CRISPR-Cas – encoding image and a movie

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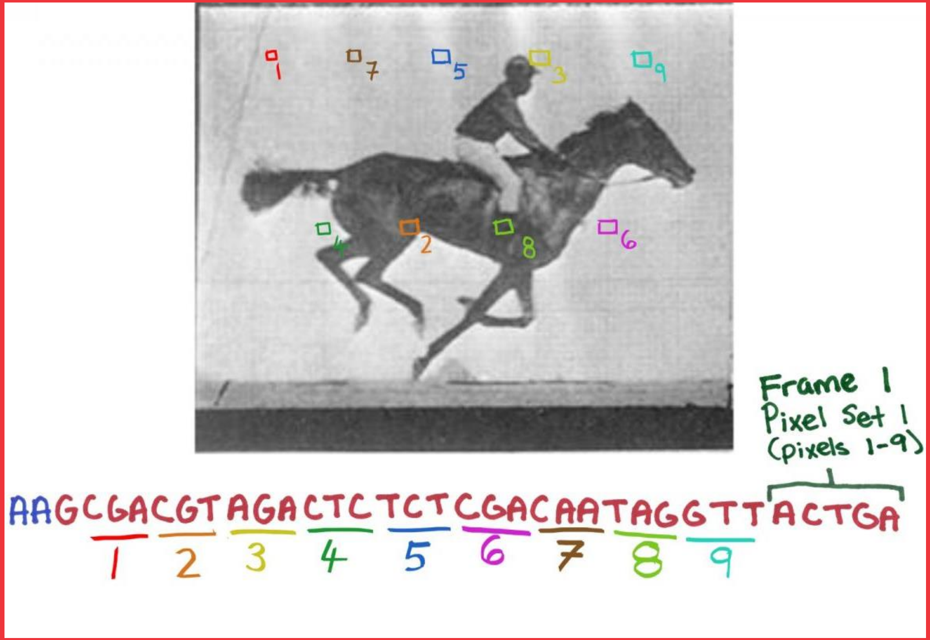


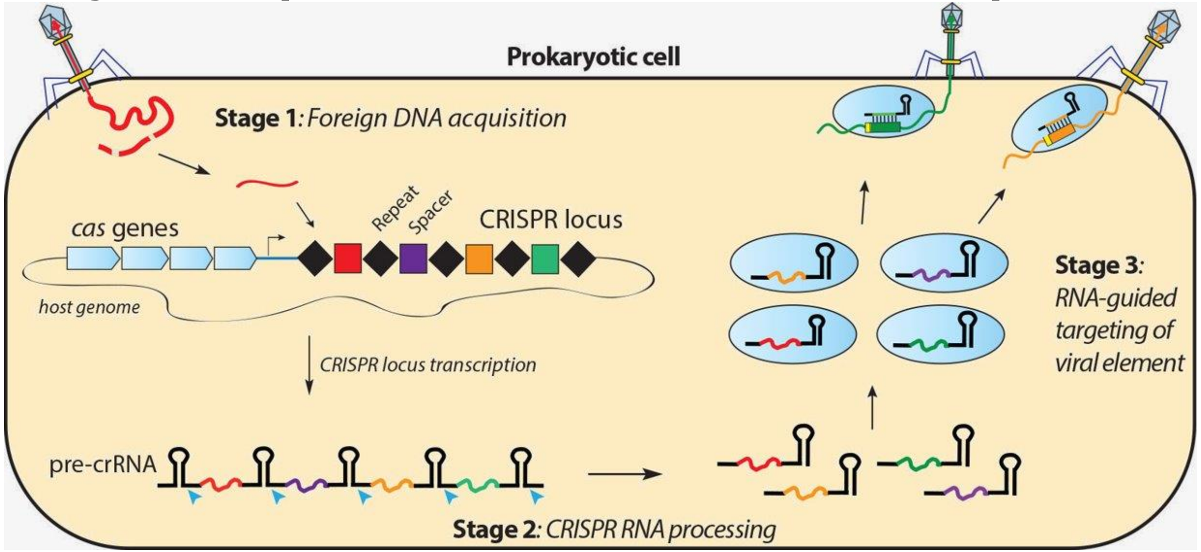
Credit: https://www.genengnews.com/

Contents

1. Basics of CRISPR

CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats

- **Adaptive immune system of prokaryotic organisms**
- **A tool for genome editing**
    - Nobel Prize in Chemistry 2020



https://doudnalab.org/research_areas/crispr-systems/

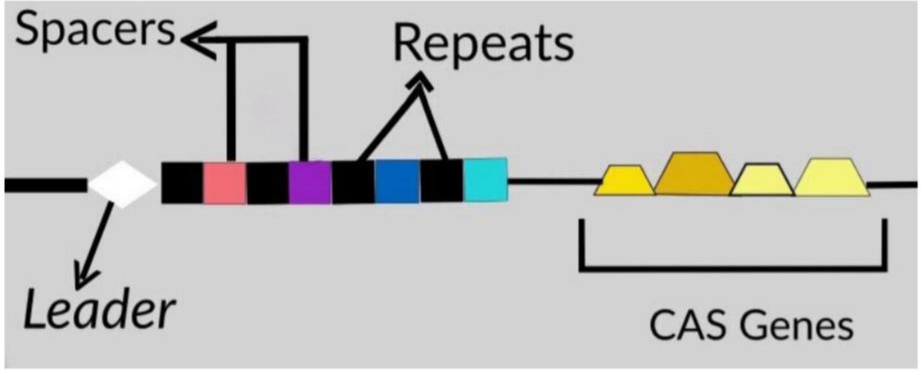
Marraffini, L. CRISPR-Cas immunity in prokaryotes. *Nature* 526, 55–61 (2015). https://doi.org/10.1038/nature15386

1. Basics of CRISPR

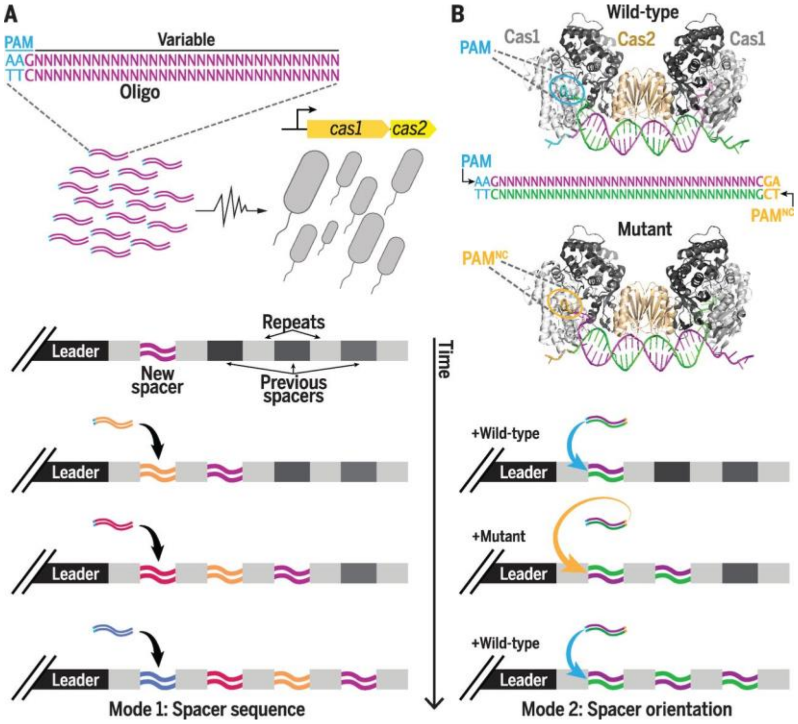
- **First identified in the *E. coli* genome in 1987**
    - The functional importance of the CRISPR loci remained elusive until 2005

- **CRISPR-associated (cas) genes encode cas proteins**
    - Helicases and nucleases
    - Cas 1 and 2 involved in spacer acquisition

CRISPR-Cas array



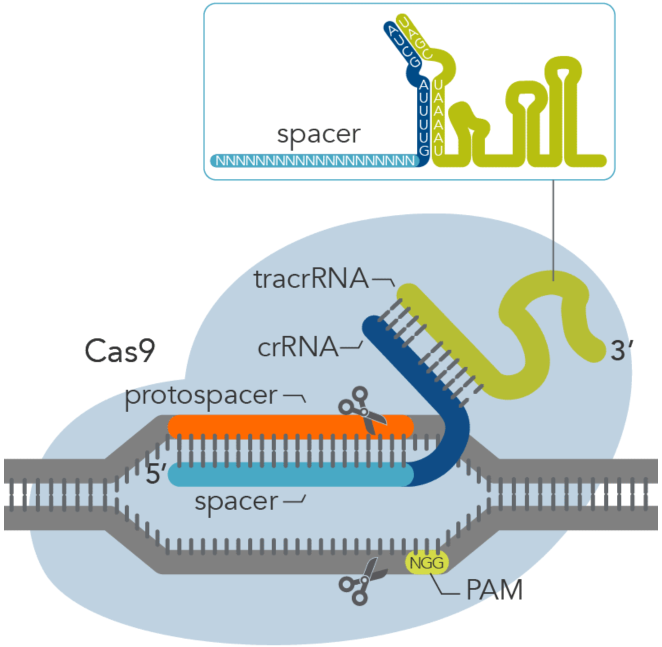
Structure of CRISPR array (Mohamadi et al,. 2020)



CRISPR-Cas array using Cas 1 and Cas 2 (Shipman, 2016)

CRISPR-Cas System

- **Spacers** = short DNA sequences between the short palindromic repeats, each segment is unique
    - **Protospacers** = sequences from which spacers are derived
- **Spacer acquisition** = adaption, integrating foreign genome into CRISPR array (McGinn & Marraffini, 2019)
- **PAM** = protospacer adjacent motif
- **AAM** = acquisition affecting motif



(Credits: idtdna.com)

2. CRISPR-Cas encoding a digital movie

LETTER

doi:10.1038/nature23017

CRISPR–Cas encoding of a digital movie into the genomes of a population of living bacteria

Seth L. Shipman[1,2,3], Jeff Nivala[1,3], Jeffrey D. Macklis[2] & George M. Church[1,3]

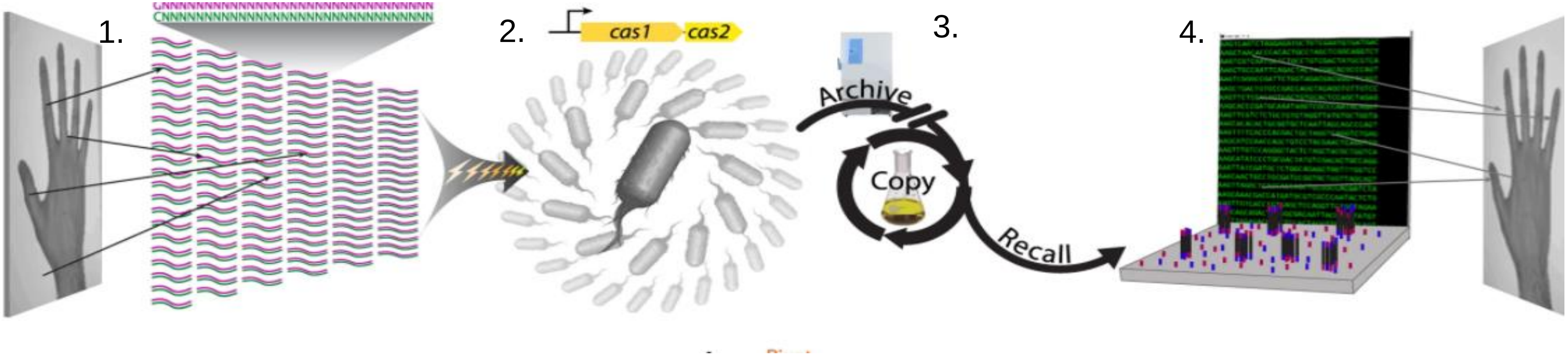
- **Encode pixel values into the genomes of a population of living bacteria**
- **Optimize delivery method, nucleotide content, and reconstruction method → DNA as a stable type of "molecular recorder"**
- **Discover principles of CRISPR-Cas adaptation system**

! This presentation mainly focuses on the methology

- Not focus: error quantification and optimization process

Materials and Methods

Encoding image into genome

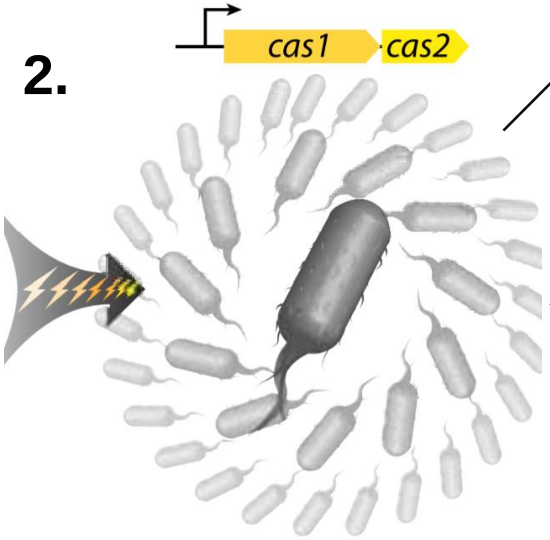


1. **Encoding of pixel values to protospacers**
2. **Electroporation of protospacers into bacteria (overexpress Cas1 and 2)**
3. **Bacteria can be archived and copied**
4. **In the final stage the bacteria are sequenced to recall the image**

S L Shipman *et al. Nature* 1–5 (2017) doi:10.1038/nature23017

Differing pixel-value-encoding strategies

2.



Hand^R
(A rigid strategy)

Hand^F
(A flexible strategy)

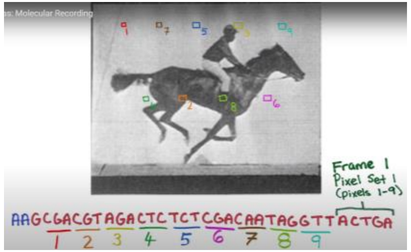
1st base	2nd base: T	C	A	G	3rd base
T	1	2	3	4	T
	5	6	7	8	C
	9	10	11	12	A
	13	14	15	16	G
C	17	18	19	20	T
	21	1	2	3	C
	4	5	6	7	A
	8	9	10	11	G
A	12	13	14	15	T
	16	17	18	19	C
	20	21	1	2	A
	3	4		5	G
G	6	7	8	9	T
	10	11	12	13	C
	14	15	16	17	A
	18	19	20	21	G

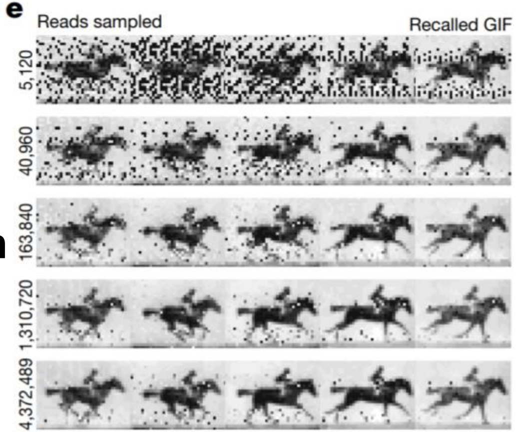
- Hand^R
  - 4 pixel colors defined by a differing base
- Hand^F
  - 21 pixel colors defined by a nucleotide table

S L Shipman *et al. Nature* 1–5 (2017) doi:10.1038/nature23017

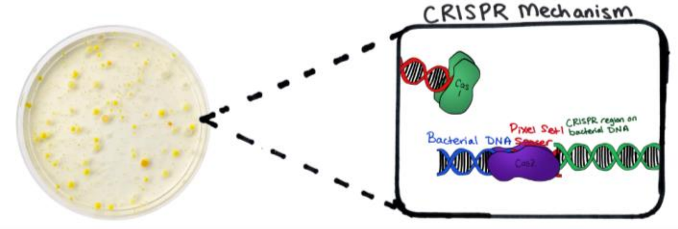
Encoding a GIF into genome

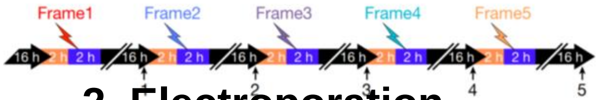
1. Encode frames using hand[F]



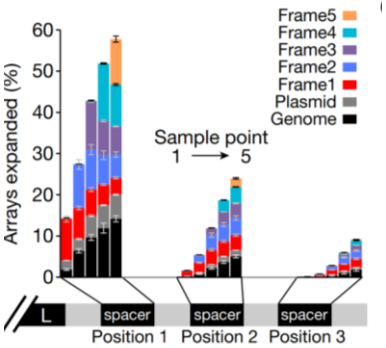


4. GIF recontruction





2. Electroporation



3. Spacer integration

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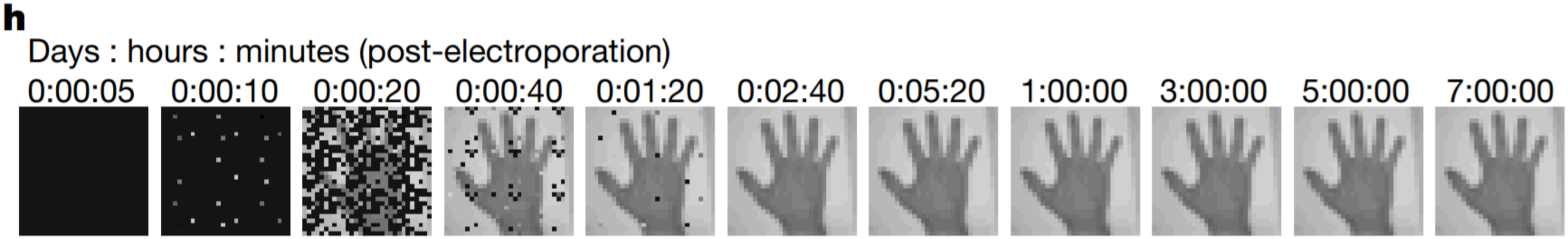
Shipman *et al. Nature* 1–5 (2017) doi:10.1038/nature23017

3. Results for encoding the image(s)

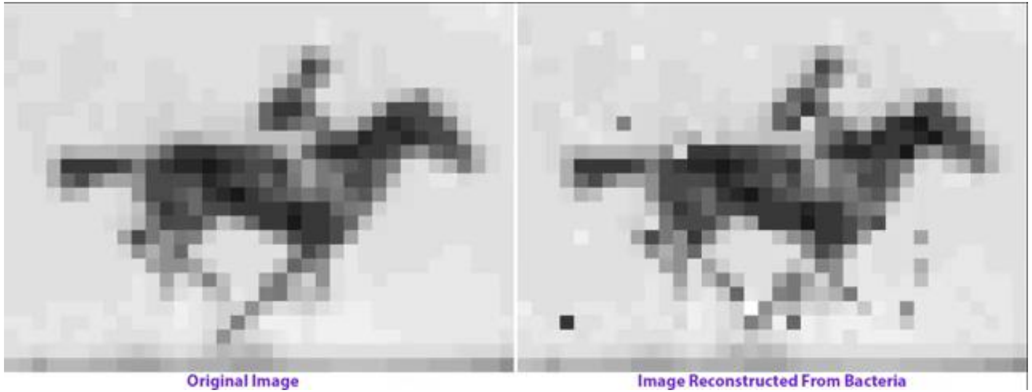
- **Reconstructed image from bacteria of hand[F]**
- **Out of 655,360 reads about 96% of pixet sequences were accurately recalled**
- **Reconstruction of GIF frames up to > 90% of overall accuracy**



Shipman *et al. Nature* 1–5 (2017) doi:10.1038/nature23017

3. Results for encoding the GIF

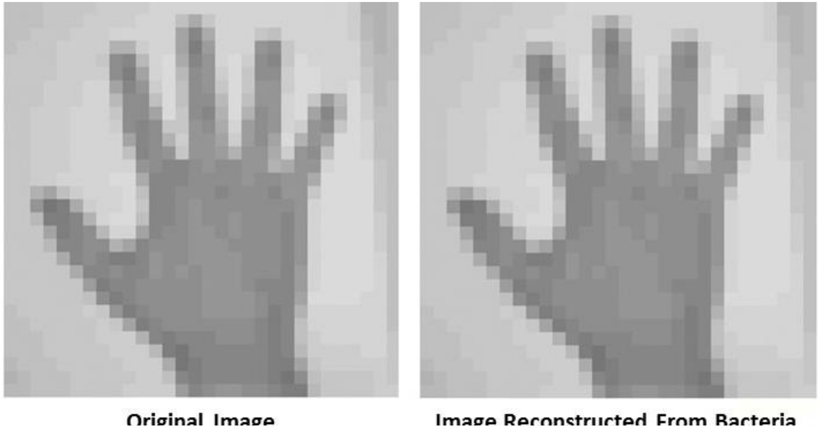
- **Not all protospacer sequences are equally effective → optimization**
- **Successfully tracked barcoded sequence elements over 5 times**
- **→ the system can record multidimensional biological information single-cell storage.**

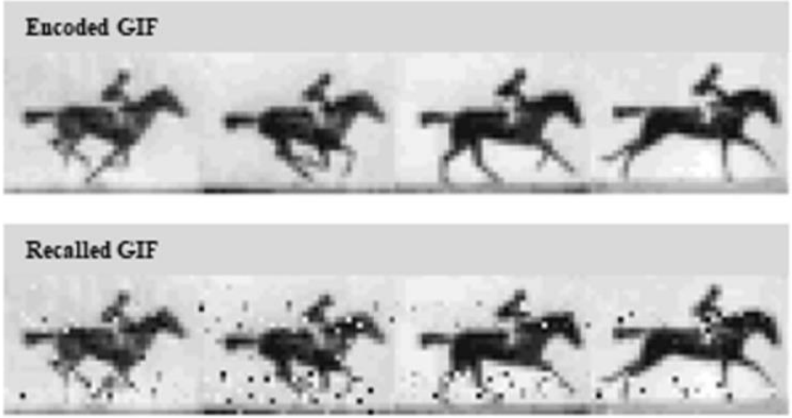


Shipman *et al. Nature* 1–5 (2017) doi:10.1038/nature23017

Summary

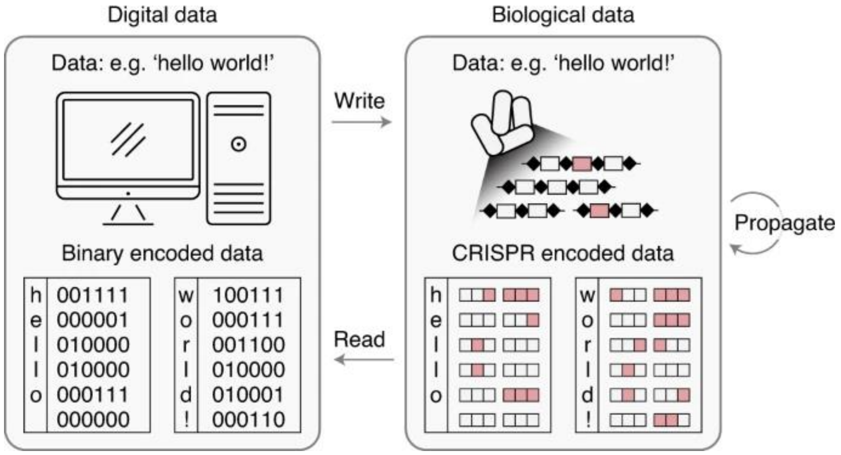
- **DNA can be utilized as a medium for storing data *in vitro***
- **Protospacer sequences differ at effectiveness of transferring data into genome**





Credit: Seth Shipman, Harvard Medical School, Boston

4. Cells (DNA) as information storage



(Yim, et al,. 2021)

Article | Open Access | Published: 22 October 2020

Low cost DNA data storage using photolithographic synthesis and advanced information reconstruction and error correction

Philipp L. Antkowiak, Jory Lietard, Mohammad Zalbagi Darestani, Mark M. Somoza, Wendelin J. Stark, Reinhard Heckel & Robert N. Grass

Nature Communications **11**, Article number: 5345 (2020) | Cite this article

3677 Accesses | **3** Citations | **19** Altmetric | Metrics

Limitation and future of DNA storage

Limitation

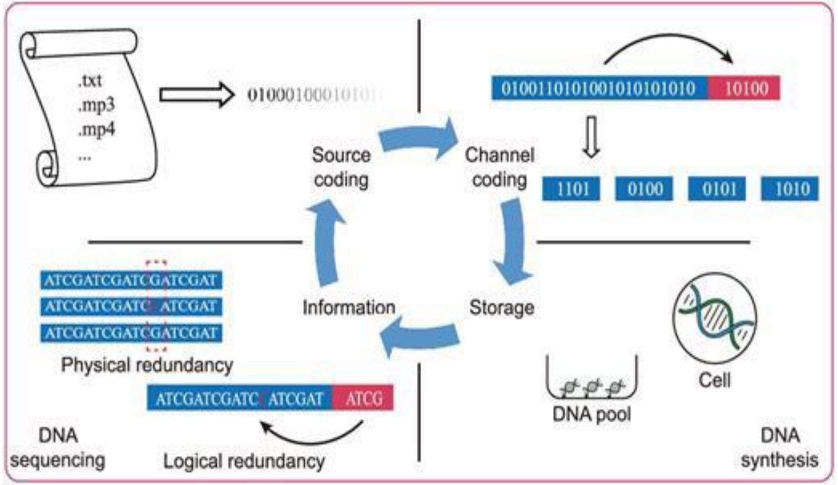
- **Time consuming to convert data**
- **Expensive to synthesize and read data (Service, 2021)**
- **Only short sequences are synthesized**
- **“Destructive process”**

Future (Lee, 2019)

- **DNA is stable**
- **Require less energy**
- **DNA has high storage capacity** *Escherichia coli* 10^{19} bits/cm^3

Conclusions

- **Ultilize the CRISPR system to integrate the encoding DNA into the genome of *E. coli*.** (Matsoukas, 2017)
    - an effective memory device.
    - scaled-up potential for recording in the genome memories/molecular experiences



General workflow of the DNA information storage process (Dong, et al., 2020)

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Questions?

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