

**Formalin fixation at low temperature  
better preserves nucleic acid integrity**

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***University of Turin***

## **Disclosure of interests:**

**G.B. was originally responsible for the invention of the Cold Fix procedure, but is not the owner of the patents and does not receive Royalties.**

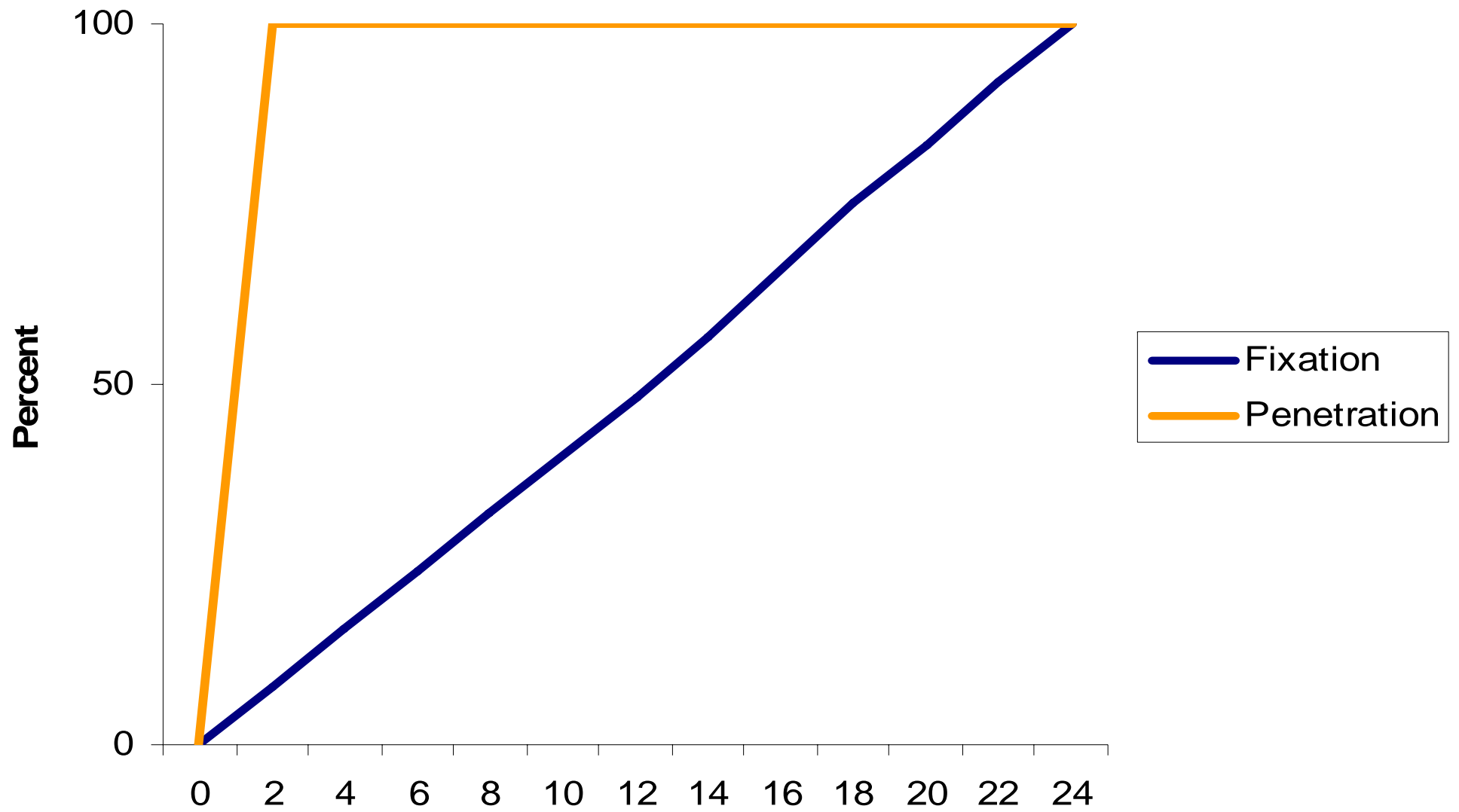
# RNA yeld from FF-PET

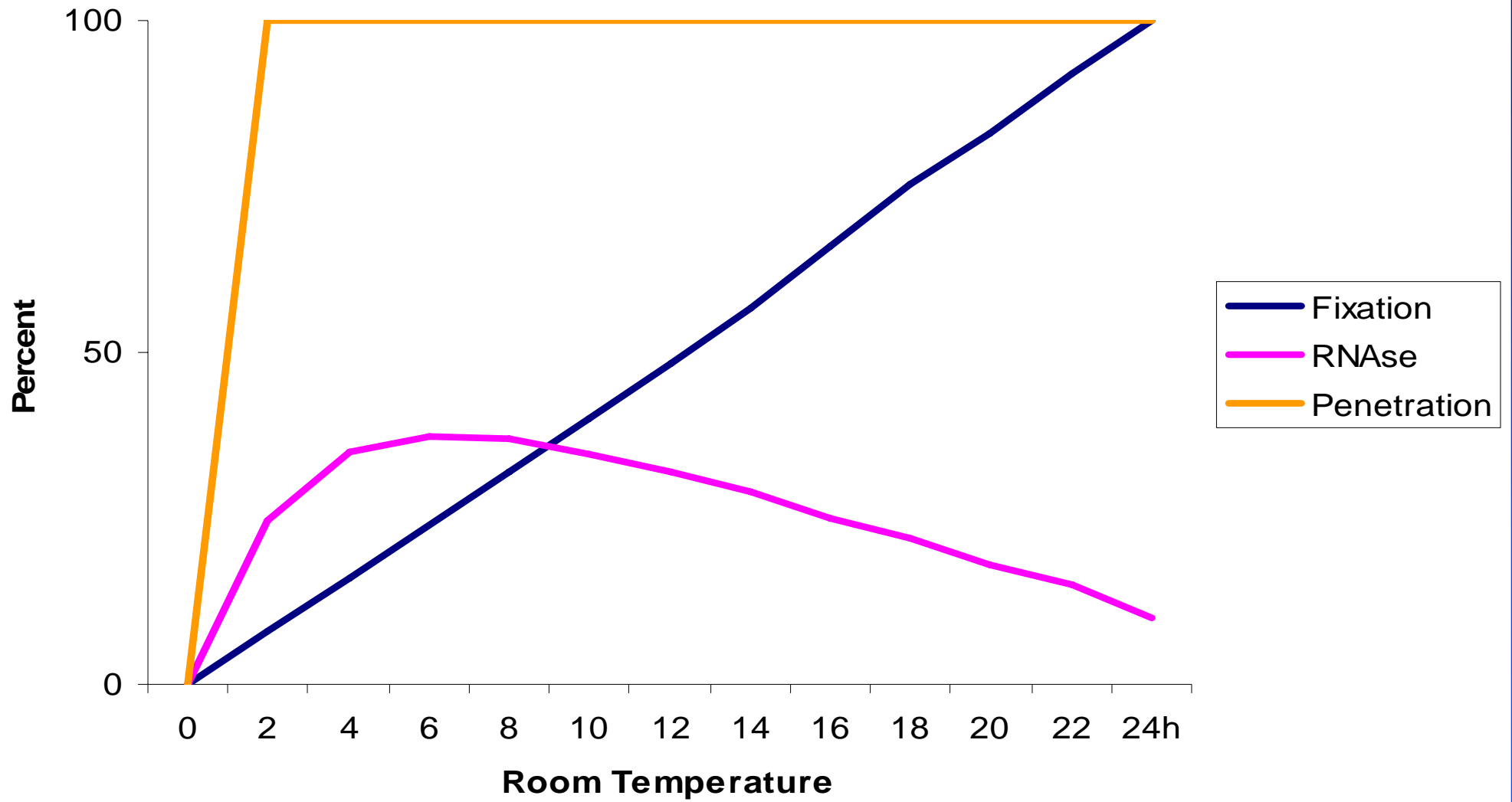
**Fragmented**

**Result of RNase activity (?)**

**...formaldehyde does not inhibit RNAses.**

**Ding J, Ichikawa Y, Ishikawa T, Shimada H.  
Scand J Clin Lab Invest. 2004;64(3):229-35**





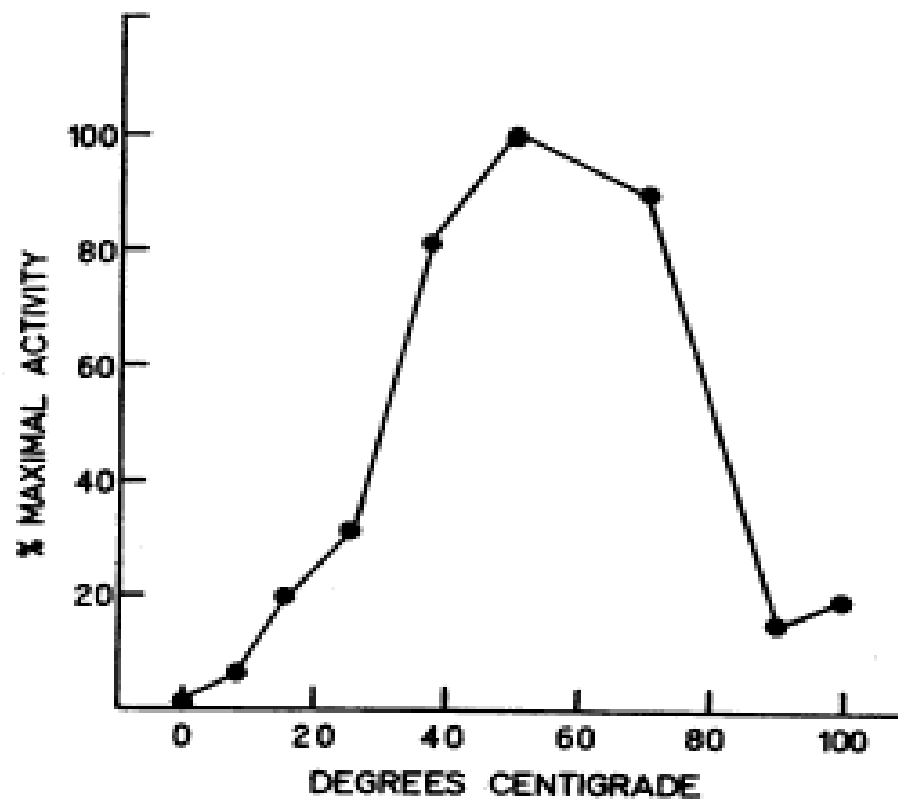
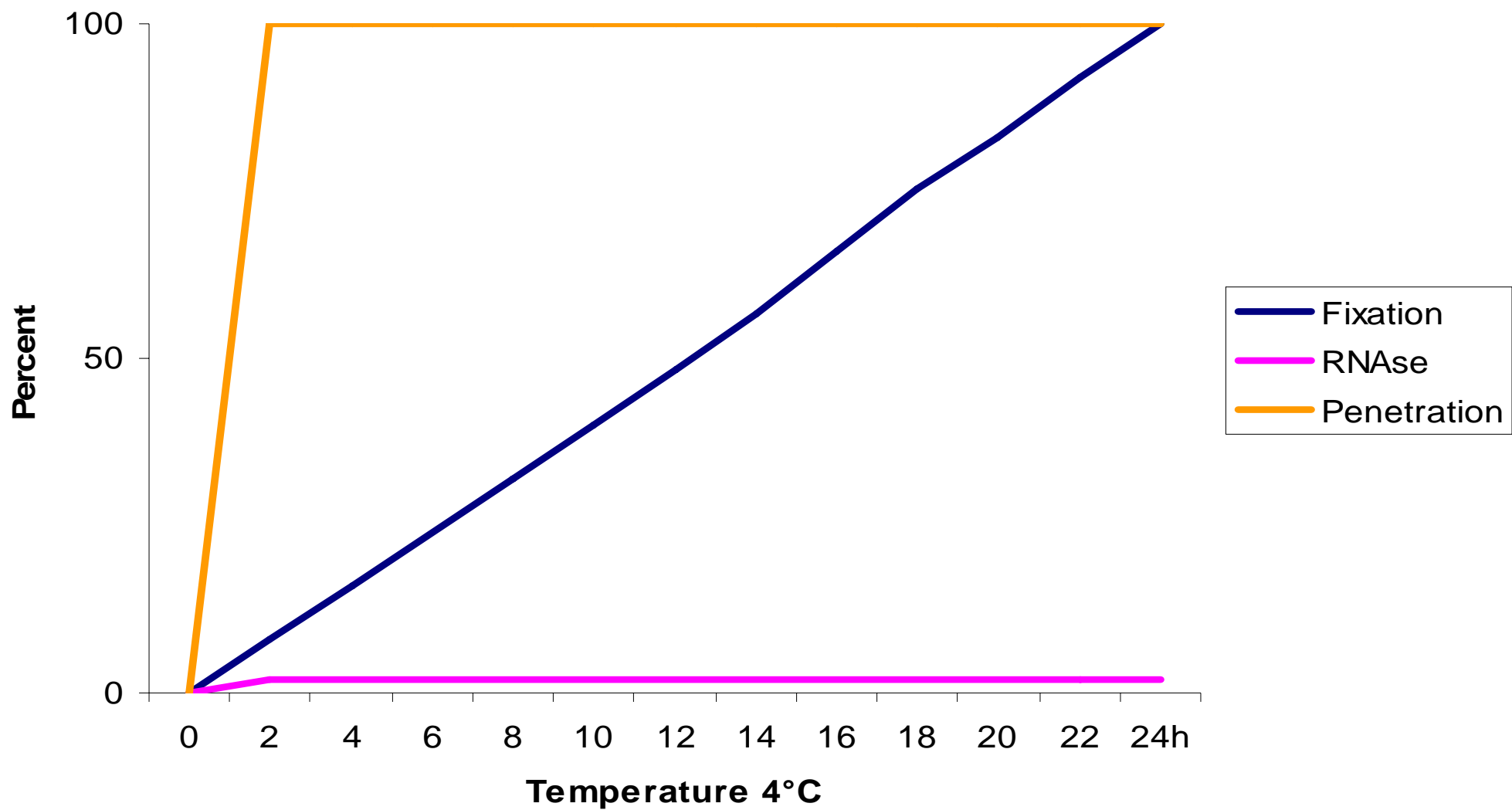


FIGURE 4 The relationship between RNase activity in whole lysates and temperature.

Burka ER ,J.Clin. Invest. 48, 1724,1969

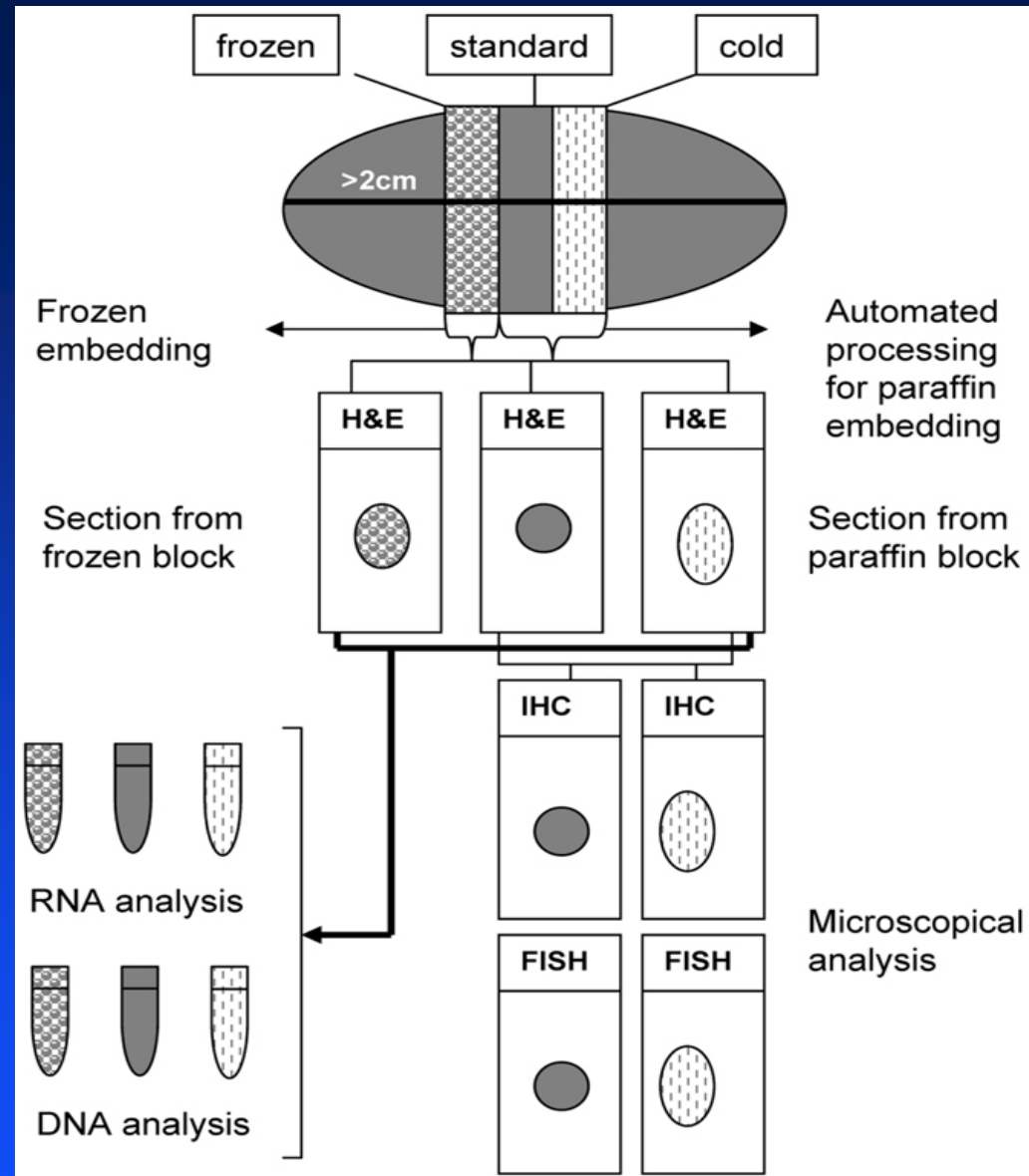




# Experimental protocols for Cold Fixation:

- 1) Fixation in PBF at 4°C for 24 h, followed by heating at 50°C for 20 min using MW, followed by Ethanol dehydration and Paraffin embedding.
- 2) Fixation in PBF at 4°C for 24 h, followed by heating at 90°C for 5 min, followed by Ethanol dehydration and Paraffin embedding.
- 3) Fixation in PBF at 4°C for 24 h, followed by 95% Ethanol at 4°C for 2 h, then standard Ethanol dehydration and Paraffin embedding.

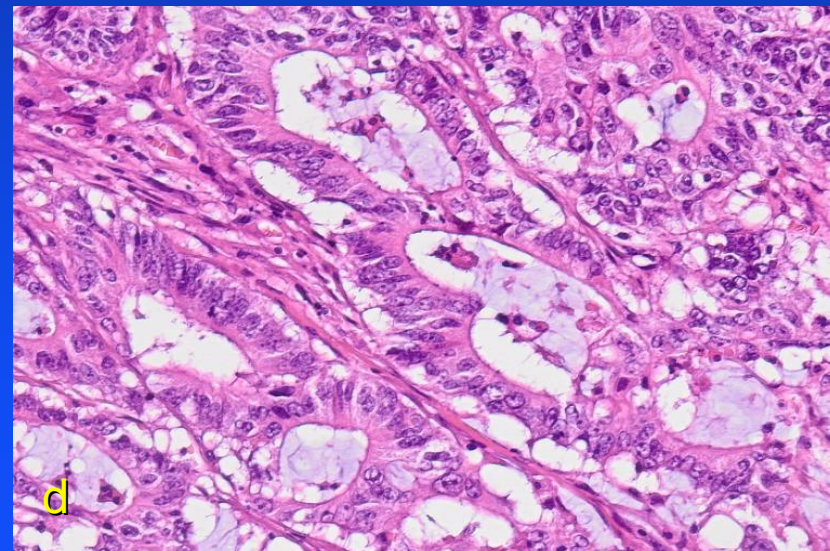
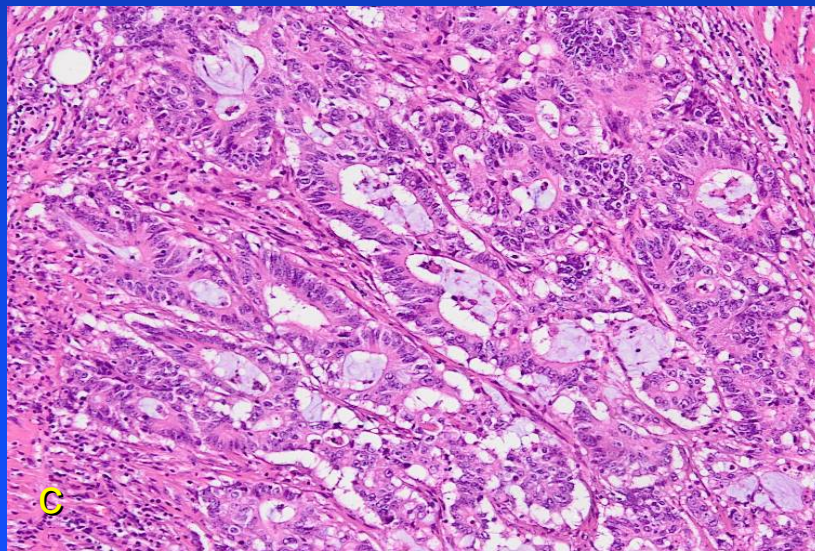
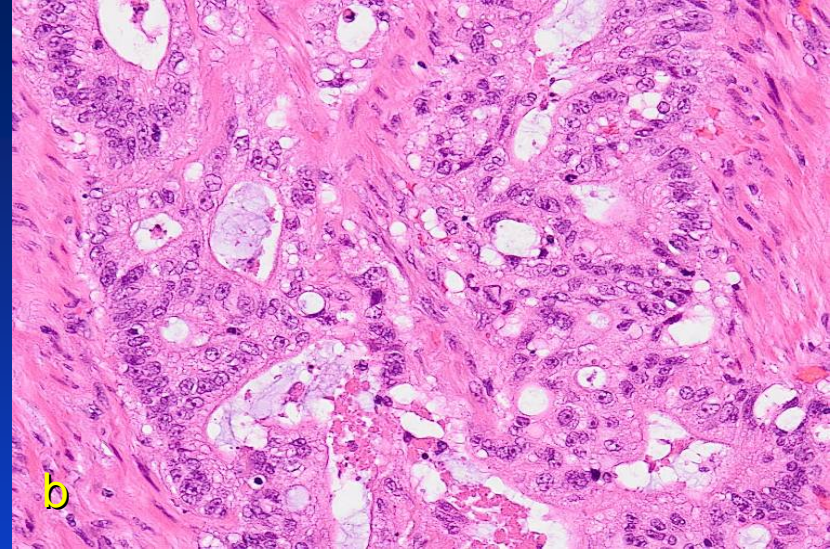
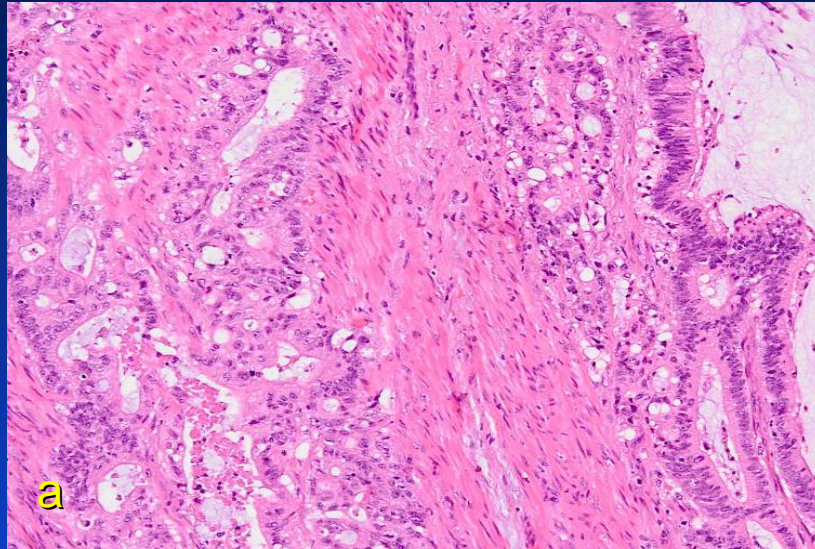
**Cases of:**  
**Breast Cancer**  
**Colon Cancer**  
**Stomach cancer**  
**Pancreatic cancer**



Study flowchart. Flowchart illustrating the design of the study.

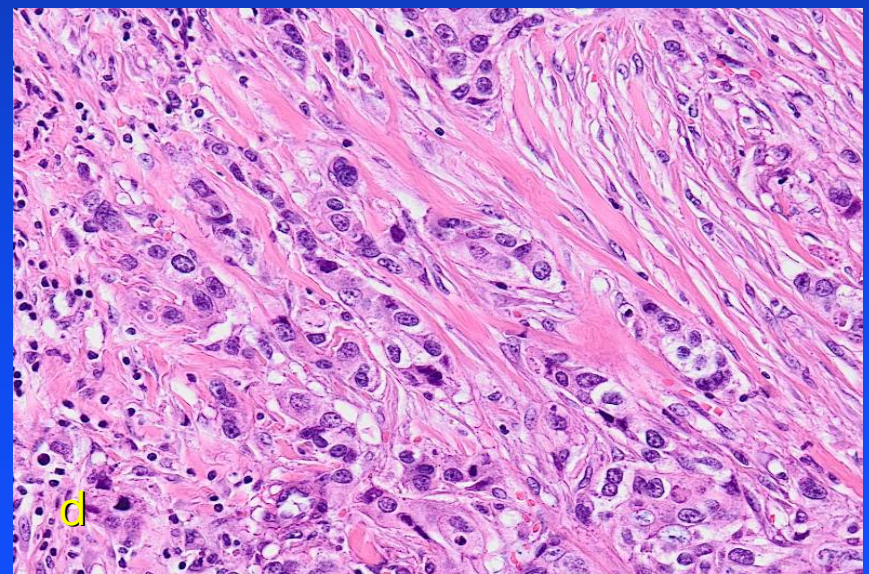
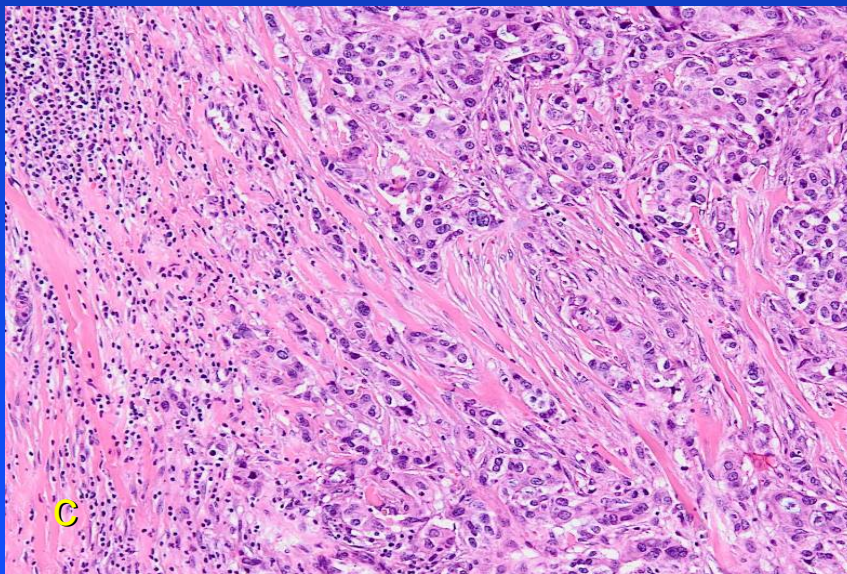
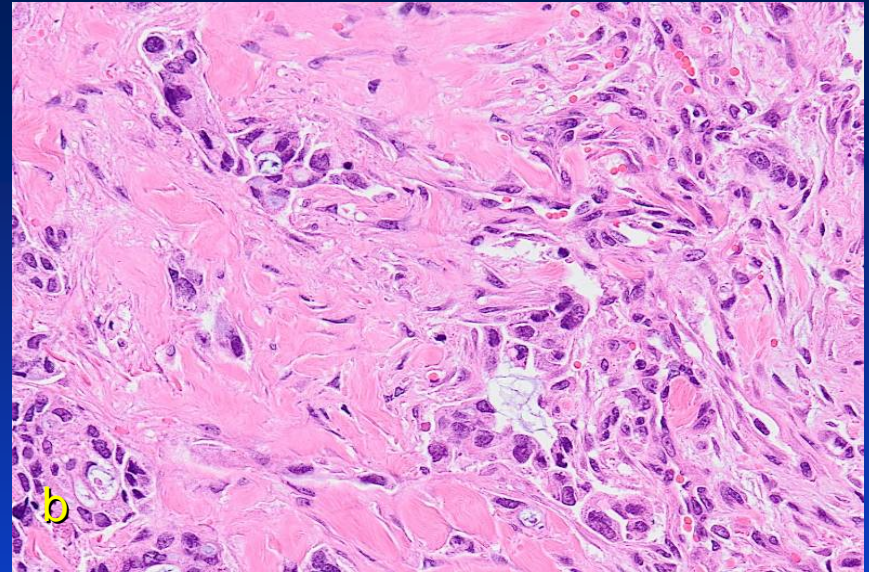
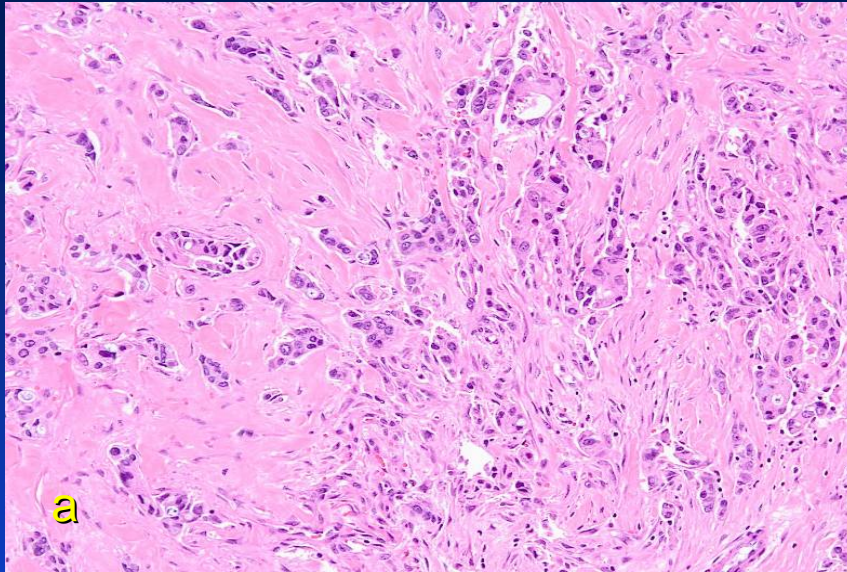


Colon cancer processed either routinely  
(c, d) or following the CF procedure (a, b)



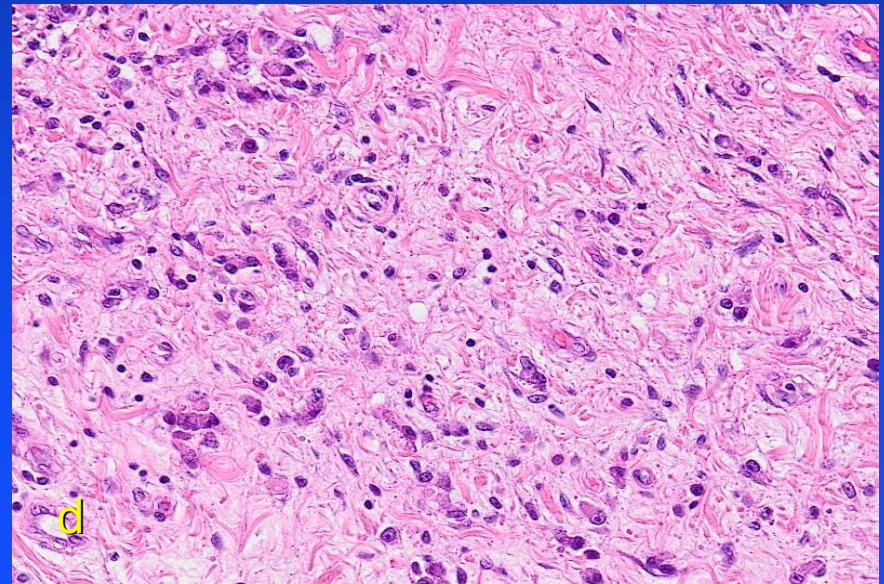
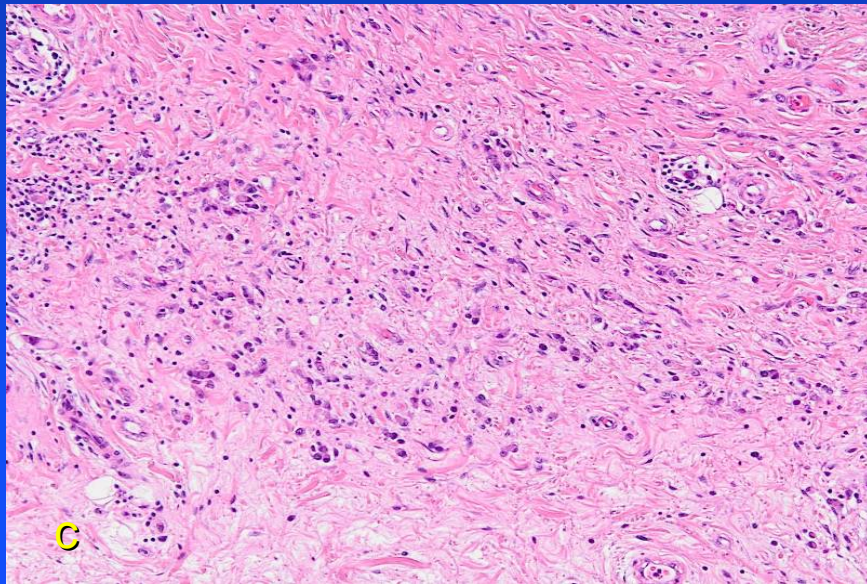
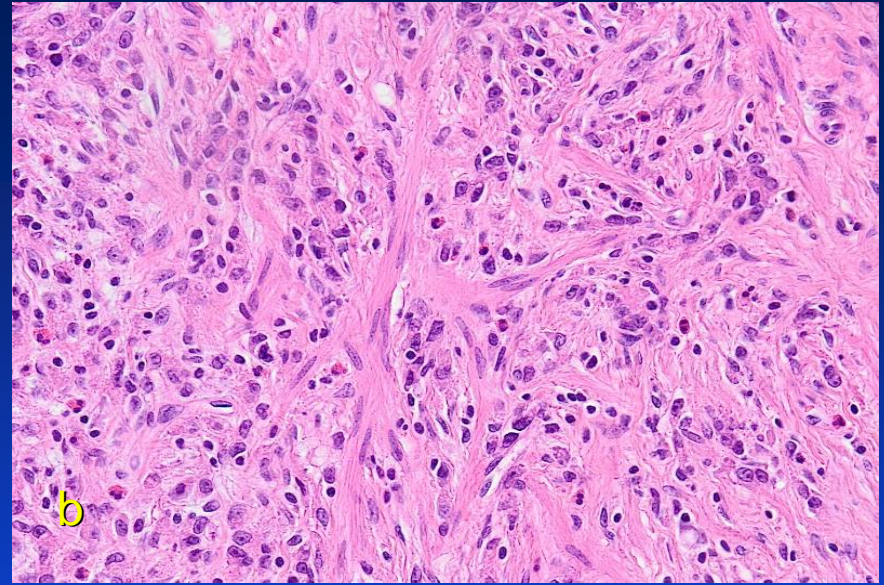
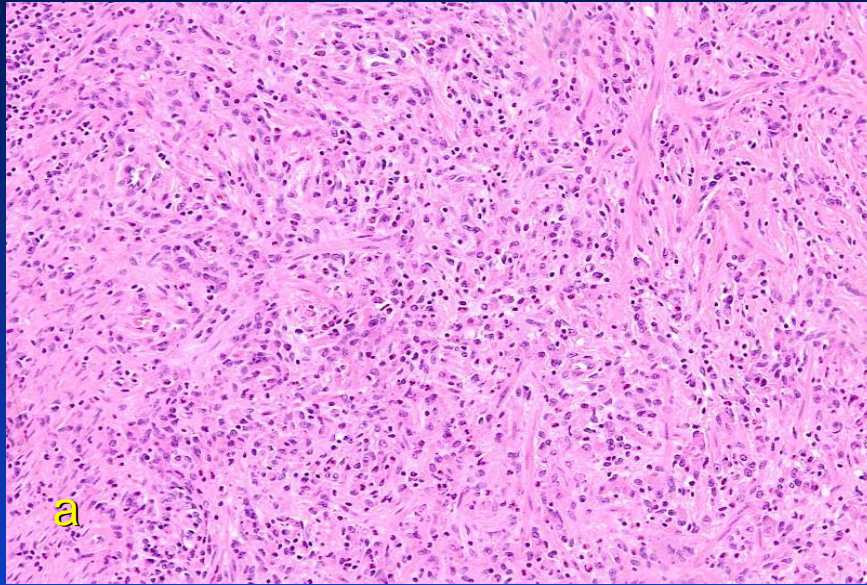


Breast cancer processed either routinely (c, d) or following the CF procedure (a, b)



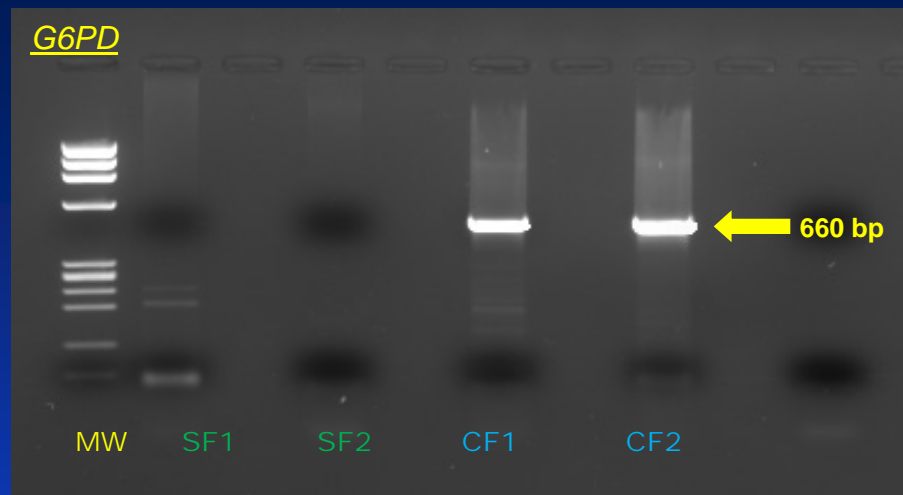


Stomach cancer processed either routinely  
(c, d) or following the CF procedure (a, b)

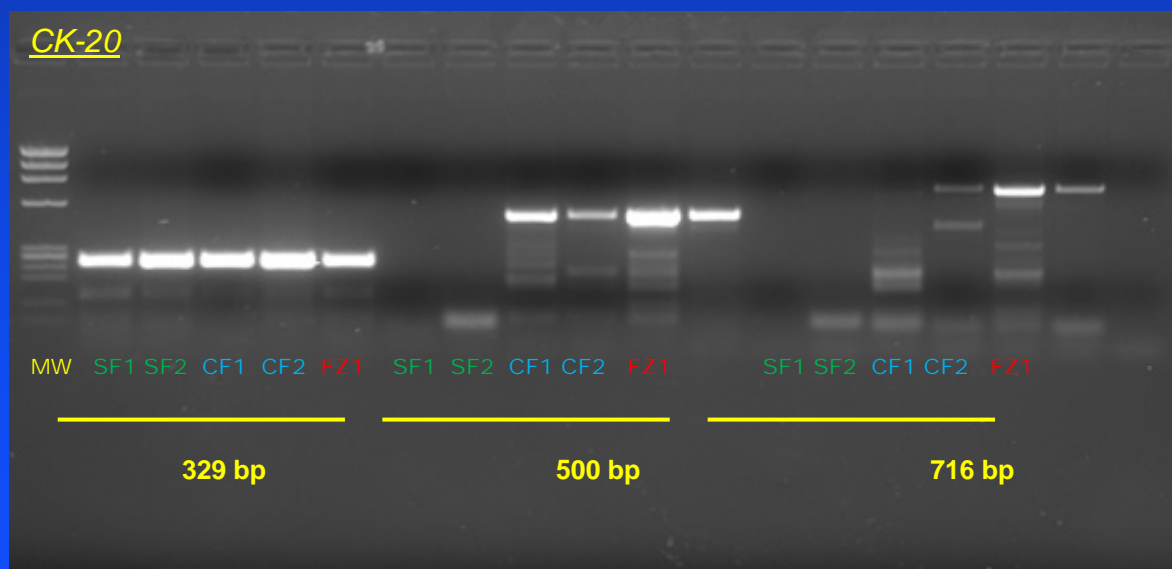


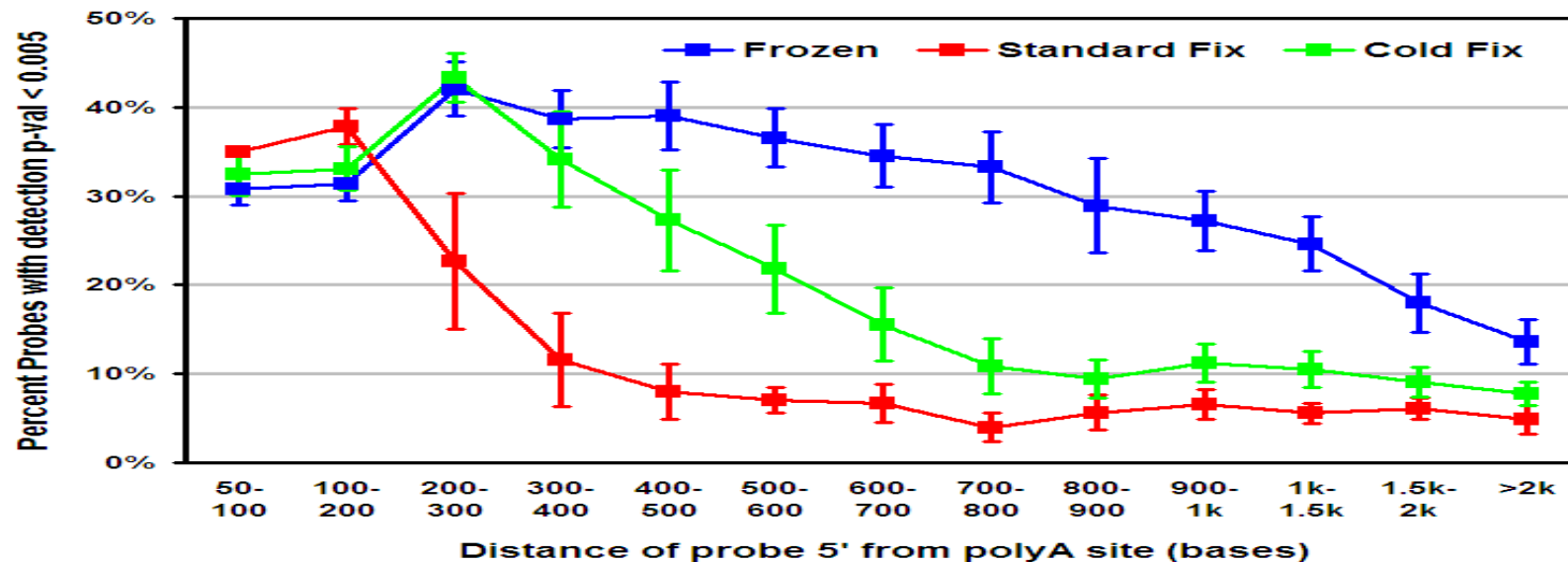


## Standard fixation (SF) procedure vs Cold fixation (CF) procedure

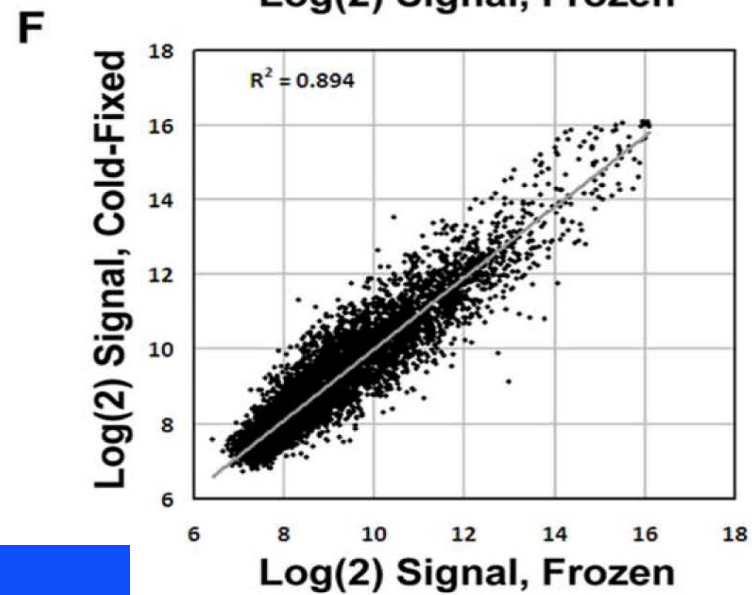
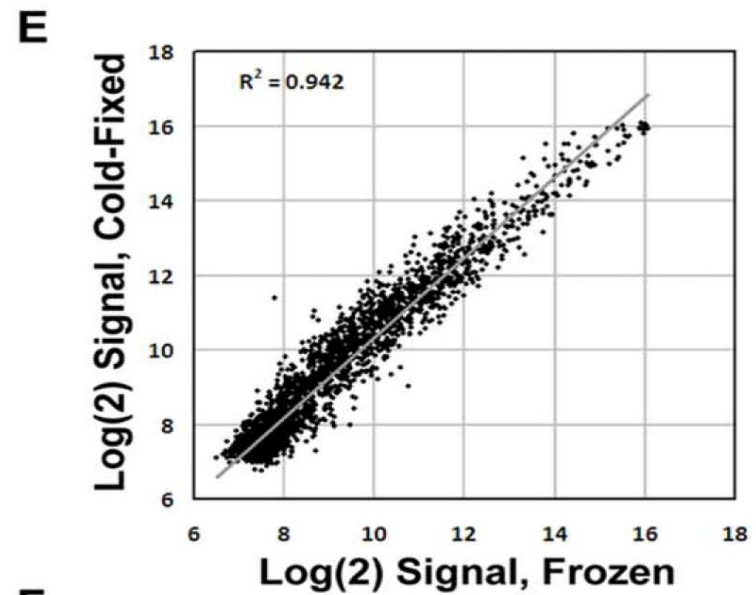
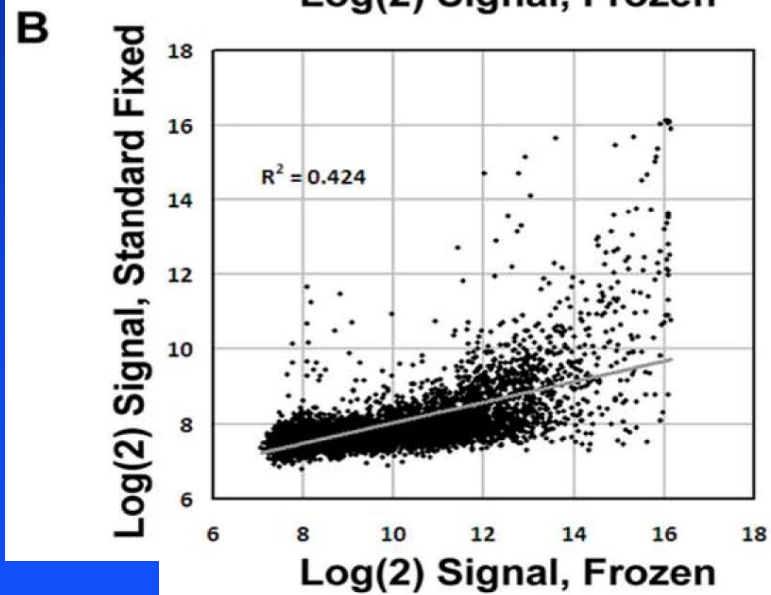
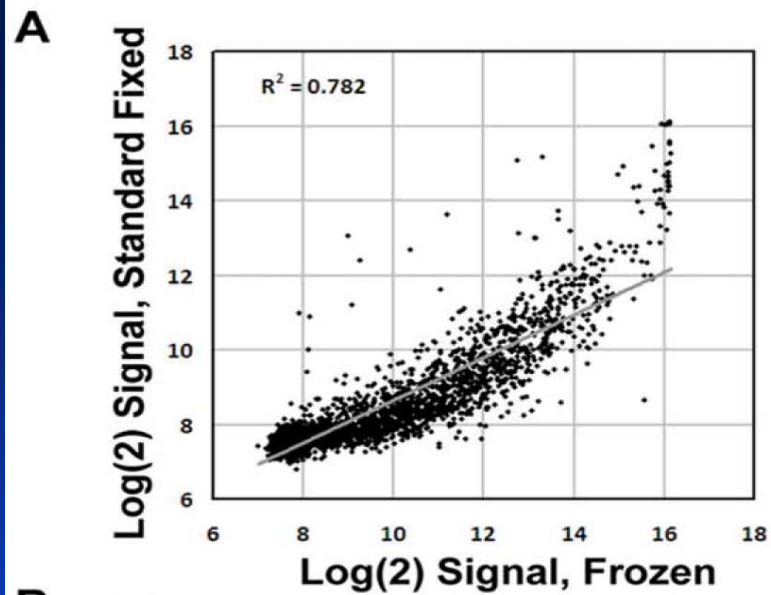


MW Molecular Weight  
 SF1 Standard Fixation Sample 1  
 SF2 Standard Fixation Sample 2  
 CF1 Cold Fixation Sample 1  
 CF2 Cold Fixation Sample 2  
 FZ1 Frozen Sample 1



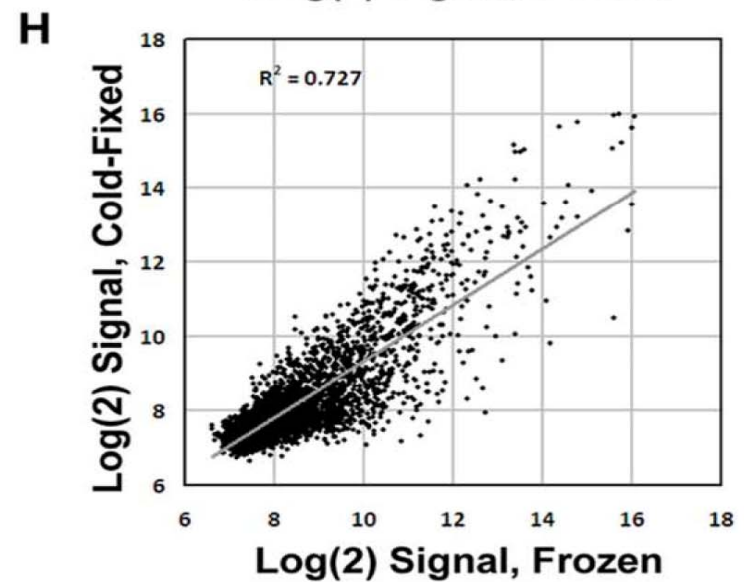
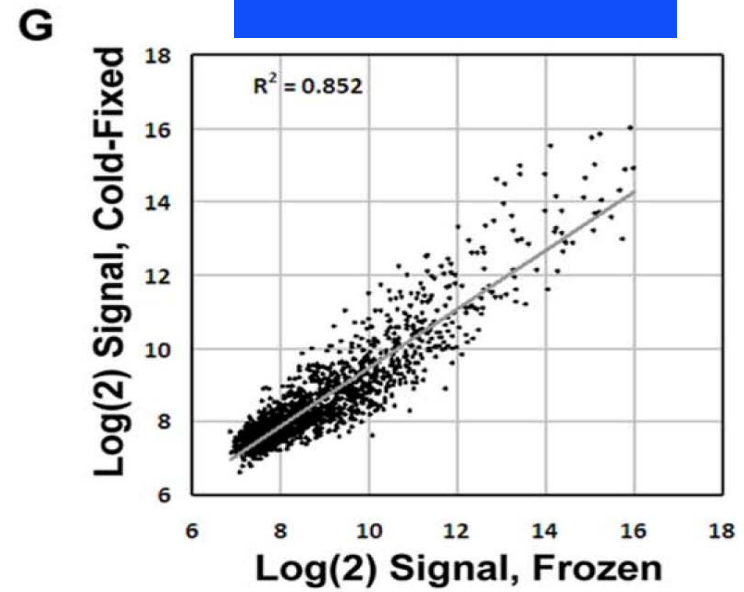
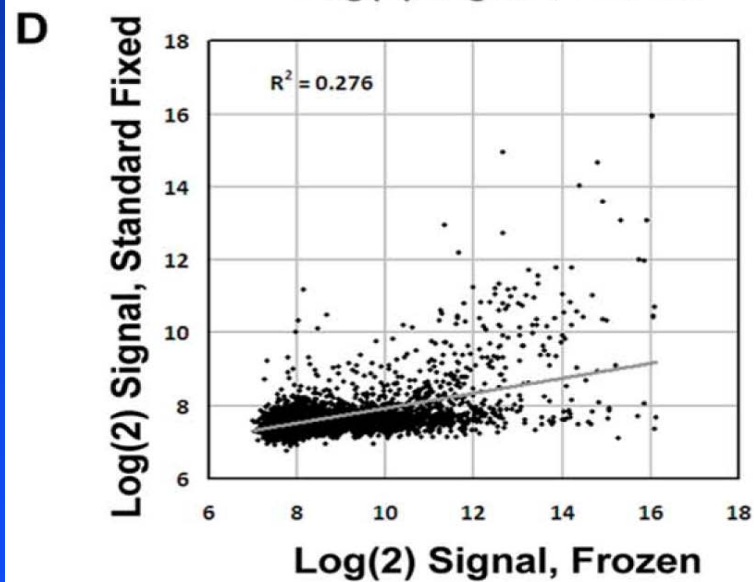
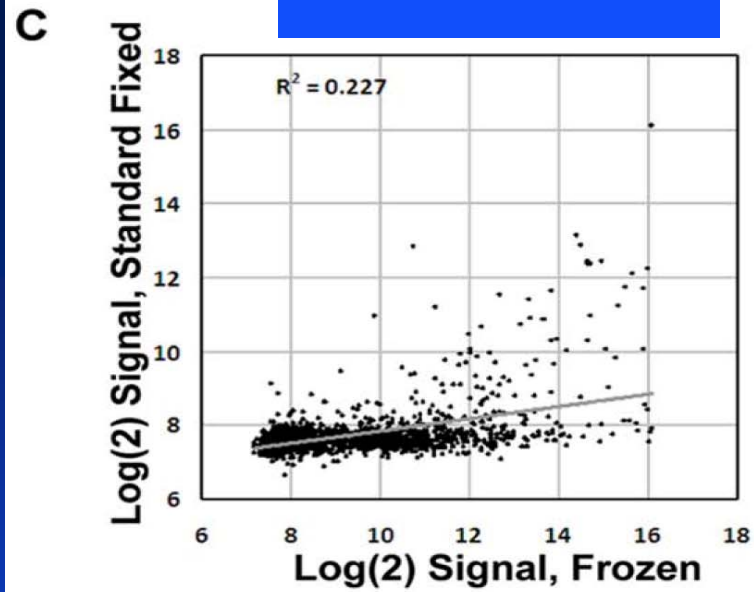


**Bussolati et al., Figure 3.** Messenger RNAs from Cold-Fixed samples are detected by microarray probes hybridizing more than 500b upstream from the reverse transcription start site. Graph showing the fraction of probes with detectable signal (y-axis) for each bin of distance of target sequence from the mRNA poly(A) site from which RT is initiated (x-axis).



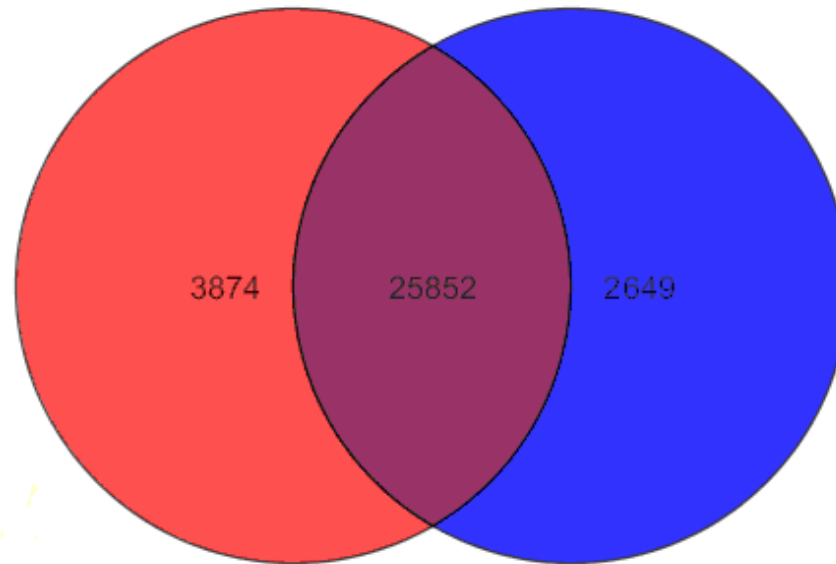
The plots show probes of less than 200 bases (A, E) and 200-500 bases (B, F).





The plots show probes of 500–700 bases (C, G), more than 700 bases (D, H)

Venn diagram comparing the genes detected in the fresh sample (red) and the cold fixation sample (blue)



**Venn diagram comparing in parallel the genes detected in 2 fresh samples (red) and 2 cold fixed (CFFPE) samples (blue) from the same cases of breast cancer. Using the software Agilent Genespring 11.5.1 we generated lists of genes detected in cold fixed and fresh sample. The number of genes detected in the fresh samples was 29726 and in the cold fixed samples was 28501 genes. The number of genes detected in the fresh samples was only slightly (4.12%) higher than in the cold-fixed ones.**

**PLoS One.** 2011;6(6):e21043. Epub Jun 15

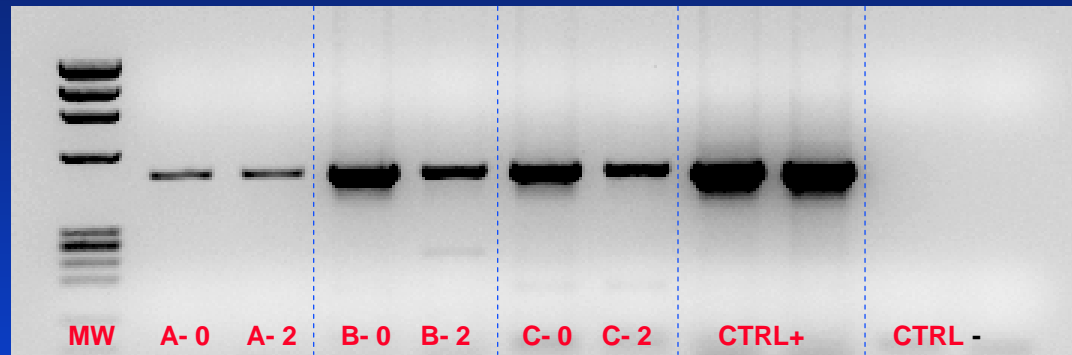
# **A formalin fixation procedure preserving nucleic acid integrity.**

**Gianni Bussolati, Laura Annaratone, Enzo Medico,  
Giuseppe D'Armento and Anna Sapino.**



**The Cold Fix  
Apparatus**

# Assessment of RNA conservation in Cold-Formalin Fixed Paraffin Embedded Tissue blocks after two years

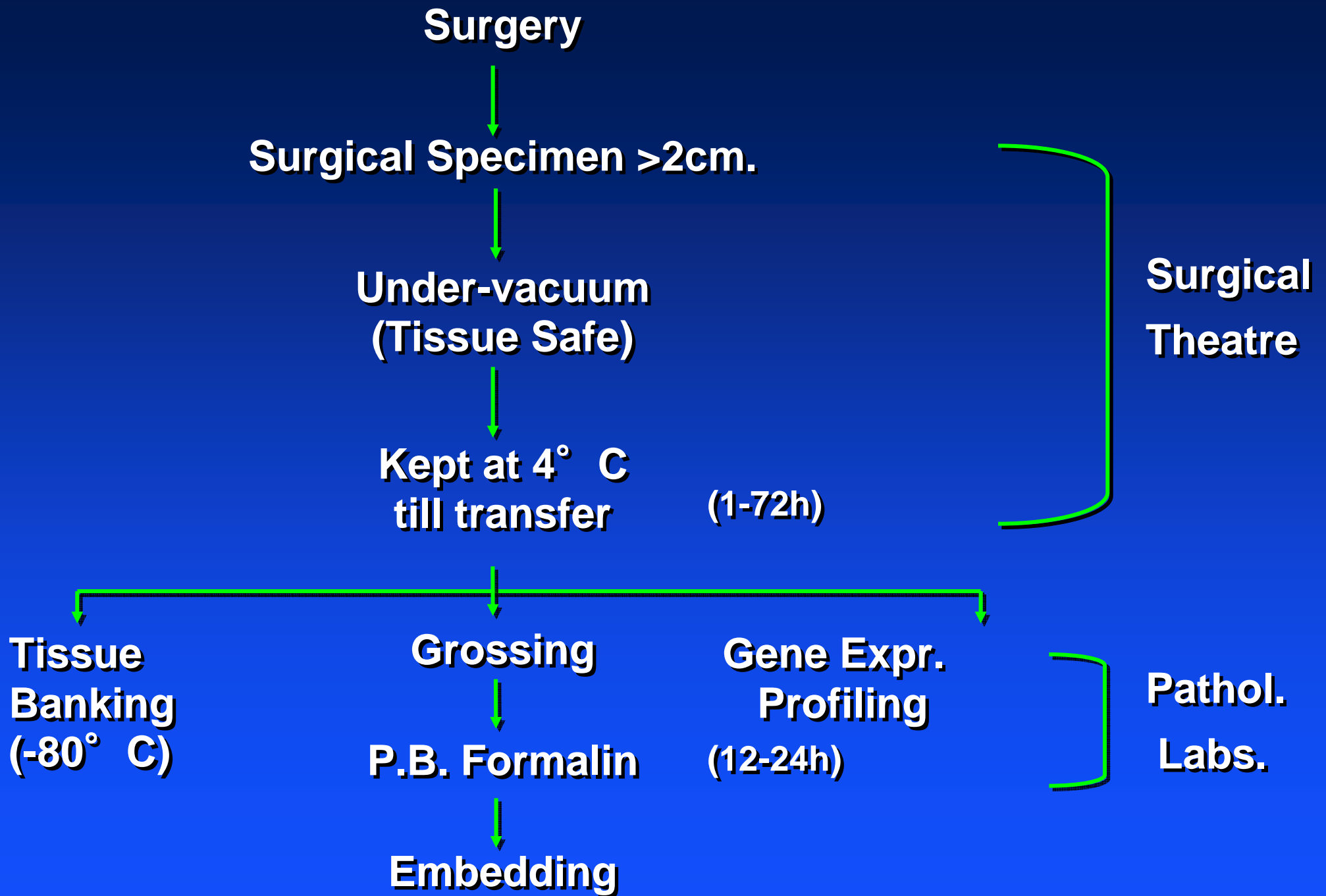


**RT-PCR for detection of CK-20 in colorectal cancer (500bp).**

## Samples:

A- 0	RNA extraction year 2010
A- 2	RNA extraction year 2012
B- 0	RNA extraction year 2010
B- 2	RNA extraction year 2012
C- 0	RNA extraction year 2010
C- 2	RNA extraction year 2012

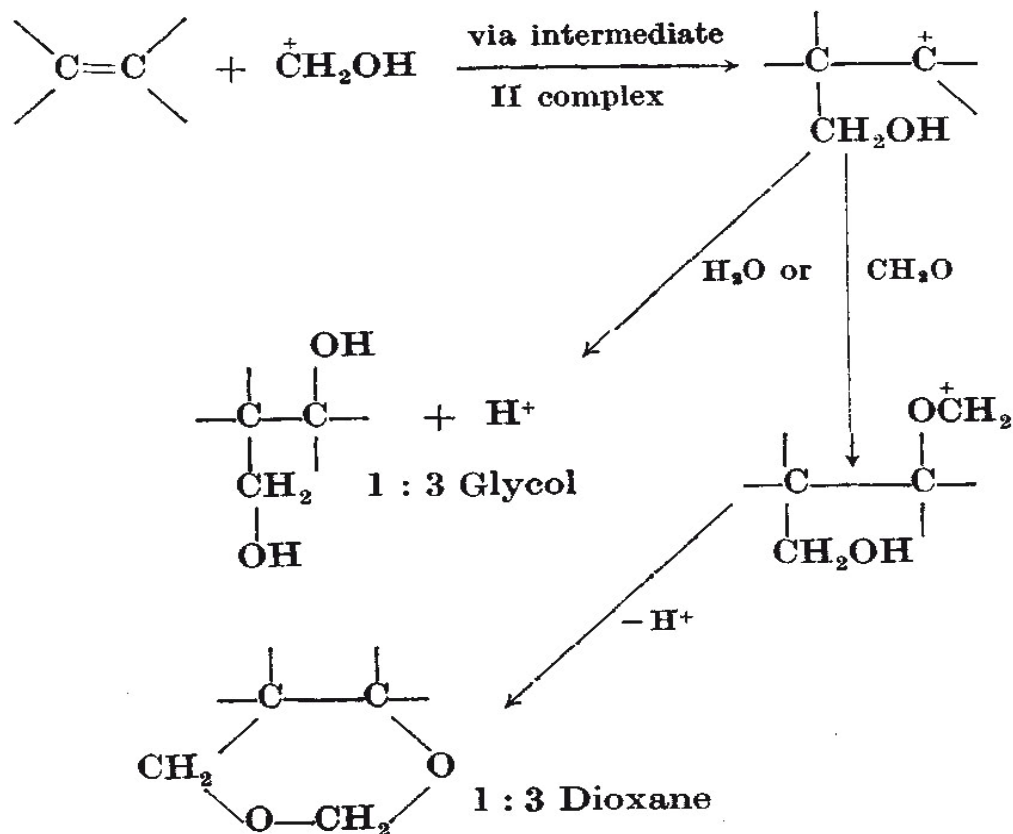




(a) Formation of electrophilic species:



(b) Reaction with double bond:



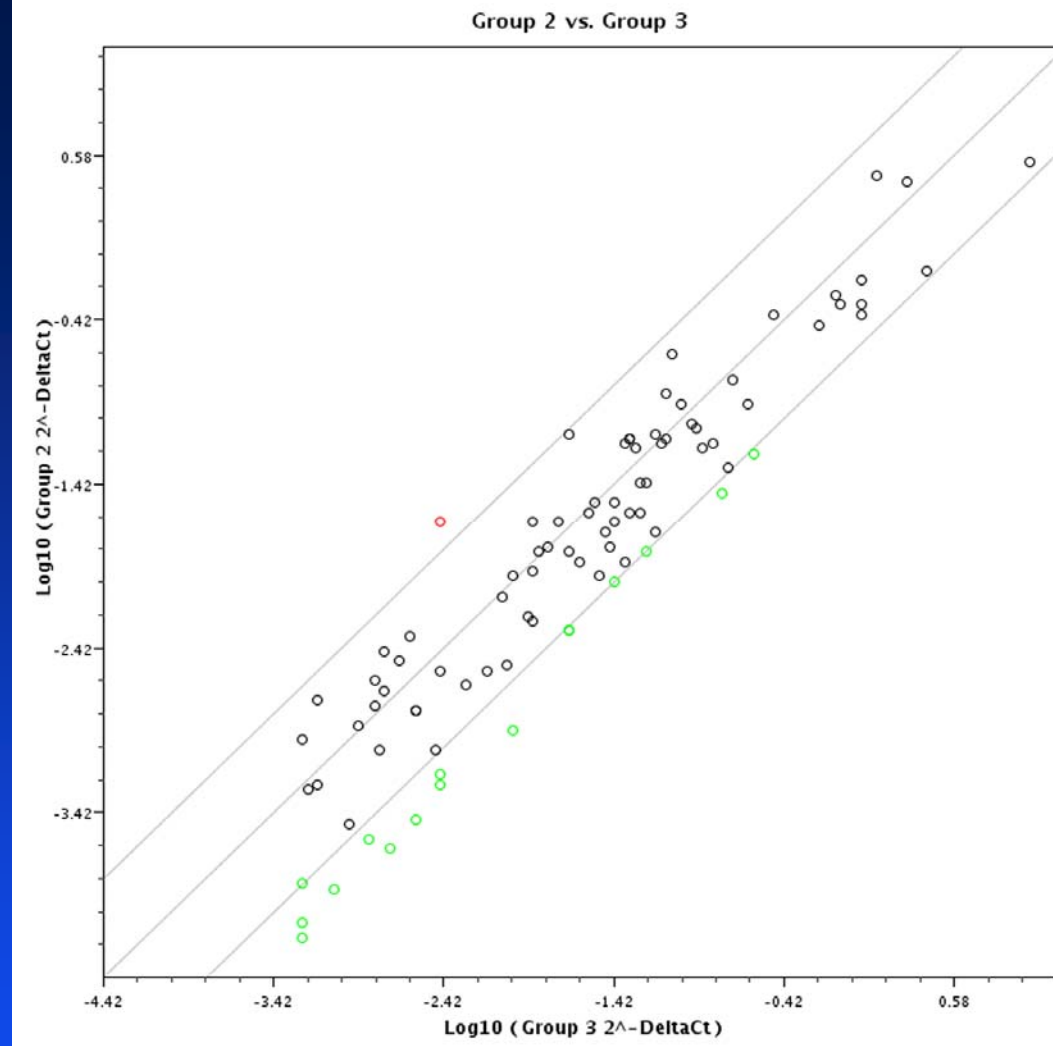
## Reaction of Formaldehyde with Unsaturated Fatty Acids during Histological Fixation

DENNIS JONES  
G. AUSTIN GRESHAM

Department of Pathology,  
University of Cambridge.

Nature. 1966 25;210:1386-8.





**The scatter plot puts in comparison 2<sup>-DeltaCt</sup> values obtained from a fixed sample with 2<sup>-DeltaCt</sup> values obtained from the same sample, but frozen, both evaluated with Breast Cancer RT2 Profiler PCR Array (QIAGEN) and analysed with QIAGEN Web-Based PCR Array Data Analysis Software.**