Abstract Book

Oral Presentations

Consideration of surrogate dam gut microbiome to improve animal model rigor and reproducibility

Professor James Amos-Landgraf[1], Craig Franklin[1], Aaron Ericsson[1]
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Session 10: Assisted Reproductive Technologies, Imperial East (Main Lecture Room), November 15, 2023, 11:00 - 23:40

During transgenic animal production, the scientific community has recognized that the host genetic background can be crucially important to the phenotype of the genetically modified animals. Recent work by our group and others has highlighted that not only the host genome but also the animal's microbiome can significantly contribute to the phenotype as well. The Mutant Mouse Resource and Research Center at the University of Missouri has now examined several well-characterized mouse models of human disease and found quantitative differences in model phenotypes in animals harboring different vendor-derived gut microbiomes (GMs). We have created CD-1 colonies that harbor the GMs of four major commercial vendors' Specific Pathogen Free (SPF) microbiomes that we use as surrogate dams for rederivation or transgenic mouse model production. While all of the resulting animals are SPF, the diversity and richness of the GMs in the animals vary significantly. Using 16s rRNA sequencing to characterize the GM revealed unique taxa in each of the GMs and significant differences of relative abundances of various shared taxa. Using predictive algorithms and untargeted mass spectroscopy-based metabolomics we identified dramatic differences in the metabolic capacity between the most diverse and complex microbiome and the least. We examined phenotypes in the IL10-/-, ApcMin mouse and ApcPirc rat models, as well as established models of behavior, and found significantly different phenotypes as determined by histology, quantitative tumor development, and behavior tests respectively in the various models. It may be beneficial for transgenic cores to inform clients about the source of the recipient dams to inform them about the GM. Additionally, if their animals will be used in crosses with other existing mutants, information about the commercial source of the animals can better inform the choosing of surrogates to more closely match GMs in their colony, leading to reproducible and less variable experiments.

Dual Flex Transgenic Systems: Paired Genetic Switches for Leakproof Conditional Transgenes and Intersection Biology

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Session 2: Precision Animal Models of Human Disease II: Tiered Talk, Imperial East (Main Lecture Room), November 13, 2023, 10:55 - 12:45

We developed a leakproof genetic switch for the purpose of providing tight control of gene expression in conditional On/Off, Off/On, or reversible transgenic alleles.

The Dual Flex system was developed as a solution to recurrent failures using traditional StopFlox (also called Lox-Stop-Lox) elements to regulate the Off/On expression of a potent transgenic oncogene. The problematic model was a conditional oncogenic fusion of CIC-DUX4 using a StopFlox targeted to the Rosa26 or endogenous Cic locus. In three attempts to generate the model, 100% (108/108) of chimeric animals carrying the targeted allele developed tumors in the absence of Cre-recombinase and died before sexual maturity. Using PCR to amplify and sequence across the StopFlox cassette coupled with immunohistochemistry, we confirmed spontaneous (Cre-independent) recombination resulting in CIC-DUX4 mis-expression and metastasis driven mortality. A similar Cre-independent loss of the StopFlox has been observed and reported by others (1).

The Dual Flex system uses two Flex elements, one Cre-based and one Flp-based, each designed to conditionally invert an anti-sense exon into the correct orientation with respect to the promoter and remainder of the CID-DUX4 oncogene. The Dual Flex genetic switch operates similar to multifactor authenticator (or dual key lock), requiring authentic recombination-mediated inversion of both anti-sense exons to activate synthesis of the proper coding transcript and expression of the oncogene. Therefore, this switch should not fail, and leaky expression of the oncogene by either simple deletion, or by accidental exposure to one of the recombinases, should not occur.

We will present the latest status and characterization the Dual Flex CID-DUX4 model under development. We will also explore possible causes of Cre-independent deletion of the StopFlox.

Reference:
1) Katerina Politi, Ana Kljuic, Matthias Szabolcs, Peter Fisher, Thomas Ludwig
and Argiris Efstratiadis ‘Designer’ tumors in mice. Oncogene 23, 1558–1565 (2004).

Efficient large knockins using rAAV donors in mouse embryos

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Session 9: Transgenic Resources, Imperial East (Main Lecture Room), November 15, 2023, 09:00 - 10:30

Large knockins in mouse embryos are usually a more challenging type of gene editing, compared to KO and introduction of SNPs and small tags, yet necessary to generate models for humanization of a mouse gene, gene overexpression or replacement, reporter lines and conditional knockins (flexing). Using recombinant Adeno-associated virus (rAAV) to deliver donor templates circumvents the need to microinject single cell embryos, when combined with electroporation of Cas9/gRNA ribonucleoprotein complexes. We report here successful creation of tens of mouse models using rAAV donors, overcoming the payload limit of rAAV by using multiple viral donors for sequential insertions in a single round targeting in the embryos, with the largest insertion so far approaching 10 kb. Additionally, we report the development of Nanopore long read sequencing for genotyping, eliminating the need for multiple sets of junction PCRs, to verify the accuracy of insertion and screen against random integration. We will also discuss the overall efficiencies and difficulties we have encountered along the way.

Long Read Sequencing reveals high frequency of bystander mutations following AAV-driven electroporation of CRISPR RNP complexes in mouse zygotes

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Session 3: Animal Models in Space Biology I, Imperial East (Main Lecture Room), November 13, 2023, 13:45 - 15:45

Over the last decade CRISPR gene editing has been successfully used to streamline the generation of animal models for biomedical purposes. However, one limitation of its use is the potential occurrence of on-target mutations that are detrimental or otherwise unintended. These bystander mutations are often undetected using conventional genotyping (i.e., PCR) and routine (i.e., Sanger) sequencing.

Recently, Long Read Sequencing (LRS) has been used in mice to identify the insertion site of randomly integrated transgenes, and to confirm integration following recombinase-mediated cassette exchange (RMCE). However, to the best of our knowledge LRS has not yet been used to perform quality control (QC) following AAV-driven gene editing in zygotes (referred to as “CRISPR-READI”), and the mechanisms by which AAVs release the single-stranded transgenes used as templates for homology directed repair (HDR) remain elusive.

To this end, we performed CRISPR-READI to generate three different types of integrations in two different murine genes (i.e., ACE2 and FOXG1). We generated and analysed these knock-ins (KI) using Oxford Nanopore Technologies (ONT) and identified instances of concatemerisation and partial backbone integration (particularly inverted terminal repeats: ITR sites) in two out of five (40%) lines generated.

Therefore, we recommend using LRS as a stringent QC for KI lines generated using CRISPR-READI (and potentially other methods). Long read sequencing is a powerful, reliable, fast and cost-effective method to assess the outcome of gene editing in animal models. We established a bioinformatics workflow to analyse these outcomes and deliver fully validated mouse lines.

Adaptive sampling long-read sequencing for characterization of CRISPR/Cas9-generated founder transgenic mice

Dr Zachary Freeman[1], Weisheng Wu[1], Laura Burger, Elizabeth Hughes[1], Thomas Saunders[1], Shipra Garg[1], Olivia Koues[1], Judith Meyers[1], Suzanne Moenter[1], Chris Gates[1]
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Session 3: Animal Models in Space Biology I, Imperial East (Main Lecture Room), November 13, 2023, 13:45 - 15:45

Complete characterization of CRISPR/Cas9-generated animal models presents many challenges that may require advanced sequencing methods. We previously used a long single stranded donor combined with a single gRNA strategy to generate an endogenous iCre knock in the Npvf gene in C57BL/6J mice. G0 founders were identified by PCR and Sanger sequencing spanning 5' and 3' ends of the insert including the arms of homology. After breeding to wild type, candidate G1 founders were identified using the same PCR strategy, but spanning PCR across the insert were unsuccessful at fully characterizing the entire allele. We used Oxford Nanopore Technology (ONT) long-read sequencing with Adaptive sampling for Chromosome 6 on an individual G1 founder to determine if the entire insert sequence and arms of homology were correct as designed. Reads were aligned to mouse reference genome GRCm38 using the wf-alignment pipeline with the minimap2 v2.24 to align reads. Structural variants (SV) were then called using Sniffles and CuteSV and consensus SVs were called using SURVIVAL v1.0.7. Adaptive sequencing resulted in 58X total coverage with 30X coverage (51.7% allele frequency) of the targeted allele. The insert sequence was identified in the SVs and aligned against the designed megamer sequence. All reads were then segregated into variant supporting and reference supporting reads, which were then assembled into separate consensus sequences using Flye v2.9.1. These two consensus sequences were then aligned individually to the megamer sequence with variant supporting reads matching the megamer sequence in the correctly targeted location. Furthermore, the junctional regions around the arms of homology were correct with no evidence of mutations. These data support the use of ONT adaptive sequencing for the characterization of CRISPR/Cas9-generated animal models.

Generation and comprehensive analysis of CRISPR-mediated NHEJ profiles in F0 founders

Jade Zhang[1], Ms Charleen Hunt[1], Michael Kelley[1], Michael Ceriello[1], Suzanne Hartford[1], Heather Brown[1], Jarrell Wiley[1], Clarissa Herman[1], Brittany Lee[1], Jessica Kuhnert[1], Thomas Kehrer[1], Marine Prissette[1], Virginia Hughes[1], Jennifer Schmahl[1], Nicolas Gale[1], William Poueymirou[1], Eric Chiao[1], Brian Zambrowicz[1], Guochun Gong[1]

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Session 3: Animal Models in Space Biology I, Imperial East (Main Lecture Room), November 13, 2023, 13:45 - 15:45

The delivery of CRISPR/Cas9 (CC9) nucleases to one-cell stage mouse embryos is a powerful approach for rapidly generating gene knockouts (KOs) bypassing the need to generate modified embryonic stem cells. One long-standing concern regarding CRISPR KOs is the generation of mosaic alleles in founder mice. Here, we describe our systematic approach to optimizing CRISPR non-homologous end joining (NHEJ) outcomes associated with high-throughput embryo electroporation. We sought to gain a better understanding of typical CRISPR-mediated NHEJ events by employing next generation sequencing technologies to thoroughly characterize KO allele sequences in eight adult F0 founder tissues with comparison to cognate P7 tail snips. We further characterized embryonic and adult F0 founder phenotypes relative to TaqMan and NGS genotypes to highlight the utility of F0 KO animals for rapid, go/no-go, decisions.

Modeling disease-associated variants in mice using prime editors

Dr Denise Grant Lanza[1], Matthew Gonzalez[1], Lan Liao[2], Angelina Gaspero[1], Dr John Seavitt[1], Center for Precision Medicine Models[3], Dr. Aleksandar Milosavljevic[1], Dr. Lindsay Burrage[1,4], Dr. Jason Heaney[1]

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Session 10: Assisted Reproductive Technologies, Imperial East (Main Lecture Room), November 15, 2023, 11:00 - 23:40

The Center for Precision Medicine Models leverages Baylor College of Medicine's genome discovery, informatics, and animal modeling programs to develop precision animal models that will end the diagnostic odyssey of patients with undiagnosed, rare, and Mendelian diseases and serve as resources for pre-clinical studies. However, modeling de novo disease-associated variants with traditional knock-in alleles has been challenging as undesired indel mutations are often generated in trans to a desired variant with biallelic editing causing embryonic or neonatal lethality of founder animals. Moreover, variants alone often cause disease-associated, early-onset phenotypes that lead to founder death prior to weaning. Consequently, time-consuming strategies, such as conditional variant knock-in alleles, are often pursued.

As an alternative, we utilized prime editing in one-cell stage mouse zygotes, employing both PE2 and PEmax editors and PE3 and PE3b approaches. We hypothesized that prime editing would (1) introduce precise genetic alterations in the absence of indel mutations, and (2) produce low-level germline mosaic founders that are viable, fertile, and can be bred to generate heterozygous N1 progeny. To date we have targeted 9 separate loci and successfully generated correctly targeted F0 mice (founders) for 6 knock-in alleles. Founder occurrence varied by locus and often were a small percentage of the total number of F0s genotyped (5-67%). Low-level mosaicism (allele contribution 10-40%) likely contributed to the survival of these founders compared to previous traditional knock-in attempts. Undesired mutations were less common (5-22%) amongst F0 animals and usually not observed in correctly targeted F0s. Of the 6 targeted loci, 4 founders have been bred to generate live-born progeny and 2 have been utilized to generate embryos for an N1 phenotyping screen. Ultimately, the use of prime editors has greatly facilitated model production of embryonic or neonatal lethal variants to produce a stable source of mice for phenotypic evaluation.

Efficiencies of Different Genetic Modification Techniques in Rat Embryos

Dr James McNew[1,2], Daniel Davis[1,3], Hongsheng Men[1,4], Elizabeth Bryda[1,2,3,4]
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Session 1: Precision Animal Models of Human Disease I, Imperial East (Main Lecture Room), November 13, 2023, 09:00 - 10:25

CRISPR-Cas9 technology has revolutionized our ability to create genetically modified animals. Many animal models require the need to insert (knock-in) preconstructed DNA templates called repair templates. DNA repair templates along with CRISPR-Cas9 reagents can be introduced into embryos by pronuclear injection (PNI), electroporation (EP), or delivery via adeno-associated virus with electroporation (AAV+EP). Currently, no published literature compares the efficiency of these delivery techniques as it relates to DNA insertions via CRISPR mediated genome editing in rats. We used a 400-base pair (bp) repair template consisting of homology arms flanking a floxed short artificial intron designed to target exon 2 of the Crh gene. Superovulated Sprague Dawley (SD) female rats mated to SD stud males were used to generate zygotes. Zygotes were randomly assigned into four groups: culture only control, PNI, EP, and AAV+EP. After manipulation, embryos were cultured to the blastocyst stage and submitted for Next Generation Sequencing (NGS) to detect evidence of genome editing. Embryo survival after one day in culture was significantly less following PNI, 58% (101/175), compared to the culture only control, 98% (109/111). Cleavage rates and development to a 4-cell stage did not differ between embryos that survived 24 hours in culture. Knock-in rates for manipulated embryos were 67% (12/18) for PNI, 0% (0/35) for EP, and 63% (22/35) for AAV+EP. We conclude that PNI decreases embryo survivability but not development, and that EP and AAV+EP do not decrease embryo survival or development. Using a 400 bp DNA repair template, we found knock-in rates were similar with PNI and AAV+EP while the template failed to be inserted into the genome with EP only.

A strategy for the efficient production of CRISPR interference mouse models

Dr. Leesa Sampson[1], Mrs. Jennifer Skelton[1], Mrs. Linda Gower[1], Ms. Katarzyna Jopek[1], Dr. Anna Osipovich[1,2], Dr. Lauren Woodard[1,3,4,5], Dr. Mark Magnuson[1,2,6]
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Session 9: Transgenic Resources, Imperial East (Main Lecture Room), November 15, 2023, 09:00 - 10:30

Introduction and Aims:
CRISPR interference (CRISPRi) is a promising alternative strategy to more conventional CRISPR knockout and Cre/loxP techniques. CRISPRi mouse models offer specific advantages, such as the ability to repress multiple genes in a single cross, more quantitative control of gene expression levels, and reversibility of gene repression. These advantages are likely to extend the utility of the mouse for studying complex, polygenic diseases like cancer, diabetes, and heart disease. However, there are currently few descriptions of the use of CRISPRi in mice and methods for the selection and genomic integration of small guide RNAs that reliably repress their targets when in complex with dCas9-KRAB require definition.

Methods, Results, and Conclusions:
We have developed a rapid and simple strategy for designing, generating, and validating CRISPRi mouse models that utilizes piggyBac transgenesis, commercially sourced reagents, and recently developed constitutive and inducible dCas9-KRAB alleles. Our protocols are easily workable in academic transgenic mouse core facilities capable of pronuclear microinjection. To demonstrate the feasibility of our strategy, we successfully generated seven CRISPRi models targeting five unique genes, resulting in gene repression levels ranging from 50% to 95%. As a proof of concept, we demonstrate the repression of glucokinase (Gck) by a dual sgRNA transposon.

FinnDisMice project: CRISPRing the Finnish Disease Heritage

Dr Satu Kuure[1], Dr Tomas Zarybnicky[1], Dr Sonja Lindfors[1], Dr Petra Sipilä[2], Dr Reetta Hinttala[3]
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Session 9: Transgenic Resources, Imperial East (Main Lecture Room), November 15, 2023, 09:00 - 10:30

Tackling rare diseases is recognized not only as a crucial aspect to pave the way for general improvement to diagnosis but also to facilitate understanding of similar groups of common diseases. Lack of preclinical models hinders identification of pathophysiological mechanisms of disease and thus development of effective disease modifying strategies. To improve this, we took the advantage of rare diseases enriched in Finland and successfully generated disease models that faithfully genocopy the disease-causing mutation in the mouse genome. Accordingly, nine new mouse models were generated representing rare human fetal/pediatric syndromes, pediatric-onset epilepsies, mitochondrial encephalomyopathy, an adult-onset motoneuron disease, and growth disorder with defective immunity. CRISRP/CAS9 genome editing with single stranded DNA templates was carried out in zygotes or 2-cell stage embryos either by microinjections or electroporation with varying efficiencies that depended on both strategy and genetic locus. The pathophysiological and cellular causes of the models are currently under active investigation. Highlights of our findings so far will be presented mainly focusing on lethal congenital contracture syndrome 1 (LCCS1), which is caused by pathogenic variant in GLE1 RNA transport mediator and in human leads to fetal death before the 32nd gestational week. Human LCCS1 fetuses are akinetic, show hydrops, arthrogryposis, pulmonary hypoplasia, and micrognathia with reported defects in motoneuron development. Phenotype characterization both at morphological and molecular level will be discussed. In generally, the results of our FinnDisMice studies will facilitate understanding of disease-causative pathomechanisms for these rare, incurable diseases. Importantly, the models we have generated are expected to serve as valuable preclinical validation instruments for potential new therapies.

Building a Better Mouse, One Base Pair at a Time

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[1], Department of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mt. Sinai, New York, United States

Session 4: ISTT Prize Lecture, Imperial East (Main Lecture Room), November 13, 2023, 16:30 - 17:30

My laboratory is very active in the field of mouse molecular genetics and has made major contributions to our understanding of how signaling pathways operate during embryonic development. In this ISTT Prize lecture, I will discuss my path over several decades that has taken me to develop techniques for building a better mouse. Our early work focused on the genetic analysis of Src family kinase genes, which showcased the effects of genetic redundancy within a mammalian multigene family and prompted the study of their involvement in growth factor and integrin signaling. We furthermore made major contributions in deciphering receptor tyrosine kinase signaling specificity in vivo, using null, conditional and intracellular effector docking sites mutations. Our work has provided evidence for individual signaling pathways in mediating specific developmental phenotypes as well as for integration of signaling pathways and prompted a reconsideration of cell signaling mechanisms in vivo. We have identified growth factor targets and downstream pathways using retroviral gene trap mutagenesis, proteomics or genomics to clarify mechanisms underlying receptor tyrosine kinase specificity. Along the way, a number of tools were developed, including some of the first gene targeting vectors, the βgeo reporter, the ROSA26 reporter lines that have facilitated recombinase mediated lineage tracing, and the codon-optimized Flpo and ΦC31o site directed recombinases. It has been a fun trajectory and hope you will enjoying coming along for the ride…

Random Insertion Transgenesis - A Fluorescence Based Selection Approach

Viktor Lang[1], ShiTing Misaki Hu[1], Juergen Klawatsch[1], Ms Tabitha Tombe[2], TienYin Yau[1], Dieter Fink[1, 3]
[1]Institute of Laboratory Animal Science, University of Veterinary Medicine Vienna, Vienna, Austria, [2]Vancouver Prostate Centre, Vancouver, Canada, [3]British Columbia Cancer Research Institute, Vancouver, Canada

Session 2: Precision Animal Models of Human Disease II: Tiered Talk, Imperial East (Main Lecture Room), November 13, 2023, 10:55 - 12:45

Random insertion transgenesis has been proven useful in the past besides its drawbacks regarding position effects, concatemer arrays, and non-controllable genomic rearrangements. Without direct readout of expression patterns, selecting the right founder animals expressing the gene of interest at constant levels, can prove challenging. To render this technique more controllable, we re-designed the lox-stop-lox (LSL) vector that now allows for removal of the fluorescent reporter, utilizing flp recombinase. This way, the mouse lines can be established, the marker removed, and the fluorescent channel is free for other applications. In-vivo imaging systems were used for quantification of fluorescence in founder animals and their offspring. Based on fluorescent levels and educated guess, we selected the respective animals for further breeding. This allows for segregation of non-linked multiple integration events and to establish the mouse lines. To further demonstrate the power of this technique, we selected low, medium, and high expressing transgenic lines. Within this work, we demonstrate a novel strategy to establish random insertion transgenic mouse lines that simplifies the comparison of transgene expression levels in living mice. Non-bred transgenic F1 animals might be cryopreserved (sperm and ovary) until the selected lines are established. Considering the 3Rs, we reduce the colony size, shorten the timeline necessary for colony establishment, altogether leading to lower animal numbers.

Advancing Translational Biomedical Research: Gene-Edited Rabbit Models at CAMTraST

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Session 3: Animal Models in Space Biology I, Imperial East (Main Lecture Room), November 13, 2023, 13:45 - 15:45

Rabbit models, characterized by their relatively larger size and extended lifespan, in comparison to rodent models, exhibit great potential in translational biomedical research. They often closely recapitulate disease pathologies observed in humans, including cardiovascular disorders, eye conditions, infectious diseases, and more. In July 2012, the University of Michigan launched the Center for Advanced Models for Translational Sciences and Therapeutics (CAMTraST) to bridge the gap between laboratory discoveries and clinical applications, and to facilitate the studies on the molecular mechanisms driving disease development and progression. Our team, consisting of specialists in molecular biology, embryology, and animal reproduction, has reached significant milestones. These accomplishments include the successful cloning of rabbits, and the creation of multiple rabbit models for human diseases through gene targeting.

CAMTraST operates as a non-profit resource dedicated to efficiently and cost-effectively producing and validating rabbit models for the broader national and international scientific community. Over the past decade, we've developed a highly efficient and cost-effective rabbit genome editing platform, resulting in the creation of over 40 knockout/knock-in rabbit models for various human diseases, predominantly utilizing CRISPR technologies. We also did rabbit whole-genome sequencing with PacBio method to provide a more continuous new assembly, UM_NZW_1.0. In the ISTT—TT2023 meeting, we will provide a comprehensive overview of the processes involved in generating and validating rabbit models. This encompasses reproductive technologies such as ovulation induction, zygote collection, and embryo transfer to pseudopregnant females in rabbits, a PacBio based whole genome sequencing of rabbits, along with considerations for rabbit genome editing technologies, including transgene construct design, donor DNA/vector synthesis, validation, microinjection, and genotyping. We will introduce several novel rabbit models that closely mimic patient symptoms, offering promising insights into unraveling the complexities of individual diseases and for pioneering innovative treatments.

Poster Presentations

P-021: A reverse genetic approach in geckos with the CRISPR/Cas9 system by oocyte microinjection

Dr Takaya Abe[1], Ms. Mari Kaneko[1], Dr. Hiroshi Kiyonari[1]
[1]RIKEN BDR, Kobe, Japan

Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Reptiles are important model organisms in developmental and evolutionary biology but are used less widely than other amniotes such as mice and chickens. One of the main reasons for this is that has proven difficult to conduct CRISPR/Cas9-mediated genome editing in many reptile species despite the widespread use of this technology in other taxa. Certain features of reptile reproductive systems make it difficult to access one-cell or early-stage zygotes, which impedes gene editing techniques. Recently, Rasys and colleagues reported a genome editing method using unfertilized oocyte microinjection that allowed them to produce genome-edited Anolis lizards. This method opened a new avenue to reverse genetics studies in reptiles. Here, we report the development of a related method for genome editing in the Madagascar ground gecko (Paroedura picta), in which we have established genome and transcriptome resources, and describe the generation of Tyr and Fgf10 gene-knockout geckos in the F0 generation.

P-080: Biallelic Variants in KMO Cause a Novel Form of Congenital NAD Deficiency

Ms Nathalie Aceves[1,2], Dr Chih-Wei Hsu[3], Dr Nanbing Li-Villareal[3], Mrs Xiaohui Li[2], Dr Seema Lalani[2,4], Ms Jill Rosenfeld[2], Ms Angelina Gaspero[2], Dr Denise Lanza[2], Dr Audrey Christiansen[3], Dr Tara Rasmussen[3], Dr John Seavitt[6], Dr Mary Dickinson[3], Dr Brendan Lee[2,4], Dr Ronit Marom[2,4], Undiagnosed Diseases Network, Dr Harmut Cuny[5], Dr Sally Dunwoodie[5], Baylor College of Medicine Center for Precision Medicine Models, Dr Jason Heaney[1,2], Dr Lindsay Burrage[1,2,4]
[1]Genetics and Genomics Graduate Program, Baylor College of Medicine, Houston, United States, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States, [3]Department of Integrative Physiology, Baylor College of Medicine, Houston, United States, [4]Texas Children's Hospital, Houston, United States, [5]Victor Chang Cardiac Research Institute, Darlinghurst NSW, Australia, [6]The Jackson Laboratory, Bar Harbor, United States

Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Genetic deficiencies of enzymes in the kynurenine pathway (KP), which synthesizes NAD+ from tryptophan, are associated with NAD deficiency, congenital anomalies, and miscarriages. To date, biallelic variants in three KP genes (KYNU, HAAO, NADSYN1) have been associated with congenital NAD deficiency disorder (CNDD). Through the Undiagnosed Diseases Network at BCM, we identified an individual with biallelic variants in kynurenine 3-monooxygenase (KMO), which encodes a KP enzyme. The patient has short stature, congenital anomalies, elevated levels of upstream metabolites, and low levels of NAD. We hypothesize that KMO deficiency is a novel form of CNDD and increases the risk for congenital anomalies. To test this, we generated a knockout mouse model (Kmo-/-) and confirmed the deletion on an RNA and protein level. The Kmo-/- mice are viable and fertile on breeder chow and they have significantly elevated serum kynurenine levels as compared to wild-type littermates. However, when placed on a niacin-free diet, the Kmo-/- mice, but not Kmo+/+ littermates, fail to thrive. This demonstrates that Kmo-/- mice cannot synthesize NAD+ from tryptophan and rely on NAD+ synthesized from niacin through the Preiss-Handler and salvage pathways. Moreover, a similar gene x environment interaction was observed during embryogenesis as Kmo-/- embryos from Kmo-/- dams fed a low niacin diet have shorter ulna, vertebral anomalies, and a higher proportion of external congenital anomalies as compared to Kmo+/- littermates. Micro-computed tomography imaging analysis is in progress to evaluate the embryos for additional abnormalities. Overall, these Kmo-/- embryos have phenotypes that are reminiscent of human CNDD: shortened long bones, vertebral anomalies, and other congenital anomalies. Studies are underway to test whether all phenotypes are preventable with niacin supplementation. Our work will provide insights into a novel form of CNDD and provide data supporting the roles of NAD and gene/environment interactions during embryonic development.

P-066: Evolving the analysis pipeline for CRISPR/Cas9 mediated, embryonic stem cell derived, mouse models

Dr Alasdair Allan[1], Dr James Cleak[1], Miss Ellen Hyde[1], Mr Akash Mukhopadhyay[1], Mr Connor Macfarlane[1], Mr Krystian Nowicki[1], Miss Hannah Dobbs[1], Mr Christy Greenwood[1], Mrs Sue Varley[1], Mr Jorik Loeffler[1], Mr Matthew Mackenzie[1], Dr Rosie K. A. Bunton-Stasyshyn[1], Dr Gemma F. Codner[1], Dr Lydia Teboul[1]
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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

The advance of transgenic techniques is revolutionising the modelling of human genetic diseases with ever greater complexity. The Mary Lyon Centre at MRC Harwell is at the forefront of generating mouse models with which to study human genetic diseases for both the UK and global research community. As part of our production pipeline, we have a laboratory dedicated to the production of materials for complex alleles via the embryonic stem (ES) cell to mouse conversion route. In order to increase efficiencies, we have been implementing traditional gene targeting vector electroporations with the aid of CRISPR/Cas9 technology to cut the genome at the desired integration site. This approach has been shown to enhance the incorporation of large and/or complex genetic modifications into ES cells in comparison to traditional electroporation techniques.
The increasing complexity of our models in turn requires an increasing complexity towards determining the accuracy of modified alleles. Here we will present our current quality control pipeline for the analysis of CRISPR-mediated ES cell lines using a range of traditional and contemporary methods. This pipeline is designed to quickly eliminate clones that are not of biological interest while also confirming, in as robust a manner as is practicable, the genetic sequence of both the targeted locus and the surrounding sequence both in ES cells and the subsequent mouse line.

P-083: Consideration of surrogate dam gut microbiome to improve animal model rigor and reproducibility

Professor James Amos-Landgraf

Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

During transgenic animal production, the scientific community has recognized that the host genetic background can be crucially important to the phenotype of the genetically modified animals. Recent work by our group and others has highlighted that not only the host genome but also the animal's microbiome can significantly contribute to the phenotype as well. The Mutant Mouse Resource and Research Center at the University of Missouri has now examined several well-characterized mouse models of human disease and found quantitative differences in model phenotypes in animals harboring different vendor-derived gut microbiomes (GMs). We have created CD-1 colonies that harbor the GMs of four major commercial vendors' Specific Pathogen Free (SPF) microbiomes that we use as surrogate dams for rederivation or transgenic mouse model production. While all of the resulting animals are SPF, the diversity and richness of the GMs in the animals vary significantly. Using 16s rRNA sequencing to characterize the GM revealed unique taxa in each of the GMs and significant differences of relative abundances of various shared taxa. Using predictive algorithms and untargeted mass spectroscopy-based metabolomics we identified dramatic differences in the metabolic capacity between the most diverse and complex microbiome and the least. We examined phenotypes in the IL10-/-, ApcMin mouse and ApcPirc rat models, as well as established models of behavior, and found significantly different phenotypes as determined by histology, quantitative tumor development, and behavior tests respectively in the various models. It may be beneficial for transgenic cores to inform clients about the source of the recipient dams to inform them about the GM. Additionally, if their animals will be used in crosses with other existing mutants, information about the commercial source of the animals can better inform the choosing of surrogates to more closely match GMs in their colony, leading to reproducible and less variable experiments.

P-009: The NCI Mouse Repository: Cancer models and miRNA-ES cell resource

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The NCI Mouse Repository, located at the Frederick National Laboratory for Cancer Research (FNLCR), Frederick, Maryland, is an NCI-funded resource of approximately 160 genetically-engineered mouse cancer models and associated strains, including mice bearing conditional and point-mutant alleles in cancer-related genes. In addition, the Repository houses a unique collection of over 1500 different mouse ES cell clones bearing conditionally-activated miRNA transgenes to facilitate in vivo exploration of miRNA functions. The NCI Mouse Repository's mouse strains and mESCs are available to all members of the scientific community (academic, non-profit, and commercial). The mouse models and ES cell clones are cryo-archived and distributed as frozen germplasm or cells.

Requests may be placed through the NCI Mouse Repository website (https://ncifrederick.cancer.gov/Lasp/MouseRepository/Default.aspx). In addition to the request form, this website includes detailed descriptions for each strain accepted into the Repository and the associated publications provided by the donating scientist. The miR-harboring ESCs, originally generated at the Cold Spring Harbor Laboratories for the NCI, are described in full detail and include validation data for each miRNA ES cell clone. These resources are available for nominal cost to NCI, NIH, and other US government-funded investigators, as well as to Investigators at non-profit organizations.

P-058: Is the custom creation of GMO mouse models using the CRISPR-Cas9 system possible today?

Mr Thomas Bagarre[1], Mme Naïs PHILIPPE[1], Mme Corine ARNOUX[1], Mme Mireille RICHELME[1], Mme Cécile GARCIA[1], Mme Diane LALAINA[1], Mr Fabien ANGELIS[2], Mr Aziz ADDA-BENATIA[2], Mr Yannis BERKANE[2], Mr Robin-Nicolas LACOMBE[2], Mr Elyes TAVERNIER[2], Mr Frédéric FIORE[3]
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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Since 2012, a new technological tool has appeared in the transgenesis market: the CRISPR-Cas9 system. In the context of providing the creation of genetically modified mice, it is now classically used on the C57BL/6 genetic background. Despite everything, we occasionally encounter drops in performance when obtaining certain models. This is observed more frequently when it comes to a less commonly exploited genetic background.

Depending on the type of genetic modification to be made (e.g. Knock-Out, Knock-In of a point mutation, conditional Knock-Out, large Knock-In) and the targeted locus, different strategic choices are proposed, which directly influence the rate of success and the effectiveness of the means implemented. In this poster, we will present a quantitative comparative study of our work carried out in a service provision context. We will evaluate the impacts of the choice of technique (microinjection or electroporation) and the type of embryos (from natural mating or cryopreserved) used on different genetic backgrounds (BALBc/J, NOD, C57BL/6N and C57BL/ 6J). We will list in our materials and methods the equipment, the reagents, the concentrations of the ctRNP complex as well as the animals that we use, which are all modifiable variables that have a major impact. This assessment now allows us to optimize our strategies by adapting our techniques and protocols according to the requested model.

P-072: A novel 61 base-pair intronic deletion of Snrpb is crucial for Snrpb regulation and normal development in mouse.

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Mutations in a common core spliceosomal factor called SNRPB causes cerebrocostomandibular syndrome (CCMS). Most CCMS patients have point mutations that increase levels of transcripts containing a pre-termination codon (PTC) containing alternative exon 2 (AE2). Herein, we generated a mouse line with a 61-base pair intronic deletion upstream of AE2 (Δ61) and show that 10% of heterozygous and homozygous embryos (Snrpb Δ61/+; Snrpb Δ61/Δ61) had abnormalities similar to those found in CCMS patients. Δ61 mutants had microcephaly, defects in the bones of the craniofacial region and ribs and 18/47 die from 4 weeks of age onwards. These embryos also had a significant increase in expression of the AE2 and a reduction in Snrpb levels. In parallel, we generated a conditional mutant mouse carrying loxp sequences flanking exons 2–3 of Snrpb. We used mesoderm-specific Mesp1-Cre to delete Snrpb, and showed that a fraction (5/40) of heterozygous Snrpb loxp/+; Mesp1-Cre+/- embryos are abnormal at E9.5. They have a narrow frontonasal prominence, a smaller 2nd pharyngeal arch, an enlarged heart, and begin to die at E12.5 where 50% are found alive. In these mutants, Snrpb expression was not changed whereas expression of the AE2 was half of controls, suggesting a potential compensatory increase of the Snrpb wild-type allele. To test if the Δ61 mutation fails to complement Mesp1-Cre mediated deletion of Snrpb, we generated Snrpb loxp/Δ61; Mesp1-Cre+/- mutant embryos. At E9.5, all of the recovered double heterozygous embryos showed an unlooped heart, misshapen somites and failed to turn. These embryos had a significant reduction in Snrpb levels without a change in AE2 expression. Our findings suggest that the 61-bp intronic region regulates AE2 inclusion and plays an important role in Snrpb regulation. Thus, these sequences should be investigated in CCMS patients that do not carry mutations in SNRPB coding exons.

P-057: Innovative mouse models by replacement or insertion of large genomic fragments (ex humanization)

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The CRISMERE technology allows to manipulate big genomic fragment to recapitulate human phenotype in rodents. We have successfully obtained deletion, duplication and inversion of big genomic fragments and generated Down, 16p11.2, 17q21.31 and Klinefelter syndromes models (Birling et al., 2017; Schaeffer et al., 2023). With a similar approach we have also managed to remove the human Chr21 non-syntenic region present on the additional minichromosome in Ts65 mouse model and obtained the Ts66 mouse line (Duchon et al., 2022).

Gene humanization by replacement or site-specific insertion is something that is much less efficient when long donor DNA and CRISPR reagents are provided directly in fertilized eggs. We show here that using BAC donor DNA, CRISPR/Cas9 and embryonic stem cells; it is possible to replace or insert very efficiently at specific location whole genomic fragment (ex: humanization of a gene). The BAC can be previously engineered by addition of LoxP (cKO) or a Knock-in (punctual mutation or addition of a reporter). We have managed to humanize whole genes or larger genomic regions (up to 200 kb) in C57BL/6N ES cells with high success rates. Recombination frequency was higher when 5 kb homology arms were used but ssODNs (with 100 nts homologies; in replacement of the homology arms) were also surprisingly efficient. After carefully validating each ESC clones, we have obtained a number of humanized mouse lines and confirmed by RT-qPCR that complete humanization of a gene by exchange of genomic fragment (genomic replacement) is effective. This approach greatly simplifies breeding steps by delivering murine gene knockout and humanization in the same animal (3Rs).

P-017: Knock-in genome engineering at novel genomic safe harbor sites in pluripotent stem cell & animal models

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Knock-in genome engineering refers to the process of precise and targeted insertion of DNA fragments or payloads and is a powerful reverse genetics tool for manipulating the genomes of human pluripotent stem cells and biomedical animal models. A handful of genomic safe harbor (GSH) sites are widely used for the integration of large DNA fragments, often several to tens of kilobases in size, that may contain regulatory elements such as enhancers, promoters or synthetic transgene circuits, or may contain genetic modifications that recreate a disease phenotype. Overall, the choice of GSH site for generating cellular and animal models will depend on the experimental criteria, but to qualify as an authentic safe harbor, the integration site should yield minimal disruption to the neighboring and endogenous gene expression patterns; and importantly, any disruption should not lead to genotoxic stress, abnormal cellular functions, developmental abnormalities, and disease. The most common GSH include: ROSA26, AAVS1, COL1A1, H11, and CCR5. Recently, Aznauryan et al, 2022 discovered two novel regions of gene insertion, eponymously named Rogi1 and Rogi2, that are GSH on human chromosomes 1 and 3, respectively. Using bioinformatics, we discover and map new Rogi1 orthologous GSH sites in both mice and swine. Additionally, we develop genome engineering reagents such as CRISPR-Cas9 high-performing sgRNAs and PASTEv3 editing with integrases Bxb1 and Bacillus cereus (BceINTa) suitable for the insertion of the large DNA payloads at Rogi1. Using pluripotent stem cells from mice including embryonic stem (ES) and iPS cells, and porcine expanded potential stem cells (EPSCs) we demonstrate high-level and ubiquitous expression at Rogi1 safe harbor using a CAG promoter driven StayGold. We have microinjected the StayGold genetic reporter into both mouse and pig zygotes to monitor transgene expression in whole tissues and validate Rogi1 as a GSH in both small and large biomedical animal models.

P-010: Rat Resource and Research Center

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

The NIH-funded Rat Resource and Research Center (RRRC) serves as a centralized repository for maintaining/distributing rat models and providing rat-related services to the biomedical community. Currently, the RRRC has over 575 rat lines; all are archived by cryopreservation to ensure against future loss. The RRRC distributes live animals, cryopreserved sperm/embryos and rat embryonic stem (ES) cell lines. Quality control measures for all materials include extensive genetic validation and health monitoring. The RRRC has expertise in rat reproductive biology, colony management, health monitoring, genetic assay development/optimization, isolation of germline competent ES cell lines from transgenic rats and can partner as consultants/collaborators. Fee-for-service capabilities include a wide variety of genetic analyses, strain rederivation and cryopreservation, isolation of rat tissues, microbiota analysis and characterization of genetically engineered rats. The RRRC, in conjunction with the MU Animal Modeling Core, makes genetically engineered rat models from start to finish using a variety of state-of-the-art technologies including genome editing (e.g., CRISPR/Cas9) as well as traditional methods such as random transgenesis and modified embryonic stem cell microinjection into blastocysts. Our website (www.rrrc.us) allows user-friendly navigation. Current research efforts include generation and characterization of a variety of new rat models and improvements to rat in vitro fertilization. The University of Missouri is home to the NIH-funded MU Mutant Mouse Resource and Research Center (MMRRC) and the National Swine Resource and Research Center (NSRRC) as well as the MU Animal Modeling Core and MU Metagenomics Center. Together, these highly collaborative groups provide a variety of animal model-related services across species to facilitate biomedical research. Funding: NIH 5P40 OD01106.

P-077: Addition of LHRH and examination of route of gonadotropin injection in the PMSG/hCG superovulation regime to increase fertilization and ovulation rates in mice and rats

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Injection of exogenous gonadotropins to induce superovulation is widely used in several mammalian species. Intraperitoneal (i.p). injection of Pregnant Mare Serum Gonadotropin (PMSG), to promote follicular growth, is followed by i.p. injection of human Chorionic Gonadotropin (hCG), to induce ovulation. This PMSG/hCG superovulation model, first developed in prepubertal mammals, works well in juvenile mice (3-5 weeks) and rats (4-5 weeks), but less well in older, cycling animals. Luteinizing hormone releasing hormone (LHRH) has been used in cycling mammals to desensitize the pituitary, suppress endogenous gonadotropins and induce cycle synchronicity upon withdrawal; and in anestrous animals to initiate pituitary gonadotropin release and induce ovulation.
This studies objective was to utilize LHRH injection to control the estrous cycle allowing for synchronization of injected gonadotropins with endogenous hormones, thereby increasing follicular development and ovulation. Ovulation and fertilization rates were determined at 0.5dpc and after overnight culture, in mature C57BL/6N (B6) mice and immature Sprague-Dawley (SD) rats, after administration of a standard PMSG/hCG superovulation protocol, with or without prior LHRH injection. We also compared i.p. vs subcutaneous (s.c.) injection of LHRH and PMSG, based on the premise that slower absorption through s.c. administration would closer mimic the sustained release of gonadotropin releasing hormone (GnRH) and follicle stimulating hormone (FSH) in vivo, while faster absorption via i.p injection would mimic the in vivo LH surge.
We have shown that s.c. PMSG injection increases ovulation rate in prepubertal B6 mice and that LHRH injection improves PMSG/hCG superovulation in adult B6 mice and immature SD rats. These refinements to standard superovulation regimes should reduce the number of mice and rats sacrificed to generate embryos, and eliminate the wastage of surplus or unresponsive mice by allowing them to be reused and superovulated as adults. This should significantly reduce animal usage in transgenic and related fields.

P-078: Production of genetically modified mice through microinjection of eggs from vitro fertilization

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Microinjection technology of embryos at pronuclear stage combined with various gene constructs is a well-established methodology for generating genetically modified animals. The conventional approach involves sourcing pronuclear stage embryos through the natural mating of superovulated female mice with their male counterparts. Subsequent microinjection introduces the genetically modified material, followed by the transfer of viable embryos to the oviduct, facilitating their successful development into viable mice. The conclusive phase entails genetic analysis to discern and validate the founder(s) harboring the intended modifications.
In recent years, advances in genetic research and the creation of immune-humanized animal models have necessitated the production of genetically modified mice within precise inbred strains. However, procuring embryos from these strains via natural breeding mandates dedicated housing for individual stud mice. Furthermore, suboptimal mating efficacy among male mice often yields a limited embryo count for subsequent microinjection procedures. In contrast, in vitro fertilization (IVF) techniques, conventionally employed to harvest embryos at the 2-cell stage for cryopreservation purposes, present an avenue to obtain ample fertilized eggs without reliance on live studs. This study exploits the potential of IVF-derived pronuclear stage eggs for microinjection procedures, effectively engendering genetically modified mice across strains such as C57BL/6JNarl, NOD.CB17-Prkdcscid/JNarl and other strains. An assortment of constructs, encompassing plasmids, BAC DNA, lsDNA, ssODN, Cas9 RNA/RNP, and mRNA, were also assessed. These constructs, formulated for diverse objectives and constituents, consistently yielded stable generation of genetically modified mice utilizing IVF-derived embryos. This investigation showcases the successful utilization of IVF-derived pronuclear stage eggs for microinjection procedures, facilitating the consistent production of genetically modified mice across various strains. The comprehensive assessment of diverse constructs underscores the versatility and reliability of this approach in generating stable genetic modifications.

P-025: Humanizing the NSG mice for adopting human blood cells and producing specific inhibitory antibodies against blood clotting factors

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Background
Humanized mouse models by transplanting human peripheral mononuclear cells (PBMCs) into NSG mice are valuable tools for they can provide an opportunity to study the mechanism(s) contributing to the eradication of the inhibitory antibodies in autoimmune or alloimmune diseases. However, these PBMC-engrafted mice typically die within 1-2 months due to graft-versus-host disease (GvHD).

Aims
This research aims to modify the hemophilia A (HA)-NSG mice to minimize GvHD and to effectively harbor anti-factor VIII (FVIII) antibody-producing immune cells for long term.

Methods
The NSG-HA mice we have generated by the CRISPR technology (Yen et at 2016, Thrombosis J 2016) were further modified to knockout mouse MHC class I (H2-K1, H2-D1) and class II (H2-IA) genes. Two guide RNAs (sgRNAs) are designed at the 5' and 3' end of each target gene respectively. Inhibitor-positive HA patients' PBMCs (3 x 106/mouse) were transplanted into the spleen of mice. Engraftment efficiency was examined by flow cytometry. Inhibitory antibodies were analyzed by neutralization assays.

Results
The novel NSG-KDIA(null)-HA mice were generated by three rounds of CRISPR/Cas9 technology. Human CD45+ cells (white blood cells) can be detected in the periphery of mice 2 weeks after engraftment into the NSG-KDIA(null)-HA mice and persist for > 20 weeks. The mean hCD45+ cells reached 17.41% at week 12. The spleen and bone marrow of the highest recipient mice were 87.8% and 32.2%, with 3.31% and 3.68% of B cells. Mean FVIII-inhibitor titers are 1.93 BU (range 0.22-9.68 BU, week 6).

Conclusion
We have established a novel NSG-HA mouse model that can harbor HA patients' PBMCs for long term without showing GvHD phenotype and can produce a significant amount of functional human antibodies.

P-056: Optimization of superovulation and zygote electroporation to edit immunodeficient NSG mouse genome

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Background

Immunodeficient NSG mice are commonly used in xenograft transplantation and humanization. Genetic modification of the NSG mice can create better xenograft models to study human diseases by improving the success rates of human cell transplantation.

Aims

This study aims to develop a protocol for efficient genome modification in NSG mice.

Methods

We tested various amounts of a superovulation reagent called CARD HyperOva® in female mice under 8 weeks (wks) of age. The gene-modified NSG mice were generated by the combination of in vitro fertilization (IVF) and the CRISPR/Cas9 technology. We used IVF to gain sufficient numbers of NSG mouse embryos for genetic manipulations. Several electroporation conditions were tested with mixtures of Cas9 protein and sgRNA into the embryos.

Results

We found that when a full dose Hyper-OVA was used, even old females (>24 wks) could give an average 22 oocytes per mouse (n=26), medium age females (8~24 wks) gave 51 oocytes (n=152) and young females (<8 wks) gave more than 3 times as many oocytes (75 oocytes/mouse, n=18) as that of the old females. Given Hyper-OVA plus PMSG at a 1:1 ratio, the number of oocytes recovered were 35~38 per mouse at either young or medium age. In terms of electroporation, we optimized several electroporation conditions for introducing sgRNA into fertilized eggs and found that the condition of 2 pulses of 1 ms at 27V with a 1000 ms interval enabled CRISPR/Cas9-based genome editing with high efficiency (37.5~50%, n=16) and high survival rate (46~50%, n=16). The birth rate of electroporated NSG embryos (n=1,693) was 24.5%, which was comparable to that of un-electroporated ones (n=1,650, 23.6%).

Conclusion

We established a novel procedure to efficiently modify the NSG mice by optimizing the superovulation protocol, IVF, and electroporation conditions to introduce the Cas9/sgRNA into zygotes.

P-003: Efficient large knockins using rAAV donors in mouse embryos

Dr Xiaoxia Cui

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Large knockins in mouse embryos are usually a more challenging type of gene editing, compared to KO and introduction of SNPs and small tags, yet necessary to generate models for humanization of a mouse gene, gene overexpression or replacement, reporter lines and conditional knockins (flexing). Using recombinant Adeno-associated virus (rAAV) to deliver donor templates circumvents the need to microinject single cell embryos, when combined with electroporation of Cas9/gRNA ribonucleoprotein complexes. We report here successful creation of tens of mouse models using rAAV donors, overcoming the payload limit of rAAV by using multiple viral donors for sequential insertions in a single round targeting in the embryos, with the largest insertion so far approaching 10 kb. Additionally, we report the development of Nanopore long read sequencing for genotyping, eliminating the need for multiple sets of junction PCRs, to verify the accuracy of insertion and screen against random integration. We will also discuss the overall efficiencies and difficulties we have encountered along the way.

P-060: Optimization of Prime Editing approach for reversion of mouse albino phenotype.

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

The discovery of programmable nucleases, in particular the CRISPR system, has revolutionized the field of genome modification. Versatile and efficient, this tool offers the possibility to precisely modify in vivo any potential locus thanks to the delivery of various exogenous genetic components. Since its implementation in gene-edited mouse model generation, there has been a constant effort to build an even easier, faster, and highly efficient system by developing new innovative CRISPR-based techniques. For instance, Prime Editing (PE) has recently emerged as a promising technique to induce small genetic changes without need of double-stranded DNA breaks nor donor DNA. Ex vivo zygote electroporation, for its part, represents a very effective delivery method of CRISPR/Cas9 components into mouse zygotes. Thus, we decided to take advantage of these new technologies to broaden our pipelines for mouse model generation within our transgenesis platform. First, we successfully managed to induce precise substitutions into the tyrosinase gene via mouse zygote microinjection of PE system under the form of RNA molecules. This technique corrected the mutation at the origin of mouse albino phenotype with good efficiencies and reduced off-target effects compared to strategies based on Homology-Directed Repair (HDR) pathway often accompanied by undesired mutations triggered by Non-Homologous-End-Joining (NHEJ) mechanism. Encouraged by these results, we then generated Tyr-edited mouse embryos by zygote electroporation delivery of PE system under the form of ribonucleoprotein complexes reaching up to 40% efficiency. Finally, we are currently refining the PE system by optimizing each component. These improvements take advantage of strategies like, for example, introduction of additional silent mutations into PE templates or use of in vitro optimized Prime Editors and PE template RNA molecules.

P-067: Increasing knock-in efficiency in mouse zygotes by transient hypothermia

Dr. Amine Bouchareb[1], Mr Daniel Biggs[1], Dr Samy Alghadban[1], Mr Chris Preece[1], Dr Benjamin Davies[1,2]

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Integration of a point mutation to correct or precisely edit a gene requires the repair of the CRISPR-Cas9 induced double-strand break by homology directed repair (HDR). This repair pathway is enhanced in late S and G2 phases of the cell cycle, whereas the competing pathway of non-homologous end joining (NHEJ) is present throughout the cell cycle. Modulation of the cell cycle by chemical perturbation or simply by the timing of gene editing has been shown to increase the rate of HDR. One simple protocol modification that has shown promise in stem cells is to modulate the cell cycle via a transient hypothermia.

Using a traffic light reporter in mouse embryonic stem cells and a fluorescence conversion reporter in human induced pluripotent stem cells, we confirm that a transient cold shock leads to an increase in the rate of HDR, with a corresponding decrease in the rate of NHEJ repair. We then investigated whether a similar cold shock could lead to an increase in the rate of HDR in the mouse embryo.

By analysing the efficiency of gene editing using 5 different sgRNAs targeting 3 different genetic loci, we found that a transient reduction in temperature after zygote electroporation of CRISPR/Cas9 RNP with an ssODN repair template did indeed increase knock-in efficiency, without affecting embryonic development. The efficiency of gene editing with and without the cold shock was first assessed by genotyping blastocysts. As a proof of concept, we then confirmed that the modified embryo culture conditions were compatible with live births by targeting the coat colour gene Tyrosinase and observing the repair of the albino mutation. Taken together, our data suggest that a transient cold shock could offer a simple and robust way to improve knock-in outcomes in both stem cells and zygotes.

P-028: Long Read Sequencing reveals high frequency of bystander mutations following AAV-driven electroporation of CRISPR RNP complexes in mouse zygotes

Dr Fabien Delerue

Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Over the last decade CRISPR gene editing has been successfully used to streamline the generation of animal models for biomedical purposes. However, one limitation of its use is the potential occurrence of on-target mutations that are detrimental or otherwise unintended. These bystander mutations are often undetected using conventional genotyping (i.e., PCR) and routine (i.e., Sanger) sequencing.
Recently, Long Read Sequencing (LRS) has been used in mice to identify the insertion site of randomly integrated transgenes, and to confirm integration following recombinase-mediated cassette exchange (RMCE). However, to the best of our knowledge LRS has not yet been used to perform quality control (QC) following AAV-driven gene editing in zygotes (referred to as “CRISPR-READI”), and the mechanisms by which AAVs release the single-stranded transgenes used as templates for homology directed repair (HDR) remain elusive.
To this end, we performed CRISPR-READI to generate three different types of integrations in two different murine genes (i.e., ACE2 and FOXG1). We generated and analysed these knock-ins (KI) using Oxford Nanopore Technologies (ONT) and identified instances of concatemerisation and partial backbone integration (particularly inverted terminal repeats: ITR sites) in two out of five (40%) lines generated.
Therefore, we recommend using LRS as a stringent QC for KI lines generated using CRISPR-READI (and potentially other methods). Long read sequencing is a powerful, reliable, fast and cost-effective method to assess the outcome of gene editing in animal models. We established a bioinformatics workflow to analyse these outcomes and deliver fully validated mouse lines.

P-016: Successful immortalization of mouse embryonic fibroblasts from C57BL/6J using two strategies

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Mouse embryonic fibroblasts (MEFs) have been widely used in biological research, such as, as feeder cells to support growth of many embryonic stem cells or as an effective system to test gene function due to their easy accessibility and rapid growth rates. However, it is time-consuming to prepare primary MEFs and their proliferation in vitro is limited by replicative senescence. In this study, we aimed to establish immortalized MEFs (iMEFs) originating from C57BL/6J mice, which is the most commonly used inbred strain for generation of genetically modified mouse models. MEFs were prepared from Day 14.5 embryos and cultured in a humidified 5% CO_2 atmosphere at 37 °C. Two strategies were applied to immortalize MEFs, including serial passaging for spontaneous immortalization (Strategy I) and transformation of primary MEFs by overexpression of SV40 Large T antigen (Tag) (Strategy II). In Strategy I, primary MEFs were cultured at 50% to 90% confluency and passaged every 3 days till they passed their growth-crisis stage. Spontaneous immortalization was achieved in these MEFs, which could be proliferated in vitro for > 40 passages with a Median Doubling Time (MDT) of 51 ± 22 h. In Strategy II, primary MEFs at passage 3 were transfected with a Tag expressing vector (Addgene plasmid #21826) via electroporation. After transfection, cells were passaged multiple rounds at a low density of 1: 10 to get rid of non-transformed cells. The resulting iMEF lines were subjected to single-cell cloning using serial dilution. Totally, we generated 5 single-cell-derived iMEF colonies, of which one had been cultured for 20 passages with an MDT of 20 ± 2 h. The integration of Tag in these colonies was determined by PCR amplification and Sanger sequencing. Applications of these iMEFs are in progress for testing knock-in of DNA constructs through CRISPR-Cas9 approach.

P-073: Comparison of Genotyping Approaches for Conditional Knock-Out and Cre Mouse Breeding Lines

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Conditional knock-out (cKO) mouse models aid in the detailed analysis of gene functions in a tissue- or temporally specific fashion. Most cKO mouse models follow the Cre/loxP system to control these genetic changes involving a floxed (Two-flanking loxP sites) mouse allele model cross-bred to a Cre mouse model that expresses the enzyme to control the tissue- or temporally-specific ablation of the floxed allele. While cross-breeding these mouse lines, it is key to identify wild-type, heterozygous or homozygous genotypes for the cKO allele and for the presence or absence of the Cre transgene. Traditional end point PCR, gel and sequencing analysis methods used for this genotyping, can be laborious and time consuming. Here we compare our efforts to use Capillary Electrophoresis (CE), multi-plexed (fluor) quantitative PCR and outsourcing for genotyping cKO and Cre mouse lines. When compared to traditional methods, these alternatives allow for quicker turnaround times on making genotype calls ultimately leading to a reduced mouse cage footprint.

P-044: Adaptive sampling long-read sequencing for characterization of CRISPR/Cas9-generated founder transgenic mice

Dr Zachary Freeman

Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Complete characterization of CRISPR/Cas9-generated animal models presents many challenges that may require advanced sequencing methods. We previously used a long single stranded donor combined with a single gRNA strategy to generate an endogenous iCre knock in the Npvf gene in C57BL/6J mice. G0 founders were identified by PCR and Sanger sequencing spanning 5’ and 3’ ends of the insert including the arms of homology. After breeding to wild type, candidate G1 founders were identified using the same PCR strategy, but spanning PCR across the insert were unsuccessful at fully characterizing the entire allele. We used Oxford Nanopore Technology (ONT) long-read sequencing with Adaptive sampling for Chromosome 6 on an individual G1 founder to determine if the entire insert sequence and arms of homology were correct as designed. Reads were aligned to mouse reference genome GRCm38 using the wf-alignment pipeline with the minimap2 v2.24 to align reads. Structural variants (SV) were then called using Sniffles and CuteSV and consensus SVs were called using SURVIVAL v1.0.7. Adaptive sequencing resulted in 58X total coverage with 30X coverage (51.7% allele frequency) of the targeted allele. The insert sequence was identified in the SVs and aligned against the designed megamer sequence. All reads were then segregated into variant supporting and reference supporting reads, which were then assembled into separate consensus sequences using Flye v2.9.1. These two consensus sequences were then aligned individually to the megamer sequence with variant supporting reads matching the megamer sequence in the correctly targeted location. Furthermore, the junctional regions around the arms of homology were correct with no evidence of mutations. These data support the use of ONT adaptive sequencing for the characterization of CRISPR/Cas9-generated animal models.

P-069: Speeding Up The Pipeline To Phenotyping Cohort Generation

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Mouse embryonic stem cell gene targeting efforts usually consist of two independent electroporations. One to introduce the targeting vector and another to remove the drug-selection cassette found within the vector. In between these two events, considerable time is required to characterize and identify correctly targeted clones, also testing their ability to contribute to chimera production & germline transmission to choose the clone which will be used for cassette removal. This process can take months to produce the cassette-deleted targeted clones desired to move forward with cohort production for phenotyping.

Here we describe a shortened timeline to produce these targeted cassette-deleted clones by removing the need to identify cassette-containing clones before proceeding to cassette removal step. After the initial electroporation and drug-selection, the resulting colonies are used directly for cassette removal. The standard process requires the picking of colonies after each electroporation while this new workflow requires colony picking only once. These colonies are then characterized using LOA Taqman screening technology to identify clones having been both targeted and having the drug-selection cassette removed. Our results indicate the cassette removal step is very efficient and the success of this new workflow is dependent on the initial targeting event taking place.

This much time-constrained timeline allows for the identification of clones desired for cohort production in weeks, compared to the months previously required. Less effort and time are required in colony picking, clone expansion and Taqman screening. Further, by eliminating the need to test clones for germline transmission, less animals are required during this process. The time savings realized throughout allows for earlier than expected phenotyping.

P-070: Implementation of Oxford Nanopore Technology targeted sequencing to screen large insertions in mouse models.

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Crispr-Cas9 technology is now routinely used to generate genetically modified animal models. Recently there has been an increased demand to generate mouse models that integrate large transgenes (over 1KB) using Crispr-Cas9 technology and a donor template. Although our ability to deliver and integrate larger transgenes in a site-specific manner has improved, it is still a challenge to correctly identify the Founder mice (F0). Traditionally, to identify potential F0 mice to establish a new mouse line, our lab would design PCR assays to amplify specific regions across the transgene insert and through the junctions of the homology arms into the endogenous locus. The PCR amplicons would be sent for Sanger sequencing to identify FO mice with a targeted insertion. Our lab has had success with this strategy but found developing reliable PCR assays for each individual project labor intensive. In addition, we wanted to be able to screen across the entire inserted transgene to verify all key elements and screen for any undesired mutations, rearrangements, or deletions. To overcome these challenges, our lab has recently incorporated Oxford Nanopore Technology targeted sequencing to screen F0 mice for large transgenes (knock-ins) and eliminated Sanger sequencing using PCR assays to screen for correct insertion. We are now directly screening the genomic DNA of Founder mice without PCR amplification using Oxford Nanopore Technology long read sequence technology Crispr-Cas9 kit. Here we will describe how we developed our workflow to screen Founder mice and their future F1 offspring and future directions on how this strategy could be implemented across different targets.

P-013: Direct Genetic Engineering of NSG Mouse Zygotes, Derivation of a Reporter Mouse Line, and it´s Use as a Model for the Study of Neoangiogenesis

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Immunodeficient mice such as NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (common name NSG) mice are essential for stem cell research, engraftment and infectious disease research. Genetic modification of mice with an immunodeficient background remains challenging due to a poor breeding performance and therefore low embryo numbers, vulnerable embryo quality and high requirements for husbandry conditions and handling.
Here we present a microinjection protocol for efficient germline transgenesis in the NSG mouse line employing the non-autonomous Sleeping Beauty Transposon System encoding a vital fluorescent reporter gene. In vivo produced zygotes were injected with specifically adapted microcapillaries, followed by an overnight in vitro culture. The next day two cell embryos were transferred to F1 foster mothers (n=5). Six transgenic mice were born, showing an ubiquitous green fluorescence, resulting from integration of the CAGGS promoter Venus transposon. The founder animals were vital, fertile, and used to establish Venus-NSG mouse line.
We use this model to study the tumor host interaction; therefore mouse induced pluripotent (iPS) stem cells were injected subcutaneously in Venus-NSG mice. Developing tumors were analyzed with histological sections and HE-staining and further more we performed cryosections to conserve the Venus-fluorescence. The results show, that migration of cells can be observed. In further investigations a detailed analysis of the origin of blood vessels will be performed. The involvement of host cells in the growth of teratomas can be shown. This involvement could be demonstrated visually by Venus fluorescence and can provide new insights in the neoangiogenesis. Based on these data, the teratoma model in Venus-NSG mice can be considered as a promising in vivo model to study neoangiogenesis and to screen for drugs which might inhibit neoangiogenesis and thus become of translational relevance.

Keywords: Transgenic NSG Mice, Sleeping Beauty, Non Autonomous Transposon System

P-040: Ectopic expression of the germ plasm organizer Bucky ball in mammalian cells and embryos

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Germ cell specification follows two general mechanisms in different species: the maternal inheritance mode or the induction mode. The maternal inheritance mode is specified by maternal cytoplasmic determinants, termed germ plasm. During oogenesis, germ plasm forms a distinct cellular structure such as the Balbiani body, an aggregate of organelles also found in mammals. However, mammals are generally known to follow the inductive mode for germline specification. In this case, the germline is specified by external signals from surrounding cells and not by maternal inheritance of germplasm. Nevertheless, the Balbiani body could be detected in many different mammals. Bucky ball is the first known gene to play an important role in the formation of the Balbiani body in zebrafish. Bucky ball homologous sequences in other vertebrates, including chicken (cBuc) and human, were found by conserved synteny and BLAST searches.
To clarify the possible function of the Balbiani body in mammals, either the zebrafish Bucky ball (zBuc) or the chicken cBuc gene was fused with the fluorescent protein Venus and expressed in murine embryos by means of microinjection. The expression patterns during pre-implantation were recorded by vital fluorescence detection of the fused Venus reporter and used to provide information on whether a Bucky ball homologous protein is active in mice. Expression of Bucky ball-Reporter fusions in mouse embryos and the ectopic expression in somatic cells (MEFs) reveals very similar and unique aggregations of Venus fluorescence signals. Our results suggest that mechanisms related to the germ plasm may still be active in mice. These results could help to explain the presence of the Balbiani body in mammals. The assumption of a mutually exclusive presence of the maternally inheritable or the inductive mechanism should be reconsidered.

P-053: Using Cas9 electroporation and AAV templates to generate knock-in and conditional alleles in mice

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Introduction: Genome editing with Cas9 significantly simplified generation of genetically modified mice. However, targeted insertion of large fragments and one-step generation of loxP-flanked conditional alleles remain challenging. Recently, co-incubation of recombinant adeno-associated virus (rAAV) with rodent embryos has been used to deliver repair templates for Cas9-mediated genome editing. Coincident or subsequent delivery of Cas9 ribonucleoprotein (RNP) by electroporation was reported to efficiently generate knock-in alleles in treated embryos.
Aim: Our aim was to test and adopt these protocols to establish a workflow to efficiently generate knock-in and conditional alleles.
Methods: We tested several variables to establish our workflow, including different rAAV-embryo co-incubation times, different Cas9 RNP concentrations for electroporation, and electroporation of zygote and two-cell stage mouse embryos. Pups born from these experiments were screened with short- and long-range PCR, Sanger sequencing, and template copy number assessment by quantitative real-time or digital PCR to identify founders. Germline transmission test breeding was done by crossing founders with wild-type mice of the same strain background.
Results: All combinations of rAAV co-incubation times to deliver repair templates with electroporation to deliver Cas9 RNP successfully generated founders with either knock-in or loxP-flanked conditional alleles. Template-specific PCR and template copy number assessment demonstrated that multicopy rAAV integration can occur both on- and off-target. Evaluation of screening results allowed us to develop a founder screening pipeline that eliminates the most mice at early steps, minimizing the number of animals that need to be screened at each stage. Using founders that passed our founder quality control for germline transmission test breeding enabled us to establish mouse lines with the desired allele in each case tested.
Conclusions: We conclude that template delivery by rAAV co-incubation and Cas9 RNP electroporation is a robust and easily adopted protocol to generate knock-in and conditional alleles.

P-049: Using Precision Animal Models to Support the Discovery of a New AXIN2-Related Disorder

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Variants associated with new disorders or phenotypic expansion in children are often de novo and heterozygous. Producing mouse models and achieving germline transmission (GLT) of such variants can be challenging as they often cause lethal, early-onset phenotypes. CRISPR founder screens provide a means to rapidly assess variant pathogenicity without GLT, but variability in variant allele dosage and phenotypes caused by indel alleles complicate phenotype interpretation. As an alternative, we utilized the inefficiency of prime editing to generate low-level mosaic founder mice harboring a de novo variant in AXIN2 (NM_004655.4:c.196G>A (p.Glu66Lys)). This variant was identified by the BCM Undiagnosed Diseases Network in a patient with global developmental delay, limb anomalies, short palate, and other phenotypes. We generated a mosaic (20%) male founder that was viable and fertile but did not produce live N1 offspring harboring the variant. However, 48% of E18.5 embryos were heterozygous for the variant with a subset having tail (36%), limb (7%), and size (21%) abnormalities. MicroCT revealed that all heterozygous E18.5 embryos had a short palate like the patient. Heterozygous p.Glu66Lys embryos did not phenocopy an Axin2 knockout mouse characterized by BCM KOMP2. Thus, neither loss-of-function nor a simple dominant negative effect are likely to be the pathogenic mechanism of the variant. In fact, overexpression of human AXIN2 p.Glu66Lys variant cDNA in Drosophila wing showed a gain-of-function phenotype while it acted as a loss-of-function in the developing fly eye, demonstrating context-specificity on Wnt-signaling. Our model organism data and identification of other patients with similar phenotypes show that p.Glu66Lys is a pathogenic variant in AXIN2. Our work demonstrates that prime editing N1 screening is a useful strategy for quickly testing pathogenicity of heterozygous de novo variants that cause severe, early-onset phenotypes. Unlike CRISPR founder screens, the N1 screen generates animals of fixed genotypes, simplifying interpretation of genotype-phenotype associations.

P-048: The Baylor College of Medicine Center for Precision Medicine Models

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Precision animal models are critical for interpreting variants of uncertain significance and for pre-clinical investigation of personalized medicine approaches. To assist clinicians, researchers, patient support groups, and large research programs with the production and phenotyping of precision animal models of undiagnosed, rare Mendelian disorders, we have established the NIH-funded Baylor College of Medicine (BCM) Center for Precision Medicine Models (CPMM). CPMM combines the expertise of fly, mouse, and nonhuman primate modeling programs with the clinical, gene discovery, and informatics expertise at BCM. Variants are nominated for modeling through the Center website and are reviewed for the feasibility and applicability of creating a precision fly or mouse model that can address the clinical question with consideration of existing models, variant, and clinical data. The Center also searches a database of genome and exome data from nonhuman primates in National Primate Research Centers to identify models with spontaneous mutations relevant to the nominated variant. To support the pathogenicity of a variant, the Center can query the Baylor Genetics clinical exome database for similar variants, perform RNA-seq using patient-derived cells, or reanalyze the patient exome or genome data. Nominators are given access to our Nomination Tracking System where their nomination information, review comments, and a decision letter can be accessed. If accepted, CPMM members work closely with the nominator to develop a modeling plan. Nominators are provided access to our Project Management System where progress can be monitored and meet regularly with CPMM members to review project status and results. To date, CPMM has received 75 nominations encompassing 118 variants and accepted 33 nominations (18 mouse, 13 fly, 2 both) involving 71 variants. The precision models currently being generated by CPMM are helping to uncover new disease mechanisms, reveal new disease biomarkers and phenotypes, and identify and test new therapeutic strategies for patients.

P-008: An Efficient Approach for Generating Genetically Modified Mice Expressing Predefined Monoclonal B-Cell Receptor Repertoires

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Genetically modified mice expressing predefined monoclonal B-cell receptor (BCR) repertoires play a pivotal role as essential tools in immunological research. Traditionally, such mice were generated through transgene insertion into zygotes, resulting in expression from nonnative loci and limitations in isotype switching and affinity maturation.
In this study, we present an innovative and efficient methodology for generating monoclonal BCR-expressing mice. Leveraging the power of Ribonucleoproteins (RNPs) and single-stranded DNA (ssDNA) repair templates, we successfully eliminated a part of the endogenous Igh locus and replaced it with a bicistronic allele encoding both the light and heavy Ig chains of the BCR, under control of an Ig-V promoter . This novel approach enabled mice capable of isoclass switching, somatic hypermutation, and affinity maturation.
Furthermore, through the utilization of RNPs and ssDNA repair oligos, we removed the coding regions of the light and heavy Ig chains, leaving the Ig-V promoter in place, creating a genetic harbor for the insertion of other heavy and light chains in a single step. By implementing this streamlined technique, we significantly reduced labor and complexity, ultimately leading to the generation of monoallelic BCR-expressing mice with precision and ease.
The implications of this methodology empower researchers to tailor BCR repertoires with specific characteristics, shedding light on intricate aspects of immune regulation and immune response adaptation to new threats.

P-061: New preclinical DMD mouse models through genome editing of existing YAC transgenic mice.

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Duchenne muscular dystrophy is caused by out-of-frame mutations, usually involving one or more exons, in the DMD gene, while the milder Becker muscular dystrophy is caused by in-frame mutations. Restoration of the reading frame using exon skipping-antisense oligonucleotides (ASOs) or genome editing is a promising therapeutic approach for Duchenne.

In the past we generated a humanized hDMD mouse model carrying the complete human DMD gene (2.3 Mb) on a Yeast Artificial Chromosome (YAC) on a mouse dystrophin-deficient background (hDMD/mdx). A patient-specific del exon 52 variant was made (hDMDdel52/mdx) using ES cells derived from the hDMD/mdx model. In this process it was found that the transgene integration site contained two complete, tail-to-tail copies of the YAC.

To generate additional preclinical models for other mutations we used PacBio whole genome sequencing to identify unique sequences to remove one of the YAC copies and make future deletion of individual exons easier. However, we were not able to assemble a complete sequence contig of the transgene integration. We then used the knowledge of the double integration event to design better (pre)screening assays and used CRISPR/Cas9 to delete both copies of hDMD exon 44 from the hDMD/mdx ES cells. Correct candidate clones lacking both exon 44 copies were easily identified. Chimeras generated with hDMDdel44/mdx ES cells readily gave germline transmission. We then successfully attempted the generation of additional mutant alleles directly in zygotes generated with hDMD/mdx fathers and electroporation of RNPs. We currently have five humanized YAC DMD models for the most common patient mutations (del44, del45, del51, del52, del53) in different stages of quality control, colony building, functional testing and pre-clinical use. Upon confirmation of restored dystrophin protein expression using appropriate ASOs for each mutation all models will be made available to academic and industrial parties for preclinical testing of new human therapeutics.

P-042: Where is my Transgene? Long-range Sequencing to Determine the Integration Site of a Transgene in the Mouse Genome

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

A triple-transgenic mouse line (3DR) carrying drug resistance for neomycin, puromycin and hygromycin was generated by co-injection of 3 transgenes into pronuclei of fertilized FVB-background mouse eggs. Incorporation of the transgenes into the genome was confirmed by PCR amplification of the individual transgenes. G0 founders were subsequently bred to wild type FVB with the 3 drug resistance transgenes co inherited on G1 offspring, confirming close linkage. This transgenic mouse line was subsequently used in preparation of mouse embryonic fibroblasts for use as feeder layers for mouse embryonic stem cells in gene-targeting experiments. We sought to identify the genomic location of transgene integration using long read sequencing. Genomic DNA was extracted from 3DR mouse embryonic fibroblasts and submitted to the University of Michigan Advanced Genomics Core for Oxford Nanopore Technology (ONT) Sequencing. Initial whole genome sequencing ONT achieved 3X coverage and after alignment identified potential integration sites in Chromosome 4 and X. ONT Adaptive sampling real time sequencing which allows for real time enrichment based on alignment to a reference sequence enhancing overall target read depth. Chromosome 4 and X were used for references and sampling confirmed all three transgenes were co integrated into Chromosome 4 at position chr4:125440180. The transgenes were each integrated in a single copy in the following order: hygromycin, puromycin, and neomycin. These methods represent a promising way to identify transgene insertion sites.

P-045: Establishing a pipeline for validating VelociGene's humanized animal models

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The VelociGene department of Regeneron Pharmaceuticals creates animal models for scientific study. Humanizations (humIns) involve the complete or partial replacement of a mouse or rat gene with human homologous sequence. These animal models are used for various study needs, such as therapeutics development, antibody testing, and modeling human disease. Proper expression from the humIn allele is therefore vital. We present here our pipeline for validation of humanized mRNA and protein expression during early cohort breeding. By evaluating F1 animals heterozygous for a humIn allele, we can confirm that engineered mRNA and protein are made in the tissue locations and amounts necessary or halt further study for redesign if the allele does not perform satisfactorily. This early validation saves time and animal cage space. Here we present mRNA and protein results from a series of humIn alleles.

P-001: Idiopathic accumulation of subcutaneous fats in CYP17A1 knockout rats

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Most obesity models, including transgenic animals (ob/ob mouse, DIO rats, etc.), are being studied in relation to visceral fat, and the association of subcutaneous fat with metabolic syndrome has yet to be thoroughly investigated due to a lack of a suitable subcutaneous research model. The CYP17A1 gene, which encodes an enzyme with 17,20-lyase and 17a-hydroxylase activity, is crucial for the body's ability to synthesize steroid hormones like androgen and glucocorticoids. Recent research revealed a connection between the CYP17A1 gene and atherosclerosis, prostate cancer, congenital adrenal hyperplasia (CAH), and polycystic ovarian syndrome (PCOS). Here, we generated CYP17A1 knockout rat using CRISPR/Cas9 via ribonucleoprotein (RNP) electroporation. Pre-incubated gRNA and Cas9 RNP was electroporated into 1-cell stage embryo, and 2-cell stage embryos (number: 20~25) were transferred to oviducts of recipients. Total 281 embryos were transferred to 19 recipients, 48 pups were borned, and 41 pups had CYP17A1 indel mutation (85.42%). In order to identify depot-specific fat accumulation, we sampled CYP17A1 knockout rats, which demonstrated a sex-reversed and obese phenotype. Inguinal fat (subcutaneous fat) was found to accumulate excessively in CYP17A1 KO rats compared to wild-type rats when body weight or visceral adipose tissue mass were normalized. A number of tests, including the insulin tolerance test, the oral glucose tolerance test, the blood biochemistry test, and the blood pressure analysis, were used to examine the metabolic symptoms of CYP17A1 KO rats. These tests revealed that CYP17A1 KO rats are normal in insulin and glucose tolerance, and also have no hypertension, hyperglycemia, hyperlipidemia, or hypercholesterolemia. In conclusion, it demonstrated that the CYP17A1 knockout rat model will aid in understanding the relationship between steroid hormones, subcutaneous fat accumulation, and metabolic syndrome.

P-050 Overview of Regeneron Genotyping Pipeline: Building Capacity through Automation and Innovation

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The Genotyping Group determines genotypes of the genetically modified rodents produced by Regeneron. To achieve rapid turnaround and high throughput, Real-Time qPCR is employed, which allows for genotype calling by a copy number counting method. Since 2012 Regeneron has significantly expanded its vivarium capacity, and consequently the demand for genotyping has increased (from 122,382 samples in 2012 to 287,799 samples in 2022). Additionally, genotype complexity has risen, as the number of modified alleles to be genotyped has increased from 2.8 alleles per animal in 2012 to 5.7 in 2022. Therefore, our group has deployed key innovations to build genotyping capacity.

We created new workflows to merge our team's workload, use reaction wells more efficiently, and digitally track samples. We automated biopsy sample processing, experimental planning, and execution, as well as data analysis.

New software tools enabled an increase in throughput. These include a database of 2D barcoded qPCR assays, permitting automated retrieval of assay sets, and a custom Excel macro to analyze data and upload results to a database, which stores and disseminates these results. We also implemented a LIMS for assay and sample tracking.

Further, we implemented several robotic platforms. An automated biobank freezer enabled storage and retrieval of qPCR assay vials, which are measured by a volume check platform. We added liquid handler capacity: a Hamilton Star for the preparation of allele specific qPCR master mixes (on average 153 mixes per day), and Biomek i7s for combining master mixes and sample DNA into 384 well plates. A hands-free platform, equipped with robotic arms and scheduling software, can distribute 288 plates per day to thermocyclers. Finally, we developed an automated platform to process and consolidate samples for DNA isolation.

These improvements have eliminated bottlenecks, improved risk management, increased the productivity of genotypers, decreased turnaround time, and substantially increased throughput.

P-035 Generation of Novel Mouse Model for GGGGCC Repeat-Expanded Humanized C9orf72 Allele and Analysis of the Repeat Instability

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Microsatellite expansion diseases are a group of genetic disorders that are characterized by an expansion of short nucleotide homopolymer. GGGGCC repeat expansion is the most common cause of familial Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD), but the mechanisms of how this microsatellite expands or contracts, i.e. repeat instability, are largely unknown. Recapitulating microsatellite expansion diseases in experimental animals is challenging due to the technical difficulty in manipulating repeat-containing sequences in vitro. In this study, we successfully generated targeted humanized C9orf72 mouse with 96x GGGGCC repeats to study repeat instability and explore mechanisms involved in expansion. Tissue analysis in mouse showed somatic repeat instability varied depending on age and tissue. When Msh2, a critical gene in Mismatch repair (MMR) pathway, was deleted from the C9orf72 humanized mouse and blocked MMR pathway, this manipulation stabilized somatic repeat expansions, indicating MMR pathway is the major driver for the GGGGCC somatic repeat expansion. In our effort to generate larger C9orf72 repeat alleles, we found a DSB adjacent to the repeats, introduced by CRISPR-Cas9, induced large-scale repeat expansion, enabling us to generate up to 550x GGGGCC alleles. Importantly, this CRISPR-Cas9-induced repeat expansion occurred independently from MMR pathway. Our findings suggested that C9orf72 GGGGCC expands through multiple pathways. Also, CRISPR-Cas9 induced repeat expansion may be applicable to model other microsatellite expansion diseases.

P-019 A new approach for gene replacement and humanization in the mouse

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

The replacement of mouse genes against human homologs is increasingly demanded to study gene function, therapies, and drug actions in humanized research models.
However, the use of donor vectors for homology-directed repair (HDR) of CRISPR/Cas9-induced double-strand breaks in zygotes limits the size of replacements to 5-10 kb.
To enable HDR-independent and large gene replacements, we explored our NHEJ-based Replace knock-in approach (1) for the direct replacement of a 27 kb segment with exons 1-9 of the Ace2 gene against a segment with human sequences. The humanized Ace2 model will be sensitive to infection with SARS-CoV2 and enable COVID-19 disease studies.

By employing Cas9 in zygotes along with a pair of gRNAs that delete mouse exons 1-9 and release the human donor sequence, we successfully obtained knock-in founder alleles and established two Ace2 humanized mouse lines that are presently further characterized.

While we provide proof of concept for this new humanization approach, it remains essential to conduct further investigations to determine the feasibility of replacing even larger gene segments, ranging from 50 to 100 kb and beyond, in zygotes.

1. Danner E, Lebedin M, De La Rosa K, Kühn R. A homology independent sequence replacement strategy in human cells using a CRISPR nuclease: Replace Targeting. Open Biol. 2021. doi:10.1098/rsob.200283

P-051 Comparison of medium formulations in derivation of mixed background mouse ES cell lines

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Although simple genetic modifications can be achieved by introducing the CRISPR/Cas9 system directly in mouse embryos, large modifications still require targeting via homologous recombination in mouse embryonic stem (ES) cells. To support and accelerate the generation of mouse models with multiple genetic modifications, we routinely derive new mouse ES cell lines that are homozygous for multiple large, engineered alleles. Historically, we have used 2i medium to derive mixed background ES cell lines that consist of various percentage contributions of the C57BL/6, 129S6, and Balb/c strains. Following microinjection of the modified ES cells into 8-cell embryos, 100% ES cell-derived mice can be produced. From 2008 to 2016, we have used those lines derived in 2i medium in more than 500 microinjections, transferring approximately 24,000 injected embryos into pseudo-pregnant surrogates, resulting in an overall average of 6% of the transferred embryos giving rise to 100% ES cell-derived live-born F0 animals.

Recently, we performed parallel ES cell derivations from C57BL/6, 129S6, and Balb/c mixed background mice, comparing the standard 2i medium with 2 newer published medium formulations: N2B27-LCM medium and N2B27-LCC medium. Here we report the details and conclusions of these experiments. In 5 out of 6 independent mouse derivations, N2B27-LCDM was the most optimal medium which yielded a 15% to 45% range of 100% ES cell-derived mice following microinjection into 8-cell embryos. From these observations, we have standardized the use of N2B27-LCDM in our derivation pipeline for C57BL/6, 129S6, and Balb/c mixed background mice.

P-055 Modeling disease-associated variants in mice using prime editors

Dr Denise Grant Lanza

Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

The Center for Precision Medicine Models leverages Baylor College of Medicine's genome discovery, informatics, and animal modeling programs to develop precision animal models that will end the diagnostic odyssey of patients with undiagnosed, rare, and Mendelian diseases and serve as resources for pre-clinical studies. However, modeling de novo disease-associated variants with traditional knock-in alleles has been challenging as undesired indel mutations are often generated in trans to a desired variant with biallelic editing causing embryonic or neonatal lethality of founder animals. Moreover, variants alone often cause disease-associated, early-onset phenotypes that lead to founder death prior to weaning. Consequently, time-consuming strategies, such as conditional variant knock-in alleles, are often pursued.

As an alternative, we utilized prime editing in one-cell stage mouse zygotes, employing both PE2 and PEmax editors and PE3 and PE3b approaches. We hypothesized that prime editing would (1) introduce precise genetic alterations in the absence of indel mutations, and (2) produce low-level germline mosaic founders that are viable, fertile, and can be bred to generate heterozygous N1 progeny. To date we have targeted 9 separate loci and successfully generated correctly targeted F0 mice (founders) for 6 knock-in alleles. Founder occurrence varied by locus and often were a small percentage of the total number of F0s genotyped (5-67%). Low-level mosaicism (allele contribution 10-40%) likely contributed to the survival of these founders compared to previous traditional knock-in attempts. Undesired mutations were less common (5-22%) amongst F0 animals and usually not observed in correctly targeted F0s. Of the 6 targeted loci, 4 founders have been bred to generate live-born progeny and 2 have been utilized to generate embryos for an N1 phenotyping screen. Ultimately, the use of prime editors has greatly facilitated model production of embryonic or neonatal lethal variants to produce a stable source of mice for phenotypic evaluation.

P-075 Evaluating Cas9 ribonucleoprotein (RNP) stability for mouse genome editing

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Introduction: Mouse genome edits are commonly introduced by electroporation (EP) of zygotes with RNA-guided endonucleases such as Cas9 or Cas12a. At The Centre for Phenogenomics, we typically use a ribonucleoprotein (RNP) complex, which we make shortly before EP; however, this workflow can be operationally limiting since the RNP is prepared outside the barrier by different staff than those treating the embryos.
Methods: RNP complexes were prepared up to seven days before EP and stored at 4ºC. Same-day RNP complexes were made up to 1 hour before EP. On the day of EP, zygotes were randomly split into two groups and each group was electroporated with same-day or earlier-day complexes. Pups were assayed by end-point PCR to identify the desired genome edit.
Results: In pilot experiments using RNP complexes prepared up to 7 days before EP, we found high genome editing rates in GFP reporter mice, as assayed by the absence of GFP signal in blastocysts and day 10.5 embryos. We then compared founder rates for targeted deletions using same-day and earlier-day RNP complexes. Both conditions produced founders in all experiments except for one that yielded no founders. While the average number of deletion founders was somewhat lower when RNP was prepared before the day of EP, we obtained sufficient founders to establish the new mouse line.
Conclusion: S. pyogenes Cas9 RNP complexes can be prepared up to seven days before EP, allowing more flexibility for scheduling mouse genome editing experiments.

P-023 Simple and Efficient Transposon Mediated BAC Transgenesis

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Introduction: Bacteria artificial chromosomes based transgenesis (BAC-TG) enables introduction of large DNA fragments into target genome to faithfully recapture the expression pattern, however, the efficiency of BAC-TG in mice by traditional linearized DNA injection into fertilized egg or pronuclei, is limited.

Aims: To efficiently generate BAC-TG mouse strains under different genetic backgrounds.

Methods: We present the combination of the cut-and-paste transposon system, PiggyBAC, with BAC construct to generate transposase-mediated BAC-TG.

Results: We have efficiently generated multiple BAC-TG mice strains with insertions up to 187.0 kbp. This approach was also demonstrated to be suitable for transgenesis in multiple inbred strains, including C57BL/6J, BALB/cJ and immunodeficient backgrounds. In addition, we found that injection of transposase expressing plasmid into the pronuclei was more efficient compared to mRNA injection in the cytoplasm of fertilized eggs.

Conclusion: The PiggyBAC mediated BAC-TG system is applicable to any BAC sequence requirement under different backgrounds for efficient transgenesis in mice.

P-026 Applicability of Oxford Nanopore Cas9-capture in the validation of transgenic mouse models

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Oxford Nanopore Technologies (ONT) sequencing is a now well-established technology applicable to a wide range of genetic research and clinical applications. Even though a major advantage of ONT sequencing is that it allows for the processing of long and ultra-long reads, amplicons multiple kilobases in size to be sequenced can be difficult to attain via conventional PCR methods. Recently we have adopted ONT Cas9 capture as an alternative, PCR-free approach that utilises the DNA-cleaving activity of Cas9 nuclease to capture and subsequently sequence regions of interest too large to enrich via conventional PCR.

Here we present the applicability of ONT Cas9 capture in the quality control and validation of transgenic animals carrying newly generated knock-in or conditional alleles spanning multiple kilobases. We show that mouse models created via combinations of CRISPR/Cas9 editing and homologous recombination on the ES cell level can successfully be validated by Cas9 capture. We also show applicability to mosaic founders and F1 generations obtained by pronuclear microinjection of one-cell embryos with CRISPR reagents and DNA donor. Resulting sequencing depths are sufficient to offset error rates inherent to the technology. Cas9 capture therefore provides a crucial step in the validation process of transgenic animals, such as in the identification of SNVs as well as potential structural rearrangements that are difficult to identify through PCR and Sanger sequencing.

Due to its complex and elaborate laboratory procedure, successful implementation as well as data acquisition can be challenging to achieve and require careful and detailed attention to a wide range of experimental parameters. We therefore also highlight several key technical choices, such as DNA extraction and preparation approaches, guide cleavage conditions and final library assembly considerations that can improve input sample quality and ultimately provide robust data output of ONT Cas9 capture.

P-006 Human diseases of single nucleotide variant modeling in mice

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Human genomic variations have a significant amount of single nucleotide variant (SNV) that is a missense or nonsense mutation causing a disease (ClinVar-NCBI data). To closely model this type of human disease with mice, a nucleotide must be exclusively mutated in their genome. The application of CRISPR-Cas9 and the SNV-containing single-stranded oligodeoxynucleotides (ssODN) template without introducing a couple of silent mutations often resulted in mice carrying the SNV and insertions or deletions (INDEL) at the targeting site due to the recurring cleavages after homology-directed repair (HDR). Or no mice were born in cases of gene disruption causing embryonic lethal. To circumvent this problem, we carried out two rounds of genetic modifications with the CRISPR-Cas9 approach to generate the solely interested SNV in mice. Firstly, intermediate founder mice were generated to carry a distinctive targeting site that differs from the wild-type allele. The newly generated targeting site contains either a short in-frame deletion or a few silent mutations. New guide RNAs were designed to target the distinctive site for the second modification round with the HDR template containing only interested SNV in the embryos produced by in vitro fertilization with the mice carrying the unique targeting site. This procedure has been successfully employed in three projects and is developing a general protocol.

P-020 Evaluating CRISPR/Cas9 guide RNA activity in rodent blastocysts

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Guide RNA (gRNAs) are a critical component to determining CRISPR/Cas9 activity when generating transgenic animal models. A bioinformatic approach is commonly used to identify gRNA with current pipelines relying heavily on in vitro results in immortalized cell lines coupled with to protospacer adjacent motif (PAM) sequence identification to predict on target activity. It remains unclear how the in vitro and in vivo results correlate with the expected success of gRNA identified by these methods. We have evaluated more than 950 unique gRNAs over the past 7 years utilizing an ex vivo pipeline to test gRNA activity prior to generating transgenic rodents. Briefly, gRNAs are microinjected into fertilized zygotes, allowed to develop to blastocysts, and then characterized at an individually blastocyst level for on target cutting activity. gRNAs are determined to be active if evidence of cutting is present in more than 33% of tested blastocysts. We aimed to understand from this data how current gRNA selection tools predicted on target cutting in zygotes. We hypothesize that the relationship between guide scoring and blastocyst cutting activity are not strongly correlated. We found that only 61.3% of gRNAs with predicted activity were active in our assay while 38.7% of guides were inactive. Furthermore, the percent active guides remained constant over time and was similar across different variants of Cas9 enzyme. We next evaluated Moreno-Mateos (MM) and cutting frequency determination (CFD) scores in active vs inactive gRNA, as metrics for gRNA cutting activity and off-target events, respectively. We found no difference in MM and CFD scores for active vs inactive gRNAs. These data suggest that current gRNA selection tools may need improvement for application to the generation of transgenic animal models.

P-005 Evidence-Based Guide to Using Artificial Introns for Tissue-Specific Knockout in Mice

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Introduction: Up until recently, methods for generating floxed mice either conventionally or by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 (CRISPR-associated protein 9) editing have been technically challenging, expensive and error-prone, or time-consuming. To circumvent these issues, several laboratories successfully used a small artificial intron to conditionally knockout (KO) a gene of interest in mice.

Problem: However, many other labs are having difficulty getting the technique to work. The key issue appears to be either a failure in achieving correct splicing after the introduction of the artificial intron into the gene or, just as crucial, insufficient functional KO of the gene's protein after Cre-induced removal of the intron's branchpoint.

Resolution: Presented here is an evidence-based guide to the selection of a suitable exon and the optimal placement of the recombinase-regulated artificial intron (rAI) within that exon. The primary objective is to avoid disrupting normal gene splicing after rAI insertion while maximizing mRNA degradation after recombinase treatment. The reasoning behind each step in the guide is also discussed. Adherence to these recommendations should significantly increase the success rate of this easy, novel, and alternative approach for generating conditional KO mice. (Published paper at https://www.mdpi.com/1422-0067/24/12/10258)

P-015 Efficiencies of Different Genetic Modification Techniques in Rat Embryos

Dr James McNew

Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

CRISPR-Cas9 technology has revolutionized our ability to create genetically modified animals. Many animal models require the need to insert (knock-in) preconstructed DNA templates called repair templates. DNA repair templates along with CRISPR-Cas9 reagents can be introduced into embryos by pronuclear injection (PNI), electroporation (EP), or delivery via adeno-associated virus with electroporation (AAV+EP). Currently, no published literature compares the efficiency of these delivery techniques as it relates to DNA insertions via CRISPR mediated genome editing in rats. We used a 400-base pair (bp) repair template consisting of homology arms flanking a floxed short artificial intron designed to target exon 2 of the Crh gene. Superovulated Sprague Dawley (SD) female rats mated to SD stud males were used to generate zygotes. Zygotes were randomly assigned into four groups: culture only control, PNI, EP, and AAV+EP. After manipulation, embryos were cultured to the blastocyst stage and submitted for Next Generation Sequencing (NGS) to detect evidence of genome editing. Embryo survival after one day in culture was significantly less following PNI, 58% (101/175), compared to the culture only control, 98% (109/111). Cleavage rates and development to a 4-cell stage did not differ between embryos that survived 24 hours in culture. Knock-in rates for manipulated embryos were 67% (12/18) for PNI, 0% (0/35) for EP, and 63% (22/35) for AAV+EP. We conclude that PNI decreases embryo survivability but not development, and that EP and AAV+EP do not decrease embryo survival or development. Using a 400 bp DNA repair template, we found knock-in rates were similar with PNI and AAV+EP while the template failed to be inserted into the genome with EP only.

P-029 Evidence of Gram-negative bacteria in the reproductive tract of embryo donor female mice after copula.

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Previous findings of our laboratory have shown bacterial contamination in the culture media drops of in vivo produced murine embryos from different mouse strains. Although all reagents and materials used were tested, no contamination could be detected. The aim of this work was to determine if the source of this bacterial contamination could come from the female's reproductive tract after male copula. Twelve C57BL/6J females (4-week-old) were superovulated with 5 IU of eCG injection followed by 5 IU of hCG injection 48 h later. Immediately, six females were mated with C57BL/6J adult males (1x1; mating group), while the others were not mated (control group). Next morning all females were euthanized by cervical dislocation, and their oviducts were individually collected in 200uL of M2 medium. Thirty minutes later, 50uL of M2 medium were placed in M16 drops under paraffin oil (two replicates/female) and maintained in 5% CO2, 37°C for 96h. M16 drops were observed under a Zeiss Stemi 508 stereomicroscope (50x), using rear-lit. After 24 h, motile bacteria were detected in the mating group samples but not in the control ones. Samples from the contaminated drops were Gram stained and a preliminary study of the 16S rRNA amplicon sequencing was performed using MinION (Oxford Nanopore). For this approach, bacteria were enriched in Luria-Bertani broth and Monarch® Genomic DNA Purification Kit was used for DNA extraction. Gram-negative bacteria were found in the sample smears. Taxonomical classification of the sequences using SINA (SILVA Incremental Aligner) with the SILVA ribosomal RNA database revealed bacteria from the Enterobacteriaceae family, particularly Escherichia-Shigella. Preliminary results of our group show Gram-negative bacteria in the reproductive tract of females after copula, which probably comes from their vagina (Parr, 1985). More studies are being performed to determine the effect of these bacteria on embryo development.

P-034 Implementing in vitro fertilization for rat model cryo-resuscitation from frozen-thawed sperm

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Intracytoplasmic sperm injection (ICSI) is currently the most commonly used method to resuscitate rat models from frozen sperm due mainly to the lack of efficient and repeatable sperm cryopreservation and in vitro fertilization (IVF) protocols. The ICSI procedure requires expensive equipment and extensive technical training. Therefore, optimization and implementation of IVF as a cost-effective alternative for cryo-resuscitation will provide the biomedical community with more accessibility to cryopreserved rat models. Since significant improvements in both sperm freezing and IVF have been accomplished in recent years, we investigated the feasibility of IVF for rat model cryo-resuscitation at the Rat Resource and Research Center which serves as a repository for a large number of rat strains/stocks generated and used by the research community. Two transgenic and two knock-in lines were used in this pilot study. Cauda epididymal sperm were frozen in an egg yolk-lactose-Equex STM based freezing medium. The frozen thawed sperm were then used to fertilize oocytes from superovulated wild type immature females in vitro. The in vitro development potential of embryos resulting from IVF was used to assess the efficiencies of the IVF procedure. Preliminary results showed that the fertilization rates ranged from 44.4% to 91.3% and blastocyst rates ranged from 0% to 71.4% with significant variation among different mutant lines and even different IVF attempts within the same mutant line. However, within three replicates, we achieved blastocyst rates ranging from 45.8% to 71.4% in all four lines. Cryo-resuscitation only requires a few animals to establish breeding pairs, therefore, the preliminary results support implementation of the use of IVF for cryo-resuscitation with ICSI as a back-up procedure for lines with repeated IVF failures. Future work will include additional mutant lines and embryo transfers to assess their in vivo developmental potential (funded by NIH grant P40 OD011062-22S1).

P-032 Use of ddPCR to Determine Copy Number in Founder mice made with AAV6

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Introduction:
Knock-in mouse models serve as tools to study in vivo biology. Generating knock-in mouse models is inefficient but the use of AAV6 to deliver template DNA has improved gene editing outcomes significantly. However, since of use AAV6 as template DNA is relatively new, mice generated in this way haven't undergone full QC. Due to the high permeability of AAV6 to enter the nucleus, the increased frequency of random integrations has not been determined. Further, we assessed the effect of volume on AAV6 infected media on copy number integrations in embryos.
Methods:
We generated multiple Dre recombinase mouse lines using AAV6 by infecting mouse zygotes at the 1-cell stage (5.33-8.64 x10^9) and electroporating them with CRISPR/Cas9.We used digital droplet PCR (ddPCR) to accurately measure the number of integrations in founder mice.
Results:
We identified positive mice with Dre integrations in the correct loci using traditional long range PCR in two lines. In these same mice we determined the number of copies of Dre with median copy number of ~ 1 per mouse. However, a few founders had more than 3 copies. We also compared different volumes of AAV6 infection media on embryos and found lower volumes increased copy number per founder mouse.
Conclusions:
Use of AAV6 at sensible concentrations does not cause excessive random integrations. In scenarios where low titre AAV is produced, reduction of total infection media may enhance AAV exposure. Overall, It is important to track copy number when choosing F0 mice to breed further.

P-027 EV-AAV Vector: A Novel Application of the Compound Vector for Efficient and Non-Invasive Delivery In Vitro and In Vivo

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In this study, we introduce a novel application of the EV-AAV vector, a compound vector that combines the strengths of extracellular vesicles (EVs) and adeno-associated virus (AAV). Our research focuses on an innovative application of this compound vector as a non-invasive and efficient delivery system into zygotes and cells. We successfully evaluated the efficiency of EV-AAV vectors as carriers for homology templates using the CRISPR-READI method and demonstrated that the EV-AAV compound vector has the potential to deliver an AAV genome exceeding the five kilobases. Additionally, we conducted further investigations into the potential of the EV-AAV vector in delivering the PiggyBac transposon system, enabling us to explore delivery efficiency and potential toxicity. Our results show that the EV-AAV vector outperforms the standard AAV vector, exhibiting superior delivery efficiency and reduced toxicity profiles. In conclusion, this study highlights the remarkable potential of EV-AAVs as an innovative delivery platform for diverse genetic materials. These vectors represent a convergence of unique advantages, encompassing simple production, characterization, and adaptable cargo delivery.

P-033 Humanized mouse models for the study of SARS-CoV2 infection and COVID19.

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Here we present two new humanized knockin mice, created by gene editing in embryos, as models for the study of COVID-19. In both models, the human hACE2 protein is expressed under the endogenous transcriptional control of the murine Ace2 gene promoter. In the first model (hACE2-TomatoKI) the Tomato fluorescent reporter is co-expressed with hACE2. In the second model (hACE2-hTMPRSS2KI) the human proteins hACE2 and the membrane protease hTMPRSS2, involved in viral Spike protein processing, are co-expressed from the same mRNA. In both models, the murine Ace2 gene is knocked-out. Therefore, they represent physiological mouse models for the study of SARS-CoV2 infection.

The expression pattern of the hACE2, Tomato and hTMPRSS2 proteins in each of the models generated has been characterized and coincides with that of the murine Ace2 gene. In lung, we have detected hACE2 in the membrane of the ciliated cells of the bronchial epithelium, mainly in the apical zone of the membrane. It is also detected in a small proportion of type II pneumocytes. In the kidney, we detected hACE2 on the luminal surface of the renal tubules. In the intestine, expression levels are very high on the luminal surface of enterocytes and in some neuroendocrine cells. Using immunofluorescence, immunohistochemistry and RNAscope techniques, we have confirmed the co-expression of hACE2 and Tomato (in the hACE2-TomatoKI model) or TMPRSS2 (in the hACE2-hTMPRSS2KI model) in the same organs and cell types.

An infection protocol for both models has been developed with a SARS-CoV-2 preparation obtained from an infective cDNA with the sequence of the SARS-CoV-2 Wuhan Hu-1 isolate (GenBank MN908947). The lung pathology caused by the infection in each of the models was analyzed, using the K18-hACE2 transgenic model as a control. The characterization of the pathologies caused by the infection in each of the models is currently being completed.

P-064 Can We Use Historical Data to Predict Optimal Targeting Strategies for Efficient Knock-in Generation of Mouse Models

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Over the last few years, a multitude of CRISPR-based methods have been developed and used to generate knock-in mouse modes. Is it possible to use historical data to predict the most effective targeting strategy for creating knock-ins at specific genomic loci? We conducted a comprehensive analysis of our transgenic core facility's data collected over the past three years. In this, we were focusing on the correlation between our success rate and the targeting techniques used, the conformation and DNA methylation status of the targeted locus as well as the assumed conformation of the inserted DNA fragment.

P-039 Management of mouse sperm freezing and quality control

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Generally, in research an increasing set of gene-modified mouse lines and intercrosses thereof is maintained in the animal facility. Due to changing research some mouse lines are not used in experimental settings. These lines have been bred in a “maintenance mode” to secure the genetic modification, especially if the gene modification will not be considered by repositories. However, even with a minimal number of breeders, offspring is constantly arising, ultimately limiting animal capacity for “active lines”, increasing costs and animal numbers. The management of gene-modified mouse lines, considering three “Rs” in project design and animal maintenance therefore requires management strategies to reduce the number of breeding lines, typing work and expenses.
To achieve these aims, systematic sperm freezing was introduced, based on the protocol of Takeo & Nakagata for mouse lines on a C57BL/6N background. Moreover, different media and conditions were evaluated. Report and data forms were developed for documentation and quality control of cryopreservation and in vitro fertilization (IVF). Currently, more than 25 lines have been cryopreserved and successfully rederived by IVF of mouse oocytes (C57BL/6N). To test sperm viability and gene modifications, PCR conditions for different alleles were optimized based on the method of Scavizzi et al., allowing typing of fertilized embryos already at the blastocyst stage. This reduces the numbers of foster mice and born offspring required for proof of sperm viability and correct genotype. Frozen sperm was used to transfer mouse lines to other facilities, reducing transport animal stress and reducing quarantine procedures. Here, storage and shipping protocols using dry ice instead of liquid nitrogen were evaluated.
Thus, we consider routine sperm freezing as a standard measure for the management of gene-modified mouse lines keeping all generated alleles available, enabling simplified transfer of mouse lines, and providing a significant reduction of animals required in research.

P-076 Comparison of numbers of pups and implantation sites between the left and right uterine horns after embryo transfer of a minimally sufficient amount to induce pregnancy

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In the field of transgenic technologies, embryo transfers (ETs) involve embryos being surgically transferred into the oviduct or uterus of pseudo-pregnant recipient females to obtain live pups. In an ideal scenario, where there is an excess of embryos to transfer, 18 embryos are typically transferred per recipient, however some difficulties can arise in obtaining embryos. These include, but are not limited to, a low fertilization rate, poor response to superovulation, or low survival post microinjection.

This studies purpose was to compare the survival of embryos in unilateral and bilateral transfers and determine whether the left or right uterine horn is more supportive of embryo development. Preliminary experiments determined that 4, 2-cell embryos appeared to be the minimally sufficient number of embryos to initiate and maintain pregnancy. 2-cell embryos (N=4) were transferred (unilaterally or bilaterally) into the oviduct of 0.5 dpc (day of plug) pseudo-pregnant 8-16-week-old CD-1 females. For unilateral transfers, all 4 embryos were transferred either into the left or right oviduct, whereas for bilateral transfers they were split evenly between both sides (N=2 per oviduct). We also examined survivability in relation to which oviduct received embryos first, either right side followed by left (right-left) or left followed by right (left-right). Recipients were euthanized at day 19 post-transfer to determine number and location of pups and implantation sites (total of reabsorption sites and pups).

We determined that the right uterine horn is more supportive of embryo implantation and survivability than that of the left. There was no significance in survivability between the right-left and left-right bilateral ETs. We conclude that when there is a suboptimal number of embryos to transfer, live births can be maximized by favoring embryo transfer into the right uterine horn. Implementing this approach will improve survivability of embryos post-transfer and reduce animal usage in transgenic procedures.

P-063 Generation of a mouse line with a cAMP FRET sensor

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CUTie (cAMP Universal Tag for imaging experiments) is a cAMP FRET-based sensor designed by computational techniques in order to be targeted to different macromolecular complexes, with equal cAMP sensitivities in different environments. This characteristic allows direct comparison of cAMP signals at multiple subcellular sites.

These sensors have been already tested in cells with good results, allowing accurate detection of compartmentalized cAMP near certain subcellular complexes [1].

Our aim is to develop a genetically modified mouse model harboring one variant of CUTie with ubiquitous expression in (nearly) every cell of the animal. If successful, this mouse model will serve as a source of reporter cells and/or organoids for multiple studies involving cAMP signaling.

In the first trial, the construction was targeted to Rosa26 locus employing the corresponding sgRNA, Cas9-mSA and a biotinylated-PCR as a template, injected in two-cell embryos. Four potential founder animals were obtained out of 30 born pups. However, three of them were random insertions and the one that was inserted on-site had a rearrangement and had to be discarded. As an alternative strategy, we are currently injecting in zygotes a plasmid with Cas9/sgRNA cutting sites flanking the template sequence and homology regions, with the aim to guide the modification to H11 safe harbor locus. One hundred and forty-seven 2-cell embryos were transferred to seven B6D2 F1 pseudo pregnant females, but no pups were born.

Improving the design in order to succeed in the generation of this model is mandatory as a starting point to produce future mouse lines harboring FRET-based sensors.

1. Surdo NC, Berrera M, Koschinski A, Brescia M, MacHado MR, Carr C, et al. FRET biosensor uncovers cAMP nano-domains at b-adrenergic targets that dictate precise tuning of cardiac contractility. Nat Commun. 2017;8. doi:10.1038/ncomms15031

P-074 A novel electroporation-based CRISPR strategy for generation of conditional knockout alleles

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Conditional knockout (CKO) mouse models provide valuable insight into the etiologies of human disease and are therefore critical for the development of novel therapies. As such, improving methods to efficiently and rapidly generate CKO mice is invaluable. Here, we compared a novel approach to previously reported strategies that utilized CRISPR technology in combination with electroporation to successfully produce floxed mice. Horii et al. (2017) reported using sequential electroporation, which involves knocking in one loxP in zygotes and retargeting the same embryos at the 2-cell stage to knock in the second loxP. Alternatively, Sentmanat et al. (2022) utilized simultaneous electroporation of zygotes to knock in both loxPs in one step. Our modified simultaneous electroporation approach in zygotes combines 5' and 3' loxP oligos, Cas protein/sgRNA #1 in a complex (RNP) and Cas9 mRNA plus sgRNA#2. Taking advantage of delayed mRNA translation, we separate the two DNA cutting events, thereby reducing dropout rates and increasing our rate of successful floxing.

P-012 Colony management services (CMS): From Administration to Validation

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The CMS STP offers Cryopreservation, Genotyping, Genetic monitoring, microbiome analysis and Strain sharing/creation services, which require efficient and timely management.

To streamline these processes over the last year we have implemented pipelines and SOPs to ensure all team members carry out the actions via the same method and to provide a constant traceability of strains across the Crick. Using software and tools such as PowerBI and Notion we were able to implement a system that tracks all colony requests through the import and rederivation process. We have also implemented a programme of testing including standard genotyping and genetic monitoring profiles for all strains entering the Crick to ensure the validity of the lines researchers were requesting. Paying particular attention to projects that have improved genotyping techniques by designing specific genotyping probes replacing the previous generic options. Finally, the introduction of a translations system that part automates our genotyping entry into the mouse record database and provided the CMS team an opportunity to review every single line in the Crick for 'best practice' validation.

The improved traceability of Strains across the Crick has minimised the time taken for the requested lines to be fully rederived into the facility. The more specific genotyping and addition of genetic monitoring checks have identified and rectified issues early on preventing unnecessary breeding and maintenance of lines. The automated translations system has resulted in reduced number of animals kept in the rederivation pipeline unnecessarily and a therefore gained space that is now being used for further project development that was previously on hold.

These advantages have allowed us to maintain the 3Rs principles and we have
been visited by multiple institutes across the UK asking us to share this knowledge and help to implement these processes at their facilities.

P-038 Transgenic Rhesus Macaques Expressing Human Na+ Taurocholate Co-Transporting Polypeptide for the Study of Chronic Hepatitis B Infection

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Introduction: Although an effective vaccine against hepatitis B virus (HBV) exists, more than 296 million individuals are living with chronic HBV. With no curative therapies available, nearly one million people die every year as a result of complications arising from chronic HBV infection. The species-specific nature of HBV leads to limited immunocompetent animal models to study HBV treatments. Though rhesus macaques (RMs) are not naturally susceptible, we have shown that HBV infects RMs transduced with an adenoviral (Ad5) vector expressing the HBV entry receptor, human Na+ taurocholate co-transporting polypeptide (hNTCP), under the liver-specific transthyretin (TTR) promoter. However, “priming” RMs with Ad5-TTR-hNTCP before HBV infection has drawbacks: 1) the number of HBV-susceptible hepatocytes is limited by Ad5-TTR-hNTCP transduction efficiency, 2) Ad5-TTR-hNTCP transduction results in transient expression due to adenoviral genome loss during hepatocyte division, and 3) Ad5-TTR-hNTCP is immunogenic and could confound HBV replication dynamics.

Aim & Methods: To overcome these drawbacks, we created the first transgenic RMs with permanent germline hNTCP expression. We inserted a TTR-hNTCP piggyBac transposon expression cassette into the genomes of RM zygotes, then transferred developing embryos into surrogate dams for gestation.

Results: Genetic testing revealed integration of TTR-hNTCP in two infant RMs. The presence of hNTCP DNA in the liver, skin, muscle, lymph nodes, and rectum confirmed genomic editing in both RMs. The high specificity of the TTR promoter facilitated mRNA expression exclusively in the liver at levels comparable to humans. Isolated transgenic primary hepatocytes were HBV-susceptible ex vivo, with viral antigens detected in the supernatant, and viral DNA and RNA detected within infected cells.

Conclusions: Utilizing gene editing technology, we have generated the first HBV-susceptible transgenic RMs. This innovative approach addresses the lack of physiological relevant animal models in HBV research and provides a unique opportunity for testing HBV therapies.

P-030 Optimizing the isolation of murine bone marrow cells: A direct comparison of three protocols

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In primary cell culture, bone marrow (BM) cells are often used to differentiate various hematopoietic cell types by cultivation with the appropriate growth factors. Optimizing the protocol for BM cell isolation increases cell viability and yield, saves time and reduces the number of mice required. Here, we evaluate three methods for the isolation of murine BM cells.
1) Flushing: Both ends of prepped femurs and tibiae are carefully severed with scissors and the BM is flushed into a 5 ml petri dish with a 23 G needle.
2) Crushing: Prepped, meticulously cleaned and dried femurs and tibiae are placed in a mortar. After cracking the bones into 2-3 pieces with a pistil, buffer of choice is added and bones are gently crushed. The liquid is then collected. These steps are repeated until the bones appear pale.
3) Centrifuging: The distal ends of prepped femurs and the proximal ends of prepped tibiae are severed with scissors. Both are placed with the open end downwards into a 0.5 ml tube which has been pierced at the bottom with a 18 G needle. This tube is inserted into a 1.5 ml tube and both are centrifuged, expelling the BM.
Time wise, centrifuging is the best option, as the bones do not need to be cleaned extensively. They can be processed in parallel and tubes can be prepared beforehand. Also, there is far less detritus, higher yield and better viability compared to the crushing protocol. While flushing also yields adequate numbers of viable cells, it is more time intensive than centrifuging.
In conclusion, in our hands centrifugation of femurs and tibiae is the ideal protocol to quickly isolate the maximum number of murine BM cells without compromising the viability. In addition, the protocol can easily be adapted for other bones.

P-071 The Laboratory Animal Biotechnology Unit – more than 15 years' experience in South America

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The LABU was created in 2006 as a regional platform for the generation of genetically modified (GM) mouse models at Institut Pasteur de Montevideo. Since then, the whole unit has been in continuous evolution, incorporating new equipment, staff, services and mouse lines. Currently, 120 scientists employ animals at some point in their research, and LABU is producing 15 times more mice than in early stages, maintaining the same SPF microbiological status. At present, more than 50 GM lines are bred at the SPF area to cover a variety of research projects in the fields of cancer, metabolic disorders, and neurodegenerative and infectious diseases. Since 2014 we have introduced the CRISPR technology that has replaced the use of ES cell microinjection, increasing the efficiency and reducing the time needed to produce GM-edited mice. Additionally, ARTs improvements, for instance, the use of IVF instead of conventional superovulation and natural breeding, and ultrasuperovulation schemes have boosted embryo and sperm cryopreservation and rederivation techniques. Special emphasis to develop a preclinical research area that includes an in vivo imaging lab, surgical procedure's room, 2 behavioral testing rooms, 2 BSL3 cabinets, blood and biochemical profile determination and polyclonal antibody production has been carried out. Last but not least, our unit has organized 11 national and 8 international courses including use and welfare of lab animals, genome editing, cryopreservation and alternative methods. We have fruitfully combined services and research that are reflected in more than 50 international articles. The continuous effort and dedication of our staff ensures the excellence in research for our country and region, being the center of reference for many scientists working in these fields.

P-081 Advancing Liver Regeneration Research through Innovative Mouse models

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Introduction: Mouse models play a vital role in advancing both basic and translational research, serving as invaluable assets for investigating gene functionality, comprehending genetic ailments, pinpointing potential drug targets, and innovating new therapeutic interventions. At the Mouse Genome Engineering Core, Children's Research Institute at UTSW, we generated multiple mouse models using CRISPR Cas technology to answer questions regarding liver regeneration and homeostasis.

Aims: The liver's ability to regenerate and maintain proper tissue mass after injury is indeed remarkable and has been recognized for centuries. However, understanding the precise mechanisms and sources of new liver cells involved in tissue growth, maintenance, and regeneration has been a subject of ongoing research and debate. One of the key debates has revolved around the regenerative capacity of different liver cell populations, specifically hepatocytes and cholangiocytes, and whether there exists a liver stem or progenitor cell population. This is in part due to the lack of diverse lineage tracing strains that label different cellular populations.

Method: At the Mouse Genome Engineering Core, Children's Research Institute at UTSW, we successfully generated eleven distinct inducible knock-in mouse models. These models are designed to label specific zonal populations along the portal-central axis within the liver lobule. Additionally, we have created conditional knock-out models to investigate gene functions.

Results and Conclusion: The innovative lineage tracing reagents and knock-out models generated by our core have proven invaluable in identifying the key regenerative cell populations, both under normal homeostatic conditions and within clinically relevant liver cancer models. Beyond advancing our understanding of liver biology, this endeavor has also set a precedent for researchers studying other tissues. It showcases how mouse engineering techniques can be harnessed to capture the functional diversity present in various cell populations.

P-018 Rodent Model Resource Center (RMRC) - An eclectic rodent resource to fulfill global and domestic needs.

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The Rodent Mouse Resource Center (NMΞ) was established in 2009, and founded by the National Laboratory Animal Center (NLAC). RMRC acts as an international non-profit core center dedicated to the deposition, archiving, and distribution of genetically modified (GM) rodents. Up to now, our center has backups near 450 strains precious rodent strains generated by NLAC and domestic researchers. Over 50% of the RMRC's strains were generated by NLAC. These strains can be categorized into five major groups.

1. Humanized mice contain 5 strains. The distinct strains are APOE4 knockin and TREM2(R47H) transgenic mice for studying late-onset Alzheimer's disease—HiBiT-ACE2 knockin mice for the research on SARS-CoV-2 infection.
2. Conditional knockout (cKO) mice contain 104 strains. It includes several disease models, such as autism (Scn2a1), Alzheimer's disease (Ngf), liver tumor (Mir122a), Tourette's syndrome (Slitrk1), Blood-brain barrier dysfunction (Mir195), and polycystic kidney disease (Pdk1 or Wdr19).
3. Knockout (KO) rodents contain 10 strains. The profound strain is Apoe KO mice and rats, which can be applied to studying atherosclerosis and hyperlipidemia.
4. Cre mice contain 50 strains. Most of our cre or creERT2 transgenic mice were made through a BAC vector to mimic the expression pattern of endogenous genes. Moreover, fluorescence protein was inserted into the construct for tracing or sorting out the cells that specifically express cre. The distinct strains are endothelial-specific cre (Tek-RFP-cre), heart-specific cre (Fgf1-RFP-creERT2), striatum-specific cre (Adora2a-RFP-creERT2), and adipocyte-specific cre (Adipoq-FusRed-creERT2).
5. Reporter rodents contain 30 strains. The fluorescent and luciferase reporter rodents are helpful tools in studies related to in vivo cell tracking and tumor implantation. NLAC has generated several reporter rodents, including green, blue, red, bioluminescence, or dual-color strains to fulfill the research demand.

Therefore, RMRC supports the development of precision medicine and medical research to accelerate Taiwan's biotech and pharmaceutical industry entering the world's trend.

P-046 Refining euthanasia methods with isoflurane before cervical dislocation for sperm cryopreservation

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Isoflurane (Iso) is commonly used prior to cervical dislocation (CD) to reduce animal stress. The use of Iso prior to CD is known to reduce the viability of pre-implantation mouse embryos and to negatively impact their development in vitro. However, to our knowledge, systematic investigation of the effects of isoflurane prior to CD on sperm function and embryo development have not been reported. To investigate the effects of Iso prior to CD, we randomly separated CD-1 and C57BL/6 sperm donors into two groups – one euthanized by CD and one for which euthanasia by CD was preceded by isoflurane anesthesia. Pre-freeze and post-thaw motility and swimming patterns were compared, and no statistically significant differences were noted. In vitro fertilization (IVF) was performed for both groups. There was no statistically significant difference in fertilization rate, implantation rate or live birth rate between Iso+CD and CD groups for both CD-1 and C57BL/6 mice. Based on these results, the use of Iso prior to CD does not negatively affect sperm quality or performance and therefore should be used to refine euthanasia by CD prior to sperm collection, in keeping with the 3Rs.

P-014 Use of "Nanoblades" to target difficult-to- transfect cells

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Delivery of Cas9 and gRNAs for gene editing in eukaryotic cell lines or primary cells can be technically challenging. Several technologies are available, such as transfection, transduction, direct injection or electroporation. Each with its pros and cons. The group of Emiliano Ricci (Mangeot et al., 2019) has developed Nanoblade technology to directly introduce proteins into cell lines or embryos. Nanoblades are engineered murine leukemia virus-like particles which serve as transgene-free protein-delivery particles that allow the transfer of Cas9-sgRNA ribonucleoproteins (RNPs). We have implemented Nanoblade technology in our Transgenic Core Facility to generate knockout cell lines and primary cells. We developed a standardized pipeline to generate knockout cells. Firstly, several gRNA combinations are selected that should generate an out of frame deletion. These gRNA combinations are transfected into LentiX cells to produce the Nanoblades which are then used to deliver their cargo to a cell line of choice. A PCR screening followed by ICE analysis is performed on a pool of cells to check for the presence of cells containing the intended deletions. These pools are single cell sorted over FACS and a 3 primer PCR screening - of which 1 primer is located in the deleted region - is performed to quickly identify which clones contain the intended deletion. We already successfully used Nanoblades on several cell types such as 293T, THP1, HaCa T, Mefs and human fibroblasts.

Genome editing in primary cells and in vivo using viral-derived Nanoblades loaded with Cas9-sgRNA ribonucleoproteins. Mangeot et al., Nat Commun 2019 Jan 3;10(1):45. doi: 10.1038/s41467-018-07845-z.

P-065 Identifying genetic modifiers to understand and reverse the clinical signs of an inherited metabolic disorder, Barth Syndrome.

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Barth Syndrome (BTHS) is a rare genetic condition. Some of the symptoms in affected individuals include an enlarged heart, muscle weakness, low blood cell counts and growth delay. Like most rare diseases a single gene defect, a mutation in Tafazzin on the X-chromosome, has shown to underlie BTHS.

Although a single gene alteration underlies BTHS, the severity of the disorder can vary considerably between patients. One explanation for the variability of symptoms observed in the clinic, is the presence of modifier genes. Modifier genes can increase or decrease the severity of the condition, and in some cases can even prevent onset and progression of the disease. This makes modifier genes potentially attractive therapeutic targets and their discovery can represent a valuable opportunity for the development of novel therapies.

In order to better understand the molecular mechanisms underlying the development and progression of BTHS, we have generated a mouse model. As a result of the Tafazzin mutation mice develop phenotypes that mirror the clinical signs of BTHS. Furthermore, we could clearly demonstrate the existence of potential modifier genes in the mouse by showing that the genetic background of the mouse strain alters the impact of the Tafazzin mutation.

Analysing the molecular basis of these differences allowed us to identify candidate genes which play a role in the progression of the condition. We have subsequently shown that altering candidate genes expression is able to reverse some of the clinical signs of BTHS in our mouse models. Importantly we have confirmed that the changes in candidate gene expression, observed in the mice, are also present in patient samples from affected individuals with BTHS. These analyses demonstrate how the identification of genetic modifiers of phenotypic outcomes in mouse models could lead to new therapeutic paths for rare diseases.

P-041 Refined Protocol for Newly Onset Identification in Non-obese Diabetic Mice: An Animal-Friendly, Cost-Effective, and Efficient Alternative

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Abstract
Therapeutic interventions for diabetes are most effective when administered in the newly onset phase. However, determining the precise onset moment is challenging in spontaneous models like non-obese diabetic (NOD) mice, as diabetes develops randomly in 60 to 90% of female mice between 12 to 32 weeks of age. Conventional blood glucose (BG) surveys require extensive sampling across the entire onset window on large cohorts to identify newly diabetic mice for further testing. In this study, we explored the use of ultrasensitive urine glucose (UG) tests as a substitute for blood glucose (BG) tests before diabetes onset, aiming for more efficient and accurate diagnoses. Our survey revealed that the appearance of a small amount of glucose in the urine (approx. 25 mg/dl) can specifically accompanied or predict BG levels reaching 240 mg/dl, the diagnostic threshold for diabetes. We refined the survey protocol to conduct UG surveys twice-weekly to identify positive candidates, who were then subjected to intensive BG measurements. This approach precisely identified every single individual near the onset of diabetes, who could then be confirmed by following BG tests within the next few days. The average confirmed BG level was 350 mg/dl, lower than conventional once-weekly BG surveys (approx. 400 mg/dl). Our refined protocol substantially reduces hundreds of blood sampling, lancet usage, labor, and animal suffering, aligning with the 3Rs principle. It serves as a convenient and animal-friendly alternative for early identification of diabetes onset, facilitating research on pathogenesis, prevention, and treatment of autoimmune diabetes.

P-052 Genome editing of a human Copy Number Variant in a novel BAC transgenic mouse model of schizophrenia

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Recent genome-wide association studies (GWAS) have identified copy number variations (CNVs) at chromosomal locus 7q36.3 that significantly contribute to the risk of schizophrenia, with all of the microduplications occurring within a single gene: Vasoactive intestinal peptide receptor 2 (VIPR2) (Vacic et al., 2011). To confirm disease causality and translate such a genetic vulnerability into mechanistic and pathophysiological insights, we have developed a series of conditional VIPR2 Bacterial Artificial Chromosome (BAC) transgenic mouse models of VIPR2 CNV with one extra copy (microduplication) or four extra copies of human VIPR2, and one fully humanized model in the murine Vipr2 null background. VIPR2 duplication in mice elicits prominent dorsal striatal dopamine dysfunction, consistent with recent human neuroimaging reporting dopamine abnormalities in schizophrenia are the greatest within dorsal striatum. VIPR2 CNV mice manifest cognitive, sensorimotor gating and social behavioral deficits preceded by an increase of striatal cAMP/PKA signaling and disrupted early postnatal striatal development. Employing a non-invasive neurotropic CRISPR/Cas9 mediated genome editing in vivo, We observed an efficient and accurate deletion of 204 kb of a human genomic fragment of the duplicated CNV in multiple brain regions. Our results provide further evidence to support the GWAS studies that the dosage sensitivity intolerance of VIPR2 is causative in the manifestation of associative striatal dysfunction, and cognitive, and social behavioral deficits. The conditional BAC transgenesis offers a novel strategy to model CNV-related neurodevelopmental disorders and facilitate the genetic dissection of when/where/how the genetic vulnerabilities affect the development, structure, and function of neural circuits. Neurotropic AAV/CRISPR-Cas9 mediated deletion of VIPR2 duplications in mice represents a new somatic genome editing strategy for probing the perturbed neurodevelopment or therapy for neurodevelopmental disorders caused by a gain-of-gene dosage or aneuploidies, such as Trisomy 21.

P-084 Random Insertion Transgenesis - A Fluorescence Based Selection Approach

Ms Tabitha Tombe

Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Random insertion transgenesis has been proven useful in the past besides its drawbacks regarding position effects, concatemer arrays, and non-controllable genomic rearrangements. Without direct readout of expression patterns, selecting the right founder animals expressing the gene of interest at constant levels, can prove challenging. To render this technique more controllable, we re-designed the lox-stop-lox (LSL) vector that now allows for removal of the fluorescent reporter, utilizing flp recombinase. This way, the mouse lines can be established, the marker removed, and the fluorescent channel is free for other applications. In-vivo imaging systems were used for quantification of fluorescence in founder animals and their offspring. Based on fluorescent levels and educated guess, we selected the respective animals for further breeding. This allows for segregation of non-linked multiple integration events and to establish the mouse lines. To further demonstrate the power of this technique, we selected low, medium, and high expressing transgenic lines. Within this work, we demonstrate a novel strategy to establish random insertion transgenic mouse lines that simplifies the comparison of transgene expression levels in living mice. Non-bred transgenic F1 animals might be cryopreserved (sperm and ovary) until the selected lines are established. Considering the 3Rs, we reduce the colony size, shorten the timeline necessary for colony establishment, altogether leading to lower animal numbers.

P-011 Using extended shelf-life HTF to replace Cook's discontinued fertilization medium in an affordable state-of-the-art mouse IVF protocol

Dr Magdalena Wigger[1,2], Marco Schneider[1,2], Anni Feldmann[1,2], Sonja Assenmacher[1,2], Prof Dr Branko Zevnik[1,2], Dr Simon Tröder[1,2]

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Sperm cryopreservation is becoming increasingly popular for the archiving of genetically engineered mouse lines, offering cost, time, and animal welfare advantages. For the rederivation of these lines by IVF, we previously developed SEcuRe, an affordable state-of-the-art IVF protocol. Based on the principles of the leading CARD method, our universal SEcuRE protocol allows efficient fertilization with sperm from all major cryopreservation protocols as well as freshly harvested sperm. A key component was Cook's® "Research Vitro Fert" (RVF) fertilization medium. However, its unexpected discontinuation disrupted the applicability of our approach and other IVF protocols dependent on this fertilization medium, necessitating a suitable alternative.

Here we present our updated SEcuRe 2.0 protocol using HTF instead of RVF as a basal fertilization medium. Comparison of RVF and HTF during IVFs with cryopreserved C57BL/6 sperm from a variety of genetically engineered mice revealed equal fertilization rates, validating our approach. In addition, we demonstrate that HTF has a substantially extended shelf-life by utilizing commercial HTF that was six months past its expiration date. Expired HTF did not affect fertilization or subsequent embryonic development, underscoring the economic value of our modified approach. In summary, we demonstrate that extended shelf-life HTF can be used in place of the now-discontinued RVF medium to ensure the applicability of SEcuRe and other IVF protocols employing Cook's® RVF. With a media composition identical to the successful CARD protocol, our enhanced SEcuRe 2.0 offers IVF laboratories an easily adaptable and 3R-compliant method to efficiently archive and distribute genetically engineered mouse models with minimal costs.

P-024 Enhancing the Efficiency of Fragment Knock-in Efficiency in CRISPR/Cas9-mediated genome edited mouse model by using of HDR enhancer.

Dr Ching-yen Tsai[1], Mei-Ling Chang, Lu-A Lu, Hao-Chun Chen, You-Xuan Zhu, Chun-Ru Lin, Chun-Feng Wu

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

The application of CRISPR/Cas9-mediated genome in mic model production was first demonstrated in 2013. Among the modifications of mouse genome, the needs of direct knockout mediated by bases insertion and deletion (Indel) are much higher than precise knock-in (KI) mutation. For the past decade, researchers have demonstrated several approaches to perform CRISPR/Cas9- mediated homology-directed repair (HDR), including PITCh (Precise-Integration into Target Chromosome), Easi-CRISPR, Homology-mediated End Joining (HMEJ) and 2C-HR CRISPR etc. However, sometimes in some difficult cases, it is still hard to get a precise KI mutant no matter which approach was used.

In this study, we first demonstrated a commercialized in-vitro HDR enhancer was able to use in-vivo, i.e. in mouse embryos. We tested the embryo toxicity of the HDR enhancer and used it at a non-toxic concentration. We tested the HDR enhancer in 54 individual cases, the results showed the HRD enhancer indeed enhances the KI efficiency significantly (24.0 ± 2.7 % vs 16.42 ± 2.8). Among the 54 cases, three of them were extreme difficult to obtain precise KI founders. By using the HDR enhancer, the KI efficiencies were dramatically increased from 0% to 61.1%.

In summary, we first demonstrated the commercial in-vitro HRD enhancer was applicable in-vivo. The precise KI efficiency is significantly increased, furthermore, in those loci that are difficult to be edited, the HDR enhancer shows a positive effect on the HDR events. This enhancement of the HDR enhancer can increase the KI efficiency therefore the less animal amount was used, which comply with the 3R’s.

P-004 Precise Plasmid Vector Integration into the Rosa26 Gene of Sheep Fetal Fibroblasts using Integrase Editing.

Dr Iuri Viotti-Perisse[1], McKaily Adams[2], Dr Kenneth White[1], Dr Irina Polejaeva[1]
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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

'Integrase editing' is a new nomenclature established here to represent a promising and innovative approach in the field of genetic engineering, particularly for large DNA integration. This technique, when combined with CRISPR/Cas9, allows for the precise integration of a large vector into the host genome independent of Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) pathways. Current integrase editing approaches may be accomplished by using either CRISPR/Cas9, TwinPE, or PASTE editing tools, which will first introduce the attB sequence in the target locus before a vector integration takes place through an integrase enzyme (i.e., Bxb1). In our study, we developed an approach using CRISPR/Cas9 to insert a 38 bp attB sequence into the Rosa26 locus of sheep fetal fibroblasts, followed by the integration of the px458-SpCas9-GFP plasmid. Initially, we transfected the cells using an electroporation method with Cas9/gRNA Ribonucleoprotein (RNP) and 138 bp of a single-stranded oligodeoxynucleotides (ssODN) to introduce the attB sequence into the Rosa26 locus. Following a 24-hour recovery period, we confirmed mutations at the Rosa26 site by using PCR-RFLP and verified the presence of the attB site through PCR amplification with the endogenous gene. Subsequently, the successfully modified cells were cultured in T-25 flasks. Upon reaching confluence, we further transfected the cells with a modified px458 plasmid, containing an attP sequence upstream of the U6 promoter, along with a pCMV-Bxb1 plasmid. After 48 hours post-transfection, the cells were analyzed and confirmed by PCR amplification and Sanger sequencing to contain the px458 vector integrated into the Rosa26 locus. Our findings demonstrate the feasibility and efficiency of Bxb1 integrase-mediated gene editing in sheep fetal fibroblasts, providing valuable insights into the targeted integration of large DNA fragments. This approach holds considerable potential for advancing gene editing technologies, offering numerous applications in various scientific fields, including agriculture, medicine, and biotechnology.

P-022 KOAP: the Largest Collection of Genome Engineered Mouse Models

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Introduction: Genome engineered mice have been extensively applied in various aspects of biomedical research due to their close evolutionary relationship with human and well-developed genetic tools, however, a easy-to-approach, ready-to-use collection of these models, especially conditional floxed allele-bearing models, is absent.

Aims: To generate a world's largest collection of genome modified mice models mainly consist of KO and conditional floxed strains.

Methods: Benefited from the rapid development of CRISPR/Cas based genome modification, the KO and floxed allele of most genes were simultaneously generated and independently screened with high efficiency through simultaneous introduction of two targeting sgRNAs and single strand DNA donors.

Results: We have generated more than 20,000 independent mouse germlines, the majority of which are KO and conditional floxed strains on C57BL/6J background. Additionally, for combined application of the floxed strains, we generated over 230 Cre-expressing transgenic and targeted knock-in strains and completed validation for most of them.

Conclusion: Our resources can be easily distributed among the scientific and industrial organizations all over the world, which may significantly accelerate the process for both basic and applied researches in biology and medicine.

P-002 The RNA receptor RIG-I binding synthetic oligodeoxynucleotide promotes pneumonia survival

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Pneumonia is a worldwide threat to public health, demanding novel preventative and therapeutic strategies. The lung epithelium is a critical environmental interface that functions as a physical barrier to pathogen invasion while also actively sensing and responding to pathogens. We have reported that stimulating lung epithelial cells with a combination therapeutic consisting of a diacylated lipopeptide and a synthetic CpG oligodeoxynucleotide (ODN) induces synergistic pneumonia protection against a wide range of pathogens. We report here that mice deficient in Toll-like receptor 9 (TLR9), the previously described receptor for ODN, still partially respond to ODN. This prompted us to seek an alternate ODN receptor, and we discovered by mass spectroscopy that the RNA sensor RIG-I can also bind DNA-like ODN. ODN binding by RIG-I results in MAVS-dependent pneumonia-protective signaling events. While RIG-I is essential to native defenses against viral infections, we report that therapeutic RIG-I activation with ODN promotes pathogen killing and host survival following both viral and bacterial challenges. These data indicate that maximal ODN-induced pneumonia protection requires activation of both TLR9 and RIG-I/MAVS signaling pathways. These findings not only identify a novel pattern recognition receptor for DNA-like molecules, but reveal a potential therapeutic strategy to protect susceptible individuals against lethal pneumonias during periods of peak vulnerability.

P-007 Improving Efficiency of CRISPR-based Gene Editing via Mouse Zygote Microinjection

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[1]Harvard University, Cambridge, US

Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Genetically modified mouse models support basic and translational research to understand disease mechanisms and identify potential treatments. Our core facility has performed nearly 700 genome editing projects in mice using CRISPR/Cas9 technology. Approximately 80% of the projects were site-specific transgene insertions and ~20% were targeted gene disruptions or deletions. We will describe our latest efforts on optimizing the development and application of CRISPR derived transgenic mouse models. We will share our experience on improving the gene editing in mice with focus on improving gene knock-in efficiency to an average of around 40% by injecting plasmid DNA donor, Cas9 protein, and gRNA into mouse zygotes rather than 2-cell embryos.

We have generated mice with gene disruptions or deletions of up to 3.2 million bases (Mb), or insertions ranging from a few bp to 12kb via mouse zygote microinjection with CRISPR technology. Based on analysis of the current data, the average efficiency for gene KO through NHEJ is ~60%. The average efficiency of gene KI through HDR or HR varies by method, from ~18% using oligo DNA donors, and ~30% using long ssDNA donors to ~40% using plasmid DNA donors. Our results from approximately 250 projects using plasmid DNA donors demonstrates that high gene KI efficiency could be achieved when CRISPR reagents are injected into mouse embryos at 1-cell stage.

P-054 CRISPR-based genome editing of Nile Grass Rat, a Diurnal Rodent Species

Dr Huirong Xie[1], Katrina Linning-Duffy[2], Dr Elena Demireva[1], Bana Abolibdeh[1], Dr. Shigeki Iwase[3,4], Dr. Lily Yan[2,5]

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

Rodents are widely used in the field of biomedical research due to their translational potential. However, typical rodents like mice and rats are nocturnal and adapted to a nocturnal chronotype, in contrast to humans with diurnal biology, especially in the context of circadian rhythm and visual systems. Despite several diurnal species having been cultivated for laboratory use, repeated attempts of genome targeting to create diurnal rodent models have failed, as currently most are not genetically tractable. Here we report the successful generation of a CRISPR-Cas9 based knockout of Rai1 (Retinoic Acid-Induced 1) Nile Grass Rats, Arvicanthis niloticus, a diurnal rodent species used in laboratories for decades. The newly developed Rai1 KO grass rat lines could serve as a model for Smith-Magenis syndrome, a neurodevelopmental disorder.

Understanding the reproductive biology of a species is critical in establishing a protocol for producing donor embryos, an efficient approach for genome targeting at the correct time window, proper conditions for maintaining term pregnancy – all requirements for successful genome editing. A series of experiments were carried out to understand the reproductive fundamentals. The results showed that in grass rats, natural mating is more efficient in generating embryos than superovulation; its mating pattern and timeline of embryo development are slightly different compared to mouse or rat; co-housing males with females after targeting surgery is crucial for maintaining pregnancies to term. Multiple Rai1 KO founders were generated by delivering CRISPR RNPs via iGONAD.

Furthermore, embryo culture test showed that the rat embryo culture media mR1ECMs could support grass rat embryo development to the blastocyst stage in vitro. High deletion efficiency from 2-cell stage embryo microinjection with RNPs targeting Rai1 indicated that this method could be utilized to generate more complex genome edits requiring insertion of larger DNA fragments, such as reporters or Cre lines.

P-043 Advancing Translational Biomedical Research: Gene-Edited Rabbit Models at CAMTraST

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Rabbit models, characterized by their relatively larger size and extended lifespan, in comparison to rodent models, exhibit great potential in translational biomedical research. They often closely recapitulate disease pathologies observed in humans, including cardiovascular disorders, eye conditions, infectious diseases, and more. In July 2012, the University of Michigan launched the Center for Advanced Models for Translational Sciences and Therapeutics (CAMTraST) to bridge the gap between laboratory discoveries and clinical applications, and to facilitate the studies on the molecular mechanisms driving disease development and progression. Our team, consisting of specialists in molecular biology, embryology, and animal reproduction, has reached significant milestones. These accomplishments include the successful cloning of rabbits, and the creation of multiple rabbit models for human diseases through gene targeting.

CAMTraST operates as a non-profit resource dedicated to efficiently and cost-effectively producing and validating rabbit models for the broader national and international scientific community. Over the past decade, we've developed a highly efficient and cost-effective rabbit genome editing platform, resulting in the creation of over 40 knockout/knock-in rabbit models for various human diseases, predominantly utilizing CRISPR technologies. We also did rabbit whole-genome sequencing with PacBio method to provide a more continuous new assembly, UM_NZW_1.0. In the ISTT—TT2023 meeting, we will provide a comprehensive overview of the processes involved in generating and validating rabbit models. This encompasses reproductive technologies such as ovulation induction, zygote collection, and embryo transfer to pseudopregnant females in rabbits, a PacBio based whole genome sequencing of rabbits, along with considerations for rabbit genome editing technologies, including transgene construct design, donor DNA/vector synthesis, validation, microinjection, and genotyping. We will introduce several novel rabbit models that closely mimic patient symptoms, offering promising insights into unraveling the complexities of individual diseases and for pioneering innovative treatments.

P-059 A new experimental animal model in mammalian genetics, “The grey short-tailed opossum”

Ms Riko Yoshimi[1], Dr. Hiroshi Kiyonari[1], Dr. Takaya Abe[1], Ms Mari Kaneko[1]
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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

Marsupials represent one of three extant mammalian subclasses with several very unique characteristics not shared by other mammals. Most notably, much of the development of immaturely born neonates takes place in the external environment.

The grey short-tailed opossum (Monodelphis domestica; hereinafter “the opossum”) is thought to be the ancestor of all marsupials. The opossums are most used as experimental marsupial model because opossums are similar in body size and breeding characteristics to popular rodent models such as mice and rats. Moreover, in 2007, the opossum was the first marsupial species to have its whole genome sequenced. Like other marsupials, the opossums give birth after a short gestation period of 14 days to immature pups that are the equivalent of 13- to 15-day embryos in mice and rats or 40-day embryos in humans. Interestingly, although the opossum is a marsupial, females don’t have a pouch. After birth, the baby clings to the mother’s nipples until weaning. As a result, it has been reported that the maturity of each organ varies greatly before and after birth, with the forelimbs, jaws, tongue, and sense of smell being highly developed at birth, and the digestive, reproductive, and lymphatic systems, ears, eyes, and hindlimbs being developed after birth.

Despite these unique characteristics, the lack of technology to manipulate their genomes has hindered the development of in vivo genetic approaches in this group of mammals. Recently, we have successfully generated tyrosinase gene knockout opossums by genome editing using CRISPR/Cas9. This is the first demonstration of the production of genetically engineered animals in marsupials. This study will be able to provide a critical foundation for the venue to expand mammalian genetics to the metatherian subclass

P-079 Facilitate Cryopreservation and Re-derivation of Genetically Modified Mice via Automated Sperm Cryopreservation

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Happy Hour, Exhibition & Poster Session 1 (Odd Numbers), Imperial West, November 13, 2023, 17:30 - 19:00

The emergence of efficient genome editing technologies significantly increased the need for fast and reliable cryopreservation methods to cryopreserve extensive number of genetically modified mouse lines which are no longer needed for breeding. The various cryopreservation tools available have significantly reduced the cost and ethical concerns associated with animal studies. However, despite the progress made in this field in the past two decades, sperm cryopreservation remains a slow manual process that requires tedious and time-consuming sample aliquoting into cryo vials or straws, which can also lead to human errors and inconsistent volume and quality of the cryopreserved samples.
Here we present the development and implementation of a novel automated sperm cryopreservation procedure which utilized a STARlet Liquid Handling System and a LabElite Automated Screw Decapper. The automated sperm cryopreservation had adapted slow rate freezing with skim milk/raffinose cryoprotectant (1) to freezing in 2D barcode 200 ul Matrix tubes. We conducted a comprehensive comparison of the automated sperm cryopreservation techniques to the original manual method, which outlined significant increase of the speed of the cryopreservation process while maintaining sperm viability and post-thaw motility parameters. The procedure can be used to freeze sperm from up to 40 males in less than 90 minutes. It minimizes the chance of human error and enhances the reliability of the aliquoting and freezing steps. Post implementation data from IVF procedures utilizing cryopreserved sperm showed that the automation can improve the fertilization rates leading to more successful outcomes in subsequent sperm cryopreservation recovery procedures. Furthermore, the automation approach allows to cryopreserve large amount of genetically modified mouse lines in a short period of time. Using it, we were able to reduce significant burden on our vivarium by cryopreserving 640 males in ten weeks in a response to the Covid19 pandemic in 2020.

P-068 Re-engineering the mouse ROSA26 targeting vector for improved efficiency

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Poster Session 2 (Even Numbers), Imperial West, November 14, 2023, 15:30 - 17:00

The ROSA26 (R26) locus is a widely used safe harbor locus for transgene integration in the mouse genome. We previously reported creating more than 35 R26 models using the pR26 CAG AsiSI/MluI targeting vector (Chu et al. 2016, PMID: 26772810) with a median of 3 transgenic founders per 100 eggs injected. We received data from one investigator demonstrating evidence of leaky expression of a cDNA cloned behind the floxed stop (LSL)cassette in the vector. mRNA expression of the cDNA was present at levels similar to the endogenous gene which interfered with the investigator's research. We responded by designing new R26 targeting vectors to overcome this limitation. We report the development of a new ROSA26 vector with 1kb arms of homology and an enhanced stop cassette that prevents unwanted expression of cDNAs. Furthermore, our new R26 vector is compatible with the sgRNA targeting the canonical ROSA26 XbaI site (Chu et al.). We present data from two projects in which knockin mice were generated with both the pR26 CAG AsiSI/MluI targeting vector and our new UMTG R26 targeting vector. We compare integration rate, efficiency, and specificity of our new vector in generating ROSA26 knockin mouse models. In one project, when a cDNA behind the LSL cassette included a suspected embryonic lethal human SNP, we identified no founders with the pR26 CAG AsiSI/MluI vector, while we successfully generated a founder with our new UMTG R26 vector. A series of UMTG ROSA26 targeting vectors: CAG-LSL-MCS-bGH pA, CAG-MCS-bGH pA and non-CAG version were cloned for mouse and rat targeting. Our results suggest that these new ROSA26 targeting vectors are a powerful new tool for transgenic animal model creation.

Establishing a Reptile Research Colony for Developmental Genetic Studies

Dr Richard Behringer

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Session 5: Alternative Animal Models, Imperial East (Main Lecture Room), November 14, 2023, 09:00 - 10:20

Reptiles are understudied for developmental genetic studies. The brown anole (Anolis sagrei) has emerged as a model reptile for genetic manipulations, using CRISPR/Cas. We have established a brown anole research colony to investigate reproductive tract organ development and function. Simple and inexpensive housing in plastic cages on plastic racks was established within a space of ~110 square feet. Twigs are placed in cages for the lizards to perch on. The lizards require a misting system for water, UVA & B light, and warm temperature. The colony is stocked with lizards collected from the wild in the Houston area. Adult lizards are fed live crickets dusted with a supplement. Small plastic containers filled with potting medium are provided for females to lay eggs. Freshly laid eggs already contain advanced embryos. The eggs are incubated in small petri dishes with vermiculite. Egg incubation time is ~1 month. Hatchlings are fed flightless flies. Time to sexual maturity is ~6 months.

The effects of mouse substrain on effective generation of animal models

Dr Fernando Benavides

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Session 8: 3Rs Awards & Lecture, Orbis Pictus Lecture & Oral Presentations, Imperial East (Main Lecture Room), November 14, 2023, 14:00 - 15:30

With this presentation, I aim to raise awareness about the existence of various substrains of mice that, if overlooked, could potentially impact the results and repeatability of experiments. Many mouse users are unaware that inbred strains of mice are distributed in "families" of related substrains originating from a common ancestral strain, each with distinct genetic differences. Over time, while permanent inbreeding eliminates some new mutant alleles, another unnoticed fraction may progressively become fixed in the homozygous state, replacing the original allele—a phenomenon known as genetic drift. Mutations hidden in substrain genomes are known as passenger mutations. However, most passenger mutations carried by substrains are silent, resulting in no obvious phenotype.

The differences between C57BL/6N and C57BL/6J substrains, separated at generation F32 in 1951, are well-documented. However, numerous examples in the literature demonstrate how substrains from other inbred strains have acquired passenger mutations due to genetic drift. This presentation highlights the importance of recognizing and understanding these subtle genetic variations within mouse substrains, emphasizing the need for meticulous consideration at the time of creating GEM lines to ensure the accuracy and reproducibility of experimental results.

Xenopus Mutant Resource

Dr Marko Horb[1], Hitoshi Yoshida[1], Kelsey Coppenrath[1], Sarah Burton[1], Zoe Reynolds[1], Gary J Gorbsky[2], Thomas Kirkland[3], Michael Slater[3], Robin Hurst[3], Nikko-Ideen Shaidani[1]
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Session 9: Transgenic Resources, Imperial East (Main Lecture Room), November 15, 2023, 09:00 - 10:30

Historically, Xenopus has not been seen as a genetic model organism; but with the advent of CRISPR-Cas9 it has now become possible to make genetic mutants in Xenopus. In 2020 we established the Xenopus Mutant Resource (XMR) at the Marine Biological Laboratory. This new resource supports visiting scientists to come to the NXR and use any of the transgenic and mutant lines, make new mutants or transgenics or to come for training in husbandry or genome editing. The goal of this resource is to enhance the use of Xenopus mutants and transgenics and provide a site where multiple labs can gather and collaborate. In the last 5 years, we has focused on generating new models of human disease in both X. laevis and X. tropicalis. To date, we have generated over 250 different mutants and continue to produce more, prioritizing the generation of germline mutants. In this talk, I outline some of the progress we have made with our mutants, including our first maternal effect and sex determination mutants. I will also discuss opportunities for scientists to visit the XMR and take advantage of this new resource. Lastly, I will describe our efforts to improve homology directed repair (HDR) in Xenopus where we utilize Cas9-HaloTag to improve HDR efficiency.

Understanding the Role of Steroid Hormone Signalling in Sex-specific Development of Reptiles

Dr Bonnie Kircher, Dr Richard Behringer[1]
[1]University Of Texas, Md Anderson Cancer Center, Houston, United States
Session 5: Alternative Animal Models, Imperial East (Main Lecture Room), November 14, 2023, 09:00 - 10:20

Reproduction modes across vertebrates are diverse. Squamates (lizards and snakes) have particularly diverse reproductive strategies. Despite this, the reproductive organs of squamates have a similar overall architecture in species that have been studied. However, the order squamata is one of the most speciose group of tetrapods and knowledge of the reproductive anatomy of many species is lacking. One understudied species is the brown anole, Anolis sagrei. This species is popular as a research model and CRISPR genome editing techniques that rely on manipulation of the reproductive tract have recently been developed. Here, we present data from the brown anole (Anolis sagrei) exploring the development and adult architecture of the female reproductive system. Using histology and 3D imaging, we show that the brown anole female oviduct has three distinct anterior-to-posterior (AP) regions, the infundibulum, the glandular uterus, and the non-glandular uterus. The infundibulum has a ciliated lip, a region where the epithelium is inverted so that cilia are present on the inside and outside of the tube. The glandular uterus has epithelial ducts that are patent with a lumen as well as extensive acinar structures. The non-glandular uterus has a heterogeneous morphology from anterior to posterior and these morphological changes, coupled with AP changes in spatial gene expression, are similar to the morphological and gene expression changes that occur in the mammalian cervix. We explore the expression of steroid nuclear hormone receptors in the reproductive tract to inform genetic manipulation of steroid hormone signaling in this species. Our data complement histological studies of reproductive organ morphology and advance our understanding of reptilian reproductive tract development. This work was funded by NIH T32 HD098068 and NSF PRFB 2209150.

Genetic Approach in New Animal Models with Genome Editing

Dr Hiroshi Kiyonari[1], Mari Kaneko[1], Dr Takaya Abe[1], Ms Riko Yoshimi[1], Ken-ichi Inoue[1], Aki Shiraishi[1], Dr Yas Furuta[1,2]

[1]1 Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [2]Mouse Genetics Core Facility, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, United States

Session 5: Alternative Animal Models, Imperial East (Main Lecture Room), November 14, 2023, 09:00 - 10:20

Since the 1990s, mammalian genetics has been the era of genetically engineered mice using the embryonic stem (ES) cell-based gene targeting. Genetically engineered mice have made it possible to analyze gene functions in vivo and have opened up a new direction in genetic research to date. However, comparative studies using a variety of animal species are important to correctly understand the complex biological phenomena that occur in vivo. In recent years, the emergence of CRISPR/Cas9-mediated genome editing technologies has accelerated targeted gene modification not only in rodents, but also in a wide range of animal species for which ES cells and conventional gene targeting approaches were not available.

In this talk, I will introduce our recent developments in genetically engineered animals, focusing on the gray short-tailed opossum (Monodelphis domestica), a marsupial, and the madagascar ground gecko (Paroedura picta), a reptile, as new animal models.

Novel Transgenic Technologies and Animal Models to Evaluate Spaceflight Hazards and Confer Human Radioresistance for Deep Space Exploration and Colonization

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Session 3: Animal Models in Space Biology I, Imperial East (Main Lecture Room), November 13, 2023, 13:45 - 15:45

As NASA prepares for future exploration missions to the Moon and Mars, the countermeasure to safeguard space flight crews against deep space radiation is still exclusive, largely due to the lack of understanding of its long-term pathogenic roles. Cell signaling network (e.g., DNA damage response) has a highly modular nature, consisting of sensor, processing, and actuator functions. Synthetic biology harnesses this knowledge to develop engineered robotic systems capable of precise sensing and programmable responses, with applications in medicine and biotechnology. We previously developed single cell-BAC transgenesis, MORF, and a novel genetic sensor, PRISM, which employed the genomic instability of a hypermutable microsatellite sequence for sensing genotoxic stress. The genetic sensor enables us to access and study space radiation-damaged cells in vivo. By subjecting the PRISM sensor to realistic ground simulations of galactic cosmic radiation (GCR), we were able to map the neural exposome and obtain single-cell readouts of space radiation-elicited neuropathology. This allowed us to computationally model neuron irradiation, spanning from mice to marmosets and humans, using the Monte Carlo Simulation. In contrast to traditional genetic perturbation in all cells, we developed a synthetic sensor-actuator circuit as a “Cellular Robot,” which enables precise genetic engineering in cells damaged by space radiation. Through this design, we were able to investigate the pathogenic role of simulated GCR-induced genotoxic stress, its interaction with cellular senescence, neuroinflammation, and the autophagy/lysosome system, ultimately contributing to accelerated premature brain aging. By employing novel transgenic technologies for genetic dissection of the “space brain,” we aimed to gain a better understanding of the pathogenic effects of space radiation exposure and develop strategies to confer radioresistance for deep space exploration.

Perspectives for Equine Chorionic Gonadotropin (eCG) in Mice: Dilemma Between Drug Production, Animal Welfare, and Synthetic Compounds

Dr Alejo Menchaca

Session 9: Transgenic Resources, Imperial East (Main Lecture Room), November 15, 2023, 09:00 - 10:30

Equine chorionic gonadotropin (eCG) is a hormone used to improve fertility with numerous applications in many species, including experimental animals. The hormone was discovered almost 100 y ago as a factor found in the blood of the pregnant mares, and when administrated in non-equid species it induces both follicle-stimulating hormone (FSH) and LH activity by binding to FSH and LH receptors. Recently, the availability of the hormone has suffered certain restrictions pushed by NGOs claiming for animal welfare concerns on mare management during hormone production. Non-availability of the hormone in many countries is thus affecting the implementation of assisted reproductive technologies in different species.

Since eCG is purified from blood of pregnant mares, it has the potential to significantly compromise animal health and welfare. Like other animal management systems, implementation of a range of strategies is needed to protect the mares during the production procedures. Appropriate regulation is required, including the 5 domains of animal welfare framework for farm animals as well as the 3Rs principles for experimental animals. Strengthened inspection processes to control the implementation of guidelines and regulation would help to assure mare welfare in those companies involved in eCG production. In addition, those corporations and industries producing, selling, and using eCG have an ethical responsibility to ensure that practices for oversight, care, collection of blood, and management of pregnant mares are conducted humanely. Drug production under these conditions is therefore possible.

Synthetic hormone compounds to substitute native eCG is not commercially available yet. Recombinant technology opened an opportunity for the synthetic production of this hormone, but no acceptable outcomes had been achieved during many years. Recently, two new synthetic compounds have been reported in Argentina and Uruguay. Promising results were obtained in ovarian superstimulation and embryo production in different mice strains, cows and sheep. Although these results open new expectations for the substitution of native eCG in experimental animals, regulatory, industrial and commercial processes are required to have the product really available. In the meantime, conventional hormone production in mares may be conducted appropriately respecting animal welfare, guaranteeing the supply of this essential compound for experimental animal facilities worldwide.

Advancing Reproductive Technology for Robust Laboratory Animal Science

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Session 10: Assisted Reproductive Technologies, Imperial East (Main Lecture Room), November 15, 2023, 11:00 - 23:40

Reproductive technology has become an important tool for managing animal facilities and enhancing research projects using genetically engineered mice and rats. Until now, we have developed a robust system of CARD Mouse Bank using advanced reproductive technologies at Kumamoto University. To share reproductive technologies, we have organized the CARD Mouse Reproductive Technology Workshop worldwide since 2000. Recently, we had online workshops on mouse reproductive technology in Japan, Thailand, and Sri Lanka. This year, the CARD workshop will be restarted this November. In addition, we launched a new project of CARD Rat Bank. Recently, we developed techniques of rat sperm cryopreservation, IVF using frozen-thawed rat sperm, and cold storage of rat sperm. Using these techniques, genetically engineered rats can be efficiently managed at animal facilities. During my presentation, I will introduce these improved techniques of mouse and rat reproductive technology, elucidate their application of these techniques in animal facility management, and provide insight into the latest activities of our CARD team.

Humanization of Drosophila genes for rare disease diagnosis and research

Dr Shinya Yamamoto[1,2], Oguz Kanca[1,2], Michael F Wangler[1,2], Hugo J Bellen[1,2]
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Session 1: Precision Animal Models of Human Disease I, Imperial East (Main Lecture Room), November 13, 2023, 09:00 - 10:25

Next-generation sequencing technology has rapidly accelerated the discovery of genetic variants of interest in individuals with rare diseases. However, showing that these variants are causative of the disease in question is complex and may require functional studies. Fruit flies (Drosophila melanogaster) enables the rapid and cost-effective assessment of the effects of gene variants, which can then be validated in mammalian model organisms such as mice and in human cells. Over the past decade, our team has been developing a suite of technologies, tools, and resources to facilitate functional annotations of rare genetic variants associated with diverse rare diseases using flies (Yamamoto et al., 2023 Nat Rev Genet, PMID: 37491400). For example, one approach allows researchers to attempt 'humanization' of Drosophila genes, a method that permits experiments to probe whether the function of the fly and human orthologs are evolutionarily conserved and to further test the functional consequences of rare coding variants in the context of the human protein of interest. These and other methodologies have been allowing us to discover >30 novel disease-causing genes and variants in collaboration with diverse clinicians who are part of large clinical genomic research consortium such as the Undiagnosed Diseases Network (UDN) and Centers for Mendelian Genomics [CMG, now known as the GREGoR (Genomics Research to Elucidate the Genetics of Rare diseases) consortium]. Furthermore, thousands of reagents, including versatile CRISPR- or RMCE (recombinase-mediated cassette exchange)-mediated gene-trap lines and phiC31-integration system based UAS-human cDNA transgenic lines and constructs, are made available to the community through public stock centers to further facilitate the use of Drosophila in rare disease research. These tools and genetic strategies can be further utilized to dissect molecular mechanisms of rare diseases and identify potential drug targets, which have further implications to common diseases.