26th NSW Stem Cell Network Workshop

Stem Cells and Gene Editing

The Brain and Mind Centre
University of Sydney, Camperdown, NSW
Wednesday 29th March 2017

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WELCOME

Welcome to the 26th Workshop of the NSW Stem Cell Network

Gene editing technology is rapidly advancing the field of stem cell research. The discovery of CRISPR/Cas9 has given scientists the ability to target and edit specific genes in the genome, with potential to treat human genetic disease—highlighting the relevance of the 26th workshop of the NSW Stem Cell Network.

We will start by discussing the science of gene editing technology. This will begin with an overview of CRISPR/Cas9 followed by how this technology can be used to screen human pluripotent stem cells. The science of editing the epigenome and generating mutations in the genome using CRISPR also will be explored.

Next we will discuss Clinical Applications, what the current state of clinical trials are in Australia and how this compares with that of the rest of the world. We will also discuss the latest clinical research in Australia utilising the CRISPR/Cas9 technology including that to treat inherited degenerative ocular diseases.

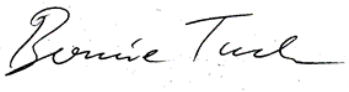
It is an important time for regulators to discuss the ethical, legal and social implications of this fast paced technology and the workshop will conclude with a session discussing the regulation and ethics of gene editing technology from leading Australian bioethicists.

By gathering key Australian experts, this event aims to provide opportunities for cross disciplinary collaborations and enquiries, leading to more efficient translation of stem cell research. Perhaps this Workshop will be the catalyst for a change in Australian laws to permit some gene editing of embryos for therapeutic purposes.

We hope that you take advantage of this unique occasion to discuss the main advances and issues in stem cell and gene editing research with proponents of the field.

This Workshop would not have been possible without the generous help from our sponsors, speakers and all of you present today. We are truly grateful for your support and contribution. We would specifically like to thank Associate Professor Greg Neely and Dr Marco Herold, who were of major assistance in planning the Program.

We hope you enjoy the Workshop and continue to support the NSW Stem Cell Network at future events!



Tamara Treleaven
NSW Stem Cell Network
Manager

Prof. Bernie Tuch
NSW Stem Cell Network
Director

PROGRAM

Practical Session	**Live Demonstration at Charles Perkins Centre** **Chair: A/Prof. Greg Neely (University of Sydney)**
9:00am	**A/Prof. Greg Neely (University of Sydney)** *Practical demonstration and overview of gene editing technology*
12:00pm	Lunch (The Brain and Mind Centre—Sydney University)
1:15pm	**Prof. Bernie Tuch (NSW Stem Cell Network)** *Welcome*
1:20pm	**Prof. Trevor Hambley (University of Sydney)** *Open*
Session 1	**The Science** **Chair: A/Prof Greg Neely (University of Sydney)**
1:30pm	**Dr. Marco Herold (Walter and Eliza Hall Institute of Medical Research)** *Overview of the CRISPR/Cas9 technology*
1:50pm	**Dr. Leslie Caron (University of Sydney)** *Genome-wide CRISPR knock-out screening in human Pluripotent Stem Cells*
2:10pm	**Prof. Ryan Lister (University of Western Australia)** *Editing the Epigenome*
2:30pm	**Dr. Gaeton Burgio (Australian National University)** *Generation of point mutations using CRISPR/Cas9 genome editing technology*
2:50pm	Afternoon Tea
Session 2	**Clinical Application** **Chair: Prof. Bernie Tuch (NSW Stem Cell Network)**
3:10pm	**Prof. John Rasko AO (The Royal Prince Alfred Hospital - University of Sydney)** *Contextualising clinical CRISPR-Cas9 - bold new paradigm or neat new trick?*
3:30pm	**Dr. Guei-Sheug (Rick) Liu (Menzies Institute for Medical Research, University of Tasmania)** *Steps Towards Ophthalmic Applications of CRISPR/Cas DNA Editing*
3:50pm	**A/Prof. Alice Pebay (University of Melbourne)** *Pluripotent stem cells to model human retinal diseases*
4:10pm	Coffee/Tea Break

PROGRAM

Session 3	Regulations and Ethics Chair: Dr. Bernadette Tobin (Plunkett Centre, St Vincents Hospital)
4:30pm	**Dr. Lynda Bolt (University of Melbourne)** *Gene Editing Technologies and Challenges for Institutional Biosafety Committees*
4:50pm	**Tess Whitton (University of Tasmania)** *Human Genome, Gene Editing and Future Directions*
5:10pm	**Prof. Rachel Ankeny (University of Adelaide)** *Fast, Accurate, Precise… So What's Wrong with Gene Editing?*
5:30pm	Networking/Close

Dr Marco Herold—Walter & Eliza Hall Institute of Medical Research, VIC

Dr Marco Herold is a Laboratory Head in the Molecular Genetics of Cancer Division at WEHI. He is an expert in developing novel mouse models of human cancer, and has brought the CRISPR/Cas9 genome editing technology to WEHI, where he has recently been established the new Genome Editing Laboratory; the task of this laboratory is to generate mutant mice using CRISPR/Cas9 methodology. Dr Herold's PhD at the University of Würzburg (Germany) addressed key questions in apoptosis and cancer. His major research interest is in the identification of novel genes in apoptosis, and in finding new targets for cancer therapy.

Overview of the CRISPR/Cas9 technology

CRISPR/Cas9 mediated genome engineering provides an easy and rapid way to edit genes in mammalian cells. I will talk about its origin and how it can be used as a tool for manipulating genes *in vitro* and *in vivo* in cells and the whole organism. Furthermore, I will present some of our own work on how we use CRISPR/Cas9 in haematopoietic cells as a whole genome knockout screening tool to identify and validate potential novel drug targets for cancer therapy.

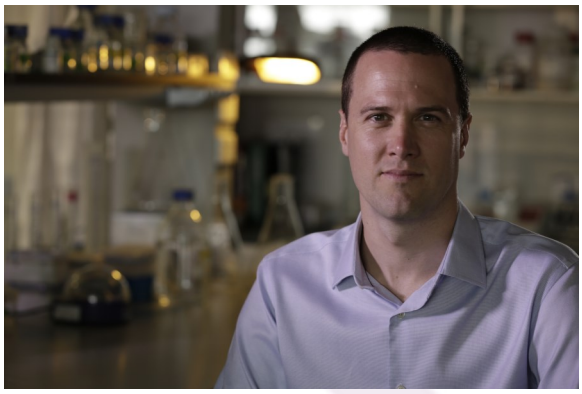
Dr Leslie Caron— Charles Perkins Centre, University of Sydney

Dr Leslie Caron received a Ph.D from the University of Nice-Sophia Antipolis in France where she worked on skeletal muscle differentiation of mouse embryonic stem cells (ESCs). Following her degree, she joined Pr Ken Chien's laboratory at Harvard Medical School in Boston, USA. Her postdoctoral studies focused on isolating cardiovascular progenitors from mouse and human ESCs and showed that a triple-marked Isl1+/Nkx2.5+/Flk1+ multipotent progenitor gives rise to the three major lineages of the heart: cardiac myocytes, smooth muscle, and endothelial cells. This study provided new insights into the lineage diversification during mammalian heart development and represents a new strategy for cardiac tissue regeneration. In 2011, Leslie Caron moved to Australia to join Genea, an IVF Clinic and Stem Cell Company based in Sydney, and was awarded a fellowship from FSHD Global Research Foundation to develop stem cell-based models for muscular dystrophies using affected human ESC lines. Lack of a suitable cellular model for muscle diseases was stunting research into muscle diseases, with very little known and no progress in therapeutics. The system she developed is now commercialized and being used by researchers in Europe and USA. In 2016, Leslie joined A/P Greg Neely's laboratory at the Charles Perkins Centre / The University of Sydney to set up a human pluripotent stem cells platform for the study of various human diseases.

Genome-wide CRISPR knock-out screening in human Pluripotent Stem Cells

Human pluripotent stem cells (hPSC) are potential models of human development and disease and an unlimited source for cell replacement therapies. The crucial challenge for these applications is to understand the molecular pathways that regulate differentiation into specific cell types. While hPSC have been extensively studied world-wide over the years, the tissue-specific differentiation processes are still poorly understood. The CRISPR/Cas9 gene editing technology is a major advance that offers the great opportunity to genetically disrupt genes in the human genome to elucidate their function in biological processes. We have extensive experience with lenti-CRISPR libraries that allow us to turn on or off each gene individually in human cells and hold great potential for finding therapeutic targets. To gain a more complete understanding of the regulatory network that can drive hPSC into different lineages, we propose to conduct a pooled genome-wide Knock-out CRISPR/Cas9 screening in hPSC differentiating into various cell types such as neurons or skeletal muscles. We expect that some of the genes, when knocked-out, will affect the differentiation capacity of hPSC into a particular cell type, some will modulate the functionality and activity of the differentiated cells and other will protect the differentiated cells from degeneration. Identifying a lineage's essential genes may hold the key to unlocking tissue-specific disease and regeneration, and can have a major impact in regenerative medicine.

Professor Ryan Lister— Harry Perkins Institute of Medical Research, UWA



Professor Ryan Lister leads a research group exploring the epigenome, at the University of Western Australia and the Harry Perkins Institute of Medical Research. After receiving his PhD from UWA in 2005, Ryan undertook postdoctoral studies at The Salk Institute for Biological Studies from 2006. There he developed new techniques to map the epigenome, the molecular code superimposed upon the genome that plays crucial roles in regulating the information contained in the underlying DNA sequence. His discoveries include generating the first accurate maps of the human epigenome, and characterizing the surprising complexity of the human brain epigenome. His research has yielded new insights into the composition and function of the epigenome in a variety of systems, including plants, the brain, stem cells, and developing vertebrate embryos. Having returned to UWA in 2012, Ryan's laboratory is focused upon understanding how the epigenome patterns are established and changed, how they affect the readout of underlying genetic information, their involvement in plant growth and human disease, their role in brain development and function, and developing molecular tools to precisely edit the epigenome.

Editing the Epigenome

DNA methylation is an essential layer of the epigenome, involved in diverse processes including regulation of gene expression, cellular differentiation, development, learning and memory. However, we still we do not clearly understand how DNA methylation states are controlled, and their role in genome regulation, cell function, development, and disease. A major obstacle in deciphering the mechanistic roles of epigenetic modifications is the inability to precisely control and change the modification state in the genome. We have developed highly effective CRISPR-Cas9 based tools that enable specific addition or removal of DNA methylation at desired locations in the genome in a controlled fashion. We have undertaken a range of analyses to assess the efficacy and specificity of these functional epigenetic tools, and have utilized them to explore the sensitivity of DNA binding proteins to DNA methylation state. Overall, the development of epigenome editing tools is critical in order to establish the causal relationships between epigenomic modifications and genome regulation.

Dr Gaétan Burgio— John Curtin School of Medical Research, ANU

Dr Gaétan Burgio MD, PhD was born in France and completed his education in Paris. He graduated from the Faculty of Medicine Xavier Bichat in Paris in 2002 with a Medical Degree. He worked as a consultant in an emergency department at Paris while he was undertaking his PhD studies at Pasteur Institute, Paris. He completed his PhD in 2008 from Pasteur Institute in France and migrated to Australia to join Professor Simon Foote as a postdoctoral researcher at the Menzies Research Institute (University of Tasmania) from 2008 to 2012, and the Australian School of Advanced Medicine at Macquarie University. In 2015 he was appointed as a Group Leader at The John Curtin School of Medical Research, and Head of the transgenesis core facility at the Australian National University. His laboratory focuses on the elucidation of the host response to infectious diseases (malaria, bacterial infection) to discover novel therapies using a genetics approach. His research is also focus on the development of CRISPR/Cas9 genome editing technology with a particular focus on a better understanding how to enhance homology direct repair (HDR). He is also provides a service to the Australian research community to generate CRISPR/Cas9 genome-edited mouse and cell lines. His research is supported by the National Health and Medical Research Council (NHMRC) and the National Collaborative Research Infrastructure Strategy (NCRIS) in Australia.

Generation of point mutations using CRISPR/Cas9 genome editing technology

Novel precision genetic technologies such as CRISPR/Cas9 genome editing technology offer novel avenues to a better understanding the mechanisms of diseases. Using CRISPR/Cas9 we are able to precisely modify the mouse or the human genome by creating knockout or a specific single nucleotide change to enable the study of the function of the gene of interest. The generation of these models lies on the ability of Cas9 to create a double strand break in the DNA and the repair to occur via the error prone Non-Homologous End Joining (NHEJ) or the precise Homology direct Repair (HDR) mechanisms. The factors contributing to NHEJ versus HDR repairs are still largely unknown. Our group aims to better understand how the choice of the repair is determined and how to direct the Double strand break DNA repair towards HDR. This presentation will give an overview on the factors that influence the likelihood of success to generate a point mutation in mouse or cell line using CRISPR/Cas9 genome editing.

Professor John Rasko—Centenary Institute, University of Sydney

Professor John Rasko is an Australian pioneer in the application of adult stem cells and genetic therapy. He directs the Department of Cell and Molecular Therapies at Royal Prince Alfred Hospital and heads the Gene and Stem Cell Therapy Program at the Centenary Institute, University of Sydney.

John Rasko is a clinical hematologist, pathologist and scientist with a productive track record in gene and stem cell therapy, experimental haematology and molecular biology. In over 150 publications he has contributed to the understanding of stem cells and haemopoiesis, gene transfer technologies, oncogenesis, human aminoacidurias and non-coding RNAs.

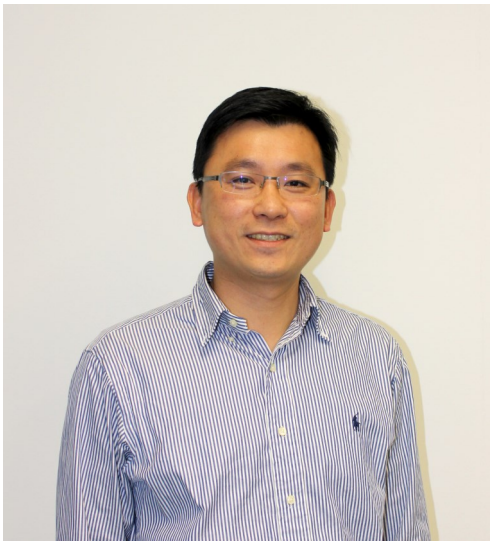
He serves on Hospital, state and national bodies including Chair of GTTAC, Office of the Gene Technology Regulator – responsible for regulating all genetically-modified organisms in Australia – and was Chair of the Advisory Committee on Biologicals, Therapeutic Goods Administration (2013-5). Contributions to scientific organisations include co-founder (2000) and past-President (2003-5) of the Australasian Gene and Cell Therapy Society; Regional Vice President of International Society for Cellular Therapy - Australia and New Zealand Region (2008-12); Scientific Advisory Committee and Board member for philanthropic foundations; and several Human Research Ethics Committees. He is a founding Fellow of the Australian Academy of Health and Medical Sciences. He is the recipient of national (RCPA, RACP, ASBMB) and international awards in recognition of his commitment to excellence in medical research, including appointment as an Officer of the Order of Australia in 2012.

Contextualising clinical CRISPR-Cas9 - bold new paradigm or neat new trick?

Since the first human clinical trial using gene technology in 1989, there have been over 2400 approved clinical trials worldwide. A few dozen clinical trails have been undertaken in Australia representing just over 1% of the trials worldwide. The overwhelming majority of human clinical trials involves short-term gene expression or random integration of a therapeutic gene.

More precise gene targeting tools were first described in the early 2000s. Targeted gene editing or replacement using Zinc Finger Nucleases or TALENS have been tested in about a dozen clinical trials since 2009. These include attempts to delete the CCR5 protein on T cells (completed 2015+) and therapeutic ZFN-mediated genome editing in mucopolysaccharidosis (recruiting 2016+) and the haemophilias (recruiting 2016+). The pace of clinical development has accelerated over nearly three decades of gene therapy. However within this context, its worth noting that the first ever (controversial) use of CRISPR to delete PD-1 in a late-stage lung cancer patient was administered in October 2016.

Dr Guei-Sheung (Rick) Liu—Menzies Institute for Medical Research, TAS



Dr Guei-Sheung Liu is a Senior Research Fellow and leads a gene-based therapy research group at Menzies Institute for Medical Research, University of Tasmania. He obtained his PhD at Kaohsiung Medical University (Taiwan) in 2006, and awarded a fellowship from "The Aim for the Top University Plan, Taiwan" for his postdoc training at National Cheng Kung University (2008-2009). Subsequently, he relocated to the O'Brien Institute (2009-2011) and then Centre for Eye Research Australia (Ophthalmology, University of Melbourne; 2012-2016) where he established gene therapy research. In 2017, Dr Liu was recruited to the Menzies Institute for Medical Research to lead the gene engineering and gene therapy research. His research has expanded from traditional "gene augmentation therapy" to emerging "gene editing" approaches. Recently, he helped lead the first application of viral-mediated CRISPR/Cas editing in the retina and a major focus of his ongoing work, will be to refine these applications thereby clearing the hurdles impeding clinical translation.

Steps Towards Ophthalmic Applications of CRISPR/Cas DNA Editing

Many blinding eye diseases manifest by the degeneration of retinal cells and collectively inherited retinal diseases are now the leading cause of blindness in working-aged adults. Despite the identification of specific mutations causing many inherited retinal dystrophies, none of these conditions are currently treatable. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated protein (Cas) has recently been adapted to enable efficient editing of the mammalian genome, opening novel avenues for therapeutic intervention of inherited diseases, especially in the eye. With a viral delivery system, we have successful transduced essential CRISPR/Cas components and modified the genome of retinal cells in adult mouse. Moreover, CRISPR/Cas genotoxicity is a significant consideration in clinical applications and, regardless of fidelity, it is foreseen that the overall chances of eventually cutting off-target sites, which result in cytotoxicity, would accumulate with time. To this end, we have rationally designed a self-destructing "kamikaze-CRISPR" system that enables destruction of CRISPR enzymes after initial activity. These works will lay the foundation for a new generation of gene therapy: "direct gene editing" for the inherited degenerative ocular diseases.

Associate Professor Alice Pebay—University of Melbourne

Associate Professor Alice Pebay holds a PhD in Neuroscience from the University of Paris (2001). She has more than 15 years of experience on disease modelling using human stem cells. Since 2012, she has been leading the Neuroregeneration Research Unit at the University of Melbourne and the Centre for Eye Research Australia. Alice was a NHMRC CDF and is currently supported by an ARC Future Fellowship.

Generating human induced pluripotent stem cells (iPSCs) directly from patients, differentiating them into specific cell types of interest are extremely valuable means by which to model and to investigate the pathogenesis of human diseases. Alice lead the first publication by an Australian team on the generation of iPSCs for disease modelling. Her group focuses on using patient cells to model monogenic diseases as well as complex genetic and environmental diseases of the eye. She aims to provide well-characterised *in vitro* human models to study human diseases, and identify phenotypes causative of diseases that will then be used for screening of new treatments to prevent or alter progression of disease.

Pluripotent stem cells to model human retinal diseases

Human induced pluripotent stem cells. combined with gene editing and automation are valuable tools for retinal disease modelling, as these cells are of patient origin and can be differentiated into cell types of interest. The use and molecular manipulations of iPSC-derived retinal pigment epithelium cells and retinal ganglion cells for modelling degenerative diseases of the retina and optic nerve will be discussed.

Dr Lynda Bolt—University of Melbourne

Dr Lynda Boldt is the Gene Technology and Biosafety Officer at The University of Melbourne. Lynda's role includes the administration and management of the University's gene technology responsibilities, coordinating the activities of the University's Institutional Biosafety Committee, and she is the primary contact for transactions with external agencies in this area. Lynda earned her PhD in Biochemistry and Molecular Biology at James Cook University, she has a background in marine science and education, and her particular areas of interest include aquatic photobiology and algae.

Gene Editing Technologies and Challenges for Institutional Biosafety Committees

New genome-editing tools such as CRISPR are inspiring researches within the university sector. Researchers are embracing new technologies with vigour and want to start working with them immediately. Identifying and advising on the management of the risks associated with new technologies can be challenging for Institutional Biosafety Committees (IBCs), and IBCs can have varying interpretations of where new technologies fit within the existing legislation and regulatory frameworks. The questions being asked are: how do we know what the risks are in relation to these advancing technologies and, can we be confident that the risks are able to be appropriately mitigated given these technologies are progressing with such speed? This presentation will explore some of the challenges faced by IBCs when assessing the risks associated with new gene-editing technologies within the current regulatory framework.

Tess Whitton—University of Tasmania

Tess Whitton is the Projects Officer at the Centre for Law and Genetics. She has an LLB (Hons) Tess Whitton LLB (Hons) is the Centre for Law and Genetic Projects Officer and the TasGRID Executive Officer (an initiative to set up a Tasmanian Biobank) at the Menzies Institute for Medical Research, both at the University of Tasmania. She is involved in a broad range of legal research across the health and genetics space. She has conducted research in the areas of biobanking, material transfer agreements, ownership in human tissue and genome editing as well as commercialization and patenting of biotechnology and genetic inventions.

Human Genome, Gene Editing and Future Directions

To use human embryos outside of the ordinary course of nature is exceptional. Although it raises profound ethical, legal and social questions, it is also full of potential. I will be speaking on the regulation of research involving Human Embryos for specific technologies like CRISPR and stem cells. I will also discuss key concerns relating to germline manipulation. At present, the Australian prohibitions are somewhat out of step with the laws in some other jurisdictions, particularly the UK and Sweden so I will address these issues and make suggestions to Australian Parliament to reconsider some of the prohibitions.

Professor Rachel Ankeny—University of Adelaide

Professor Rachel A. Ankeny is an interdisciplinary teacher and scholar whose areas of expertise cross three fields: history/philosophy of science, bioethics and science policy, and food studies. She is well-recognized as a scholar who can translate academic findings in ways that are relevant for students and the broader community. In the history and philosophy of science, her research focuses on model organisms, the philosophy of medicine, and the history of contemporary life sciences including genomics. Her research in bioethics examines ethical and policy issues in genetics, and embryo and stem cell research, among other topics. She also has expertise and ongoing research on health and science policy, particularly regarding public engagement. Professor Ankeny holds grants from the Australian Research Council in association with several of her research projects including on the history of genetic modification science, activism, and public engagement, and on animal welfare in the red meat industry. She was a member of the Expert Working Group on gene drives for the Australian Academy of Science and of the Gene Technology Ethics and Community Consultative Committee for the Office of the Gene Technology Regulator of the Commonwealth of Australia.

Fast, Accurate, Precise… So What's Wrong with Gene Editing?

Popular media and scientific fora are abuzz with news of novel techniques for genomic editing for various purposes including altering genes to prevent human disease particularly for single gene disorders, modifications of non-human organisms to develop better animal models for some diseases, and more generally for developing deeper understanding of the structure, function, and regulation of genes. Despite numerous calls for engaging the public before these techniques become even more well-established, such efforts have been limited to date. This paper explores a range of ethical issues associated with genomic editing techniques in concert with an analysis of recent media coverage on these technologies, in order to underscore the need to focus on the potential value conflicts underlying use of these technologies and to anticipate public reaction in a thoughtful and productive manner, unlike what arguably occurred in the case of debates over genetic modification and other emerging technologies.

Posters

1. Mechanism of UBE3A silencing by an antisense transcript

Ingrid Macindoe, Henriette O'Geen, Victoria Le, Ashley Lee, Dave Segal, Joel Mackay
University of Sydney, University of California, Davis, Foundation for Angelman Syndrome Therapeutics Australia

2. **Human Model to Identify Genetic Drivers for Leukaemia in Down's Syndrome Children**

Melinda Tursky[1], Crisbel Artuz[1], Tim Molloy[1,2], To Ha Loi[1], Dmitry Ovchinnikov[3], Helen Tao[1,2], Ernst Wolvetang[3], David Ma[1,2]

AFFILIATIONS:
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[2]St Vincent's Clinical School, Faculty of Medicine, UNSW Australia.
[3]Stem Cell Engineering Group, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD Australia.

Notes

Be a Member of the NSW Stem Cell Network

The NSW Stem Cell Network is a professional community with an interest in all forms of stem cells.

Our all inclusive, free membership makes this network unique in consisting of not only researchers and practitioners, but members of the public, industry and government bodies. Our aim is to ensure effective communications between diverse sectors for the advancement of stem cell research. As a member you will receive invitations to upcoming network and external stem cell related events, as well as the latest stem cell news. Sign up at:

www.stemcellnetwork.org.au

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NSW Stem Cell Network

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