UCLA Statistics

Statistical Rigor in Genomics Data Analysis

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Statistical rigor challenges in genomics data analysis

What is statistical rigor?

- Performance guarantee of statistical methods, e.g.,
  - p-values: uniformly distributed between 0 and 1 under the nulls
  - confidence intervals: coverage probabilities $\geq$ the claimed level (e.g., 95%)
  - false discovery rate (FDR): average (# false discoveries)/(# discoveries) in permutation analysis $\leq$ the claimed level (e.g., 5%)

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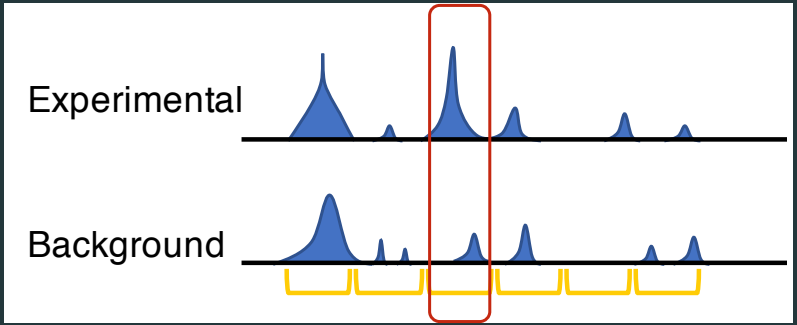
Why is statistical rigor challenging in genomics data analysis?

- New and complex data types
- Fast method development

Common causes of invalid p-values

1. **Mis-formulation of a two-sample test as a one-sample test**
   - Peak calling from ChIP-seq data



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	a region	background count	experimental count
–	random variable (hypothetical)	X	Y
	random observation (data)	x	y

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  - p-value $= \mathbb{P}(Y \geq y)$ where $Y \sim \text{Poisson}(x)$ — correct?
  - No, because it assumes $Y \sim \text{Poisson}(\lambda)$ and tests

$$H_0 : \lambda = x \quad \text{vs.} \quad H_1 : \lambda > x\,,$$

    which treats x as a fixed parameter and ignores its randomness

Common causes of invalid p-values

1. **Mis-formulation of a two-sample test as a one-sample test**
   - How to perform a two-sample test when the sample size is 1 vs. 1?
     - p-value calculation is difficult...

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Our solution: Clipper

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Clipper: p-value-free FDR control on high-throughput data from two conditions

Xinzhou Ge, Yiling Elaine Chen, Dongyuan Song, MeiLu McDermott, Kyla Woyshner, Antigoni Manousopoulou, Ning Wang, Wei Li, Leo D. Wang, Jingyi Jessica Li

doi: https://doi.org/10.1101/2020.11.19.390773

— accepted by *Genome Biology*

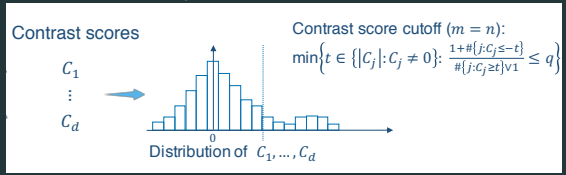
Our solution: Clipper

- **Does not**
  - require high-resolution p-values
  - assume parametric distributions
  - require many replicates
- Two components
  - **contrast scores**
  - **cutoff**
- Applications
  - ChIP-seq
  - mass spectrometry
  - bulk and single-cell RNA-seq
  - Hi-C

sample size m vs. n

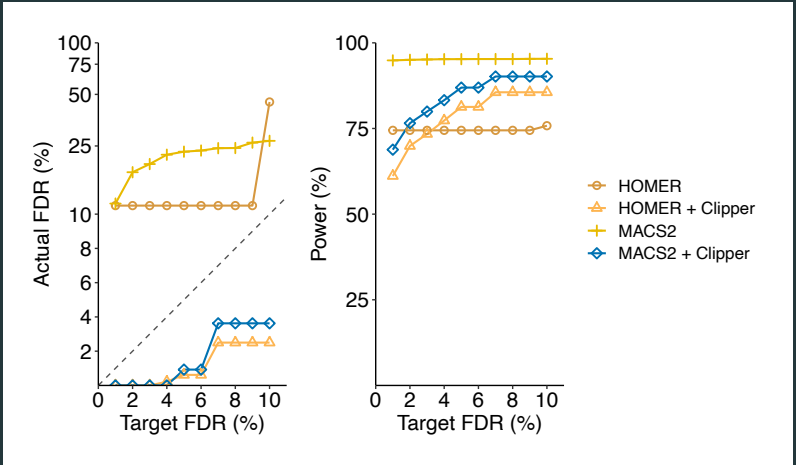
d features

FDR threshold q



Our solution: Clipper

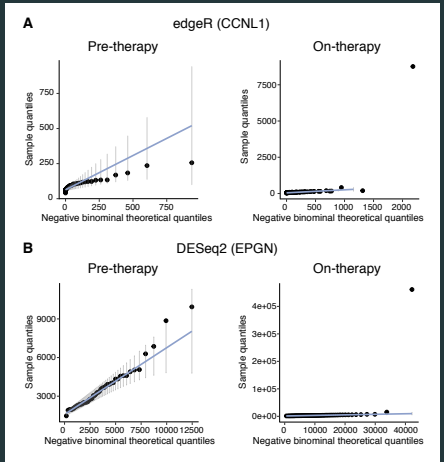
- Peak calling from ChIP-seq data
  - as an add-on, Clipper improves the FDR control of MACS2 and HOMER



Common causes of invalid p-values

2. Mis-specification of a parametric model that does not fit data well

- Identification of differentially expressed (DE) genes from RNA-seq data

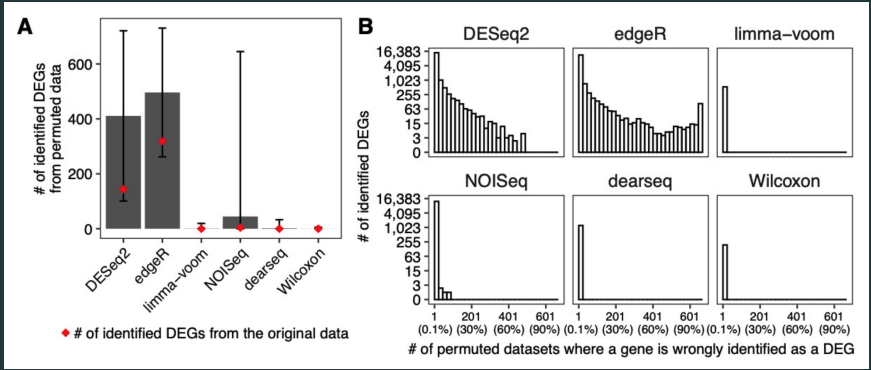


data from Riaz et al. *Cell* 2017

Common causes of invalid p-values

2. Mis-specification of a parametric model that does not fit data well

- Identification of differentially expressed genes (DEGs) from RNA-seq data
  - FDR Check: permute samples between conditions (no true DEGs)



Our recommendation: Mann-Whitney-Wilcoxon rank-sum test

2. **Mis-specification of a parametric model that does not fit data well**
   - Recommendation: consider non-parametric methods when sample size is large: (sample size)/(# number of parameters) ≥ 20

— collaboration with Dr. Yumei Li in Dr. Wei Li's lab (UC Irvine)

Common causes of invalid p-values

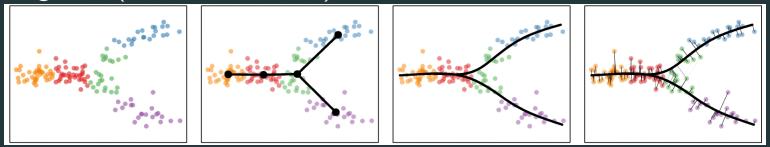
3. Mis-treatment of inferred covariates as observed

- Identification of DEGs along cell pseudotime from scRNA-seq data
  - Pseudotime: a latent "temporal" variable that reflects a cell's relative transcriptome status among all cells
  - Pseudotime inference (trajectory inference): estimate the pseudotime of cells, i.e., order cells along a trajectory based on transcriptome similarities
  - Popular methods:

    `Monocle3` (Trapnell *et al.* 2014)

    `TSCAN` (Ji *et al.* 2016)

    `Slingshot` (Street *et al.* 2018)



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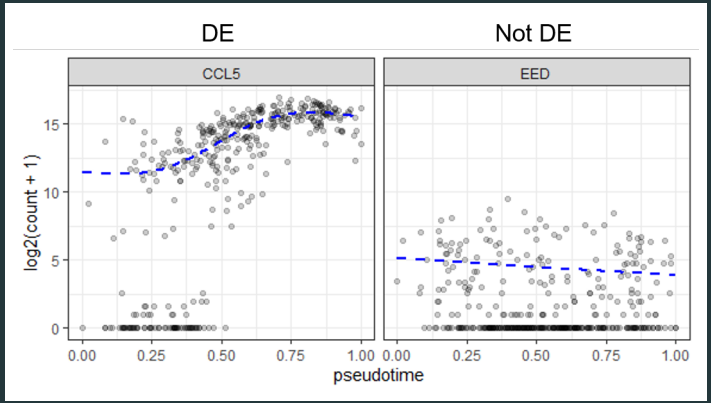
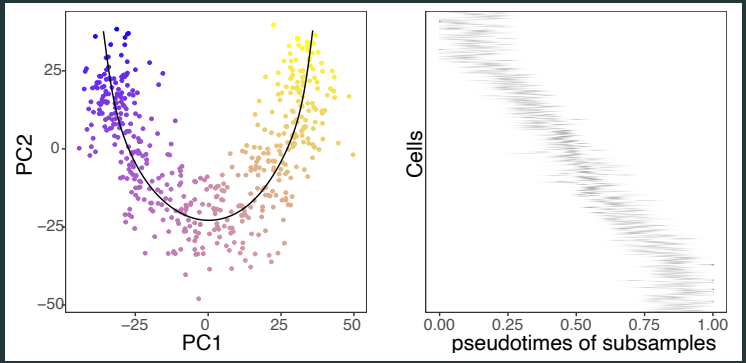


Common causes of invalid p-values

3. Mis-treatment of inferred covariates as observed

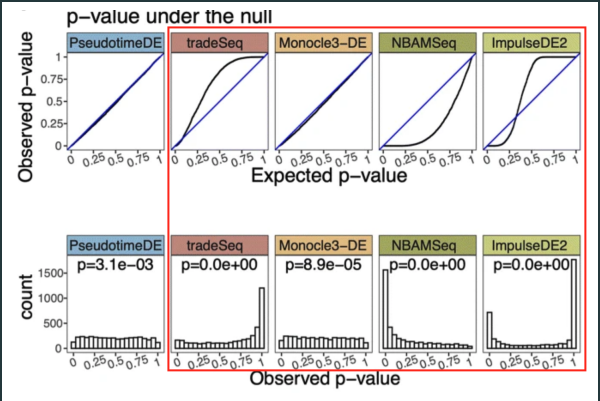
- Identification of DEGs along cell pseudotime from scRNA-seq data
  - Cell pseudotime is random



Common causes of invalid p-values

3. Mis-treatment of inferred covariates as observed

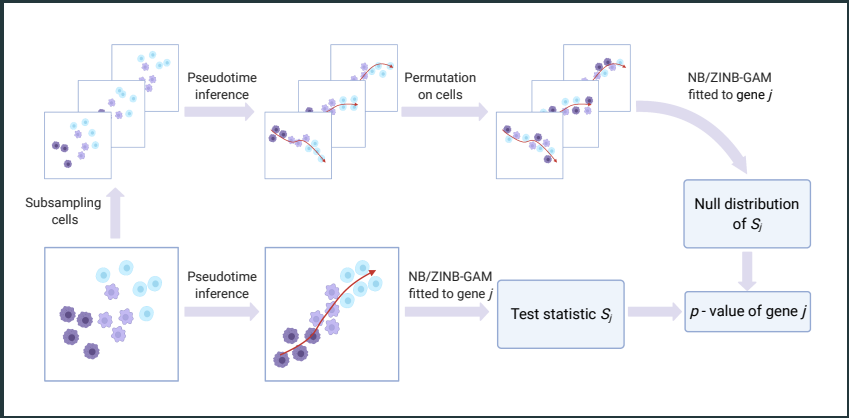
- Identification of DEGs along cell pseudotime from scRNA-seq data
  - Existing methods treat cell pseudotime as a observed covariate



Our solution: PseudotimeDE

3. Mis-treatment of inferred covariates as observed

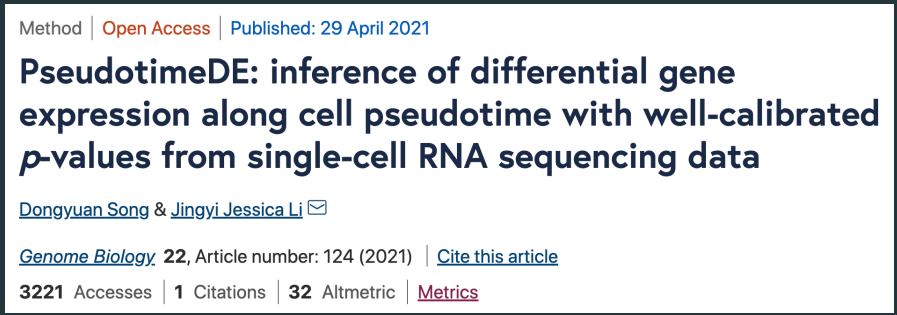
- Identification of DEGs along cell pseudotime from scRNA-seq data
  - PseudotimeDE considers the uncertainty of pseudotime inference



Our solution: PseudotimeDE

3. Mis-treatment of inferred covariates as observed

- Identification of DEGs along cell pseudotime from scRNA-seq data
  - PseudotimeDE generates well-calibrated p-values for FDR control & uses a generalized additive model for good power



Method | Open Access | Published: 29 April 2021

PseudotimeDE: inference of differential gene expression along cell pseudotime with well-calibrated *p*-values from single-cell RNA sequencing data

Dongyuan Song & Jingyi Jessica Li

Genome Biology **22**, Article number: 124 (2021) | Cite this article

3221 Accesses | **1** Citations | **32** Altmetric | Metrics

Summary

Three common causes of invalid p-values in genomics data analysis

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Summary

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1. Mis-formulation of a two-sample test as a one-sample test
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3. Mis-treatment of inferred covariates as observed

Our proposals

1. Clipper: a p-value-free FDR control framework
2. Renaissance of classical non-parametric methods (e.g., Mann-Whitney-Wilcoxon rank-sum test) when sample sizes are large
3. PseudotimeDE: a method that identifies DEGs along cell pseudotime by considering pseudotime inference uncertainty

Hypothesis testing vs. binary classication

Patterns

CellPress
OPEN ACCESS

Perspective

Statistical Hypothesis Testing versus Machine Learning Binary Classification: Distinctions and Guidelines

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Clipper
large-sample DE

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Clipper

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large-sample DE

Dr. Wei Li (Collaborator PI @ UCI)
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PseudotimeDE