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

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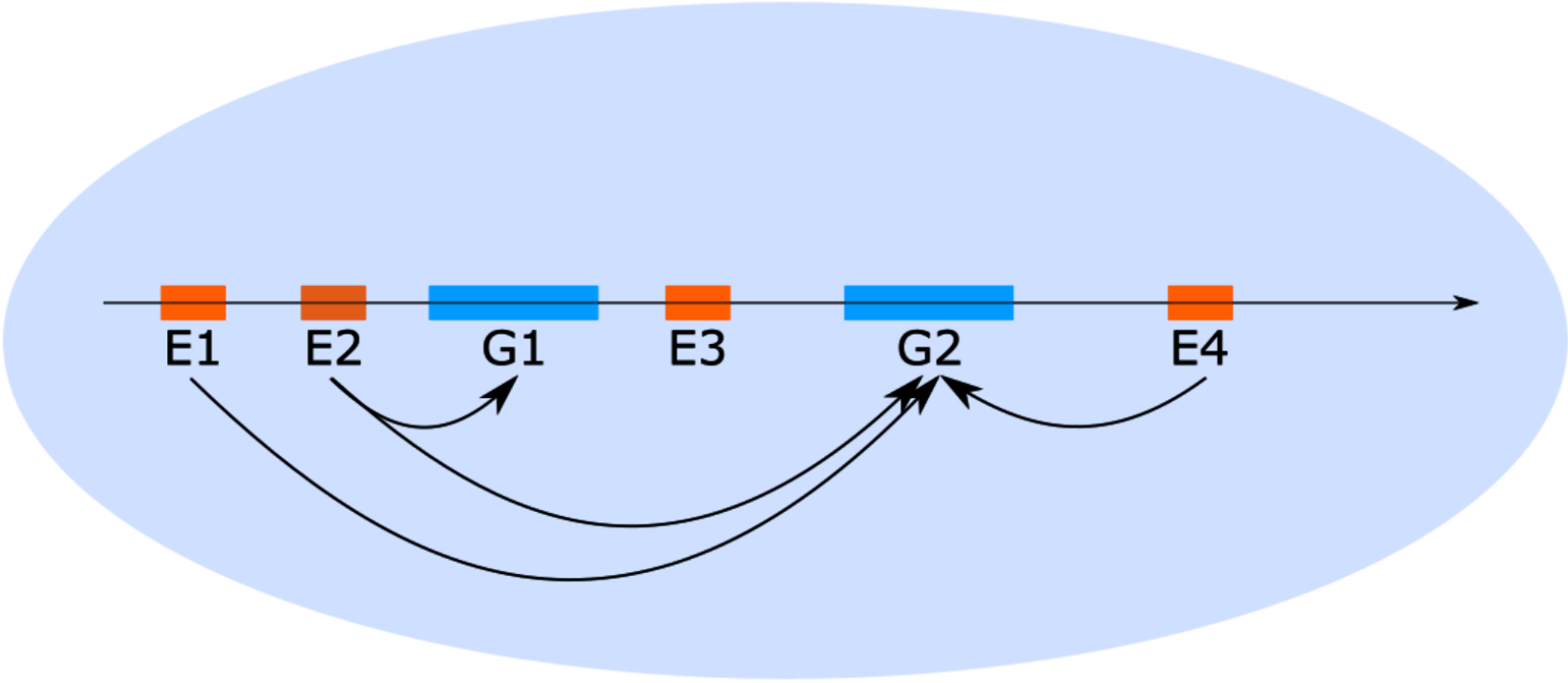
September 2021

Overview

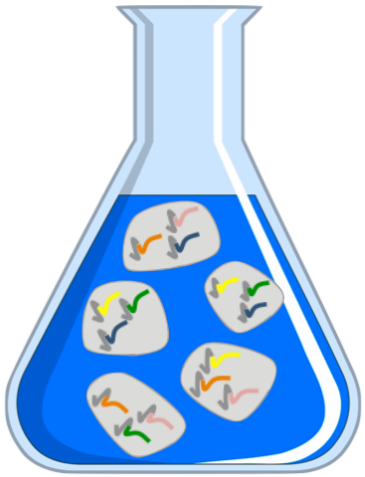
- **Background**
- Analysis objective and challenges
- Existing approach
- Proposed method
- Simulation results
- Real data results

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.

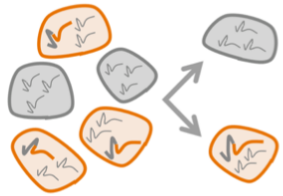
Perturb cells with gRNAs

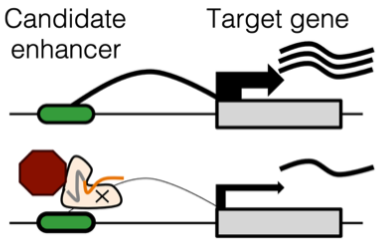


Sequence single cells

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

Differential expression



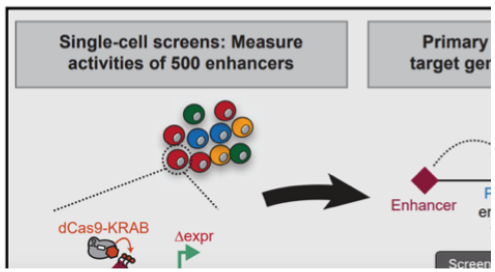


We investigate single-cell CRISPR screen datasets produced by [Gasperini et al., 2019] and [Xie et al., 2019].

Cell Reports

Global Analysis of Enhancer Targets Reveals Convergent Enhancer-Driven Regulatory Modules

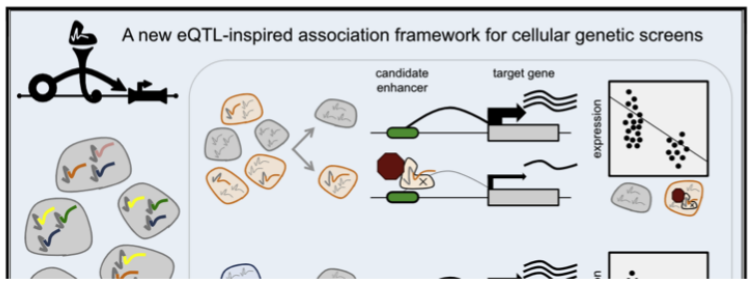
Graphical Abstract



Cell

A Genome-wide Framework for Mapping Gene Regulation via Cellular Genetic Screens

Graphical Abstract



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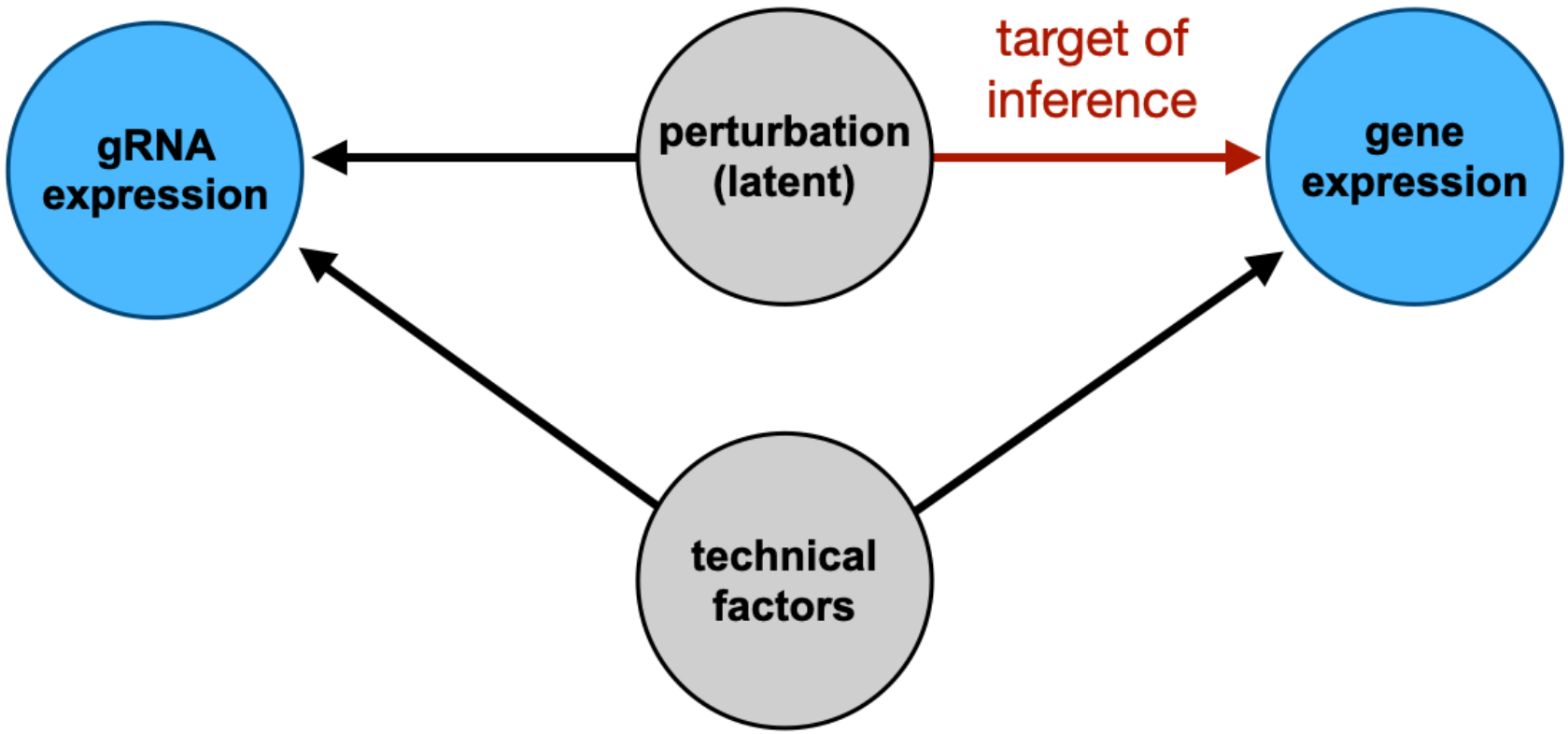
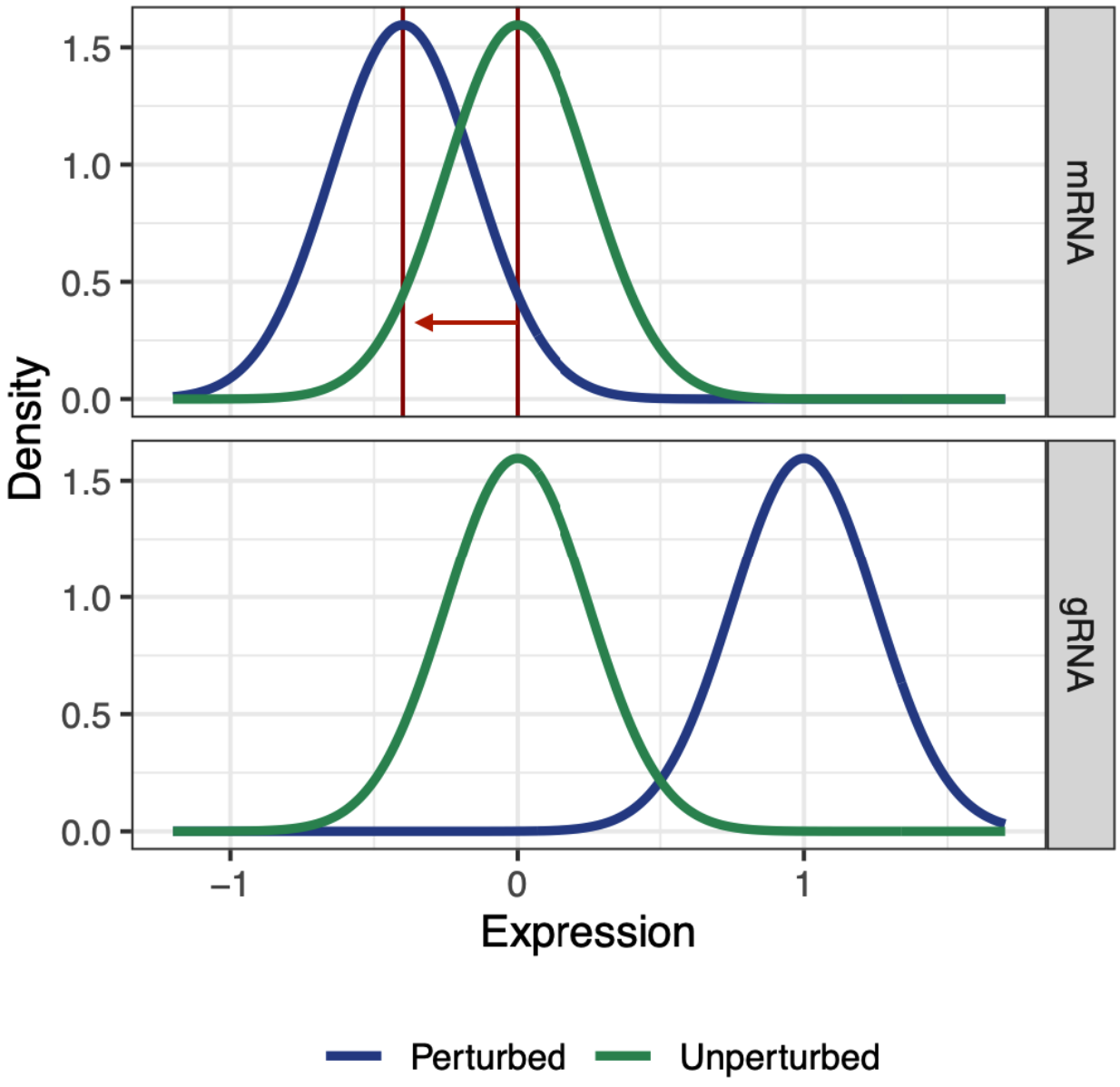
There are several challenges to the analysis of single-cell CRISPR screen data:

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.

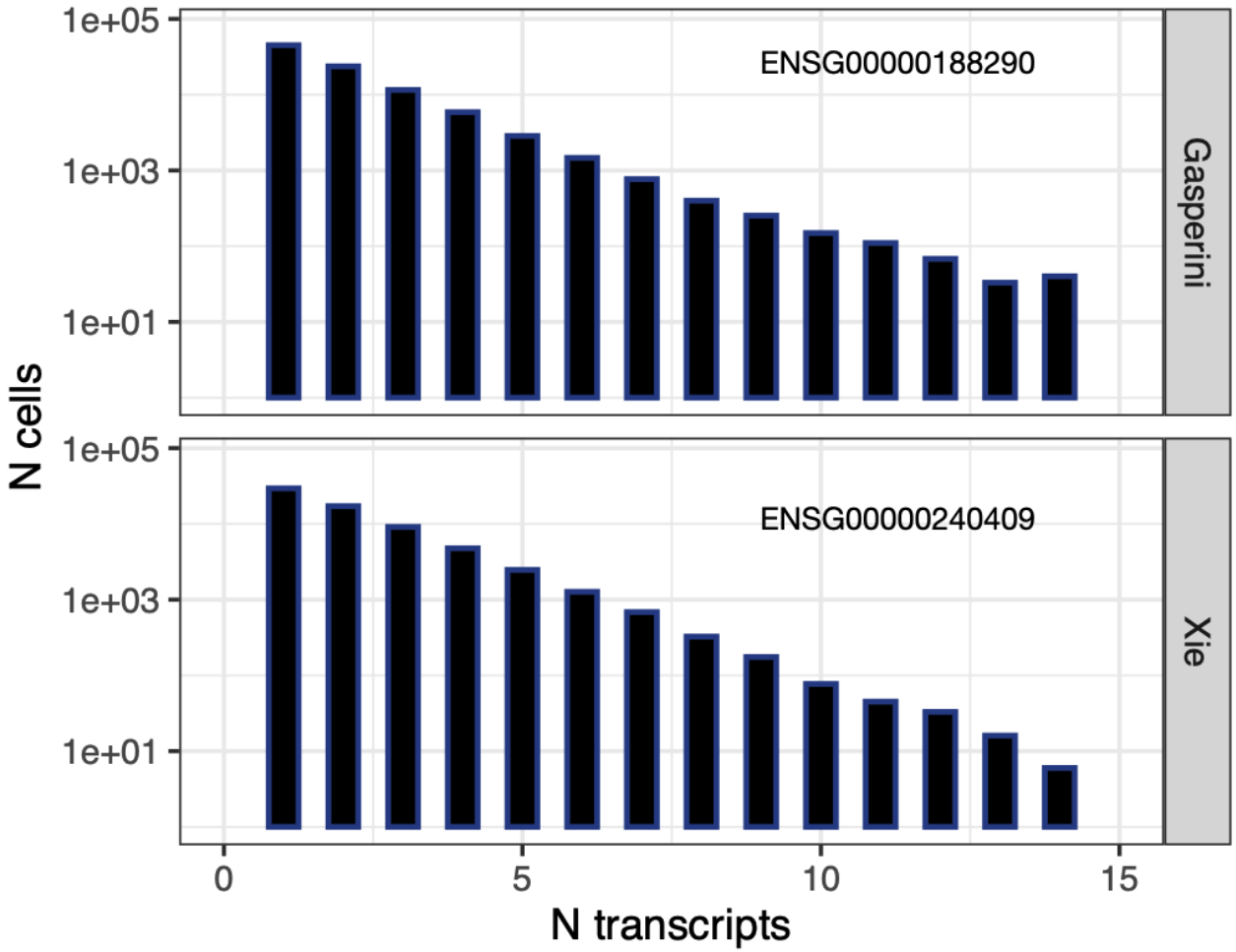
(1) The perturbation is unobserved, and (2) technical factors are present.



(3) Unperturbed cells exhibit background gRNA reads.



(4) The count data are highly discrete.



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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.
- We measure $\approx 5,000$ 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]

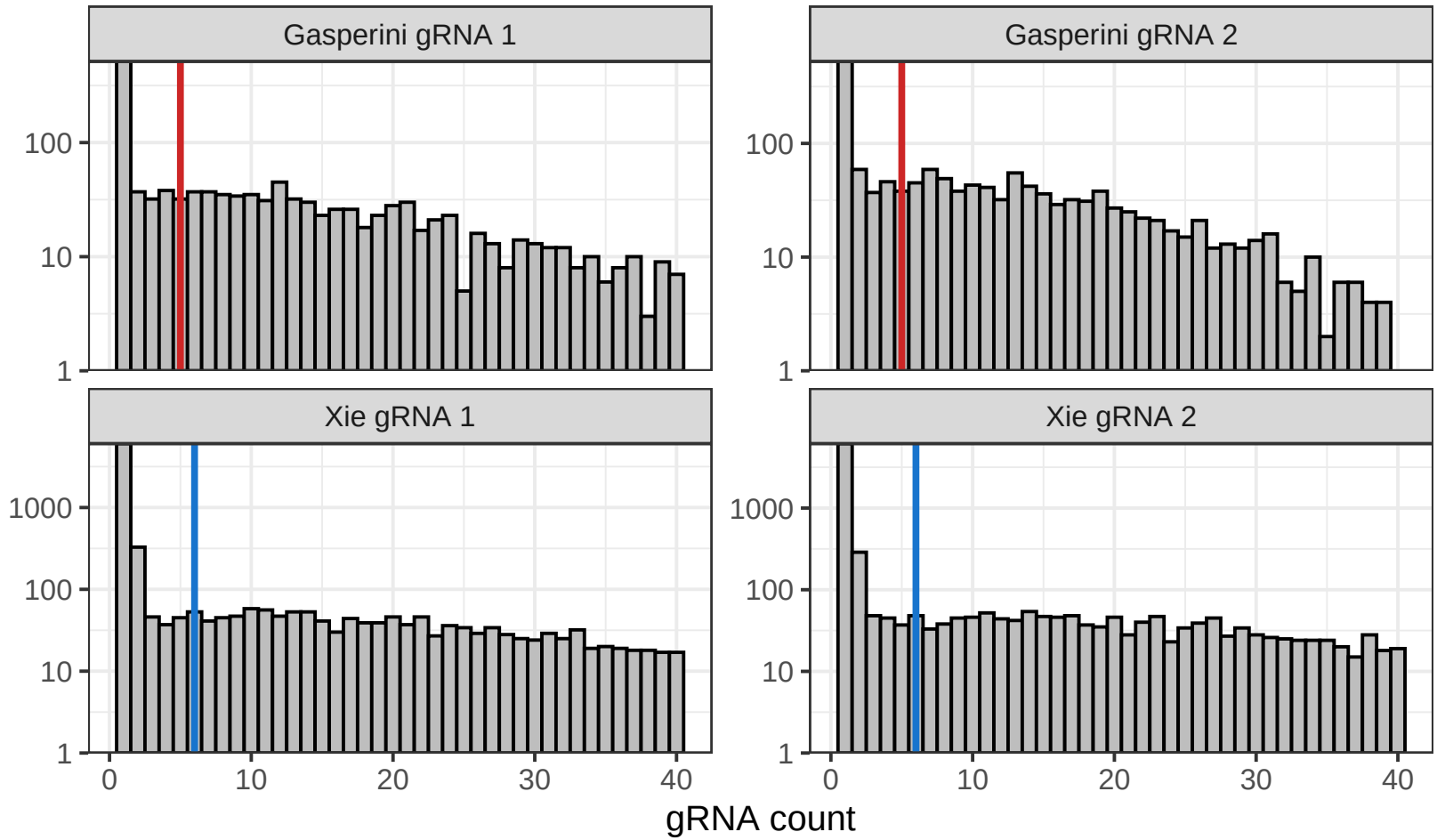
$$m_i | (z_i, l_i^m) \sim \mathrm{NB}_\theta(\mu_i),$$

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 in the data at which to draw the threshold.



Problem 2: Thresholding can lead to attenuation bias.

As a simple example, suppose

$$\begin{cases} p_1, \ldots, p_n \sim \text{Bern}(\pi) \\ y_i = \beta_m p_i + \epsilon_i \\ x_i = \beta_g p_i + \tau_i, \end{cases}$$

where $\epsilon_i \perp\!\!\!\perp \tau_i$ and $\epsilon_i, \tau_i \sim N(0, 1)$. We observe

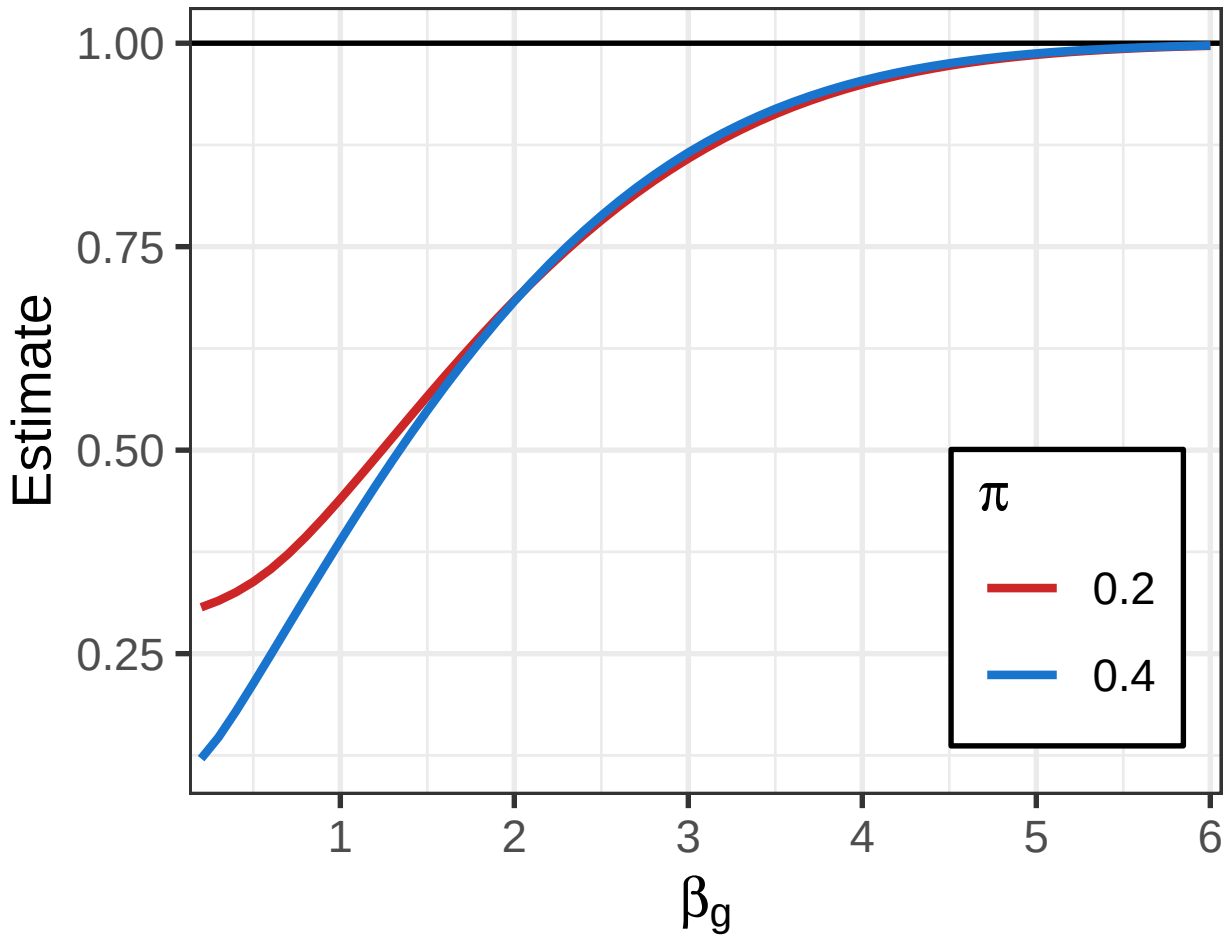
$$\{(x_1, y_1), \ldots, (x_n, y_n)\},$$

and we want to estimate β_m using the thresholding method. Assume β_g and π are known. We select the threshold c so as to minimize the misclassification rate, i.e.,

$$c = \arg\min_{c \in \mathbb{R}} \frac{1}{n} \sum_{i=1}^{n} \mathbb{I}(\hat{p}_i \neq p_i).$$

The thresholding method shows clear attenuation bias.

- We set β_m to 1.



Research question

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

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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.

Consider the mRNA model.

- Let $g_m : \mathbb{R} \to \mathbb{R}$ be the link function, i.e.

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

- The canonical parameter for the ith cell, η_i^m, is given by

$$\eta_i^m = \left[\psi_m'\right]^{-1}(\mu_i) = \left[\psi_m'\right]^{-1}\left(g_m^{-1}\left(\beta_m p_i + \gamma_m^T z_i + \log(l_i^m)\right)\right).$$

- Thus, the model is defined by (i) the cumulant-generating function ψ_m, and (ii) the link function g_m.

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 use statistical tricks to produce an accurate pilot estimate of the parameters, enabling us to run the EM algorithm using only one restart.

- Naive approach (random parameter initialization):

$$(15 \text{ EM restarts}) \left(\frac{20 \text{ iterations}}{\text{EM restart}} \right) \left(\frac{2 \text{ GLMs}}{\text{iteration}} \right) \approx 600 \text{ GLMs}.$$

- GLM-EIV approach:

$$(1 \text{ EM restart}) \left(\frac{3 \text{ iterations}}{\text{EM restart}} \right) \left(\frac{2 \text{ GLMs}}{\text{iteration}} \right) \approx 6 \text{ GLMs}.$$

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

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

We develop a pipeline to deploy the method across hundreds or thousands of processors on HPC and cloud.

nextflow

Method summary

- We extend the parametric model of [Sarkar and Stephens, 2021] to model both mRNA counts and gRNA counts.
  - Arbitrary exponential family distributions and link functions are supported.
- We (i) propose a fast EM algorithm to fit the model, (ii) derive an analytic expression for the observed information matrix, and (iii) implement a computational pipeline to deploy the method on HPC and cloud.
  - These features enable GLM-EIV to scale to large datasets.

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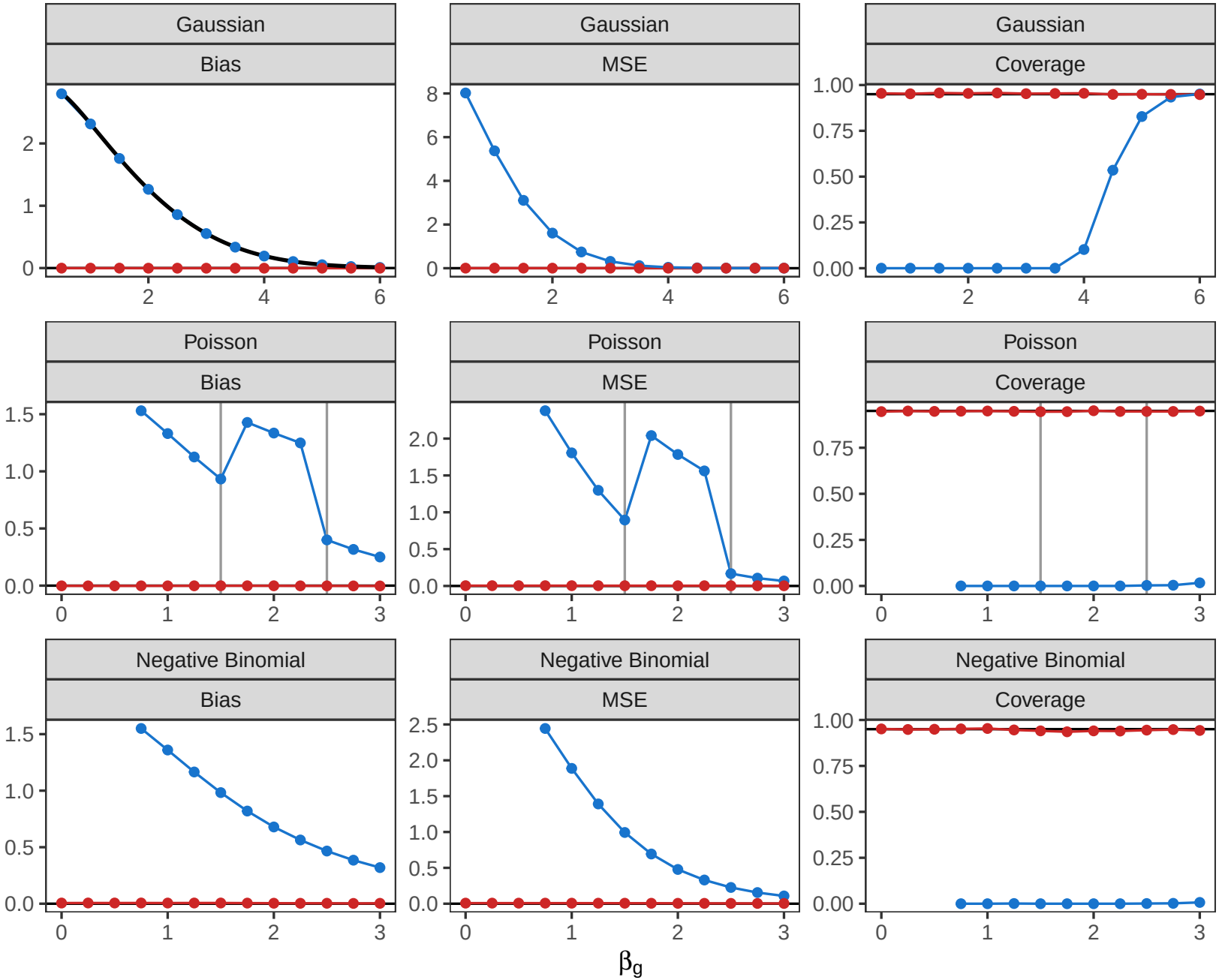
Simulation setup

- No covariates
- No offset terms (i.e., library size fixed at one)
- Intercept terms fixed
- π fixed
- β_m fixed (and of moderate size)
- β_g varied over an interval
- Gaussian, Poisson, and negative binomial distributions


Gaussian
Bias
Gaussian
MSE
Gaussian
Coverage
Poisson
Bias
Poisson
MSE
Poisson
Coverage
Negative Binomial
Bias
Negative Binomial
MSE
Negative Binomial
Coverage
β_g
Method
GLM−EIV
Thresholding

GLM-EIV outperforms the thresholding method on the simulated data for two main reasons:

1. GLM-EIV leverages information from *both* modalities to assign perturbation identities to cells.
2. GLM-EIV generates *soft* rather than *hard* assignments, capturing the inherent uncertainty in whether a perturbation occurred.

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We followed recommendations of [Choudhary and Satija, 2021] for quality control and modeling.

- Quality control
  - Lowly-expressed genes filtered
  - Cells with library sizes below 5th percentile or above 95th percentile filtered
- mRNA model
  - used negative binomial distribution (with log link)
  - size parameter θ estimated from data
- gRNA model
  - used Poisson distribution (with log link)

The estimate $\hat{\beta}_g$ for β_g was large on both datasets across all site types.

Gasperini

Site type	Mean $\exp(\beta_g)$ 95% CI
Candidate *cis*	$(4453, 5353)$
Negative control	$(4484, 5408)$
TSS-targeting	$(3605, 4205)$

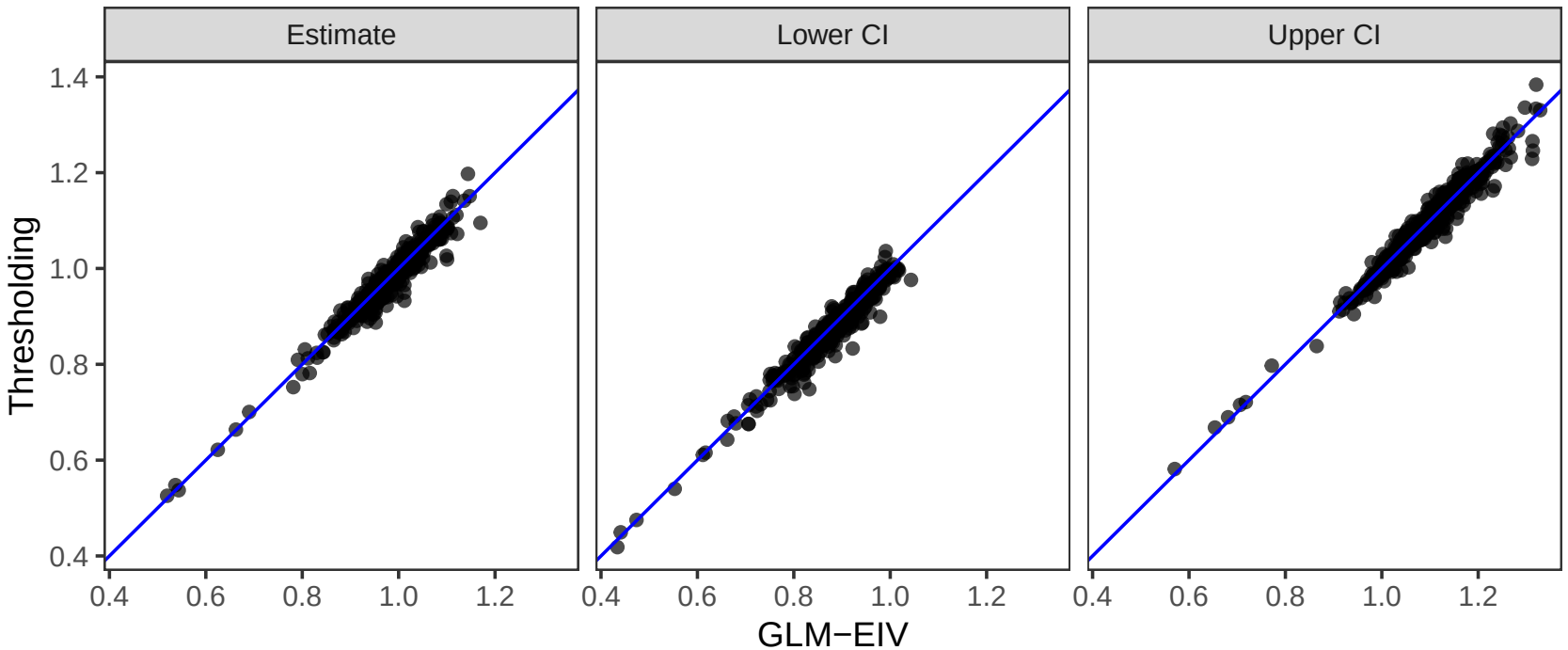
Xie

Site type	Mean $\exp(\beta_g)$ 95% CI
Candidate *cis*	$(307, 324)$
Negative control	$(299, 316)$

GLM-EIV and thresholding exhibited similar CI coverage rates on the negative control pairs.

Dataset	GLM-EIV	Thresholding
Xie	93.7%	93.2%
Gasperini	90.6%	91.4%

Estimates and CIs produced by GLM-EIV and the thresholding method were similar.



Candidate *cis* pairs in Xie et al. data.

We now can answer our core research question.

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

- Yes, if the problem is in a "sufficiently challenging" setting.
- The real data that we analyzed, surprisingly, were in an "easy" setting.
- Therefore, GLM-EIV and the thresholding method performed similarly on the real data.

The proposed method could help solve practical challenges.

- Selecting cell-specific thresholds
- Identifying “problem difficulty” and thus whether thresholding is appropriate

Together, GLM-EIV and SCEPTRE shed light on core analysis challenges posed by single-cell CRISPR screens, paving the way for the development of new methods.

1. Perturbation unobserved
2. Confounders and nuisance variables
3. Possible model mispecification
4. Background reads
5. Highly discrete data
6. Ineffective gRNAs

Thank you.

Acknowledgments:

- Xuran Wang helped with the Xie data preprocessing.
- All analyses were run on Pittsburgh Supercomputer.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