# Fifteen years of real-time stability data at room temperature and accelerated aging: Validation of DNAshell® encapsulation for sustainable DNA data storage

Marthe COLOTTE, Aurélie LUIS, Delphine COUDY, Magali MILHAU, Sophie TUFFET, Jacques BONNET

Marthe COLOTTE, PhD
Imagene CTO
colotte@imagene.eu

# DNA data storage

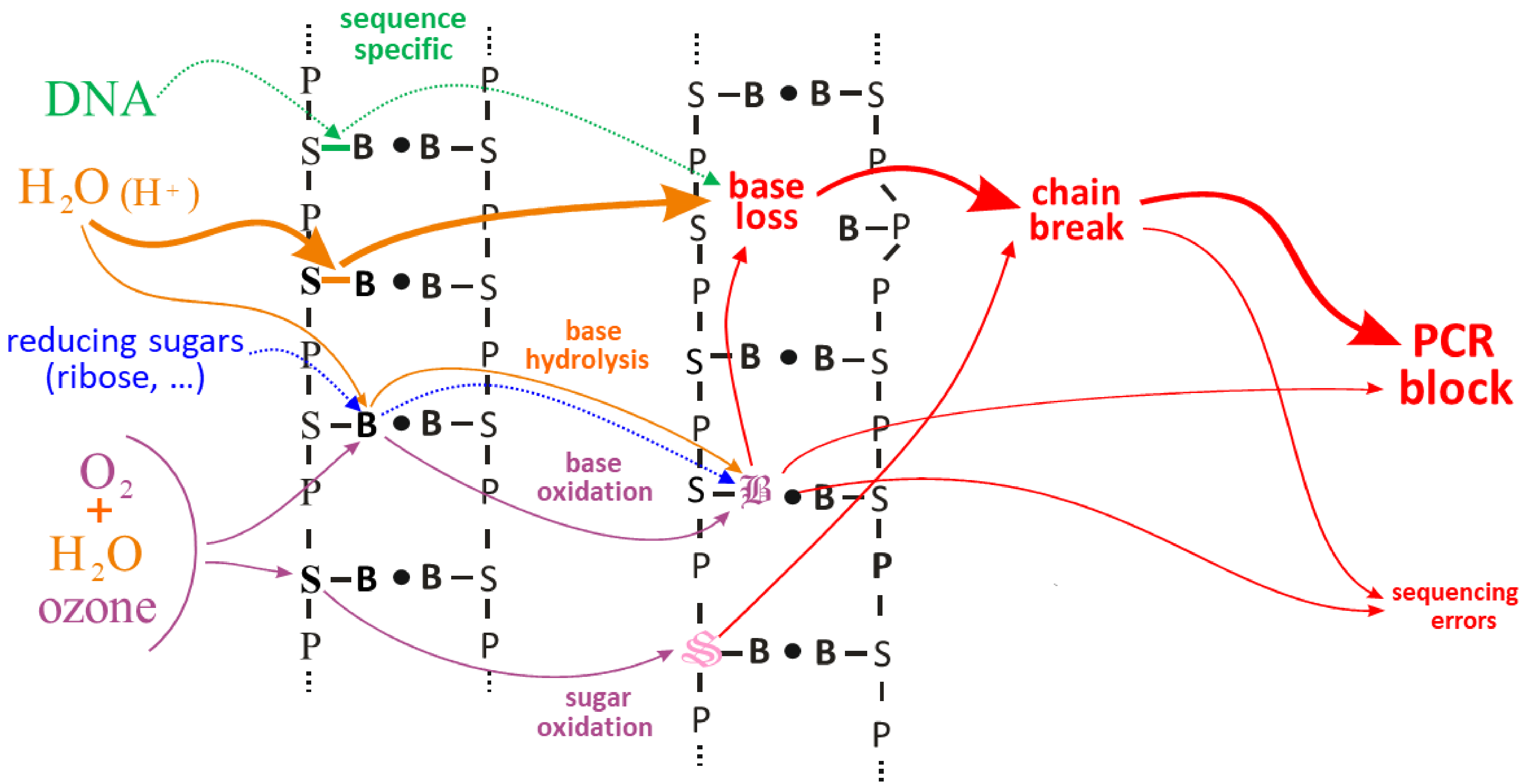
- Will contribute to resolve the data volume explosion crisis
- Parameters to take into account / challenges to address
  - Scale of storage
  - Information density
  - DNA stability* / data retrieval

Stability often overlooked

- DNA degradation mechanisms and factors
- DNAshell® / RNAshell® technology
- Evaluation of stability
  - Accelerated aging (Arrhenius model)
  - Real time aging @RT

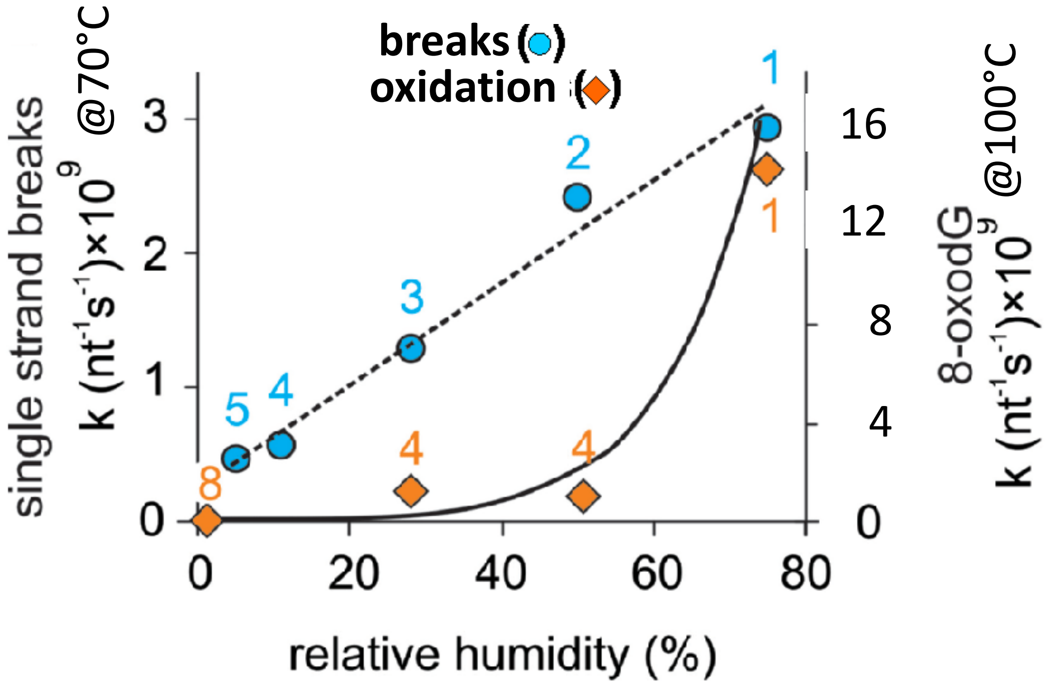
*We'll talk about data encoded in DNA sequence, not in its structure

# Main mechanisms of degradation



# Consequences

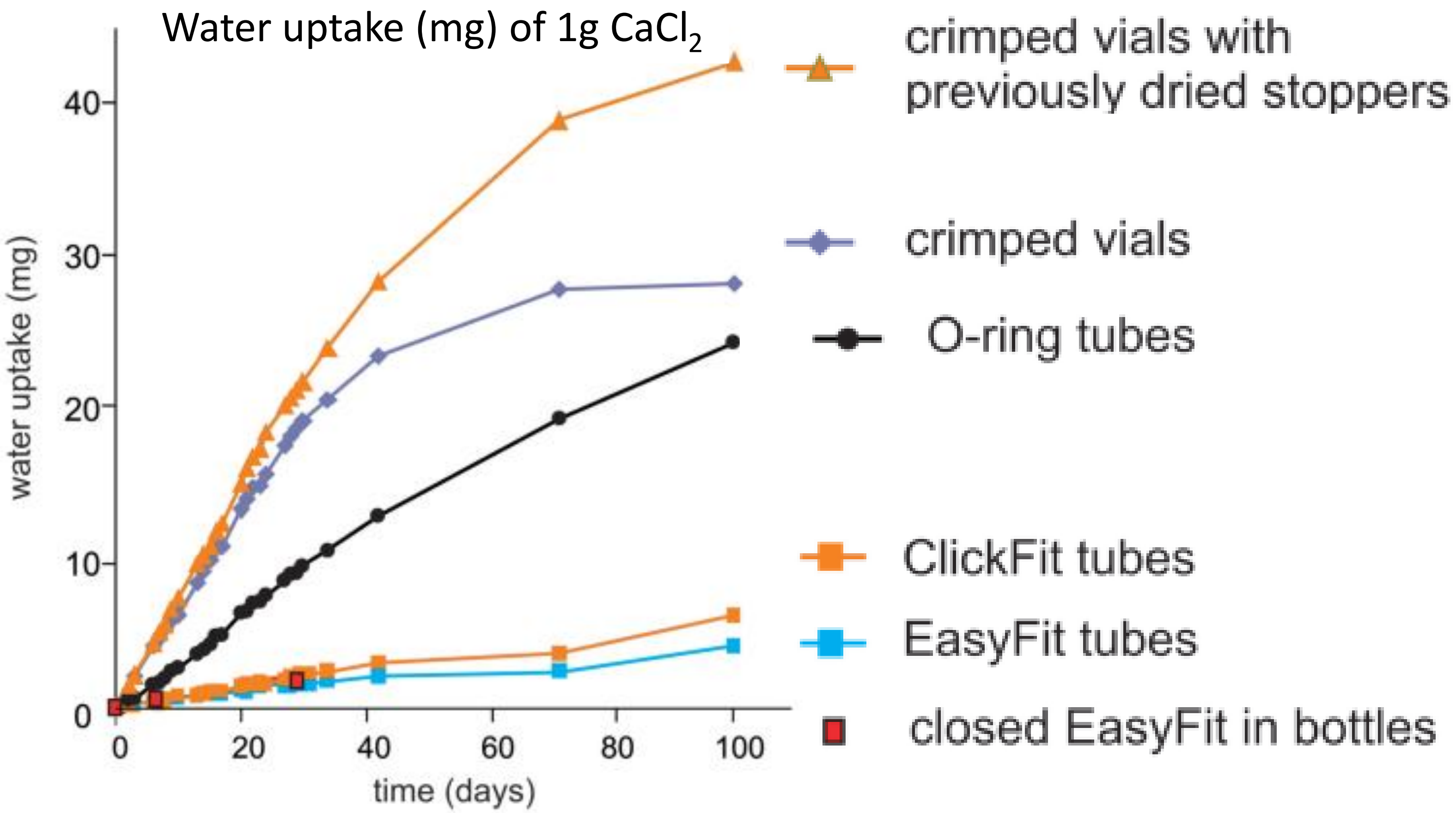
- The main and most deleterious event: chain breakage
- The relative importance of hydrolysis and oxidation vary according to:
  - Water content
  - Environment (contaminants, co-extractants, …)




Bonnet *et al.* Nucleic acids research 2010
10.1093/nar/gkp1060 (corrected)

Water is the enemy

# How hermetic is a container/sealing?




Bonnet *et al.* Nucleic acids research 2010
10.1093/nar/gkp1060

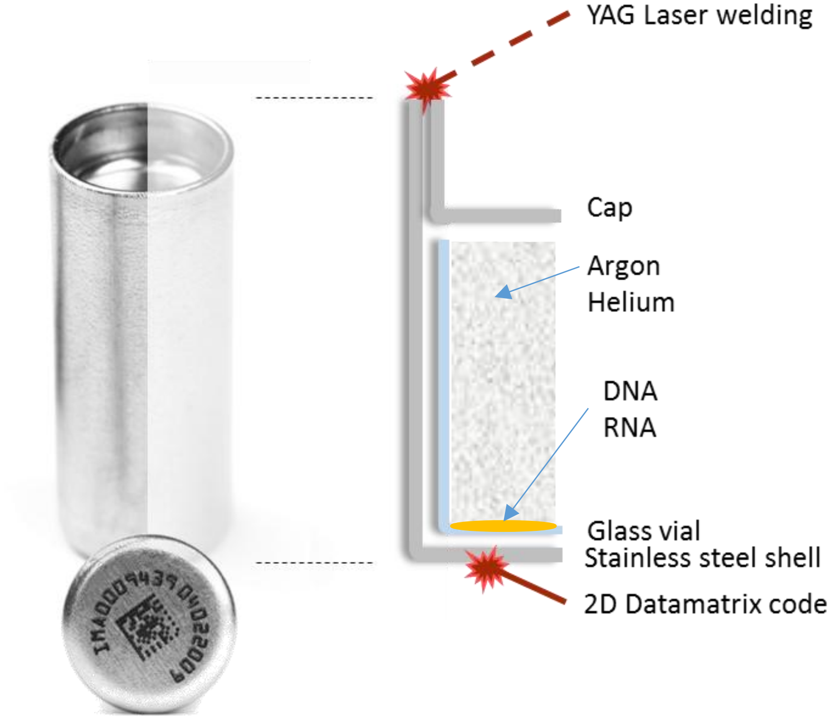
- Counter intuitive results!
- Inner RH will gradually equilibrate with outer RH
- In a closed but non hermetic system, water uptake has to be taken into account during stability testing (modeling can be tricky)

New container highly needed!

# The Imagene technology

- Imagene's technology: completely remove water, oxygen and ozone and maintain these conditions for efficient and long-term storage.
- Imagene stainless steel capsules: DNAshell® and RNAshell®
  - Stainless steel shell
  - Glass vial
  - Stainless steel cap
- The capsule is sealed by YAG laser welding allowing to maintain the desiccated sample under an inert atmosphere.
- A unique 2D barcodes is engraved on each capsule for full traceability.
- Target markets: biobanking, molecular diagnostics, DNA data storage

DNA shell RNA shell

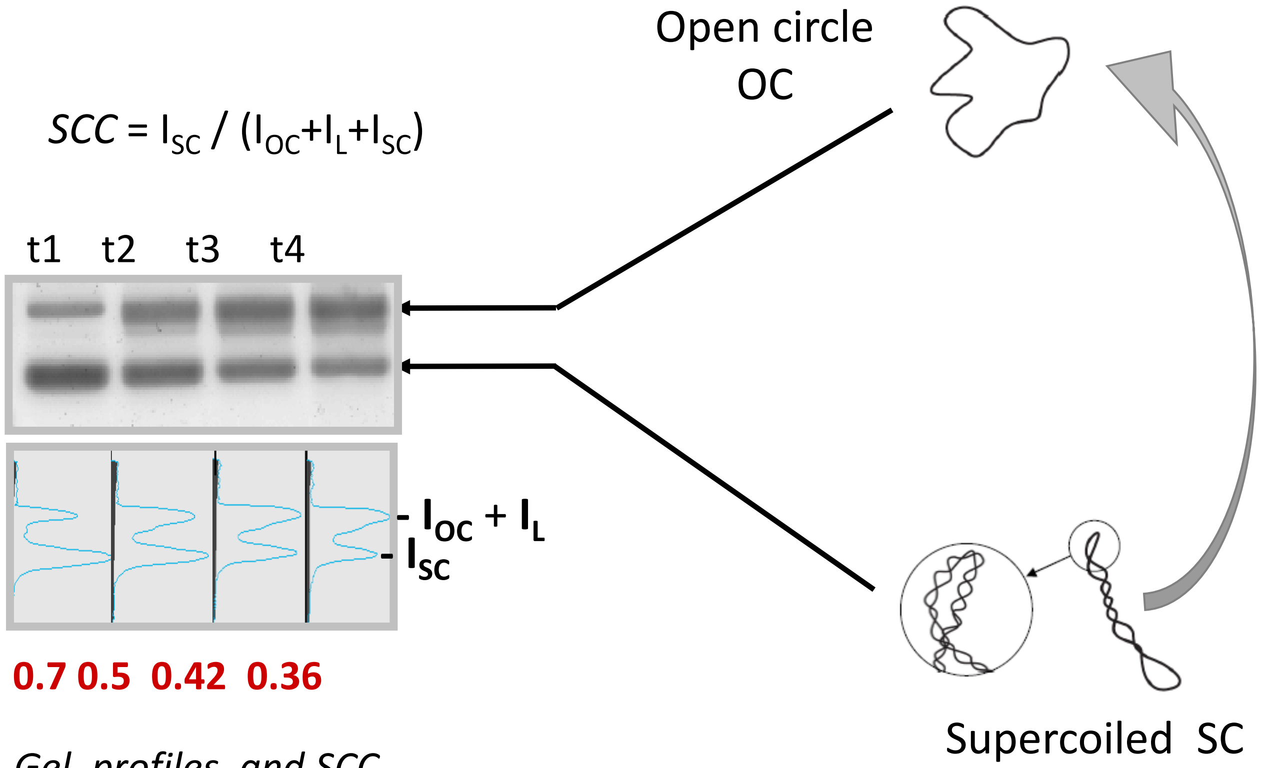



1.3 g
Space 0.7 $cm^3$
Useful volume 0.2-0.3 mL

# Monitoring chain breakage with pDNA

Only one chain break on one of its two strands is sufficient to relax a supercoiled plasmid. The supercoiled plasmid content (SCC) is thus the fraction of intact DNA in the sample. The relaxation is a first order reaction (exponential).

$SCC = I_{SC} / (I_{OC}+I_L+I_{SC})$

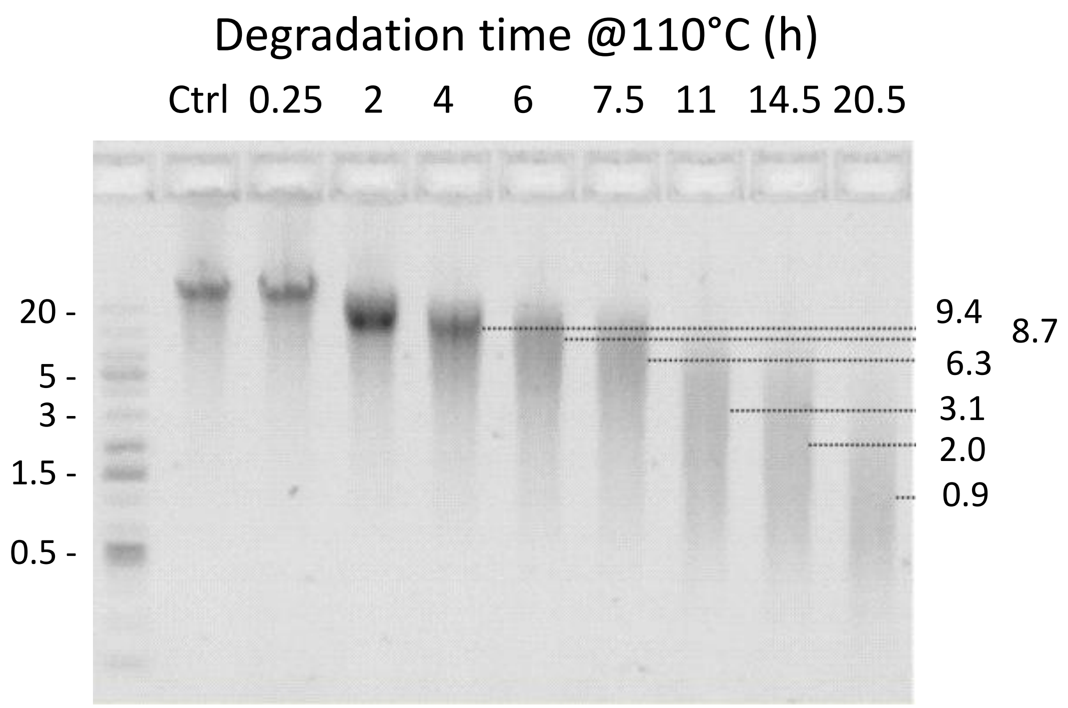


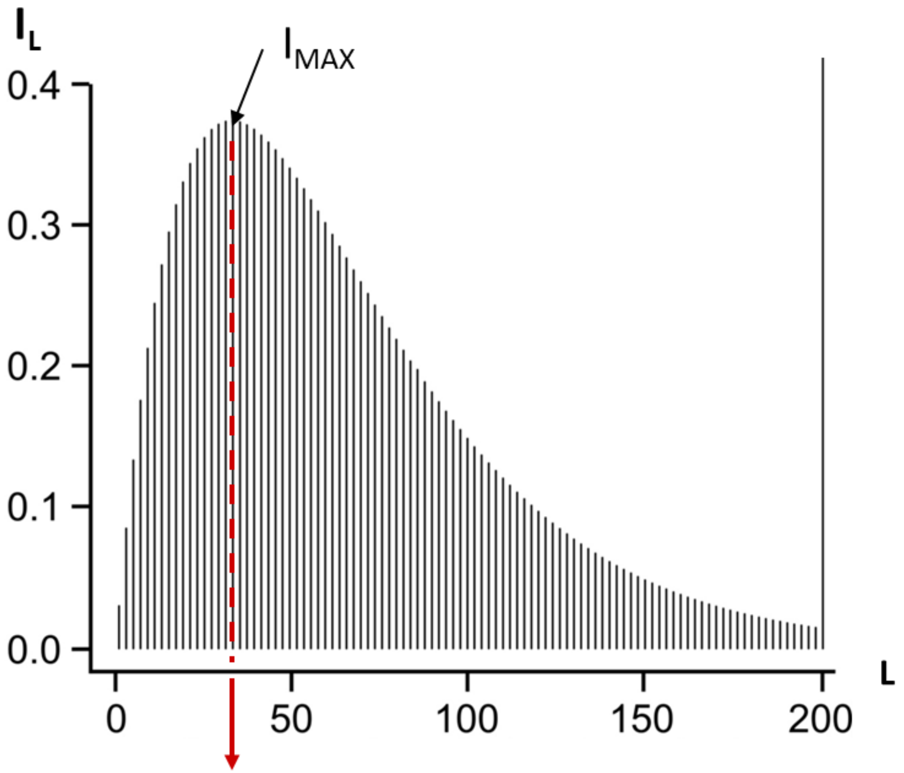

*Gel, profiles, and SCC determination*

$SCC = e^{-k_T \times t \times (n-1)}$

- *SCC*
- $k_T$: $s^{-1}$. $nt^{-1}$
- t: s
- n: plasmid size (nt)

# Monitoring chain breakage with gDNA




$L_{MAX} = 1 / [1 - \exp(-k_T \times t)]$

$L_{MAX}$ = size of the maximum of intensity on the gel (single strand)

→ $L_{MAX}$ : from $k_T$ and time t (estimation after Arrhenius equation)

→ $k_T$ from time t and $L_{MAX}$ (from gel)

# Long term stability evaluation

- Accelerated aging
  - Conduct degradation kinetics at several high temperatures T
  - Measure the degradation rates $k_T$ (chain breaks/nt /s)
  - Plot -log $k_T$ vs 1/T

    => Arrhenius equation

    => extrapolation of degradation rates @RT (or any temperature)

*Bonnet, J., *et al.*, Nucleic acids research 2010 10.1093/nar/gkp1060
*Coudy, D., *et al.*, Long term conservation of DNA at ambient temperature. Implications for DNA data storage. PLoS One, 2021. 16(11)
* Fabre, A-L. *et al.*, An efficient method for long-term room temperature storage of RNA. Eur J Hum Genet 2014 Mar;22(3):379-85
* Fabre, A-L. *et al.*, High DNA stability in white blood cells and buffy coat lysates stored at ambient temperature under anoxic and anhydrous atmosphere PLOSOne 2017 10.1371/journal.pone.0188547

# Long term stability evaluation

- Real time evaluation of degradation rate at room temperature
  - To verify the accelerated aging results
  - To take potential unforeseen parameters into account

* Colotte *et al.* Biopreserv Biobank. 2011 10.1089/bio.2010.0028
This work (unpublished)
* Fabre, A-L. *et al.*, High DNA stability in white blood cells and buffy coat lysates stored at ambient temperature under anoxic and anhydrous atmosphere PLOSOne 2017 10.1371/journal.pone.0188547

# Material and methods

- Source biospecimens and extraction methods

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

- 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)

# Material and methods

- Accelerated aging – simulated 25-100 yrs: no degradation (not shown)
- Real-time long term storage

Capsules stored at room temperature without moisture and temperature control (15°C - 30°C / 50 % RH)

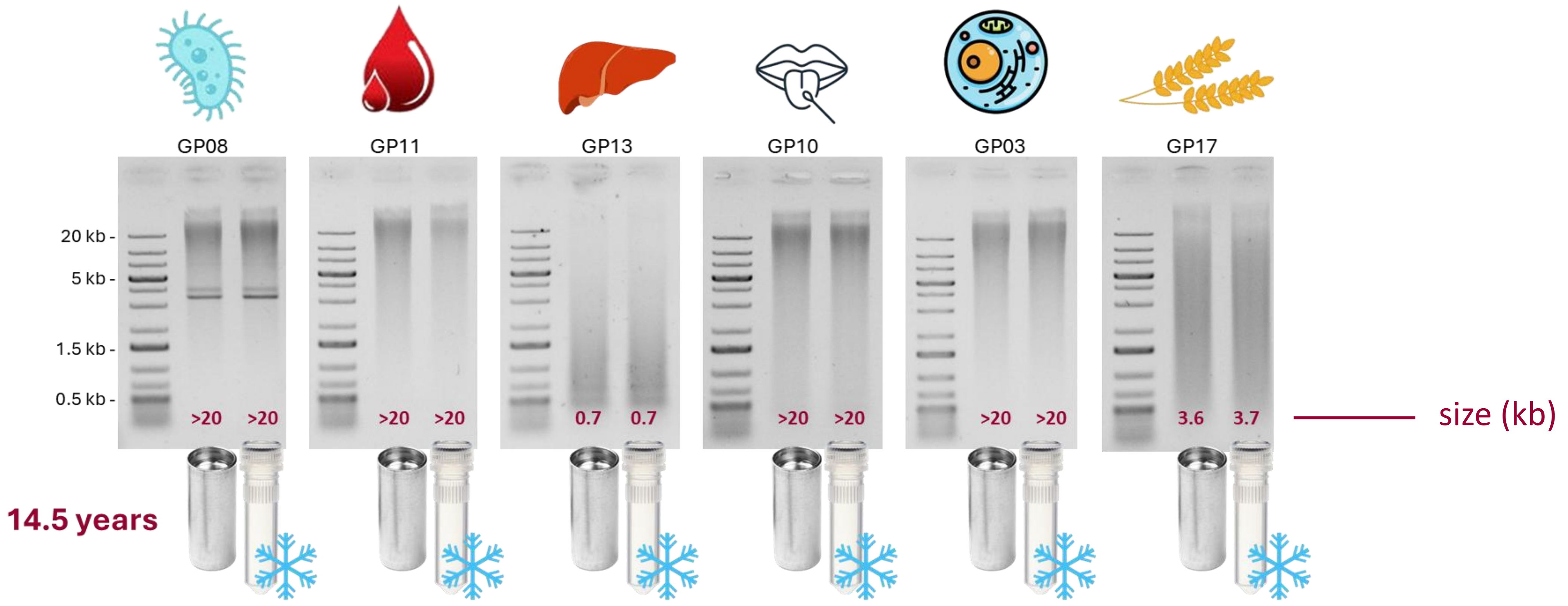
Control capsules stored at -20°C

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

- 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)

# Results

# Purified DNA

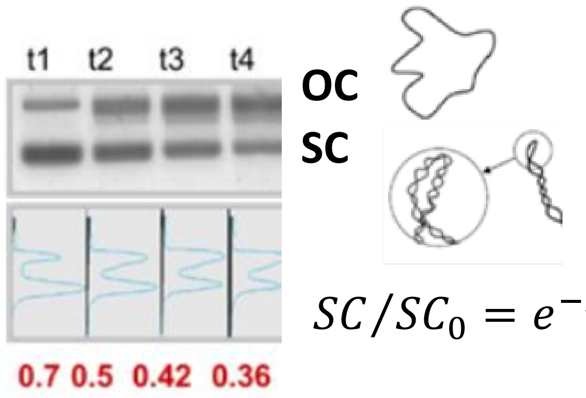



*Heat denaturation + 0.8% agarose gel – TAE buffer – EtBr staining - Size in kb*

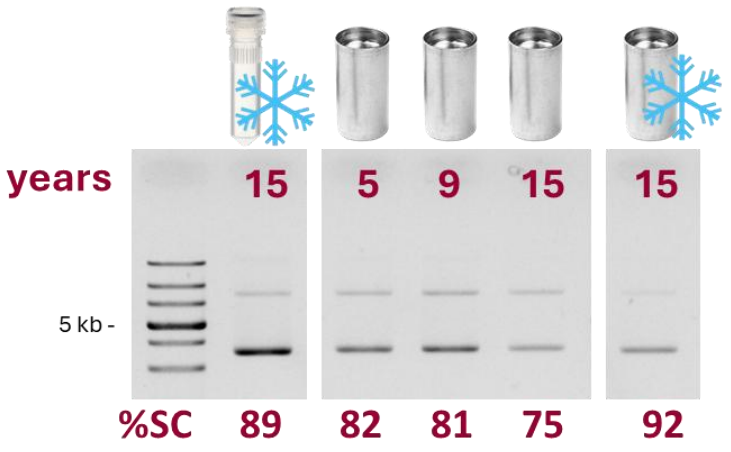
$L_{max}$ > 20 kb after t= 14.5 yrs => $k_{25°C} < 1.09 \times 10^{-13}$ /nt/s

No difference between the frozen controls and the capsules (same as initial sizes, not shown).

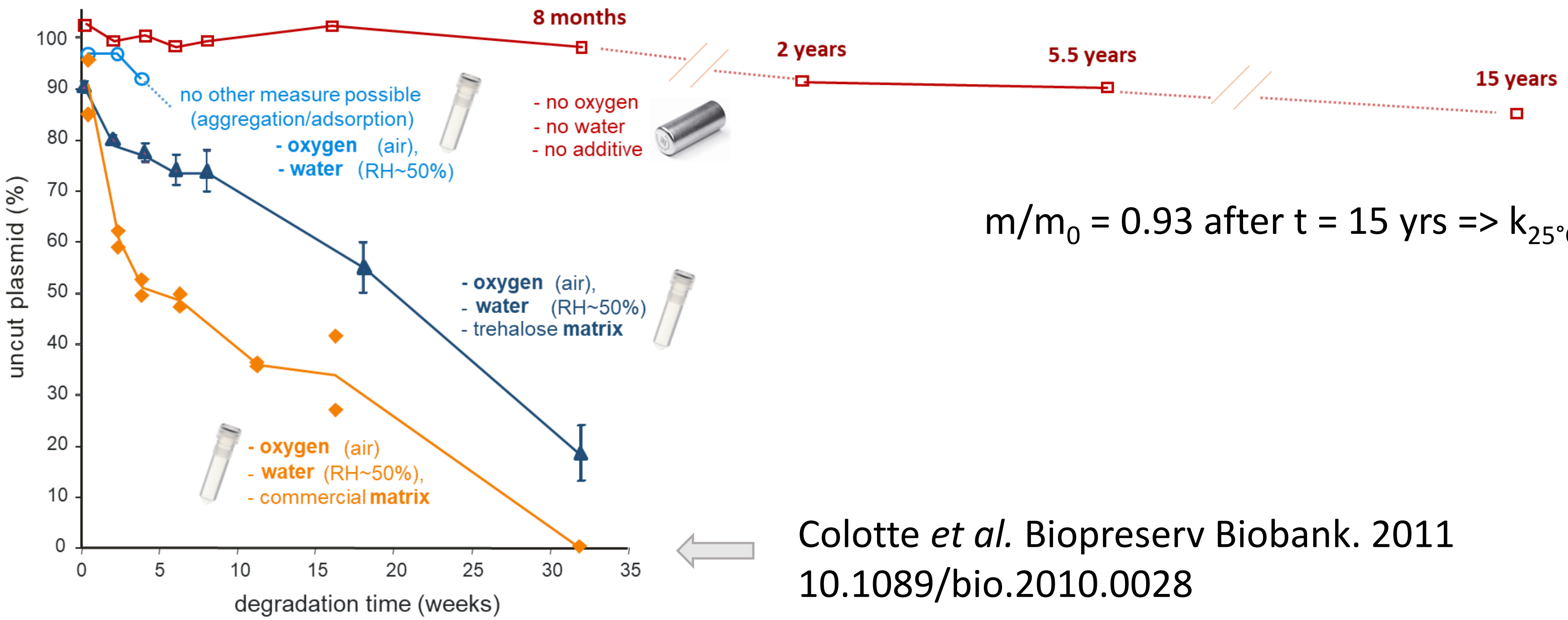
# Plasmid DNA




$$SC/SC_0 = e^{-k_T \times n \times t}$$



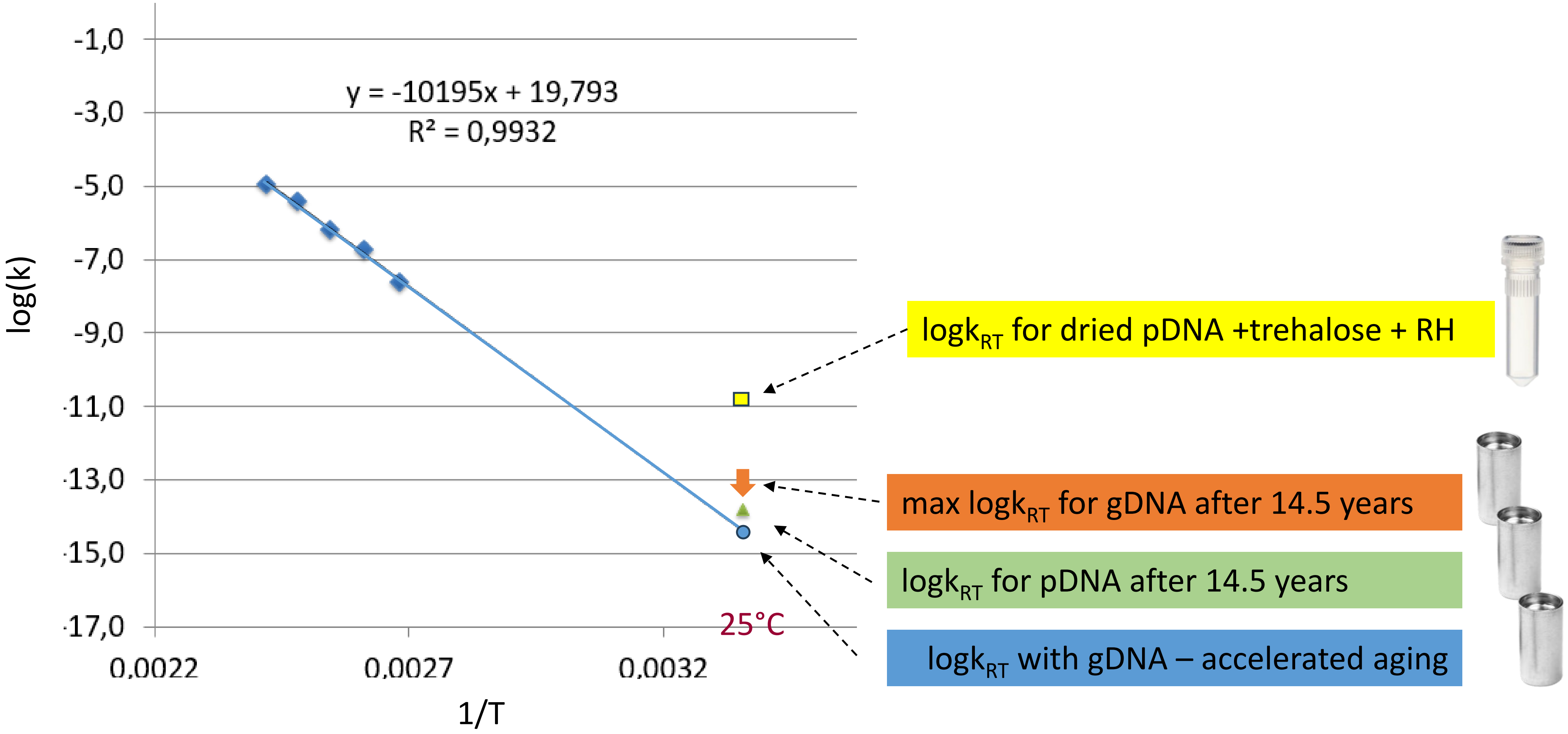

*0.8% agarose gel – TAE buffer – EtBr staining*




$m/m_0$ = 0.93 after t = 15 yrs => $k_{25°C} = 1.4 \times 10^{-14}$ /nt/s

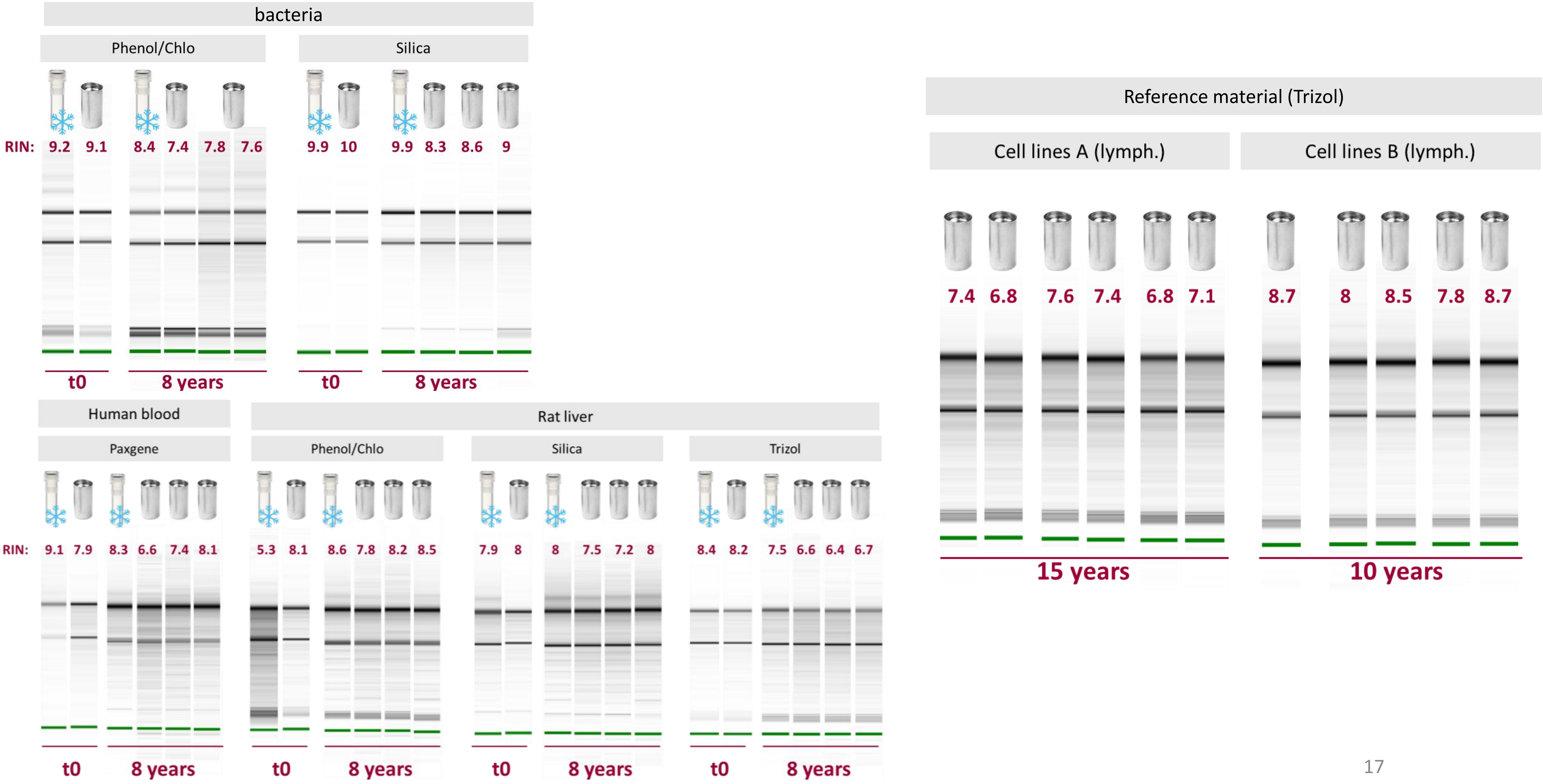
Colotte *et al.* Biopreserv Biobank. 2011
10.1089/bio.2010.0028

# Accelerated vs real-time

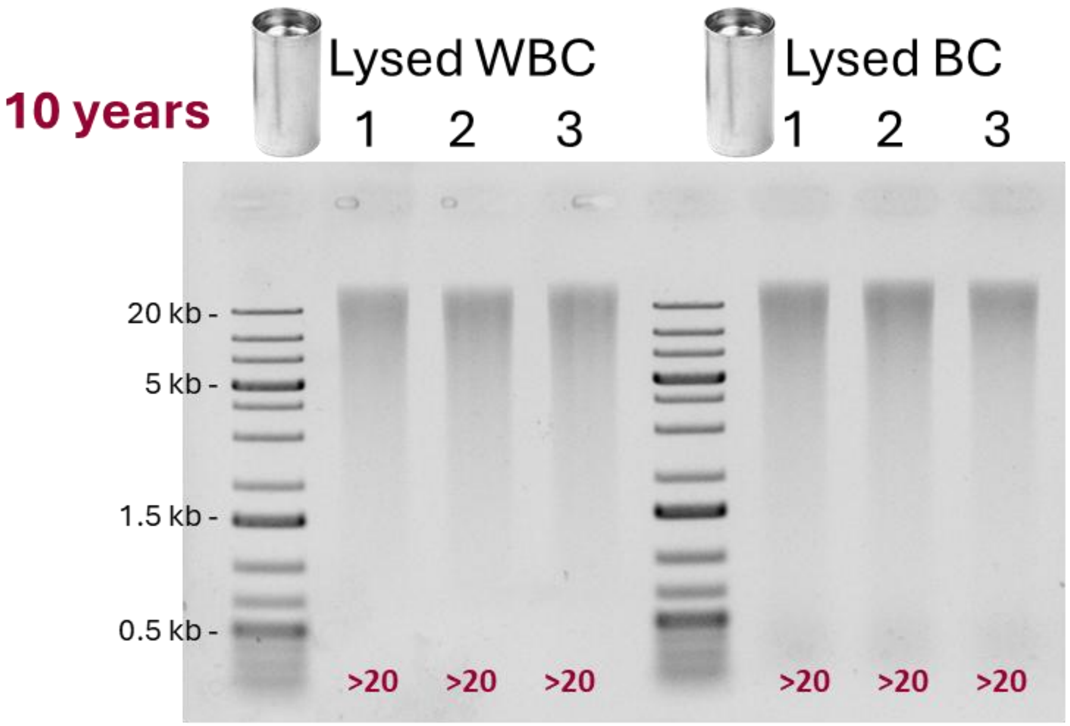



Redrawn from Coudy, D., *et al.*, Long term conservation of DNA at ambient temperature. Implications for DNA data storage. PLoS One, 2021. 16(11)

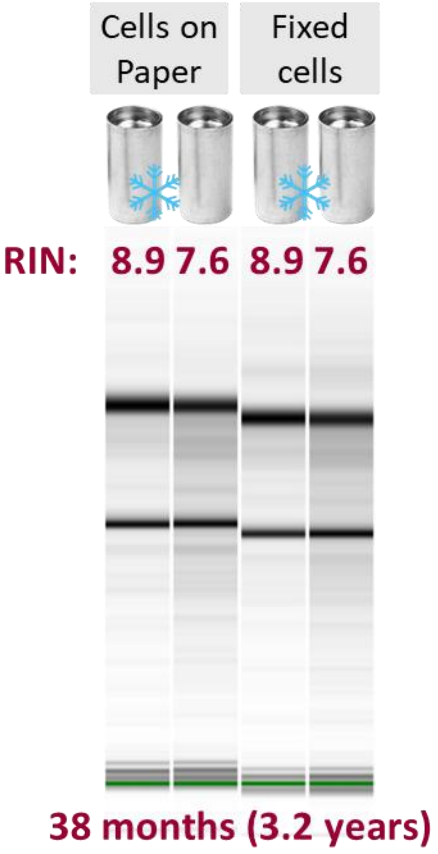
# Purified RNA



# DNA and RNA in biospecimen




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



# Conclusions

- DNAshell /RNAshell is the most advanced available technology for DNA/RNA stabilization, with 15 years of recorded performance

- To build confidence in DNA data storage, it is imperative to:
  - Use validated methods (SNIA standard) & run all necessary controls
  - Use high-sensitive methods to demonstrate stability (easy to hide poor performance)

imagene

Smart Biopreservation

# Thank you !


Marthe COLOTTE, PhD

Imagene CTO

colotte@imagene.eu

# Publications for DNA preservation

External

In house

## DNA

2008 — Stabilité chimique et conformationnelle de l'ADN à l'état sec et à température ambiante
Thèse de Marthe Colotte (2008)

2009 — Chain and conformation stability of solid-state DNA: implications for room temperature storage
DOI: 10.1093/nar/gkp1060

2009 — Simultaneous assessment of average fragment size and amount in minute samples of degraded DNA - DOI: 10.1016/j.ab.2009.02.003

2010 — Adverse effect of air exposure on the stability of DNA stored at room temperature
DOI: 10.1089/bio.2010.0028

2011 — Novel procedure for high yield recovery of traces amounts of DNA stored at room temperature
Poster - ESBB

2013 — Assessment of DNA encapsulation, a new room-temperature DNA storage method
DOI: 10.1089/bio.2013.0082

2014 — Evaluation of DNA/RNAshells for room temperature nucleic acids storage
DOI: 10.1089/bio.2014.0060

2015 — Preservation of biospecimens at ambient temperature: special focus on nucleic acids and opportunities for the biobanking community
DOI: 10.1089/bio.2015.0022

2016 — Quality Matters: 2016 Annual Conference of the National Infrastructures for Biobanking
DOI: 10.1089/bio.2016.0053

2018 — Ensuring the Safety and Security of Frozen Lung Cancer Tissue Collections through the Encapsulation of Dried DNA
DOI: 10.3390/cancers10060195

2018 — DNAshell Protects DNA Stored at Room Temperature for Downstream Next-Generation Sequencing Studies
DOI: 10.1089/bio.2018.0129

## DNA reference material

2015 — A novel method for room temperature distribution and conservation of RNA and DNA reference materials for guaranteeing performance of molecular diagnostics in onco-hematology: A GBMHM study
DOI: 10.1016/j.clinbiochem.2015.04.004

## White Blood Cells & Buffy coat

2017 — High DNA stability in white blood cells and buffy coat lysates stored at ambient temperature under anoxic and anhydrous atmosphere
DOI: 10.1371/journal.pone.0188547

## Synthetic DNA

→ DNA data storage

2021 — Long term conservation of DNA at ambient temperature. Implications for DNA data storage
DOI: 10.1371/journal.pone.0259868

2021 — An Empirical Comparison of Preservation Methods for Synthetic DNA Data Storage
DOI: 10.1002/smtd.202001094

# Publications for RNA preservation

External

In house

## ▪ RNA

2014
An efficient method for long-term room temperature storage of RNA
DOI: 10.1038/ejhg.2013.145

2014
Evaluation of DNA/RNAshells for room temperature nucleic acids storage
DOI: 10.1089/bio.2014.0060

2017
Long-term room temperature storage of dry ribonucleic acid for use in RNA-Seq analysis
DOI: 10.1089/bio.2017.0024

## ▪ RNA Reference material

2015
A novel method for room temperature distribution and conservation of RNA and DNA reference materials for guaranteeing performance of molecular diagnostics in onco-hematology: a GBMHM study
DOI: 10.1016/j.clinbiochem.2015.04.004

## ▪ Synthetic RNA

→ Reference material SARS-CoV-2

2023
Reference materials for SARS-CoV-2 molecular diagnostic quality control: validation of encapsulated synthetic RNAs for room temperature storage and shipping
DOI: 10.1101/2023.08.28.555008

→ Certified Reference material SARS-CoV-2

2024
Certification of the identity and the copy number concentration of synthetic single-stranded RNA including fragments of the SARS-CoV-2 genome and part of the human RNAse P gene: EURM®-014k – JRC Reference Material report.

# Publications for Biospecimen

External

In house

- Blood biomarkers (for diagnostics)

*2017*

Stability of newborn screening markers in dried-blood spot (DBS): the innovative imagene solution.
Poster - SFEIM

- Live bacteria & viruses

*2017*

ANVBIS3 – Acides nucléiques, virus & bactéries d'intérêt en stockage standardisé et sécurisé
Poster - 6ème Forum DGA innovation

- Spermatozoa *(for nucleus transfert)*

*2023*

Reviving Vacuum-Dried Encapsulated Ram Spermatozoa via ICSI after 2 Years of Storage
DOI: manuscript accepted

*2025*

Fertility preservation of vacuum-dried ram spermatozoa stored for four years at room temperature
Theriogenology – Vol 239, June 2025, 117390