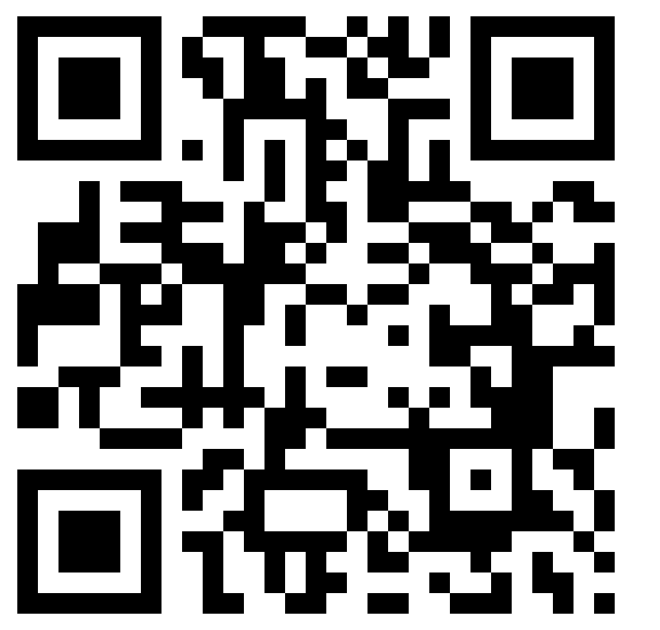


Perturbation-expression association analysis in low-MOI single-cell CRISPR screens with SCEPTRE

Timothy Barry, Kaishu Mason, Kathryn Roeder & Eugene Katsevich

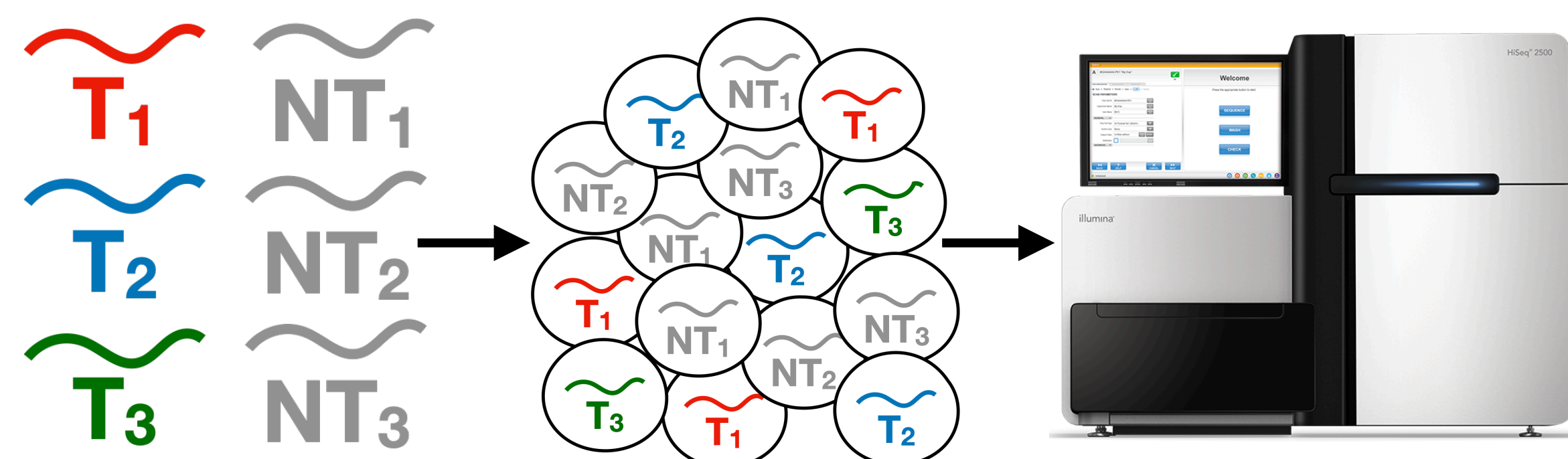


Manuscript
& Software

Single cell CRISPR screens

Simultaneous profiling of CRISPR perturbations and whole transcriptome in single cells.

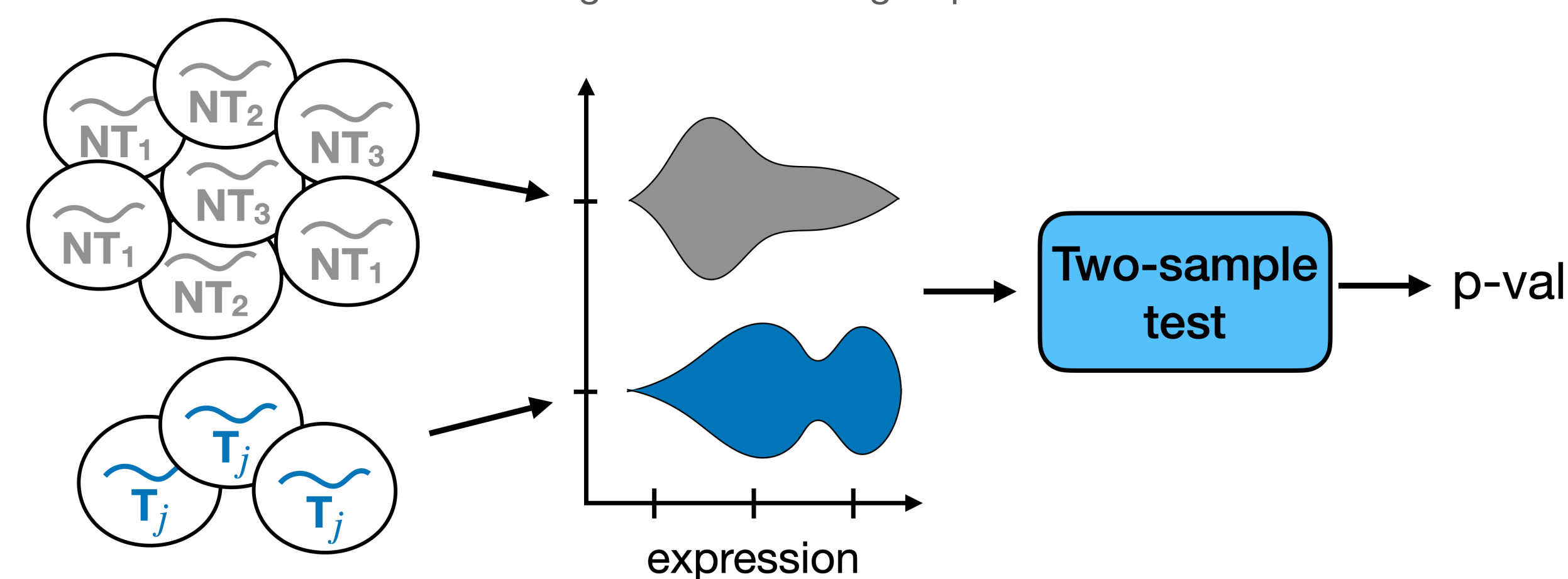
1. Library of targeting (T) and non-targeting (NT) guide RNAs
2. Infect cells at low multiplicity of infection
3. Single-cell RNA sequencing of gRNAs and transcriptome



Perturbation-gene association

Differential expression based on perturbation.

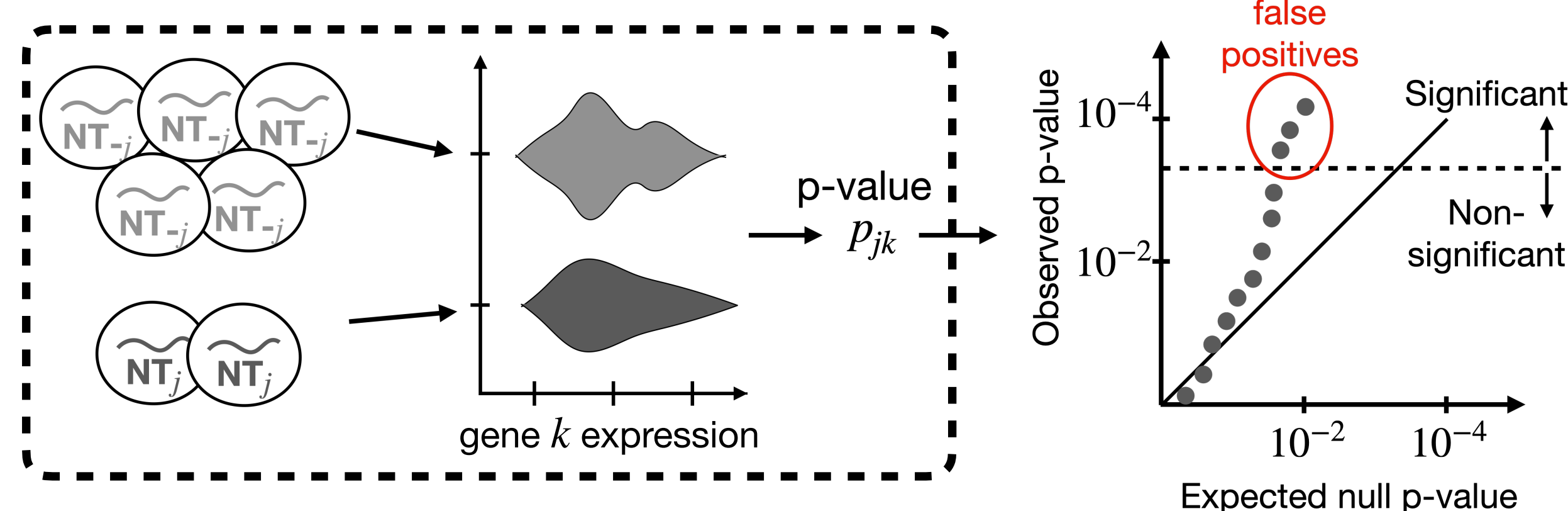
1. Extract cells with NT or T_j
2. Extract expression of gene k for each group
3. Apply two-sample test for differential expression



Calibration check framework

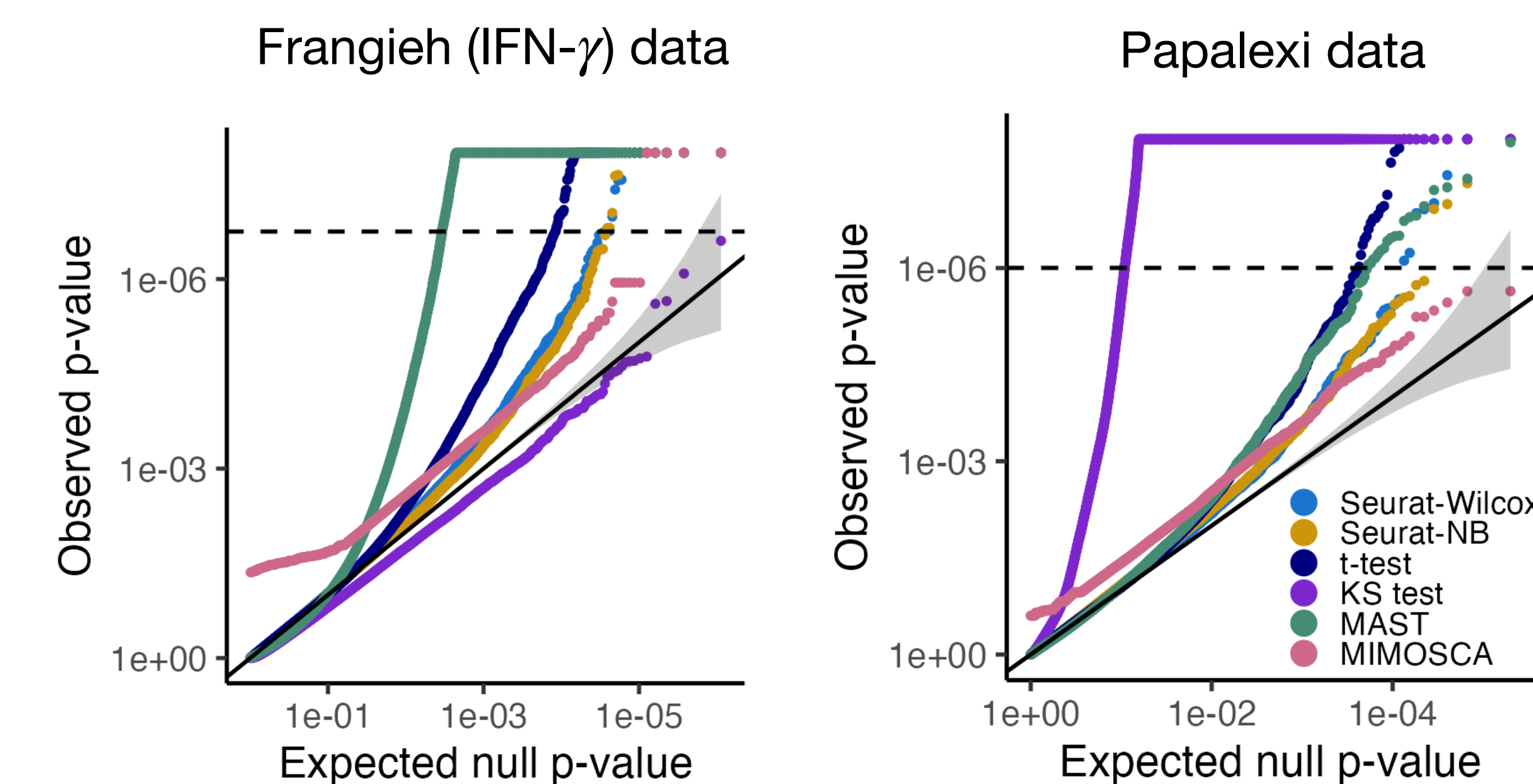
Apply DE method to each (NT gRNA, gene) pair.

1. For each j and k , test association between NT_j perturbation and gene k expression
2. Multiplicity correction; significant hits are false positives



Benchmarking calibration

Existing methods suffer excess false positives.

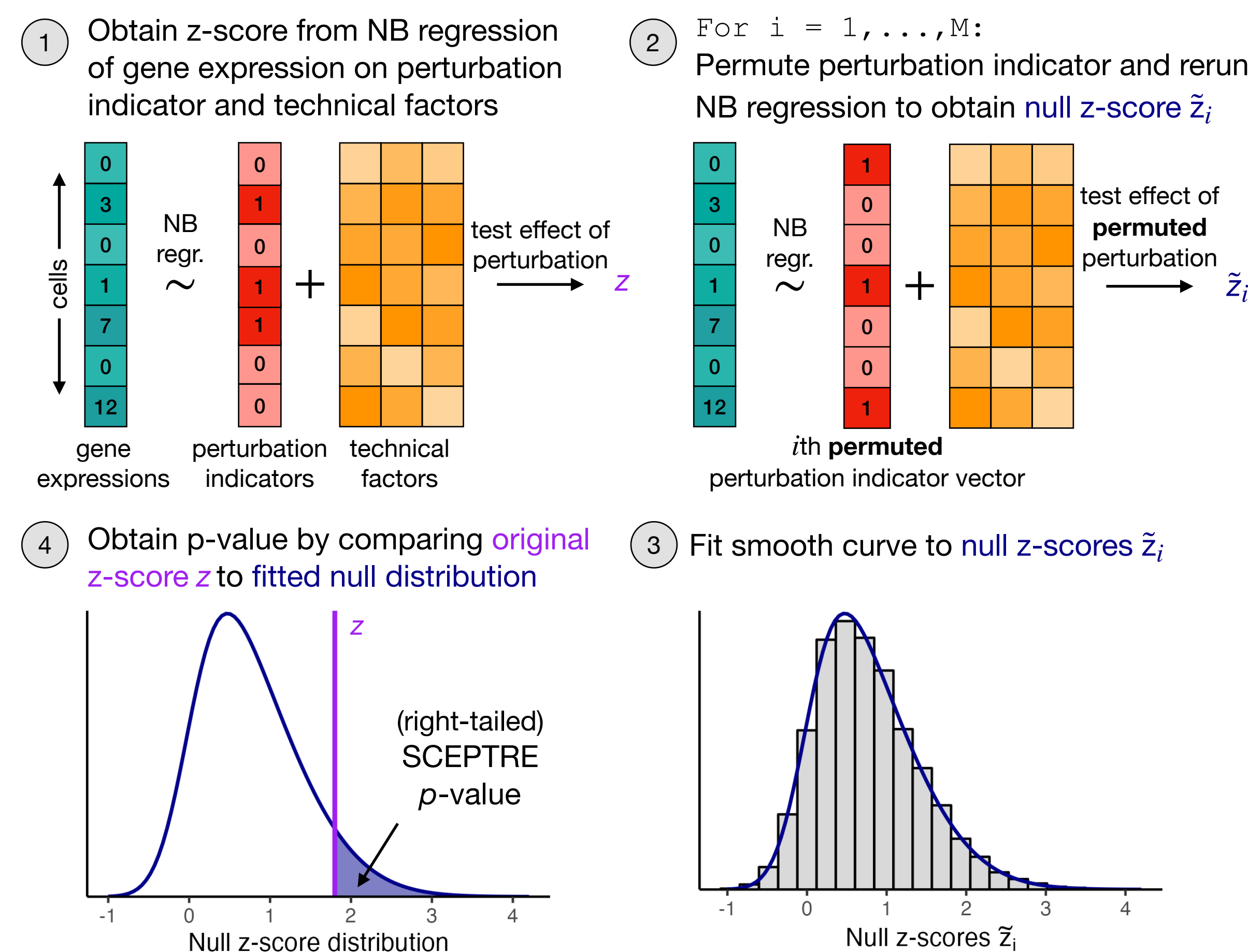


Reasons for miscalibration include:

- Sparsity breaks asymptotic approximations
- Confounding due to technical factors
- Model misspecification

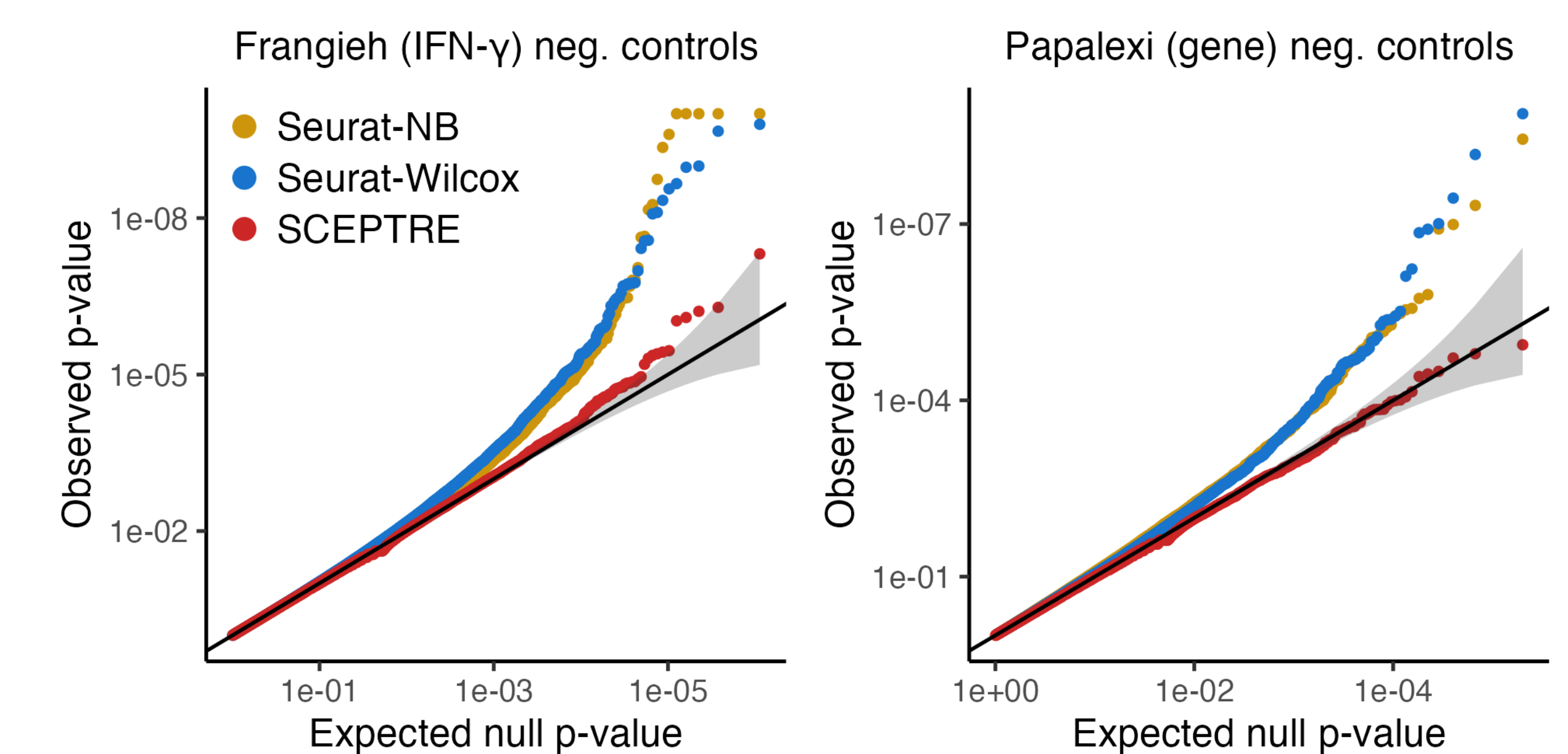
SCEPTRE methodology

Permutation test based on NB score statistic.



Calibration on control data

SCEPTRE improves calibration and sensitivity on six real datasets.



Number of false positives

Dataset	SCEPTRE	Seurat-Wilcox	Seurat-NB	t-test	MAST	KS test	MIMOSCA	NT pairs
Frangieh (Co Culture)	1	13	10	89	2083	0	4	596344
Frangieh (Control)	0	7	16	69	1873	0	0	528239
Frangieh (IFN-γ)	1	15	15	67	1933	0	5	565502
Papalexi (Gene)	0	8	4	24	19	9191	0	100458
Papalexi (Protein)	0	2	0	1	2	0	0	36
Schraivogel	3	2	3	4	1	1	19	4357
Simulated	0	0	0	7	16	0	1	96944
Average	0.7	6.7	6.9	37.3	846.7	1313.1	4.1	

Number of true positives

Dataset	SCEPTRE	Seurat-Wilcox	Seurat-NB	t-test	MAST	KS test	MIMOSCA	PC pairs
Frangieh (Co Culture)	103	98	94	-	-	90	5	181
Frangieh (Control)	77	74	72	-	-	70	4	170
Frangieh (IFN-γ)	94	89	81	-	-	81	8	181
Papalexi (Gene)	13	12	13	11	11	-	0	25
Papalexi (Protein)	2	2	2	2	2	2	2	2
Schraivogel	22	22	21	23	22	19	0	25

Take home message

SCEPTRE improves the quality of single-cell CRISPR screen analysis, paving the way for functional genomics discovery.