Engineering Light Controllable CAR T-Cells

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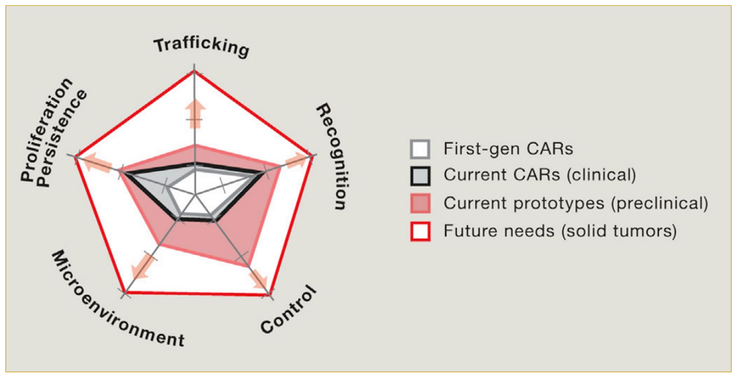
Background

- Common Cancer Treatments: Surgery, Chemotherapy, and Radiotherapy.
- Surgery: residual tumor lead to potential resurgence of cancer [1].
- Chemotherapy: risks of cancer recurrence than direct surgery but high risk with leukopenia, abnormally low white blood cell count and regenerability [2].
- Cancer therapy called chimeric antigen receptor (CAR) T-cell was developed as an alternative that uses T-cells to attack the tumor [3].
- Current CAR T-Cell: high efficiency of targeting tumor [4].
- However, the therapy can be too sensitive which results in CAR T-cells attacking patient's body [5].



Objectives

- Develop and implement an optogenetic circuit into human T cells to achieve spatial and temporal control over the CAR expression.
- Use fluorescent protein as a reporter to represent and monitor the expression of CAR through fluorescence imaging.
- Use programming to quantify the expression profile of reporter gene in cells in response to light-induction over time.
- Optimize the efficiency and minimize the cost of CAR-T therapy.

Design

1. Plasmid and primer design

Use online Benchling tool to design recombinant plasmids through inserting circuit components into templates.

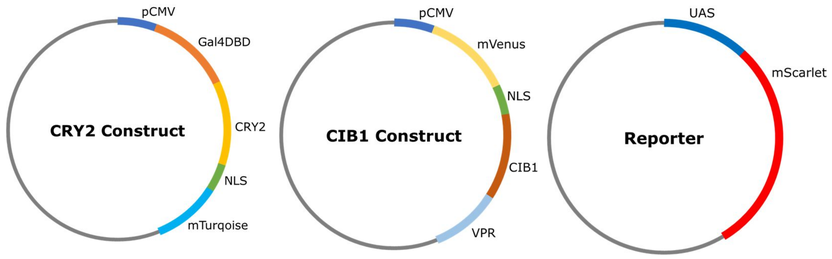


Fig 1. Plasmid constructs used for experimental group

2. Wet lab experiment

PCR amplify fragments and make constructs through Gibson Assembly. Clone into E. coli to replicate. After sequencing verification, transfect into HEK293T cells. Use fluorescence microscope to image cells that have desired constructs.

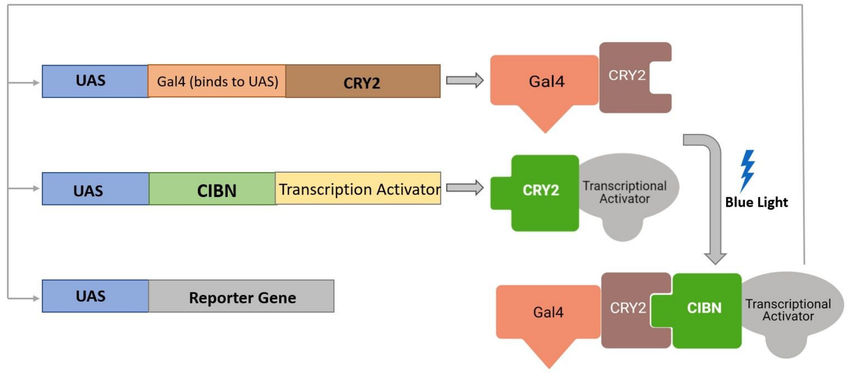


Fig 2. Schematic diagram of the interactions among different circuit components

3. Computational analysis

Quantify the fold-change of mean fluorescence intensity in cell images over time.

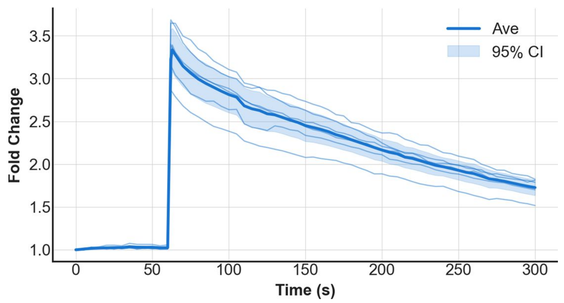


Fig 3. Timelapse of mScarlet expression fold-change. Blue light pulse is given at t=60s for 1s.

4. ODE modeling

Combine Hill's equation and circuit behaviors to fit differential equation model to experimental results.

Expected Outcomes

- mScarlet expression is greatly induced by shining blue light into the cells, whereas CRY2 and CIB1 expression stays constant.
- The optogenetic circuit exhibits positive feedback loop function of the fold-change as the time goes
- The timelapse fold-change of mScarlet expression can be fitted by Hill's Model

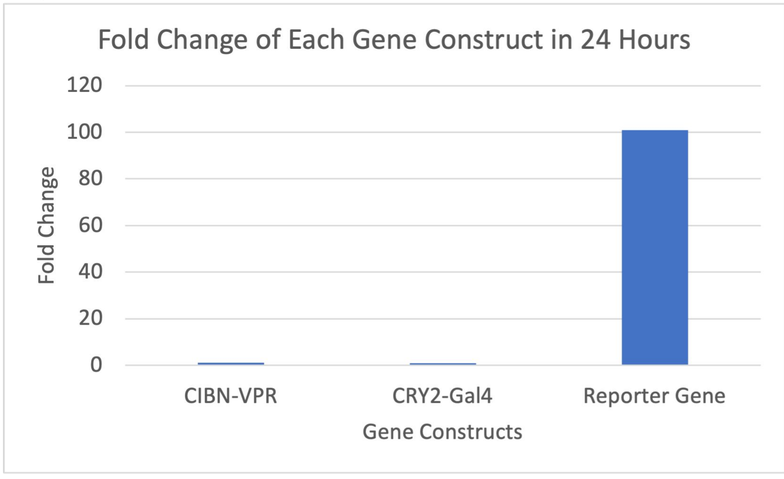


Fig 4. Fold-change between t=0 and t=24h in the fluorescence intensity by CIBN-VPR, CRY2-Gal4, and the reporter gene respectively.

Conclusions

- Overall, significant fold change in mScarlet reporter gene expression compared to negative control,
- Blue light in the cells can induce a high increase in gene expression, indicating that our design is able to achieve spatial and temporal control over gene expression
- The behavior of the reporter gene can be characterized and validated by the Hill function.
- In the future, expand our design toward controlling the expression of real CAR and test the therapy in cancer patients at clinical trials.

Acknowledgements

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References

[1] Tohme, Samer et al. [2] Liang, Yi-Hsin et al. [3] Lim WA et al. [4] Jayaraman J et al. [5] Titov A et al.