# NEW TECHNOLOGIES FOR FFPE SAMPLES: Improved RNA Isolation and novel cDNA priming for qPCR and for universal mRNA amplification

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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. Although the RNA is severely degraded and poses additional challenges due to inter- and intramolecular cross-linking and base modifications. Mining of gene expression data is still possible and extracted information about differential gene expression is comparable to data from Fresh-Frozen samples, even at a quantitative level. Our novel FFPE RNAready kits provide a novel procedure for RNA liberation and demodification, resulting in highly reproducible data in RT-qPCR studies [1] and derived gene expression profiles of cancer samples [2,3] are useful for molecular risk assessments [3].

**TR priming/amplification of FFPE RNAs** (incorporated in ExpressArt TR mRNA amplification kits) combines advantages of oligo-dT and random priming: **Like oligo-dT**: preferential priming near the 3'end **Like oligo-dT**: selection against rRNAs **Like random**: mRNA fragments without poly(A). **Superior to random**: 3'-preference for full-length "cDNA-fragments", no further "subfragmentation" **Like random:** internal priming for **whole transcript** coverage. **Superior to random**: preferential starts at pause sites

**Applications:** ◊ qPCR analyses ◊ mRNA amplification and microarray analyses ◊ **Unique advantages for Exon Arrays**

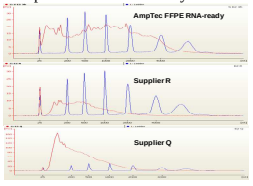
[1] Oberli et al. (2008) Expression profiling with RNA from formalin-fixed, paraffin-embedded material. BMC Medical Genomics 2008, 1:9. / [2] Schobesberger et al. (2008) Gene expression variation between distinct areas of breast cancer measured from paraffin-embedded tissue cores. BMC Cancer 2008, 8:343.

[3] Antonov et al. (201) 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 commercially available kits




### Experimental Flow Chart

**Place up to 5 FFPE sections in a reaction tube**

**Deparaffinisation**
Time required: 1 h

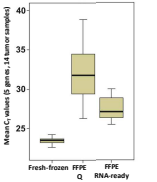
**Lysis**
Time required: 3 h

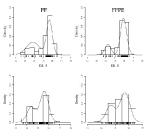
**Demodification**
Time required: 0.5 h

**Spin column purification, including DNA digestion**
Time required: Approximately 0.75 h

**Purified RNA** in ~50 µl eluate
Expected yield: up to 2 µg per slide
Total time required: **Approximately 5.5 h**

### Variability in the qPCR performance of FFPE RNA

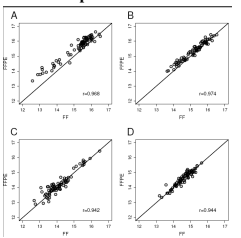


Histograms of ER and PGR scores with fitted mixtures of Gaussian distributions. Fresh-Frozen and FFPE samples result in very similar biphasic distributions. Results of 82 matched samples are shown

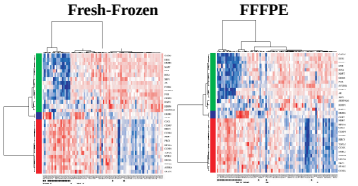
## FFPE RNA as RT-qPCR template

### Comparison of Molecular Scores derived from Fresh-Frozen vs FFPE tissues




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.

**A:** ER_8, with 8 genes representing estrog receptor function, **B:** PGR_5 for progesterc receptor, **C:** HER2_2 for Her2 and **D:** PRO_ with 10 genes for proliferation status. Pears correlations are indicated.




Unsupervised hierarchical clustering of data from FF- and FFPE-derived RNA. Shown are heat maps based on normalized expression from RNA of FF and FFPE tissues. Proliferation (red box), Her2 (blue box) and ER or PgR related genes (green box) are indicated.
The hormone receptor status of each tumor was also assessed by IHC. ER negative (black circles) and Her2 positive tumors (open circles) are indicated.

**Ziplex Automated Workstation**
Samples and reagents are pumped through probe array channels in microplate wells for efficient & **fast** mixing/hybridisation.
Numerical data are available within 3 hours. Automatic Quality Control metrics ensure the output of only high quality data: failure samples with too low signal/background ratios are flagged.

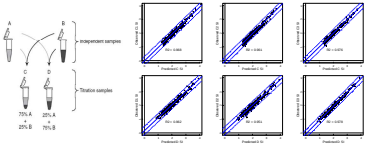
## FFPE RNA and Microarrays

## Conclusions

- The results demonstrate the feasibility of amplifying and quantifying sequences at any position within transcripts in degraded mRNA from FFPE samples.
- Results accurately reflect transcript abundance in total RNA samples.
- Expression differences of two-fold or less may be analyzed with tens or hundreds of probes for translational research and clinical assay development on the Ziplex Automated Workstation.

### "MAQC"- like Sample Titration with FFPE RNAs

Analogous to the MAQC study (Nature Biotechnol. 2006; 24(9):1151-61), FFPE RNAs from breast cancer (sample A) and colorectal cancer (sample B) were mixed to create the C and D titrations. Aliquots of the 4 samples were amplified with the ExpressArt TR kit and hybridised on the Ziplex Workstation. There was good agreement ($R^2 > 0.95$) between the observed and predicted results for C and D samples, calculated from the A and B expression values. Median CV's of the A, B, C and D samples were 19.9, 18.7, 24.3 and 19.6%, respectively.




Observed normalized signal intensities for C and D samples vs. predicted intensities that were calculated from A and B sample data.
Similar performance was observed for 3'-biased probes and for probes several hundred bases away from the 3'end of the mRNA, confirming the lack of 3' bias in the TR amplified RNAs.