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RNA isolation and gene expression profiling of formalin fixed and paraffin embedded tissue

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**GERMAN
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1. Using FFPE samples for Microarray analysis
2. RNA quality from FFPE tissue samples
3. Deparaffinization and RNA isolation
4. RNA amplification, Hybridization and Analysis
5. References

Why FFPE?

- Millions of biopsies are currently stored in hospitals/laboratories but the majority is in paraffin blocks and formalin-fixed
- The use of archived samples from retrospective clinical trials with well-documented clinical follow-up will accelerate the discovery of potentially useful clinical gene expression signatures

•FFPE expression studies are strongly dependent on sample preparation (e.g. fixative, fixation time...).

1. Type of fixative

- 4% phosphate-buffered formaldehyde (formalin)
(„chemical networking“ is reversible, in contrary to e.g. glutaraldehyde)
- „HOPE“ ...

2. Changes in transcriptome may occur during formalin fixation

Penetrance of formalin (temp. dependent):
± 0.5mm/h at RT

3. Duration of formalin fixation may affect quality of mRNA

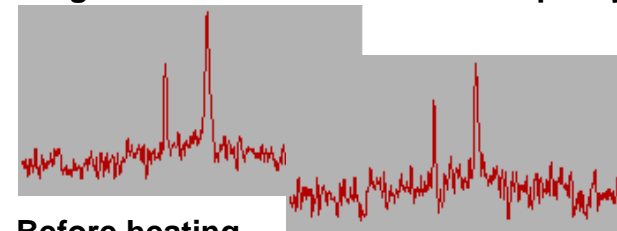


•Formalin fixed, paraffin-embedded samples, by nature, are partially degraded and contain low amount of total RNA

1. Mechanical shearing
2. Degradation
3. Chemical modification
Bridging dimers of amino bases
Methylol additions (N-CH₂OH)

Reversible through heating at 70°C

Heating does not influence the RNA quality



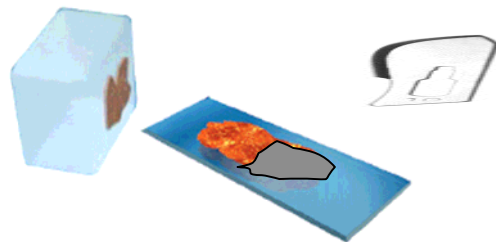
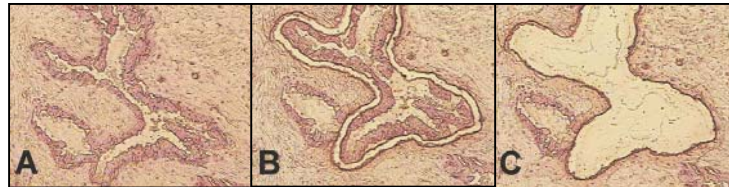
Before heating

After heating (1h, 70°C)

Deparaffinization, RNA isolation and measurement

Deparaffinization:

- Xylene (Microdissection / Scrape)
- Proteinase K (Tissue block / Tissue slice)

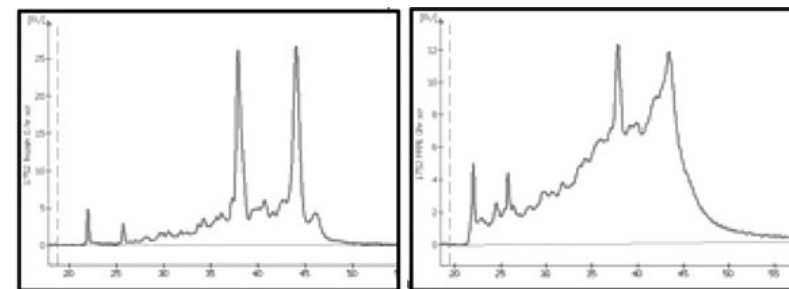


RNA isolation:

Because of the tiny amounts of RNA that are vulnerable to degradation tissue should be processed as fast as possible with a well established protocol

RNA measurement:

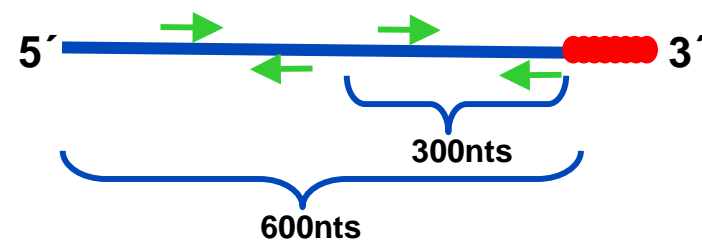
Agilent



Frozen

FFPE

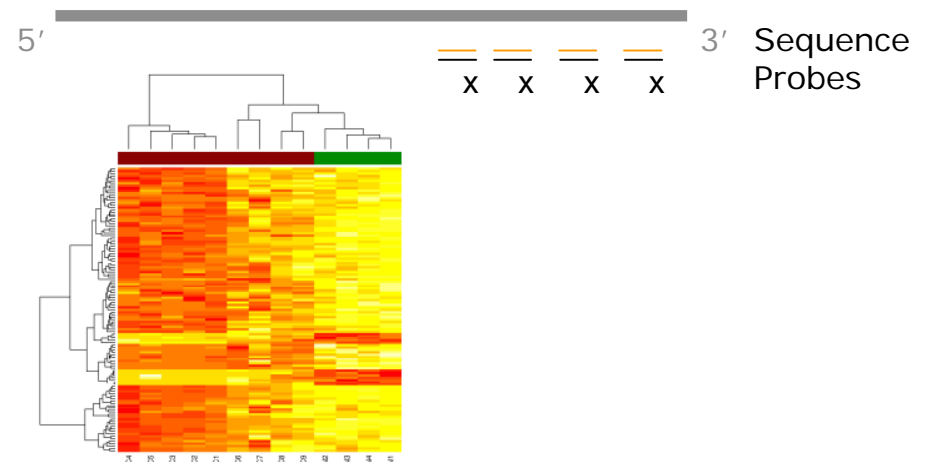
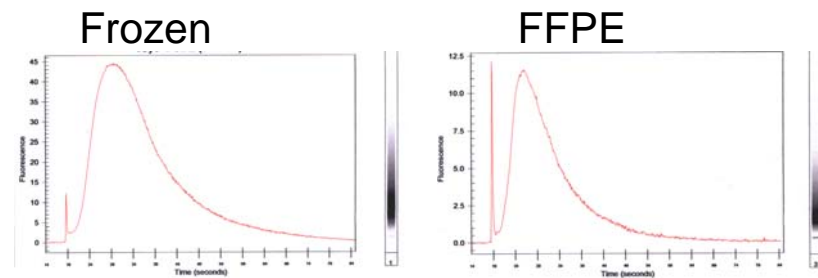
qRT-PCR on a housekeeping gene



Ratio 600 / 300

RNA amplification, Hybridization and Analysis

- RNA amplification efficiency is dependent on RNA quality and amount
- Poly-T amplification shortens RNA with each amplification step
- Average length of amplified RNA might influence the later analysis
- Probes of Microarray for FFPE should be concentrated to the 3'-end (X3P Affymetrix)
- Single probe analysis is important (avoid Custom CDF)



Isolation and amplification

- Paradise (Arcturus)
- RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Ambion)
- High Pure FFPE RNA Micro Kit (Roche)
- ArrayGrade™ FFPE RNA Isolation Kit (SuperArray Bioscience Corporation)
- PureLink™ FFPE RNA Isolation Kit (Invitrogen)
- FormaPure System (Agencourt)
- Message Amp II (Ambion)
- Paradise (Arcturus) combined with IVT (Roche) or Megascript (Ambion)
- WT-Ovation FFPE System (NuGEN)

Microarray Platforms

- Affymetrix
- Agilent
- Applied Biosystems
- GE Healthcare

RNA quality and quantity

- Agilent Bioanalyser (Agilent Technologies)
- Nanodrop (NanoDrop)
- RiboGreen RNA Quantitation (Molecular Probes)

Literature

- RNA expression analysis of formalin-fixed paraffin-embedded tumors, [Penland SK](#) et al., Lab Invest. 2007 Apr;87(4):383-91
- Successful application of microarray technology to microdissected formalin-fixed, paraffin-embedded tissue, [Coudry RA](#) et al., J Mol Diagn. 2007 Feb;9(1):70-9
- The HOPE-technique permits Northern blot and microarray analyses in paraffin-embedded tissues, [Goldmann T](#) et al., Pathol Res Pract. 2004;200(7-8):511-5
- Preliminary comparison of quantity, quality, and microarray performance of RNA extracted from formalin-fixed, paraffin-embedded, and unfixed frozen tissue samples, [Scicchitano MS](#) et al., J Histochem Cytochem. 2006 Nov;54(11):1229-37
- Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples, [Masuda N](#) et al., Nucleic Acids Res. 1999 Nov 15;27(22):4436-43