DIGITAL ISOLATION WITH SINGLE-CELL PRECISION: ASSESSMENT OF GENOTYPING ASSAYS PERFORMANCE ON LOW-COUNT, PURE CELL POPULATIONS FOR BIOLOGICAL MIXTURE RESOLUTION

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Background

Genomic characterization of minute biological samples can be achieved with the latest technologies and genotyping assays. Today, the biggest challenge in forensic genetics comes from biological mixtures, as DNA profiling produces in most cases a mixed genetic profile. DEPArray™ technology affords digital separation of single-cells, and has been reported to be enabling the resolution of forensic mixtures, by precise separation of pure cell populations from different biological fluids.
Using standard genotyping and next generation sequencing methods, here we characterize the genotyping profile completeness and concordance of 100%-pure digitally isolated cell populations.

Materials and Methods

Aliquots of blood, saliva and semen obtained from multiple donors, were collected on swabs and stored at +4°C (mean days=16). Cells were then reconstituted in a cell suspension, fixed and stained with cell type-specific fluorescent antibodies. Precise numbers of cells (lymphocytes, epithelial or sperm cells) were digitally isolated using the DEPArray™ system and lysed using a single tube method.
Genotyping with AmpFlSTR®NGM SElect Kit was performed from pools of 10 (n=8) and 20 (n=27) cells split in two aliquots to obtain a replicate as required in forensic genetics, along with equivalent quantity of gDNA obtained by serial dilution (66pg for diploid, n=12 or 33pg for haploid cells, n=4) as comparison.
Additionally, single sperm cells (n=8) isolated with the DEParray™ system , were genotyped with PowerPlex Fusion Kit 6C and pools (n=3) of 10 cells were analyzed with MiSeq FGx NGS platform.

Results

Genotyping profile completeness and concordance with respect to gDNA showed, respectively:
10 cells: 92%, 100%
20 cells splits: 89%, 99%
Single cells: 53%, 99,5%
66pg: 98%, 98% and for 33pg: 73%, 97%.
The MiSeq FGx analysis of 10 cell pools was able to detect ≥ 85% of gDNA Short Tandem repeats (STRs) at 35x coverage. All SNP alleles called had 100% concordance in gDNA, regardless of coverage, sensitivity ranged from 90% to 81%, at 15x and 35x coverage, respectively.

Highlights

We report, using standard forensic methods, the systematic performance assessment of genetic analysis from low-count pure cell populations isolated with DEPArray™. We demonstrate that this workflow allows one to obtain pure profiles, highly complete (~90%) down to 10 cells. With single sperm cells, profile completeness decreases. However, since drop-outs are random, profile completeness may be restored, in-silico, using multiple single-cell data.