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ABSTRACTS

Program and Abstracts of the 7th Transgenic Technology Meeting (TT2007)

Elizabeth Williams

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12–14 February 2007, Brisbane, Australia
Institute for Molecular Bioscience.
The University of Queensland.
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Dmitry Ovchinnikov (IMB)
Frances Lemckert (Children's Hospital Westmead, Sydney)
Julie Scott (WEHI, Melbourne)
Graham Kay (QIMR, Brisbane)
Ric Broadhurst (AgResearch, New Zealand)
Johannes Wilbertz (Karolinska Institutet, Stockholm)
Lluís Montoliu (CNB-CSIC, Madrid)

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TT2007 Program

Monday, 12th February, 2007

12.00–2.00 pm	Registration for all participants. Posters to be set-up
2.00–2.15 pm	Welcome address— **Elizabeth Williams** (Chair of Organizing Committee)

Session I: Assisted Reproduction—Applications in Transgenic and Knock-out Production

Chair: **Ric Broadhurst** (AgResearch, New Zealand)

2.15–2.45 pm	Mouse cloning-methods and applications **Bjorn Oback** AgResearch, Hamilton, New Zealand
2.45–3.15 pm	New Transgenesis and Transfection with Transposase Enzymes **Stefan Moisyadi** University of Hawaii, USA
3.15–3.45 pm	Overview of Mouse IVF **George Thouas** Monash University, Melbourne
3.45–4.15 pm	Afternoon Tea

Session II: genOway prize for transgenic technologies

4.15–4.30 pm	Presentation of genOway Prize for Transgene Technologies **Kader Thiam** genOway, Lyon, France
4.30–5.30 pm	Talk given by the TT2007 genOway prize Winner **Charles Babinet** Unite de Biologie du Developpement, Institut Pasteur
6.00–8.00 pm	Welcome Reception- Drinks and Snacks IMB Rooftop

Tuesday 13th February

Session III: Embryonic Stem Cells—Designs, Strategies and Novel Applications for their use in Knock-out Studies

Chair: **Dmitry Ovchinnikov** (IMB, Brisbane)

9.00–9.30 am	Tissue and Temporal Control of Gene Expression in vivo **Klaus Mathieu** The Australian National University, Canberra
9.30–10.00 am	Development of a transposon insertional mutagenesis system in rats **Richard Behringer** UT MD Anderson Cancer Center, Houston
10.00–10.30 am	Transcription Factors, Non-coding RNAs and ES cell Differentiation in vitro and in vivo **Andrew Perkins** IMB, Brisbane
10.30–11.30 am	Poster Session- presenters must be with posters Morning Tea

Tuesday 13th February

Session IV: New Trends in Transgenic Production

Chair: **Graham Kay** (QIMR, Brisbane)

11.30–12.00 pm	Targeting Vector Construction Platform **Kader Thiam** genoway, Lyon
12.00–12.30 pm	**Frank Koentgen** Ozgene, Perth
12.30–1.00 pm	Experimental Approaches to Study Gene Function in Whole Mouse Embryos **Satomi Tanaka** Kumamoto University, Japan

Session V: Running a Transgenic Unit. Round-table discussion

Chair: **Lluís Montoliu** (CNB-CSIC, Madrid)

1.45–3.30 pm	**Ric Broadhurst** (AgResearch, New Zealand) **Karen Brennan** (Children's Hospital, Westmead) **Johannes Wilbertz** (Karolinska Institutet., Stockholm) **Kazuki Nakao** (Riken, Kobe)
3.30 pm–4.00 pm	Afternoon Tea

Session VI: New Work/Short Presentations

Chair: **Johannes Wilbertz** (Karolinska Institutet, Stockholm)

4.00 pm–5.30 pm	Oral Presentations to be selected among submitted abstracts
5.30 pm–6.30 pm	Meeting for General Assembly of ISTT members
6.30 pm	Buses leave for Toowong/City
7.30 pm	Conference Dinner ''Oxley's'' On the River 330 Coronation Drive, Milton

Wednesday 14th February

Session VII: Use of Transgenics in Research

Chair: **Julie Scott** (WEHI, Melbourne)

9.00 am–9.30 am	The use of Transgenes to Study Macrophage Differentiation and Function **David Hume** IMB, Brisbane
9.30 am–10.00 am	Working with Transgenic Animals to Advance Health Care **Kerry Fowler** Murdoch Children's Research Institute, Melbourne
10.00 am–10.30 am	Transgenic Zebrafish: Real-time Imaging in a Living Vertebrate **Phil Crosier** University of Auckland, New Zealand
10.30 am–11.00 am	Structural and Functional Analysis of Casein gene Expression Neighbourhood **Satish Kumar** CCMB, Hyderabad, India
11.00 am – 12.00 pm	Poster Session- presenters must be with posters Morning Tea

Session VIII: Services for Transgenic Research

Chair: **Elizabeth Williams** (IMB, Brisbane)

12.00 pm–12.30 pm	NHMRC Australian Phenome Bank **Stuart Read** Australian Phenomics Facility, Canberra
12.30 pm–1.00 pm	Speed Congenics **Darryl Irwin** Sequenom, Brisbane
1.00 pm–1.30 pm	Genetically Engineered Animals- Outreach and Education **Jan Parker-Thornberg** MD Anderson Cancer Centre, Houston
1.30 pm–2.15 pm	Lunch—IMB Rooftop

Session VIIII: Gamete and Embryo Cryopreservation

Chair: **Frances Lemckert** (Children's Hospital Westmead, Sydney)

2.15 pm–2.35 pm	Cryopreservation of Mouse Sperm **Carlisle Landel** Thomas Jefferson University, Philadelphia
2.35 pm–2.55 pm	Cryoloop Vitrification of Mouse Oocytes and Embryos Maintains Cellular Integrity and Increases Embryo Viability. **Michelle Lane** Adelaide University
2.55 pm–3.15 pm	Cryopreservation of Ovarian Tissue **Jillian Shaw** Monash University, Melbourne
3.15 pm–4.00 pm	Cryopreservation Discussion time
4.00 pm–4.10 pm	Concluding Remarks – **Elizabeth Williams** (Chair of Organizing Committee)
4.10–4.30 pm	Presentation by TT2008 Host

End of the TT2007 meeting

1 Mouse cloning: methods and applications

Fleur C. Oback, David N. Wells, Björn Oback*
AgResearch, Reproductive Technologies, Ruakura Research Centre, Hamilton, New Zealand

Nuclear transfer (NT) cloning was developed in the 1950s in frogs and first applied to mammals in the mid 1970–1980s. The first four decades of NT experiments were dominated by the idea that cloning success depends on the donor cell being embryonic, however, this notion changed radically with the cloning of Dolly in 1996 from a differentiated mammary gland cell by somatic cell NT (SCNT). Since then, SCNT cloning has expanded into a world-wide research field, spanning over 160 laboratories across at least 37 nations and resulting in cloned offspring in 15 mammalian species to date. This growing list could easily detract from the fact that SCNT remains a labor-intensive procedure that requires high technical skills and still fails to generate viable offspring in about 95–99% of attempts, depending on the species.

While the initial objective behind SCNT was commercially driven—namely to multiply genetically superior animals with desired phenotypic traits and to produce transgenic livestock for agriculture and biomedicine—researchers now use SCNT as a method to address diverse questions in developmental and cell biology. This includes the nature of epigenetic information, imprinting during gametogenesis, the mechanisms regulating cell plasticity, differentiation and regeneration, and epigenetics of cancer. Naturally, such basic questions are best answered using the laboratory mouse with its short gestation period, large litter size, precisely described embryonic development and well-characterised, easily manipulated genome. However, for all its obvious advantages, there is also one major drawback—mice are very difficult to clone. Although first reported in 1998, still only a handful of laboratories worldwide can reproducibly clone viable mice from somatic cells. Cloning from embryonic stem (ES) cells is often more reliable, enabling, in principle, the production of transgenic animals from ES cells with targeted mutations in a single generation.

This paper reviews current mouse cloning methodology and presents a simplified zona-free mouse NT procedure that increases ease of operation, reproducibility and throughput in cloned embryo and cloned offspring production. It also discusses the biological causes underlying low cloning efficiency. These causes seriously limit the use of NT to produce transgenic animals from ES cells for basic research.

This work was funded by the New Zealand Foundation for Research, Science and Technology and AgResearch.

2 New transgenesis and transfection with transposase enzymes

Ryota Suganuma[1], Kazuto Morozumi[1,5], Pawel Pelczar[2], Joseph M. Kaminski[3], Ryuzo Yanagimachi[4] Stefan Moisyadi[4]*
[1]Department of Obstetrics and Gynaecology, Fukushima Medical University, 1 Hikarigaoka, Fukushima, Japan; [2]University of Zurich, Institute of Laboratory Animal Services, CH-8091 Zurich, Switzerland; [3]Center for Molecular Chaperone, Radiobiology, and Cancer Virology, Medical College of Georgia, Augusta, GA 30912, USA; [4]Department of Anatomy, Biochemistry and Physiology, John A. Burns School of Medicine, Honolulu, Hawaii 96822, USA

Several methods have been developed for producing transgenic animals, including pronuclear microinjection, ICSI-Tr, virus-mediated insertion, and ES cell-mediated approaches. Of these only the virus-mediated insertion (Lentiviral) employs an active mode of transgene insertion, with the others relying on the repair mechanism of the oocyte for transgene integration. Since the development of ICSI-Tr which works optimally only with freeze-thawed sperm, we concentrated on improving the integration of transgenes in mice by prodding active transgenesis procedures. Among approaches with protein recombinases and transposases, the hyperactive Tn5 transposase protein (*Tn5p) was by far the most efficient method when introducing the transgene in a transposon along with spermatozoa into unfertilized oocytes (TN:ICSI). The technique also proved effective with round spermatids (TN:ROSI). In our hands, this approach dramatically increased the efficiency

of producing transgenic mice, with 11% of eggs injected and 22% of live births resulting in transmission of the transgene DNA and over 75% of transgenic mice expressing the EGFP transgene. Of these transgenic animals, 25% had one or two copies of the transgene inserted in their genome, making them ideal for mutagenesis experiments where the revealing of gene function is important.

However, because TN:ICSI and TN:ROSI methods suffer from cumbersome enzyme preparation techniques, we have now moved away from the enzymatic insertions of transgenes and developed DNA based procedures that allow synthesis of the transposase in-situ. During four mammalian cell transfection experiments using several in-situ synthesized transposases, we found that *piggyBac*, a transposase isolated from the cabbage looper moth *Trichoplusia ni*, is the most effective for stable insertion of transgenes. To achieve target specificity, we fused the GAL4 DNA binding domain to the N-terminal of the *piggyBac* protein and determined the activity of the chimeras by chromosome integration assays. The GAL4-*piggyBac* transposase displayed an activity similar to that of wild-type *piggyBac* and inserted at its regular tetranucleotide site (TTAA). We therefore suggest that this transposon system, because of its flexibility for molecular engineering and its relatively high transposition activity, could be ideal for mammalian transgenesis and pre-clinical gene therapy experiments.

3 Overview of mouse IVF

George Thouas*
[1]Monash University Biomedical Engineering and Technology Alliance (MuBeta), Department of Mechanical Engineering, Clayton, Australia; [2]Monash Immunology and Stem Cell Laboratories (MISCL), Monash University, Wellington Road, Clayton, VIC 3800, Australia

In vitro production (IVP) of pre-implantation embryos by oocyte in vitro fertilization (IVF) is an important laboratory process in animal biotechnology and clinical assisted reproduction. Laboratory mice are amongst the first mammalian species in which successful IVF and continuous zygote-to-blastocyst stage embryo development has been achieved. Key experimental advantages of the mouse model include its amenability to breeding and genetic manipulation, the variety and availability of unique strains and the accessibility of the female reproductive tract for tissue isolation and embryo replacement.

In the mouse, IVF essentially involves the ex-vivo insemination of mature oocytes isolated from hormonally stimulated female animals. Removal of attached ovarian support cells from oocytes enables the assessment of meiotic maturity, indicated by the presence of the first polar body. Mature oocytes are then inseminated by short-term co-incubation with known concentrations of purified, capacitated epididymal spermatozoa. This procedure is referred to as "standard IVF". An equally effective alternative method is intra-cytoplasmic sperm injection (ICSI), whereby single spermatozoa are microinjected directly into the oocyte cytoplasm. In clinical IVF laboratories, ICSI has surpassed standard IVF as the preferred method of insemination. Although labour intensive, ISCI circumvents the incidence of abnormal fertilization caused by polyspermy, a common observation in mouse oocytes inseminated using epididymal extracts. Following insemination, a series of morphological changes to the oocyte indicate normal fertilization and formation of a zygote (one-cell embryo). Sequentially, these events include second polar body extrusion (signifying sperm penetration, cell-cycle activation, and oocyte haploidization); formation of a male and a female pronucleus (signifying gamete chromosomal reassembly and compartmentalization), and syngamy (fusion of pronuclei to form a diploid genome). Zygotes are routinely cultured to the two-cell stage (shortly after syngamy) or further to the blastocyst stage (96 h after fertilization), at which times embryo transfer is most commonly performed.

Assisted breeding of genetically modified mice routinely involves the manipulation of IVF/IVP pre-implantation embryos. Direct introduction of gene constructs has been performed previously by microinjection into zygotes or blastocysts, although inefficiencies arise due to the randomness of construct integration. More recent alternatives include sperm mediated transgenesis of

oocytes, and the construction of chimeric cleavage-stage embryos and blastocysts using genetically modified embryonic stem cells. These more precise methods represent valuable improvements in the efficiency of site-directed transgenesis and mutagenesis of IVF/IVP embryos.

4 Tissue and temporal control of gene function in vivo

Klaus I. Matthaei[1]*, Kristy E. Lam[1], T. Hilton Grayson[2], Heidi Scrable[3] Caryl E. Hill[2]
[1]Division of Molecular Bioscience, The John Curtin School of Medical Research, The Australia National University, Canberra 0200, Australia; [2]Division of Neuroscience, The John Curtin School of Medical Research, The Australia National University, Canberra Australia 0200; [3]The University of Virginia Department of Neuroscience, Charlottesville, VA 22908, USA

The use of genetically modified (gene deficient or transgenic) mice for the understanding of gene function in vivo has been the premier tool for more than two decades. However, the method suffers from a number of potential as well as real deficiencies. These include embryonic lethality, genetic strain variation, transgenic ''leakiness'' as well as epigenetic mechanisms (1). A number of different methods (e.g. Cre/LoxP, tetracycline controlled) have been devised to overcome some of these problems but all of these are either not reversible or suffer from ''leakiness'' of transgenes. Recently a new method based on the *E. coli* Lac Operon system was reported that promised tightly regulated tissue and temporal specific control of gene function in mice (2). Our latest results using this technique will be described.

References

1. Klaus I Matthaei (2004)
''Caveats of Gene Targeted and Transgenic Mice''
in Handbook of Stem Cells, Volume 1: Embryonic Stem Cells, Elsevier
Academic Press, (Lanza et al. ed) pp. 589–598.
2. Carolyn A Cronin, Wendy Gluba and Heidi Scrable (2001)
''The lac operator-repressor system is functional in the mouse.''
Genes and Development 15: 1506–1517.

5 Development of a transposon insertional mutagenesis system in rats

Chuan-Wei Jang[1,2], Paul A. Overbeek[1,3], Richard Behringer[1,2]*
[1]Program in Developmental Biology, Baylor College of Medicine, Houston, Texas, USA; Department of Molecular Genetics, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA; [3]Department of Cellular & Molecular Biology, Baylor College of Medicine, Houston, Texas, USA

Rats are very important laboratory animals. These rodents are especially useful for physiological, neurobehavioral, transplantation, and toxicological studies. Although not traditionally used for genetic studies like the mouse, extensive genomic resources for the rat have recently become available. Thus, the rat model system is now primed for functional genomic studies. However, one particularly valuable resource to facilitate studies of gene function that is sorely lacking in the rat system is a large collection of mutants and a continuous source of new mutations. There are currently no embryonic stem (ES) cells for this species that could be used to generate targeted mutations. The ability to easily generate new mutant rat strains and to efficiently identify the mutated genes would significantly advance the use of this central laboratory animal for biomedical research.

We have developed a transposon-mediated insertional mutagenesis system in rats. Transgenic rats were generated by very efficient transposition-mediated integration of a Piggy Bac transposon carrying a *tyrosinase* gene. Expression of the tyrosinase transgene cures the albinism of a Sprague-Dawley outbred stock. In addition, we have generated transgenic rats carrying an *Oct4*-transposase gene construct for germ line transposase expression. These two types of transgenic rats are used in a breeding scheme to mobilize the transposon for insertional mutagenesis. The *tyrosinase*-carrrying

transposon also has gene trap sequences to increase mutagenicity. Genes mutated by transposon insertions can be quickly identified by inverse PCR and comparison with the rat genome sequence. This simple mutagenesis system should enhance the rat system for functional genomic studies.

6 Transcription factors, non-coding RNAs and ES cell differentiation in vitro and in vivo

Andrew Perkins*, Stephen Bruce, Marcel Dinger, Brooke Gardiner, Anita Steptoe, Lez Burke, John Mattick, Sean Grimmond
Institute for Molecular Bioscience, & ARC Special Research Centre in Functional and Applied Genomics, University of Queensland, Brisbane, Australia

ES cell differentiation systems provide an accessible model system for stem cell self-renewal and differentiation into tissues from all germ layers. We have performed extensive expression profiling of ES cell differentiation in semisolid cultures systems containing serum or serum-free chemically-defined media using illumina® 48 K chips. We have also examined the expression of a large custom set of non-coding RNAs including microRNAs and non-coding RNAs which are less easily classified. Expression of most of the genome (both coding and non-coding) varies in a very dynamic way during 16 days of differentiation. Using a number of clustering algorithms, we have identified novel genes with syn-expression patterns suggestive of a role in stem cell self-renewal and mesoderm differentiation. Validation has been performed using a number of complimentary methods and functional testing is being performed using lentiviral RNAi in ES cells and in mice. Lastly, approaches to the study of transcription factor interacting networks in ES cells and in vivo will be discussed.

7 Kader Thiam

8 Frank Koentgen

9 Experimental approaches to study gene function in whole mouse embryo and fetal ovary in vitro

Satomi S. Tanaka[1*], Yasuka L. Yamaguchi[1*], Patrick P. L. Tam[1,2]
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The conventional approach of studying gene function in the mouse has been focused on transgenesis or targeted mutation in the whole organism. An expeditious alternative is to analyze the impact of modulating genetic activity in vitro on whole mouse embryos and fetal organs. Both gain and loss of function tests could be performed efficiently by introducing expression vectors or short interference RNA by electroporation or lipofection. Another efficacious strategy is to alter gene activity by expressing short hairpin RNAs which are targeted to specific genes in the embryonic stem (ES) cells and testing the effect on cell differentiation and embryonic development in completely ES cell-derived embryos generated by the tetraploid aggregation technique. We will review the outcome of studies on the function of *Ifitm1, Ifitm3* and *Importin13* (*Ipo13*) using a combination of these techniques. Our results show that Ifitm1 and Ifitm3 play distinct roles in the navigation of migrating mouse PGCs and that Ipo13 is required for meiotic mouse germ cell differentiation in a stage-specific manner.

10 The use of transgenes to study macrophage differentiation and function

David A. Hume
ARC Special Research Centre for Functional and Applied Genomics & CRC for Chronic Inflammatory Diseases, Institute for Molecular Bioscience, University of Queensland, Brisbane 4072, Australia

The mononuclear phagocyte system is a family of cells comprised of precursors in bone marrow, blood monocytes and tissue macrophages. Their growth and differentiation is controlled by macrophage colony-stimulating factor (CSF-1), which

acts through a receptor, c-fms or CSF-1R. The CSF-1R gene is expressed specifically in mononuclear phagocytes. The only other site of expression is in placental trophoblasts. Our research on CSF-1R has focussed on two areas. Firstly, we have used conventional transgenes, in which promoter fragments are connected to reporters, to elucidate the regions of the CSF-1R locus that are required to direct approach expression in a copy-number and position independent manner. Additionally, we are dissecting the distinct regions required for macrophage and trophoblast expression. We have produced mice (the MacGreen line) in which all of the macrophages express EGFP and used these animals in many studies of macrophage biology in vivo. Secondly, we have used the CSF-1R promoter region to generate a range of tools to permit constitutive, inducible or amplified expression of transgenes in the macrophage lineage, to disrupt gene expression in macrophages, or to ablate macrophage lineage cells.

11 The use of transgenic animals in biomedical research

Kerry J. Fowler
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In recent decades, transgenic animals have provided a rich biological resource for understanding the functional role of mammalian genes in health and disease. This powerful technology has enabled the generation of numerous mouse models of human disease for preclinical testing of therapeutic compounds (for review, see Benson et al., *Nature*, 441:451–456, 2006) and for in vivo assessment using state-of-art tools such as micro-imaging (reviewed by Rudin and Weissleder, *Nature Rev* 2:123–131, 2003). Furthermore, genetically engineered animals have been used to produce human proteins for drug development and testing. Thus far, more than 30 purified transgenic products have been approved for clinical trials in humans (Lonberg, *Nature Biotech*, 23(9):1117–1125, 2005; News in Brief, *Nature*, 441: 681, 2006).

At the MCRI in Melbourne, scientists and clinicians working in specific teams have made use of GM mice to help explain the pathogenesis of multifactor diseases such as asthma and arthritis. In addition, humanised mouse models of thalassemia and methyl malonic aciduria have been developed to trial cell-based therapies whereas humanised Freidreich ataxia mice are being used for high-throughput chemical screening for identifying effective molecules that target and regulate gene expression.

Collectively this small snapshot of mouse mutants highlight the important role that transgenic animals play in unravelling bedside observations at the laboratory bench with a view to returning to the patient with new insight for improved health care and management.

12 Transgenic zebrafish: real-time imaging in a living vertebrate

Chris Hall, Maria Flores, Enid Lam, Kathy Crosier, Phil Crosier*
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The value of transgenic model systems in the investigation of developmental processes is well recognised. This technique, coupled with the intrinsic attributes that the zebrafish offers as a model system, principally its exquisite optical transparency and ex utero development, have enabled this model to provide significant insights into novel developmental mechanisms. Numerous stable transgenic zebrafish lines have been generated providing a unique opportunity to study specific aspects of development previously inaccessible within a living vertebrate model system, from neuronal tracking to blood vessel formation. Historically, a major obstacle in generating stable transgenic zebrafish was the extremely low frequency at which exogenous genetic material is integrated into chromosomal DNA and the high germline mosaicism within the injected founder

population. Although these problems could be overcome by injecting large numbers of embryos, a new nonautonomous transposon-mediated approach has significantly addressed these issues. By injecting transgenic constructs flanked by sequence of the *Tol2* transposable element, in the presence of co-injected in vitro transcribed transposase transcript to catalyse transposition, germline transgenic founders can be routinely generated with high efficiency (in some cases up to 50% of injected embryos) (reviewed in Kawakami, 2005). In our research group we have successfully adopted the *Tol2* system to generate stable germline transgenic zebrafish.

We are interested in applying zebrafish transgenesis to answer questions specific to haematopoiesis and immune cell function. We are in the process of generating transgenic GFP reporter lines for the Runx transcription factors to facilitate lineage tracing of definitive haematopoietic precursor-cell populations. We are also interested in generating a bank of transgenic lines in which specific immune cell compartments are fluorescently labelled facilitating real-time in vivo characterisation of an immune cell response within various tissue compartments. In particular we are interested in the mucosal immune response within the zebrafish gut of both wild-type and pathogen-challenged animals. Details of these transgenic cell lines will be discussed in this presentation.

Kawakami K. 2005. Transposon Tools and Methods in Zebrafish. Dev. Dyn. 234:244–254.

13 Structural and functional analysis of casein gene expression neighbourhood

Satish Kumar
Centre for Cellular and Molecular Biology, Hyderabad 500007, India

Co-expressed and co-regulated genes are frequently closely linked in the genome and are often found in a defined order of preference. The casein locus represents one such example. We have taken a transgenic approach to understand the functional significance of the organization of this locus. We have shown that the targeted disruption of the *k*-casein gene leads to failure of lactation, and thus, indirectly results in the interruption of the process of reproduction. We have proposed that organisation of a *k*-casein gene would have been a significant event during the mammalian evolution. Currently, we are examining whether *k*-casein gene of *Echidina*, an egg-laying mammal, would restore lactation in the mutant mice. More interestingly, the apparent failure of lactation in the *k*-casein-deficient mice is manifested during mid-pregnancy itself, indicating one of the functional constraints on the organization of the casein locus. We are addressing this question by (i) a conditional rescue of the *k*-casein mutant phenotype through a temporally regulated transgene, (ii) relocation of the *k*-casein gene ~450 kb away from its native position through engineering a cre-mediated inversion, and (iii) removal of all the remaining casein genes using cre-mediated deletion spanning ~250 kb of DNA at this locus.

14 NHMRC Australian Phenome bank

Stuart Read[1]*, David Porter[1], Moira O'Bryan[2], Chris Goodnow[1]
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Australian database of genetically modified murine strains

Until now, it has been quite difficult for researchers to determine whether a particular strain of mouse already exists in Australia. Several societies have put considerable effort into establishing databases of murine strains however they have not had the resources to maintain or expand these databases.

Through an NHMRC Enabling grant the Australian Phenomics Facility (APF) has developed an Internet accessible database of murine strains housed in Australia. This database has been titled NHMRC Australian Phenome Bank. The Phenome Bank database provides Australian researchers with a central location to gain information about strains of mice maintained in Australia, whether alive or cryopreserved.

The database can be used to identify whether mice carrying mutations in a particular gene exist

within Australia. Future versions of the database will allow researchers interested in a particular disease or developmental stage to search the Phenome Bank database for mice harbouring abnormalities within specific organs, tissues or cell types or biological functions resulting in disease or developmental abnormalities.

The databases established by ANZSLAS and ANZSCDBI have been incorporated into the Phenome Bank. The strains housed at APF and the ENU mutants generated from library screens have and are also being incorporated into the database as they are identified.

The Phenome Bank database will foster exchange and expertise and minimise duplication of mouse resources.

Cryopreservation of murine sperm

As part of our services the APF is offering cryopreservation of murine sperm. Through the support of an NHMRC Enabling grant the cryopreservation is available at no charge for strains that are distributable to other researchers within Australia. The depositor retains ownership of the strain and MTAs with requestors are permitted, if required. The APF offers to cryopreserve, store, rederive and distribute the strains. Duplicate samples are stored at both ANU and Monash to prevent accidental loss occurring from equipment failure.

Federation of International Mouse Resources (FIMRe)

The APF is a founding member of FIMRe. This organisation represents a global effort to coordinate access and archiving of mouse genetic variants.

This presentation will further discuss the database and cryopreservation service and how researchers and managers of animal facilities can access these.

15 Genetically engineered animals—outreach and education

Jan Parker-Thornburg
Department of Biochemistry and Molecular Biology, M. D. Anderson Cancer Center, Houston, TX, USA

From the push to teach creationism in the schools, the protests against GM foods, and the failure to acknowledge global warming as a problem, the world public (and, particularly the American public) has demonstrated an abysmal failure to understand science. As practicing scientists, and especially as the creators of genetically engineered animals, we are in a unique position to educate our co-workers, in addition to the general public regarding the need for sound science, as well as for the use of GM animals. This presentation will describe publicly available materials that can be used for public outreach and education, provide examples of presentations made to diverse public groups, and discuss common questions that the public raises when presented with material about genetic modifications in animals.

16 Speed congenics

Darryl L. Irwin
Sequenom Inc. Asia Pacific, 300 Herston Rd, Herston 4006, QLD, Australia

Mouse models play an important role in understanding the genetics and pathophysiology of human disease and other traits determined by either single genes or sets of multiple loci. The completion of the sequencing of inbred mouse strains and subsequent databases of single nucleotide polymorphisms (SNP) between inbred strains have provided a valuable tools for genetic mapping in the mouse. Using SNP markers the time involved in creating congenic mice can be shortened greatly through the use of marker assisted selection (so-called speed congenics). Mice at the second backcross generation are typed for markers distributed across the entire mouse genome and only those mice that carry the highest percent of the genome of the desired strain are used to produce the next backcross generation. This process is again repeated at each successive backcross generation. Moreover, selecting offspring containing donor genetic material close to the centromere increases the chance of recombination in subsequent generations. In this way, it is possible to

create congenic lines in only three to four backcross generations (~1 year) as opposed to 10 backcross generations for normal congenic strains.

We use the Sequenom MassARRAY iPlex system for high-throughput, low cost custom SNP genotyping. By consulting public databases we constructed a panel of 680 evenly spaced SNPs providing informative markers at a 3–5 cM level in crosses of all common inbred mouse strains. This highly multiplexed panel provides sufficient density to facilitate 'speed congenics' in the F2 to F4 generations, requiring only small amounts (500 ng) of genomic DNA. Furthermore the flexibility of the Sequenom system allows low cost development of custom panels to further characterise ongoing generations. Using this approach the time and cost required to develop congenic strains is significantly reduced.

17 Cryopreservation of mouse sperm

Carlisle P. Landel
Kimmel Cancer Center, Thomas Jefferson University, 233 S 10th Street, Philadelphia, PA 19107, USA

The adoption of sperm cryopreservation for archiving strains of mice has been hampered by a general lack of success in applying current techniques to sperm of inbred mouse strains. We have investigated modifications of these techniques, and identified a number of modifications in the cooling rate during freezing and in handling after thawing that allow successful cryopreservation of sperm from inbred strains. This allows the reliable recovery of mice from frozen C57BL/6J sperm and has enabled the production of embryos by in vitro fertilization from a number of inbred strains, including 129S1/SvImJ, BALB/cByJ, A/J, NOD/LtJ, NON/LtJ, SJL/J and DBA/1J. We have also begun to investigate the genetic basis for the strain variation in the recovery of cryopreserved sperm, and have identified a number of regions in the genome that may harbor genes regulating this phenomenon.

18 Cryoloop vitrification of mouse oocytes and embryos maintains cellular integrity and increases embryo viablility

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With the increasing number of transgenic mouse lines being created there is a need for a reliable method for the cryostorage of mouse gametes and embryos that will result in high rates of subsequent survival and development after transfer to pseudopregnant recipients. Ultrarapid vitrification involves vitrifying samples in high concentrations of cryoprotectants in small volumes that are cooled at rates around 20,000°C/min. This technique also has the advantage over conventional slow-freezing procedures in that it is quick (around 1 min) and does not require specialised equipment.

We have used one such ultra-rapid vitrification procedure using the cryoloop to successfully vitrify mouse oocytes and embryos. We have determined that cryoloop vitrification results in reduced damage to cellular organelles compared to slow freezing. Specifically slow-freezing of oocytes causes unrecoverable perturbations to mitochondrial distribution and membrane potential. In contrast, vitrification caused fewer perturbations to the mitochondria. As a result vitrification resulted in increased fertilization and development rates and more normal gene expression. Furthermore, we have determined that the inclusion of an osmolyte to the vitrification solutions results in further reduction in cellular stress so that mitochondrial measurements were not different to control oocytes that were not cryopreserved and significantly increased subsequent development to the blastocyst stage. Transfer of resultant blastocysts also increased viability after transfer.

The use of the cryoloop vitrification combined with an osmolyte is therefore a useful method for the rapid and routine cryo-storage of mouse oocytes and embryos which results in high pregnancy rates and fetal development after embryo transfer.

19 Cryopreservation of ovarian tissue

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In cases where it is important to store a female's germline, ovarian tissue cryopreservation can offer an attractive alternative (or adjunct) to the preservation of mature oocytes.

Although live offspring were obtained from frozen thawed ovarian grafts in 1960, ovarian cryopreservation received little attention in comparison to either oocyte or embryo cryopreservation until about 1990, when its potential to save the germ cells of young human females with cancer was realized. Ovarian cryopreservation is however also used in animal husbandry, e.g. for mouse strains and germ cell banking for rare and endangered species. Its use is not restricted to mammals as frozen ovarian grafts have also been successful in insects.

The main differences between ovarian and oocyte cryopreservation are:

(A) mature oocytes are usually only available for short periods of time in small numbers and, unless superovulation has been used, only in females of reproductive age. By contrast ovaries contain significant numbers of follicles (with oocytes) from fetal life until the end of her reproductive life. Ovaries contain follicles irrespective of puberty, cycle stage or superovulatory drugs. A single ovarian tissue sample, collected by means of a relatively simple surgical procedure or at autopsy, should therefore contain many thousand primordial (dormant) and developing follicles.

(B) Mature oocytes are very sensitive to environmental, procedural and cryopreservation conditions, and there are very significant species specific differences. Follicles within ovarian tissue of most species appear by contrast to be relatively resistant to most stressors including cryoinjury.

(C) Cryopreservation of mature oocytes is becoming more effective, but an individual oocyte rarely gives rise to more than one progeny. Ovaries and ovarian pieces which are collected and processed under appropriate conditions may by contrast restore full fertility to a recipient for several months or years and give rise to multiple offspring/litters.

(D) Cryopreserved mature oocytes or embryos are easy to insert non-surgically and may implant in the uterus of any receptive female of the same species. Ovarian tissue is significantly more antigenic than an isolated oocyte, embryo or fetus and is likely to be rejected (immunologically) unless the recipient and the donor are histocompatible with each other (or autologous). It is however possible to subdivide a single (fresh or cryopreserved) donor ovary and then graft these pieces to multiple compatible recipients and thereby have several recipients producing young derived from the original donor ovary.

In recent years an improved understanding of the impact of collection, processing, cryopreservation and grafting strategies has emerged. One important advance is the development of strategies for cryopreserving and re-anastomosing whole ovaries of both small and large species. Another is the realization that the outcome can be influenced by the graft site, possibly because of qualitative and quantitative differences in blood supply.

In summary: Ovarian tissue cryopreservation can provide a very convenient strategy for storing female gametes.

20 Animal facility in CDB

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Mutant mouse is extensively used as a powerful tool in biological and medical science nowadays. Easy production of mutant mice contributes to high quality and efficacy of researches. Major activity in our laboratory is to produce mutant mice for researches in the fields of developmental biology and regenerative science. This activity supported by the CDB and NBRP (National Bio Resources Project) is done in collaboration with researchers in the field within the CDB, the others in Japan and Asia-Pacific developmental biotechnology. In addition to this major activity, we provide a great deal of services such as rapid propagation of mutant mouse colonies by in vitro fertilization and cryopreservation of mutant mouse strains, as a CDB support laboratory.

Besides this lab performs a great number of maintenance and logistical functions: housing mice in SPF conditions, cleaning infected animals, production and distribution of animals.

21 Factors affecting rat transgenic efficiency

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The rat is an important animal model for physiology, cancer, and pharmacological research. The use of transgenic technology to overexpress proteins in a cell specific fashion promises to increase the utility of rats as models of human disease. Anecdotal evidence suggests that the production of transgenic rats is more difficult and less efficient than transgenic mouse production. In order to increase access to transgenic rats we evaluated methods to improve rat transgenic efficiency in three key areas: superovulation, microinjection, and pseudopregnant recipient identification.

Five superovulation treatments were compared. The most effective superovulation treatment was 30 IU PMSG followed by 20 IU HCG 48 h later. Modified and conventional microinjection needle geometries were compared. Needles that were longer and thinner than conventional needles increased the ease of egg microinjection and increased egg survival. Four methods for preparing pseudopregnant recipients were compared. The preferred method for pseudopregnant recipient preparation combined estrus synchronization with LHRH agonist treatment and mating with vasectomized males. The optimized procedures reduced the numbers of rats needed to produce fertilized eggs and to produce pseudopregnant recipients.

We made three Sprague-Dawley Crl:(CD)SD and four Fischer 344 F344/NHsd transgenic rat models by DNA pronuclear microinjection. The efficiency of SD transgenesis was one transgenic founder per four egg donors or 1.9% of injected eggs developed into transgenic founders. F344 transgenesis was less efficient: one transgenic founder per eight donors or 1.4% of injected eggs developed into founders. The transgenic efficiency with outbred SD rats is comparable to transgenesis in inbred mouse lines such as C57BL/6J (one founder per six donors or 1.0% transgenic eggs). Transgenesis in inbred F344 rats required more effort because of lower egg yields and lower birth rates.

In our hands, the efficiency of SD rat transgenesis is similar to that of inbred C57BL/6J mice. We expect that transgenic units experienced in C57BL/6J transgenesis will have little difficulty in preparing transgenic SD rats. Identification of rat strains with desirable superovulation characteristics may further improve the efficiency of rat transgenesis. The combination of extensively characterized rat genetic backgrounds with transgenic technology will increase our ability to model human diseases with transgenic rat models.

22 The production of male of-only offspring in beef cattle—a proof of principle project

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The Australian beef industry places the greatest value in bulls, in comparison to cows, for prime beef production. Male carcasses can be sold for a larger profit due to their increased muscle mass. This project aims to demonstrate the feasibility of producing male animals that can sire male only offspring, through a transgenic approach in mice that could later be translated into livestock production systems.

The mouse *Sry* (Sex determining region on the Y) gene has been shown to provide the initiating molecular signal leading to male sex determination in mammals. *Sry* has also been shown to cause sex reversal in XX mice transgenic for the gene. In this project *Sry* will be targeted to a locus

not subject to X-inactivation on the X chromosome of XY mice. These mice will be bred to determine how the transgene is passed on, to determine expression of the transgene, and to assess its activity in causing XX sex reversal.

The male mice transgenic for the *Sry* gene on their X chromosome will be produced using tetraploid aggregation, which in a single step produces 100% ES cell derived embryos. The same target locus can later be used to introduce the bovine *SRY* gene onto the X chromosome of *bovidae* species and using germ cell transplantation produce sex reversed animals. This would bypass the need for expensive chimera crosses and provide farmers with a stud bull capable of producing only sons.

23 Validation of SLC7A9 knockout model for pharmacological studies

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Cystinuria is a common recessive disorder of renal reabsorption of cystine and dibasic amino acids that results in urolithiasis of cystine. Cystine precipitates in the urinary system, forming calculi, which can cause obstruction, infection, and ultimately, renal failure. Cystinuria is caused by defects in the amino acid transporter rBAT/ $b^{0,+}$AT. Mutations in *SLC3A1* (rBAT) cause cystinuria type A, characterized by a silent phenotype in heterozygotes (phenotype I). Mutations in *SLC7A9* ($b^{0,+}$AT) cause cystinuria type B, in which heterozygotes in most cases hyperexcrete cystine and dibasic amino acids (phenotype non-I). To facilitate in vivo investigation of $b^{0,+}$AT in cystinuria, we have generated *Slc7a9* knockout mice (*Stones*). These mice show also urinary hyperexcretion of dibasic amino acids and cystine, and formation of cystine calculi in the urinary system. Calculi develop during the first month of life and grow throughout the life span of the animals. Histopathology in kidney reveals typical changes for urolithiasis (tubular and pelvic dilatation, tubular necrosis, tubular hyaline droplets and chronic interstitial nephritis). Thus, *Stones* provide a valid model of cystinuria which can be used in the study of genetic, pharmacological and environmental factors involved in cystine urolithiasis. In humans, cystinuria treatment is based on the prevention of calculi formation and its dissolution or breakage. We have developed a new protocol with D-penicillamine to validate our mouse model for the study of the therapeutic effect of other drugs to treat cystinuria lithiasis.

We performed a 5-week treatment of lithiasic mutant mice individually caged, with a DP dose previously tested. Mice were X-ray analyzed before and after treatment to appraise the evolution of the lithiasis. We also performed metabolic cage experiments to collect urine to quantify amino acids in DP treated and non-treated mice. We found clear differences between DP treated and non-treated mice in calculi size and in urinary cystine excretion.

Histopathological analysis did not show significant differences in urinary damage between DP treated versus non-treated mice.

Our results validate the use of *Slc7a9* KO mouse model to test the efficacy of potential new cystinuric drugs.

24 Complementary applications of microsatellite and SNP genotyping

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There are currently two common types of mouse background strain genotyping. Microsatellite markers, also known as simple sequence length repeat markers (SSLP), consist of repeated runs of dinucleotides, trinucleotides and tetranucleotides. These are generally detected via PCR amplification from conserved sequences flanking the repeat.

The number of repeated units can be highly variable. When a repeat is identified that is different in two or more mouse strains, it can be incorporated into genotyping panels to distinguish these strains. Note that the number of repeats can be multiallelic, with several different strains each generating a distinct marker size, thus individual microsatellite markers are more highly informative than SNPS.

With complete sequences (albeit in varying degrees of coverage) of 15 common inbred mouse strains, there are more than six million known SNPs. In the human genome, it has been estimated that there is one SNP approximately every 1 kB, and the mouse genome is expected to be similar. It is more difficult to accurately assess the number of microsatellite polymorphisms

For genome scanning using SNPs, it is necessary to choose 'representative' markers. Genotyping all or most SNPs would be cost-prohibitive and would also result in unmanageable datasets. Certain SNPs can be selected as haplotype markers, in which it is assumed that the presence of the SNP correlates with the presence of other SNPs in a congruent piece of DNA. However, the limits of haplotype blocks can be difficult to define.

Microsatellite markers are the preferred markers for genome scanning applications, such as speed congenics and genetic background confirmation. These processes are performed most efficiently with a small number of markers per chromosome. For isolating quantitative trait loci or attempting to identify polymorphisms associated with disease, the finer scale of SNP analysis is more appropriate, although it is worth noting that initial genome scans to identify target loci can be efficiently performed via microsateliite analysis.

25 Description of chromosomal loci controlling breeding capacity of female mice—possible applications in transgenic technology and research

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We have recently dicovered a number of polymorphic chromosomal regions (loci) controlling breeding proerties in female mice (Liljander et al., *Genetics*, June 2006). Two of these loci (*Fecq3* and *Fecq4*) affect litter size and four other loci (*Pregq1–4*) show influence on pregnancy rate (frequency of successful pregnancies). We have in our genetic studies used crossings between a C57Bl strain (B10Q) and an inbred NMRI mouse of NIH origin denoted NFR/N. It is well known that C57Bl is a fairly ''moderate breeder'', while the NFR/N mice are known for their extraordinary good breeding and nursing properties. Although the final aim of our investigation is to identify individual genes that significantly promote female breeding capacity, it should be possible to apply some of our congenic C57/Bl strains (congenic with a small chromosomal fragment of NFR/N origin) to optimize egg production and breeding performance in C57Bl mice, which are becoming increasingly important as ''reference mice'' in transgenic research. We wish to present breeding data from some of our congenic strains and discuss their possible use in transgenic technology. Those strains that only contain one small NFR/N locus could quickly be back-crossed to an ordinary C57Bl mouse again (two back-crossings confirmed by simple PCR screening).

26 A green fluorescent protein expressing cassette as a negative selector in PNS targeting vectors

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Homologous recombination (HR) is a low frequency event in mammalian cells, above all in somatic cells, and targeted integration of the vector only occurs in a very small proportion of cells, therefore a selection strategy is required.

Positive-negative selection (PNS) is one of the most used strategy to enrich for homologous recombination among vector integration events in cell culture. This is the technique of choice to target genes that are not actively transcribed in the chosen cell type. The classical negative selection cassette usually expresses the Herpes Virus Thymidine kinase to inhibit the grow of cells underwent to random integration event. Nevertheless this enrichment has a low efficiency due to the negative selection cassette: typically, this strategy yields a 2–10-fold enrichment for targeted clones. Moreover, selection of recombinants with Ganciclovir has the drawback of a general toxic effect on the whole cell population. In this work we designed a targeting vector containing an EGFP (enhanced green fluorescent protein) expressing cassette as a negative selector. The negative selection cassette derived from a plasmid containing the EGFP cDNA under the control of the chicken beta-actin promoter and cytomegalovirus enhancer, beta-actin intron and bovine globin poly-adenylation signal. The GFP from the jellyfish *Aequorea victoria* has become an important reporter in eukaryotic cells: the fluorescence is stable, species-independent and can be monitored noninvasively using techniques of fluorescence microscopy. In our work, most of transfected cells bearing random integration exhibit green fluorescence, in contrast with cells underwent to homologous recombination. The employ of such GFP vector allows to isolate and analyse only the non-fluorescent cells, using a single selective drug, resulting in less toxic culture conditions.

27 ‘‘COMPARTIR’’: a Spanish academic network initiative for sharing genetically modified mice

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Today, most research institutions and centres within the biological, biomedical or animal biotechnological fields, have investigators or facilities generating and/or analysing genetically modified mice, from ‘‘classical’’ transgenic animals to the most sophisticated knockout/knockin mice. Often, researchers require the use a reported transgenic/knockout mouse line for their experiments, in order to test their animal model or to challenge their transgenes/knockout alleles in the presence of an additional genetic modification. A quick Medline search will easily tell who originated and first published those mice. But obtaining them it is another history, frequently full of endless and tedious administrative and governmental paperwork, besides being very expensive and time consuming. As an alternative solution, the researcher might decide to check whether the desired mouse is available in their own country (i.e. in another centre, due to previous agreements) thus avoiding the complicated importation procedures. Asking around (i.e. using generic-type of e-mail lists) might not be the most helpful solution since, usually, researchers might refrain from sharing, openly, the existence of a given animal model in their facilities or their interest in a given mouse. Using public databases of transgenic animal models can be also problematic since they need to be updated. A few years ago, a first networking initiative was created in UK (‘‘Mouse Locator-UK’’; Bugeon L & Rosewell I, Transgenic Res. 2003, 12: 637) with the aim of sharing transgenic mice and knowledge, while respecting the privacy of their owners. The system was simple and was based on responsible persons in each institution receiving and sharing the requests from their colleagues, locally, and distributing the request to other homologous responsible persons, whose task would be to check whether any of their own users has, or not, the requested animal model. If the animal model is found, then the responsible person puts in contact the initial requester and the owner of the requested mouse and it is then up to them to establish the arrangements. A similar system has been also reported in France (Holzenberger M et al., Transgenic Res. 2005, 14: 801–802). Here, I am introducing the ‘‘COMPARTIR’’ (‘‘share’’, in Spanish) initiative, which I have established in Spain, within the working group of ‘‘Transgenesis in Mammals’’ (http://www.cnb.uam.es/~transimp/), founded in 1999, with its corresponding e-mail list (transgenicos@cnb.uam.es). The COMPARTIR network is currently made of 19 nodes (institutions) with a

responsible person in each centre, whose task is to keep an updated list of animal models existing in his centre and to facilitate a direct contact between the requester and the owner, following the schemes described in UK and France.

28 Phenotyping the visual and auditory systems of transgenic mice: an animal model of oculocutaneous albinism type I

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Albino mammals display a number of retinal-visual abnormalities, including rod-photoreceptor cell deficiency, underdevelopment of central retina and incorrect retinal-brain connections. All these abnormalities result in an impaired visual system, with reduced visual acquity and reduced stereoscopic vision. Oculocutaneous albinism type I (OCAI) is a congenital hypopigmentation syndrome whose molecular basis is a mutation in the tyrosinase gene, resulting in alterations in gene expression or the production of abnormal protein product with absent or suboptimal enzymatic function. Lack or reduction in tyrosinase function appears to be the primary cause of retinal and visual abnormalities recorded in albino animals.

Hearing impairment is often also present in hypopigmented congenital diseases, such as albinism. Different types of albinism have also been related to auditory defects although its precise relevance and pathophysiological mechanism have not been yet established. There have been some reports describing auditory defects, for example, abnormal decussation of auditory pathways in albino animals, presumably correlated with its lack of pigmentation. Inside the cochlea, at the stria vascularis, there are neural crest-derived melanocytes. Cochlear hypopigmentation might cause these auditory alterations. In albino animals, these inner ear melanocytes are also non-pigmented.

Recently, we have shown that the ectopic expression of tyrosine hydroxylase in albino transgenic mice allows the correction of all albino visual abnormalties in the absence of melanin, thus demonstrating the important role of early melanin precursors (i.e. L-DOPA) in the normal development of mammalian retina (Lavado et al., J. Neurochem. 2006, 96(4):1201–1211). Here, we have analysed the tyrosine hydroxylase (TyrTH) transgenic mice in deeper details, using electrophysiological (electroretinographic recordings, ERG), visual (optomotor test) and histological (detection of photoreceptor cells in sections and whole-mounted retinae) experimental approaches to ascertain whether the observed rescue of the visual system in these mice correlates with proper photoreceptor cell function. In addition, to specifically address the potential role of tyrosinase in ear development and hearing function, as it has been established in the retina, we have analysed these TyrTH transgenic mice by testing the auditory brainstem response (ABR), at different ages. Observed differences will be presented and discussed.

29 Generation of yeast artificial transgene mice by Intracytoplasmic sperm injection

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Genomic-type transgenes are usually expressed in appropriate spatial- and temporal-specific manners. The largest genomic transgenes can be prepared using yeast artificial chromosomes (YACs). Normally, YAC transgenic mice are produced by standard pronuclear microinjection although other methods, involving the use of

embryonic stem (ES) cells, have been also devised. To overcome the difficultness and time extension of ES cell-type approaches and to improve the rather usual low efficiency of YAC DNA transgenesis by pronuclear microinjection, mostly dependent on the YAC DNA quality of samples, we have devised an updated intracytoplasmic sperm injection (ICSI) method for the stable incorporation of YACs into the germ line of mice. DNA transgenesis efficiencies achieved are in the range of 10 times higher than those usually obtained by standard microinjection, thus enabling the identification of either more founder animals and/or the use of reduced numbers of individuals in animal experimentation.

30 Cost efficiency considerations in managing a transgenic mouse facility

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Transgenic mouse technology has been widely used for decades to create animal models in biomedical research area. The transgenic mouse facility has become a critical infrastructure to support research projects. There are some differences among individual transgenic mouse facilities in term of efficiency in transgenic mouse production. Some facilities use more materials/animals to generate transgenic founder mice from each DNA construct. Others take longer time to produce transgenic mice for investigators. In order to reduce the cost in transgenic mouse production and provide the high quality service for investigators who use the transgenic mouse facility, some key factors affecting the efficiency in operating a transgenic mouse facility are discussed. These factors include minimizing the number of mice in the facility in order to reduce the cost per diem, optimizing superovulation by adjusting the hormone dosage and the light cycle in the animal room in order to get the maximum number of fertilized embryos, controlling the quality of injected DNA by using the correct protocol in order to improve the transgenic frequency, and improving personal technical skills by training to enhance the embryo viability from microinjection and the percentage of live born from embryo transfer.

31 Applications of high-resolution in vivo imaging for small animal research

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The purpose of the talk is to introduce participants to high-resolution in vivo imaging using micro-ultrasound as a research tool for small animal models. This innovative imaging modality is a novel approach to investigate and quantify physiological and anatomical phenomena longitudinally in mice, rats, chick embryos, zebra fish and other small animal models from embryonic stages (i.e. from E5.5 in mice) through to adulthood. This in vivo imaging modality is applicable for multiple small animal research application areas including cancer research, cardiovascular research, developmental biology, gene therapy, neurobiology, and preclinical drug development. Recent results in the areas of embryology, cardiology and cancer research will be illustrated. Visualization of image-guided needle injection procedures using high-resolution imaging will also be described.

32 Research animal management system

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1. Problem statement
Management of research animal facilities, particularly mouse and rat facilities, typically involves significant populations of animals requiring a range of procedures to maintain their welfare and provide the scientific results required.

Animal Ethics Committees (AECs) are required to report annually on individual research proposals.

They also need to assess the adequacy of facilities, staff training requirements and any administrative difficulties experienced. Collation of the necessary information on populations and welfare checks for AECs from cage cards is difficult and ineffective in any reasonably sized facility.

Managing projects in concordance with the 3 R's (Replacement, Reduction, Refinement) is not a measurable goal without the collection of accurate data. It is also difficult to allocate vivarium operating costs without proper recording of activities.

2. Approach
In operating a vivarium, both recurrent and sequential processes were identified. These provided the framework used to recognize the actions required to maintain animal welfare, husbandry and scientific processes. The philosophy adopted was to give processes crucial to animal welfare priority over other activities.

The Ozgene Research Animal Management System (OzMouse) identifies the tasks required on any date. Supplementary welfare activities can be initiated by vivarium staff. Barcode technology linked to OzMouse dictates and records the animal welfare, husbandry and research processes undertaken.

Accurate record keeping allows for better resource planning and this reduces the number of animals required in support and project colonies.

3. Results
The OzMouse database allows instant access to population figures and a log of welfare information. There is also a reduction in operating costs through better scheduling and therefore staffing levels required, and a reduction of animal populations through better management. OzMouse was also used to facilitate a successful audit of vivarium population by Ernst & Young.

4. Conclusions
It is our contention that the use of cage cards as a collection mechanism is thoroughly inadequate to meet AEC requirements of record keeping. A database system is required for any reasonably populated facility.

The OzMouse database work in progress will be presented at the Conference.

33 The use of cryopreserved mouse embryos as hosts for the production of chimeric mice

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The use of in vitro fertilization is a convenient way to produce large numbers of 2-cell mouse embryos. Such embryos can then be cryopreserved and recovered at a later date at high efficiencies of survival. Recovered embryos can then be used to produce chimeric mice by both microinjection and aggregation. This approach provides a convenient method for providing a consistent and known quantity of embryos for chimera production.