STORAGE OF NUCLEIC ACIDS and PROTEINS

Basic Molecular Pathology Course
ASM Istanbul
19-21 September 2013

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http://www.istanbul.edu.tr/english/

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We wi

Dr. Philipp Schwartz

Prof.DrTalia Bali Aykan
1918-2003

- We isolated DNA/RNA/Protein
- Calculate them
- Now we need to store them

Why do we need to store nucleic acid

- Usually we perform analysis right after isolation
- We have aqueous
  - DNA (200 µlt)
  - RNA (50 µlt)

    or
  - cDNA (50 µlt)
  - PCR product

Storing DNA

- After cell death enzymes start to break down the bonds between the nucleotides that form the backbone of DNA , and micro-organisms speed the decay.
- In the long run, reactions with water are thought to be responsible for most bond degredation.

DNA

- Storing nucleic acids in long term manner; becoming more and more important
- Due to the development of different genetic methods for clarifying biological and medical problems

DNA

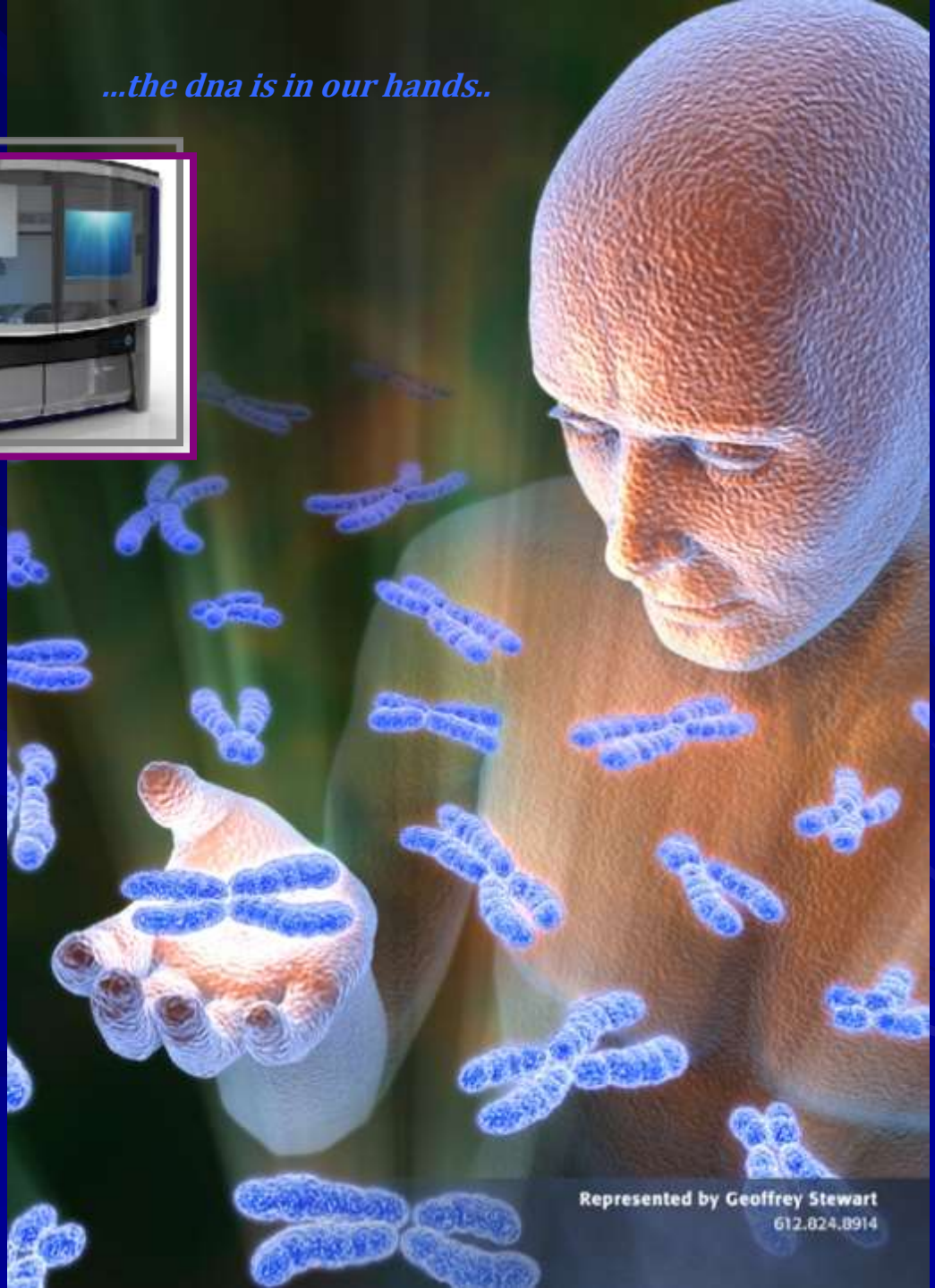
- The importance of the nucleic acid analyzes for both the biological and medical research increasing due to new methods such as
- Next generation sequencing
- Improved quantitative RT-PCR
- But fixed or inappropriate cryopreserved material is only accessible to a very limited number of genetic methods.

- Personalised Medicine improving, new drugs are coming
- New targets, new biomarkers coming each year
- Initial tissue do not exist anymore; since
- could be used (for isolation, IHC, ISH, or clinical trials etc.
- After chemotherapy patient do not have tumor (therapy effect, necrosis etc.

- In our molecular pathology lab,
- Our oncologists ask another test for same patient from same material after 3-4 years
- For example CRC case; it was requested kras mutation analysis 4 years ago
- KRAS was negative. 2 years later they want BRAF mutation.
- And this year they want MGMT metilation analysis

...the dna is in our hands..

Highly accurate, massively parallel next generation sequencing approaches..



DNA

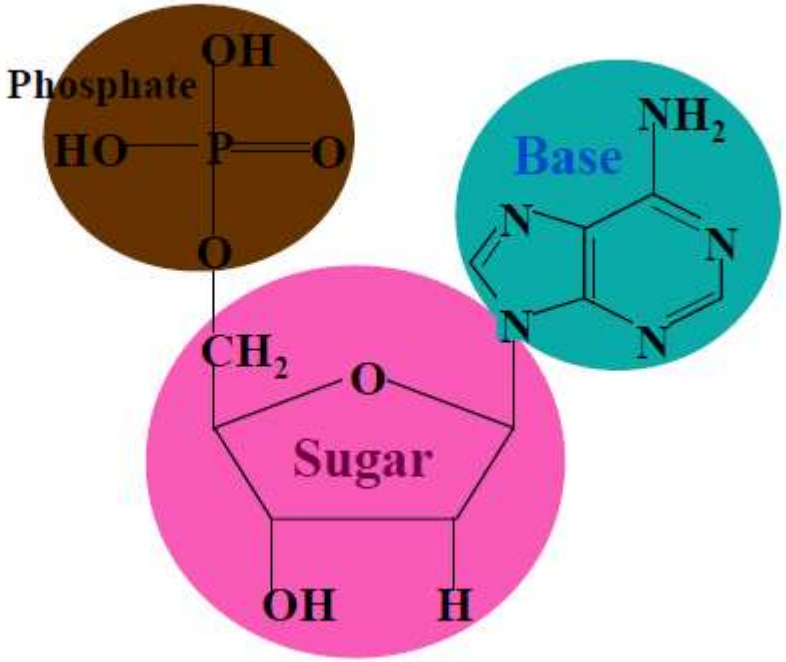
- Storing DNA is important
- To preserve nature historically valuable material
- Nucleic acids stemming from tumors to maintain these for later analysis
- For future genetic approaches as effectively as possible
- For cancer patients an optimal storage of genetic material from tumor tissue for further prospective analysis can be advantageous and positively influence their course of therapy

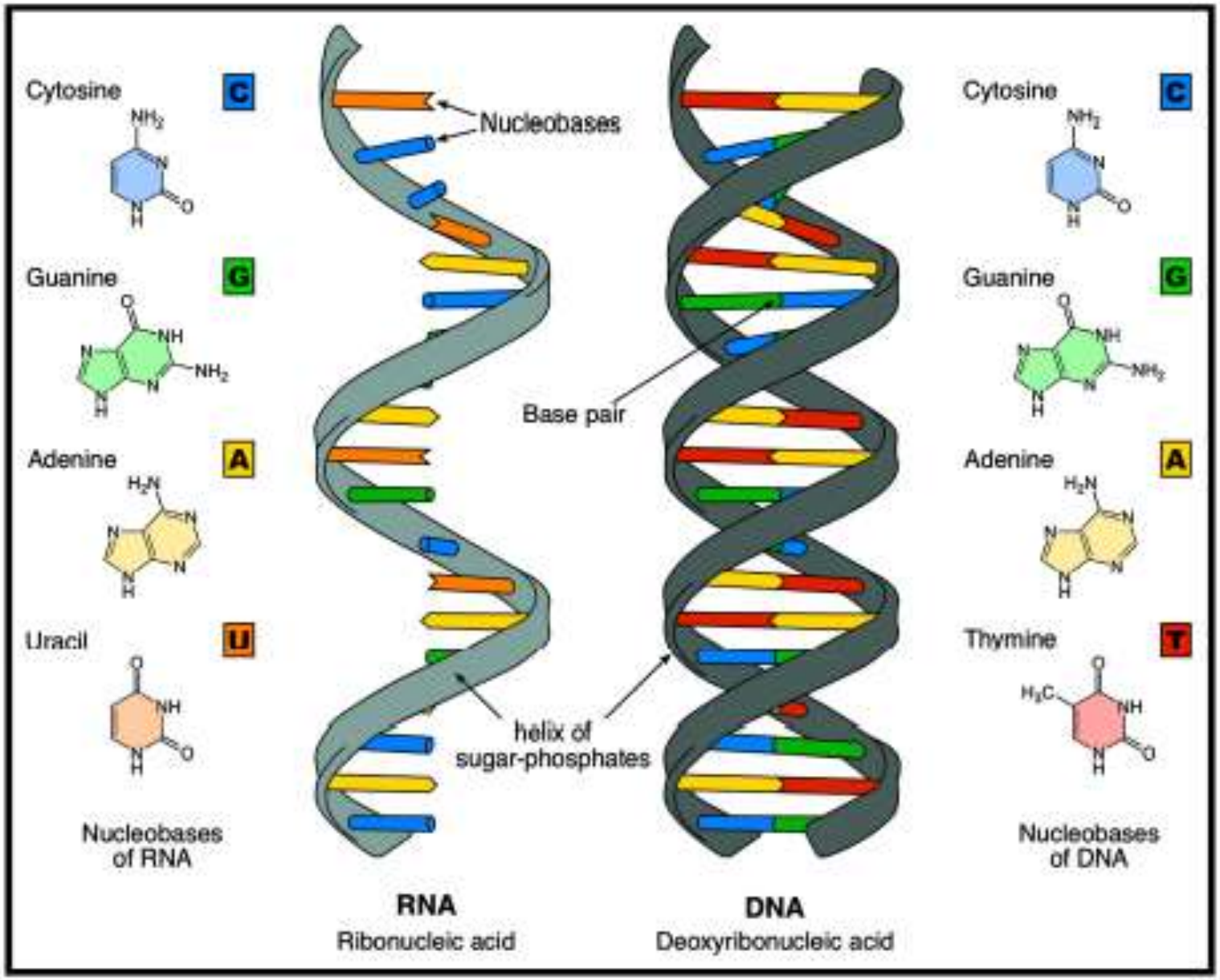
- So we have to keep those DNA,RNA and proteins properly

problems

- Degradation
- Contamination

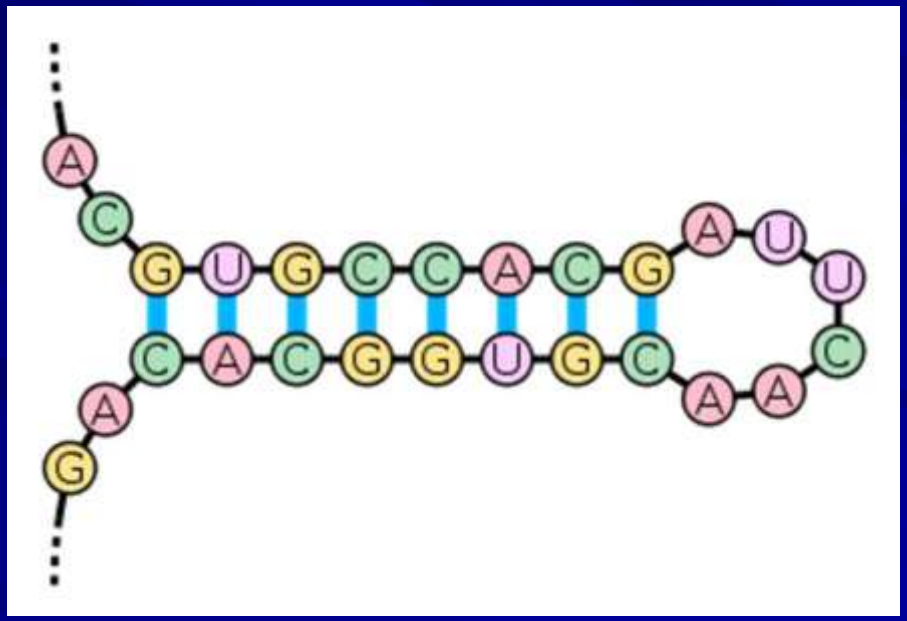
- DNA
- RNA




Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
Nucleobases of DNA

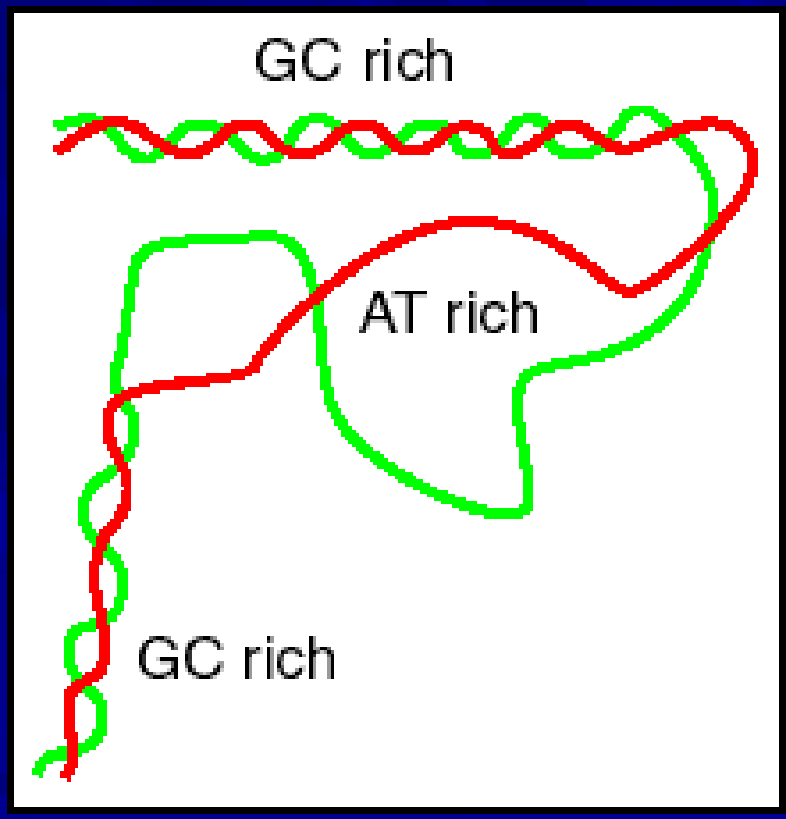
Stability of DNA

- Double stranded DNA more stable than single stranded
- Sugar-phosphate bound
- Hydrogen bounds
- Aggregation effects (hydrophobic)



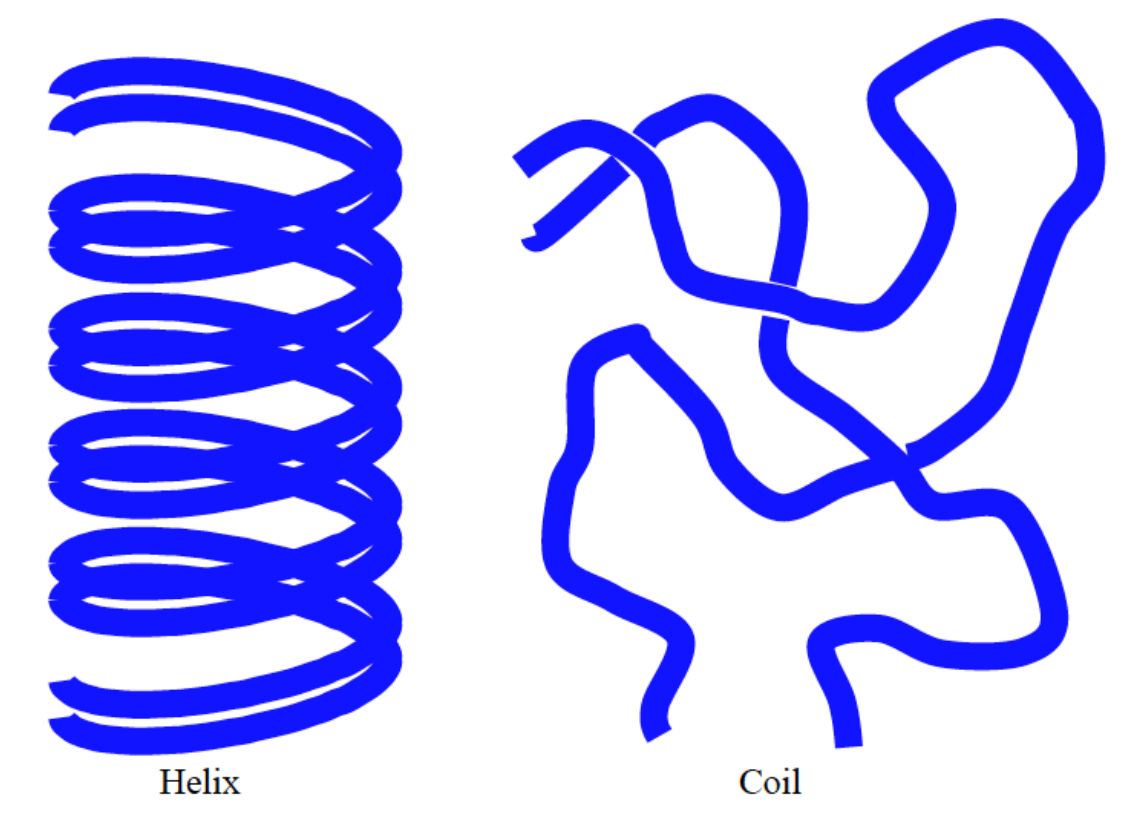
Factors effecting DNA stability

- Content of bases (G≡C > A=T)
- The ration of single stranded DNA amount
- Temperature
- pH level
- Salt level of medium
- Phenol-chlorophorm



- DNA usually resistant alkaline solution
- Strong acidity cause hydrolyse to base,sugar and phosphate groups

High temperature cause denaturation



- Ionized radiation
- Free radicals
- inappropriate plastic tubes
- Inappropriate solution
- Agresive pipetting and vortex
- Presence of trace elements

- The rate of oxidation is enhanced by presence of trace metals (e.g.$Fe^{3}+$, $Cu^{2}+$) due to the production of free radicals via Fenton-type reactions

The Fenton Reaction

- Was discovered in 1894 by HJH Fenton
- The breakdown of hydrogen peroxide by metal ions such as iron
- $Fe^{2}+ + H2O2$ ----- $Fe^{3}+ + OH + OH-$
- $Fe^{3}+ + H2O2$-------$Fe^{2}+$ OOH + H+
- Free radicals react with other compounds and cause damage to DNA

- Demetalation of all components (DNA, buffers, water) can significantly reduce degradation during storage
- it is imposible to measure very low levels of metal contamination
- Highly purified DNA can contain iron levels of 30-40 ppb (> 5ppb)

chelation

- Chelation which can have elation into DNA preperations to limit metal-catalysed reactions
- Chelating agents can inhibit nucleases which can have an adverse effect on downstream
- Chelating agents do not completely prevent fenton-type chemistry, it may include anti oxidants or scavengers in the storage medium

In rutin practice

- Quality of the DNA is dependent on the temperature and the buffering conditions used
- DNA eluted and stored in Buffer AE
- Buffer AE : 10mM Tris
  - 0.5 mM EDTA
  - ph 9.0
- Stable at least 8 years at 2-8 ^{0}C or -20 ^{0}C

suggestion

- Short term storage: (weeks): at 4 ^{0}C in Tris-EDTA
- Medium-term storage (months): at -80 ^{0}C in Tris-EDTA
- Long term storage (years): -80 ^{0}C as a precipitate under ethanol
- Long term storage (decades): at -164 ^{0}C or dried (storage in liquid nitrogen)

- According to Roder et all:
- Optimal long-term storage of standart DNA in solutions
- Containing 50% glicerol in ddH20 at 20 ^{0}C

Long term DNA preservation

- Dried and stored at room temperature is alternative to store in very low temperatures
- DNA is maintained in glassy (vitreous) state
- In glassy state, molecules lose the ability movement of proton, thereby prevent chemical and nuclease degradation
- if moisture is added to the “dry state” or temperature is raised above the glass transition temperature of water, movement and reactivity of protons is reestablished and damage to DNA can occur


Liquid Stabilization
Stored
(up to 1 year)
Ready
for analysis
Add
Purified
Dry
Recover
& Use
Store
Downstream
Analyses
Liquid-to-Dry Stabilization

Dry storage

- Removing water from liquid preparations:
  - Spray drying
  - Spray freeze drying
  - Air drying
  - Lyophilization
- Save money! ($100 million)

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Green Technologies for Room Temperature Nucleic Acid Storage

Table 1. Characteristics of Biomatrica and GenVault nucleic acid storage platforms. *Manufacturer specifications, not tested here.

	Biomatrica	GenVault
DNA Storage		
Product Name	DNA SampleMatrix®	GenTegra™ DNA
Product Formats	1.7 ml Snap-Cap Individual Tubes, 96 and 384 Well Plates	96 Well Cluster Tubes
Max DNA Per Tube or Well*	30 µg	50 µg
Minimum Recoverable DNA*	5-10 pg	50 ng
Drying Method	Air Dry, Speed Vacuum	GenVault FastDryer, Air Dry
Storage Environment	Desiccating Box, Moisture-Barrier Bag	GenVault Storage Cabinet, Desiccating Box
Room Temp Storage Time*	26 Months Real Time Tested, 30 years Simulated at 60°C	4 Months Real Time Tested, 10 Years Simulated at 76°C
Recovery	Add Water or TE Buffer	Add Water
Reusability*	Up to 3 Rehydration/Drying Cycles	Up to 4 Rehydration/Drying Cycles; May also perform additional cycles by transferring to a new GenTegra tube
Sample Tracking	Barcoded Tubes or Racks	Barcoded Tubes or Racks
RNA Storage		
Product Name	RNAstable™	NA
Product Formats	1.5 ml Screw-Cap Tube, 96-Well Plate	NA
Max RNA Per Tube or Well*	100 µg	NA
Minimum Recoverable RNA*	10 pg (using RNAconcentrator)	NA
Drying Method	Air Dry, Speed Vacuum	NA
Storage Environment	Desiccating Box, Moisture-Barrier Bag	NA

Table 2. Estimated DNA yields, measurement of A_{260}/A_{280}, and measurement of A_{260}/A_{230} following room temperature storage using Biomatrica and GenVault platforms.

Biomatrica Sample	Expected Conc. (ng/ L)	Measured Conc. (ng/ L)	% DNA Recovery	A_{260} / A_{280} (Before / After)	A_{260} / A_{230} (Before / After)
1	250	249.0	99.6	1.88 / 1.91	1.57 / 0.73
2	250	259.8	103.9	1.88 / 1.87	1.76 / 0.77
3	250	334.7	133.9	1.89 / 1.89	1.74 / 0.87
4	250	254.7	101.9	1.90 / 1.92	1.70 / 0.75
5	250	227.2	90.9	1.86 / 1.89	1.74 / 0.73
6	250	243.6	97.4	1.86 / 1.84	1.87 / 0.75
7	250	281.7	112.7	1.89 / 1.91	1.95 / 0.82
8	250	264.8	105.9	1.87 / 1.85	1.99 / 0.83
			Median: 103	**Mean: 1.88 / 1.89**	**Mean: 1.79 / 0.78**
GenVault Sample	**Expected Conc. (ng/ L)**	**Measured Conc. (ng/ L)**	**% DNA Recovery**	**A_{260} / A_{280} (Before / After)**	**A_{260} / A_{230} (Before / After)**
1	100	94.6	94.6	1.88 / 1.83	1.57 / 0.33
2	100	138.7	138.7	1.88 / 1.85	1.76 / 0.38
3	50	61.5	123.1	1.89 / 1.85	1.74 / 0.18
4	50	47.9	95.7	1.90 / 1.86	1.70 / 0.15
5	100	108.9	108.9	1.86 / 1.86	1.74 / 0.28
6	100	200.5	200.5	1.86 / 1.88	1.87 / 1.94
7	100	170.9	170.9	1.89 / 1.88	1.95 / 0.39
8	50	44.9	89.9	1.87 / 1.85	1.99 / 0.14
			Median: 116	**Mean: 1.88 / 1.86**	**Mean: 1.79 / 0.47**

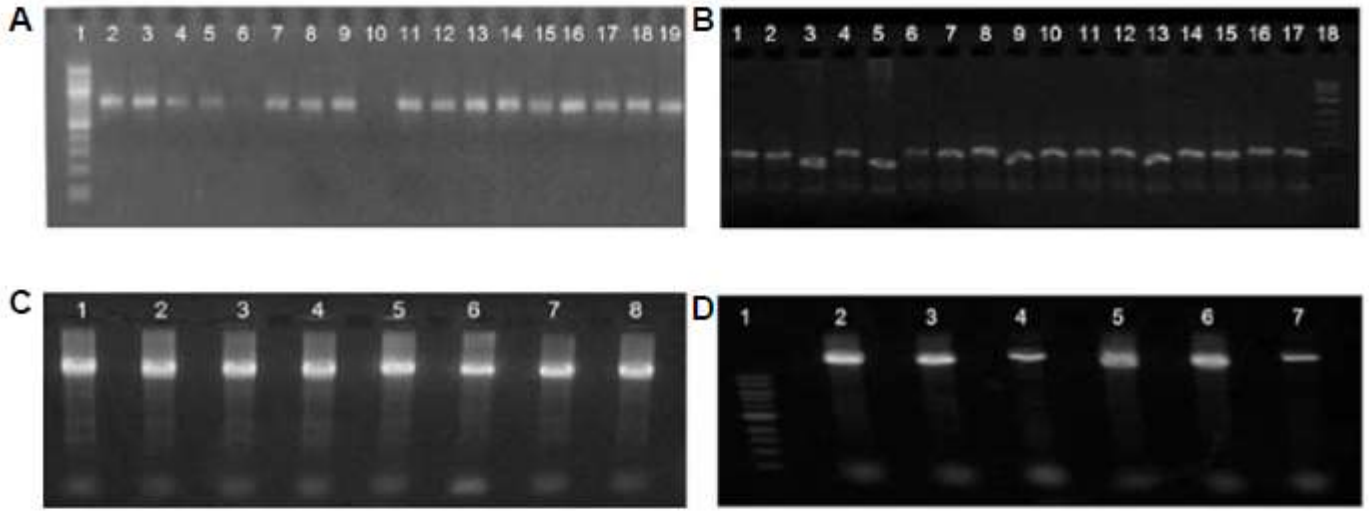

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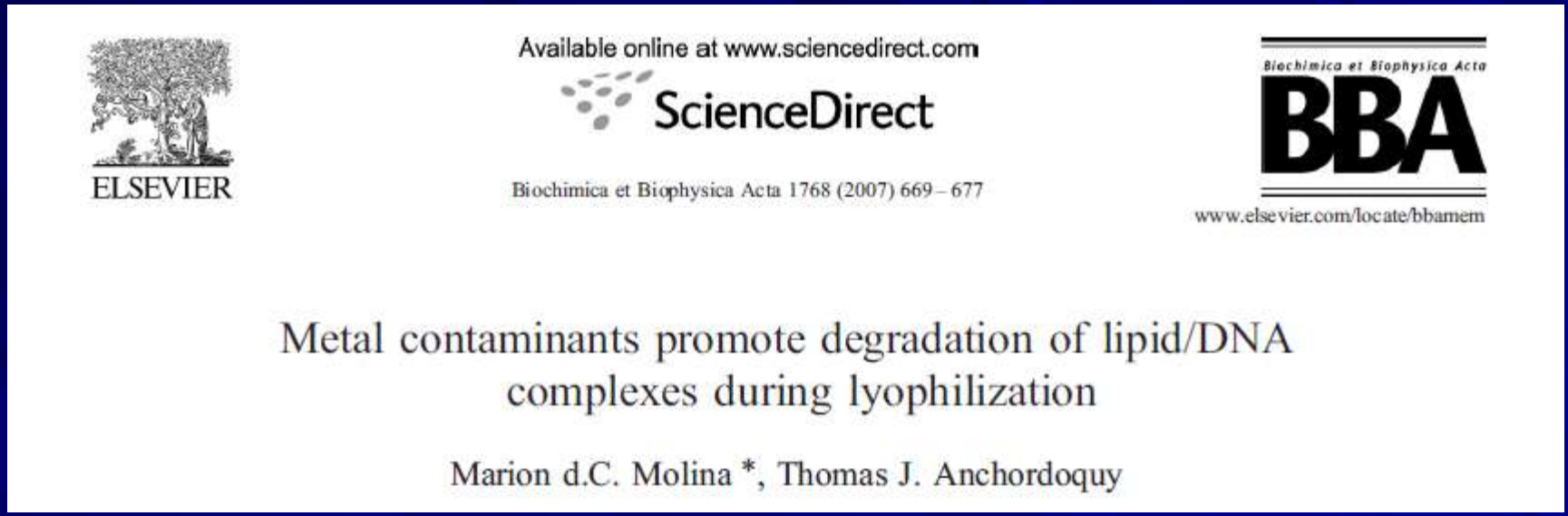
Table 5. Comparison of features for room temperature nucleic acid storage and freezer storage.

Feature	**Freezer (-80°C)**	**Room Temperature Nucleic Acid Storage (Biomatrica, GenVault)**
Footprint	Large, Typically in a Separate Lab Area / Room	Small to Large, Desktop Storage Possible
Energy Consumption	7,000 kWh/year	0
Annual Energy Cost	Approx $1,000	0
Fluorocarbon and CO_2	Yes, Some Models	No
Risk of Sample Loss with Power Outage	Yes	No
Sample Shipping	Dry Ice or Cold Packs	Room Temperature
Sample Storage Ease	Freeze-Thaw within Minutes	Overnight Drying; Instant Rehydration



Their results clearly demonstrate that trace amounts of transition metals (Cu2+, Fe2+) increase formation of single and double strand breaks, presumably through ROS (reactive oxygen species) production.

Their data also show that both the type of metal and its concentration influence the extent of DNA damage during acute lyophilization.

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Notes & Tips

Effect of storage regime on the stability of DNA used as a calibration standard for real-time polymerase chain reaction

Ellen Podivinsky [a,*], John L. Love [a], Loraine van der Colff [a], Laly Samuel [b]

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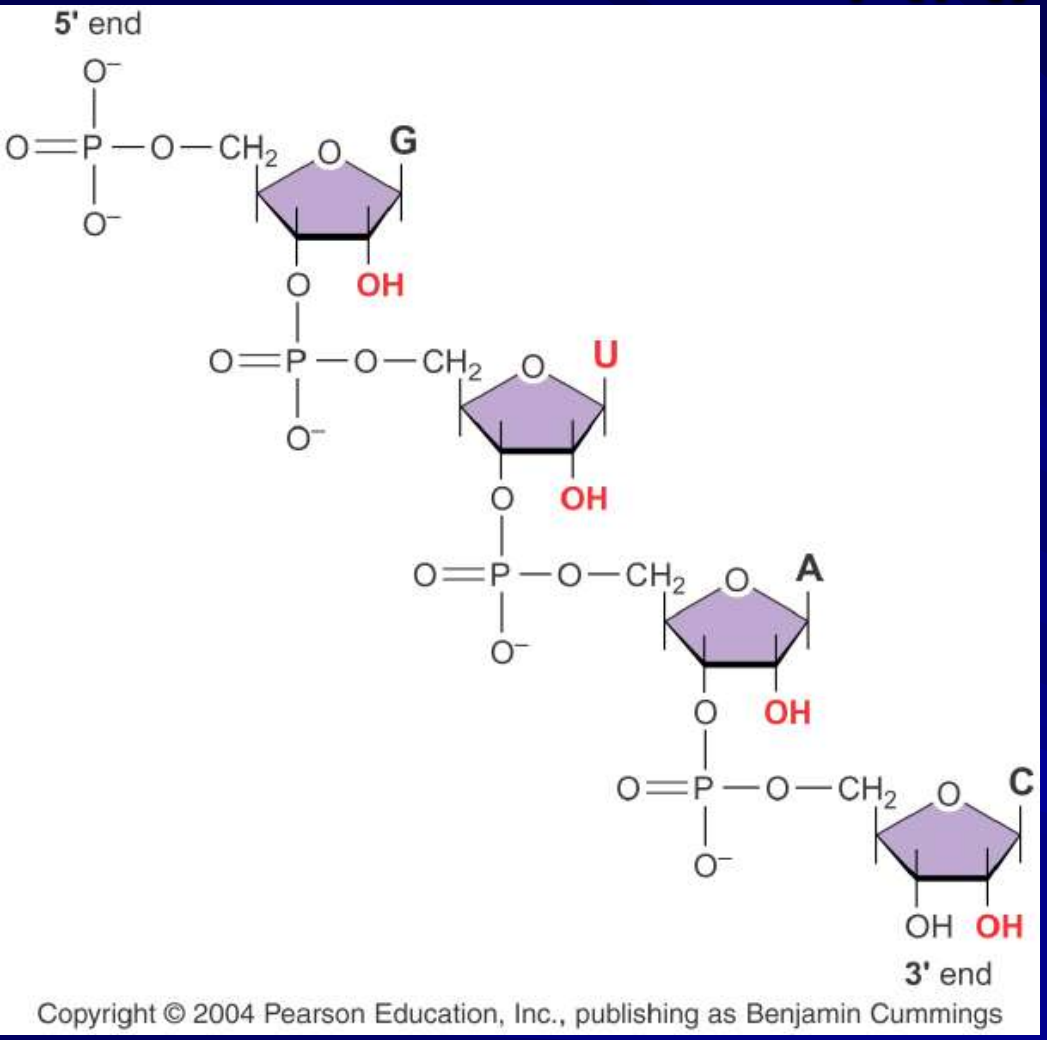
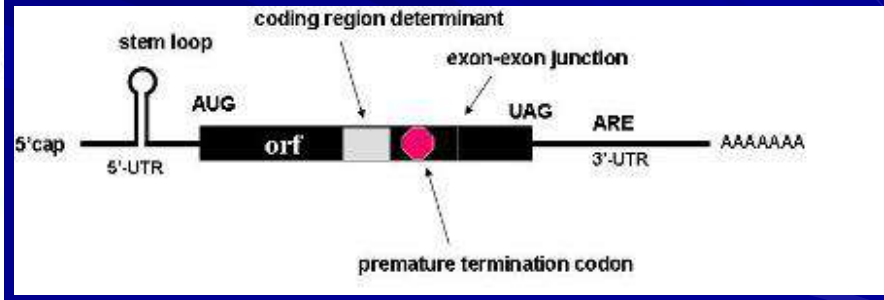
[b] Measurement Standards Laboratory of New Zealand, Christchurch 8543, New Zealand

a b s t r a c t

This article looks at storage factors influencing the stability of potential DNA calibration standards for use in quantitative polymerase chain reaction (PCR). Target sequences from the bacteria Campylobacter jejuni were cloned into a plasmid vector. Samples of these potential calibration standards were stored at +4, 20, and 80 C as aqueous and lyophilized samples and were prepared as both single-use aliquots and multiple-use preparations. Results showed that **the samples stored as single-use aqueous solutions at +4 C and lyophilized samples stored at +4 and -20 C were the most stable. Samples stored as frozen aqueous solutions at -20 C were the least stable**.

RNA





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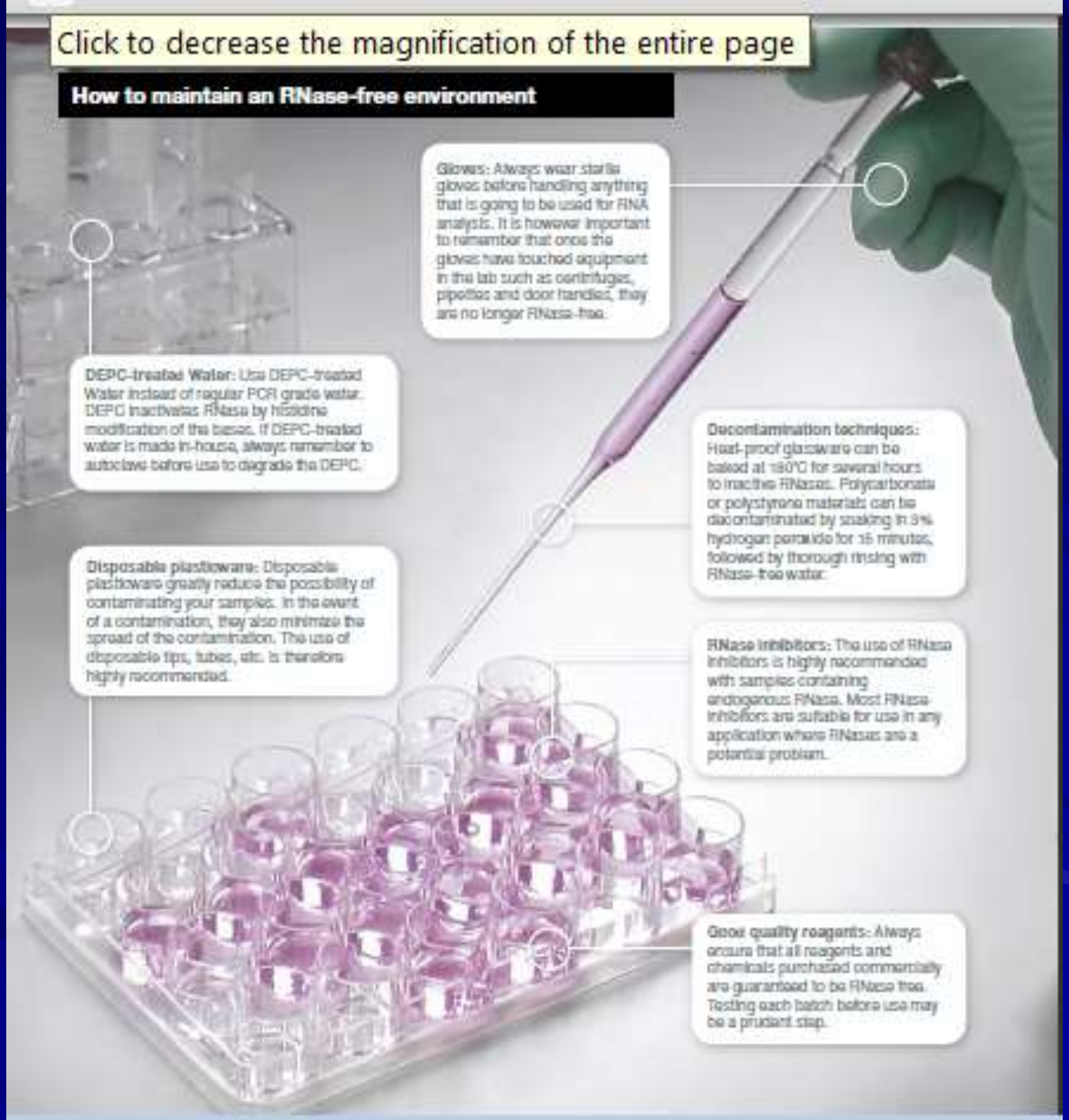


RNA

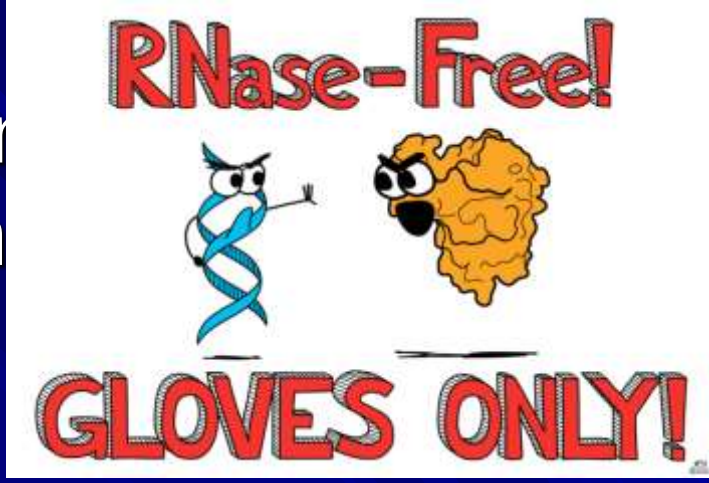
RNase

- Play important roles in nucleic acid metabolism
- Found every cell type
- Human body uses Rnases to defend against invading microorganisms by secreting these enzymes in fluids such as tears, saliva, mucus and perspiration
- Also present in flaked skin, on hair thay may fall on to a bench, and in pet hair that may cling to clothing

RNase

- Small compact proteins containing several cystein residues that form numerous disulfid bonds
- Denaturated Rnase tend to regain their native structure and partial fuction after being cooled to room temperature in the absence of a denaturant
- Rnase can retain substantial activity after freeze-thaw cycles and even autoclaving

- Wear gloves during experimer to prevent contamination from Rnase found human hands
- Change gloves after touching skin (e.g. Your face), doorknobs and common surfaces
- Have a dedicated set of pipett that are used soley for RNA work
- Use tips and tubes re tested a guranteed to be Rnase-free



- Use RNAase-free chemicals and reagents
- Designate as an “Rnase free zone” or low-traffic area of the lab is away or shielded from air vents or open windows
- “fingerases”
- Tip and tubes

- Water and buffers
- Diethylpyrocarbonate (DEPC) treatment is the most common method used to inactivate RNases in water and buffers. (0.05-0.1%)
- However certain reagent such as Tris cannot be DEPC treated.
- Commercially avaible buffers exist.(Quigen, Ambion, Introgen etc.

- The presence of trace amount of Rnase can compromise RNA integrity, even if the samples are stored frozen in an enviroment
- Short term storage:
- RNA samples can be resuspended in

  Rnases-free water with 0.1 mM EDTA

  or

  TE buffer (10mM Tris, 1mM EDTA)

  stored -80 ^{0}C

- Also using a buffer solution that contains a chelating agent is a better way to store RNA. Chelation of divalent cations such as Mg^{+2} and Ca^{+2} prevent heat induced strand scission.
- (RNA can be chemically cleaved when heated in the presence of Mg^{+2})

- Purified RNA can be stored at -20 ^{0}C or -80 ^{0}C
- Most common solutions
- 1mM sodium citrate, pH:6,4
- 0.1 mM EDTA (treated with DEPC)
- 10 mM Tris-HCl, 1 mM EDTA pH 7.0
- For long term precipitate in ethanol (or isopropanol) and sodium-acetate and stored at -80 ^{0}C

In practical use

- Use DEPC treated water (Rnase free) to dissolve RNA and store it in -80 ^{0}C
- Do RT-PCR to generate cDNA (which is more stable than RNA) same within a day or two; store it -20 ^{0}C in aliquots;

  and main vials in -80 ^{0}C to avoid freeze thaw cycles (freeze and thawing repeatedly can degrade the samples)

proteins

- pH and temperatures are very important
- Total protein extraction or compartmental protein isolation
- in house or commercial kits are available for isolation
- Protein degradation is a problem
- Lysis buffer with protease inhibitors
- Aliquot and Keep protein lisate in -80 C

Acknowledgement:

Cerrahpasa Medical College
Molecular Pathology and FISH Lab

Prof Dr Sibel Erdamar MD
Prof Dr Buge Oz
Asc Prof Dr Hilal Aki

Mol Biol Canan Unlu Ozkurt
Mol Biol Esra Simsir

Thanks for your kind attention

DNA

RNA

- Keep on ice
- 4 C
- -20 C
- -80 C
- Trizol (invitrogen) works well for stabilising, but also inhbitor of reverse transcriptase and not always recommended
- Room temperature


DNA

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Drying Method	Air Dry, Speed Vacuum	GenVault FastDryer, Air Dry
Storage Environment	Desiccating Box, Moisture-Barrier Bag	GenVault Storage Cabinet, Desiccating Box
Room Temp Storage Time*	26 Months Real Time Tested, 30 years Simulated at 60°C	4 Months Real Time Tested, 10 Years Simulated at 76°C
Recovery	Add Water or TE Buffer	Add Water
Reusability*	Up to 3 Rehydration/Drying Cycles	Up to 4 Rehydration/Drying Cycles; May also perform additional cycles by transferring to a new GenTegra tube
Sample Tracking	Barcoded Tubes or Racks	Barcoded Tubes or Racks
RNA Storage		
Product Name	RNAstable™	NA
Product Formats	1.5 ml Screw-Cap Tube, 96-Well Plate	NA
Max RNA Per Tube or Well*	100 µg	NA
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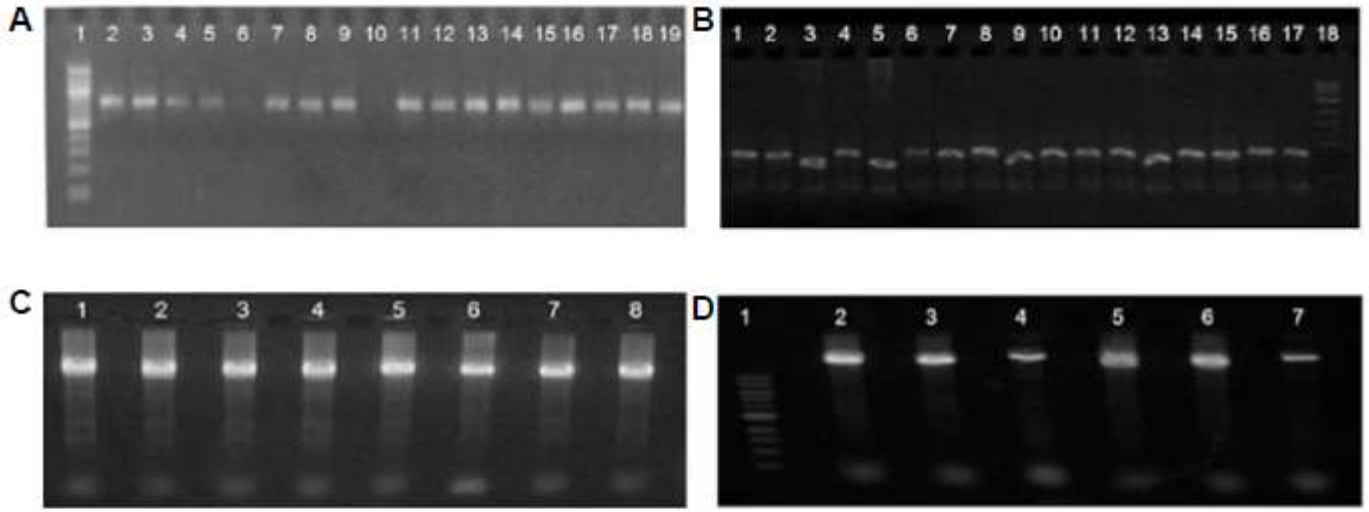

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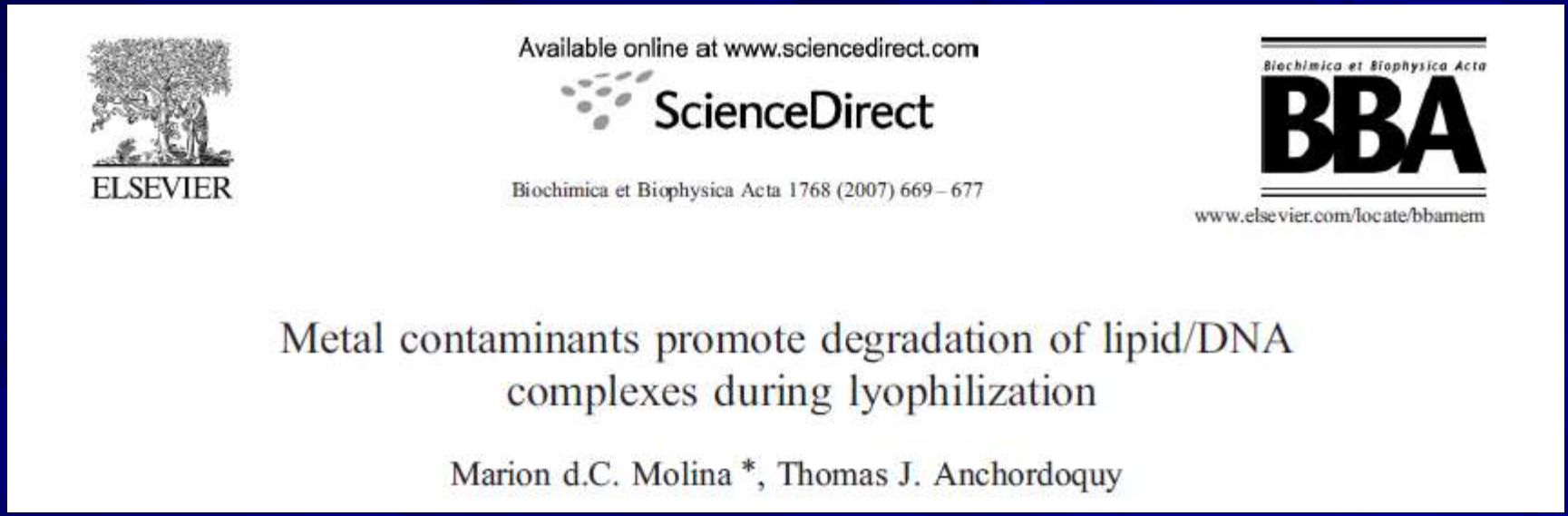

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Notes & Tips

Effect of storage regime on the stability of DNA used as a calibration standard for real-time polymerase chain reaction

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a b s t r a c t

This article looks at storage factors influencing the stability of potential DNA calibration standards for use in quantitative polymerase chain reaction (PCR). Target sequences from the bacteria Campylobacter jejuni were cloned into a plasmid vector. Samples of these potential calibration standards were stored at +4, 20, and 80 C as aqueous and lyophilized samples and were prepared as both single-use aliquots and multiple-use preparations. Results showed that **the samples stored as single-use aqueous solutions at +4 C and lyophilized samples stored at +4 and 20 C were the most stable. Samples stored as frozen aqueous solutions at 20 C were the least stable**.