A generalized errors-in-variables model, with application to single-cell CRISPR screens

Tim Barry[1], Eugene Katsevich[2], Kathyrn Roeder[1]

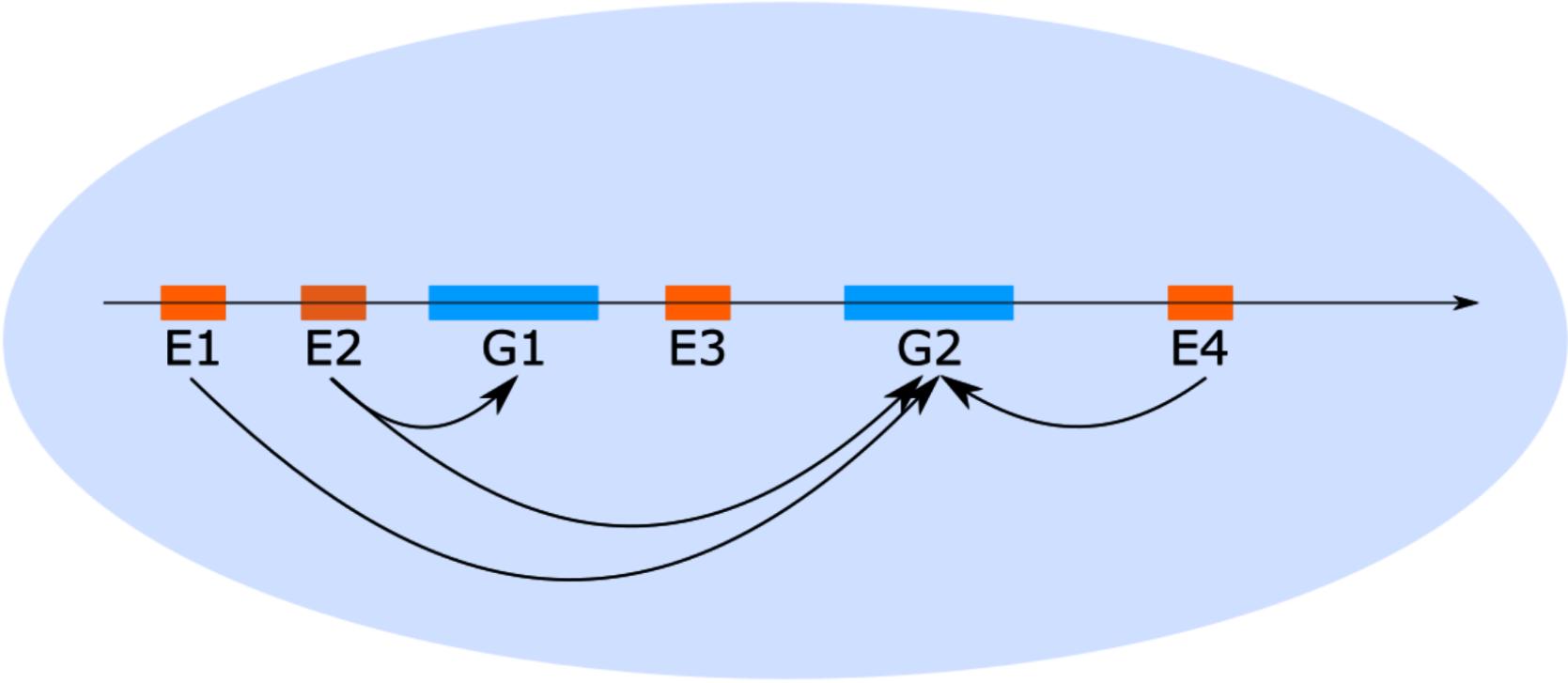
[1]CMU Statistics and Data Science
[2]Wharton Statistics and Data Science

September 2021

Overview

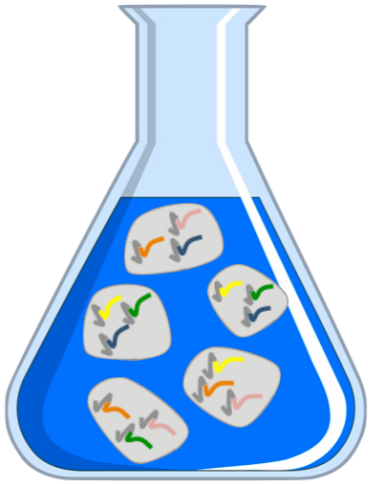
- **Background**
- Analysis objective and challenges
- Existing approach
- Proposed method
- Simulation results
- Real data results

Single-cell CRISPR screens are a powerful technology for mapping the regulatory wiring of the genome.



Single-cell CRISPR screens entail sequencing gRNAs and mRNAs in individual cells.

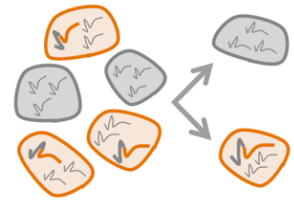
Perturb cells with gRNAs

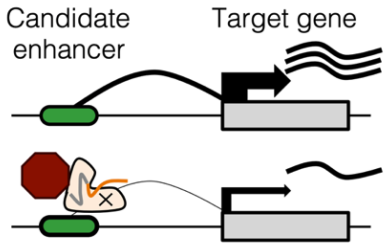


Sequence single cells

For each cell, measure:
1.gRNAs
2.gene expression

Test for differential expression





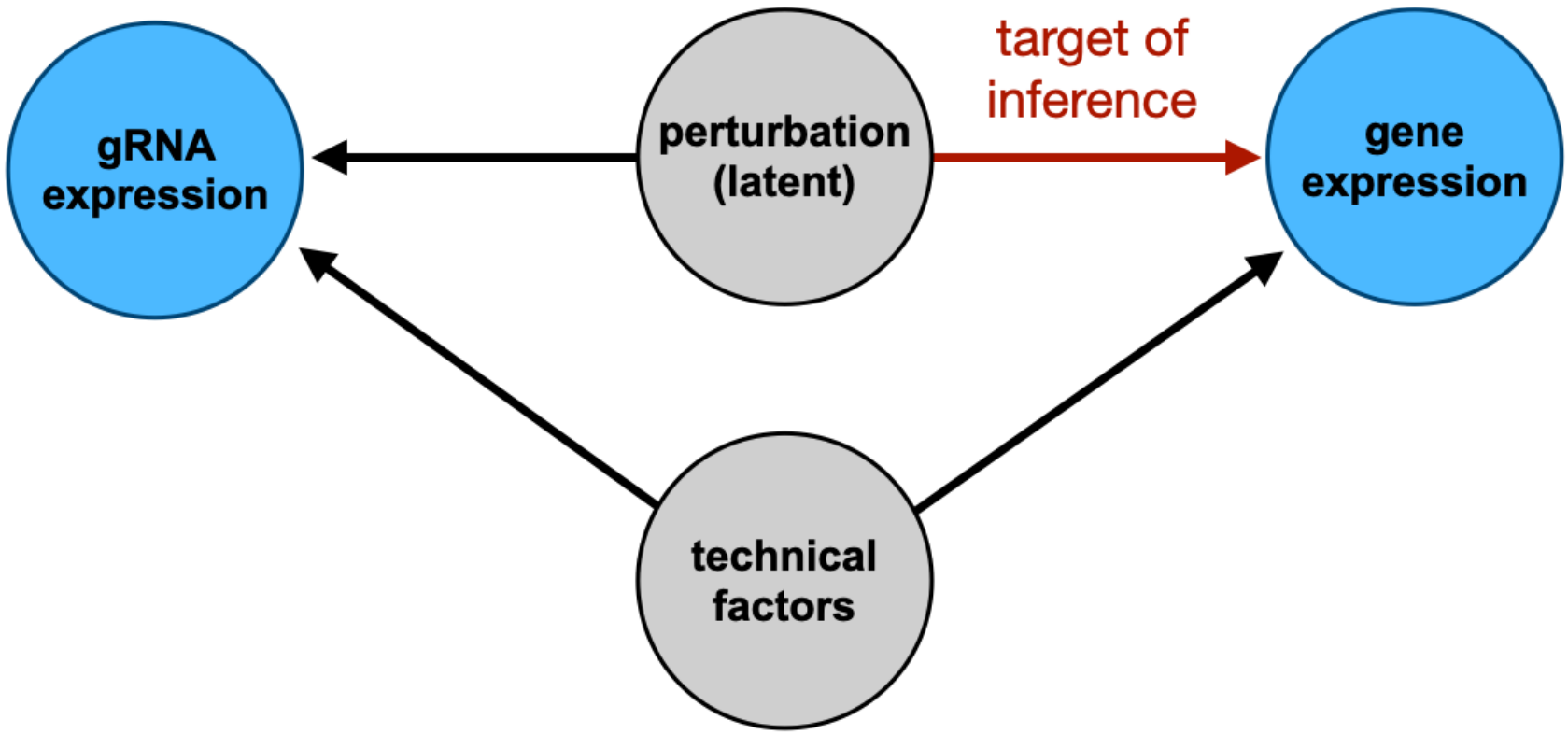
Overview

- Background
- **Analysis Challenges**
- Existing approach
- Proposed method
- Simulation results
- Real data results

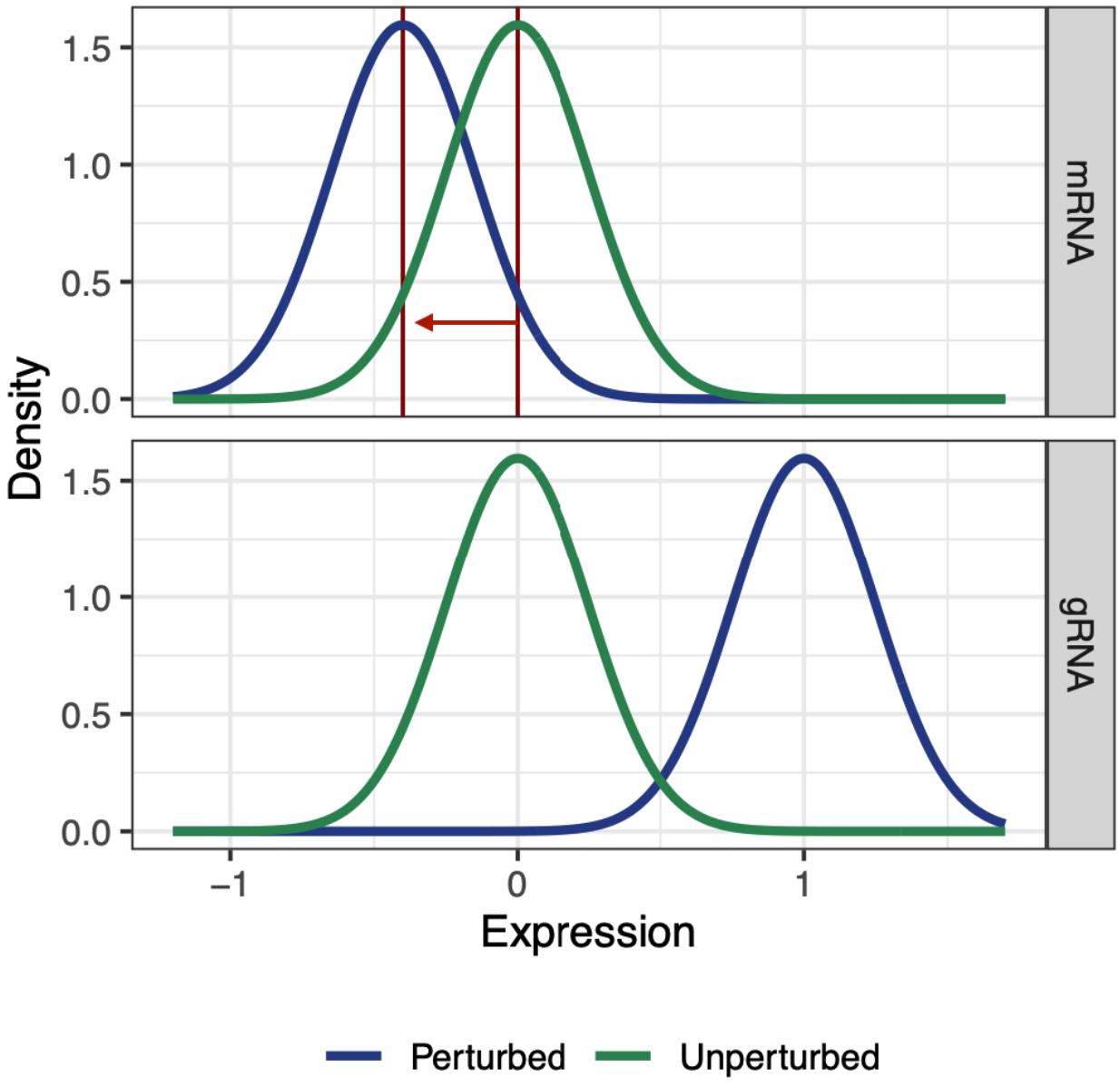
There are several challenges to the analysis of single-cell CRISPR screen data:

1. The perturbation is unobserved.
2. Technical factors, such as batch and sequencing depth, explain variability in mRNA and gRNA counts.
3. Unperturbed cells exhibit "background gRNA reads." [Schraivogel et al., 2020]
4. The mRNA and gRNA expression data are highly discrete.

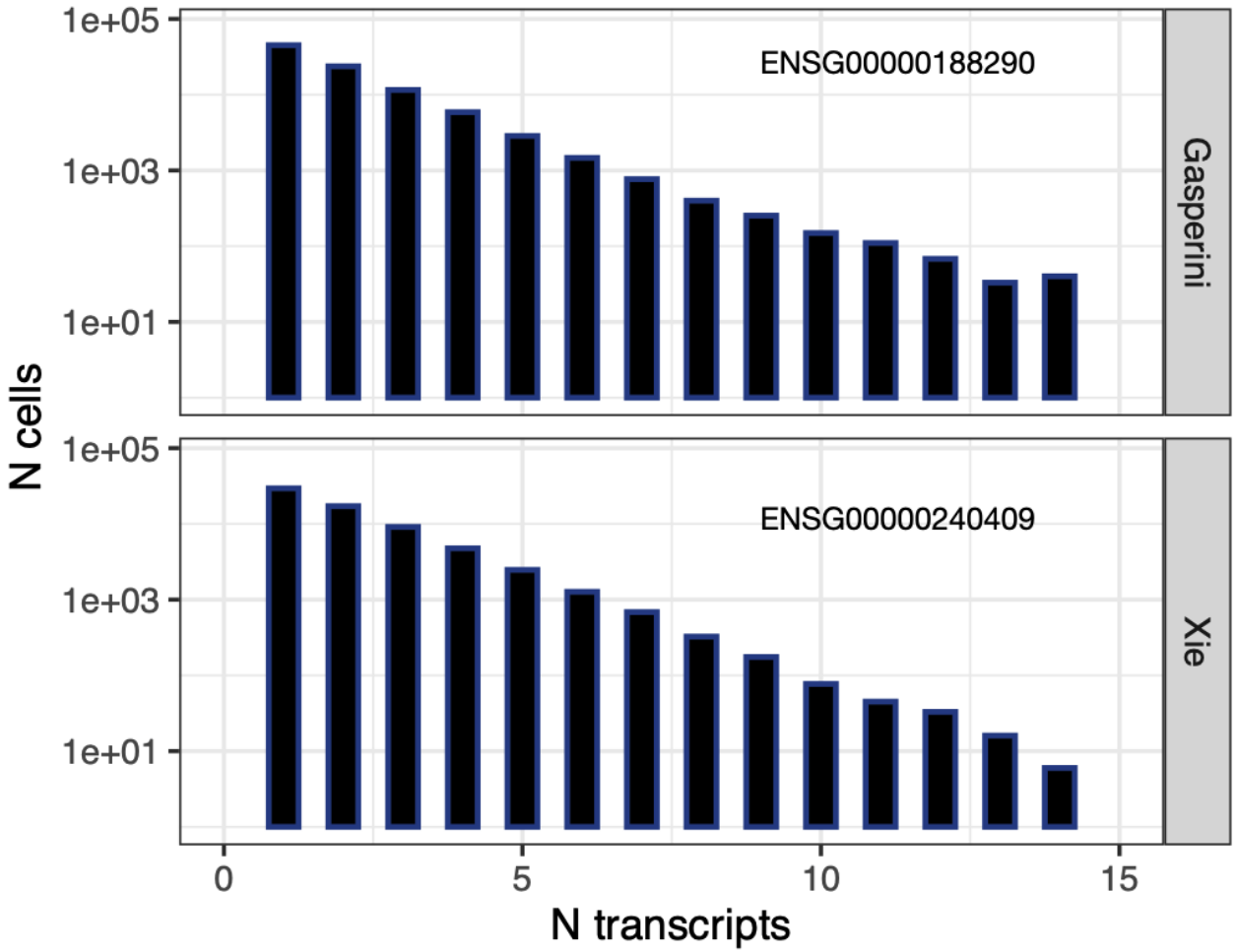
(1) The perturbation is unobserved, and (2) technical factors are present.



(3) Unperturbed cells exhibit background gRNA reads.



(4) The count data are highly discrete.



Overview

- Background
- Analysis Challenges
- **Existing approach**
- Proposed method
- Simulation results
- Real data results

Data and notation

- Observe $n \approx 100,000 - 250,000$ cells.
- Consider a given mRNA and gRNA of interest.
- For cell $i \in \{1, \ldots, n\}$, let
  - $p_i \in \{0, 1\}$ indicate whether a perturbation occurred.
  - $m_i \in \mathbb{N}$ be the mRNA count.
  - $g_i \in \mathbb{N}$ be the gRNA count.
  - $l_i^m \in \mathbb{N}$ be the mRNA library size.
  - $z_i \in \mathbb{R}^{d-1}$ be a vector of technical factors, possibly including an intercept term.
- $\approx 5,000$ genes, $\approx 500 - 5,000$ gRNAs

The "thresholding method"

1. For given threshold $c \in \mathbb{N}$, estimate P_i by

$$\begin{cases} \hat{P}_i = 0 \text{ if } g_i \geq c, \\ \hat{P}_i = 1 \text{ if } g_i < c \end{cases}.$$

2. Fit the regression model [Sarkar and Stephens, 2021]

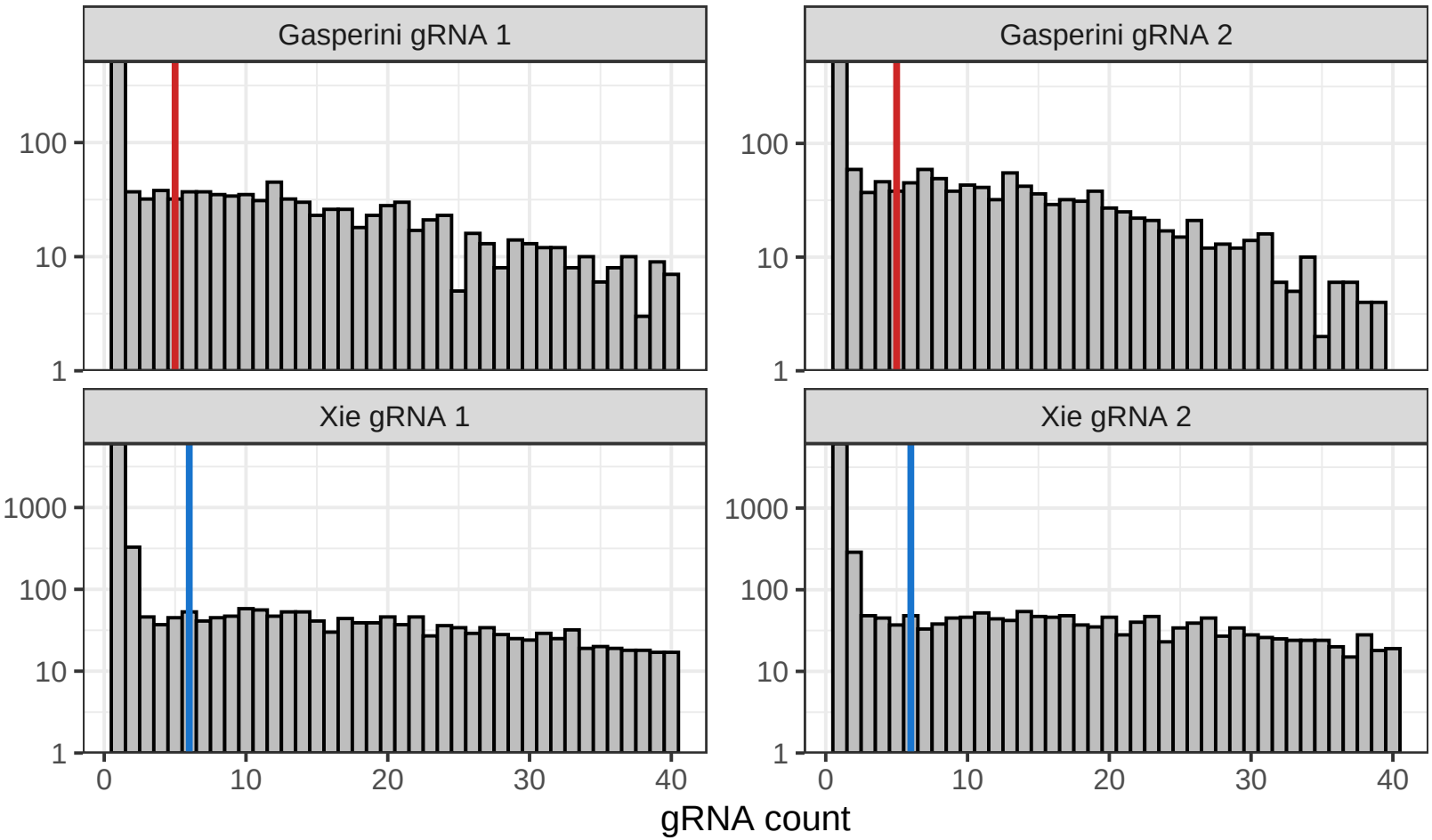
$$m_i | (z_i, l_i^m) \sim \mathrm{NB}_\theta(\mu_i),$$

where $\theta > 0$ is the NB size parameter and

$$\log(\mu_i) = \beta_m \hat{P}_i + \gamma_m^T z_i + \log(l_i^m).$$

3. Obtain an estimate $\hat{\beta}_m$ of β_m and compute a CI for β_m.

Problem 1: There is no clear location at which to draw the threshold.



Problem 2: Thresholding leads to attenuation bias in many measurement error models [Stefanski, 2000].

Research question

Does modeling the gRNA count distribution directly (thereby bypassing thresholding) improve estimation and inference?

Overview

- Background
- Analysis Challenges
- Existing approach
- **Proposed method**
- Simulation results
- Real data results

A bit more notation

For cell $i \in \{1, \ldots, n\}$, let l_i^g be the gRNA library size.

We extend the parametric model of [Sarkar and Stephens, 2021] to model both mRNA *and* gRNA counts.

1. **mRNA**: $m_i|(z_i, l_i^m) \sim \mathrm{NB}_\theta(\mu_i^m)$, where

$$\log(\mu_i^m) = \beta_m P_i + \gamma_m^T z_i + \log(l_i^m).$$

2. **gRNA**: $g_i|(z_i, l_i^g) \sim \mathrm{NB}_\theta(\mu_i^g)$, where

$$\log(\mu_i^g) = \beta_g P_i + \gamma_g^T z_i + \log(l_i^g)$$

3. **Perturbation**: $P_i \sim \mathrm{Bern}(\pi)$, where $\pi \in [0, 1/2)$. P_i is latent.

Generalizing the NB model to arbitrary exponential family response distribution and link function yields the "GLM-EIV" (GLM errors-in-variables) model.

This extension is important, because authors have used

- Negative binomial, [Choudhary and Satija, 2021]
- Poisson, [Schraivogel et al., 2020]
- and Gaussian [Lin et al., 2021]

distributions to model single-cell data.

Generalizing the NB model to arbitrary exponential family response distribution and link function yields the "GLM-EIV" (GLM errors-in-variables) model.

1. **mRNA density**:

$$f_m(m_i; \eta_i^m) = \exp\left\{m_i \eta_i^m - \psi_m(\eta_i^m) + c_m(m_i)\right\}.$$

2. **gRNA density**:

$$f_g(g_i; \eta_i^g) = \exp\left\{g_i \eta_i^g - \psi_g(\eta_i^g) + c_g(g_i)\right\}.$$

3. **Perturbation density**:

$$f(p_i) = \pi^{p_i}(1-\pi)^{1-p_i}.$$

Generalizing the NB model to arbitrary exponential family response distribution and link function yields the "GLM-EIV" (GLM errors-in-variables) model.

The canonical parameter of the mRNA distribution for the ith cell, η_i^m, is given by

$$\eta_i^m = h_m\left(\beta_m P_i + \gamma_m^T z_i + \log\left(l_i^m\right)\right).$$

If the canonical link function is used, then h_m is the identity. The case for η_i^g is similar.

We derive an EM algorithm to fit the model.

E step:

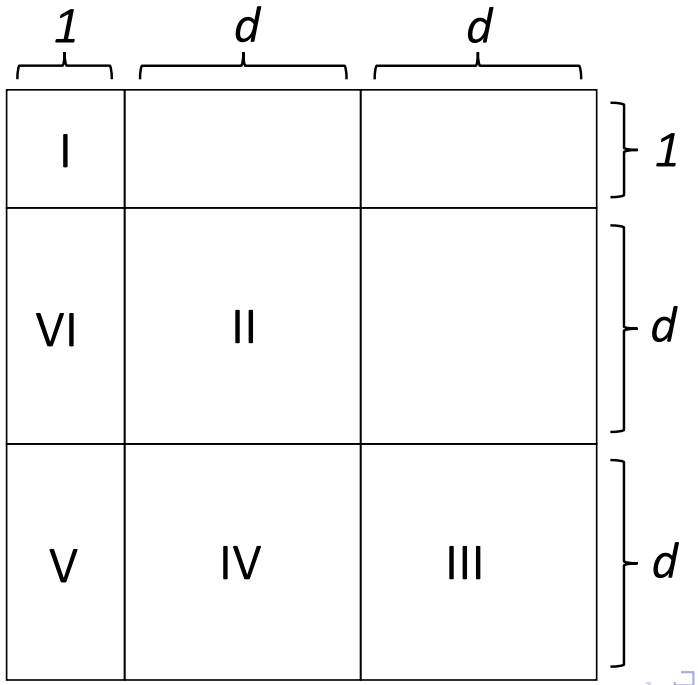
- Compute membership probabilities $T_1, \ldots, T_n$ using the model.

M step:

- Augment count vectors $m \to [m, m], g \to [g, g]$.
- Augment offset vectors $l_m \to [l_m, l_m], l_g \to [l_g, l_g]$.
- Augment covariate matrix $Z \to [Z, Z]$; append column of 1s and 0s for perturbation indicators.
- Fit weighted GLM to both modalities using membership probabilities $[T_1, \ldots, T_n, 1 - T_1, \ldots, 1 - T_n]$ as weights.

We derive an analytic expression for the observed information matrix to enable fast inference (CIs, p-values).

$$J(\hat{\theta}; m, g) = -\mathbb{E}\left[\nabla^2 \mathcal{L}(\theta; m, g, p) | g, m, \hat{\theta}\right]$$
$$+ \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) | g, m, \hat{\theta}\right] \cdot \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) | g, m, \hat{\theta}\right]^T$$
$$- \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) \nabla \mathcal{L}(\theta; m, g, p)^T | g, m, \hat{\theta}\right].$$



We implement several statistical accelerations to make the method fast.


Choudhary, S. and Satija, R. (2021).
Comparison and evaluation of statistical error models for scRNA-seq.
bioRxiv, (8):2021.07.07.451498.


Gasperini, M., Hill, A. J., McFaline-Figueroa, J. L., Martin, B., Kim, S., Zhang, M. D., Jackson, D., Leith, A., Schreiber, J., Noble, W. S., Trapnell, C., Ahituv, N., and Shendure, J. (2019).
A Genome-wide Framework for Mapping Gene Regulation via Cellular Genetic Screens.
Cell, 176(1-2):377–390.e19.


Lin, K. Z., Lei, J., and Roeder, K. (2021).
Exponential-Family Embedding With Application to Cell Developmental Trajectories for Single-Cell RNA-Seq Data.
Journal of the American Statistical Association, 0(0):1–32.


Sarkar, A. and Stephens, M. (2021).
Separating measurement and expression models clarifies confusion in single-cell RNA sequencing analysis.

Nature Genetics, 53(6):770–777.

Schraivogel, D., Gschwind, A. R., Milbank, J. H., Leonce, D. R., Jakob, P., Mathur, L., Korbel, J. O., Merten, C. A., Velten, L., and Steinmetz, L. M. (2020).
Targeted Perturb-seq enables genome-scale genetic screens in single cells.
Nature Methods, 17(6):629–635.

Stefanski, L. A. (2000).
Measurement Error Models.
Journal of the American Statistical Association, 95(452):1353–1358.