Imperial College London

15th September 2021

Encoding Information into DNA

MatBio 2021, King's College London

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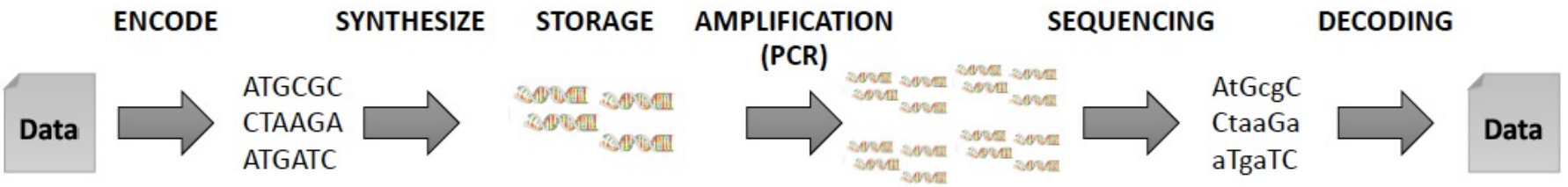
Why Encode Information into DNA?

- **Yearly global production of data growing exponentially**
  – outpacing our ability to store this data
  – hardware and software obsolescence, storage medium decay
  – risk is that information currently stored on magnetic or optical media will not be recoverable in a century or less

- **Benefits of DNA**
  – theoretical possibility of storing 455 EB/g
  – un-treated, DNA has a half-life of approximately 520 years
  – when treated (desiccated, chilled), can be stable for thousands of years
  – currently could be used for high-latency storage, i.e. cold storage, archiving
  – numerous encodings for mapping data to DNA's Base 4 (A,T,G,C)
  – reagents (Adenine, Thymine, Guanosine and Cytosine) are readily available and abundant

What's involved in the end-to-end workflow?



Phase	
Writing	**Encoding the data** • apply **error-correction** codes • ensure sequence meets **biological constraints** **Synthesise the DNA**
Storage	**Store DNA in/on appropriate medium**
Reading	**Sequence the DNA** • raw sequence trace undergoes **base-calling** **Decoding the data** • **apply error detection, error correction** to recover any corrupted blocks

What are the considerations?

Constraint	Reason
Biological Constraints	**Homopolymers (i.e. runs of same base letter, i.e. GGGGG)** – difficult to synthesise and often recalcitrant to sequencing **GC extremes** – unstable and difficult to sequence
Error detection & Error Correction	**Synthesis and Sequencing** – both prone to errors i.e. insertions, deletions, substitutions Error detection alone insufficient, require **error correction**
Information Density	**Synthesis cost.** Metadata (**primers, error correction**) costs nucleotides and thus reduce information density
Data retrieval method	**Sequencing whole DNA pool** inefficient **vs Random access**
Storage medium	**In vivo (in cells)** – not ideal for storage (has been done) **Microplate / wells / immobilised** (more practical)

Early approaches and encodings

- **Mostly focused on the novelty, artistic projects**

Microvenus (1996) [1]	Encoded Germanic Rune for Life and the Female Earth (5 x 7, or 7 x 5 figure, totaling 35 bits). Stored in E. coli (transfected)
Genesis Project (1999) [2]	Encoded sentence from Genesis, using morse code mapping
Hiding data in DNA microdot (1999) [3]	Ternary code. Stored in microdot
Organic memory using DNA (2003) [4]	Ternary code. Stored in E. coli and extremophile Deinococcus Radiodurans

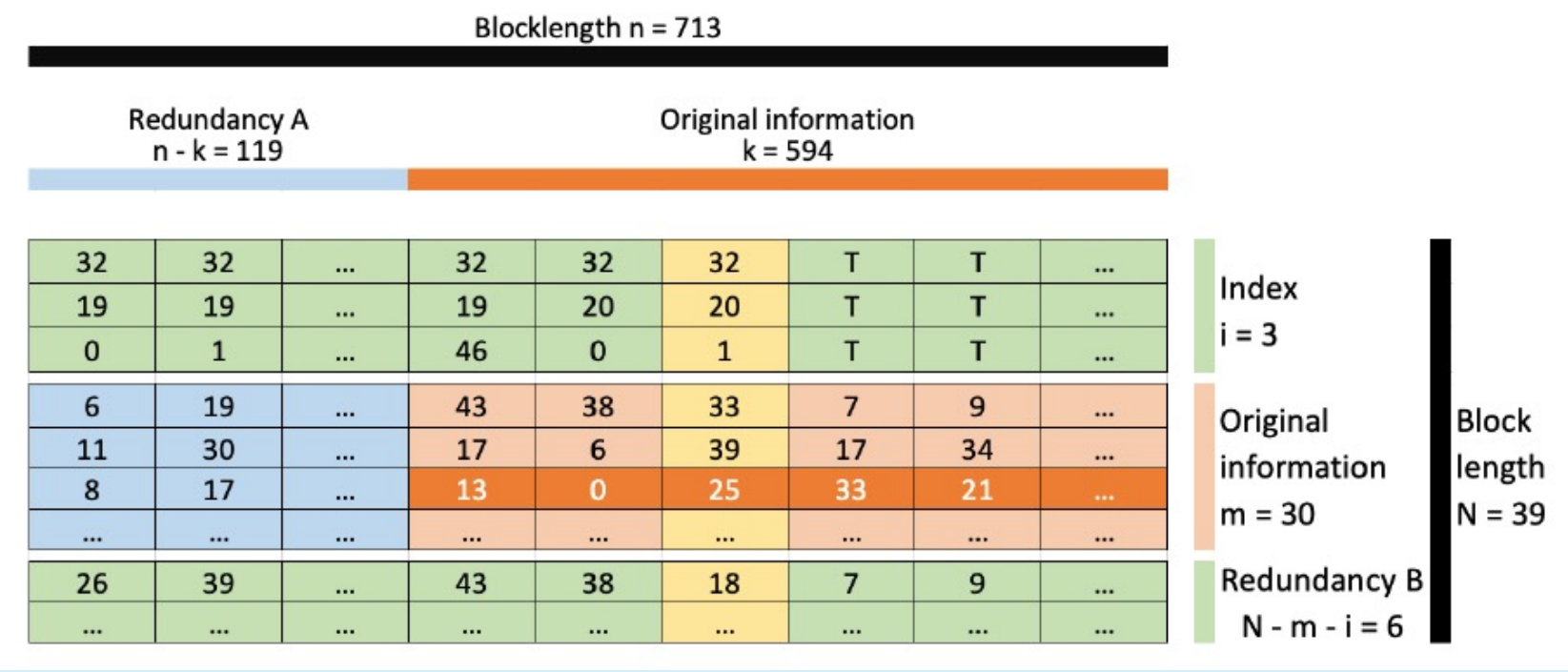
- **Used trivial encoding schemes**
  - **Morse code –** mapping Dash → T, Dot → C, and space → A.
  - **Phase-change encoding –** codes bit repeats until a change occurs
  - **Ternary → Quaternary mapping –** A → 0000 → AAAA, B → 0001 → AAAC, C → 0002 → AAAT, …
  - **Ignorant of biological constraints i.e. homopolymers, extreme GC**

Huffman, Comma and Alternating codes [5]

Huffman code	**Most frequent character is encoded with the least number of symbols**, and the least frequent character the most symbols, such that **e → T, z → CCCTG** • As the frequency decreases, the codeword increases in size • Varying codeword length makes it difficult to distinguish between artificial and natural sequences • No error-detection or error-correction is incorporated
Comma code	Uses **G nucleotide as a comma to separate all other codewords of length 5**. G is never used in any codeword • Proposed code uses G as the comma every six nucleotides encoding is easily identified as synthetic. 5 nucleotide codeword uses A,T,C but not G. Balances GC content, more efficient amplification process. • General **format of codeword is CBBBC where B in {A,T}, with B's C's in any permutation** (80 possible)
Alternating code	**64 codewords, six nucleotides long** • In the form **XYXYXY, where X in {A,G}, Y in {C,T}** • XYXYXY / YXYXYX avoids homopolymers of three • Encoding used has suboptimal GC content, could be improved using: X in {C,G}, Y in {A,T}

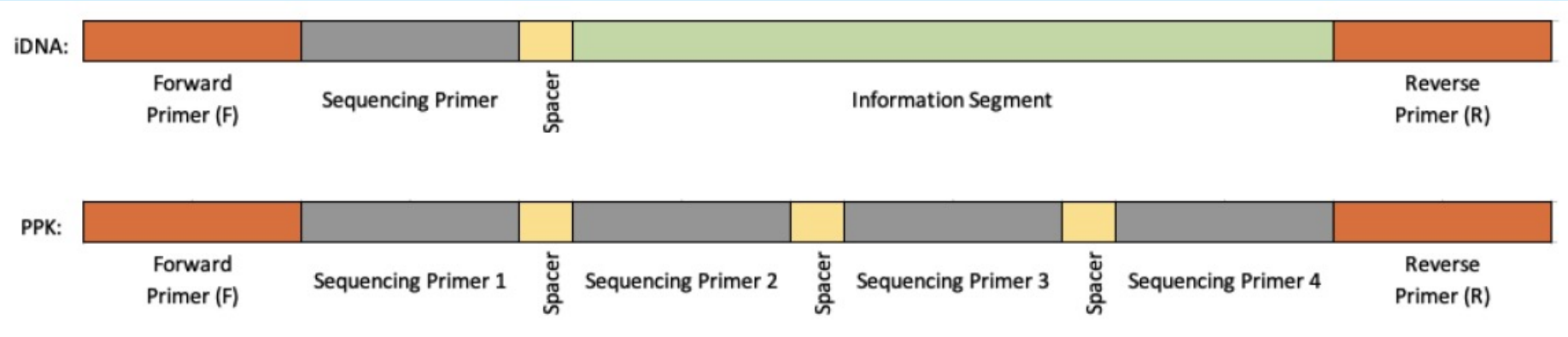
Reed-Solomon error-correcting codes [6]

- Most newer approaches implement Reed-Solomon codes
- An inner and outer code add error-checking/correction, redundancy
- Corrupted blocks can be recovered 😄



Random access using Primers [7]

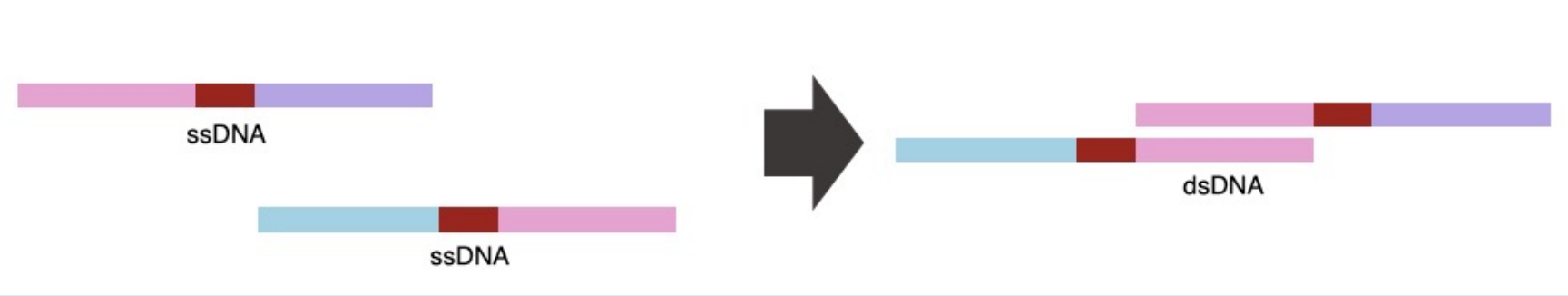
- Sequential access requires that the whole DNA pool of Oligonucleotides is read – this is inefficient and time consuming
- **Solution:** Use a separate Oligo (PPK – Poly-Primer Key) comprising multiple Primers to index blocks (sequences) on data-bearing Oligonucleotides (iDNA – information DNA)
- This method allows for a *subset* of the information (i.e. sequences in a subset of iDNAs) to be retrieved from the DNA Oligo pool



Inter-molecular computation (DB join example) [8]

- OligoArchive is an archival tier built on a Postgre SQL relational DB
- Performs schema-aware encoding & decoding of relational data
- Object Identifier (Primer) uniquely identifies subset of Oligos (similar to PPK and iDNA we saw earlier)
- *get* operation uses Primer to filter and extract all oligos for the Primer

- *Inter-molecular (in-vitro) join* operation leverages molecular biology
  - the annealing of two complementary single-stranded (ssDNA) sequences to form a double-stranded oligo (dsDNA)
  - resulting oligos are then sequenced to retrieve the data



Challenges and relevant areas to explore

- DNA Synthesis is still more expensive than sequencing
  - sequencing costs driven down by clinical diagnostics, R&D etc not so with synthesis
- Choice of Sequencing technology
  - Illumina vs Nanopore (very different profiles)
  - optimisation of base-calling using ML
- Standardisation of encoding
- Miniturisation of the synthesis/sequencing technologies
- Xeno nucleic acids (XNA) – synthetic (distinguishable from DNA)
- Biosecurity
  - prevention/flagging of generation of pathogenic sequences
    - requires a sequence check against a database

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We are open to collaboration

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