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LABORATORY FOR FORENSIC TECHNOLOGY DEVELOPMENT & INTEGRATION



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

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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.





PRELIMINARY WORK : FOUR DIRECT-TO-PCR EXTRACTION METHODS LFTDI



ORIGINAL ARTICLE

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

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EXTRACTION TREATMENT COMPARISON USING DEPARRAY

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EXTRACTION TREATMENT COMPARISON USING DEPARRAY

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ORENSIC SCIENCES

TECHNICAL NOTE Criminalistics

PP1

PP2

PP3

PP4

2% 4% 2% High-quality data from a forensically relevant single-cell 100% 0% 100% 11% pipeline enabled by low PBS and proteinase K concentrations 17% PP2 1-49% 0% **PP1** Nidhi Sheth MS¹ | Ken R. Duffy PhD² | Catherine M. Grgicak PhD^{1,3} 4% 1-49% **Extraction Treatment Variations** 83% 50-99% 77% Higher Pro K and PBS (1X) 50-99% 13% Lower Pro K and PBS (1X) 8% 20% 100% 14% 2% 0% 0% 100% 1-49% Lower Pro K and PBS (0.5X) 0% 1-49% **PP3** PP4 Lower Pro K and PBS (0.25X) **Pie charts.** Percentage of profiles exhibiting full (100%), 76% 67% 50-99% most (50-99%), few (1-49%), and no alleles across 50-99% heterozygous loci where the known alleles are at least two STR units apart



Separation,

Analysis

SINGLE CELL EPG SIGNAL QUALITY

رورورو 2 8000 ا **RF Genotypes: 7** 70000 **Genotypes: 6** 60000 **Fotal Intensity/Cell** -0.01 50000 -0.02 -0.03 40000 'alue 29530 **Genotypes: 5** 30000 -0.01 -0.02 20000 23822 œ. -0.03 10000 0 8658 -0.01 -0.02 Leukocyte **Epithelial** Sperm -0.03 20000 80000 40000 60000 0 **Experimental Conditions Total Intensity [RFU]** DDT Conc. for Sperm Extraction : **Extraction** 1.5mM Lower peak height indicates decreased β -value for the epithelial Global Filer[™] (30 Cycles), 25 cell, which could result from DNA damage. Amplification,

sec injection on 3500 Genetic

analyzer, and Osiris(10.3.1)

Analysis

Moving forward, 1X PBS and Lower ProK will be implemented in the experimental pipeline.

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SINGLE-CELL CLUSTERING AND INTERPRETATION STRATEGY LFTDI

CAMDE



ALTERNATIVE CLUSTERING TECHNIQUES COMPARISON: LEUKOCYTE LFTDI

CAMDEN



Model Based Clustering performs better, no misclusters



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



COMPUTING THE LIKELIHOOD RATIO USINGLFTD

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	Analytical Thresholds		Analytical Thresholds			Less than Contains I	Less than log _e (POI LR) Greater than log _e (POI LR) Contains log _e (POI LR)				Less than log _m (POI LR) Contains log _m (POI LR)			
Add Batch			Add Batch											
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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

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Heat maps show that most admixtures resulted in Log₁₀LR(C,s_{true}) of at least 25, regardless of sample complexity. C=cluster



MODEL-BASED CLUSTERING: OVER ALL LOGLR LFTDI

Number of Contributors 2 5 3 4 40 LR_{avg}(A,S_{true} ect 0.1% vercluste Prop. ç -40 Tests 40 Miscluster 45% -40 Н Η н н Proportion Smallest Contributor [L<0.2; H≥0.2]

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)



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