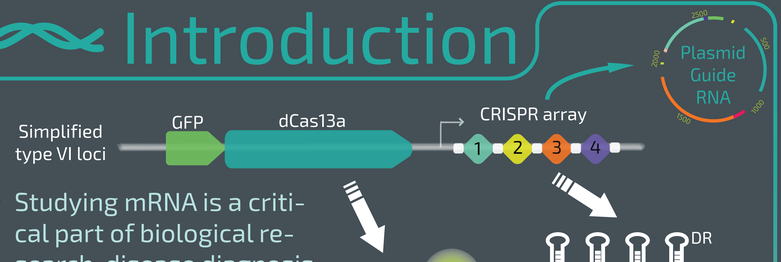
LuCAS

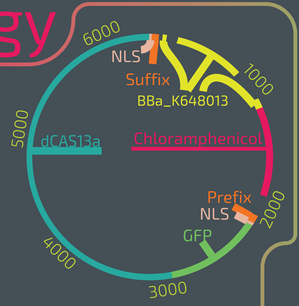
"A novel way to track mRNA localization in vivo"

Introduction

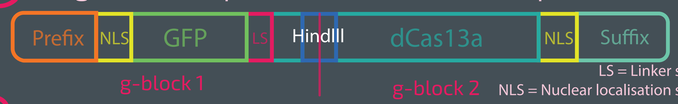


- Studying mRNA is a critical part of biological research, disease diagnosis and treatment [1,2]
- LuCAS is a research tool that visualizes mRNA localization in vivo using dCas13a and a GFP. This is an improvement over current techniques as it does not modify the target RNA and can easily be adjusted for another target

Methodology



- Gibson Assembly of pSB1C3 with GFP (BBa_K648013) and 'dCas13a' derived from *L. Buccalis* containing four point mutations resulting in a HEPN nuclease domain 1 and 2 inactive mutant, yielding our BioBrick (BBa_K2340000)
- Design of fusion protein for mammalian expression:

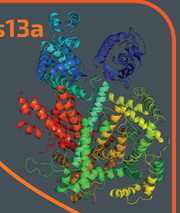


- Ligation of fusion protein via HindIII and amplification
- Design of guide RNA sequences (BBa_K2340001 - BBa_K2340011) for crRNA synthesis derived from human pkp4, Rab13, inpp1, and β-Actin expressing mRNA:

- Ligation of whole fusion protein with a) pSB1C3 bacterial expression vector and b) pcDNA 3.1 mammalian expression vector via EcoRI and XhoI
- Transformation of bacterial fusion protein construct into DH5α cells and transfection into *HEK293* cells followed by imaging

Modelling

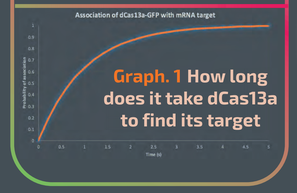
This is a statistical model that shows the movement of a dCas13a enzyme via diffusion which has the probability of colliding with a free floating mRNA molecule over a given amount of time.



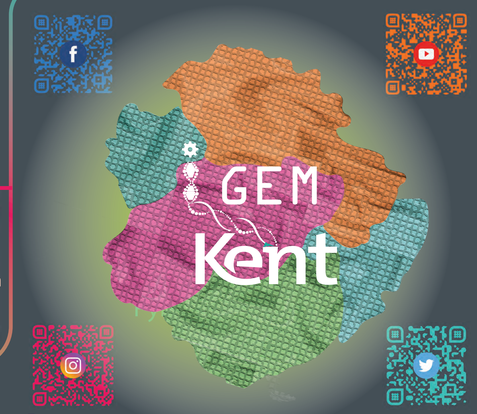
- Probability of enzyme-mRNA molecule collision using a normalized integral $\int_0^T e^{-nv\sigma\Delta t} nv\sigma\Delta t = 1$
- T is defined as the average amount of time for a given enzyme to collide $T = \frac{1}{nv\sigma} = 2.8$ milliseconds
- Maximum no. of enzymes in the given amount of time yielding a 100% collision rate for the fibroblast and neurons was **24**.
- Einstein-Stokes theorem to find the diffusion constant $D = \frac{kT}{6\eta\pi r} = 9.10\text{x}10^{-15}\text{m}^2\text{s}$
- Raleigh criterion was used to distiguish between two GFP molecules

$$R = \frac{1.22\lambda}{0.95 + 1.45}$$

- Where λ is 510nm, The minimum distance we can resolve for 2 GFP molecules is: R = 256 nm



Graph. 1 How long does it take dCas13a to find its target



Human Practices

Liaising with the public was incredibly important to us and we wanted to exercise the connections we had to our community through different outreach opportunities.

b[O]ing! 2017 Family Festival: Created a science stall for children where we showcased different experiments and activities whilst gathering survey data from their parents

LuCas' Adventure: An interactive game aimed at young teens incorporating our dCas13a

Open days:

- Spoke with aspiring science students about our project
- Gathered data to integrate into our project through surveys and communication

- Consulted with professionals in their field
- Gained feedback through presenting in the Bioscience Symposium

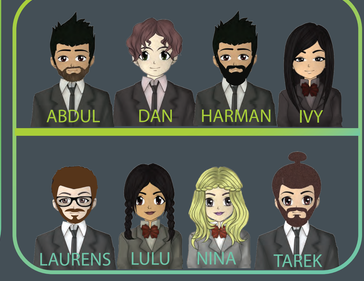
Collaboration

We collaborated with team Judd_UK on the interlab study

We provided lab space and equipment required for the standardized study

Judd provided DNA from Distribution Kit plate 6 as we weren't successful in transforming the DNA from our kit

Team



Results

- We built one biobrick, validated by screening primers (Fig 1 and 2), by fusing together a wtGFP gene using NEB Gibson Assembly[3] a 'dead' Cas13a gene, adding a flexible linker and two nuclear localization sequences to improve stability and reduce background noise.
- We transfected our construct (BBa_K2340000) with a guide RNA plasmid (BBa_K2340001 - BBa_K2340011) (1:2 ratio) into HEK293[4] cells using Lipofectamine™ 2000 in a 24 well plate in media and incubated for 24 hrs at 37°C.
- The function of our construct was not proven (Fig 3) perhaps due to the poor folding of the wtGFP at 37°C and the short time frame.
- Furthermore, we designed 11 biobricks which could not be sent due to time constraits.

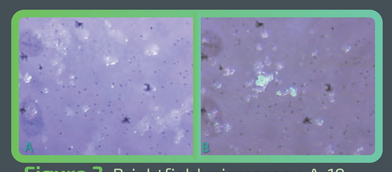


Figure 3: Brightfield microscopy. A-19: Cas13 + β-actin (1); no expression after 24 hrs. After 30/48 hours - no expression (not shown), B-18: GFP Control; GFP expression

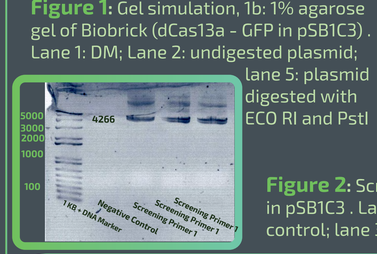


Figure 1: Gel simulation, 1b: 1% agarose gel of Biobrick (dCas13a - GFP in pSB1C3) . Lane 1: DM; Lane 2: undigested plasmid; lane 5: plasmid digested with ECO RI and PstI

Figure 2: Screening PCR products of dCas13a - GFP in pSB1C3 . Lane 1: DNA Marker; Lane 2: negative control; lane 3 - 5; Screening PCR;

References

1. Briley WE, Bondy MH, Randeria PS, Dupper TJ, Mirkin C a. Quantification and real-time tracking of RNA in live cells using Sticky-flares. Proc Natl Acad Sci. 2015;112(31):201510581. doi:10.1073/pnas.1510581112.
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3. 1New England Biolabs (2017). Gibson Assembly® Master Mix. [WWW Document]. URL https://www.neb.com/products/e2611-gibson-assembly-master-mix#Product%20Information. 2Thomas P.,
4. Smart, T.G. (2005). HEK293 cell line: A vehicle for the expression of recombinant proteins. Journal of Pharmacological and Toxicological Methods, 51: 187-200.

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