

Whole Genome Sequencing (WGS) in Cancer -- Why Fresh Frozen tissue provides the patient with improved genomic results{1ITALIC}

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Formalin fixation is universally used by histopathology laboratories to preserve tissue for subsequent diagnostic analysis such as haematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) tests. It is known that formalin preserves tissue by forming protein-protein cross links which prevents DNases, RNases and proteinases digesting the tissues. As a result any DNA extracted from such tissue requires harsh pre-treatment prior to extraction to reverse the cross linking, resulting in fragmented and degraded DNA. This is sub-optimal for downstream genomic testing.

Early results from the 100,000 Genomes project for cancer demonstrated marked differences in the quality of data obtained from DNA extracted from fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tissue taken from the same prostate tumour. FFPE samples gave sub-optimal results including AT and CG dropout and unevenness of coverage. As a result national sample handling guidance was introduced to prioritise the implementation of formalin free pathways. Other guidance included upstream handling of fresh tissue including the use of refrigeration to preserve tissue as well as vacuum packing. However it was recognised that in some circumstances fresh tissue was not an option therefore an optimized DNA extraction from FFPE tissue protocol was provided which included recommendations such as controlled processing schedules and fixation. Protocols for DNA extraction were recommended including time and temperature for reversing cross links. Some improvement in the DNA quality was observed from routine FFPE processed samples but there was still inflation in likely artefactual somatic calls. The WGS results from fresh tissue clearly demonstrated the superior quality of WGS results with the lack of background noise. This benefits the patients by minimising inaccurate calling of variants, copy number and structural variants providing a more accurate, reliable WGS service which can aid patient care.