

EID 325: Science and Application Bioengineering Technology (ie. Eukaryotes for Engineers).

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The genetic modification of lifeforms (including ourselves!) using the growing toolkit of biotech is progressing at a rapid pace. In this project based course, we will be holding laboratory sessions throughout the semester, where the tools of genetic engineering and molecular biology will be learned and applied to eukaryotic organisms such as *Saccharomyces cerevisiae* (brewer's yeast) and *Arabidopsis thaliana* (a small flowering plant widely used in genetics research) to construct novel genetic circuits. Bi-weekly scientific journal club sessions will also be held, covering the latest topics in synthetic biology, biosecurity, gene therapies and the controversies underlying the biosafety of genetically modified organisms (GMOs).

Week 1/Journal Discussions (Introduction to course and background to synthetic biology)

Fred Sherman, Getting Started with Yeast, *Methods in Enzymology* Vol 350, 2002

Fred Sherman, An Introduction to the genetics and molecular biology of the yeast *Saccharomyces cerevisiae*. , Modified from F. Sherman, *Yeast Genetics*, 1998

Johnston, et al. Gene Disruption, *Methods in Enzymology*, Vol. 350. 2002

Clontech Laboratories, *Yeast Protocols Handbook*

Beth A. Montelone. Yeast Mating Type. *Encyc. Of Life Sci.* 2002

Week 2/Laboratory Session 1

Quantitation of *S. cerevisiae* using hemocytometer and spectrophotometer and plating.

Week 3/Journal Discussions (The Yeast 2-Hybrid System)

Andrew Mendelsohn. An enlightened genetic switch. *Nature Biotechnology* Vol 20, 2002

S. Fields and O. Song. A novel genetic system to detect protein-protein interactions. *Nature*. Vol 340. 1989

Mark Johnston. A model fungal gene regulatory mechanism: The GAL genes of *Saccharomyces cerevisiae*. *Microbiological Reviews*, Vol 51, No. 4. 1987

Quail et al. Universal Light-switchable gene promoter system. United states Patent 6,858, 429 B2. 2005

Shimizu-Sato, et al. A light switchable gene promoter system. *Nature Biotechnology*. Vol 20. 2002

Week 4/ Laboratory Session 2

Amplification of light inducible PhyB-Pif3 inserts and restriction digests of backbone plasmids.

Week 5/Journal Discussions (Optogenetics and light mediated gene regulation)

Boyden et al. Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience*. Vol 6. No. 9. 2005

Joseph Duffy. GAL4 system in *Drosophila*: A fly geneticist's Swiss Army Knife. *Genetics* 34:1-15. 2002

Lima and Miesenböck. Remote Control of behavior through Genetically Targeted Photostimulation of Neurons. *Cell*. Vol 121 . 2006

Levkaya, et al. Spatiotemporal control of cell signaling using a light switchable protein interaction. *Nature*. 2009

Week 6/ Laboratory Session 3

Restriction digests of amplified products along, analysis using gel electrophoresis and silica column purification of digested inserts.

Week 7/Journal Discussions (Cas9 CRISPR Gene targeting system)

Jinek, et al. A programmable Dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* Vol. 337. 2012

Mali, et al. RNA-guided human genome engineering via Cas9. *Science* Vol 339. 2013

Bahya et al. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Ann. Rev. Genetics* Vol 45 2011

David J. Segal. Bacteria herald a new era of gene targeting. *eLIFE*. 2013

John van der Oost. New tool for Genome surgery. *Science*. 339. 2013

Week 8/ Laboratory Session 4

Gel isolation of digested plasmid backbones and concentration determination of all purified products by UV spectroscopy. Calculate amounts for overnight ligations.

Week 9 (Plant Biotechnology)

Paine et al. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology*. Vol 23. 2005.

Ye et al. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid free) rice endosperm. *Science* Vol. 287. 2000

Bradford, et al. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology*. Vol 23. 2005.

Tzfira and Citovsky. Agrobacterium-mediated genetic transformation of plants: biology and biotechnology. *Current opinion in Biotechnology*. Vol. 17 2006

Nathanial Johnson. *Grist*. Online GMO review.

Amy Harmon. A lonely quest for facts on genetically modified crops. *New York Times*. Jan 4, 2014.

Week 10/ Laboratory Session 5

Colony PCR of transformants and preparation for sequencing of constructs.

Week 11/Journal Discussions (Policy reports on biosecurity and dual use research)

Biotechnology research in an age of terrorism. Executive Summary of the Committee on research standards and practices to prevent the destructive application of biotechnology, National Research Council.

Fauci and Collins. Benefits and Risks of Influenza research: Lessons Learned. *Science* Vol 336 2012

Lipsitch et al. Evolution, safety and highly pathogenic influenza viruses. *Science* Vol 336 2012

Carrie Wolinetz. Implementing the New U.S. Dual-Use Policy. *Science* Vol 336 2012

Imai, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortment H5 HA/H1N1 virus in ferrets. *Nature*. Vol 486. 2012

Sander Herfst, et al. Airborne transmission of Influenza A/H5N1 virus between ferrets. *Science* Vol. 336. 2012

Week 12/ Laboratory Session 7

Validate sequence data of constructs, lithium acetate transformation into *S. cerevisiae*.

Plan experiments for our light inducible dual plasmid system.

Week 13/Journal Club Session (Human Stem Cell Technologies)

Tachibana et al. Mitochondrial gene replacement in primate offspring and embryonic stem cells *Nature* Vol 461:17 2009

Yamada et al. Nature 2014 Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells

Lo, et al. Cloning Mice and Men: Prohibiting the use of iPS Cells for human reproductive cloning. *Cell Stem Cell*. Vol 6. 2010.

Insoo Hyun. Regulate embryos made for research. Nature Vol. 509 2014.

Week 14/ Laboratory Session 8

Microplate Bradford assay to determine beta-galactosidase expression levels of our light inducible yeast expression system.

Week 15/Laboratory Notebook Turn In and Final Journal Discussions