

MAPNET 2022 ABSTRACTS

DAY 1

Māori Data Sovereignty: An introduction and implementation using Te Tiriti principles

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An introduction to Māori Data (Digital and Biological) and Māori Data Sovereignty through a Te Ao Māori perspective using the most up to date and widely recognised knowledge, and how the principles can be introduced by researchers. Te Tiriti, United Nations Declaration of the Rights of Indigenous Peoples and the Mataatua Declaration are discussed and practicable steps to ensure Crown agencies recognise and implement appropriate protocols.

Genomic Medicine Pathfinder

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A pathfinder programme to develop capability and infrastructure for research into personalised genomic-data informed medicine for Aotearoa New Zealand. Demonstrating genuine co-design and integration of mātauraka Māori and whakapapa knowledge within the research programme. Our action learning approach to development of a scalable precision medicine programme, is dynamic. Moving theory into using demonstration to understand integrated practice. Indications are for the importance of locally applied solutions and nationally applied support. We have realized the need for federation of access, analysis and research function from multiple data storage sources under localized authority, meeting localized need. We are not so much working with 'Māori data sovereignty' theory but rather applied 'Ngāti Porou data sovereignty' practice. More closely aligned to a genuine Treaty authority compliant approach. We are attempting to understand the 'ecosystem' and environment within which practice sits - is it supportive? Does it provide public trust? What improvements are needed? We have developed functional understandings for a managed access data facility which will meet research and governance needs for Ngāti Porou Hauora our mana whenua research partner. Lessons learnt may provide a basis for scale. The programme continues in finding current research environmental settings and a pathway toward new practice.

Capturing global genetic diversity in a white clover core collection using pooled GBS

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White clover (*Trifolium repens*) is a key component of New Zealand's pastoral agriculture, providing both quality forage and plant-available nitrogen fixed from the atmosphere via symbiosis with soil-borne *Rhizobium bacteria*. It is grown in mixed sward pastures with grasses, such as perennial ryegrass, throughout temperate regions. White clover is an allotetraploid resulting from a recent (15K – 28K years ago) hybridisation in western Europe. It has retained the genomes from both progenitors, and this expanded genomic tool kit may underpin the adaptive success of this species and its global spread. Development of new cultivars targeted for environmental traits and climate adaptation relies upon discovery and subsequent utilisation of natural genetic variation. Germplasm centres, such as the Margot Forde Germplasm Centre (MFGC) based at AgResearch Grasslands, Palmerston North, are repositories of plant genetic variation that may be accessed and exploited. An initial step in utilising this material is characterisation based on genetic diversity to support the identification of a core set of accessions that is a genetically-representative subsample of the collection. This core then provides a practicable-sized set for trait characterisation. A set of 689 accessions was obtained from the MFGC spanning white clover's original geographic range across western Europe/North Africa to Central Asia, as well as elsewhere around the globe. An additional set of legacy and modern cultivars was incorporated into the analysis. The accessions were characterised genetically using pooled genotyping-by-sequencing (pooled GBS) based on 30 individuals per accession. Approximately 30,000 SNPs were identified and, using discriminant analysis of principal components (DAPC) to investigate population structure, 11 clusters were described across Europe and Central Asia. All ecotypes from outside this area were members of four western European clusters, and most cultivars were derived from two of these clusters. These results indicate that white clover cultivars are derived from a narrow genetic base and that there is much untapped genetic diversity across a wide range of ecogeographical zones. Using two methodologies (k-medoids and Core Hunter), a set of 157 accessions were identified across the clusters that represent a core subsample of the assessed collection. This core collection will provide a basis for developing diverse pre-breeding populations that will be assessed for environmental traits and will support development of new cultivars providing greater climate resilience and improved environmental performance from NZ pastures.

Accelerating genetic gains in New Zealand dairy sheep

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The New Zealand dairy sheep industry has grown markedly in the last decade. With better genetics, dairy sheep companies in New Zealand have been able to increase milk yield, protein and fat content and achieve several other key outcomes. In this talk, we will present a case study for a routine genetic evaluation program in a large commercial dairy sheep operation and showcase the partnership that AgResearch has developed to implement genomic selection in this industry. The genetic evaluation now includes over 100,000 phenotypic records and 10,000 genotyped animals and its outputs are not only informing selection decisions at a commercial scale but also allowing genome-wide association studies and population genetics analysis in New Zealand dairy sheep.

DAY 2

Investigating genotype-phenotype relationships for tuber bruising in autotetraploid potatoes

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Tuber bruising of tetraploid potato is an important quality trait as it affects the appearance and flavour of the tubers and thus impacts their fitness for sale. The development of potato lines that are more resistant to bruising is therefore a desirable objective for breeding programs, rendering the genetic analysis of this trait an important task. In this study, we investigated the biological mechanisms underlying tetraploid potato tuber bruising using multi-omics data. Genotype by sequencing using exon capture obtained from a breeding population of half-sibling families was used to uncover regions of interest for the bruising phenotype, as well as other agronomic traits of interest. In addition, we employed a Systems Biology approach to obtain a more holistic and comprehensive view of the molecular mechanisms involved in tuber bruising, and bridge the gap between genetic variations and phenotype. To this end, RNA sequencing and metabolomics data were obtained, and GWAS, differential expression analysis and multi-omics data integration were leveraged in order to detect molecular features (i.e. genomic variants, genes and metabolites) involved in tuber bruising. We demonstrate that even as capture sequencing only allows us to measure genetic variations in a subset of the genome, it is possible to uncover interesting and biologically meaningful genotype phenotype associations, especially when combining the GWAS results with other omics datasets. Moreover, these associations were obtained with samples selected from a breeding program, demonstrating that available data from populations not specifically designed for association study can be used to uncover genomic regions potentially associated with a trait of interest.

Building R Statistical Genetics Workflows as Task Graphs with the Targets Package

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Development of statistical genetics models for GWAS, breeding value prediction and mapping typically require the preparation, QC and integration of multiple data sources, followed by iterative fitting, QC, and exploration of models. Traditional linear script or notebook approaches for development scale poorly and can pose major challenges, especially where high performance computing is required. The modern alternative approach to encoding data and modelling workflows is to express these as task graphs, where the nodes are data objects connected by user-defined functions (UDFs). These graphs can then be executed by a workflow engine which can selectively execute steps in the workflow and manage caching and integration with workload managers. Currently the most mature pipeline toolkit for R is the targets package, which was initially developed in Eli Lilley to enable Bayesian modelling at scale. We will describe the changes in mindset and development practice needed to adopt usage of such toolkits, and experience gained in building pipelines for breeding value estimation at scale.

Genomics-based breeding of radiata pine: are we there yet?

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A decade of investment in genomics has made it possible to test if this technology can currently accelerate and improve decision making in tree breeding. We used a large data set to demonstrate that (1) the accuracy of pedigree information was improved dramatically using a moderate number (<10k) of single-nucleotide polymorphism (SNP) markers; (2) genomic prediction was possible across populations for two of the most important traits currently targeted by radiata pine breeding in Australasia (diameter and wood density); and (3) single-step blended prediction combining historical and contemporary data sets can be effective. Operational implementation of genomic and single-step blended prediction is currently in progress and economic feasibility will be assessed directly over the next few years. In addition, gene discovery through genome-wide association studies is gaining momentum, making it possible to use gene editing in addition to genomic selection to increase the agility and resilience of tree improvement.

Developing a model for genomic selection of low epitope wheat

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Wheat is a vital component of the modern human diet. The value in wheat comes primarily from its unique grain proteins that make up gluten. Gluten proteins are the components in wheat flour that allow it to produce a strong, elastic, springy dough and it's those characteristics that are required to produce the quality bread and other baked products we all know and love. Although the perception of wheat based products has been somewhat tainted with lower quality, high additive breads, wheat continues to provide a significant proportion of many vital nutrients to the average human diet, including proteins, vitamins and dietary fibre. The value of gluten in wheat based products is difficult to understate, however there is also a growing awareness with regards to the downsides of gluten. Coeliac disease is an autoimmune disease caused by gluten consumption where affected individuals develop a permanent intolerance to dietary gluten. The disease affects around 1% of the general population, making it the most common food sensitive enteropathy in humans. There is also strong evidence that the incidence of coeliac disease is increasing globally which may well be linked to increased gluten consumption per capita. The symptoms of coeliac disease are triggered by gluten epitopes. These epitopes are relatively small and specific parts of the overall gluten protein. It is known that the level of gluten epitopes in wheat can differ significantly from cultivar to cultivar. This means that there is an opportunity for breeders to utilise this diversity in genetics and potentially breed towards lower gluten epitope wheats. The long term benefits of this could be significant to both industry, in the form of higher value/niche products as well as to consumers, in the form of reduced incidence of coeliac disease or even reduced symptoms of gluten intolerance.

Genome-wide association study for milk traits in New Zealand dairy sheep

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This study performed a genome-wide association (GWA) analysis to identify the genotype-phenotype association on dairy sheep in New Zealand. The sample included 4,590 ewes with records for milk yield and 2,823 ewes for fat and protein. There were 14,409 single nucleotide polymorphisms (SNP) available for all the analyses. We included the top three principal components in the linear mixed model as fixed effects to account for population structure and relatedness. GWA analysis for fat and protein showed no significant markers. Five polymorphisms reached the significant threshold identified in the study for milk yield. Each of those markers came from chromosomes 4, 13, 26, while two of the significant markers were from chromosome 3. Importantly, significant variants were mapped to seven genes and validated in previous dairy sheep studies. Genes shown in this study could be promising candidate genes, requiring future study to identify the causative mutations associated with milk yield.

Transposable element dynamics in Sparidae genomes

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Teleost fish represent the largest group of vertebrates, and this diversity is reflected in their genomes. Recent approaches to assessing structural genome changes, instead of only single nucleotide variants (SNPs), have revealed that most genome diversity within tāmure/snapper (*Chrysophrys auratus*, family Sparidae) is due to these structural variants. The genomic context of structural variants has been investigated in terms of genic content, but not yet in relation to other genomic elements. Transposable elements (TEs) are structural genetic elements which can move throughout the genome using either “cut and paste” or “copy and paste” mechanisms. TE patterns can be inherited, but additionally, TE movements can also occur during the lifetime of a cell, and often disrupt genes and other functional regions. Here, we investigate the TE content of the tāmure genome, and compare it to its sister species (i.e. red seabream, *Pagrus major*). Both *C. auratus* and *P. major* contain similar levels of TEs (25.27% and 24.26% respectively), and most (13.55% and 15.79% respectively) are currently unclassified. Of the classified TEs, the greatest proportion of the genome is taken up by DNA transposons (*C. auratus*: 6.49%; *P. major*: 4.36%), with a few families being found up to 1000s of times each. This fits within the wide range of TE proportions in teleost fish genomes, from about 5% in pufferfish (*Tetraodon nigroviridis*) to 56% in zebrafish (*Danio rerio*). This work forms the basis of extensive TE curation which will produce specific libraries for Sparidae. TE curation and quantification will also support ongoing work focused on the genomic consequences to various performance traits (e.g. growth), and their relationships to structural variant hotspots.

DAY 3

Low-cost methods of quantifying disease challenge infection whilst simultaneously genotyping the host via sequencing-based assays

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Genomic selection is particularly effective where full siblings are exposed to a disease challenge, either naturally via field outbreaks or in challenge experiments, whilst the breeding population is retained in an unchallenged environment and measured for growth and production traits. In many cases only survival or time-to-death phenotypes are available. More recently qPCR and associated Ct values for quantifying infection levels have also been proposed. This is especially valuable in field challenges where multiple pathogenic organisms may be present or when only a low percentage of fish suffer disease signs. However, the qPCR methodology is expensive, cannot be sample multiplexed, and typically lacks multiple internal controls. For example, this includes having several amplicons of the host and pathogen(s) coupled with DNA sequence-based validation of each amplicon. Here we present examples in Arctic charr (*Salvelinus alpinus*) of simultaneous multiplex quantification and host genotyping of the bacterium *Aeromonas salmonicida* inducing furunculosis and *Renibacterium salmoninarum*, inducing bacterial kidney disease (using sequencing-based assays, namely restriction enzyme reduced representational sequencing (RE-RRS) and/or genotyping-in-thousands by sequencing (GT-seq). Separately, in Atlantic salmon (*Salmo salar*) we use a modified GT-seq protocol, by adding a reverse transcriptase step (rtGT-seq), to quantify infection levels of the ds RNA piscine myocarditis virus (PMCV) causing cardiomyopathy syndrome (CMS) and ds RNA piscine orthoreovirus (PRV) causing heart and skeletal muscle inflammation (HSMI). For all methods where values are expressed as $\log_{10}(\text{disease count}/\text{host count})$ there was typically high repeatability ($r^2 > 0.95$) and the approach could be easily calibrated to the sensitivity required. For example, we achieved a linear relationship over a 3.5 \log_{10} dilution range with a r^2 greater

than 0.98 using Arctic char samples spiked with *A. salmonicida* DNA in a GT-seq assay. Using arctic char samples naturally infected with *A. salmonicida*, the GT-seq and RE-RRS assay had a high linear correlation ($r^2 = 0.87$) over a \log_{10} infection range of slightly less than 3.5. For Atlantic Salmon artificially infected with CMS and assayed using rtGT-seq a correlation (r^2 of 0.95) was achieved in separate technical replicates. A correlation (r^2 of 0.65) was achieved for the rtGT-seq method with CMS Ct values using an independent qPCR assay which had no internal controls. Given the challenged fish are concurrently genotyped for genomic selection, the cost of these novel assays varies. There is nil additional cost for the RE-RRS method, NZ\$10/sample for the GT-seq method and NZ\$20/sample for the rtGT-seq assay.

Systematic evaluation of assays to profile DNA methylation: application for livestock breeding

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Advances in “omics” technologies have fuelled investigation into the epigenome as a tool to enhance livestock selection and breeding practices. DNA methylation is an important epigenetic mark that is essential for genomic stability and maintenance throughout development and serves as a biomarker of chronological age and a biological fingerprint of the stress response. In order to successfully incorporate DNA methylation data into current genetic merit predictions for livestock the approaches that are used need to be high throughput, robust and cost-effective. This study reviews four DNA methylation profiling assays (1) whole-genome bisulphite sequencing (WGBS); (2) reduced-representation bisulphite sequencing (RRBS); (3) Oxford Nanopore Technology long-read sequencing; and (4) methylation microarray technology via the recently released custom mammalian methylation array “HorvathMammalMethyl40”. The relative accuracy of each assay is benchmarked against the WGBS dataset, the gold standard methodology, and the cost benefits are discussed with particular consideration for industry application. Two exemplar studies are outlined to demonstrate the potential applications of methylation profiling for livestock breeding. The first uses RRBS to profile genome-wide methylation levels across a relatively small cohort of sheep (*Ovis aries*; n=54) exposed to a controlled facial eczema disease challenge and identifies stress-imposed changes to DNA methylation across three time points; day 0 (pre-challenge), day 21 (post-challenge) and day 275 (post-challenge). The second study employs the mammalian methylation microarray to construct epigenetic clocks for livestock that are highly predictive of chronological age. The concept of so-called “epigenetic clocks” has emerged from a large body of literature describing the correlation between genome-wide methylation levels and age. Presented here are the first of its kind for domesticated goat (*Capra hircus*), as well as cattle (*Bos taurus*), Red (*Cervus elaphus*) and Wapiti deer (*Cervus canadensis*) and composite-breed sheep. In addition to the species-specific clocks is a New Zealand livestock ‘farm animal epigenetic clock’ for all animals included in the study, which will enable robust predictions to be extended to various breeds, species and environments. The farm animal clock shows similarly high accuracies to the

individual species' clocks ($r > 0.97$), utilising only 217 CpG sites to estimate age (relative to the maximum lifespan of the species) with a single mathematical model. Overall, this research is directed towards the discovery of individual methylation markers or marker combinations that may be useful in the selection and breeding of livestock for continued improvement.

Development of a Recombinase Polymerase Amplification assay for rapid in-field detection of *Oryctes rhinoceros nudivirus* in coconut rhinoceros beetle

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Coconut rhinoceros beetle (CRB) is an important pest of palm plantations across the Pacific. A natural pathogen of CRB, *Oryctes rhinoceros nudivirus* (OrNV) is used as a biocontrol agent to suppress the pest. As part of regular field monitoring, a PCR-based assay has been used to determine the presence and spread of the virus in CRB populations. However, samples need to be sent to well-resourced laboratories to perform these diagnostics, adding to the time for obtaining results. While the conventional PCR-based approach has proven reliable and effective, it requires purified DNA, sophisticated instrumentation and takes up to 3 hours. Recombinase Polymerase Amplification (RPA) has become a powerful tool for 'point of care' molecular diagnostics because of its simple set-up and shortened timeframes. Similar to PCR, RPA amplifies DNA but operates at a constant temperature (between 25°C to 42°C). RPA is also tolerant to numerous PCR-inhibitory substances, which reduces the time and cost involved in obtaining purified DNA from test samples. Additionally, RPA requires minimal instrumentation (e.g. a simple heating block and/or a portable fluorometer) with results obtained within 30-40min, making it suitable for field conditions in the Pacific. We have developed an RPA assay to detect OrNV within 20 min using fluorescence-based chemistry. Results show a high correlation between the RPA- and the PCR-based assay using purified DNA. Testing of crude tissue lysates from CRB gut tissue is in progress. We will present progress and discuss the opportunity that RPA-based assays present within the context of efforts to improve CRB management for our Pacific Island neighbours.

Rumen microbiome information as a proxy phenotype for enteric methane emissions in the New Zealand livestock industry

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Collecting measurements on various important economic and environmental livestock traits, such as greenhouse gas emissions and feed intake, are often expensive and time-consuming, necessitating the need for proxy measures. One suitable proxy is the rumen microbiome information as it has been shown to be associated with traits such as methane emissions and residual feed intake. However, implementation of rumen microbiomes as a proxy phenotype at an industry level requires a method that is scalable and provides robust predictions across different farms and environments, whereas most studies to-date have been on small scales, with predictions only evaluated within flocks. Using a restriction enzyme reduced representation sequencing approach, we generated several datasets from sheep, which were from a range of genetically linked research and commercial flocks and feed a variety of diets, to investigate the potential of using rumen microbiome data to predict methane traits. We used a linear mixed modelling (LMM) approach, similar to what is commonly used for genomic selection in industry but with the genomic relationship matrix replaced by a microbial relationship matrix and a scaling approach that enables phenotype predictions across environments and flocks. Several datasets consisting of older sheep that were on different diets were used to train the LMM and prediction of methane traits on younger animals were made, with prediction accuracies ranging from 5.7% to 68% (mean accuracy of $30\% \pm 12\%$) across a variety of research and commercial flocks used as validation sets. The genetic and phenotypic correlations between methane predicted from the rumen microbiome and direct methane measurements was also estimated in a bivariate LMM analysis, with moderate to high genetic correlation (0.76 ± 0.14) and moderate phenotypic correlation being obtained (0.35 ± 0.03). We also investigated various dimension reduction and machine learning methods for predicting methane traits and found prediction accuracies were similar or slightly improved compared to the LMM approach. Overall, our results show potential for rumen microbiome information to be used for phenotype prediction in

breeding applications potentially enabling further genetic gains to be made in New Zealand's livestock industry.

Snapper like it hot: growth and gene expression in response to temperature

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Organisms are constantly exposed to changes in their environment. Ectotherm fish are highly dependent on environmental temperature to regulate their body temperature. Hence, changing water temperatures due to a changing climate are directly impacting important life history traits. While fish are able to cope with seasonal and daily temperature variations, permanent exposure to more extreme temperatures close to the species' thermal tolerance limits may detrimentally impact the organism's health and ultimately its survival. Here we test gene expression changes that allow individuals to be more resilient to changing temperatures, which allows for adaptive responses due to phenotypic plasticity. Moreover, we test if gene expression profiles are similarly affected under long-term exposure to different temperature regimes to gain insight into the role of chronic temperature conditions on individual growth and transcriptomic responses. Juvenile Australasian snapper, *Chrysophrys auratus* (Forster, 1801), were exposed to two different temperature regimes (warm versus cold) over a period of three months. Phenotypic measurements were collected for 576 individuals to identify fitness consequences. Furthermore, pooled transcriptomic data was obtained from head kidney and liver tissue of 48 individual fish. Fish exposed to warm temperatures showed significantly higher growth rates than fish kept under cold temperatures. Gene expression profiles between treatments were compared to identify differentially expressed genes. Our initial results indicate that some genes are differentially regulated between both tissues, head kidney and liver, as well as between the warm and cold treatment, demonstrating the distinct effect of temperature on gene expression. Our study will provide a better understanding of the transcriptomic underpinnings of thermal plasticity in snapper and will inform future aquaculture breeding programs of this and related species.