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 fluids1 (Fig.1).

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 AmpFISTR®/NGM SSelect 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 (Fig.2).

STR Analysis
Sorted 10 cell pools allow the correct detection of:
- ≥ 85% of gDNA Short Tandem repeats (STRs) at 35x coverage;
- 35x coverage represents the best trade-off between sensitivity (sn) and positive predictive value (ppv), both reaching 85% (Fig.3).

SNP Analysis
The whole set of SNP alleles detected from the sorted pools of 10 cells shows:
- 100% concordance (n=255 total loci – mean 85/sample) with the corresponding genomic DNA;
- sensitivity ranged from 90% (at 15x coverage) to 81% (at 35x coverage) (Fig.4).

Genetic Characterization Results
Genotyping mean profile completeness and concordance with respect to gDNA showed (Fig.5):
- ≥ 66g gDNA dilution (10 diploid cell-equivalent):
  - 92% completeness 98% concordance
- 100% concordance

- ≥ 33g gDNA dilution (10 haploid cell-equivalent):
  - 89% completeness 73% concordance
- 99% concordance

Conclusions
We report, using standard forensic methods, the systematic performance assessment of genetic analysis from low-count pure cell populations digitally 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.

1 Fontana F et al, Poster ISFG 2015 “DEPArray™ Digital sorting of biological mixtures achieves isolation of 100% pure cell populations for clear-cut genetic analysis”