Fifteen years of real-time stability data at room temperature: validation of encapsulation for sustainable biobanking

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A. Background and previous work

1. Classical nucleic acid storage in frozen state:

- leads to space and security issues,
- necessitates high maintenance and energetic costs,
- requires dry ice shipping of RNA, expensive and subjected to transportation hazards

> Highly needed: a procedure allowing room temperature storage and shipping

2. Main nucleic acid degradation mechanisms and factors:

Hydrolysis:

- phosphodiester bond breaking (RNA ++)
- N-glycosidic bond breaking (depurination) (DNA ++)
- base deamination

Oxydation of bases and sugars by:

- Reactive Oxygen Species (ROS) produced from O_2 and H_2O in the presence of heavy metal ions
- Ozone O_3

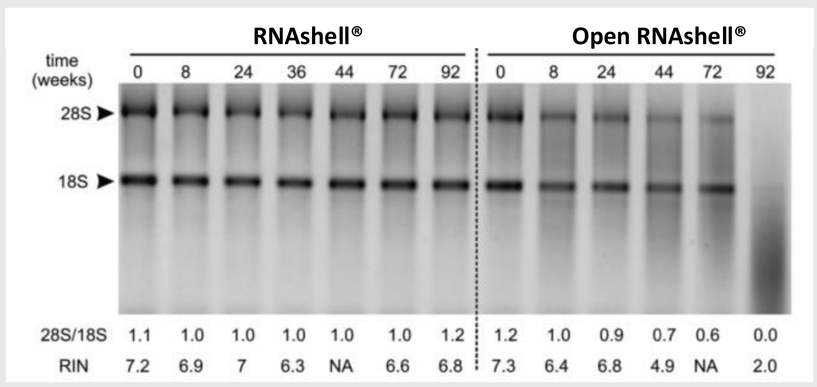
3. Dehydration of DNA and RNA should:

- inhibit both hydrolysis and oxidation
- slow down chemical processes via the reduction of molecular mobility due to the vitrified state

> Enable room temperature storage

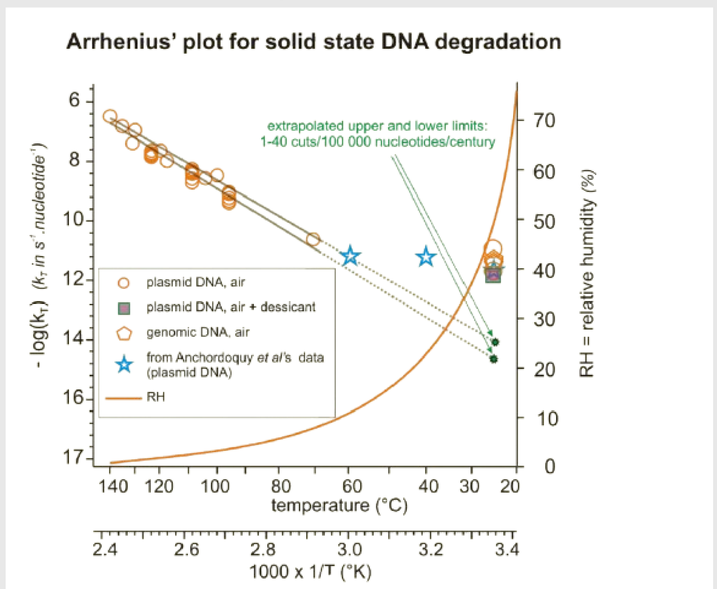
4. Previous work

4.1 Deleterious influence of the atmosphere on solid state nucleic acids



> Protection from air is needed for efficient room temperature storage of nucleic acids

4.2 Accelerated aging used to estimate shelf life at room temperature (Arrhenius model)



Redrawn from Bonnet et al. NAR 2010 Mar; 38(5): 1531-46.

> Very high stability of encapsulated DNA and RNA in minicapsules

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B. Aim of the work

- Confirm in real time at room temperature the stabilities obtained with accelerated aging
- Show that stability is the same as in frozen controls
- Show that in the dry sate, the stability is the same whatever the sample source and extraction methods

C. Material and Methods

1. Source biospecimens and extraction methods

	Purified		In biospecimen	
Biological material	**DNA**	**RNA**	**DNA**	**RNA**
Cell lines	Organic Salt precip Silica	Phenol/Chlo Trizol	-	Fixed cells Cells on paper
Bacteria	Organic Salt precip Silica Ion exchange	Phenol/Chlo Silica Trizol	-	-
Rat liver	Organic Salt precip	Phenol/Chlo Silica Trizol	-	-
Blood	Organic Salt precip	Paxgene	Lysed WBC Lysed BC	-
Saliva	Organic Salt precip	-	-	-
Plant	Organic Silica	-	-	-

2. Processing

- Collection of the biospecimens
- Purified NA: extraction + QC
- NA in biospecimen: lysis for DNA in blood cells / Cell fixation or deposition on paper for RNA
- Addition of stabilization proprietary solutions
- Aliquoting in the minicapsules
- Desiccation
- Encapsulation (laser sealing under argon)

3. Real time storage

Capsules stored at room temperature without moisture and temperature control (15°C --- 30°C)

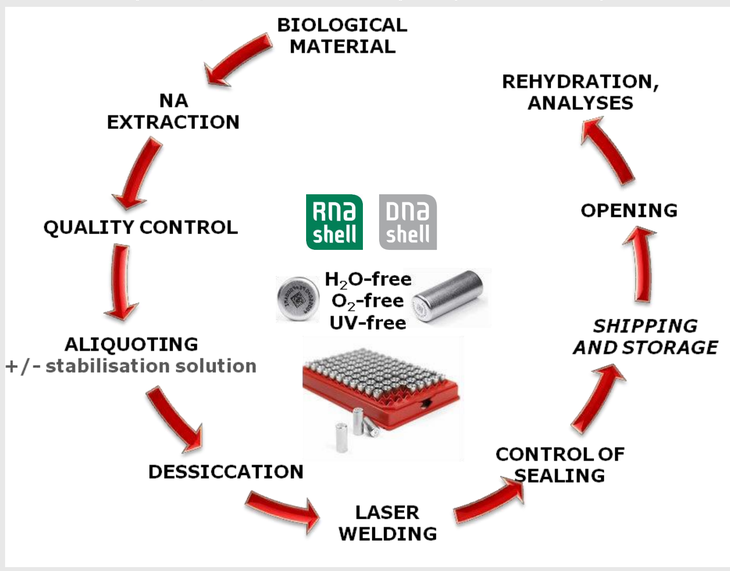
Control capsules stored at -20°C

Controls in solution stored at -20°C (DNA) or -80°C (RNA)

For a given storage time

4. Post-storage analysis

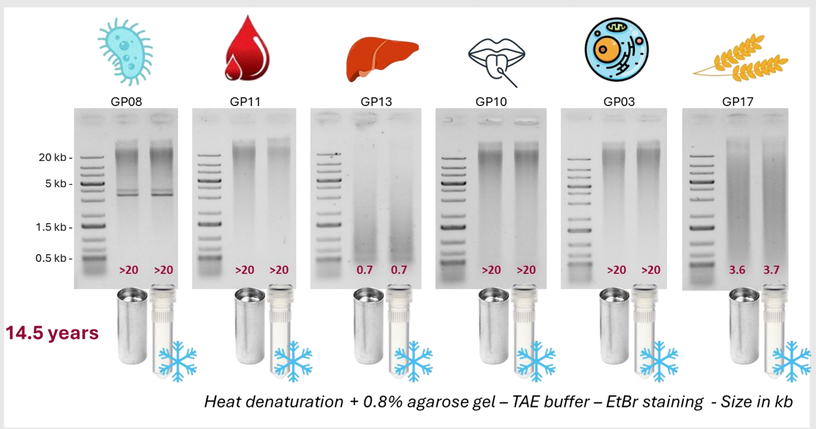
- Opening of the minicapsules with a shellOpener
- Rehydration
- Extraction for biospecimen (salt precipitation for DNA / Trizol for RNA)
- QC (not shown)
- Electrophoresis agarose gels/ Bioanalyzer (RIN)
- RT-qPCR / RT-dPCR analysis (not shown)



D. Results

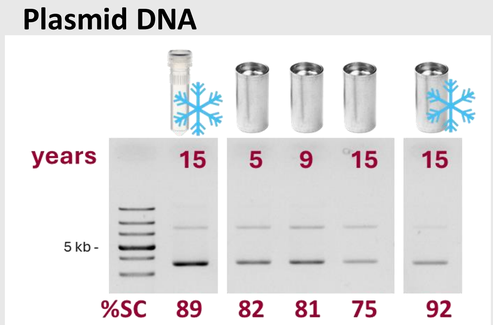
1. Purified DNA

Genomic DNA *representative results after 14.5 years*



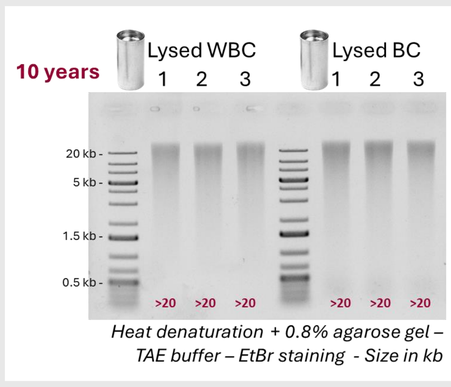
Heat denaturation + 0.8% agarose gel – TAE buffer – EtBr staining - Size in kb

Plasmid DNA



0.8% agarose gel – TAE buffer – EtBr staining

2. DNA and RNA in biospecimen



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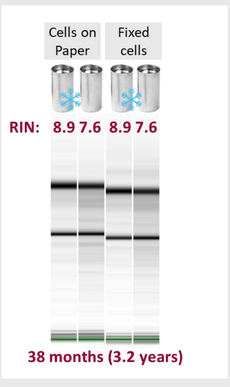
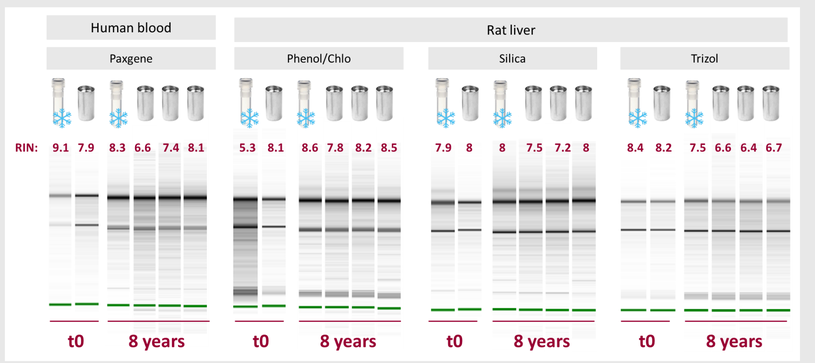
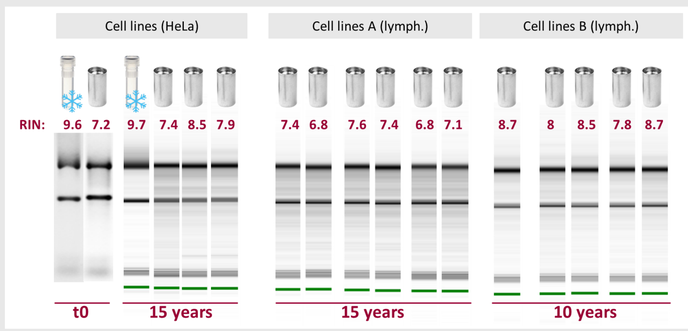


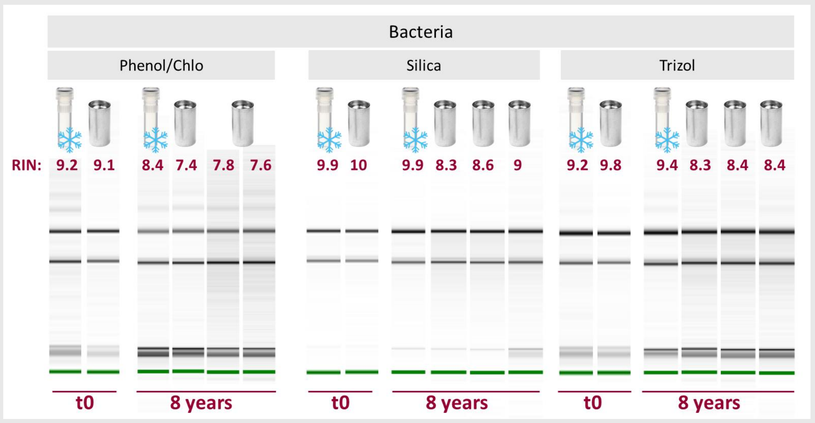
3. Purified RNA

Eukaryotes total RNA





Prokaryotes total RNA



E. Conclusions

Unparalleled data over 3-15 years in true ambient conditions

→ great NA stability (in biospecimen or purified)

→ limited influence of NA source and extraction procedure.

> Encapsulation enables storage and shipping of nucleic acids at RT, regardless of climatic hazards.