

Robust molecular profiling from RNA derived of archival tissue

Janine Antonov
Molecular Biology Lab
Department of Clinical Research
University of Bern
Switzerland

Why are archival samples interesting?

- Routine cancer diagnosis relies on descriptive histopathological data
- "Gene Signatures" are very powerful for further discrimination of clinically relevant subtypes of cancer

Clinical relevant questions

- good \leftrightarrow bad prognosis
- therapeutic response

Optimal samples:

- Clinical data available
- Long observation periods
- Prospectively randomized trials

**Archival Material:
formalin-fixed-
paraffin-embedded
(FFPE)**

A method to quantify gene expression in archival tissue makes many valuable tumor samples available for molecular analysis

Agenda

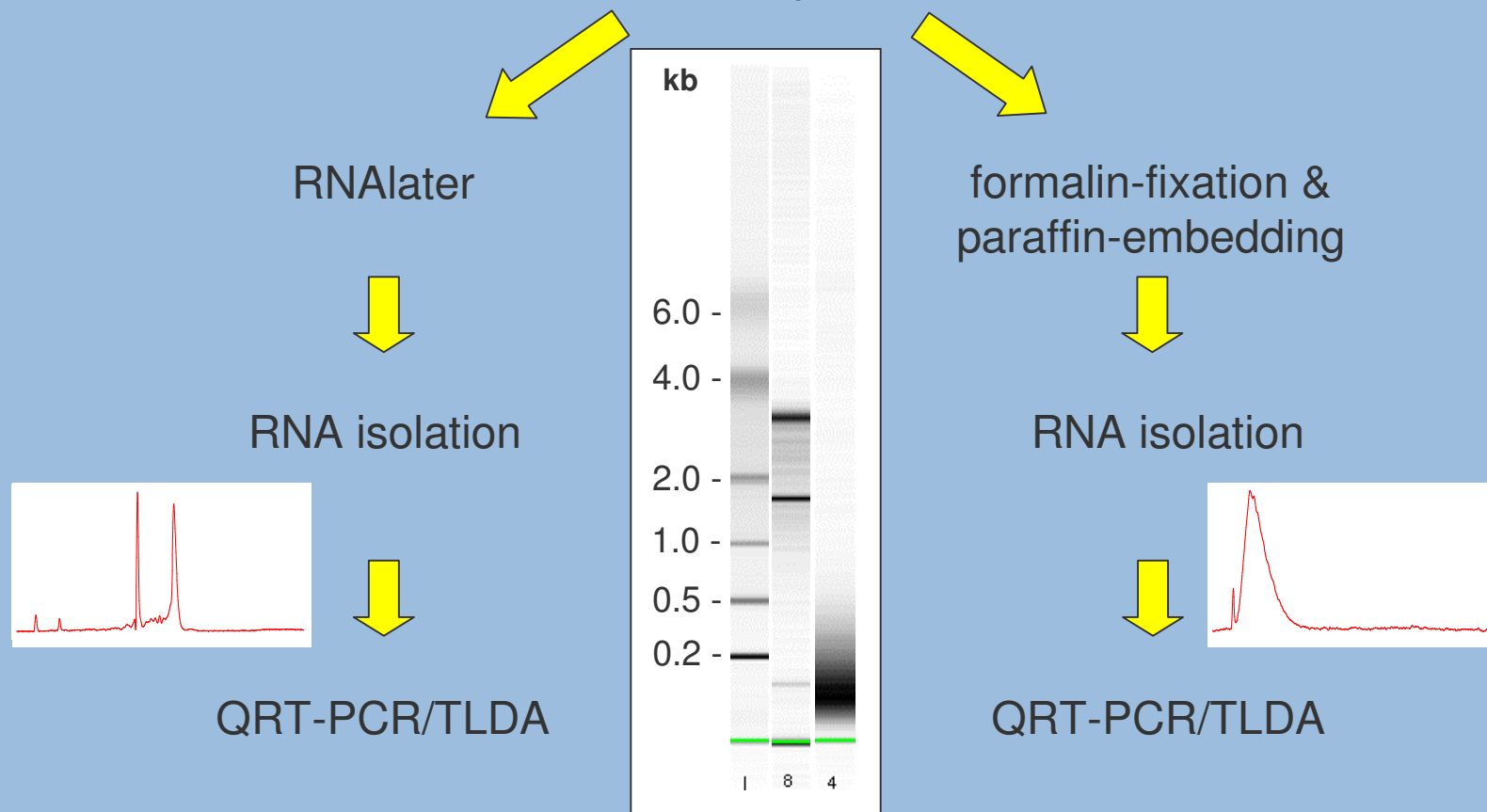
- > Introduction
 - Gene expression measurements in archival samples with **standard protocols**

- > Optimization
 - RNA extraction
 - cDNA synthesis
 - qPCR parameters

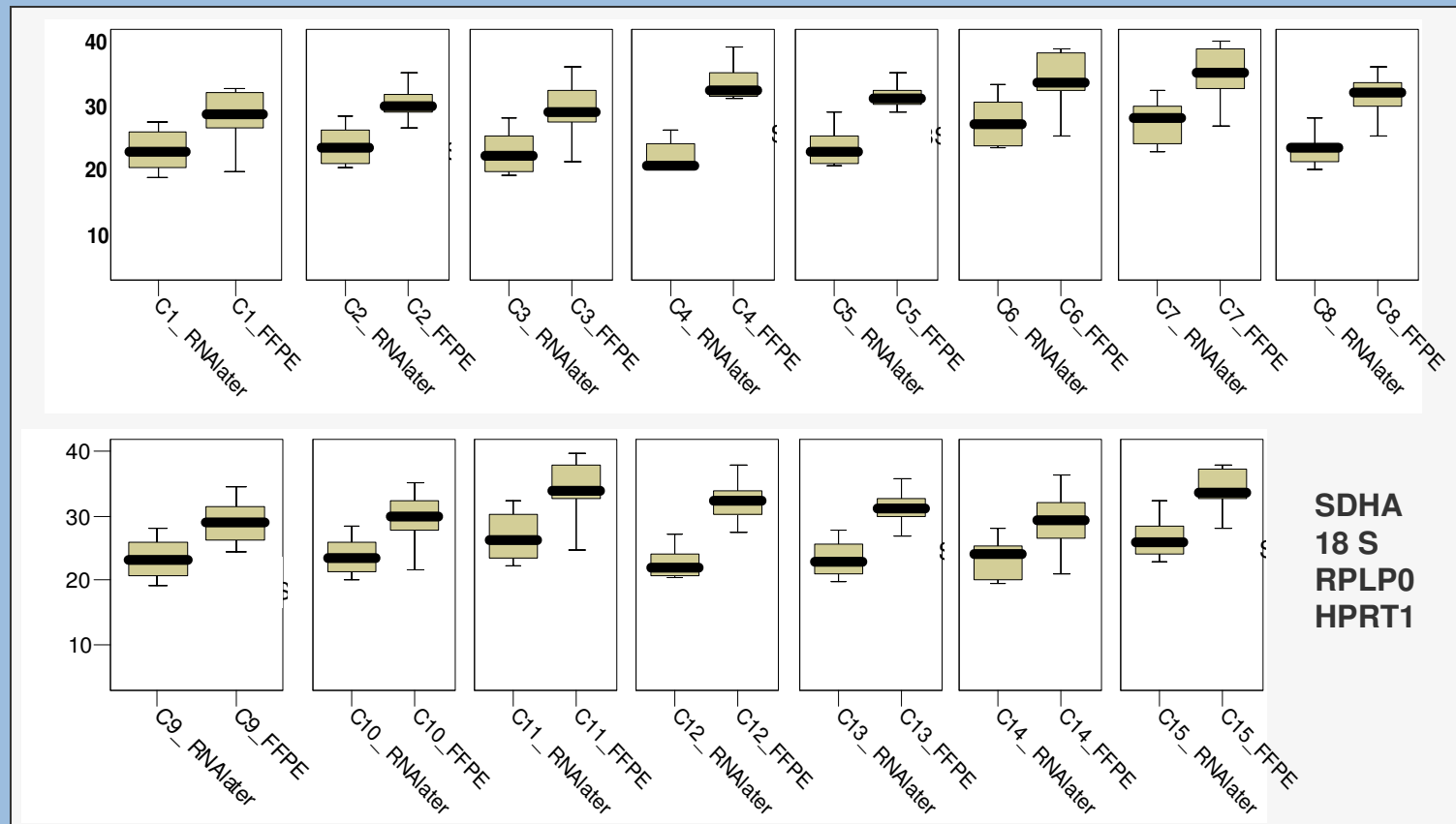
- > Validation
 - Implementation of **optimized procedure** on 22 tumor samples

Comparison of RNA integrity of matched tumor samples

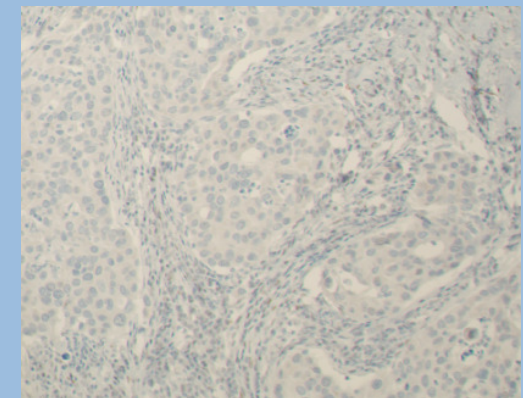
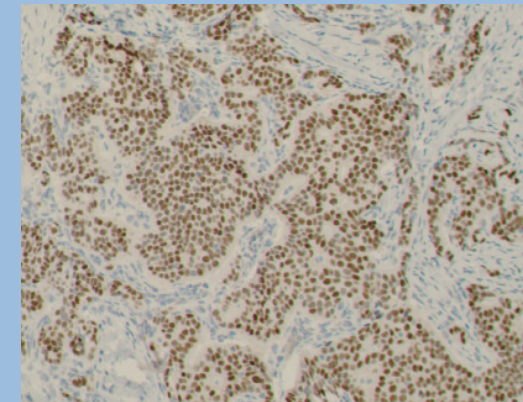
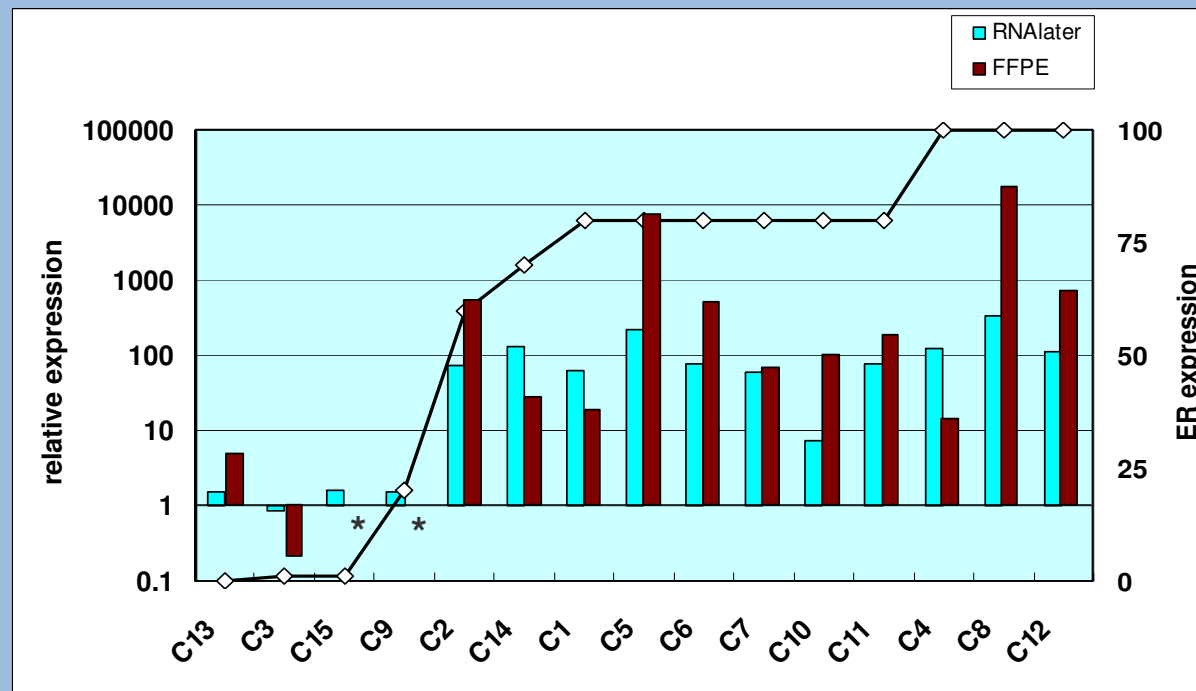
matched human breast cancer samples



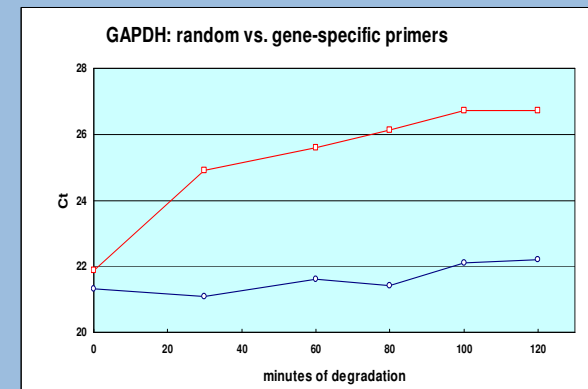
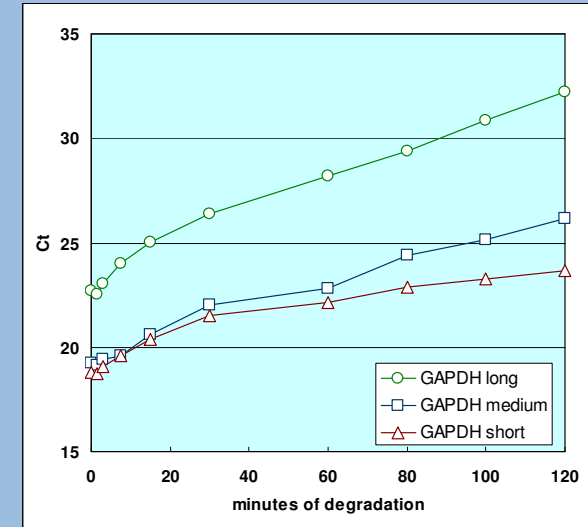
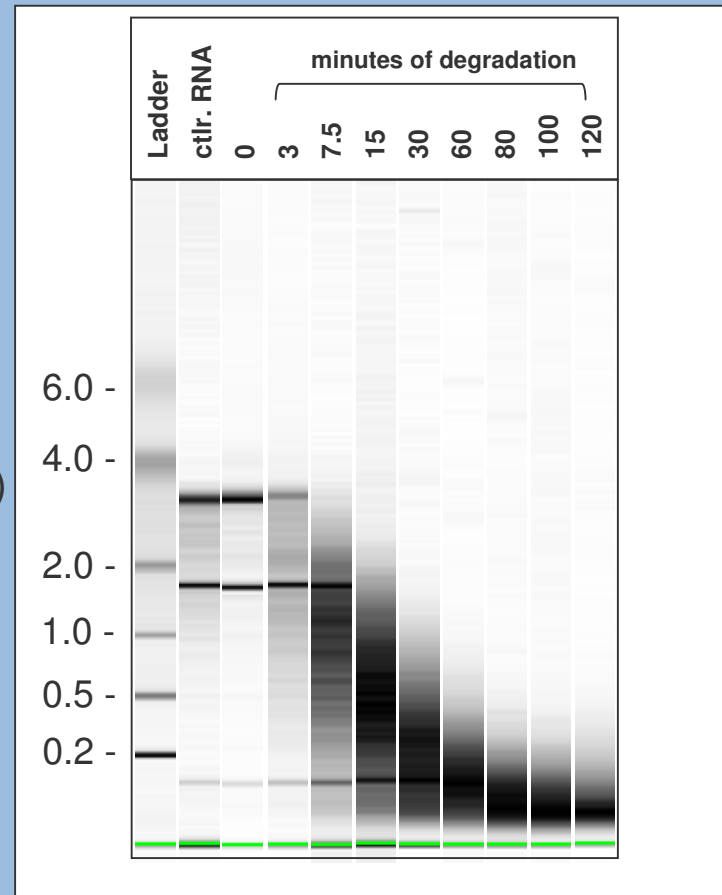
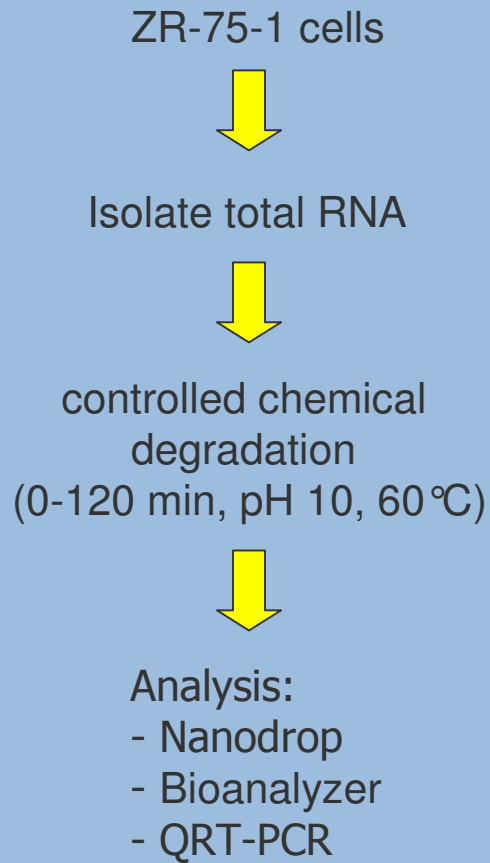
Variability of control gene expression RNAlater versus FFPE



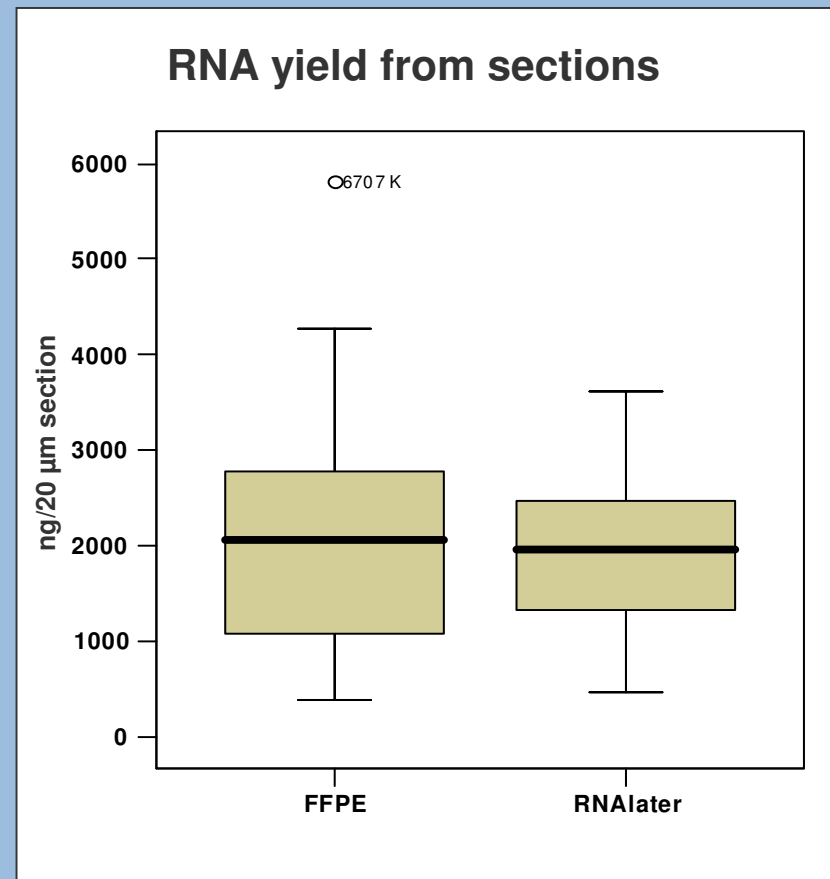
Measurement of estrogen receptor levels by semiquantitative immunohistochemistry and QRT-PCR



QRT-PCR optimization with an experimental test system based on ZR-75-1 breast cancer cells

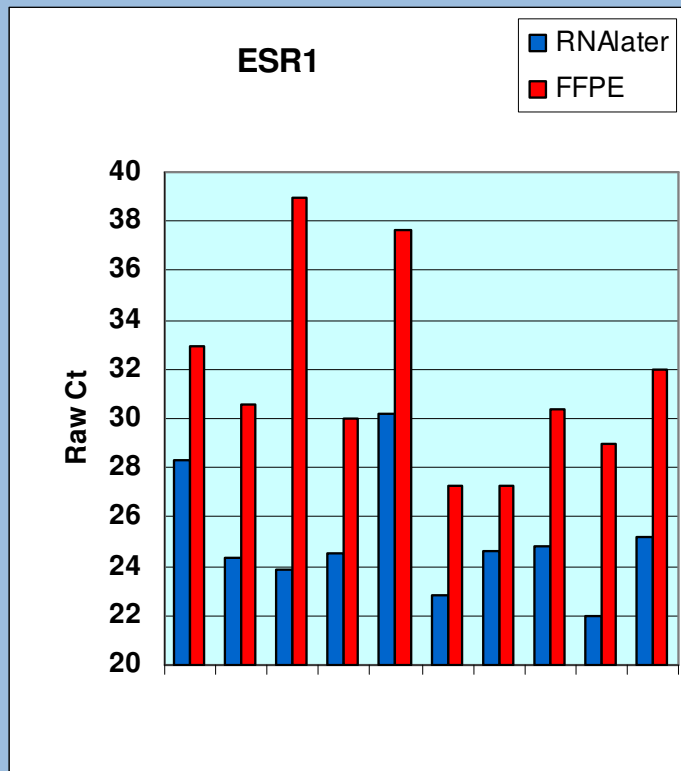


RNA recovery of 22 matched breast cancer tumors with optimized protocols

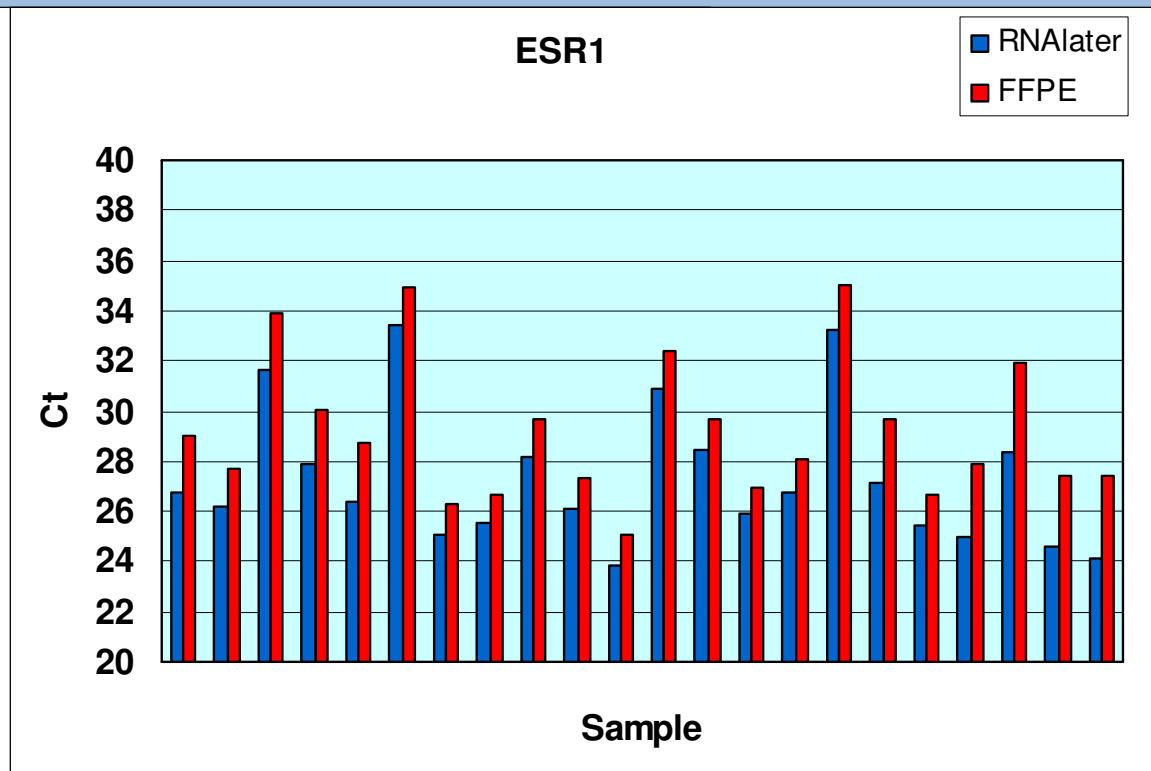


Comparison of gene expression measurements of standard with optimized procedure

Standard procedure

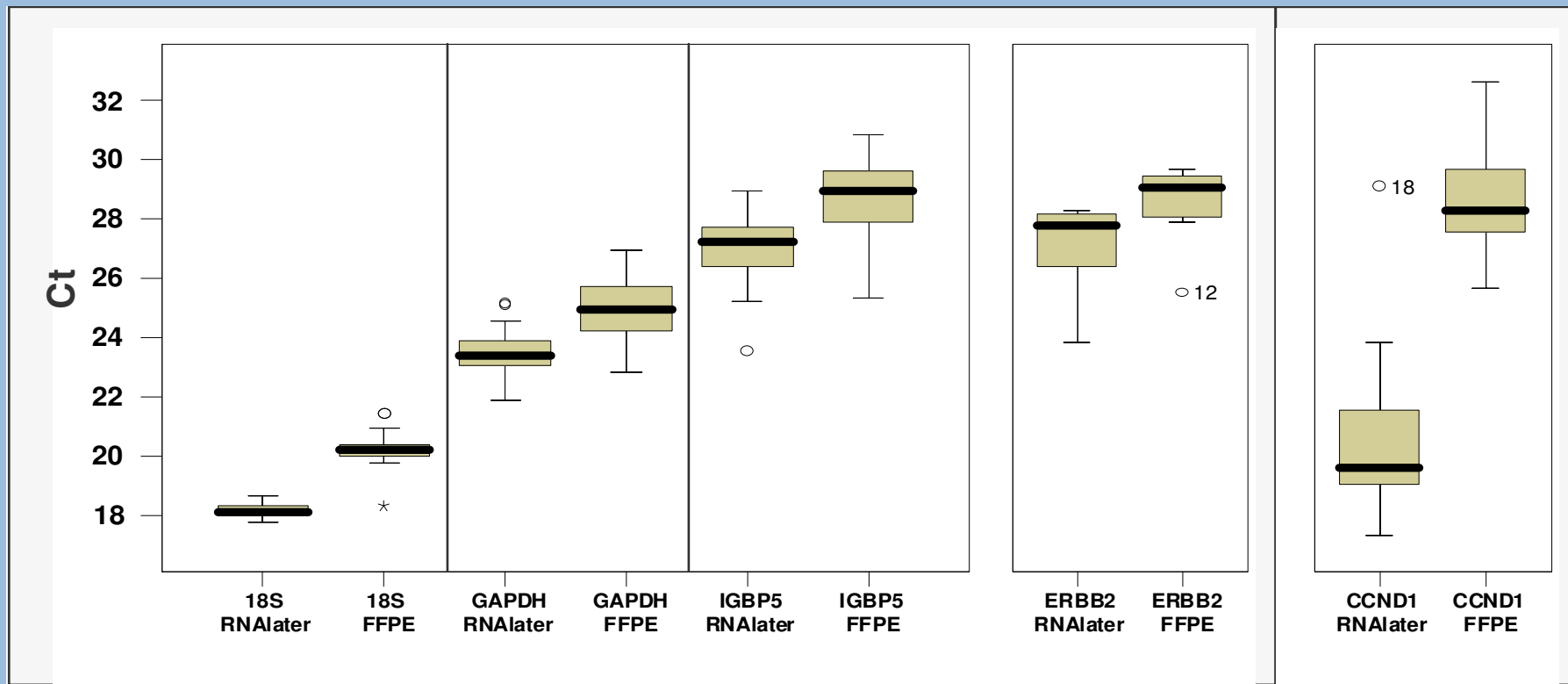


Optimized procedure



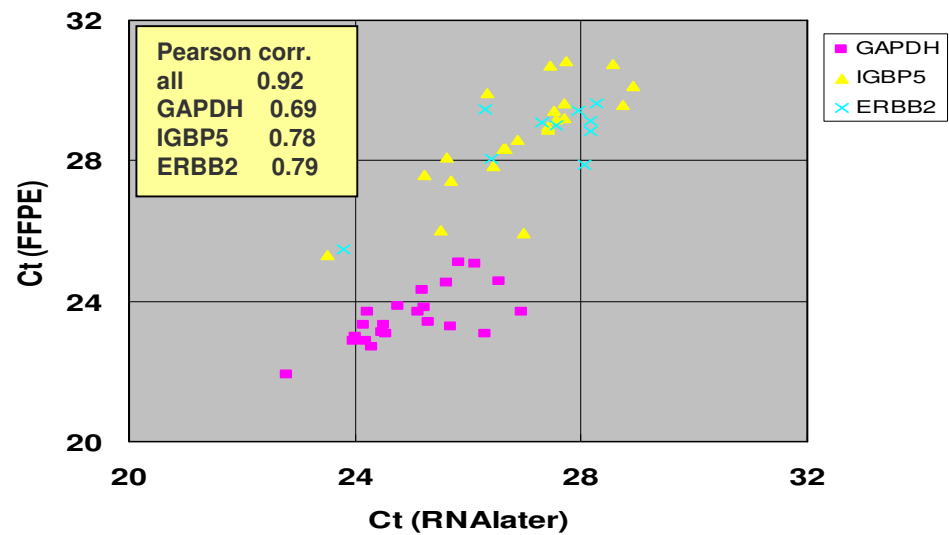
Ct values from good and poor quality RNA with optimized protocols

22 matched tumor samples → optimized RNA extraction → gene specific cDNA → qPCR



Pearson correlations:

Assay	Gene	Delta Ct	SD	Pearson corr.
random	GAPDH	3.306	0.984	0.733
	IGBP5	3.571	0.959	0.804
	ERBB2	4.983	0.487	0.938
gene-specific	GAPDH	1.440	0.731	0.690
	IGBP5	1.755	0.960	0.783
	ERBB2	1.404	0.866	0.791



Conclusions:

- > FFPE: RNA quality and yield can be improved with optimized RNA extraction protocols
- > The method of cDNA synthesis and amplicon size are critical for gene expression measurements from FFPE
- > Each assay must be tested individually before it can be used for archival material.

Outlook

- > Extend test system to at least 100 matched breast cancer samples (RNAlater-FFPE)
- > Design primer probe assays for diagnosis relevant genes
- > Validate gene signatures on matched samples
- > Validate gene signatures on archival tumor tissues of clinical trials

Collaborators

- > Molecular Biology group Rolf Jaggi, PhD
Andrea Oberli, Anna Baltzer, Technicians
Departement of Clinical Research, Bern

- > Pathology Hans Jörg Altermatt, MD
Pathologie Länggasse, Bern

- > Tumorbank Bern
(TBB) Daniel Muellener, Coordinator

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