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Enrichment of circulating tumor DNA from cell-free DNA of hematopoietic origin.

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Authors:
Jason Terrell, Priscilla Van den Ackerveken, Alison Lobbens, Mark Eccleston, Clotilde Hannart, Jean-Philippe Deglasse, Jean-Valery Turatsinze, Marielle Herzog, Jake Micallef; University of Texas at Austin Dell Medical School, Austin, TX; Volition, Namur, Belgium; Belgian Volition Sprl, Isnes, Belgium; Belgian Volition, Namur, Belgium; Voltion, Isnes, Belgium; Belgian Volition, Isnes, Belgium; Volition RX, Isnes, Belgium; Volition, Isnes, Belgium

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Background:
In liquid biopsy, circulating tumor DNA (ctDNA) is more fragmented than background cell free DNA, peaking at 147bp (equivalent to a mono-nucleosome) instead of 165bp (nucleosome with an additional 20bp of linker DNA). Isolation of these shorter cell free DNA fragments from longer, extracted cell-free DNA improves detection of ctDNA as demonstrated by enrichment of tumor specific mutations. Nuclease protection of the additional 20bp of linker DNA, conferred by bound linker proteins such as Histone 1, would account for the 165bp peak in host background DNA. We hypothesised that extracting intact nucleosomes with linker DNA using a novel, H1 antigen based, immunocapture approach would enrich the ctDNA fraction in the remaining nucleosomes.

Methods:
We expressed H1.0 protein in E. coli and following extraction, purification and chemically immobilised it to tosyl-activated magnetic beads. The beads were first used to immunodeplete mono-nucleosomes from HeLa cell digests and the level of immunocaptured nucleosomes was determined by immunoassay targeting intact nucleosomes. The level of nucleosome levels determined before and after depletion was further determined by ELISA targeting H3.1 containing intact nucleosomes. DNA

was extracted from the H1 immunocaptured “long” nucleosomes and size profiles compared with the remaining nucleosomes in the supernatant by BioAnalyzer. Then, the method was applied to clinical plasma samples and the size distribution of NGS Libraries (Illumina system) prepared from five colorectal cancer and three healthy samples, their immune depleted supernatants and the immunocaptured nucleosomes were then compared. Enrichment of specific genomic regions was also evaluated.

Results:

We observed relative enrichment of nucleosomes with short DNA in supernatants following H1 immuno-depletion of the cancer samples as evidenced by a change in size distribution by Bioanalyzer and NGS-sequencing. We also observed potential enrichment of TSS is the H1 immunocaptured nucleosomes consistent with linker DNA positioning of TF binding sites.

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Conclusions:

Histone 1.0 has the highest affinity of H1 mammalian isoforms and successfully immunodepleted plasma samples containing cell free circulating nucleosomes with DNA longer that 147bp. Immobilized H1.0 effectively formed a pseudo-chromatosome by binding to free linker DNA or displacing endogenous H1 and other linker associated proteins. H1 antigen based immuno-depletion offers a simple way to enrich tumour derived nucleosomes and thus cell free DNA.