Session V-4: Replacement and Reduction in the use of genetically-engineered animals

Session V-4: Oral presentations

V-4-671

Gene supplementation and editing in livestock for biomedical and agricultural applications

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The conservation of gene-function and physiology between people and livestock species advocates for their utility in modeling human disease. Furthermore, agricultural animal production will play a pivotal role in providing high-quality protein to advance human health in the face of a burgeoning global population. Both the development of animal models and the improvement of livestock for food can be greatly facilitated by genetic engineering. Historically, genetic modification has largely been restricted to mice due to technological and logistical challenges. Recent developments have essentially eliminated the technological barriers to livestock genetic modification. Transposons provide an efficient, non-viral method for gene addition in livestock, without the use of an antibiotic resistance gene that could confound regulatory acceptance. The facile use of engineered nucleases to inactivate genes and to stimulate gene conversion in livestock will also be described. Finally, the loci to be engineered, and the species-dependent and geopolitical constraints on implementing genome engineering into animal production paradigms will be discussed.

V-4-109

Optimizing fluorescent protein choice for transgenic embryonic medaka models

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Transgenic medaka (*Oryzias latipes*) have proven to be pertinent models for a variety of ecotoxicological and research applications. Very early stages of aquatic vertebrates, just after hatching of the eggs, using non-feeding embryonic fish, represent an alternative non-compliant with the European regulatory definition of a laboratory animal. However, a number of technical issues needed to be solved, such as limiting the impact of autofluorescence at different embryonic stages, which could inhibit

the read out of biomarkers *in vivo*. To overcome this problem, we have determined the spectrum of emission wavelengths of different developmental stages of medaka submitted to a broad range of excitation wavelengths and various experimental conditions.

For each developmental stage tested, ten medaka embryos were individualized in a 384 well plate and each well was subjected to excitation wavelengths from 350-670 nm in 5 nm increments. For each excitation wavelength, emitted light was quantified from 20 nm above the excitation wavelength to 700 nm in 5 nm increments. The results show a fairly homogenous level of autofluorescence across the spectrum prior to hatching, with almost undetectable levels at 6 days post fertilization. After hatching, higher levels of autofluorescence were observed in specific regions of the spectrum from blue to red (375-700 nm) emission with emission wavelengths close to the excitation wavelength.

This information will allow selection of optimal reporter genetic constructs, providing high signal to noise ratio for the quantification of fluorescence. Furthermore, this will pave the way to combining multiple biomarkers with different fluorescent proteins to detect various signals within the same organism.

V-4-067

The importance of genetic background in mouse and rat models

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It is increasingly recognized that the genetic background (i.e. all genomic sequences other than the gene(s) of interest) can have profound influences on the phenotype of an animal model. It has been shown that mutations (spontaneous and induced), transgenes, and targeted alleles (knock-outs and knock-ins) that are "moved" onto a different background can show a change in phenotype. One of the first cases involved the classical diabetes (Leprdb) mutation that presented transient diabetes on a C57BL/6 background but overt diabetes on C57BLKS. In order to highlight the importance of this issue, I will present a selection of recently published articles showing the influence of genetic background on different mouse and rat models. I will also discuss some of the problems arising from the use of genetically engineered mice, like mixed backgrounds after breeding chimeras, the genetic variability among 129 sub-strains (ES cells), and the "flanking genes" concern. Finally, I will present different ways to avoid or resolve these drawbacks, including the development of congenic strains by marker-assisted backcrossing and the use of newly available ES cells from strains other than 129. In order to stay away from confounding or unreliable experimental results, particularly with the increasing number of mouse and rat strains, attention to the genetic background and genetic monitoring is crucial.

V-4-714

Breeding: a tool to improve genetically engineered mouse welfare

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The use of proper breeding schemes, appropriate genetic background and sound monitoring of mouse colonies contribute positively to reduction and refinement. The presentation gives a résumé of some of the best practice in mouse breeding and strategies to increase animal welfare.

Session V-4: Poster presentations

V-4-525

Reducing and refining animals used in transgenesis with the use of frozen embryos

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A key step in targeted transgenesis line creation is the production of injectable blastocysts for ES cell injections. To make fresh blastocysts available, most model creation labs must set up an in-house colony of breeder males and purchase breeding age females. This requires significant resources, including dedicated space and equipment, training, and time to provide basic animal care duties to the in-house colony. Also, with this approach, there can be variations in the quantity and quality of blastocysts. Even for experienced teams these variations are challenging to control. Additionally, a full colony is usually maintained for the purpose of producing fresh embryos.

Here we are reporting the development of a commercial product to provide frozen morulas, supported with technical recommendations and appropriate culture media (BlastoKit®, Charles River, Lyon, France) that allows reduction of the number of animals during model creation. After embryo thawing and overnight culture, the BlastoKit® allows researchers to produce injectable blastocysts, using less space and animals, with the benefits of standardisation.

Moreover, this technique allows centralization of embryo production and decreases animal needs for the same production level. In particular, only one colony of males is used more efficiently compared to local low-employed colonies. The final number of embryos produced per breeding male is higher. Through superovulation, this system reduces the risk of having to euthanize females with no embryo production and to thaw valuable ES cells clones without blastocysts ready to perform injections. By using frozen embryos, injection technicians can save time and resources and animals needed in embryo production. Also, by knowing in advance the number of injectable embryos, the transgenic facility team can prepare pseudopregnant females as recipients only when needed, and with a refined number, which again reduces unused female production.

Currently, germ line transmission using BlastoKit®-derived embryos is validated by a range of users in private and academic laboratories for both C57BL/6NCrl and BALB/cAnNCrl embryos. Along with highlighting the reduction of animals used, analysis of blastocyst development rate (at least 70% and 50% for C57BL/6NCrl and BALB/cAnNCrl embryos, respectively), microinjection efficiency, and birth rates (up to 40% for C57BL/6NCrl embryos) obtained during beta-testing will be presented and analysed at this conference.

V-4-587

Reduction of transgenic animal use by simultaneous assessment of *lacZ* and *Pig-a* mutations, micronuclei and DNA adducts in Muta™Mouse

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This study used the transgenic Muta™ Mouse to show that multiple genotoxicity endpoints can be measured in the same animals, thus reducing the use of genetically engineered animals. Muta™ Mouse were exposed to benzo[a]pyrene (BaP) daily for 28 days via oral gavage. Endpoints measured include DNA adducts, indicative of internal dose, micronuclei (MN), indicative of chromosome damage, and gene mutations. Mutations were measured at the lacZ transgene in several tissues, and at the *Pig-a* gene, evaluated as GPI-anchor deficient reticulocytes (RETs) and red blood cells (RBCs).

Dose-dependent increases in DNA adducts were observed in all tissues examined (liver > glandular stomach > small intestine > bone marrow). Dose-related increases in *lacZ* and *Pig-a* mutant frequency (MF) were also observed; doubling dose was the same for these two endpoints; however the *lacZ* MF in bone marrow was approximately 25x higher than that observed for *Pig-a* in RETs. This difference may be related to differences in target size and/or differences in the cell populations examined. Dose-related increases in % MN were also observed, and the doubling dose for % MN was approximately 2.5x higher than the mutation endpoints.

The results of this study demonstrate that measurements of mutation, chromosome damage and internal dose can easily be integrated into a 28-day mouse study. Matching *in vitro* analyses are currently comparing the kinetics observed here to those of cultured cells derived from Muta™ Mouse. The results will contribute to the use of cultured transgenic cells for quantitative hazard assessment, and to the validation of the *Pig-a* assay.