Micro Nuwa

A Multi-target Editor for DNA Data Storage

iGEM Tianjin 2022
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IGEM tianjin2022's project is based on more possible applications of DNA storage technology. The team built a single base editor and gRNA array to realize the editing technology of base multi-target, and realize the simultaneous modification of multiple sites on the DNA sequence. Through programming, a piece of text, music and other information to be stored can be transformed into a sequence that can be edited by a single base editor with multiple targets. Maximize the potential of editing tools through appropriate coding.

Abstract

Background

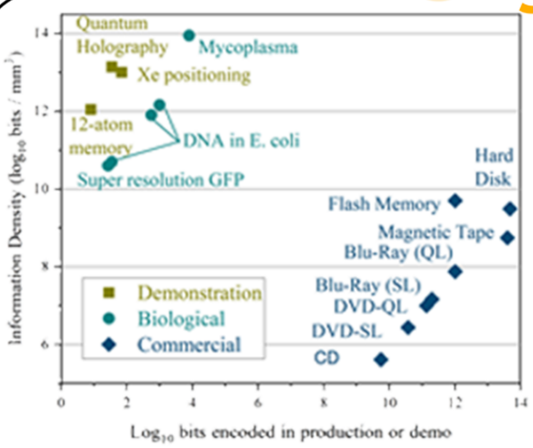


Figure 1: Comparison of information density of different storage media

Nowadays DNA information storage technology can only writing, locating and reading. So we barely get the deadinformation. It makes DNA information storage inconvenient and function less.Therefore we want to put 'Editing' into the process ,which we can convert dead information into live information easily.

Design&Muse

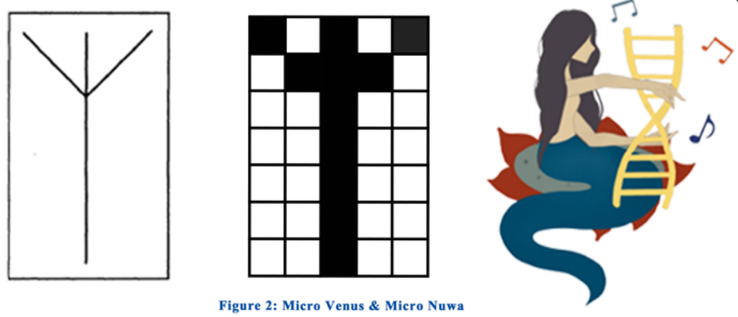


Figure 2: Micro Venus & Micro Nuwa

In 1996, artist Joe Davis stored the first picture in DNA and named it micro Venus. IGEM Tianjin combined with the story of the Chinese myth Nuwa , designed the concept of micro Nuwa. It aims to show that data changes from static to dynamic. Nuwa creates life out of mud and wicker while Micro Nuwa uses a base editor called CBE to modify the bases in the DNA sequence that carry the information, turning "dead" stored information into "live" information.

Wet Lab

Experiment Materials

CBE

The editing window of the CBE is located at the 1-5 bases of the gRNA.

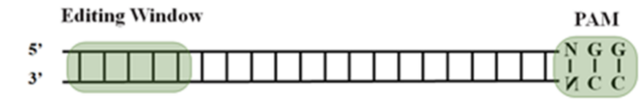


Figure 3: dCDA1Δ198-BE3

gRNA array

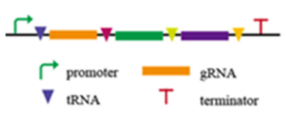


Figure 3: A polycistronic unit expresses three gRNAs, which are cleaved by four tRNAs.

The tRNA sequence is inserted between two gRNAs, and every three gRNAs are transcribed using a promoter and a terminator. RNase cut tRNA sequences to release

Experiment Methods

The project transforms three plasmids into the yeast. They are gRNA array、CBE and DNA information plasmid.

There are sequences in the gRNA array that target to the ade2, which suggests that the reddish colonies may have been edited. Send the reddish colonies to be sequenced to see if experiments achieve multi-target editing.

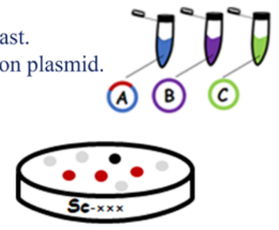
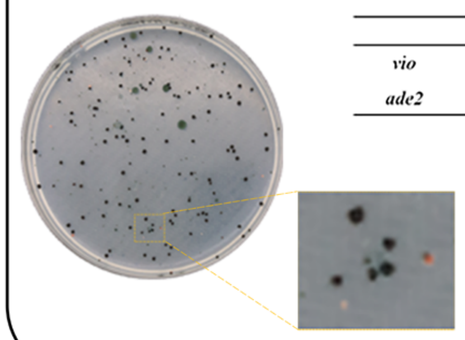


Figure 5: Schematic diagram of experiment

Edit Results



	Black	Red	White
vio	×	√	√
ade2	×	√	×

The successfully edited yeast is red. Although the current efficiency isn't very high, it proves that this path is feasible.We consider integrating CBE into the genome to induce expression.

Dry Lab

Part 1 Picture-editing

We use 5*7 pixel lattice for bitmap storage, and the information of each pixel lattice is stored by the following sequence:

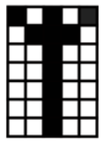


Figure 8: MicroVerus

TAGAAGAAGTGACAGTTTGTCAGCCGTTATCACGG
ATCTTCTTCACTGTCAAACAGTCGGCAATAGTGCC

Positioning windows | Row of pixel grid | gRNA
Color of pixel grid | Line of pixel grid | PAM

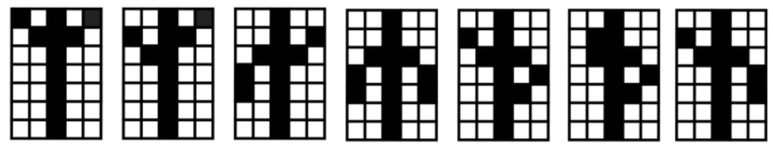
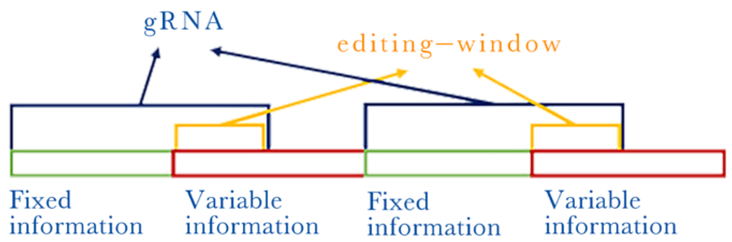


Figure 9: Through the reserved editing windows, the bases can be changed as needed,and finally achieve the effect of changing the content of the picture. Finally, a short film can be obtained by playing each edited picture in a certain order.

Part 2 Music-editing

If two editing windows are close and exist on different chains, there will be four situations for this DNA data which can be the variable data:

5` CGTAGTACG 3` 5` CGTAGTATG 3`
3` GCATCATGC 5` 3` GCATCATAC 5`
5` CATAGTACG 3` 5` CATAGTATG 3`
3` GTATCATGC 5` 3` GTATCATAC 5`



Fixed information: $x_1 = 0,1,2,3,4,5,6,7$
First variable information: $x_2 = 0,1,2,3$
Second variable information: $t = 1,-1$
Any note: $F(x_1,x_2) = (x_1 + t \cdot x_2) * 8n, n \in Z^+$

Ex:
Change C to A
$x_1 = 1, x_2 = 3,$
$t = -1, F = 6$

Possible relative position of CAS protein during multi-target editing

Form 1: X1 value and corresponding sequence of C major scale

8n-3	8n-2	8n-1	8n	8n+1	8n+2	8n+3	8n+4
G=5	A=6	B=7	Rest=0	C=1	D=2	E=3	F=4
TA	TT	TG	TC	AA	AT	AG	AC

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