# QMB Abstracts Applied Genetic Technologies

## G1: Beyond CRISPR/Cas9 genome editing to genome engineering

Stewart, A.F.
Center for Molecular and Cellular Bioengineering, University of Technology, Dresden, Germany

Sequence specific cleavage of the genome with designer nucleases is fundamentally changing genome engineering. The advent of Cas9/CRISPR has further simplified the technology and many genomes are now accessible for genome editing by site-directed damage. Similarly site-directed point mutations can be inserted at workable frequencies by including oligonucleotides for incorporation via homologous recombination at the cleavage site.
We are working to apply Cas9 to more challenging genome engineering applications, in particular to establish complex alleles such as humanizations, large protein tags or the introduction of loxP sites for conditional mutagenesis. These exercises start with the building of targeting constructs using recombineering, which is particularly suited to complex recombinant DNA exercises (1,2). The length of homology for optimal targeting is a critical question that we have examined using the classic HPRT assay in embryonic stem cells. We also applied the HPRT assay to evaluate other Cas9-assisted strategies and parameters. Furthermore we have applied Cas9 to replace a mouse gene with its human counterpart. This exercise involved building a 42kb replacement section in a BAC and targeting in mouse ES cells (3).

1. Hofemeister, H, Ciotta, G, Fu J, Seibert P, Schulz A, Maresca M, Sarov M, Anastassiadis K and Stewart AF. (2011) *Recombineering, transfection, Western, IP and ChIP methods for protein tagging via gene targeting or BAC transgenesis.* Methods 53, 437-52.
2. Baker O, Gupta A, Obst M, Zhang Y, Anastassiadis K, Fu J and Stewart AF (2016) *RAC-tagging: Recombineering And Cas9-assisted targeting for protein tagging and conditional analyses.* Sci Rep. 6, 25529.
3. Baker O, Tsurkan S, Fu J, Klink B, Rump A, Obst M, Kranz A, Schröck E, Anastassiadis K and Stewart AF (2017) *The contribution of homology arms to nuclease-assisted genome engineering.* Nucleic Acids Res, 45:8105-8115.

## G2: Genetically modified pigs for biomedicine

Konrad Fischer, Angelika Schnieke
Livestock Biotechnology, Technische Universität München, Germany

Pigs are increasingly recognised as a valuable adjunct to pre-clinical research, and their value is considerably increased by the engineering of precise genetic modifications that replicate lesions responsible for human disease conditions or by providing modified organs for the pig to human xenotransplantation. The production of genetically modified pigs has been technically challenging, but can now be significantly streamlined by using gene editing enzymes.

In our group combinations of genetic engineering technologies are being employed to follow two main aims.

The first aim is to provide a series of genetically-defined pigs that model serious and common human cancers which will allow new diagnostic and therapeutic strategies to be investigated at human scale, and longitudinal studies under conditions that mimic the human patient. To model colorectal cancer we have generated gene-targeted cloned pigs carrying a nonsense mutation in the adenomatous polyposis coli tumour suppressor gene (*APC*$^{1311}$), orthologous to a mutation responsible for the inherited predisposition, familial adenomatous polyposis (FAP). Histological and molecular analyses showed that the porcine model recapitulates all major features of early stage FAP.

Our second aim is to alter the porcine genome which will enable us to provide xeno-organs for transplantation into humans. To overcome the hurdles of hyperacute and acute vascular rejection mechanisms, these pigs require a large number of modifications including transgene expression of complement regulatory genes, anti-apoptotic and anti-inflammatory genes as well as inactivation of porcine genes such as *GGTA1*, *CMAH* and *B4GalNT2*. For addition of transgenes we have established several approaches for the co-expression of multiple transgenes and for a precise targeting and re-targeting approach. Inactivation of porcine genes is performed by using multiple knockout CRISPR/Cas9 constructs. These animals are currently being characterised and further modified to inhibit cellular rejection mechanisms.

## G3: Gene editing in cattle

Van Eenennaam, A.L.
Department of Animal Science, University of California, Davis, CA, USA.

In 2016, the global cattle population of 1.5 billion head produced 6.5 billion tons of cows' milk, and 66 million tons of beef. In the past century, cattle breeding programs have greatly increased the yield per animal with a resultant decrease in the GHG emissions intensity per unit of milk or beef, but this has not been true in all regions. Gene editing using site-directed nucleases (e.g. CRISPR/Cas9) offers an opportunity to precisely edit or change the genetic code. Gene editing could be integrated into conventional cattle selection programs to introduce useful alleles into elite germplasm without the lengthy process of introgressing those same alleles from distant breeds[1]. To date, gene editing research in cattle has focused on disease resistance, production, elimination of allergens and welfare traits such as introducing the polled (hornless) allele from beef breeds into horned dairy cattle breeds.

| **Target** | **Targeted Trait/Goal** |
|---|---|
| Intraspecies *POLLED* allele substitution | No horns/welfare trait |
| Intraspecies *SLICK* allele substitution | Heat tolerance |
| Myostatin (*MSTN*) gene knockout | Increased lean muscle yield |
| Beta-lactoglobulin gene knockout | Elimination of milk allergen |
| Prion protein (*PRNP*) knockout | Elimination of prion protein |
| *CALPAIN* & *CAPASTATIN* allele substitution | Improved meat tenderness |
| Insertion of lysostaphin/lysozyme transgene | Resistance to mastitis |
| *CD18* gene edit | Resistance to bovine respiratory disease |
| Insertion of *SP110, NRAMP1* | Resistance to tuberculosis |
| *SRY* translocation onto Y chromosome | All male offspring |
| *NANOS3* gene knockout | Infertile cattle (for germline surrogacy) |

As with earlier genetic engineering approaches, whether livestock breeders will be able to employ genome editing in cattle genetic improvement programs will very much depend upon global decisions around regulation and governance of genome editing for food animals[2].

1. Van Eenennaam AL (2017). Genetic Modification of Food Animals. Curr Opin Biotech 44: 27-34. 2. Van Eenennaam AL (2018). The Importance of a Novel Product Risk-Based Trigger for Gene-Editing Regulation in Food Animal Species. The CRISPR Journal 1: 101-106

## G4: Generation of enhanced cattle genotypes by genome editing

Brigid Brophy[1], Sally Cole[1], Jingwei Wei[1], Stefan Wagner[1,2], Paul Maclean[1], Grant Smolenski[1,3], Dan Carlson[4], Scott Fahrenkrug[4], David Wells[1] and Goetz Laible[1,5]

[1]AgResearch, Ruakura, Hamilton, New Zealand
[2]Rowett Institute, Aberdeen, United Kingdom
[3]MS3 Solutions Ltd., Hamilton, New Zealand
[4]Recombinetics, St. Paul, United States of America
[5]School of Medical Sciences, University of Auckland, New Zealand

Cell- and embryo-mediated approaches are two alternatives for genome editing livestock species. Editing in cells allows full characterization of the edit and generation of validated, non-mosaic animals. However, production of live animals from edited cells by somatic cell nuclear transfer (SCNT) is hampered by low efficiencies. By contrast, zygote-mediated approaches do not compromise production efficiencies of live animals but lack control over the extent of editing with the potential to produce mosaic animals.

Naturally occurring mutations which impact on important traits are of particular relevance for agricultural applications of the technology. Such variants can be introduced by homology-directed repair (HDR) editing. The two approaches, zygote- and cell-mediated, were tested for their suitability to produce cattle with precisely, HDR-edited genotypes. By embryonic editing, we introduced a nine bp deletion generating a premature stop codon in the signal peptide to eliminate the allergenic cow milk protein beta-lactoglobulin. Analysis of the genotypes of three edited calves demonstrated the feasibility of generating precisely biallelically edited cattle by zygote-mediated editing and verified the functionality of the nine bp deletion genotype to disrupt the production of the allergenic protein[1].

To reduce radiative heat gain as a factor of heat stress levels in dairy cattle we have introduced a three bp deletion in the PMEL gene that has been associated with coat colour dilution in Highland and Galloway cattle[2] into Friesian cattle. Two edited calves that were produced with the cell-mediated/SCNT approach displayed marked coat colour dilution compared to controls, validating the causative nature of the introduced mutation.

Although precision editing of livestock genomes works with both approaches, deciding on the approach of choice will depend on the specifics of the mutation to be introduced and the particular demands of the intended application for the edited livestock and hence, needs to be considered case by case.

1. Wei, J., Wagner, S., Maclean, P., Brophy, B., Cole, S., Smolenski, G., Carlson, D.F., Fahrenkrug, S.C., Wells, D. N. and Laible, G. (2018). *Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin*. Scientific Reports. 8: 7661
2. S.M. Schultz and D.L. Dreger (2012). *Interaction of MC1R and PMEL alleles on solid coat colors in Highland cattle*. Animal Genetics. 44: 9-13

## G5: Genetic Tools for Pest Eradication – Opportunities and Challenges

Gemmell, N.J.
Department of Anatomy, University of Otago, Po Box 56, Dunedin 9054, New Zealand.

New Zealand has set an ambitious goal to eradicate mammalian predators from our shores by 2050. The key targets are possums, rats and stoats; species that cause enormous damage to our flora and fauna and in some cases are an economic burden to our productive sectors. As all of these species were introduced to New Zealand from elsewhere there is little sympathy nationally for any of them and their control and eradication has been a key component of conservation and animal health management in this country for decades. Thanks to the work of many, over many decades of incremental gain, we can control and even eradicate many of these species at increasingly large scales. The success of these programs has seen a variety of "pest-free" sanctuaries formed where many native species, including kiwi, kokako, and kaka now have a realistic chance for population persistence and recovery. Pest control with current technologies over significant spatial scales is definitely possible, but its time-consuming and expensive. Thus if we want to reach a goal of a pest free New Zealand by 2050 we need to come up with smart ways to control our pest problem – new gene technologies are one possible solution to our pest problem. In this talk I will explore some of the genetic solutions currently being considered, including gene drives, the opportunity and challenges associated with each, and some directions for future endeavours.

## G6: Possums and primordial germ cells- a novel method of pest control

Laird, M.K.[1], Gemmell, N.J.[1], Hore, T.A.[1]
[1]Department of Anatomy, University of Otago, Dunedin, NZ

Brushtail possums pose a serious environmental and economic threat to New Zealand, despite extensive control effort by trapping and baiting. Innovative gene-editing techniques have the potential to revolutionise pest species control through introduction of mutations into the germline to perturb fertility or sex ratios[1,2]. Nevertheless, it is assumed that rodent-optimised gene manipulation, which focuses on the pre-implantation embryo, will work for possum. We propose an alternative method of accessing the possum germline using primordial germ cells (PGCs)- the undifferentiated precursors to adult eggs and sperm- to provide proof-of-principle for gene-editing techniques in possums. Unlike most mammals, possum gonads are undifferentiated at birth, meaning that PGCs can be readily accessed from the immature gonads of pouch young[3]. We aim to exploit this unique aspect of possum developmental biology to develop novel, tailored strategies for possum control.

Here we present our preliminary data on possum gonad ontology and PGC isolation techniques. Our ultimate aim is to create transgenic possums for strategic release into wild populations, an effort that could potentially result in eradication of possums from New Zealand in a humane and strategic way.

1. Harvey-Samuel T, Ant T, Alphey L. 2017. *Towards the genetic control of invasive species*. Biol Invasion 19:1683-1703.
2. Dearden PK, Gemmell NJ, Mercier OR, Lester PJ, Scott MJ, …. & Penman DR. 2017. *The potential for the use of gene drives for pest control in New Zealand: A perspective.* J Roy Soc New Zealand, 1-20.
3. Suzuki S, Shaw G, Renfree MB. 2013. *Postnatal epigenetic reprogramming in the germline of a marsupial, the tammar wallaby.* Epigen Chromatin 6:14-21.

## G7: Precise disruption of *DAZL* abrogates the male germline in sheep

McLean, Z.L.[1, 2], Snell, R.G.[2], Oback, B.J.[1, 3]

[1]Reproduction, AgResearch, Ruakura, NZ,

[2]School of Biological Sciences, University of Auckland, NZ,

[3]School of Medical Sciences, University of Auckland, NZ

Deleted in Azoospermia-like (DAZL) is a conserved RNA-binding protein that functions in gametogenesis in many species from insects to man. *DAZL* homozygous null (-/-) male mice are sterile because their germ cell specification is disrupted. We aim to produce chimeric 'absolute transmitter' rams whose own germline has been replaced with that of a genomcially selected elite donor embryo. This will be achieved by genetically disabling spermatogenesis and complementing the vacant sperm niche with germline-competent embryonic donors. For proof-of-concept, *DAZL*$^{-/-}$ somatic cell nuclear transfer (SCNT) cloned male host embryos and animals were produced by genome editing.

We employed CRISPR/Cas9 genome editors to disrupt DAZL in male ovine fetal fibroblasts (OFFs). A single-stranded homology-directed repair (HDR) template was designed to mediate a small insertion, introducing a stop codon and Taq1 restriction site. After OFF sub-clones were isolated, DNA sequencing and Taq1 digest confirmed biallelic editing in 10% of clones. Proven OFF clones were used as nuclear donors for SCNT to generate embryos for transfer into surrogate recipients. Following lambing, newborn edited animals were analysed for their testis phenotype. Paraffin-embedded sections of DAZL$^{-/-}$ testis cords comprised histologically normal somatic support cells but lacked spermatogonia. Immunohistochemistry on frozen sections of wild-type testis cords showed cells with clear cytoplasmic signals for DDX4, a specific marker of spermatogonia. By contrast, DAZL$^{-/-}$ mutants contained no DDX4-positive cells within the testis cords. This confirms the conserved role of DAZL in sheep germline specification. Genetically sterilised male DAZL$^{-/-}$ host embryos could be complemented with elite wildtype NT donor embryos to reconstitute the germline and create chimeric absolute transmitter rams as an alternative to artificial insemination in extensive farming systems

## G8: The implications of gene editing technology for New Zealand

Barry Scott
Institute of Fundamental Sciences, Massey University, Palmerston North, NZ

The revolution in gene editing technologies is making it easier to make targeted changes in the genomes of animals, plants and microorganisms. The development of these new technologies has huge potential benefits in many sectors including healthcare, agriculture and conservation. However, the technology to carry out gene editing and the ideas about how it might be applied are, in many cases, moving well ahead of public understanding and consideration of the proposed changes, and any consensus on how this technology might be used.

To explore the implications of gene editing technology for New Zealand, the Royal Society Te Apārangi has convened a multidisciplinary panel of experts, supported by a Māori reference group, to consider the social, cultural, legal and economic implications of gene-editing technologies for New Zealand.

The terms of reference for the panel are to:

- Raise awareness of the current gene editing technologies, their recent development and what they are being used for
- Outline the technologies' opportunities and risks, including current global practice
- Provide insight and advice for public, business and government audiences on the future implications of these new technologies for New Zealand

The approach taken by the panel has been to generate a series of discussion papers containing various gene-editing scenarios to initiate a conversation with the NZ public. To date, papers on gene editing in healthcare and pest control have been released and a series of meetings held to canvas views of policy makers, industry, scientific and community organisations, and high school students. Two additional pieces of work are in progress on *The use of gene editing in the primary industries* and the *Implications for the New Zealand regulatory framework*.

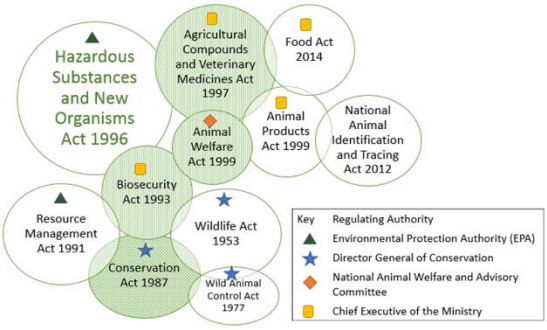
This talk will provide an overview of the activities of the panel, progress to date, major issues encountered and future challenges that lie ahead.

Reference

- https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

## G9: Gene editing in Aotearoa – legal considerations

Julie Everett-Hincks and Mark Henaghan.
Law Faculty, University of Otago, Dunedin, New Zealand.




Gene edited crops and animals pose significant new challenges for regulation. Under current NZ legislation (Hazardous Substances and New Organisms Act, 1996) and a judicial ruling on interpretation of the legislation and regulations, the status of gene-edited crops and animals in New Zealand are considered genetically modified. A precautionary approach is employed for regulating these new organisms. The implications from the effects of differing legislation and regulatory authorities has been investigated, identifying legal and policy issues requiring consideration for gene editing use in our primary industries.

Professor Mark Henaghan and Dr Julie Everett-Hincks have been working with the Gene Editing Panel for Royal Society Te Apārangi, providing legal advice as to the potential use of gene editing in the areas of human healthcare, pest control and primary industries. Royal Society Te Apārangi is encouraging New Zealanders to consider and share their views on some potential uses of gene editing in New Zealand. To assist public discussion, two papers have been produced outlining scenarios for the use of gene editing for both pest control and healthcare. A further paper with scenarios for the use of gene editing in primary industries will be published soon, along with a paper examining current legislation and regulation. The papers have been produced by a multidisciplinary expert panel convened by the Society and co-chaired by Professor Barry Scott, who is also Vice President of the Society and a Professor of Molecular Genetics at Massey University.

For further information please contact Dr Julie Everett-Hincks jeh@otago.ac.nz
More information can be found by visiting the Royal Society Te Apārangi website: https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

## G10: Social License or Cultural License: Is there a difference?

Maui Hudson
University of Waikato

Gaining a social license from the public for the adoption of new biotechnologies has become the focus of increasing attention. The level of trust the public has in the scientific community to make responsible choices in the public interest has seemingly declined over a number of years. Gene editing is the most recent addition to the genetic engineering toolbox to challenge public perceptions and ethical sensitivities. Understanding and mitigating their concerns is an important part of social license but how are cultural rights and interests reflected in this dialogue? This presentation will reflect some of the emerging Māori perspectives on gene editing and discuss what would be required to gain a cultural license.

## G11: Social license in the marine environment: Dissecting the discourse

Sinner, J.[1], Newton, M.[1], Farrelly, T.[2]
[1]Cawthron Institute, [2]Massey University

The term “social license to operate”, or SLO, has increasingly featured in public discussion about commercial operations in the marine environment. Choice of wording and sentence structure can affect power relations between people and groups, so how the term is used matters. We analysed grey literature documents such as company reports, government policy documents and press releases to examine the implications of how SLO is defined and deployed with respect to NZ’s marine industries. We show that this discourse has been dominated by industry and central government voices, who frequently vest agency over SLO with industry and then state or imply that industry already has SLO and just needs to maintain or improve it. Whether inadvertent or intentional, this choice of language empowers industry at the expense of communities and iwi. Industry and government could change their wording to send a different, more empowering message to iwi and community groups about seeking their acceptance and trust. This would help achieve the vision of a blue economy, increasing benefits from the ocean environment while sustaining communities and marine ecosystems.

# G12: Conflicts between agricultural and tourism sectors: evidence for solutions

Damien Mather
University of Otago

[1]Department of Marketing, University of Otago, Dunedin, NZ

Synthesising our research findings on international importers' trust in NZ agricultural exports[1, 2], crises recovery in food products[3], international market acceptance of food production technologies[4, 5] and country food production technologies on inbound tourism markets[6] with pervasive current affairs discourses surrounding NZ tourism sector and proposed agricultural production strategies[7] we noted evidence for solutions that might met all sectors' needs. We found no evidence that the choice of either agricultural, or any other sector amongst production technologies supressed inbound tourist demand[6].

Evidence for consumer or inbound tourist resistance to some agricultural production technologies offered by actors outside of the agricultural sector based on stated preferences we found to be seriously flawed[8]. This has long distracting discourse from more substantial issues such as damage to recreation land and water ecologies by pests and other agricultural management strategies which are far riskier to inbound tourist demand and highly incongruous with the positioning of New Zealand to international tourists. We find that, paradoxically, opportunities to efficiently deal with those real problems with biotechnology have been needlessly overlooked.

Furthermore, we find evidence that the profitability of both sectors can be substantially enhanced and protected with the judicious application of biotechnology to agricultural production, land and water ecology management.

1. Knight, J., Holdsworth, D. & Mather, D. Determinants of trust in imported food products: perceptions of European gatekeepers. *British Food Journal* **109**, 792-804 (2007).

2. Knight, J.G., Holdsworth, D.K. & Mather, D.W. Country of Origin and Choice of Food Imports: An In-Depth Study of European Distribution Channel Gatekeepers. *Journal of International Business Studies* **38**, 107-125 (2007).

3. Chen, S., Knight, J., Gao, H. & Mather, D. 289-290 (Springer International Publishing, Cham; 2018).

4. Mather, D., Vikan, R. & Knight, J. Marketplace response to GM animal products. *Nature Biotechnology* **34**, 236-238 (2016).

5. Knight, J.G., Mather, D.W., Holdsworth, D.K. & Ermen, D.F. Acceptance of GM food - an experiment in six countries. *Nature Biotechnology* **25**, 507-508 (2007).

6. Knight, J.G., Clark, A. & Mather, D.W. Potential damage of GM crops to the country image of the producing country. *GM Crops & Food* **4**, 1-7 (2013).

7. Knight, J., Holdsworth, D. & Mather, D. 1-89 (University of Otago, Dunedin, NZ; 2003).

8. Mather, D.W. et al. Social Stigma and Consumer Benefits Trade-Offs in Adoption of Genetically Modified Foods. *Science Communication* **34**, 487-519 (2012).

## G13: The regulation of GMOs in New Zealand

Strabala, T. J.

New Organisms, Environmental Protection Authority, Wellington, NZ.

The concept of a 'new organism' in the New Zealand context came into being with the Hazardous Substances and New Organisms Act, 1996 (The HSNO Act), and is defined as any organism not present in New Zealand on or immediately before 29 July 1998, as well as any genetically modified organism. The Environmental Protection Authority is responsible for administering the HSNO Act, and as such regulates all New Organisms, including GMOs.

A GMO in New Zealand has a broad definition, but refers to Regulations specifying those organisms that meet the definition, but are considered exempt. These regulations were reviewed and redrafted in 2016 in response to a High Court challenge to the result of another of EPA's functions, the statutory determination of whether or not any organism is a New Organism under the HSNO Act.

The EPA has the power to grant various types of approvals for GMOs, which mostly involve the importation into containment, or the development of GMOs in containment. However, the EPA also has given approvals for the field trials and release of various GMOs since the inception of the HSNO Act. I will discuss the aspects of the law that EPA must consider in coming to a decision for any approval for the release of a GMO, or a determination as to whether or not an organism is or is not a GMO for the purpose of the HSNO Act, using recent EPA decisions as examples.

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## G14: Gene editing technologies: how they fit in New Zealand's policy framework

Wouters, M.
Hazardous Substances & New Organisms Policy team, Ministry for the Environment, Wellington, NZ

Withdrawn

## G15: Genomic Selection for Improvement of Populations

Garrick, D.J.[1]

[1]AL Rae Centre of Genetics and Breeding, Massey University, Ruakura Research Centre, Hamilton, NZ.

Genetic improvement is easy to achieve – it is a simple matter of selecting above average candidates to use as parents in each successive generation. However, the challenge in practice is in cost-effectively predicting the merits of the candidates at as young an age as possible. Mass prediction uses individual phenotypes to predict genetic merit, pedigree selection relies mostly on phenotypes from close relatives sometimes including the selection candidate itself to predict genetic merit, whereas genomic prediction sums up the predicted effects of all the chromosome fragments inherited by the selection candidate. The accuracy of genomic prediction is influenced by our ability to reliably predict the effects of all the chromosome fragments.

Predicting the effects of chromosome fragments depends upon population aspects such as effective population size, which determines the size and number of segregating chromosome fragments; the size of the so-called training population used to predict the effects of each and every chromosome fragment; the heritability of the traits which effects the amount of data required to partition phenotypic performance into genetic and residual effects; the structure of the training population which affects the extent to which chromosome fragments inherited by the selection candidate were confounded in the training population; and the genetic architecture of the selection traits, which influences the genomic location and number of chromosome fragments influencing the trait as well as their mode of gene action.

Beyond these biological, genetic, genomic and statistical factors, the single most important factor determining the utility of genomic prediction in a given circumstance is the value proposition. The value proposition determines the level of investment that can be attracted to collect relevant phenotypic and genomic information that is critical to effective genomic prediction. These aspects will be described in the context of implementing genomic selection in some agricultural circumstances.

## G16: Optimised selection strategies in New Zealand aquaculture breeding schemes

Hely, F.S.[1], Symonds, J.E.[2], Walker, S.P.[2], Amer, P.R.[1]
[1]AbacusBio Ltd, Dunedin, NZ, [2]Cawthron Institute, Nelson, NZ

Selective breeding schemes have been implemented successfully in both research and commercial aquaculture species in New Zealand over the past 20 years. In aquaculture breeding schemes with high levels of individual fecundity compared with many other organisms, there is a significant challenge to maintain a genetically diverse population within limited infrastructure, while also driving genetic gains as rapidly as possible. An elegant solution is to deploy optimised parent and mating selection pair selection methods which maximise genetic gain while constraining inbreeding accumulation. A simulation model was initially developed to test the efficacy of applying a two-stage optimisation approach, combining optimised parent selection with minimum inbreeding mating[1], and showing the superiority of this approach in terms of increased genetic gain at similar or lower rates of inbreeding accumulation when compared to non-optimised methods. A challenge in aquaculture breeding schemes can come from the difficulty in maintaining equal proportions of offspring from mating pairs with potentially high, but very erratic levels of fecundity. The simulation model was extended to model these unequal family proportions within an aquaculture breeding scheme, to test the impact on inbreeding accumulation and the efficacy of the optimised parent and mate selection methods under these conditions. The simulation results showed that it was possible to use optimal parent selection and minimum inbreeding to maintain reasonable levels of inbreeding in a small aquaculture breeding scheme with moderate to severely unequal family proportions, although at the cost of some genetic gain.

## G17: Delivering Genomic solutions to New Zealand's Biological Economy

Clarke, S.M., Dodds, K.G., Brauning, R., Hess, A.S., Ashby, R.L., Van Stijn, T.C., Anderson, R.M., Caulton, A.J., Rowe, S.J., Bilton, T.P., McCulloch, A.F. and McEwan, J.C.
AgResearch Ltd, Invermay Agricultural Centre, Private Bag 50034, Mosgiel 9053, New Zealand

To enhance the value, productivity and profitability of the New Zealand biological economy, AgResearch has developed a suite of genomic tools. In addition to developing a suite of SNP array based genotyping tools, AgResearch has also invested in genotyping by sequencing (GBS) methods, both targeted and restriction enzyme based. For restriction enzyme based GBS, combining low-depth sequencing with algorithms that produce bias free genomic relationship matrices we can estimate: breed composition, pedigree, traceability, inbreeding and co-ancestry as well as using directly in existing mixed models (GBLUP) to estimate breeding values. In addition, further developments for GBS analysis has established methods to undertake GWAS, linkage mapping, estimation of linkage disequilibrium and derivatives such as the effective population size, Ne. I will present the development and implementation of genomic tools in the NZ livestock, forage and aquaculture industries which have been extended for use in genetic diversity studies.

## G18: Genomics of New Zealand trevally: identifying the genetic basis of quantitative traits to inform a newly developed breeding programme.

Valenza-Troubat, N.[1,2], Ashton, D.[1], Ritchie, P.[2], Wellenreuther, M.[1,3]
[1]The New Zealand Institute for Plant and Food Research, Nelson, NZ, [2]School of Biological Sciences, Victoria University of Wellington, Wellington, NZ, [3]Faculty of Science, University of Auckland, Auckland, NZ.

Most diversity in phenotypic traits is due to a combination of variation alleles at multiple Quantitative Trait Loci (QTL) and environmental effects (Mackay, 2001). Understanding the complex network of genes underlying phenotypic variation and their external modulation has been a major and longstanding challenge in genetics (Fisher, 1930). In animal breeding, one of the main goals is to identify individuals that have high breeding values for traits of economic interest and use them to produce offspring carrying these particular traits within short time frames (Dekkers, 2012). Modern genetic marker-informed breeding programmes can help accelerate these gains by focusing directly on the inherited components of traits and using parentage assignment to maximise family representation and control inbreeding (Vandeputte and Haffray, 2014).
Aquaculture has been slow to add genomic information to breeding programs. Here, we present the first efforts to identify the key genes that influence growth-related traits in a new aquaculture species, the white trevally (*Pseudocaranx georgianus*). We will generate the first genotype-phenotype map for this species to identify QTLs and understand their distribution and effects sizes. Ultimately, our research will combination of genome-wide DNA sequence information and phenotypic trait data to gain fundamental insights into the genetic architecture of traits and the extent to which these by genetic variation.

1. Mackay TFC (2001) The genetic architecture of quantitative traits. Annual Review of Genetics, 35, 303–339.
2. Fisher R 1930. The genetical theory of natural selection, Clarendon.
3. Dekkers JCM (2012) Application of genomics tools to animal breeding. Current Genomics, 13, 207–212.
4. Vandeputte M., Haffray P. (2014). Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. Front. Genet. 5:432. 10.3389/fgene.2014.00432

## G19: Using CRISPR genome editing tools for animal modelling human rare diseases

Almudena Fernández, Santiago Josa, Diego Muñoz, Andrea Montero, Marcos Rubio, Marta Sánchez-Díez, Marta Cantero, Julia Fernández, Lluis Montoliu
CNB-CSIC and CIBERER-ISCIII, Madrid, Spain

Genome editing techniques have transformed the way we envisage and address experiments in Biomedicine. Particularly with the prokaryote-derived CRISPR-Cas9 tools[1] and the ease by which it is now possible to engineer subtle mutations in any desired genome, it has become possible to reproduce in cellular and animal models those mutations that are commonly found in humans. Our laboratory focuses its research in investigating albinism, a rare genetic condition whose main trait is a characteristic profound visual deficiency, which can appear associated, or not, with an obvious hypopigmentation phenotype. To date we know of at least 20 different types of albinism, associated with mutations in the corresponding 20 independent loci[2]. Traditionally, the field has been using generic-type of mutations in mice to approach the associated human rare disease. However, now it is possible to reproduce in an animal model exactly the same mutation observed in a patient, thanks to the CRISPR-Cas9 genome editing technology.

In this talk, we will summarize our efforts and achievements towards a better understanding of the pathophysiology of albinism. Using CRISPR-based genome editing we have generated numerous new mouse models of several types of albinism[3]. These animal models will be instrumental not only for our comprehension of this complex genetic condition but also as unique recipients for testing novel and innovative therapeutic approaches that are being currently explored.

1. Mojica, F.J.M. and Montoliu, L. (2016) *On the Origin of CRISPR-Cas Technology: From Prokaryotes to Mammals.* Trends Microbiol. 24:811-20.
2. Montoliu, L. and Marks, M.S. (2017) *A new type of syndromic albinism associated with mutations in AP3D1.* Pigment Cell Melanoma Res. 30:5-7.
3. Seruggia, D., Fernández, A., Cantero, M., Pelczar, P. and Montoliu, L. (2015) *Functional validation of mouse tyrosinase non-coding regulatory DNA elements by CRISPR-Cas9-mediated mutagenesis.* Nucleic Acids Res. 43:4855-67.

## G20: Getting the Balance Right: Targeting Excitatory Dysfunction in the ALS Cortex

Clark R, Brizuela M, Joseph-Daas S, Clark C, Blizzard CL, Dickson T
Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

In amyotrophic lateral sclerosis (ALS), hyperexcitability of the motor cortex is a prominent event, often preceding motor neuron degeneration. While many factors may be attributed to this excitatory pathophysiology, a possible candidate, the interneuron, has largely been overlooked. Our previous research has identified that degeneration in the transgenic G93A SOD1 mouse model of ALS is marked by progressive and dynamic interneuron involvement. Changes in the number of both calretinin- (CR) and neuropeptide Y-expressing (NPY) interneurons in the motor cortex of this familial model were identified, suggesting their potential involvement in motor neuron circuitry defects. However, it remains unclear if these changes represent a primary or secondary disease mechanism. To further explore the underlying mechanisms we utilised a primary culture approach. We found that mutant G93A SOD1 significantly altered the intrinsic firing properties and neuronal morphology of cortical interneurons. We also found a differential vulnerability of bipolar versus multipolar interneurons to disease. In addition, the neurite morphology of bipolar interneurons was unaltered while multipolar interneurons had significantly increased neurite complexity observed as increased branch number and neurite tree path length. Our results have shown for the first time that cortical interneurons are innately vulnerable to the human G93A SOD1 mutation and suggest that differential priming of interneurons may be an early step in the initiation of disease. Collectively our work indicates that inhibitory involvement in ALS may not be a static phenomenon, but instead involves dynamic changes throughout disease, which determine the susceptibility and vulnerability of MNs to disease. Therefore, the inhibitory system may represent a viable early target for the prevention and treatment of ALS.

## G21: Generation of a sheep model of CLN7 Batten disease using the CRISPR/Cas9 genome editing system – preliminary results and ethical reflections

Tammen, I.[1,2], Grupen, C.G.[1], James, A.[1], Pollard C.L.[1], Morey, N.[3], Delerue, F.[3]

[1]The University of Sydney, Sydney School of Veterinary Science, Camden, AU, [2] Batten Animal Research Network (BARN), [3]Transgenic Animal Unit, University of New South Wales, Sydney, AU

The neuronal ceroid lipofuscinoses (Batten disease) are a group of fatal neurodegenerative inherited diseases in humans and animals[1]. Animal models have been instrumental to further the understanding of genetics, of the underlying disease mechanism, and most importantly are crucial for safety and proof of concept studies for therapeutic interventions[2]. Considerable progress has come from studying three naturally occurring ovine models[3]. There is no cure, but studies in animal models and clinical trials suggest that enzyme replacement therapy and gene therapy can be beneficial for variants that are caused by mutations in genes coding for soluble proteins. Variants that are caused by mutations in genes coding for membrane proteins are likely to require other therapeutic interventions and large animal models are essential to develop and validate such therapies. We thus proposed using CRISPR/Cas9 technology to generate an ovine CLN7 research flock. After evaluation of different transfection methods in sheep fibroblasts, we are currently optimising electroporation as a method of transfection in the *in vitro* produced sheep embryos. In addition to technical constraints relating to the use of genome editing in large animals, regulatory and ethical issues need to be considered.

1. Mole SE, Williams RE, Goebel HH (eds). *The Neuronal Ceroid Lipofuscinoses* (Batten Disease) 2nd edition. New York: Oxford University Press Inc.; 2011.
2. Bond M, et al. *Use of model organisms for the study of neuronal ceroid lipofuscinosis*. Biochim Biophys Acta. 2013; 1832(11):1842-65.
3. Palmer DN, et al. *Recent studies of ovine neuronal ceroid lipofuscinoses from BARN, the Batten Animal Research Network*. Biochim Biophys Acta. 2015; 1852:2279-86.

## G22: Brain urea increase is an early pathogenic event in Huntington's disease

Handley, R.R.[1], Reid, S.R.[1], Brauning, R.[2], Maclean, P.[2], Mears, E.R.[1], Fourie, I.[1], Patassini, S.[1,3], Cooper, G.J.S.[1,3], Rudiger, S.R.[4], McLaughlan, C.J.[4], Verma, P.J., Gusella, J.F., MacDonald, M.E.[5], Waldvogel, H.J.[1], Bawden, C.S., Faull, R.L.M.[1], Snell, R.G.[1]

[1]Centre for Brain Research, University of Auckland, Auckland, NZ, [2]Invermay Agricultural Centre, AgResearch Ltd, Mosgiel, NZ, [3]Centre for Advanced Discover and Experimental Therapeutics, University of Manchester, Manchester, UK, [4]South Australian Research and Development Institute, Adelaide, Australia, [5]Centre for Genomic Medicine, Massachusetts General Hopstial, Boston, USA.

The neurodegenerative disorder Huntington's disease (HD) is characterized by extensive loss of striatal neurons and the midlife onset of debilitating and progressive chorea, dementia, and psychological disturbance. HD is caused by the expansion of a poly-glutamine coding CAG repeat in the Huntingtin (HTT) gene. The pathogenic mechanism resulting in cell dysfunction and death beyond the causative mutation is not well defined and currently there is no therapy that can prevent or slow the disease. To further delineate the early molecular events in HD, we performed RNA-sequencing (RNA-seq) on striatal tissue from a cohort of 5-y-old OVT73-line sheep (n = 6 OVT73, 6 control) expressing a human CAG-expansion HTT cDNA transgene. Our HD OVT73 sheep are a prodromal model and exhibit minimal pathology and no detectable neuronal loss. We identified significantly increased levels of the urea transporter *SLC14A1* in the OVT73 striatum, along with other important osmotic regulators. Further investigation using a biochemical assay revealed elevated levels of the metabolite urea in the brain (striatum and cerebellum) of the same OVT73 sheep. In parallel, we discovered that levels of urea are also increased in post-mortem human brain from HD cases, including those with low-level neuropathology (Vonsattel grade 0/1). This elevation in urea indicates increased protein catabolism, possibly as an alternate energy source, given the generalized metabolic defect in HD. Increased urea and ammonia levels due to dysregulation of the urea cycle are known to cause neurologic impairment. Taken together, our findings indicate that aberrant urea metabolism could be the primary biochemical disruption initiating neuropathogenesis in HD.

## G23: Genomics Aotearoa: Aiming to improve the use of genomics in New Zealand

Dearden, P.K.[1]

[1]Genomics Aotearoa and Biochemistry Department, University of Otago. P.O. Box 56, Dunedin, New Zealand.

Genomics Aotearoa is an MBIE funded partnership between 3 Universities and 4 CRIs tasked to improve the use and uptake of genomics in New Zealand. Genomics Aotearoa aims to build capacity and capability in genomics and bioinformatics through exemplar projects that develop tools and technology to solve problems of importance to New Zealand. Genomics Aotearoa is developing both bioinformatics infrastructure, and skilled personnel to develop the use of genomics in health, environment and primary production. Many of the issues to be addressed are of importance to Māori, so Genomics Aotearoa will work in partnership with Māori to ensure benefit sharing and the development of trust.

This talk will present the principles of Genomics Aotearoa, introduce its current suite of funded activities and indicate upcoming opportunities for New Zealand researchers.

## G24: A step towards unifying health genomic activity in NZ introducing Genomic Health Alliance New Zealand (GHANZ)

Neas K.[1], Bromhead, C.[2], Felix, C.[3], Gamet K.[4],Hewett R.[5] King, R.[6], Print, C.[7], Robertson, S.[8], Wihongi, H.[9], Yap, P[4]

[1]GHSNZ, Central Hub, Wellington, NZ, [2]NZORD, Wellington, NZ, [3]Wellington Regional Genetics Laboratory, CCDHB, Wellington, NZ, [4]GHSNZ, Northern Hub, Auckland, NZ, [5]Lab Plus, ADHB, Auckland, NZ, [6]Canterbury Health Labs, CDHB, Christchurch, NZ, [7]Auckland University, Auckland, NZ , [8]University of Otago, Dunedin School of Medicine, NZ, [9]Waitemata DHB, Auckland, NZ.

The GHANZ vision is to improve the health of all NZers by integrating genomics into routine healthcare. In the talk I will discuss the key objectives and current outcomes of GHANZ.

## G25: Functional genomic approaches to understanding responses to cytotoxic anti-cancer therapies

Hunter, F.W.[1,2], Lipert, B., Tsai, P.[3], Kakadia, P.[3], Li, D.[1], Mirinargesi, M.[1], Xu, Y.[1], Khan, A.[1], Bohlander, S.K.[2,3], Print, C.G.[2,3], Wilson, W.R.[1,2]
[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand, [2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand, [3]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand

Despite major advances in molecular and immune targeted therapies over the recent two decades, cytotoxic interventions such as chemotherapy and radiotherapy remain mainstays in the management of most advanced cancer presentations. Patients with histologically equivalent disease can show dramatic variation in response to cytotoxic therapy, creating challenges for the individualisation of management and underscoring the need for an improved understanding of genomic determinants of treatment sensitivity.
We have employed whole-genome CRISPR-Cas9 knockout functional screens in cultured cancer cells as an incisive tool to identify and characterise genomic modifiers of sensitivity to anti-cancer agents. Focusing on DNA-crosslinking drugs (platinating agents and nitrogen mustards), we identified an ensemble of known and novel sensitivity-modifying genes. Principal among these was the RNA methyltransferase *NSUN2*, which conferred marked drug resistance when knocked out in isogenic cells, with an effect magnitude equivalent to the well-characterised platinum sensitivity gene *SLFN11*. Clinically, high-grade serous ovarian carcinoma patients stratified by *NSUN2* mRNA expression showed significantly different response rates to first-line platinum-based chemotherapy.

In related screens, we identified CYREN (*C7orf49*) as a novel mediator of cellular response to ionising radiation. Isogenic CYREN-null cells were moderately hypersensitive to radiation, though less so than cells carrying mutations in the canonical DNA repair genes *PRKDC* or *XRCC4*. CYREN regulates DNA repair pathway choice in S/G2-phase cells in favour of engaging high-fidelity homologous recombination repair for resolution of radiation-induced DNA double-strand breaks. Accordingly, CYREN-null cells are reliant on error-prone non-homologous end joining and show greater *de novo* induction of thiopurine resistance-conferring mutations when irradiated. Intriguingly, this observation suggests that radiation-induced mutational burden may be enhanced in CYREN-low tumours, with implications for the subsequent use of immunotherapies for progressive or recurrent disease.
A functional genomic platform is in place at the University of Auckland for probing clinically-relevant gene/treatment interactions and other cell intrinsic phenotypes.

## G26: Immunogenomic engineering of MHC alleles

Kelton, W.J.[1], Waindok, A.C.[1], Pesch, T.[1], Pogson, M.[1], Ford, K.[1], Parola, C.[1], Reddy, S.T.[1]

[1]Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland,

The major histocompatibility complex (MHC) locus plays an important role in immunity by distinguishing 'self' from 'non-self'. In the context of host defence this system is indispensable in protecting against disease but can actively work against some medical therapies such as transplantation, leading to rejection. Finding sufficient MHC matched donors is challenging and new approaches are required to address supply shortages. We have demonstrated proof of concept of *ex-vivo* exchange of 5kb MHC alleles in immune cells using CRISPR/Cas9. For initial evaluation, we used murine antigen presenting cells expressing high levels of surface MHC (H2-Kd). Repair templates encoding the H2-Kb allele were supplied with Cas9 RNP to induce paired double stranded breaks at the MHC locus. Following allelic exchange, flow cytometry was used to enrich populations of cells containing the exchanged and newly expressed allele. Engineered cells behaved like native H2-Kb expressing cells in T cell activation assays demonstrating a gain-of-function that is absent in MHC knockout approaches. We have continued to develop our *in vitro* MHC exchange system as a technology platform for the discovery of neo-antigens. In future we envisage such an approach could be adapted to improve MHC matching in cellular transplantation.

## G27: Use of gene editing technology to accelerate xenotransplantation research.

Nottle M. B.[1,2], Salvaris E.[2], Fisicaro N.[2], McIlfatrick S.[1,2], Vassiliev I.[1,2], [3]Hawthorne W., O'Connell P.[3], Brady J.[4] , Lew A. [4,5], Cowan P [6,7].

[1]Robinson Research Institute & Adelaide School of Medicine, University of Adelaide, Adelaide, Australia.[2]Immunology Research Centre, St. Vincent's Hospital Melbourne, Melbourne, Victoria, Australia.[3]Westmead Millennium Institute, University of Sydney, Sydney, Australia.[4]Walter and Eliza Hall Institute, Melbourne, Victoria, Australia.[5]Department of Microbiology & Immunology, University of Melbourne, Melbourne, Victoria, Australia. [6]Department of Medicine, University of Melbourne, Victoria, Australia.

We have recently reported the production of GGTA1 knockout/knock-in pigs containing an anti CD2 monoclonal antibody using *Fok*1-dCs9[1]. The GGTA1 knock-in backbone was designed for versatility by incorporating a multiple cloning site to enable simple interchange of transgene regulatory elements and/or coding regions. The relative ease with which a 8.4kb hTBM transgene was knocked in a preliminary study suggests that the upper size limit for HDR using short homology arms (1.06 and 0.74kb) is yet to be reached. Suggesting that additional genes can be incorporated into the construct either as separate transcriptional units or by using 2A for mult-cistronic expression. This will greatly accelerate xenotransplantation research by allowing multiple modifications to be incorporated into the genome in the space of one generation as well as having other biomedical and agricultural applications. In other work we have isolated a new pluripotent stem cell type which is isolated earlier in development than existing primed human ESCs. While differentiating these cells to different cell types may provide an alternative to xenotransplantation, by combining these two technologies the possibility exists of producing humanised pigs which provides another dimension to solving one of medical sciences greatest challenges.

1. Nottle MB, Salvaris EJ, Fisicaro N, McIlfatrick S, Vassiliev I, Hawthorne WJ, O'Connell PJ, Brady JL, Lew AM, Cowan PJ. Targeted insertion of an anti-CD2 monoclonal antibody transgene into the GGTA1 locus in pigs using *Fok*I-dCas9. Scientific Reports. 2017 16; 7(1): 8383. doi: 10.1038/s41598-017-09030-6.

## G28: Generating immune-compatible sheep for xenotransplantation

Appleby, S.J.[1,2], Davidson, A.J.[1], Oback, B.J.[1,2]

[1]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ, [2]Reproduction, AgResearch Ruakura, Hamilton, NZ.

Sheep have a similar physiology and anatomy to humans, making them potentially widely acceptable donors for organ xenotransplantation. However, one critical barrier to the use of organs from foreign species is immune rejection. To overcome this, we have been targeting two main xenoantigens involved in hyperacute immune reaction: galactose-α(1,3)-galactose (α-Gal) and N-glycolylneuraminic acid (Neu5Gc).

The CRISPR-Cas9 system was used to disrupt the enzymes responsible for the formation of α-Gal and Neu5Gc in sheep, α(1,3)galactosyl transferase (GGTA) and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH), respectively. Ovine fetal fibroblasts (OFFs) were transfected with two plasmids, containing CRISPR guide RNA sequences for targeting GGTA or CMAH and a puromycin selection marker. Following transient antibiotic selection, isolated OFF clones were grown for one week prior to analysis. Editing was determined by TIDE analysis[1], flow cytometry of fluorescently-labelled isolectin $B_4$ against α-Gal, and droplet digital PCR with a wild-type drop-off probe against CMAH. These assays detected GGTA and CMAH edits in 97±0% and 77±4% of cells, respectively. Mitotic OFF doublets were manually selected and individually seeded, resulting in 54 clones with 11 different homozygous edited genotypes. Within each genotype, identical edits on both alleles occurred in 64% of GGTA and 55% of CMAH clones, with 27% of clones containing identical mutations for both genes. Frameshift mutations were frequent for GGTA and CMAH (73 and 64%, respectively), with 55% of OFF clones containing frameshifts in both genes. Two cell clones without random plasmid integration and minimal off-target editing were chosen for serial somatic cell nuclear transfer (SCNT). Following SCNT into abattoir-derived oocytes, development into blastocysts was 19±3% (89/476, n=12). At day 35 of development, ultrasonography scanning confirmed that 21±6% of embryos (11/53, n=10) established a viable pregnancy. Resulting lambs will be analysed to confirm loss of α-Gal and Neu5Gc and evaluated as donors for xenotransplantation.

*Brinkman, E.K., Chen, T., Amendola, M., and van Steensel, B. (2014).* Easy quantitative assessment of genome editing by sequence trace decomposition. *Nucleic Acids Research. 42(22): e168*

## G29: Functional outcomes of fine tuning the expression of PD-L1 and PD-1 proteins to the melanoma immune response

Storm Taurere[1], Yale Michaels[2], Daniel Verdon[1], Ashleigh Gaines[1], Tudor Fulga[2], Rod Dunbar[1], Hilary Sheppard[1]

[1]School of Biological Sciences, University of Auckland, NZ, [2]Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.

Immune checkpoint inhibitors have proved effective in clinical trials targeting a number of cancers including melanoma. A key example is the blocking antibody pembrolizmab (marketed as Keytruda™) which targets the inhibitory PD-L1/PD-1 pathway. It is proving effective in the clinic with responsive patients showing durable results. One predictor of patient response is PD-L1 positivity within the tumour. However some studies have shown that patients which score negative for PD-L1 expression can have durable responses and others with high PD-L1 levels do not respond at all [1]. Both PD-L1 and PD-1 expression levels can be dynamic which can confound these types of studies. Therefore questions still remain regarding the levels of PD-L1 within the tumour and the effectiveness of blocking antibodies such as Keytruda. We aim to answer some of these questions using an *in vitro* system in which we can fine-tune expression of both PD-L1 on melanoma cells and PD-1 on melanoma specific T cell clones. Our collaborators in Oxford University, UK have engineered a system to fine-tune mammalian gene expression based on engineered, synthetic microRNA response elements that can be appended downstream of any gene of interest [2]. Here the microRNA silencing-mediated fine-tuners (miSFITs) have been engineered into the 3' UTRs of both human PD-L1 and PD-1 to generate panels of lentivirus constructs which produce graded expression of these proteins. We will present our preliminary results assessing the functional outcomes of varying expression of both of these key proteins on the immune response to melanoma. We will also discuss the progress of our recent foray into using CRISPR/Cas9 ribonucleic proteins to edit endogenous genes in primary human T cells and primary human keratinocytes.

1. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, Ribas A, Hodi FS, Joshua AM, Kefford R, Hersey P, et al.: P*rogrammed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma*. *J Clin Oncol* 2016, 34:4102-4109.
2. Michaels Y, Mike B. Barnkob, Hector Barbosa, Toni A. Baeumler, Mary K. Thompson, Violaine Andre, Huw Colin-York, Marco Fritzsche, Uzi Gileadi, Hilary M. Sheppard, David D.J.H.F. Knapp, Thomas A. Milne, Vincenzo Cerundolo, Tudor A. Fulga: *Precise tuning of gene expression levels in mammalian cells*. *Submitted* 2018.

## G30: New breeding technologies for fruit trees

Allan, A.C.[1,2] and Varkonyi-Gasic, E.[1]
[1]The New Zealand Institute for Plant & Food Research Limited (PFR), Private Bag 92 169, Auckland, New Zealand
[2]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

Many studies have focused on manipulating levels of secondary metabolites in fruit through both conventional breeding and GM approaches. However, New Breeding Technologies (NBTs) offer ways of providing large changes consumer traits (in addition to grower traits) if the public will accept the resulting fruit.
Annualization of woody perennials has the potential sped the breeding and production of fruit crops and rapidly improve horticultural species. Kiwifruit (*Actinidia chinensis*) is a recently domesticated fruit crop with a short history of breeding and tremendous potential for improvement. We used CRISPR/Cas9- mediated manipulation to target mutation of CENTRORADIALIS (CEN)-like genes. Targeting these genes transformed a climbing woody perennial, which develops axillary inflorescences after many years of juvenility, into a compact plant with rapid terminal flower and fruit development. These changes have made kiwifruit amenable for accelerated breeding, indoor farming and cultivation as an annual crop. Using these plants, crossing has begun targeting higher levels of anthocyanins and carotenoids for future cultivars.

## G31: Biotechnology and Forest Trees in New Zealand

Charleson Poovaiah, Sanjeev Raikar, Lorelle Phillips, Steffi Fritsche, Catherine Reeves and Glenn Thorlby.
Scion, Rotorua, NZ,

The large stature of forest trees, long periods of juvenile (pre-reproductive) growth, and delayed expression of traits, such as wood quality result in breeding programmes being long and expensive. Biotechnology provides solutions to mitigate these breeding challenges, particularly through the use of new breeding technologies such as gene editing which allow rapid and precise trait modifications. We will illustrate the potential benefits of integrating gene editing into forest tree breeding programmes using engineered sterility as an example trait. Wildings, non-native invasive conifer tree species that have spread from planted forests, are a major problem in New Zealand. Their control costs in excess of $15M per year and they have been described by the government as “the most significant weed problem New Zealand faces”. The production of trees that are not able to generate wildings would provide a tool to mitigate the environmental and socio-economic damage they cause. Uncertainly over the global regulatory status of gene editing remains a barrier to its integration into breeding programmes. New Zealand is one of the few countries where the regulatory status of gene editing has been clarified. In 2014 the NZ Environmental Protection Authority ruled that plants produced via gene editing, where no transgene was used in the editing process, would not be regulated as GMOs. However, following a challenge in the High Court, this decision was overturned such that NZ currently regulates the products of gene editing as GMOs. The regulatory process which led to gene editing’s current GMO classification in NZ will be discussed.

## G32: Developing Gene editing technology in conifers

Charleson Poovaiah[1], Sanjeev Raikar[1], Glenn Thorlby[1]

Scion, Rotorua, NZ.

The CRISPR/Cas9 nuclease system is a powerful and flexible tool for genome editing. CRISPR/Cas9 has been demonstrated to edit genomes in various plant species including woody species but has not yet been developed in conifers. Scion is developing gene editing technology in conifers, as we believe it will be an invaluable tool in accelerating breeding in these relatively undomesticated species. For a commercial release, it is likely that it will be beneficial to produces edited trees that do not contain a transgene. The long breeding cycles of conifers would make it challenging to uses crossing to remove a transgene. We are, therefore, developing protocols to directly edit protoplasts using a non-transgenic procedure and to regenerate trees from these edited protoplasts. We have consistently isolated protoplasts and have carried out successfully transformation. We will test the efficiency of various RNA polymerase III promoters for use in gene editing. Preliminary results show protoplasts can be used for gene targeting in conifers and will provide mutations for tree improvement and functional studies.

## G33: CRISPR Fruit: three bites at the problem

Drummond, R.S.M.[1], Wang, T.[1], Foster, T[2].

[1]The New Zealand Institute for Plant and Food Research, Auckland, NZ. [2]The New Zealand Institute for Plant and Food Research, Motueka, NZ.

*Actinidia* is a long lived woody perennial with excessive vegetative vigour that can contribute to an unacceptably low yield in an otherwise promising cultivar. Creating a dwarfed cultivar via GA insensitivity is a potential route to improving yield in such a plant. We are using three CRISPR-Cas9 based strategies that will produce respectively; a small deletion event, a gene replacement event and a base editing event, that lead to the removal or inactivation of the DELLA domain from an otherwise functional RGL gene. In this plant the T-DNA locus is likely to be unlinked to the RGL gene allowing its segregation away from the edited allele during subsequent breeding. The regulatory constraints on the use of these plants will vary between both the methods used and the jurisdictions where the plants are grown despite similarly precise and limited changes to the plant's genome.

# Summary of Abstracts for the Poster Session Template

| No. | Title | Presenter | Institutions |
|---|---|---|---|
| G34 | Double cytoplast nuclear transfer improves in vitro but not in vivo development from mitotic embryo-derived pluripotent stem cells in cattle | Pavla Turner | AgResearch, NEW ZEALAND |
| G35 | Modification of Bovine Coat Colour Using CRISPR-Cas9 Homology Directed Repair | Brigid Brophy | AgResearch, NEW ZEALAND |
| G36 | Using low-depth genotyping-by-sequencing data for genome-wide association studies | Andrew Hess | AgResearch, NEW ZEALAND |
| G37 | Optimised conditions for bovine zygote-mediated genome editing by electroporation | Jingwei Wei | AgResearch, NEW ZEALAND |
| G38 | *Grainyhead-like 2* is required for inner ear-like organoid formation from mouse embryonic stem cells | Blaise Forrester-Gauntlett | University of Waikato, NEW ZEALAND |
| G39 | Genotyping-By-Sequencing: Applications from Conservation Genetics to Genomic Selection | Jeanne Jacobs | AgResearch, NEW ZEALAND |

## G34: Double cytoplast nuclear transfer improves *in vitro* but not *in vivo* development from mitotic embryo-derived pluripotent stem cells in cattle

Appleby, S.J.[1,2], Turner, P.M.[1], Oback, F.C.[1] and Oback, B.[1,2]
[1]Reproduction, AgResearch, Ruakura, New Zealand
[2]School of Medical Sciences, University of Auckland, New Zealand

Producing multiple animals from one genomically selected embryo in a single generation would reduce cost and accelerate genetic gain in dairy cattle breeding. We previously converted bovine embryos into embryo-derived pluripotent stem cells ('ePSCs') using chemically-defined culture medium with two kinase inhibitors ('2i')[1]. Kinase inhibitor concentrations were titrated to further promote expression of pluripotency markers in bovine ('2i$^{PLUS}$' medium)[2]. Here we tested ePSCs for embryonic cell nuclear transfer (ECNT) and cloning of ePSC-derived cattle.

Following immunosurgical isolation from the inner cell mass of *in vitro* produced (IVP) blastocysts, adherent ePSCs were derived with an efficiency of 86±6% (blastocysts *N*=105, replicates *n*=5). After six days of feeder-free culture in 2i$^{PLUS}$, colonies were treated with 500 nM nocodazole overnight. Immunofluorescence against phosphorylated histone 3 (P-H3) showed 40±2% of cells within a nocodazole-treated colony in metaphase (*N*=16, *n*=4) compared to 11±3% in DMSO controls (*P*<0.01). Within each colony, an average 43±13% of cells expressed the pluripotency markers SOX2 and NANOG (*N*=25, *n*=4). For ECNT, on average 27 mitotic ePSCs per colony (N=48) were harvested. Karyotyping revealed that 21% of ePSCs had 60 chromosomes, while 64% deviated ±5% from diploid (127 spreads, 28 colonies, *n*IVP=6). Following chemical activation in 6-DMAP, mitotic ePSCs developed into blastocysts at 16±4% (*n*IVP=258, *n*=10), similar to NT with fibroblasts that were arrested in 100 nM nocodazole (10±5%, *n*IVP=146, *n*=5). Double cytoplast NT ('DCNT'), whereby another enucleated oocyte was fused to the first reconstruct, increased blastocyst development from ePSCs and fibroblasts to 24±5% (*n*IVP=574, *n*=14, *P*<0.01) and 25% (*n*IVP=28, *n*=1, *P*=0.06), respectively. Only 1% (1/69, n=6) of mitotic ePSC-derived blastocysts held pregnancies until Day 70 compared to 27% (3/11, n=2) of serum-starved SCNT controls (P<0.05). This poor post-blastocyst development was probably due to genetic errors arising from nocodazole-arrested donor cells.

1 Verma, V., Huang, B., Kallingappa, P. K. & Oback, B. *Dual kinase inhibition promotes pluripotency in finite bovine embryonic cell lines*. Stem Cells Dev **22**, 1728-1742 (2013).
2 McLean, Z., Meng, F., Henderson, H., Turner, P. & Oback, B. *Increased MAP kinase inhibition enhances epiblast-specific gene expression in bovine blastocysts.* Biol Reprod **91**, 49 (2014).

## G35: Modification of Bovine Coat Colour Using CRISPR-Cas9 Homology Directed Repair

Brigid Brophy, Sally Cole, Jingwei Wei, David Wells and Goetz Laible

AgResearch, Ruakura, Hamilton

Selective breeding has long been used to develop desirable traits in livestock however this process is very inefficient and time consuming. We are interested in applying CRISPR-Cas9 gene editing technology, specifically homology directed repair (HDR), to directly introduce beneficial naturally occurring traits into high worth dairy cows.

Previous studies have shown that a 3bp deletion in the PMEL gene (PMEL:c50_52delTTC), resulting in the deletion of a leucine from the signal peptide, causes dose dependent dilution in coat colour in Highland and Galloway cattle[1]. It has been proposed that this lighter coat may provide better heat tolerance by reducing radiative heat gain.

Using cell-mediated HDR-editing, combined with somatic cell nuclear transfer (SCNT), we have generated colour-diluted Friesian cattle. Briefly, the PMEL target gene sequence was screened to identify potential CRISPR sites using the http://crispor.org website. Three CRISPRs were chosen based on proximity to the desired edit, predicted efficiency and off target sites. Initial co-transfection of cells with CRISPR and donor DNA identified the CRISPR with the best HDR efficiency. Individual mitotic cells were picked to generate clonal cell lines which were initially screened by a 3bp deletion-specific PCR. Positive clones were screened by a HDR-specific Droplet Digital™ PCR to identify homozygous edited clones which were confirmed by sequencing. Cell clone CC14 was selected for SCNT along with control wild type (WT) cells. Two edited calves were born that displayed a marked coat colour phenotype with grey and white coat colouring as opposed to the black and white pattern of the three WT controls.

1. S.M. Schultz and D.L. Dreger (2012). *Interaction of MC1R and PMEL alleles on solid coat colors in Highland cattle*. Animal Genetics. 44: 9-13

## G36: Using low-depth genotyping-by-sequencing data for genome-wide association studies

Hess, A.S.[1], Caulton, A.[1], Foote, B.[2], Foote, J.[2], Dodds, K.G.[1], Brauning, R.[1], McEwan, J.C.[1], Clarke, S.M.[1]

[1]AgResearch Limited, Invermay Agricultural Centre, Mosgiel, New Zealand, [2]Foote Goat Farm, Northland, New Zealand.

Genotyping-by-sequencing (GBS) is a sequencing method that uses restriction enzymes to target portions of the genome to obtain genomic information. To reduce costs, low depth (2-4x) sequencing is often used, however, this generates a level of uncertainty in the SNP genotype scores obtained. This uncertainty is particularly evident when distinguishing between homozygous and heterozygous genotypes at loci where only one allele is observed for an individual. Therefore, methods for genome-wide association studies (GWAS) that account for uncertainty in GBS genotype calling are desirable when searching for loci associated with a trait.

We used probability-based genotypes to account for uncertainty in genotyping due to low-depth sequencing. Using this approach, we demonstrated the utility of this method for GBS data by performing a GWAS on milk fat percent in dairy goats, whereby we identified a strong functional candidate gene associated with milk fat percent.

Once a gene of interest is identified and the causal mutation has been found, it may be of interest to genotype this causal mutation en masse on a large number of individuals while capturing variants in the rest of the genome. Previous studies in French dairy goats have identified a causative mutation in the *DGAT1* gene associated with milk fat percent in dairy goats. Our GWAS identified a peak near this gene. We developed primers to test the effects of this mutation on milk fat percent in our population – an approach that could be incorporated into our GBS workflow. We successfully obtained genotypes at this locus, which subsequently allowed us to confirm association of this causative mutation with milk fat percent in New Zealand dairy goats.

## G37: Optimised conditions for bovine zygote-mediated genome editing by electroporation

Wei, J.[1], Gaynor, P.[2], Cole, S.[1], Brophy, B.[1], Oback, B.[1,3], Laible, G.[1,3]

[1] Reproduction, AgResearch Ruakura, Hamilton, New Zealand
[2] Department of Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand
[3]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ

The current strategy for animal breeding by marker-assisted selection to genetically improve livestock is inherently too slow to respond to rapid global climate changes and challenges arising from an increasing human population. Genome editing with programmable nucleases can introduce beneficial natural sequence variants or entirely novel variants known from other breeds or species into elite livestock genotypes within a single generation. In combination with traditional genomic selection, it can accelerate genetic gain by increasing the numbers of desirable variants assembled in an individual high-merit animal. Thus, it offers an exciting new targeted breeding approach to quickly adapt livestock to changing conditions. Genome editing relies on the introduction of site-specific DNA double strand breaks by chimeric designer nucleases. In the presence of an exogenous donor template, the cell can repair the damage by homology-driven repair (HDR). This enables precise control of the repair outcome and introduction of sequence variants specified by the repair template.

Presently, the main approach uses microinjection of the editing tools into individual zygotes, which is labour- and cost-intensive. Here we investigated simultaneous introduction of genome editing tools into numerous bovine zygotes by electroporation. Using an *EGFP* reporter plasmid, we established conditions for both electroporating bovine zygotes with a custom-made electroporation set-up and achieving embryonic development. We introduced CAS9/gRNA ribonucleoprotein complexes and single-stranded HDR repair templates at different concentration ratios into up to 60 bovine zygotes simultaneously. This approach generated site-specific mutations in one target locus, both by random non-homologous end joining and precise HDR-mediated editing with up to 100% and 6% editing efficiency, respectively. At the same time, blastocyst development from electroporated zygotes was not compromised compared to non-treated controls. Compared to microinjection, electroporation with microfabricated co-planar film electrodes[1] was easier to perform, faster and scalable, providing a useful alternative for genome editing livestock.

1 Clow A., Gaynor P. & Oback, B. *Coplanar film electrodes facilitate bovine nuclear transfer cloning.* Biomed Microdevices ***4****, 851-9 (2009)*

## G38: *Grainyhead-like 2* is required for inner ear-like organoid formation from mouse embryonic stem cells

Forrester-Gauntlett, B.[1,2], Henderson, H.[3], Peters, L.[1] and Oback, B.[2,4]

[1]Faculty of Science and Engineering, University of Waikato, New Zealand

[2]Reproduction, AgResearch, Ruakura, New Zealand

[3]Statistics, AgResearch, Ruakura, New Zealand

[4]School of Medical Sciences, University of Auckland, New Zealand

Single nucleotide polymorphisms (SNPs) in the transcription factor *grainyhead-like 2* (*Grhl2*) gene are associated with progressive non-syndromic sensorineural deafness autosomal dominant type 28 (DFNA28)[1]. To study its role in hearing loss, we have disrupted the *Grhl2* gene in mouse embryonic stem cells (ESCs) using CRISPR/Cas9. For a complete knockout (*KO*), *wild-type* (*WT*) ESCs were transfected with pools of three different gRNA/*Cas9* expression vectors and homology-directed repair (HDR) plasmids, targeting insertion of a red fluorescent protein (RFP) and puromycin resistance gene into exons 2, 3 and 4. We identified 16 RFP-positive, puromycin-resistant clones with targeted HDR events. These included two monoallelic and five biallelic clones with insertions into exon 3, the latter lacking full-length *Grhl2* mRNA transcripts.

To elucidate the role of *Grhl2* in DFNA28, we differentiated floating embryoid bodies (EBs) into three-dimensional epithelial organoids that contain mammalian inner ear-like support and sensory hair cells[2]. Using CellProfiler™ image analysis, we quantified development from homozygous vs heterozygous *KO* vs *WT* organotypic ESC cultures. On day 1 after seeding, heterozygous and *WT* ESCs aggregated into spherical tightly packed EBs with a smooth surface. By contrast, homozygous ESCs formed significantly bigger aggregates with a larger surface area, a more eccentric shape and a less compact structure. These differences indicate a role for *Grhl2* in cell-to-cell interactions during initial EB formation. Stable *Grhl2* overexpression in homozygous clones restored normal EB development. On day 8, aggregates were transferred into minimal maturation medium to allow self-guided organogenesis for two weeks. During this period, homozygous and *WT* lines developed similarly but maintained their initial differences in size and shape. However, heterozygous lines underwent different cellular rearrangements and all degraded by day 16. In summary, N-terminal truncation of GRHL2 altered inner ear organoid differentiation, consistent with a role of *Grhl2* in organising epithelial integrity during development.

1. Peters, LM et al. (2002) *Hum Mol Genet*. 11(23):2877-85;

2. Koehler, KR., Hashino, E. (2014). *Nat Protoc.* 9(6):1229-1244

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## G39: Genotyping-By-Sequencing: Applications from Conservation Genetics to Genomic Selection

Jeanne Jacobs[1], Shannon Clarke[2], Marty Faville[3], Andrew Griffiths[3], Rachael Ashby[2], Timothy Bilton[2], Rudiger Brauning[2], Ken Dodds[2], John McEwan[2]

AgResearch, New Zealand;
[1]Lincoln Science Centre, Private Bag 4749, Christchurch 8140,
[2]Invermay Agricultural Centre, Private Bag 50034, Mosgiel 9053,
[3]Grasslands Research Centre, Private Bag 11008, Palmerston North 4442.

Genotyping-by-Sequencing (GBS) is used for generating high-density genetic marker data in a cost-effective manner across a range of applications. We have developed the infrastructure and skill base required to apply (high throughput) GBS for different purposes across a wide range of species. Thus far, we have optimised GBS in over 50 different species encompassing plants, mammals, shellfish, fish, birds, and insects. Several of these species have no prior genetic and/or genomic information, making GBS the method of choice. The methodology has been scaled from small sample sizes (< 100) for diversity studies, up to many thousands in animal and plant breeding programmes where GBS underpins genomic selection. We have used GBS for environmental metagenomics, population genetics and conservation genetics studies. Continuous improvements in wet-lab methods have enabled increased quality and quantity of data generated. Data analyses have been enhanced through improved bioinformatic workflows tailored to each application, including statistical methods designed specifically for (low depth) GBS data such as; 'KGD' for developing genomic relationship matrices[1], 'GUS-LD' to estimate pairwise linkage disequilibrium while accounting for under-called heterozygous genotypes[2], and 'GUSMap' for constructing linkage maps[3]. The components of the data analysis workflow are available in a public Github repository (https://github.com/Agresearch). Examples of the various applications will be highlighted. Many studies are in collaboration with universities and research organisations, as well as commercial entities, both nationally and internationally.

1. Dodds et al., *Construction of relatedness matrices using genotyping-by-sequencing data* BMC Genomics 16:1047, 2015 doi 10.1186/s12864-015-2252-3

2. Bilton et al., *Linkage Disequilibrium Estimation in Low Coverage High-Throughput Sequencing Data* Genetics 209: 389-400, 2018. doi 10.1534/genetics.118.300831

3 Bilton et al., *Accounting for Errors in Low Coverage High-Throughput Sequencing Data When Constructing Genetic Maps Using Biparental Outcrossed Populations.* Genetics 209: 65-76, 2018. doi 10.1534/genetics.117.300627