DEPArray™ digital sorter resolve any interference of contaminating stromal cells after FFPE macrodissection

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Introduction
We have previously shown reliability in isolating pure populations of cells from complex tissues using the DEPArray™. Macrodissection, the gross manual dissection of FFPE samples guided by a histologic section, is used to isolate areas of interest within a specimen for optimal downstream analysis. Though better defined than a non-dissected tissue sample, the actual percentage of targeted cells obtained by macrodissection is dependent on the degree to which such architecture and admixed cellular components allow. Here we demonstrate preliminary results showing the degree of stromal cell contamination following macrodissection and the concurrent recovery of 100% pure tumor cell populations using the DEPArray™.

Methods
FFPE macrodissected sections (n=9) originating from prostate; breast, pancreatic and lung primary tumors were disaggregated down to single cells suspensions and stained with Keratin, Vimentin and DAPI. The analysis with DEPArray™ technology, an image-based cell sorting platform with single cell resolution, identified the multiple cell sub-populations, considering both cells staining and ploidy. Pools of pure sorted cells (mean=174, range: from 37 to 280), along with an aliquot of the corresponding unsorted cells and the corresponding DNA extracted from FFPE macrodissected tissue, were used as input for the AmpliSeq™ Cancer Hotspot Panel v2, sequenced with Ion Torrent™ PGM and data analyzed with Torrent Suite v4.2 with a custom low-stringency set of parameters.

Results
DEPArray™ analysis allowed identification of well separated cell populations, including tumor (Vim+/Ker- ) and stromal (Vim-/Ker-) cells, in 7 out of 9 samples. We were thus able to estimate the % of tumor cells (mean 23% range 4-54%), demonstrating an unexpected low frequency of tumor cells remaining following macrodissection. In fact, 61% (21 to 93%) of the cells analyzed were of stromal origin. For subsequent NGS analysis, groups of pure cells (mean 174 cells, range 37 to 280) for each population were recovered on the basis of DNA index that allow to estimate the cells DNA content. Among the tumor cells isolated from the lung cancer specimen, we observed 3 non-synonymous somatic variants in TP53 and APC gene (p.G245C, p.D245V and p.E303K). The variant frequency of APC:p.E303K suggests the presence of a population in stromal sample. This situation can not be highlighted in sorted population where the variant frequency looks like background noise. Moreover, pure tumor population allows to describe 3 LOH events on R81, APC and CSF1R genes, 2 copy-number gains of the mutant allele on IDH1 and 1 2 copy-number gains of the wild-type allele on KIT. Though in unsorted populations (prior to DEPArray™) these variants are detectable, with DEPArray™ sorting it is possible to describe variants with a precision level such as to determine the exact mutational event.

Conclusions
DEPArray™ technology can be used to isolate pure tumor cells from heterogeneous FFPE samples that have undergone macrodissection. Thus, the DEPArray™ platform brings digital precision to detection, quantification and recovery of pure target cells for subsequent downstream molecular analysis that can improve cancer diagnosis and treatment decisions.

References
[Polzer 2014] Molecular profile of single circulating tumor cells with diagnostic intention, Embo Molecular Medicine, September 2014
[Conver 2011] High-Resolution Multiparameter DNA Flow Cytometry for the Detection and Sorting of Tumor and Stromal Subpopulations from Paraffin-Embedded Tissues

Fig. 1 Comparison of conventional and proposed method. Besides getting pure tumor DNA, the method applies also to getting pure stromal cells. This proves useful to tell apart germ-line from somatic mutation.

Fig. 2 Scatter plot of disaggregated FFPE microdissection (2A), with ploidy analysis based on integral intensity in DAPI (2B), showing a different peak for the Tumor Cells relative to internal control of Stromal Cells. In fact, the calculated DNA index (DI) is about 1.68 suggesting the presence of hyperdiploid cell lines in the Tumor population.

Fig. 3 Summary of digital quantification of V+K- and V+K+ events in the 9 macrodissection samples analyzed. DEPArray™ digital analysis allow to estimate the number of events Vim+K+Vim+, Vmin+K+Vim, Vmin+K+Vim and VminK- in, in this way it’s possible evaluate the percent of Stromal and Tumor amount. In particular: Tumor% = V+K+(V+K+ + V+K-), Stromal% = 1 - Tumor%.

Fig. 4 Summary of variant frequencies in unsorted (unsr), Tumor Cells (V+Hyperdiploid), Stromal Cells (V+ Diploid) populations in a lung-cancer patient. GVC (Genetic Variant Class) column describes different mutational events: somatic mutations (black), LOH (red), CNV (green) and germline variants (light blue). Effect column shows the variant effect on protein: “M” (missense), “S” (splice site), “U” (UTR) and “blank” (synonymous or intron variants). Annotations with COSMIC (C) or dbSNP (s) are shown on annotation column.

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