In Vitro Cellular & Developmental Biology - Animal
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# Plenary Symposia

## PS-1

Use of Short Homology for Targeted Integration in Zebrafish. Wesley A. Wierson[1], Jordan M. Welker[1], Maira P. Almeida[1], Carla M. Mann[1], Dennis A. Webster[2], Melanie E. Torrie[1], Trevor J. Weiss[1], Sekhar Kambakam[1], Macy K. Vollbrecht[2], Merrina Lan[1], Kenna C. McKeighan[1], Jacklyn Levey[1], Zhitao Ming[1], Alec Wehmeier[1], Christopher S. Mikelson[1], Jeffrey A. Haltom[1], Kristen M. Kwan[3], Chi-Bin Chien[4], Darius Balciunas[5], Stephen C. Ekker[6], Karl J. Clark[6], Beau R. Webber[7], Branden S. Moriarity[7], Stacy L. Solin[2], Daniel F. Carlson[2], Drena L Dobbs[1], Maura McGrail[1], and JEFFREY ESSNER[1]. [1]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA; [2]Recombinetics, Inc., St. Paul, MN; [3]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; [4]Department of Neurobiology and Anatomy, University of Utah Medical Center, Salt Lake City, UT; [5]Department of Biology, Temple University, Philadelphia, PA; [6]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, NY; and [7]Department of Pediatrics, Masonic Cancer Center, University of Minnesota, Minneapolis, MN. Email: jessner@iastate.edu

Efficient precision genome engineering requires high frequency and specificity of integration at the genomic target site. Here, we describe a set of resources to streamline reporter gene knock-ins in zebrafish and demonstrate the broader utility of the method in mammalian cells. Our approach uses short homology of 24–48 bp to drive targeted integration of DNA reporter cassettes by homology-mediated end joining (HMEJ) at high frequency at a double strand break in the targeted gene. Our vector series, pGTag (plasmids for Gene Tagging), contains reporters flanked by a universal CRISPR sgRNA sequence which enables in vivo liberation of the homology arms. We observed high rates of germline transmission (22–100%) for targeted knock-ins at eight zebrafish loci and efficient integration at safe harbor loci in porcine and human cells. Our system provides a straightforward and cost-effective approach for high efficiency gene targeting applications in CRISPR and TALEN compatible systems.

## PS-2

Base Editing Strategies for Consumer Traits in Fruits and Vegetables. Y. BILL KIM. Pairwise Plants, 807 East Main Street, Suite 4–100, Durham, NC. Email: bkim@pairwise.com

Consumption of fruits and vegetables is one of the most effective solutions to malnutrition and global obesity epidemic. However, despite wide availability of fruits and vegetables, Americans for example consume only half of the daily recommended intake of fruits and vegetables. Pairwise aims to tackle these challenges by making healthy food more convenient, affordable, and sustainable. We have assembled a portfolio of CRISPR technologies and a state-of-the-art gene editing capability including CRISPR base editing tools and deployed them in multiple plant species at editing efficiencies as high as 85%. We also extended target sequence accessibility through gene and protein engineering. We are applying these tools for novel consumer traits in cane berries. This presentation will describe Pairwise progress in developing genome editing tools as well as our vision for opportunities in fresh produce.

## PS-3

Targeting RNA Regulation with Programmable Technologies. BRYAN. C. DICKINSON. University of Chicago, Department of Chemistry. Chicago, IL. Email: Dickinson@uchicago.edu

RNA transcribed from the genome in the nucleus bears little resemblance to the RNA polymer it will ultimately become in the cytoplasm where it is translated into protein. Well-known processes such as capping, splicing and polyadenylation, as well as the recently discovered and ever-expanding list of diverse chemical modifications and editing, significantly alter the properties and fates of a given RNA during the course of its lifetime. These alterations regulate critical aspects of RNA function such as stability, transport, protein binding, and translation. Especially in mammalian systems, these post-transcriptional gene expression regulatory processes are often

a key determinant of genetic information flow. Moreover, from an engineering and therapeutic perspective these RNA regulatory processes represent new ways to control or retune gene expression at the RNA level, if they can be harnessed. I will present technologies our group has developed to measure the chemical composition and localization of RNAs, and to measure and control protein-RNA interactions with an eye toward therapeutic development.

**PS-4**

Synthetic Biology Research and Development at the Joint Genome Institute. NIGEL J. MOUNCEY, I. Blaby, J.-F. Cheng, and Y. Yoshikuni. DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA 94720. Email: nmouncey@lbl.gov

As a result of the tremendous advances in DNA sequencing, we have accumulated vast amounts of sequence information from all types of organisms and environments. However, the rate of functional protein characterization has lagged behind, resulting in a pressing need for scalable and high-throughput annotation methods. At the JGI, we seek to close this gap through deploying integrative genome science from sequencing, synthetic biology, functional genomics, metabolomics to large computational analyses and modeling to generate biological insights relevant to energy and environmental research. The JGI DNA Synthesis Platform and Program develops and applies novel synthetic biology approaches to design and build constructs and genome-wide libraries, engineer a broad range of microbial hosts and conduct research with users to explore biological systems in the areas of microbial metabolism, gene regulation, microbial community and host-interaction biology and biogeochemistry.

**PS-5**

A Novel Plant Cell-free Synthetic Biology Platform and Its Applications. KRISHNA MADDURI[1], Matthias Buntru[2], Simon Vogel[2], Stefan Schillberg[2], Prasanth Maddipati[1], Paul Ketterer[1], Jennifer Arruda[1], and Robbi Garrison[1]. [1]Corteva Agriscience, 9330 Zionsville Road, Indianapolis, IN 46268 and [2]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstr. 6, 52074 Aachen, GERMANY. Email: Krishnamurthy.madduri@corteva.com

Recent advances in cell-free expression systems have enabled the emergence of the cell-free synthetic biology field. Cell-free synthetic biology technology is expanding at a rapid pace. It is being deployed in both basic and applied research for metabolic pathway optimization, on-demand, point-of-care vaccine production, diagnostics application, reconstitution and mechanistic evaluation of complex membrane protein complexes, to name a few. In microbial product development, cell-free synthetic biology approaches are reducing the need for intact cell-based systems to accelerate the design-build-test cycle and significantly increasing the throughput. However, the application of cell-free synthetic biology approaches in plant biotechnology to speed up the design-build-test cycle is limited by the unavailability of plant cell-free expression systems. We recently developed a robust plant cell-free expression system and demonstrated its potential to produce a wide range of functional proteins. We accomplished this by a coupled transcription and translation process, which is an energy-independent system and capable of producing amino acids needed for protein synthesis using active metabolic pathways and functional organelles. This cell-free system contains the intact organelles and therefore is, in essence, an "open-cell" system. We developed applications using plant cell-free systems to establish an early version of a plant cell-free synthetic biology platform. We demonstrated its utility for evaluating alternate gene designs, screening of transit peptides, and identification of cis-acting translation control elements, to name a few. This talk will discuss the background and commercial availability of this system.

**PS-6**

Extruded Collagen Graft Intergration with Biphasic Gene Electrotransfer in a Subcutaneous Small Animal Model. K. Christensen[2], C. Boye[1], K. Asadipour[1], M. Francis[2], and A. BULYSHEVA[1]. [1]Old Dominion University, Electrical and Computer Engineering Department, 1210 W. 45th Street, Norfolk, VA 23529 and [2]Emobdy, Inc., 4111 Monarch Way, Norfolk, VA 23508. Email: abulyshe@odu.edu

Tendon injuries are common athletic injuries that have been increasing in prevalence. While there are current clinical treatments for tendon injuries, they have relatively long recovery times and often do not restore native function of the tendon. Tissue engineering approaches of collagen-based grafts for tendon and ligament repair can benefit from strategies mimicking native angiogenesis and wound healing. In this study we examined a gene therapy approach for fibroblast growth factor (FGF-2) delivery and expression at the site of a collagen graft implantation for tendon repair. We have developed a biphasic electrotransfer gene delivery protocol for optimized delivery to the skin using reporter genes. The biphasic approach allows minimizing heating and twitching at the electrode/skin interface, while enhancing gene delivery to the epidermis, dermis and underlying muscle. Bioluminescence imaging was utilized to quantify gene expression, while immunofluorescence staining of skin samples 48 hours after gene delivery was used to determine the location of expression within the skin layers. Gene expression with biphasic electrotransfer was enhanced over ten-fold compared to plasmid DNA injection only,

without any skin damage visible macroscopically or histologically. Once pulsing parameters were optimized, intradermal gene delivery of plasmid DNA encoding human FGF-2 was quantified. Biofabricated, aligned, collagen scaffolds mimicking tendon structure were implanted subcutaneously for biocompatibility and angiogenesis analyses. GET of human fibroblast FGF-2 significantly increased angiogenesis and biocompatibility. The combination of aligned collagen scaffold and angiogenic GET therapy leads to improved graft biocompatibility in tendon repair.

**PS-7**

Nanosecond Pulsed Electric Field Stimulation: From Small Pores to Big-time Applications. A. G. PAKHOMOV, S. Xiao, I. Semenov, E. Gudvangen, U. Mangalanathan, V. Kim, G. Slkuniene, M. Silkunas, and O. N. Pakhomova. Frank Reidy Research Center for Bioelectrics, Old Dominion University, 4211 Monarch Way, Suite 300, Norfolk, VA 23508. Email: apakhomo@odu.edu

Nanosecond pulsed electric field (nsPEF) is a novel modality for electromanipulation of living cells. The principal primary mechanism responsible for diverse nsPEF bioeffects is the formation of anomalously stable nanopores in cell plasma membrane and intracellular membranous structures. These pores have "smart" functional properties which previously were thought to be unique for endogenous ion channels and transporters, such as voltage and current sensitivity, ion selectivity, and inward rectification. Once induced, nanopores oscillate between open and quasi-open (electrically silent) states, followed by either gradual resealing or abrupt breakdown into larger pores, with immediate loss of nanopore-specific behaviors. Nanopores appear adequately equipped for certain functions that are traditionally ascribed to ion channels. The talk will focus on nanopore properties and diverse consequences of nanoporation for cell function, from the activation of calcium transients, nerve and muscle excitation, modulation of ion channel activities, to the induction of necrotic or apoptotic cell death. Next, we will highlight the phenomenon of bipolar cancelation, which stands for a reduced efficiency of bipolar pulses compared to unipolar ones. The bipolar cancelation phenomenon comes in sheer contrast to conventional electroporation, where bioeffects are proportional to the time duration when the pulse voltage exceeds a critical level. The efficiency of bipolar nsPEF can be restored by the superposition of two properly shaped and synchronized bipolar waveforms into a unipolar pulse remotely from stimulating electrodes. This approach uniquely enables remote targeting of nsPEF effects, to assist such existing and emerging medical applications of nanosecond pulses as cancer and atrial ablation, immune and neuroendocrine stimulation, and deep brain stimulation.

**PS-8**

Characterizing Novel BRD4-dependent Nuclear Proteins in the Heart. M. STRATTON[1], A. Canella[1], L. Marcho[1], O. Bermeo[2], N. Shamama[2], A. Francois[1], E. McGrail[1], B. Whitson[3], P. Janssen[1], and R. Gumina[2]. [1]Department of Physiology and Cell Biology, [2]Division of Cardiovascular Medicine, and [3]Division of Cardiac Surgery, Ohio State University Wexner Medical Center, Columbus, OH 43210. Email: matthew.stratton@osumc.edu

Heart failure (HF) affects over 6 million Americans and its prevalence is projected to increase 46% by 2030. The 5-year mortality rate for HF is near 50%. Excess extracellular matrix (ECM) deposition, or fibrosis, in the ventricular wall is a clinical hallmark of HF, regardless of the initiating pathology. In rodent models of HF, cardiac function is preserved by targeting pro-fibrotic mechanisms. These strategies have not been amenable for clinical use, in part because the targeted proteins were critical for basic physiologic function in multiple organ systems (i.e. on-target toxicity). Thus, effective therapies targeting cardiac fibrosis remain an urgent unmet need. The epigenetic reader protein and transcriptional co-activator, BRD4, is necessary for cardiac fibroblast activation in vitro, and for cardiac fibrosis in rodent models of heart failure. Small molecule inhibitors of BRD4 are clearly protective against pathologic remodeling in the heart and improve function. However, BRD4 is essential for normal physiological function. Induced genetic disruption of BRD4 in mouse cardiomyocytes rapidly leads to death while chemical inhibition impedes learning/memory and telomere maintenance. Thus, we began searching for BRD4-activated genes as potential novel therapeutic targets. We uncovered a nuclear protein, Sertad4, as our leading candidate for which no molecular functions have been described. Our preliminary data indicate that like BRD4, Sertad4 is a positive regulator of fibroblast activation. This presentation will detail specific roles we have identified for Sertad4 in fibroblast biology, including proliferation and myofibroblast differentiation.

**PS-9**

Epigenetic Regulation of Genomic Stability During DNA Replication. Y. JACOB[1], J. Dong[1], Y. Huang[1], H. Davarinejad[2], C. LeBlanc[1], A. Poulet[1], B. Mermaz[1], G. Villarino[1], K. M. Webb[3], V. Joly[1], J. Mendez[1], P. Voigt[3], and J. F. Couture[2]. [1]Yale University, Department of Molecular, Cellular and Developmental Biology, Faculty of Arts and Sciences, 260 Whitney Avenue, New Haven, CT 06511; [2]Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, CANADA; and [3]Wellcome Centre for Cell Biology, School

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Epigenetic mechanisms play diverse roles in the regulation of genome stability in eukaryotes. In plants, genome stability is maintained during DNA replication by the plant-specific histone methyltransferases ATXR5 and ATXR6, which catalyze the deposition of the histone post-translational mark lysine 27 mono-methylation (K27me1) specifically on replication-dependent histone H3.1 variants (H3.1K27me1). Loss of H3.1K27me1 in *atxr5 atxr6* double mutants leads to heterochromatin defects, including transcriptional de-repression and genomic instability, but the molecular mechanisms responsible for these phenotypes remain largely unknown. In addition, no molecular role has been assigned to K27me1 or H3.1 variants to explain their involvement in transcriptional de-repression and genomic instability associated with DNA replication. Finally, the absence of ATXR5 and ATXR6 orthologues in animals raises questions about the conservation of mechanisms working at the chromatin level that ensure the maintenance of DNA copy number in eukaryotes. The work that will be presented reveals our new insights into the roles of histone modifications and histone variants in preventing heterochromatin instability in plants, and the conserved mechanisms in plants and animals that are used to maintain genomic stability during DNA replication.

**PS-10**

Epigenetic Regulatory Mechanisms Associated with Neural Cell Specification. HEHUANG XIE. Epigenomics and Computational Biology Lab, Fralin Life Sciences Institute of Virginia Tech, Blacksburg, VA 24060 and Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24060. Email: davidxie@vt.edu

Epigenetic dynamics in DNA methylation and histone modifications allow genetically identical stem cells to differentiate into distinct types of cells with various phenotypes. They also enable differentiated cells to change gene expression profiles in response to environmental stimuli without the alterations in genomic DNA sequence. These facts inspired my lab to explore where in genomes, epigenetic differences may occur to allow brain cells having different neural cell fates, and how do these epigenetic differences arise in cells during brain development. This talk will focus on our recent studies identifying epigenetic regulatory mechanisms that underlie brain cell specification and brain functions.

**PS-11**

New Technologies as Game-changers in Drug Development and Safety Sciences. THOMAS HARTUNG. Johns Hopkins Center for Alternative to Animal Testing (CAAT), Baltimore, MD and CAAT-Europe, University of Konstanz, GERMANY. Email: thartun1@jhu.edu

The areas of drug development and regulatory safety testing are seeing in part over-due changes in technologies. The reason is that we can obtain the needed information at the same quality or better from new means than traditional animal testing and cell culture. Increasingly, the limitations of animal-based toxicology to predict human health threats are recognized. Drug development becomes more and more aware of how much animal models misled product development. However, we also recognize more and more shortcomings of traditional (human) cell culture. These include cell identity, differentiation, genetic stability and mycoplasma infection as well as non-homeostatic and non-physiological culture conditions. The increasing pace of technological developments of modern cell culture and their integration leads to what is called “disruptive technologies”. The development of alternatives to traditional approaches for product development and safety assessment benefits from this. The creation of large toxicological databases (“big data”) and data-mining technologies (“artificial intelligence”) allow predictive computational approaches on a new scale. As an example, our new automated read-across (RASAR, i.e. read-across-based structure activity relationships) is given. At the same time, the combination of cell culture with bioengineering has led to a number of technologies to make cell culture more organo-typic, such as 3D culture, human stem cell-derived systems, perfusion, co-cultures, combinations with scaffolds and sensors etc.. Increasingly, they lead to “organ-on-chip” or even multi-organ “human-on-chip” solutions. By recreating organ architecture, homeostasis of the cell environment and organ functionality, these models mirror more closely the physiological situation. The example of our human iPSC-derived brain organoids is used to illustrate this. The commercial availability of organoids also improves standardization and reproducibility. Such technological advances promise to be real “game-changers”. Combined with an increased mechanistic base of reasoning (e.g. Adverse Outcome Pathway concepts), Integrated Testing Strategies and evidence-based methods of data evaluation and integration, a revolutionary change for how we assess the biological effects of substances has been set into motion.

**PS-12**

Regulations & Transparency: Implications for Plant-based Genome Editing. GARY A. BANNON. Benson Hill, Inc., 1001 N. Warson Rd., St. Louis, MO 63132. Email: gbannon@bensonhill.com

Crops grown today are the result of selection and improvements due to a variety of conventional breeding methods and genetic modifications. Regulatory agencies have applied a science-based risk assessment process to ensure food/feed and environmental safety via a pre-market assessment process. New plant varieties produced through conventional breeding techniques are considered safe by almost all global regulatory agencies and are not subject to the pre-market assessment process. This is due to their long history of safe use and use of the plant's own genetic diversity to produce improved varieties. Conversely, genetically modified crops (GMOs), defined as containing "foreign" DNA, are universally subject to regulation by global regulatory agencies prior to market introduction. Genome editing is a new breeding tool that can efficiently and rapidly develop improved plant varieties that, in many cases, produces a final product that is indistinguishable from what could be produced through conventional breeding techniques. Genome edited plants have forced regulatory agencies to apply their science-based risk assessment to determine if these varieties should be exempt from regulation or whether they should be subject to GMO regulations. Depending on geographic region, regulatory agencies have come to different conclusions regarding regulation of genome edited plants. Some countries have designated some genome edited plants as exempt from regulation while others have decided that they should be subject to the same pre-market assessment as applied to GMOs. The science behind these decisions and the implications of a non-harmonized global policy on transparency around international trade and public acceptance will be discussed.

**PS-13**

Regulation of Gene Editing in Livestock. ALISON L. VAN EENENNAAM. University of California, Davis, Davis, CA. Email: alvaneenennaam@ucdavis.edu

Risk assessments of genetically modified (GM) animals over the past 20 years have focused on characterizing the structure and expression of a heterologous gene(s) and their effect on the health of the animal, assessing the toxicological and potential allergenicity of the novel protein(s), as well as of the whole food derived from the GM animal; and evaluating whether food and feed derived from the GM animal is as nutritious to humans and/or animals as traditionally-bred animals. In relation to the food and feed risk assessment, the underlying assumption of this comparative approach is that traditionally-bred animals have a well-established history of safe use. These traditionally-bred animals serve as a baseline for the food and feed safety assessment of GM animals or their products and the welfare of the GM animals (EFSA Journal 2012;10:2501). It is less clear that this approach is appropriate when gene editing is used to knockout a gene, make single-base changes without any foreign DNA contribution, to add additional copies of an endogenous gene, or to convert one wild-type allele to another wild-type allele from the same species. These alterations are often identical to the DNA sequences present in traditionally-bred animals. Several jurisdictions categorically exempt deletions, single base pair substitutions, insertions from compatible relatives, and null segregants from GM regulatory oversight. However, others are requiring full GM risk assessments for these same alterations. The fact that such studies are not required or performed on animals developed through traditional breeding techniques makes this requirement disproportionate. Traditional animal breeding over the past century has an exceptional safety record. Products with the same level of risk should be treated equally irrespective of the process used to produce them. This unique regulatory burden will disincentivize the use of gene editing in livestock breeding programs, and favor less-efficient methods (e.g. introgression) to introduce useful genetic variation purely for their immunity to GM regulatory hurdles.