NEW TECHNOLOGIES FOR FFPE SAMPLES

Gene Expression profiles from FFPE samples with improved RNA decrosslinking technology

A case study: Molecular profiling of breast cancer from formalin-fixed, archival material

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Archival FFPE samples have been collected over decades in routine clinical procedures and they harbour a great wealth of information, including mRNA expression profiles. A novel demethylation/decrosslinking protocol for RNA recovery from archival FFPE material was developed. The resulting FFPE RNA quality is superior to RNA obtained with other commercial FFPE RNA isolation kits. Larger RNAs can be recovered, and RT-qPCR data demonstrate less variability and lower Ct values. This FFPE RNA is suitable for differential gene expression measurement by qPCR, with high concordance with parallel RNA samples from fresh-frozen tissues was observed [1, 2].

Prognosis of breast cancer is determined by clinicopathological and molecular factors. "Molecular scores" were developed and validated that reflect the hormone status (ER, PGR, HER2 scores) and the proliferation status (PR0 score) of breast cancer cells. The scores can be combined to an overall RISK score. Molecular scores are independent prognostic parameters, they were validated in postmenopausal patients with estrogen receptor positive breast cancer. Multivariate analysis revealed that PR0 and RISK scores outperform conventional parameters (histological grading and Ki-67 labeling index). Molecular scores are based on routine pathological material, testing can be implemented easily into routine diagnosis [3].


Molecular Scores

**Comparison of Fresh-Frozen vs FFPE tissues**

Combination of several genes into Molecular Scores

Relative expression with 3 reference genes (GUSB, RPLP0, UBB)

PRO_10 with 10 genes for proliferation

PGR_5 with 5 genes for progesterone response

HER2_2 with 2 genes for Her2 response

**Risk assessment**

by expression profiling with FFPE RNA

Histological grading is an important factor in estimating the risk of recurrence. Our findings show that PRO_10 scores (analysis of 10 genes) were prognostic for DFS in the entire patient population and histological grade II tumors could be further classified into low and high risk of recurrence or into 'grade 1 like' and 'grade 3 like' tumors.