**Introduction**

Studying mRNA is a critical part of biological research, disease diagnosis and treatment. 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 sequence.

LuCAS is a mammalian expression vector via adjusted for another target the target RNA and can easily be 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. Bucillatis containing four point mutations resulting in a HEENT nuclear domain 1 and 2 inactive mutant, yielding our BioBrick (BBa_K2340000).**
- **Design of fusion protein for mammalian expression:**
  - Prefix = 5' block 1 | U6 Promoter | SLS = Serine linker sequence | RCS = RNA coding sequence | Suffix = terminator
  - Prefix = 5' block 2 | LS = Linker sequence | NS = Nuclear localization sequence
- **Ligation of fusion protein via HindIII and amplification**
- **Design of guide RNA sequences (BBa_K2340001 - BBa_K2340010) for crRNA synthesis derived from human pKp4, Rab13, in1p, and β-Actin expressing mRNA:**
  - Prefix = U6 Promoter | SLS = SLS coding sequence | RCS = RCS coding sequence | Suffix = terminator
  - Prefix = 5' block 1 | SLS = SLS linker sequence | NS = NS coding sequence
- **Ligation of whole fusion protein with pSB1C3 bacterial expression vector and β) pcDNA 3.1 mammalian expression vector via ScORI and XhoI**
- **Transformation of bacterial fusion protein construct into DH5α cells and transfection into HEK293 cells followed by imaging**

This has been made possible by the support of:

**References**

2. Judd_UK on the interlab study
7. Judd_UK on the interlab study
10. Judd_UK on the interlab study
12. Judd_UK on the interlab study
14. Judd_UK on the interlab study
16. Judd_UK on the interlab study
18. Judd_UK on the interlab study
20. Judd_UK on the interlab study
22. Judd_UK on the interlab study
24. Judd_UK on the interlab study
26. Judd_UK on the interlab study