Gemini: a dual reporter of promoter activity

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Context

Biologically encoded memory devices underpin numerous potential applications, ranging from sensing to counting. Building upon work by Ham et al [1], a two-state memory device has been designed to controllably flip a small segment of DNA using recombinase enzymes. This "*Flipee*" requires no energy to maintain state, and a promoter on the *Flipee* sequence will drive a PoPS signal [2] to a read-out device that indicates its state.

Two requirements

Gemini is the read-out device that reports the state of the *Flipee*. The device will be used to evaluate *Flipee* performance (such as the speed of switching), and has two requirements.

1. Sensitive reporter

Flipee output may be weak, so the reporter signal must be sensitive. LacZ produces a detectable signal that is sensitive to promoter activity, but the cell requires a substrate and must be in a metabolically active state to produce assayable product (right panel). Florescence, though less sensitive, enables in-situ and non-disruptive monitoring (left panel). GFP-LacZ fusion reporters has been shown to encapsulate the positive properties of both into a single gene: GFP enables non-invasive monitoring of promoter activity and LacZ reports promoter activity if the GFP signal is weak (undetectable). The full LacZ gene, over 3kb, has been used in the dual reporter demonstrated in the literature [3].

GFP | ß-galactosidase (ß-gal)

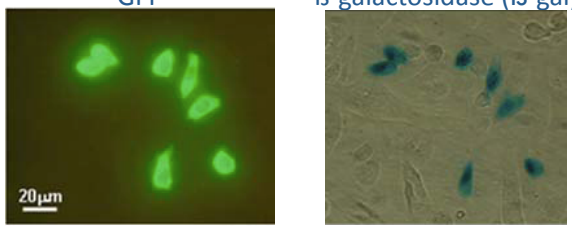

Figure 1: Cells expressing both GFP and ß-gal

2. Small sequence length

Because transformation efficiency decreases with plasmid size, it may be preferable to replace full length LacZ with a small yet critical fraction of the LacZ gene. This polypeptide, known as the ß-gal alpha fragment (shown below), has been shown to compliment an inactive ß-gal monomer in trans, resulting in a functional enzyme [4].

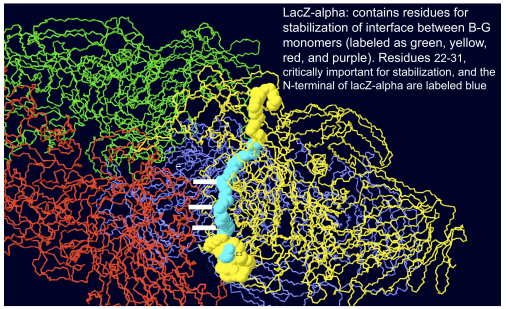

Figure 2: LacZ alpha complementation restores function to inactive ß-gal

Design

The aim is to build a dual LacZ-GFP reporter capable of of alpha-complementation so that it does not have to carry the full LacZ gene.

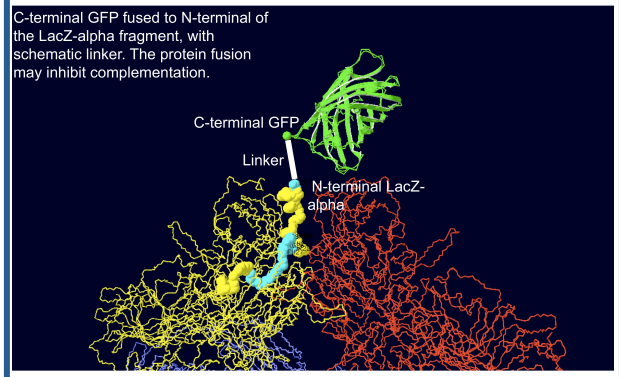

Figure 3: GFP fused to LacZ alpha fragment capable of complementation

Risks

GFP fusion may hinder alpha complementation. In addition, the linker used for full length LacZ GFP fusion reported by Hwang [3] contains a restriction site (EcoR1) incompatible with Bio-Brick assembly standard.

Hwang et al. (2006) LacZ-GFP fusion

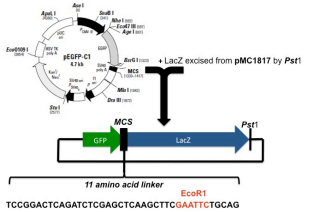

Figure 4: Proven fusion linker incompatible with assembly standard

Assembly strategy

The design must enable efficient testing of many possible linkers and florescent proteins. Staged construction enables testing of both reporters prior to fusion. Once full-length LacZ fusion is validated, fusion with LacZ alpha will be tested, first with possible linker variants and potentially with GFP variants [5] or other florescent reporters.

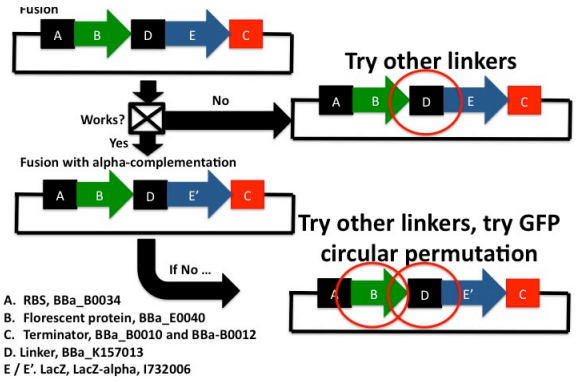

Figure 5: Staged assembly with modular components eases troubleshooting

[1] *Design and construction of a double inversion recombination switch for heritable sequential genetic memory*
Ham TS, Lee SK, Keasling JD, Arkin A.
PLoS ONE. 2008.
[2] PoPS (polymerase per second) - A measure of promoter activity at a specific point on DNA
[3] *Dual reporter genes enabling cell tracing with viable and reliable selection of various cell types*
C.N. Hwang, S. Hong, S.S. Choi, K.S. Lee, S.S. Park & S.H. Lee
Biotechnology Letters. 2006.
[4] *High resolution refinement of ß-galactosidase provides a structural basis for alpha-complementation*
Matthews, Brian et al.
Protein Science 2000.
[5] *Circularly permuted variants of the green fluorescent protein*
Topell S, Hennecke J, Glockshuber R.
FEBS Lett. 1999.

SynBERC Synthetic Biology Engineering Research Center