QMB Abstracts

Q1: Enhancing crop yield and composition to reduce environmental impacts of agriculture

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Plant photosynthesis is regulated by feedback mechanisms based on the carbohydrate status and the carbon and nitrogen balance. Stable accumulation of micro-organelles containing fatty acids in photosynthetic tissues via co-expression of cysteine oleosin and diacylglycerol acyl-transferase (DGAT1) confers a novel carbon sink that increases photosynthesis and biomass. Efficacy has been demonstrated in soybean and perennial ryegrass in multiyear field trials.

We are proving the efficacy of this novel technology in diverse crop species (soybean, hemp, rice, alfalfa and perennial ryegrass) and demonstrating the benefits of increased carbon dioxide assimilation to crop productivity, composition, and animal nutrition.

We developed genetically modified soybean, hemp, perennial ryegrass, rice and Arabidopsis expressing cysteine oleosin (a modified fatty acid encapsulating protein) and DGAT1 in photosynthetic cells. Proof of concept for some crops has been demonstrated in both containment and in the field.

Experiments in containment in perennial ryegrass demonstrated increased leaf fatty acid levels, photosynthesis, plant growth and gross energy. Field trials in both soybean and perennial ryegrass have revealed increased carbon and nitrogen capture and translation of the technology from lab to field. In multiyear/site field trials, transgenic soybean maintains yield parity with improved seed composition (up to 17% more oil and 7% more protein). This provides increased value for soy meal with greatly improved protein composition. This meal will be tested in pig and chicken nutrition trials in early 2022. The additional oil extracted during processing has significant market value. Transgenic perennial ryegrass had increased plant fat, and greater gross energy with no growth penalty in multi-year field trials.

Improving soybean seed composition has benefits in improving farm efficiency, capturing more carbon and nitrogen and providing an improved product for animal nutritionists. In perennial ryegrass the improved composition is expected to benefit ruminant nutrition by providing more energy, reducing nitrogen excretion and potentially decreasing methane emissions while maintaining productivity. Details of these benefits and the pathway to market for these novel crops will be discussed.

Q2: Climate-smart cattle – rapid adaptation to warmer environmental conditions using modern genetic technologies

Laible, G. [1,2], Cole, S.[1], Brophy, B.[1], Wells, D.N.[1], Wei, J. [1]

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Current genetic improvement strategies are unsuitable to confront the rapidly emerging challenges through global warming and environmental pressures for greater sustainability. Genome editors can rapidly recreate favourable genotypes that otherwise would not be possible or would take decades of breeding. This makes them exceptionally valuable tools for transforming animal agriculture under the realities of climate change.

We have applied precision genome editing to introduce two naturally occurring sequence variants associated with increased tolerance of elevated temperatures. Calves generated by cell-mediated editing for a coat colour dilution phenotype had a lighter coat colour which for the first time validated the causative nature of the sequence variant and can be expected to reduce solar heat gain and associated heat stress in these animals.

As an alternative approach, embryo-mediated editing offers higher efficiencies for producing live calves compared to the cell-mediated approach. However, full conversion into the intended edited genotypes can be challenging with embryo-mediated genome editing and often results in mosaic genotypes. Analysis of a small biopsy taken from edited embryos proved to be a powerful screening tool to identify fully edited embryos. We have successfully applied this strategy to generate embryos fully edited for the coat colour dilution variant and a sequence variant associated with thermotolerance known from tropical cattle. The results showed the feasibility and potential of genome editing to generate cattle with improved milk production and cow comfort under heat stress, enhancing resilient genotypes in a single generation.

Q3: Is the kea really an alpine parrot? A genomic investigation into the fate of the kea in a warming world.

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Climate warming, in particular in island environments, where opportunities for species to disperse are limited, may become a serious threat to cold adapted alpine species. In order to understand how alpine species may respond to a warming world, we need to understand the drivers that have shaped their habitat specialisation and the evolutionary adaptations that allow them to utilize alpine habitats.

The endemic, endangered New Zealand kea (*Nestor notabilis*) is considered the only alpine parrot in the world. As a species commonly found in the alpine zone it may be highly susceptible to climate warming. But is it a true alpine specialist? Is its evolution driven by adaptation to the alpine zone or is the kea an open habitat generalist that simply uses the alpine zone to – for example – avoid lower lying anthropogenic landscapes? We use whole genome data of the kea and its close, forest adapted sister species, the kākā (*N. meridionalis*) to reconstruct the evolutionary history of both species and identify the functional genomic differences that underlie their habitat specialisations.

Our analyses do not identify major functional genomic differences between kea and kākā in pathways associated with high-altitude. Rather, we find evidence that selective pressures on adaptations commonly found in alpine species are present in both Nestor species, suggesting that selection for alpine adaptations has not driven their divergence. Strongly divergent demographic responses to past climate warming between the species nevertheless highlight potential future threats to kea survival in a warming world.

Q4: The chemical microbiology behind infectious disease and the microbiome

Hards, K.[1,2,3]

[1]Biosecurity Intelligence Team, Intelligence & Biosecurity Support Services, Biosecurity New Zealand, Ministry for Primary Industries, NZ, [2]Department of Microbiology and Immunology, University of Otago, NZ, [3]Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, NZ.

Studies of microbial physiology in pure cultures do not explain the dynamics of the human microbiome. A key factor is that microbes extensively share metabolites and metabolic machinery. These communal resources play many roles across both host and microbe, but a knowledge gap is how this affects opportunistic pathogens within the microbiome. Two opportunists, Enterococcus faecalis and Streptococcus agalactiae, are thought to have inactive and vestigial machinery for cellular respiration. This viewpoint is at odds with the genetic essentiality of said respiratory machinery during infection. I will present evidence that chemical electron carriers, produced by non-pathogenic and commensal bacteria, reconstitute, and activate the respiratory machinery of opportunists. Genes from other bacteria may therefore be essential for opportunists in vivo. At the end of the talk, I will briefly discuss my new work in the field of scientific intelligence, as part of an early warning system for offshore biosecurity threats.

Q5: He reo tuku iho, he reo ora: Living language transmitted intergenerationally

Jones, P-L. [1], Mahelona, K. [1]

[1]Te Reo Irirangi o Te Hiku o te Ika (Te Hiku Media), Kaitaia, NZ.

Te Reo Irirangi o Te Hiku o te Ika (Te Hiku Media) is a charitable media organization, collectively belonging to the Far North iwi of Ngāti Kurī, Te Aupōuri, Ngāi Takoto, Te Rarawa and Ngāti Kahu. Te Hiku Media is an iwi communications hub for iwi radio, online TV and media services with a core focus of Māori language revitalization. Since its inception over 30 years ago, Te Hiku Media have embarked on a range of projects to support the use of te reo Māori and indigenous languages, in particular, in the digital world.

Everyday tasks can be completed using your voice and speaking to your devices, but due to the absence of the large datasets required for machine learning, speakers of many indigenous languages cannot engage with this technology. Indigenous communities are also understandably cautious when engaging with technology because of the ongoing issue of exploitation of indigenous knowledge and data, often with no consent, compensation, or recognition for iwi, hapū and whānau.

In order to provide opportunities for indigenous peoples to engage with the digital world whilst also protecting indigenous knowledge and ensuring data sovereignty, Te Hiku Media saw a need for innovative and indigenous solutions. This presentation will discuss some of the work Te Hiku Media has been involved in, including the Papa Reo project, a multilingual language platform grounded in indigenous knowledge and ways of thinking and powered by cutting edge data science. It will highlight the importance of sovereignty over data, platforms, and technologies and provide examples of how Te Hiku Media is challenging the issues indigenous communities have been facing.

Q6: Connectivity: a sovereign authority perspective

Wood, W.[1]

[1]Tangata Whenua Rōpū, Kauri Dieback Programme.

Increasingly mana whenua hapū and traditional iwi are requiring a higher level of engagement from researchers and scientists who work with natural biodiversity and the impacts of incursions and diseases that are contributing to its decline. If they occur at all, Te Tiriti strategic relationships are often homogenized to 'community' and relegated to a meeting where scientists relate their work that they will undertake on taonga or in a hapū or traditional iwi tribal territory.

As research, science and technology advances, scientists without the understanding of their obligation to the indigenous peoples of Aotearoa and without any critical checks in place to regulate cultural license, determine based on their lived experience and the value they attribute to mana whenua engagement, the level at which they (scientists and researchers) will engage.

This presentation will discuss the legitimacy of hapū and traditional iwi in the research and science system and discuss the importance of whakapapa (provenance) of information, data and taonga.

Q7: Biocultural Labels: disclosing Indigenous rights in genomic data

Hudson, M.[1,2], Anderson, J.[2,3], Barbarich, M.[2,4], Moore, J.[2,4], Hamilton-Pearce, J.[1,2], Waiwiri, R.[2,4], Liggins, L.[5], Patuawa, T.[6], Hohaia, H.[7]

[1]Te Kotahi Research Institute, University of Waikato, [2]Equity in Indigenous Research and Innovation Coordinating Hub (ENRICH), [3]New York University, [4]Indigenous Design and Innovation Aotearoa, [5]School of Natural and Computational Sciences, Massey University, [6]Te Roroa Iwi Trust, [7]Manaaki Whenua Landcare Research.

Concerns over Indigenous Data Sovereignty[1,2,3] and addressing the provisions of the Nagoya Protocol underpin the application of Biocultural (BC) Labels/Notices. Developed to be part of the Local Contexts system (www.localcontexts.org) that has successfully instilled information regarding the Indigenous provenance, protocols, and permissions associated with collections into digital infrastructures, through the use of Traditional Knowledge (TK) Labels and Notices[4], the BC Labels and Notices extend the recognition of Indigenous interests to biological research[5]. The Labels and Notices are designed to provide a persistent and durable connection between collaborating Indigenous communities and researchers, research projects, genetic resources, Digital Sequence Information (DSI), and associated traditional knowledge, that exist as metadata in sample/data repositories[6]. The BC Labels support Nagoya Protocol expectations around the disclosure and origins of genetic resources (i.e. Provenance Label) and help to define and communicate Indigenous community expectations and consent about appropriate and future use of genetic resources and derived benefits. Importantly BC Labels may only be applied by an Indigenous community, and they are both human readable and machine readable. The icons are generic in appearance, each Label has a persistent unique identifier, and the Label metadata (as text) is customized to each use-case. These community customized Labels are hosted on the Local Contexts Hub and offer a practical mechanism to ensure transparency and integrity in research engagements with Indigenous communities[5].

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2. Garrison, N. A. et al. (2019) *Genomic Research Through an Indigenous Lens: Understanding the Expectations*. Annu. Rev. Genomics Hum. Genet. 20, 495–517.
3. Hudson, M., Nanibaa'A, G., Sterling, R., Caron, N.R., Fox, K., Yracheta, J., … Taualii, M., (2020). *Rights, interests and expectations: Indigenous perspectives on unrestricted access to genomic data.* Nature Reviews Genetics, 21(6), 377–384. doi: 10.1038/s41576-020-0228-x
4. Anderson, J. (2012). *Options for the future protection of GRTKTCES: the traditional knowledge license and labels initiative.* Journal of World Intellectual Property Organization, 4(1), 66–75.
5. Anderson, J., & Hudson, M. (2020). *The Biocultural Labels Initiative: Supporting Indigenous rights in data derived from genetic resources.* Biodiversity Information Science and Standards, 4, e59230. doi: 10.3897/biss.4.59230
6. Liggins, L., Hudson, M., Anderson, J. (2021). *Creating space for Indigenous perspectives on access and benefit-sharing: Encouraging researcher use of the Local Contexts Notices.* Molecular Ecology, 30(11), 2477-2482. doi: 10.1111/mec.15918

Q8: The Indigenous Genomics Institute

Warbrick, L.[1,2]

[1]Indigenous Genomics Institute, [2]Ngātī Awa, Ngātī Rangitihi, Ngātī Maru, Te Atihaunui a Pāpārangi.

Where do whānau go to ensure the protection of taonga species and data?

Where do whānau go to discuss complex genomic matters in a safe and informed forum?

How do whānau access genomic research information to determine one's own Tino Rangatiratanga?

How do tangata whenua ensure the rights and interests of taonga species are protected at a time when commercial imperatives and social inequities collide?

The Indigenous Genomics Institute or IGI, provides a forum to engage, advise, educate, and protect the mauri of our taonga species. The IGI strives to advance the objective of Māori-lead responses to engagement with genetic and genomic research and the difficulties of working to establish culturally safe and tikanga based spaces within non-Māori institutions. As genomics continues to evolve and influence human health, ecology, agriculture, horticulture, climate change monitoring and more, an opportunity arises to acknowledge and further embed the teachings of mātauranga Māori alongside western science.

Q9: Kiore and their contribution to understanding the movement of people across the Pacific

Collins, C.[1], Gosling, A.[1], Lisa Matisoo-Smith, L.[1]

[1]Department of Anatomy, University of Otago, NZ.

While we have a reasonable understanding of the timing of the settlement of the Pacific, the complexities of the origins of Pacific populations and post-settlement interactions are not fully understood. One way to improve our understanding of these settlement processes is to take a holistic approach, combining multiple sources of data. The transportation of culturally important plants and animals, such as the kiore (*Rattus exulans*) was part of the settlement strategies of Pacific people as they settled uninhabited islands. Analysing genetic data from modern and ancient kiore is one source of data we can contribute to this approach – by better understanding the phylogeography of kiore across the Pacific, we can learn more about the migration of the people who transported them.

Q10: Explainable deep transfer learning model for disease risk prediction using high-dimensional genomic data

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Building an accurate disease risk prediction model is an essential step in the modern quest for precision medicine. While high-dimensional genomic data provides valuable data resources for the investigations of disease risk, their huge amount of noise and complex relationships between predictors and outcomes have brought tremendous analytical challenges. Deep learning model is the state-of-the-art methods for many prediction tasks, and it is a promising framework for the analysis of genomic data. However, deep learning models generally suffer from the curse of dimensionality and the lack of biological interpretability, both of which have greatly limited their applications.

We have developed a deep neural network (DNN) based prediction modelling framework. We first proposed a computationally efficient group-wise feature importance score for feature selection, where genes harbouring genetic variants with both linear and non-linear effects are efficiently detected. We then designed an explainable transfer-learning based DNN method, which can directly incorporate information from feature selection and accurately capture complex predictive effects. The network architecture is flexible, allowing researchers to easily incorporate prior knowledge. The proposed DNN-framework is biologically interpretable, as it is built based on the selected predictive genes. It is also computationally efficient and can be applied to genome-wide data. Through extensive simulations and the analysis of the dataset obtained from Alzheimer's Disease Neuroimaging Initiative, we have demonstrated that our proposed method can not only efficiently detect predictive features, but also accurately predict disease risk, as compared to many existing methods.

Q11: From Genotype to Phenotype: Reconstructing Causal Multi-Omics Networks in the Autotetraploid Potato

Angelin-Bonnet, O.[1], Thomson, S.[2], Baldwin, S.[2], Biggs, P.J.[1,3], Vignes, M.[1]

[1]School of Fundamental Sciences, Massey University, Palmerston North, 4442, NZ, [2]The New Zealand Institute for Plant & Food Research Limited, Christchurch, 8140, NZ, [3]School of Veterinary Sciences, Massey University, Palmerston North, 4442, NZ.

Understanding the biological mechanisms by which variations at the DNA level impact an Organism's phenotype, through the actions of complex molecular networks, is an important topic in biology. Such knowledge would lead to exciting advances in numerous fields, from plant and animal breeding to medicine. Importantly, it would permit an improvement of models for phenotype prediction from omics data. In order to bridge genotype and phenotype, statistical methods must be leveraged to extract information from multi-omics datasets. In particular, methods that can reconstruct regulatory networks spanning the different molecular layers (genome, transcriptome, proteome, metabolome) are needed. In this talk, I present how I leveraged genomics, transcriptomics, metabolomics and phenotypic data to reconstruct a causal multi-omics network for tuber bruising in the autotetraploid potato. Combining traditional single-omics analyses such as genome-wide association study and differential analysis with the multi-omics integration package mixOmics, I selected, across the different omics datasets, features involved in shaping the phenotype of interest. I then applied causal inference methods to discover causal relationships between the selected features. The reconstructed causal multi-omics network can be used to understand the mechanisms and pathways through which genetic variants impact tuber bruising, and can help develop a prediction model for tuber bruising from omics measurements. This talk will also highlight some limitations that need to be addressed when integrating multi-omics datasets.

Q12: Non-additive mutation discovery in sequenced cattle populations

Reynolds, E.[1], Neeley, C.[2], Lopdell, T.[2], Harland, C.[2], Wang, Y.[2], Keehan, M.[1], Dittmer, K.[1], Johnson, T.[2], Tiplady, K.[1,2], Worth, G.[2], Couldrey, C.[2], Walker, M.[2], Davis, S.[2], Sherlock, R.[2], Carnie, K.[2], Harris, B.[2], Charlier, C.[3], Georges, M.[3], Spelman, R.J.[2], Garrick, D.J.[1], Littlejohn, M.D.[1,2]

[1]Massey University, NZ, [2]Livestock Improvement Corporation, Hamilton, NZ, [3]University of Liege, Liege, Belgium.

Mutation discovery for recessive disorders has generally comprised a forward genetics approach, where a disease or trait with Mendelian presentation motivates focussed genotyping and phenotyping of the population of interest. We recently described an alternative, hypothesis-free approach that uses sequence-resolution GWAS of proxy phenotypes to reveal major effect recessive alleles in cattle. Here, phenotypes such as body weight—routinely derived in animal breeding programs and known to represent a wide array of null alleles in mice, can be used in conjunction with non-additive association models to highlight loss of function deleterious mutations. Applying this approach in a population of 80,027 New Zealand dairy cattle, we identify six novel recessive mutations with effects generally exceeding the largest-effect variants identified from additive GWAS.

We further show that other traits such as lactation phenotypes can serve as similar proxies of recessive disorders, where analysis of over 124,000 lactating cattle highlights an additional five, hitherto unknown deleterious QTL. The majority of these loci present compelling missense and nonsense mutations as candidates for these effects, where cumulative frequencies suggest that >50% of all purebred NZ dairy animals will be carriers for one or more of these recessive mutations.

These results present new selection opportunities to minimize the incidence of genetic disease in cattle and suggest similar effects may be resolvable in other selected species.

Q13: Modelling drug-induced apoptosis to rationalize multi-agent chemotherapy in high-risk neuroblastoma

Han, J.Z.R.[1], Phimmachanh, M.[1], Latham, S.L.[1], Kamili, A.[2], Fletcher, J.[2], Croucher, D.R.[1]

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High-risk neuroblastoma is an aggressive and invasive paediatric malignancy, with few actionable somatic mutations. As such, intense multi-agent chemotherapy remains the standard-of-care. Failure to effectively activate apoptosis, or the ability to evade apoptosis, has been established as a key mechanism of chemoresistance in neuroblastoma. Despite this, there is little understanding of the apoptotic mechanism-of-action of individual standard-of-care chemotherapeutic agents, let alone their combined mechanism of action. Here we apply a network-wide, systems level approach to identify drug-specific apoptotic signaling axes which will inform patient-specific, synergistic drug combinations.

A functional genomics screen was performed on a high content cellomics platform with a siRNA library of 200 apoptotic genes with current standard-of-care chemotherapy and preclinical drugs. Multi-dimensional analysis of this dataset elegantly demonstrated that synergy between any two chemotherapy drugs is proportional to the magnitude of divergence in apoptotic signaling between individual drugs. Identified drug-specific apoptotic signaling nodes were validated using genetically engineered stable cell lines harboring fluorescent biosensors and/or CRISPR-Cas9 mediated endogenously tagged proteins on our high content cellomics platform. These tools have allowed us to perform high-throughput kinetic live cell analysis at the single cell resolution and will help establish how synergy arises due to differential apoptotic signaling at the single cell level.

The application of our systems biology approach to rationalize mechanism-based drug selection will address fundamental questions about the network level functioning of apoptotic signaling pathways which has clinically relevant implications. Our data has demonstrated that it is differences in single agent drug-induced apoptotic signaling that will give rise to synergistic drug combinations. This is contrary to the current dogma of utilizing drugs with different molecular targets in combination chemotherapy. This research will inform the development of precision medicine approaches with the aim to improve patient outcomes for high-risk neuroblastoma.

Q14: Making progress with big data in radiata pine (Pinus radiata)

Dungey, H.S.[1], Klápště, J.[1], Slavov, G.[1,2], Graham, N.J.[1], Frickey T.[1], Sturrock, S.[3], Freeman, J.[1,4], Rippel, L.[1,5], Mercier, C.[1], Telfer, E.J.[1,6], Ismael, A.[1,7], Stovold, G.T.[1]

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Radiata pine is the world's most widely planted exotic conifer, making up 90% of the planted forest estate in New Zealand and representing over four million hectares of planted forests worldwide. Over the last decade, Scion and the Radiata Pine Breeding Company have partnered to develop genomic resources for this commercially important species as the breeding generation intervals of radiata are at least 17 years and genomics has the potential to reduce this interval by 50% or more. Progress has been good. We have been successful in the development of significant resources, including a high-density SNP exome-capture probe panel with Rapid Genomics LLC 1 and a 36,285 SNPs genotyping array (NZPRAD02 2). Genotyping in two linkage mapping families has shown that SNPs are well distributed across radiata pine's 12 linkage groups. A quality genome assembly has been completed and is now undergoing annotation. Re-sequencing of key progenitors has also commenced using 40 megagametophytes and 40 diploid genotypes. The genotyping array has been tested on more than 20,000 trees across the breeding programme. This represents the first operational implementation of genomics in radiata pine. The use of genomic tools will only help accelerate the delivery of targeted genetics for fit-for-purpose forests of the future. Examples and application of these resources will be discussed in the context of big data and the challenges for the future.

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2. Graham, N., Telfer, E., Frickey, T., Slavov, G., Ismael, A., Klápšte, J. and Dungey, H. (2022). *Development and Validation of a 36K SNP Array for Radiata Pine (Pinus radiata D.Don)*. Forests, 13: 176. https://doi.org/10.3390/f13020176

Q15: Communicating the pandemic

Wiles, S.[1]

[1]Bioluminescent Superbugs Lab, University of Auckland, Auckland, NZ.

In this era of 'fake news' and ill-informed influencers, effective science communication is vital for maintaining public trust. A microbiologist by training, Siouxsie has spent the last decade learning how to better communicate science to the public. That is including tweeting, blogging, as well as working with artists, animators, and illustrators. In her presentation, Siouxsie will talk about her journey from ivory-towered academic to becoming an engaging and trusted public communicator of science. She will also reflect on the good, the bad, and the ugly of communicating science during the pandemic.

Q16: Making science palatable for our communities

O'Sullivan, J.M.[1]

[1]Liggins Institute, The University of Auckland, Auckland, NZ.

How can we communicate palatable and unpalatable information to the community, policy makers and the people who need to know? I will share the highs and lows of trying to communicate: 1) research on faecal microbiome transfer to the public; 2) research on complex and rare diseases (e.g. Parkinson's, Anorexia, Autism); and 3) the utility of and genomics in medicine to government and policy makers. These examples draw on experiences from my recent research career and as a fellow within the office of the Prime Minister's Chief Science Advisor. It is my hope my experiences aid others as we continue to embrace our responsibility to communicate our work accessibly, accurately and in a timely manner to our communities.

Q17: Community engaged and community driven research – and example from PNG

Matisoo-Smith, L.[1], Gosling, A.L.[1]

[1]Department of Anatomy, University of Otago, Dunedin, NZ.

As part of our research using the commensal model to address Pacific origins, we needed to sample *Rattus exulans* populations from New Ireland Province in PNG to identify likely origins for Pacific rat populations, including the kiore populations in Aotearoa. This fieldwork presented unanticipated issues for sample collection that resulted in a new approach. The engagement of the community in the collection and recording of samples, brought greater interest in the research, which led to requests for further research addressing key questions identified by the community. This research, engagement and the relationships that resulted demonstrate the value of such an approach to both identifying key questions and interpreting results of genetic analyses investigating origins and interactions in the past and the implications of this on modern health of Pacific peoples.

Q18: Making genomics relevant

Belov, K.[1]

[1]School of Life and Environmental Sciences, University of Sydney, NSW 2006, Australia.

As the cost of sequencing decreases, genome sequencing and use of genomic markers increases. My team at the University of Sydney sequence genomes of Australian animals to better understand immune response to disease, for conservation management and for discovery of novel bioactives. We have actively communicated our work and findings to the public throughout, generating considerable interest in the power of genomic tools for wildlife conservation. During this talk I will give an overview of our research with koalas and Tasmanian devils, highlighting ways we have translated this work for outreach. In particular, I will provide examples of 1) use of genomics to understand the spread of a contagious cancer in Tasmanian devils, 2) use of genomics for genetic rescue of populations with low levels of genetic diversity, 3) discovery of novel antimicrobial peptides in the pouch that provide a new arsenal against the rise of drug-resistant bacteria.

Q19: Community Engagement, Biobanks, Accessible Data and Deterrents – how important are these elements in a rapidly developing eDNA landscape?

Bunce, M.[1,2]

[1] Institute of Environmental Science and Research (ESR),[2] University of Otago (Honorary Professor).

Environmental DNA (eDNA) is a tool that is being rapidly adopted around the globe for a variety of biomonitoring applications. Over the past decade the research community has focused on technique development, informatics, validation and applying the eDNA toolkit to a wide variety of applications. This presentation will explore the areas of eDNA that have had less 'air-time'; community engagement, data accessibility, sample archives and use of eDNA as a deterrent.

I will advocate that to grow as a field eDNA needs to make its data more accessible and to demonstrate the value of long-term chronosequences. How we take people on their 'eDNA journey' will likely be a key driver on the integration and acceptance of eDNA as a biomonitoring tool. Moreover, if we mobilise community, iwi/hapū and industry behind eDNA technologies can we positively influence collaboration, resourcing, and environmental stewardship.

Finally, this presentation will explore how eDNA, including connections to the Covid-19 pandemic, can be a 'springboard' for deeper and far wider discussions on the uptake and acceptance of genetic and genomic technologies within Aotearoa New Zealand.

Q20: Learning from the past to protect and guide lake restoration – a national scale study in New Zealand

Wood, S.[2], Vandergoes, M.[1], Pearman, J.[1], and the Lakes380 team (www.lakes380.com/home/the-team/)

[1]Cawthron Institute, Nelson, NZ, [2]GNS Science, Lower Hutt, NZ.

The health of our lakes is central to New Zealand's environmental, economic, and cultural wellbeing. Yet we cannot robustly assess the water quality or ecological health of our 3,800 (>1 ha) lakes because over 95% of them are not monitored. Even for the few lakes that are monitored, datasets are short (<10 years), and assessment incomplete because monitoring started after those lakes had deteriorated. With increasing pressure to improve national water quality, it is now vital to have informed benchmarks of natural lake state and an enhanced understanding of causes of degradation. Lake sediments are natural archives that continuously record environmental history, providing measures of current and historical aquatic communities and water quality – equivalent to many centuries of environmental monitoring. The 'Our lakes' health; past, present, future (Lakes380)' project in a national scale project that has samples water, surface sediment and sediment cores from over 300 of our lakes. In this talk we use examples from a subset of lakes which we have already analysed to demonstrate how sedimentary DNA can be combined with traditional approaches including pollen, diatoms, and pigments to determine current lake health and reconstruct water quality and identify the timing of changes over the past 1000 years. This approach is allowing us to define natural states and reveal historic resilience mechanisms to inform restoration, as well as to quantify the risk to lake health on a national scale.

Q21: Industry adoption of eDNA for biodiversity monitoring and natural capital accounting: EnviroDNA perspective

Barclay, H.[1], Noble, L.[1]

[1]EnviroDNA, Brunswick, Victoria, Australia.

eDNA-based methods can help meet a long-sought need for scalable and scientifically robust ways to directly measure the condition of ecosystems. EnviroDNA was formed to realise this potential, facilitating principled adoption of applications, collaboration among research and industry, and increased regulatory confidence in eDNA methods. We give a brief overview of current and future activity in this space, covering major projects and the Southern eDNA society. SeDNAs is a collective of experts with a mission to "promote collaboration across science and industry in Australia and NZ for the advancement of best practice eDNA methods that enables adoption across government, private and community sector". An upcoming workshop will be assisting with next steps to more formally establish the group. Projects include catchment-scale monitoring programs, with adoption of eDNA into the Accounting for Nature Framework, and a national citizen science survey aimed at prioritising conservation and restoration efforts.

Q22: Biodiversity on the fly: Carrion fly iDNA metabarcoding to monitor mammals in a fragmented terrestrial ecosystem

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Severely fragmented habitats increase the risks towards native mammal populations through isolation, increased edge effects, and predation. Therefore, monitoring the movement of mammal populations through anthropogenically-altered landscapes can be a valuable tool for conservation. Here we use metabarcoding of invertebrate-derived DNA (iDNA) from carrion flies to track mammal populations in the Wheatbelt Region of southwestern Australia, where widespread clearing for agriculture has removed most of the native perennial vegetation and replaced it with an agricultural system. We investigated whether the localization of the iDNA signal reflected the predicted distribution of four native species – echidna (*Tachyglossus aculeatus*), numbat (*Myrmecobius fasciatus*), woylie (*Bettongia penicillata*), and chuditch (*Dasyurus geoffroii*) - and two non-native mammal species - fox (*Vulpes vulpes*) and feral cat (*Felis catus*) - in this landscape. We collected iDNA samples from three conservation reserves and road edges between them and detected 14 of the 40 mammal species known from the region, including our target species. Detections of target native taxa were centered on conservation reserves, with some detections from road edges nearby. We detected foxes and feral cats throughout the study area, including all conservation reserves. The diversity and composition of taxa on road edges and conservation reserves were significantly different: conservation reserves hosted more native biodiversity than road edges. Our data suggest that the signals from iDNA are highly localized and reflect the known distribution of mammals in this region. The development of iDNA methods shows promise for the future of monitoring mammals.

Q23: Detection of exotic Khapra beetle, Trogoderma granarium, in dust samples collected from shipping containers in Australia

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Khapra beetle (*Trogoderma granarium*) poses a prominent biosecurity risk to Australia, where a widespread incursion could cost the country $15.5 billion over 20 years. Environmental DNA (eDNA) and RNA (eRNA)-based methods could offer non-invasive, sensitive detection tools to inform biosecurity officers on the presence of high-risk pests across the biosecurity continuum. Khapra beetle has been detected as a contaminant pest on imported goods such as furniture, household appliances and associated packaging imported to Australia via shipping containers. This study used two Real Time PCR TaqMan assays to detect khapra beetle environmental DNA/RNA in dust samples collected from shipping containers arriving to Australia. A total of 2119 dust samples were collected from 2000 shipping containers arriving at an empty shipping container park in Brisbane during May-August of 2021. These containers were not subject to biosecurity conditions and were selected randomly for sampling on a daily basis by staff at the site. Dust samples were then shipped to the University of Canberra to be processed and tested for khapra beetle eDNA/RNA. A total of 2112 dust samples were tested for khapra beetle eDNA/RNA, of which 229 samples (10.08%) showed positive detection for eDNA and 16 (0.75%) tested positive for eDNA/RNA. Of note, two shipping containers with positive eDNA/RNA results were recommended to undergo further examination and were confirmed to contain either live khapra beetle specimens or khapra beetle larvae skins. This study shows how eDNA/RNA- based detection could improve detection of priority pests in future Australian biosecurity responses.

Q24: Marine plastic-degrading bacteria are rare and exhibit little substrate preference for attachment

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The ubiquity of plastic debris in marine environments raises the question, what impact do plastics have on our marine microbiota? To investigate this, we applied bacterial 16S rRNA gene and fungal ITS2 region sequencing to identify changes in microbial biofilm community compositions on marine plastic, over time. Biofilm on polyethylene (PE), nylon (PA) and glass were sampled after 2, 6 and 12 weeks of immersion in Lyttelton Harbour, New Zealand, alongside seawater. Although *Proteobacteria* and *Bacteroidetes* were predominant in all samples, *Verrucomicrobiota* were most abundant in mature biofilms. Microbial communities on the three substrate types were significantly distinct from the seawater, regardless of age, but not between attachment substrates. Indicative species analyses suggest that the highest abundances of plastic-specific bacterial taxa occur 2 weeks after immersion, whereas plastic-specific fungal taxa are detected at 6 weeks. Taxa closely related to species previously reported as plastic degraders were found in low abundance across all material types. This suggests these microorganisms are not selecting to attach to plastic substrates for metabolic benefit, but as a surface to form generalist biofilm communities. Metagenomic analyses of plastisphere communities may further illustrate the genetic mechanisms used by different communities to colonize and potentially degrade marine plastics.

Q25: Towards optimisation of molecular surveillance techniques for wide uptake in marine biosecurity

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Environmental DNA and RNA (eDNA and eRNA) represent the organisms' blueprint in an ecosystem and have proven useful for detection and identification of marine species, including those posing biosecurity risks. Technological advances make the capture, extraction, and detection of even early incursions increasingly realistic at time scales where management actions can make a difference to the establishment and spread of nuisance organisms. However, to harness the full potential of eDNA/eRNA-based methods for biosecurity, analytical protocols, sampling approaches, designs and strategies must be optimized to ensure they are fit-for-purpose and deliver robust and quality assured data crucial for the biosecurity sector. Data on species presence/absence, which is not properly caveated with an understanding of the limitations of the technology, particularly around its accuracy and precision, may trigger unwarranted management responses, cause widespread reluctance of the uptake of the technology by end-users, or contribute to the ongoing propagation of unwanted pests. Therefore, the research community should work closely with biosecurity practitioners to ensure protocol steps are feasible, and that data-associated caveats are clearly understood and communicated. This is particularly important in New Zealand, where the current biosecurity strategy calls for building a team of 5 million to aid in protecting the country from the spread of unwanted organisms. The emergence of simple, low-cost (yet scientifically robust) biosecurity tools opens the prospect of engaging the wider community and citizen science groups to undertake monitoring and surveillance. A time when eDNA/eRNA-based surveillance data might be collected by school children, community groups, and local agencies directly using devices that produce geospatially tagged genotype or sequence data in the field is becoming a reality. As molecular data acquisition becomes more routine, we need to ensure the preparedness of the wider end-user communities (i.e. familiarity with marine ecology, molecular surveillance, biosecurity and kaitiakitanga concepts) and infrastructure (i.e. capacities for molecular data verification, storage and integration into biosecurity management pipelines).

Q26: Global surveillance using environmental DNA

Gemmell, N.J.[1]

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Ecological degradation, species loss and extinction, the emergence and spread of invasive species, and the spread of microbial agents that threaten our health and those of the productive and natural sectors upon which we depend are issues that face Aotearoa New Zealand and the world. The need for near real-time data to detect and manage such threats has never been more obvious in the light of the Covid-19 pandemic. In response to this threat new approaches to speed detection of this virus at community and national scale, such as wastewater monitoring, have emerged as important additional safeguards in our global battle with this pandemic. These initiatives are impressive, but what if we could extend such approaches to develop near real time information systems that could inform our response to the myriad challenges imposed on our planet. Here I will outline the possibility of embarking on a new national and global ambition to sequence at scale to protect ourselves from future incursions of pests and disease and better secure our ourselves, our productive industries and our biodiversity.

Q27: Frogs, ponds and plants: DNA extraction technologies for field deployment

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There is a growing call for in-field, real-time molecular screening across a range of applications such as infectious disease surveillance, biosecurity and environmental monitoring. Platforms capable of detecting molecular targets at the point-of-need are available. To be useful, however, these detection platforms must be presented with DNA free from inhibitors, obtained from crude samples quickly, produced using minimal infrastructure and in a form compatible with downstream molecular diagnostic processes. This talk will review how we have approached in-field DNA extraction, focusing on the PDQeX and prepGEM systems from MicroGEM, for sequencing and qPCR detection methods. Sample types include swabs, saliva, water and plant material.

Q28: Squeezing out ecological insights from contemporary and historic sponge borne eDNA

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Anthropogenic pressures are modifying our natural world at an unprecedented pace, radically affecting ecosystem stability and health. To understand the consequences of these changes, monitoring biodiversity trends is crucial. Aquatic environmental DNA (eDNA) surveys have provided accurate biodiversity assessments in recent years. Time-intensive field and laboratory protocols, however, are hindering large-scale implementation. Recently, eDNA was retrieved from marine filter feeders, a discovery that potentially circumvents scalability issues surrounding aquatic eDNA approaches. Here, we determined the ability and efficiency of filter-feeding organisms to accumulate eDNA. We compared vertebrate diversity obtained from sponges and mussels to aquatic eDNA and traditional diver surveys along a vertical transect in Doubtful Sound. By detecting migratory (e.g., blue whale – *Balaenoptera musculus*) and terrestrial organisms (e.g., brown kiwi – *Apteryx rowi*), as well as residing fish populations (e.g., butterfly perch – *Caesioperca lepidoptera*), eDNA from water and filter feeders detected more vertebrates compared to the diver survey. Furthermore, both eDNA surveys displayed similar beta-diversity patterns and identified the zonation pattern induced by the near-permanent halocline. Our results demonstrate the accumulation of eDNA in filter-feeding organisms. By circumventing the long process of water filtration, filter feeders might facilitate large-scale eDNA survey implementation, thereby providing essential information for effective management responses.

Q29: Moving environmental DNA (eDNA) technologies from benchtop to the field using passive sampling and PDQeX extraction

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Environmental DNA (eDNA) metabarcoding have shown great promise as an effective, non-invasive monitoring method for marine biomes. However, long vacuum filtration times and the need for state-of-the-art laboratories are restricting sample replication and *in situ* species detections. Methodological innovations, such as passive filtration and self-contained DNA extraction protocols, have the potential to alleviate these issues. We explored the implementation of passive sampling and a self-contained DNA extraction protocol by comparing fish diversity obtained from active filtration (1L; 0.45 µm cellulose nitrate [CN] filters) to five passive substrates, including 0.45 µm CN filters, 5 µm nylon filters, 0.45 µm positively charged nylon filters, artificial sponges, and fishing net. Fish diversity was then compared between the PDQeX Nucleic Acid Extractor and the conventional Qiagen DNeasy Blood & Tissue protocol. Experiments were conducted in both a controlled mesocosm and *in situ* at the Portobello Marine Laboratory, New Zealand.

No significant differences in fish diversity were observed among active filtration and more porous passive materials (artificial sponges and fishing net) for both the mesocosm and harbor waters. For the *in situ* comparison, all passive filter membranes detected a significantly lower number of fish species, resulting from partial sample drop-out. While no significant differences in fish eDNA signal diversity were observed between either DNA extraction methods in the mesocosm, the PDQeX system was less effective at detecting fish for the *in situ* comparison. Our results demonstrate that a passive sampling approach using porous substrates can be effectively implemented to capture eDNA from seawater, eliminating vacuum filtration processing. The large variation in efficiency observed among the five substrate types, however, warrants further optimization of the passive sampling approach for routine eDNA applications. The PDQeX system can extract high abundance DNA in a mesocosm with further optimization to detect low abundance eDNA from the marine environment.

Q30: Community science provides an effective catalyst for the uptake of regulatory eDNA monitoring in Aotearoa New Zealand

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Aotearoa's rivers, lakes and wetlands have degraded steadily over several decades, driven largely by population growth and land-use intensification. A history of fragmented data collection and reporting has prompted reforms to broaden the scope and scale of freshwater ecological monitoring through the new National Policy Statement on Freshwater Management (NPS-FM). However, traditional monitoring techniques still impose prohibitive costs and lack the scalability required for national-scale reporting. eDNA metabarcoding offers a powerful and scalable solution, but despite being widely used overseas, Aotearoa's government agencies have been slow to adopt this technology.

To catalyse the uptake of environmental DNA monitoring across Aotearoa, Wilderlab partnered with the NZ Environmental Protection Authority (NZEPA) to develop the Wai Tuwhera o te Taiao community science and engagement programme. This initiative aims to increase environmental awareness and demonstrate the benefits of whole-ecosystem monitoring for freshwater stakeholders, including government, communities, iwi, hapū, catchment/stream-care groups and kura. Over 200 community groups enrolled in the programme following its launch in 2020, and members have since collected over 1000 geolocated eDNA samples from around Aotearoa. These data are searchable via the Open Waters interactive map at wilderlab.co.nz/explore), and contain more than 6,000 fish records, 10,000 insect records, and many records of other taxa including birds, mammals, invertebrates, plants, microeukaryotes and bacteria. As further testament to the programme's effectiveness, all 11 of Aotearoa's Regional Councils have now adopted eDNA as an ecosystem monitoring tool. This programme provides an excellent example of how community-based science can engage and enable stakeholders to broaden our understanding of ecological and environmental change.

Q31: The game-changing nature of Nanopore sequencing

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The current 'climate' is full of buzzwords, such as: AI (artificial intelligence); deep learning; cloud computing, the bleeding edge and the 'Internet of Things'. Add to that the sudden public awareness around genomics and its importance and utility it's a lot to take in. As consumers, and even research specialists, this can all be overwhelming. ESR endeavours to provide it's staff, clients, and the wider community, with some insight into the technologies behind this jargon. In this talk I will discuss ESR's experiences with Nanopore sequencing, particularly focusing on the potential of GPUs (graphics processing units) and their implementation into the workflow, highlighting the many facets of genomics that it unlocks in the process. I will do this through a series of case studies and will also touch on a couple of personal anecdotes. I hope to highlight just what an exciting and disruptive technology Nanopore sequencing is, even more so when paired with affordable GPU compute. This journey has led me to form international, collaborative, cross-programme studies, with the singular goal of deploying disruptive, portable and affordable sequencing technology into the hands of the wider community to empower their health, well-being and curiosity. In a plot twist I am now the first New Zealand based Oxford Nanopore Technologies employee. I will round out this talk with some of the exciting work that I have been doing in this new role. The current 'climate' is full of buzz words, such as: AI (artificial intelligence); deep learning; cloud computing, and the 'Internet of Things'. Add to that the sudden public awareness around genomics and its importance and utility it's a lot to take in. As consumers, and even research specialists, this can all be overwhelming. At ESR we are endeavoring to provide our staff, clients, and hopefully the wider community, with some insight into the technologies behind this jargon. In this talk I will discuss our experiences Oxford Nanopore sequencing, including the many facets of genomics that it unlocks. I will do this through a series of case studies and will also touch on a couple of personal anecdotes. I hope to highlight just what an exciting and disruptive technology Nanopore sequencing is, particularly its ability to form international, collaborative, cross-programme studies to develop and deploy an innovative, disruptive, portable and affordable sequencing technology into the hands of the community to empower their health and well-being.

Q32: Decoding Alaska's Soil Resistome through the Lens of Nanopore Sequencing

Drown, D.M.[1]

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Temperatures in high-latitude regions are inducing widespread changes to the environment. In permafrost-associated soils, climate change and human-driven forces augments near surface thaw which alters physical and chemical properties of the overlying active layer. The changing permafrost can have direct effects on the associated soil microbial communities by altering community composition and activity. With increasing disturbance, we expect to find decreased microbial diversity, increased antibiotic resistance, and less stable bacterial communities all of which have major implications in terms of human and environmental health in Alaska. Soils have also long been implicated as a reservoir of antibiotic resistance. Here, we used a combination of sequencing technologies to characterize the widespread abundance of antibiotic resistance genes (ARG) across a permafrost thaw disturbance gradient. We found that antibiotic resistance was widespread with a positive correlation in the abundance of ARGs with permafrost thaw. However, the types of ARGs cluster more strongly by bacterial taxa rather than permafrost thaw. These results emphasize evolutionary origins of resistance, and the role vertical gene transfer has in shaping the core resistome. Overall, our results contribute to a better understanding of the role of environmental disturbance in shaping the resistome. Studies have consistently found participation in land-based culture as important for community well-being or as a major protective factor for mental health outcomes in the circumpolar North. A shift of the image of the land and soil from a nurturing to a threatening entity can therefore affect human health beyond the physical impacts of pathogens. Alaskan communities are at the center of these rapid environmental changes.

Q33: A long road to the wide adoption of 3rd-gen genomics: haps and mishaps of nanopore sequencing in Africa

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The generation of long reads of native DNA and RNA molecules represents a revolution in the world of genomics. With the MinION, Oxford Nanopore Technologies (ONT) offers lightweight, inexpensive devices that promise the sequencing of "anything, by anyone, anywhere". This includes the possibility to sequence de novo complete genomes with higher contiguity than ever, or to develop DNA-based assays and deploy them on the field with a drastically reduced time from sample to results. But the ability to effectively transform such a promise into reality is dependent upon the availability of qualified research staff with a thorough understanding of advanced molecular biology and bioinformatics, equipped with the ability to use high-performance compute.

Being a continent with an incredible amount of molecular diversity for many species but with reduced access to financial resources, the successful implementation of third-generation genomics in Africa constitutes the perfect use case for ONT technologies. It is of prime importance that we develop the necessary human capacity to handle those. The stakes are high: affordable genomics and bioinformatics are key to solving major continental issues of food security through crop and breed improvement, climate change mitigation and zoonotic pathogen surveillance.

At the International Livestock Research Institute (Nairobi, Kenya), with partners from the John Innes Centre and the Earlham Institute (Norwich, UK), we embarked on a journey of building capacity in genomics and bioinformatics in Africa for the development of agriculture on the continent. In this talk, we will walk you through our training programs and their successes. We will show how, together with a very diverse cohort of early-career African researchers, we harnessed the power of long-read sequencing to provide the first draft genome for an African orphan crop, to improve drastically another draft genome, and to participate in the global epidemiological surveillance of SARS-CoV-2 strains.

Q34: Long-read sequencing technology enables comprehensive profiling of transposon expression and reverse transcription

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Transposable elements (TEs) can cause genetic impacts when mobile and trigger epigenetic cascade when transcribed, affecting chromatin structure and gene expression, irrespective of the mobilization competency of the expressed TE loci. Despite their various influences on the host genomes, the identification of expressed TE loci and their characteristics remained elusive with the short-read sequencing method due to TE's self-proliferation and repetitive features.

Here, we utilized Oxford Nanopore Technology cDNA sequencing (ONT cDNA-seq) and Illumina RNA sequencing (RNA-seq) to explore TE transcription profile in grapevine (*Vitis vinifera*) embryogenic callus exposed to the biotic stress known to induce TE expression[1]. Genome-wide analysis reveals a strong location bias of expressed TE loci towards transcribed genes, suggesting the prerequisite for TE expression[2]. We also found that gene-TE fusion transcripts are more frequently associated with LINEs and DNA transposons than LTR-retrotransposons. The intronic TE sequence as part of the host gene's intron-retention isoform tends to coincide with the presence of premature termination codon, which prophesies the poor protein-coding productivity of the isoform. Furthermore, a low level of full-length TE transcripts was detected using ONT cDNA-seq, suggesting the mobilization potential of these TE loci, including a retrotransposon. Retrotransposon has been frequently reported to contribute to new traits in plants exposed to stress[3,4]. In addition to profiling TE transcription, detecting retrotransposon's reverse transcription product, cDNA, within the virus-like particles (VLPs) can help identify the retrotransposons promoting autonomous and non-autonomous mobilisation[5]. Therefore, we conducted ONT DNA sequencing to explore VLP DNA accumulation in hop (Humulus lupulus) plantlets subjected to heat shock and pharmacological inhibitors of epigenetic silencing key factors. We've successfully identified a Copia retrotransposon family producing VLP DNAs, suggesting its potential to fuelling new insertions. Overall, this research demonstrates a comprehensive way to investigate TE's transcription and retrotransposon's reverse transcription activity with ONT sequencing. These approaches facilitate TE-oriented study in non-model crops.

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5. Lee, S. C. et al. *Arabidopsis retrotransposon virus-like particles and their regulation by epigenetically activated small RNA*. Genome Res. 30, 576–588 (2020).

Q35: Anything but the SNP- what third generation sequencing can teach us about genomic variation

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Most genomic studies focus on SNPs to capture variation that impacts phenotypes. Recently it has become clear that other forms of genetic variants, including transposable elements (TEs), structural variants (SVs), and 3D DNA structures are major modifiers of phenotypic traits due to their impacts on gene regulation, TE suppression, and chromatin regulation. SVs and TEs have not simply been ignored, but they have been technically difficult to detect, while 3D structures, like G-quadruplexes (G4s), are difficult to detect with NGS because of their high-GC content and the structures they form.

Here we evaluate the utility of long-read, or third-generation sequencing (TGS) using two case studies. First, we use a new genome assembly for Tāmure/Australasian snapper (*Chrysophrys auratus*) using TGS and explore its ability to aid TE and SV identification. Second, we use high quality genome assemblies of a range of bird species to predict G4 structures in the chicken genome and include these in an evolutionary analysis considering their conservation and selection.

Analysis of the snapper genome assembly showed that the new version is more contiguous, has fewer gaps, and about 2.6% more of the genome identified as TEs, and approximately 250 more TE copies found, which may be active. This presents an excellent resource for the ongoing genetic research underpinning the snapper breeding programme at PFR. Analysis of the bird genomes proved useful to identify conserved regions, G4s, and TEs, and showed that G4s were more likely to be found on microchromosomes. Evolutionarily constrained G4s frequently overlapped with exon-associated features, and less frequently with lncRNAs and TEs. This suggests that G4s may be involved in gene regulation and evolution. Overall, these case studies highlight the utility of TGS technologies in resolving difficult genomic features to shed light on biological function and evolutionary history in a variety of contexts.

Q36: Long read sequencing and understanding the genome and biology of the New Zealand Brushtail Possum

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The New Zealand brushtail possum (*Trichosurus vulpecula*) is an invasive marsupial pest that was introduced from Tasmania and mainland Australia in the 19th- and early 20th-century[1]. In collaboration with the Vertebrate Genomes Project (VGP), we recently completed sequencing of a male brushtail possum joey from the Otago peninsula. The resulting chromosome-level assembly is arguably the best of any marsupial genome to date, a success largely due to optimised VGP protocols involving long-read sequencing[2]. Here we present further long-read sequencing experiments that explore enigmatic aspects of possum and marsupial biology, including the evolution and reprogramming of epigenetic traits such as genomic imprinting and composition of the Y-chromosome. This work, alongside other genome-based studies, provide a basis to better understand possum biology and ultimately, help control or eradicate their population.

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Q37: Insights from DNA methylome

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Our team has developed some of the first pipelines for genome-scale DNA methylation analysis. Our work on neutrophils provided a comprehensive resource for understanding altered disease susceptibility and phenotype due to variable DNA methylation and has broader implications for the selection of DNA methylation biomarker for common human diseases. My research also highlighted the epigenetic landscape of zebrafish and mangrove rivulus and opened new avenues for using these models to further understand the biology of methylation. We are applying whole genome scale DNA methylation analysis to investigate several biomedical research questions (e.g., methylation map of chronic fatigue syndrome, colorectal, prostate and lung cancer patients). Our work in epigenetic editing has implication in revealing causal function and new epigenetic regulation of genes in cancer cell. Our work has revealed aberrant methylation and expression patterns in several cancer types and revealed new mechanism of epigenetic regulation in cancer cells. In this talk I will present the key findings from some of our works over the years and briefly elaborate on some future directions of our work in understanding role of methylation events in metastasis and treatment resistance and early detection in cancer.

Q38: From host-microbe associations to holobionts: what ecological and evolutionary drivers facilitate this transition?

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Holobionts are assemblages of microbes and hosts, where each individual holobiont, comprising a single host and its associated microbial complement (or microbiome), is a unit of selection. Although there has been considerable discussion on the value of the "holobiont" concept (and its attendant "hologenome"), there is little doubt that different groups of hosts and their microbiomes exist along a continuum from "loose and opportunistic" at one end, to "intimate and specific" at the other. What has been missing, so far, is any explicit model that identifies how holobionts, as units of selection, might emerge from loose and opportunistic associations of hosts and microbes and -- once they have emerged -- how they might continue to exist over evolutionary time. In this presentation, I will review a selection of recent research that seeks to address this issue. I will conclude with some proposals on how we might make progress on understanding the evolutionary dynamics of host-microbe associations and identify how holobionts might emerge and persist.

Q39: Host density drives microbiome diversity patterns in southern bull kelp

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Microbiome structure and composition can be driven by a range of ecological factors, from broad scale factors such as temperature, or local scale factors such as wave intensity. Here, we examine the effects of a major ecological disturbance, the 2016 Kaikoura earthquake, on southern bull kelp (*Durvillaea*) microbiomes. The 2016 earthquake led to large scale extirpation of *Durvillaea* populations across the Canterbury, New Zealand region, and thus provides a natural system to examine how host microbiomes assemble during recolonization.

We find that host associated microbiomes are significantly different from both the surrounding seawater and from substrates adjacent to the host. In locations where bull kelp was largely wiped out, and is now recolonizing, microbiomes have greater alpha and beta diversity than flanking populations which were not affected by the earthquake. Furthermore, these disturbance driven changes are more important in determining microbiomes than host specific variables such as tissue type or host age.

We suggest that that these changes in diversity are the result of ecological selection dominating community assembly in existing populations, while ecological drift dominates community assembly in recovering *Durvillaea* populations. Finally, we outline our current use of *in situ* microfluidic experiments to show how we can differentiate between ecological drivers in early stages of community assembly, and how recolonizing algae can provide a source of potential microbial seeds for community assembly.

Q40: The drivers of viral diversity and movement in New Zealand ecological communities

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Viral disease emergence via cross-species transmission is a significant threat to wildlife, domestic animals, and humans, yet our understanding of the processes driving viral movement between species is limited. To put disease emergence in it's true context, viruses need to be studied as part of ecological communities, which is now possible via metagenomic sequencing allowing the entire virome of a sample to be sequenced. Herein, we show how viral metagenomics can be applied using an ecosystem level approach, rather than focusing on individual viruses or host species. We use examples from four studies we have conducted over the past three years on New Zealand animals and environments to illuminate the drivers behind viral diversity, and the implications this has for cross-species transmission.

Q41: Large scale shotgun sequencing study of the NZ dairy cow milk microbiome

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[1] Livestock Improvement Corporation (LIC), Hamilton, NZ.

Cattle diseases caused by microorganisms cost the New Zealand dairy industry about $300 million per year. Invasive foreign organisms, such as Mycoplasma bovis, can have high costs associated with eradication if not detected early. To date, published research examining the cow milk microbiome has been limited to 16S rRNA sequencing of 10s of samples. Here we present data from the first large scale whole genome shotgun sequencing study of the cow milk microbiome. We have sequenced more than 6000 herd test milk samples from 1689 cows across five farms. Using an optimised subset of NCBI's RefSeq database and the Kraken2 classifier, we identified ~4000 bacterial and 500 viral and fungal species in milk samples from NZ cows. Within the species identified, common mastitis-causing bacteria are readily detected. Gold standard tests such as bacteriology and PCR for these species, including Staphylococcus aureus and Streptococcus uberis, have been used to validate this sequencing-based approach to animal health. We have identified species showing significant quantitative differences in high and low-producing cows, and further investigation of these species showed correlations between bacterial species that thrive in similar environments. Principle component analysis of a time series from one farm showed a seasonal effect on the milk microbiome. We have also been able to create a model of the cows' predicted immune response to their milk microbiota.

Our large dataset offers a great deal of potential insight into the microbiome as well as scope for nationwide surveillance and biosecurity for the NZ dairy cow microbiome. Over the coming year we intend to improve the geographic spread of our sampling and focus more on bulk milk tank sampling in order to provide farm-level analysis of the microbiome.

Q42: Sinonasal and gastrointestinal bacterial composition and abundance are stable after 1 week of once-daily oral antibiotic treatment for chronic rhinosinusitis

Wagner Mackenzie, B.[1], Siu, J.[1], Klingler, L.[2], Biswas, K.[1], Wang, Y.[2], Hung, C.-T.[2], Jeong, S.H.[3], Barnett, D.[4], Tingle, M.D.[3], Douglas, R.G.[1]

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Despite the widespread prescription of antibiotics for the treatment of chronic rhinosinusitis (CRS), their efficacy remains uncertain. Limited penetration of systemic antibiotics into the sinonasal mucosa was reported previously by our group. This study investigated the effects of antibiotics on the sinus and gut microbiota and any relationships with drug distribution.

Thirty subjects with CRS were randomized to one of three groups: doxycycline (100mg daily, 7 days); roxithromycin (300mg daily, 7 days); or control. Sinonasal and stool samples collected before and after treatment were analysed using amplicon sequencing of the bacterial V3V4 hypervariable region of the 16S rRNA gene and ddPCR for community composition and bacterial load, respectively.

There were no significant bacterial community shifts or load following the treatment period in all groups. For the roxithromycin group, sinonasal bacterial diversity was negatively correlated with serum drug levels when compared to controls ($p<0.05$). The relative abundance of *Staphylococcus*_ASV129 in sinonasal samples reduced with increasing mucus doxycycline levels ($p=0.01$).

These data add to the existing evidence that antibiotics for the treatment of CRS is not recommended.

Q43: The microbiome of mānuka (Leptospermum scoparium)

Ridgway, H.J.[1], Larrouy, J.L.[2], Nieto-Jacobo, F.[2], Jones, E.E.[2], Dhami, M.[3], Biggs, E.[3], Thrimawithana, A.[1], Sansom, C.E.[1], van Klink J.W.[1], Perry, N.B.[1]

[1]The New Zealand Institute for Plant and Food Research Limited, NZ, [2]Department of Pest Management and Conservation, Lincoln University, NZ, [3]Manaaki Whenua Landcare Research, Lincoln, NZ.

Associations between plants and microorganisms have shaped the evolution and diversification of both partners. The microbial partners provide a second genome that influences the development, physiology, and health of the host. Mānuka (Leptospermum scoparium) is taonga and we explored the role of the microbiome in this iconic native plant. The role of the microbiome was assessed through a combined approach of culturome, plant assays, metabarcoding and metagenomics. A collection of 330 bacteria isolated from leaves was assessed for their effect on plant growth and essential oil production. Isolates of Pseudomonas sp. and Erwinia. increased the shoot dry weight of seedlings by 77–130% (P=0.02) and affected relative amounts of the leaf phytochemicals grandiflorone and nor-grandiflorone. Similarly, arbuscular mycorrhizal fungi increased shoot height and dry weight (P=0.034, P=0.011) and changes to the relative amounts of □-selinene, □-selinene, and flavonoids.

The effect of the microbiome on plant growth and phytochemical composition raised the question of whether microorganisms in the floral nectary could influence honey quality. In nectar, dihydroxyacetone (DHA) is the precursor from which the unique mānuka factor, methylgloxal, is derived. Metagenomics showed the nectary of high DHA plants contained unique bacteria and fungi. Some of these could independently synthesise DHA in vitro from glycerol. Metabarcoding of microbes associated with six stages (immature bud, bud, bud burst, mature flower, spent flower and seed) showed that there was a core microbiota that persisted across all stages despite high microbial turnover. This research demonstrated the functional significance of the microbiome in mānuka.

Mānuka is taonga and this work was completed as part of a research partnership with mana whenua aiming to uphold the spirit of Te Titiriti and aligns to PFR's Mātauranga and Taonga Principles. The mānuka nectary work was done in partnership with Ngāi Tahu Farming Ltd and Ngāti Porou Miere Ltd.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
Q44	Transcriptome analysis of *Cellaria immersa* (Phylum: Bryozoa) provides insights into marine invertebrate biomineralization and consequences of climatic changes	Achilleos, K.[1,2], Smith, A.M.[1], Kenny, N.[2], Brown, C.M.[2]	1 - Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, NZ 2 - Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin 9054, NZ
Q45	Preptin deficiency does not protect against high-fat diet induced bone loss in mice	Tan, J.[1*], Buckels, E.J.[1, 2*], Hsu, H.-L.[1], Buchanan, C.M.[1, 2], Lee, K.L.[1, 2], Matthews, B.G.[1, 2] *Contributed equally to this work	1 - Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ 2 - Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, NZ
Q46	Preptin deficiency has sexually dimorphic effects on trabecular bone volume in mice with advancing age	Buckels, E.J.[1], Watson, M.[2], Hsu, H.-L.[1], Buchanan, C.M.[1], Lee, K.L.[1], Matthews, B.G.[1]	1 - Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ 2 - Department of Medicine, University of Auckland, Auckland, NZ
Q47	High ratios of GDF9:BMP15 during *in vitro* maturation improved oocyte competency via maintenance of cumulus cell-oocyte coupling	Clark, Z.L.[1], Singh, A.R.[1], Morbeck, D.E.[2], Pitman, J.L.[1]	1 - School of Biological Sciences, Victoria University of Wellington, NZ 2 - Fertility Associates Ltd.
Q48	Synthesis and Validation of Fluorescence *in situ* Hybridisation Probes (FISH) for Genotyping Mouse	Derecourt, N.J.[1], Pitman, J.L.[1], Clark, Z.L.[1]	1 - School of Biological Sciences, Victoria University of Wellington, NZ

	Models of Embryonic Lethal Aneuploidies		
Q49	Isothermal titration calorimetry analysis of amino acid binding aptamers	Dunham, H.[1], Soundy, J.P.[2], Pitman, J.L.[1]	1 - School of Biological Sciences, Victoria University of Wellington, NZ 2 - Auramer Bio Ltd, Te Toki a Rata building, Victoria University of Wellington, Wellington, NZ
Q50	Snapper like it hot: growth and gene expression in response to temperature	Flammensbeck, C.K.[1,2], Ashton, D.[1], Anastasiadi, D.[1], Wylie, M.[1], Santure, A.W.[2], Wellenreuther, M.[1,2]	1 - The New Zealand Institute for Plant and Food Research Limited, Nelson Research Centre, NZ 2 - The School of Biological Sciences, University of Auckland, NZ
Q51	PlasticDB: a database of microorganisms and proteins linked to plastic biodegradation	Gambarini, V.[1], Pantos, O.[2], Kingsbury, J.M.[2], Weaver, L.[2], Handley, K.M.[1], Lear, G.[1]	1 - School of Biological Sciences, University of Auckland, 3a Symonds Street, Auckland 1010, NZ 2 - The Institute of Environmental Science and Research, 27 Creyke Road, Ilam, Christchurch 8041, NZ
Q52	Molecular and Cellular Characterisation of the Parkinson's Disease Olfactory Bulb: An Origin of Disease Pathology	Garton, A.[1], Lehnert, K.[1], Curtis, M.[1], Jacobsen, J.[1]	1 - University of Auckland, NZ
Q53	Autism spectrum disorder: understanding the impacts of SNPs on biological pathways in the human fetal and adult cortex	Golovina, E.[1], Fadason, T.[1,2], Lints, T.J.[3], Walker, C.[4], Vickers, M.H.[1,2], O'Sullivan, J.M.[1,2,5,6,7]	1 - Liggins Institute, University of Auckland, Auckland, NZ 2 - Maurice Wilkins Centre, University of Auckland, Auckland, NZ 3 - School of Medical Science, University of

			Auckland, Auckland, NZ 4 - School of Population Health, University of Auckland, Auckland, NZ 5 - Brain Research New Zealand, University of Auckland, Auckland, NZ 6 - MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK 7 - Australian Parkinsons Mission, Garvan Institute of Medical Research, Sydney, Australia
Q54	Effect of *Alexandrium* spp. on the early life stages of *Perna canaliculus*	Greenhough, H.E.[1,2], Smith, K.F.[2], Vignier, J.[2], Peychers, C.[2], Rolton, A.[2], Kenny, N.J.[1]	1 - Department of Biochemistry, University of Otago, Dunedin, NZ 2 - Cawthron Institute, Nelson, NZ
Q55	What's your poison? Differential transcriptomic responses to diverse stressors in the Lake Baikal sponge *Lubomirskia baikalensis*	Hawkes, M.[1], Kenny, N.J.[1], Itskovich V.B.[2]	1 - Department of Biochemistry, University of Otago, Dunedin, NZ 2 - Limnological Institute, Siberian Branch of the Russian Academy of Science, Irkutsk, 664033, Russia
Q56	Allelic diversity of the pharmacogene *CYP2D6* in New Zealand Māori and Pacific peoples	Hitchman, L.M.[1], Faatoese, A.[2], Merriman, T.R.[3,4], Miller, A.L.[1], Liau, Y.[1,5], Graham, O.E.E.[1], Kee, P.S.[1], Pearson, J.F.[1], Fakahau, T.[6,7],	1 - Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ 2 - Christchurch Heart Institute, Department of Medicine, University of Otago, Christchurch,

		Cameron, V.A.[2], Kennedy, M.A.[1], Maggo, S.D.S.[1]	NZ 3 - Biochemistry Department, University of Otago, Dunedin, NZ 4 - Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Alabama, US 5 - Current address: Auckland District Health Board, LabPLUS, Auckland City Hospital, Auckland, NZ 6 - Pacific Trust Canterbury, ntreal Street, Christchurch, NZ 7 - Current address: Auckland City Council, Auckland, NZ
Q57	Machine learning identifies six genetic variants and alterations in the Heart Atrial Appendage as key contributors to PD risk predictivity	Ho, D.[1], Schierding, W.[1,7], Farrow, S.[1,7], Cooper, A.A.[2, 3], Kempa-Liehr, A.W.[4], O'Sullivan, J.M.[1,5,6,7]	1 - Liggins Institute, The University of Auckland, Auckland, NZ 2 - Australian Parkinsons Mission, Garvan Institute of Medical Research, Sydney, New South Wales, AUS 3 - St Vincent's Clinical School, UNSW Sydney, Sydney, New South Wales, AUS 4 - Department of Engineering Science, The University of Auckland, Auckland, NZ 5 - Brain Research New Zealand, The University

			of Auckland, Auckland, NZ 6 - The Maurice Wilkins Centre, The University of Auckland, Auckland, NZ 7 - MRC Lifecourse Epidemiology Unit, University of Southampton, UK
Q58	Investigating the link between rimu fruit and successful kākāpō breeding	Hughes, A.K.[1], Harrington, D.[2], Hinkley, S.F.R.[2], Pitman, J.L.[1]	1 - School of Biological Sciences, NZ 2 - Ferrier Research Institute, Victoria University of Wellington, NZ
Q59	*Ex vivo* gene therapy for epidermolysis bullosa; severe skin fragility disorders	Hunt, J.[1], du Rand, A.[1], Samson, C.[1], Meidinger, S.[1], Lehnert, K.[1], Dunbar, R.[1], Knapp, D.[2], Mahon, C.[4], Purvis, D.[3], Feisst, V. [1], Sheppard, H.[1]	1 - Department of Biological Science, The University of Auckland, Thomas Building, Auckland 1050, NZ 2 - Institute for Research in Immunology and Cancer, University of Montreal, Montreal Quebec, Canada 3 - Starship Children's Hospital, Auckland 1142, NZ, [4]Christchurch DHB, Christchurch 8031, NZ
Q60	Regulatory networks reveal interactions between SARS-CoV-2 and complex disorders	Jaros, R.K.[1], Fadason, T.[1,2], Golovina, E.[1], O'Sullivan, J.M.[1,2,3,4,5]	1 - The Liggins Institute, The University of Auckland, Auckland 1023, NZ 2 - Maurice Wilkins Centre for Molecular Biodiscovery, Auckland 1010, NZ 3 - MRC Lifecourse Epidemiology Unit, University of Southampton, UK

			4 - Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore 5 - Australian Parkinson's Mission, Garvan Institute of Medical Research, Sydney, New South Wales, AUS
Q61	Single cell RNA-seq data analysis of the Huntington's disease cerebellum and OVT73 HD sheep model striatum using our new interactive webserver, ICARUS.	Jiang, A.[1], Mears, E.[1], Handley, R.[1], Hawkins, V.[1], Bawden, S.[2], Rudiger, S.[2], Mclaughlan, C.[2], Kelly, J.[2], Verma, P.[2], Waldvogel, H.J.[3], Faull, R.L.M.[3], You, L.[4], Lehnert, K.[1], Snell, R.G.[1]	1 - Centre for Brain Research, School of Biological Sciences, The University of Auckland, Auckland, NZ 2 - Molecular Biology and Reproductive Technology Laboratories, South Australian Research and Development Institute, Adelaide, AUS 3 - Centre for Brain Research, Faculty of Medical and Health Science, The University of Auckland, Auckland, NZ 4 - Department of Human Anatomy & Histoembryology, School of Basic Medical Sciences, Fudan University, Shanghai, China
Q62	Improving abundance estimates in metabarcoding analyses through CRISPR-Cas enrichment	Kardailsky, A.[1], Jeunen, G.J.[2], Matthaei, C.D.[1], Gemmell, N.[2], Dowel, E.[2]	1 - Department of Zoology, University of Otago, Dunedin, NZ 2 - Department of Anatomy, University of

			Otago, Dunedin, NZ
Q63	Stretched Mussels: tracing the genetic basis of resilience to climate change and ocean acidification in cultured green-lipped mussels (kuku) from genome to embryo	Kenny, N.J.[1]	1 - Department of Biochemistry, University of Otago, Dunedin, NZ
Q64	Single cell RNAseq and ATACseq analysis uncovers the transcriptional landscape underpinning cell type specification in the flatworm Schmidtea mediterranea	Kenny, N.J.[1,2], Arias-Baldrich, C.[1], García Castro, H.[1], Emili, E.[1], Mason, V.[1], Solana, J.[1]	1 - Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK 2 - Department of Biochemistry, University of Otago, Dunedin, NZ
Q65	Investigation of the relationship between the milk microbiome and SCC based on the comparison of healthy and unhealthy cows	Ling, H.[1], Wallace, A.J.[1], Neeley, C.E.[1], Gatenby, S.P. [1], Harland, C.S.[1], Couldrey, C.[1]	1 - Department of Research and Development, LIC, Hamilton, NZ
Q66	Genetic/genomic resources for biogeographic research in terrestrial Antarctica	Liu, X.P.[1], Duffy, G.A.[1], Pearman, W.S.[1], Pertierra, L.R.[2], Fraser, C.I.[1]	1 - Department of Marine Science, University of Otago, Dunedin, NZ 2 - Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa
Q67	Legacy land use effects on soil microbial community composition and functional potential	Louisson, Z.[1], Hermans, S.M.[2], Buckley, H.L.[2], Case, B.S. [2], Taylor, M.[3], Curran-Cournane, F.[4], Lear, G.[1]	1 - School of Biological Sciences, University of Auckland, Auckland, NZ 2 - School of Science, Auckland University of Technology, Auckland, NZ 3 - Waikato Regional Council, Hamilton, NZ 4 - Joint Evidence Data and Insights, Ministry for the Environment, Auckland, NZ

Q68	An experimental approach to understanding virgin plastic leachates and their impacts on plastisphere microorganisms	Maday, S.D.M.[1], Handley, K.[1], Kingsbury, J. M.[2], Lear, G.[1]	1 - School of Biological Sciences, University of Auckland, Auckland, NZ 2 - Institute of environmental science and research, Christchurch, NZ
Q69	Dissecting the PD-1/PD-L1 axis in melanoma using a microRNA-based fine-tuning approach.	Malhi, C.[1,2], Meidinger, S.[1,2], Didsbury, A.[1,2], Michaels, Y.[3], Dunbar, R.[1,2], Fulga, T.[4], Sheppard, H.[1,2]	1 - School of Biological Sciences, University of Auckland, Thomas Building, Auckland 1050, NZ 2 - Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland 1050, NZ 3 - School of Biomedical Engineering, University of British Columbia, Vancouver BC V6T 1Z3, Canada 4 - Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DS, UK
Q70	Aminoacylation of Indole Diterpenes by Cluster Specific Monomodular NRPS-like Enzymes	McLellan, R.[1], Cameron, R.[1], Nicholson, M.[1], Parker, E.[1]	1 - Ferrier Research Institute, Victoria University of Wellington, NZ
Q71	Population genomics of New Zealand pouched lamprey (kanakana; *Geotria australis*)	Miller, A.K.[1], Baker, C.[2], Kitson, J.[3], Gillum, J.[1], Sharif, S.[4], Clarke[5], Timoshevskaya, N.[6], Smith, J.J.[6], Gemmell, N.J.[1*], Alexander, A.[1*]	1 - Department of Anatomy, University of Otago, Dunedin, Otago, NZ 2 - National Institute of Water and Atmospheric Research, Hamilton, Waikato, NZ 3 - Kitson Consulting Ltd, Invercargill/

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Q72	Identification and comparison of RNA modifications across natural isolates of *E. coli*	Morampalli, B.R.[1], Silander, O.[1]	1 - School of Natural and Computational Sciences, Massey University, Auckland, NZ
Q73	An MLL-AF9 Zebrafish Acute Leukaemia Model	Saberi, M.[*,1], Delfi, O.[1], Kakadia, P.M.[1], Bohlander, S.K.[1]	1 - Leukaemia & Blood Cancer Research Unit, Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, NZ
Q74	Characterising models of embryonic triploidy	Stepney, E.[1], Olds, M.E.[1], Clark, Z.L.[1], Pitman, J.L.[1]	1 - School of Biological Sciences, Te Herenga Waka, Victoria University of Wellington, Wellington, NZ
Q75	Polycistronic gene expression for fungal biosynthetic engineering	Stevenson, L.J.[1,2], Parker, E.J.[1,2]	1 - Ferrier Research Institute, Victoria University of Wellington, Wellington, NZ 2 - Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, NZ
Q76	Contrasting patterns of single nucleotide polymorphisms and structural variations across multiple invasions	Stuart, K.C.[1], Edwards, R.J.[2], Sherwin, W.B.[1], Rollins, L.A.[1]	1 - Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW

			Sydney, Sydney, New South Wales, AUS 2 - Evolution & Ecology Research Centre, School of Biotechnology and Biomolecular Sciences, UNSW Sydney, Sydney, New South Wales, AUS
Q77	Pinpointing and targeting novel drivers of pancreatic cancer progression, invasion and metastasis using TRAP-seq.	Trpceski, M.[1,2], Ip, K.[1,2], Chambers, C.[1,2], Herzog, H.[1,2], Murphy, K.[1,2], Chtanova, T.[1,2], Timpson, P.[1,2], Herrmann, D.[1,2]	1 - The Garvan Institute of Medical Research & The Kinghorn Cancer Centre, Sydney, NSW, AUS 2 - St Vincent's Clinical School, Faculty of Medicine, University of New South Wales Sydney, Sydney, NSW, AUS
Q78	Low maternal vitamin C during pregnancy in guinea pigs results in persistent epigenetic dysregulation in the hippocampus of offspring together with an ADHD-related phenotype	Vissers, M.[1], Smith-Diaz, C.[1], Coker, S.[2], Dyson, R.[2], Hore, T.[3], Berry, M.[2]	1 - Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ 2 - Department of Paediatrics and Child Health, University of Otago, Wellington, NZ 3 - Department of Anatomy, University of Otago, Dunedin, NZ
Q79	Genomics of *Staphylococcus aureus* strains in New Zealand dairy cows	Voss, E.M.[1,2], Williamson, J.[1], Gatenby, S.[1], Pruden, S[1], Ling, H.[1], Wallace, A.[1], Harland, C.[1], Ferguson, S.A.[2], Morales, S.[2], Cook, G.M.[2], Couldrey, C.[1]	1 - Livestock Improvement Corporation, Hamilton, NZ 2 - Department of Microbiology and Immunology, University of Otago, Dunedin, NZ

Q80	To haline back: Investigating freshwater sponge hologenomes and mitogenomics using draft genomic sequencing	Walker L.A.[1], Kenny N.J.[1], Itskovich V.B.[2]	1 - Department of Biochemistry Te Tari Matū Koiora, University of Otago, Dunedin, Aotearoa New Zealand 2 - Limnological Institute, Siberian Branch of the Russian Academy of Science, Irkutsk, 664033, Russia
Q81	Correlation of stem cell frequency with the immunophenotype during serial transplantation in a CALM/AF10 leukaemia model: evidence of phenotypic plasticity	Zandvakili, N.[1], Lee, H.M.[1], Desai, R.[1], Oryshchuk, A.[1], Browett, P.J.[1], Kakadia, P.M.[1], Bohlander, S.K.[1]	1 - Leukaemia & Blood Cancer Research Unit, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ

Q44: Transcriptome analysis of *Cellaria immersa* (Phylum: Bryozoa) provides insights into marine invertebrate biomineralization and consequences of climatic changes

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One of the most salient features of marine bryozoans is their well-calcified skeleton, and many species in this phylum are important reef-builders. To date, the molecular machinery responsible for skeletal formation in these key animals, and how it will be affected by environmental stressors, remains unknown. In this study we performed *de novo* transcriptome assembly from erect articulated *Cellaria immersa* colonies collected in New Zealand. *Cellaria* species form erect, heavily calcified arborescent colonies which when abundant can create micro forests or meadows on the ocean floor.

RNA was extracted separately from younger distal and older proximal parts of 10 colonies, aiming to identify the key genes involved in biomineralization. Differential expression analysis was carried out to identify genes that are expressed at a higher rate in distal and proximal parts of the colonies. The assembly resulted in a set of 21,219 transcripts. Of the proteins translated from these, 11,148 had predicted function when tested for protein similarity against a range of databases using Interproscan. Over 50 proteins were identified as candidates involved in the biomineralization process and skeletal resorption in *C. immersa*. Many represent structural proteins, transmembrane transport channels and enzymes known to be involved in skeletal processes in other metazoans. This is the first such study on a heavily calcified species from the phylum Bryozoa, significantly increasing the amount of 'omic data available for *C. immersa* and the phylum. Identifying and cataloguing this 'biomineralization toolkit' is critical for understanding the potential effects of environmental stressors and climatic changes on these vital ecosystem engineers.

Q45: Preptin deficiency does not protect against high-fat diet induced bone loss in mice

Tan, J.[1*], Buckels, E.J.[1, 2*], Hsu, H.-L.[1], Buchanan, C.M.[1, 2], Lee, K.L.[1, 2], Matthews, B.G.[1, 2]
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Preptin is a 34-amino acid peptide derived from the E-peptide of pro-IGF-II. Preptin is co-secreted with insulin from β-cells, can increase glucose-stimulated insulin secretion, and promotes proliferation and differentiation of osteoblasts. We discovered that preptin deficiency has sexually dimorphic effects on bone microarchitecture in a preptin knockout (KO) mouse model, whereby several trabecular and cortical bone parameters are increased in male KO mice as they age compared to male wild-type (WT) mice. A high-fat diet (HFD) adversely affects bone in rodents. Therefore, we tested the hypothesis that an HFD would negatively affect bone microarchitecture but that preptin deficiency would protect against this phenotype.

Nine-week-old WT and KO C57BL/6J mice were placed on a chow diet (CD) or HFD for 14 weeks. Metabolic phenotypes were evaluated by intraperitoneal insulin tolerance tests (ITT) performed at 9-, 16-, and 20-weeks of age and an oral glucose tolerance test (GTT) at 22-weeks of age (n=12-15/sex/genotype). Femurs were excised at 23-weeks of age and analysed using micro-computed tomography.

Bodyweights were increased in all HFD-fed mice and tended to be lower in male KO vs. WT mice independent of diet ($p=0.06$). The glucose response to a GTT was increased in male mice fed an HFD vs. CD, with no genotype effects. There were no metabolic differences in female mice.

HFD affected both cortical and trabecular indices with no genotype effects. HFD decreased trabecular bone volume in male WT and KO mice by 40% and 29%, respectively, and in female mice by 51% and 44%, respectively. Cortical bone area was decreased in male WT and KO mice by 13% and 6%, respectively, and in female mice by 7% and 9%, respectively.

Therefore, although we had observed a protective effect of preptin deficiency in aging, there was no protective effect of preptin KO in response to an HFD.

Q46: Preptin deficiency has sexually dimorphic effects on trabecular bone volume in mice with advancing age

Buckels, E.J.[1], Watson, M.[2], Hsu, H.-L.[1], Buchanan, C.M.[1], Lee, K.L.[1], Matthews, B.G.[1]

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Preptin is a 34-amino acid peptide derived from the E-peptide of pro-IGF-II. Preptin is co-secreted with insulin from β-cells, can increase glucose-stimulated insulin secretion, and promotes proliferation and differentiation of osteoblasts. We tested the hypothesis that preptin deficiency alters bone metabolism by evaluating a preptin knockout (KO) mouse.

Experimental KO and wild type (WT) mice were generated by heterozygous breeders. Adult livers (n=4-9) had similar *Igf2* mRNA expression between genotypes, with undetectable preptin expression in KO mice. Metabolic phenotypes were evaluated by weekly fasting blood glucose measurements, intraperitoneal insulin tolerance tests (ITT) at 9, 29, and 44-weeks of age, and oral glucose tolerance test (GTT) at 45-weeks of age (n=12-14/sex/genotype). Bone phenotypes were evaluated by femoral microCT at 6-weeks (n=8-12/sex/genotype; immature), 14-weeks (n=10-12/sex/genotype; peak bone mass), and 47-weeks of age (n=12-14/sex/genotype; aging).

Bodyweights were similar between genotypes at all ages. Blood glucose concentrations returned to baseline quicker following ITT in female KO than WT mice at 9-weeks of age only. Female KO had increased blood glucose concentrations 15- and 30-minutes post-glucose during GTT compared to WT mice. There were no metabolic differences in males.

There were no differences between genotypes in bone microarchitecture at 6-weeks of age. By 14-weeks of age, trabecular bone volume fraction (BV/TV) was increased by 21%, trabecular number was increased 17%, and cortical bone area was increased 8% in male KO vs. WT mice. These effects were absent in females. At 47-weeks of age, males had similar bone microarchitecture; however, trabecular bone volume fraction was increased by 29% (p=0.09), and trabecular number was increased by 30% in female KO vs. WT mice.

Male preptin KO mice had increased trabecular bone volume at 14-weeks of age only, whereas female preptin KO mice developed this phenotype as they aged. Mechanistic evaluation of this phenotype is ongoing.

Q47: High ratios of GDF9:BMP15 during *in vitro* maturation improved oocyte competency via maintenance of cumulus cell-oocyte coupling

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The ratio of growth differentiation factor 9 (GDF9): and bone morphogenetic protein 15 (BMP15) mRNA in oocytes has been implicated in the regulation of litter size in a range of mammalian species (1). Supplementation with either of these proteins during *in vitro* maturation (IVM) increased the proportion of oocytes that reach the blastocyst stage, a measure of oocyte competency (2). We hypothesised that supplementing IVM media with a high, compared to a low, GDF9:BMP15 ratio (characteristic of high and low ovulation rate species, respectively) would improve oocyte competency in sheep oocytes. Furthermore, we suggest that this would be mediated via the closely associated cumulus cells (CC).

The aim of Study 1 was to investigate the effects of high (6:1) and low (1:6) GDF9:BMP15 ratios on CC gene expression and blastocyst rate using the gold-standard IVM system. The aim of Study 2 was to investigate the effects of these divergent GDF9:BMP15 ratios on CC and oocyte gene expression levels, gap junction communication, and nuclear maturation rates using a modified version of this gold-standard IVM system.

Overall, the high GDF9:BMP15 ratio improved blastocyst rates ($P<0.05$). In both culture systems, expression levels were altered in several CC genes (*BAX*, *CX43, EGFR, HAS2, VCAN, FSHR, PGR, EREG*; all $P<0.05$) in response to treatment with either ratio. Study 2 demonstrated that a higher degree of gap junction communication between the oocyte and CC was maintained during IVM with high GDF9:BMP15 ($P<0.05$). However, there were no differences in oocyte gene expression levels or rates of nuclear maturation ($P>0.05$).

In conclusion, high ratios of GDF9:BMP15 increased critical gap junction communication which likely contributed to improved oocyte competence and blastocyst formation.

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Q48: Synthesis and Validation of Fluorescence *in situ* Hybridisation Probes (FISH) for Genotyping Mouse Models of Embryonic Lethal Aneuploidies

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Approximately 50% of all embryos produced in fertility clinics have an abnormal number of chromosomes (i.e., aneuploid)[1]. Of these, 25% have aneuploidies that are embryonic lethal[2] and result in spontaneous abortions in the first trimester[3]. Our overall goal is to develop a non-invasive method for detecting these aneuploidies in preimplantation human embryos. For this goal to be achieved, we require a number of embryonic lethal aneuploidy models that can be developed and validated in the laboratory.

One way to produce embryos that exhibit embryonic lethal aneuploidies is through mouse breeding programmes that make use of Robertsonian translocations. Robertsonian translocations (RobT) are one of the most common forms of chromosomal rearrangements in humans and can cause whole chromosome duplications/deletipons[4]. The long (q) arms of two chromosomes recombine and fuse, creating an additional chromosome, whilst the short (p) arms are lost[5,6,7]. A balanced carrier of this translocation is viable and produces normal gametes, carrier gametes, as well as gametes that will result in aneuploid (trisomy or monosomy) embryos[8].

As these RobT mouse models are being developed in our laboratory, an accurate method for identifying the trisomic and monosomic embryos produced will be critical. This project aims to synthesise and validate fluorescent *in situ* hybridisation (FISH) point probes for mouse chromosomes 4, 10, 11, and 15 using a cost-effective, PCR-based method[9]. Herein, we present data from validation experiments for FISH probes for mouse chromosome 4 and 10, in two different tissue types, namely metaphase spreads produced from mouse lymphocytes and zygotes (fertilised oocytes).

From this study, FISH probes for the identification of aneuploidies involving chromosome 4 and 10 have been validated. Moreover, an in-house, reliable methodology for constructing FISH probes for mouse chromosomes has been developed for the purpose of identifying aneuploid embryos.

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Q49: Isothermal titration calorimetry analysis of amino acid binding aptamers

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The systemic concentration of free amino acids is gaining attention in New Zealand and internationally as indicators of nutritional, metabolic and fertility status. A rapid, cheap, and easy measurement of free amino acids from a biological fluid would provide individuals with a snapshot of their metabolic status. Aptamers represent next generation bioreceptors and are capable of recognizing and binding target molecules in complex biological samples. Small-molecule aptamer biosensors often rely on the aptamer changing structure upon ligand binding. Ligands exhibiting an intercalation binding mode cause the aptamer to restructure into a form that is more rigid where ligands with a groove binding mode do not.

The aim of this study was to utilize isothermal titration calorimetry (ITC) to investigate the specific physical properties of the binding interactions of arginine, phenylalanine & tryptophan aptamers to their target ligands and determine their suitability in a biosensor. ITC analysis of the arginine aptamer Arg12-28 shows there are two binding sites, the small enthalpy and favorable entropy of the binding interaction indicates a DNA groove binding mode. ITC analyses for two phenylalanine binding aptamers, Phe1 K_d = 5.4 µM, the favorable enthalpy ΔH = -25 kcal/mol and unfavorable entropy -TΔS = 17.9 kcal/mol, and Phe3 K_d = 10.9 µM, the favorable enthalpy ΔH = -16.42 kcal/mol and unfavorable entropy -TΔS = 9.7 kcal/mol indicates a DNA intercalation binding mode for both of these aptamers. For the Tryptophan aptamer K_d = 154 µM the favorable enthalpy ΔH = -5.2 kcal/mol and unfavorable entropy ΔS = 0.09 kcal/mol indicates a DNA intercalation binding mode.

This study demonstrates that ITC may be used to define the binding properties of aptamers and may inform their usefulness for downstream integration into biological assays. This information may be used to fine-tune aptamer binding properties and improve biocompatibility, thus advancing their use in biosensors, diagnostic tools, and therapeutics.

Q50: Snapper like it hot: growth and gene expression in response to temperature

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Ectotherm fish are highly dependent on ambient water temperatures to regulate their own body temperature and functions. While fish have evolved to cope with seasonal and daily temperature variations across their natural range[1], permanent exposure to more extreme temperatures close to the species' thermal tolerance limits may detrimentally impact the organism's health and ultimately survival[2, 3, 4]. One way that species can respond to temperature changes is by adjusting the expression of their genome, which can facilitate phenotypic plasticity and thus adaptation.

Here we apply a manipulative temperature experiment on the Australasian snapper (*Chrysophrys auratus*) to describe the gene expression changes occurring following exposure to cold and warm water for three months. Phenotypic measurements were collected for 539 individuals to identify fitness consequences including growth and survival. 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 (~35% and ~27% higher gain in weight and peduncle length, respectively) than fish kept under cold temperatures. Gene expression profiles between treatments were compared to identify differentially expressed genes (DEGs). When comparing warm versus cold treatments, we found 353 and 223 DEGs in the liver and head kidney, respectively ($\log_2$FC > 1.0, p-adjusted < 0.01), indicating that gene expression is substantially impacted by temperature. From the total 576 DEGs, only 25 genes were shared between both tissues, indicating considerable differences in their gene expression profiles.

Our study will provide a better understanding of the gene expression changes underpinning thermal plasticity and growth performance in snapper. This will inform future aquaculture breeding programmes of this and related species and provide insights into mechanisms available to wild snapper stocks to cope with a warming world.

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Q51: PlasticDB: a database of microorganisms and proteins linked to plastic biodegradation

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The number of presumed plastic-degrading microorganisms reported is rapidly increasing, making it possible to explore the conservation and distribution of plastic-degrading traits across the diverse microbial tree of life. After assessing the international scientific literature, we centralized information on known plastic-degrading microorganisms in an interactive and updatable phylogenetic tree and database (http://plasticdb.org/). The database currently has 562 species of microorganisms reported in the scientific literature to have plastic-degrading capabilities and 112 enzymes described to break down plastics. With these data, we verify that plastic degradation is, in general, not phylogenetically conserved. Instead, plastic-degrading microorganisms are widely distributed across the bacterial and fungal branches of the tree of life, although polylactic acid (PLA) degradation is dominated by the bacterial family Pseudonocardiaceae. Our database is freely available to the scientific community in the form of a web server, such that researchers can now analyze various aspects of plastic biodegradation via genome and metagenome annotation and comparison, species identification in taxonomic tables, and metabolic pathway analysis. We predicted in silico the structures of all presumed plastic degrading enzymes in the database. These can now be visualized in the user's browser or downloaded and incorporated into any downstream analysis. Our database and the tree are regularly updated, reflecting rapid developments in this area of science. Our research benefits the field by providing an updated 'library' of presumed plastic-degrading taxa and traits. Further, it expands our understanding of microbial plastic-degrading traits' genetic diversity and evolution.

Q52: Molecular and Cellular Characterisation of the Parkinson's Disease Olfactory Bulb: An Origin of Disease Pathology

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms that results in the progressive degeneration of striatal dopaminergic neurons in the substantia nigra pars compacta. It is a heterogenous and complex disorder, with more than 20 genes associated with PD; however, for the vast majority of cases the underlying cause remains unknown. Interestingly, up to 95% of PD cases have olfactory dysfunction prior to motor symptom onset, and alpha-synuclein pathology has been identified in the early stages of PD in the olfactory bulb. Despite this, the human olfactory bulb remains largely uncharacterized, particularly in the context of disease processes.

Therefore, the aim of my project is to evaluate the human olfactory bulb as a source of disease origin in PD by integrating genomic (whole genome sequencing), transcriptomic (total RNA sequencing and spatial RNA sequencing), and proteomic (RNAscope®) evidence from 26 PD brains from the New Zealand Neurological Foundation of New Zealand Human Brain Bank. Whole genome sequencing of DNA extracted from post-mortem brain tissue revealed one causal variant (a heterozygous mutation in LRRK2, p.Gly2019Ser), and one susceptibility variant (a heterozygous mutation in GBA, p.Leu444Pro) in two separate cases. Structural variant analysis is currently underway. Total RNA sequencing and spatial RNA sequencing will be performed on the olfactory bulb of at least three PD cases and three age and sex matched controls to characterize and compare cell types and their transcriptional profiles. These results will be overlayed with RNAscope® and related immunohistochemical techniques on the same cohort to determine the pathogenic cascade from gene to protein and identify novel disease mechanisms in the olfactory bulb in PD. These analyses will provide a level of characterisation of the PD olfactory bulb from the transcriptome through to whole tissue pathology not previously seen in the human brain.

Q53: Autism spectrum disorder: understanding the impacts of SNPs on biological pathways in the human fetal and adult cortex

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by significant and complex genetic etiology. Genome-wide association studies (GWAS) have identified hundreds of single nucleotide polymorphisms (SNPs) associated with ASD. The majority of these SNPs are non-coding, and thus they can mark regulatory regions (expression quantitative trait loci or eQTLs). Yet, the regulatory mechanisms by which these SNPs can influence the development of ASD remain poorly defined.

In the present study, we integrated four distinct levels of biological information (GWAS, eQTLs, spatial genome organization and protein-protein interactions) to identify potential regulatory impacts of ASD-associated SNPs ($p < 5\times10^{-8}$) on biological pathways within human fetal and adult cortical tissues. We found 80 and 58 SNPs that mark regulatory regions in the fetal and adult cortex, respectively. These eQTLs were also linked to other psychiatric disorders (*e.g.* schizophrenia, attention-deficit hyperactivity disorder, bipolar disorder). Functional annotation of ASD-associated eQTLs revealed that they are involved in diverse regulatory processes. In particular, we found significant enrichment of eQTLs within regions repressed by Polycomb proteins in the fetal cortex compared to the adult cortex. Furthermore, we constructed fetal and adult cortex-specific protein-protein interaction networks and identified that ASD-associated regulatory SNPs impact on immune pathways, fatty acid metabolism, ribosome biogenesis, aminoacyl-tRNA biosynthesis and spliceosome in the fetal cortex. By contrast, in the adult cortex, they primarily impact on immune pathways.

Collectively, these findings highlight potential regulatory mechanisms and pathways through which ASD-associated variants can contribute to the development and maintenance of ASD. The integrative approach used in this study, coupled with clinical studies on ASD, will contribute to an individualized mechanistic understanding of ASD.

Q54: Effect of *Alexandrium* spp. on the early life stages of *Perna canaliculus*

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Perna canaliculus (Kuku or Greenshell™ Mussels (GSM)) are a key aquaculture species for New Zealand (NZ), generating more than $350-million-dollars in revenue annually[1]. Kuku are exposed to a multitude of stressors including those from climate change and following from this phenomenon, a growing incidence of harmful algal blooms (HABs)[2]. The animal health implications of HABs on the early life stages of Kuku are poorly understood, with most research being focused on adult Kuku. This research investigated the effects of the HAB species *Alexandrium pacificum* (a paralytic shellfish toxin (PST) producer) and *Alexandrium minutum* (non-PST producer) on gametes, embryos, and D-stage larvae of Kuku. Exposures to field relevant concentrations of whole cell, lysate and filtrate treatments were carried out. Exposure of embryos to whole cells of *A. pacificum* impaired development into D-stage larvae by 85%. Exposures of D-stage larvae to both *A. pacificum* and *A. minutum* halved larval growth rates in the 4-day exposure period, which remained impeded during the 4-day recovery period.

In the wild, HABs can last for several months, reaching cell densities higher than those investigated in this research. This data indicates that HABs are detrimental to vulnerable early life stages of Kuku, which could have implications on sources of seed spat in the wild and contribute to losses on mussel farms.

In upcoming experiments, I aim to use molecular techniques to investigate the effects of multiple stressors (i.e., increasing ocean temperatures in conjunction with HAB exposure) on Kuku, to indicate how this impacts global DNA methylation patterns, up- or down-regulation of specific genes, and importantly, whether these changes are passed on from stressed mothers to offspring.

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Q55: What's your poison? Differential transcriptomic responses to diverse stressors in the Lake Baikal sponge *Lubomirskia baikalensis*

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Lake Baikal is the oldest and deepest freshwater environment on earth. Despite its status as a World Heritage site, its unique ecosystem is now under a variety of threats, both from directly anthropogenic pressures (climate change and pollution)[1], and downstream consequences of these (disease)[2]. One of the keystone species for this ecosystem is *Lubomirskia baikalensis*, but to date there is little data regarding how this species can adapt to the threats facing it. To address this issue, we have sampled independent *L.baikalensis* from a variety of conditions (control "normal" samples direct from wild habitat, specimens suffering from Brown Rot Syndrome, those under thermal stress, some exposed to nitrate pollution, and treatment controls).

A reference transcriptome was assembled *de novo.* Assays of completeness (BUSCO) indicate a well-assembled dataset, with in excess of 95% of the expected metazoan and eukaryotic gene complement. Differential expression analysis (edgeR) of individual conditions revealed highly variable gene expression between *L. baikalensis* samples. Comparison of transcriptional profiles showed that while thermal stress had less impact, disease and pollution dramatically changed the transcriptional profile of sponges compared to natural conditions. These results suggest that *L. baikalensis* may be resilient to thermal changes, which reflects their broad tolerance in the wild. Our results will inform conservation of these key species, which are foundational for the Baikal ecosystem, and provide a raft of information on the means by which sponges can adjust to a range of external stresses, and the extent to which these adjustments share a common transcriptional cassette.

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Q56: Allelic diversity of the pharmacogene *CYP2D6* in New Zealand Māori and Pacific peoples

Hitchman, L.M.[1], Faatoese, A.[2], Merriman, T.R.[3,4], Miller, A.L.[1], Liau, Y.[1,5], Graham, O.E.E.[1], Kee, P.S.[1], Pearson, J.F.[1], Fakahau, T.[6,7], Cameron, V.A.[2], Kennedy, M.A.[1], Maggo, S.D.S.[1]

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The enzyme cytochrome P450 2D6 (CYP2D6) metabolises approximately 25% of commonly prescribed drugs, including analgesics, anti-hypertensives, and anti-depressants, among many others. Genetic variation in drug metabolising genes can alter how an individual will respond to prescribed drugs, including predisposing to adverse drug reactions. The majority of research on the *CYP2D6* gene has been carried out in European and East Asian populations, with indigenous and minority populations greatly underrepresented. However, genetic variation is often population specific, and analysis of diverse ethnic groups can reveal differences in alleles that may be of clinical significance. For this reason, we set out to examine the range and frequency of *CYP2D6* variants in a sample of 202 Māori and Pacific people living in Aotearoa (New Zealand). We carried out long PCR to isolate the *CYP2D6* region, and then carried out nanopore sequencing to identify all variants and alleles in these samples. We identified eleven novel variants, three of which were exonic missense mutations. Six of these occurred in single samples (<0.5% each) and one was found in 19 samples (9.4%). The remaining four novel variants were identified in two samples each (0.99%). In addition, five new suballeles of *CYP2D6* were identified. One striking finding was that CYP2D6*71, an allele of unknown functional status which has been rarely observed in previous studies, occurs at a relatively high frequency (9.2%) within this cohort. These data will help to ensure that *CYP2D6* genetic analysis for pharmacogenetic purposes can be carried out accurately and effectively in this population group.

Q57: Machine learning identifies six genetic variants and alterations in the Heart Atrial Appendage as key contributors to PD risk predictivity

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Parkinson's disease (PD) is a complex neurodegenerative disease with a range of causes and clinical presentations. Over 76 genetic loci (comprising 90 SNPs) have been associated with PD by the most recent GWAS meta-analysis[1]. Most of these PD-associated variants are located in non-coding regions of the genome and it is difficult to understand what they are doing and how they contribute to the aetiology of PD. We hypothesised that PD-associated genetic variants modulate disease risk through tissue-specific expression quantitative trait loci (eQTL) effects. We developed and validated a machine learning approach that integrated tissue-specific eQTL data on known PD-associated genetic variants with PD case and control genotypes from the Wellcome Trust Case Control Consortium[2], the UK Biobank[3], and NeuroX[4]. In so doing, our analysis ranked the tissue-specific transcription effects for PD-associated genetic variants and estimated their relative contributions to PD risk. We identified roles for SNPs that are connected with *INPP5P*, *CNTN1*, *GBA* and *SNCA* in PD. Ranking the variants and tissue-specific eQTL effects contributing most to the machine learning model suggested a key role in the risk of developing PD for two variants (rs7617877 and rs6808178) and eQTL associated transcriptional changes of *EAF1-AS1* within the heart atrial appendage. Similarly, effects associated with eQTLs located within the Brain Cerebellum were also recognized to confer major PD risk. These findings warrant further mechanistic investigations to determine if these transcriptional changes could act as early contributors to PD risk and disease development.

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Q58: Investigating the link between rimu fruit and successful kākāpō breeding

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Kākāpō (*Strigops habroptilus*) are a critically endangered parrot endemic to Aoteoroa New Zealand. Despite the Kākāpō Recovery Team's efforts, the population remains at 197 individuals. One rate-limiting factor is that Kākāpō breed only once every 2-5 years, coinciding with mass fruiting of the NZ rimu tree (*Dacrydium cupressinum).*

The mechanistic link between successful breeding and rimu fruiting is unknown. One hypothesis is that phytooestrogens in rimu fruit act on oestrogen receptors (ESR1) in the liver to stimulate egg yolk protein and mature ovarian follicle production.[1] We have reported oestrogenic activity in crude rimu fruit extracts.[2] In addition, we have identified an extra eight amino acid indel in the ligand-binding domain of Kākāpō *ESR1* (as compared ot the human receptor) that may confer heightened receptor activity to phytoestrogens.[3] The objectives of this study were to:

(i) Identify mutations within the *ESR1* gene of 171 individual Kākāpō using the Kākāpō125+ genome database, and;

(ii) Use a yeast bioassay transfected with human *ESR1* to screen rimu extracts for oestrogenic activity.

Despite SNPs within introns of most individuals, only one SNP (TCC<TCG) was present in the coding region in 33 individual Kākāpō, but did not result in an amino acid change (S<S). Eleven out of eighteen extracts from rimu fruit, seeds and unfertilised ovules expressed oestrogenic activity, with, unexpectedly, the water-based extracts exhibiting the highest activity.

This study showed no functional mutations in *ESR1* that may impair an individual's breeding success. This work also demonstrated the presence of rimu-derived phytoestrogens in aqueous extracts which mirrors the birds eating habit of crushing the rimu fruit and discarding the chews.

1. Fidler et al., (2008). Wildlife Research 35, 1-7.
2. Davis CE (2013). PhD thesis. Victoria University of Wellington. b) Hudson D (2022). MDDD Thesis, Victoria University of Wellington
3. Davis CE… & Pitman JL (2018). Reproduction, Fertility and Development. 30(2), 262-271.

Q59: *Ex vivo* gene therapy for epidermolysis bullosa; severe skin fragility disorders

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The development of specific, efficient, and versatile genome editing tools such as CRISPR/Cas9 has accelerated the field of genome editing. However, the progression of these technologies into personalized therapeutic applications are hindered by the low efficacy of correction and safety concerns surrounding 'off-target' genotoxicity. This research aims to correct patient-specific mutations in human skin cells and develop a low-risk, proof-of-principle genome editing application. Epidermolysis bullosa (EB) encompasses a group of monogenic skin disorders characterized by severe blistering in response to minor friction. *Ex vivo* gene therapy using gene-engineered skin may significantly decrease disease burden and provide a novel cure for people with EB.

We hypothesize that *ex vivo* gene therapies for recessive dystrophic EB will be most effective when delivered as bilayered skin equivalents, so will require the genetic correction of patient epidermal keratinocytes and dermal fibroblasts. Using CRISPR/Cas9 delivered as ribonucleoproteins and short ssDNA repair templates, we achieve up to 50% allele-specific homology-directed repair (HDR) in epidermal keratinocytes. Preliminary data indicates similar rates of HDR in dermal fibroblasts. To ensure the safety of CRISPR/Cas9 editing we intend to analyze editing outcomes, including structural variants, in pooled heterogeneous DNA using Oxford Nanopore Technology sequencing. We will assess the functional consequences of gene correction by digital-droplet PCR (mRNA) and immunocytochemistry (protein) assays. Finally, we have developed novel methods to generate bilayered skin equivalents to help facilitate their transition into the clinic. We utilize both commercially available skin substitutes and a novel synthetic mesh designed for burns patients to generate robust bilayered skin sheets. These methods replicate the EB blistering phenotype so may act as a functional model of gene correction. Overall, this research aims to pave the way for clinical-grade therapeutic genome editing in New Zealand.

Q60: Regulatory networks reveal interactions between SARS-CoV-2 and complex disorders

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Comorbidities greatly increase SARS-CoV-2 health burdens, but the landscapes of their individual and combined genetic risks have not been systematically investigated. The mechanism(s) that underlie the associations during the acute and post-acute stage, are also not entirely clear. Genome-wide association studies have contributed to the identification of reproducible genomic regions associated with SARS-CoV-2 and thousands of common traits. However, translating our genetic knowledge of disease-associated genetic variants into functional understandings of individual and shared disease processes remains a major challenge. Here we identify putative mechanisms for associations between SARS-CoV-2 and comorbidities without *a priori* assumptions; using a *de novo* protein diffusion network analysis coupled with tissue-specific gene regulatory networks. We identified variants, genes and biological pathways that link inherited risk factors for SARS-CoV-2 and coronary artery disease for the first time. The network and pathway-based approach we used anchors known SARS-CoV-2 comorbidities (e.g. diabetes and cardiovascular disease) and previously undescribed genetic predispositions (e.g. Parkinson's disease) in their shared genetic aetiology, identifies molecular insights, and highlights potential therapeutic targets. These findings pave the way for patient stratification through an in depth understanding of genetic impacts on traits that collectively alter an individual's predisposition to acute and post-acute SARS-CoV-2 infection outcomes. Such information also has important applications in clinical medicine and drug discovery by using our approach to understand the nature of many factors found to associate with risk in epidemiological studies.

Q61: Single cell RNA-seq data analysis of the Huntington's disease cerebellum and OVT73 HD sheep model striatum using our new interactive webserver, ICARUS.

Jiang, A.[1], Mears, E.[1], Handley, R.[1], Hawkins, V.[1], Bawden, S.[2], Rudiger, S.[2], Mclaughlan, C.[2], Kelly, J.[2], Verma, P.[2], Waldvogel, H.J.[3], Faull, R.L.M.[3], You, L.[4], Lehnert, K.[1], Snell, R.G.[1]

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Huntington's disease (HD) is a debilitating neurodegenerative genetic disorder caused by an expanded polyglutamine (CAG) trinucleotide repeat in the Huntingtin (HTT) gene resulting in a triad of behavioural, cognitive, and motor defects. Current knowledge of disease pathogenesis remains unclear and no disease modifying interventions have been discovered. We present the results of our cerebellar single cell RNA-seq data performed on 5 human HD cases and 5 controls. We will also present the analysis of striatal samples taken from 6-year-old HD sheep (OVT73) and 6 controls. The datasets reveal further supporting evidence for neuroinflammation, excitotoxicity and mitochondrial dysfunction in the HD brain.

The dataset was analyzed with ICARUS, a new web server tool designed and implement to enable users without experience in R to undertake single cell RNA-seq analysis [1]. The focal point of ICARUS is its intuitive tutorial-style user interface, designed to guide logical navigation through the multitude of pre-processing, analysis, and visualization steps (Available here, https://launch.icarus-scrnaseq.cloud.edu.au/).

In summary, we will present our latest findings incorporating both the sheep model and the human results.

1. Jiang, A., et al., *ICARUS, an interactive web server for single cell RNA-seq analysis.* Nucleic Acids Research, 2022.

Q62: Improving abundance estimates in metabarcoding analyses through CRISPR-Cas enrichment

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Environmental DNA (eDNA) surveys have the potential to revolutionise freshwater macroinvertebrate biomonitoring. In New Zealand, the Macroinvertebrate Community Index (MCI) is often considered to be the 'gold standard' of freshwater biomonitoring. This index relies on expensive and time-consuming microscopic analyses for taxonomy assignment, which are heavily reliant on specialist taxonomic expertise and subject to human bias. As a result, monitoring in New Zealand is currently restricted to yearly surveys for only major rivers and streams in NZ.

As an alternative, eDNA surveys have the capability of identifying species presence and community assemblages in a matter of days for a fraction of the cost. However, laboratory workflows that rely on a PCR based approach are, even with countless attempts to optimise this technique, hampered by failures to detect some species and quantify abundances of each species in a community due to the inherent biases in the methods used[1, 2].

Here, we use CRISPR-Cas enrichment on bulk eDNA samples of freshwater macroinvertebrates collected from local streams to determine the feasibility of generating quantitative biomass estimates, increasing the sensitivity of rare species identification, and decreasing biomonitoring costs. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is a bacterial immune system that has been adapted for both DNA editing and enrichment, due to its highly specific DNA targeting ability. By comparing CRISPR-Cas enrichment to traditional metabarcoding enrichment (PCR amplification) we aim to assess accuracy, sensitivity, and opportunities for the application of the CRISPR technique in molecular biomonitoring. This research presents an exciting opportunity to expand the field of genomic-based biomonitoring techniques with the potential for faster, cheaper, and more accurate measures of stream health.

1. Elbrecht, V. and Leese, F., *Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol*. PLOS ONE. 10(7):e0130324
2. Van der Loos, L.M. and Nijland, R., *Biases in Bulk: DNA Metabarcoding of Marine Communities and the Methodology Involved*. Molecular Ecology.

Q63: Stretched Mussels: tracing the genetic basis of resilience to climate change and ocean acidification in cultured green-lipped mussels (kuku) from genome to embryo

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Molluscs such as kuku (Perna canaliculus) are vital to our ecosystems, and taonga of cultural and economic importance. Climate changes, including temperature extremes and ocean acidification, threaten this species, but some populations are resilient to these problems. The source of this resilience is unknown, but differences in early development are strongly implicated. In New Zealand, bivalves are of key economic interest, with the aquaculture industry growing in importance. In particular, culture of the kuku is worth more than $348 million a year. The New Zealand aquaculture community has an innovative reputation and has been an early adopter of cutting-edge methods for improving husbandry, including selective breeding programmes. Novel genetic and molecular tools have emerged which have the potential to be transformative in industry, while allowing significant scientific breakthroughs in mollusc growth, development, and genetics. Some of these are already in development - genotyping by sequencing, for instance, has been applied to the kuku in the past. Other technologies, such as nanopore genome sequencing, RADseq, bulk and scATACseq and transient expression via lipofection/electroporation, are yet to be trialled, but will allow us to understand how some populations are resilient to climatic effects. Using cutting-edge approaches, we will pinpoint the key differences exhibited by resilient kuku. These could be genetic signals provided by the mother, or those activated in initial stages of development. Knowing the source of this resilience will greatly assist conservation and aquaculture.

Q64: Single cell RNAseq and ATACseq analysis uncovers the transcriptional landscape underpinning cell type specification in the flatworm Schmidtea mediterranea

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Single cell methods are revolutionizing biology. We are leveraging these advances using a multi-omic single-cell approach to understand cellular identities in the planarian species Schmidtea mediterranea. Using single cell RNAseq (SPLiT-seq) we have studied the cell type atlas and lineage tree of this planarian1 , and have noted key markers of individual cell types. With single cell ATACseq (10X Genomics) we can leverage this data to elucidate the regulatory code underlying the specification of a range of cell types in this species. We have identified open chromatin regions specific to all major cell types, and analyzed the motifs contained in them. We have correlated these open chromatin regions with markers from our scRNAseq data using canonical correlation analysis and integration. Genes known to denote well-conserved tissues and cell types in metazoan model species show cell-type specific expression in our scRNAseq data. This has allowed us to cross-correlate overrepresented open chromatin regions with the transcription factors known to be expressed in individual tissues, which generally support conserved metazoan programmes for cell type specification in tissues such as neurons and muscle. We have also been able to note the transcription factors and open chromatin regions involved in specifying cell types such as parenchymal tissue, which are perhaps not as well conserved across the animal tree of life. This work adds greatly to our knowledge of the process of differentiation in both evolutionarily well-conserved tissues and more specialized cell types. This data will provide a key comparison point with other species in the future, adding empirical data for understanding the molecular signals underlying cell type origins across evolutionary time.

1. García-Castro H., N.J. Kenny, P. Álvarez-Campos, V. Mason, A. Schönauer, V.A. Sleight, J.Neiro, A. Aboobaker, J. Permanyer, M. Iglesias, M. Irimia, A. Sebé-Pedrós, and J. Solana (2021) *ACME dissociation: a versatile cell fixation-dissociation method for single-cell transcriptomics.* Genome Biology 22: 89.

Q65: Investigation of the relationship between the milk microbiome and SCC based on the comparison of healthy and unhealthy cows

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Understanding the milk microbiome and its relationship to somatic cell count (SCC) is becoming increasingly relevant for farmers to manage cow well-being efficiently. High-throughput DNA sequencing technologies provide an excellent opportunity to study the genomes of all microorganisms in a given environment. This study investigates the milk microbiome on four farms on the North Island of New Zealand. Cows are separated into two groups for each farm: healthy cows - SCC consistently below 50 and not being culled or dying during the season and unhealthy cows - SCC consistently higher than 200. We focus on comparing microbiome species between the healthy and unhealthy animal groups to identify the potential microbial species related to cow mastitis across the farms. The results reveal that although there are variations in the microbial species between the selected farms, three species (Staphylococcus aureus, Streptococcus uberis and Staphylococcus chromogenes) are frequently observed in the animals in the unhealthy animal group on all farms. These species are already well-known as mastitis causing organisms. Therefore, it confirms the appropriateness of our methodology. Furthermore, we also investigate the similarity of the critical microbial species in the two low-input farms (mainly grass based feeding) with that of the higher input farms. Results indicate that cow diet may be a factor influencing which microbe species may cause health issues under different management practices.

Q66: Genetic/genomic resources for biogeographic research in terrestrial Antarctica

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Genetic and genomic data can provide powerful insights into the evolutionary processes that have shaped – and will shape in the future – spatial patterns of biodiversity. Biogeographic research requires data from many different locations, yet the challenges of working in the Antarctic mean that most studies only include a limited number of sites and samples, and often only one or a few genetic markers are sequenced. As a result, much of Antarctica's biodiversity has yet to be discovered.

Biogeographic studies that bring together disparate data sets can maximize our capacity to infer key processes. Data from all genetic and genomic studies are normally required, on publication, to be made freely and publicly available, although in some cases data are not made available with essential metadata. Here, we present a meta-analysis of Antarctic terrestrial species using pre-existing genetic and genomic data from hundreds of studies over recent decades. We highlight the taxonomic groups and regional locations that are well- versus poorly represented in the data, as well as the most common genetic markers used across studies. We examined diversity patterns and phylogenetic relationships for key markers and groups, enabling us to carry out spatial environmental analyses to reveal the likely drivers of biodiversity patterns in Antarctica.

Q67: Legacy land use effects on soil microbial community composition and functional potential

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Soil microbial communities are strongly impacted by land use change due to their sensitivity to the physiochemical conditions of their environment. Although microbial communities are crucial to ecosystem functioning, our understanding of how microbial communities and their functions are impacted by land use change remains understudied. We used shotgun metagenomic and 16S rRNA gene amplicon sequencing to assess the functional and taxonomic responses of soil microbial communities to land use change. We compared 29 converted sites that have transitioned from grassland to either exotic forest or horticulture and from exotic forest to grassland, to 28 long-term sites representing each land use, and an additional ten indigenous sites for reference.

We found community composition and functional profiles of the transitional sites significantly differed from both the historic and current land use reference sites ($P = 0.001$). The sites that transitioned more recently on average, were compositionally more like their historic land uses, while sites with older conversion events, were more like to, or displayed a shift towards, the reference sites with their current land use. For the functional profiles, this trend was only seen for the grassland to exotic converted sites. Our findings help us understand the long-term consequences of land use change on soil microbial communities and the implications of legacy effects on soil health and ecosystem functioning.

Q68: An experimental approach to understanding virgin plastic leachates and their impacts on plastisphere microorganisms

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Developed initially as a cheap and efficient alternative to other conventional materials during the early 20th century, plastics are now heralded as one of the key pollutants defining the Anthropocene. Despite recent efforts in the manufacturing of alternative plastics, and the attempt to remove macro plastics from the environment, the persistent organic pollutants associated with plastics may have already greatly impacted the health of ecosystems through leaching. Specifically, in the marine environment, many additives introduced during the manufacturing process, including additives that increase plastics malleability, durability, and resistance to degradation, have been shown to leach out and impact the life processes of organisms. However, little remains known about what constitutes plastic leachates and how they impact marine microbial life. To address these knowledge gaps, we artificially aged linear low-density polyethylene, polyethylene terephthalate, polylactic acid and polyamide plastic in an artificial seawater medium and used the resulting aqueous phase to spike seawater mesocosms. Minimal differences in leachate composition between the plastic types were detected via gas chromatography-mass spectrophotometry, while propidium iodine and syto9 fluorescence microscopy identified small differences in cell viability and community composition. At the end of the incubation period (7 days), material for 16S rRNA/fungal ITS amplicon sequencing and shotgun transcriptomic data was also collected. To date most studies, focus on characterizing microbial communities growing on plastic surfaces, termed the 'plastisphere', rather than communities growing on/in plastic leachates. This study provides interesting insight into impacts of the substances leaching out of one of the world's most notable pollutants.

Q69: Dissecting the PD-1/PD-L1 axis in melanoma using a microRNA-based fine-tuning approach.

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Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of certain cancers such as melanoma. ICIs unshackle our immune cells to kill cancer. One type of ICI works by blocking the interaction of the PD-1 receptor on immune T cells with its ligand PD-L1 on the cancer cell. ICIs targeting the PD-1/PD-L1 axis are an effective treatment in approximately 30% of melanoma patients[1], however the underlying biology remains poorly understood. PD-L1 expression alone is a poor biomarker for patient response to ICIs. 30% of PD-L1+ patients fail to respond[2], while up to 15% of PD-L1- patients do respond to ICI therapy[3]. Additionally, the role of a second PD-1 ligand (PD-L2) in the response to treatment is poorly characterized. Therefore, a fuller understanding of the PD-1 checkpoint pathway is needed to better predict which patients will benefit from ICI treatment.

Here we aim to fine-tune endogenous PD-1 and PD-L1/PD-L2 expression in T cells and melanoma respectively, at physiologically relevant levels. We hypothesize that there are optimal expression levels of PD-1/PD-L1 axis proteins which tip the balance towards either immune escape or cancer cell killing. MicroRNA based fine-tuning of endogenous gene expression is achieved through CRISPR/Cas9-mediated insertion of synthetic microRNA response elements into the 3'UTR of target genes[4].

To-date we have demonstrated up to 98% targeted insertion of a miRNA response element into the target genes PD-1 and PD-L1 in CD8+ T cells and a melanoma cell line, respectively. Flow cytometric analysis of PD-L1-edited cells showed a concurrent decrease in cell surface protein expression indicative of fine-tuned gene expression. Additionally, we have generated highly efficient (>90%) PD-L1 and PD-L2 knockout melanoma cells. Experiments are underway to assess the functional impact of melanoma PD-L1/PD-L2 modulation on melanoma-specific T cells. We expect our findings will have implications for many cancer types.

1. Gellrich, F.F., et al., *Anti-PD-1 and Novel Combinations in the Treatment of Melanoma-An Update.* J Clin Med, 2020. **9**(1).
2. Morrison, C., et al., *Predicting response to checkpoint inhibitors in melanoma beyond PD-L1 and mutational burden.* J. Immunother Cancer, 2018. **6**(1): p. 32.
3. Sunshine, J. and J.M. Taube, *PD-1/PD-L1 inhibitors.* Curr Opin Pharmacol, 2015. **23**: p. 32-8.
4. Michaels, Y.S., et al., *Precise tuning of gene expression levels in mammalian cells.* Nat Commun, 2019. **10**(1): p. 818.

Q70: Aminoacylation of Indole Diterpenes by Cluster Specific Monomodular NRPS-like Enzymes

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Development of biologically active fungal secondary metabolites into potential therapeutics is often hindered by naturally low abundance and structural complexity. Understanding the biosynthesis of these metabolites can facilitate efficient access to these valuable metabolites. Indole diterpenes (IDT) are a large and structurally complex class of secondary metabolites produced by filamentous fungi that have a wide variety of bioactivities, including potent insecticidal and anti-cancer activity, making them enticing candidates for agrichemical or pharmaceutical development. Recent advances in molecular and synthetic biology have provided the tools to produce these previously inaccessible secondary metabolites in heterologous hosts through reconstitution of their biosynthetic pathways.

We identified IDT clusters in *Aspergillus alliaceus* (*ACE* cluster) and *Aspergillus nomiae* (*NOM* cluster), which are the first to contain genes encoding non-ribosomal peptide synthetase (NRPS)-like enzymes. Through heterologous pathway reconstruction, we have illuminated the genetic and biochemical basis for the only reported examples of aminoacylation in IDT biosynthesis, demonstrating the unusual involvement of monomodular non-ribosomal peptide synthetase (NRPS)-like enzymes in IDT decoration.[1] The iterative nature of this pathway reconstitution created fungal strains that efficiently accumulated pathway intermediates, enabling their isolation and structural characterisation. These discoveries significantly expand the understanding of IDT biosynthesis and will form the basis for future research on these metabolites and their drug development.

1. McLellan, R. M., Cameron, R. C., Nicholson, M. J. & Parker, E. J. *Aminoacylation of Indole Diterpenes by Cluster-Specific Monomodular NRPS-like Enzymes.* Organic Letters **24**, 2332-2337, doi:10.1021/acs.orglett.2c00473 (2022).

Q71: Population genomics of New Zealand pouched lamprey (kanakana; *Geotria australis*)

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Pouched lamprey (*Geotria australis*) or kanakana/piharau is a culturally and ecologically significant jawless fish that is distributed throughout Aotearoa New Zealand. Despite its importance, much remains unknown about historical relationships and gene flow between populations of this enigmatic species within New Zealand. To help inform management, we assembled a draft *Geotria australis* genome and completed the first comprehensive population genomics analysis of pouched lamprey within New Zealand using targeted gene sequencing (Cyt-b and COI) and restriction site-associated DNA sequencing (RADSeq) methods. Employing 16,000 genome-wide single nucleotide polymorphisms (SNPs) derived from RADSeq (n=186) and sequence data from Cyt-b (766 bp, n=94) and COI (589 bp, n=20), we reveal low levels of structure across 10 sampling locations spanning the species range within New Zealand. F-statistics, outlier analyses, and STRUCTURE suggest a single panmictic population, and Mantel and EEMS tests reveal no significant isolation by distance. This implies either ongoing gene flow among populations or recent shared ancestry among New Zealand pouched lamprey. We can now use the information gained from these genetic tools to assist managers with monitoring effective population size, managing potential diseases, and conservation measures such as artificial propagation programs. We further demonstrate the general utility of these genetic tools for acquiring information about elusive species.

Q72: Identification and comparison of RNA modifications across natural isolates of *E. coli*

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It is known that bacteria use RNA modifications for post-transcriptional regulation These modifications are known to affect antibiotic resistance (Brakier-Gingras and Phoenix, 1984), translation termination, and translation efficiency of transcripts (Hoernes et al., 2016). Traditional methods used to identify RNA modifications are laborious and require previous knowledge of the type of modification. Identifying RNA modifications on a transcriptome-wide level is a daunting task, preventing systematic identification and investigation of such modifications. Here we use direct RNA sequencing with the Oxford Nanopore platform to identify RNA modifications. We use *in vitro* transcription to create a transcriptome that is devoid of modifications and compare this to the native transcriptome. As proof-of-principle, we highlight results for ribosomal RNAs in the lab strain E. coli K12. We then compare the modifications detected in this strain with modifications detected in two natural isolates of *E. coli.* Finally, we show that this method can be extended to identify modifications across the entire transcriptome provided we have enough depth.

1. Brakier-Gingras, L., and Phoenix, P. (1984). *The control of accuracy during protein synthesis in Escherichia coli and perturbations of this control by streptomycin, neomycin, or ribosomal mutations.* Can. J. Biochem. Cell Biol. *62*, 231–244.
2. Hoernes, T.P., Clementi, N., Faserl, K., Glasner, H., Breuker, K., Lindner, H., Hüttenhofer, A., and Erlacher, M.D. (2016). *Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code.* Nucleic Acids Res. *44*, 852–862.

Q73: An MLL-AF9 Zebrafish Acute Leukaemia Model

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The *MLL-AF9* fusion, a consequence of the t(9;11)(p22;q23), is one of the most common translocations found in Acute Myeloid Leukaemia (AML).
Our aim was to establish an *MLL-AF9* zebrafish leukaemia model.
Two transgene constructs (pTol2-Runx1+23: MLL-AF9-IRES-EGFP or -mCherry) were injected into one cell stage zebrafish embryos with Tol2 transposase mRNA. The murine Runx1+23 enhancer was used to direct *MLL-AF9* expression to haematopoietic stem cells.
30% (100 of 340 embryos) of the transgenics reached adulthood. After 6 to 24 months, 77% of them developed signs of sickness. Dissection of the sick fish showed pale kidneys and pale and enlarged spleens. Histological sections revealed increased cellularity of the kidney, spleen and liver with massive cellular infiltration of these organs. In flow cytometry, the kidney marrow showed an expansion of lymphoid (12 fish), precursor cells (9 fish) or myeloid cells (6 fish). These data suggested the development of a haematological malignancy in the MLL-AF9 transgenic fish.
Whole mount *in situ* hybridization (WISH) with RNA probes for early haematopoietic markers (*gata1, …*) on 24 and 48 hpf F1 transgenic embryos showed a shift from erythrocytes towards myelocytes in early haematopoiesis..
We also performed transplantation experiments with the putative malignant kidney marrow cells to test whether the disease was transplantable. The disease was serially transplantable into secondary and tertiary recipients. Transplanted fish had a significantly shorter latency to disease development of only 2 to 6 weeks.
Our data strongly suggest that we succeeded in establishing an *MLL-AF9*-driven acute leukaemia in zebrafish.

Q74: Characterising models of embryonic triploidy

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Approximately half of human embryos produced by *in vitro* fertilisation (IVF) worldwide contain an incorrect number of chromosomes (aneuploidy). Preimplantation genetic testing for aneuploidy (PGT-A) is available but relies on a biopsy taken from an embryo's trophectoderm cell layer, which is invasive, expensive, and time-consuming. We are developing a non-invasive PGT-A that instead uses the genetic material secreted from an embryo to determine its ploidy status. The development of such a test relies on good models of aneuploidy. Triploid embryos, which contain an entire extra chromosome set, are a common form of aneuploidy, of which several models can be developed in the laboratory.

One model creates digynic triploid embryos that have an extra maternal haploid chromosome set by manipulating meiosis[1,2]. We have optimised the *in vitro* generation of digynic triploid embryos by exposing mouse oocytes to the mycotoxin cytochalasin-B following fertilisation. Cytochalasin-B inhibits the expulsion of the second polar body, resulting in an embryo with two maternal and one paternal pronuclei.

A second model creates diandric triploid embryos that have an extra paternal haploid chromosome set through polyspermy[3]. We are currently developing this method in our laboratory by injecting two sperm cells into a single mouse oocyte using intracytoplasmic sperm injection (ICSI). This results in an embryo that has one maternal and two paternal pronuclei.

To further characterise these triploidy models, the developmental progression of these embryos will be recorded using Primovision cameras enabling early embryonic cytokinetic events to be compared. In addition, the expression levels of several developmental genes will be quantified by qPCR. The information provided by the digynic and diandric triploid embryos, in comparison to their chromosomally-normal (euploid) controls, will enable the characterisation of these two models of triploidy for use in future studies.

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Q75: Polycistronic gene expression for fungal biosynthetic engineering

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Fungi have evolved in nature to produce secondary metabolites diverse in structure and bioactivities. These natural products have many applications across health and agriculture industries. Synthetic biology can provide access to fungal metabolites that are minimally produced, or even not expressed all, by fungi in laboratory environments. A major focus of our research is the production of difficult to access indole diterpene compounds (IDTs) in *Penicillium paxilli*, a fungus that natively produces large amounts the IDT paxilline.

Expression of large biosynthetic pathways requires the coordinated expression of many genes. Repeated use of the same promoters is not desirable, as this can result in instability of the genetic constructs, however promoter options can be very limited in non-model organisms. Viral 2A peptides have been used in many eukaryotic expression systems to produce polycistronic operons, wherein multiple independent proteins can be expressed under the control of a single promoter. This can allow many genes to be expressed from few promoters, with temporal coordination, and without repetition in the DNA constructs.

We have used this expression technique to produce IDTs in *P. paxilli*, with key genes in the pathway linked by 2A peptide sequences. Preliminary results suggest this design method is an effective means of reducing repetition in our synthetic biology constructs, while preserving our ability to express the genes required for compound production.

Q76: Contrasting patterns of single nucleotide polymorphisms and structural variations across multiple invasions

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Adaptive divergence is a fundamental process that shapes genetic diversity within and across species. Structural variants (SVs) are large-scale genetic differences (insertion, deletions, and rearrangements) within a species or population. SVs can cause important functional differences in the individual's phenotype. Characterising SVs across invasive species will help fill knowledge gaps regarding how patterns of genetic diversity and genetic architecture shape rapid adaptation in response to new selection regimes. In this project we seek to understand patterns in genetic diversity within the globally invasive European starling, Sturnus vulgaris. We use whole genome sequencing of eight native United Kingdom (UK), eight invasive North America (NA), and 33 invasive Australian (AU) starlings to examine patterns in genome-wide SNPs and SVs between populations and within Australia. The findings of our research demonstrate that even within recently diverged lineages or populations, there may be high amounts of structural variation. Further, patterns of genetic diversity estimated from SVs do not necessarily reflect relative patterns from SNP data, either when considering patterns of diversity along the length of the organism's chromosomes (owing to enrichment of SVs in sub telomeric repeat regions), or interpopulation diversity patterns (possibly a result of altered selection regimes or introduction history). Finally, we find that levels of balancing selection within the native range differ across differed across SNP and SV groupings. Overall, our results demonstrate that the processes that shape allelic diversity within populations is complex and supports the need for further exploratory SV classification across a range of taxa to better understand correlations between oft well studied SNP diversity and that of SV.

Q77: Pinpointing and targeting novel drivers of pancreatic cancer progression, invasion and metastasis using TRAP-seq.

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Pancreatic Cancer (PC) has a low 5-year survival rate of 11%, which can be attributed to its' rapid metastatic spread and resistance to standard-of-care chemotherapy. Therefore, there is an urgent need to identify novel 'druggable' candidates that become de-regulated during PC progression and metastasis and can be co-targeted with standard-of-care chemotherapy to improve patient survival. Here, we aim to use innovative Translating-Ribosome-Affinity-Purification followed by RNAsequencing (TRAP-seq) to enrich in a cell-type-specific manner for mRNAs, which are actively being translated into proteins in metastatic PC and may therefore represent valid 'druggable' targets. Our genetically engineered mouse models closely mimic the mutational landscape, histopathology and progression of human PC. Both models are driven by an initiating Kras mutation and either loss of p53 (poorly metastatic) or a gain-of-function mutation in p53 (highly metastatic). These were crossed to the Cre-inducible Rpl10a-GFP mouse to activate expression of the GFP-tagged ribosomal protein Rpl10a specifically in PC cells, which can be immunoprecipitated with associated translating mRNAs for downstream RNA-seq analysis. We isolated tumours for TRAP-seq analysis from end-stage mice of both models (120-220 days of age) and also established primary cell lines, which retain Rpl10a-GFP expression ex vivo. Genomic DNA profiling has verified the presence of a Kras mutation and either loss of p53 or a gain-of-function mutation in p53 in both cultured cell types. Optimization has also shown that TRAP-seq purification results in high-quality, enriched mRNA transcripts. This technology will be coupled with validation of deregulated candidate targets in our libraries of murine and human PC samples, as well as functional assessment using our established in vitro and in vivo PC models. This will allow us to identify and validate novel 'druggable' candidates to target PC progression and metastasis in conjunction with standard-of-care chemotherapy in a pre-clinical setting.

Q78: Low maternal vitamin C during pregnancy in guinea pigs results in persistent epigenetic dysregulation in the hippocampus of offspring together with an ADHD-related phenotype

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Background: Recent advances in epigenetics have highlighted mechanisms whereby changes in maternal nutrition could affect development. A pertinent example is the ten-eleven translocation (TET) dioxygenase family of enzymes, which play a key role in mammalian developmental epigenetics, as established by gene deletion and overexpression studies. The activity of these enzymes is particularly sensitive to the availability of metabolic intermediates including vitamin C (ascorbate). Dependency on ascorbate, an essential micronutrient for humans, provides a potential mechanism whereby human nutrition can impact epigenetic processes.

Methods and results: Our study investigates how suboptimal maternal vitamin C intake during pregnancy affects hippocampal function and behaviour of postnatal offspring in a guinea pig model. We found that offspring from low vitamin C pregnancies had decreased plasma and brain vitamin C concentrations. This effect had normalized in the plasma by 1-week of age and in the brain by 4-weeks of age. During behavioural assessments, female offspring from low vitamin C pregnancies were found to exhibit behavioural changes in open field consistent with a hyperactive phenotype that closely resembles attention-deficit/hyperactivity disorder (ADHD). This behavioural phenotype was matched by gene expression changes data from the hippocampus of offspring from low vitamin C pregnancies. Key findings from the hippocampal transcriptome include the downregulation of GABAergic signalling genes as well as genes associated with hyperactivity and cognitive disorders. A statistically significant proportion of the downregulated genes were also downregulated in a recent study investigating the repression of Tet1 in postnatal hippocampal neurons, supporting our hypothesis that reduced vitamin C availability mimics the effects of compromised TET activity in neurodevelopment.

Conclusions: These findings provide a further layer of evidence implicating epigenetic dysregulation of the GABAergic signaling system in the aetiology of ADHD. Our research also suggests a potential adverse consequence for offspring following inadequate maternal vitamin C intake during pregnancy.

Q79: Genomics of *Staphylococcus aureus* strains in New Zealand dairy cows

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Mastitis is a significant economic problem for dairy farming across the world. *Staphylococcus aureus (S. aureus)* is a common mastitis-causing bacterium in dairy cattle; studies in New Zealand and worldwide have focussed on its genetic diversity, virulence, clonal nature, and antimicrobial resistance (AMR) patterns. However, no studies have been conducted into the patterns of bovine *S. aureus* on a New Zealand scale. To analyse and understand AMR and virulence profiles in *S. aureus* nationwide, we have used whole-genome sequencing to classify 161 bovine *S. aureus* isolates taken from aseptic quarters and non-sterile bulk tank milk samples. Isolates were identified as *S. aureus* through colony morphology on Eschulin blood agar and coagulase/catalase testing. All identified *S. aureus* isolates were sequenced via Illumina sequencing and analysed using a modified Nullarbor bioinformatic pipeline to classify isolates based on multilocus sequence typing (MLST) and provide details on the genes associated with virulence and AMR. From 161 sequenced isolates, 59% were classified as the human-derived lineage - strain type 1 (ST1). ST1 is rare in overseas studies, with the majority of bovine mastitis isolates typically belonging to CC97 or CC151/CC705[1]. The dominance of ST1 suggests an unexpected New Zealand-specific trend that could be linked to our unique pasture-based system or potential inter-host transmission[2]. 34% of all isolates harboured the penicillin resistance encoding genes *blaZ*. All isolates carrying *blaZ* displayed phenotypic resistance determined by a Kirsty-Bayer disc diffusion assay. This research indicates a unique population of *S. aureus* within the New Zealand dairy sector compared to overseas *S. aureus* populations. Our approach highlights the importance of undertaking nationwide studies investigating a broad range of genetic and phenotypic characteristics.

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Q80: To haline back: Investigating freshwater sponge hologenomes and mitogenomics using draft genomic sequencing

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Freshwater sponges (Porifera: Demospongiae: Spongillida) are an astonishingly successful but often overlooked clade of animals. They are found in a wide range of freshwater habitats around the globe. As with marine sponges, one of the keys to their success are a range of complex relationships with microorganisms. We are only just beginning to catalogue and understand these relationships, but many sponges operate as "holobionts", playing host to an array of other organisms, to their mutual benefit.

Two examples of these sponges are *Stratospongilla* sp and *Metschnikovia tuberculata,* from India and the Caspian Sea respectively. These species are believed to occupy a key position in the Spongillid radiation and are vital for understanding what components of the sponge microbiome are possessed widely across freshwater sponges, and which are lineage-specific. *M. tuberculata,* a brackish species, also allows us to infer the impact of changes in salinity on holobiont content. To date, however, we have lacked "omic" tools to investigate the phylogenetic position and microbial content of these sponges.

Here we have used whole genome shotgun sequencing on the Illumina platform to construct novel mitochondrial assemblies, and both Bayesian and maximum likelihood phylogenetic methods to confirm the placement of these sponges within the Spongillida. We have also used this data to gain the first insight into the hologenomes of these species and have contrasted these with the microbial content of other freshwater sponges, from established databases.

This adds significantly to our understanding of the diversity and evolution of the hologenome of freshwater sponges, as well as the phylogenetic structure of this clade more generally, providing a basis for future, targeted investigations in this fascinating group of animals.

Q81: Correlation of stem cell frequency with the immunophenotype during serial transplantation in a CALM/AF10 leukaemia model: evidence of phenotypic plasticity

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Background: Leukaemia stem cells (LSC) are rare cells possessing self-renewal and leukaemia-propagating abilities. We established a CALM/AF10 retroviral-transduction murine bone-marrow transplantation acute myeloid leukaemia model (MBMTLM). Previously, we had shown that LSCs are enriched in the lymphoid-positive (B220-positive)/myeloid-negative (Mac1-negative) population. Recently, we showed primary CALM/AF10-MBTLM leukaemias with high B220-expression (1˚B220high) have a higher LSC frequency (LSC-F) than leukaemias with low B220-expression (1˚B220low). We aimed to explore variation of the B220-immunophenotype of LSCs in a CALM/AF10-B220low leukaemia after serial transplantation.

Methods: LSC-F were measured with limiting dilution assays (LDAs). Flow cytometry was used to determine the immunophenotype and retroviral insertion sites, and clonality were monitored with splinkerette analysis.

Results: $5x10^5$ cells from 1°B220low with 4% B220-expression were transplanted into secondary recipients. The secondary leukaemia had 6.5% B220-expression (2°B220Low). Two LDAs of 1°B220low and 2°B220Low showed the LSC-F had not changed significantly. However, B220-positivity among tertiary leukaemias (n=14) in the 2°B220Low-LDA, ranged from 2% to 50%. Moreover, splinkerette indicated our leukaemias originated from one transduced cell.
Then, we measured the LSC-F of B220-positive and B220-negative cell-fractions from the leukaemias with the highest (50%) and the lowest (2%) B220-expression (3°B220-2% and 3°B220-50%), which had been generated from only one LSC. We expected the highest LSC-F in the B220-positive cell fraction of 3°B220-50%. However, 3°B220-2% had about the same LSC-F in its B220-negative cell fraction like the 3°B220-50% in its B220-positive cell-compartment. We could not detect any LSC in the B220-negative cell fraction of 3°B220-50%. After transplantation, >95% of the B220-negative cells remained negative while, on average, 50% of the B220-positive cells lost B220-expression.

Our results indicate that, despite initiating from a single cell, our CALM/AF10-MBTLM shows variable B220-expression, which changes after serial transplantation and is not directly linked to LSC-F. Our models might allow to study phenotypic plasticity independent of genetic changes.