SOME POSSIBLE ALTERNATIVES TO FORMALIN

- METHACARNOY: MeOH 60ml; $CHCl_3$ 30 ml, HAc 10 ml
- FINEFIX®
- ALCOLIN™
- RCL2®
- HOPE®
- PaxGene®
- Glyoxal

Table 1. Examples of Commercially Available or Published Reagents Tested in the Screening Program

Reagent	Vendor or composition
RNAlater	Life Technologies, Carlsbad, CA
Michel's	Zeus Scientific, Raritan, NJ
HistoChoice	Amresco Inc., Solon, OH
Prefer	Anatech Ltd, Battle Creek, MI
Z5	Anatech Ltd, Battle Creek, MI
FineFIX	Milestone, Bergamo, Italy
Hope	DCS, Hamburg, Germany
RCL2	Alphelys, Plaisir, France
Streck Tissue Fixative	Streck, Omaha, NE
PreservCyt	Hologic, Bedford, MA
AquaPreserve	MultiTarget Pharmaceuticals, Salt Lake City, UT
RNAsecure	Life Technologies, Carlsbad, CA
S.T.A.R. buffer	Roche Molecular Biochemicals, Penzberg, Germany
UMFix	Sakura Finetek, Torrance, CA
Ethanol	70% ethanol p.a. (pro analysis)
Methacarn	Methanol, chloroform, acetic acid
Carnoy's fixative	Ethanol, chloroform, acetic acid
Farmer's	Ethanol, acetic acid
Wolman's solution	Ethanol, acetic acid
Delaunay's	Acetone, ethanol, trichloroacetic acid
FAA	Formalin, acetic acid, ethanol
FPA	Formalin, acetic acid, propionic acid
Davidson's fixative	Formalin, ethanol, acetic acid, water

NEW GENERATION FIXATIVES

HOPE

HOPE fixation is based on a incubation of the fresh tissue in a aqueous protection solution, ON at 0-4C.

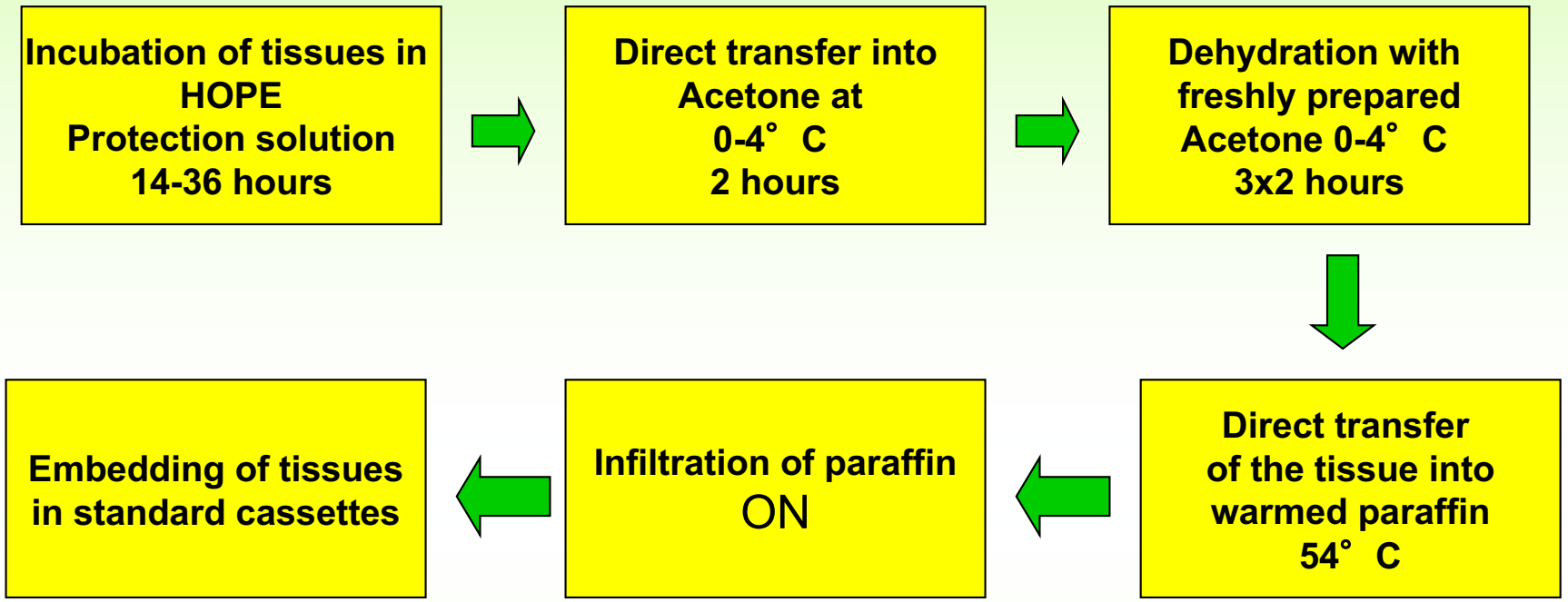
Afterwards tissues are incubated in acetone at 0-4° C.

Later on tissues can be paraffin embedded.

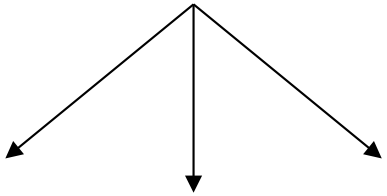
PROTECTION SOLUTION

- It contains a mixture of different amino-acids (with concentrations of 10–100 mM), and exhibits a pH of 5.8–6.4 at room temperature. It contains HEPES and glutammic acid.
- **It penetrates the tissues by diffusion, in the manner of immersion fixatives.**
- This step reduces the destructive effects of the next step, i.e., incubation with organic solvent such as acetone.
- **This procedure has been optimized to precipitate the compounds of the protection solution during infusion with acetone in the second step. This removes most of the HOPE solution from the tissue, leaving only small amounts which are necessary for further protection in the next fixation step.**

TISSUE PROCESSING WITH HOPE



"SIMPLE" Alcoholic fixatives



RCL2:
FIXATION ON 4° C

Alcolin:
FIXATION rt ON

MethaCarn:
60% MeOH, 30% $CHCl_3$,
10% GLACIAL HAc
FIXATION rt ON

RSH-2 REDUCTION OF WORKING TIMES

Times are reduced to 30 min for both fine needle biopsies and endoscopic biopsies, to 120-180 minutes for surgical biopsies of more than 3 mm thick. RSH-2 combines microwave radiation with a computerized temperature and time control system in the clearing dehydration phase.

MICROWAVE AND TISSUE CONSERVATION

Diffusion is a key factor in all phases of tissue processing. Exposure to microwaves increases the diffusion coefficient by stimulating it. The result is a significantly lower processing time.

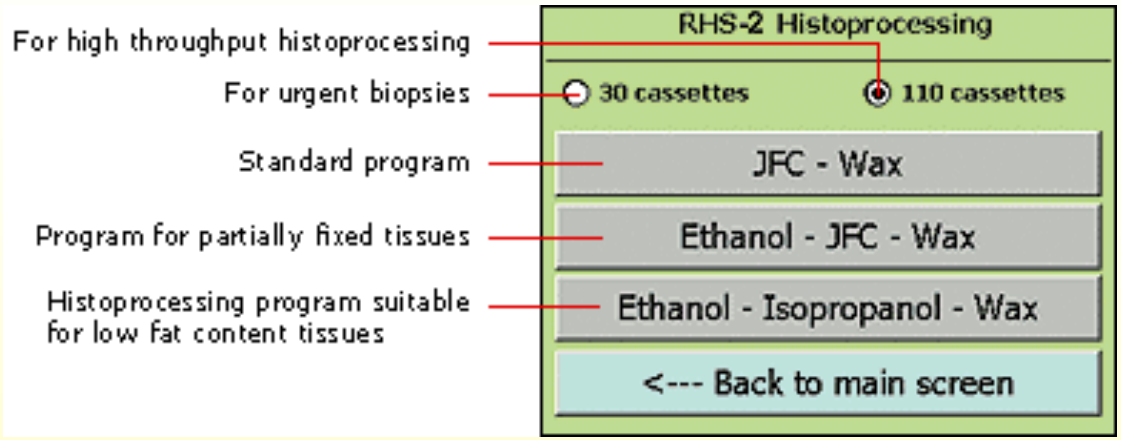
✓ **Tissue processing depends on the fixative used.**

✓ **For the microwave technique a particular coagulant fixative -Kryofix is used. It is a mixture of absolute ethanol and PEG300. The use of this fixative is favored because the tissue is directly immersed in the first processing bath (absolute ethanol).**

✓**The time period required to fix by the use of coagulant fixatives is shorter if compared to formalin.**

ADVANTAGES

- ✓ Biopsies of various sizes can be processed
- ✓ It can process simultaneously different type of tissues
- ✓ No use of toxic reagents
- ✓ A unique solution for dehydration and clearing
- ✓ It can process 110 biopsies in 30-40 min.
- ✓ It can process 110 standard cassettes every 2-3 hours



DEHYDRATION SOLUTION

JFC

- It is used in RSH instruments that combine vacuum with microwave.
- The JFC solution contains absolute EtOH, isopropanol and a long chain hydrocarbon. The last component is a non-toxic and chemically inert organic solvent.
- Dissolves and removes lipids from tissues.
- Under MW the mixture becomes particularly efficient in the simultaneous removal of H_2O and lipids
- The 3 components are miscible.
- The action of the mixture under MW irradiation can be explained in terms of polarity.

DETAILS

- To achieve a good extraction, the polarity of the solvent used must be similar to the polarity of the impurities to be removed, here lipids and H_2O.
- Tissue lipids are mainly composed of glycolipids and phospholipids. The former are moderately polar, the latter are highly polar.
- Under MW irradiation tissue lipids and the molecules of H_2O acquire energy as EtOH and Isopropanol from JFC do so. As a result, impurities leave the tissues and EtOH and Isopropanol from JFC enter it.
- The third component is non-polar but its activity in eliminating lipids is enhanced by MW and the presence of EtOH and Isopropanol.
- The non-polar component acts on non-polar fat chains while the JFC's EtOH and Isopropanol act on the polar portions of lipids $\Rightarrow$Efficient lipids extraction.

Paraffin

- ✗ It absorbs the energy of the MW
- ✗ It's transparant to MW

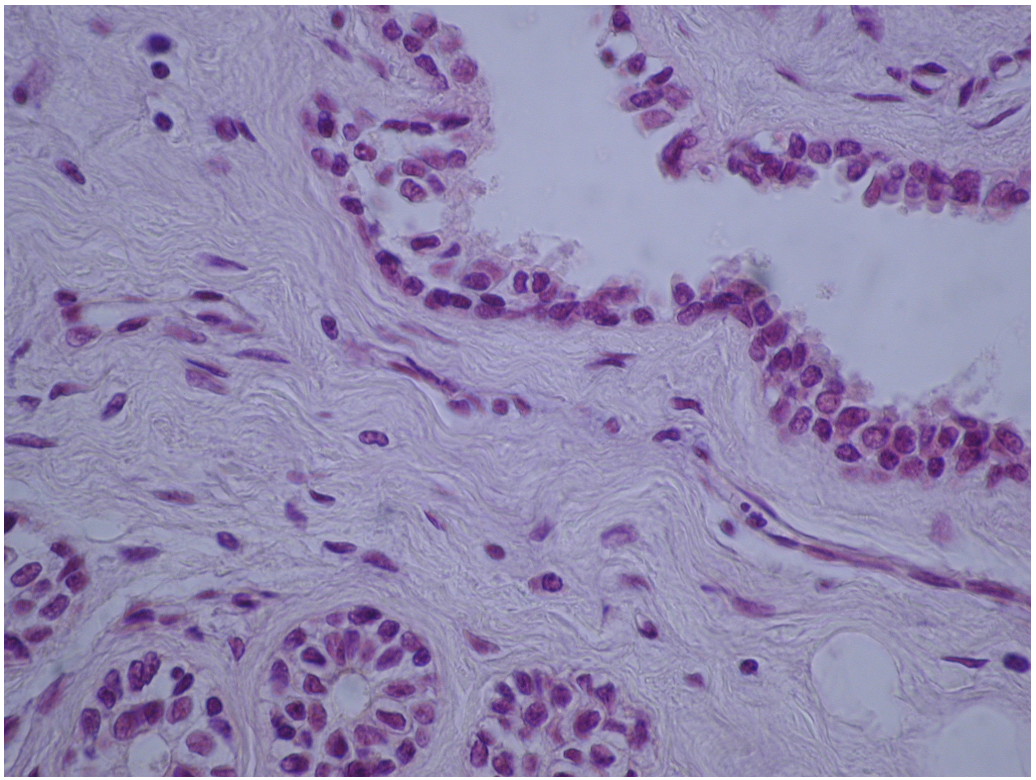
Vuoto

- ✗ This procedure allows the isopropanol to be removed in the inclusion step.
- ✗ Vacuum at the same time as the MW to eliminate traces.

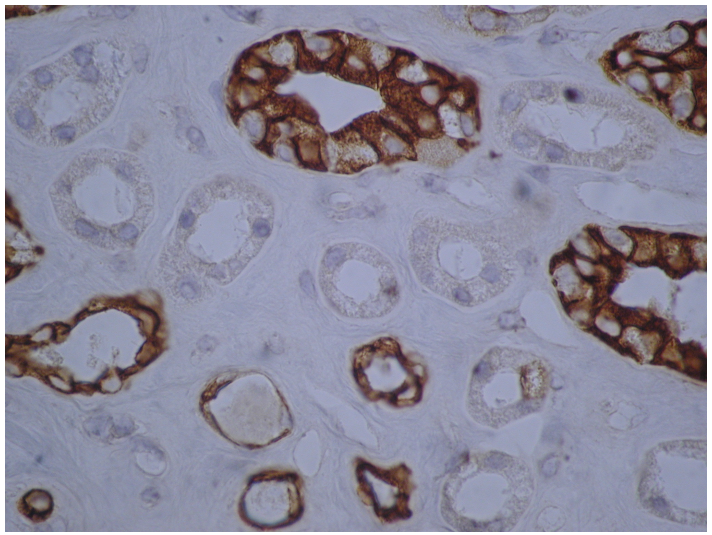
Vantaggi

- ✗ Under Vacuum temperatures are lower
- ✗ In the inclusion EtOH and isopropanol are removed in the gaseous phase from the tissues (sucked away by the means of transport: air)

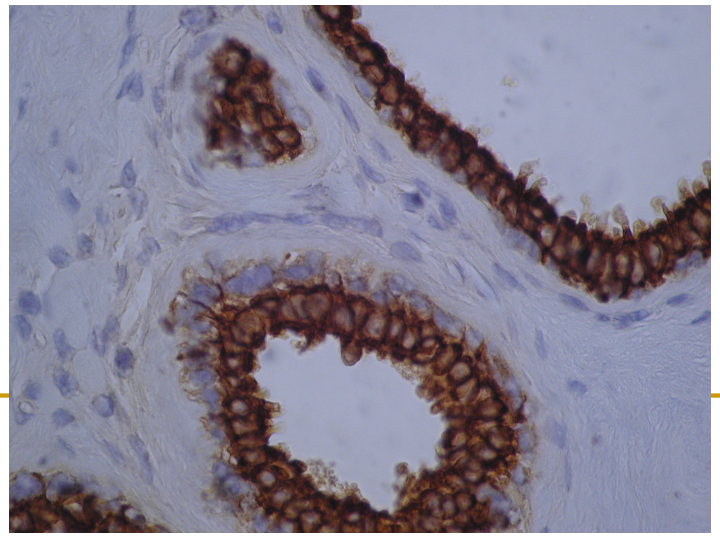
Morphology and immunohistochemistry



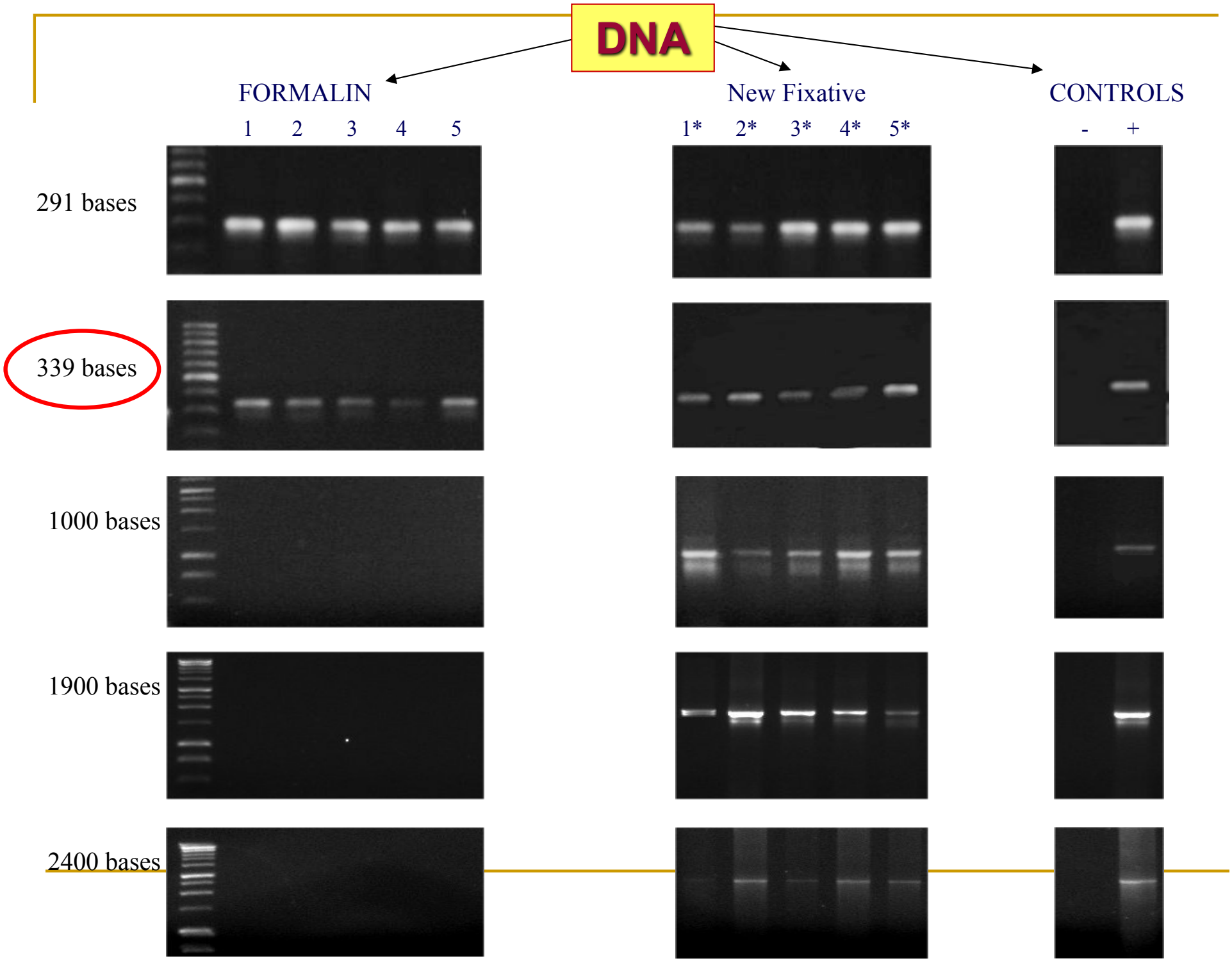
Breast, H&E

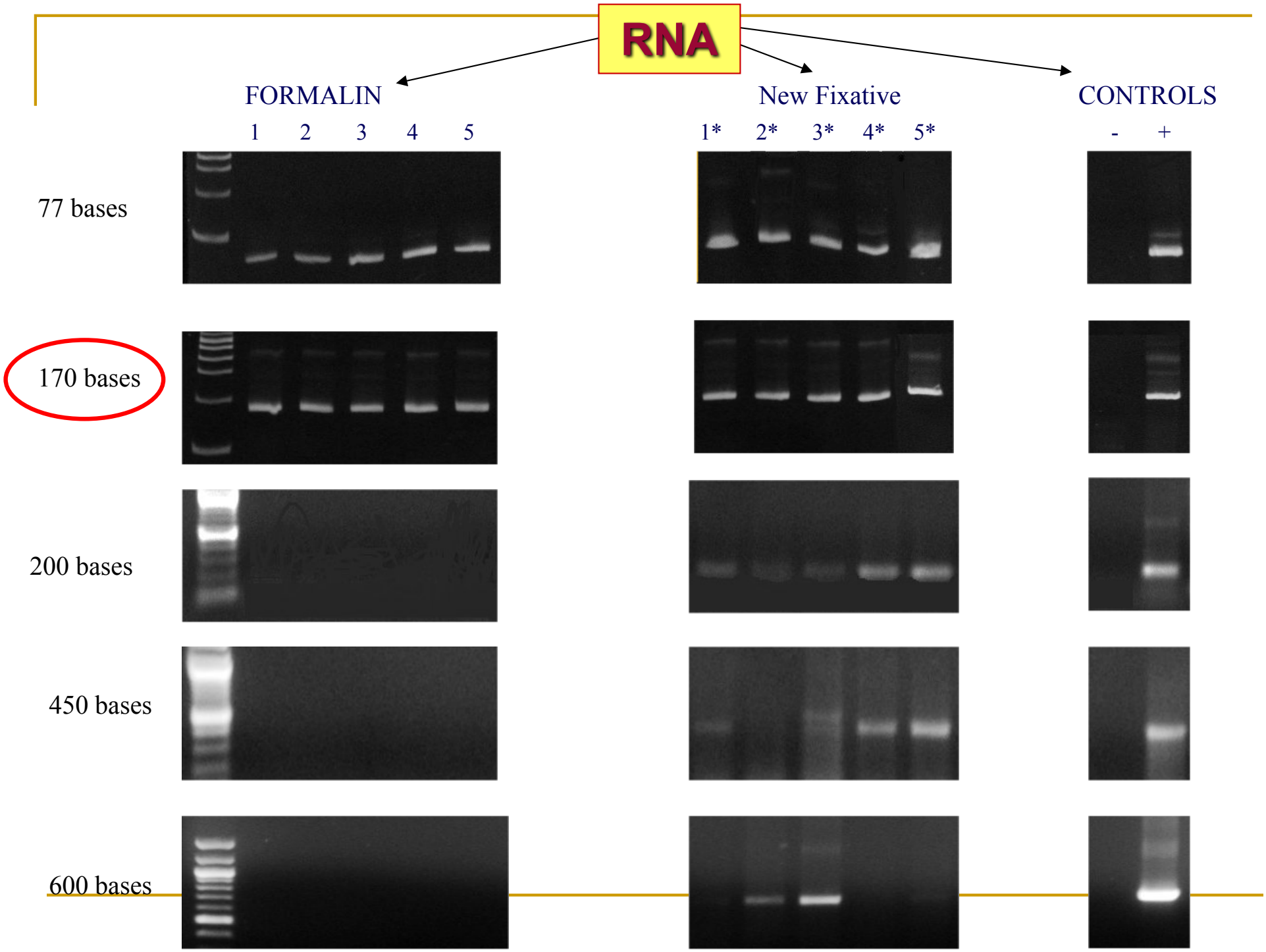


Kidney, IHC

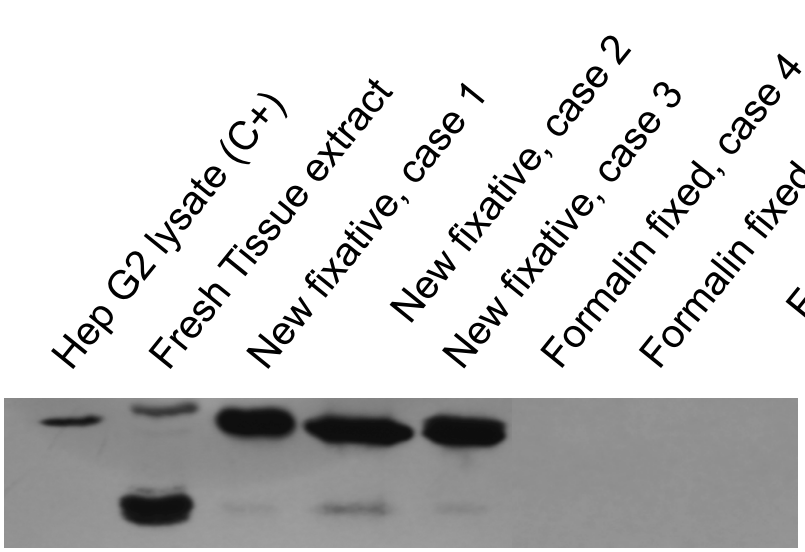


Breast, IHC

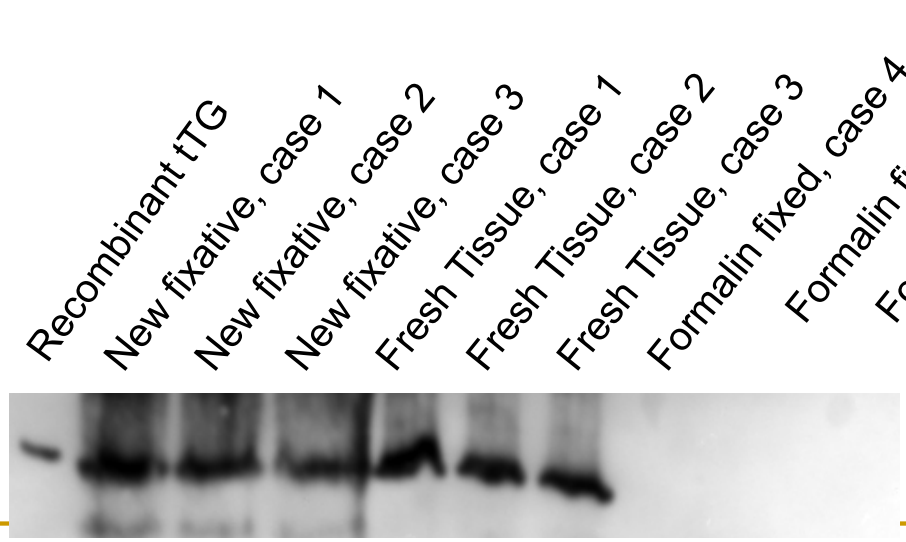

DNA
FORMALIN
New Fixative
CONTROLS
1 2 3 4 5
1* 2* 3* 4* 5*
- +
291 bases
339 bases
1000 bases
1900 bases
2400 bases


RNA
FORMALIN
New Fixative
CONTROLS
1 2 3 4 5
1* 2* 3* 4* 5*
- +
77 bases
170 bases
200 bases
450 bases
600 bases

Western Blot analysis

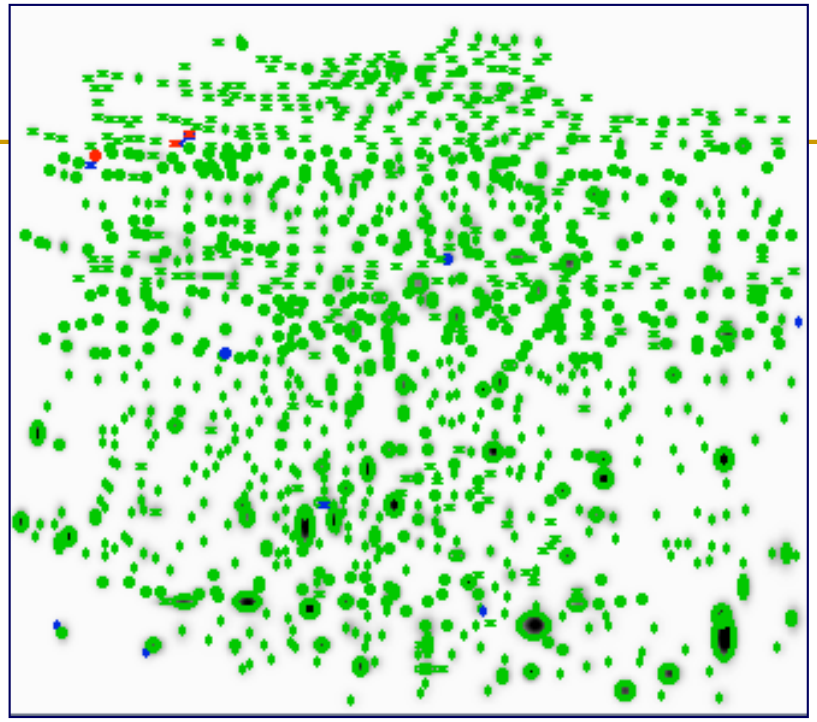


WB against α-tubulin, around 50 kDa



WB against human tTG, 77 kDa

New Fixatives



Fresh Tissue

Stanta G, Pozzi Mucelli S, Petrera F, Bonin S, Bussolati G, "A novel fixative improves opportunity of nucleic acids and proteomic analysis in human tissues", Diagn. Molec. Pathol 15:115-23;2006

DNA

Lenght (bases)	Formalin	RSH
291	5/5	5/5
339	5/5	5/5
1000	0/5	5/5
1900	0/5	5/5
2400	0/5	5/5

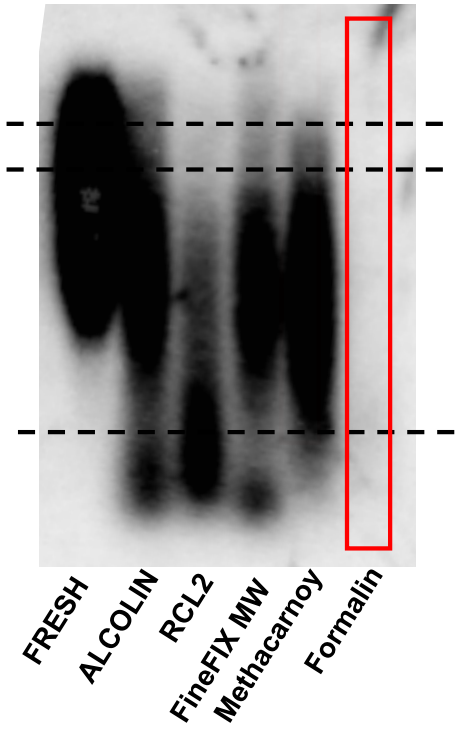
RNA

Lenght (bases)	Formalin	RSH
77	5/5	5/5
170	5/5	5/5
200	0/5	5/5
450	0/5	5/5
600	0/5	3/5

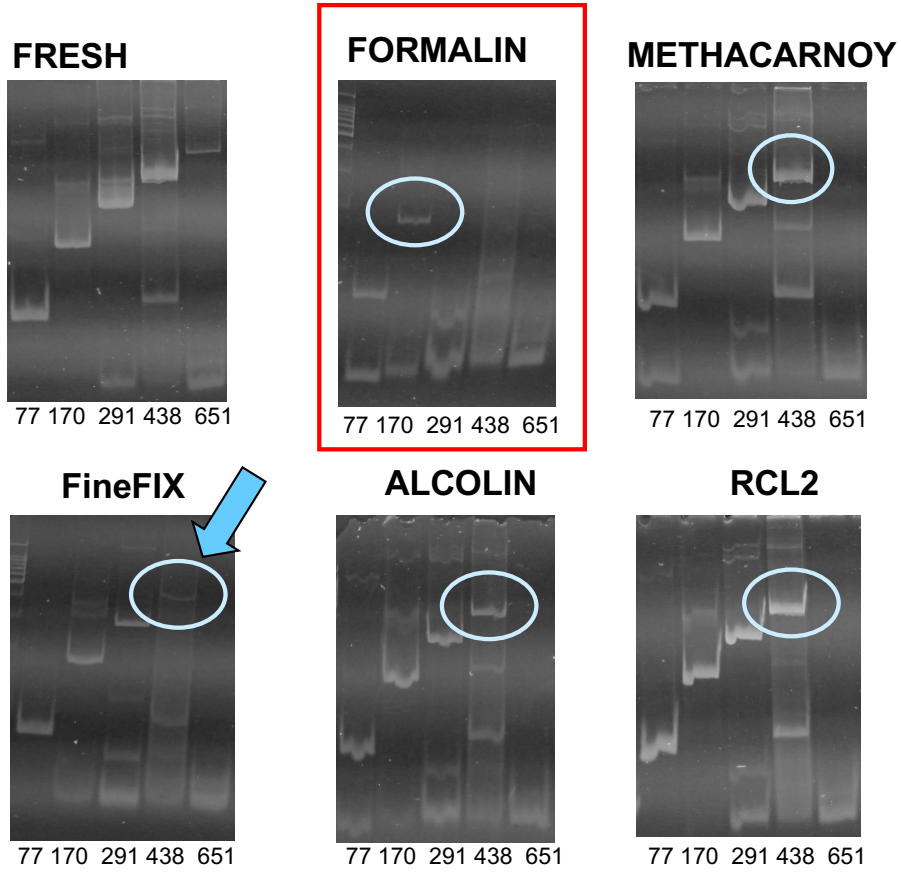
DIFFERENCES WITH CONVENTIONAL PROCESSING

- Fixation is optimized and is conducted before histological processing
- Dehydration in a single step instead of 2 to 6 used in conventional procedures.
- No hydrated alcohols are used
- JFC is not very active at RT. The intermediate used in the traditional technique involves from 2 to 4 changes. Here 1.
- Dehydration and clearing in a single step
- The paraffin bath reaches 82 C, about 20 ° higher than the traditional technique. One impregnation is enough.

NORTHERN BLOT with poly-dT PROBE



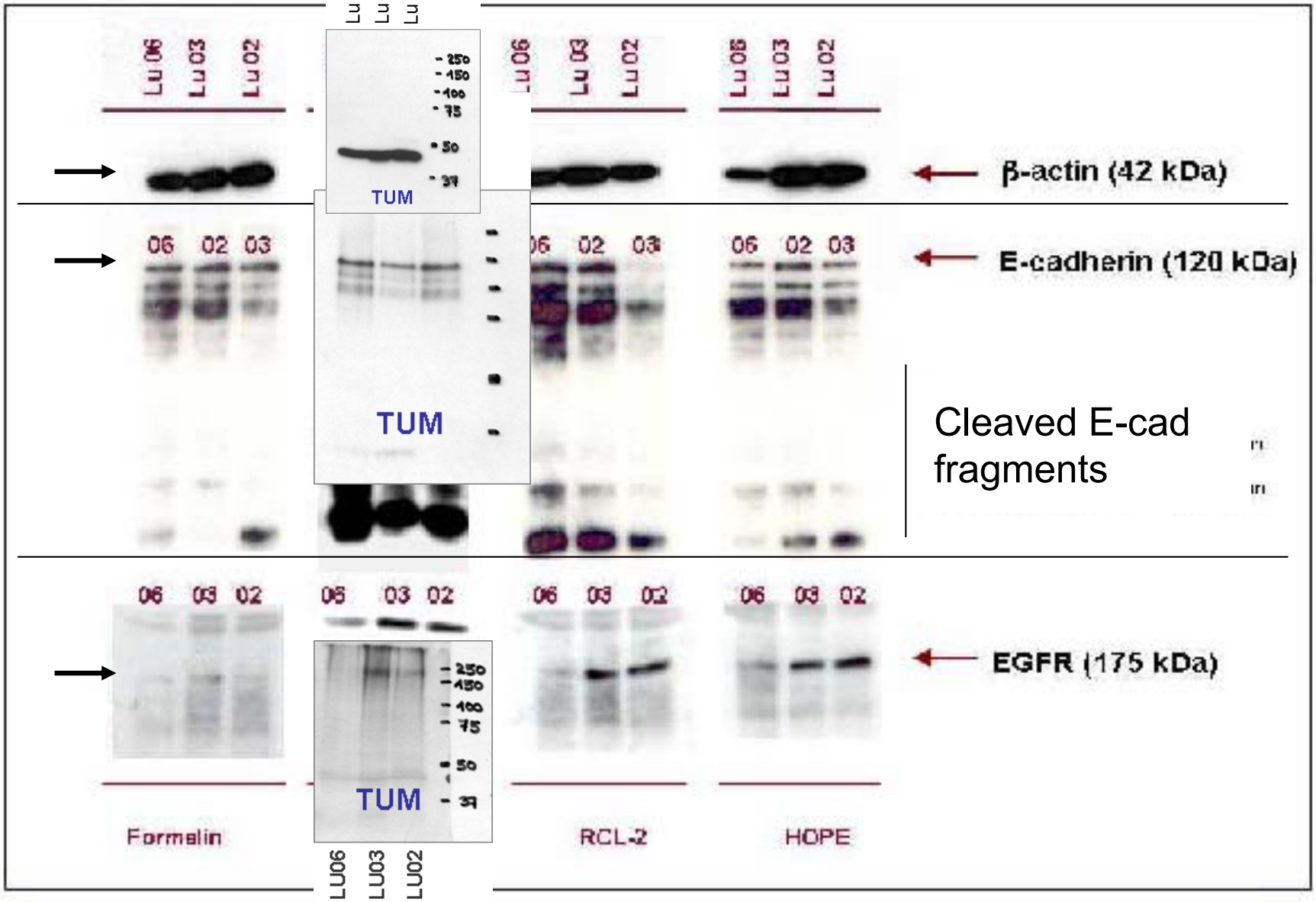
Endpoint RT-PCR on fragments of increasing length



All alcoholic fixatives are conservative of mRNA integrity (up to 438bp)

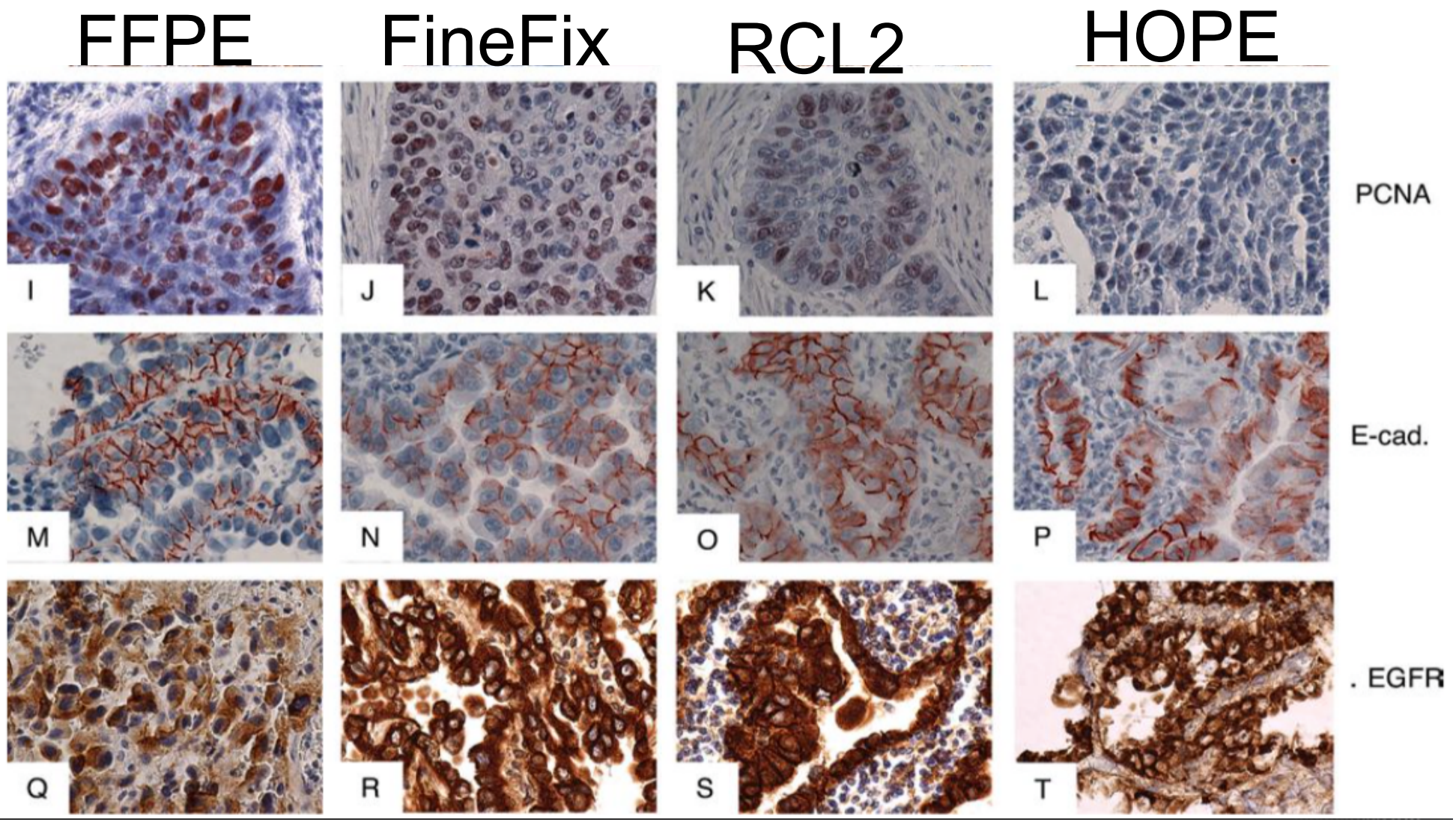
Protein extraction from formalin-, FineFix-, RCL-2-, and HOPE-fixed and paraffin-embedded lung cancer samples from Graz

Qproteome FFPE tissue kit



Courtesy of IMPACTS group

Arch Pathol Lab Med—Vol 135, June 2011 Comparison of Formalin-Free Fixatives—Kothmaier et al



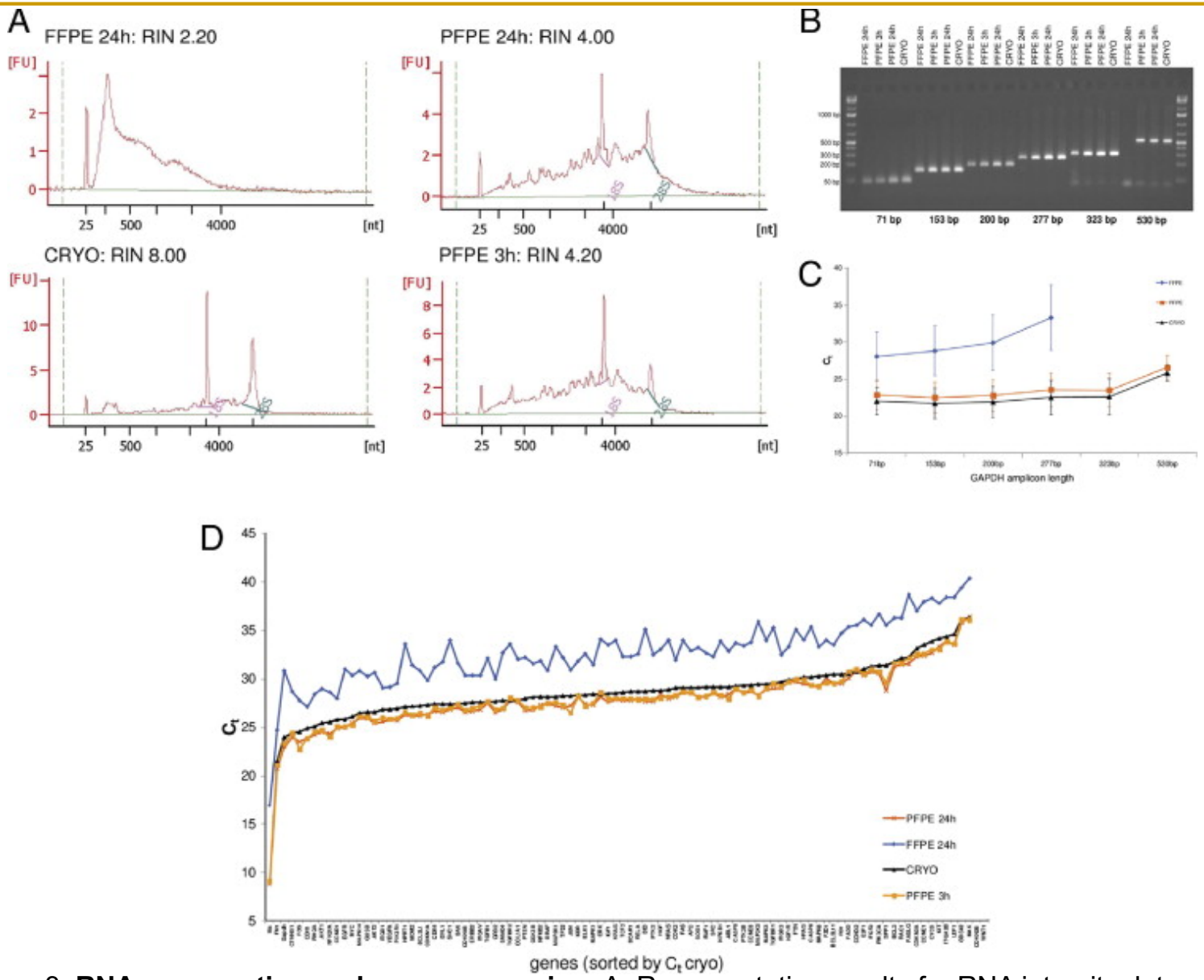


Figure 3. **RNA preservation and gene expression**. A: Representative results for RNA integrity determined on an Agilent Bioanalyzer 2100 platform. The electropherogram of RNA from FFPE tissue shows strongly degraded RNA. For PFPE tissue (fixed for 3 or 24 hours, ...

http://dx.doi.org/10.1016/j.jmoldx.2012.05.002
The Journal of Molecular Diagnostics, Volume 14, Issue 5, 2012, 458–466

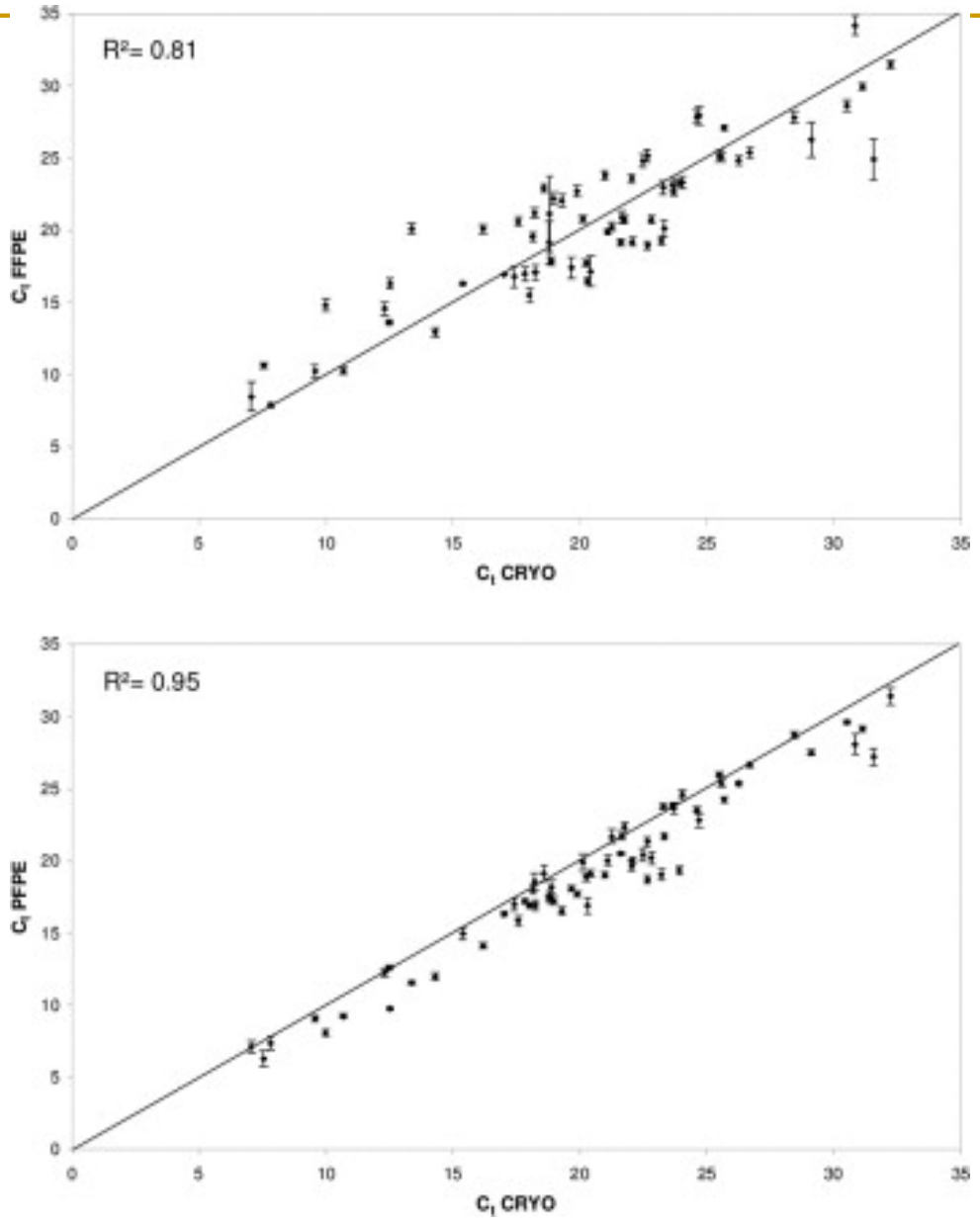


Figure 4. **Correlation of miRNA expression between PFPE, FFPE, and snap-frozen samples**. miRNAs from corresponding aliquots of three colon cancer cases were quantified by qRT-PCR on a TaqMan ABI Prism 7700 sequence detection system. miRNAs 10a, 16, 29a, 30b, 103...

http://dx.doi.org/10.1016/j.jmoldx.2012.05.002

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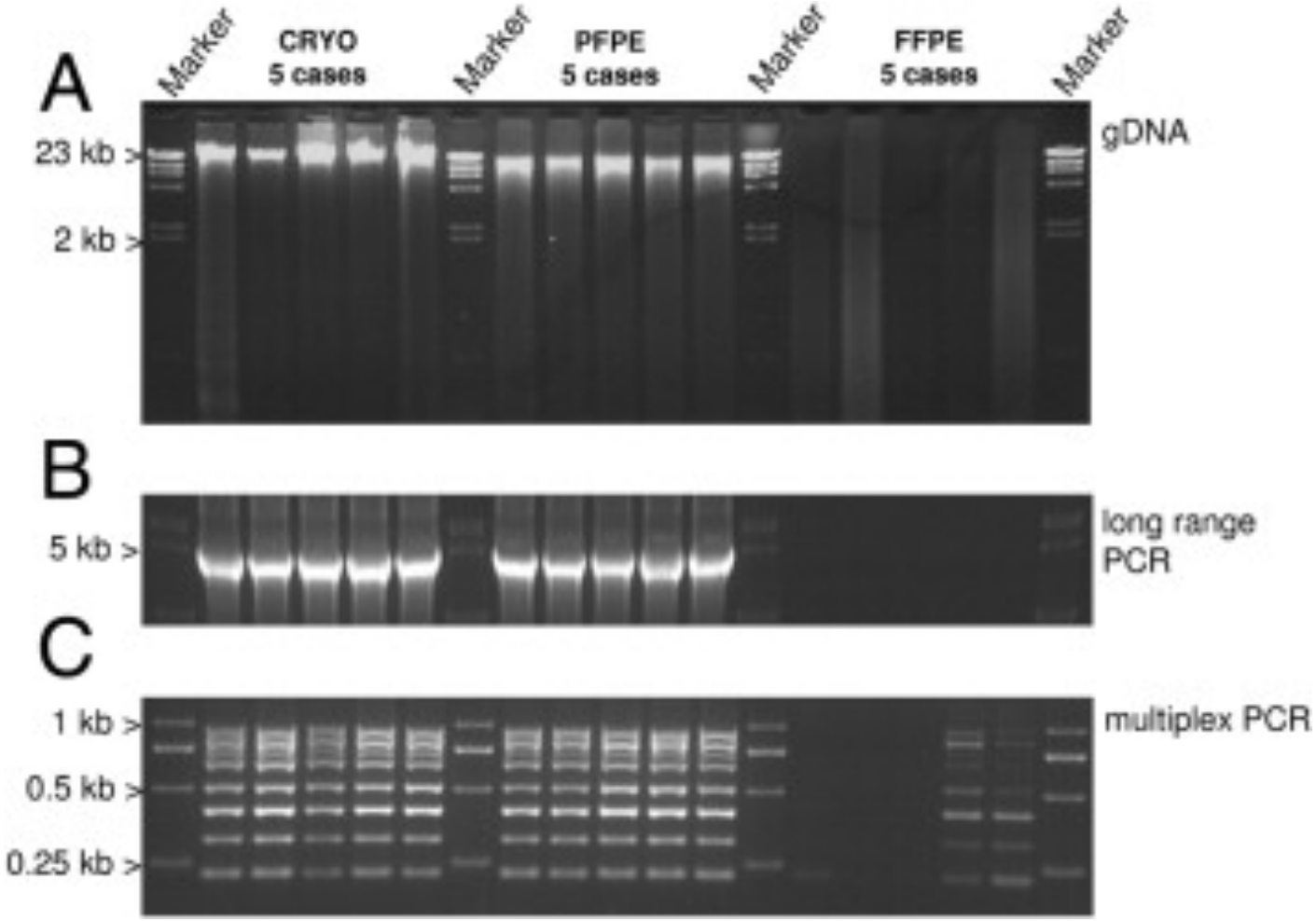

Figure 5**. DNA integrity and performance in long-range and multiplex PCR**. A: Genomic DNA extracted from corresponding FFPE, PFPE, and snap-frozen (CRYO) samples from five human colorectal cancer cases was separated on 1% agarose gels and visualized with ethidium bromide

http://dx.doi.org/10.1016/j.jmoldx.2012.05.002
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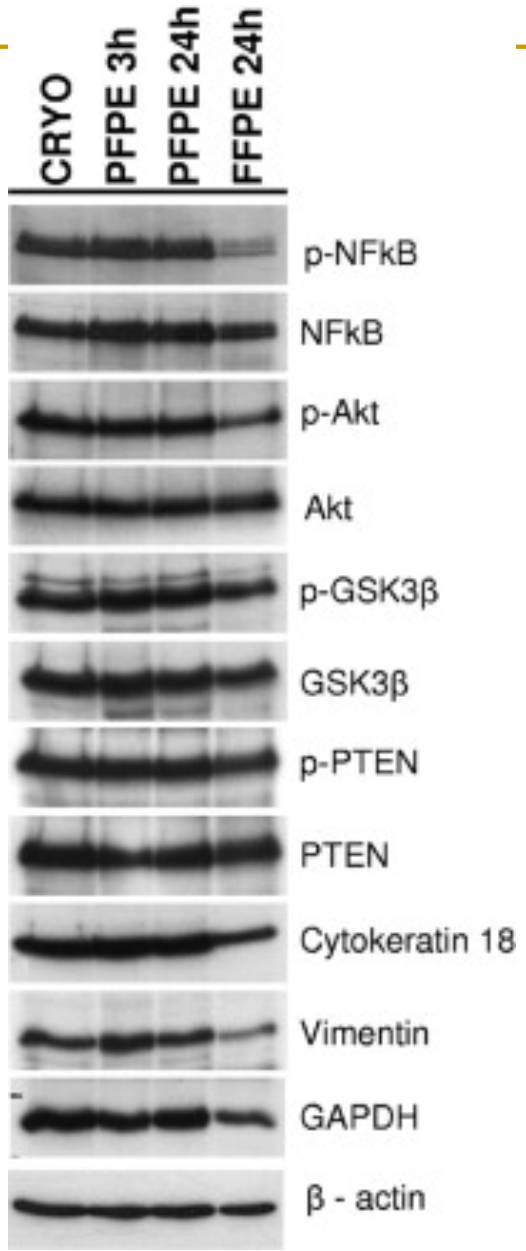

Figure 6. **Western blot analysis** of protein preservation. Proteins were extracted from corresponding snap-frozen (CRYO), PFPE (fixed for 3 or 24 hours, followed by 24 hours stabilization), and FFPE (fixed for 24 hours) human liver samples. Equal amounts (20 μg)...

http://dx.doi.org/10.1016/j.jmoldx.2012.05.002

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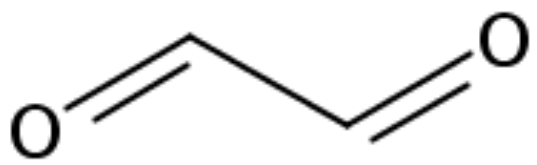
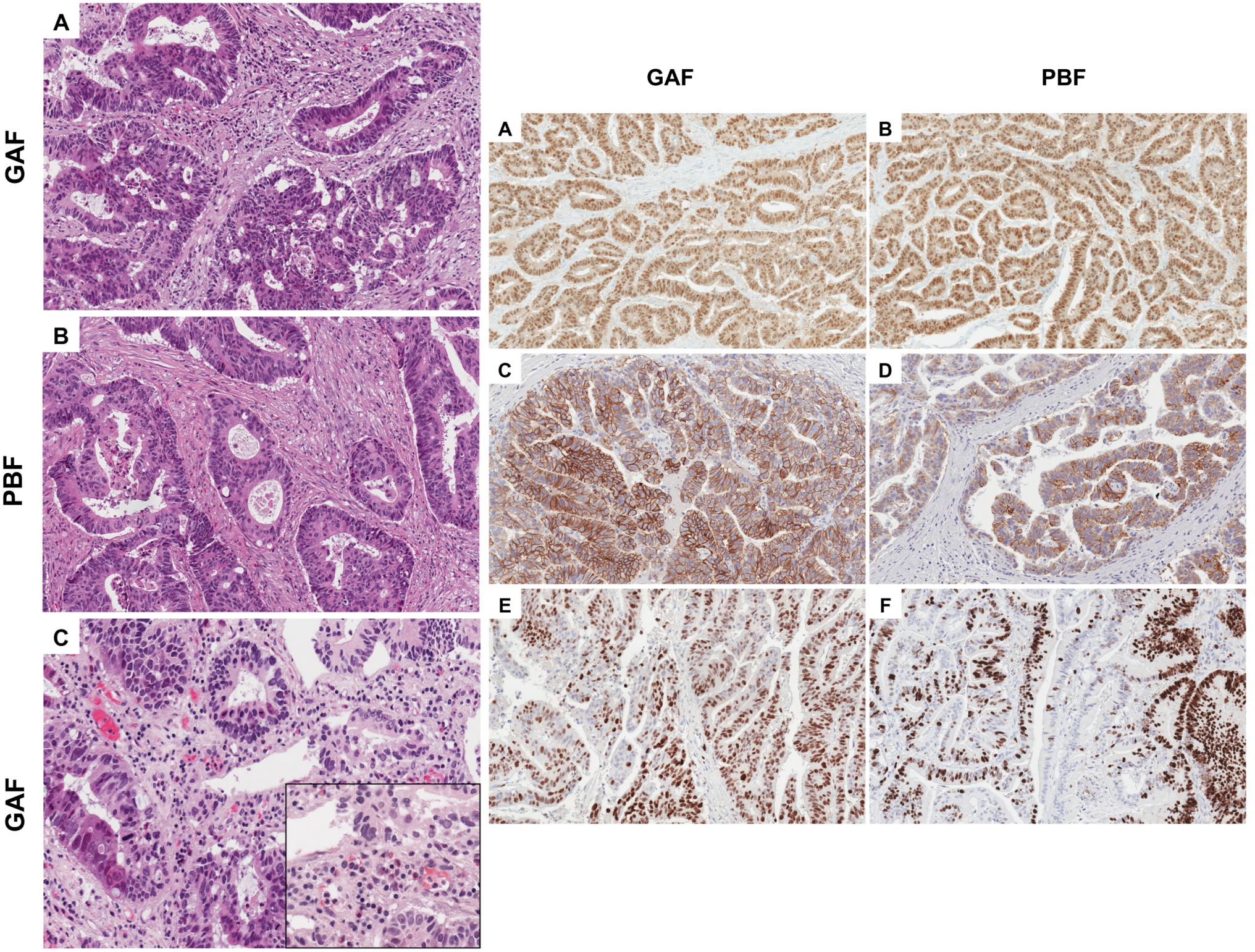


Acid free glyoxals fixative for histological preparations

A 2% GAF solution in 0.1 M phosphate buffer pH 7.3 **is stable for a few weeks**, then gradually undergoes **oxidation** producing an acidic reagent with **sub-optimal fixation properties**.

To overcome the stability problem, a stock solution containing 20% GAF in 50% ethanol (Carlo Erba, Milan, Italy) added with 0.1 g insoluble calcium carbonate (Sigma- Aldrich) in 100 ml of the solution (stock solution) can be used.

The final (working) solution employed as GAF fixative is obtained by diluting the stock solution (exempted of calcium carbonate) 1:10 in 0.11 M phosphate buffer pH 7.3.


GAF
PBF
A
B
C
GAF
PBF
A
B
C
D
E
F