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LABORATORY FOR FORENSIC TECHNOLOGY DEVELOPMENT & INTEGRATION

SINGLE CELLS PRODUCE HIGHLY INFORMATIVE FORENSIC COMPARISONS ACROSS MULTIFARIOUS ADMIXTURES

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Single cell genetic pipelines extract DNA one cell at a time and use direct amplification

Two features common to all single-cell experiments:

intact cells or nuclei are isolated before the cell is lysed; and

that the extraction and amplification (or library preparation) occurs in the same vessel to which the cell was added

These make single cell forensics unique:

by isolating the cell before lysis, DNA from each cell are resolved from other types, and alleles remain coupled during isolation; and

by extracting and amplifying in the same vessel, signal drop-out from fractionating the extract into two parts – one that is stored and one that is amplified – is abated

INFORMATION LIMIT IS DEFINED ONLY BY THE NUMBER OF CELLS COLLECTED, WHICH IS FLEXIBLE

Since we sample without replacement, the probability of isolating at least one cell from a total of t cells, where t_d is the number of cells from d, and when m cells are isolated is,

$$\Pr(r \geq 1) = 1 - \Pr(r = 0)$$

$$= 1 - \frac{\binom{t - t_d}{m}}{\binom{t}{m}}$$

e.g., t=100; t_d=5 (1 in 20 mixture); m=40 cells, this evaluates to 92%.

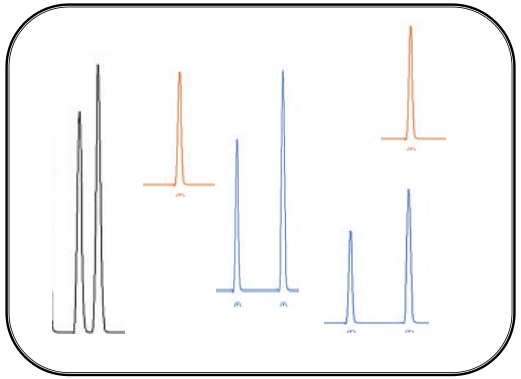
By isolating m=80 cells the probability increases to 99.8%

Supports the position to accelerate research into high throughput single-cell forensics

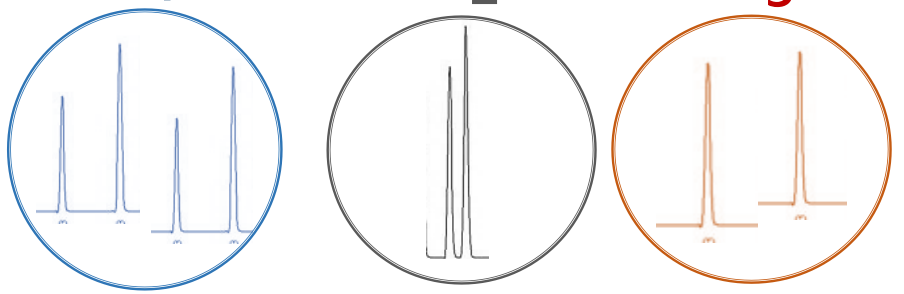
Single cell analysis addresses both investigative and evaluative aims

- 5 scEPGs
- One locus
- Colors=different donors

Admixture, *A*

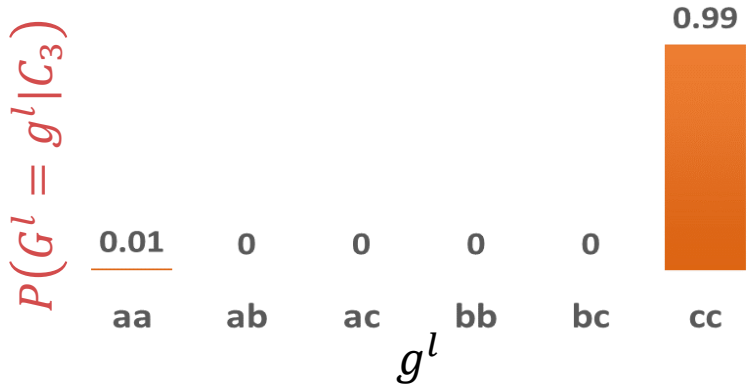


Suspect-agnostic clustering



Investigative (no suspect)

$$P(G^l = g^l|C) = \frac{\{\prod_{i=1}^{v} P(E_i^l|G^l = g^l)\}P(G^l = g^l)}{\sum_{g^l}\{\prod_{i=1}^{v} P(E_i^l|G^l = g^l)\}P(G^l = g^l)}$$



Evaluative (suspect)

Sub-sub-source evaluation, i.e., cluster evaluation

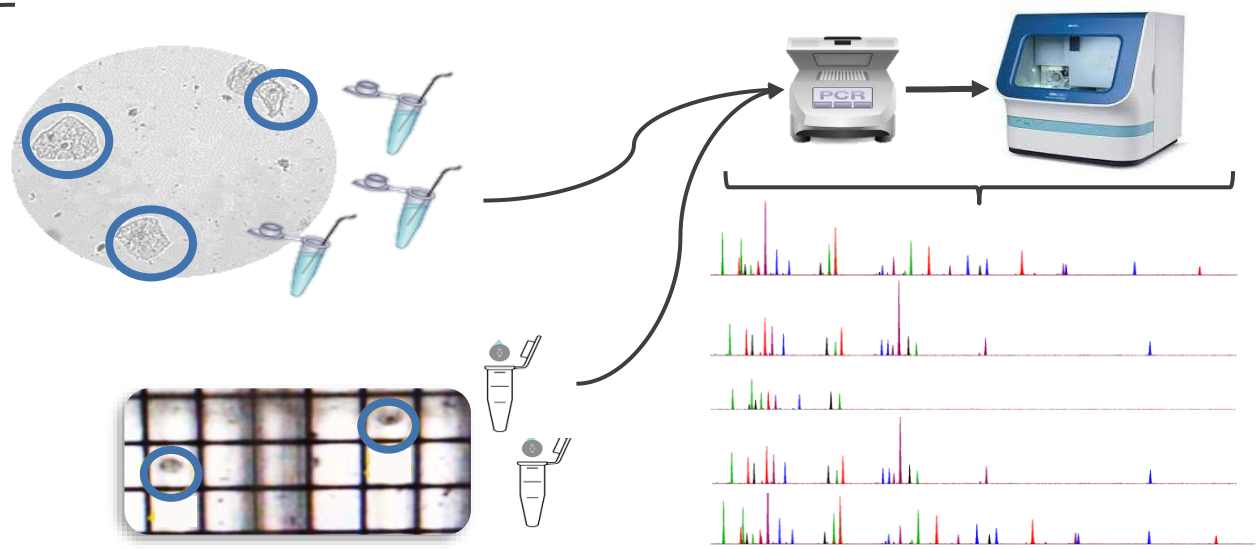
$$\mathrm{LR(C, s)} = \frac{\prod_{l=1}^{L}\prod_{i=1}^{v} P(E_i^l|G^l = s^l)}{\prod_{l=1}^{L}\sum_{g^l}\prod_{i=1}^{v} P(E_i^l|G^l = g^l)P(G^l = g^l)}$$

Sub-source evaluation, i.e., for the entire admixture, *A*, of cells continues by averaging the LR across clusters

$$\mathrm{LR(A, s)} = \frac{1}{n}\sum_{i=1}^{n} \mathrm{LR}(C_i, s)$$

e.g., For suspect, *s*, $\mathrm{LR}(A, s) = \frac{1}{3}[10^{-40} + 10^{-40} + 10^{30}] = 10^{29}$

scEPGs IMPROVES EVALUATIVE AND INVESTIGATIVE OUTCOMES



Isolate epithelial or blood cells from single source samples by way of manual or fluidic treatments. Follow with direct-to-PCR extraction, STR amplification, electrophoresis and fragment analysis. Each scEPG is, therefore, of known genotype allowing performance evaluations

Performance assessment

Sensitivity

Donor genotype
-Included
-Excluded

Robustness

Specificity

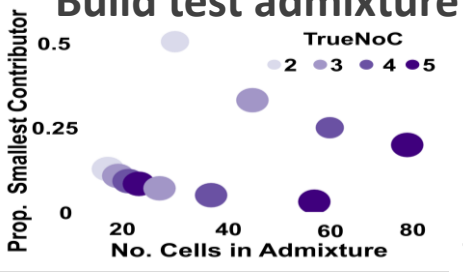
Speed

Fit EESCIt™ models to scEPG calibration dataset

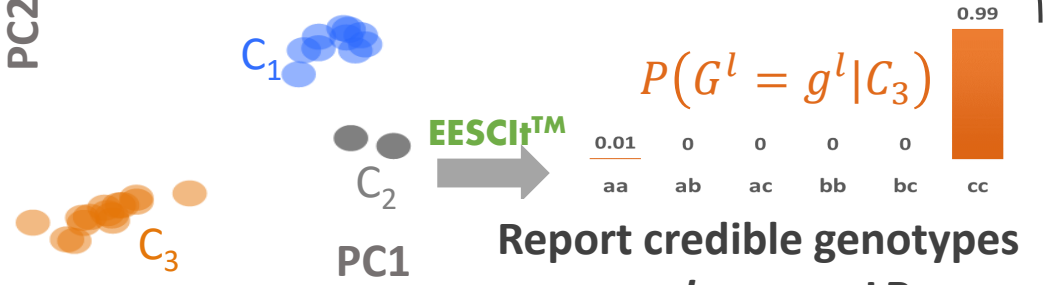
Calibration **N=1420**

Build test admixture dataset

Test **N=643**

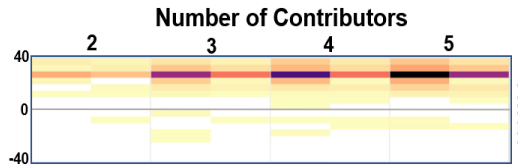


630 mixtures
2 to 5 donors
17 to 75 cells
3.5 to 50% minor



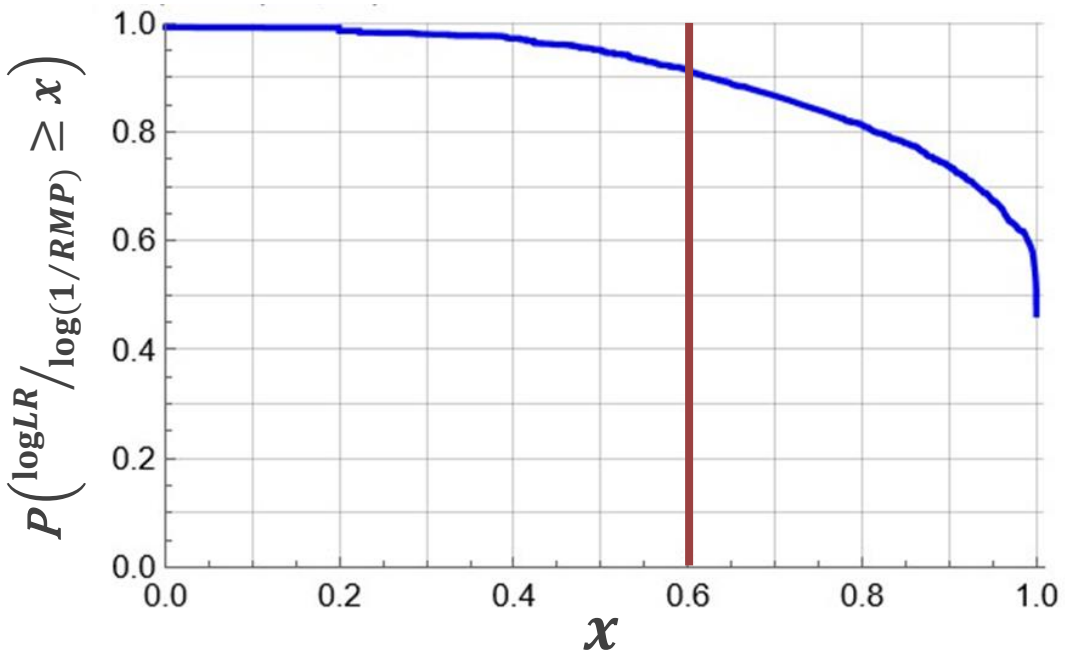
Cluster scEPGs in each test sample by similarity using mclust, **without reference to a suspect**

Report credible genotypes or *sub*-source LRs



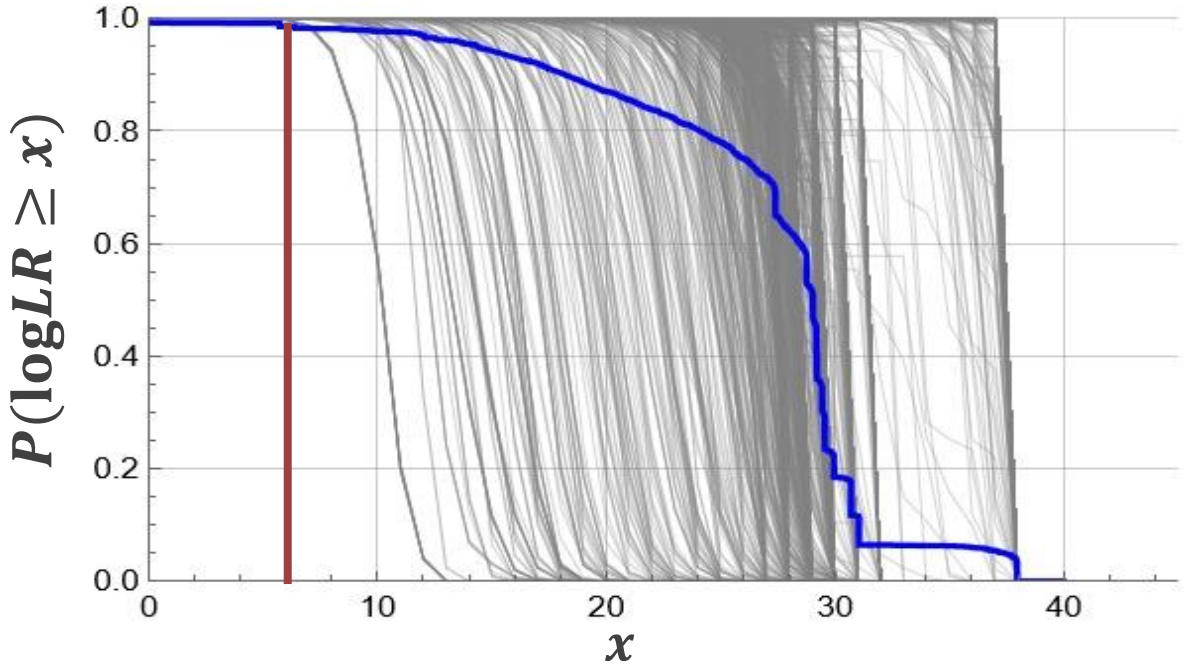
INVESTIGATIVE SINGLE CELL GENETICS: P(LOGLR ≥ X)

Proportion of 2,519 clusters for which $\frac{logLR}{log(1/RMP)} \geq x$



91% of the clusters give at least 60% of the maximal amount of information that could have been returned, which is LR of 10^{18}

For each cluster 10,000 LRs were simulated to get $P(\log LR \geq x)$



$P(\log LR > 10^6) \cong 1$ for all clusters, meaning every cluster was of a searchable state

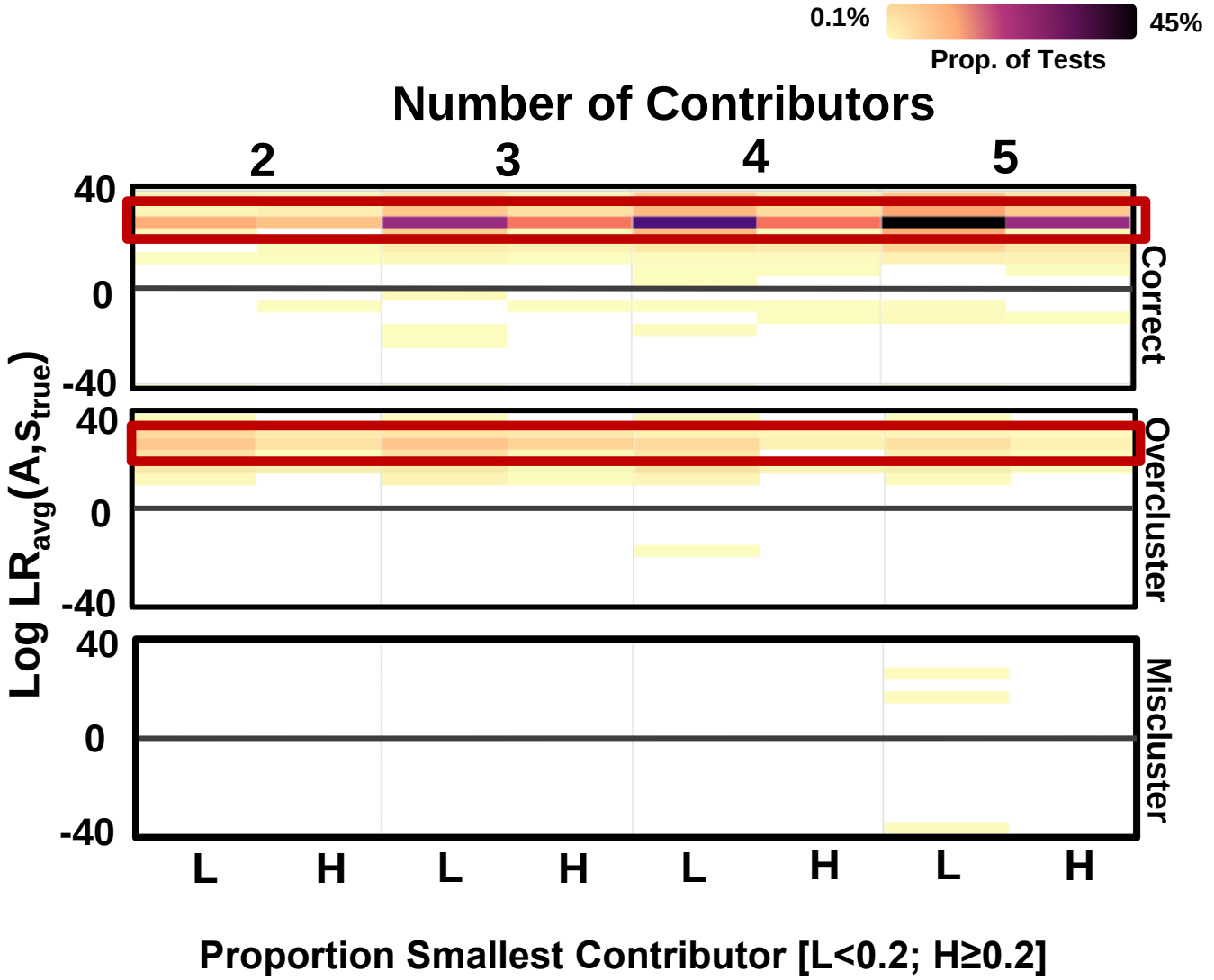
Sub-source (suspect) evaluations are well resolved across mixture complexities

Likelihood ratios for all cells in the admixture by EESCIt™,

$$\mathrm{LR}(\mathrm{A}, s) = \frac{1}{n}\sum_{i=1}^{n} \mathrm{LR}(C_i, s)$$

Out of 2,310 suspect-mixture comparisons all but 21 gave LR>1

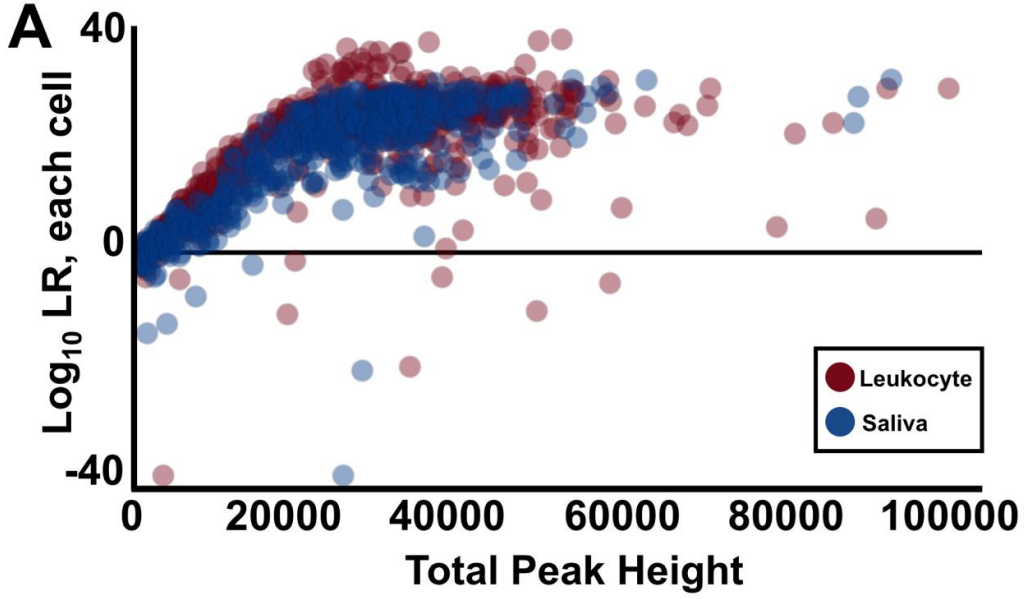
High density of $\log_{avg}$LRs at [25-30) across TrueNOC shows robustness across all complexities



EVALUATIONS ARE NOT INFLUENCED BY FEATURES OF THE MIXTURE, EVEN AT THE EXTREMES

The logLR of one scEPG can be just as informative as a single-source high-template EPG

Slope in linear region (0.001 [$\frac{logLR}{RFU}$]), shows that for every 1000 RFU — ca. 2 alleles — logLR will, on average, increase by 1

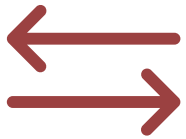


The logLR of true donor of a twelve person, 643 cell mixture is approx. equal to 1/RMP (2 hours on a laptop)

Person	Log LR (based on genotype)	Single cell log LR
1	30.59	29.88
2	29.09	28.39
3	29.58	28.69
4	29.55	28.79
5	29.41	26.59
6	31.04	30.29
7	29.00	28.29
8	29.11	28.39
9	27.37	26.69
10	28.70	27.99
11	29.78	29.09
12	38.44	37.19

In Summary, single cell data:

supports efficient database searches and investigations for all components in all mixtures



ability to discriminate hypotheses is independent of the qualities of the mixture
- i.e., LRs do not decrease with NoC or contributor proportion

require few computational resources

can address other pertinent questions like *source*
- i.e., DNA originates from the blood?

Future work will address laboratory treatments and extracellular DNA

FUNDERS AND COLLABORATORS

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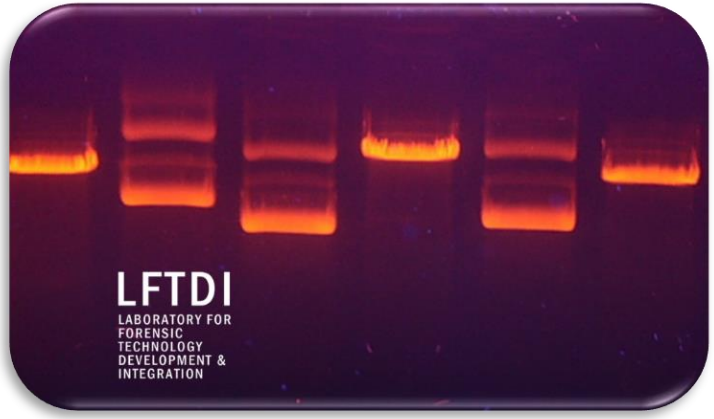

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