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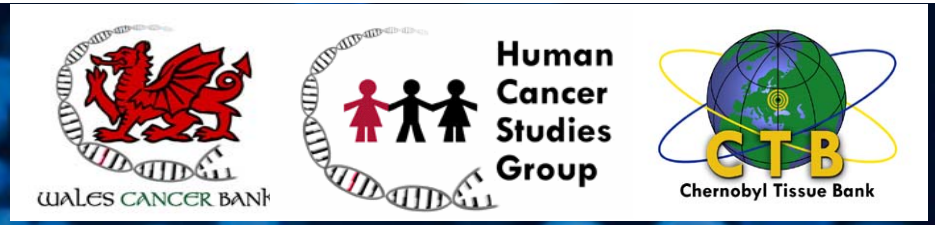

Practical Applications of Biospecimen Science in Biobanking

Gerry Thomas, Professor of Molecular Pathology, Imperial College, London
14th March 2008 BRN Symposium: Advancing Cancer Research Through Biospecimen Science

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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

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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

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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.

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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).

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Quality Assurance

The sample MUST be what we say it is

It MUST be fit for purpose

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Garbage in…

…*garbage* out

Diamonds in……

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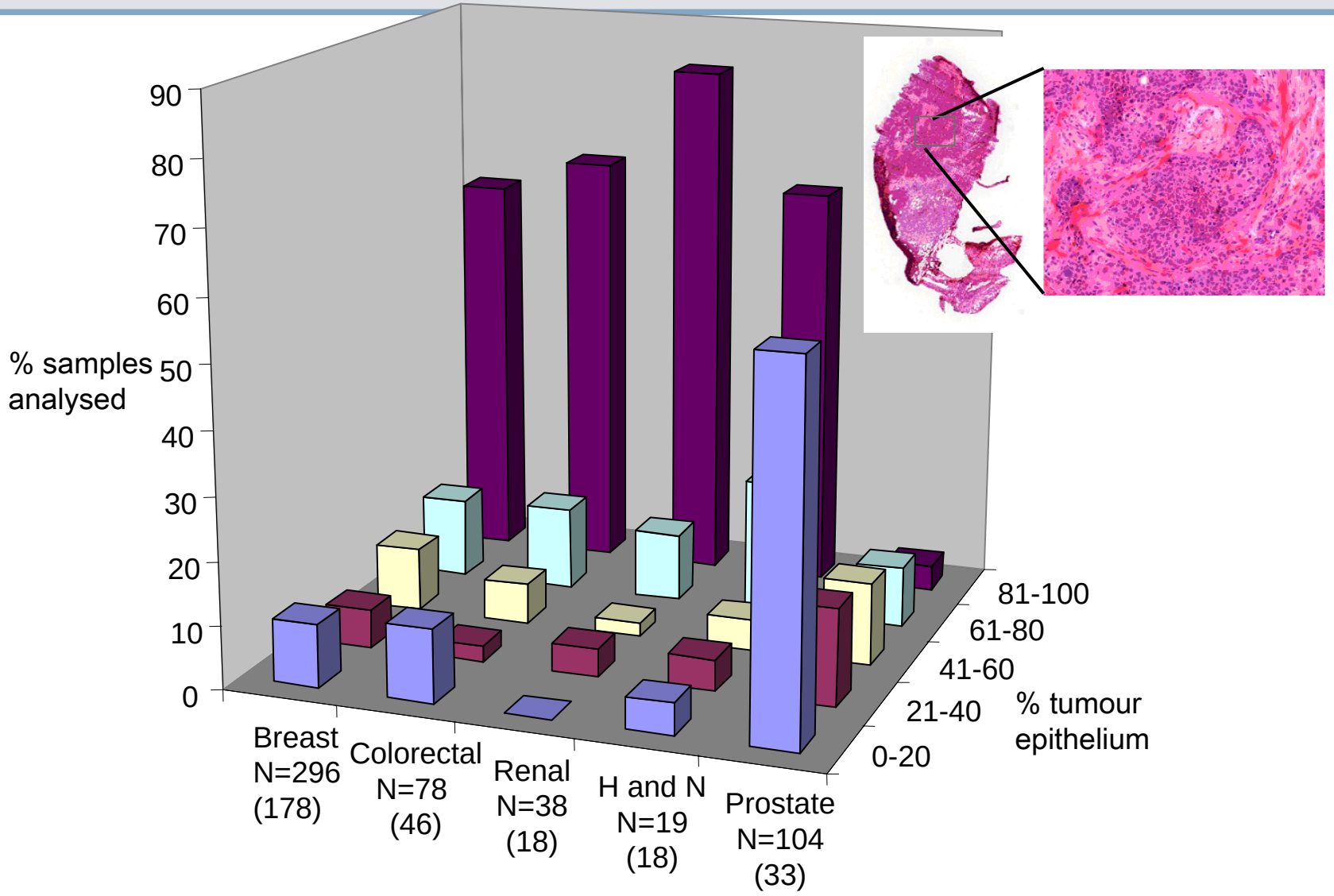
Is it tumour? How much of it is tumour?



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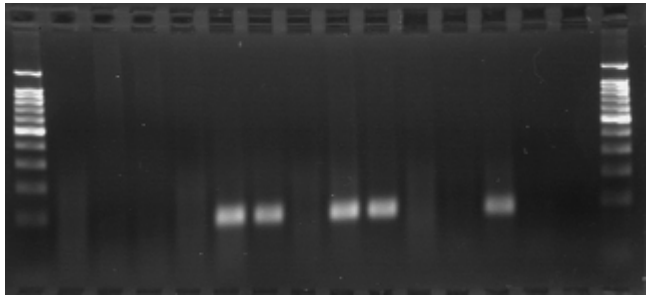
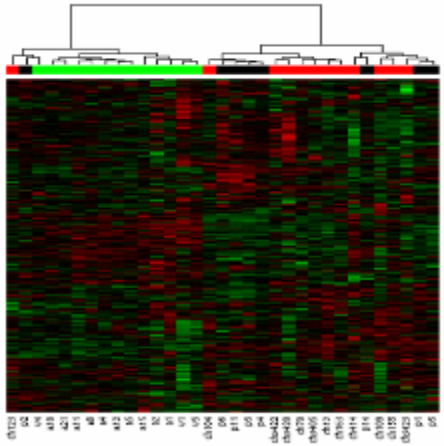
What are you going to do with it?
QA must reflect the use to which the sample will be put

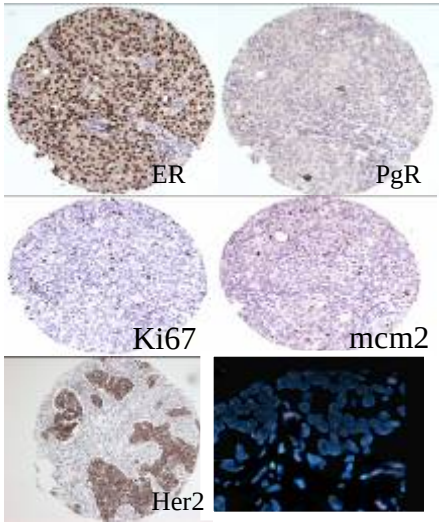


Single gene expression – RT-PCR or qRT-PCR?



Microarray – Affymetrix 3', exon array or Agilent cDNA?

BAC aCGH?



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What potentially affects RNA quality from frozen tissue?

- 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?

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Methods to assess RNA quality

- 5'/3' ratio using Affy chip
  - Expensive
- Gel electrophoresis for ribosomal RNA
  - Wasteful of valuable material
- Microfluidics - Agilent Bioanlyser
  - Relatively inexpensive – easy and high throughput

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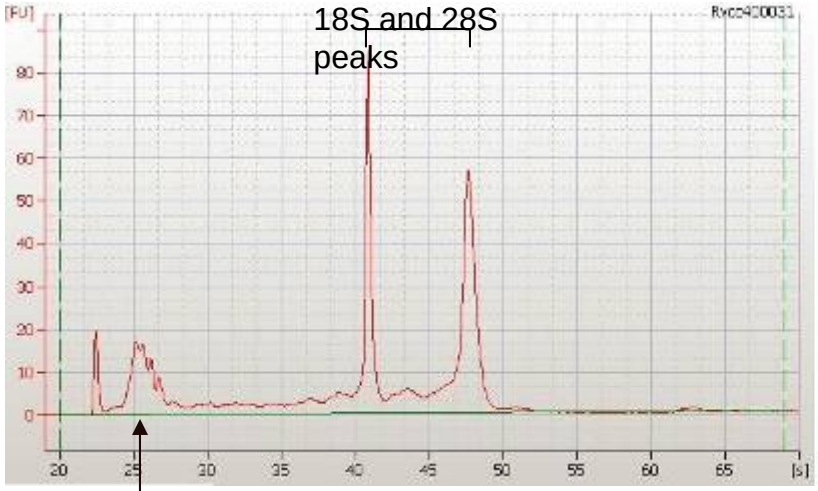
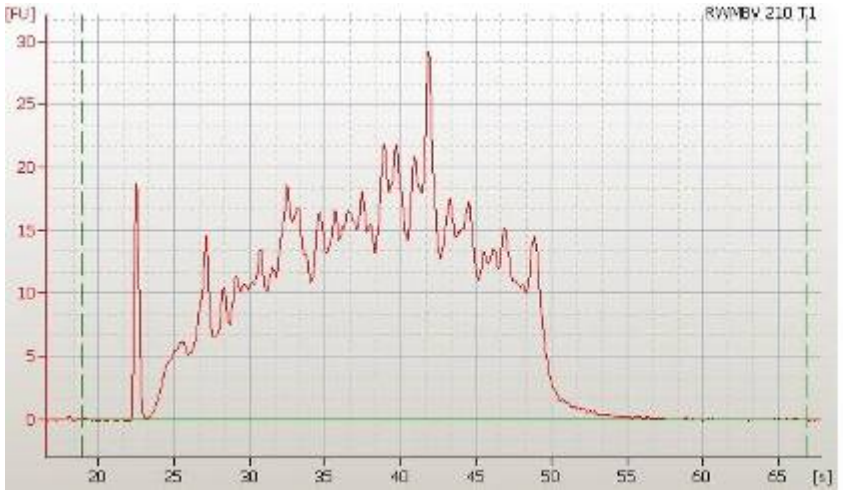
Agilent Bioanalyser QA



Gel view



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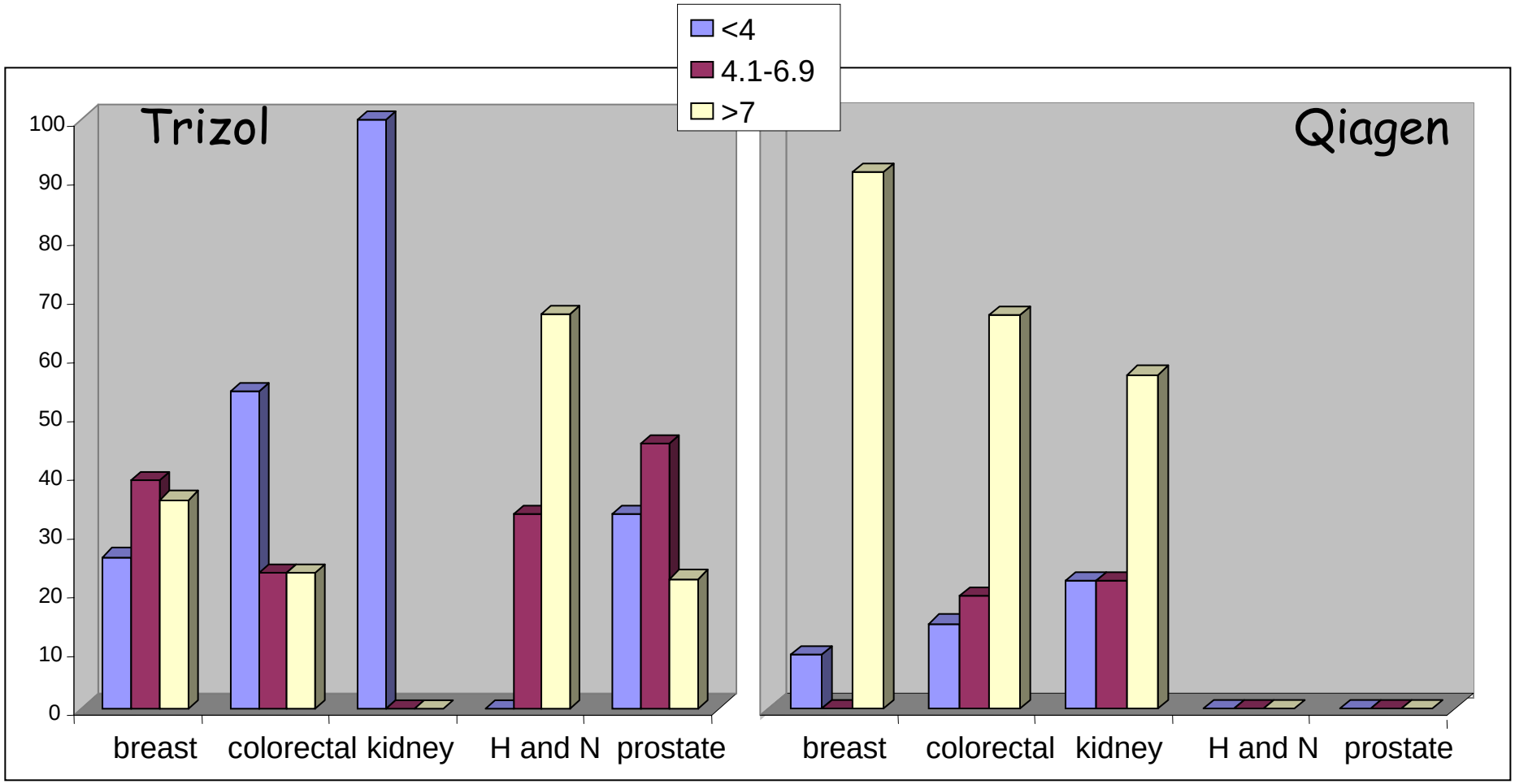
RNA Quality – Is it fit for purpose? Extraction Methods Compared with respect to RNA Integrity



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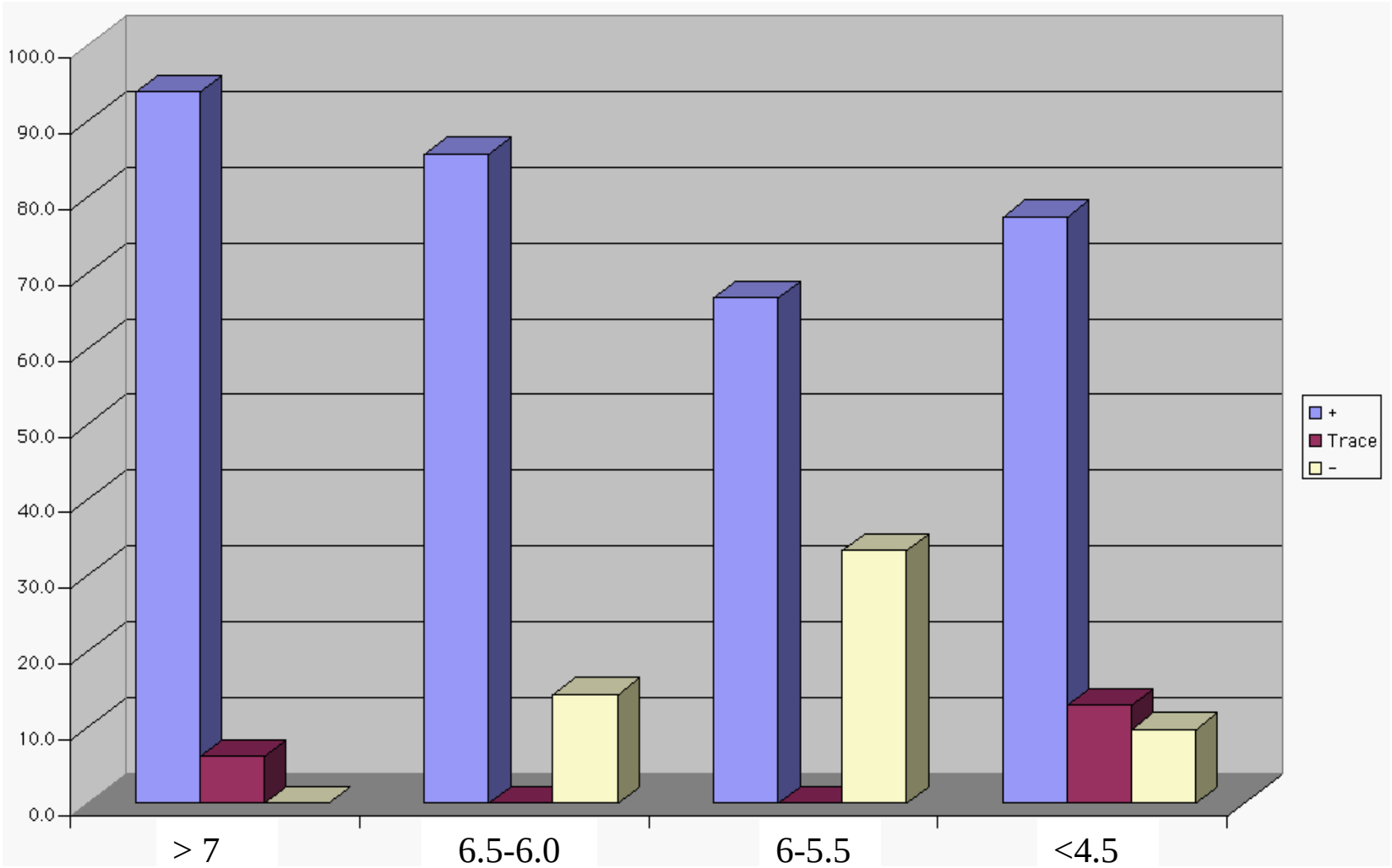
Agilent vs RT-PCR - 1kb PBDG



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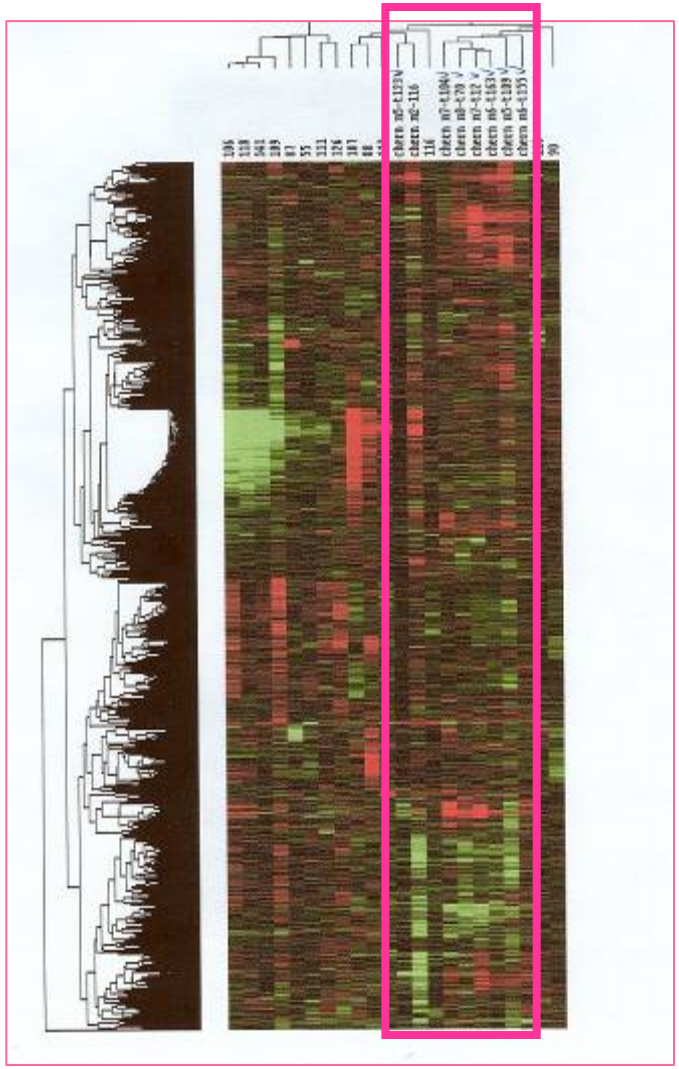
Effect of RNA quality on gene expression analysis



Is this a Nature paper or ……

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What potentially affects RNA quality from FFPE tissue?

- Type of fixative used?
- Length of time in fixative?
- Length of time/temperature used for deparaffinisation?
- Whether RNA extracted from sections stored prior to processing?
- Temperature of storage?

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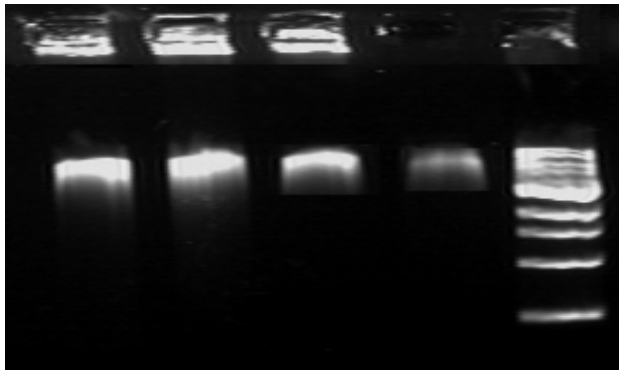
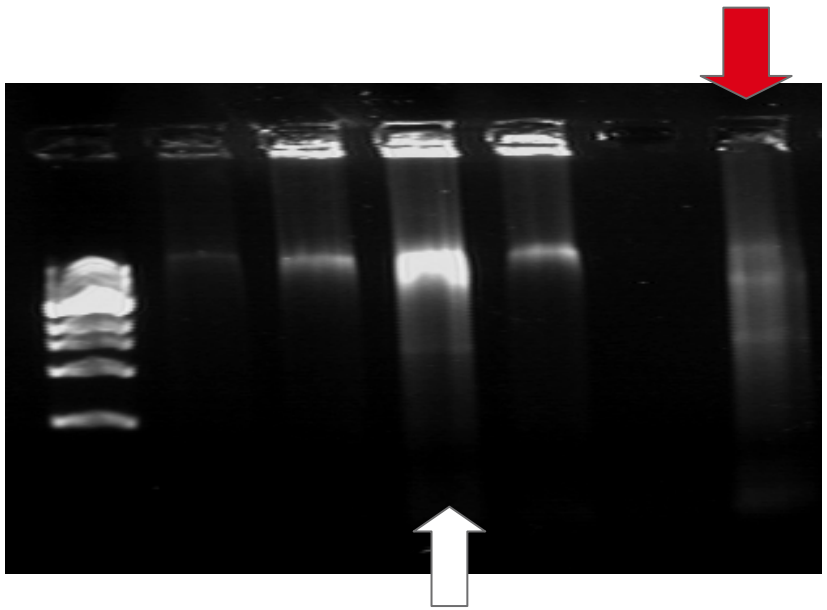
QA for DNA from frozen tissue – 10kb gel electrophoresis





Degraded DNA

Protein contamination

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QA for BAC aCGH from FFPE tissue using Multiplex PCR

Largest product in multiplex PCR	Success (%)	Good aCGH	Failed aCGH	Not done	N
400 bp (100%)	100	2	0		2
300 bp	100	5	0		5
200 bp	97	38	1		39
100 bp	16	6	31		37
No product	ND	0	0	10	10
Totals		51	32	10	93

Van Beers et al., BJC, 2006

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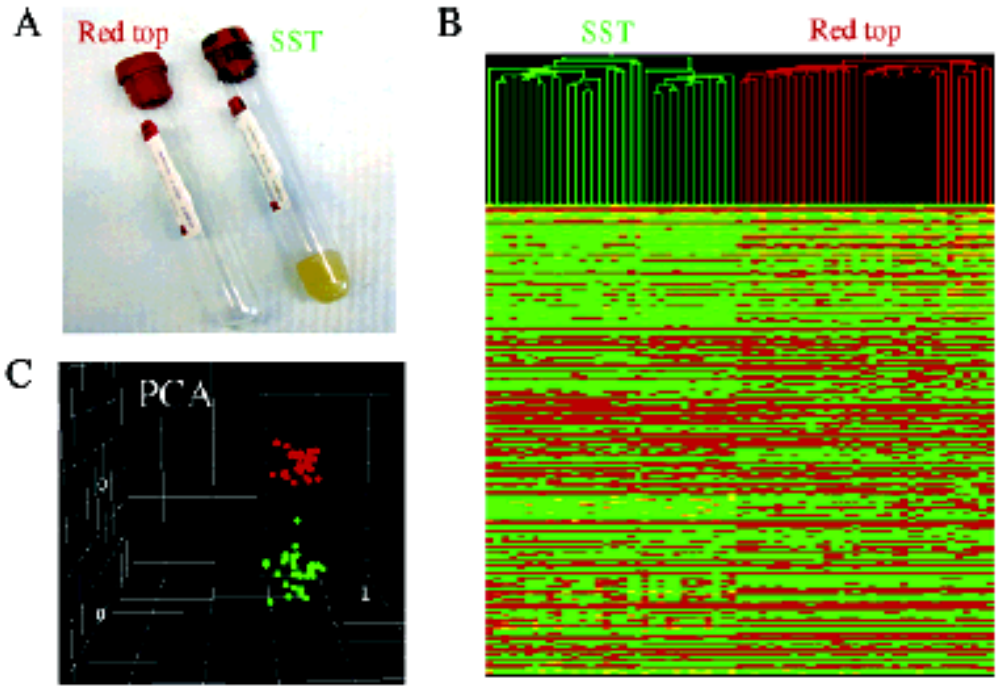
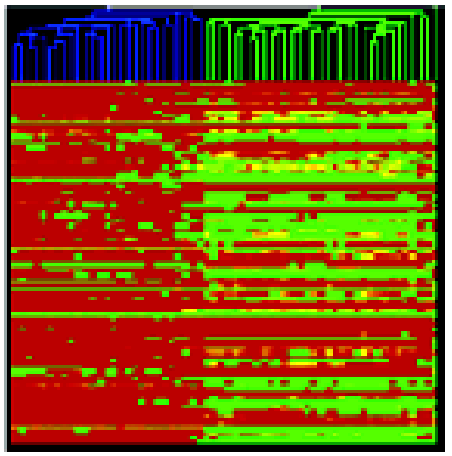
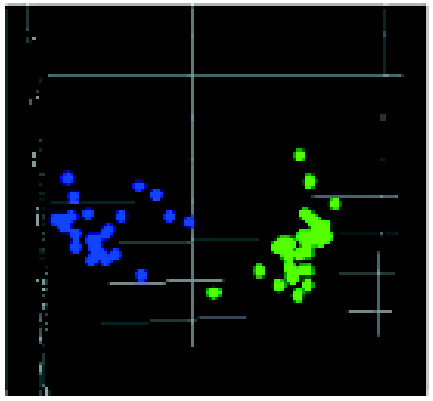
Effect of method of storage on serum proteomics



TC C





Villanueva et al., 2005

J Proteome Res 4: 1060-1072

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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!

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More information?

- Wales Cancer Bank website - SOPs available from the website (www.walescancerbank.com)
- Or email me (gerry.thomas@imperial.ac.uk)

IEM, Kiev and MRRC RAMS Obninsk

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Prof Gordon Stamp

Dr Kristian Unger

Dr Bill Mathieson

Dr Sileida Oliveros

Miss Mahrokh Nohadani