DNA copy number profiling: from bulk tissue to single cells

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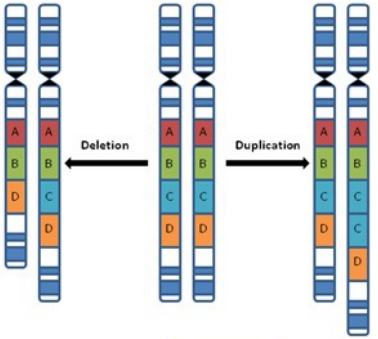
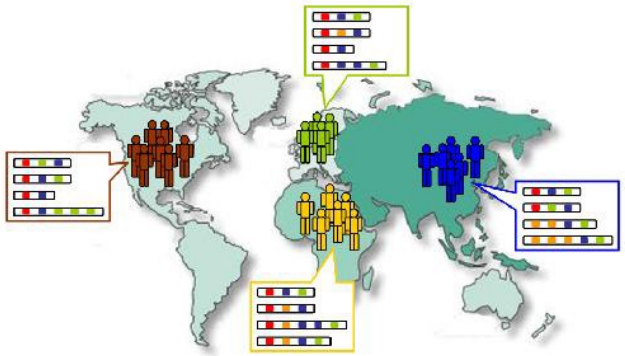
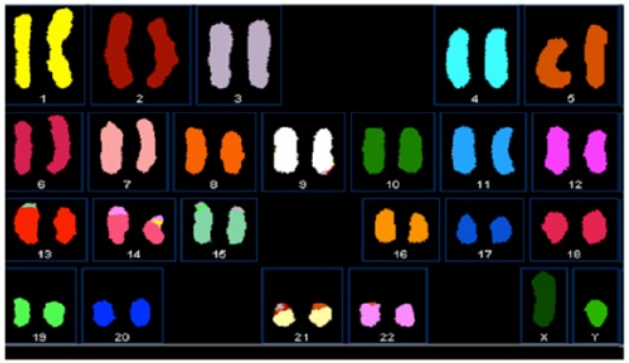
Copy number variation (CNV)

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





Normal



Tumor

CNV profiling: from bulk tissue to single cells

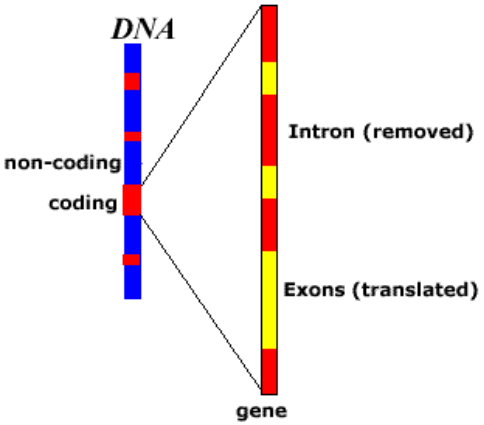
- Bulk DNA-seq
  - Whole-genome sequencing (WGS)
  - Whole-exome sequencing (WES) & targeted sequencing
- Single-cell DNA-seq
  - Conventional whole-genome amplification
  - 10X Genomics Chromium Single Cell CNV Solution / DLP+

Population of cells (bulk sequencing)

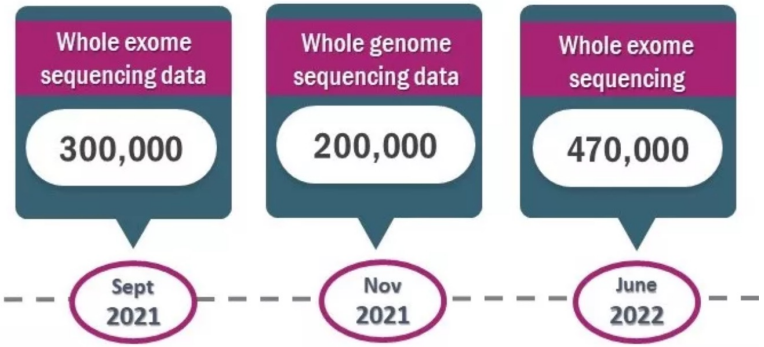
Single cell (single-cell sequencing)

Whole-exome sequencing

- Exome/exons: “functional” protein coding regions (1%) of the genome



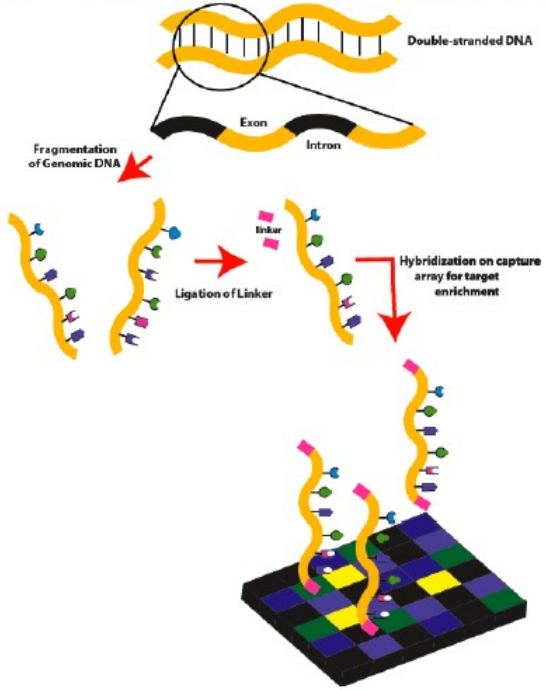
- Whole-exome sequencing
  - A cheaper, faster, but still effective alternative to whole-genome sequencing
  - Method of choice for large-scale studies



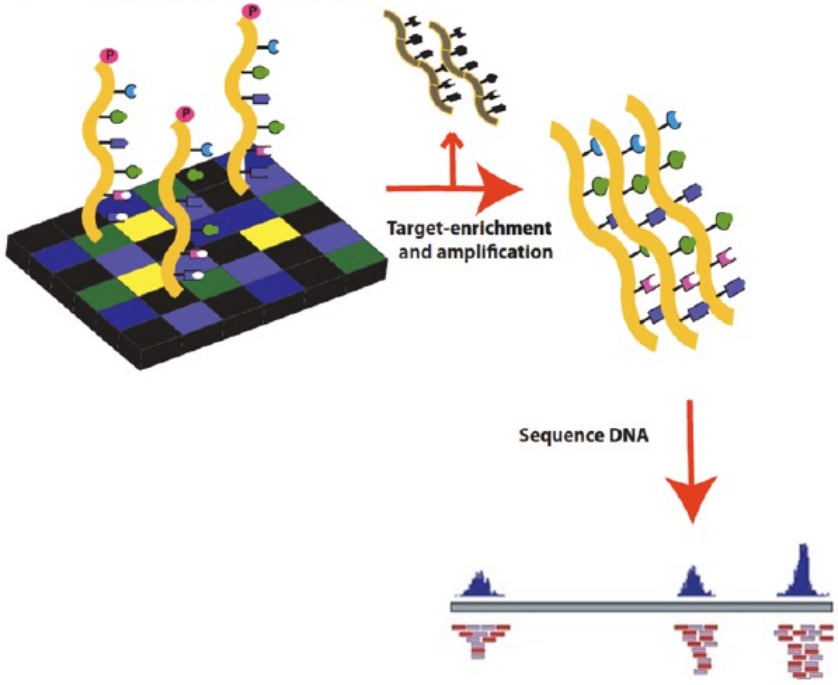
(https://www.ukbiobank.ac.uk)

Whole-exome sequencing protocols

1. Fragment DNA.
2. Capture exonic regions.



3. Amplify captured/exonic regions.
4. Sequence DNA.

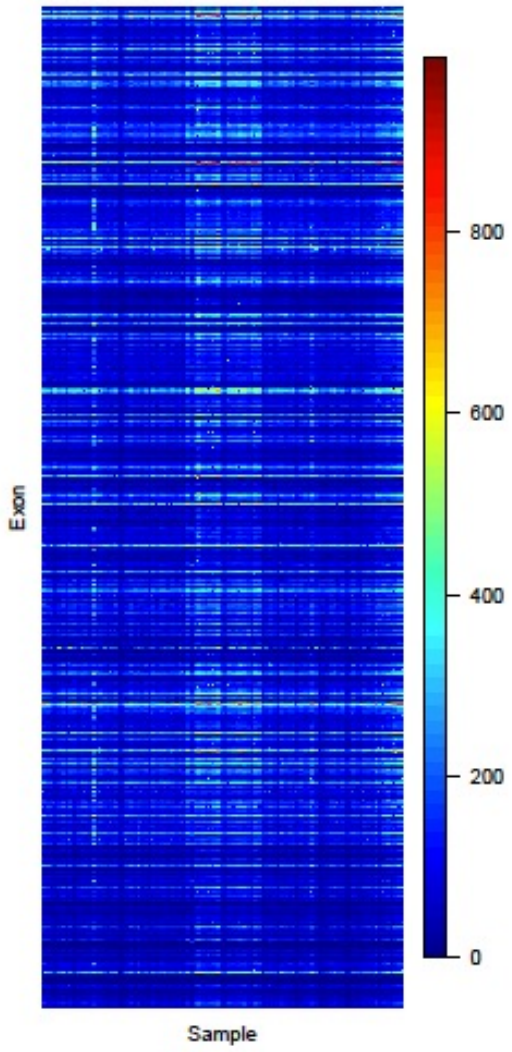


(https://en.wikipedia.org/wiki/Exome_sequencing)

Our goal

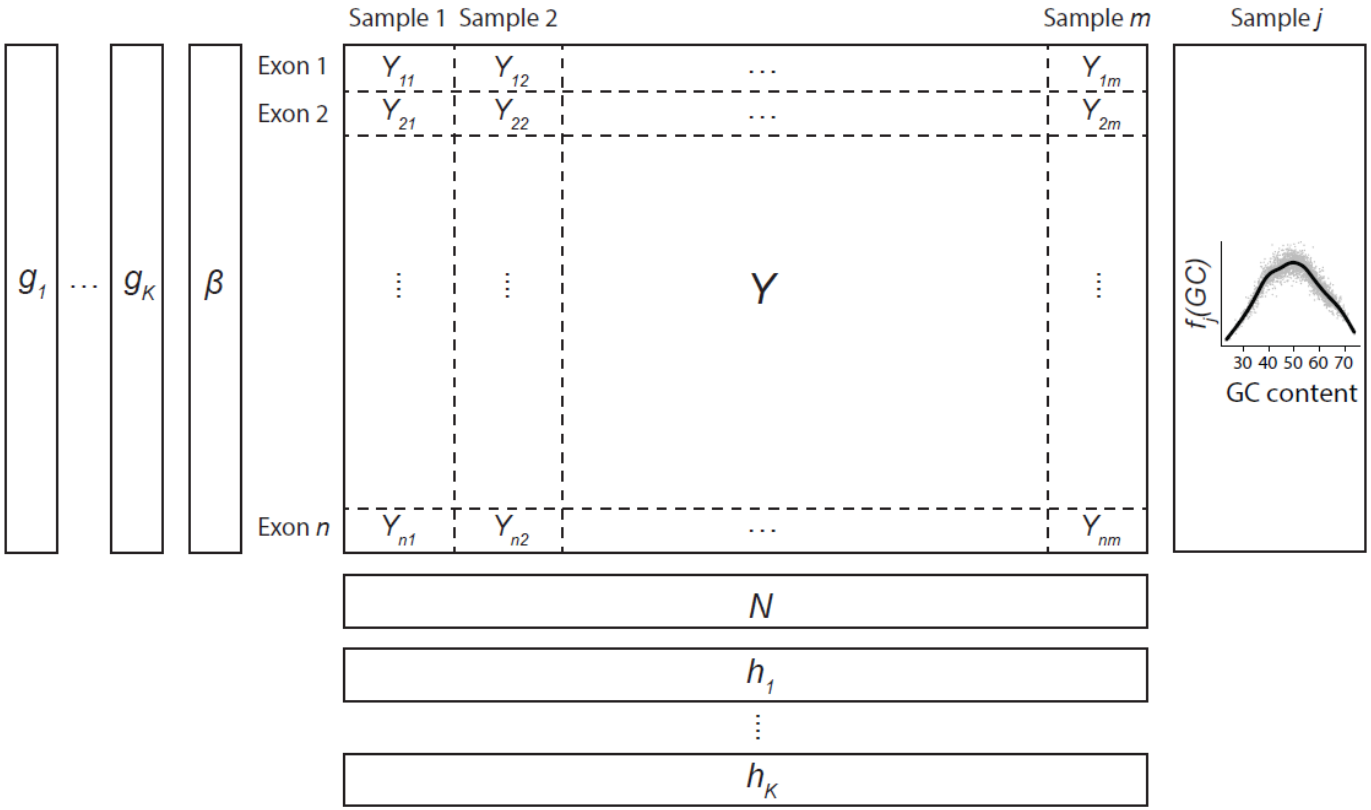
- Use bulk sequencing to accurately detect CNV
  - Based on depth of coverage
    (i.e., number of times a genomic region is “read”)
- What are the biases?
  - GC content (ease of segmentation)
  - Exon capture and amplification efficiency
  - Batch effect, population stratification
  - Latent factors
  - …

Cross-sample normalization model



- What has been done: PCA
  - Continuous measurements
  - Assume Gaussian noise structure
  - Ignores known quantifiable biases, such as GC content, exon lengths, etc.
  - GC content bias cannot be captured by a linear PC

Poisson latent factor model



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
$g_{ik} h_{jk}$ $(1 \leq k \leq K)$: kth latent Poisson factors

Poisson latent factor model

- CNV detection:

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

(Jiang et al., Nucleic Acids Research, 2015)

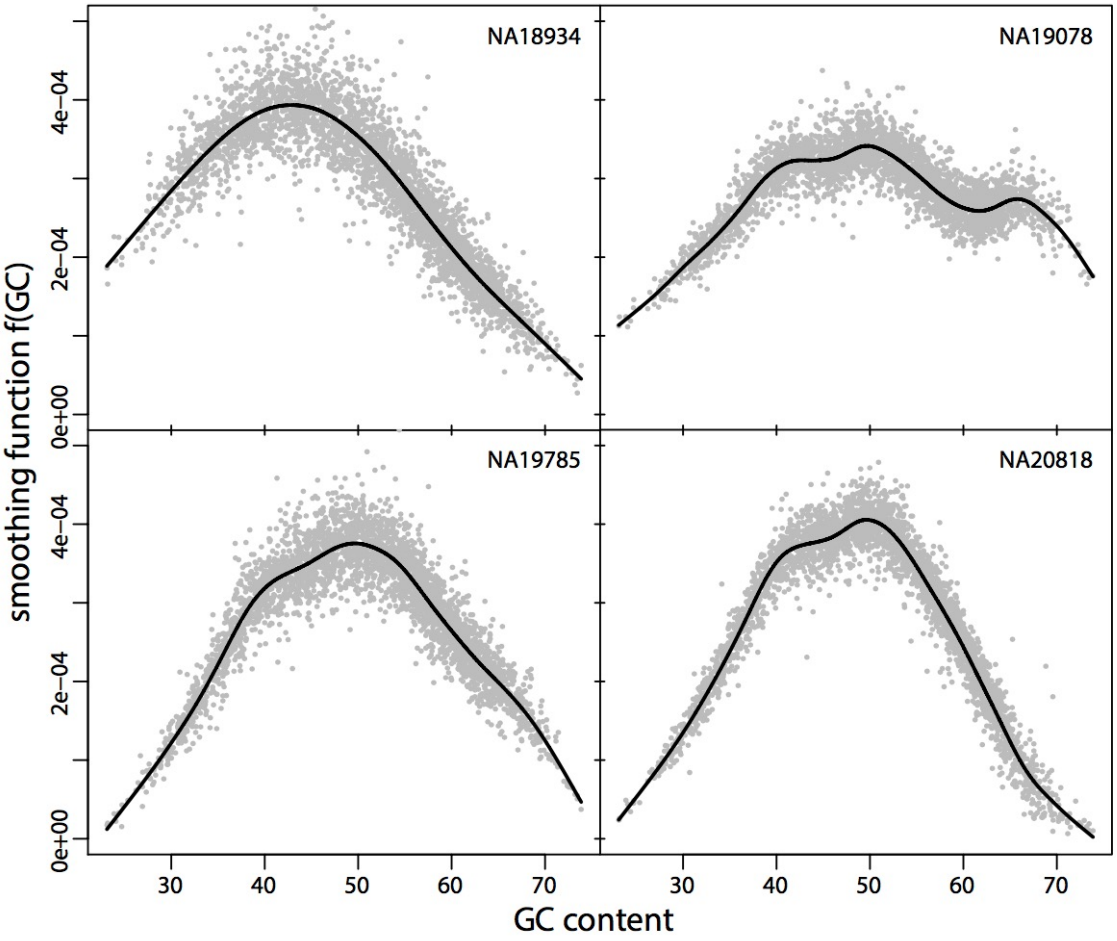
- Spatial transcriptomics:

$$Y_{i,j} \mid \lambda_{i,j} \sim \text{Poisson}\left(N_i \lambda_{i,j}\right)$$
$$\log\left(\lambda_{i,j}\right) = \alpha_i + \log\left(\sum_{k=1}^{K} \beta_{i,k}\mu_{k,j}\right) + \gamma_j + \varepsilon_{i,j}$$

(Cable et al., Nature Biotechnology, 2022)

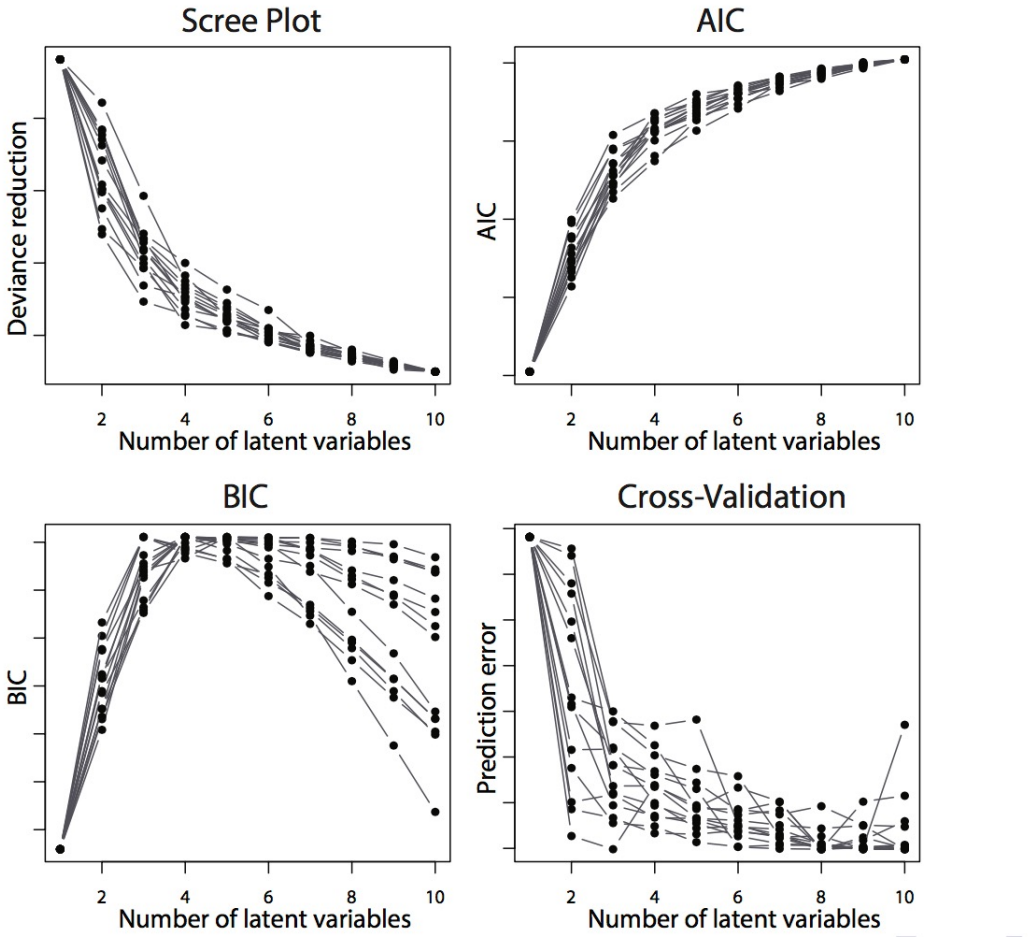
GC content bias

Whole-exome sequencing data from the 1000 Genomes Project

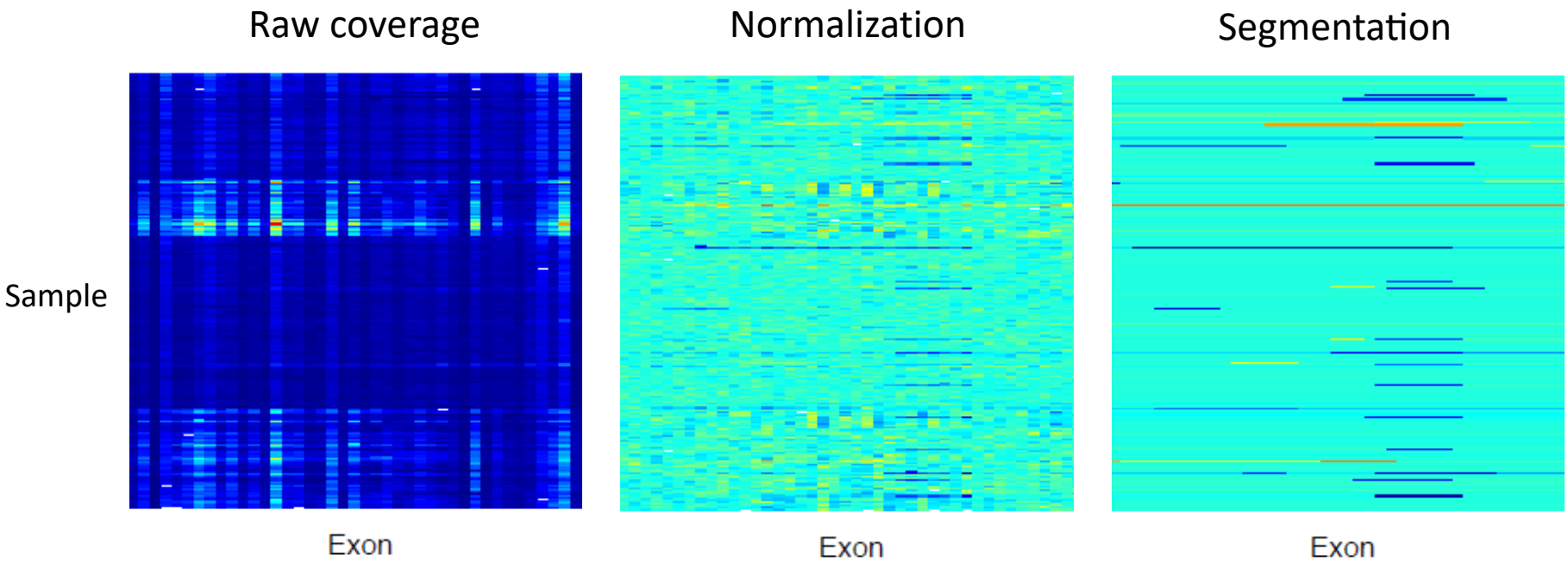


How to choose the number of latent factors?

Whole-exome sequencing data from the TARGET initiative

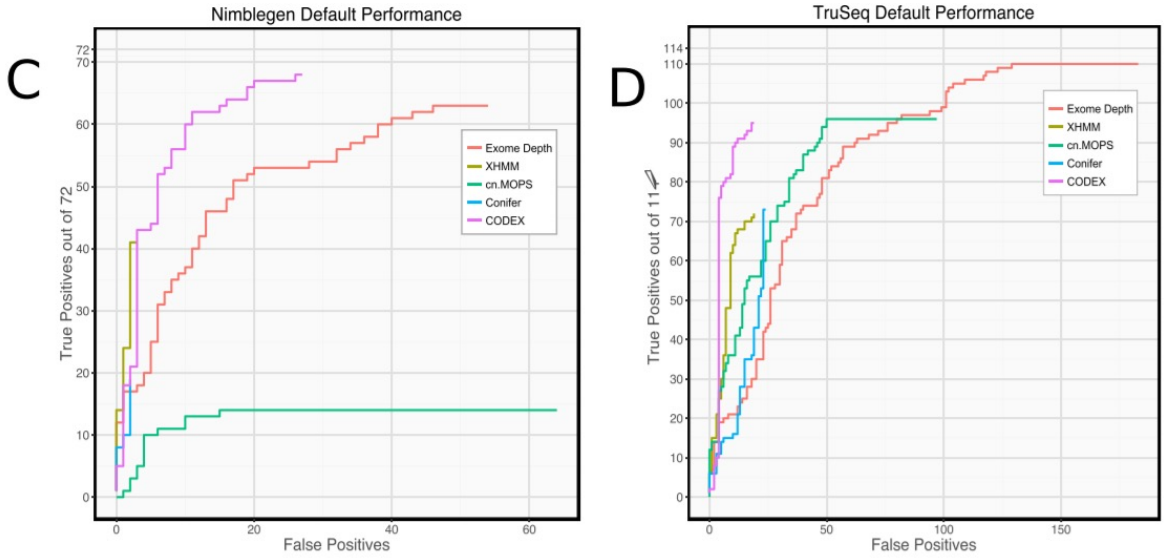


CODEX: COpy number Detection by EXome-seq



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

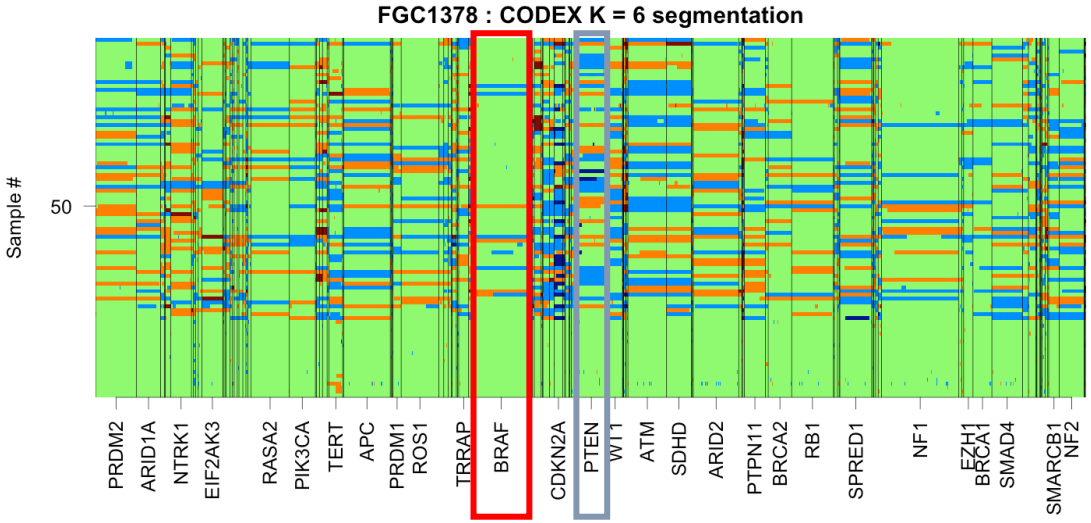
Independent benchmark results



(Sadedin et al., GigaScience, 2018)

Melanoma targeted sequencing

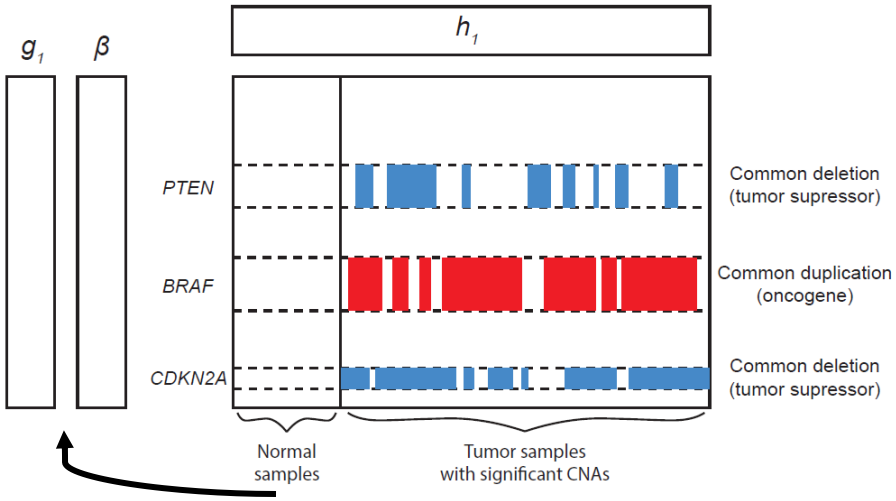
High amp
Amp
Null
Hemi. del
Homo. del

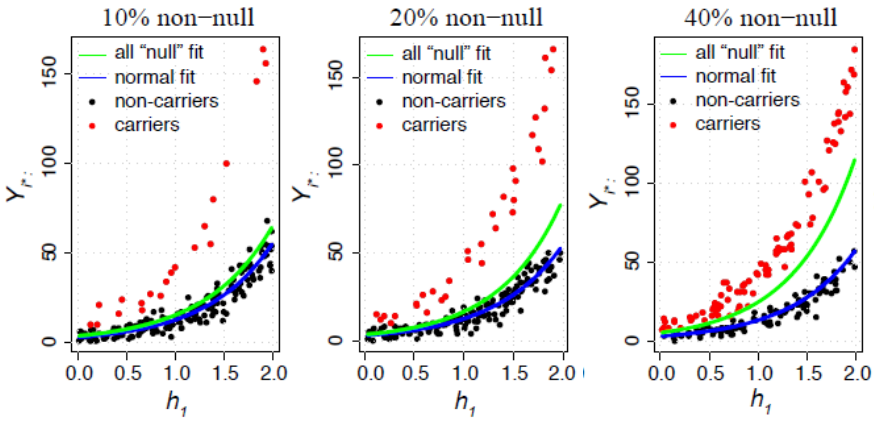


CODEX

Common CNV signals are attenuated by the Poisson latent factors!

Low sensitivity for common CNVs





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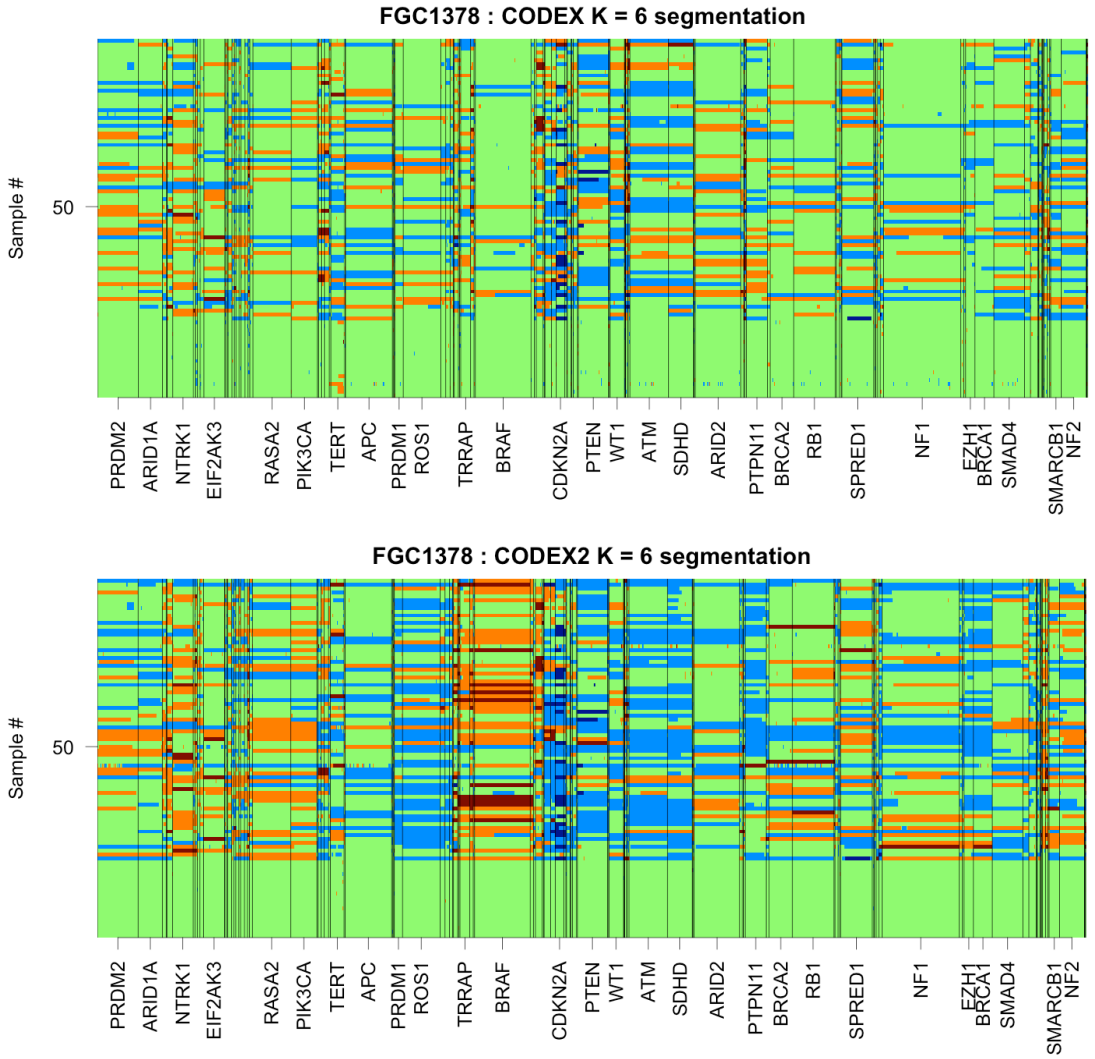
Solution:
ONLY use normal samples to estimate exon-specific bias and latent factors!

Melanoma targeted sequencing: sanity check

High amp
Gain
Null
Hemi. del
Homo. del

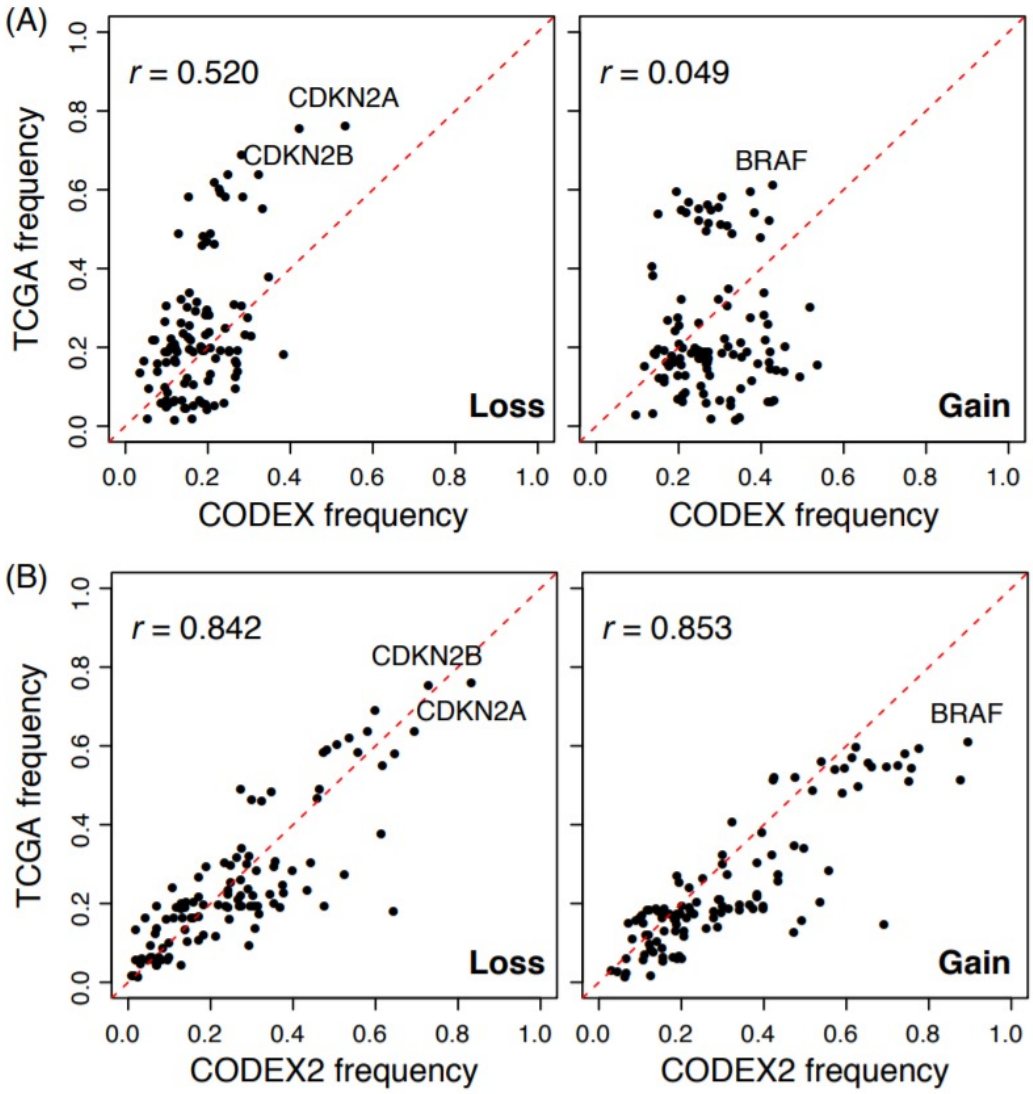


CODEX

CODEX2

(Garman et al., Cell Reports, 2017)

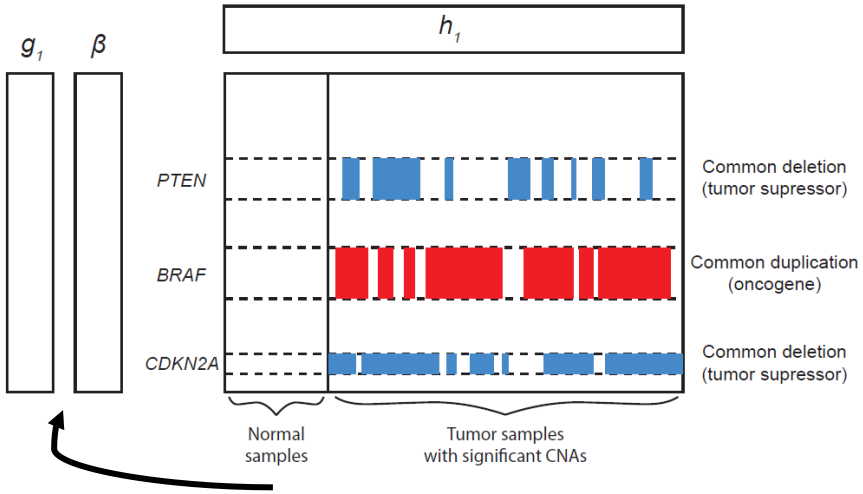
TCGA validation

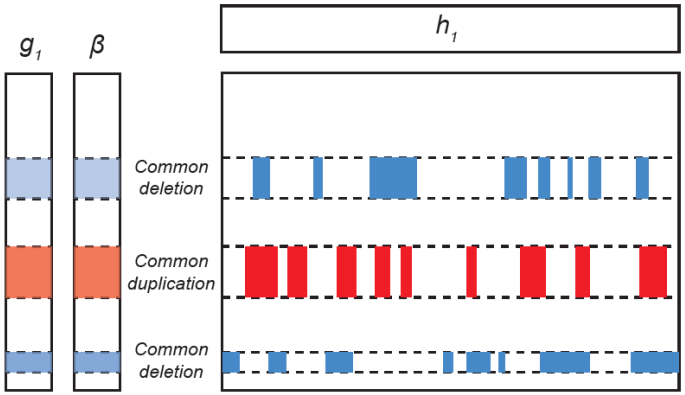


CODEX

CODEX2

Recovering common CNV signals

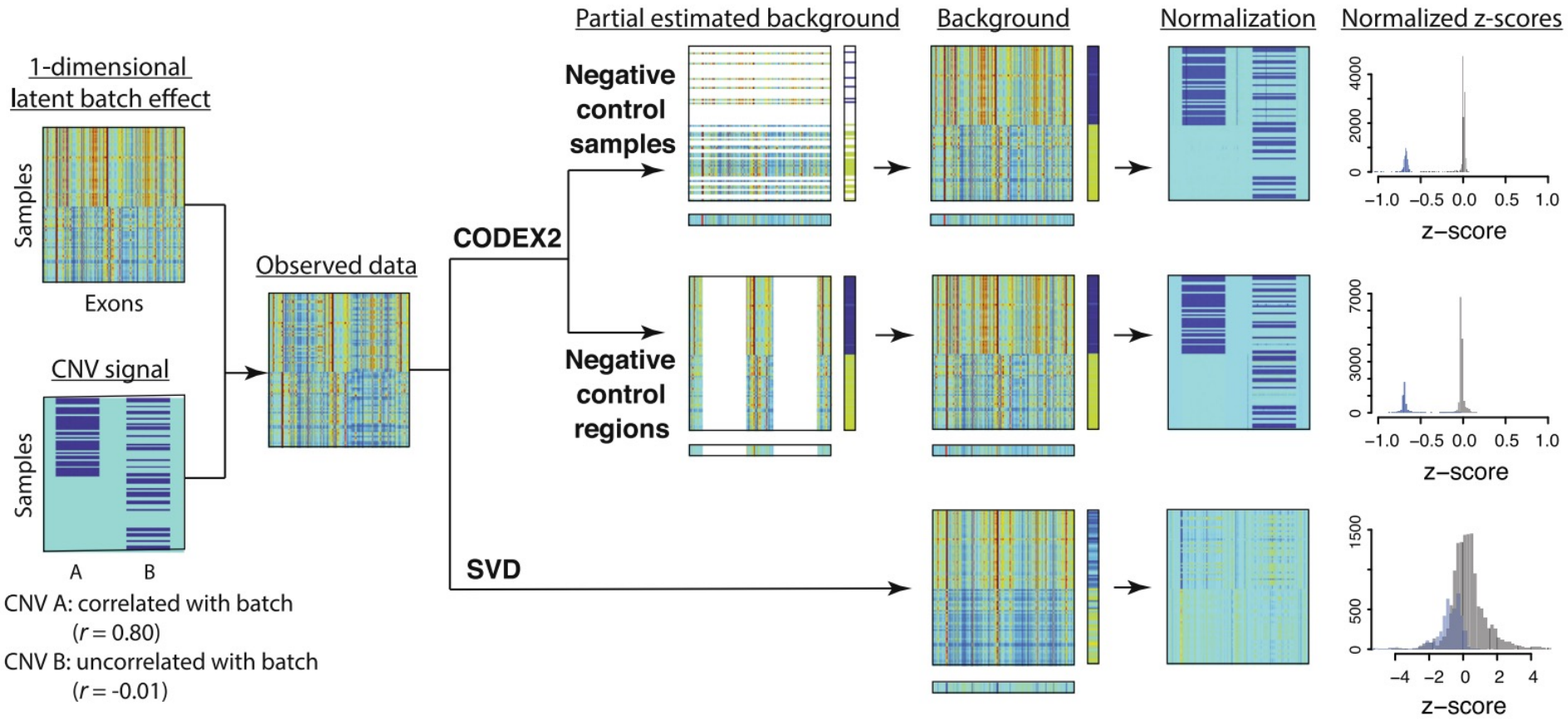




ONLY use normal samples to estimate exon-specific bias and latent factors.

What if we don't know which samples are "normal" (common germline CNV detection)?

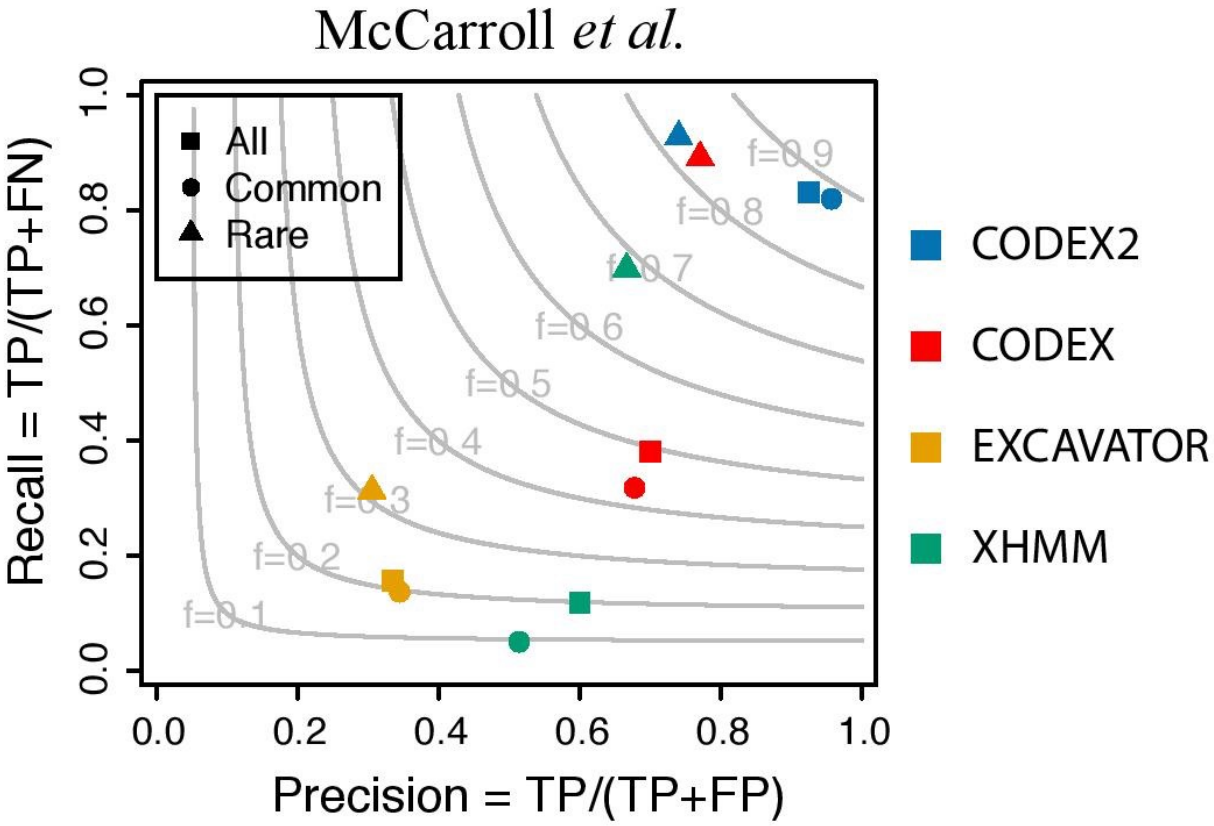
CODEX2: full-spectrum CNV detection by NGS

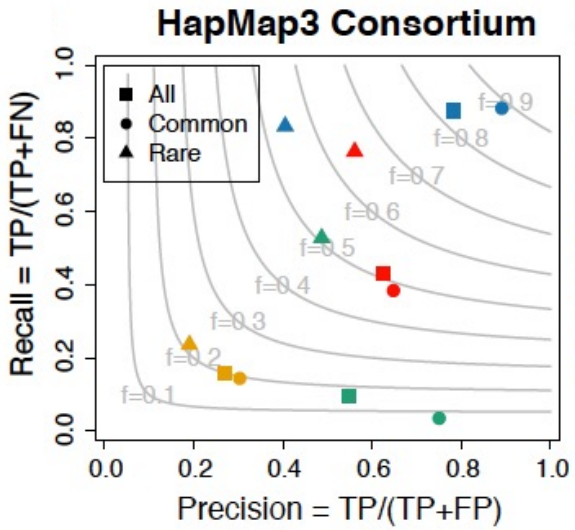


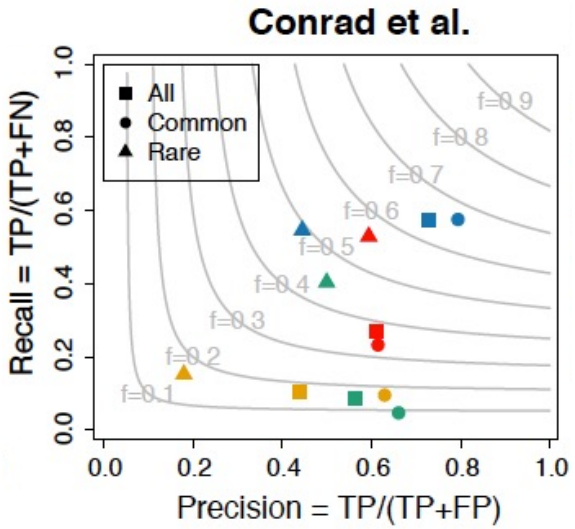
https://github.com/yuchaojiang/CODEX2

(Jiang et al., Genome Biology, 2018)

HapMap samples: SNP-array validation







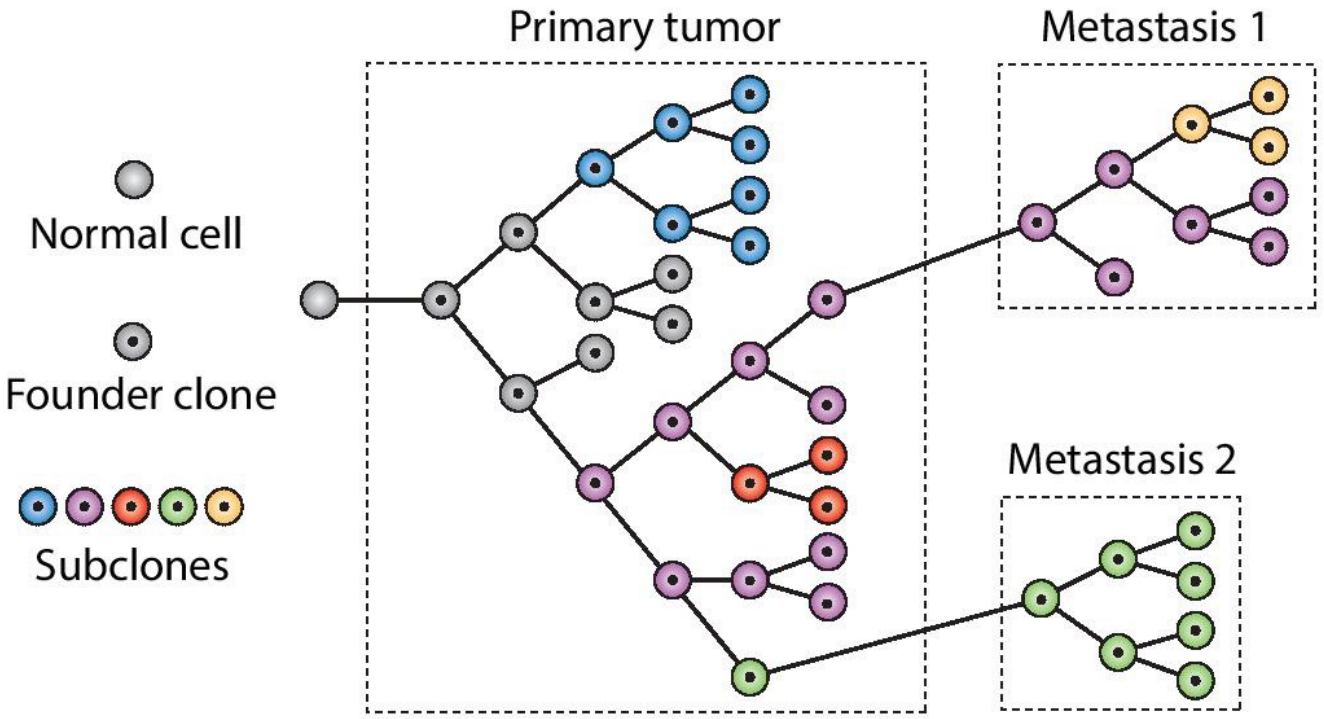
CNV profiling: from bulk tissue to single cells

- Bulk DNA-seq
  - Whole-genome sequencing (WGS)
  - Whole-exome sequencing (WES) & targeted sequencing
- Single-cell DNA-seq
  - Conventional whole-genome amplification
  - 10X Genomics Chromium Single Cell CNV Solution / DLP+

Population of cells (bulk sequencing)

Single cell (single-cell sequencing)

Profiling somatic copy number aberrations by scDNA-seq

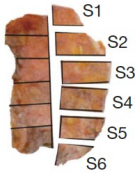


(Jiang et al., *PNAS,* 2016)

- Goal: Use scDNA-seq to assess intra-tumor heterogeneity by profiling somatic copy number aberrations with single-cell resolution.

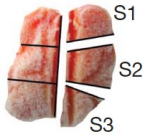
scDNA-seq data breast cancer patients

Patient T10

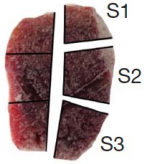


Polygenomic tumor

Patient T16 Primary Tumor



Patient T16 Metastasis

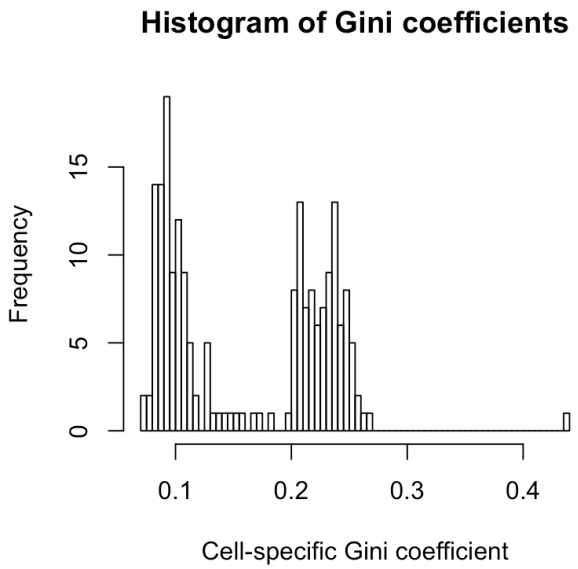


Monogenomic tumor

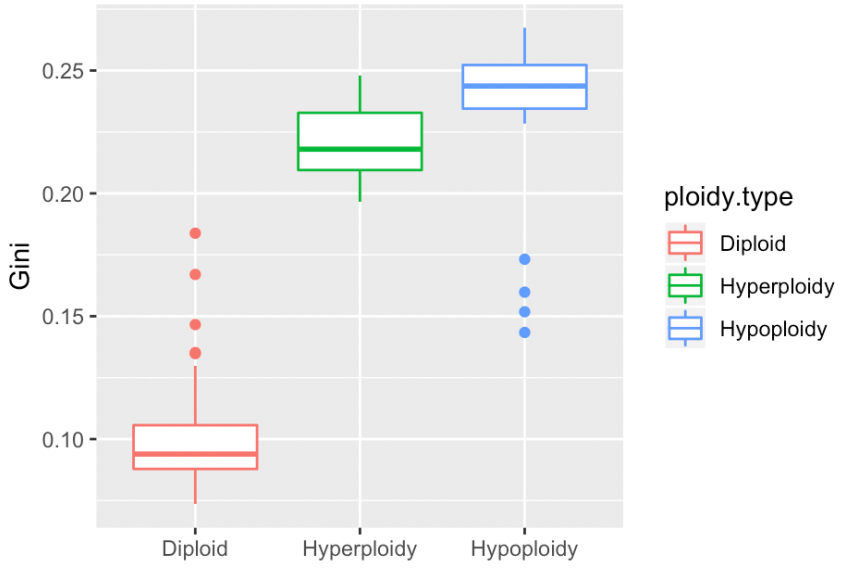
FACS sorting followed by copy number profiling by scDNA-seq revealed three distinct subclones in patient T10 and a single clonal expansion in patient T16.

Using Gini index to identify normal cells

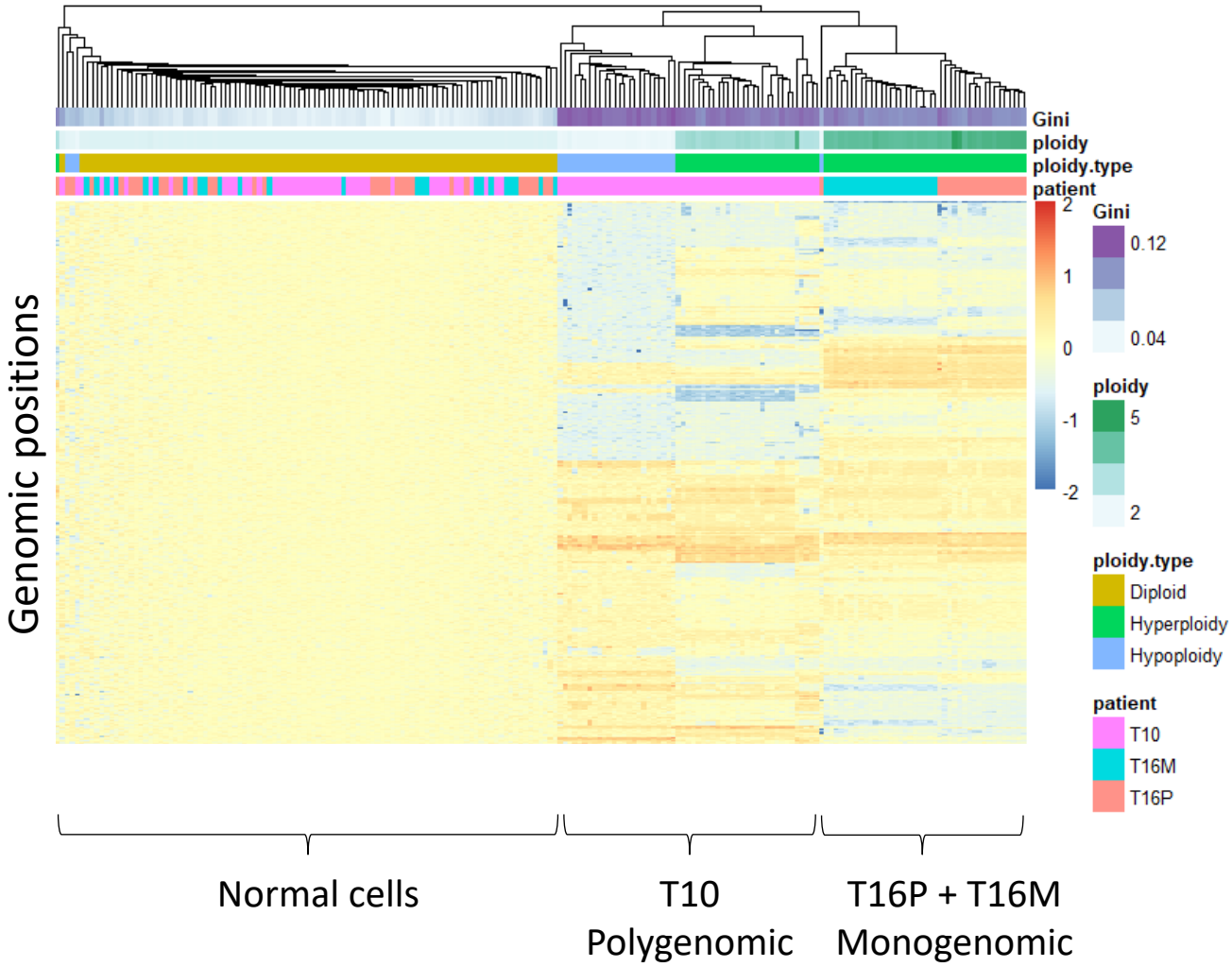
- Cell-specific Gini index distribution.

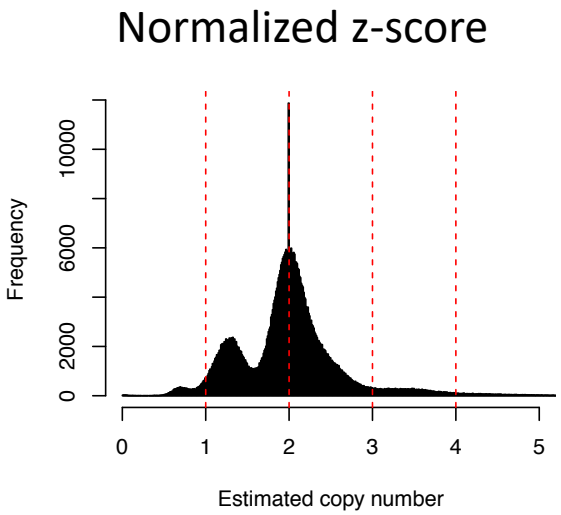


- Cancer cells have higher Gini index compared to normal cells by FACS sorting.



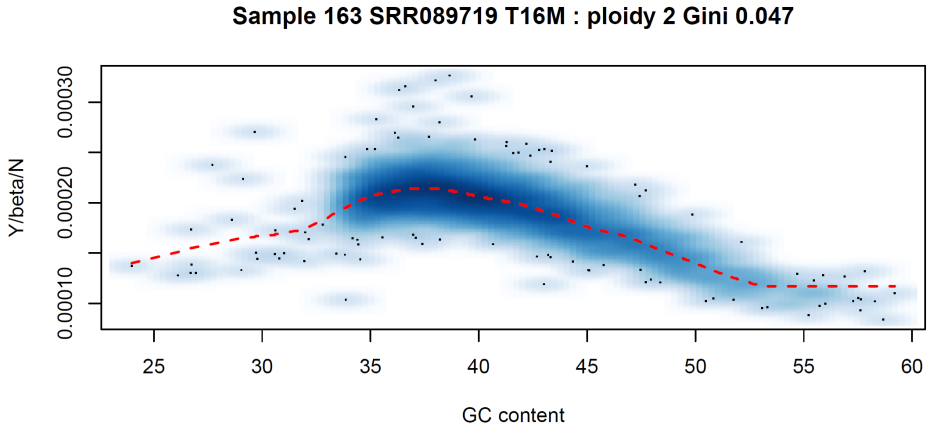
CODEX2 with negative control samples (i.e., normal cells)



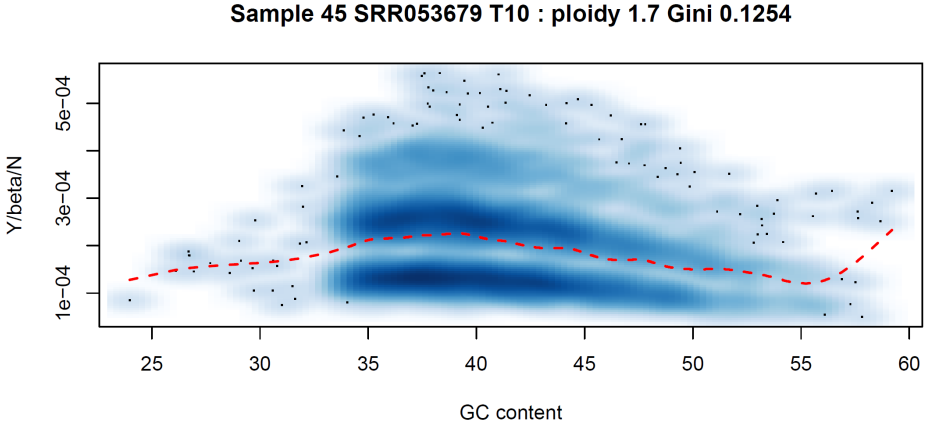


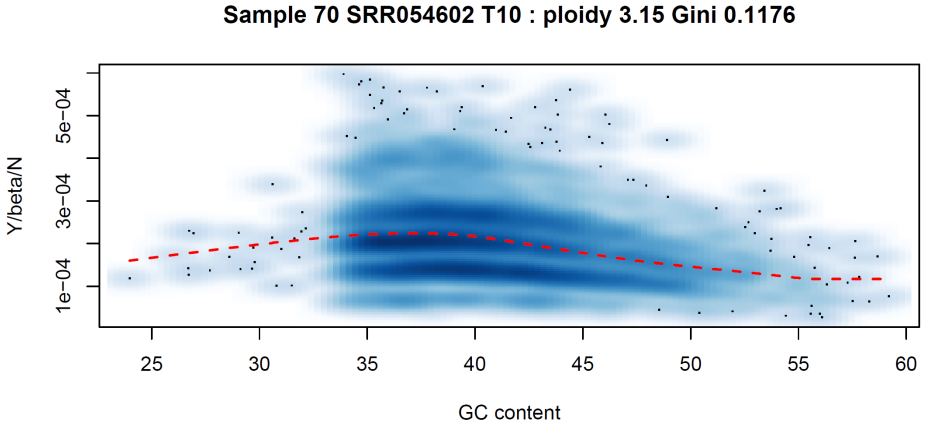
Sanity check: GC content bias *f(GC)* by CODEX2

- Good fit:



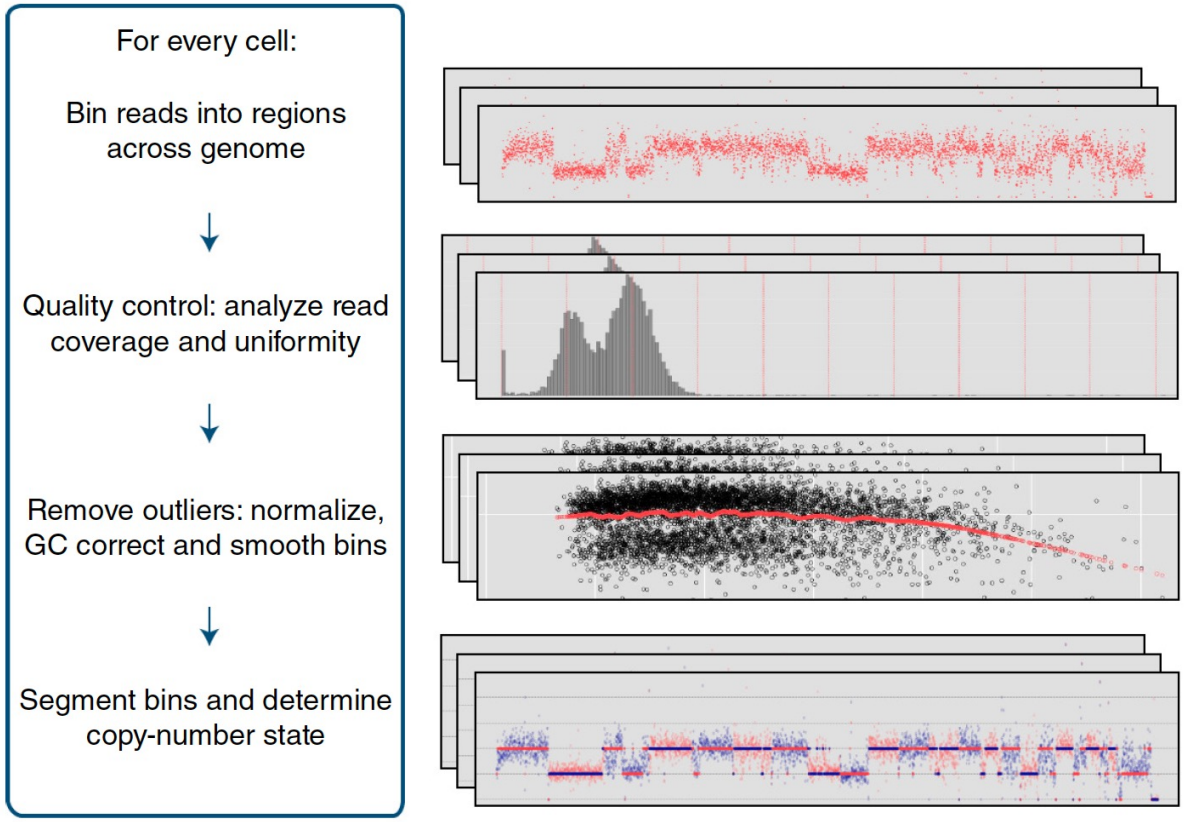
- Poor fit: multiple different copy number states contribute to multiple increments in $f(GC)$





Benchmark against existing method

- Ginkgo (Garvin et al., Nature Methods, 2015)
  - Normalization: Lowess fit to correct for GC bias
  - Segmentation: Cell-specific circular binary segmentation



(Figure 1: Gavin et al., *Nature Methods*, 2015)

EM algorithm for GC bias

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

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

- If there are significant genome-wide copy number changes,

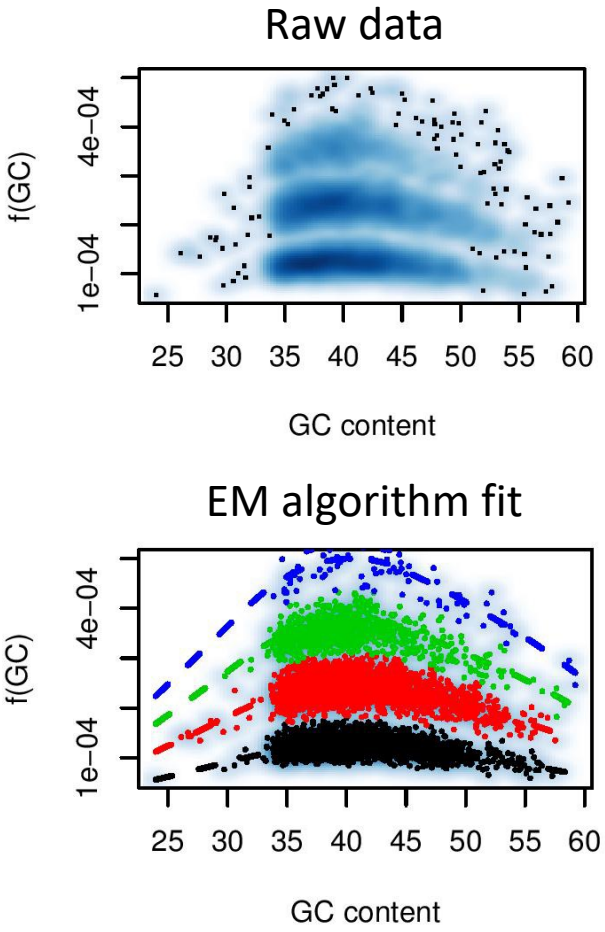
$$\boldsymbol{\alpha_{ij}} = \begin{cases} 1/2 & \text{with probability } \pi_1^{(j)} \\ 2/2 & \text{with probability } \pi_2^{(j)} \\ \vdots & \vdots \\ T_j/2 & \text{with probability } \pi_{T_j}^{(j)} \end{cases},$$

  where T_j is the number of copy number groups and $\sum_{t=1}^{T_j} \pi_t^{(j)} = 1$.

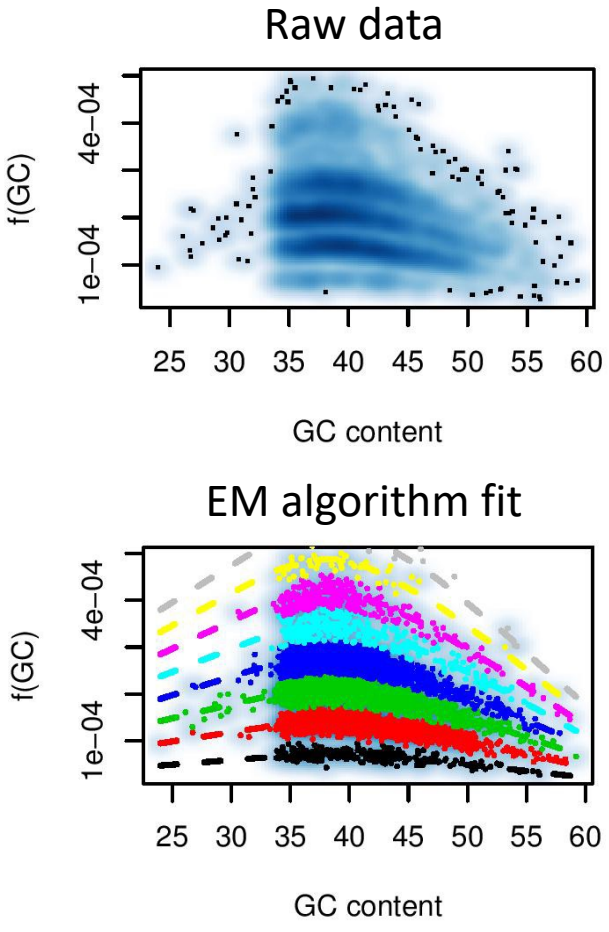
- $\phi(Y_{ij}) = \sum_{t=1}^{T_j} \pi_t^{(j)} \phi_t(Y_{ij}) =$
  $\sum_{t=1}^{T_j} \pi_t^{(j)} \text{pPoisson}\left(Y_{ij}; N_j \beta_i f_j(GC_i) \frac{t}{2} \exp\left(\sum_{k=1}^{K} g_{ik} h_{jk}\right)\right)$.

EM algorithm for GC bias: empirical fit

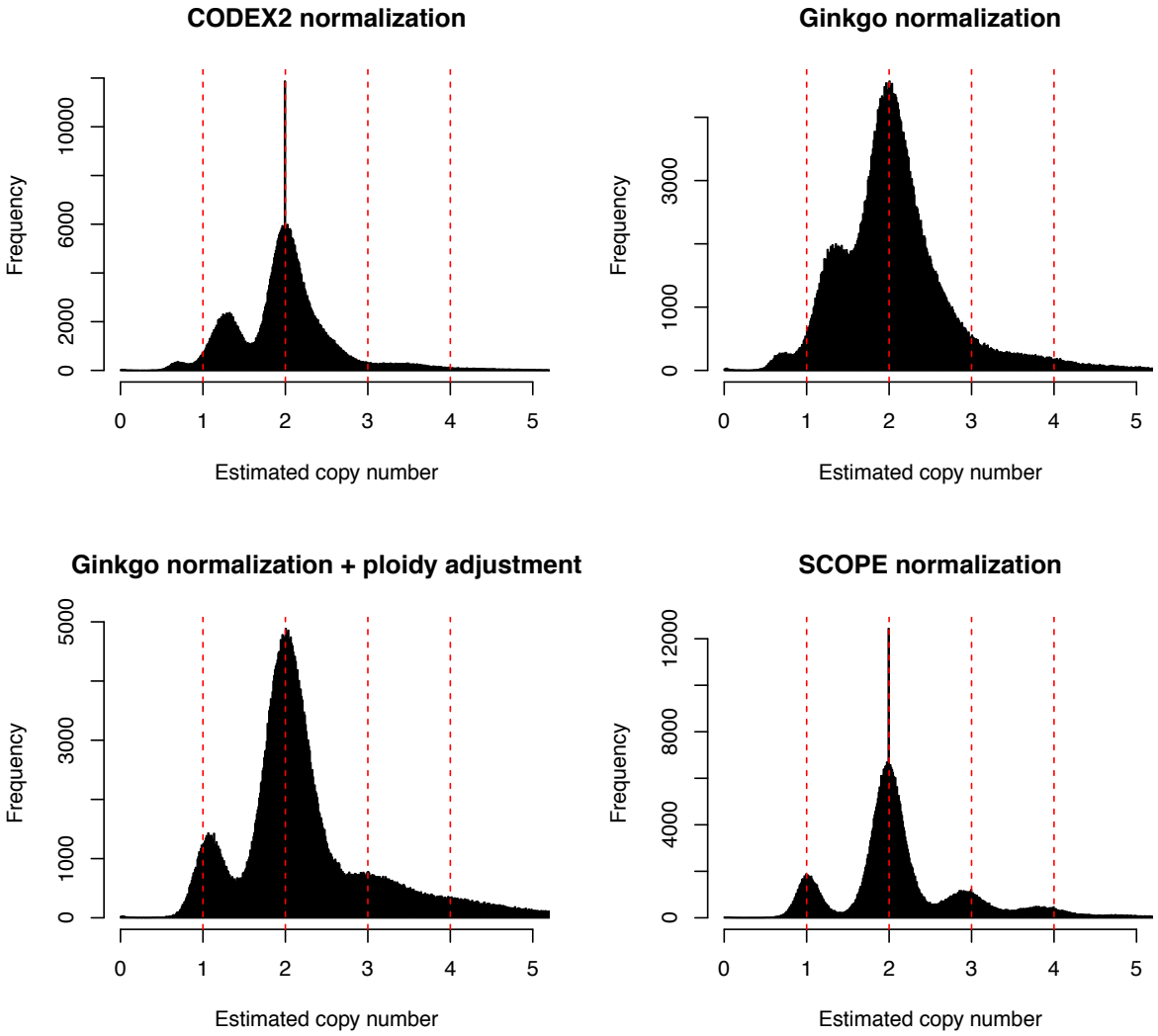
Hypodiploid cell (cell ploidy < 2)



Hyperdiploid cell (cell ploidy > 2)

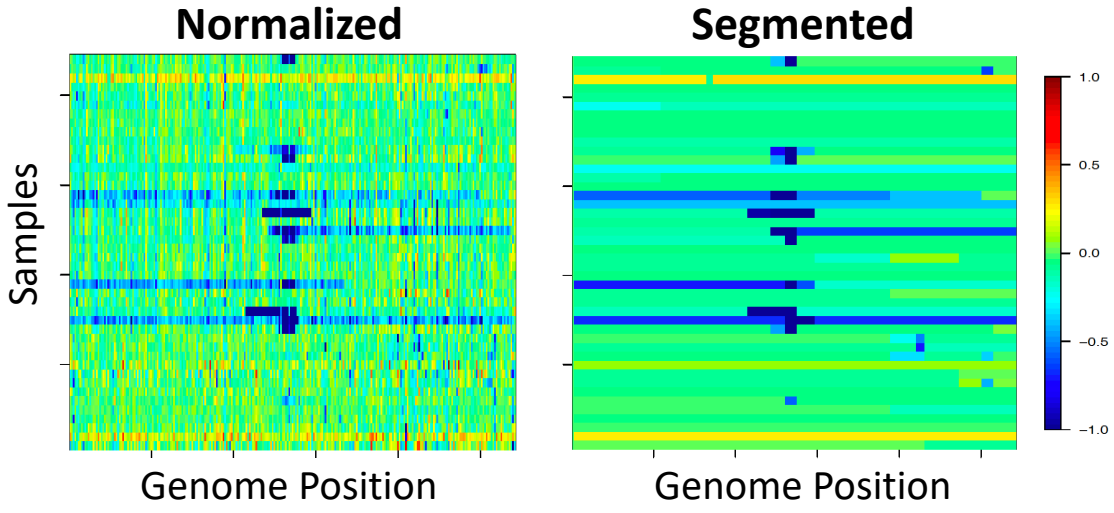


Normalization results for patient T10



Cross-sample segmentation

- Why across samples? Breakpoints are shared across cells from the same clone.



- The scan statistic <u>across all cells</u> is $\max_{s,t} Z_{s,t}$:

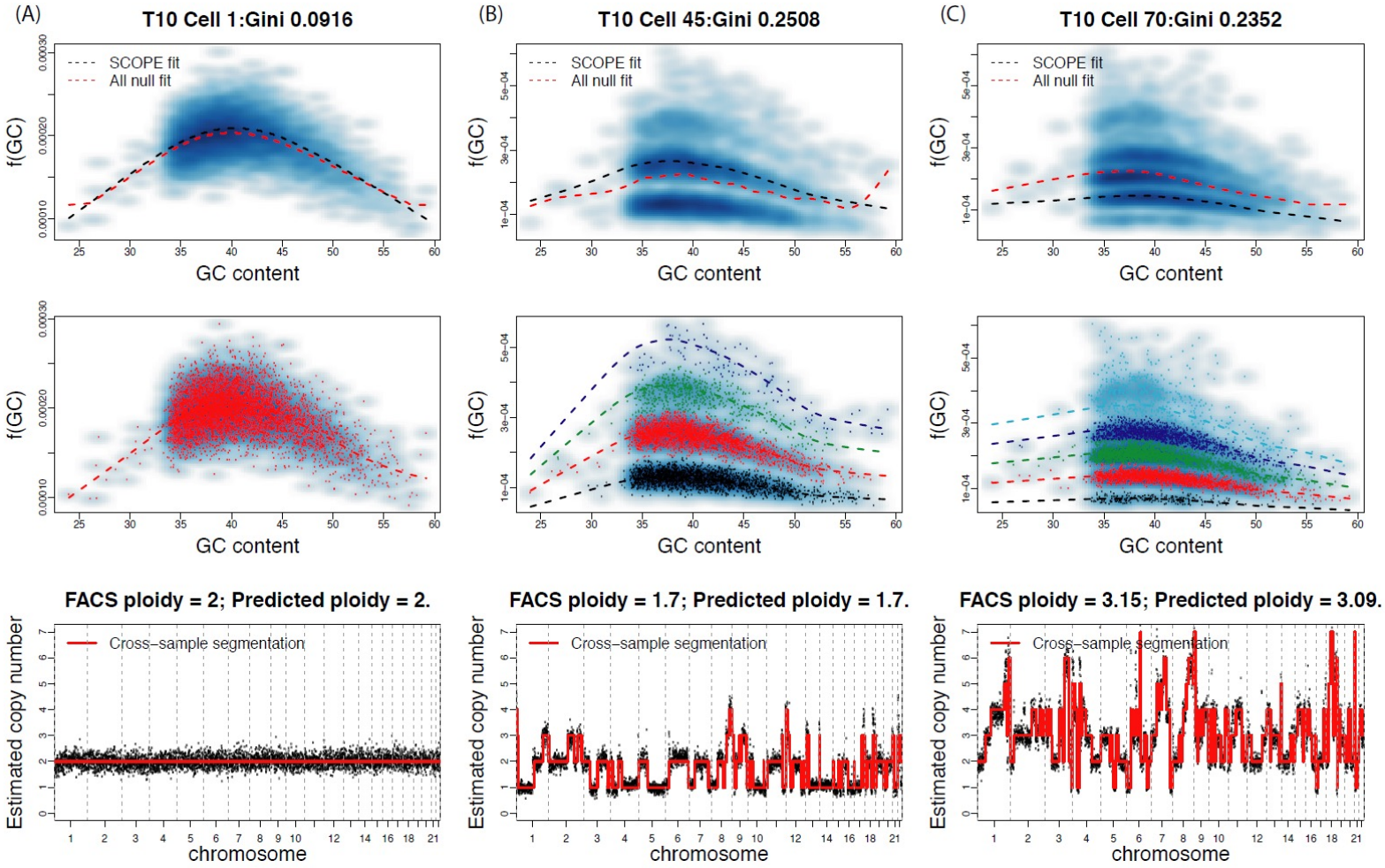
$$Z_{s,t} = \sum_j U_j(s,t) = \sum_j Y_{s:t,j} \log\left(\frac{Y_{s:t,j}}{\lambda_{s:t,j}}\right) - \left(Y_{s:t,j} - \lambda_{s:t,j}\right).$$

- Recursively find the maximizing cut in the sub regions, using a cross-sample mBIC as stopping rule:

$$\mathrm{mBIC}(p) = \log\left(\frac{L_\tau}{L_0}\right) - \frac{P}{2}\log\frac{2\log(L_\tau/L_0)}{P} - \frac{P}{2} - \log\binom{m}{p} - p(\kappa_1 - \kappa_2)$$

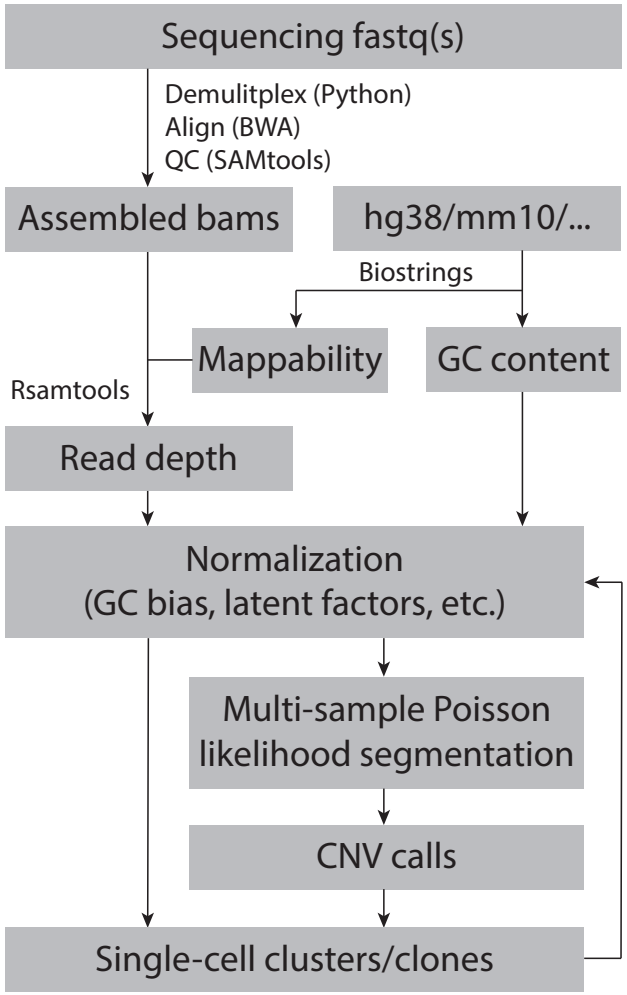
$$- \sum_{\rho=1}^{p} \log\left(\sum_{j\text{th carrier}} \hat{\delta}^2_{\rho,j}\right) + P\log\pi + (np - P)\log(1-\pi).$$

Embed EM for fitting GC bias in Poisson latent factor model with negative control samples



Estimated ploidy is taken as the mean estimated copy number across all bins. Close to previous reports.

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

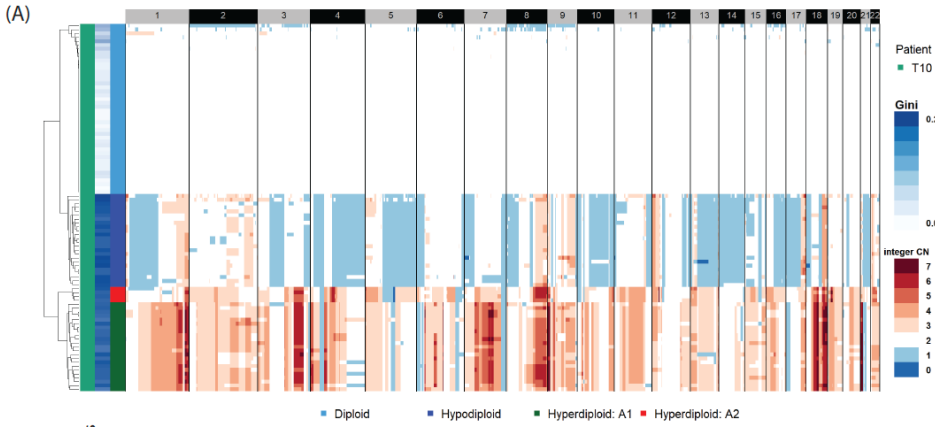


https://github.com/rujinwang/SCOPE

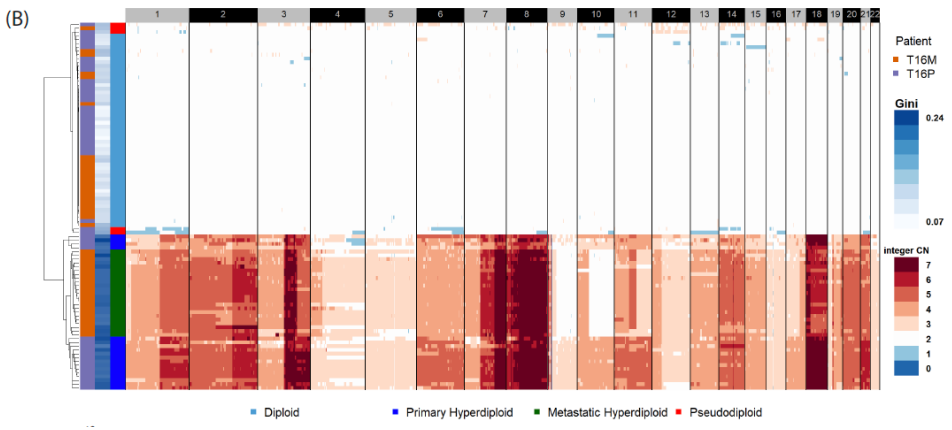
(Wang et al., *Cell Systems*, 2020)

CNV profiles of breast cancer patients T10, T16

Polygenomic tumor

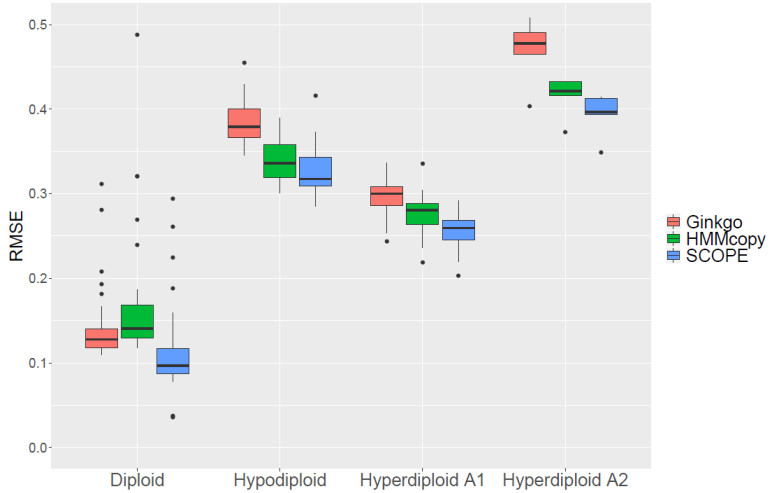


Monogenomic tumor

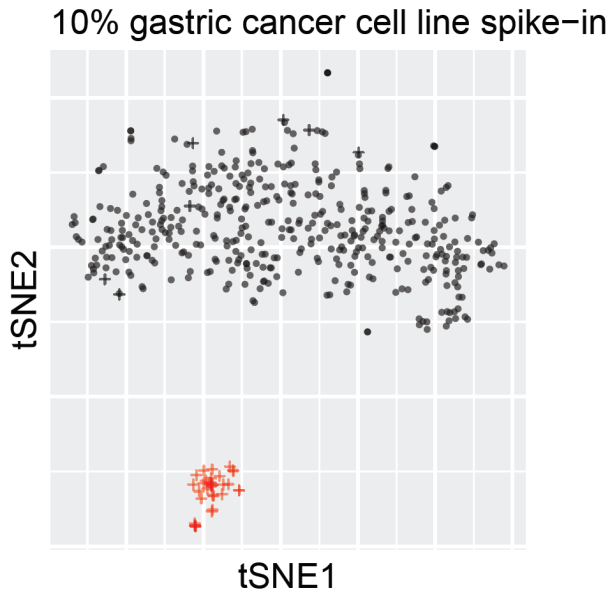


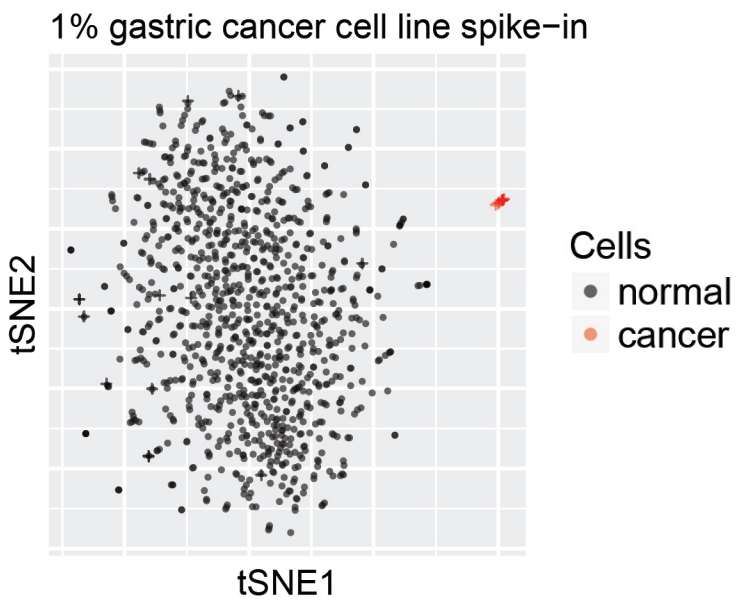
Gold standard for orthogonal validation:

CNV calls by aCGH of purified bulk samples (Navin et al., *Genome Research*, 2010)



10X Genomics: gastric cancer cell line spike-in



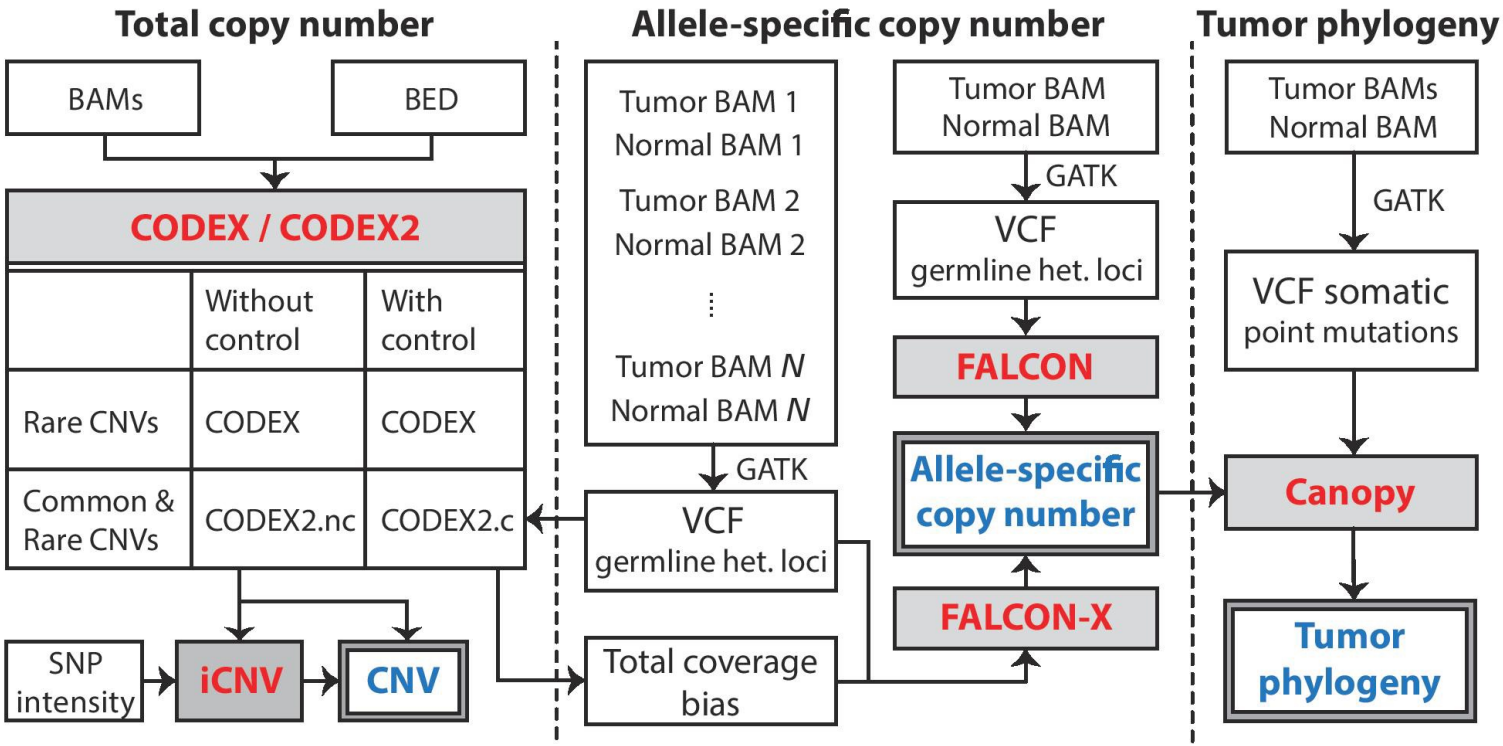


Estimated % cancer cells: 36/462 ≈ 8%.

Estimated % cancer cells: 11/1055 ≈ 1%.

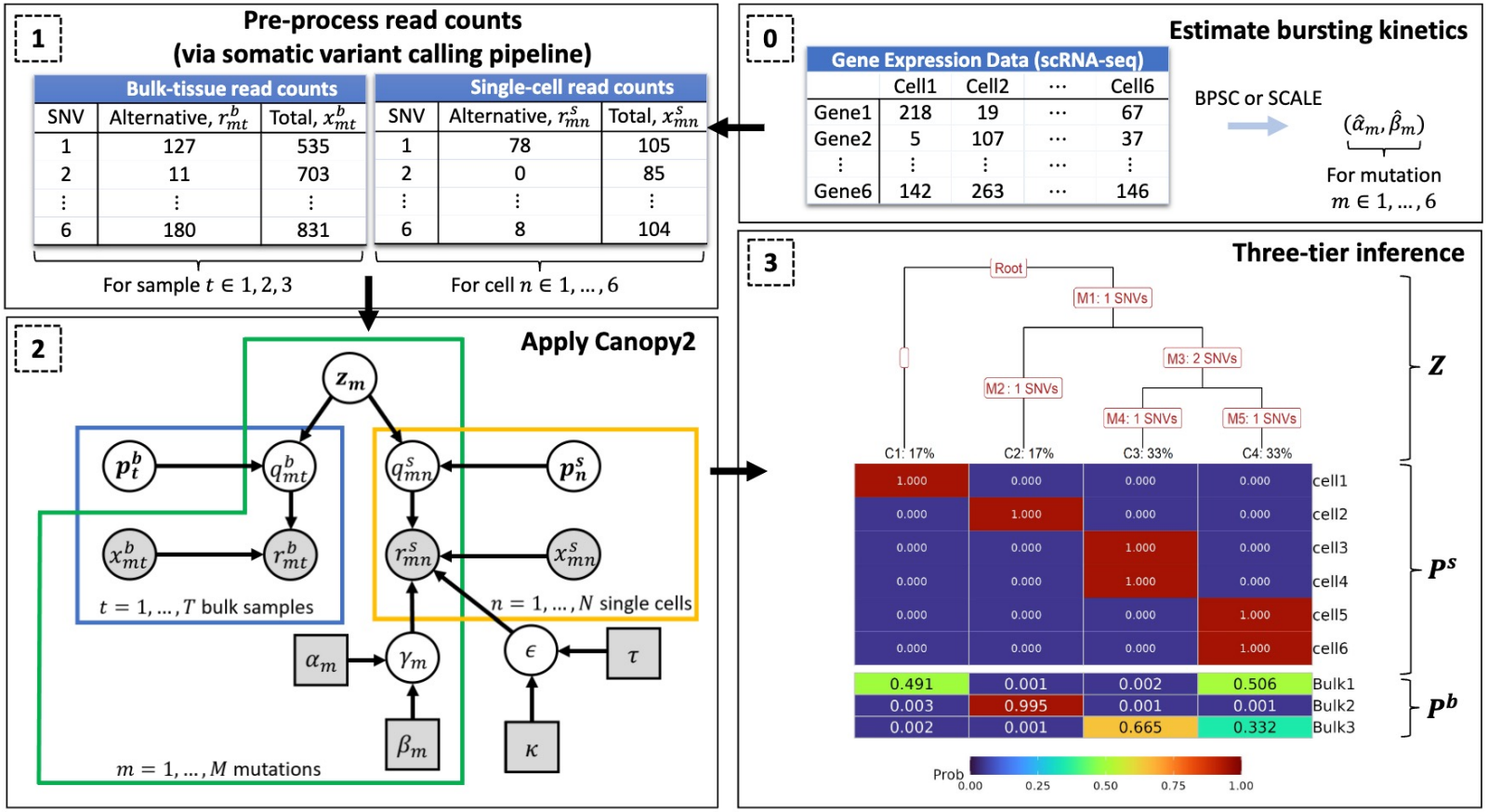
Other lines of research

Intratumor heterogeneity by bulk-tissue DNA-seq



Canopy: https://CRAN.R-project.org/package=Canopy
(Jiang et al., PNAS, 2016)
MARATHON: https://github.com/yuchaojiang/MARATHON
(Urrutia et al., Bioinformatics, 2018)

Intratumor heterogeneity by bulk-tissue DNA-seq and single-cell RNA-seq



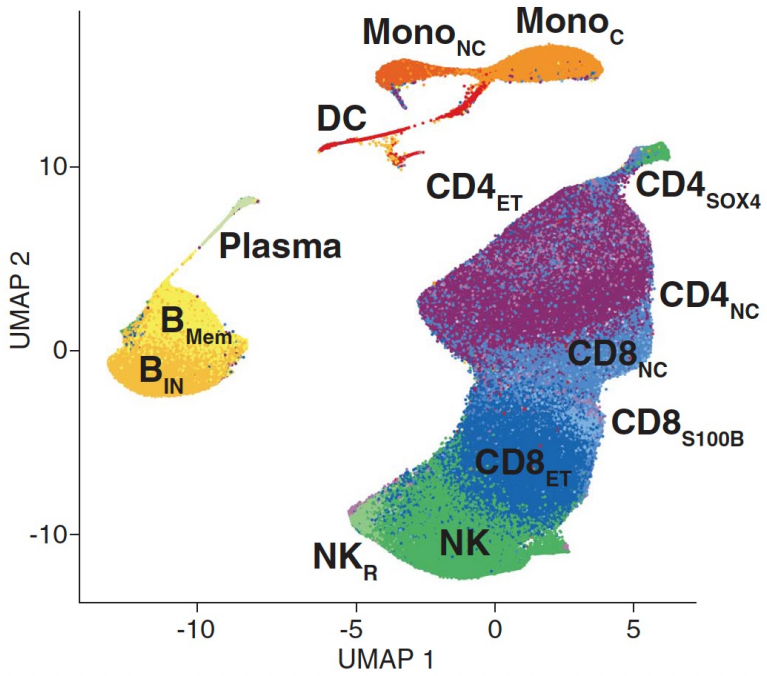
Canopy2: tumor phylogeny inference by bulk DNA and single-cell RNA sequencing

https://github.com/annweideman/canopy2

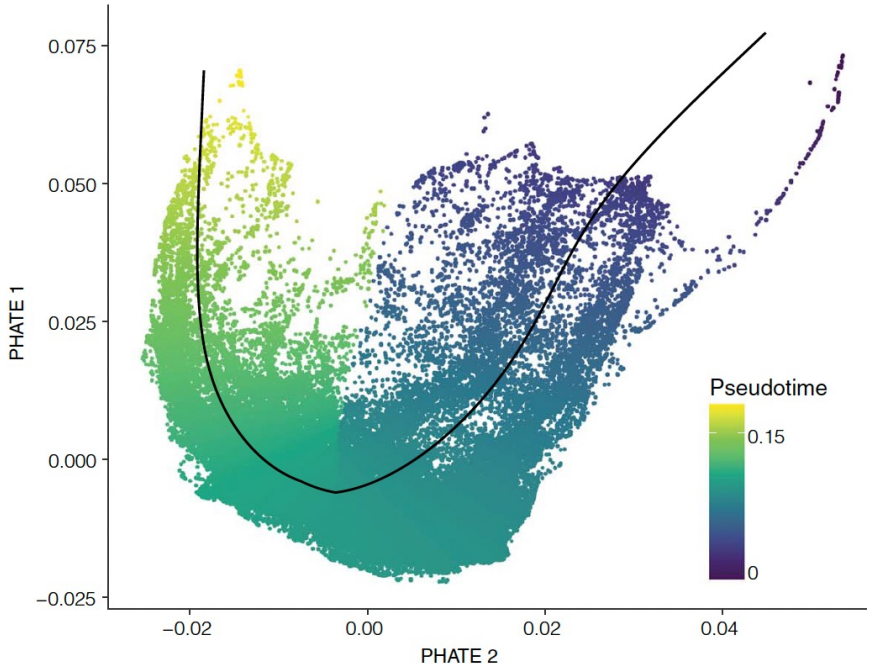
TAMU Biomedical Engineering Seminar: Nov 16th 2023, 2:20pm-3:35pm

Population-Scale Single-Cell Sequencing Data

- Population-Scale Data: 1.27 million PBMCs collected from 981 donors



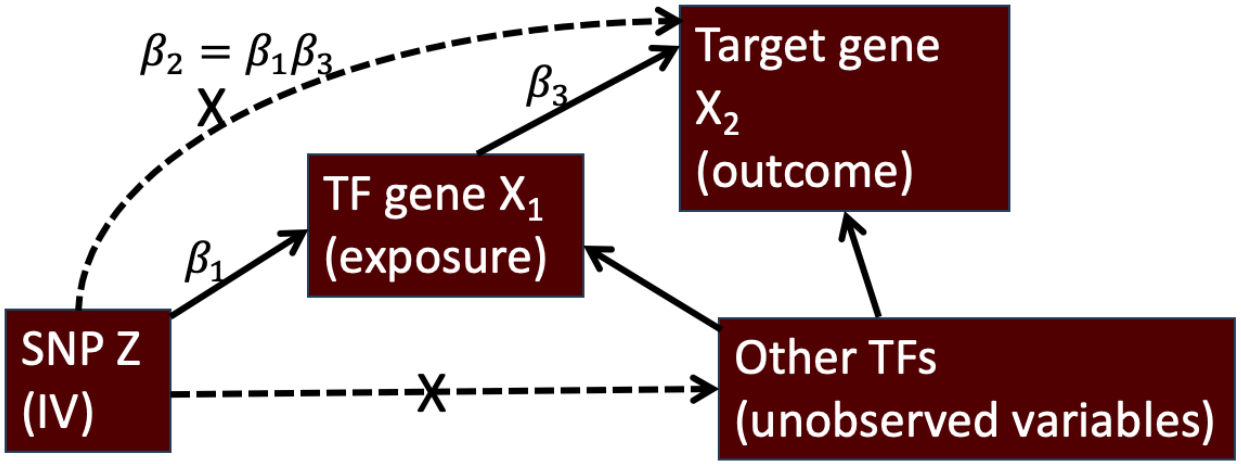
31 discretized blood cell types



B cell trajectory (125K B cells)

(Joint with Yang Ni)

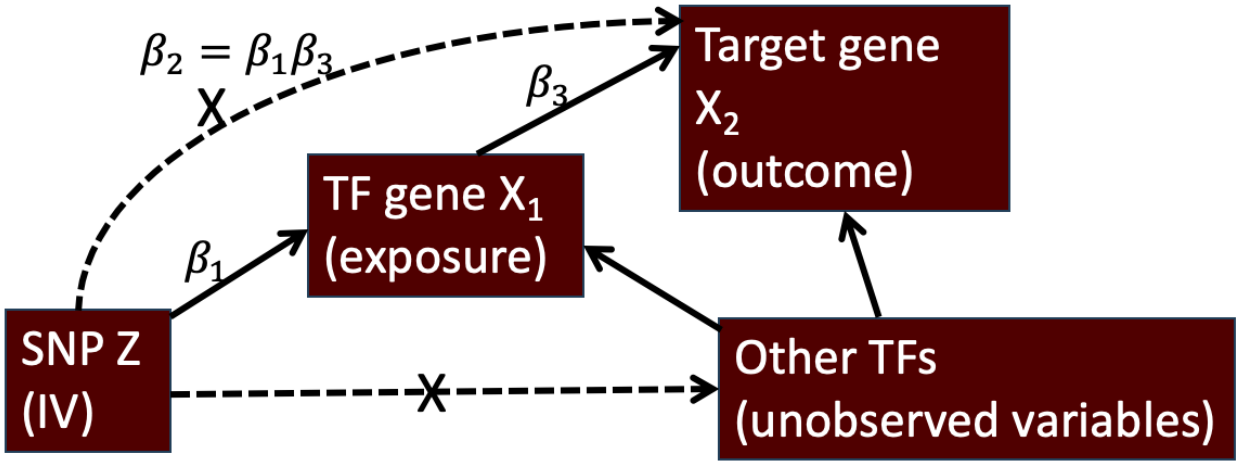
Mendelian Randomization for Cell-Type- and Cell-State-Specific Inference of Gene Regulation



- Using genetic variant (SNP) as an instrumental variable (IV):
  - First scan for significant cis-eQTL for the TF gene.
  - The cis-eQTL has no direct effect on the target gene (only mediated by the TF).
  - The cis-eQTL is not associated with unobserved confounders, such as other TFs.

(Joint with Yang Ni)

Mendelian Randomization for Cell-Type- and Cell-State-Specific Inference of Gene Regulation



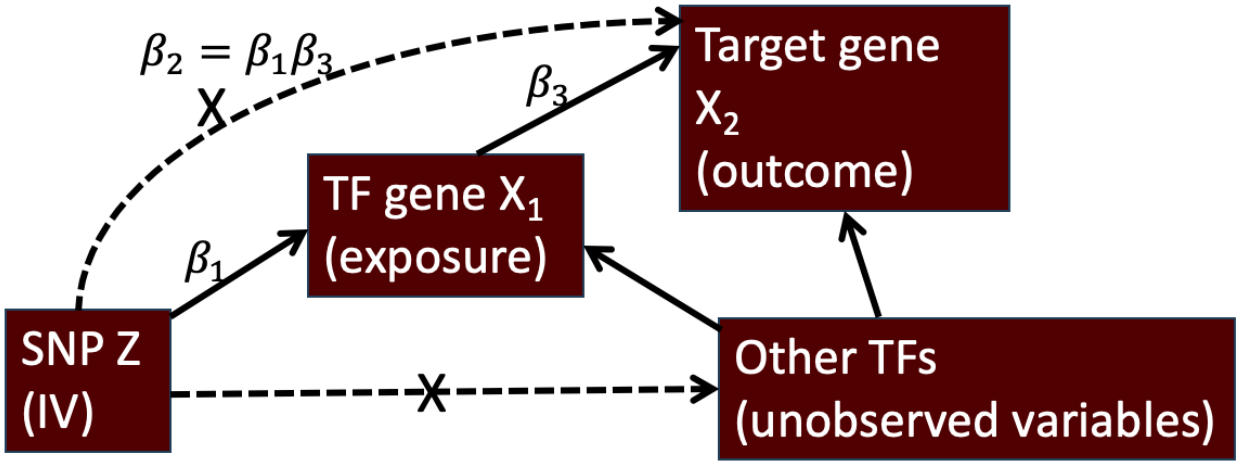
- For discretized cell type k:

$$X_1^k = \beta_1^k Z + \epsilon_1^k,$$
$$X_2^k = \beta_2^k Z + \epsilon_2^k.$$

Test $H_0: \beta_3^k = \frac{\beta_2^k}{\beta_1^k} = 0$ or regress across SNPs $\hat{\beta}_{2j}^k = \beta_3^k \hat{\beta}_{1j}^k + \epsilon_{\beta_j^k}$ to establish a TF-gene causal relationship.

(Joint with Yang Ni)

Mendelian Randomization for Cell-Type- and Cell-State-Specific Inference of Gene Regulation



- For transient cell states with pseudotime $t \in (0,1)$:

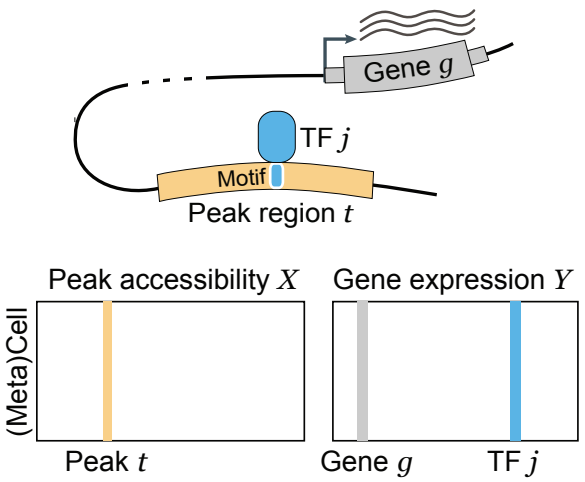
$$X_1(t) = \beta_1 Zt + \gamma_1 Zt^2 + \epsilon_{X_1}(t),$$
$$X_2(t) = \beta_2 Zt + \gamma_2 Zt^2 + \epsilon_{X_2}(t).$$

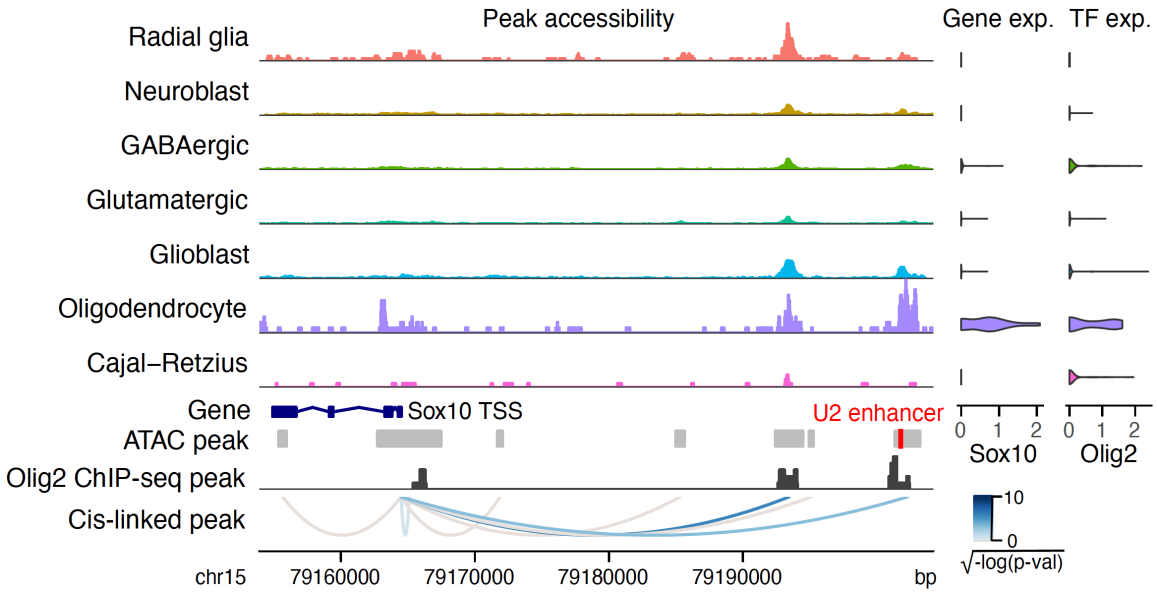
Or more generally using functional regression:

$$X_1(t) = \beta_1(t)Z + \epsilon_1(t),$$
$$X_2(t) = \beta_2(t)Z + \epsilon_2(t),$$
$$X_2(t) = X_1(t)\beta_3(t) + \epsilon_3(t).$$

(Joint with Yang Ni)

Nonparametric interrogation of transcriptional regulation in single-cell RNA and ATAC data



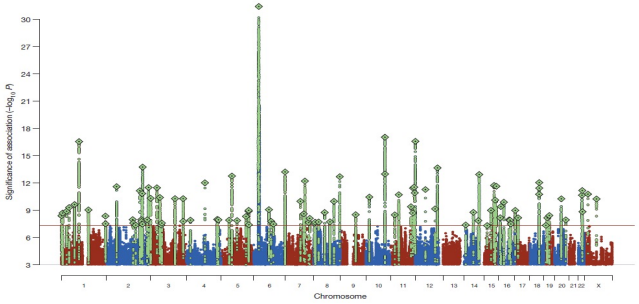


One peak-TF-gene trio:
(Jiang et al., Cell Systems 2022)
https://www.youtube.com/watch?v=Z8apanXAIS0&t

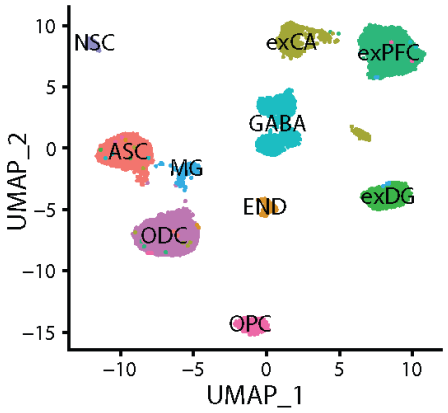
Multiple peaks, TFs, and genes:
Joint with Saptati Datta & Valen Johnson

Genome-wide associate studies + single-cell sequencing

Schizophrenia GWAS
(15,358,497 SNPs x 79,845 individuals)



Single-cell RNA sequencing
(17,698 genes x 14,137 cells)



How should GWAS summary statistics be integrated with single-cell data to **prioritize trait-relevant cell types** and to elucidate disease etiology?

GWAS + scRNA-seq: Wang et al., PLOS Genetics, 2022
https://www.youtube.com/watch?v=Z8apanXAIS0&t

GWAS + scATAC-seq: ongoing

Thanks! Questions?