DNA DATA STORAGE ALLIANCE
A SNIA Community

A standard method and metric for measuring the media durability claims of a DNA Containment System (DCS) for digital data storage

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Acknowledgements
Jacques Bonnet, Marthe Colotte, Lee Organick, Chris Takahashi

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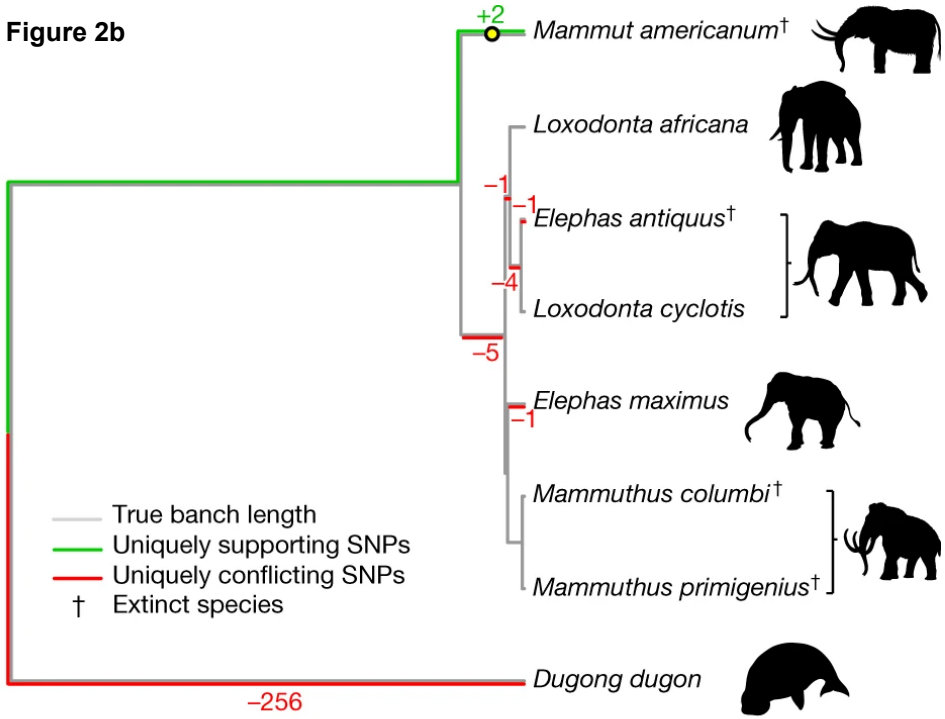
DNA bits are very durable …

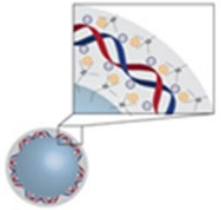


Kjær, K.H., Winther Pedersen, M., De Sanctis, B. et al. A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. Nature 612, 283–291 (2022). https://doi.org/10.1038/s41586-022-05453-y

.. but we won't store digital data in DNA using fossils

Must create trust that manufactured DNA Containment Systems (DCS) work

- In an ecosystem, different DCS's have different cost, complexity, and duration characteristics
- Apples-to-apples comparison of durability requires
  - Standard method(s) for conducting media aging experiments
  - Standard metric(s)
- Challenges to creating standard methods & metrics
  - DNA ages very slowly so we need to believe that an accelerated wear methodology won't skew results
  - Can a DCS be evaluated independently of the other steps in the DNA data storage pipeline?



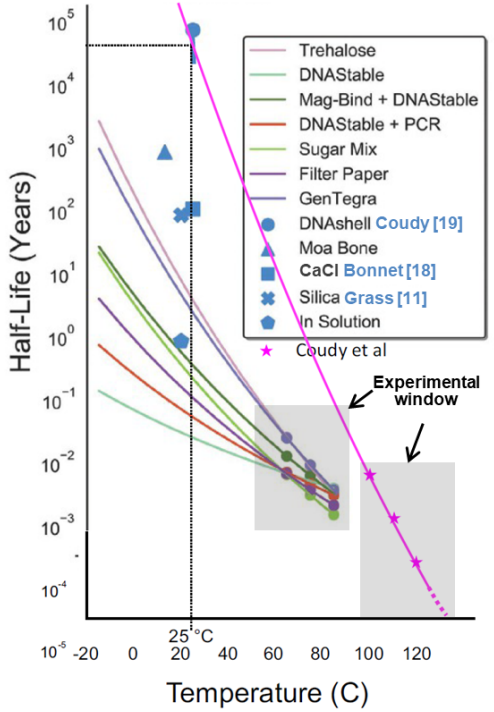
Silica nanoparticles (x years)

Vials (y years)

Sealed stainless steel (z years)

Research on preservation/containment

- In general, possible to extend the durability of DNA media by using various additives and containment mechanisms [table]
- In particular, a DCS that shields DNA media from the atmosphere preserves molecular stability for long periods at room temp (i.e., 25°C), Grass [11], Coudy [19]



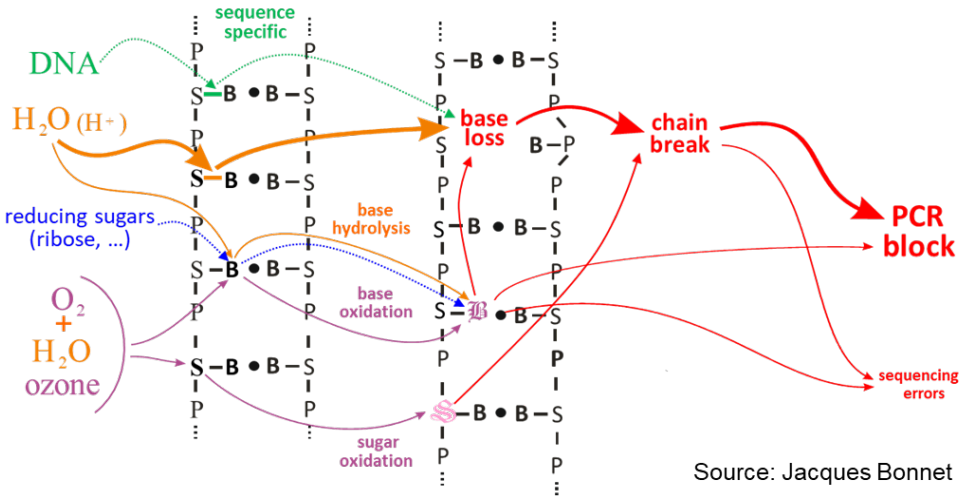
Edited from figure 2b in Organick et al [41]

Preservation Category	Preservation Substrate/Method	Drying	Protection from atmosphere	Stability estimation method
Chemical encapsulation	Encapsulation in salts[1,2,3]	✓		Accelerated aging
	Degradable Polymer[4,5]		✓	Accelerated aging
	Cationic Diblock Copolymer[6]		✓	
	Silica nanoparticles[7,8,9,10,11]	✓	✓	Arrhenius
	Magnetic silica nanoparticles[12]		✓	Accelerated aging
Lyophilization	3D-printed microfluidic chip[13]	✓		
	DNA movable type blocks[14]			
	Living Memory Microspheroid[15]	✓		3 months at RT
	Storage Platform, Physical Data Partitioning[16]	✓		
	DNA Data Storage in Perl[17]	✓		Accelerated aging
Physical encapsulation	Stainless steel capsules[18,19]	✓	✓	Arrhenius
Inclusion in a matrix	DNAstable[41]	✓		Arrhenius
	Gentegra DNA[41]	✓		Arrhenius
	Pullulan[20]	✓		
	Silk[21]	✓		
	composite nucleic acid-polymer fibers[22]	✓		Accelerated aging
	300K matrix inclusion[23]	✓		
	Hierarchically structured polymeric microparticles[24]			
Absorption on paper	FTA paper[41]	✓		Arrhenius
	Chitosan treated paper[25]	✓		
Dehydration on solid supports	Glass[26,27]	✓		
	Silicon[28]	✓		
Dissolution in liquid salts	Imidazolium ammonium pyridinium cations[29]			
	Ammonium-Based Ionic Liquid[30]			>1 year at rt
Living organism	yeast genome[31,32]	✓		
	E.coli genome[32,33,34]	✓		
	yeast cells[35]	✓		
	Bacteria[30,33]	✓		
	Bacillus spores[36]	✓		
	Living Memory Microspheroid[15]	✓		3 months at RT
DNA beads	Magnetic Bead Spherical Nucleic Acid[31]	✓		Arrhenius
storage in long DNA molecules	Experimental DNA storage platform[37]			
	Storage in an Extremophile Genomic DNA[38]			
	construction, sequencing, long artificial DNA sequence[39]			
	DNA as a universal chemical substrate[40]			

Source: Jacques Bonnet, Marthe Colotte

What did the research tell us about defining a durability verification method and metric?

- The predominant form of DNA degradation events in storage is a strand break, which can be detected and quantified with qPCR or dPCR, since broken chains will not polymerize. [diagram]
- Strand breaks during storage appear to be independent of the sequences in the DNA strands stored. [11, 41] In other words, no observed sequence bias for DCS studied to date.
- If a DNA strand survives storage intact, the data stored in that strand appears to be recoverable (even at elevated temp during accelerated wear). [11, 41]



Source: Jacques Bonnet

Conclusion

A standard accelerated wear methodology measuring the time by which only 50% of the original strands remain intact in a DCS gives us a durability metric (half-life) that is independent of synthesis, retrieval, and sequencing.

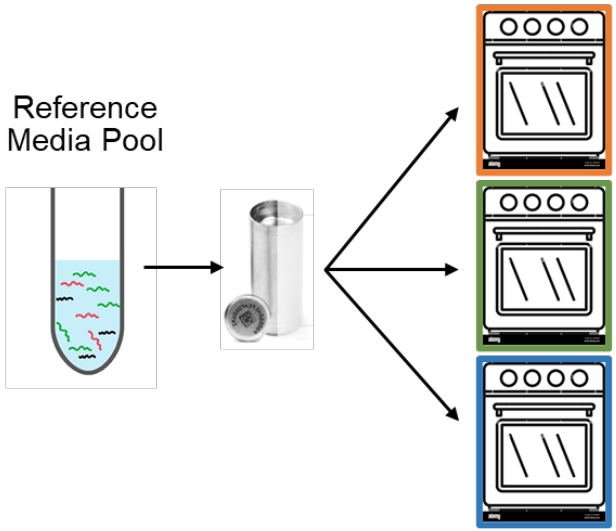
We took the goal to standardize this flow

DNA Containment System (DCS)

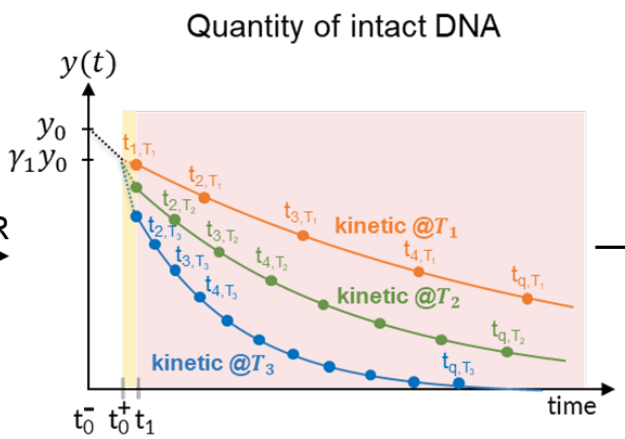
Accelerated aging at various temperature, humidity conditions

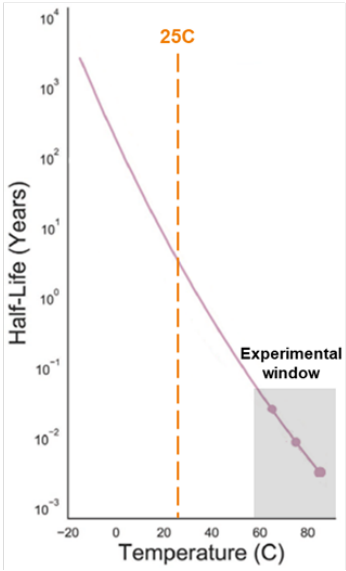
Measured % remaining material (i.e., uncut strands over time)

Arrhenius curve fit of degradation data set

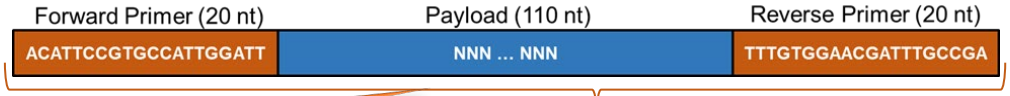


qPCR





Outline of the spec



Recommended, but not required.

Reference Media Pool (5)

Rest of Talk

Accelerated Aging (6)

Real-time Aging (7)
Same flow as Accelerated, but with actual storage conditions

Experiment Parameter Design (6.1)

Storing the Media in the DCS (6.2)

Pre-Aging Media Loss Quantification (6.3)

Aging and Measurement (6.4)

Half-Life Calculation (6.5)

Highly recommended to run a realtime test even if no measurable degradation detected. E.g., for a DCS with a claimed half-life of 10 years at 25°C, for 150 nt strands, 87% of the strands should be intact after 2 years. A significant deviation from this should lead to re-evaluation.

Alternative Methods (8)

Permissible to vary Reference Pool format and measurement techniques for specific cases; must be documented

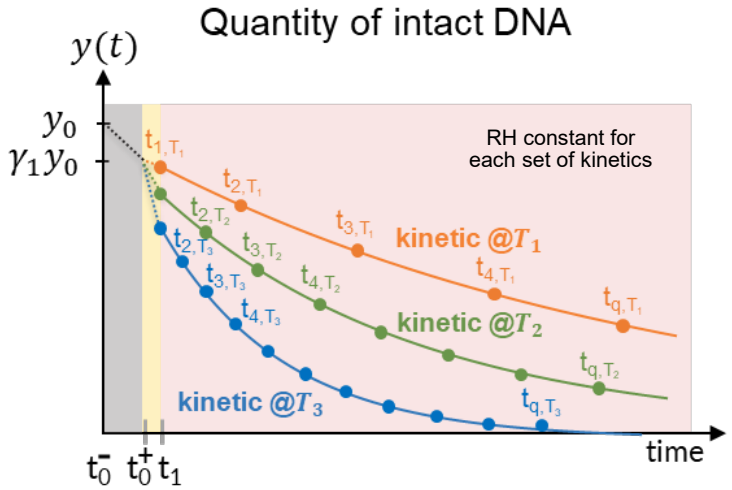
E.g., properties of a DCS may cause short (150nt) oligos to result in very long test times, even at accelerated temp. This may require longer oligos or other forms of DNA (e.g., supercoiled plasmids) that are more sensitive to degradation at lower temperatures. This may require measurement methods more sensitive than qPCR or dPCR (e.g., gel electrophoresis).

The experiment model (section 6.1)

- Humidity (RH) is the controlled parameter
  - Constant for each set of temperatures ($T_{1\text{-}n}$) defined in experiment
  - Two mandatory RH points (50% and 75%).
  - More RH points optional; ≥ 20% from the two mandatory points and each other
- Temperature (T) is the acceleration parameter
  - Set of T values for each value of RH
  - Minimum of 3 temperatures ($T_{1\text{-}n}$ where n ≥ 3)
  - Each temperature point ≥ 10°C from each other
- Nonlinear degradation, plus constants for pre-aging material loss:
  - $\gamma_1 = y(t_0^+)/y_0$ - material loss prior to aging conditions being applied
  - $\gamma_{2,T} = y(t_{1,T})/(t_0^+)$ - material loss while degradation rate not at steady state

$$\text{Concentration at time } t \quad y(t) = \begin{cases} y_0 & \text{for } t = t_0^- \\ \gamma_1 y_0 & \text{for } t = t_0^+ \\ \gamma_1 \gamma_{2,T} y_0 \exp\left(-nA \exp\left(\frac{-E}{T}\right) t\right) & \text{for } t \geq t_{1,T} \end{cases} \quad (1)$$

- Two pre-aging phases defined by three time-values
  - t_0^- = time at which material is prepared, but not yet stored in the DCS
  - t_0^+ = time at which aging conditions are applied to media in the DCS
  - $t_{1,T}$ = time by which aging conditions have been applied long enough so the degradation rate has reached steady state for the kinetic at T.
- Aging phase
  - For each T, DNA is sampled at time points, $\{t_0^-, t_0^+, t_1, t_2, \ldots, t_q\}$, $q_T \geq 4$
  - Δt is constant for each kinetic (i.e.,$t_{q,T} = t_{1,T} + (q_T - 1)\Delta t_T$), but each kinetic can have a different Δt



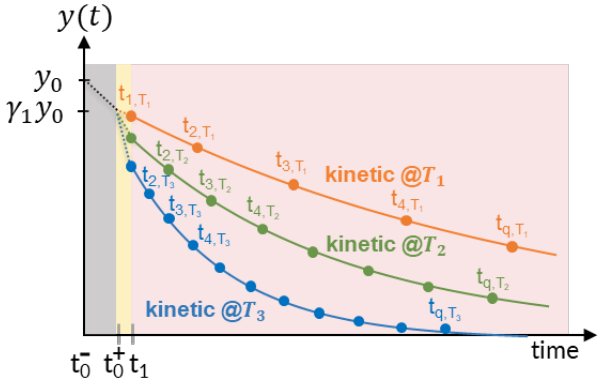
Analysis and Reporting (Section 6.5)

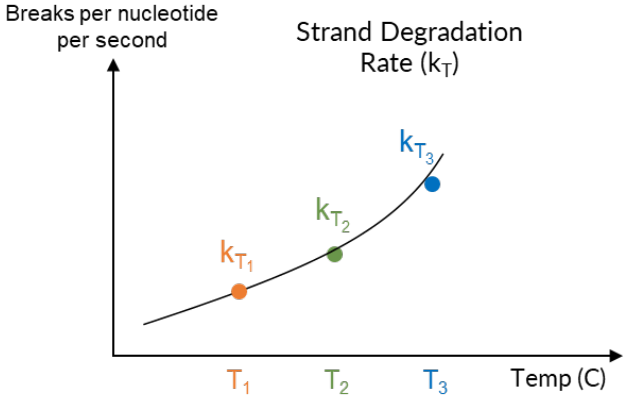
The measured data series for each kinetic, y(t) for t = $t_{1,T}, t_{2,T}, \ldots, t_{q,T}$ for each value of T, are fit to:

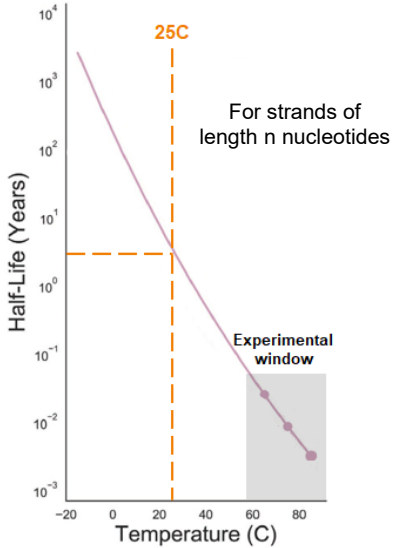
$$y(t_T) = y(t_{1,T})\exp(-nk_T(t_T - t_{1,T})) \quad (2)$$

From each exponential equation, the degradation rate k_T (i.e., strand degradation rate at temperature T), is estimated in units of breaks per nucleotide per second

The k_T are used to plot an Arrhenius relation (-log10(k_T) plotted 1/T) that is then used to extrapolate the half-lives ($t_{1/2}$) of DNA fragments, of length n nucleotides, to 25°C



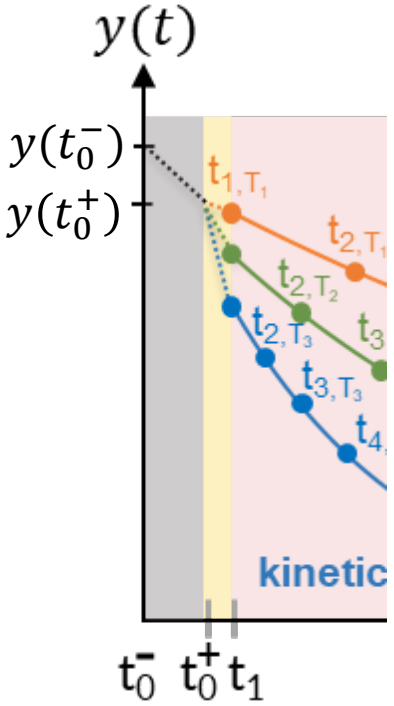




- Testers must report on the quality of all curve fits (i.e., residuals) and the method used to calculate the fits (e.g., R-square)

Pre-Aging media loss quantification (section 6.3)

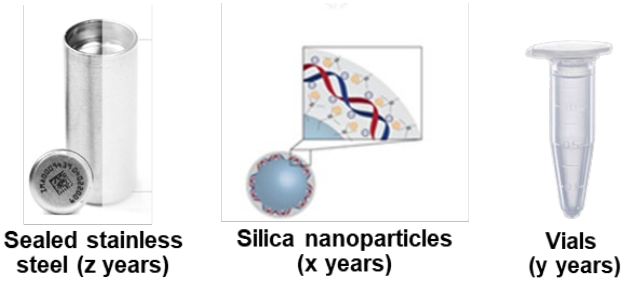
- Amount of material lost before aging starts (expressed in model as γ_1), is also a valuable measure of the quality of a DCS (ease of use, estimate of how much material one should start with, etc.).
- Spec recommends strongly to capture this quantity, "DCS Recovery Rate %", $\gamma_1 = y(t_0^+)/y(t_0^-)$):
  - $y(t_0^-)$: Amount of intact DNA before the media stored in DCS
  - $y(t_0^+)$: Amount of remaining intact DNA after media stored in DCS but before experimental conditions applied
- The data collected to calculate the DCS Recovery Rate % use same methods as for data collected in Aging Phase, but are not considered in degradation rate calculations



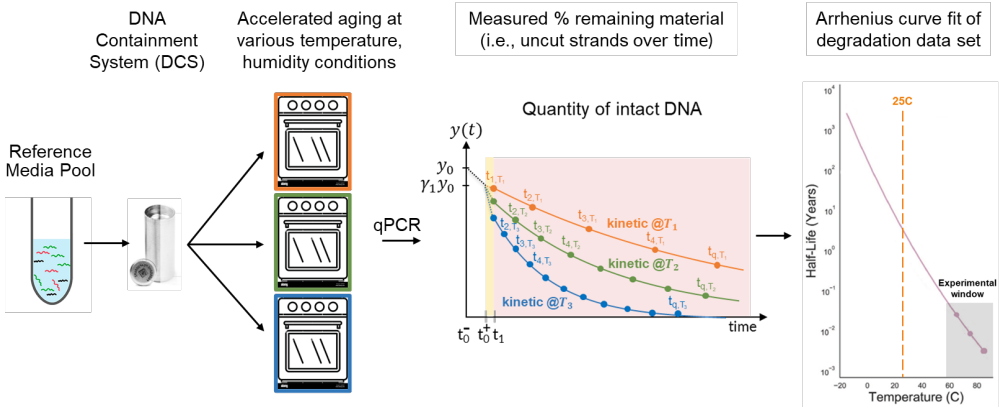
We needed a standard method and metric

We offer the DNA Data Stability Evaluation Method v1.0 to meet this challenge

- In an ecosystem, different DCS's have different cost, complexity, and duration characteristics



- Apples-to-apples comparison of durability requires
  - Standard method of conducting media aging experiments
  - Standard metric



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THANK YOU

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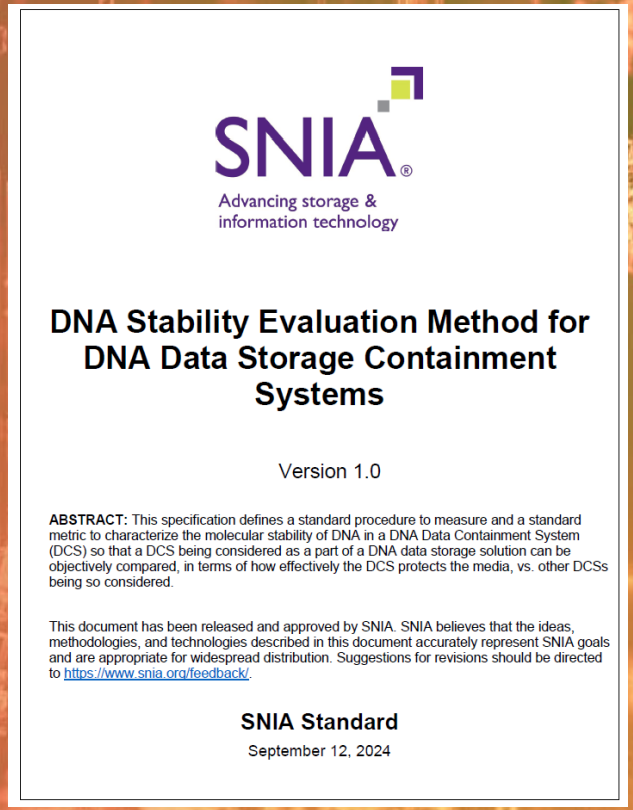
SNIA
Advancing storage & information technology

DNA Stability Evaluation Method for DNA Data Storage Containment Systems

Version 1.0

ABSTRACT: This specification defines a standard procedure to measure and a standard metric to characterize the molecular stability of DNA in a DNA Data Containment System (DCS) so that a DCS being considered as a part of a DNA data storage solution can be objectively compared, in terms of how effectively the DCS protects the media, vs. other DCSs being so considered.

This document has been released and approved by SNIA. SNIA believes that the ideas, methodologies, and technologies described in this document accurately represent SNIA goals and are appropriate for widespread distribution. Suggestions for revisions should be directed to https://www.snia.org/feedback/.

SNIA Standard

September 12, 2024

BACKUP

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Grass et al [11]

Can we recover data in uncut strands that survive accelerated wear?

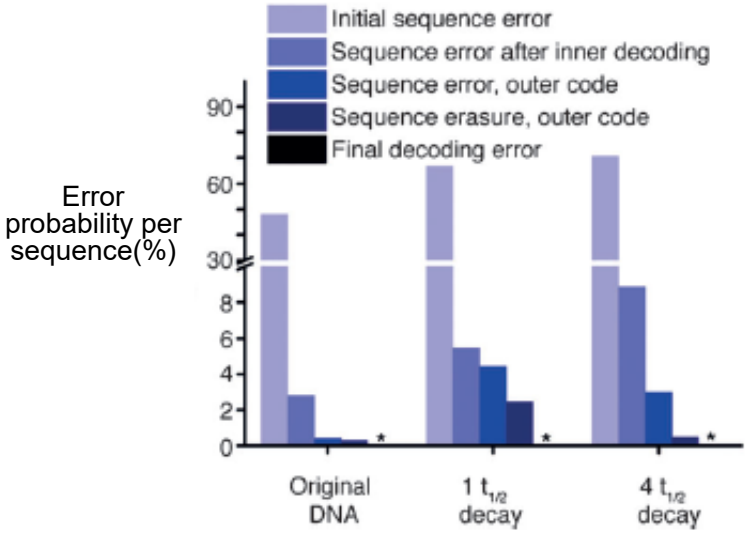
Study observations

- "Inner and outer code of the error correcting scheme had to correct significantly more errors than in the non-heat-treated sample, [but] in both cases the original information could be recovered without final error"

Answer: Yes

- Enough strands survived temperatures used in accelerated wear to validate high temperature stress method

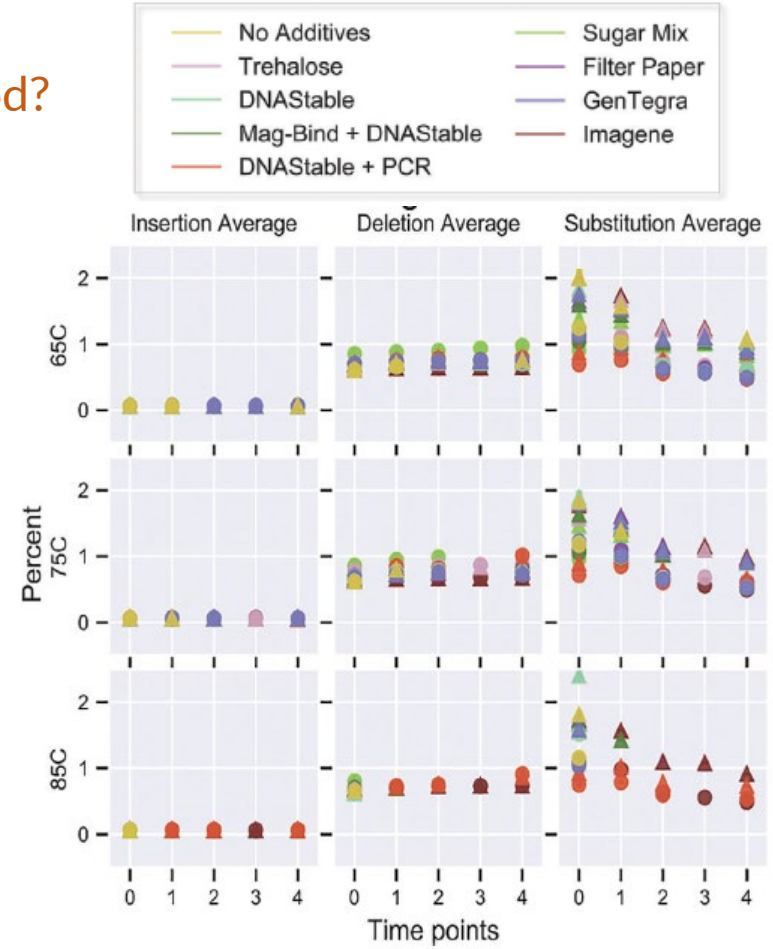
Figure 3
Recovering original data from silica substrate



Organick et al [41]

Do read errors vary due to preservation method?

Figure 4 – Observed error rates



Study observations:

- Minimal (< 1%) variation in error rates across preservation methods, temps, and time points
  - Even substitutions, which show most variance, show this variance before any aging begins
- No one preservation method showed consistently more or fewer errors than any other method across different temperatures and time points
  - Suggests insertion, deletion, substitution errors are independent of storage method

Answer: No

- For the purposes of evaluating and comparing a DCS, errors introduced/corrected by synthesis, retrieval, and sequencing can be ignored

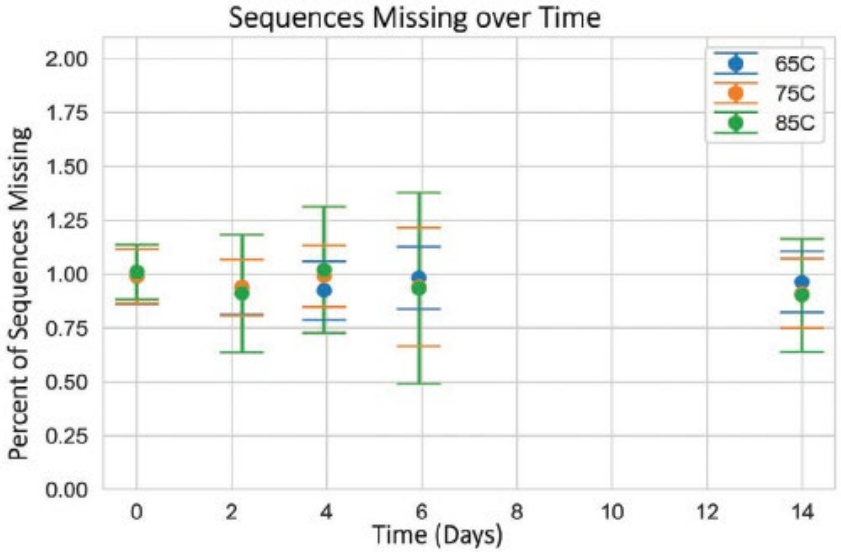
Organick et al [41]

Do certain sequences cause read errors with specific preservation methods?

Study Observations

- Total # of sequences found missing during sequencing (across all methods, time points, temperatures) were analyzed for sequence loss
  - Total # missing sequences did not increase over Time 0, indicating no sequence dependent degradation caused by preservation method (i.e., no "storage bias")
  - This finding reinforced by further finding that individual sequences missing at a particular timepoint had > 90% probability of reappearing and being successfully sequenced later

Figure 5a



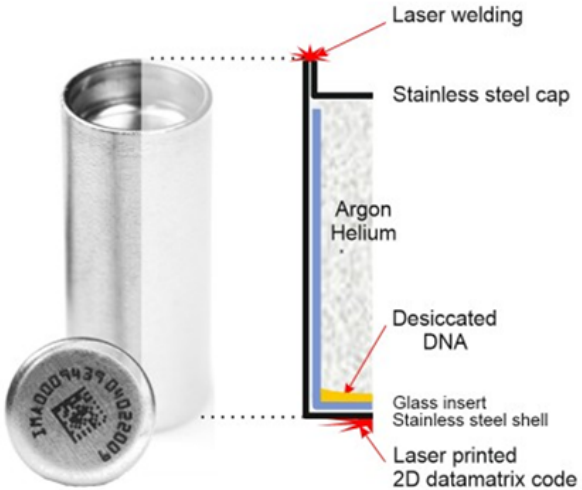
Answer: No

- Further reinforces the conclusion one can define a standard stability evaluation methodology that is independent of the effects of synthesis, retrieval, and sequencing

DNA sealed in inert atmosphere

Coudy et al [19]

- Methods which completely seal media from atmosphere yield very high durability



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