

## Storing digital data on DNA via CRISPR mediated base editing

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Global digital data will surpass 1.4 Yottabits by 2025<sup>1</sup>. While demands for data storage increases, the conventional digital storage systems are reaching their physical limits, have limited retention, require huge infrastructure cost, and consume power unsustainably<sup>2</sup>. Therefore, alternative information storage materials including DNA storage systems that could provide viable energetics, spatial capacity, data retention, and economics solutions are becoming a trend<sup>2</sup>. DNA as an alternative for data storage potentially enables great physical density, scalability, energy consumption, reproducibility, and durability<sup>1,2</sup>. Additionally, rapid development of DNA synthesis and sequencing in recent decades<sup>1,2</sup>, has helped the community to seriously consider DNA as an alternative information storage medium<sup>2</sup>. Considerable challenges that need to be addressed for DNA to become a main-stream DNA storage include, current limitations for sustainability, latency, and scalability. While majority of the community are attempting to provide solutions for scalability, we believe *de novo* DNA synthesis –which in most cases results in large amounts of toxic waste– is a major bottle neck<sup>1,2</sup>.

In this report, we used DNA Mutational Overwriting Storage (DMOS) to overwrite the sequence content (state) of greenly synthesized template DNA domains (bits) to write the digital data. Our DMOS DNA templates contain 16-bit domains and addressing strings. We wrote the digital information by simply allowing or prohibiting localized mutations the DNA templates via guided activity of synthetic chimeric enzymes (hereby called punchers). Our puncher enzyme (APOBEC3A) recognizes the desired domain by aid of our predefined library of ribonucleic protein complexes (CRISPR-deactivated Cas9 and guide RNAs)<sup>3</sup>. The developed cell-free punchers perform cytidine deamination of single-stranded DNA strands, resulting in base substitution mutations of base-C (intact state) to base-T (mutated state) in the targeted domains<sup>3</sup>. Also, we developed error-correction and DNA sequencing codes that enable high data retention in our DMOS system. As schematically demonstrated in Fig. 1, we performed mutational edits to write the data, added addressing strings to the encoded DNA pools and combined them to store the data, and performed nanopore sequencing to read the data<sup>4</sup>.

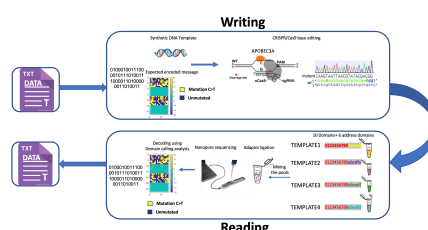


Figure1: Storing digital data on DNA using CRISP/Cas9 base editing tool

- 1- Zhirnov, V., Zadegan, RM., Sandhu, GS., Church, GM., Hughes, WL. Nucleic acid memory. *Nat Mater.* 2016;15(4):366-370. doi:10.1038/nmat4594.
- 2- Zhirnov, V., Rasic, D. 2018 Semiconductor Synthetic Biology Roadmap. (Retrieved on 03/14/2020 from <https://www.src.org/library/publication/p095387/p095387.pdf>).
- 3- Marshall, R., Maxwell, C. S., Collins, S. P., Jacobsen, T., Luo, M. L., Begemann, M. B., Gray, B. N., January, E., Singer, A., He, Y., Beisel, C. L. & Noireaux, V. Rapid and Scalable Characterization of CRISPR Technologies Using an E. coli Cell-Free TranscriptionTranslation System. *Molecular cell* 69, 146-157.e143 (2018), doi:10.1016/j.molcel.2017.12.007.
- 4- Timp, W., Comer, J. & Aksimentiev, A. DNA base-calling from a nanopore using a Viterbi algorithm. *Biophysical Journal* 102, L37-L39 (2012), doi:10.1016/j.bpj.2012.04.009.