EID 320: Special Topics in Bioengineering (ie. Prokaryotes for Engineers).

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Using microbes to produce valuable products is one of humanity's earliest technological endeavors. In this project-based course, we will be holding laboratory sessions throughout the semester, where the tools of genetic engineering will be applied to bacteria to build and test novel genetic circuits, such as biosensors. We will also be holding regular scientific journal club sessions, covering articles on the latest progress in synthetic biology, issues related to biosecurity and controversies underlying the patenting of genes, along with the commercial entrance of genetically modified organisms (GMOs).

Week 1/Introduction to Foundations in Synthetic Biology

Week 2/Laboratory Session 1

Transformation of Control plasmids and PCR of Vector Backbones.

Week 3/Journal Club (Cloning Genetic Parts: BioBricks, Golden Gate, and Gibson Assembly)

Tom Knight. Idempotent Vector Design for Standard Assembly of Biobricks. 2006.

Engler, C., Kandzia, R. and Marillonnet, S. A One Pot, One Step, Precision Cloning Method with High Throughput Capability. PLos One. Vol 3 (11) 2008.

Gibson, D.G., Young, L., Chuang, R., Venter, J.C., Hutchison III, C.A. and Smith, H. Enzymatic assembly of DNA Molecules up to several hundred kilobases. Nature Methods. Vol. 6 (5) 2009.

Joska, T. M., Mashruwala, A., Boyd, J.M. and Belden, W. J. A Universal cloning method based on yeast homologous recombination that is simple, efficient and versatile. Journal of Microbilogical Methods. 100 (46-51) 2014.

Week 4/ Laboratory Session 2

Restriction digests of PCR products, silica column purification and concentration determination.

Week 5/Journal Discussions (Biosensors and Genetic Switches)

Gardner, T.S., Cantor, C.R. and Collins, J.J. Construction of a genetic toggle switch in Escherichia coli. Nature. 403. 2000.

Kotula, J.W., Kerns, S.J., Shaket, L.A., Siraj, L., Collins, J.J., Way, J.C. and Silver, P. Programmable bacteria detect and record an environmental signal in the mammalian gut. PNAS. 111 (13) 2014.

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 (Cloning and Optimization of Drug Biosynthetic Pathways)

Galanie, S., Thodey, K., Trenchard, I., Interrante, M.F. and Smolke, C.D. Complete biosynthesis of opioids in yeast. Science 349 (6252) 2015.

Thodey, K., Galanie, S. and Smolke, C.D. A microbial biomanufacturing platform for natural and semisynthetic opioids. Nature Chemical Biology Vol. 10 2014.

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 (Multiplex Automated Genome Engineering and Gene Drives)

Esvelt, K., Smidler, A.L., Catteruccia, F. and Church, G.M. Concerning RNA-guided gene drives for the alteration of wild populations. Vol. 3 eLife 2014.

Wang, H.H., Isaacs, F.J., Carr, P.A., Sun, Z.Z., Xu, G., Forest, C.R. and Church, G.M. Programming cells by multiplex genome engineering and accelerated evolution. Vol 460 (13) Nature. 2009.

DiCarlo, J.E., Chavez, A., Dietz, S.L., Esvelt, K.M. and Church, G.M. RNA-guided gene drives can efficiently and reversibly bias inheritance in wild yeast. bioRxiv 2015.

Week 10/ Laboratory Session 5

Colony screening and Sequence analysis of constructs

Week 11/Journal Discussions (Genome Assembly and Patenting Life)

Chakrabarty. United States Patent 4,259, 444. Microorganisms having multiple compatible degradative energy generating plasmids and preparation thereof.

Robinson, D. and Medlock, N. Diamond v. Chakrabarty: A Retrospective on 25 years of Biotech Patents.

Vol 17 (10) Intellectual Property and Technology Law Journal. 2005.

Lartigue, C., Glass, J.I., Alperovich, A., Pieper, R., Parmar, P.P., Hutchison III, C.A., Smith, H.O. and Venter, J.C. Genome transplantation in bacteria: Changing one species to another. Vol. 317. Science. 2007.

Gibson, D.G. et al. Creation of a bacterial cell controlled by a chemically synthesized genome. Vol. 329 Science. 2010.

Week 12/ Laboratory Session 7

Microplate assay of Green and Red Fluorescence Proteins and Beta-galactosidase expression

Week 13/Journal Club Session (Phage Assisted Continuous Evolution)

Esvelt, K.M., Carlson, J.C. and Liu, D.R. A system for the continuous directed evolution of biomolecules. Vol. 472. Nature. 2011.

Carlson, J.C., Badran, A.H., Guggiana-Nilo, D.A. and Liu, D.R. Negative selection and stringency modulation in phage-assisted continuous evolution. Vol. 10. ZNature Chemical Biology. 2014.

Week 14/ Laboratory Session 8

Design of new experiments, repeat readings from last week and design of new constructs.

Week 15/Laboratory Notebook Turn In and Final Journal Discussions