LFTDI

LABORATORY FOR FORENSIC TECHNOLOGY DEVELOPMENT & INTEGRATION

SELECTIVELY ANALYZING AND INTERPRETING DNA FROM MULTIPLE DONORS WITH A FULL SINGLE-CELL STRATEGY

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NIJ Forensic Science R&D Symposium

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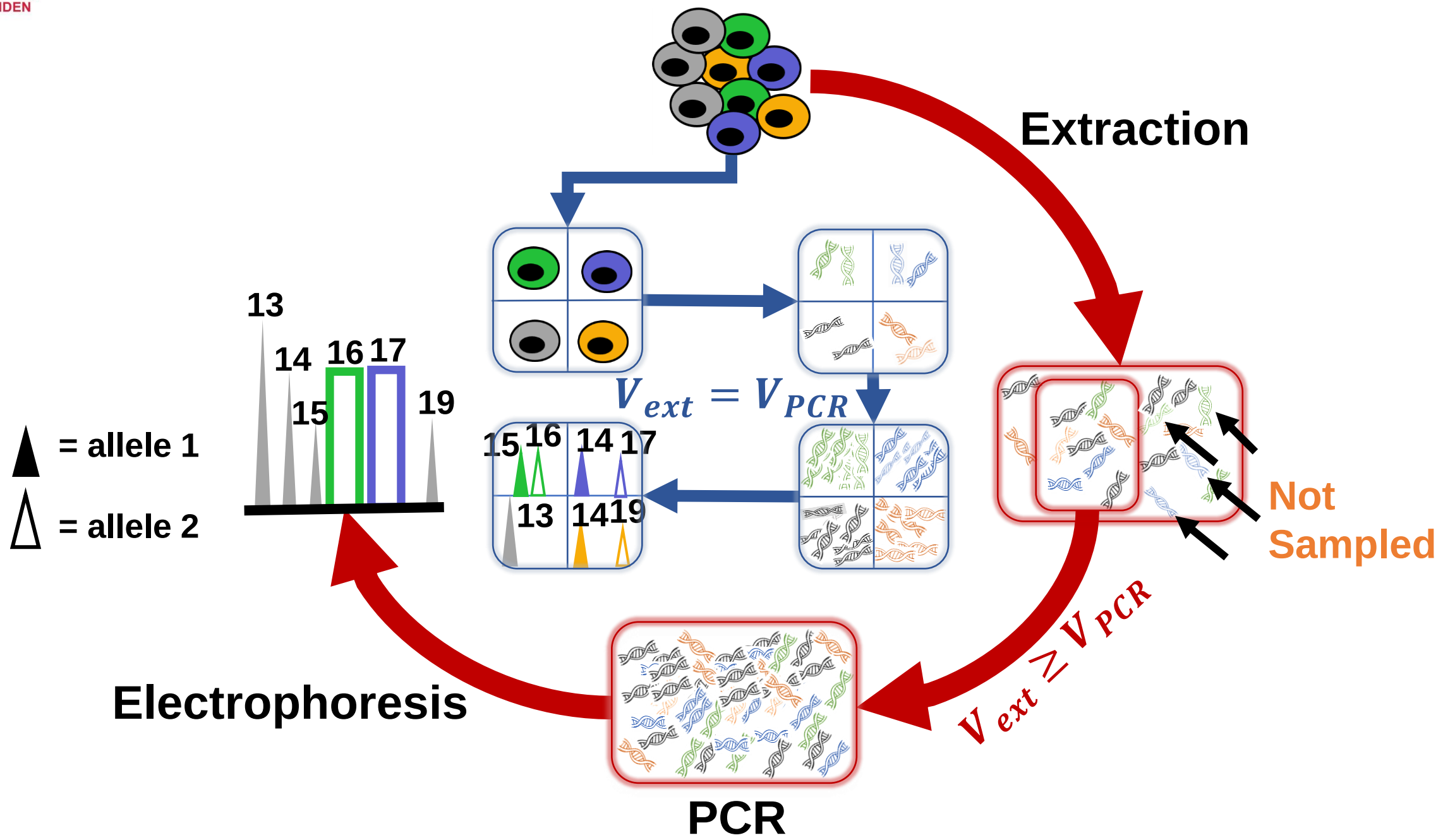
Presentation Aims

To develop a method by which we can compute the weight of evidence for an admixture using a single-cell pipeline

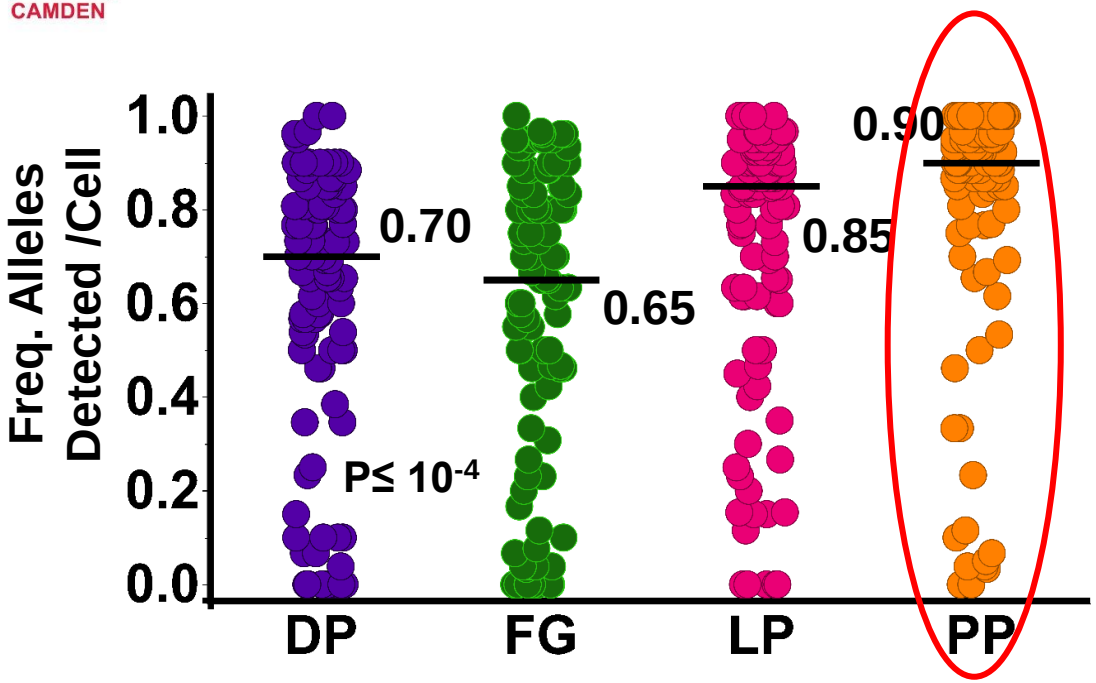
I. Implement a suspect agonistic clustering method.

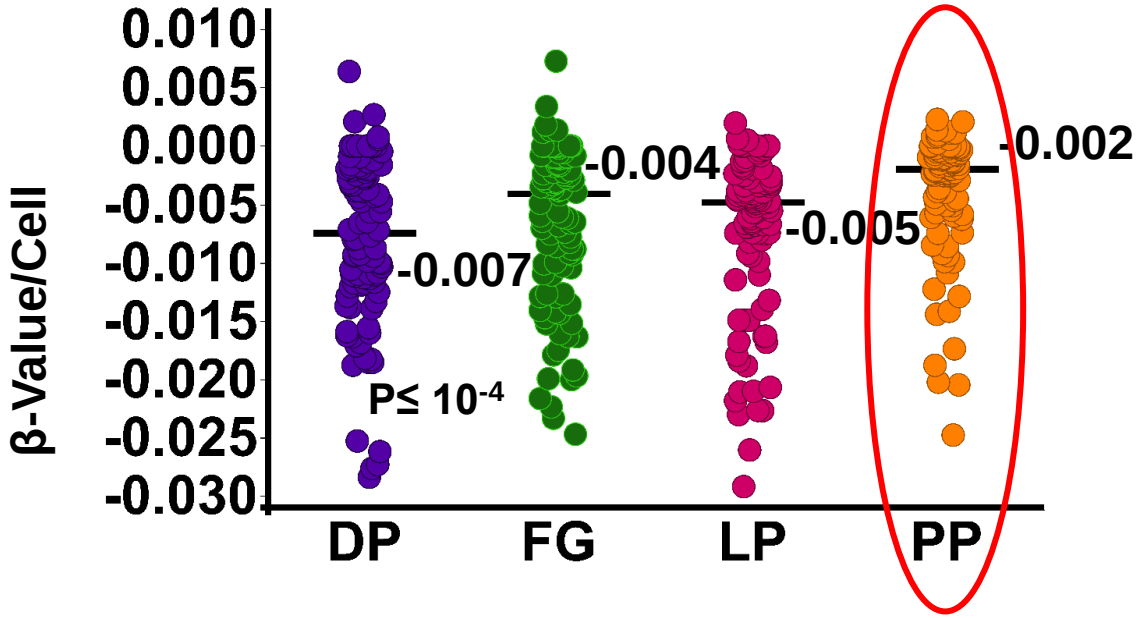
II. Combine information from single cells that is relevant to forensic science.

Single-Cell Vs. Bulk Mixture Pipeline



Preliminary Work : Four Direct-To-PCR Extraction Methods





ORIGINAL ARTICLE

Towards developing forensically relevant single-cell pipelines by incorporating direct-to-PCR extraction: compatibility, signal quality, and allele detection

Nidhi Sheth[1] · Harish Swaminathan[2] · Amanda J. Gonzalez[3] · Ken R. Duffy[4] · Catherine M. Grgicak[1,2,3]

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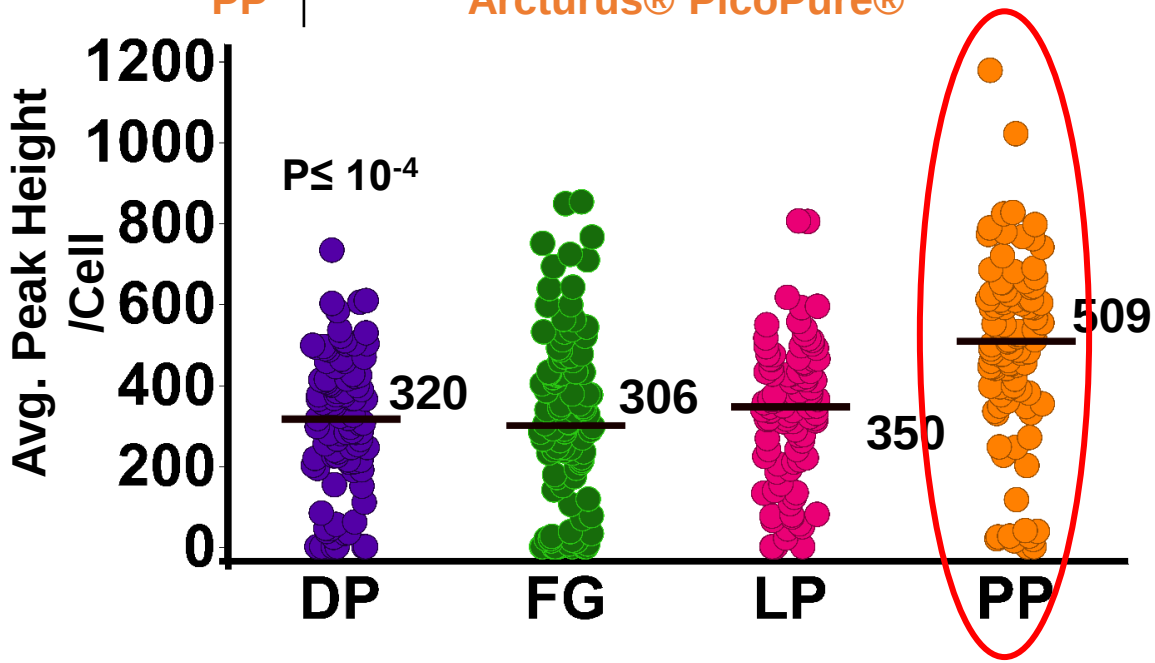
Direct-To-PCR Extraction Treatment	
DP	Viagen Direct PCR Lysis Solution
FG	forensicGEM® Saliva
LP	DEPArray™ LysePrep
PP	Arcturus® PicoPure®



Extraction Treatment Comparison Using DEPArray

TECHNICAL NOTE
Criminalistics

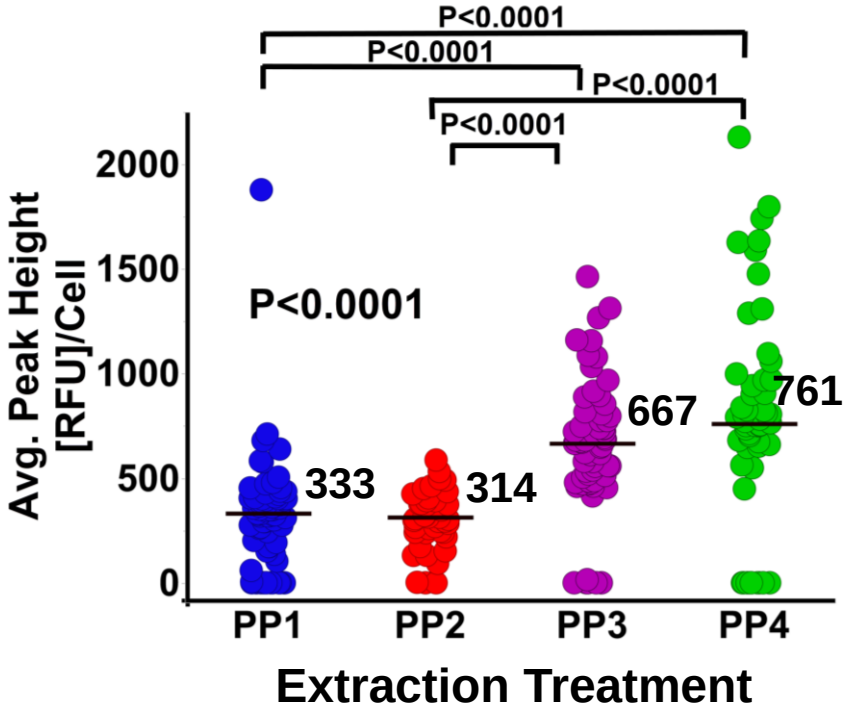
JOURNAL OF FORENSIC SCIENCES

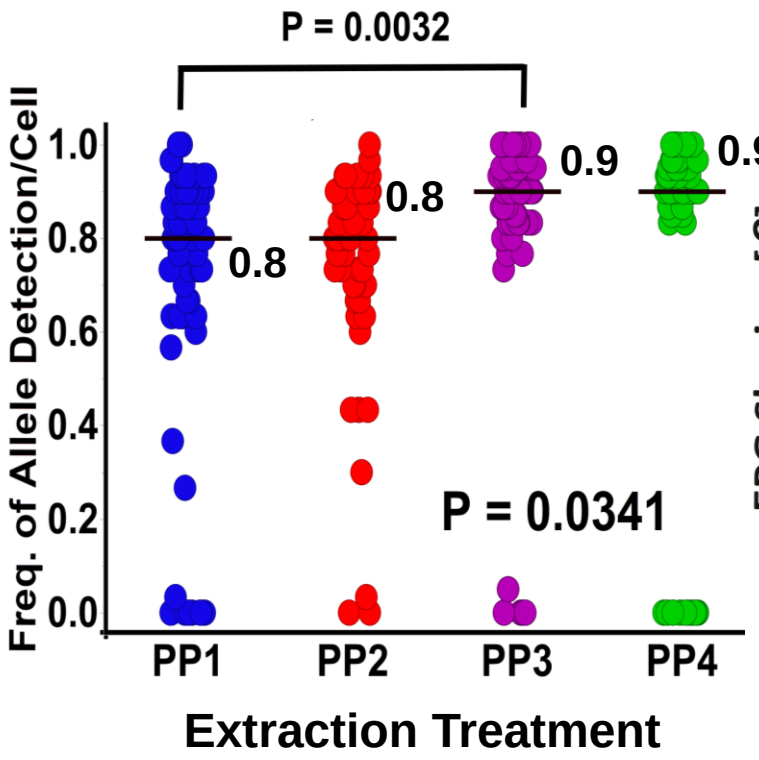
High-quality data from a forensically relevant single-cell pipeline enabled by low PBS and proteinase K concentrations

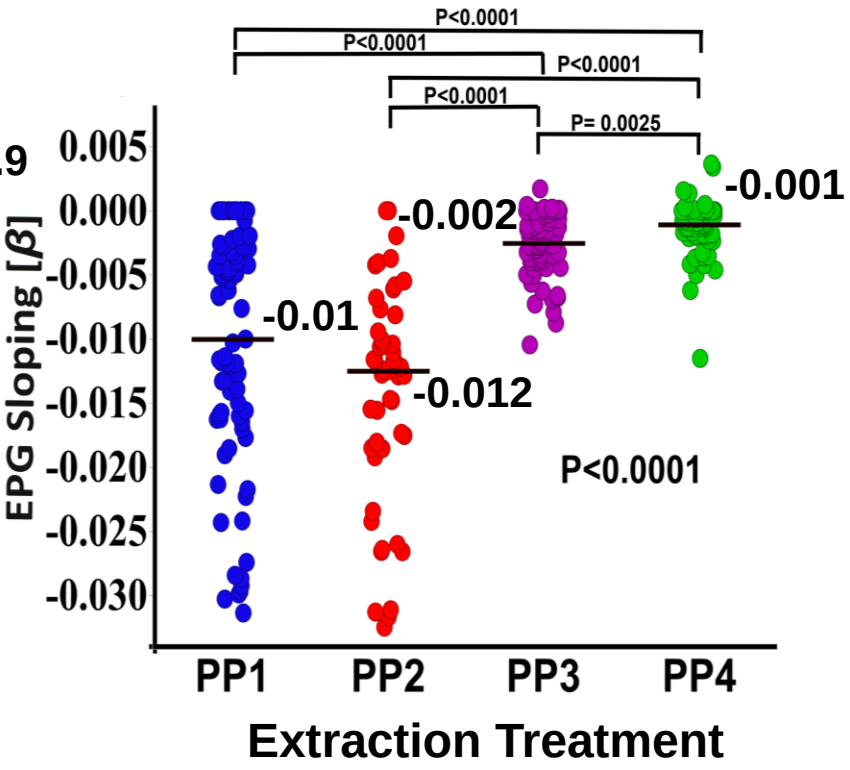
Nidhi Sheth MS[1] | Ken R. Duffy PhD[2] | Catherine M. Grgicak PhD[1,3]

Extraction Treatment Variations

PP1	Higher Pro K and PBS (1X)
PP2	Lower Pro K and PBS (1X)
PP3	Lower Pro K and PBS (0.5X)
PP4	Lower Pro K and PBS (0.25X)







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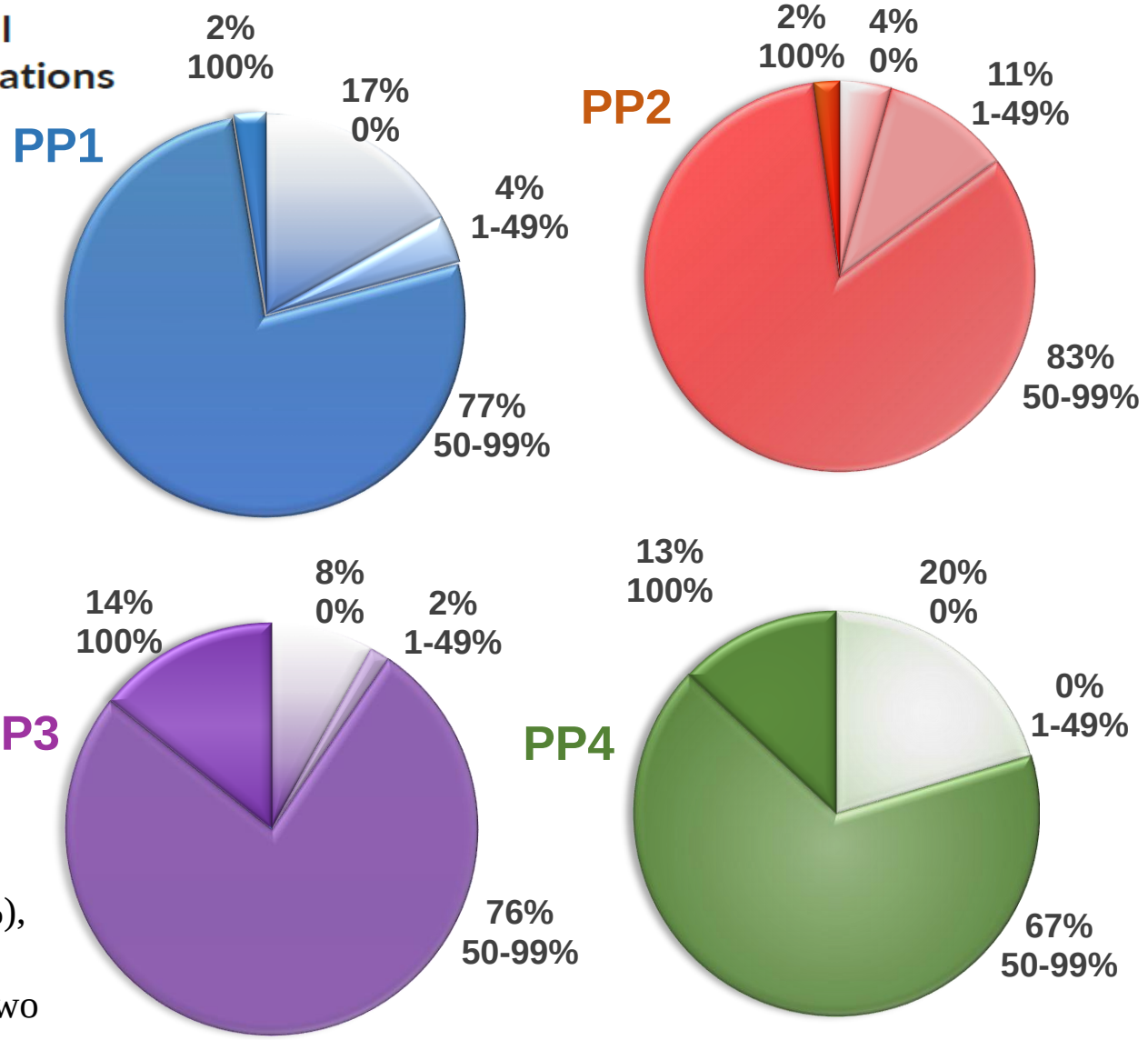
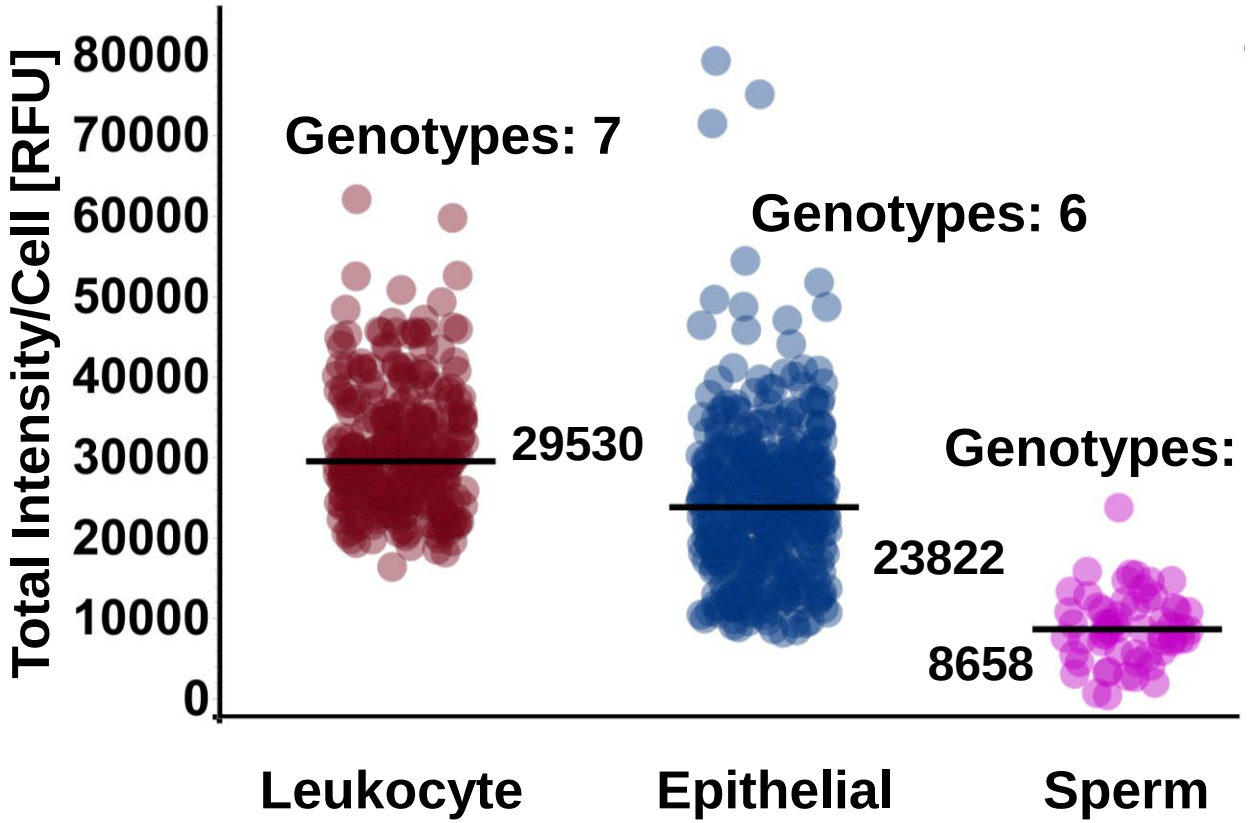
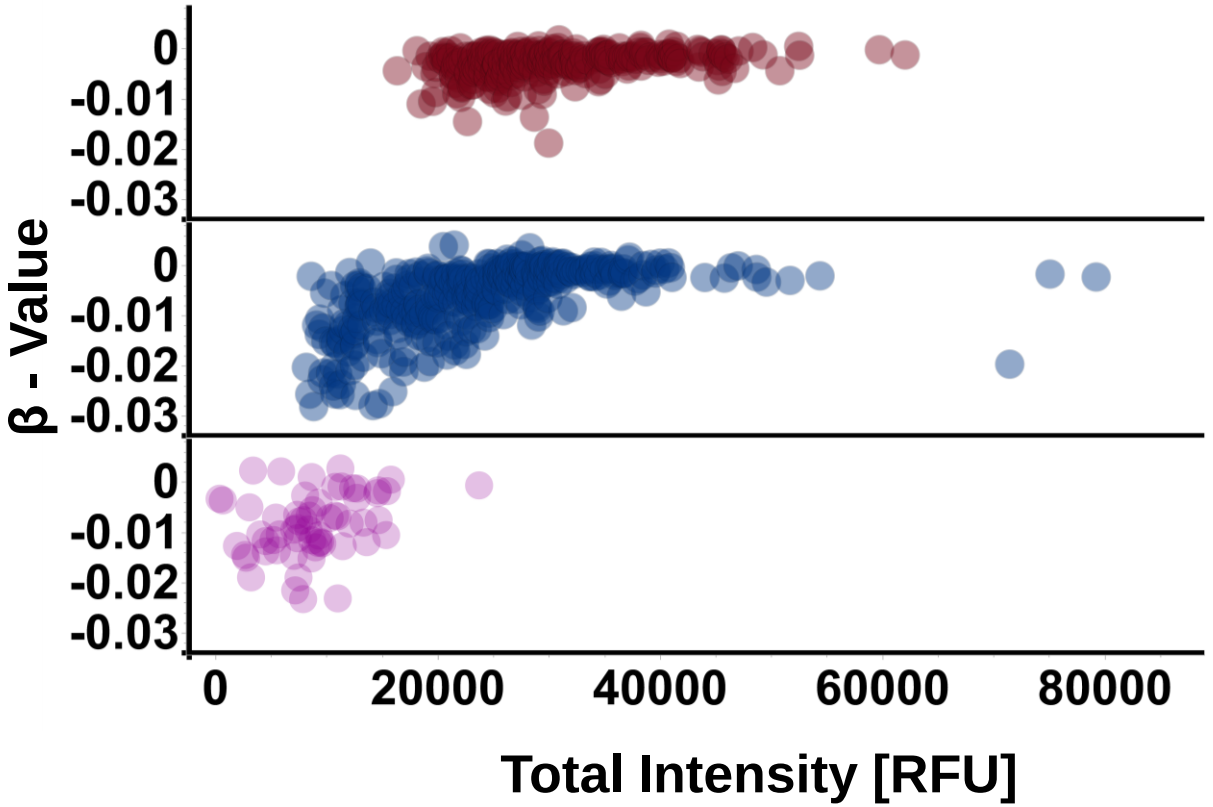


Pie charts. Percentage of profiles exhibiting full (100%), most (50-99%), few (1-49%), and no alleles across heterozygous loci where the known alleles are at least two STR units apart

Single Cell EPG Signal Quality





Experimental Conditions

Extraction	DDT Conc. for Sperm Extraction : **1.5mM**
Amplification, Separation, Analysis	**Global Filer™ (30 Cycles), 25 sec injection on 3500 Genetic analyzer,** and **Osiris(10.3.1) Analysis**

- Lower peak height indicates decreased β-value for the epithelial cell, which could result from DNA damage.
- Moving forward, 1X PBS and Lower ProK will be implemented in the experimental pipeline.

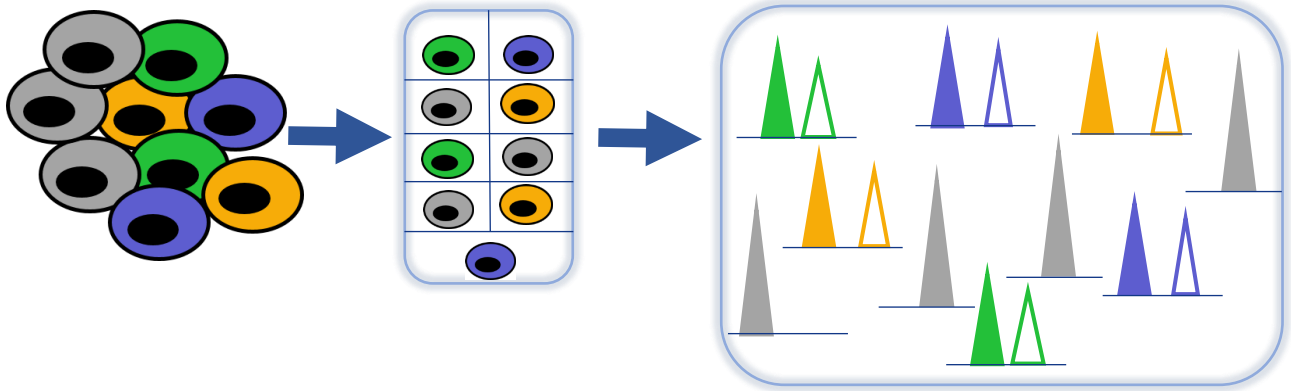
Single-Cell Clustering and Interpretation Strategy

LFTDI

Admixture

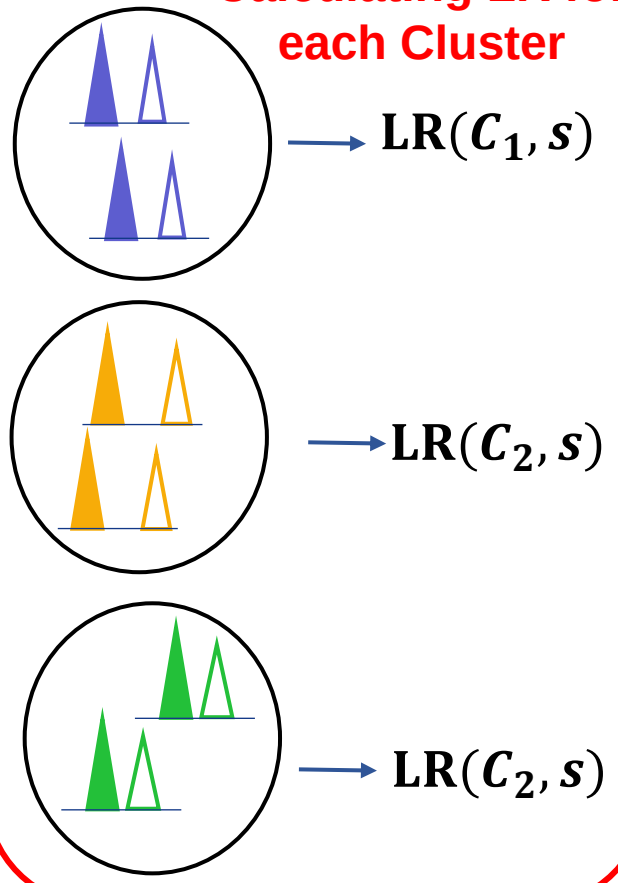
Single-cell per tube

Single-cell EPGs



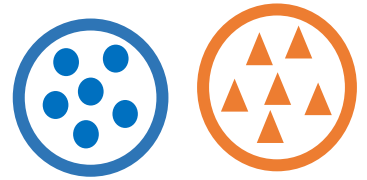
Clustering scEPGs based on similarity

Calculating LR for each Cluster



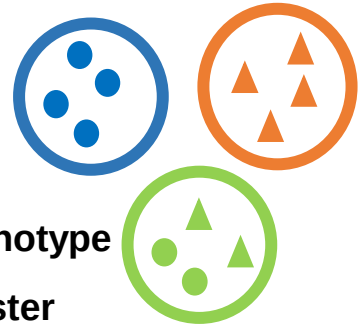
Clustering Performance

1. Correct cluster



2. Over cluster

3. Miscluster



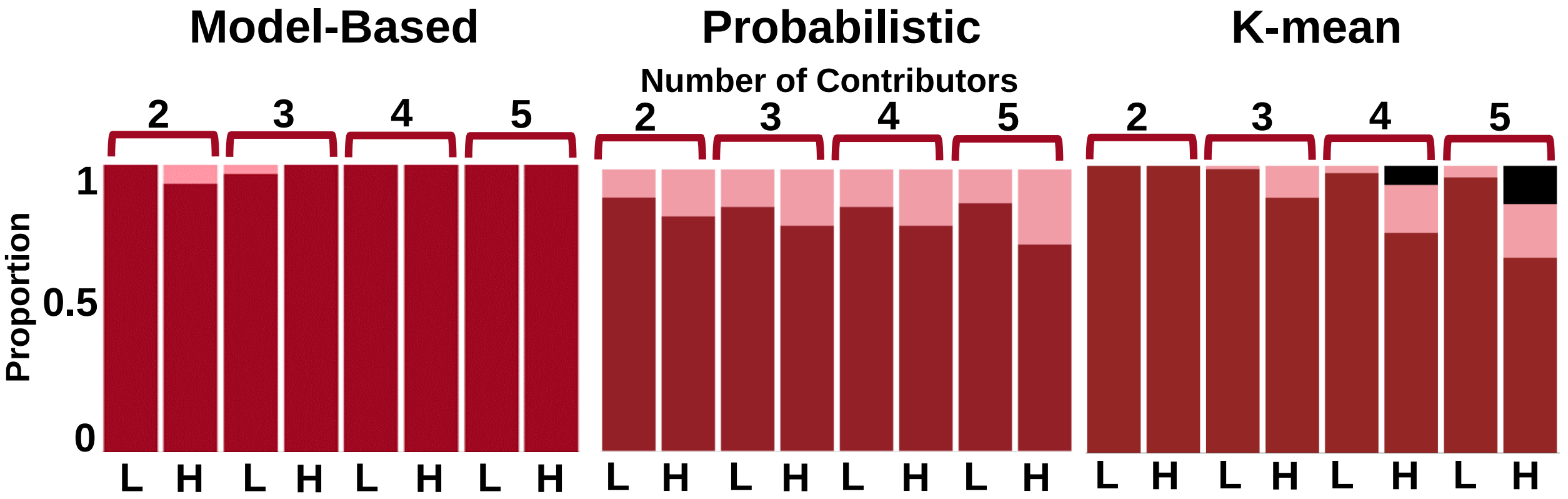
Shape = Genotype

Color = Cluster

Determine the LR for **admixture by averaging LR** from individual clusters

$$\mathbf{WoE} = \mathbf{Log}\,\mathbf{LR}_{avg}(A, s_{true})$$

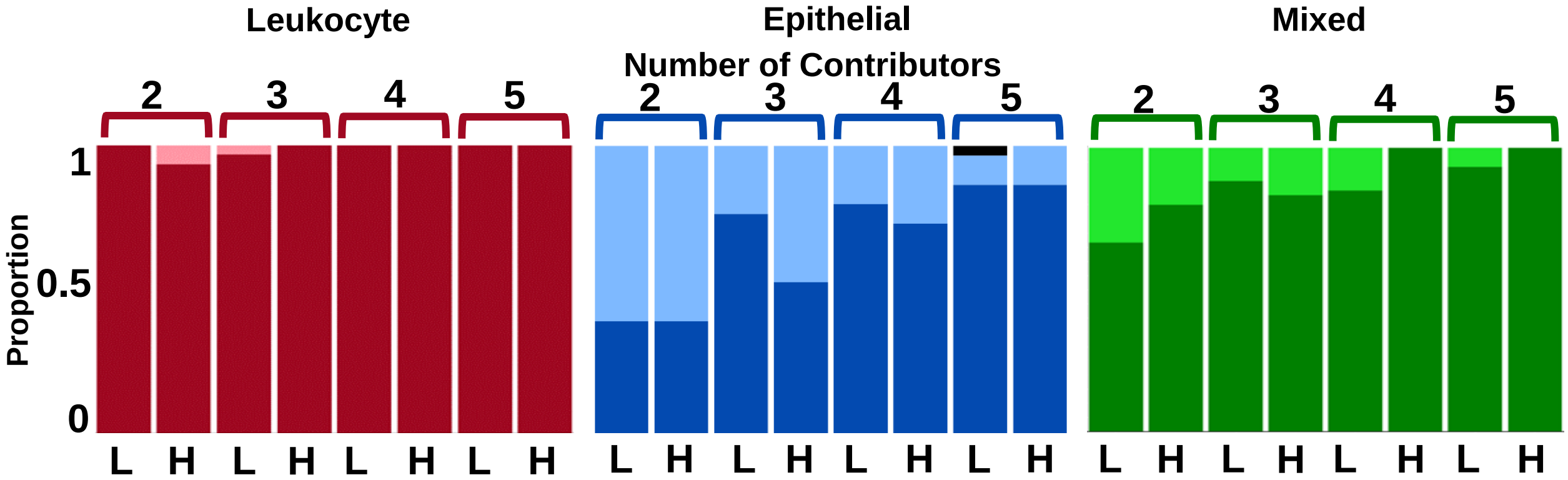
Alternative Clustering Techniques Comparison: Leukocyte



Smallest Contributor: L < 0.2, H ≥ 0.2

Model Based Clustering performs better, no misclusters

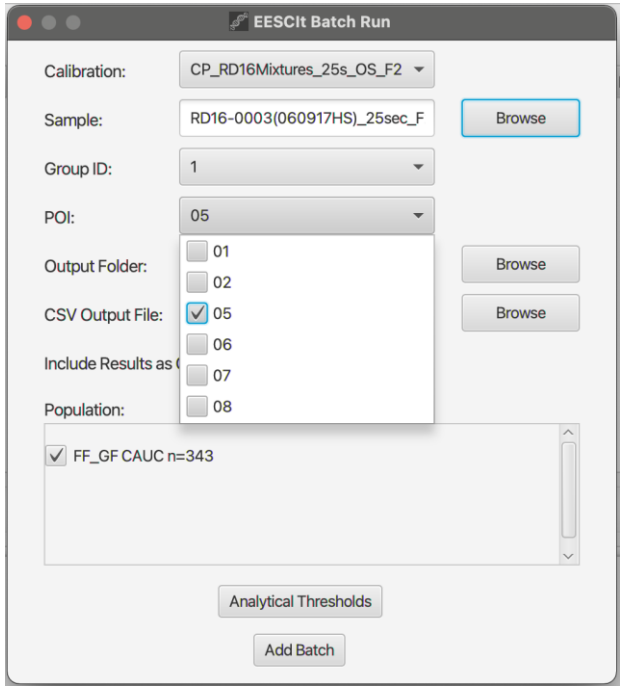
Model-Based Clustering: Results

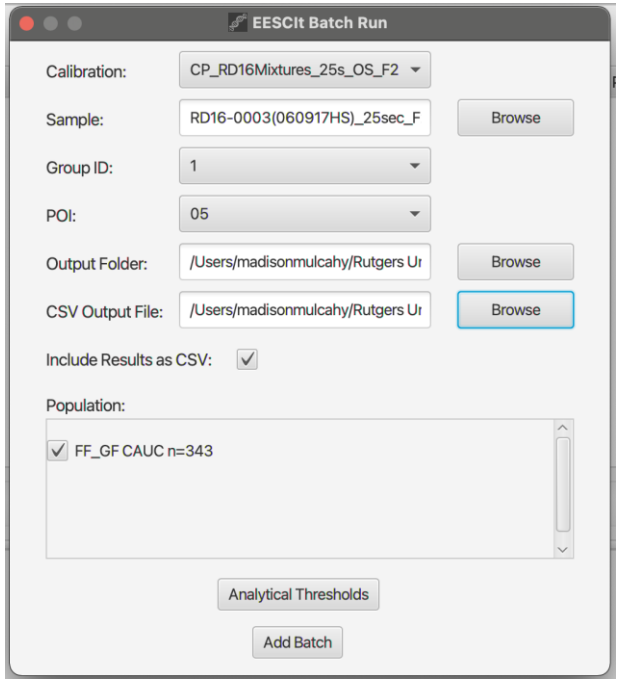


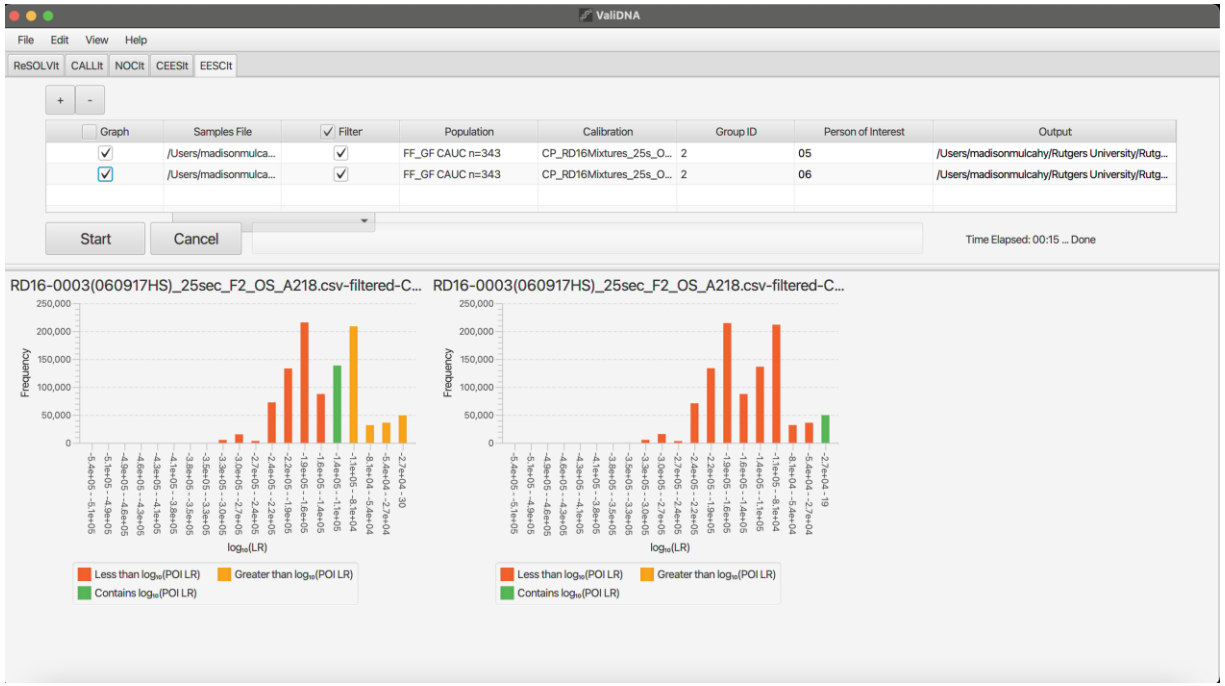
Model Based Clustering was performed using “mclust” as implemented in R.

Smallest Contributor	L < 0.2 H ≥ 0.2

Computing The Likelihood Ratio Using EESCIt (Evidentiary Evaluation of Single Cells)

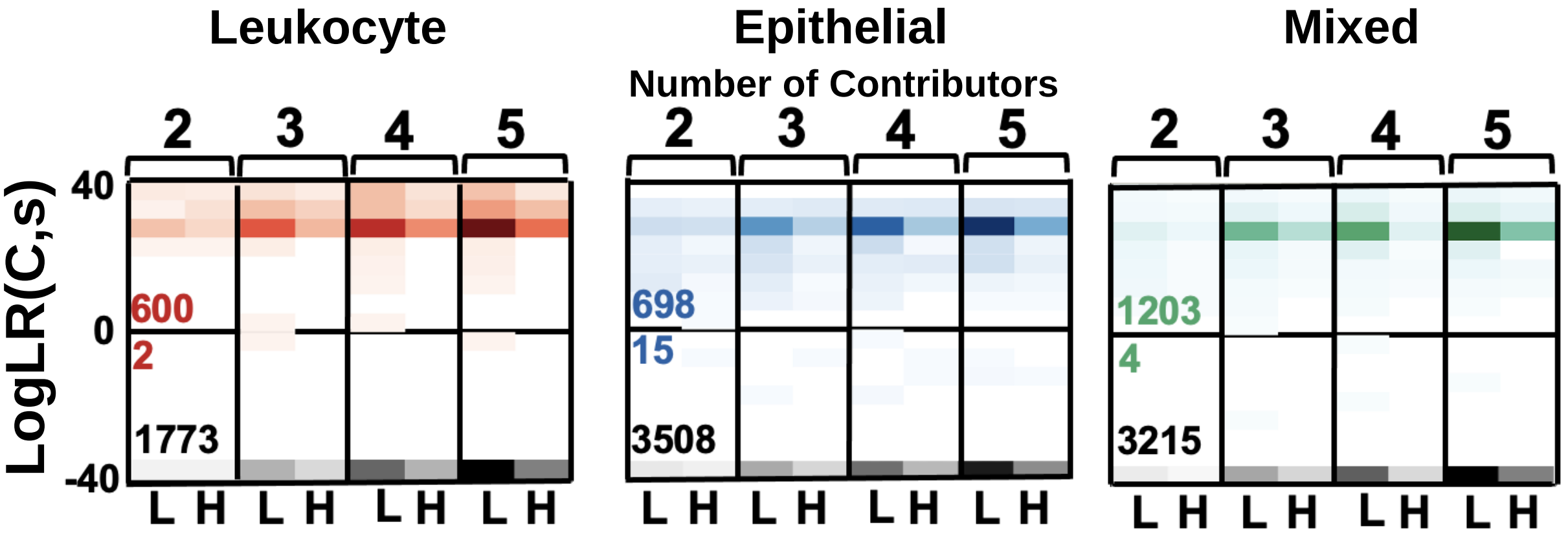






Snapshot of the EESCIt software that computes Likelihood Ratio(LR) for single-cells - Each cluster was run in EESCIt against the true contributor.

LR RESULTS FOR EACH CLUSTER



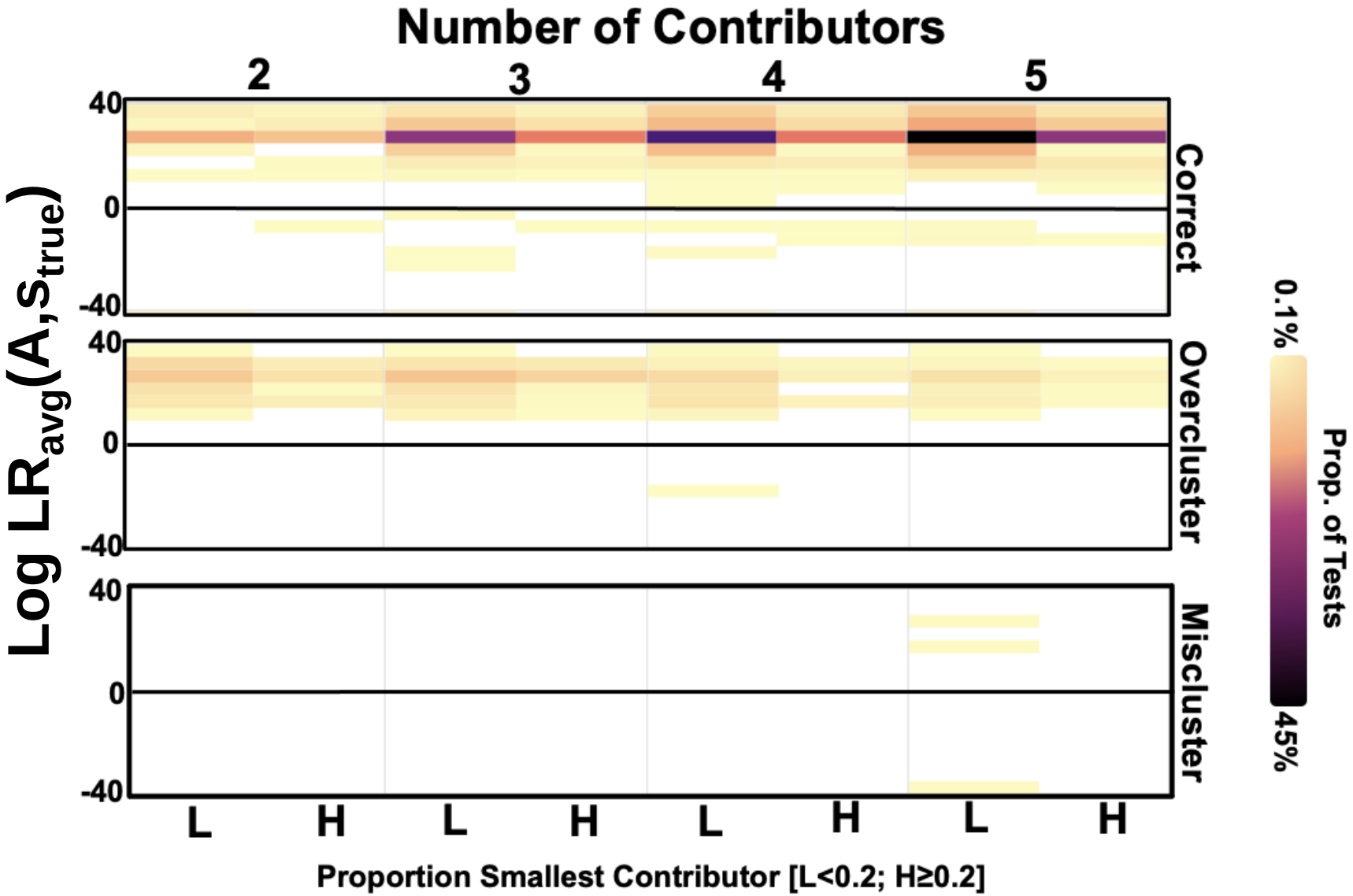
Smallest Contributor: L < 0.2, H ≥ 0.2

Prop. Admixtures

0.1% — **35%**

Heat maps show that most admixtures resulted in $Log_{10}LR(C,s_{true})$ of at least 25, **regardless of sample complexity.** C=cluster

Model-Based Clustering: Over All LogLR



Heat maps show that most admixtures resulted in **LogLR$_{avg}$ (A,s$_{true}$) >5**, regardless of sample complexity and clustering performance. Notably the highest proportion of LogLR$_{avg}$ (A,s$_{true}$) falls within [25,30)

ACKNOWLEDGMENTS

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