Cancer genomics: one cell at a time

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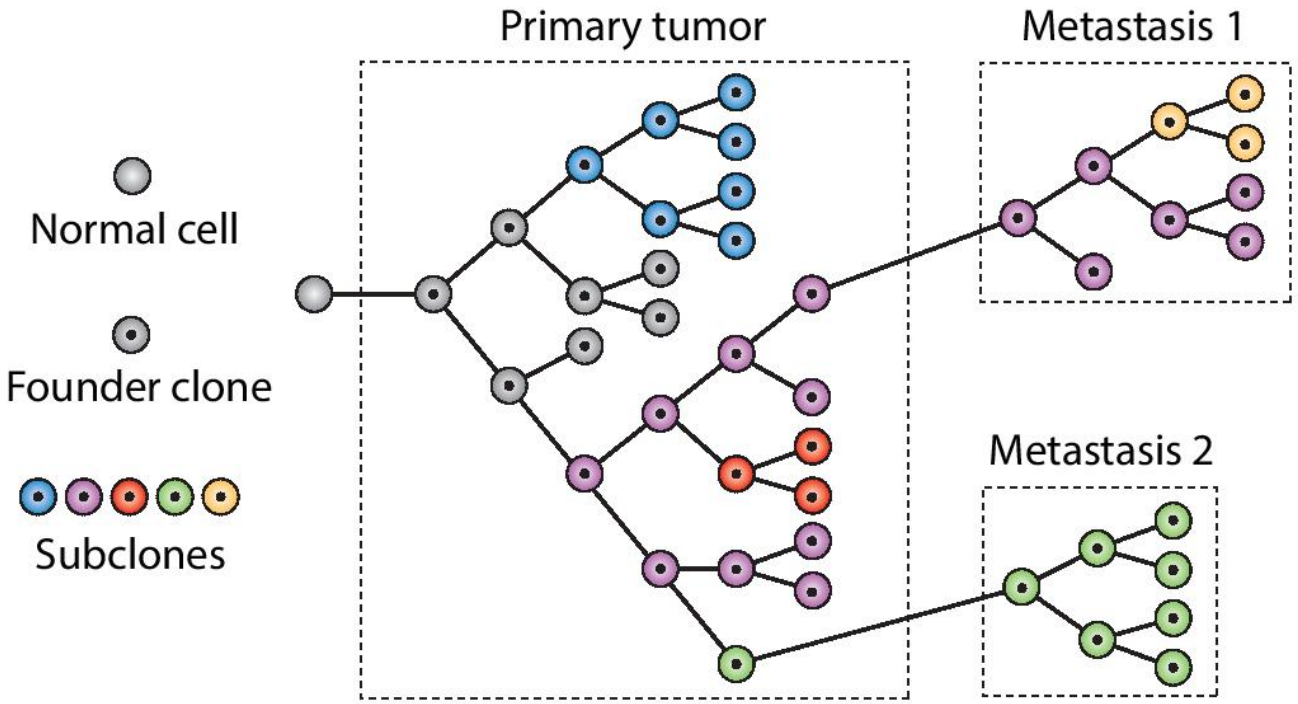
Background: tumor progression

- Follows Darwinian evolution

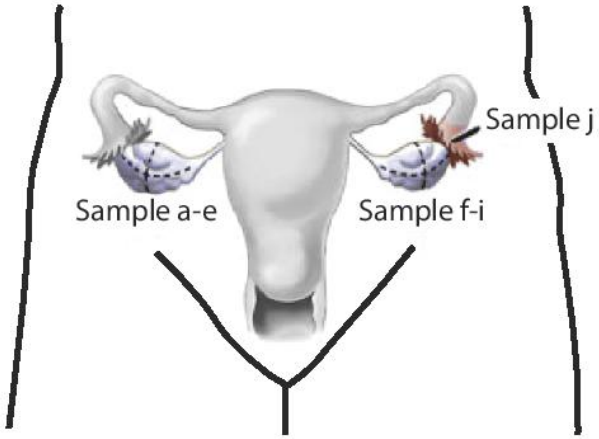


- Clones: genotypically distinct cell populations
- Longitudinal and spatial subclonal dynamics lead to failures of targeted therapies and disease recurrence

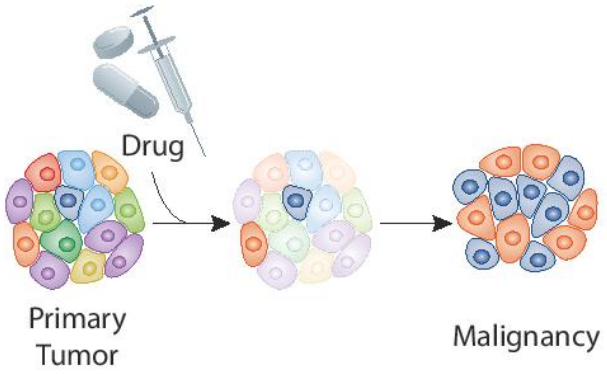
Tracking clonal evolution by bulk sequencing

- Study design:
  repeated sequencing of temporally and/or spatially separated tumor specimens from the same patient.

Spatially separated samples



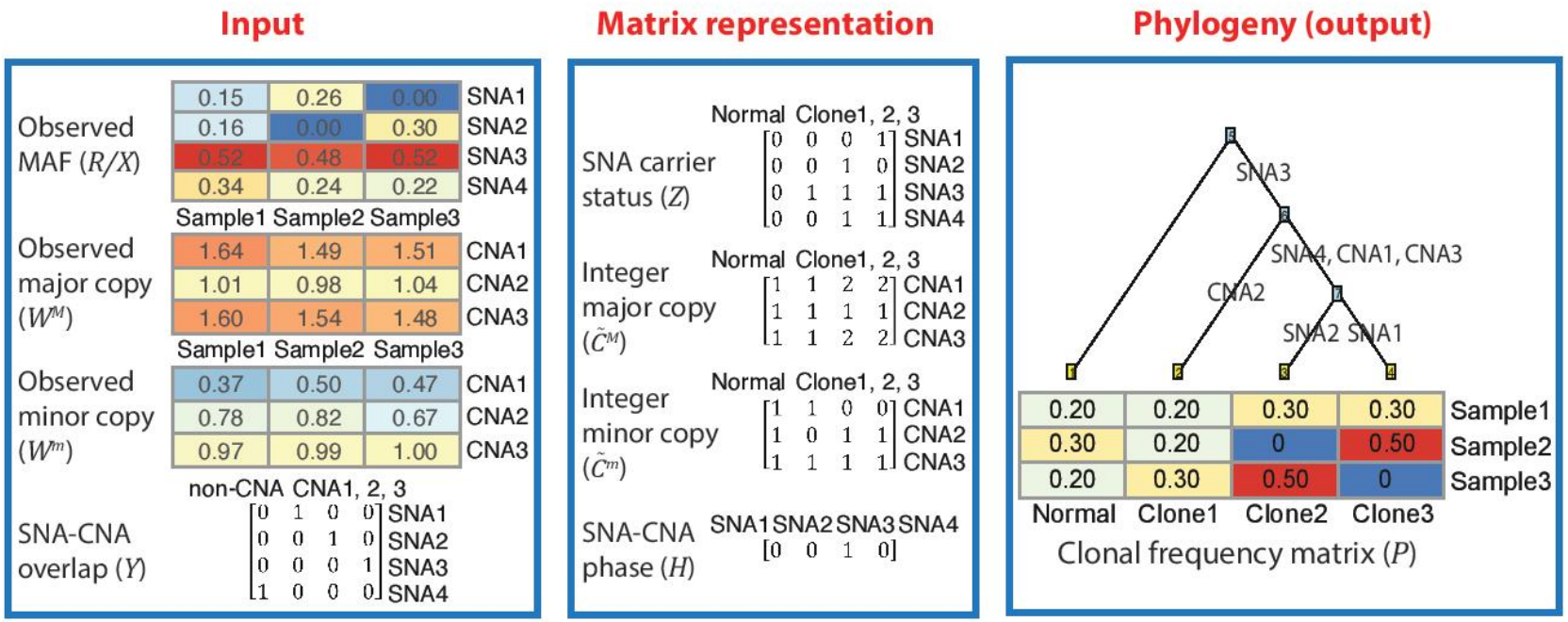
Temporally separated samples



Canopy for repeated bulk DNA sequencing

https://CRAN.R-project.org/package=Canopy

https://github.com/yuchaojiang/Canopy

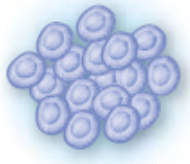


(Jiang et al. 2016 PNAS)

Tumor heterogeneity: from bulk tissue to single cells

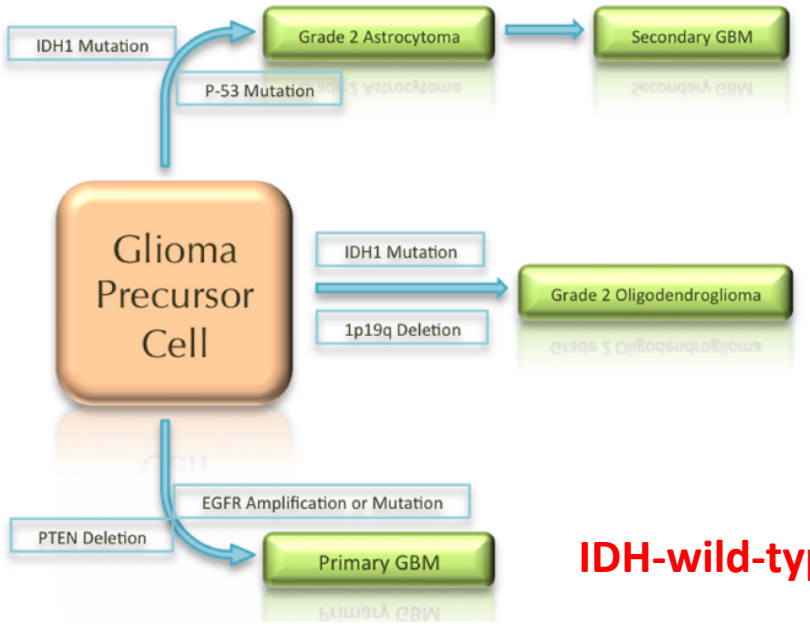
- **Repeated bulk-tissue DNA sequencing**: identify clonal mixtures and recover clonal history through deconvolution.
- **Single-cell RNA sequencing**: study cellular heterogeneity on expression level with single-cell resolution.

Population of cells (bulk sequencing)



Single cell (single-cell sequencing)

Primary glioblastoma & IDH-mutant gliomas



IDH-A

IDH-O

IDH-wild-type GBM

CANCER GENOMICS

Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq

9879 single cells from 10 IDH-A tumors
(Venteicher et al., Science, 2017)

LETTER

doi:10.1038/nature20123

Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma

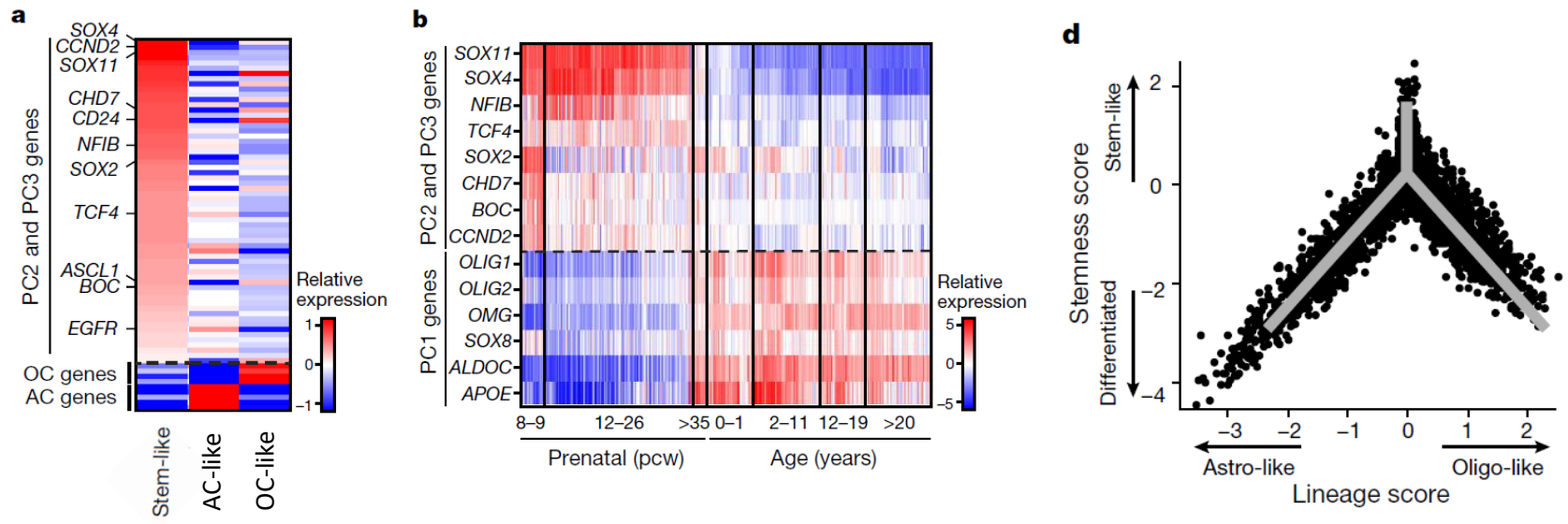
4347 single cells from 6 IDH-O tumors
(Tirosh et al., Nature, 2016)

CANCER GENOMICS

Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma

430 single cells from 5 primary GBMs
(Patel et al., Science, 2014)

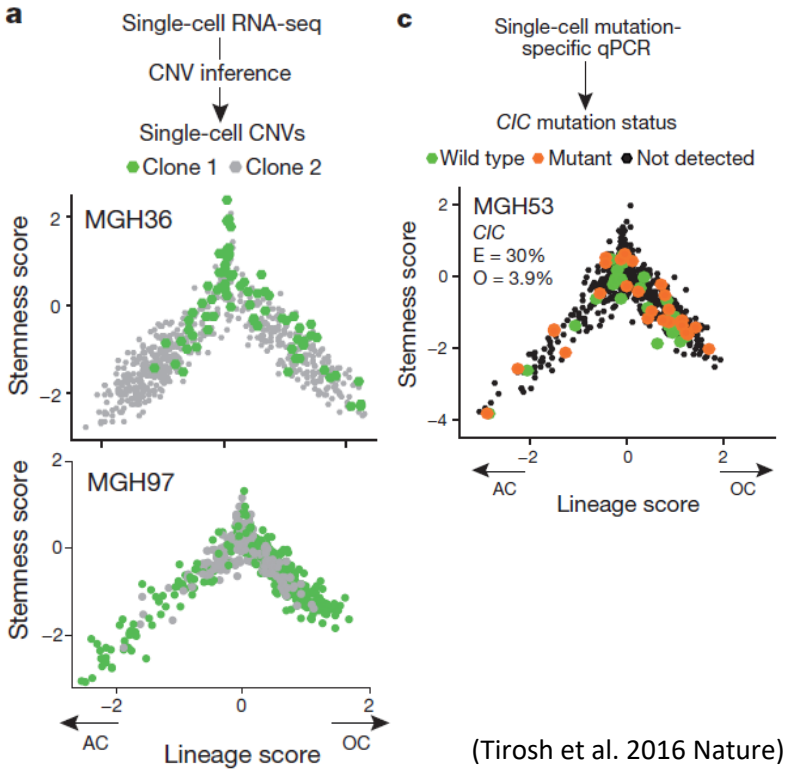
Major findings: three subpopulation of malignant cells



(Tirosh et al. 2016 Nature)

- PC1 associated genes separate two subpopulations of glial cells, with oligodendrocytic and astrocytic markers. **“Lineage Score”**
- PC2 and PC3 associated genes represent stemness signatures. **“Stemness Score”**

Role of genetics on tumor architecture?



(Tirosh et al. 2016 Nature)

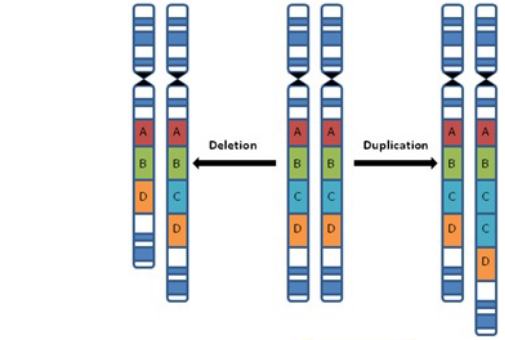
- Overall tumor architecture was preserved across clones reconstructed by CNVs and point mutations.
- Tirosh et al., Nature 2016:
  "Many subclonal mutations can accrue within the hierarchy (but not drive it), although **without a comprehensive phylogenetic reconstruction, we cannot categorically rule out a genetic influence**."
- Venteicher et al., Science 2017:
  "… patterns of differentiation and proliferation can be partially modulated by genetics and subject to selection. **Future studies should further investigate the modulation of our inferred cellular architecture by genetic evolution**"

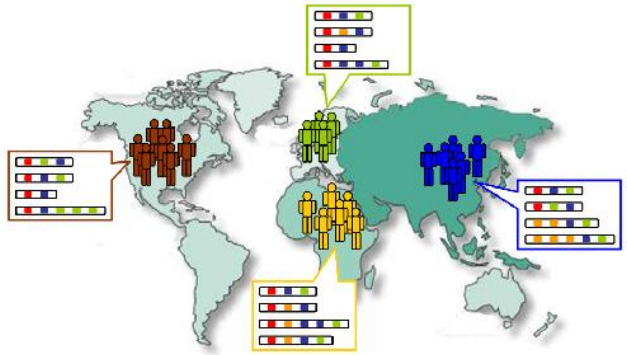
Two broad types of mutations

- Copy number aberrations (CNAs)
  - Bulk and single-cell DNA sequencing: How to remove biases and artifacts?
  - Single-cell RNA sequencing: How to infer copy number from expression?
- Single-nucleotide variants (SNVs)
  - Bulk DNA sequencing: MuTect, VarScan 2, etc., fairly standardized.
  - Single-cell RNA sequencing: Technical noise? Transcriptional bursting?

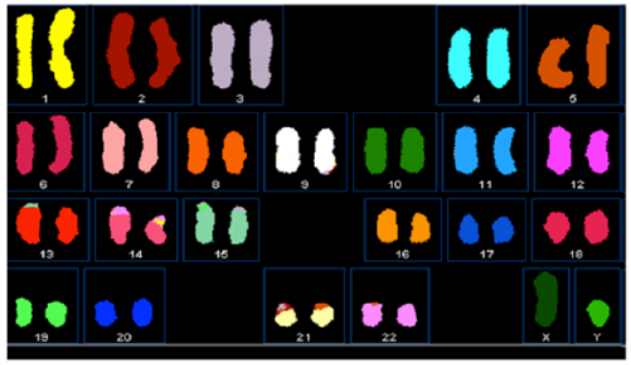
Copy number variation (CNV)

- Large deletions or duplications
- Abundant source of variations
- Associated with diseases



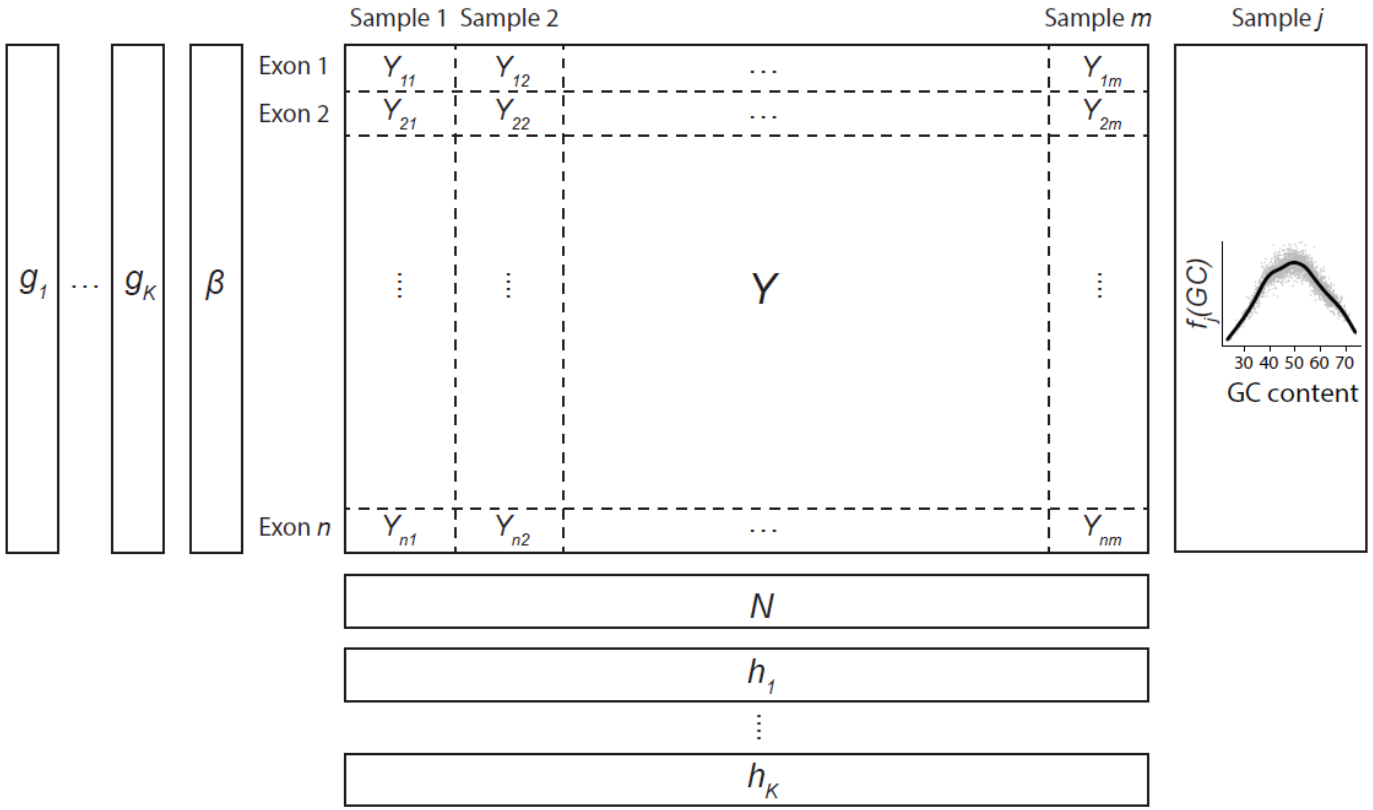


Normal



Tumor

Poisson latent factor model for normalization



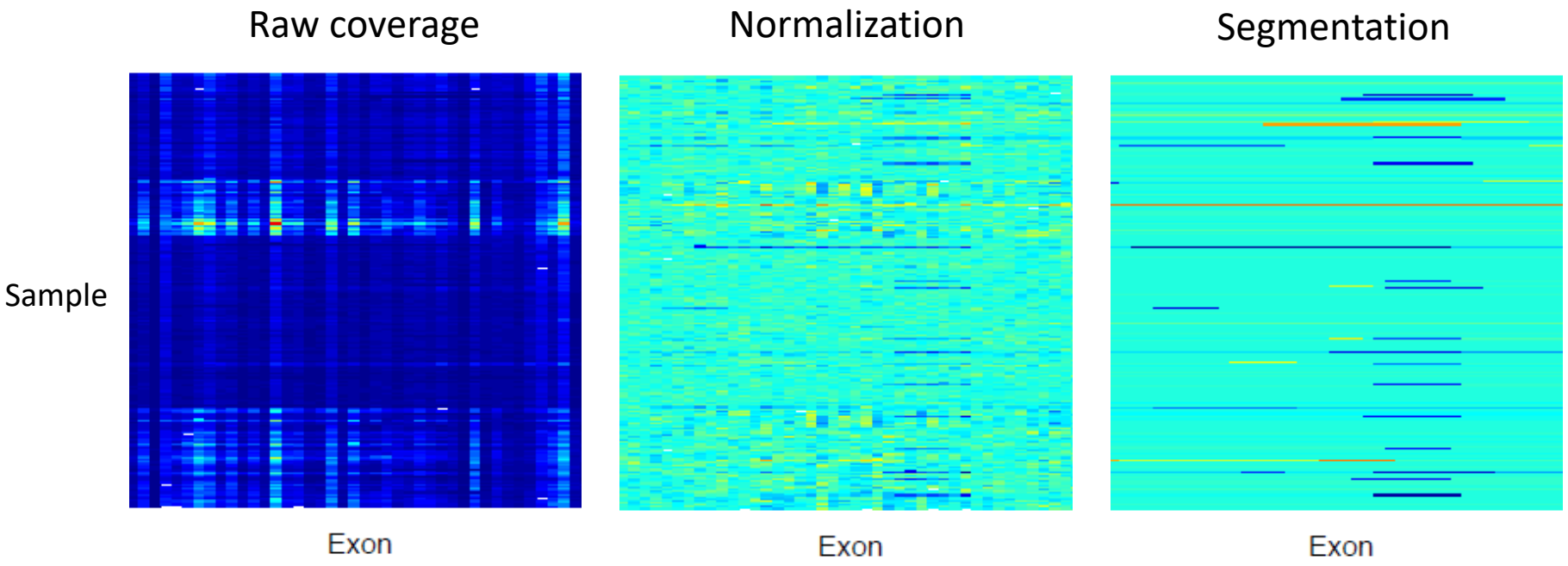
Null Model:

$$Y_{ij} \sim \text{Poisson}(\lambda_{ij})$$

$$\lambda_{ij} = N_j \beta_i f_j(GC_i) \exp\left(\sum_{k=1}^{K} g_{ik} h_{jk}\right)$$

i : exon number; j : sample number
Y : raw coverage
λ : expected coverage (no CNV)
$N_j = \sum_{i=1}^{n} Y_{ij}$
β_i : exonic-specific bias for exon i
$f_j(GC_i)$: bias due to GC content
$g_{ik} h_{jk}$ $(1 \le k \le K)$: kth latent Poisson factors

CODEX: COpy number Detection by EXome-seq

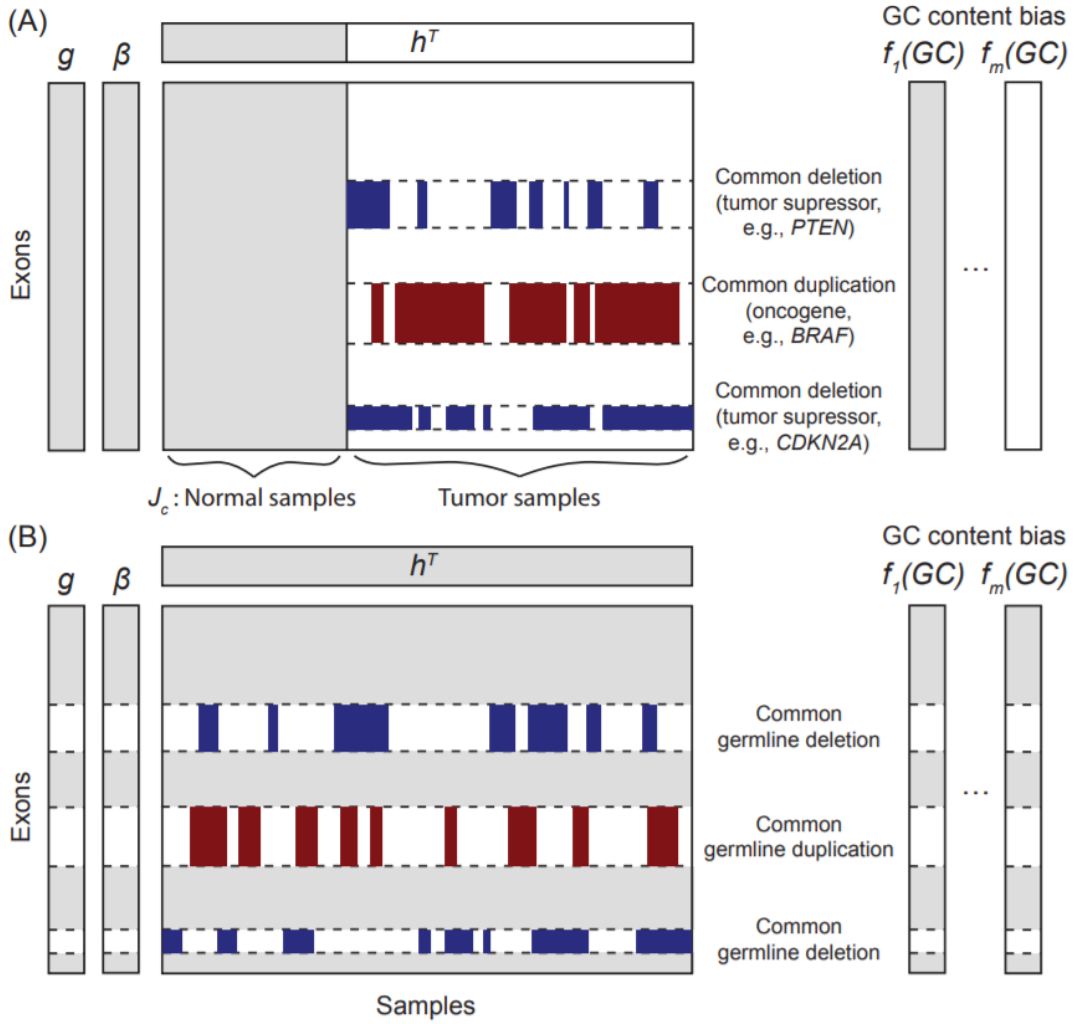


http://bioconductor.org/packages/CODEX/
(Jiang et al., Nucleic Acids Research, 2015)

CODEX2: full-spectrum CNV detection by NGS

Negative control samples

Negative control regions

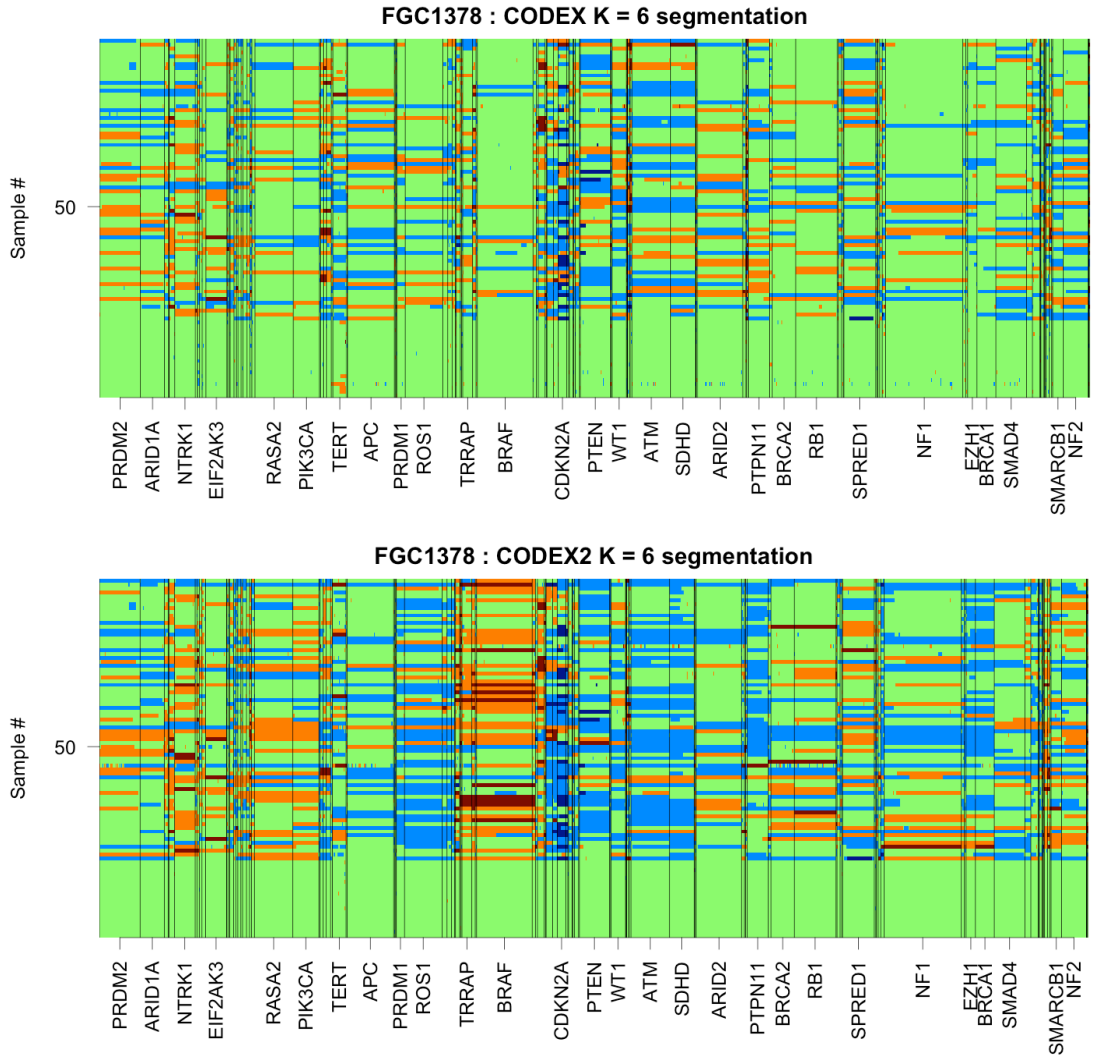


https://github.com/yuchaojiang/CODEX2

(Jiang et al., Genome Biology, 2018)

Melanoma targeted sequencing: sanity check

High amp
Gain
Null
Hemi. del
Homo. del

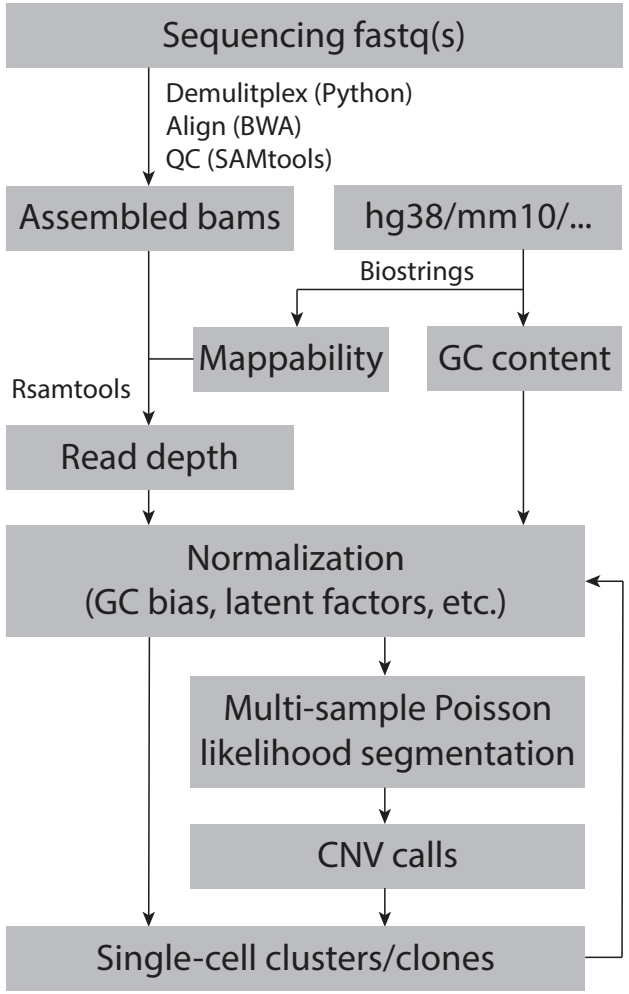


CODEX

CODEX2

(Garman et al., Cell Reports, 2017)

SCOPE: *S*ingle-cell *COPy* number *E*stimation

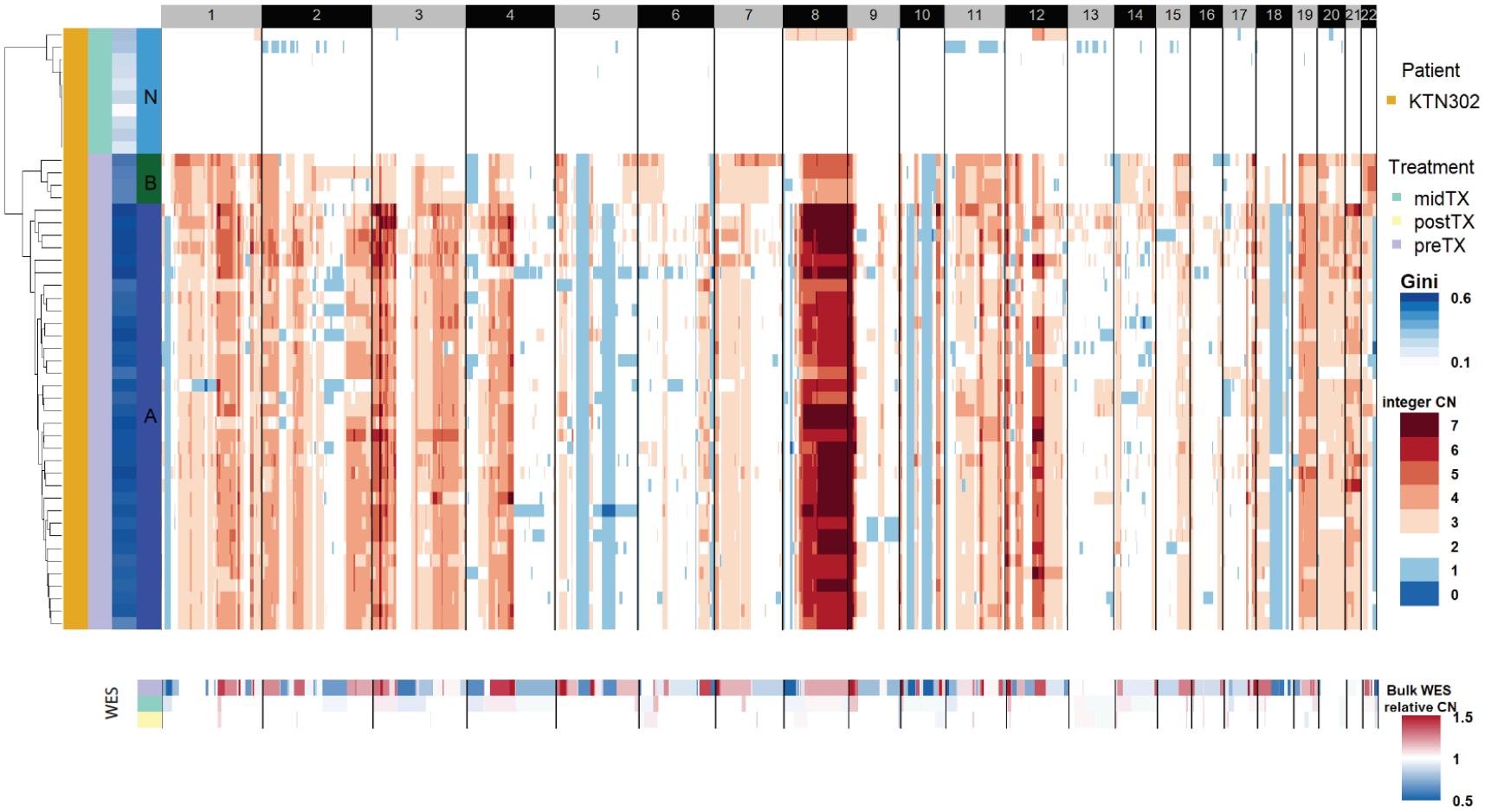


https://github.com/rujinwang/SCOPE

Wang et al., bioRxiv, 2019

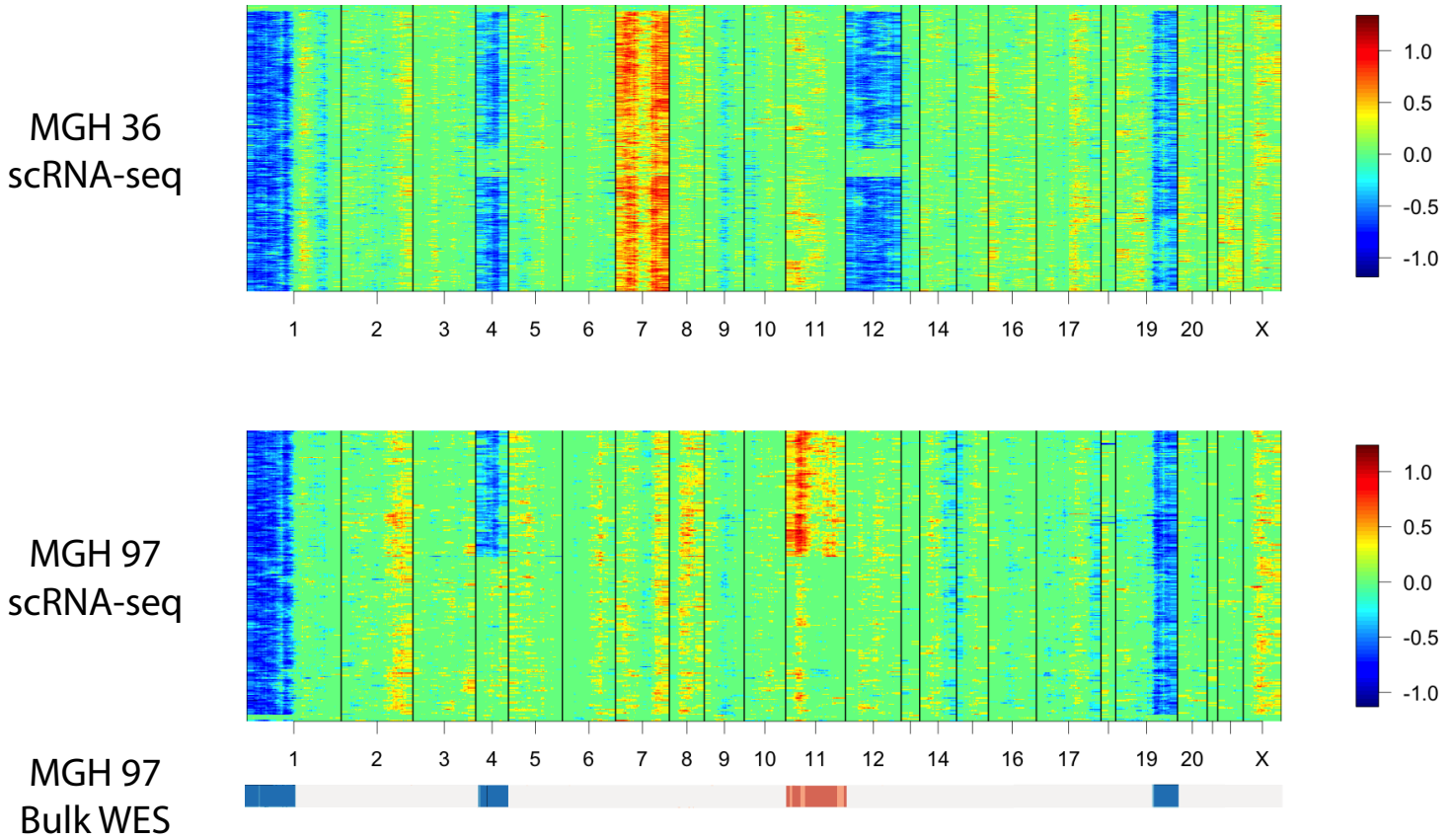
Copy number profiles by scDNA-seq, WES, scRNA-seq

Triple negative breast cancer patient from Kim et al. (Cell, 2018)



Sliding window approach for copy number profiling by scRNA-seq

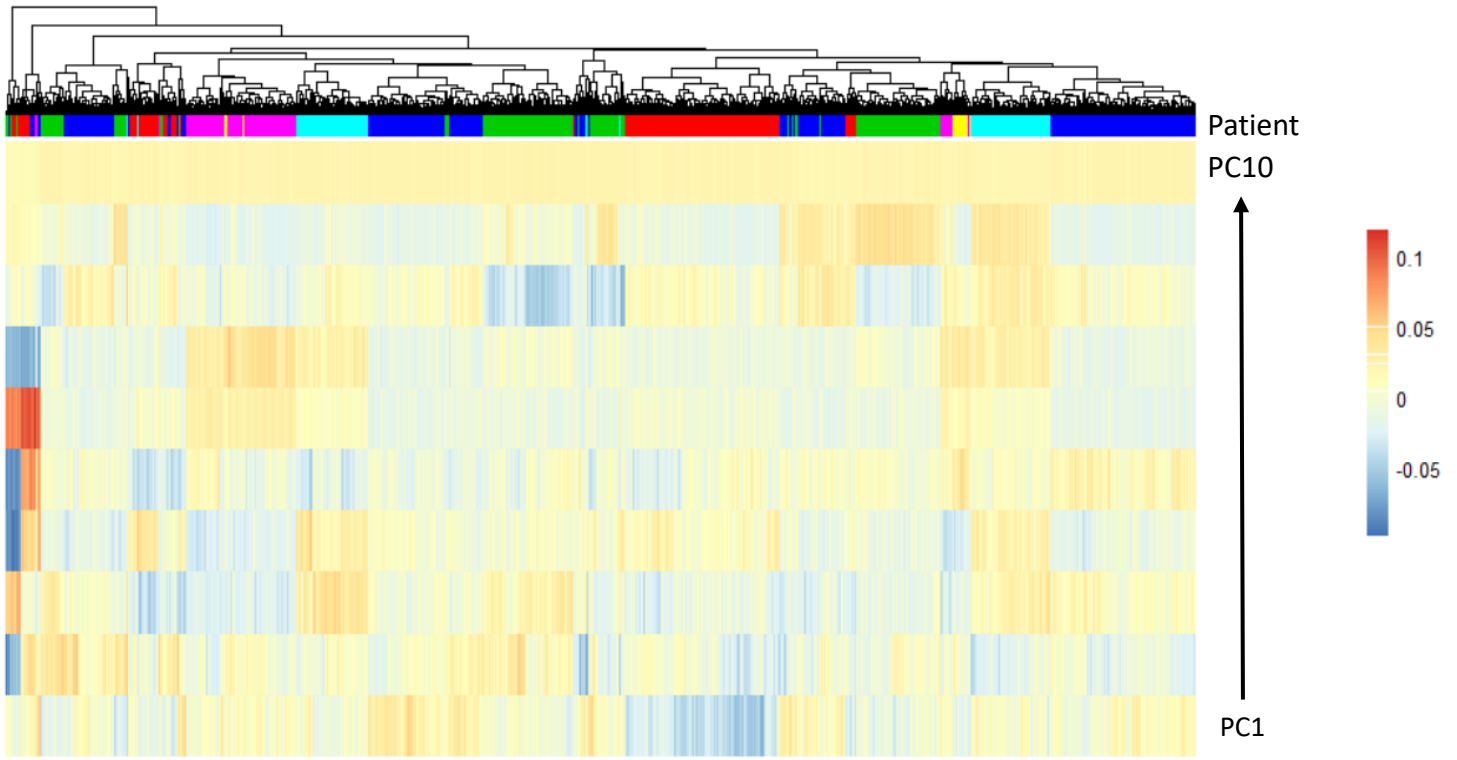
Processed glioblastoma scRNA-seq dataset from Tirosh et al., Nature, 2016

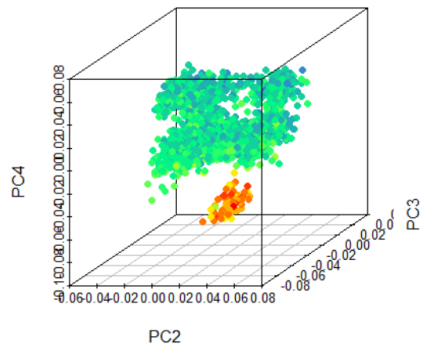


https://github.com/broadinstitute/infercnv

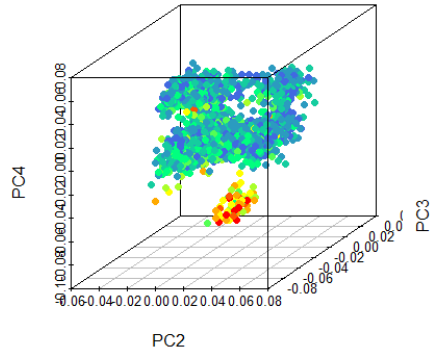
How to identify non-malignant cells?

PCA on log(TPM/10+1)

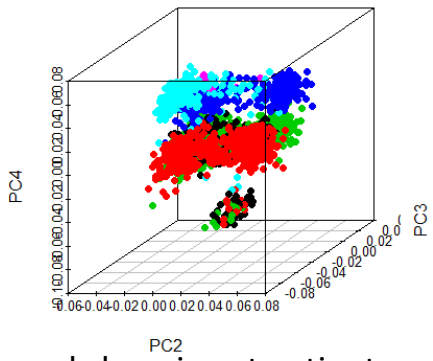




Chr1p expression (known del)



Chr11q expression (known del)

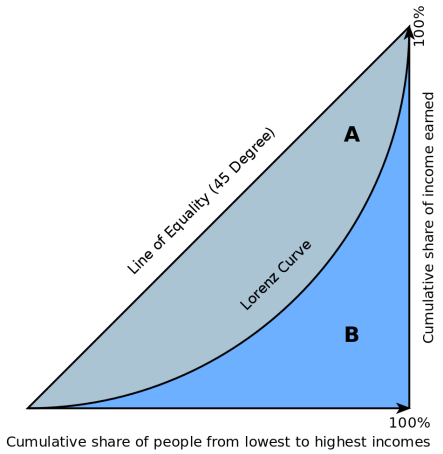


Normal clone is not patient specific!

How to identify normal cells?

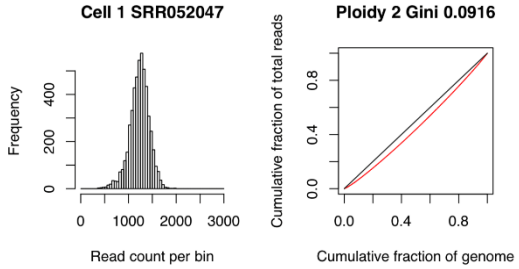
- Lorenz curve: assess coverage uniformity for each cell.
- Gini coefficient: two times the area between the Lorenz curve and the diagonal. Equivalently,

$$\text{Gini}_j = \frac{\sum_{i=1}^{m}\sum_{k=1}^{m}|Y_{ij}-Y_{kj}|}{2m\sum_{i=1}^{m}Y_{ij}}.$$

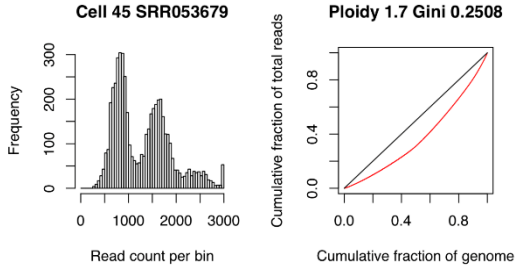


(https://en.wikipedia.org/wiki/Gini_coefficient)

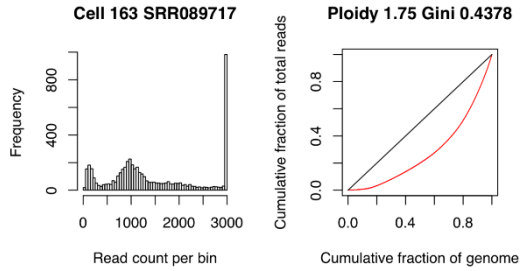
Normal cell



Tumor cell

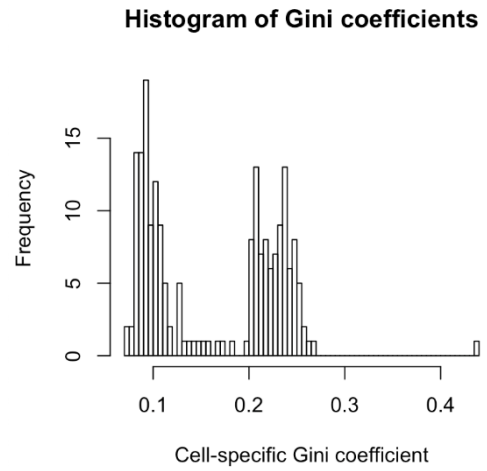


Outlier cell

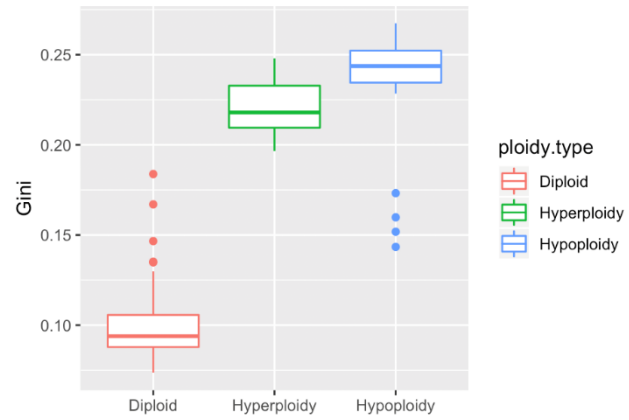


Gini index can be used to identify normal cells

- Cell-specific Gini index distribution



- Ploidy measured from FACS:
  - Hyperploidy: ploidy >= 2.1
  - Hypoploidy: ploidy <= 1.9
  - Diploid: 1.9 < ploidy < 2.1



- Hyperploidy and hypoploidy cells have higher Gini index compared to diploid cells.

Genomic and transcriptomic heterogeneity

Method

Linking transcriptional and genetic tumor heterogeneity through allele analysis of single-cell RNA-seq data

Jean Fan,[1,5] Hae-Ock Lee,[2,5] Soohyun Lee,[1] Da-eun Ryu,[2] Semin Lee,[1] Catherine Xue,[1] Seok Jin Kim,[3] Kihyun Kim,[3] Nikolaos Barkas,[1] Peter J. Park,[1] Woong-Yang Park,[2] and Peter V. Kharchenko[1,4]

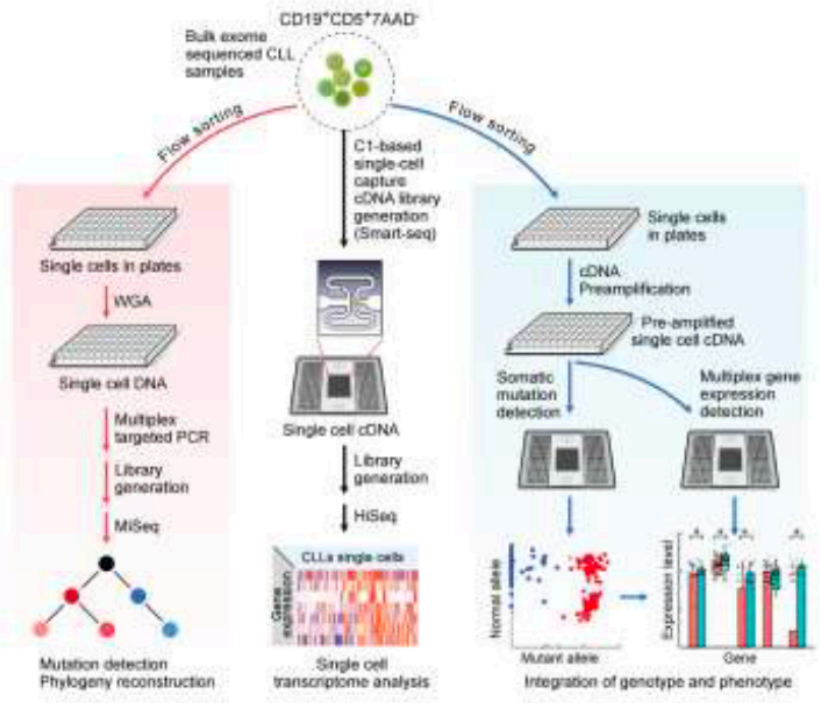
Cardelino: Integrating whole exomes and single-cell transcriptomes to reveal phenotypic impact of somatic variants

Davis J. McCarthy[1,4,*], Raghd Rostom[1,2,*], Yuanhua Huang[1,*], Daniel J. Kunz[2,5,6], Petr Danecek[2], Marc Jan Bonder[1], Tzachi Hagai[1,2], HipSci Consortium, Wenyi Wang[8], Daniel J. Gaffney[2], Benjamin D. Simons[5,6,7], Oliver Stegle[1,3,9,#], Sarah A. Teichmann[1,2,5,#]

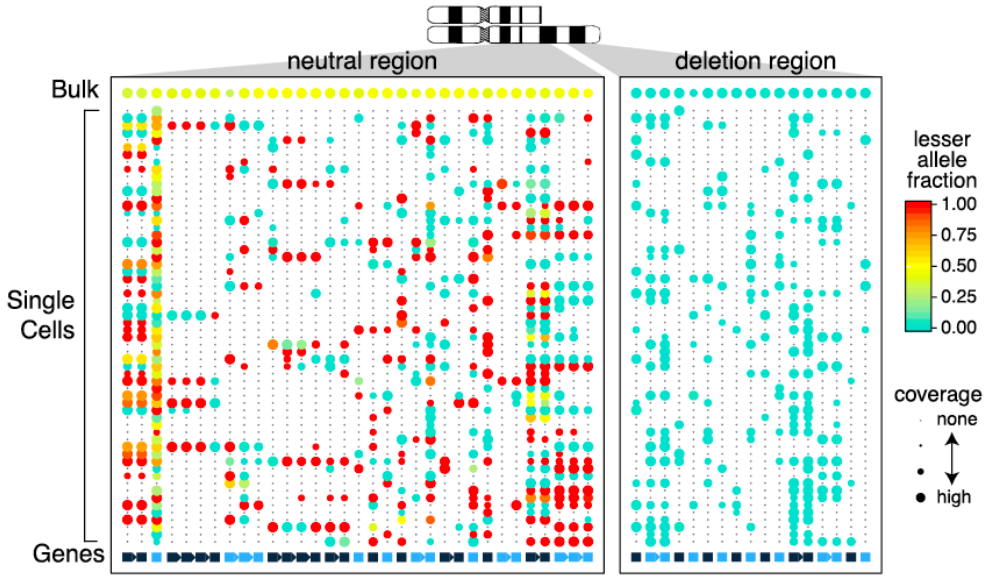
HoneyBADGER: CNAs by scRNA-seq

How to simultaneously assess transcriptional and genetic heterogeneity at the single cell level?

1. Technology Development Approach (Targeted RT-QPCR)



2. Computational Methods Approach (HoneyBADGER)

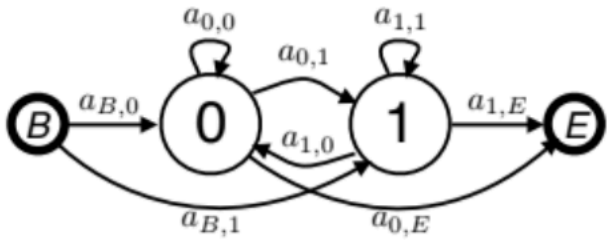


https://jef.works/HoneyBADGER/

Hidden Markov model (HMM)

- Let $\boldsymbol{X} = (X_1, ..., X_L)$ indicate whether YJ bikes on day i ($X_i = 1$) or not ($X_i = 0$)
- Suppose YJ bikes on day i with probability $\theta_0 = 0.25$ if it is cloudy ($Z_i = 0$) and with probability $\theta_1 = 0.75$ if it is sunny ($Z_i = 1$)
- Further suppose the Z_is are *hidden;* we see only $\boldsymbol{X} = (X_1, ..., X_L)$
- This *hidden Markov model* is a mixture model in which the Z_is are correlated
- We call $\boldsymbol{Z} = (Z_1, ..., Z_L)$ the *path*

Transition probability



Emission probability

$$P(x_i = 1|z_i = 0) = 0.25$$
$$P(x_i = 1|z_i = 1) = 0.75$$

$\boldsymbol{Z}$ = ? ? ? ? ? ? ? ? ? ? ? ?

$\boldsymbol{X}$ = 0 1 0 0 1 1 0 1 0 0 1 0

HMM for HoneyBADGER

HoneyBADGER implements an expression-based HMM as well as an allele-based HMM to identify regions potentially affected by CNVs. For the expression-based HMM, a transition matrix is defined on three hidden states representing deletion, neutral, and amplification:

$$\begin{pmatrix} 1-2t & t & t \\ t & 1-2t & t \\ t & t & 1-2t \end{pmatrix},$$

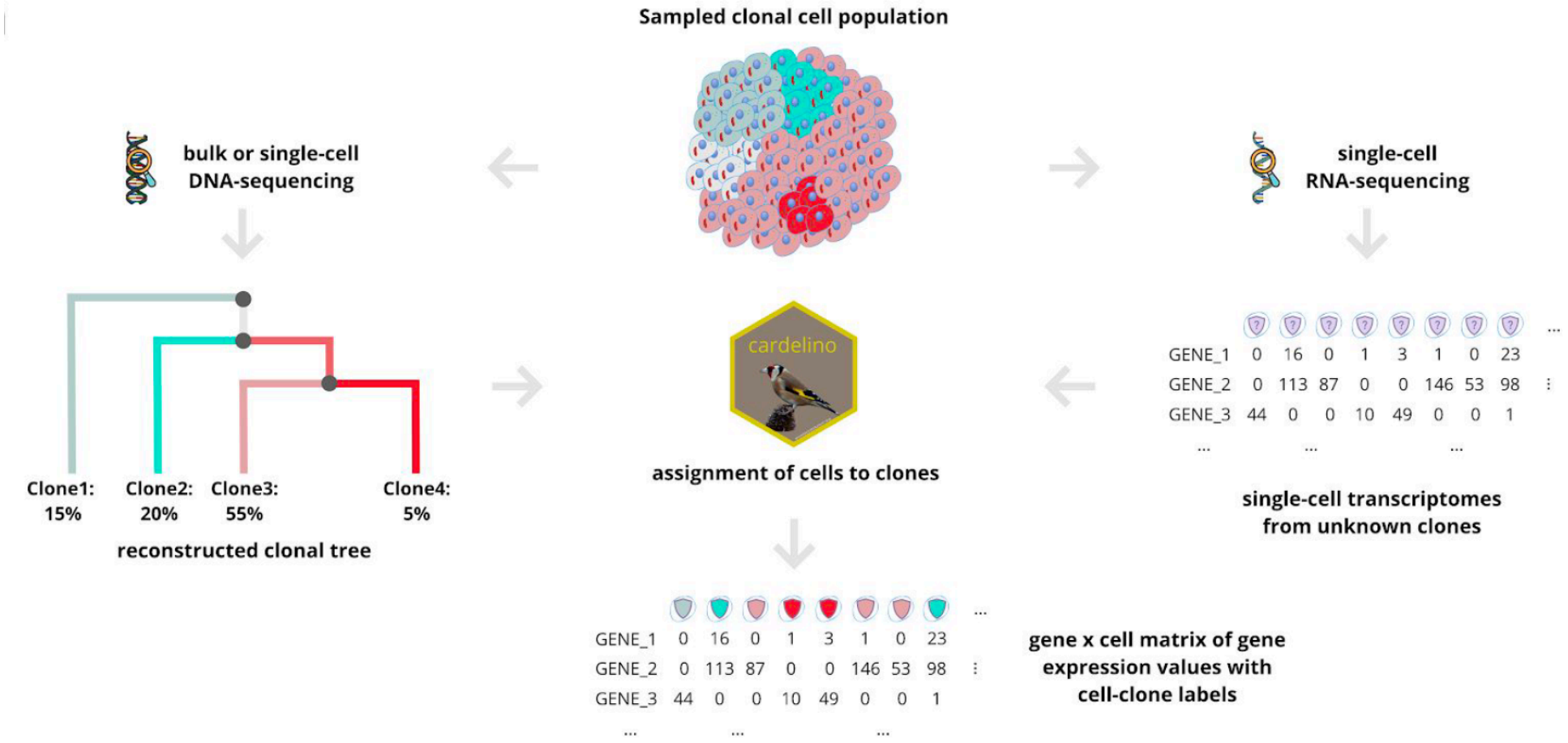
where $t = 1 \times 10^{-5}$ by default. Emission probabilities are defined by a normal distribution with means and variance estimated from the normalized expression data (see Supplemental Methods). For the allele-based HMM, a transition matrix is defined on two hidden states representing deletion or LOH, and neutral:

$$\begin{pmatrix} 1-t & t \\ t & 1-t \end{pmatrix},$$

where $t = 1 \times 10^{-5}$ by default. Emission probabilities are defined by a binomial distribution with the size parameter given by the pooled coverage at the SNP position and an expected $P = 0.1$ for the lesser allele in the case of deletion or LOH and $P = 0.45$ for neutral.

Cardelino: SNVs by scRNA-seq

Probabilistic mapping of single-cell transcriptomes to reconstructed clones



https://github.com/PMBio/cardelino

Cardelino: two-step approach

- First, a clonal tree is inferred using variant allele frequencies from bulk or single-cell DNA sequencing data.
- Subsequently, cardelino performs probabilistic assignment of individual single-cell transcriptomes to inferred clones, using variant information extracted from scRNA-seq.

References

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