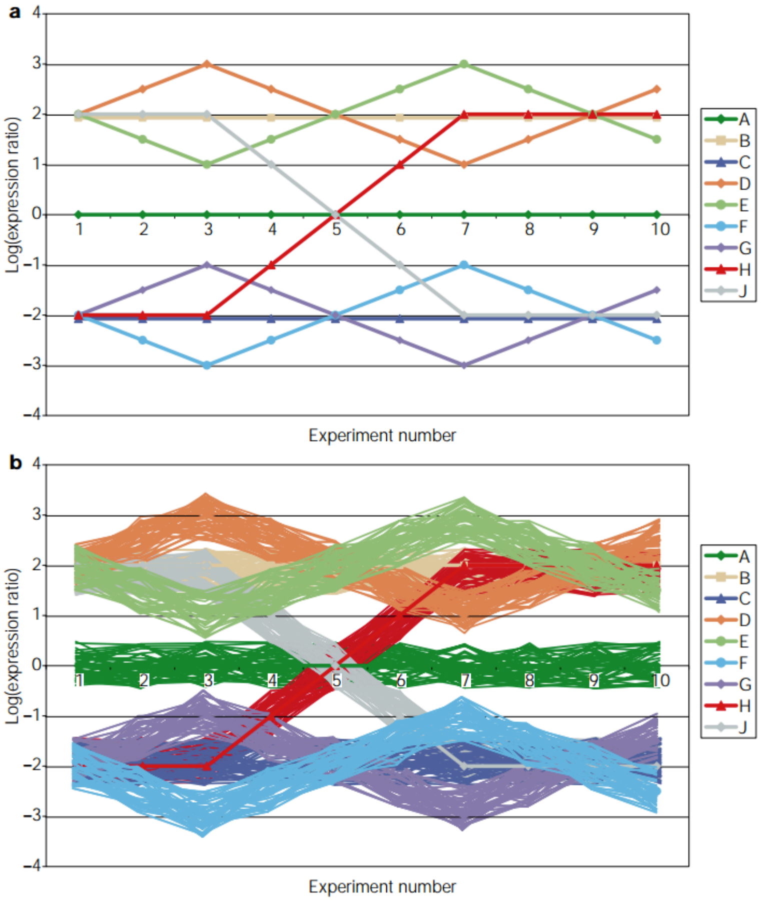
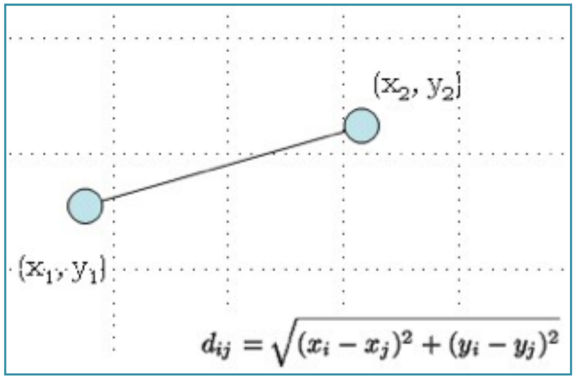
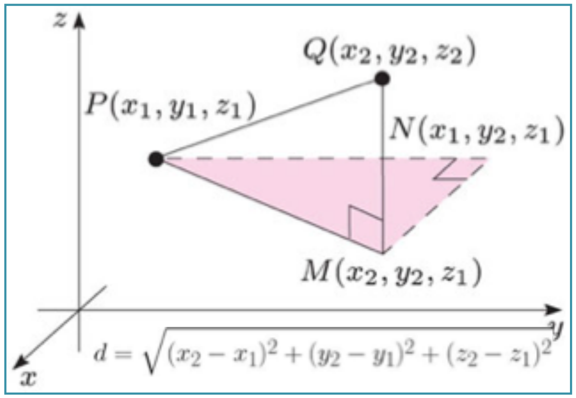


Figure 2 | **A synthetic gene-expression data set.** This data set provides an opportunity to evaluate how various clustering algorithms reveal different features of the data. **a** | Nine distinct gene-expression patterns were created with $\log_2$(ratio) expression measures defined for ten experiments. **b** | For each expression pattern, 50 additional genes were generated, representing variations on the basic patterns.

Euclidean Distance



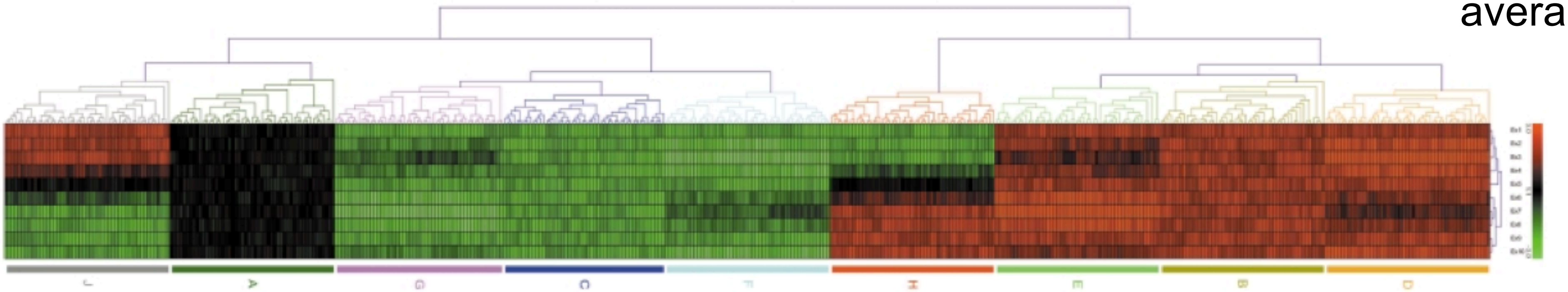


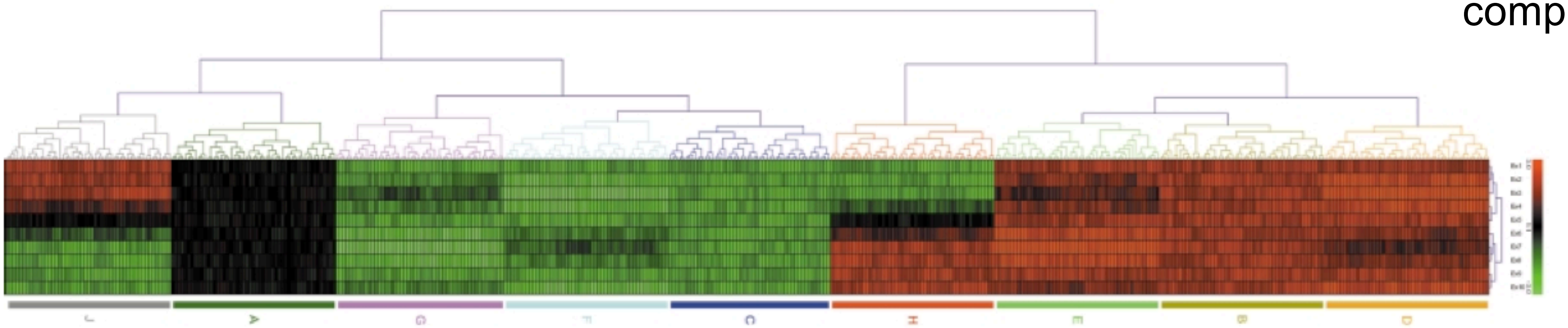
$$\mathrm{d}(\mathbf{p},\mathbf{q}) = \mathrm{d}(\mathbf{q},\mathbf{p}) = \sqrt{(q_1 - p_1)^2 + (q_2 - p_2)^2 + \cdots + (q_n - p_n)^2} = \sqrt{\sum_{i=1}^{n} (q_i - p_i)^2}.$$

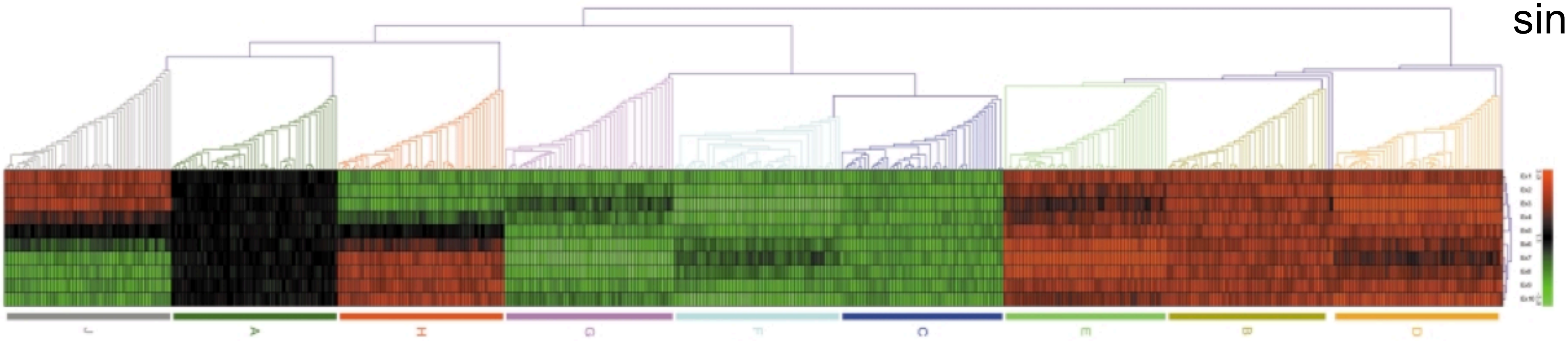
Computational genetics: Computational analysis of microarray data

Quackenbush (2001) *Nature Reviews Genetics.* doi:10.1038/35076576

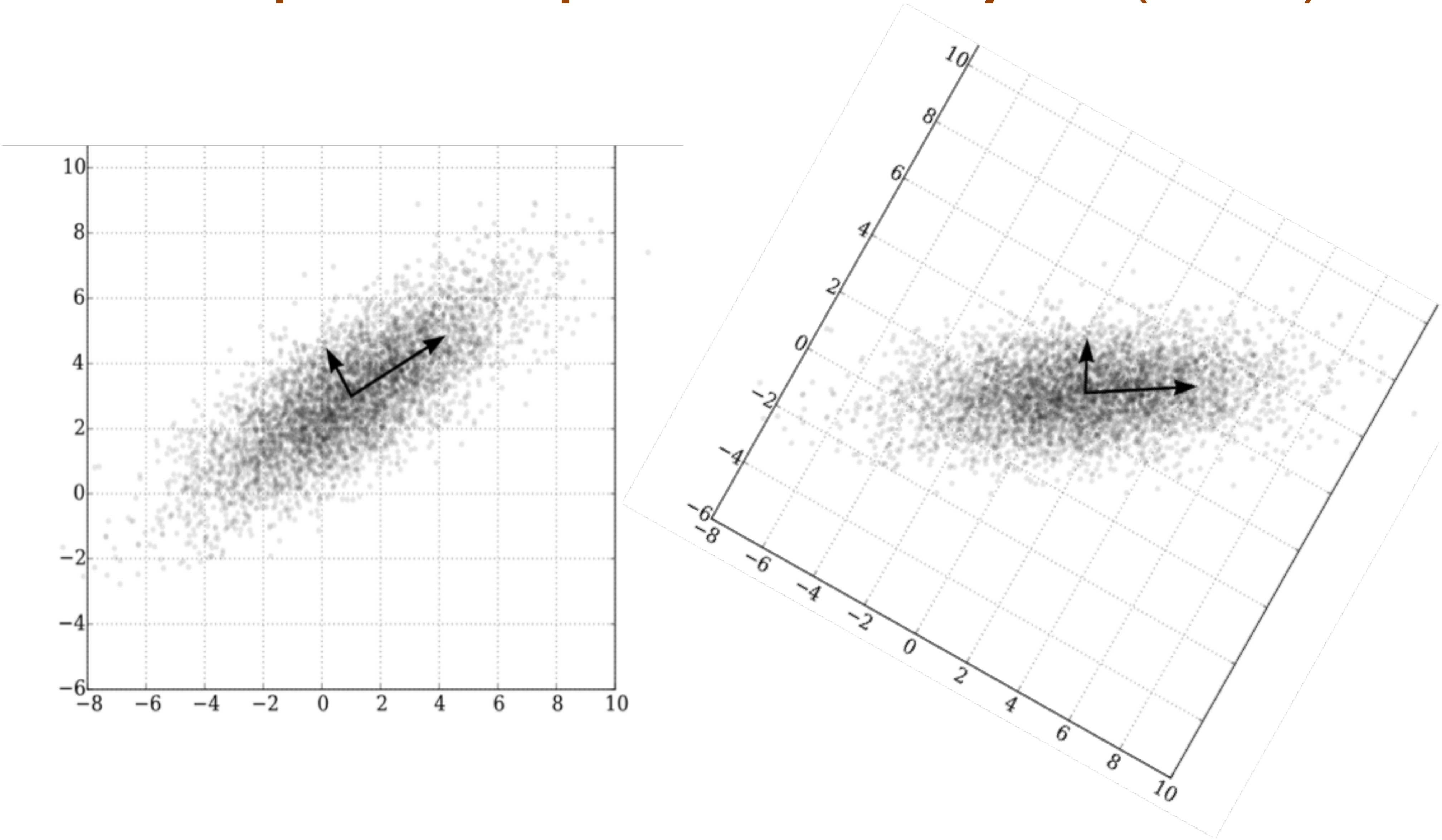
Hierarchical Clustering


average


complete


single

Principle Components Analysis (PCA)



PC1: “New X”- The dimension with the most variability
PC2: “New Y”- The dimension with the second most variability

Principle Components Analysis (PCA)

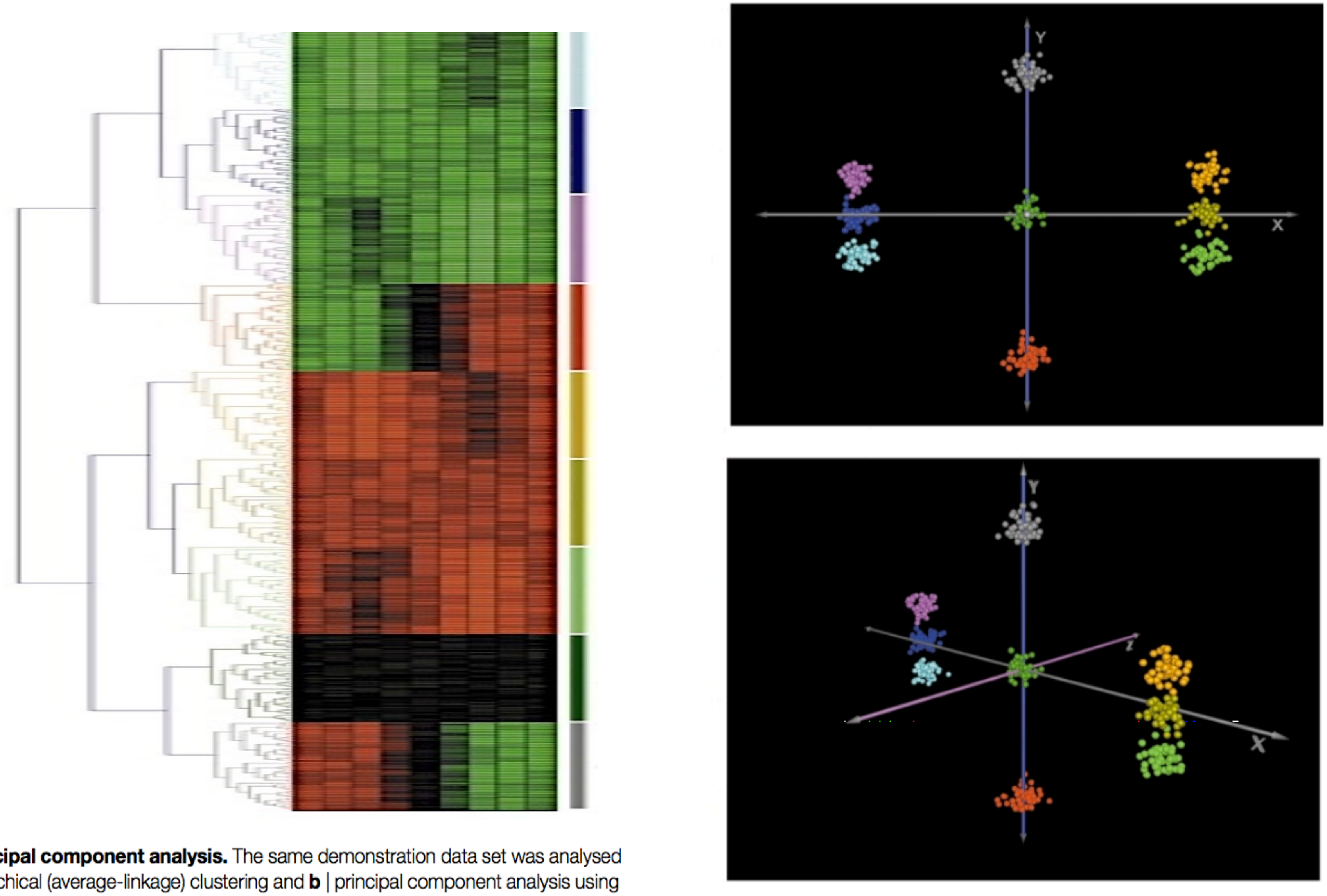
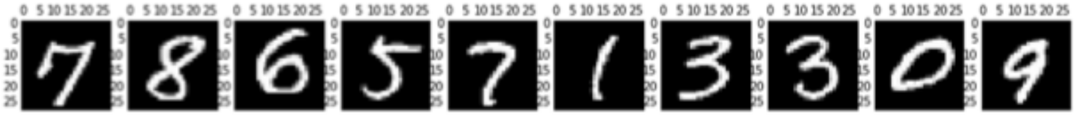
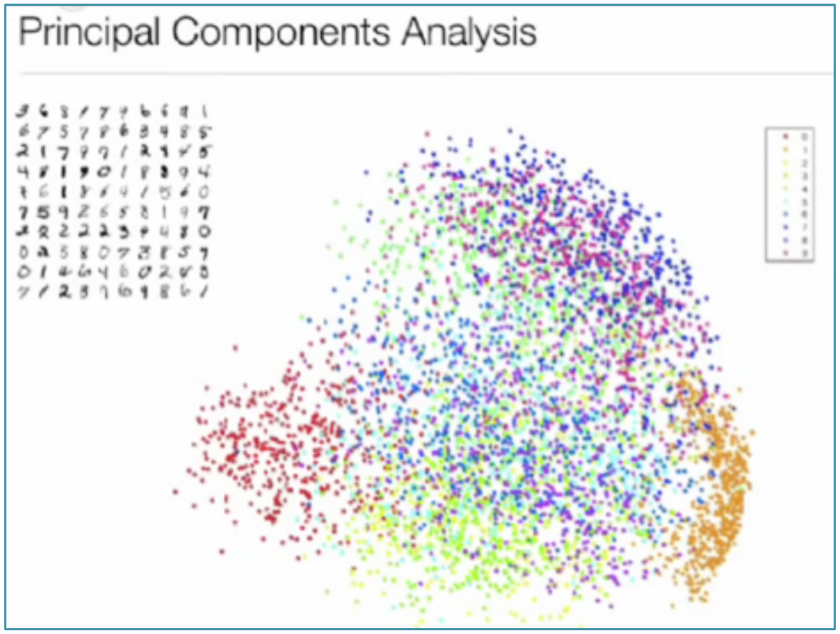
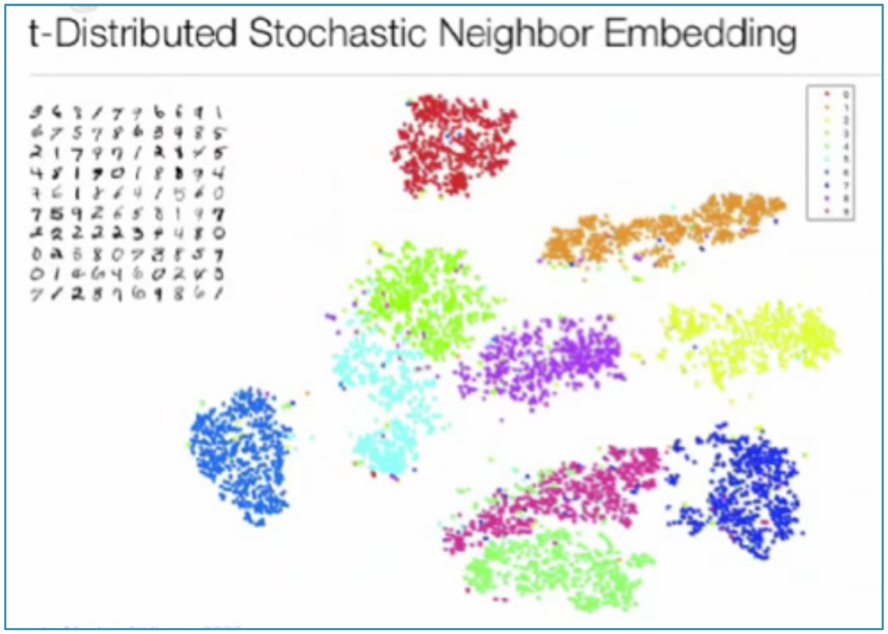


Figure 4 | **Principal component analysis.** The same demonstration data set was analysed using **a** | hierarchical (average-linkage) clustering and **b** | principal component analysis using Euclidean distance, to show how each treats the data, with genes colour coded on the basis of hierarchical clustering results for comparison.

PCA and t-SNE





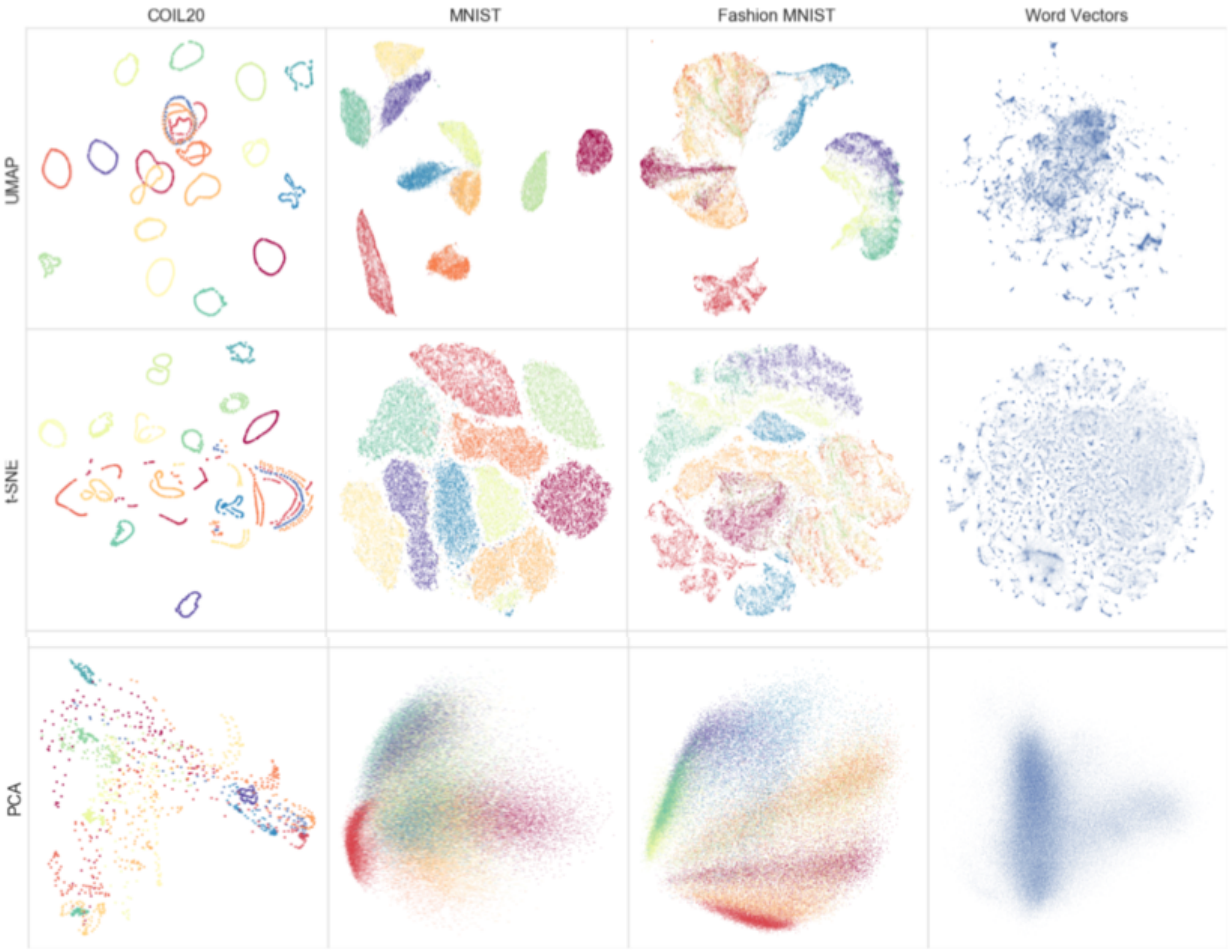


t-distributed <u>S</u>tochastic <u>N</u>eighborhood <u>E</u>mbedding

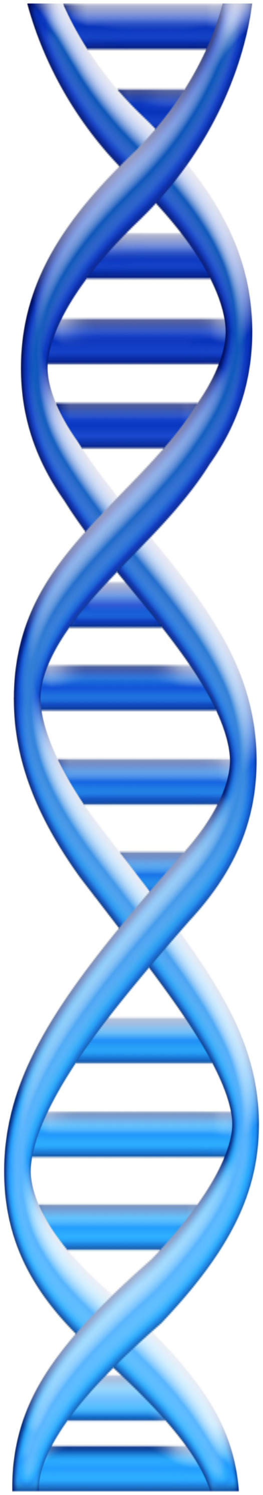
- Non-linear dimensionality reduction technique: distances are only locally meaningful
- Rather than Euclidean distances, for each point fits a Gaussian kernel to fit the nearest N neighbors (perplexity) that define the probabilities that two points should be close together
- Using an iterative spring embedding system to place high probability points nearby

Visualizing Data Using t-SNE
https://www.youtube.com/watch?v=RJVL80Gg3lA

UMAP



UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction
McInnes et al (2018) arXiv. 1802.03426



Single Cell Analysis

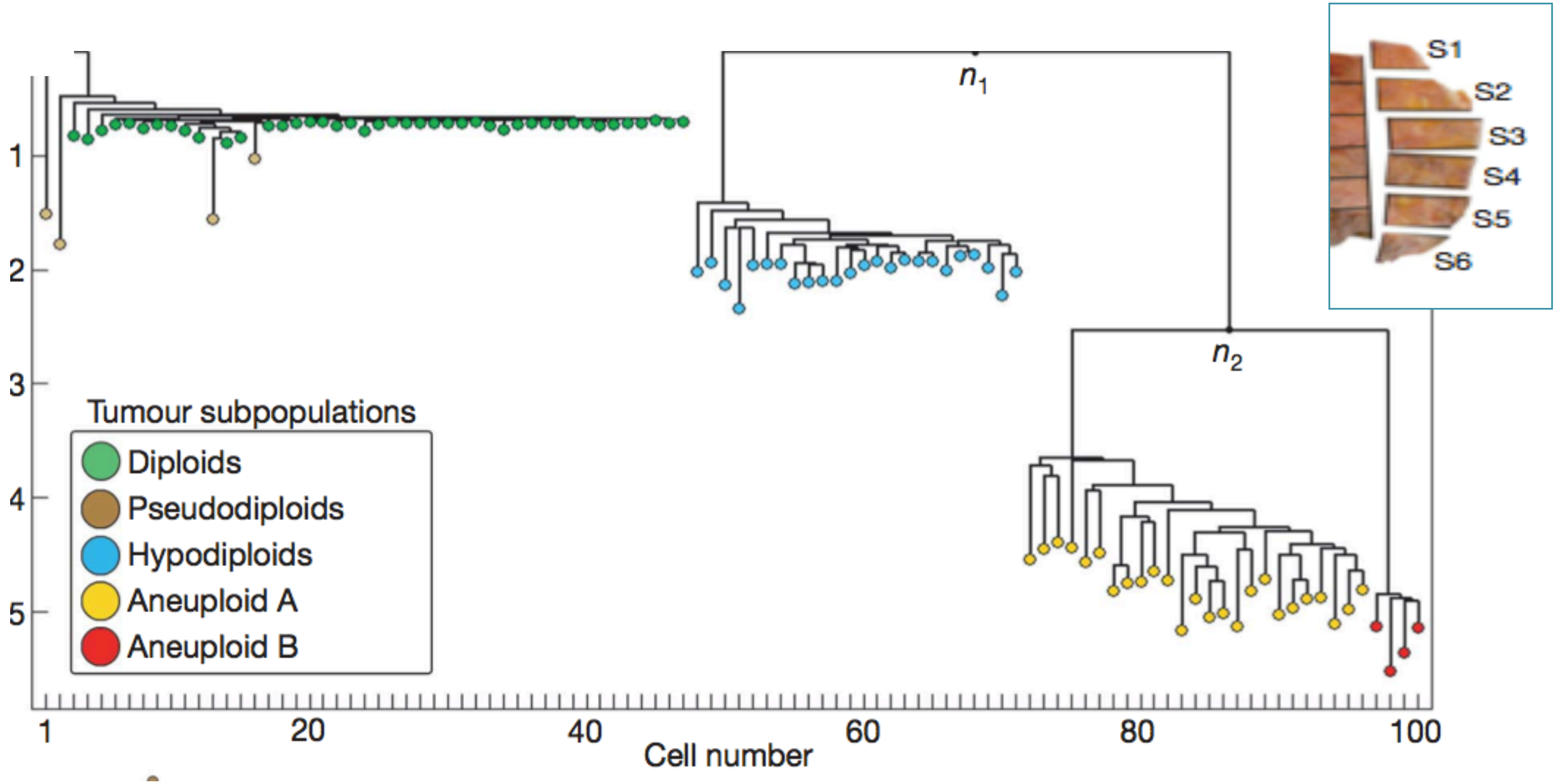
1. Why single cells?
2. scDNA
3. scRNA and other assays

LETTER

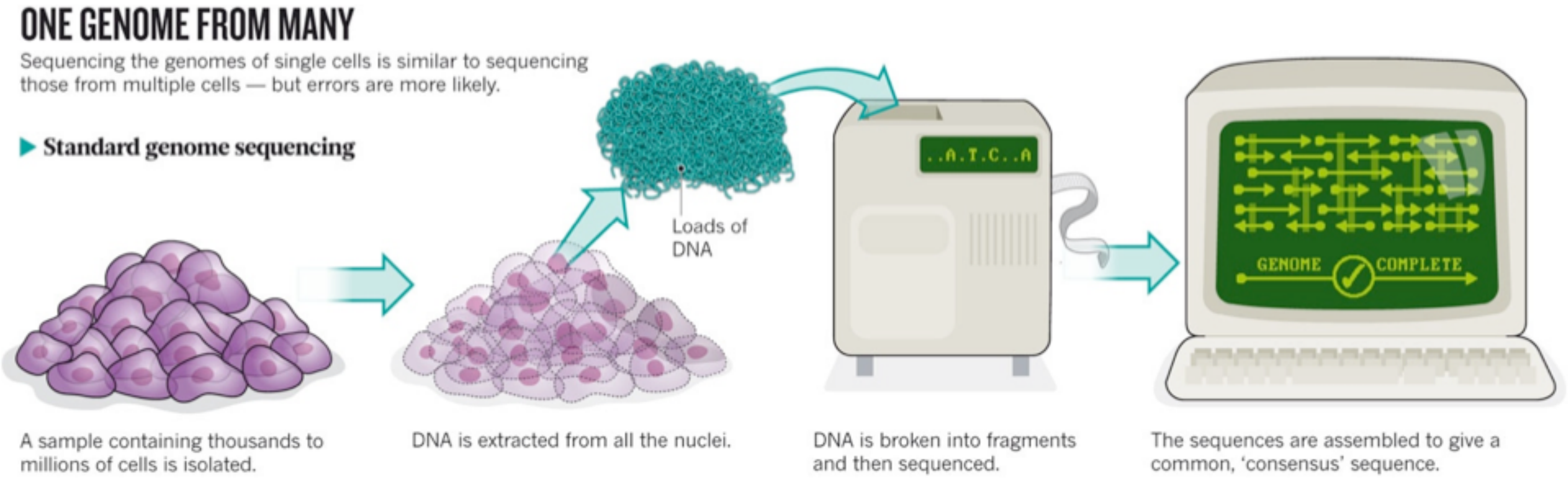
doi:10.1038/nature09807

Tumour evolution inferred by single-cell sequencing

Nicholas Navin[1,2], Jude Kendall[1], Jennifer Troge[1], Peter Andrews[1], Linda Rodgers[1], Jeanne McIndoo[1], Kerry Cook[1], Asya Stepansky[1], Dan Levy[1], Diane Esposito[1], Lakshmi Muthuswamy[3], Alex Krasnitz[1], W. Richard McCombie[1], James Hicks[1] & Michael Wigler[1]



Single-cell vs. bulk sequencing



ONE GENOME FROM MANY

Sequencing the genomes of single cells is similar to sequencing those from multiple cells — but errors are more likely.

▶ **Standard genome sequencing**

A sample containing thousands to millions of cells is isolated.

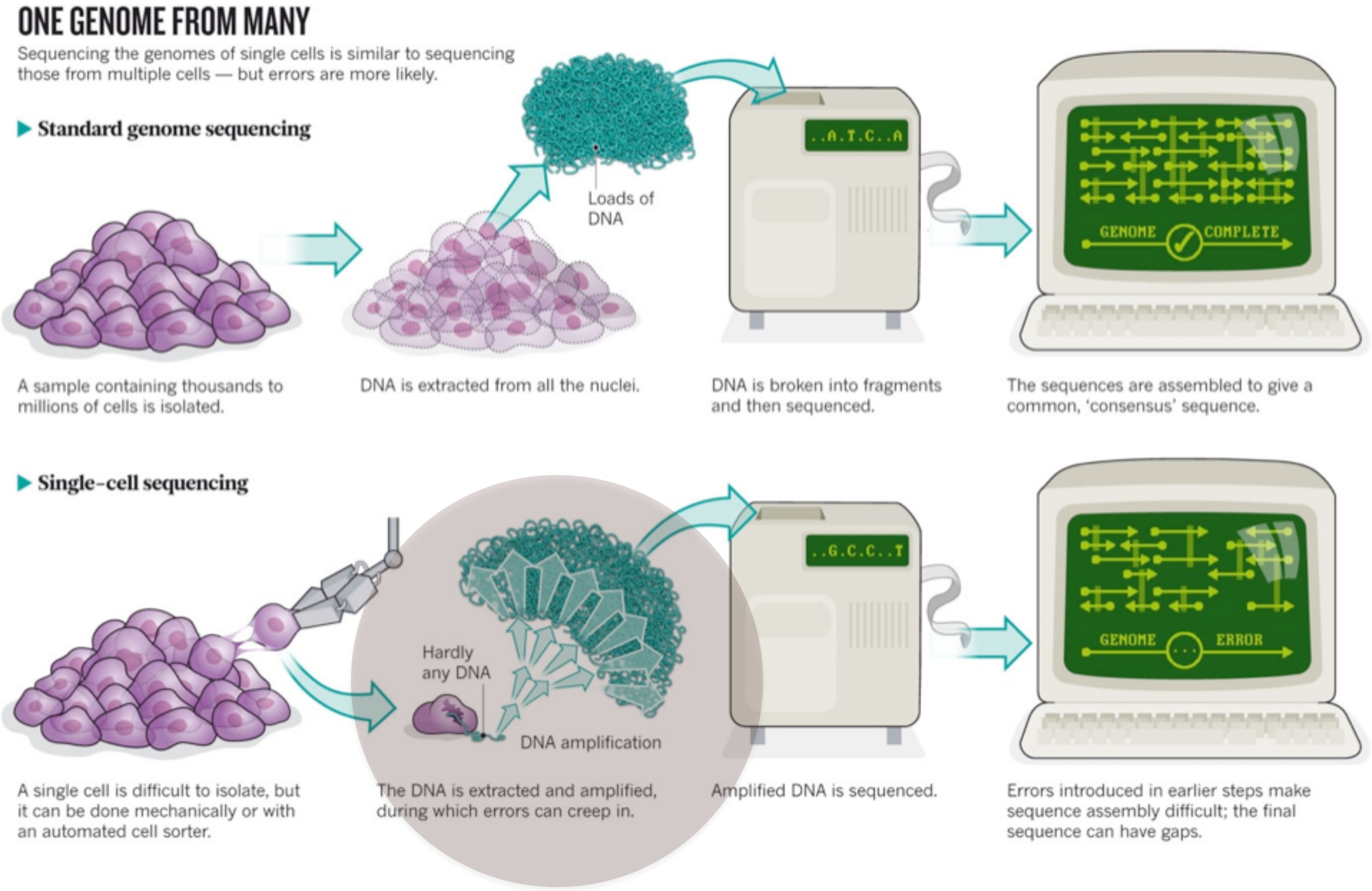
DNA is extracted from all the nuclei.

DNA is broken into fragments and then sequenced.

The sequences are assembled to give a common, 'consensus' sequence.

Brian Owens, Nature News 2012

Single-cell vs. bulk sequencing



ONE GENOME FROM MANY

Sequencing the genomes of single cells is similar to sequencing those from multiple cells — but errors are more likely.

▶ **Standard genome sequencing**

A sample containing thousands to millions of cells is isolated.

DNA is extracted from all the nuclei.

DNA is broken into fragments and then sequenced.

The sequences are assembled to give a common, 'consensus' sequence.

▶ **Single-cell sequencing**

A single cell is difficult to isolate, but it can be done mechanically or with an automated cell sorter.

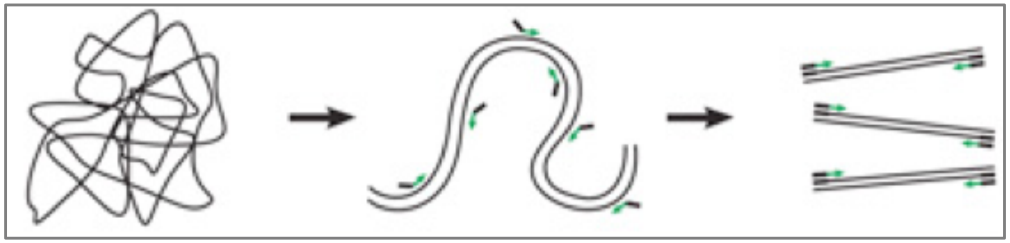
The DNA is extracted and amplified, during which errors can creep in.

Amplified DNA is sequenced.

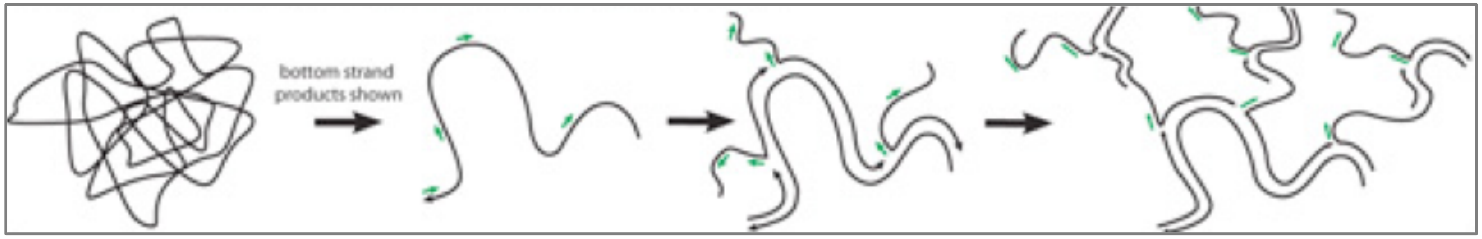
Errors introduced in earlier steps make sequence assembly difficult; the final sequence can have gaps.

Brian Owens, Nature News 2012

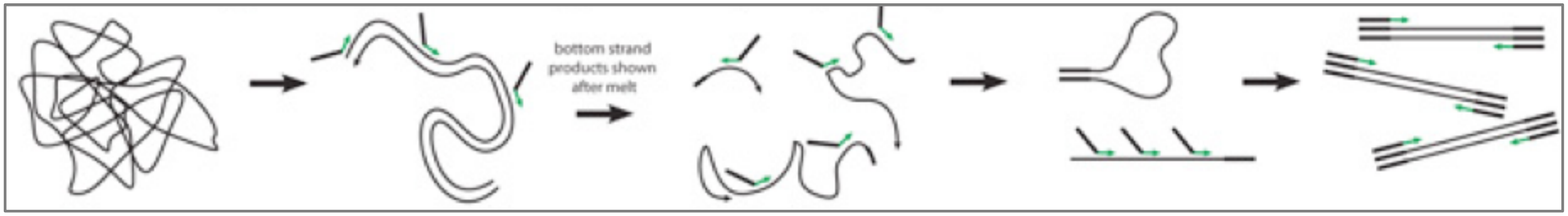
Whole Genome Amplification Techniques



DOP-PCR: Degenerate Oligonucleotide Primed PCR
Telenius et al. (1992) Genomics

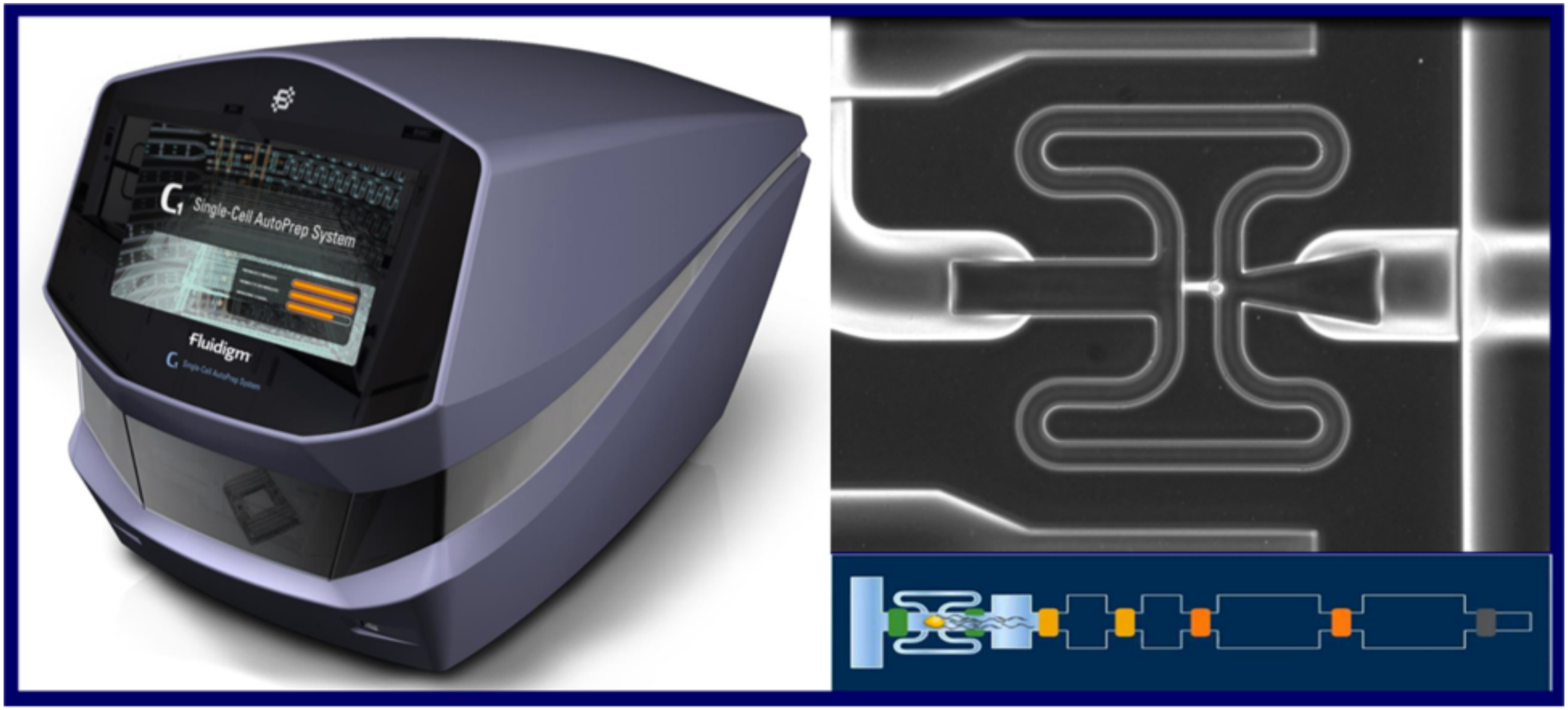


MDA: Multiple Displacement Amplification
Dean et al. (2002) PNAS



MALBAC: Multiple Annealing and Looping Based Amplification Cycles
Zong et al. (2012) Science

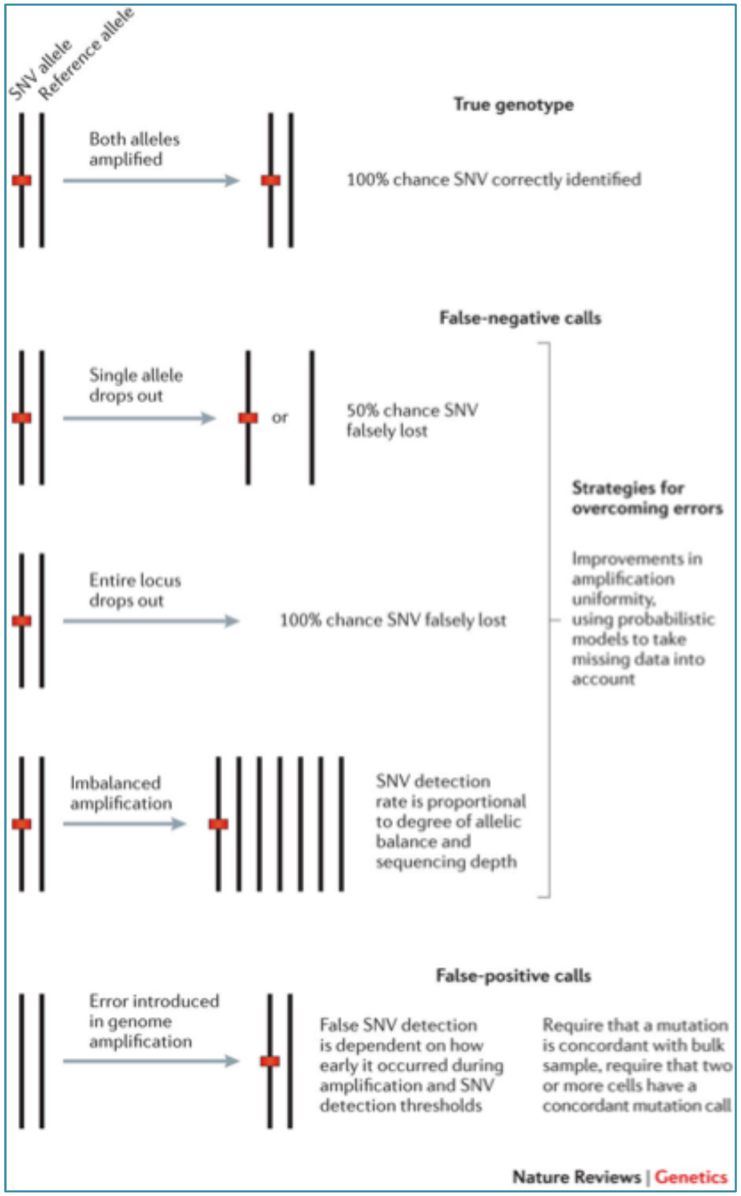
Paul Blainey, FEMS Microbiol Rev. 2013

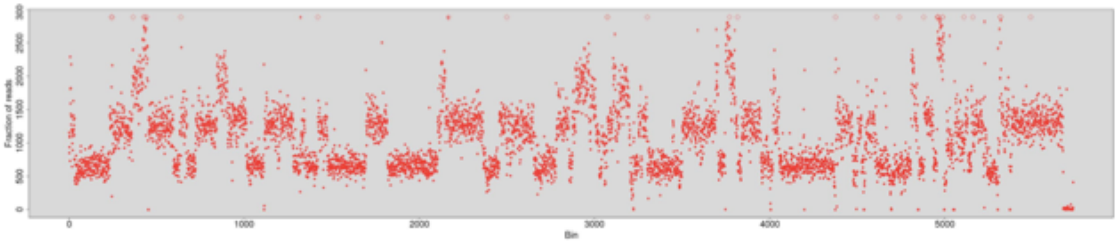

C1 Single-Cell AutoPrep System
Fluidigm
C1 Single-Cell AutoPrep System

Fluidigm C1

Benchtop automated single-cell isolation and preparation system(lysis and pre-amplification) for genomic analysis. The C1 System provides an easy and highly reproducible workflow to process ***96 single cells*** for DNA or RNA analysis.

scCNVs


SNV allele
Reference allele
True genotype
Both alleles amplified
100% chance SNV correctly identified
False-negative calls
Single allele drops out
or
50% chance SNV falsely lost
Strategies for overcoming errors
Improvements in amplification uniformity, using probabilistic models to take missing data into account
Entire locus drops out
100% chance SNV falsely lost
Imbalanced amplification
SNV detection rate is proportional to degree of allelic balance and sequencing depth
False-positive calls
Error introduced in genome amplification
False SNV detection is dependent on how early it occurred during amplification and SNV detection thresholds
Require that a mutation is concordant with bulk sample, require that two or more cells have a concordant mutation call
Nature Reviews | Genetics



Potential for biases at every step

- WGA: Non-uniform amplification
- Library Preparation: Low complexity, read duplications, barcoding
- Sequencing: GC artifacts, short reads
- Computation: mappability, GC correction, segmentation, tree building

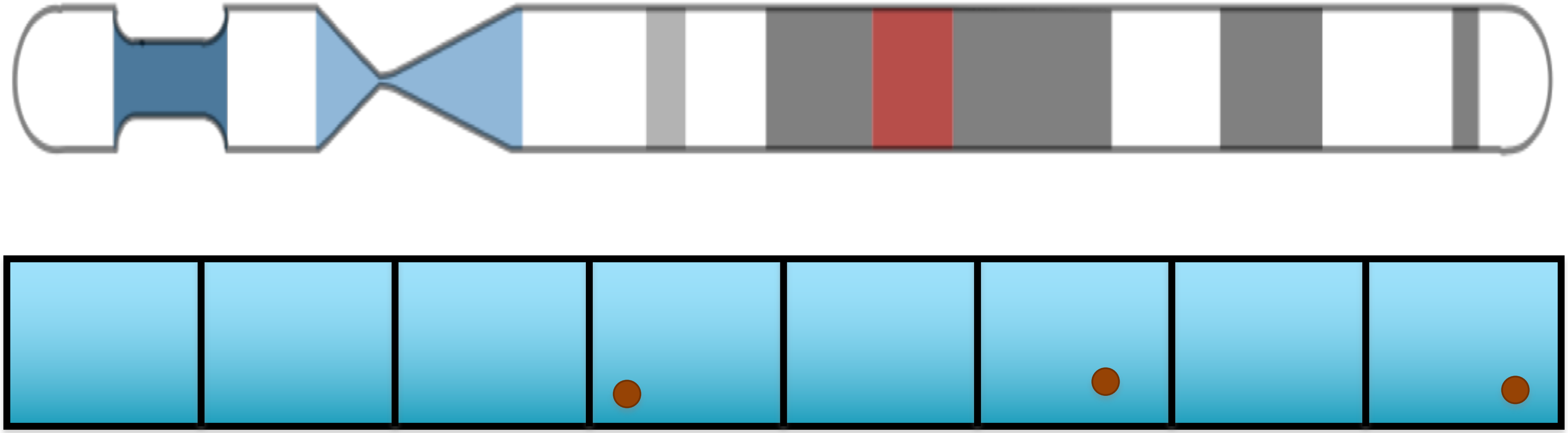
Coverage is very sparse and noisy

-> requires special processing

Single-cell genome sequencing: current state of the science

Gawad et al (2016) Nature Reviews Genetics. doi:10.1038/nrg.2015.16

1) Binning

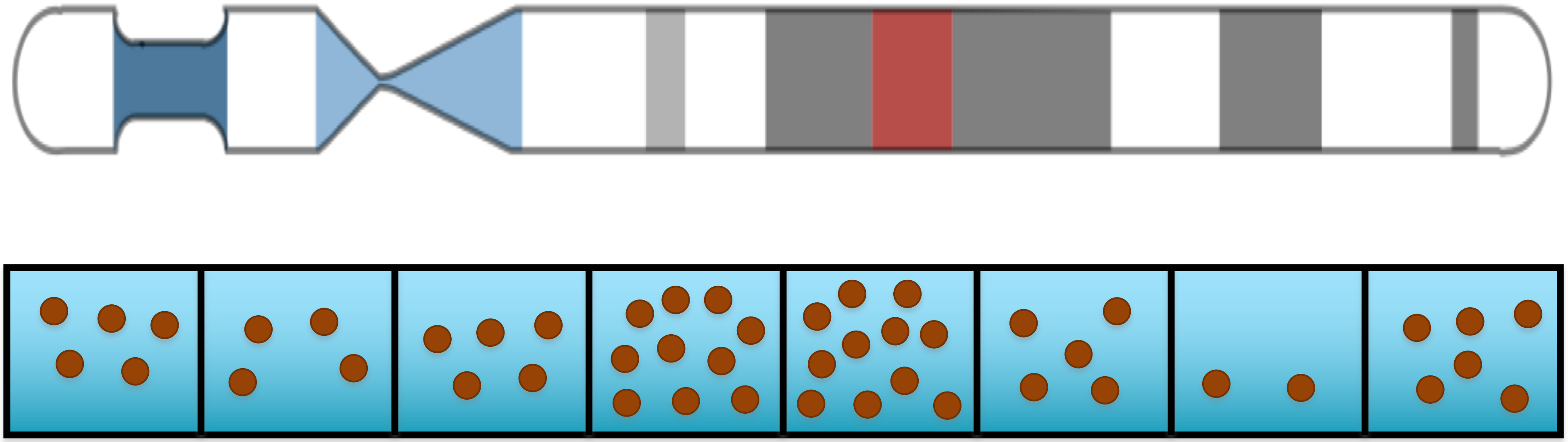


Single Cell CNV analysis

- Divide the genome into "bins" with ~50 – 100 reads / bin
- Map the reads and count reads per bin

Use uniquely mappable bases to establish bins

1) Binning



Single Cell CNV analysis

- Divide the genome into “bins” with ~50 – 100 reads / bin
- Map the reads and count reads per bin

Use uniquely mappable bases to establish bins

1) Binning

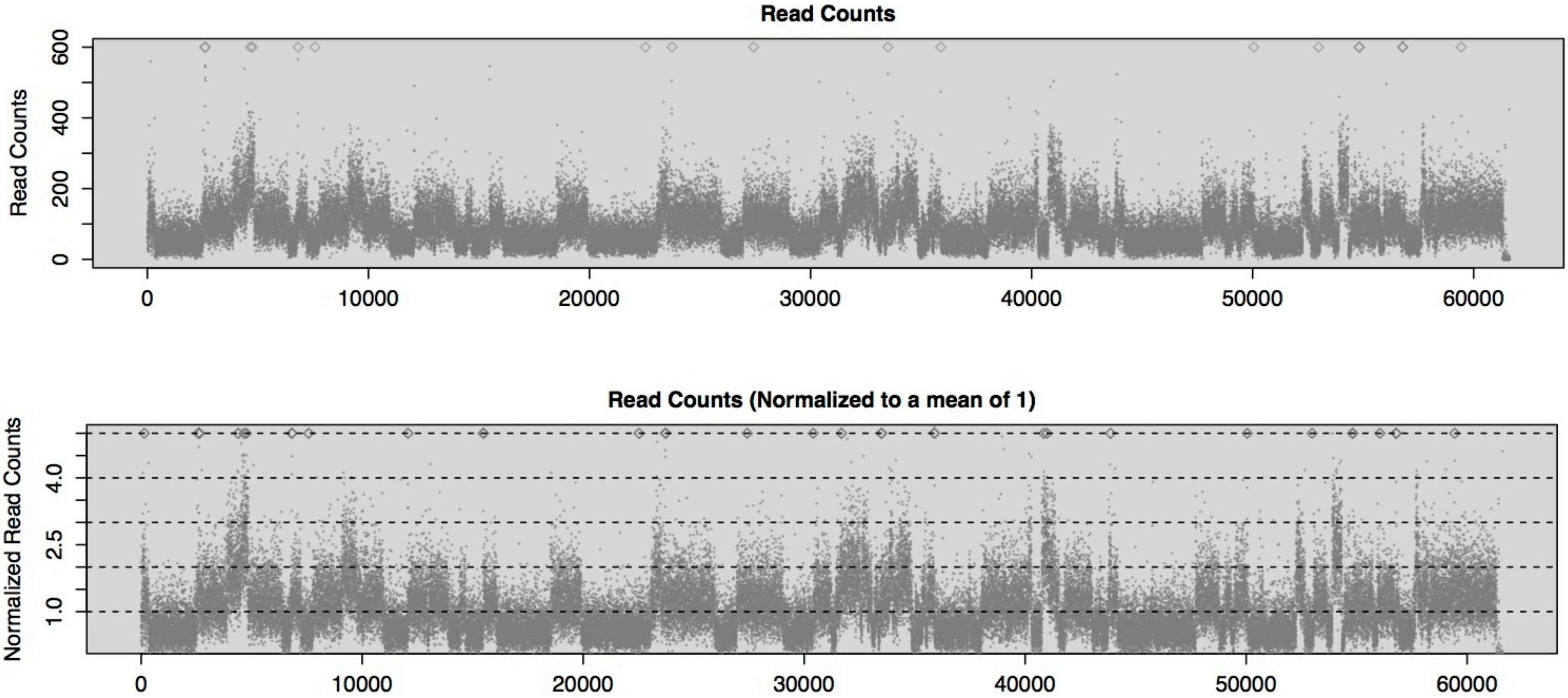
5	4	5	10	11	5	2	5

Single Cell CNV analysis

- Divide the genome into "bins" with ~50 – 100 reads / bin
- Map the reads and count reads per bin

Use uniquely mappable bases to establish bins

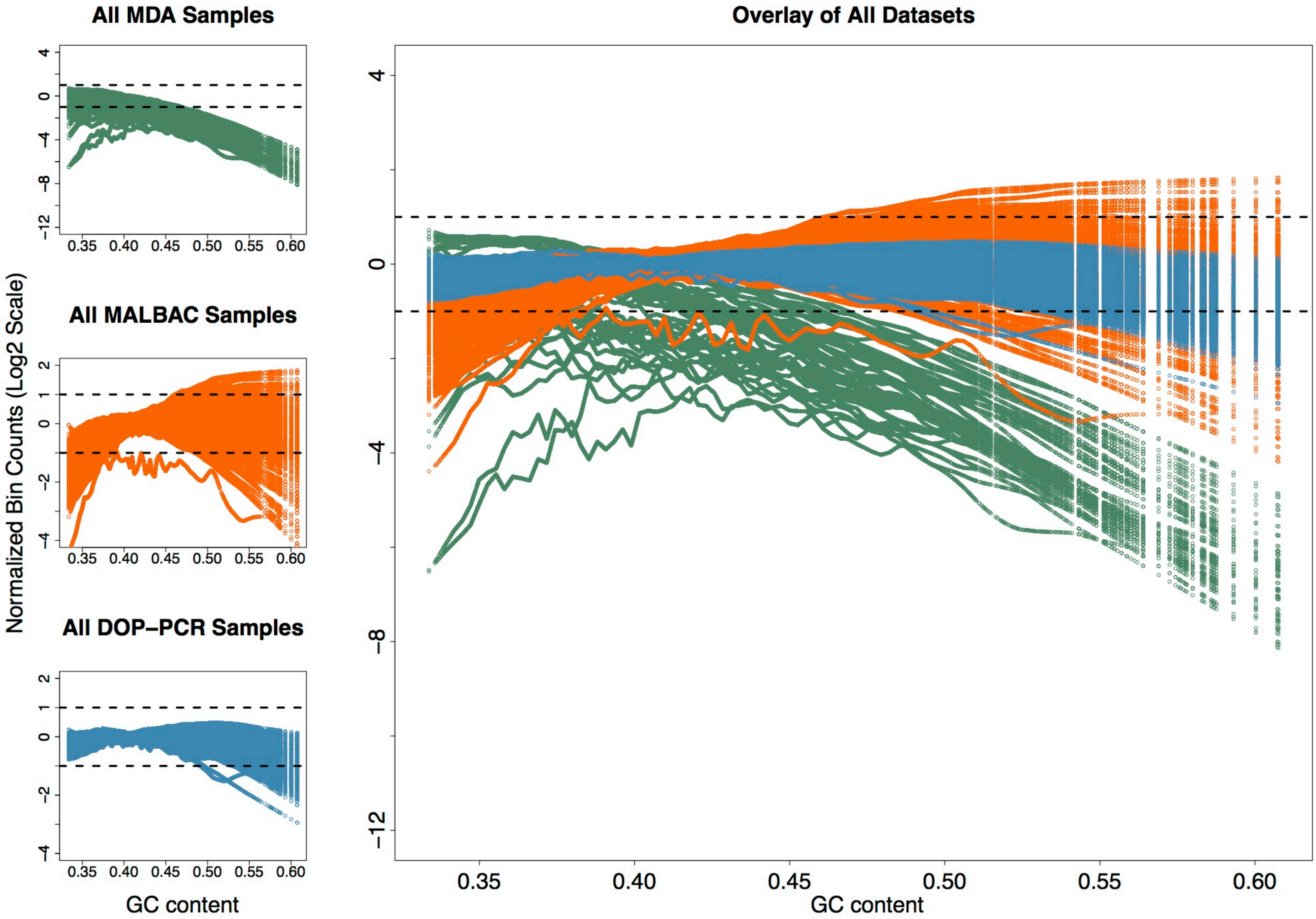
2) Normalization


Read Counts

Read Counts (Normalized to a mean of 1)

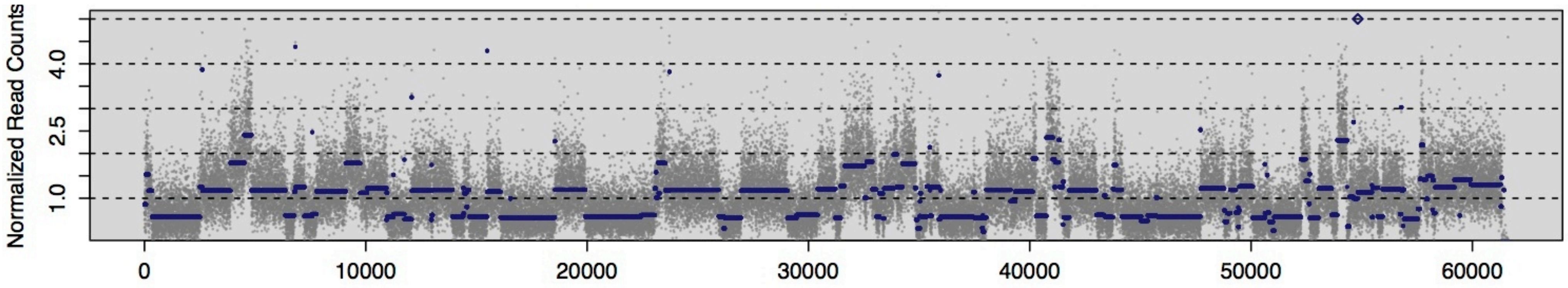
Also correct for mappability, GC content, amplification biases

GC Bias

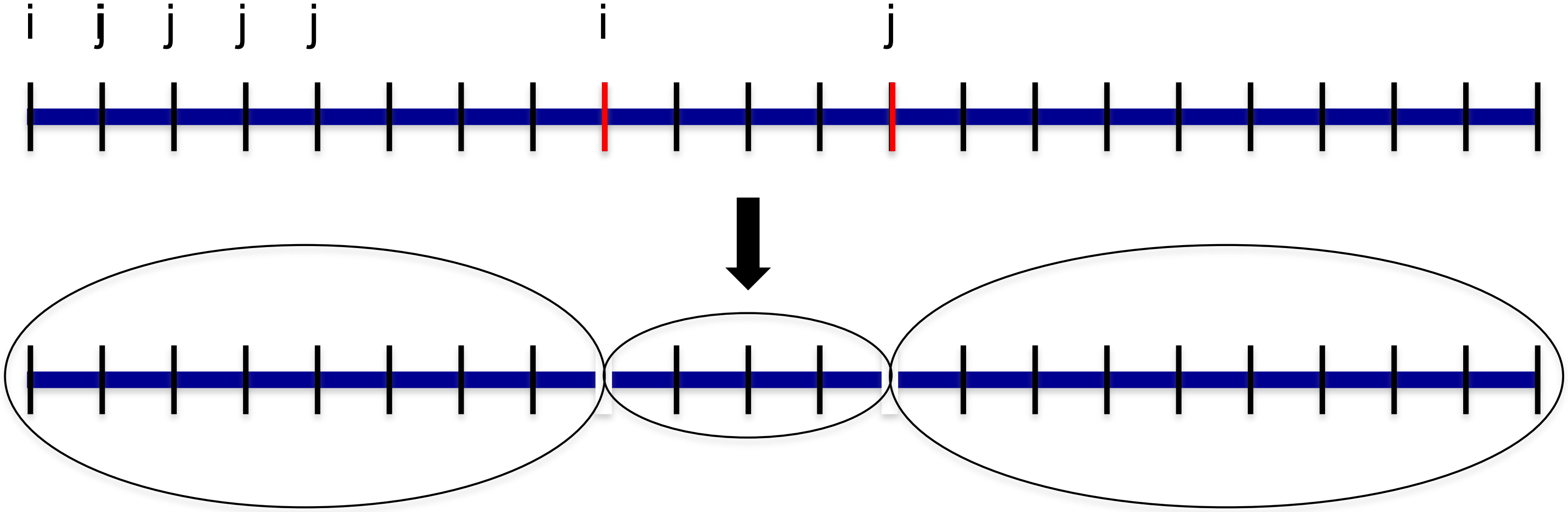


Garvin and Aboukhalil et al., Nature Methods, 2015

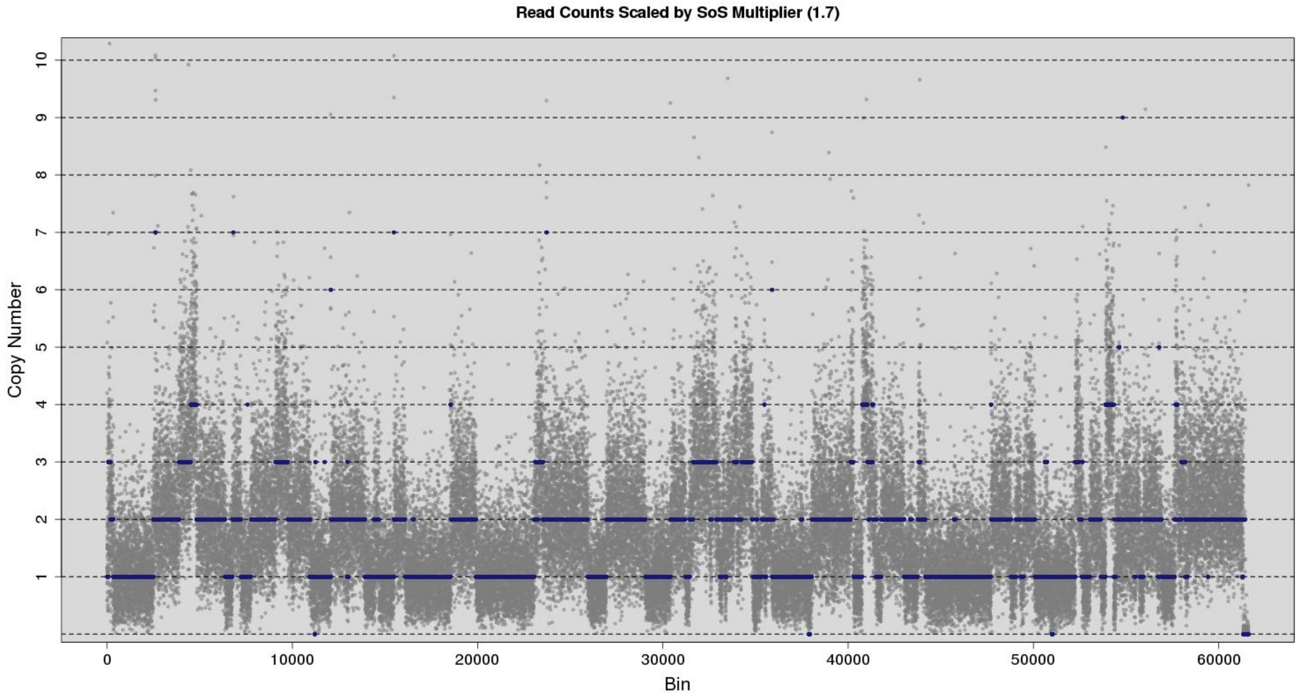
3) Segmentation



Circular Binary Segmentation (CBS)

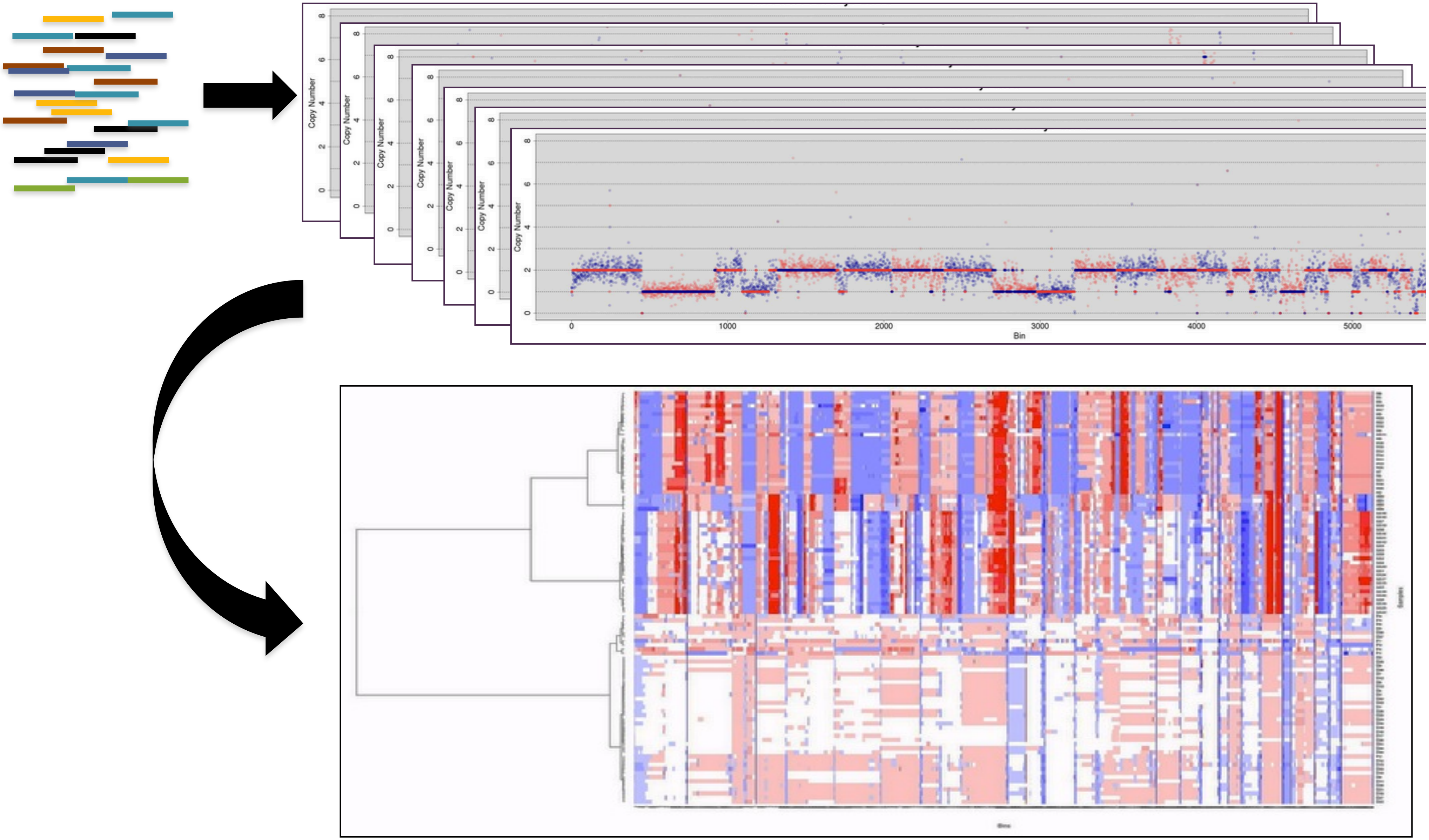


4) Estimating Copy Number



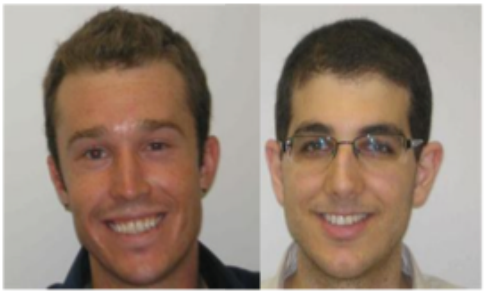
$$CN = argmin\left\{\sum_{i,j}(\hat{Y}_{i,j} - Y_{i,j})^2\right\}$$

5) Cells to Populations



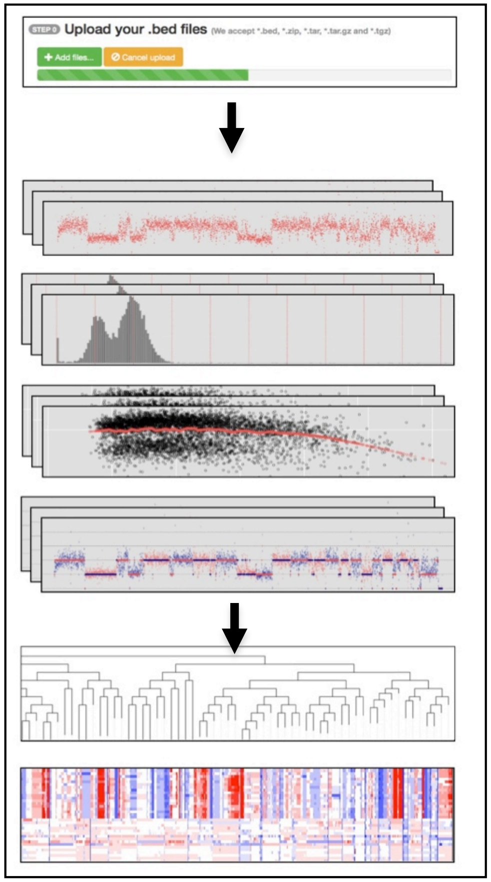
Gingko

http://qb.cshl.edu/ginkgo



Interactive Single Cell CNV analysis & clustering

- Easy-to-use, web interface, parameterized for binning, segmentation, clustering, etc
- Per cell through project-wide analysis in any species



STEP 0 Upload your .bed files (We accept *.bed, *.zip, *.tar, *.tar.gz and *.tgz)

Compare MDA, DOP-PCR, and MALBAC

- DOP-PCR shows superior resolution and consistency

Available for collaboration

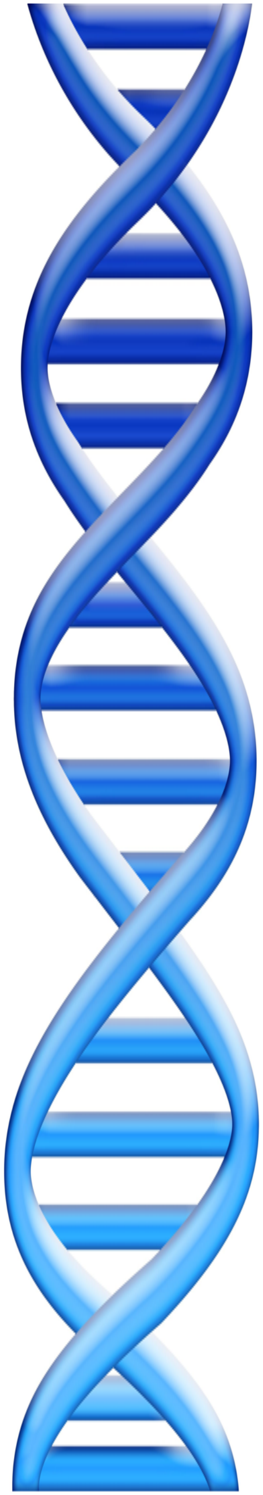
- Analyzing CNVs with respect to different clinical outcomes
- Extending clustering methods, prototyping scRNA

Interactive analysis and assessment of single-cell copy-number variations.
Garvin T, Aboukhalil R, Kendall J, Baslan T, Atwal GS, Hicks J, Wigler M, Schatz MC (2015)
Nature Methods doi:10.1038/nmeth.3578

scCNV-Seq

Single Cell CNV-Seq

- Reveal genomic heterogeneity
- Understand clonal evolution
- Determine pathogenesis and cancer progression
- Scalable from 100s-1000s of cells
- Single-cell CNV calling
- Call CNVs down to 100kb resolution
- CNV-Seq specific software pipeline



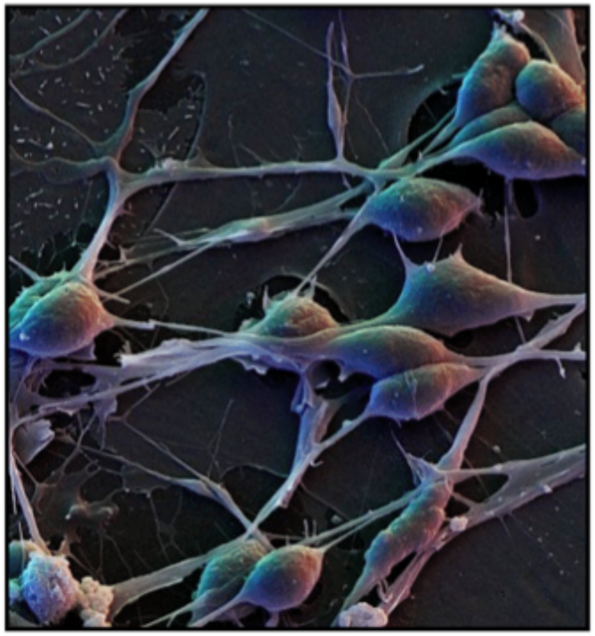
Single Cell Analysis

1. Why single cells?
2. scDNA
3. scRNA and other assays

- **Single-cell RNA sequencing,** “the bioinformatician’s microscope”

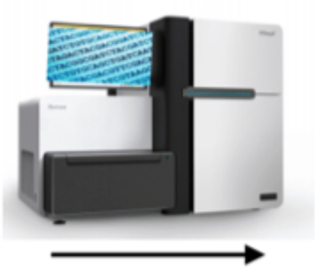
— a snapshot of the underlying biology in a data matrix.

Brain cells



Biological sample

number of times **gene i** was expressed in **cell j**



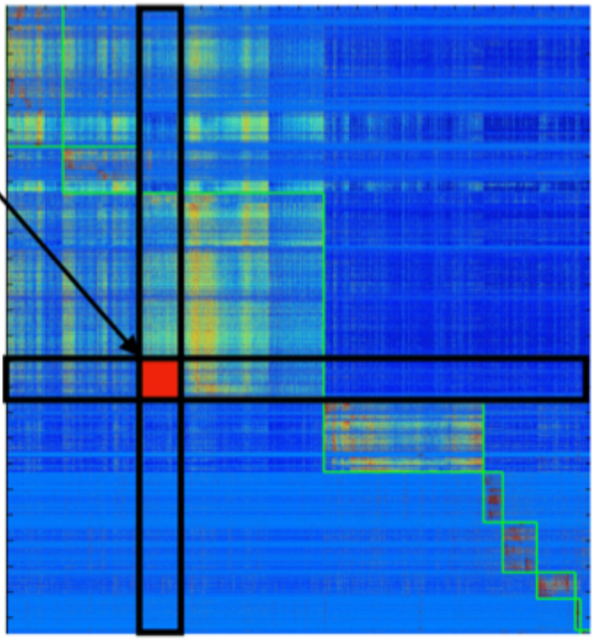
sequencing

Single Cells (samples)

cell j

Genes (features)

gene i

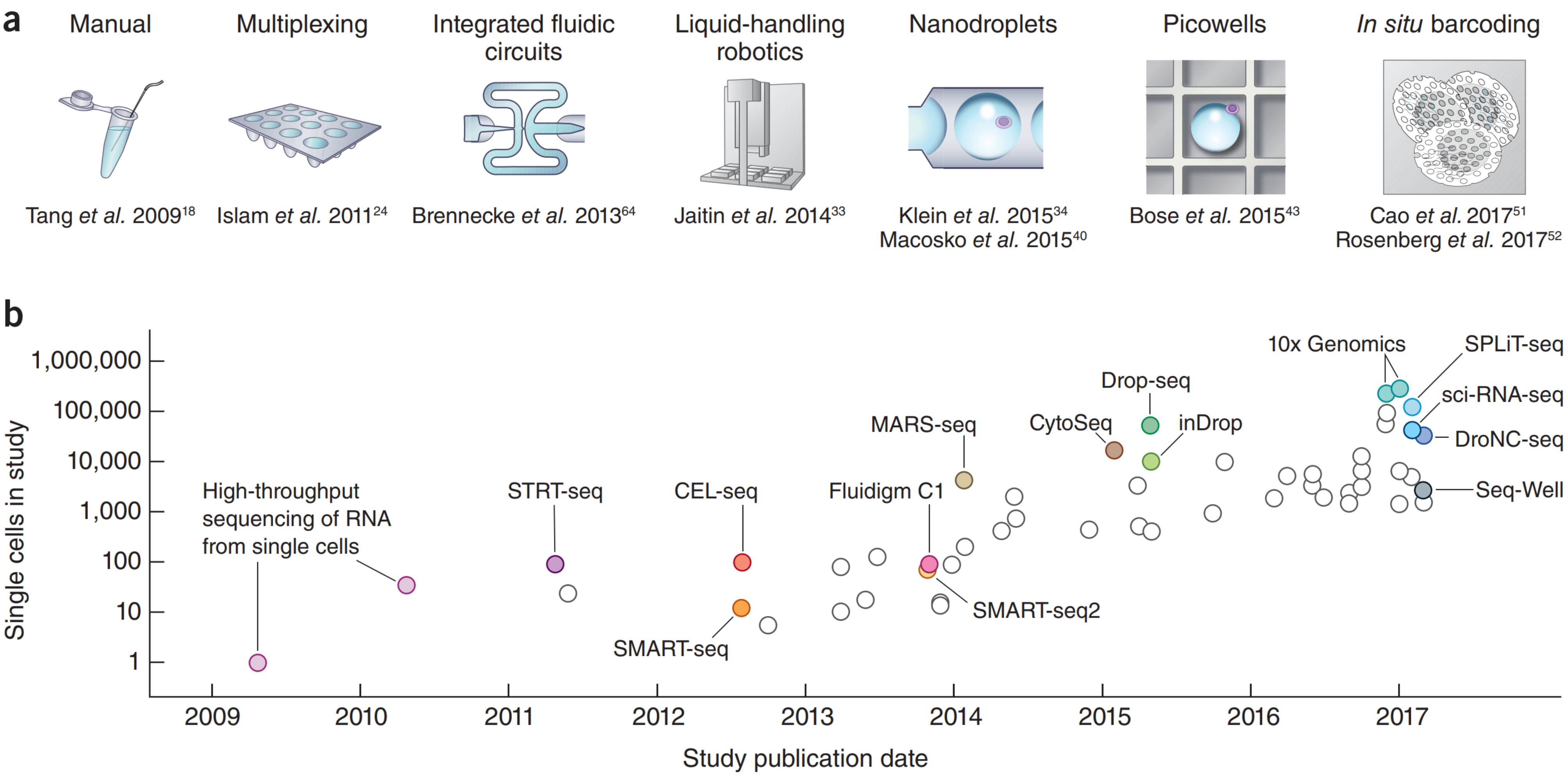


Gene expression matrix

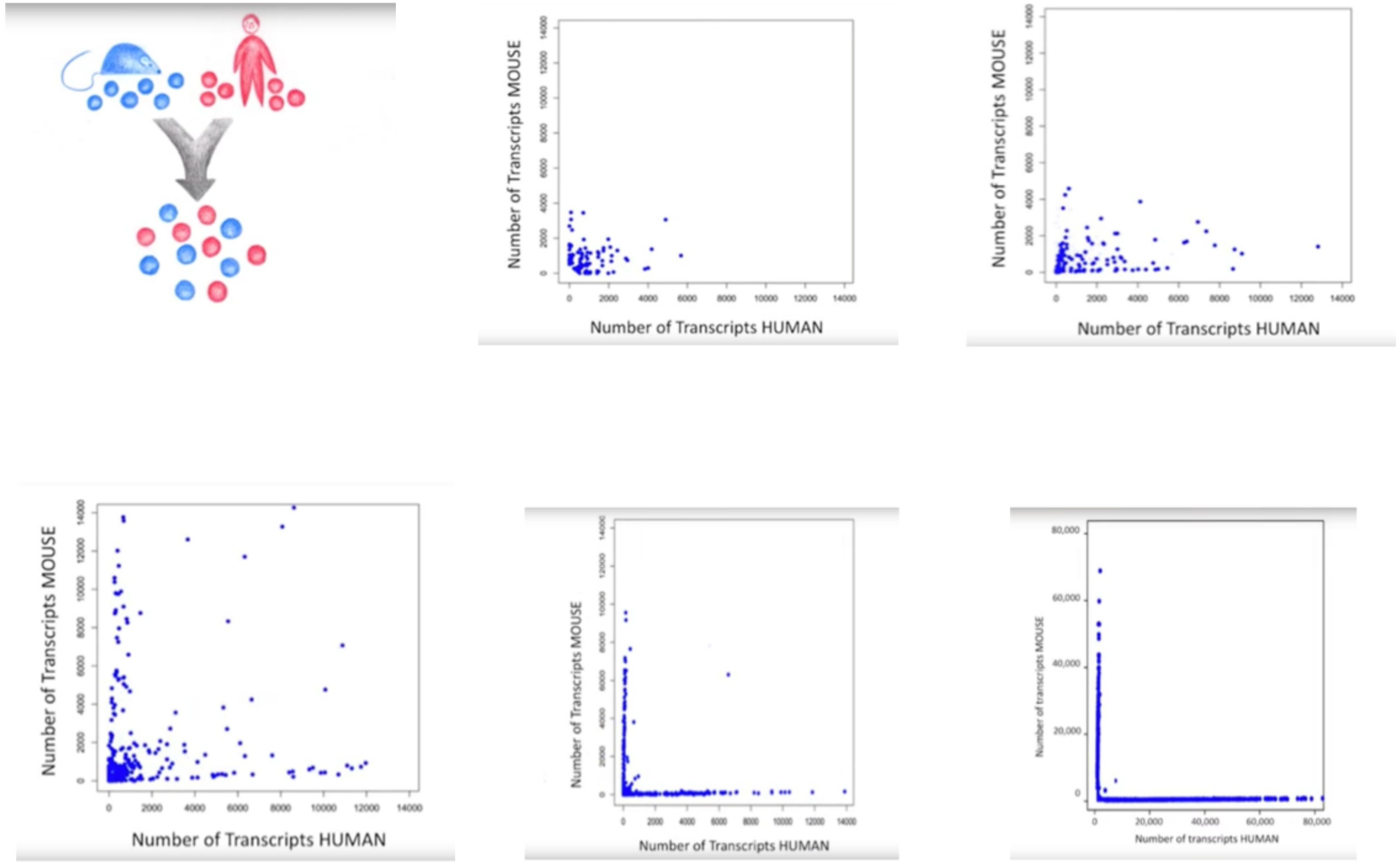
computationally explore complex biological systems

Martin Zhang

A decade of single-cell RNA-seq

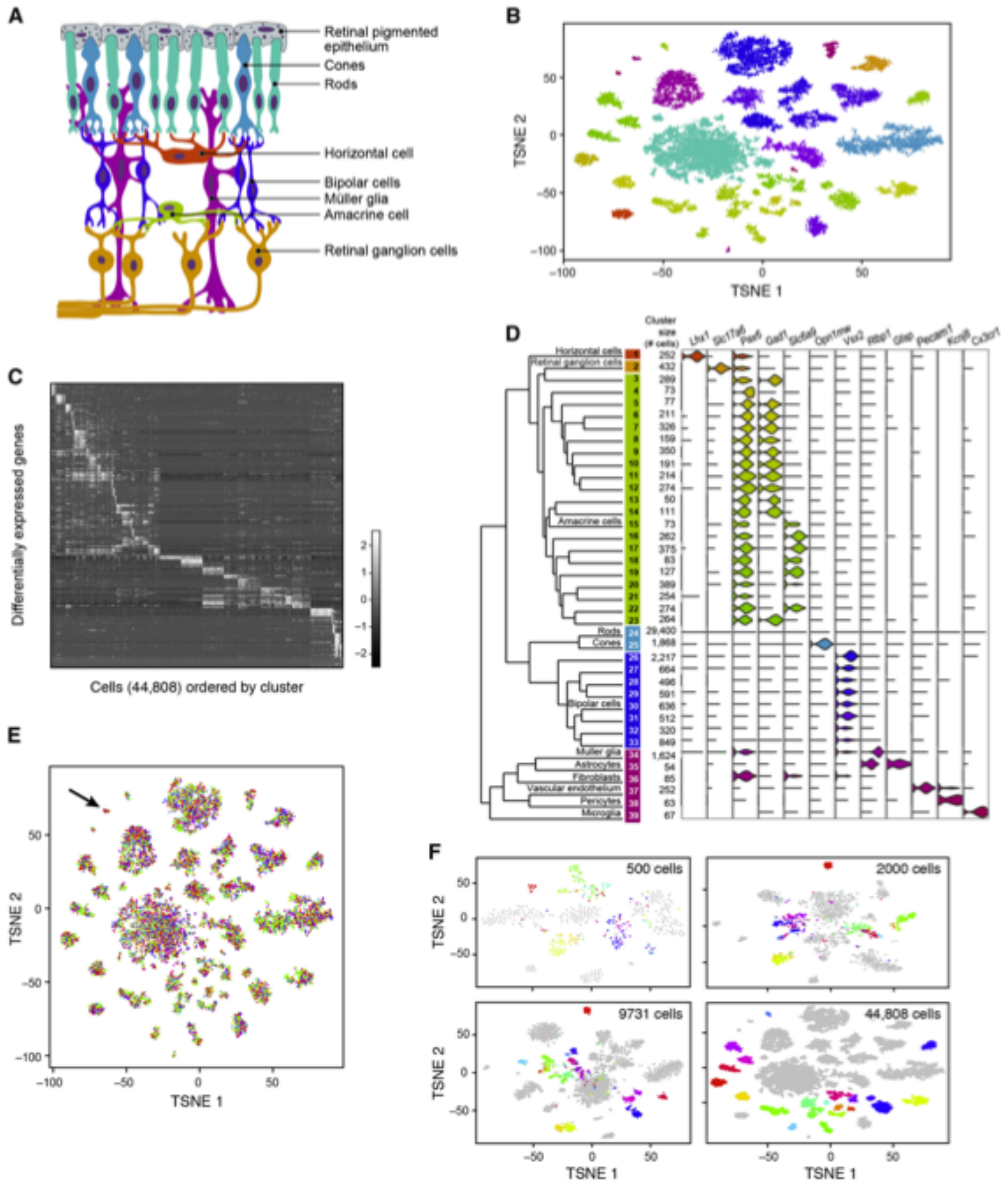


Svensson et al. 2018. DOI:10.1038/nprot.2017.149



Drop-seq: Droplet barcoding of single cells

https://www.youtube.com/watch?v=vL7ptq2Dcf0



Key Results

(a) schematic of known cell populations in retina

(b) 44,808 Drop-Seq profiles clustered into 39 retinal cell populations using tSNE

(c) Differentially expressed genes in each cluster

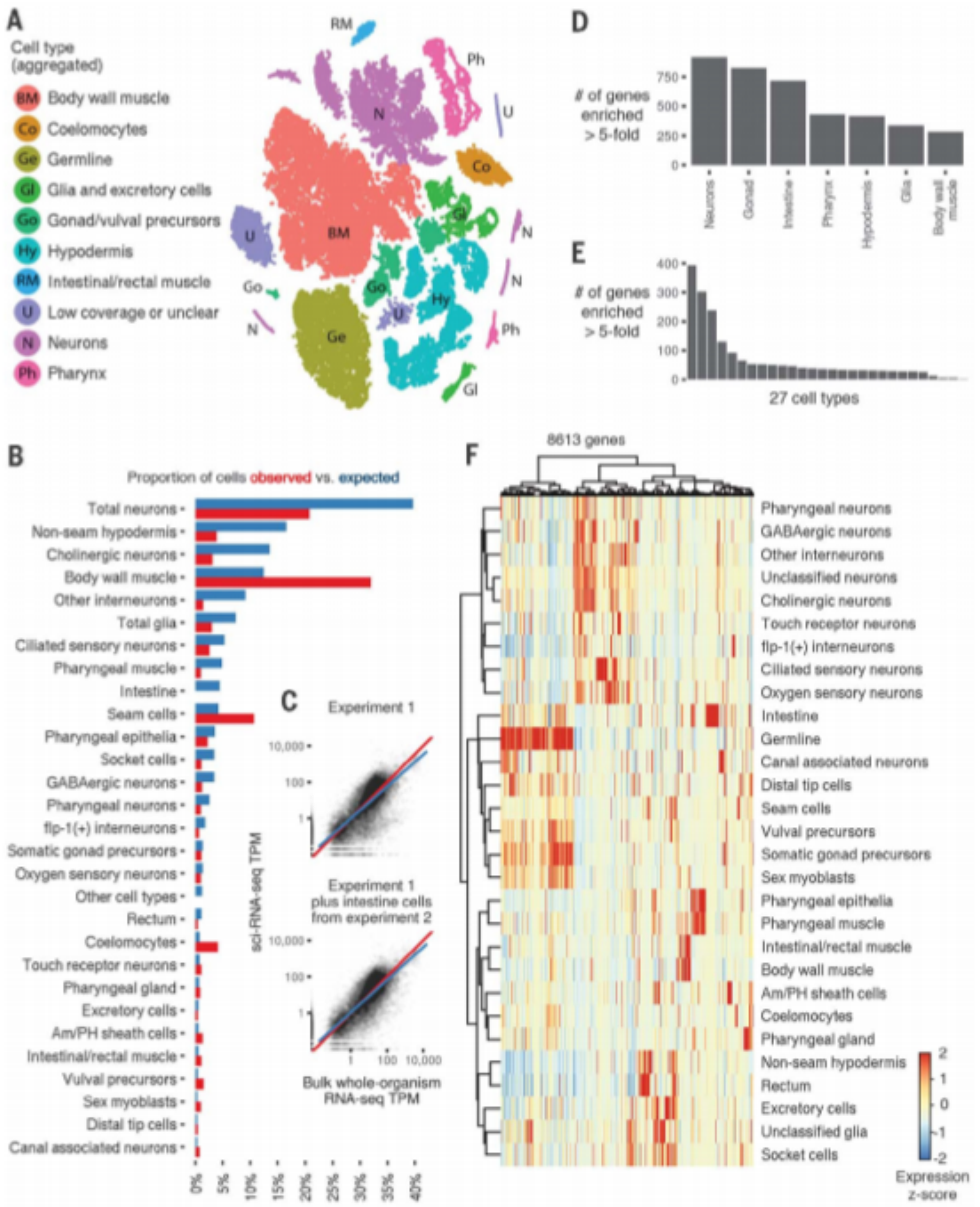
(d) Different cell types can be recognized using marker genes

(e) replicates well

(f) robust to down sampling

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Macosko et al (2015) Cell. https://doi.org/10.1016/j.cell.2015.05.002



Key Results

Profile every cell of C. elegans larva using combinatorial indexing

(a) t-SNE visualization of clusters

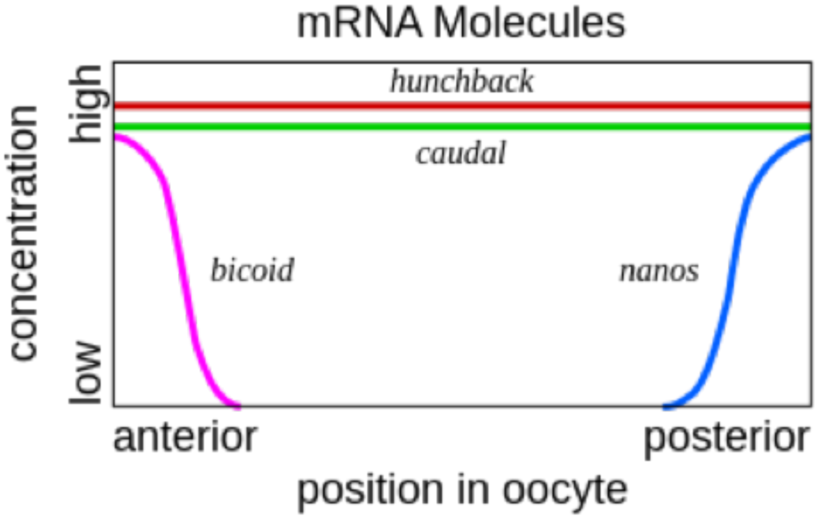
(b) Proportion of cells observed vs expected match well (including cells that only occur once or twice in the animal)

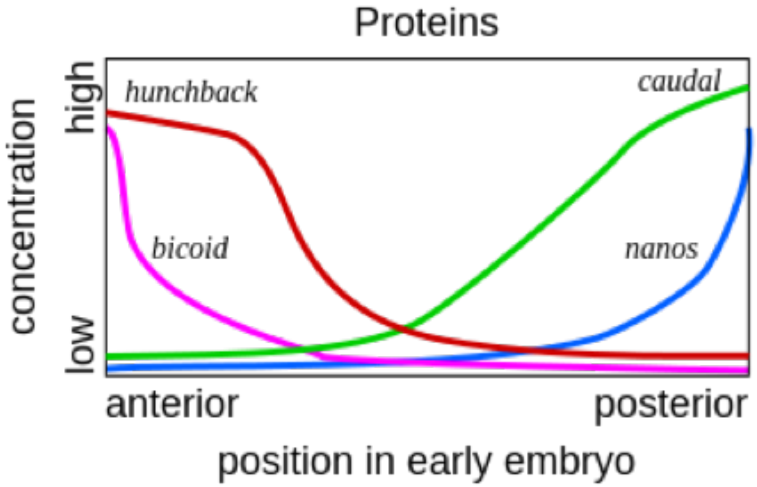
(c) Good correlation between single cell and bulk analysis of selected cell types

(d-f) Analysis of key genes per cell type

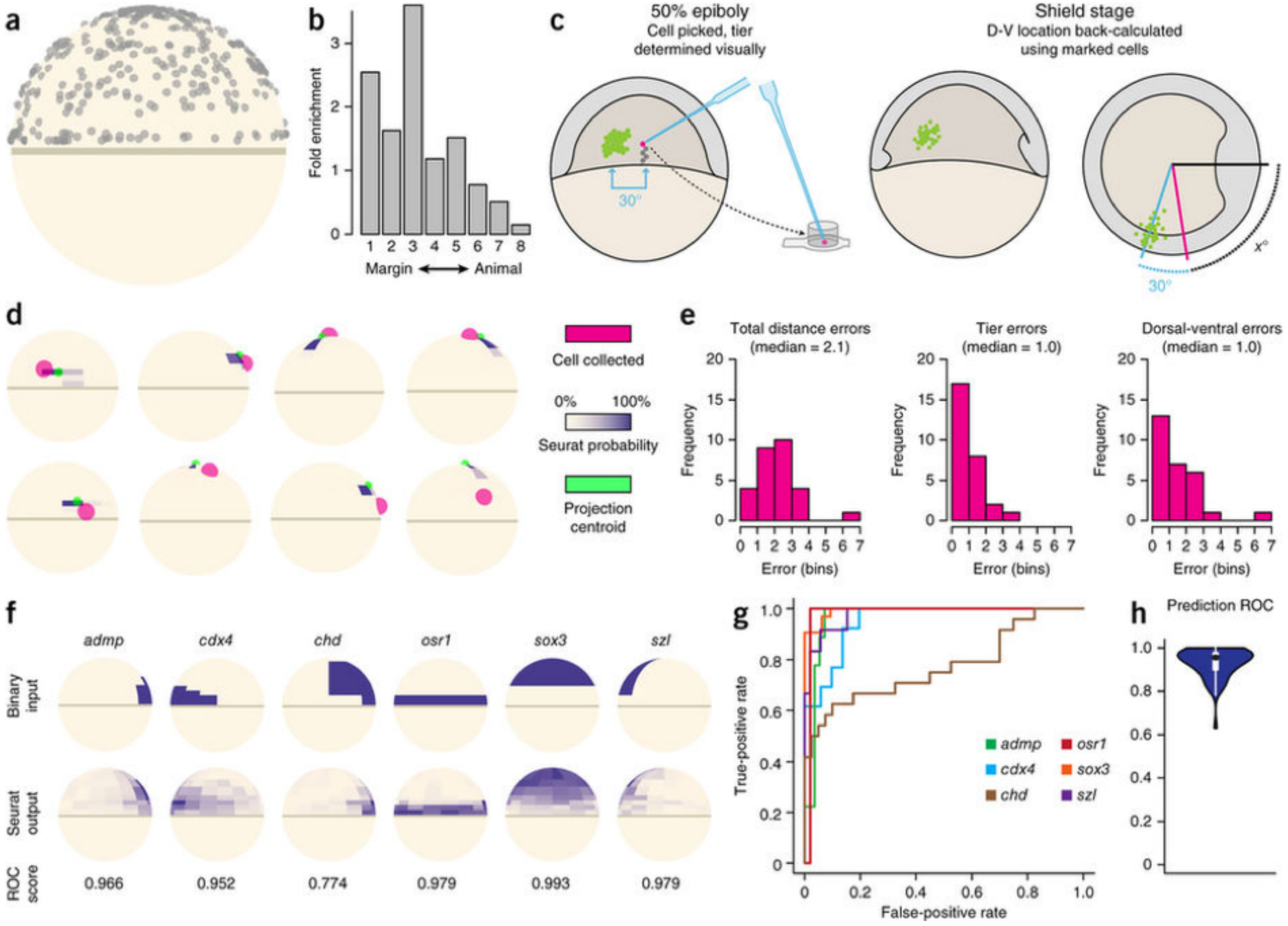
Comprehensive single-cell transcriptional profiling of a multicellular organism

Cao et al (2017) Science. 357:661-557



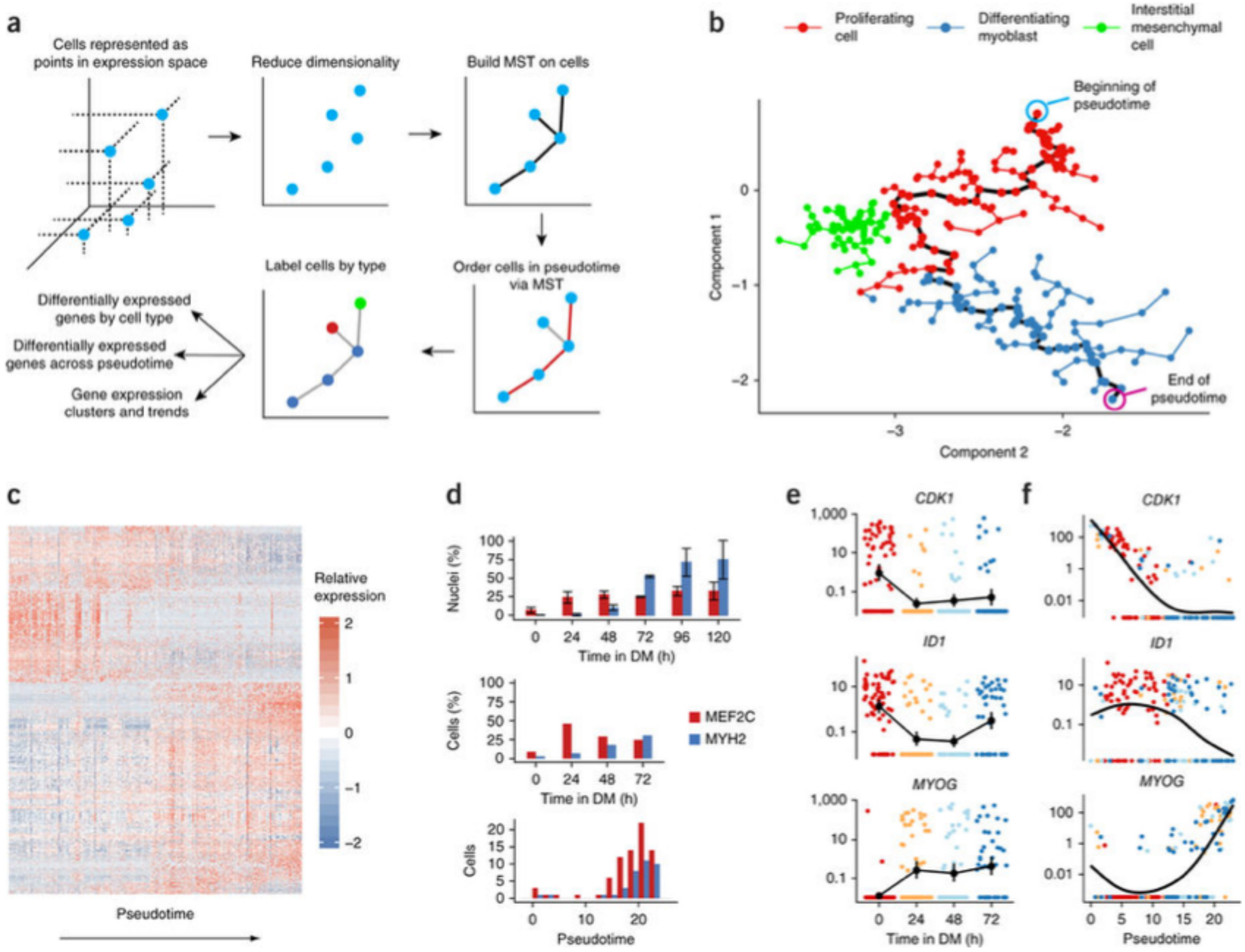


https://en.wikipedia.org/wiki/Drosophila_embryogenesis



Spatial reconstruction of single-cell gene expression data ("Seurat")

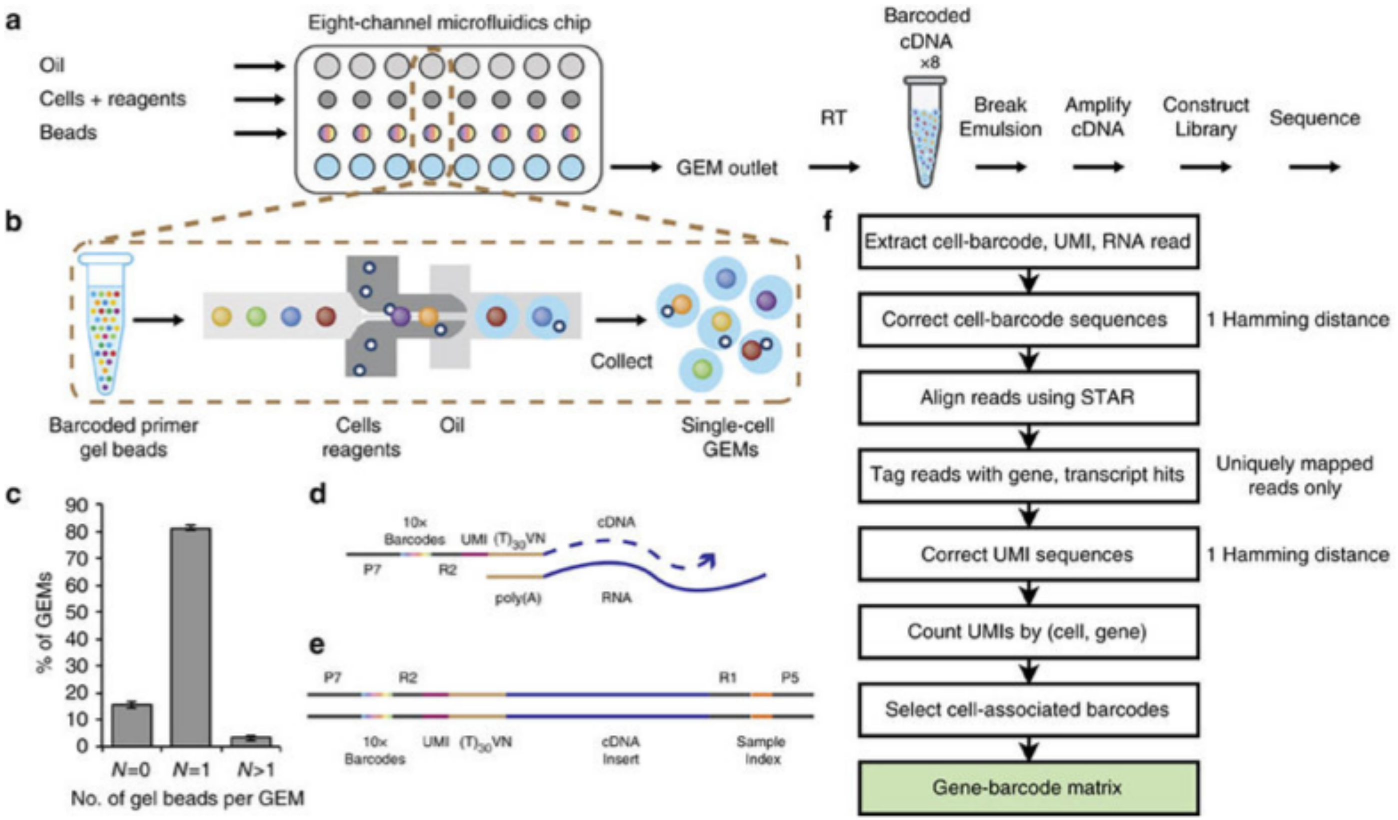
Satija et al (2015) Nature Biotechnology. doi:10.1038/nbt.3192



The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells ("Monocle")

Trapnell et al (2014) Nature Biotechnology. doi:10.1038/nbt.2859

10x GENOMICS



Up to 1M cells in a single analysis

Massively parallel digital transcriptional profiling of single cells
Zheng et al (2017) Nature Communication. doi:10.1038/ncomms14049

scATAC-Seq

Single Cell ATAC-Seq

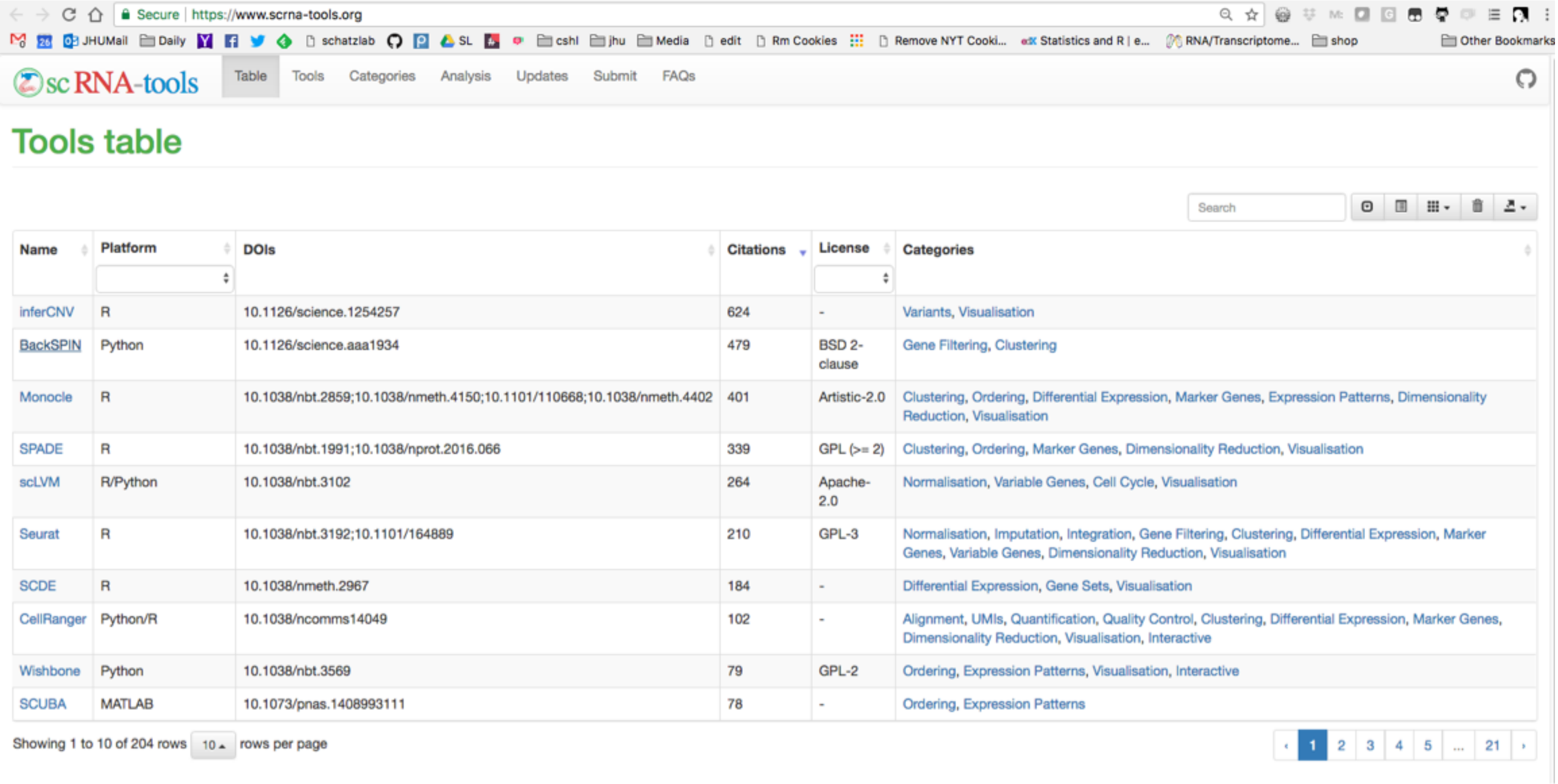
- Interrogate epigenomics at single-cell resolution
- Define cell types and states
- Investigate regulatory mechanisms
- Scalable from 1000s of cells
- High cell capture efficiency
- High transpososome capture sensitivity
- ATAC-Seq specific software pipeline

10X GENOMICS® sc-Feature Barcoding

Single Cell Feature Barcoding

- Reveal protein abundance and gene expression from the same cell
- Understand diverse CRISPR perturbations at single-cell level
- Feature barcoding reagents and protocols
  - Custom antibody conjugation
  - Preferred partners for pre-conjugated antibodies
- Scalable from 100s-1000s of cells
- Interactive visualization in Loupe cell browser
- CNV-Seq specific software pipeline

scRNA Analysis Tools: 204 and counting….



Tools table

Name	Platform	DOIs	Citations	License	Categories
inferCNV	R	10.1126/science.1254257	624	-	Variants, Visualisation
BackSPIN	Python	10.1126/science.aaa1934	479	BSD 2-clause	Gene Filtering, Clustering
Monocle	R	10.1038/nbt.2859;10.1038/nmeth.4150;10.1101/110668;10.1038/nmeth.4402	401	Artistic-2.0	Clustering, Ordering, Differential Expression, Marker Genes, Expression Patterns, Dimensionality Reduction, Visualisation
SPADE	R	10.1038/nbt.1991;10.1038/nprot.2016.066	339	GPL (>= 2)	Clustering, Ordering, Marker Genes, Dimensionality Reduction, Visualisation
scLVM	R/Python	10.1038/nbt.3102	264	Apache-2.0	Normalisation, Variable Genes, Cell Cycle, Visualisation
Seurat	R	10.1038/nbt.3192;10.1101/164889	210	GPL-3	Normalisation, Imputation, Integration, Gene Filtering, Clustering, Differential Expression, Marker Genes, Variable Genes, Dimensionality Reduction, Visualisation
SCDE	R	10.1038/nmeth.2967	184	-	Differential Expression, Gene Sets, Visualisation
CellRanger	Python/R	10.1038/ncomms14049	102	-	Alignment, UMIs, Quantification, Quality Control, Clustering, Differential Expression, Marker Genes, Dimensionality Reduction, Visualisation, Interactive
Wishbone	Python	10.1038/nbt.3569	79	GPL-2	Ordering, Expression Patterns, Visualisation, Interactive
SCUBA	MATLAB	10.1073/pnas.1408993111	78	-	Ordering, Expression Patterns

Showing 1 to 10 of 204 rows 10 rows per page

‹ 1 2 3 4 5 ... 21 ›

Single Cell Analysis Summary

Single cell analysis is a powerful tool to study heterogeneous tissues

- Overcomes fundamental problems that can arise when averaging
- scCNV analysis used for understanding tumor progression, other mutational processes
- scRNA analysis used to identify novel cell types, understand the progression from one cell type to another across development or disease
- Many other sc-assays in development, expect 1000s to 1Ms of cells in essentially any assay

Major challenges

- Very sparse amplification and few reads per cell
  - Find large CNVs, identify major cell types; hard to find small variants or perform differential expression
- Allelic-dropout and unbalanced amplification hides or distorts information
  - Use statistical approaches to smooth results based on prior information or other cells from the same cell type
- Need new ways to process and analyze millions of cells at a time