QMB Abstracts

Q1: I Contain Multitudes: The Microbes Within Us and A Grander View of Life

Ed Yong

The Atlantic

Every individual animal is a teeming ecosystem, full of trillions of microbes. These microscopic companions sculpt our organs, protect us from diseases, guide our behaviour, and bombard us with their genes. This talk will introduce the science of the microbiome, explore how microbial communities shape the lives, health, and evolution of animals, and look at our nascent attempts to manipulate the human microbiome for our own benefit.

Q2: Distinct microbiome patterns associate with colorectal cancer molecular subtypes

Rachel V Purcell[1], Martina Visnovska[2], Patrick J Biggs[3], Sebastian Schmeier[2], Frank A Frizelle[1*]

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Colorectal cancer (CRC) is a heterogeneous disease and recent advances in subtype classification have successfully stratified the disease using molecular profiling. The contribution of bacterial species to CRC development is increasingly acknowledged, and in this study, we sought to analyse CRC microbiomes and relate them to tumour consensus molecular subtypes (CMS), in order to better understand the relationship between bacterial species and the molecular mechanisms associated with CRC subtypes. We classified 34 tumours into CRC subtypes using RNA-sequencing derived gene expression and determined relative abundances of bacterial taxonomic groups using 16S rRNA amplicon metabarcoding. 16S rRNA analysis showed enrichment of Fusobacteria and Bacteroidetes, and decreased levels of Firmicutes and Proteobacteria in CMS1. A more detailed analysis of bacterial taxa using non-human RNA-sequencing reads uncovered distinct bacterial communities associated with each molecular subtype. CMS1 tumours were associated with an oral pathobiont signature that included bacterial species with known mechanisms in carcinogenesis, biofilm-forming bacteria and bacterial species previously associated with CRC. Targeted quantitative PCR validated these findings and also showed an enrichment of the oral bacteria *Fusobacterium nucleatum, Parvimonas micra* and *Peptostreptococcus stomatis* in CMS1. In this study, we have successfully associated individual bacterial species to CRC subtypes for the first time, based on metagenomic analysis of tumour tissue.

Q3: Disturbance within the human microbiome: lessons from Nature

David A. Relman

Stanford University

All complex ecosystems display multiple, alternative stable states. These alternative states can be conceptualized as discrete domains of attraction on a stability landscape, where the likelihood of a persistent state relates to the depth of the valley and the energy required to displace the system. Regime shifts, or transitions from one state to another, occur in response to disturbance or change in the environment. In humans, these environmental changes might include the effects of pregnancy, aging, loss of salivary flow, or of immunosuppressing drugs. Resilience refers to resistance to ecosystem state shift or to recovery of prior ecosystem services following disturbance. Given the known and suspected benefits that humans derive from their microbiota, the stability and resilience of this ecosystem are critical properties that deserve attention. We have undertaken longitudinal studies in human subjects, some of whom are monitored before and after a standardized disturbance, with the goals of describing the temporal dynamics of the human microbiome, and identifying features that are associated with stability in the face of disturbance as well as recovery of a prior state. A predictive understanding of the microbiome and the mechanisms that underlie resilience will inform effective strategies for its manipulation, so as to maintain or restore health, and avoid or mitigate disease.

Q4: Quantifying sources of variation in host-associated communities

Xochitl Morgan

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The microbial communities of the human gut are affected by many host factors, including but not limited to early life exposures, disease, medication, host transcripts, and biogeography. These factors may affect either the abundance of specific organisms, or the abundance of disease-associated metabolic pathways. Understanding these effects is critical to data interpretation and mechanistic understanding of disease, but they are often inadequately addressed. In cohort of children, IBD patients, and model organisms we explore and quantify how the human gut is shaped by environmental exposure.

Q5: Effects of a 'Baby-led' Approach to Complementary Feeding on Infant Gut Microbiota – A randomised controlled trial

Leong, C.[1], Taylor, R.[2,4], Tannock, G.[3,4], Haszard, J.[1], Lawley, B.[3], Szymlek-Gay, E.[5], and Heath, A-L.M.[1,4]

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Recently, there has been an increasing interest in gut microbiota and its influence on human health. However, little research has investigated the influence of the largest change in diet that humans experience – the transition from a milk-based diet to solid foods – on the gut microbiota.

This study aimed to investigate the influence of a 'baby-led' approach to complementary feeding - BLISS (Baby-Led Introduction to SolidS) - on the gut microbiota of infants. In this randomised controlled trial, 206 New Zealand infants were randomised before birth to either BLISS (introduction of solids at 6 months of age, followed by self-feeding of family foods) or Control (traditional spoon-feeding of purées). Faecal samples (n=37 from each group) and 3-day weighed dietary records were collected at 7 and 12 months of age. The composition of the microbiota of faecal samples was determined by sequencing 16S rRNA genes amplified from DNA extracted from the faecal samples. Dietary data were used to estimate dietary fibre intake, fibre variety scores, and intake of nine food groups.

At 12 months, the BLISS group faecal microbiota had a lesser complexity (lower alpha diversity; mean±SD 176±58 OTUs) than the Control group (mean±SD 203±58 OTUs) (mean difference: 31 OTUs; 95% CI: 3.4 – 58.5 OTUs; p=0.028). Mediation models demonstrated that intake of 'fruit and vegetables' and 'dietary fibre' explained 32% and 25% respectively of the link between group and alpha diversity. In summary, infants consuming a more adult diet had lower alpha diversity at 12 months than infants following traditional weaning practices, which was explained in part by variation in intake of fruits and vegetables, and dietary fibre.

Q6: Discovering the cause of phenotype in real time

Bruce Beutler

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Random germline mutagenesis in mice has led to many breakthrough discoveries of gene function, but was once an arduous process, requiring several years from the observation of a phenotype to the identification of the causative mutation. By combining massively parallel whole exome sequencing, targeted genotyping, and genetic computation, we have made it a real-time process. Approximately one third of the mouse genome has been damaged or destroyed under close observation to identify genes required for defined aspects of immunity, neurobehavioral function, metabolism, and other biological phenomena. More than 1,000 mutations responsible for phenotype have been declared, and several hundred have been verified by gene targeting or re-creation of the original ENU induced allele. This new approach to mammalian forward genetics is limited only by the speed at which mutations can be created and screened for effect.

Q7: Pathogen targeting of cell death signalling

Elizabeth L Hartland

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Cell death pathways can provide innate protection from pathogens through the elimination of infected cells. We have discovered that the bacterial pathogen, enteropathogenic *E. coli* (EPEC), uses the bacterial virulence proteins NleB and EspL to block extrinsic apoptosis and necroptosis respectively. NleB mediates a novel post-translational modification of Arg-GlcNAcylation in the death domain of cell death signalling proteins whereas EspL is a novel cysteine protease that degrades the signalling proteins, RIPK1, RIPK3, TRIF and ZBP1/DAI in the RHIM domain. *In vivo*, EspL contributed to persistent colonization of mice by the EPEC-like mouse pathogen *Citrobacter rodentium* although it is not clear if this can be attracted to blocking necroptosis. The activities of EspL and NleB define new families of bacterial effector proteins found in a range of bacteria and reveal the mechanisms by which gastrointestinal pathogens directly target apoptotic and necroptotic signalling pathways.

Q8: One Genome to Rule Them All: Sequence Diversity within Single *Helicobacter pylori* Strains

Draper, J.[1,2], Hansen, L.[3], Bernick, D.[2], Abedrabbo, S.[2], Underwood, J.[4], Kong N.[3], Huang B.[3], Weiss A.[3], Weimer B.[3], van Vliet A.[5], Pourmand, N.[2], Solnick, J.[3], Karplus, K.[2], Ottemann, K.[2]

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Although it is well known that many bacterial genomes are highly variable, it is nonetheless traditional to refer to, analyse, and publish "the genome" of a bacterial strain. When these 'reference' genomes are published, variation in the sequenced population is typically deliberately minimised ("only sequence from a single colony") or ignored ("just publish the consensus"). Experiments tracking bacterial genome evolution rarely assay the variation present at given point in time, focusing instead on change over time or in response to environmental pressures.

The human pathogenic bacterium *Helicobacter pylori* is well known for having a highly variable genome with a high mutation and recombination rate. Nonetheless, little is known about the degree of variation to expect within typical working stocks of *H. pylori*, and researchers rely heavily on single fixed reference genomes for their strains.

Here, I will discuss the variability seen in typical laboratory cultures of the mouse-passaged *H. pylori* strain SS1 and its parent strain PMSS1, as revealed by a combination of high-depth sequencing and traditional laboratory techniques. Within SS1 alone, the variation includes multiple inversions, nearly 50 SNPs at over 5% prevalence, movement of the transposable element IS607, and copy-number variation of the *cagA* gene. In addition, we observed 46 differences between SS1 and PMSS1 that were distinct from the within-genome variation. In sum, this work reveals that reliance on a single-colony reference genome or consensus assembly may be misleading, even at the level of a typical laboratory working stock.

Q9: Storytelling & sense-making: narrative in science communication

Liz Neeley

Story Collider

Storytelling is among the most hyped and least appreciated concepts in public engagement. For many audiences, stories are more interesting, understandable, convincing, and memorable than evidence-focused communications - and these qualities are often highlighted in science communication discussions. But stories are too often positioned as convenient delivery mechanisms for desired messages. They are both products of and processes for humans to collectively resolve ambiguity and construct meaning from information. It is precisely because of their power that scientists should use narratives thoughtfully, with intellectual honesty and ethical care. This talk will explore research on storytelling and persuasion, highlight the value of personal stories in science, and critically consider how and why busy researchers might approach adding something like "narrative competency" to their repertoire.

Q10: Science and the Media: Q&A with Jamie Morton and Dacia Herbulock

Morton, Jamie[1], Herbulock, Dacia[2]

[1]The New Zealand Herald, [2]The NZ Science Media Centre

In a Q&A session, Dacia Herbulock, a senior media advisor with the New Zealand Science Media Centre, will interview the New Zealand Herald's Jamie Morton about the realities of working as a science journalist in 2017. The session will cover what it's like to work in a modern newsroom, how stories are selected, developed and produced, the changing media landscape, working with scientists and what makes for an appealing science article. The session will also include how journalists might avoid "bad science" and other pitfalls in the world of "fake news" and high demand for content. Herbulock helped launch the Science Media Centre and has shaped the direction of the SMC as a core member of its team since 2008. Prior to this she worked in radio, film, television and science writing in the US, China and New Zealand, most recently at Radio New Zealand, where she was science features producer and presenter. She designs and leads Science Media SAVVY, a national programme of training workshops and events that encourage more effective communication between researchers, media and the public. Morton has been the Herald's science journalist since 2012 and has won several journalism awards, including Science and Environment Reporter of the Year (Canon Media Awards 2013) and Science and Technology Reporter of the Year (Canon Media Awards 2016). Morton writes about all facets of the science sector and his work has taken him to disaster zones around the world, to Antarctica, and to the UN Paris climate change summit in 2015. Before joining the Herald he spent a decade working in daily newspapers around New Zealand.

Q11: The REANNZ network - helping you move your research data anywhere in the world - and FAST!

Kim Partridge, REANNZ

REANNZ: Research and Education Advanced Network New Zealand

If you are at QRW your organisation is probably already connected to the REANNZ network – a high-speed, high-capacity network built to send research data around the country and the world. If you have research data you want to share with colleagues, send to supercomputers, do sequencing on or you just want to back your data up to a datacentre you will do that via your organisation's network and REANNZ.

Do you need to send a terabyte of data to Germany? Do you need to send a weekly update to a collaboration team in the US? Or Japan? Or Sydney? We receive Government funding to assist researchers to send your valuable research data to your colleagues and collaborators.

One of our members was regularly sending several hundred gigabytes of data to Brazil to be processed and returned. It took 72 hours each way. With REANNZ it now takes 55 minutes and, because we're connected to other research networks around the world, we can monitor your data from desktop to destination.

In this talk I will explain who REANNZ are, why we exist (to support research) and give some examples of how we have helped make researchers' lives easier. I'm also here to answer questions you may have about high-speed data transfer, or put you in touch with people who can help.

Q12: How Microbes keep their CRISPR memories up to date

Stan J.J. Brouns

Delft University of Technology, Department of Bionanoscience, Kavli institute of Nanoscience, Netherlands

The CRISPR immune system protects bacteria and archaea from invading viruses and plasmids. Immunity depends on protein complexes that use small RNA molecules to find matching viral or plasmid DNA. I will show how viruses escape immunity by mutating their DNA, and how a mechanism called priming takes care of these escaped viruses and will quickly update the memory of the immune system leading to rapid co-evolution between host and phage. All of these insights into the biology of CRISPR have led to some of the most revolutionary molecular genetics tools to date, with Cas9 being the most well known example. I will highlight some new CRISPR tools for genome engineering approaches to edit genomes and to knockdown gene expression.

Q13: Natural variation in the transcriptional behaviour of the lac operon

Olin Silander

Massey University, Auckland, New Zealand

Many phenotypic differences between species are driven by changes in transcriptional regulation, frequently detected as a change in mRNA transcript levels. Here we use a plasmid-based system and flow cytometry to explore how the regulation of the lac operon differs among natural isolates of *E. coli*. We find both cis- and trans- changes that affect regulation. These changes not only affect transcript levels, but inducer sensitivity, the speed of transcriptional change, and the level of variation between individual cells (transcriptional noise). We then focus specifically on two SNPs within the lac operon that differ between two closely related natural isolates, and quantify the effects each of these polymorphisms on regulation. The substantial differences we find in the regulatory behaviour of the *E. coli* lac operon suggests that there is significant, on-going selection on this phenotype in nature.

Q14: Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity

Mariann Landsberger[1], Adair Borges[2], Hélène Chabas[1], Edze R. Westra[1], Joseph Bondy-Denomy[2], Stineke van Houte[1*]

[1] Biosciences, University of Exeter, Penryn Campus, UK; [2] University of California, San Francisco, USA

In response to CRISPR-Cas adaptive immune systems, phages evolved a range of diverse anti-CRISPR (*Acr*) genes that inhibit bacterial immunity, but the (co)evolutionary implications of Acr remain unexplored. We examined this question using *Pseudomonas aeruginosa* strain PA14 and variants of phage DMS3vir carrying different *Acr* genes. We demonstrate that partial immunity of bacterial CRISPR-Cas against *Acr* phage causes extinction tipping points that are phage density-dependent. Furthermore, higher levels of partial immunity shift the tipping point towards higher phage densities. Our data demonstrate that these epidemiological tipping points are the result of cooperation between sequentially infecting *Acr* phages.

Q15: Bioinformatic discovery of noncoding RNA genes

Chris M Brown, D Chyou, A Biswas, P Baltazar, T Nicholson, B Croft and P Fineran

University of Otago, Dunedin, New Zealand

Noncoding RNA (ncRNA) genes have been shown to have critical roles across all domains of life. We are interested in the structure and function of diverse ncRNA genes. These studies have included the computational discovery and prediction of ncRNA genes, the RNAs they produce, the functions of these RNAs, and their evolution over short timeframes.

In a few model organisms many ncRNAs have been annotated on the genome sequence. However, for most genomes only a few types of RNAs (e.g. tRNA, rRNA) are automatically annotated by current pipelines (e.g. NCBI, JGI, Ensembl) other classes are inconsistently and poorly described. Major challenges in predicting ncRNA genes that are functional is that they may be small and the evolutionary conservation is more difficult to detect than protein coding genes.

Most archaea and about half of bacteria use a recently discovered adaptive immune system that is ncRNA based. The CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats, and CRISPR associated proteins) system evolved as a defense against foreign genetic elements (notably viruses or bacteriophages). The CRISPR-Cas9 system has recently been repurposed as a precision genetic targeting tool in many organisms. We have developed a suite of programs to investigate the CRISPR adaptive immune system of prokaryotes (CRISPRSuite). The bioinformatic challenges of developing these tools and applying them to ~40,000 prokaryotic genomes will be described. Insights gained into the distribution structure, function and evolution of these systems will be presented.

Q16: Evolutionary ecology of CRISPR-Cas

Edze Westra

Bacteria have a range of sophisticated immune mechanisms to protect against virus infections, but it is unclear why all these different mechanisms evolved in the first place. Under laboratory conditions, bacteria typically evolve de novo virus resistance using either surface modification or CRISPR-Cas adaptive immune systems. In this talk I will discuss ecological factors that can tip the balance in the evolution of these two immune mechanisms and examine their distinct co-evolutionary implications.

van Houte S, Buckling A, Westra ER. *Evolutionary Ecology of Prokaryotic Immune Mechanisms.* 2016 Microbiol Mol Biol Rev. 80(3):745-63.

van Houte S, Ekroth AK, Broniewski JM, Chabas H, Ashby B, Bondy-Denomy J, Gandon S, Boots M, Paterson S, Buckling A, Westra ER. 2016 *The diversity-generating benefits of a prokaryotic adaptive immune system*. Nature 532(7599):385-8.

Westra ER, van Houte S, Oyesiku-Blakemore S, Makin B, Broniewski JM, Best A, Bondy-Denomy J, Davidson A, Boots M, Buckling A. 2015 *Parasite Exposure Drives Selective Evolution of Constitutive versus Inducible Defense*. Curr Biol. 25(8):1043-9.

Q17: Cooperative autoregulation of the Type IV *abiE* toxin-antitoxin operon involves a charged surface on AbiEi

Hannah G. Hampton[1], Simon A. Jackson[1], Robert D. Fagerlund[1], Anne I.M. Vogel[1], Ron L. Dy[1], Tim R. Blower[2] and Peter C. Fineran[1]

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Abortive infection (Abi) systems provide bacteria with population-level protection from phage predation at the expense of infected hosts, via 'altruistic' cell suicide. Some Abi systems function as toxin-antitoxin (TA) systems. The *abiE* operon is a bicistronic Abi system that acts as a Type IV TA system with a target that has not yet been identified. AbiEi, the antitoxin, belongs to a large family of putative transcriptional regulators and has a conserved N-terminal winged-helix-turn-helix (wHTH) domain. This wHTH motif is essential for transcriptional repression of the *abiE* operon. The C-terminal domain (CTD) of AbiEi contains a unique fold that is sufficient for toxin neutralization but is also required for transcriptional repression. We demonstrate that a conserved positive charge along the length of AbiEi contributes to DNA binding and to negative autoregulation without influencing antitoxicity. Furthermore, we show that AbiEi binds cooperatively to two operator sites within the *abiE* promoter region. We identified critical positively charged surface residues, which, when mutated to a neutral charge, result in strictly obligate cooperative binding to the native operator. These findings provide new insight into the dual functionality (autoregulation and antitoxicity) of the unique CTD domain found in this widespread family of transcriptional regulators.

Q18: Insights into the settlement of New Zealand utilising modern and ancient DNA datasets

Collins, C.J.[1], Posth, C.[2], Krause, J.[2], Matisoo-Smith, E.A.[1].

[1]Department of Anatomy, University of Otago, Dunedin, NZ, [2] ,Max Planck Institute for the Science of Human History, Jena, Germany.

While humans first arrived in the Pacific region approximately 50,000 years ago, New Zealand and other islands in East Polynesia were settled much more recently, within the last 1,000 years[1]. However the path that these humans took on the way to New Zealand, and the genetic contribution of the various Pacific populations, is not yet fully understood. To date, human migration in the Pacific region has primarily been assessed using mitochondrial DNA and Y chromosome markers, however recent research is now incorporating genome-wide data[2]. We have combined SNP data from modern-day individuals from populations across the Pacific from new and published datasets, resulting in ~120K loci to study the migration of people into the Pacific region, and ultimately New Zealand. In addition to contemporary data, we have further mitochondrial sequences and preliminary shotgun sequence data from individuals buried at Wairau Bar, an early archaeological site in New Zealand, dated to A.D. 1285-1300[3] and thought to be representative of the founding population of Maori in New Zealand. Together, these datasets assess the ancestry of Pacific populations, with a focus on New Zealand, and provides further insights into the movement of people through this region.

1. Matisoo-Smith, E.A. (2015). *Ancient DNA and the human settlement of the Pacific: A review. Journal of Human Evolution* 79: 93-104

2. Skoglund, P., Posth, C., Sirak, K. *et al.* (2016). *Genomic insights into the peopling of the Southwest Pacfic. Nature* 538:510-513.

3. Brooks, E., Jacomb, C. Walter, R. (2009). *Archaeological investigations at Wairau Bar. Archaeology in New Zealand* 52: 259-268.

Q19: Divergent methods of encoding robustness to mutation in RNA and protein: how that relates to their evolution and our ability to rationally design robust biomolecules

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[1]Department of Biology, University of Canterbury, Christchurch, NZ, [2]Biomolecular Interaction Centre, University of Canterbury, Christchurch, NZ

Despite their essential nature, non-coding RNAs are typically difficult to trace across large phylogenetic distances. This may mean that RNAs evolve and devolve rapidly, leaving little evolutionary trace, or that they are able to continue to function despite changes to sequence, leaving little conservation for homology searches to detect distant family members. To begin addressing this, we consider how mutations in DNA affect the structure and function of individual RNA and protein molecules. Mutant libraries of the fluorescent RNA aptamer (Broccoli) and fluorescent protein (mCherry) were produced to determine their response to sequence change. We found Broccoli to be more robust than mCherry to mutation at all levels of mutation tested. We then compared the sequence divergence of RNA and protein components of ribonucleoproteins (RNPs) from *Neisseria meningitidis* and *Escherichia coli*. As they interact to perform the same overall essential functions in the cell, the RNAs and proteins in the RNP are under similar selection pressures, have similar taxonomic distributions, and function in similar environments, though their individual functions can differ. We find that these RNA and proteins encode robustness on multiple levels, from intramolecular epistasis to wobble base pairing in RNA and the genetic code in protein. Each differentially contributes to overall robustness of the molecule. There is no perfect quantitative comparison of robustness in RNAs and proteins, but this analysis serves as a starting point for investigating the range of ways in which mutational robustness can be encoded at the molecular level. We will discuss how differences between RNA and protein robustness informs our understanding of sequence divergence and de novo evolution of biomolecules and the application of it toward the smart engineering of biomolecules that are more resistant to mutation.

Q20: Akoranga i te pakiaka o te harakeke - lessons from the flax roots: genomic sciences and genome biology research involving indigenous biota and Māori communities

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[1]Department of Mathematics and Statistics, University of Otago, Dunedin, New Zealand; [2]Faculty of Māori and Indigenous Studies, University of Waikato, Hamilton, New Zealand.

The renaissance of Māori culture, Treaty of Waitangi settlements, and increasing participation of Māori entities in health, primary production and environmental management, collectively mean Māori are more frequently interacting with genomic sciences. The response of genomics researchers to this trend – almost all of whom are non-Māori – has been highly varied, with some valuable lessons from such interactions. In this presentation I will:
(a) provide a Māori view of genomic sciences in the context of the New Zealand science system;
(b) review examples of past interactions between Māori communities and genomic scientists – both positive and negative;
(c) outline some key lessons for both Māori communities and genomics researchers; and
(d) describe how these are being implemented in current and future research involving genomic information from Māori communities and indigenous biota.

Q21: Genome editing to the fittest/healthiest?

Alexei Drummond

Centre for Computational Evolution, University of Auckland, New Zealand

It has been claimed by some that CRISPR-Cas9 genome editing produces results indistinguishable from natural and artificial breeding. Furthermore, recent success in using CRISPR-Cas9 to edit the genomes of human embryos has laid the foundation for the first gene-modified people and led some researchers to claim that "gene editing could be used as a preventive tool to lower a newborn's lifetime risk of a wide range of diseases, including cancer". Here I investigate both of these claims from a theoretical perspective.

First I will review the literature on genetic load in humans. I will show some theoretical results under models of genetic load for parameter values relevant to the human population. In doing so I will demonstrate a simple and reasonable scenario in which genome editing will produce genomes that could never be produced by normal population genetic processes, despite utilising only existing genetic variation.

I will also describe genetic models of pleiotropy and epistasis - processes fundamental to understanding the genetic basis of complex diseases. These models suggest that genome-editing may not be able to simultaneously lower the risk of all complex diseases, thus converting the use of genome editing as a preventive tool into a difficult multi-objective optimisation problem.

I will finish by describing research my group is pursuing to understand the potential population-level consequences of the application of genome editing to improving human health.

Q22: New technologies for an old problem - the role of ancient DNA and functional genomics in bird conservation

Knapp, M.[1]

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

Conservation management relies on high quality genetic data, but the availability of such data has long been a limiting factor. Recent advances in DNA sequencing technology have the potential to overcome these limitations. Improvements in ancient DNA technologies for example allow us to study extinct species on a population level and over tens of thousands of years to reconstruct potential causes for extinction. Such information can provide valuable insights into potential threads for endangered species today. Furthermore, the ongoing reduction in costs of genome sequencing now allows us to replace neutral genetic markers as proxies for functional variation in a threatened species with actual information on functional genomic diversity. Such data can directly be used in conservation management, for example when making translocation decisions.

I will present ongoing research into extinct and endangered bird species and discuss implications of these studies for the future of conservation genetic research.

Q23: The illumina™ Emerging Researcher Award 2017

The arms race between bacterial CRISPR-Cas adaptive immune systems, phages and mobile genetic elements.

Jackson, S.A., Birkholz, N., Taylor, C., Fineran, P.C.

Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin 9054, New Zealand.

CRISPR-Cas systems are adaptive immune defences found in bacteria and archaea. They function as sequence-specific nucleases, which can identify and destroy viral (phage) infections and invading mobile genetic elements (MGEs). CRISPR-Cas systems typically consist of CRISPR loci and CRISPR-associated (Cas) genes. CRISPR loci (arrays) store genetic memories of prior invaders, in the form of 'spacers'. Spacer sequences determine immune specificity. Thus, immunity can be updated by the addition of new spacers – a process termed 'CRISPR adaptation'. CRISPR adaptation represents the first major step in CRISPR-Cas defence. Next, CRISPR arrays are transcribed and processed to generate CRISPR-RNAs (crRNAs), which complex with Cas proteins. The resulting Cas-crRNA surveillance complexes utilise the crRNAs to facilitate sequence-specific recognition of cognate nucleic acids. Once identified, targets are destroyed by Cas nuclease activity. Phage and MGE variants, with genetic mutations, can escape/evade sequence-specific recognition and destruction by host CRISPR-Cas defences. Therefore, a perpetual arms race exists between hosts and invader evolution. The fundamental principal of all CRISPR-Cas systems is adaptation – hosts must update their immunity to respond to new and divergent threats. Many CRISPR-Cas systems respond to phage and MGE escape mutants by stimulating CRISPR adaptation (i.e. increasing the host's spacer repertoire). The resulting diversity in CRISPR loci, both the number and sequence of spacers, is important for assuring lasting immunity in individual hosts and populations. Here, I will discuss our recent discovery of a new pathway that pre-emptively stimulates CRISPR adaptation, before escape mutants are encountered.

Q24: The Thermo Fisher Scientific™ Award 2017

Why heart disease runs in families

Pilbrow, A.P.

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Genetic factors interact with lifestyle risk factors in the development of coronary heart diease. Over the last decade, 60 regions of the genome have been robustly associated with susceptibility to coronary heart disease by genome-wide association studies. Only ~7% of these risk regions (single nucleotide polymorphisms, or SNPs) alter protein-coding sequences and almost all remaining SNPs are located in non-coding regions of the genome. Consequently, our research aims to understand the mechanisms through which non-coding genetic risk variants impact coronary heart disease. I will present recent work investigating associations of genetic variation with global gene expression in human heart tissue (expression quantitative trait locus, eQTLs), and new risk markers for coronary heart disease among non-coding RNAs.

Q25: Withdrawn

Q26: Withdrawn

Q27: *MSMEG_5243*: A Succinate dehydrogenase flavination factor

Liam K. Harold[1, 2], F. Hafna Ahmed[3], James Antoney[3], Kiel Hards[1], Chris Greening[4], Colin J. Jackson[3], and Gregory M. Cook[1, 2]

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Understanding the molecular mechanisms that *Mycobacterium tuberculosis* (MTB) uses to adapt to the hypoxic host environment may lead to the discovery of new drug targets to more effectively treat MTB infections. Flavin deazaflavin oxidoreductases (FDORs) are a superfamily of proteins, highly abundant in mycobacterial species, characterised by a split β-barrel. FDORs can be broadly classified into two distinct groups, the FDOR-As and FDOR-Bs. FDOR-As primarily have F_{420} as a cofactor whereas FDOR-Bs have a more diverse range of cofactors such as F_{420}, FMN, FAD, and Heme. While limited molecular and biological characterisations of FDOR-Bs have been reported, they have been shown to be upregulated in response to various environmental changes.

In this work, we investigated the biochemical and physiological role of *MSMEG_5243* from *M. smegmatis*, a FDOR-B with a FAD cofactor which is upregulated in response to hypoxia. When a *MSMEG_5243* markerless deletion was grown under hypoxia the long-term survival of the bacteria markedly decreased. In order to further establish the physiological effect that *MSMEG_5243* had on the cell, *MSMEG_5243* was aberrantly expressed during aerobic growth, under these conditions we observed an increased growth rate caused by increased succinate dehydrogenase (Sdh) activity, indicating that MSEG_5243 has a role in succinate metabolism.

An unpublished X-ray crystal structure of MSMEG_5243 revealed that the FAD co-factor was bound tightly, preventing electron transfer. To investigate if MSEG_5243 was a FAD flavination factor for Sdh we co-overexpressed MSMEG_5243, and the SdhA subunit of Sdh from *M. smegmatis*. The resulting lower levels of SdhA flavination indicated that MSMEG_5243 has a role in the flavination state of Sdh. Furthermore, addition of purified MSMEG_5243 to purified Sdh1, or inverted membrane vesicles effected Sdh activity. Based on this data we propose that *MSMEG_5243* is a Sdh specific flavination factor.

Q28: Next Generation Inhibitors to Combat Drug Resistant Tuberculosis Infections

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Tuberculosis (TB) remains one of the leading worldwide causes of infectious disease mortality globally with over one million annual deaths. The emergence of extensively drug-resistant (XDR), and totally drug-resistant (TDR) strains of *Mycobacterium tuberculosis*, threatens to return us to the pre-antibiotic era for this disease. The discovery of the imidazopyridine amide series (e.g. Q203), nanomolar inhibitors currently in clinical trials that inhibit mycobacterial respiration, has sparked interest in the targeting of terminal respiratory oxidases to treat drug-resistant TB disease[1]. Mycobacteria are obligate aerobes and respire using two terminal respiratory oxidases, a cytochrome bc_1-aa_3-type cytochrome *c* oxidase (*qcrCAB* operon – bc_1 complex) and a cytochrome *bd*-type menaquinol oxidase (*cydAB* operon). The cytochrome bc_1 complex is documented as being an essential part of M. *tuberculosis* electron transport chain with inhibition of both terminal oxidases causing rapid cell death[2]. Here we report on the mode of action (MOA) of TB47, a newly synthesized derivative of Q203. In contrast to Q203, TB47 was cidal against replicating cells of *M. tuberculosis* (wild-type and *ΔcydAB* mutants). Similar experiments with *Mycobacterium smegmatis* revealed that TB47 was only static against wild-type cells with an MIC of >5µM compared to 0.125µM in *ΔcydAB.* TB47 inhibited respiration of the *ΔcydAB* mutant, but was without effect on wild-type cells. TB47 dissipated the delta pH gradient in energized membrane vesicles of *M. smegmatis* in both wild-type and *ΔcydAB* mutants. Spontaneous mutants resistant to TB47 could be isolated in the *ΔcydAB* background. Whole genome sequencing of these mutants mapped the mutations to the QcrB (H190Y), the cytochrome *b* subunit of the cytochrome bc_1 complex. Respiration of the QcrB mutant was not inhibited by TB47. Taken together these data demonstrate that TB47 targets the QcrB subunit of the cytochrome bc_1-aa_3-type cytochrome *c* oxidase at residue 190 of QcrB in contrast to Q203 which targets residue T313A in the QcrB subunit leading to accelerated cell death in the *ΔcydAB* mutant. The molecular basis for cidality of TB47 against *M. tuberculosis* will be discussed.

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Q29: Screening NZ fungi for new antibiotics

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Antibiotic-resistant bacteria are a leading cause of difficult-to-treat infections, especially in patients with weakened natural defences. Several species have emerged that are resistant to multiple antibiotics, including *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* We are currently screening a large collection of NZ-isolated fungi for new kinds of antibiotics against these organisms. To do this, we grow constitutively bioluminescent strains of target bacteria alongside test fungi. Suppression of luminescence is taken as a possible indication of fungal antibiotic production. Crude extracts are then prepared from active fungi and tested for antibacterial activity. Active components of crude extracts are isolated via thin layer chromatography and bioluminescence-based bioautography. Compounds present in active fractions are identified via MS-NMR.
So far, we have screened 238 fungi, identifying 18 that selectively inhibited *S. aureus* luminescence. A further 23 isolates inhibited the luminescence of all target bacteria. Out of 10 crude extracts prepared from these fungi, the most active have been from a common plant pathogenic fungus called *Cercospora* Fresen. These extracts showed a minimum inhibitory concentration of 16 µg/ml against *S. aureus,* but greater than 1 mg/ml against *E. coli* and *P. aeruginosa.* Fractionation and MS-NMR revealed that the active compound was a cercosporin. While the antibacterial properties of cercosporins have already been reported, our data demonstrates that our bioluminescence-based screening method works, and has the potential to discover new kinds of antibiotics in the future. We are currently expanding the screening to include two additional pathogens of clinical importance, *Klebsiella pneumoniae* and *Acinetobacter baumanii*.

Q30: *withdrawn*

Q31: A genetic analysis of Japanese PSA strain with New Zealand PSA outbreak strain

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Pseudomonas syringae pv. *actinidiae* (PSA) is a bacterial pathogen that infects kiwifruit. PSA can cause widespread damage, impacting on vine health and fruit yield. In recent years, a highly virulent strain of PSA has spread across multiple countries, including New Zealand. To explore its evolution, the study compared the Japanese strain ICMP 9853 with the New Zealand strain ICMP 18708. The Japanese strain was isolated in 1984 and belongs to the Psa-1 lineage. It is almost identical to the PSA 'type strain', ICMP 9617. The New Zealand strain belongs to the Psa-3 lineage which is attributed to the recent pandemic. Both strains were sequenced using PacBio and Illumina sequencing platforms. The utilisation of both sequencing technologies allowed for accurate genome assemblies, which in turn provided a high resolution for genome comparisons.

The comparison revealed large scale deletions/acquisitions and rearrangements between the Japanese and New Zealand strains. It was noted that despite the rearrangements, genes maintained a similar distance relative to the origin of replication/replication terminator site (*ter*). It is postulated that this conservation may be due to mechanisms involved in chromosome segregation. The suggested mechanism involves multiple short sequences termed KOPs (FtsK Orientating Polar sequences)[1].

Several of the large sequence deletions/acquisitions consisted of mobile elements such as integrative and conjugative elements (ICEs) and integrative and mobilisable elements (IMEs). The original New Zealand strain carries an ICE (Pac_ICE1)[2]. Comparison of the two strains revealed that the ICE and IMEs are absent from the Japanese strain. The analysis also allowed us to confirm the presence and limits of the IMEs in the New Zealand strain. Confirming the presence of the IMEs allowed the identification of genes within the elements. This is of significance as some genes were recognised to potentially contribute to virulence and may be transmissible to other PSA.

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Q32: Antibiotic Resistant Bacteria (ARB): Trends in conventional dairy farms compared with organic counterparts in New Zealand and China

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Antibiotic resistance (AR) among a wide range of infectious agents is an internationally recognised public health threat (1). Certain agricultural practices have been implicated in the rapid emergence of antibiotic resistant bacteria (ARB) (2-5). Treatment and control of mastitis, enteric and other infectious diseases in ruminants is predominantly by the use of antibiotics, and the link between antibiotic resistant microbes from agricultural animals and human infection outbreaks appears well documented (6-10). The development and possible spread of resistant foodborne pathogens including *Escherichia coli* is a major concern (2, 11-13).

We examined conventional and organic sheep farm soil in New Zealand and China for *E. coli* and characterised their antibiotic resistance profiles, to assess any differences that may occur between these countries and farming practices. Our preliminary studies did not find any significant differences between the antibiotic resistance profiles of *E. coli* from organic farm soil compared to conventional sheep farm soil, which was similar to results of a trial conducted in China on organic and conventional dairy farms. However, the Chinese organic dairy farms had significantly more isolates showing intermediate susceptibility to tetracycline, streptomycin and cefepime compared to the New Zealand organic farm isolates ($p<0.01$). Conversely, the New Zealand organic farm had significantly more isolates showing resistance to azithromycin compared to their Chinese counterpart ($p<0.01$). The conventional farm in China showed significantly ($p<0.01$) more intermediate-resistant isolates to tetracycline and cefepime than the conventional farm New Zealand isolates.

This trial showed the tendency for *E. coli* to show increased resistance to frequently used antibiotics and this trend is higher in China compared to New Zealand.

These preliminary studies will contribute towards more detailed assessment of the prevalence and molecular epidemiology of foodborne pathogens including *E. coli* from organic and conventional dairy farms in New Zealand.

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Q33: Protozoan Predation Can Influence Bacterial Lineages to Evolve Coccal Cell Shape

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Cell shape is a fundamental characteristic of single-celled organisms, affecting nutrient acquisition, replication and growth. Ancestrally rod-like, bacteria have adopted spherical cell shape over 14 times in the phylogenetic record, losing a single gene, *mreB* in each case [1-3]. Work to understand the genetic aspects of this transition is ongoing. Ironically, spherical cell shape is a disadvantage in terms of motility, nutrient exchange and DNA segregation. Most model bacteria are inviable as *mreBΔ* mutants. However, *Pseudomonas fluorescens* SBW25 is a rod-like bacterium in which *mreBΔ* cells are slow growing, spherical, and have a dramatic 4-fold increase in cell volume. Suggesting an alternative narrative for the adaptation conferred by deletion of *mreB*: large cell size.

Large size is an advantage under protozoan predation [4, 5]. We are therefore experimentally assessing the potential of protozoan predation as a driver of cell shape evolution. We have tested *Naegleria gruberi* and *Dictyostelium discoideum* for predation preferences between WT, *mreBΔ*, and small evolved *mreBΔ* cells. Our experimental results suggest predation by physically smaller protozoa, has an 5-fold preference for WT cells over their larger *mreBΔ* derivatives. However, the larger predator prefers larger cells, giving smaller evolved cells an advantage under predation.

Experimental conditions in the laboratory can only hint at the effect of protozan predation to drive evolution of cell shape in nature. We developed an agent-based model, allowing us to take a wide range of possible conditions including predator preference, growth disadvantage and predator abundance into account and determined a 4-fold preference of WT over *mreBΔ* cells, and a minimum predator population of 10 will favour the increase of *mreBΔ* cells in mixed populations. This work allows us to speculate about ecological parameters in which *mreBΔ* cells might have a transient adaptation, leading to long-term changes in cell shape in bacteria.

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Q34: Effectiveness of CRISPR-Cas systems in response to diverse phage infections

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In every environment one of the factors that highly modulate bacterial populations is phage infection and propagation. To counteract viral infection bacteria have developed a variety of strategies, among which CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas systems constitute the adaptative immune response of bacteria. CRISPR arrays are composed of short conserved sequences (repeats), regularly interspaced by diverse sequences of similar length (spacers). In the adaptation step, new spacers are acquired from foreign genetic elements, such as plasmids and phages, and inserted into the array. The CRISPR array is then expressed and processed into crRNAs, containing the foreign DNA fragments. In case of a new phage infection, the crRNA, in conjoint action with the Cas protein complex, recognises and cleaves the targeted DNA in the interference step. Bacteria can harbour different CRISPR-Cas systems; such is the case of *Serratia* sp. ATCC 39006, our bacterial model of study.

Although mechanism of action and function of CRISPR-Cas systems have been widely described, little is known about the phage-host interaction with these systems. In this work we study the effectiveness of *Serratia* ATCC sp. 39006 CRISPR-Cas systems in response to different phage infection strategies. In order to achieve this, the acquisition of spacers targeting phages JS26, LC53 and PCH45 was evaluated showing that CRISPR-Cas systems provide a variable range of infection resistance against different phages. This study will provide a better understanding of the bacterial defence mechanisms towards different phage infections strategies and will increase our understanding of phage-bacterial interactions.

Q35: Analysis of the type I-F CRISPR Cas complexes from *Pectobacterium atrosepticum in vitro*

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CRISPR-Cas are prokaryotic adaptive immune systems that protect cells from foreign invading elements, such as viruses or plasmids. The type I-F CRISPR-Cas system is comprised of two major protein complexes, the Cas1-Cas2-3 adaptation complex and the Csy interference complex. The Csy complex is a ribonucleoprotien complex comprised of Csy1 (Cas8f), Csy2 (Cas5), Csy3 (Cas7), Cas6f and an associated CRISPR RNA (crRNA). The crRNA molecule is derived from a previous invader and functions as a guide for the Csy complex which can target invaders for destruction with remarkable specificity. Destruction of invading DNA is largely conducted by the Cas3 helicase-nuclease protein. Interestingly, in type I-F systems the Cas3 protein is physically fused to the Cas2 protein. Cas2 also interacts with a Cas1 protein forming the Cas1-Cas2-3 adaptation complex, which can acquire short sequences of invading DNA into the own genome. Invasive elements can evade initial targeting by the Csy complex by acquiring point mutations at the site of recognition. However, this can initiate a process known as priming where partial recognition by the Csy complex results in the rapid acquisition of new spacers, which limit the liklihood of further mutational escape. In these experiments we expressed and purified both the Cas1-Cas2-3 and Csy complexes from *Pectobacterium atrosepticum* and assess their interactions with DNA. Using electrophoretic mobility shift assays we have investigated the ability of the Cas1-Cas2-3 complex to bind both ssDNA, dsDNA and the ability of the Csy complex to bind target specific, non-specific and escape mutant DNA respectively.

Q36: Imprecision during spacer acquisition by type I CRISPR-Cas systems increases CRISPR diversity in bacterial populations

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CRISPR-Cas systems provide bacteria with sequence-specific immunity. This is achieved through the action of Cas proteins, which assemble to form acquisition and interference complexes. The Cas1-Cas2 acquisition complex detects small sequence portions, termed protospacers, in phage or plasmid DNA and incorporates them into a CRISPR array in the host chromosome. These newly acquired spacers are then utilized by the interference complex to identify and destroy the same foreign genetic element. To discriminate between target and non-target sequences, type I CRISPR-Cas systems use protospacer-adjacent motifs (PAMs). PAMs are also cues for the Cas1-Cas2 complex that trims pre-spacer substrates prior to their integration into CRISPR arrays. The Cas1 protein possesses a PAM-sensing domain and cleaves pre-spacer substrates adjacent to PAMs (or within PAMs for type I-E). This ensures crRNAs produced from spacers are compatible with the PAM-sensing domains of interference complexes. However, in the *Pectobacterium atrosepticum* type I-F CRISPR-Cas system approximately one in 15 acquired spacers results from cleavage staggered by one nucleotide relative to the canonical position. This imprecision is termed 'slipping'. Here, we show that slipping typically leads to spacers that are not functional for interference. Instead, slipped spacers strongly stimulate primed CRISPR adaptation (priming), resulting in increased CRISPR diversity within bacterial populations. We propose that slipping-induced priming pre-empts escape mutations in phage and mobile genetic elements. Thus, the diversity-generating characteristics of slipping might account for why higher fidelity Cas1-Cas2 activity has not arisen.

Q37: Discovery of regulators of CRISPR-Cas adaptive immunity in *Serratia*

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The CRISPR-Cas systems represent an adaptive and heritable prokaryotic immunity that confers the ability to withstand invasion by selfish genetic elements and promote host survival. Over the past decade the majority of CRISPR-Cas research has been dedicated to unravelling the molecular function and composition of these systems. To effectively counteract the infection, bacteria are proposed to employ timely and coordinated management of these systems, but the mechanisms of which are yet to be thoroughly elucidated. The central aim of the current study is to determine genetic elements that direct the expression of the CRISPR-Cas systems in *Serratia*. We have investigated the role of a number of genes in *Serratia* that play a role in modulating the bacterial adaptive immune system operation. This was performed by analysing the activity of the three specific CRISPR-Cas systems through a reporter gene-fusion system based in different mutant backgrounds. In addition, to test whether changes in the expression of the CRISPR-Cas systems result in an altered resistance profile, a number of phenotypic assays were performed, including adaptation and interference assays. The identity and mechanism of action of these potential regulators will be presented.

Q38: Loss and gain of CRISPR-Cas systems and genomic islands: a delicate balancing act?

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Bacteria, constantly challenged with foreign genetic elements, have evolved multiple mechanisms to avoid invasion. They include innate systems and adaptive systems. CRISPR-Cas (clustered regularly interspaced short palindromic repeats and CRISPR associated) are adaptive systems that provide protection against invading mobile genetic elements. The mechanism consists of three phases: 1) adaptation, with acquisition of memory, in which spacers are incorporated to the CRISPR array; 2) expression of *cas* genes and CRISPR arrays to generate the guide RNA components; and 3) interference, in which target nucleic acids are recognized and destroyed. CRISPR-Cas systems are found in approximately less than half of sequenced bacteria. It has been proposed that horizontal gene transfer is the mechanism of transfer of these systems between bacteria. However, this hypothesis has not been experimentally demonstrated yet. Several studies have shown that CRISPR-Cas systems are often found as part of mobile genetic elements, such as Genomic Islands (GIs) and Pathogenicity Islands (PAIs). In particular, *Serratia* sp. ATCC39006, a plant pathogen that has 3 CRISPR-Cas systems, possess its type III-A system in a Genomic Island that is excisable. Several different mutants have been constructed to test the ability and frequency of this GI to excise and transfer to other strains. Bioinformatic analysis have shown the presence of similar type III-A CRISPR-Cas systems in other strains, both containing these system as part of different GIs. These GIs, likewise, have been found in closely related *Enterobacteria* lacking the *cas* genes and CRISPR arrays, suggesting the high mobility of these systems.

Q39: Investigating the role of integration host factor in the function of multiple CRISPR-Cas systems

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CRISPR-Cas adaptive immune systems provide heritable protection against foreign genetic elements. Integration host factor (IHF) is a small histone-like architectural protein involved in cellular processes such as replication, gene regulation and the transposition of mobile elements. The IHFα and IHFβ subunits, encoded separately in the genome, form the IHF heterodimer which binds a consensus motif to induce a U-turn in DNA. This sequence-specific interaction is essential for the leader-proximal integration of spacers by the I-E CRISPR-Cas adaptive immune system. Our group has shown that the addition of IHF promotes the integration and specificity of leader-proximal integrations by the *Pectobacterium atrosepticum* type I-F CRISPR-Cas system in a sensitive *in vitro* spacer acquisition assay. Further analysis of various CRISPR-Cas systems revealed additional putative IHF binding sites within the promoter regions of CRISPR arrays and *cas* genes, suggesting IHF also has a role in regulation. To investigate this, Δ*ihfα* strains have been created through allelic exchange mutagenesis to further elucidate the role of IHF on the regulation, adaptation and interference of multiple CRISPR-Cas types.

Q40: DNA capture and integration by the type I-F CRISPR-Cas adaptation complex from *Pectobacterium atrosepticum*

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CRISPR-Cas systems are diverse RNA-based defence mechanisms that provide prokaryotes heritable adaptive immunity against mobile genetic elements, such as bacteriophages and plasmids. The basis of CRISPR-Cas immunity involves capture of foreign DNA and insertion into specialised CRISPR 'memory' loci by proteins Cas1 and Cas2 (a process termed adaptation)[1]. These memories are expressed through transcription and form the guides within effector complexes with other Cas proteins, which target and degrade complementary invading sequences (interference). There are six types of CRISPR-Cas systems, and the subtype I-F system is of particular interest as it contains a unique fusion of Cas2 with the type I effector helicase and nuclease involved in invader destruction, Cas3, thereby linking the adaptation complex with interference. Here we demonstrate that Cas1 and Cas2-3 from the I-F system in *Pectobacterium atrosepticum* form a 400 kDa complex with 4:2 stoichiometry and present a structural solution of the complex bound to substrate DNA[2]. We invented a sensitive in vitro integration assay and demonstrated that Cas1:Cas2-3 catalysed DNA integration into CRISPR loci via the integrase domain of Cas1. Integration required at least partially duplexed DNA with free 3'-OH groups and precise integration was stimulated by integration host factor (IHF). In a coupled capture and integration assay, Cas1:Cas2-3 processed and integrated DNA independently of Cas3 activity. These results provide insight into the structure of DNA-bound type I adaptation complexes and their integration mechanism.

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Q41: Developing SorTn-Seq: a state-of-the-art method to study CRISPR-Cas regulation

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Despite the extensive metabolic and morphological diversity present among bacteria, one common necessity is the ability to manage exposure to foreign genetic elements, such as viruses or plasmids. Certain bacteria may retain a "memory" of past infections, which are stored within a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) array. Upon subsequent exposure to the same element, the bacteria can utilize sequences found within the array to target and neutralize the invader, with the assistance of CRISPR-associated (Cas) proteins. Currently there is a lack of knowledge concerning how these systems are regulated. A typical approach to pinpoint genes involved in bacterial networks is transposon mutagenesis. However, caveats associated with this method include the labor-intensive screening required to isolate mutants of interest. Therefore, we are developing a method utilizing fluorescence activated cell sorting to isolate cells affecting CRISPR-Cas expression from within a saturated (~one million mutants) transposon library. Initial libraries and sorted pools can be deep-sequenced to locate mutations of interest. Initial transposon mutagenesis experiments in *Serratia* have shown that a large-scale transposon library is achievable. Fluorescent-reporter fusions were constructed to measure CRISPR-Cas expression. A measurable shift in fluorescence is observed between wildtype and strains with previously identified regulatory mutations, due to the differential regulation of *cas* promoter. Preliminary sorting experiments reveal that a mutant strain with altered *cas* expression can be enriched via FACS from a mixed pool of wildtype and mutant cells. Once verified, this technique will be adaptable for studying regulation in a wide range of bacteria.

Q42: A novel Cas9 mutant confers reduced off-target gene editing while maintaining on-target potency

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Precise genome editing by the CRISPR/Cas9 system has proven to be groundbreaking in basic medical research and has great implications for the treatment of human disease. The CRISPR/Cas9 system demonstrates unparalleled editing efficiency in a broad range of host species and cell types, but suffers from concerns related to target site specificity. Modified RNA guides and mutant Cas9 proteins have been developed to reduce off target editing by Cas9, but in many cases these remedies introduce significant on-target editing problems. In addition, many of these solutions were developed for plasmid DNA-based delivery of the Cas9-gRNA complex which can lead to unwanted stimulation of the immune system. Here we present a novel mutant Cas9 protein that has been evolved to reduce off-target gene editing while maintaining on-target potency. This mutant is ideally suited for RNP delivery allowing highly efficient genome editing without the complications associated with transfecting plasmid DNA. We analyzed the genome-wide editing profiles of this mutant in both cell free and *in vitro* cell culture methods, and find that it has superior properties to the wild-type nuclease. Finally, we demonstrate reduced off-target editing while preserving on-target editing in human CD34+ hematopoietic stem and progenitor cells when targeting the HBB locus, the locus involved in beta-hemoglobinopathies, including sickle cell disease.

Q43: Genome-editing based molecular tools for super-resolution and light sheet microscopy

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Recent advances in fluorescence imaging, especially super-resolution and light sheet microscopy, have revolutionised our ability to visualise biological structures and functions in cells and organisms at much increased spatial and temporal resolution. A critical prerequisite to exploit these powerful new imaging methods is to quantitatively label the proteins of interest with suitable dyes without compromising their expression or function. To achieve this, we have implemented new genome engineering technologies such as Crispr/Cas9 in order to homozygously replace endogenous genes with fluorescently tagged versions in human cell lines and mice. We illustrate the power of this approach by revealing the molecular architecture of the cell in situ and discovering new mechanisms of early mammalian development.

First we created a set of carefully validated genome edited cell lines containing endogenously tagged (with GFP, SNAP or Halo tags) nucleoporins and employed 4Pi-STORM to investigate the 3D molecular architecture of the nuclear pore complex at nanoscale resolution inside whole human cells, thereby visualising stable as well as dynamic components of the pore that have been inaccessible to structure determination so far. This advance will now allow us to investigate different functional as well as assembly states of the nuclear pore.

By creating transgenic mouse models to image the crucial divisions right after fertilization by inverted light sheet microscopy, we could gain new insights into the causes of aneuploidy at these early stages of development. Imaging live embryos for the first time with very high spatial and temporal resolution allowed us to discover the formation of two spindles in the fertilized zygote that keep the parental genomes apart during the first division.

These examples illustrate how the combination of the latest molecular biological tools with the most advanced microscopy will allow us to shine light onto many previously refractory biological questions.

Q44: Discovering new disease causing genes using Chromium whole-genome sequencing

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The advent of next-generation sequencing has changed the landscape of rare genetic disease research, with causative genes being identified at an accelerating rate [1]. Research on the rare autosomal recessive disorder Meier-Gorlin syndrome (MGS), a type of microcephalic primordial dwarfism affecting approximately 100 individuals worldwide, has identified hypomorphic gene mutations affecting essential components of the pre-replication and pre-initiation complexes, which regulate DNA replication [2-4]. We aim to identify new disease causing genes in MGS patients for which no causative gene has yet been identified. Our hypothesis is that MGS is due to dysfunction of both established and unappreciated DNA replication machinery, and through this research we hope to advance our understanding of basic mechanisms regulating growth relevant to human health.

To discover new disease causing genes we have utilised Chromium whole-genome sequencing (10X Genomics, Inc.), a sequencing technology that uses barcodes and microfluidics to retain long-range information while maintaining the power and accuracy of short read sequencing. Chromium whole-genome sequencing generates linked-reads that can be phased to resolve haplotypes and structural variants. Through this approach it is possible to establish *cis* or *trans* relationships between variants without trio sequencing thereby simplifying the identification of compound heterozygous mutations inherited in an autosomal recessive model in a cost effective manner. Here we describe our experience using Chromium whole-genome sequencing, addressing phase block size, number of phased SNPs, and ability to phase known polymorphisms, in our attempts to discover new genes causing the rare genetic disorder Meier-Gorlin syndrome.

1. Boycott, K.M., et al., (2013) *Rare-disease genetics in the era of next-generation sequencing: discovery to translation.* Nature Reviews Genetics. 14, 681–691.
2. Bicknell, L.S., et al., (2011) *Mutations in the pre-replication complex cause Meier-Gorlin syndrome*. Nature Genetics. 43(4), 356-359.
3. Bicknell, L.S., et al., (2011) *Mutations in ORC1, encoding the largest subunit of the origin recognition complex, cause microcephalic primordial dwarfism resembling Meier-Gorlin syndrome*. Nature Genetics. 43(4), 350-355.
4. Fenwick, A.L., et al., (2016) *Mutations in CDC45, encoding an essential component of the pre-initiation complex, cause Meier-Gorlin Syndrome and craniosynostosis*. American Journal of Human Genetics. 99(1), 125-38.

Q45: Unravelling the telomeres: HP1α recruitment by TERRA

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Heterochromatin formation at telomeres is imperative for stability of mammalian chromosomes. The abundance of Telomeric Repeat-containing RNA (TERRA) is inversely related to the extent of heterochromatin formation at telomeres [1]. Given the role of Heterochromatin Protein 1 alpha (HP1α) in heterochromatin formation, and also the presence of an RNA binding site [2], we propose that TERRA recruits HP1α to telomeric regions. Due to the guanine-rich nature of the TERRA molecule, G-quadruplex (G4) structures may be responsible for the telomeric RNA binding ability of HP1α.

To explore the binding of HP1α to TERRA, biolayer interferometry is being used. To map the RNA binding region previously described in the HP1α hinge region, a hinge domain mutant was produced for further investigation. A dissociation constant (KD) of 70 nM was observed for HP1α and TERRA, whereas a far greater KD was observed with TERRA and RNA-binding-mutant HP1α. To conclude that this interaction is due to the G4 structure of TERRA, further binding assays will be done with oligonucleotides incapable of forming G4's.

Future work includes Nuclear Magnetic Resonance (NMR) and Small-Angle X-ray Scattering (SAXS) to map the HP1α-TERRA interaction, and also RNA immunoprecipitation and FISH to confirm the interaction in mammalian cells. Finally, to characterise the functional significance of this interaction, telomere length and structure will be assessed by terminal restriction fragment southern blotting, and telomere FISH.

Elucidating the way in which heterochromatin is regulated at chromosome ends will provide insights into telomere degradation through the lifetime of an organism, and also shed light on the way in which telomeres are maintained in infinitely proliferating cancer cells.

1. Deng, Z., et al., *TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres.* Mol Cell, 2009. **35**(4): p. 403-13.
2. Muchardt, C., et al., *Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha.* EMBO Rep, 2002. **3**(10): p. 975-81.

Q46: Inhibition of growth hormone receptor signal transduction in a panel of cancer cell lines

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Growth hormone (GH) is essential for normal growth during childhood and puberty. However, elevated levels of GH have been observed in a variety of cancer types. Expression of GH in tumours is linked to a poorer survival outcome for breast and endometrial cancer patients. GH mediates actions through binding to a cell surface GH receptor (GHR), activating key signal transduction pathways including JAK/STAT, ERK and PI3-kinase/AKT. Furthermore, GH can activate the prolactin receptor (PRLR), and these two receptors can form receptor heteromultimers which may impact on the effectiveness of GHR antagonism. This study aimed to characterise a panel of cancer cell lines for components of GH signal transduction, and to determine responsiveness to GH and GHR antagonism.

Twelve breast, prostate, liver and colon cancer cell lines were characterised for GH-mediated signal transduction. STAT5 phosphorylation was assessed by western blot. Quantification of STAT5, ERK1/2 and AKT phosphorylation was carried out using AlphaScreen assays. A specific GHR antagonist, B2036 was used to determine which cells responded to GHR inhibition. The expression level of GH-related genes (*GH1, GH2, GHR*, *PRL* and *PRLR*) was also measured. A subset of cancer cell lines which respond strongly to GH treatment and to GHR antagonism was identified, as determined by STAT5 phosphorylation (e.g. prostate cancer cell line LNCaP). However, others exhibited a strong response to GH which was only partially inhibited by B2036 (e.g. cancer cell lines HepG2, 22Rv1 and ZR-75-1). This may be due to additional activation of the PRLR by GH in these cell lines. With the increasing interest in antagonising GHR signalling for therapeutic purposes, there will be a need for careful characterisation of the cancer cell lines used in preclinical studies. Future studies will determine the effectiveness of GHR antagonism in reducing tumour burden, as a monotherapy and in combination with radiation.

Q47: Anticancer Activity of New Zealand (NZ) Surf Clam Extracts

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Almost all the cancer chemotherapy drugs available today are known to possess serious side effects. Previous studies have revealed that some non-toxic bioactive peptides, particularly from ocean organisms, possess anticancer activities or can increase the efficacy of conventional chemotherapy drugs in a synergistic manner [1, 2]. Hence, natural anticancer compounds with little or no side effects have become a focus for the development of effective therapeutics for various human cancers. Our previous study shows that the extracts of three NZ surf clam species have high antioxidant activities [3]. The main objective of this study is to screen anticancer properties of those extracts from three NZ surf clam species in a range of cancer cells *in vitro*. MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was used to determine cancer cell viability after treatment with various doses of clam extracts and at different incubation times (24, 48 and 72 hours). Results show that when prostate cancer (PC-3), breast cancer (MCF-7), and pancreatic cancer (MIA PaCa-2) cell lines were treated with the petroleum ether extracted fraction of raw Diamond shell species clam at 1000µg/mL, high inhibition values of 43%, 20% and 18% were obtained after 48-hour incubation. We also compared extracts of oven dried and raw clams. Heat treatment on clams before extraction reduced extracts' ability to inhibit cancer cell growth by 12-24%. In conclusion, extracts from NZ surf clams possess antineoplastic effects, however, further studies is required to identify the active component(s) and elucidate the mechanism of action.

[1]. Je, J. Y., Park, P. J., Byun, H. K., Jung, W. K., & Kim, S. K. (2005). *Angiotensin I converting enzyme (ACE) inhibitory peptide derived from the sauce of fermented blue mussel*, *Mytilus edulis*. *Bioresource Technology*, 96, 1624–1629.
[2]. Wijesekara, I., Pangestuti, R., & Kim, S. K. (2011). *Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae*. *Carbohydrate Polymers*, 84, 14–21.
[3]. Odeleye, T., Li, Y., White, W. L., Nie, S., Chen, S., Wang, J., & Lu, J. (2016). *The antioxidant potential of the New Zealand surf clams*. *Food Chemistry*, 204, 141–149.

Q48: Urinary metabolome analysis of Crohn's disease (CD) patients and healthy controls (HC) undertaking short-term exclusive enteral nutrition (EEN).

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The treatment of inflammatory bowel diseases (IBD) relies on appropriate and timely use of therapies to reduce or eliminate inflammation and prevent complications. Unfortunately, the presence and severity of symptoms correlates poorly with intestinal inflammation making symptom palliation an unsuitable long-term goal. Currently methods for assessing inflammation are invasive, expensive and/or insufficiently sensitive/specific. The recognition of new biomarkers of disease activity may provide improved means of monitoring response to therapy.

EEN is a nutritional approach to CD therapy, comprising a formula administered as the sole nutritional intake. Preliminary investigations showed that two weeks of EEN treatment removed metabolic 'noise' in urine that confounds specific biomarker discovery.

The aim of this project is to detect substances in urine that differentiate between CD patients and controls at a stage when EEN has not yet resulted in improved disease status.

Urine samples were collected from three cohorts. CD patients on total EEN (n = 14), CD patients on partial EN diet (n = 9), and healthy controls on total EEN (n = 17). Each participant provided urine at baseline, after two weeks on EEN, and two weeks after returning to a normal diet.

All samples were normalised for creatinine content and were analysed using an untargeted hydrophilic metabolite detection method using HILIC-NH_2 columns on a nanoflow LC-MS system (LTQ-Orbitrap) in positive ion mode. Data processing and peak picking was performed using the xcms software package for R. Comparison of metabolite profiles between groups was performed using multivariate methods including partial least squares discriminant analysis and Random Forest classification in R.

This approach allows discrimination of CD and HC groups regardless of dietary phase and provides a start point for the discovery of candidate biomarkers for monitoring luminal inflammation. Further work will include untargeted metabolomic analysis using C18 columns and kit-based targeted metabolomics analysis.

Q49: The functional evaluation of intestinal microbiota by yuzu (*citrus junos*)

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Our research is focused on the species Citrus junos (yuzu) as a fruit for improving health[1]. We reported that yuzu in Japan produces large quantities of limonoids and polyamines (PAs). Recent studies have shown that bilateral signals between the brain and intestine are important for maintaining homeostasis in the living body and extending the life span[2]. Especially, the functions mediated by PAs may be involved in metabolism by indigenous intestinal bacteria and the health of the host[3]. We fed limonoid aglycones extracted from yuzu seeds, or commercial spermine as a polyamine to Sandhoff disease (SD) mice with abnormal glycolipid metabolism and autosomal recessive inheritance. Ingestion of limonoid aglycones or spermine by SD mice extended their longevity by about up to 12%, where it alleviated inflammation of the central nervous system. 16S rDNA analysis was performed using the T-RFLP analysis method and determine the bacterial flora in feces from SD mice and wild-type mice. The bacterial flora in the feces indicated changes in the relative ratio between Bacteroidetes (Bacteroidales and Lactobacillus) and Firmicutes (Clostridiales and Erysipelotrichaceae).

Thus in this reports, we determined the short-chain fatty acid composition and IgA productivity of feces from the same mice. In both SD and wild-type mice, the amount of short-chain fatty acids and IgA produced by the bacterial flora increased according to the intake of limonoid aglycones or spermine. Hence, we attempt that detailed bacterial species were identified by the next generation sequencer on 16S rDNA extracted from the feces. In particular, members of the genus Clostridium was shown very interesting behavior.

Members of the genus Clostridium elicit an immune response in the intestinal mucosa, which promotes the differentiation of regulatory T cells that contribute to suppression, and it is considered that changes in these intestinal bacteria are closely related to inflammatory bowel disease[4].

1. Minamisawa, M., Yoshida, S., & Uzawa, A. (2014). *The functional evaluation of waste yuzu (Citrus junos) seeds.* Food & function, 5, 330–336.
2. García-Villalba, R., Giménez-Bastida, J. A., García-Conesa, M. T., Tomás-Barberán, F. A., Carlos Espín, J., & Larrosa, M. (2012). *Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples*. Journal of separation science, 35, 1906–1913.
3. Kurihara, S., Suzuki, H., Oshida, M., & Benno, Y. (2011). *A novel putrescine importer required for type 1 pili-driven surface motility induced by extracellular putrescine in Escherichia coli K-12.* Journal of Biological Chemistry, 286, 10185–10192.
4. Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., & Kim, S. (2013). *Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota* Nature, 500, 232–236.

Q50: Differential growth of bowel commensal *Bacteroides* species on plant xylans of differing structural complexity

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The human diet is rich in complex plant polysaccharides, but only a small fraction can be digested by the host. Most of these dietary fibres reach the distal gut undegraded, where they act as food sources for bowel microorganisms (the gut microbiota). Imbalances in the composition of the gut microbiota (dysbioses) are associated with several health conditions, and a promising way to rectify these may be the use of prebiotics (indigestible food ingredients that selectively stimulate the growth of a limited number of commensal gut bacteria). There is a need to discover new indigestible polysaccharides that can be used as prebiotics, to widen the window of possibilities for prebiotic intervention in dysbiosis, as structurally different polysaccharides can influence different microbial groups. A highly branched xylan derived from New Zealand flax was used as a substrate to screen a collection of common bowel bacteria for growth. Mucilage exuded from the base of New Zealand flax leaves was traditionally used by Maori for the treatment of burns and wounds, but also for some intestinal disorders, such as diarrhoea. Two closely related *Bacteroides* species, *Bacteroides ovatus* ATCC 8483 and *Bacteroides xylanisolvens* DSM 18836, showed good growth on this substrate. Chromatographic and constituent sugar analyses showed that the two species utilized this xylan in different ways. The growth of *Bacteroides* species on xylans possessing different chemical structure and complexity was also examined, showing a differential utilization of structurally different xylans. Structurally diverse xylans can elicit different ecological outcomes with respect to the human gut *Bacteroides* population, representing potential new prebiotics to redress gut dysbiosis.

Q51: Profiling Complex Population Genomes with Highly Accurate Single Molecule Reads: Cow Rumen Microbiomes

Mio Tonouchi[1], Cheryl Heiner[1], Itai Sharon[2], Steve Oh[1], Alvaro G. Hernandez[3], Itzhak Mizrahi[4] and Richard Hall[1]

[1]PacBio, 1305 O'Brien Drive, Menlo Park, CA; [2]Tei-Hai College, Upper Galilee, and MIGAL Galilee Research Institute, Israel; [3]University of Illinois at Urbana-Champaign; [4]Ben-Gurion University of the Negev, Israel

Determining compositions and functional capabilities of complex populations is often challenging, especially for sequencing technologies with short reads that do not uniquely identify organisms or genes. Long-read sequencing improves the resolution of these mixed communities, but adoption for this application has been limited due to concerns about throughput, cost and accuracy.

The recently introduced PacBio Sequel System generates hundreds of thousands of long and highly accurate single-molecule reads per SMRT Cell.

We investigated how the Sequel System might increase understanding of metagenomic communities. In the past, focus was largely on taxonomic classification with 16S rRNA sequencing. Recent expansion to WGS enables functional profiling as well, with the ultimate goal of complete genome assemblies.

Here we compare the complex microbiomes in 5 cow rumen samples, for which Illumina WGS sequence data was also available. To maximize the PacBio single-molecule sequence accuracy, libraries of 2 to 3 kb were generated, allowing many polymerase passes per molecule. The resulting reads were filtered at predicted single-molecule accuracy levels up to 99.99%.

Community compositions of the 5 samples were compared with Illumina WGS assemblies from the same set of samples, indicating rare organisms were often missed with Illumina. Assembly from PacBio CCS reads yielded a contig >100 kb in length with 6-fold coverage. Mapping of Illumina reads to the 101 kb contig verified the PacBio assembly and contig sequence. Scaffolding with reads from a PacBio unsheared library produced a complete genome of 2.4 Mb.

These results illustrate ways in which long accurate reads benefit analysis of complex communities.

Q52: A journey of GMO regulation in New Zealand

Safavi M.

Environmental Protection Authority, New Zealand

Achieving a predator-free New Zealand by 2050 is likely to require genetic based approaches such as CRISPR/Cas9 based gene drives, the Trojan female technique, species-specific toxins, and non-genetic based approaches such as novel bio control agents. Many of these approaches are likely to be regulated by the Hazardous Substances and New Organisms Act 1996 (HSNO Act). 'New organisms' are those organisms that were not present in New Zealand immediately before 29 July 1998, including any genetically modified organisms (GMO). Under the HSNO Act, any applications to import, develop, field test, or release any new organisms are assessed by the Environmental Protection Authority (EPA). Each application is considered on a case-by-case basis, reflecting the associated potential positive or adverse effects of the new organism on the environment. In this talk, I will take you through the regulatory journey of some previously-approved GMOs, the matters that were taken into account for their risk assessment, and the requirements for field testing or releasing any new organisms. The necessity to obtain social licence for new technologies will also be discussed, as public notification is a statutory requirement for the applications that are likely to be of significant public interest.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
Q25	*withdrawn*		
Q26	Withdrawn		
Q27	MSMEG_5243: A succinate dehydrogenase flavination factor	Liam Harold[1,2], Hafna Ahmed[3], James Antoney[3], Kiel Hards[1], Chris Greening[4], Colin Jackson[3], Gregory Cook[1,2]	[1]University Of Otago, Dunedin, New Zealand, [2]University of Auckland, Auckland, New Zealand, [3] Australian National University, Canberra, Australia, [4]Monash University, Melbourne, Australia
Q28	Next Generation Inhibitors to Combat Drug Resistant Tuberculosis Infections	Zoe Williams[1], Gregory Cook[1]	[1]University Of Otago, Dunedin, New Zealand, [2]University of Auckland, Auckland, New Zealand
Q29	Screening NZ fungi for new antibiotics	Mulholland, D.[1], Uy, B.[1], Swift, S.[1], Copp, B.[2], Weir, B.[3], Wiles, S.[1]	[1]University of Auckland, Auckland, NZ. [2]School of Chemistry, University of Auckland, Auckland, NZ. [3]Landcare Research, Auckland, New Zealand
Q30	*withdrawn*		
Q31	A genetic analysis of Japanese PSA strain with New Zealand PSA outbreak strain	Joycelyn Ho[1], George Poulter, Margi Butler, Russell Poulter	[1]University Of Otago, Dunedin , New Zealand
Q32	Antibiotic Resistant Bacteria (ARB): Trends in conventional dairy farms compared with organic counterparts in New Zealand and China	Omega Amoafo[1], Stephen On[1], Ravi Gooneratne[1], Nikita Bary[1]	[1]Lincoln University, Lincoln, new Zealand
Q33	Protozoan Predation Can Influence Bacterial Lineages to Evolve Coccal Cell Shape	Danielle Kok, Heather Hendrickson	Massey University, Albany, New Zealand

Q34	Effectiveness of CRISPR-Cas systems in response to diverse phage infections	Lucia M Malone, Peter C Fineran	University Of Otago, Dunedin, New Zealand
Q35	Analysis of the type I-F CRISPR-Cas complexes from *Pectobacterium atrosepticum in vitro*	Howard Maxwell, Robert Fagerlund, Peter C Fineran	University Of Otago, Dunedin, New Zealand
Q36	Imprecision during spacer acquisition by type I CRISPR-Cas systems increases CRISPR diversity in bacterial populations	Nils Birkholz Simon A. Jackson, Corinda Taylor, Peter C Fineran	University Of Otago, Dunedin, New Zealand
Q37	Discovery of regulators of CRISPR-Cas adaptive immunity in *Serratia*	Mariya Yevstigneyeva, AG Patterson, Corinda Taylor, Peter C Fineran	University Of Otago, Dunedin, New Zealand
Q38	Loss and gain of CRISPR-Cas systems and genomic islands: a delicate balancing act?	Aroa Rey Campa[1], Andrew Pitman[2], Peter Fineran[1]	[1]University Of Otago, Dunedin, New Zealand, [2]The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand
Q39	Investigating the role of integration host factor in the function of multiple CRISPR-Cas systems	Timothy Ferguson, Robert Fagerlund, Peter Fineran	[1]University of Otago, Dunedin, New Zealand
Q40	DNA capture and integration by the type I-F CRISPR-Cas adaptation complex from *Pectobacterium atrosepticum*	Robert D. Fagerlund[1], Max E. Wilkinson[1], Oleg Klykov[2], Arjan Barendregt[2], F. Grant Pearce[3], Howard W.R. Maxwell[1], Sebastian N. Kieper[1], Angela Capolupo[2], Albert J.R. Heck[2], Kurt L. Krause[1],	[1]University of Otago, Dunedin, New Zealand, [2]Utrecht University, Utrecht, Netherlands, [3]BUniversity of Canterbury, Christchurch, New Zealand

		Mihnea Bostina[1], Richard A. Scheltema[2], Raymond H.J. Staals[1] and Peter C. Fineran[1]	
Q41	Developing SorTn-Seq: a state-of-the-art method to study CRISPR-Cas regulation	Leah Smith[1], Paul Gardner[2], Simon Jackson[1], James Ussher, Peter Fineran[1]	[1]University of Otago, Dunedin, New Zealand, [2]University of Canterbury, Christchurch, New Zealand
Q42	A novel Cas9 mutant confers reduced off-target gene editing while maintaining on-target potency	Simon Dunbar	Integrated DNA Technologies, Sydney, Australia
Q43	Genome-editing based molecular tools for super-resolution and light sheet microscopy	Bianca Nijmeijer[1], Judith Reichmann[1], Vilma Jimenez-Sabinina[1], Birgit Koch[1], Julius Hossain[1], Mark Bates[2], Stefan Hell[2], Jan Ellenberg[1]	[1]European Molecular Biology Laboratory, Heidelberg, Germany, [2]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
Q44	Discovering new disease causing genes using Chromium whole-genome sequencing	Karen Knapp, Louise Bicknell	Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand
Q45	Unravelling the telomeres: HP1α recruitment by TERRA	Ruby Roach, Sarah Bond, Elena Harjes, Vyacheslav Filichev, Tracy Hale	Massey University, Palmerston North, New Zealand
Q46	Inhibition of growth hormone receptor signal transduction in a panel of cancer cell lines	Yue Wang[1] Langley R[2], Lu M[1], Jamieson SM[2], Perry JK[1]	[1]Liggins Institute, University of Auckland, Auckland, New Zealand, [2]University of Auckland, Auckland, New Zealand

Q47	Anticancer Activity of New Zealand (NZ) Surf Clam Extracts	Tinu Odeleye, Lindsey White, Yan Li, Jun Lu	Auckland University Of Technology, Auckland, New Zealand
Q48	Urinary metabolome analysis of Crohn's disease (CD) patients and healthy controls (HC) undertaking short-term exclusive enteral nutrition (EEN)	Blair Lawley[1], Catherine Wall[2], Andrew Day[2,3,5], Richard Gearry[2,3,5], Wayne Young[4], Gerald Tannock[1]	[1]University Of Otago, Dunedin, New Zealand, [2]University of Otago, Christchurch, New Zealand, [3]Christchurch Hospital, Christchurch, New Zealand, [4]Agresearch, Palmerston North, New Zealand
Q49	The functional evaluation of intestinal microbiota by yuzu (citrus junos)	Mayumi Minamisawa[1], Akira Yamaguchi[2], Gota Kawai[1]	[1]Chiba Institute of Technology, Narashino, Shibazono, Japan, [2]Yokohama City University, Yokohama, Japan
Q50	Differential growth of bowel commensal Bacteroides species on plant xylans of differing structural complexity	Manuela Centanni[1], Jennifer Hutchison[2], Susan Carnachan[2], Alison Daines[2], William Kelly[3], Gerald Tannock[1,4], Ian Sims[2]	[1]University of Otago, Dunedin, New Zealand, [2]Victoria University of Wellington, Wellington, New Zealand, [3]Donvis Limited, Palmerston North, New Zealand, [4]Riddet Institute Centre of Research Excellence, Palmerston North, New Zealand
Q51	Profiling Complex Population Genomes with Highly Accurate Single Molecule Reads: Cow Rumen Microbiomes	Mio Tonouchi[1], Cheryl Heiner[1], Itai Sharon[2], Steve Oh[1], Alvaro G. Hernandez[3], Itzhak Mizrahi[4], Richard Hall[1]	[1]PacBio, USA, [2]Tei-Hai College, Upper Galilee, and MIGAL Galilee Research Institute, Israel, [3]University of Illinois at Urbana-Champaign, , USA, [4]Ben-Gurion University of the Negev, Israel
Q52	A journey of GMO regulation in New Zealand	Manda Safavi	Environmental Protection Authority, New Zealand