

Development of Clinical Biomarkers – the importance of SOPs and quality assurance

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- Clinical Biomarkers must work in the clinical setting – this can be a very different environment from the research setting
 - Little high quality scientific research done on effect of time delays in processing specimens, handling of specimens – we simply don't know our safe limits

SOPs rule!

- Material must be collected, documented and stored according to SOPs
- Adherence to SOPs must be regularly checked
- If you can't control quality at input level, must control quality at output

The Importance of SOPs

- SOPs enable us to collect data on how a specimen is obtained and manipulated.
- If SOPs are too rigid or impractical, human nature means that either specimens will not get collected or people will not tell the truth.
- SOPs should be developed with team involved in collection of material to ensure they are practical.

The way you collect your tissue may depend on the question you want to answer....

- Is my gene/protein of interest phosphorylated – is it labile? If so, delays in getting hold of tissue in a routine diagnostic setting may affect your results
- Am I going to be able to use this as a diagnostic/predictive marker in a routine setting? If yes – marker needs to be robust to survive the operative process and processing through pathology (both in terms of time delays and chemical onslaught).

Quality Assurance



The sample **MUST** be what we say it is

It **MUST** be fit for purpose

Garbage in...



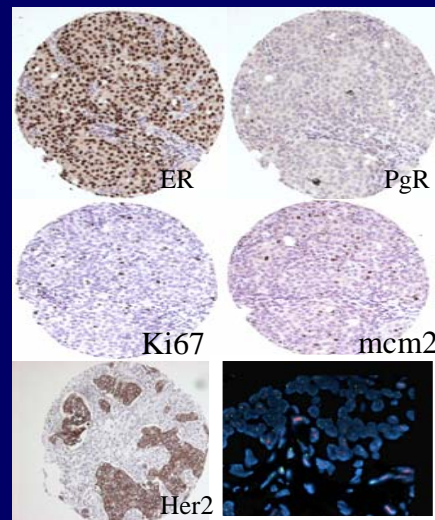
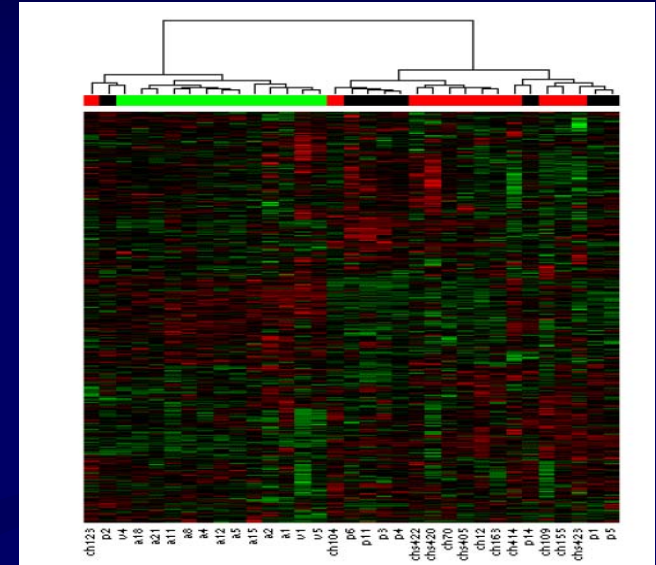
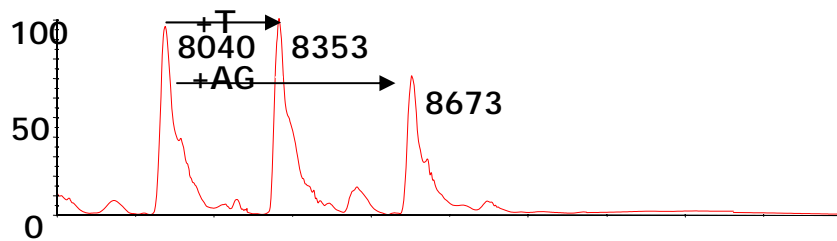
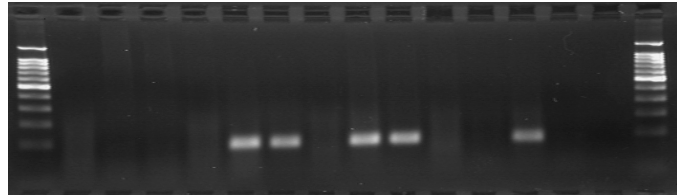
...garbage out



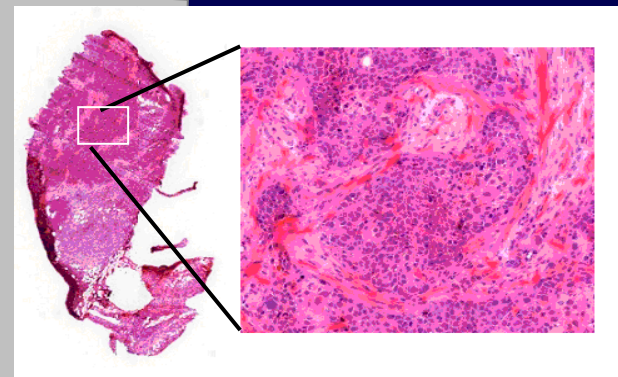
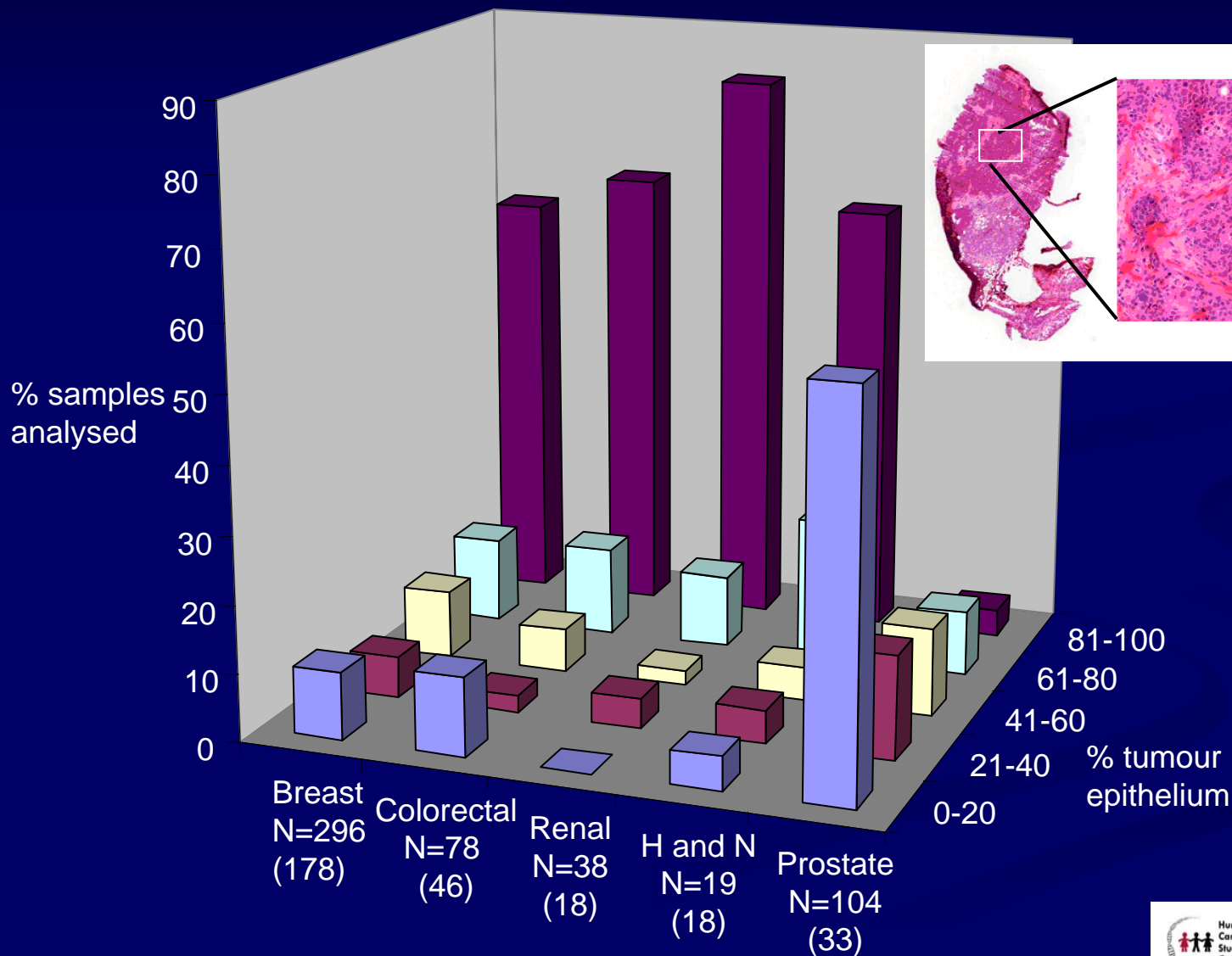
Diamonds in.....



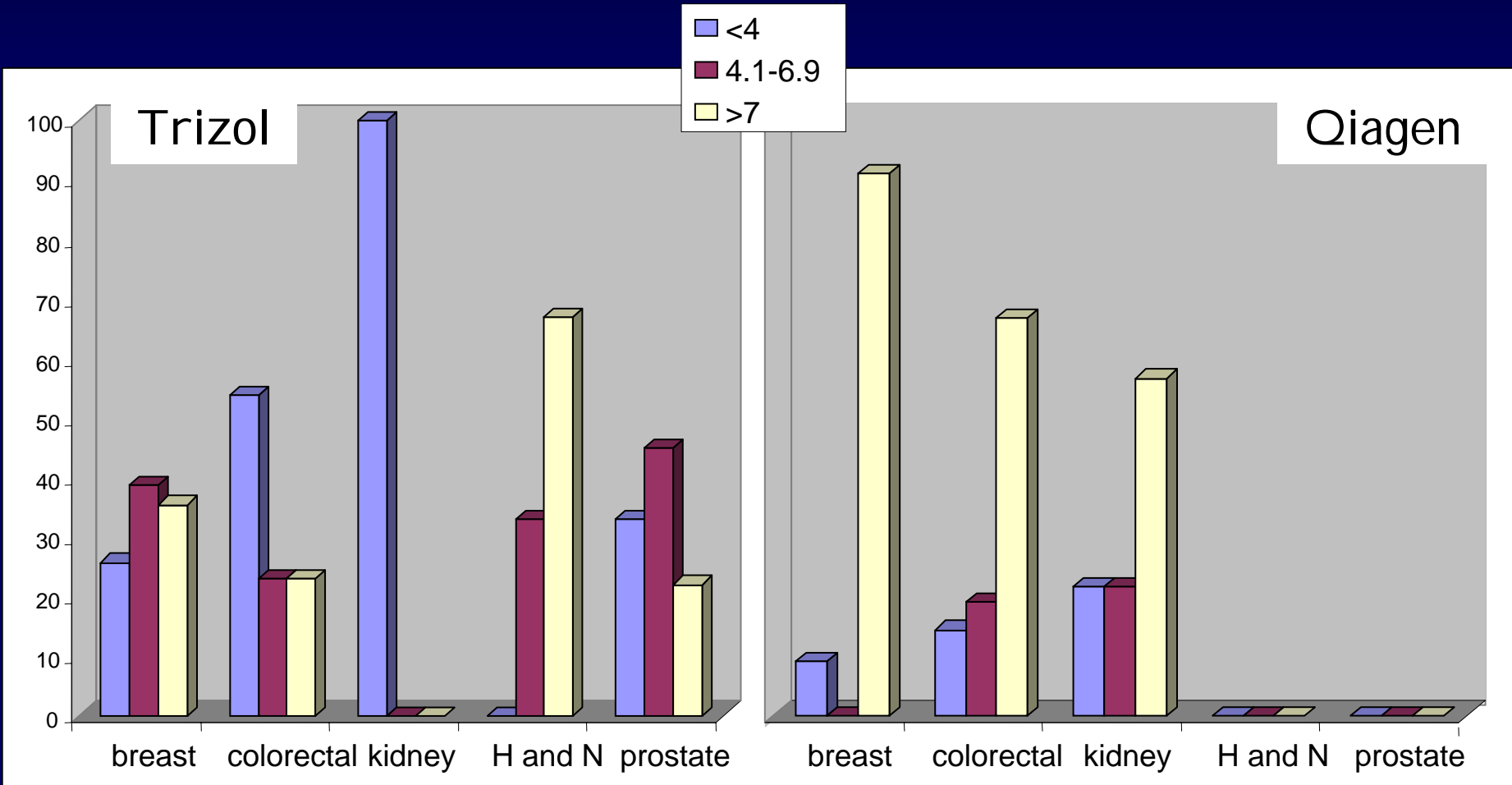
QA must reflect the use to which the sample will be put

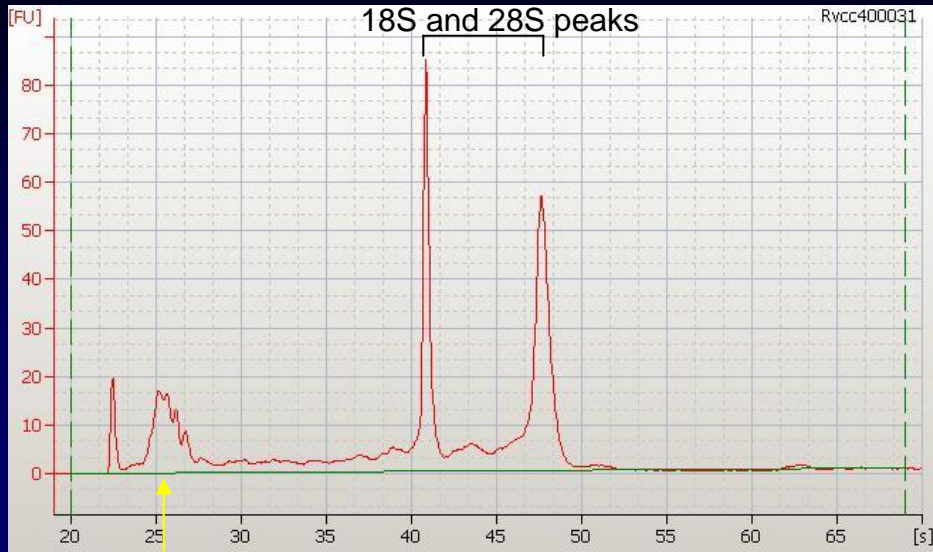


Pathology QA: Is it what we say it is?



RNA Quality – Is it fit for purpose? Extraction Methods Compared with respect to RNA Integrity

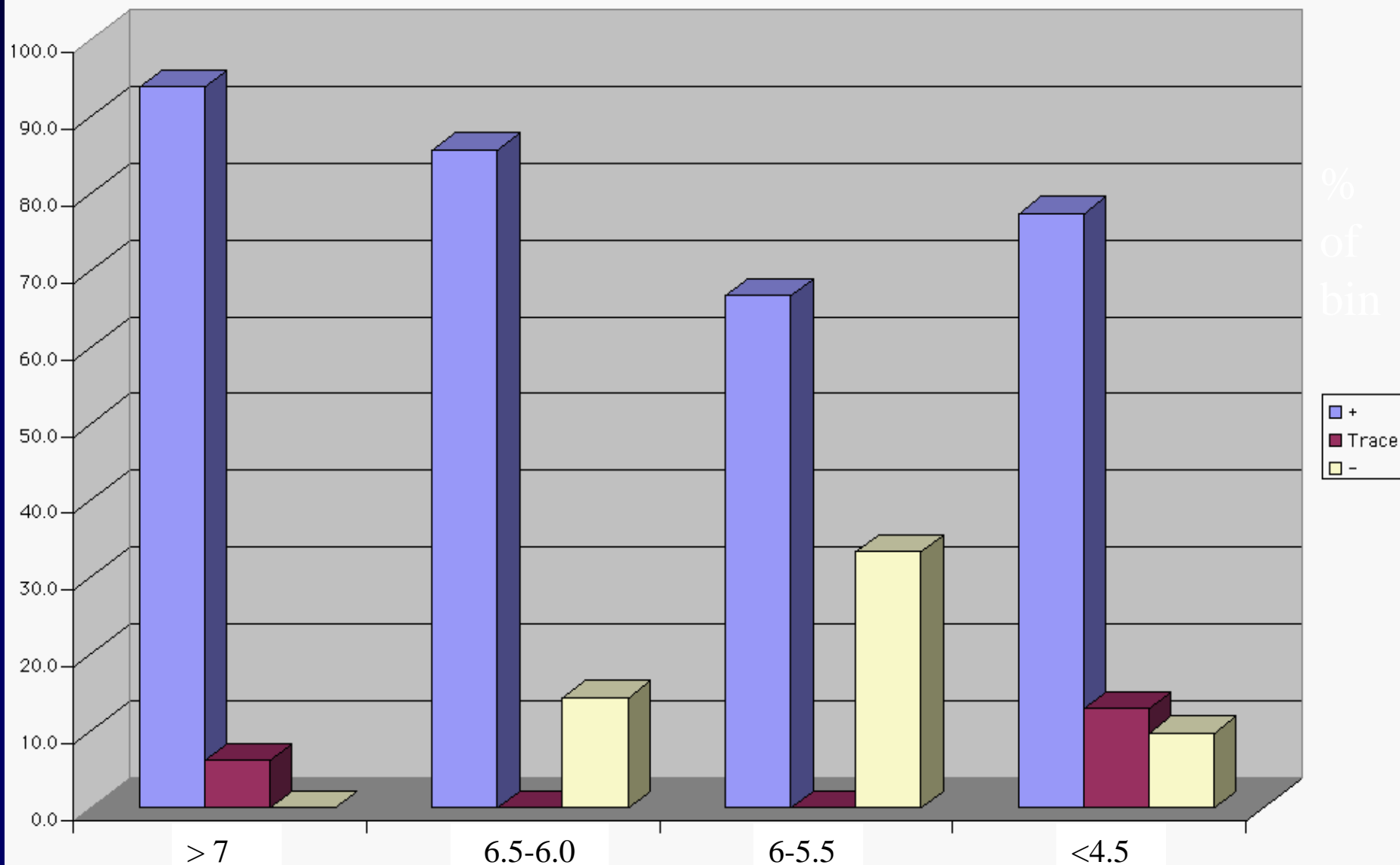




Degraded RNA

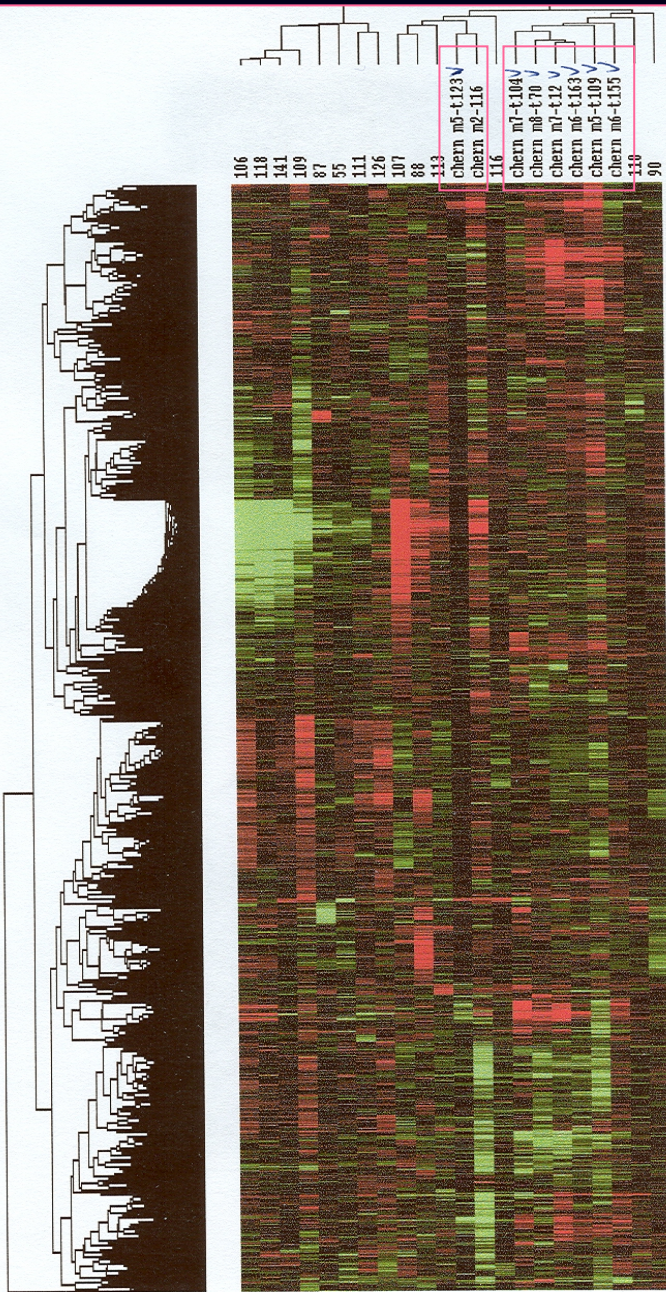


Agilent vs RT-PCR - 1kb PBDG



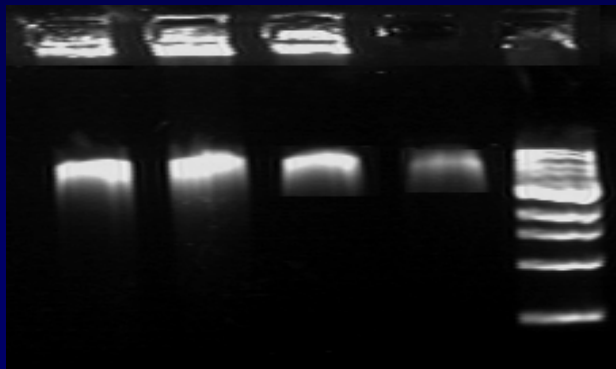
What potentially affects RNA quality?

- Ischaemic time during surgery?
- Length of time between surgery and freezing?
- Way in which tissue is frozen?
- Length of storage time as frozen tissue or RNA?
- Protocol for extraction?
- Skill of person doing extraction?

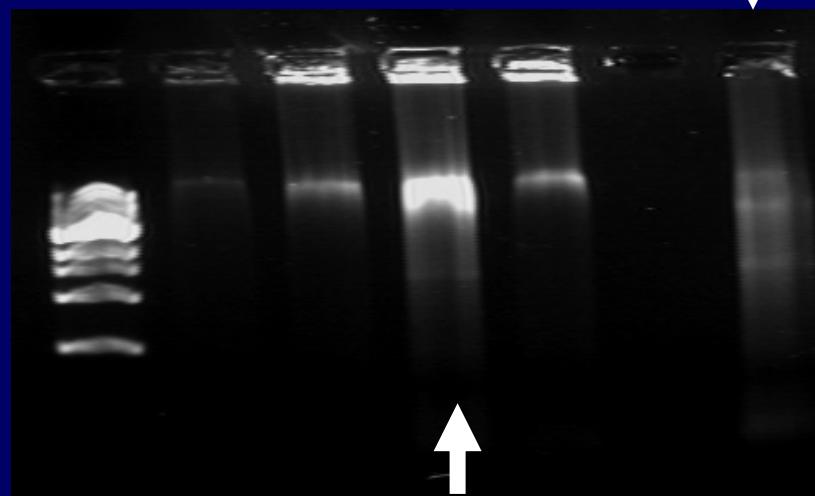


Effect of RNA quality on gene expression analysis

QA for DNA – 10kb gel electrophoresis

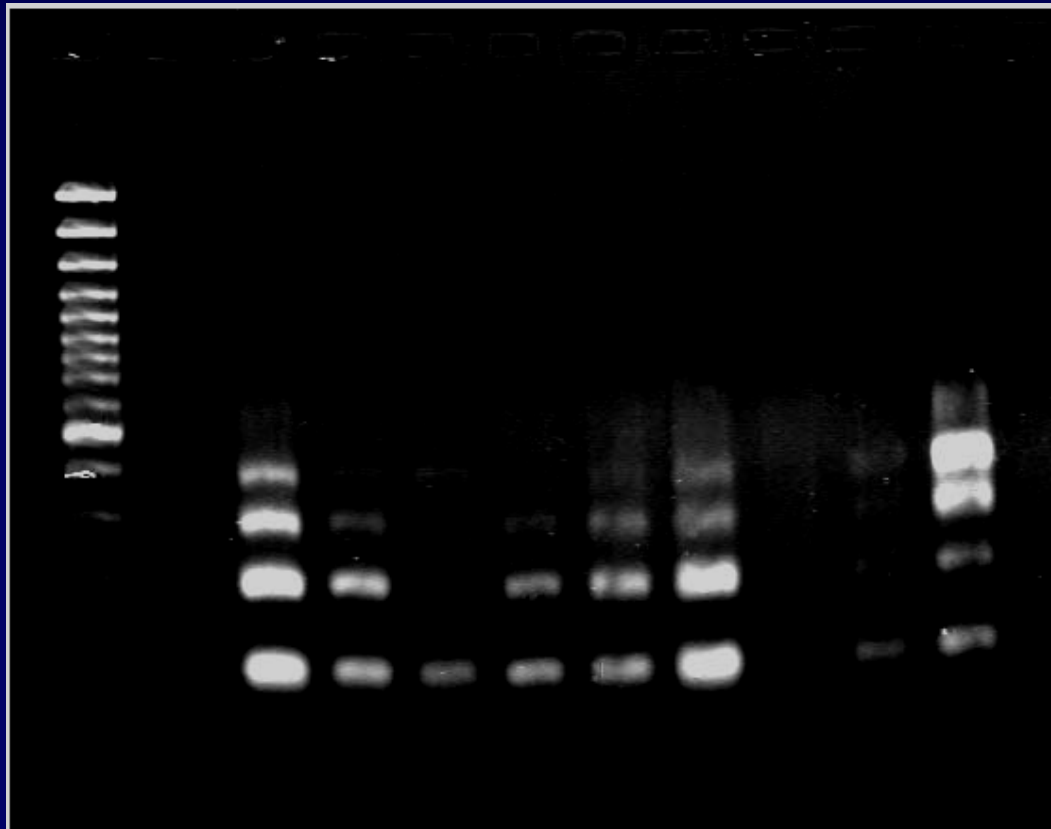


Degraded DNA



Protein contamination

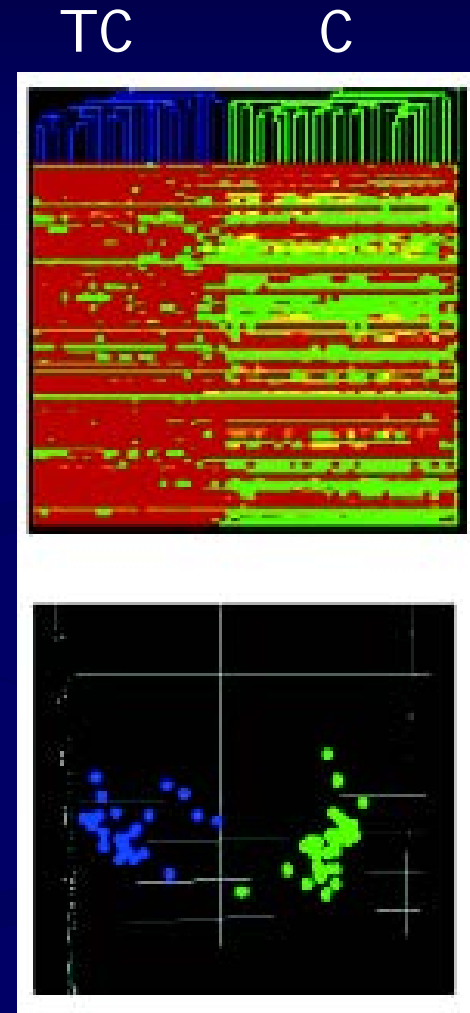
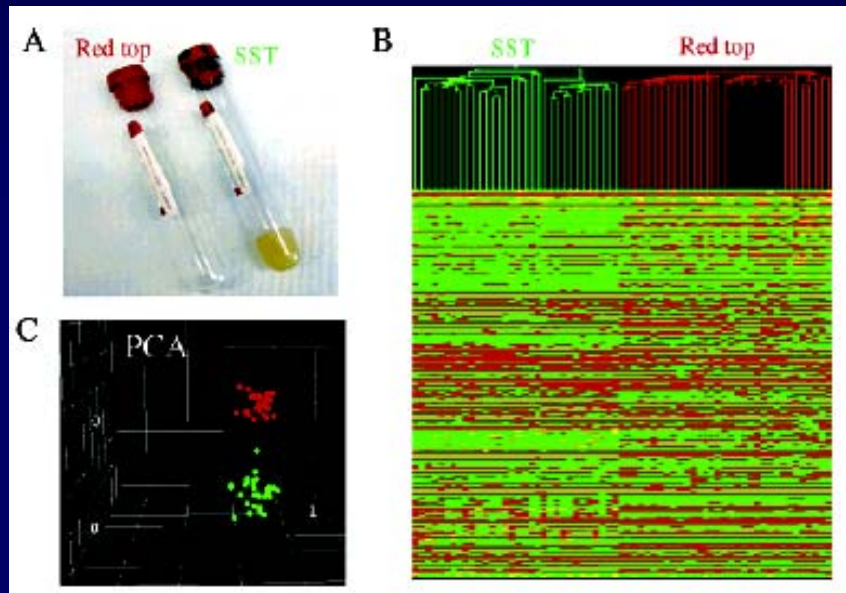
QA for DNA from FFPE tissue



Multiplex PCR to assess quality for bac array CGH
- amplicon sizes 100, 200, 300 and 400 bp

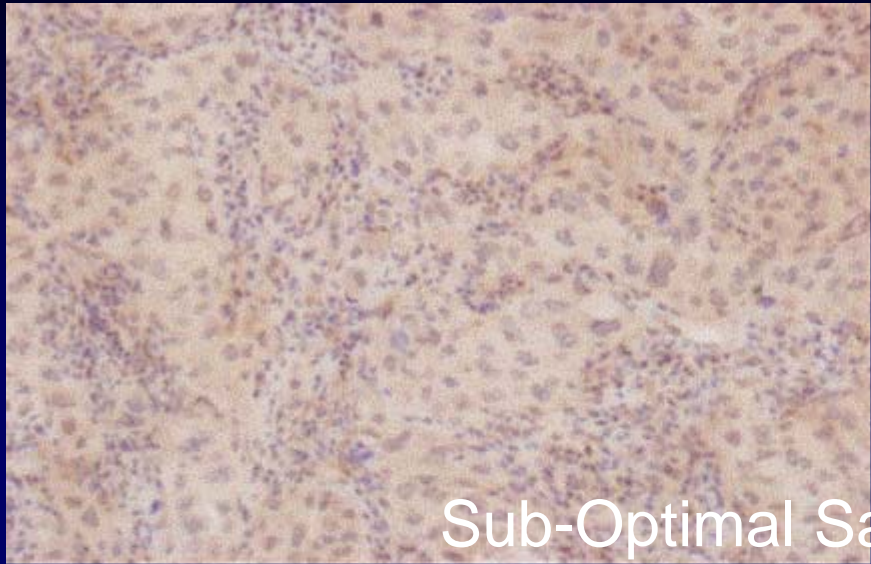
Only samples positive for 300 bp and above pass QA for bac array CGH

Effect of method of storage on serum proteomics

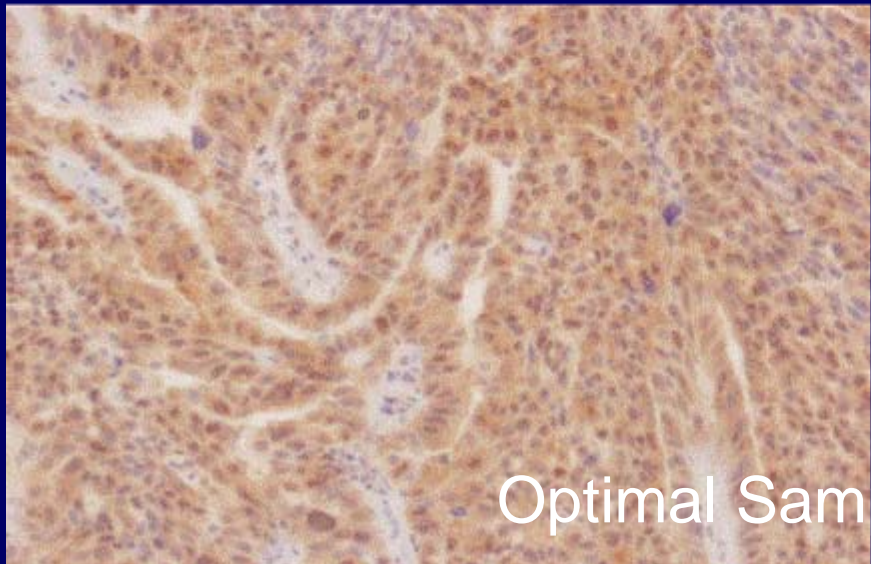
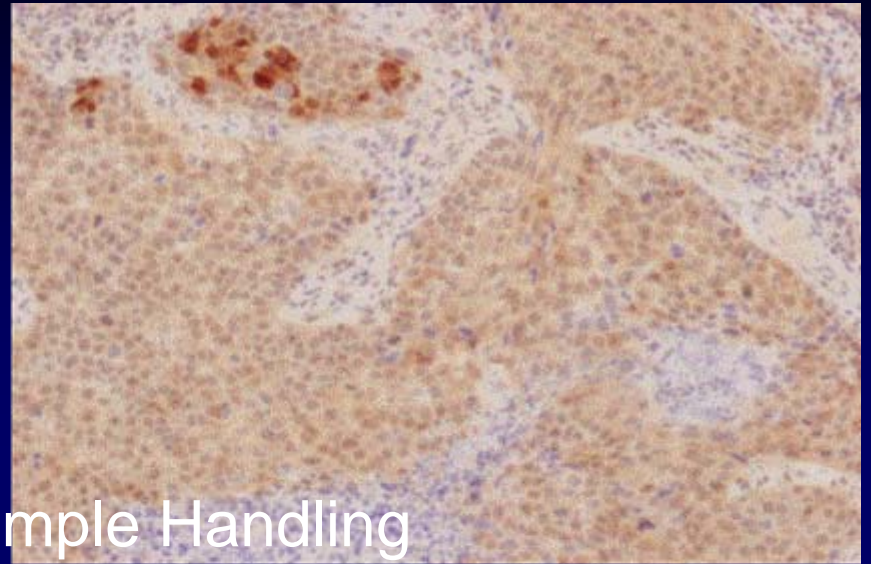


Villanueva et al., 2005

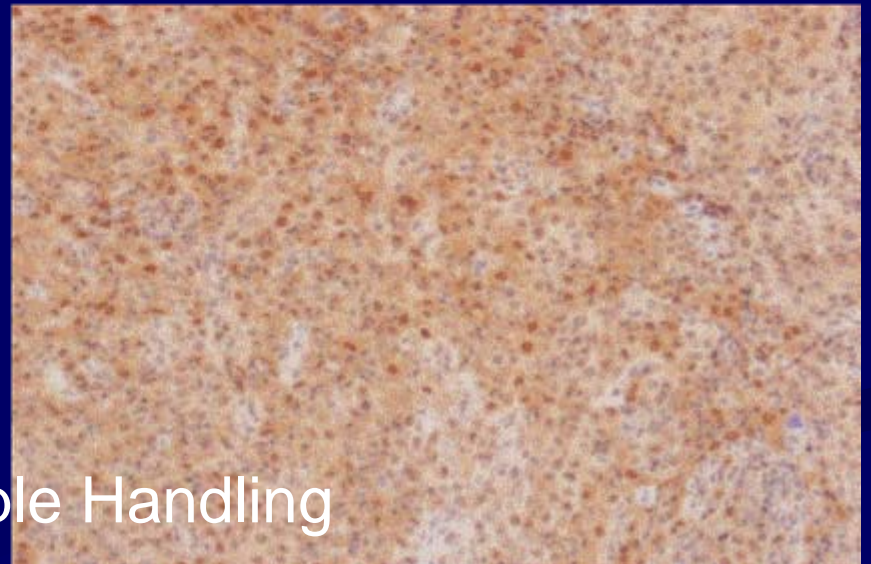
J Proteome Res 4: 1060-1072



Sub-Optimal Sample Handling



Optimal Sample Handling



Recommendations for QA

- SOPs should be written and adhered to – if protocol changed, check quality equal or improved
- If you cannot control input (i.e. alter surgical practice etc) must assess quality at all stages – from pathology to extracted material
- Need to assess the effect of time/method in storage more carefully

- QA must reflect the use to which material is put – Affymetrix arrays require higher quality RNA than straight forward RT-PCR
- All samples may not be useful for all technologies
- Need to second guess developments in science – difficult!
- One role of tissue banking is education, education, education – talk to your researchers!

More information?

- NCRI Workshop on Quality Matters 15th/16th January 2008 Wellcome Building, Euston Road London – register via the NCRI website (www.ncri.org.uk/ccb)
- Wales Cancer Bank website - SOPs available soon from the website (www.walescancerbank.com)
- Or email me (gerry.thomas@imperial.ac.uk)

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Wales Cancer Bank



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