194 Zinc-finger nucleases: Innovations in custom-designed modification of the swine genome. J. J. Whyte*, J. Zhao, K. D. Wells, M. S. Samuel, K. M. Whitworth, E. M. Walters, M. H. Laughlin, and R. S. Prather, *University of Missouri, Columbia.*

Genetically modified swine hold great promise in the fields of medicine and agriculture. Pigs are similar to humans in anatomy and physiology and they reproduce rapidly to provide a reliable source of research animals. By combining emerging gene modification technologies with the completion of the swine genome, custom-designed pigs will provide urgently needed organs and therapeutic proteins for patients, realistic models of human disease, and high quality, efficiently produced food to meet the nutritional demands of the ever-expanding world population. Conventional gene targeting (adding foreign DNA via homologous recombination) is highly inefficient in mammalian somatic cells and provides little control over the site of transgene integration. The landscape of gene modification has recently changed with the use of zinc-finger nucleases (ZFNs) to enhance targeting efficiencies up to 10,000-fold. ZFNs consist of a nonspecific endonuclease domain and a sequence-specific zinc-finger DNA binding domain. Custom pairs of ZFNs heterodimerize upon binding DNA at predetermined gene loci to form a catalytically active nuclease complex. The resulting cleavage triggers DNA repair pathways that can be exploited to either disrupt gene coding or enhance insertion of exogenous DNA constructs. ZFNs have been used to genetically alter organisms such as plants, insects, zebra fish and rats. Recent publication of the first genetic modification in pigs by combining ZFN technology with somatic cell nuclear transfer has opened the door to genome targeting with a precision that was not previously possible in a large animal model. A preliminary report describing ZFN-based knockout of the α1,3-galactosyltransferase gene in porcine fibroblast cells raises the possibility of model pigs with selective knockout of endogenous genes without introducing any transgenic sequence into the genome. This presentation will provide an overview of ZFNs, emphasizing their potential to accelerate the production of genetically modified pigs of agricultural and biomedical importance. Current methods of ZFN design, important considerations for their safe and effective use in modification of the swine genome, and future innovative applications of this technology in pigs will be discussed.

195 Improved RNA quantitation and applications to animal science. C. D. Haudenschild*, *Illumina Inc., Hayward, CA.*

In the past few years we have seen numerous significant changes in the way we approach the study of organism development and the biology of diseases in model and non model organisms. What was mostly a gene-centric method that relied on following simple expression patterns is now largely a statistically based science. With the introduction of next generation sequencing methods to the study of gene expression, we can now rely on whole genome views of the transcriptome leading to ever increasing data volumes and compare large sample numbers. Due to the variety of information sources we can now interrogate, we are able to start appreciating the true complexity of cells and the significance of the variables involved. In the study of gene expression, one now considers temporal expression in conjunction with what particular alleles are transcribed in what tissue and which splice variants are predominant. The analysis of translation state also became available and can provide essential pertinent information. Analyzing whole genome sequences in conjunction with sequence based expression profiles on large segregating populations should also allow for better QTL characterizations. The data permits the enhancement of usability of newly assembled genome by better defining gene structure annotation and thus allowing for better comparison across closely related species. Numerous protocols are available and examples of applications will be described.

196 Informatics-driven biological research: Infectious diseases as an example. B. Sobral*, *Virginia Bioinformatics Institute at Virginia Tech, Blacksburg.*

Infectious disease researchers have to deal with diverse types of data to develop hypotheses about candidate macromolecules that can be used to design countermeasures (diagnostics, vaccines and therapeutics). Even considering only molecular data, it is a challenge to access all the public information available to them and implement workflows that support their analysis needs. I will use the example of *Brucella* spp. to illustrate how public, open, freely available resources can be designed, developed, and implemented in support of such infectious disease research and development goals. There are now 40 genomes of *Brucella* (Alphaproteobacteria; Rhizobiales) sequenced, sampling all known species and biovars of this facultative intracellular pathogen. A phylogenomic analysis of these genomes united all *Brucella* when they were compared with outgroups including *Ochrobactrum*, *Bartonella*, *Mesorhizobium* and *Agrobacterium*. Although the *Brucella* genomes are united, there is some interesting diversity. A well-studied group of *Brucella* species are united in a clade separated by a long branch. This large clade has little phylogenetic depth, but species within this group are known to have specific host preferences. Sub-branching patterns within this group reflect these host preferences and specific protein families unique to, and absent from, these groups were identified. Two novel strains, *Brucella inopinata* BO1T and BO2, were recently identified and isolated from human patients. Genomes from these strains, as well as 2 new isolates isolated from Australian rodents (*Brucella* spp. NF2653 and 83/13), are quite unique and separated from the rest of the *Brucella*. Although they share many similarities with the other *Brucella* species, they are missing many large areas of their genomes that can be seen in other *Brucella*. Analyses of these areas using new bioinformatic tools and data resources have shown that some of these missing areas include previously identified genomic islands and virulence factors, and there are novel findings as well that impact biochemical pathways and unique changes in the synthesis of lipopolysaccharide.