

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 demodification/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, 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 (PRO 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 PRO 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].

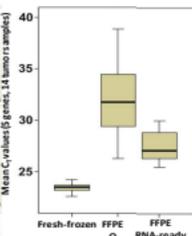
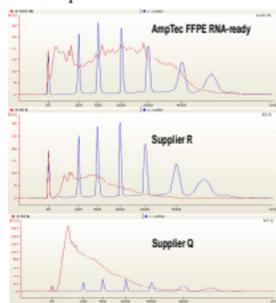
[1] Oberli et al. (2008) Expression profiling with RNA from formalin-fixed, paraffin-embedded material. BMC Medical Genomics 2008, 1:9. [2] Schobesberger et al. (2008) Gene expression variation between distinct areas of breast cancer measured from paraffin-embedded tissue cores. BMC Cancer 2008, 8:343.

[3] Antonov et al. (2011) Molecular risk assessment of BIG 1-98 participants by expression profiling using RNA from archival tissue. BMC Cancer 2010, 10:37

Isolation of FFPE RNA

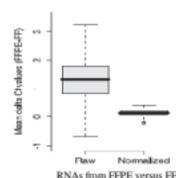
Size distribution of FFPE RNA
Comparison of commercial kits

Variability in the qPCR performance of FFPE RNA



Shown are the means of raw Ct values of five reference genes (GAPDH, GUSB, RPLP0, TFRC, UBB) with 14 samples with intact RNA (FF) and RNA from matched FFPE material.

Effect of normalization



The mean increase of raw Ct values was 1.3 for the complete panel of 34 genes with 82 RNA samples. Considering the low variability in the reference gene panel, normalization effectively compensated for this shift of Ct values.

Molecular Scores Comparison of Fresh-Frozen vs FFPE tissues

Combination of several genes into Molecular Scores

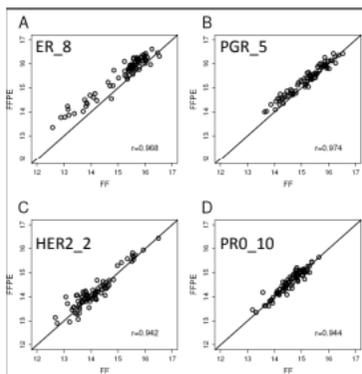
Relative expression with 3 reference genes (GUSB, RPLP0, UBB)

PRO_10 with 10 genes for proliferation

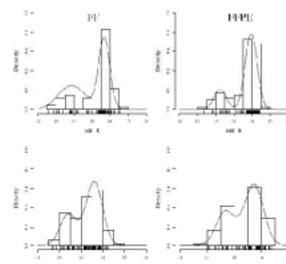
ER_8 with 8 genes for estrogen response

PGR_5 with 5 genes for progesterone response

HER2_2 with 2 genes for Her2 response



Comparison of scores with intact and FFPE RNA, determined for 82 patients. Scatter plots are shown for scores for each tumor, derived from FF (fresh-frozen) and FFPE tissues. Pearson correlations are indicated.



Histograms of ER and PGR scores with fitted mixtures of Gaussian distributions. Results of 82 matched patient samples are shown

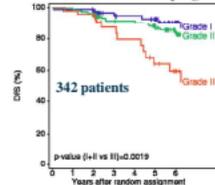
Risk assessment

by expression profiling with FFPE RNA

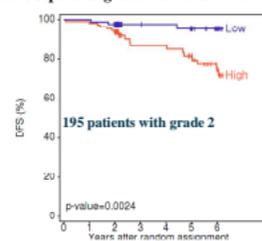
Histological grading is an important factor in estimating the risk of recurrence.

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.

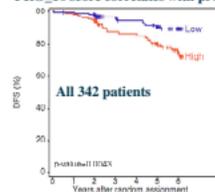
Grade correlates with prognosis



PRO_10 score separates grade 2 tumors in Low and High risk



PRO_10 score correlates with prognosis



NEW
FFPE Quick RNAready kit
Adapted for FUJIFILM QuickGene
No spin columns
No centrifugation
No multiple flow-through discarding steps