

Utilizing a novel microfluidic technology to optimize enzymatic DNA synthesis for information storage applications

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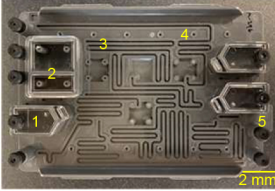
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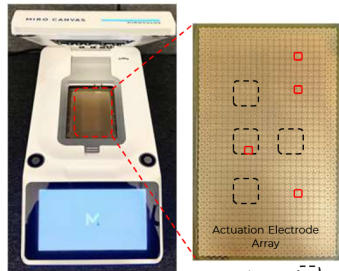
Introduction

- Conventional DNA synthesis using phosphoramidite chemistry is limited to strand lengths of ~300 bases, whereas enzymatic synthesis has no such inherent limitation.
- DNA storage applications can employ enzymatic DNA synthesis strategies using terminal deoxynucleotidyl transferase (TdT) to build ssDNA without the requirement of blocked nucleotides. This is done with Free-Running Synthesis (FRS), which generates short homopolymer stretches of ssDNA; FRS is faster, more efficient, and able to generate datastreams 1000s of bases long. Nanopore sequencing can be employed to retrieve the stored information.
- Miro Technology performs all FRS and nanopore library prep steps on a cartridge using digital microfluidics in a tabletop device; process includes dNTP and enzyme additions, incubations, thermocycling (PCR) and magnetic bead clean-ups.
- We report successful polymerization of A, C, G, and T onto an initiator strand tethered to paramagnetic beads to yield a long synthetic ssDNA fragment that can be prepared for nanopore sequencing.

Miro Technology

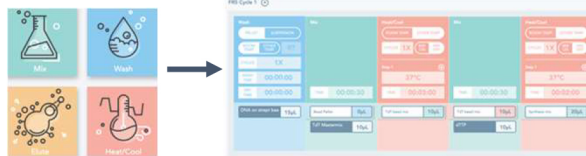
- Miro Technology consists of three distinct components: **a)** single-use cartridge, **b)** control system, and **c)** software for automated FRS.

- a)**  1. Reagent Reservoirs
2. Waste Reservoirs
3. Dispensing/mixing Channels
4. Reagent inlet holes
5. Interface ports to control system
- b)** Miro Technology utilizes electromechanical forces to manipulate fluids across a surface of patterned electrodes in an automated fashion. It integrates key operations to perform a wide range of processes:

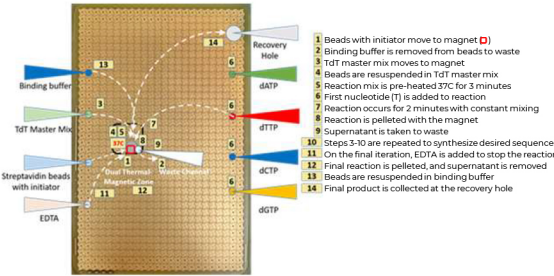


- Dispense, merge and mix
- Multiphase reagent control
- Isothermal and thermocycle control
- Magnetic control

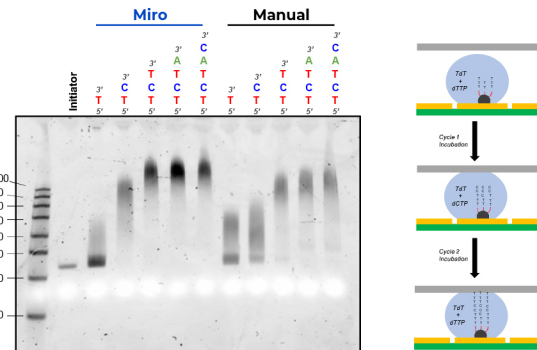
- c)** Intuitive software with drag-and-drop interaction for building protocols.



Free-running DNA synthesis on Miro Canvas



- Five bases were incorporated to the 3' end of a Biotin-TEG 42nt initiator tethered to magnetic streptavidin beads. 5x 2-minute FRS cycles were carried out on Miro and compared to a fully manually-executed FRS.



- Miro Technology can reduce the cycle time down to seconds, which is impractical and error prone when performed manually.

Library preparation on Miro Canvas for nanopore sequencing

- Input gBlock DNA was designed to contain homopolymer sequences mimicking FRS products.
- Miro-prepared libraries generated quality sequencing data that are comparable to manual workflow.

Alignment Statistics Data (using minimap2)

Metrics	Miro	Manual
Sequences	1000	1000
Reads Mapped	375 ± 18	380 ± 8
Error Rate	0.136 ± 0.005	0.130 ± 0.006
Average Length	797 ± 187	910 ± 223

- ONT Ligation Sequencing Kit.

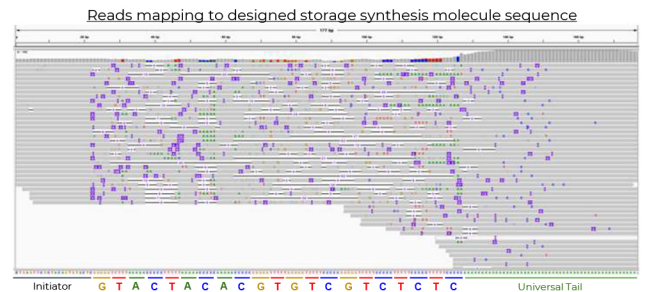
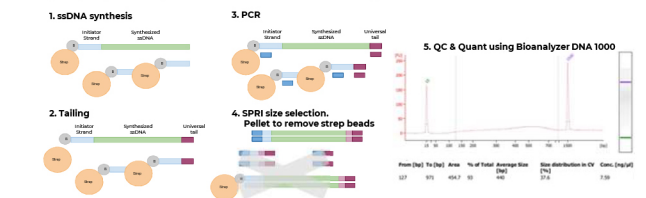


Encoding and decoding the message "MIRO" in an integrated tabletop device

- The message "MIRO" was converted into binary format using 8-bit ASCII encoding. Binary data was converted into ternary representation encoded in non-repetitive A,G,C,T resulting in a 21 nucleotide sequence



- ONT-Medaka tool can find the best consensus sequence. Constrained code and ASCII decoding retrieve encoded data. Decoding can handle sequencing errors that may misrepresent the homopolymer length and focuses on finding the base transition of the homopolymers.
- 21 consecutive 5min FRS cycles with alternating dNTP inputs followed by a poly(A) tail produce a ~600-base long molecule.



- Proof of concept experiment encoding and decoding "MIRO". Ongoing work focuses on making the amplification of long homopolymers more efficient.

Conclusions

Miro Technology allows for hands-off, free-running enzymatic DNA synthesis and library preparation to be hosted in the same device. It is an ideal platform for optimizing Free Running Synthesis reaction conditions and converting the product to nanopore sequence-ready libraries.

It is the first tabletop device that provides an automated and fully integrated DNA storage synthesis and retrieval solution combined in one. Future research is needed to increase throughput with parallelization on a single cartridge and to optimize coding and decoding strategies.

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