A High-Throughput Optogenetic Toolkit for Screening of Medical Genetic Therapeutic Targets

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The Next Generation of Medical Genetics

- Functionality – genes & proteins
- *Synthetic biology* - faster, cheaper and more accurate genomic assays
- Applications in *medicine* – finding therapy for ailments heavily rooted in genotype

Genotype specific therapy could help to slow the onset of neurodegenerative disorders, like Alzheimer’s disease.

**Source:** TestoForte Plus®, 2012
Clinical Significance of Flow Cytometry & Yeast

- **Yeast** advantages
  - genetic analogy/homology to mammalian cells
  - Rapid growth period
  - Larger culture size

- Flow Cytometry + Rapidly Growing Yeast  ➔ *lots of data*

- Screen more genes with less time
Overview of Optogenetics

- Controlling organisms with a light

- Neuroscience - Light gated ion channels control of neuronal synapses

- Cell Biology - Light inducible protein interactions
  - Phytochromes
  - Cryptochromes

Advantages of Optogenetics

• **Faster**
  - Standard activation of GAL promoter – 2 day waiting
  - Optogenetics – 5-10 second waiting

• **Cheaper**
  - Don’t need to change expensive media

• **Real-Time**
  - See gene induction from basal state
  - Dose Dependent Control

Source: Lumencor
Optogenetic Yeast Two-Hybrid

“control of DNA transcription using a light stimulus”
Fluorescent Reporters

- Traditionally used as feedback in cell biology
- Expression, Maturation, and Degradation
- GFP, YFP, BFP, mCherry
- Flow Cytometer Analysis

Above: A bacterial “painting” created using eight common phenotypic variations of fluorescent protein.

Research & Engineering Goals

1. Characterize and optimize reporters for use as feedback;

2. Develop a computational model for prediction of our network behavior *in silico*;

3. Engineer a software package for rapid analysis of flow cytometric data in optogenetic studies

4. Compare optogenetic system (described by Hughes et al) to GAL1 promoter to verify if it is experimentally robust;
Design of Reporter Plasmids

4 Reporters of different inducible and output wavelength controlled by GAL1 promoter and integrated into yeast genome at auxotrophic locus.

- GAL1-yEVenus: Yellow
  - (Top Left)
- GAL1-yEGFP: Green
  - (Top Right)
- GAL1-mCherry: Red
  - (Bottom Left)
- GAL1-tagBFP: Blue
  - (Bottom Right)
Experimental Methods

**Reporter Characterization:**
- We transformed a GAL1pr controlled construct of mCherry (Red), yEVenus (Yellow), yEGFP (Green), and tagBFP (Blue) into the MMY116 yeast strain.

**Optogenetic System Characterization:**
- Through personal correspondence with Tucker lab, we acquired CIB1-Cry2 plasmids described by Hughes, et al. We transformed into PJ694A (ΔGal4, ΔGal80)
Characterization of BFP

- Introduction
- Experimental
- Computational
- Engineering
- Application
Characterization of mCherry
Computational Methods

• We used the equations described by Gillespie et al for analysis of chemical kinetics of type \( A+B \rightarrow AB \)

• We imported these into TinkerCell, a CAD tool for synthetic biology, to develop a stochastic model of network behavior.
Computational Results

Blue Light OFF

Blue Light ON
FC Analyze Software Package

- FCS -> CSV
- Gate Data to User Specificity
- Pass through algorithmic logic
- Set Boolean value
- Forward Amino Acid string
- BLASTp returns Human Ortholog
Design of Loss of Function Assay

Optogenetic screening accelerates time consuming processes, such as long growth time in galactose media and small molecule gene activation. Yeast for gene identification: Giorgini, et al.
Alzheimer’s Disease & Amyloid-β

- **26.6 million** suffer from Alzheimer’s worldwide
- Maintaining **Amyloid-β** at median level could slow onset of Alzheimer’s

- Apply screening principles from Giorgini’s studies
- Identify post transcriptional enzymatic suppressors of Amyloid-β

**Source:** PBS News Hour
Future Goals

- Compare optogenetic system to GAL1 promoter to **verify** if it is experimentally robust

- Build upon works by Bolger et al to develop a light generator for very specific **delivery** of light pulses to our yeast samples
Human Practice

- Former members founded NCSSM High School iGEM Team
- Compiled online resource guide for high schoolers
- Contacted other local high schools about possibility of starting a team.
# Acknowledgements

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