Part 1: DNA-Based Data Storage and Computing

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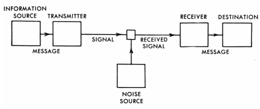
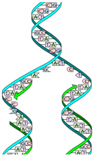
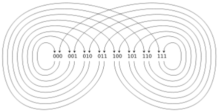
University of Illinois, Urbana-Champaign

North American School of Information Theory, Texas, 2018

May 2018

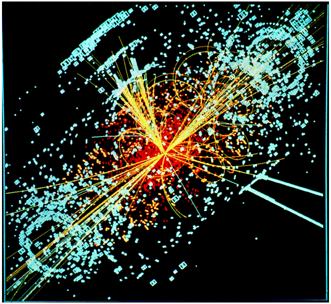






The Era of Massive Data

- Large Hadron Collider: 600 million collisions/s, 0.5 PB per week.



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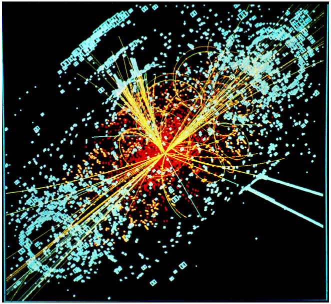

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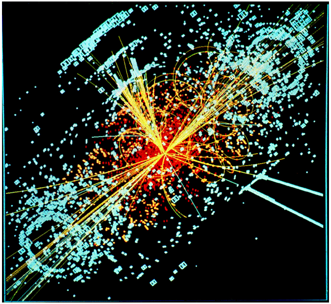

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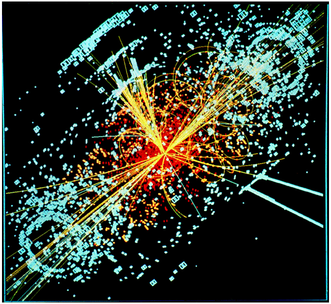
- Social networks (Twitter, Facebook, LinkedIn), NASA weather surveys, consumer and stock market data, Internet sources...



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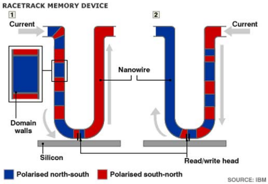
- Pushing the limits of existing storage media: Magnetic tapes, disks, flash, 3D flash,...



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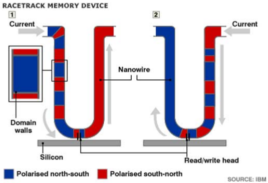
- Data compression: New initiatives by NIH (BD2K Targeted Software Development for Genomic Data Compression) and other efforts.



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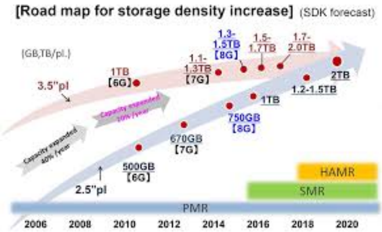

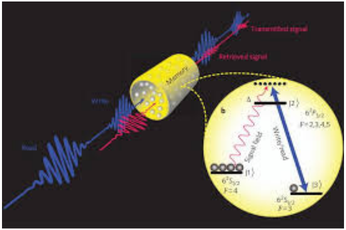

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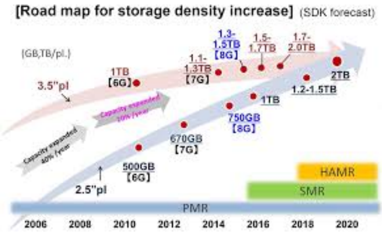
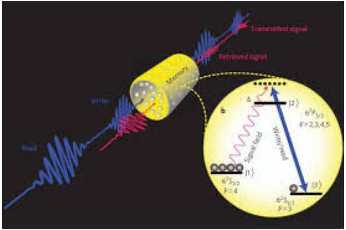
- Data compression: What densities are possible?





DNA-Based Data Storage

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- Can one store information in DNA?
- This question has been raised before: "There is plenty of room at the bottom," R. Feynman.

We can write...

We can "write" in DNA using what is called the process of DNA Synthesis.

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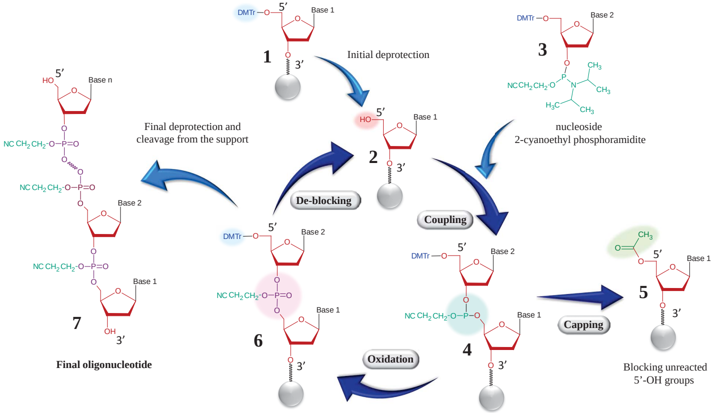
Biochemistry of synthesis: Stitching together bases from the set $\{A, T, G, C\}$ through deprotection & coupling cycles.



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Commercial synthesis: Agilent, Gen9, IDT, Twist Bioscience (recent feature on "Rewriting DNA Synthesis on Silicon").

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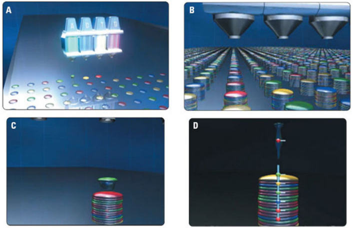
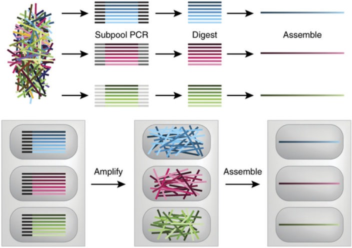
- DNA microarray-based short string (oligo) pool synthesis (left): Cost effective, large scale. Moderate error rates.
- Long strand (gBlocks) synthesis (right): Assembles short blocks. Chemical error-correction.





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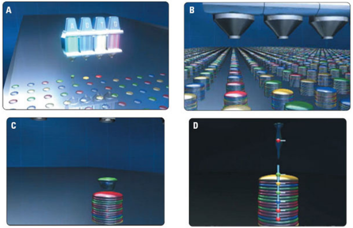
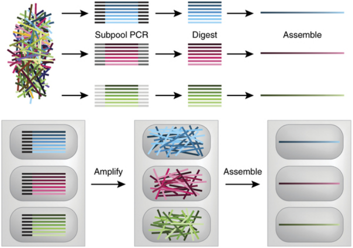
- DNA microarray-based short string (oligo) pool synthesis (left): Cost effective, large scale. Moderate error rates.
- Long strand (gBlocks) synthesis (right): Assembles short blocks. Chemical error-correction.
- Types of synthesis errors: Deletions, insertions, substitutions.





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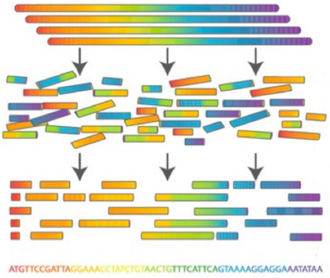
Steps: Cloning /// Shearing /// Reading of unordered pool /// Computer aided alignment of overlapping fragments /// Consensus



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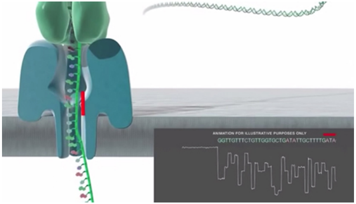
Key properties: Biological pore(s) and motor, base calling using deep learning techniques.



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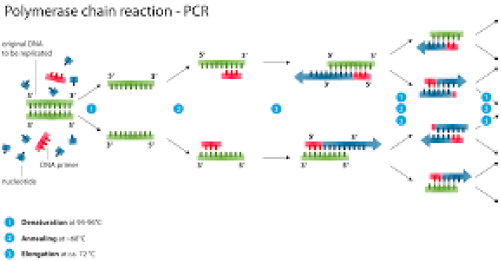
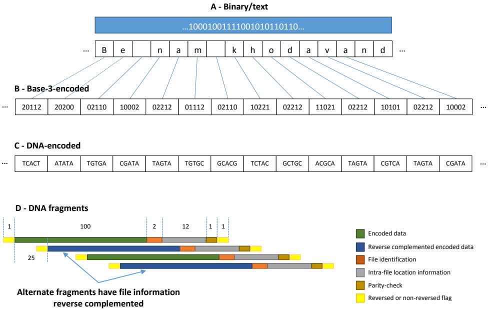


DNA Data Storage Platforms

“Double Helix Serves Double Duty”, NY Times, Jan 2013

- Church *et al.* (Science, 2012) and later Goldman *et al.* (Nature, 2013) stored 739 KB of data in synthetic DNA, mailed it and recreated the original digital files using Illumina readers.



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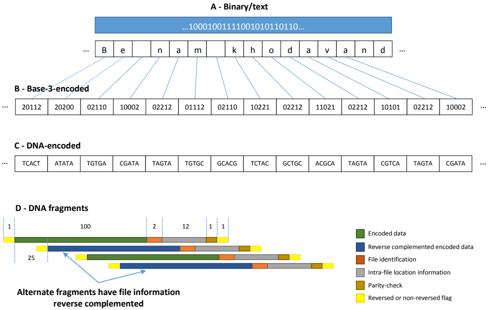
- Digital archival storage systems that will safely store the equivalent of one million CDs in a gram of DNA for 10,000 years.

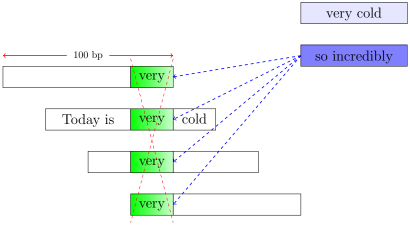


Can one Randomly Access and Rewrite the Data?

- Problem 1: Random access was impossible in first implementation - need to "read" whole book to find one sentence.
- Problem 2: To perform editing, need to change large number of reads (fragments).
- Problem 3: The first schemes were sensitive to contextual errors.

Storage format of Goldman *et al.*: overlapping reads akin to sequencer output.

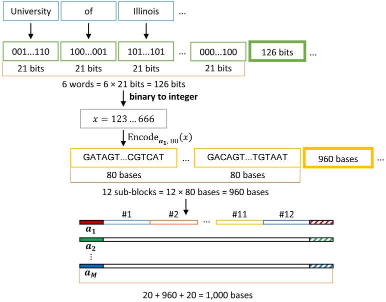
AAATTTTGCGCTATTGCCCAATT**GCC**GGGTTAAAATATATGAGACTCTAAA...



"Data Storage on DNA Can Keep it Safe for Centuries," NY Times, Dec 2015

A fully operational random access and rewritable DNA-based memory with *Sanger sequencing*.

- Yazdi et.al., 2015 - First random access, rewritable DNA-based storage system. Encoded Wikipedia entries for six US universities.



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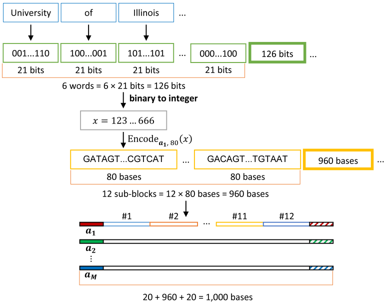
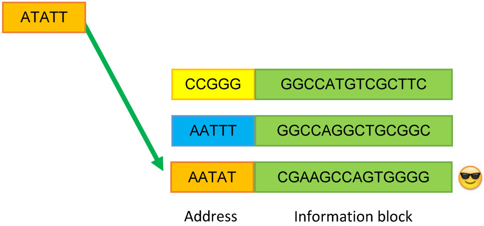
- Large scale testing of our methods: Microsoft Research/Twist Bioscience, 2016. Coverage by Spectrum, Nature, New Scientist etc.



Random Access via Addressing and PCR

- The addressing system: Primers=Addresses, used in PCR reaction. Random access equals exponential amplification.



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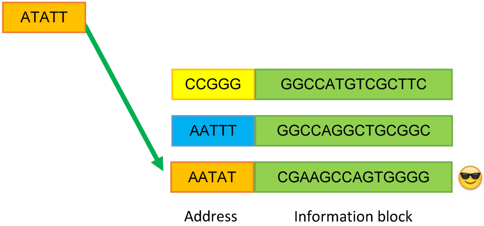
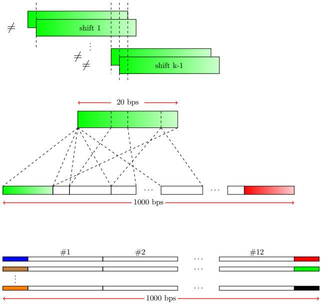


- How to avoid addressing errors?

Address Properties

- Addresses need to be sufficiently different (Hamming, Levenshtein distance) and avoided elsewhere in the blocks.



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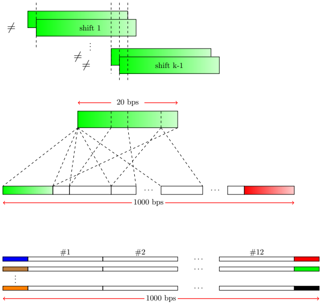
- Addresses should not fold: Needed for accurate amplification.



Address Properties

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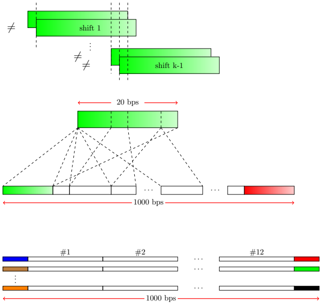
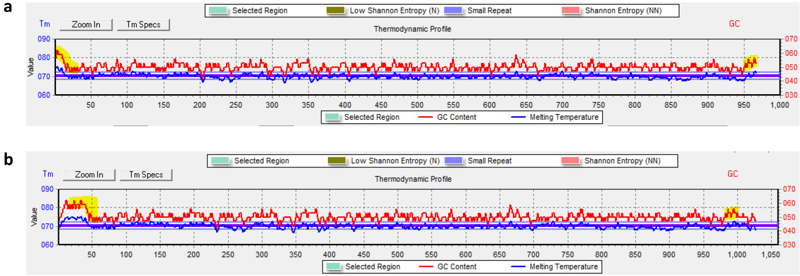
- Addresses should have balanced GC content: Needed for stable melting temperature.



GC Imbalance Hurts!

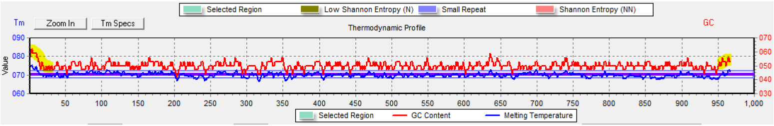
- Synthesis constraints identification with IDT.



GC Imbalance Hurts!

- Synthesis constraints identification with IDT.
- Balancing constraints: GC-content has to be balanced in small block-lengths at the $3'$ and $5'$ ends of the strings, longer blocks allowed within the sequence (blocklength$=8$).

a



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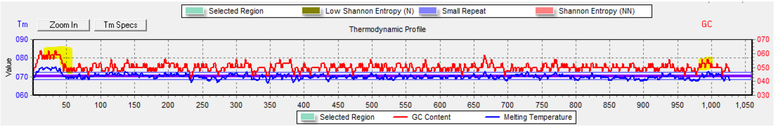
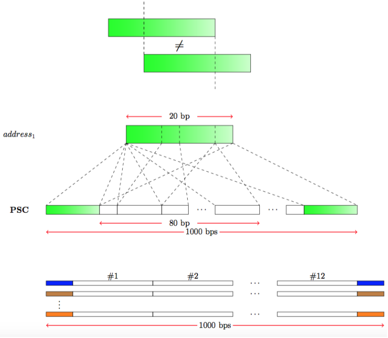


The Constrained Coding Components

Definition. A sequence $\mathbf{a} = (a_1, \ldots, a_n) \in \mathbb{F}_q^n$ is self uncorrelated if no proper prefix of $\mathbf{a}$ matches its suffix, i.e., $(a_1, \ldots, a_i) \neq (a_{n-i+1}, \ldots, a_n)$, for all $1 \leq i < n$.

Extension: A mutually uncorrelated (cross-bifix-free) code is a set of sequences such that for any two sequences $\mathbf{a}, \mathbf{b} \in \mathbb{F}_q^n$ in the code no proper prefix of $\mathbf{a}$ appears as a suffix of $\mathbf{b}$ and vice versa [L70, G60, B12].



Address Sequence Construction

Enumeration and construction of strings of a given length that contain no elements of some fixed set of strings as subwords [GO80's]:

Take addresses as "forbidden words" to ensures specific random access. Relax constraints.

MU vs. Weakly MU: A k-weakly mutually uncorrelated (WMU) code is a set of sequences such that for any two sequences $\mathbf{a}, \mathbf{b} \in \mathbb{F}_q^n$ in the code no proper prefix of $\mathbf{a}$ of length $\geq k$ appears as a suffix of $\mathbf{b}$ and vice versa [TKM16].
Construction of balanced WMU codes Hamming distance constraints?

For $\mathbf{a} = (a_1, \ldots, a_s), \mathbf{b} = (b_1, \ldots, b_s) \in \{0,1\}^s$, define

$$\Psi(\mathbf{a}, \mathbf{b}) : \{0,1\}^s \times \{0,1\}^s \to \{\mathtt{A}, \mathtt{T}, \mathtt{C}, \mathtt{G}\}^s$$

according to:

$$\text{for } 1 \leq i \leq s,\ c_i = \begin{cases} \mathtt{A} & \text{if } (a_i, b_i) = (0,0) \\ \mathtt{C} & \text{if } (a_i, b_i) = (0,1) \\ \mathtt{T} & \text{if } (a_i, b_i) = (1,0) \\ \mathtt{G} & \text{if } (a_i, b_i) = (1,1) \end{cases}$$

Address Sequence Construction

Decoupling the construction: Let $\mathcal{C}_1, \mathcal{C}_2 \subseteq \{0,1\}^s$ be two binary block code of length s. Encode all pairs $(\mathbf{a}, \mathbf{b}) \in \mathcal{C}_1 \times \mathcal{C}_2$ using $\mathcal{C}_3 = \{\Psi(\mathbf{a}, \mathbf{b}) \mid \mathbf{a} \in \mathcal{C}_1, \mathbf{b} \in \mathcal{C}_2\}$. Then:

1. $\mathcal{C}_3$ is balanced if $\mathcal{C}_2$ is balanced.
2. $\mathcal{C}_3$ is a k-WMU code if either $\mathcal{C}_1$ or $\mathcal{C}_2$ is a k-WMU code.
3. If d_1 and d_2 are the minimum Hamming distances of $\mathcal{C}_1$ and $\mathcal{C}_2$, respectively, then the minimum Hamming distance of $\mathcal{C}_3$ is at least $\min(d_1, d_2)$.

See also [LY17] for MU codes.

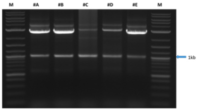
Information sequence encoding?

Information Sequence Encoding for Texts

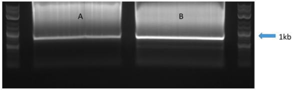
Modification based on [WI90's], and new approach in [TGM17].

Random Access and Rewriting Experiments

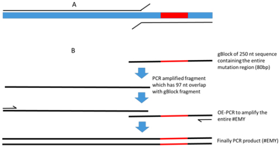
- Random access achieved via PCR, addresses used as primers.



PCR of five primers



PCR of selected string from pool (A) and in individual well (B)



Rewriting via the gBlock process

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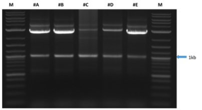

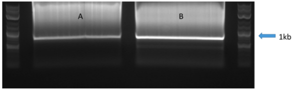

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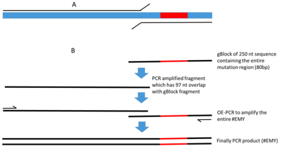

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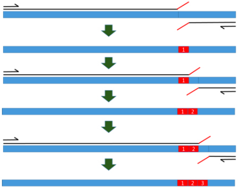
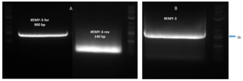


Cheap 80-primer sequential rewriting



A) Two PCR products of rewrite. B) The generated PCR rewrite with correct size of 1kb.

Sequencing results of 10 plasmids (5 from original, 5 from rewrite) with primer in forward direction of the insert. The rewritten region is covered in the red square

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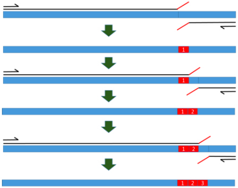

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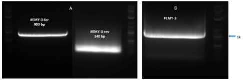

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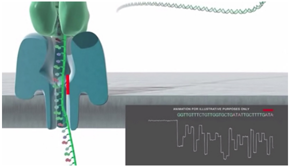
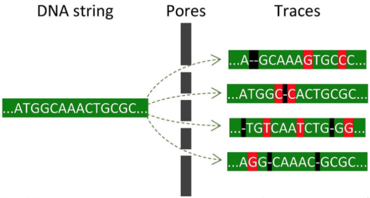
Sequencing results of 10 plasmids (5 from original, 5 from rewrite) with primer in forward direction of the insert. The rewritten region is covered in the red square

Reading with Nanopores

MinION Oxford Nanopore (R7): Sequence traces (reads).





Reading with Nanopores

Major Problem: Very large number of sequence-dependent indel and substitution errors (R.7 flowcell, ~ 10%, R 9.4 flowcell ~ 4%)!

Example Statistics:

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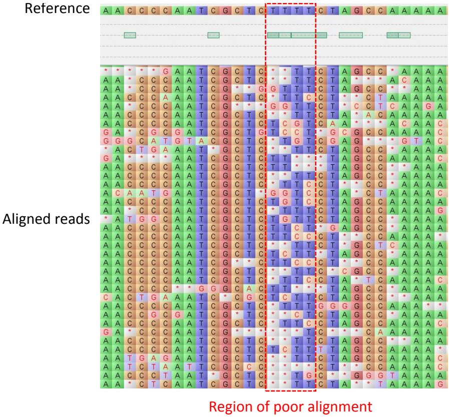

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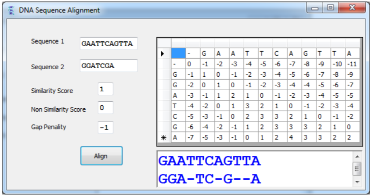

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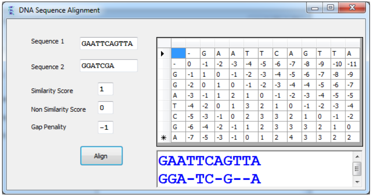

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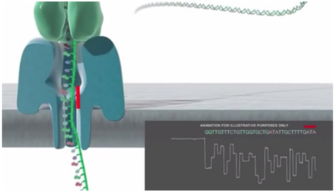
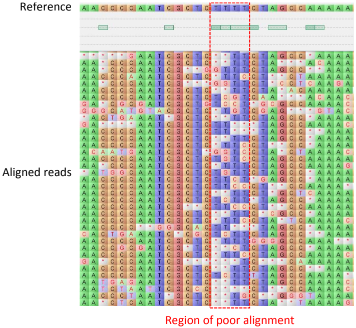
How Do We Handle Deletions?

- Key idea: Use addresses (tags) as pilot sequences to identify good quality reads.





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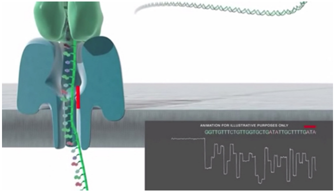
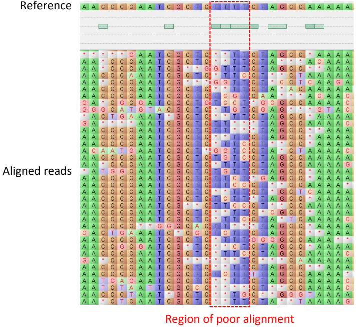
- Align good reads. Use balancing property to correct errors, repeat. Use homopolymer coding.





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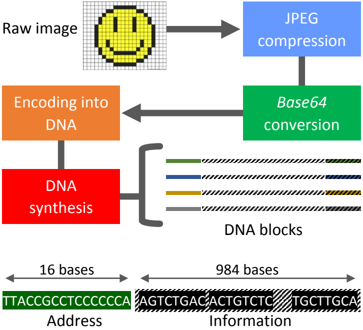
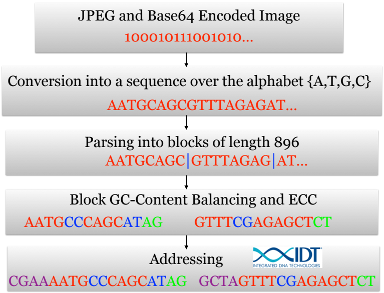
Encoded images in compressed format into DNA.

- Compression, Base64 conversion, error-correcting and constrained coding (balancing GC content and forbidden address sequences).





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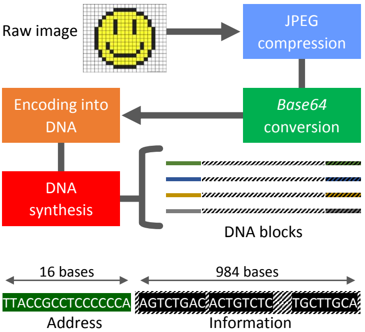

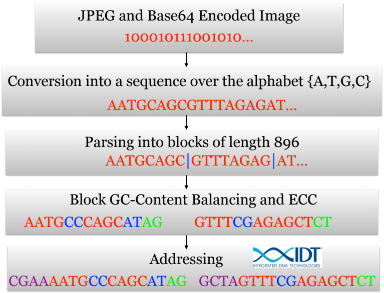

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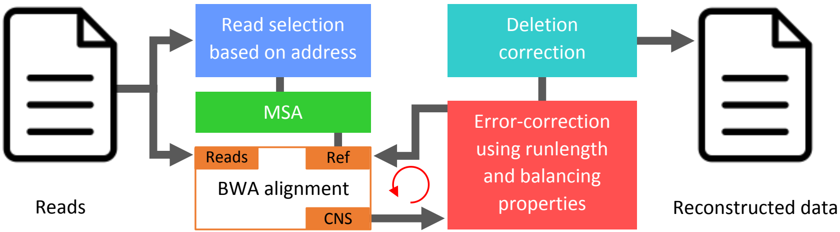

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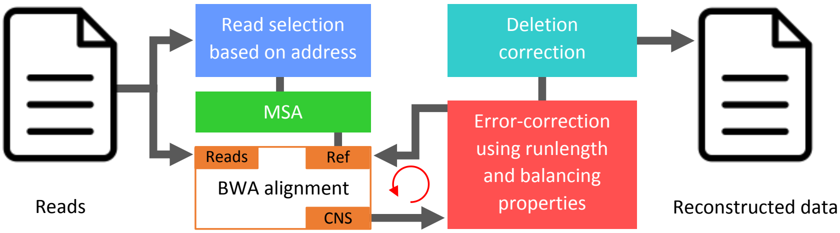

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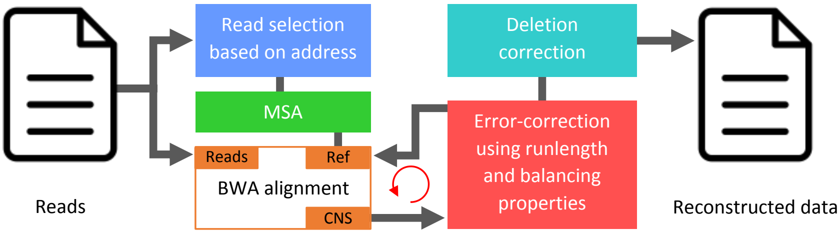

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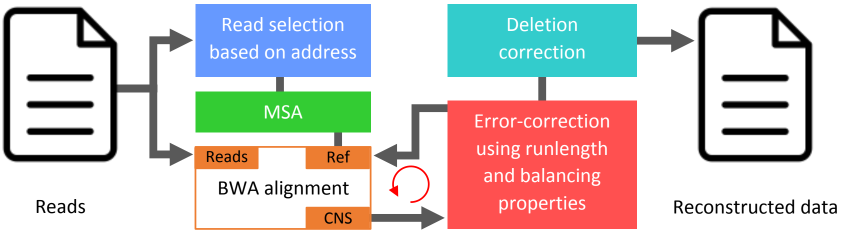

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Reading with Nanopores

Consensus may still have errors: Runlengths of As increase or decrease (protein-A interaction). Runlengths of Gs may form G quadruplexes.

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- Definition. The integer sequence of a vector $\mathbf{x} \in \mathbb{F}_4^n$ is the sequence of the length of the runs in $\mathbf{x}$.

  Example: $\mathbf{x} = (0, 0, 1, 3, 3, 2, 1, 1) \rightarrow I(\mathbf{x}) = (2, 1, 2, 1, 2)$.
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- Related to sticky deletions [B90's], [DA05].

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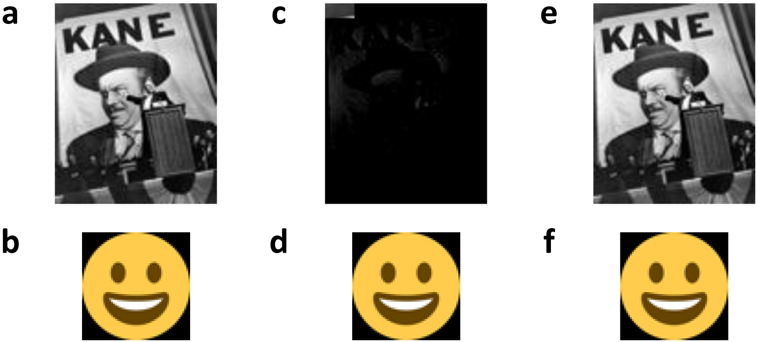
- Hybrid reconstruction: One is given a small number M of "long" asymmetric traces ($o(n)$ deletions). What is the smallest value of k for a k-deck that along with the M long traces ensures unique reconstruction?

Resolving the Portability Problem

Example images of Citizen Kane poster (1946) and Smiley. Only three deletions left after iterative alignment.
Error-free decoding is possible with coding efficiency 88%



Native DNA-Based Data Storage

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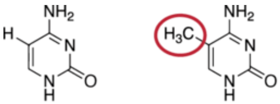

Cytosine **methylated Cytosine**

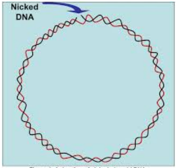

Figure 1: Animation of nicked plasmid DNA.
A nick can be enzymatically induced or caused by shearing during plasmid preparation.

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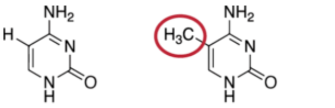

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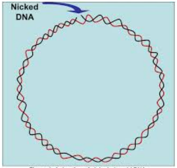

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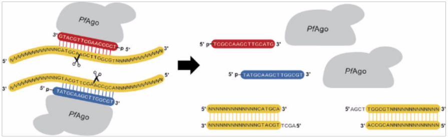

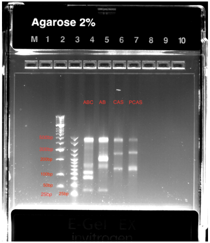

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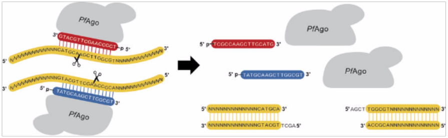

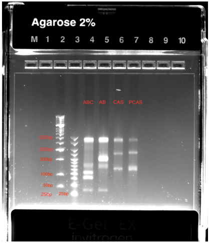

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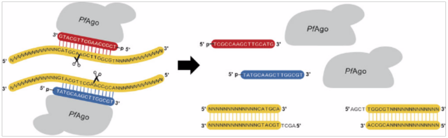

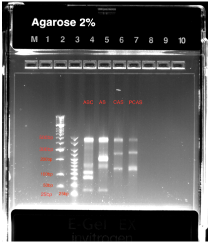

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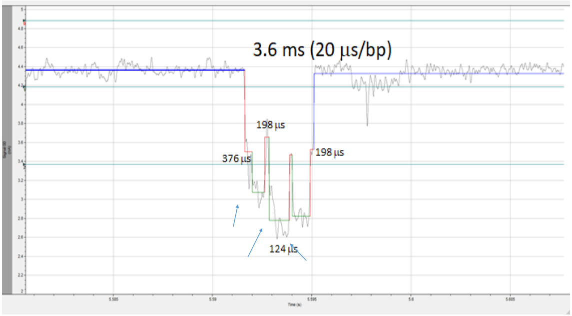

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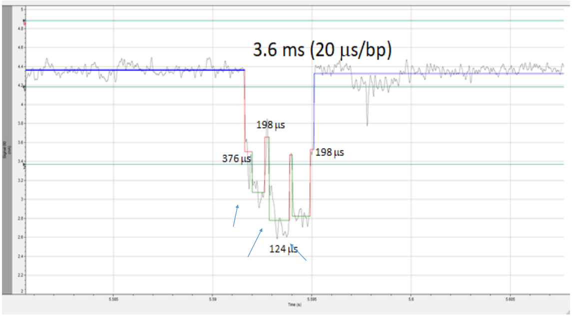

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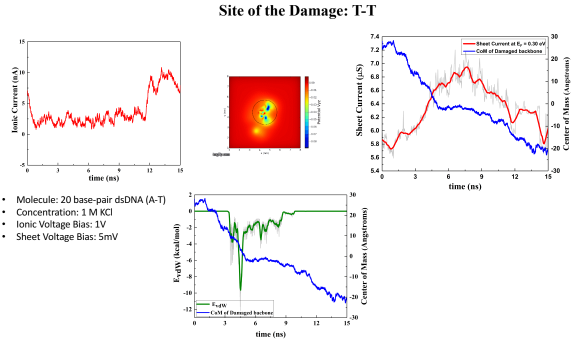

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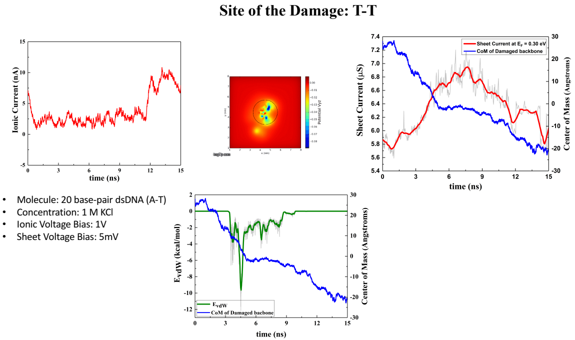

Sources of Errors

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- Instability Errors: Do not want to nick one strand exclusively, as it may cause the backbone to break down.

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- Introduced by Babai and Frankl: Let q be a prime power and $1 \leq s \leq k \leq q$. Set $n = kq$. Let ξ be a primitive element of $\mathbb{F}_q$ and $\mathcal{A} = \{0, 1, \xi, \ldots, \xi^{k-2}\}$. Then $|\mathcal{A}| = k$. For each polynomial $f \in \mathbb{F}_q[x]$, define

$$A_f := \{(a, f(a)) : a \in A\}.$$

We also have $|A_f| = k$. Let

$$\mathcal{C}(k, q, s) := \{A_f : f \in \mathbb{F}_q[x], \deg(f) \leq s - 1\}.$$

Then $\mathcal{C}(k, q, s)$ is a collection of q^s k-subsets of the set $X := \mathcal{A} \times \mathbb{F}_q$ and satisfies the property that every two sets intersect at at most $s - 1$ elements.

Set Discrepancy Theory

- Set discrepancy problem [Spencer'85, Lovasz'86]: Given a set of m subsets $\{A_1, \ldots, A_m\}$ of fixed size k over a ground set $[n-1]$, find a labeling $\ell : [n-1] \to \{-1, +1\}$ which minimizes

$$\max_{1 \le i \le m} \Big| \sum_{x \in A_i} \ell(x) \Big|, \text{ i.e.}$$

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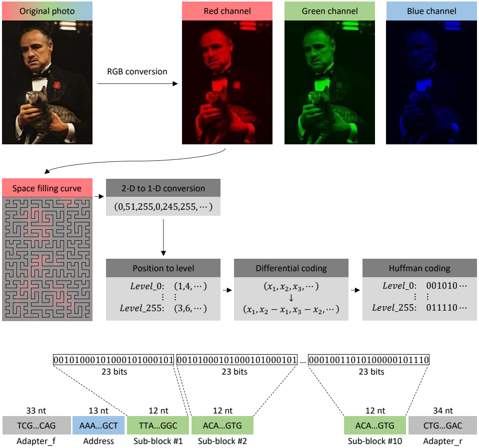
- Babai-Frankl sets can be shown to have zero discrepancy!
- Can extend the results further using Steiner systems.

Other Directions: Concentration Based Coding

- Concentration based encoding: Image processing in DNA.



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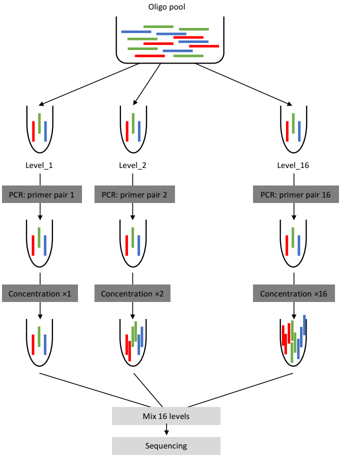

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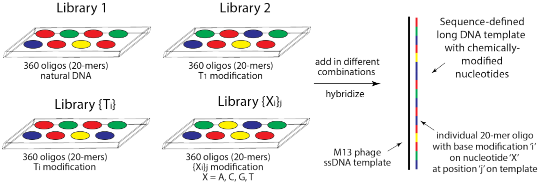

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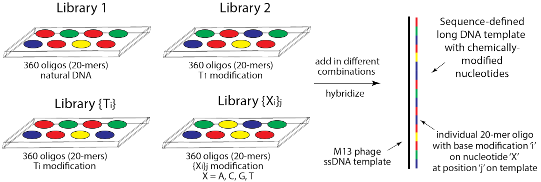
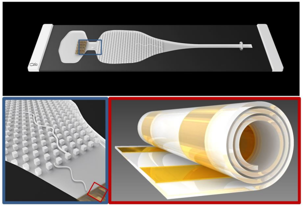


- Integration with nanoelectronics: Changing random access approaches (with Li lab).



DNA Storage in Living Cells, Nature, 2017

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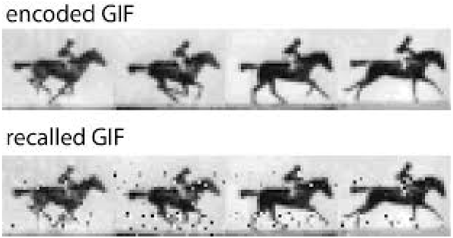

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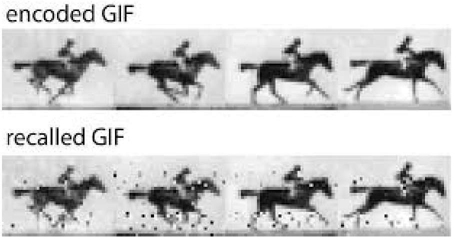
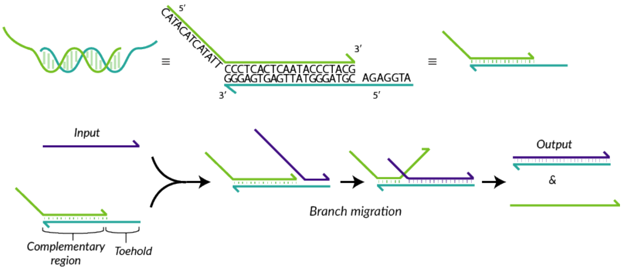


- Fountain DNA Storage: Erlich et al., 2017 (Reed-Solomon in Grass et.al.: oligos treated as symbols over a large alphabet, redundancy at the oligo level).

Native DNA-Based Computing

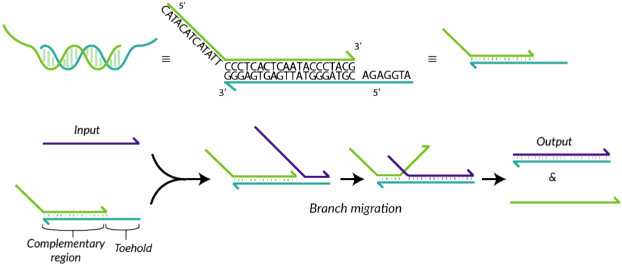
Some Computing Schemes

- Nick displacement: New computing paradigm akin to strand displacement.



Some Computing Schemes

- Nick displacement: New computing paradigm akin to strand displacement.
- Easy-to-implement operations: Incrementing/decrementing, comparison (with Soloveichik lab).



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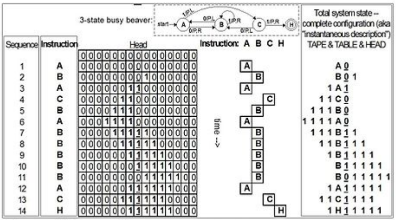

Progress of the computation (state-trajectory) of a 3-state busy beaver

Some Computing Schemes

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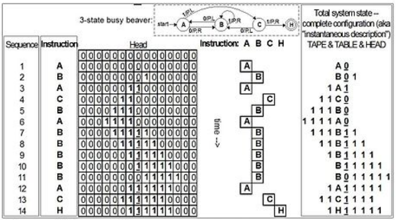
- Large number of registers: Copies of the same native genomic sequence (e.g., *E. coli*).



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Huimin Zhao, UIUC.
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