The Gene Editing Research Lab — a new classroom-based research experience at UC Merced

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Research Project Outline

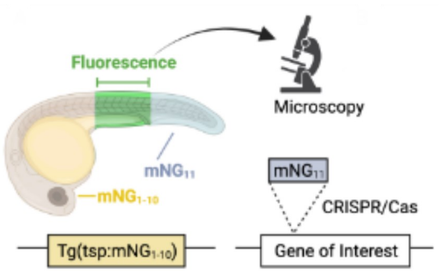

Figure 1: Schematic of split-fluorescent mNeonGreen project. The first component (mNG_{1-10}) consists of a tissue specific reporter. The second component (mNG_{11}) tags an endogenous protein. Tg, transgenic. tsp, tissue specific promoter.

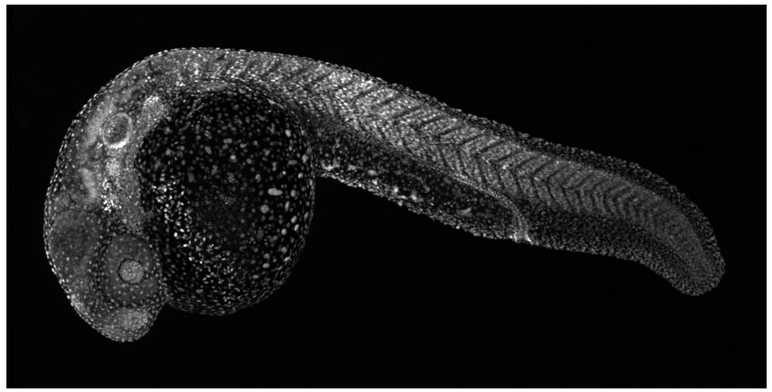

Figure 2: Transgene expression of mNG_{1-10} results in tissue specific expression. Tg(Ubb: mNG_{1-10}) fish tagged with h2az2b-mNG_{11} mRNA to show tissue specific expression of tagged proteins. Ubb, ubiquitin. Tg, transgenic.

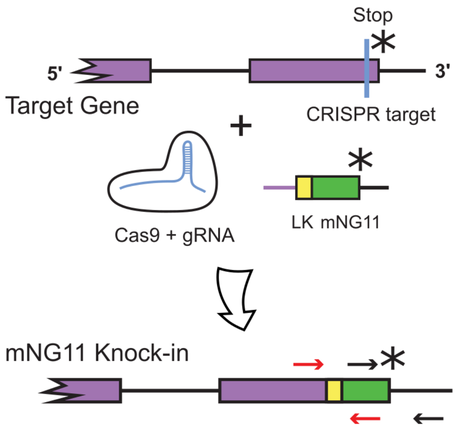

Figure 3. mNG_{11} Donor/KI DNA Schematic. Design of the knock-in strategy for mNG_{11} performed in this CURE course. The knock-in is created by homologous recombination. The homology arms target a gene of interest, which surround a double-stranded break made by CRISPR/Cas9.

Course Context

- Prerequisites: Introduction to Molecular Biology, General Chemistry I & II
- Enrollment: 6 students in their final year. All biology majors with little to no research laboratory experience.

Course Learning Outcomes (CLO's)

CLO 1 - Understand and describe how CRISPR/Cas9 is used to create gene mutations and knock-ins

CLO 2 - Experience the process of scientific discovery, including the iterative nature of biological research. This includes applying background knowledge to design experiments with clear testable hypotheses and appropriate controls and drawing conclusions from analysis of novel data

CLO 3 - Learn common scientific practices employed by biologists by performing PCR, in vitro transcription, gel electrophoresis, and T7 endonuclease assays and interpreting resulting data

CLO 4 - Communicate their research and discoveries at both technical and non-technical levels by presenting their results in a poster presentation

CURE Timeline

Introduction (Weeks 1-2)
CLO 1

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Designing, synthesizing, and testing CRISPR guide RNAs (Weeks 3-8)
CLO 1-3

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Creating Knock-Ins (Weeks 9-14)
CLO 1-3

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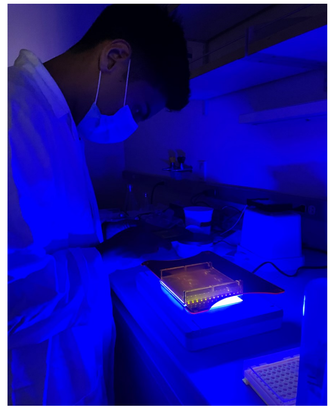
Presentations (Finals Week)
CLO 1,2,4

We have designed the course to meet twice a week for 3-hour laboratory time blocks. At the start of every session, we dedicate 30 minutes on a lecture covering fundamental concepts related to the project. Each student worked on their own gene from a list of curated targets.

Skill Development: Molecular Biology Techniques

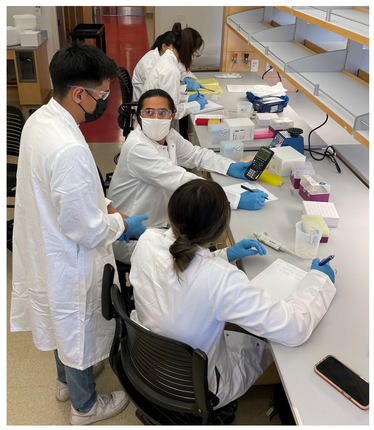
The skills developed in this class are similar skills taught in molecular biology laboratories.



Observations and Conclusions

- Increased time spent in discussion than following laboratory manuals.
- Increased ownership of project / work assigned.
- More collaborative atmosphere.
- Communal troubleshooting.

Overall, all students felt that this course tied together everything they have learned in all their biology coursework while introducing them to the iterative nature of research.



Acknowledgements

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