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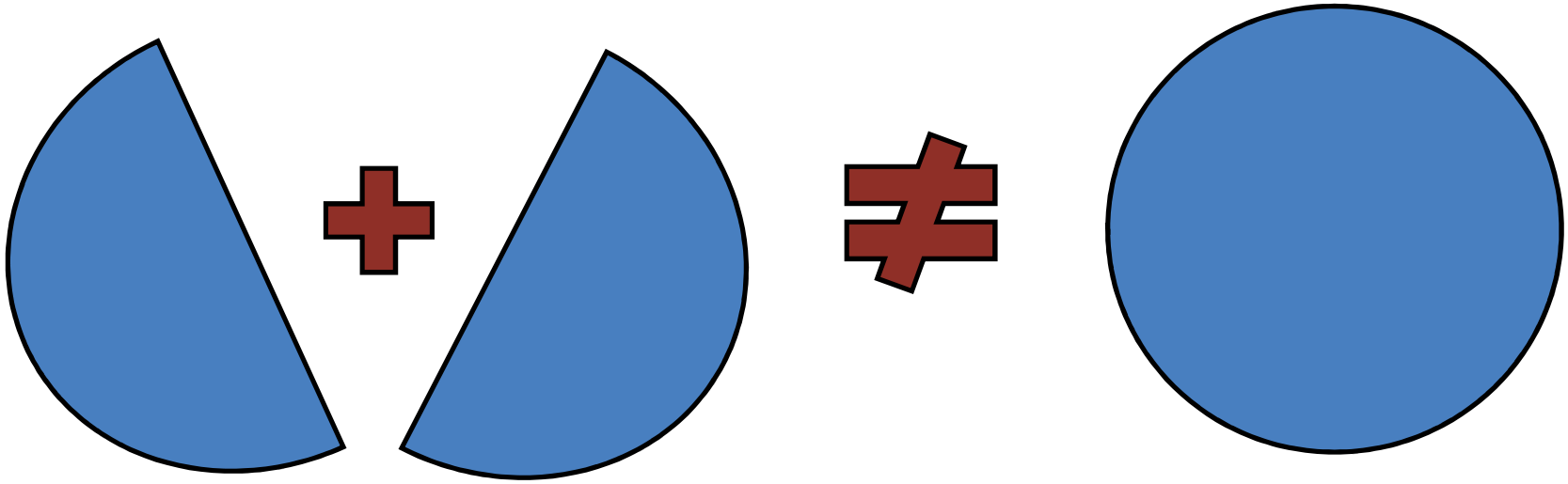
The sum of two halves may be different from the whole. Effects of splitting sequencing samples across lanes.



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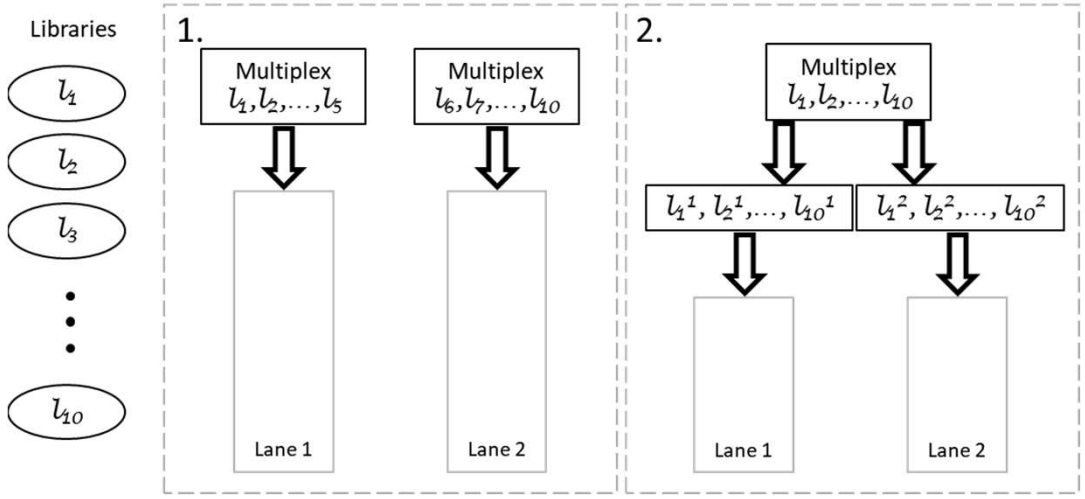
Aims



- Simulating across-lane sample splitting to illustrate variability introduced through sequencing design
- For bulk experiments, differences are observed in differential expression and enrichment analysis
- At the single cell level, we see changes in the identification of cell subpopulations
- We identify changes in biological interpretation when splitting across lanes is performed

Statistical Background

Sample 1
'Ground Truth'

AATC	10
TACT	7
GGAT	5
ATGC	5
GCGT	2
CCGT	1

Sequencing Depth: 30

Subsample at 50%

Subsample 1

AATC	5
TACT	3
GGAT	2
ATGC	5
GCGT	0
CCGT	0

Subsample 2

AATC	7
TACT	5
GGAT	1
ATGC	1
GCGT	0
CCGT	1

Subsample 3

AATC	4
TACT	6
GGAT	1
ATGC	3
GCGT	1
CCGT	0

Add 2
subsamples

SS1 + SS2

AATC	12
TACT	8
GGAT	3
ATGC	6
GCGT	0
CCGT	1

SS1 + SS3

AATC	9
TACT	9
GGAT	3
ATGC	9
GCGT	1
CCGT	0

SS2 + SS3

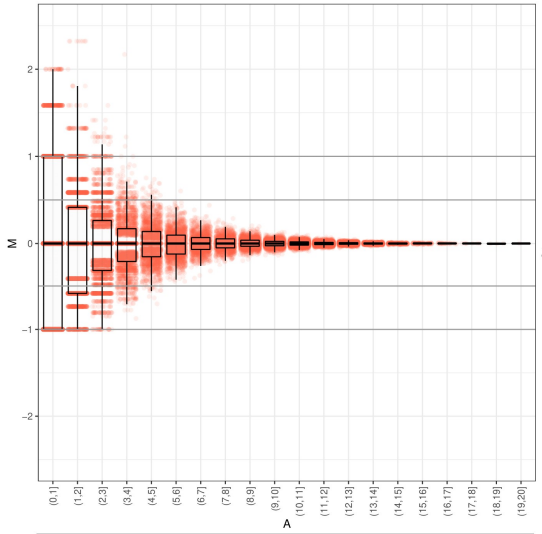
AATC	11
TACT	11
GGAT	2
ATGC	4
GCGT	1
CCGT	1

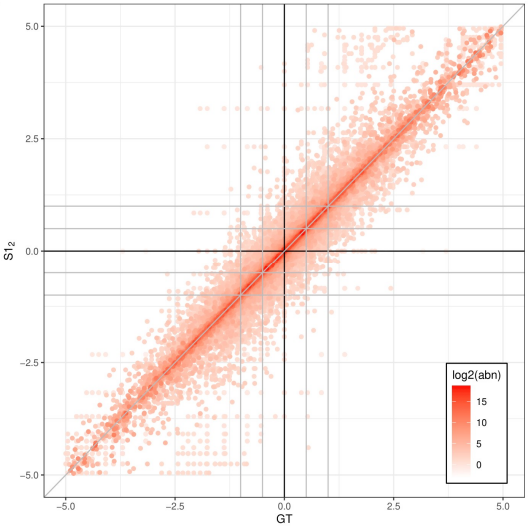
mRNAseq Case Study

Yang et al, Cell Systems, May 22;8(5):427-445.e10. (2019)
Multi-omic Profiling Reveals Dynamics of the Phased Progression of Pluripotency

GT v $S1_2$

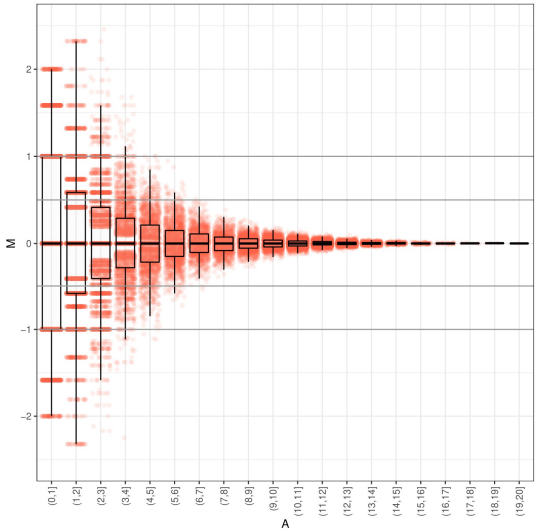
Ground truth vs simulated k=2 sample

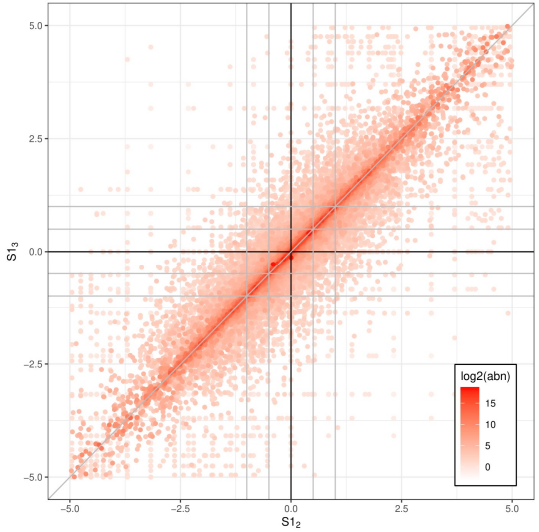


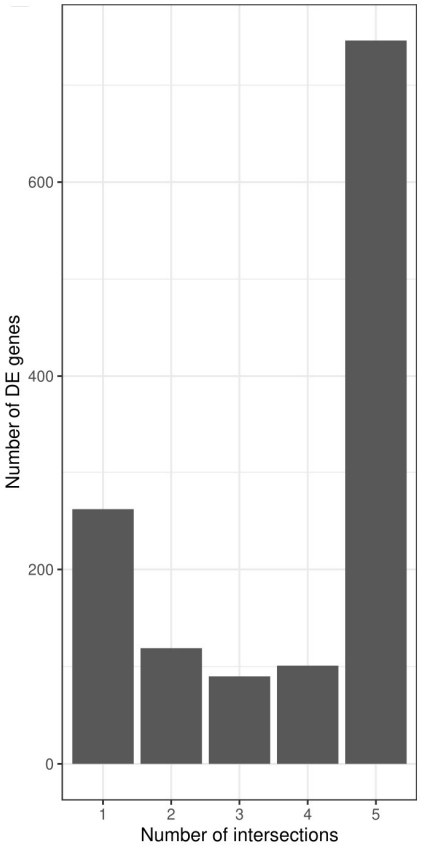


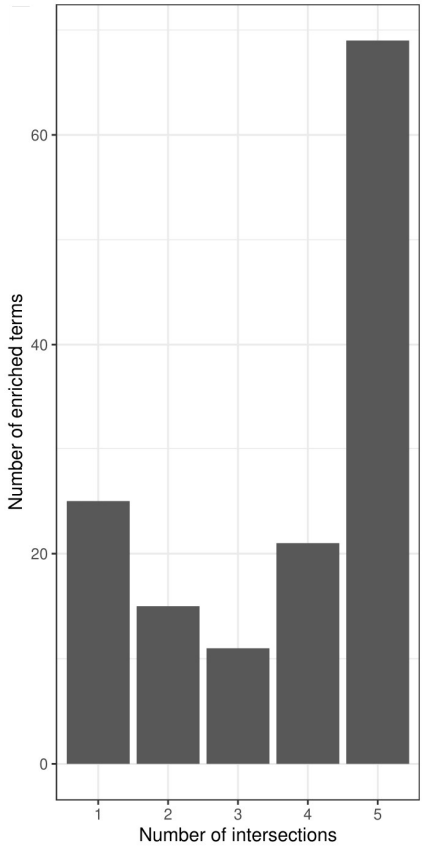
$S1_2$ v $S1_3$

Simulated k=2 sample vs simulated k=3 sample



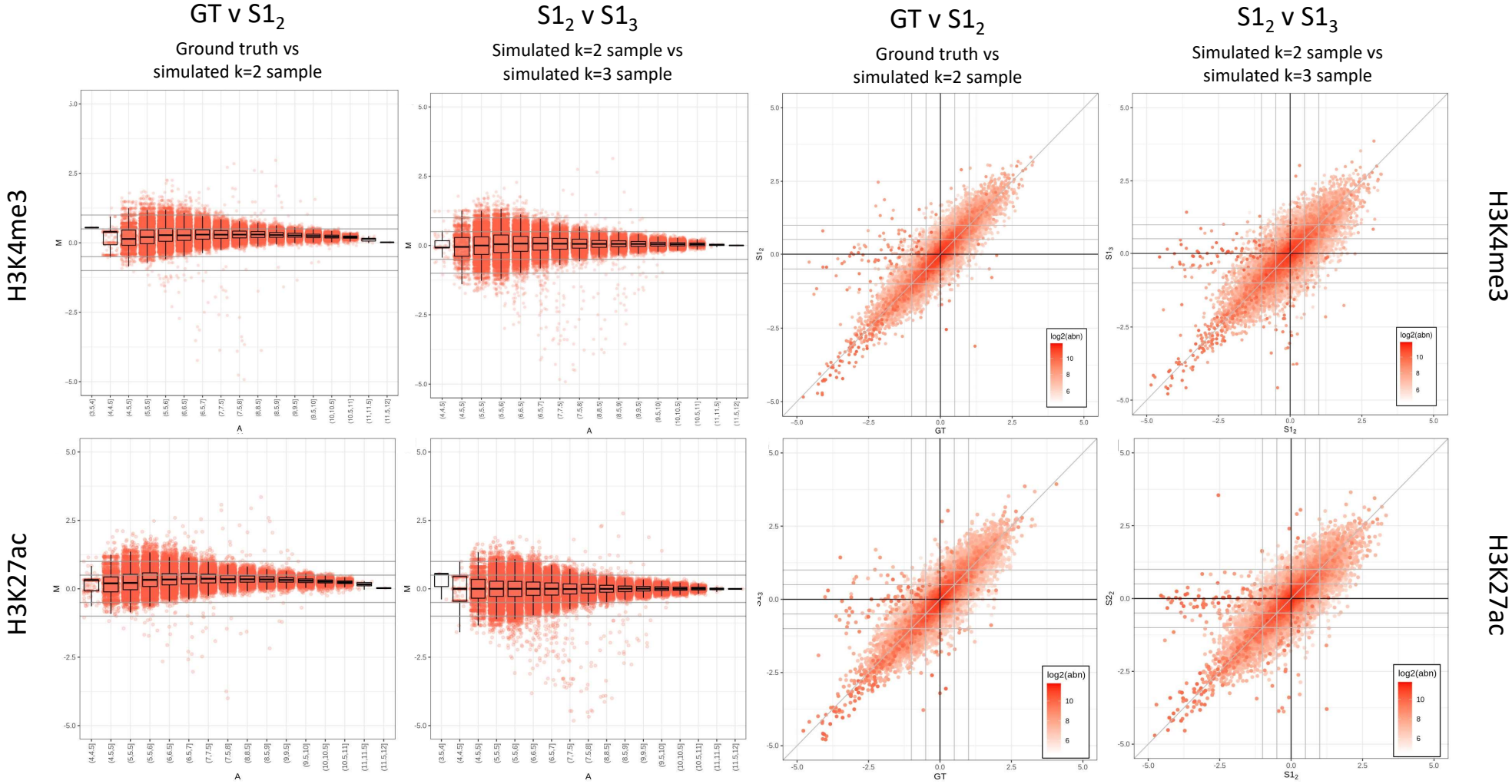






ChIPseq Case Study

Yang et al, Cell Systems, May 22;8(5):427-445.e10. (2019)
Multi-omic Profiling Reveals Dynamics of the Phased Progression of Pluripotency



mRNAseq Case Study

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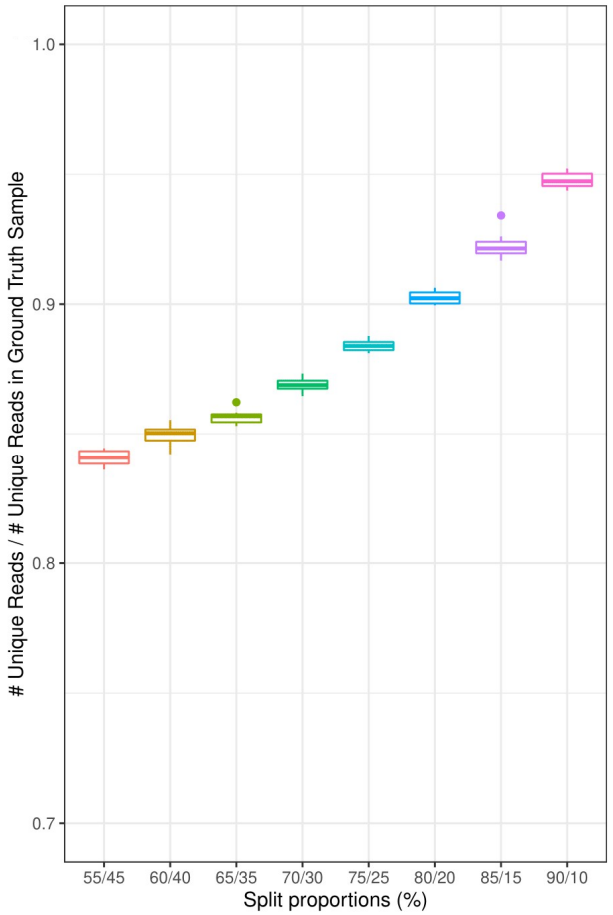
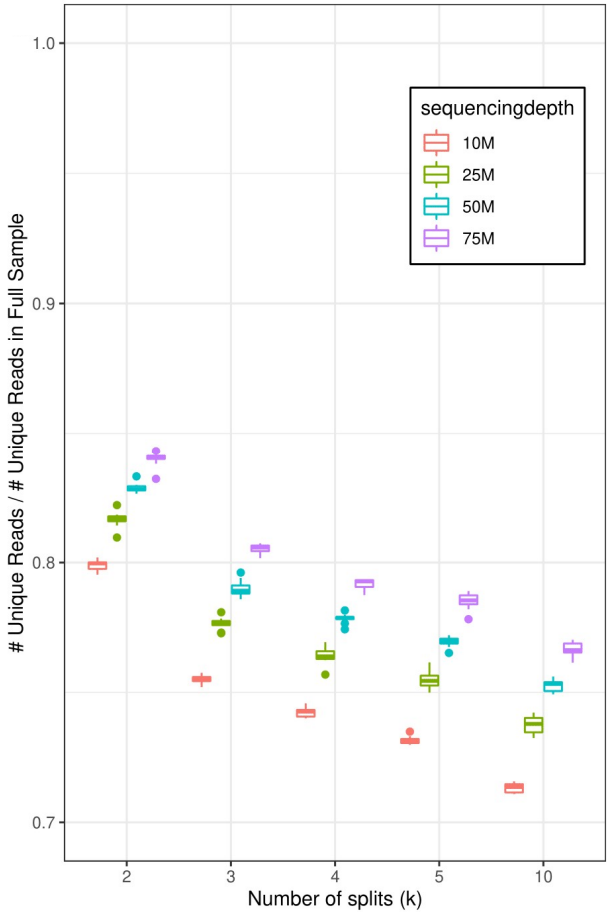
Varying split proportion



Varying number of splits and sequencing depth



ChIPseq Case Study

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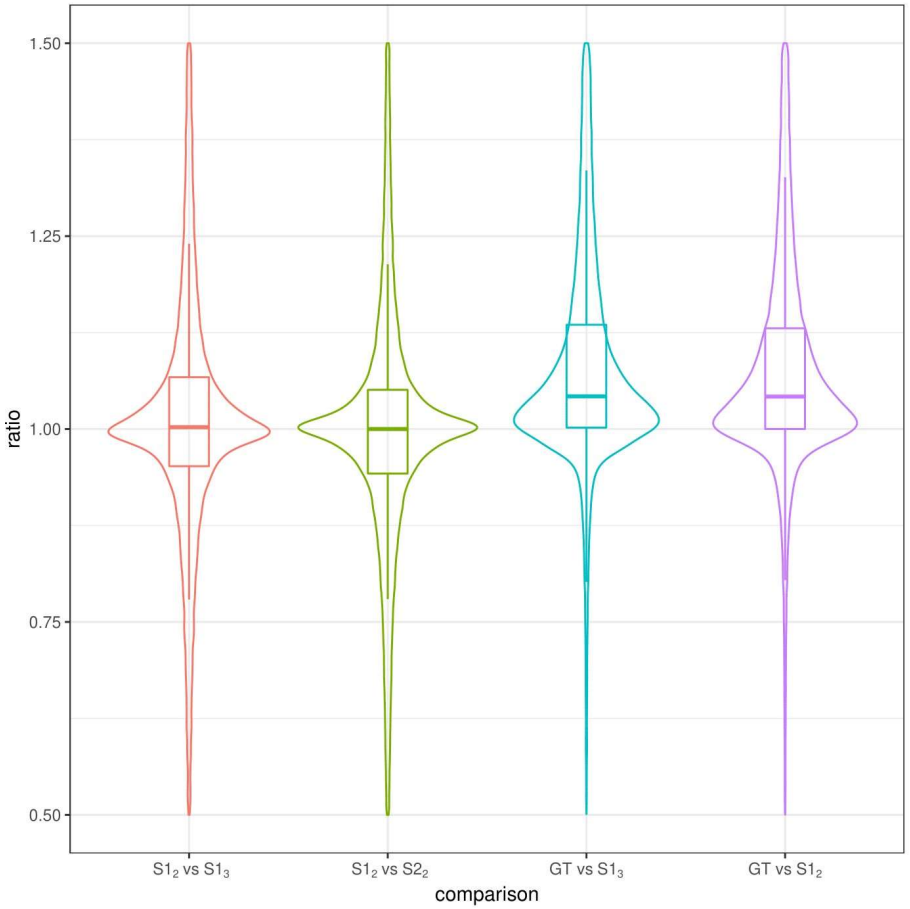
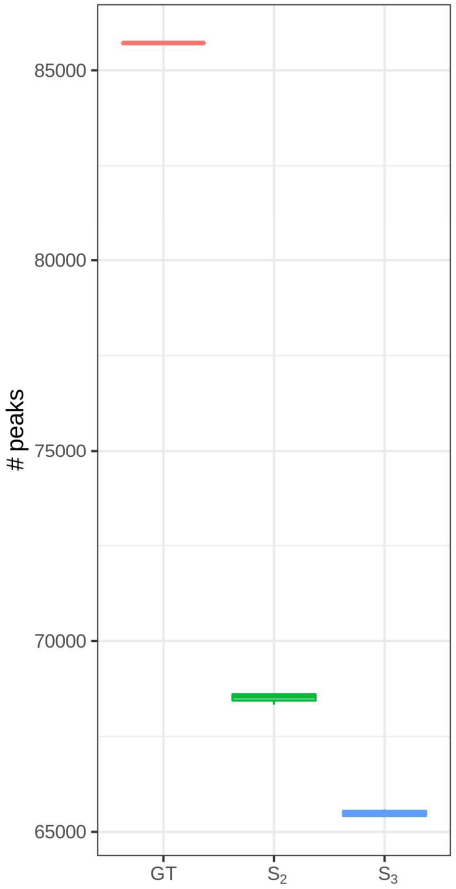
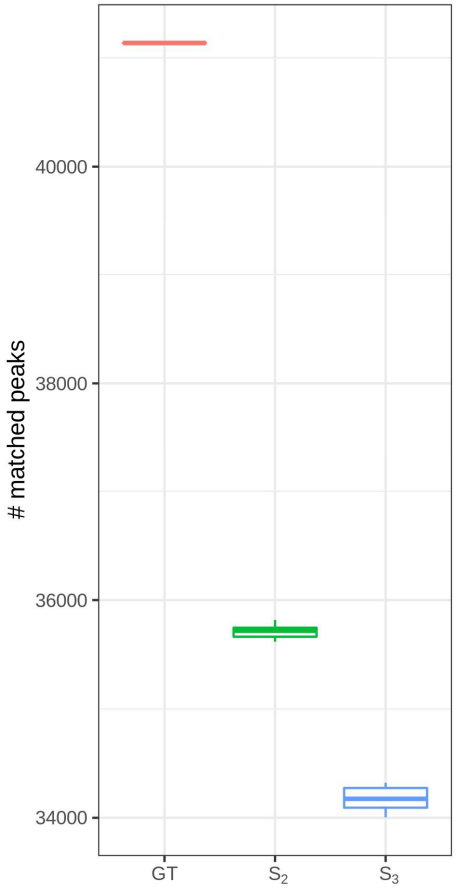
Ratio of peak lengths



Number of peaks



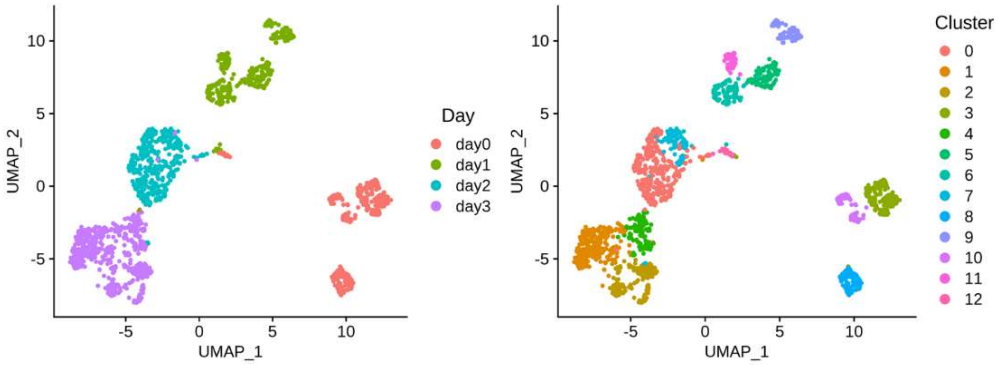
Number of peaks matched between time points

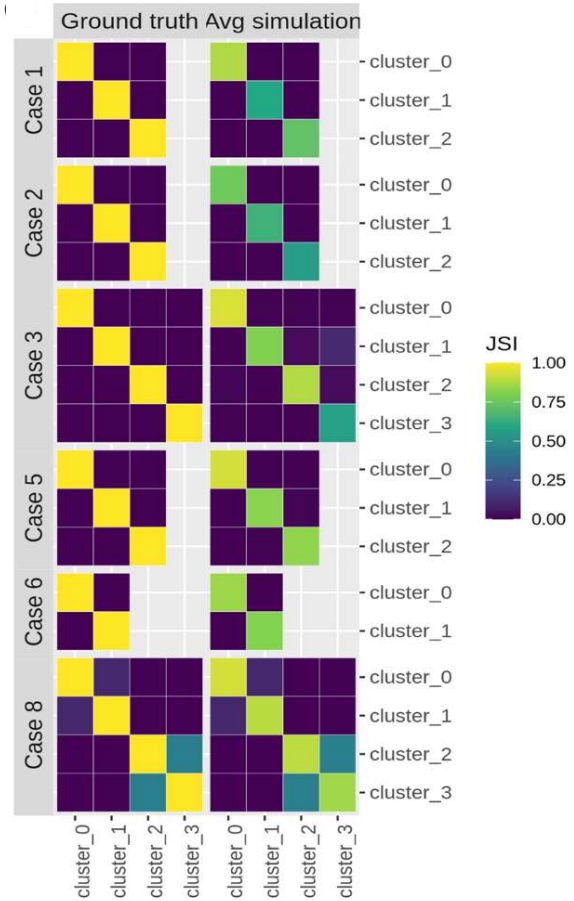


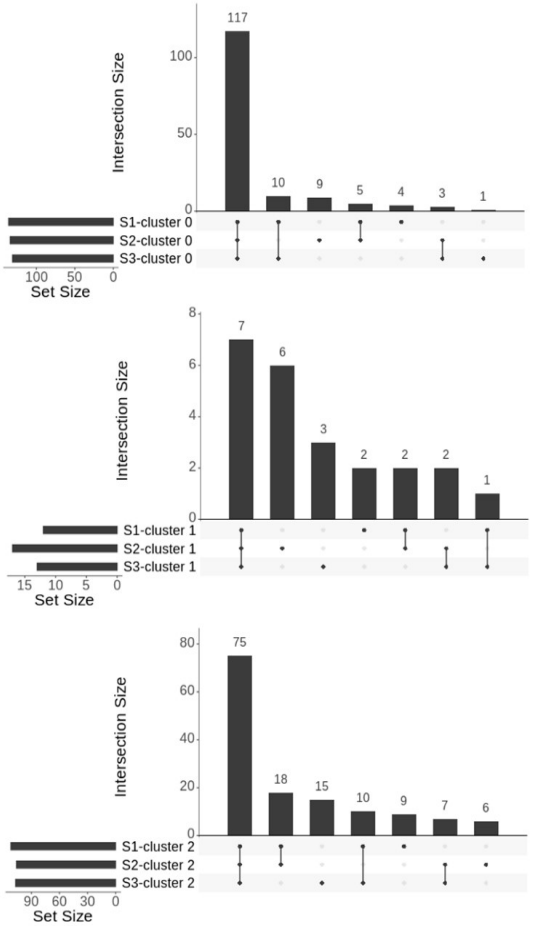
Smart-Seq2 Case Study

Cuomo et al; Nature Communications volume 11, Article number: 810 (2020)
Single-cell RNA-sequencing of differentiating iPS cells reveals dynamic genetic effects on gene expression

	Study case	N cells	Donor	N cells (donor)	Time point	N cells (time point)	Cluster	N cells (cluster)
1	Case 1	105	hayt	105	day2	105	0	105
2	Case 2	106	pahc	106	day3	106	1	66
							4	40
3	Case 3	168	melw	94	day0	168	3	168
			qunz	74				
4	Case 5	168	hayt	168	day1	61	9	61
					day3	107	1	107
5	Case 6	95	melw	47	day1	95	5	45
			vils	48			6	50
6	Case 8	217	melw	95	day0	95	3	95
			naah	122	day3	122	2	122



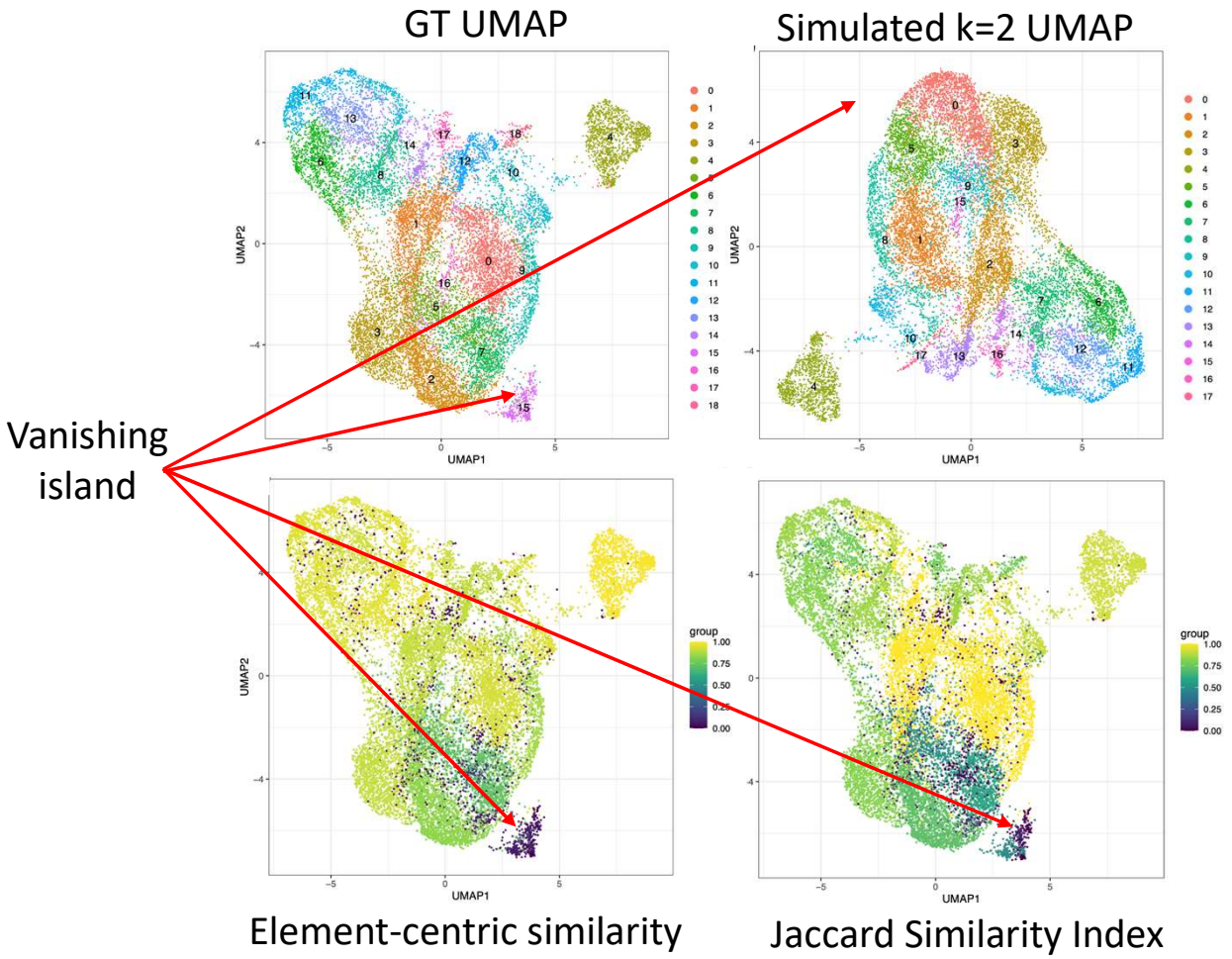




10x Case Study

Mende et al; bioRxiv (2020)

Quantitative and molecular differences distinguish adult human medullary and extramedullary haematopoietic stem and progenitor cell landscapes



Conclusions

- Splitting across lanes typically reduced read diversity, leading to over-representation for higher abundance fragments and under-representation or complete loss of lower abundance fragment
- While these changes may be difficult to see pre-alignment, we see knock-on effects in downstream analysis

- In bulk mRNAseq experiments this could lead to false positives in differential expression
- In bulk ChIPseq experiments, changes in peak calling and properties can be observed
- In single cell experiments, the topography of the UMAP can change, along with inferred cell type identities

- Ideally, don’t split across lanes but if it can’t be avoided then consistency is key

Acknowledgements

Ruben Chazarra-Gil

Arash Shahsavari

Irina Mohorianu

Core Bioinformatics Group

Ilias Moutospoulos

Alex Calderwood

Yu Zhang

Andi Munteanu

Chris Ellis

Antonella Santoro

Ingo Ringshausen

Ludovic Vallier

Rory Stark

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Preprint on bioRxiv

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