South Australian Health and Medical Research Institute Submission

2016 National Research Infrastructure Roadmap Capability Issues Paper

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Question 1: *Are there other capability areas that should be considered?*

Yes, we think that a proposal which combines elements of Issues Paper item 5.1.2 Large Bio Molecules, 5.1.3 Translation – opportunities for biopharmaceutical and other novel therapies and 5.2.1 Biologic Capability is required to fill a major gap in Australia's research capability.

The ability to genetically modify the mouse genome has revolutionised biomedical research resulting in numerous scientific breakthroughs and commercial developments. The advent of CRISPR-Cas9 technology now means that these similar opportunities exist for the livestock species which are widely used in biomedical research, including gene discovery, the development of large animal disease models, commercial applications including xenotransplantation, antibody production and drug development.

Australian researchers have already embraced the use of this technology with many groups producing genetically modified mice for their own research purposes. To improve access to this technology the University of Adelaide together with its partner organisations have established the South Australian Genome Editing facility which provides genetically modified mice to researchers throughout Australia and have produced over 30 different mouse models for various applications already.

http://www.adelaide.edu.au/robinson-research-institute/services/facilities/sage/

Researchers at the University of Adelaide have now extended the use of this technology to pigs as well as sheep on an individual basis. However, the unprecedented scientific and commercial potential this technology offers means that Australia needs to develop a large animal genome editing facility to provide access to this technology to all researchers in order for Australian biomedical and agricultural research to remain at the forefront. The establishment of a National Large Animal Bioresource Centre addresses this challenge and can build on a proven track record in providing a commercial service.

As this submission will demonstrate large animal models of human disease have great potential to contribute to the major fields of medical research (eg. cardiology, oncology, neurodegenerative disease) as well as agricultural research. Pigs (and sheep) are widely used in biomedical research because of similarities in organ size, anatomy, physiology, metabolism and genetics with humans. Pigs are also excellent models of human disease including cardiovascular and neurodegenerative diseases. The ability to genetically modify these animals provides a plethora of new and exciting possibilities explore the mechanistic basis of disease (such as Alzheimer's disease), develop new therapies (humanised polyclonal antibodies) and engineer organs suitable for transplantation to name a few. Additionally, a colony of germ free pigs, that would maintained as a basis for some of the genetic modifications envisioned, would in itself be a valuable model to

study the microbiome. This emerging field of research has provided valuable insight into the role gut bacteria have on physical and mental health, but there is still much to be learned.

Of critical note, it is impossible to import these models from overseas due to Australia's strict quarantine laws which prohibit even the import of embryos, semen or cells that could be used for animal cloning. Therefore, the only way Australian researchers could remain competitive and on the cutting edge of science is to produce these animals here in Australia. A large capital investment is required to set up even a basic facility to produce and maintain germ free and/or genetically modified large animals. Hence, to make this accessible to all Australian researchers, a dedicated Centre is essential to capitalise on the substantial investment in infrastructure. Such a Centre would be an invaluable asset to medical researchers from all the major fields of health as well as agricultural. Furthermore, the Centre would undoubtedly attract and generate industry funding on top of academic pursuits thus leading to a self-sustaining financial model.

Question 2: *Are these governance characteristics appropriate and are there other factors that should be considered for optimal governance for national research infrastructure.*

The proposed National Large Animal Bioresource Centre would be administered by a Board similar to the governance of the existing CRC system. It would have representatives from the founding institutions and an International Scientific Advisory Committee to ensure that the Centre remains at the leading edge of developments. Engagement with related NCRIS facilities will be an important element of the model to ensure a coherent direction to efficiently utilise NCRIS funding. To facilitate cooperation the board may include positions for representatives of relevant facilities.

Question 3: *Should national research infrastructure investment assist with access to international facilities?*

When the Centre is established, it will foster collaboration with a vast range of International funding agencies as well as with individual researchers overseas. This collaboration will stem from the fact that the Centre will produce modified livestock species for research purposes and export them anywhere in the world. This will enable Australian researchers and their collaborators to access international facilities to perform experiments and create new commercial opportunities which are not currently available.

Question 4: *What are the conditions or scenarios where access to international facilities should be prioritised over developing national facilities?*

Australia cannot rely upon international facilities because of the necessary quarantine restrictions to the import of animals or germ lines from overseas. In contrast, owing to the high health status of our animals we have a unique capability to generate clean' animals here and export them overseas with minimal red tape. Importantly this affords Australia an exceptional competitive advantage as a site for Industry development based on biologics derived from large animals.

For Australian research and commercial developments to become world leaders in large animal models of human disease, which have greater relevance than rodent models, we must develop the capacity here in Australia. The time is now to capitalise on the unique opportunity presented by progressive genetic technologies and be a world leading Centre for producing genetically modified large animals. It is anticipated that this would generate substantial IP.

Question 5: *Should research workforce skills be considered a research infrastructure issue?*

Yes, it is essential that we have the necessary skills and expertise in Australia to develop the animal models. This expertise is in part already available in Australia, however we need to ensure that the skills are not lost to Australian researchers. In addition the Centre would recruit skilled personnel from overseas facilities that have a track record of large animal biomedical models and gene editing. Both aspects will be supported by creating a world leading gene editing facility.

A workforce with a range of complementary skills will be required. The vision of the Centre would be driven by a Director who would draw on expertise from interested parties through consultation. The Centre would also have a Lead Scientist to ensure that the level of science and methods employed are at the cutting edge.

Support staff in a number of areas would be required to maintain the Centre, generate the animals and provide research support. Separate animal houses would be required for each species (sheep and pigs) to cater for their specific needs. Furthermore, within these animal houses there would be the need for various levels of biocontainment ensuring a closed colony of 'clean' defined germ free animals as the basis for breeding and genetic modification, each staffed by Animal Technicians

Laboratories and staff would be required for rederivation, cryopreservation (of gametes, embryos and somatic cells) and *ex vivo/in vitro* aspects of the genetic modification process. Liquid Nitrogen storage facilities would also be required for the storage of material generated through these processes.

A Research Department that would endeavour to improve the methods used by the Centre. For example, the processes of cryopreservation, detection and elimination of microbial pathogens and work towards the improvement of methods for the development genetically modified animals. The Centre would also include a department responsible for Distribution of animals to Institutes around Australia and Internationally. This department would also import reagents that may be required for experimental processes. It will be critical for staff to have an in-depth and current knowledge of regulatory requirements for import/export and shipping.

In summary, approximate staff required would include the Director, Manager and 2-3 Lead Scientists, Manager and 5 staff (sheep Animal Houses and paddocks), Manager and 5 staff (pig Animal Houses), Manager and 2 staff (Health Monitoring), Manager and 2 PhD level scientists, 2 RA level (Research Department), 3 Administrative staff (general Administration including importation/distribution).

Question 6: *How can national research infrastructure assist in training and skills development?*

There is a strong workforce in Australia on which to capitalise. Australia is at the forefront of reproductive biology and translation of this into high impact research and commercial out is dependent on availability of suitable research infrastructure. The National Large Animal Bioresource Centre will provide considerable training opportunities across a range of areas including animal and veterinary sciences, commercialisation and regulatory affairs.

A collaborative effort toward training will be required to ensure a comprehensive approach across animal care, pathogen management, research integrity and the application of globally competitive scientific methods. The Centre would propose to work with the Australian Phenomics Facility to draw on their experience in small animals and apply the relevant approaches to a large animal setting. This will be integrated with the gene editing and reproductive technology expertise in large animals that exists in Australia. The approach will be to build on this existing large animal expertise by integrating the knowledge and insights gained from small animals, draw on the

expertise of similar international facilities and ensure staff are constantly engaged with current training and conference attendance. The Centre would also aim to link in with existing ARC training Centres, such as the ARC Training Centre for Biopharmaceutical Innovation to maximise on the potential for cutting edge research, industry engagement and innovation.

Question 7: *What responsibility should research institutions have in supporting the development of infrastructure ready researchers and technical specialists?*

Our universities and Medical Research Institutes (MRI) have a responsibility to nurture a culture of research excellence as well as foster the driving of engagement with commercial industries. Our research training environment must include the sentiment that commercialising scientific output is as important as the scientific discovery which created the opportunity. The mutual dependency needs to be celebrated and honoured with equal enthusiasm.

Question 8: *What principles should be applied for access to national research infrastructure, and are there situations when these should not apply?*

The Centre will be the facilitator of a huge range of scientific endeavours with the potential to aid advances in the major areas of health (such as cardiology, transplantation, oncology, neurological disease) and agricultural research. This has the capacity to service the Medical Research sector, from fundamental academic research to final stage preclinical testing for Pharmaceutical companies. Therefore the principles must be defined to be adaptable to the needs of this range of clients, with an appropriate management structure to optimise, and extend the use of resources. For example members of the Centre will have the opportunity to collaborate with those who use its services and facilities, or the Centre can produce animals on a fee-for-service basis and for those animals to be sent to the institution which paid for them.

The Access Policy will be similar to that adopted by many NCRIS capabilities. That being a cost recovery mechanism which has a schedule of fees which represents a subsidised service to Australian researchers and Australian based companies, and a full economic cost (FEC) recovery rate and margin for overseas entities.

Question 9: *What should the criteria and funding arrangements for defunding or decommissioning look like?*

Capabilities should be able to demonstrate KPIs such as:

- A commitment to research and service excellence
- The expertise and drive necessary to stay on the forefront of the relevant research area
- True engagement with industry
- Fair and equitable access policies

If capabilities are not performing to these KPIs, then strong evidence that a move in that direction would be required for funding to be continued.

Question 10: *What financing models should the Government consider to support investment in national research infrastructure?*

Upfront payment for capital works, running costs and core staff costs of the facilities for at least 10 years is necessary. The funding model to sustain the operation will include revenues to offset variable costs, and a governance structure which will manage the uptake of the Centre's output.

Question 11: *When should capabilities be expected to address standard and accreditation requirements?*

The Centre could be set up and run according to ISO 9001 (2015) standards, its preclinical studies could be according to OECD GLP standards, and the laboratory work and postgraduate student work will be to NATA R&D accreditation status.

The proponents of the Centre know the value of accreditation status and the maintenance of Quality Systems in order to engage with Industry to attract NIH funding (for example) and to produce results which will be accepted automatically by regulating bodies. Time will be necessary to establish protocols and for bedding in. Accreditations may be expected after 1 year of operation.

Question 12: *Are there international or global models that represent best practice for national research infrastructure that could be considered?*

The US National Pig Centre is a model we could learn from and build upon. The Australian Centre would focus upon models for Biomedical Research, and to resolve basic biological questions in order to dovetail existing NCRIS nodes and local expertise. This will capitalise upon the gene editing and reproductive technology expertise in Australia. There are key drivers within the proponents to ensure success, and there are substantial links to the end-users of the novel biomedical models in livestock species to ensure that the output is user-driven.

Question 13: *In considering whole of life investment including decommissioning or defunding for national research infrastructure are there examples domestic or international that should be examined?*

Elements of the Centre could be taken over by spin off companies. However, in order to support biopharmaceutical innovation well into the future, some of the infrastructure would need to remain supported in the public domain. The partner Universities could take over responsibility for further elements of the Centre, but at its core will be the need for continued National investment.

Question 14: *Are there alternative financing options, including international models that the Government could consider to support investment in national research infrastructure?*

Local funding raised by participant organisations could be used to establish a smaller infrastructure, however, to be internationally competitive significant “up-front” funding is required to ensure coherent building and equipment needs are addressed, which will require coordinated national level financial commitment.

Health and Medical Sciences

Question 15: *Are the identified emerging directions and research infrastructure capabilities for Health and Medical Sciences right? Are there any missing or additional needed?*

The overall thrust for new investment to solve existing deficiencies as identified in the Issues Paper are correct. What the Centre will do is provide the mechanism by which the identified gaps can be filled, and the Centre will be based upon what is needed to provide new opportunities for Australian Science and for Commercial exploitation of that research output.

For example, the Centre will:

- Produce gene-edited sheep to develop a library of models of neurodegenerative diseases such as in Alzheimer’s disease or Huntington disease. These models will enable fundamental insights into the mechanism by which known mutations affect brain function, and will also be used to screen therapeutics and validate biomarkers of disease.

- Produce gene-edited pigs under SPF or germ free conditions to enable the production of xeno organs and tissues for human transplantation.
- Edit the genes of sheep to enable human antibody to be produced so that the demonstrated benefits of therapeutic polyclonal antibody can be far more widely exploited.
- Produce germ free piglets for vaccine development, and for human microbiome researchers who need to go to a larger animal than the mouse before clinical studies are conducted.

Question 16: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 17: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Health and Medical Sciences capability area?*

Environment and Natural Resource Management

Question 18: *Are the identified emerging directions and research infrastructure capabilities for Environment and Natural Resource Management right? Are there any missing or additional needed?*

Question 19: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 20: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Environment and Natural Resource Management capability area?*

Advanced Physics, Chemistry, Mathematics and Materials

Question 21: *Are the identified emerging directions and research infrastructure capabilities for Advanced Physics, Chemistry, Mathematics and Materials right? Are there any missing or additional needed?*

Question 22: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 23: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Advanced Physics, Chemistry, Mathematics and Materials capability area?*

Understanding Cultures and Communities

Question 24: *Are the identified emerging directions and research infrastructure capabilities for Understanding Cultures and Communities right? Are there any missing or additional needed?*

The public debate on gene editing needs to be informed to identify the opportunities from advances in the technology leading to increasing precision and hence more predictable and controlled outcomes, which distinguishes editing from traditional genetic modification.

Question 25: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 26: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Understanding Cultures and Communities capability area?*

National Security

Question 27: *Are the identified emerging directions and research infrastructure capabilities for National Security right? Are there any missing or additional needed?*

Therapies which can be used against bioterrorism threats are of great interest to governments via their role in Counter Measures preparedness. As they are to military planners as they plan to manage the potential infection of personnel by endemic or bioterrorism threats.

The Counter Measures officials in Canberra are highly motivated to see Australia contribute to this field in new and highly advantageous ways. Currently the NH&MRC has invested in projects to enhance the accuracy of epidemiological modelling of disease spread. One key successful outcome from the Centre will provide solutions to the problem not just defining the problem.

Question 28: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 29: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the National Security capability area?*

Underpinning Research Infrastructure

Question 30: *Are the identified emerging directions and research infrastructure capabilities for Underpinning Research Infrastructure right? Are there any missing or additional needed?*

Question 31: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 32: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Underpinning Research Infrastructure capability area?*

Data for Research and Discoverability

Question 33: *Are the identified emerging directions and research infrastructure capabilities for Data for Research and Discoverability right? Are there any missing or additional needed?*

Question 34: *Are there any international research infrastructure collaborations or emerging projects that Australia should engage in over the next ten years and beyond?*

Question 35: *Is there anything else that needs to be included or considered in the 2016 Roadmap for the Data for Research and Discoverability capability area?*

Other comments

Executive summary

The National Large Animal Bioresource Centre will:

- Enable Australian scientists to be globally competitive across the fields of medical and agricultural research through access to cutting-edge gene editing technology which will enable creation of modified sheep and pigs for a large range of scientific endeavours
- Consolidate the impressive gene editing capability in pigs, coordinated by proponents of the Centre, and extend the technology to other species to create new models, e.g. cancer, heart disease, neurodegenerative disease, and to perform basic research e.g. in the microbiome
- Create a Discovery Platform where genes of interest for livestock can be studied to inform traditional breeding systems
- Create an environment where commercialisation opportunities are identified and supported to generate wealth, industry and improve health in the human population
- Create a springboard from which basic science can lead to therapeutic opportunities, and to allow Australia to be competitive in the next generation of value-added biological industries, for example humanised polyclonal immunotherapy
- Transform the way in which cellular, genetic, and microbiological systems can be studied
- Strengthen translational potential by enabling Australian researchers to take the next step in oncology, cardiology, neurodegenerative diseases, xenotransplantation, genetic disorders, microbiome and animal science research by using tailored large animals which bridge the gap between mouse and man
- Create scientific and business opportunities for IP generated in Australia
- Create the environment where by staff and students within the Centre, and within groups who collaborate with or use the services of the Centre, have a clear focus on what commercial opportunities may arise from their basic science research
- Create a culture which leads to the combination of excellence in research output, collaboration and facilitation of service provision on a national and international scale
- Engage with Industry to provide new preclinical services not currently undertaken in Australia, and which take full advantage of the large investment already made in the NCSRIS/NIF-funded Large Animal Research and Imaging Facility (LARIF) at SAHMRI
- Enable Australian researchers to collaborate with overseas colleagues on the basis that they have an enabling technology which is not available elsewhere

National Large Animal Bioresource Centre (NLABC)

Australian Science needs to maintain or improve its scientific world ranking, in order to improve commercialisation opportunities, competitiveness and wealth outcome. An investment in the NLABC will enable all three priorities to be achieved. The Centre will generate new large animal models of human disease using the latest technology, and will be at the forefront of new editing and reproductive biology technologies. The Centre will have embedded within its culture a determination to create commercial outcomes and jobs from the scientific expertise and IP which will be developed to ensure that Australia has a scientific and commercial lead in the field.

Experience with large animal models of human disease

Australia has a long history of using sheep and pigs for experimental physiology research and in the past was well serviced with large animal research facilities. As research questions moved from the understanding of physiological principles to the desire to understand mechanisms of disease states at the cellular and more recently genetic level, the use of large animals diminished for logistic and practical reasons. Mouse-based research began to predominate especially when transgenesis and later knock-out methods enabled the genetic basis of disease mechanisms to be explored. However, mouse models have limitations in that they do not accurately mimic human physiology. Therefore information gained from rodent models needs to be carefully validated before being transferred to humans.

This NLABC proposal builds upon the wealth of research background knowledge and impressive investment in mouse facilities, and is cooperatively consistent with the continued need to invest in infrastructure to support the mandate of the NCRIS-funded Australian Phenomics Network. There is acknowledgement amongst those involved in genetically modified mouse usage that several fields of research have reached a road block where some questions can only now be asked and answered by using appropriate large animal models.

The material and staff infrastructure required to create large animal gene edited models for biomedical research is not in place in Australia with sufficient critical mass to reduce costs and optimise success rates. Importantly however, the proponents have the expertise to transfer the technology from mice to large animals. It is seen as a great strength that there is cooperation, and common interests certainly exist, between the APN and the NLABC. A differentiation exists however in that the commercialisation potential which stems from the NLABC requires a focus which is separate but complementary.

Gene editing technology

Currently, CRISPR-Cas9 genome editing technology is transforming biomedical research by enabling rapid, cost-effective generation of genetically modified animal models of human disease. Indeed, recent studies indicate that the genome of virtually any species can be modified and the technology is advancing rapidly to improve accuracy, complexity of edits and success rates. Importantly, optimized strategies for the generation of small animal disease models (eg. mice) can be adapted to larger animals, enabling generation of disease models that better approximate human physiology and pathology. Thus, it is now possible to generate sophisticated sheep models of genetic disorders in a matter of months through direct modification of somatic cells or the zygotic genome.

Gene editing in health and medical research

Pigs and sheep are widely used in biomedical research because of similarities in organ size, anatomy, physiology, metabolism and genetics with humans and hence are excellent models of human disease including cardiovascular and neurodegenerative diseases. Furthermore pigs are considered ideal organ and tissue donors for xenotransplantation. The ability to modify livestock genomes using gene editing technology will revolutionise the production of biomedical large animal models.

In the United States the National Institutes of Health (NIH) now provides this service to American Researchers through funding of the National Swine Research and Resource Centre (NSRRC) (http://nsrrc.missouri.edu/). The NSRRC was established in 2003 to develop the infrastructure to ensure that biomedical investigators across a variety of disciplines have access to critically

needed swine models of human health and disease. The NSRRC also serves as a central resource for reagents, creation of new genetically edited swine and information and training related to use of swine models in biomedical research.

While Australia continues to produce breakthroughs in this area no such facility exists here. However such a facility is needed if Australian researchers are to remain competitive. This is exacerbated by the fact that it is impossible to import these models from overseas due to Australia's strict quarantine laws which prohibit even the import of modified pig cells which could be turned into pigs here using animal cloning. Paradoxically Australia's high herd health status means that these biomedical models can be sent to most countries either as cells, semen or embryo or live animals.

The current proposal aims to address this roadblock by developing such a facility based on the existing US model. This includes having an ongoing capability to further develop existing technology as well as develop new ones. This Centre will provide a range of services including the provision of inbred pig cell lines, animal tissue, and facilities to conduct trials in large animals.

By way of example researchers at the University of Adelaide have isolated porcine embryonic stem cells which will allow embryonic stem cell based therapies to be developed as well as trialled in disease specific (genetically modified) large animal models for the first time anywhere in the world. This need has been highlighted by the NIH to progress stem cell research.

The Centre would include barrier technology to establish specific pathogen free (SPF) pigs for use in clinical trials of xenotransplantation for example.

As well as specific applications the ability to gene edit the livestock genome will provide researchers with a powerful research tool which until now has only been available in mice using gene targeting.

Xenotransplantation in health and medical research

There is a worldwide shortage of donor organs and tissue for transplantation. For example the annual cost of Type 1 diabetes to the US healthcare system alone has been estimated by the Juvenile Diabetes Research Foundation to be approximately $20 billion. For more than two decades researchers at the University of Adelaide, St Vincent's Hospital Melbourne, the Westmead Millennium Institute, the Walter and Eliza Hall Institute and the Harvard Medical School have collaborated on examining whether genetically modified pigs can be used as kidney and islet donors for humans. This Xenotransplantation Group is recognised as one if not the leading one of its type in the world. This is highlighted by the fact that as a direct consequence of their research the National Health and Medical Research Council lifted its moratorium on clinical trials on xenotransplantation several years ago.

Integral to the Group's success has been an ongoing program to develop the necessary technologies to modify the porcine genome to overcome rejection following transplantation. This includes the development of gene targeting and somatic cell nuclear transfer or animal cloning to produce Gal knockout pigs. This gene controls the immediate or hyperacute rejection of organs and tissues. By knocking this gene out in the pig, kidneys transplanted into non-human primates are not rejected immediately and survive for up to 4 days in the absence of immunosuppression. With immunosuppression the Group have achieved long term survival (up to 12 months) with islet transplants in non-human primates (Hawthorne WJ, et al, Am J Transplant 14:1300-9, 2014).

Gal knockout pigs have been bred with other animals to produce pigs containing multiple modifications designed to improve survival and afford local immunosuppression. These pig models are being sent to an increasing number of overseas collaborators in Europe, America and Asia. However the generation of these models by crossbreeding animals containing different modifications takes considerable time and resources. The advent of CRISPR-Cas9 technology now means that the necessary multiple modifications required to advance xenotransplantation to the clinic can be produced in the one generation. In a world first this Group has produced pigs where both copies of the Gal gene have been knocked out and a transgene which affords local immunosuppression been inserted at the Gal locus at the same time to demonstrate the power of this technology. As a direct result of this breakthrough the Group's work has now reached the stage where they need to establish specific pathogen free pig facilities for preclinical as well as clinical trials, the NLABC would enable this critical next step.

Sheep as a superior model of late onset neurodegenerative disease

One area of biomedical research that is particularly well positioned to benefit from advances in gene editing technology are those investigating neurodegenerative diseases such as Alzheimer's disease. Alzheimer's disease is currently the largest unmet need in neurology (Citron M, Nat Rev Drug Discov. 2010 9(5):387-98). This neurodegenerative disease is the most important cause of dementia, which affects over 350,000 people in Australia (Australian Institute of Health and Welfare, Dementia in Aust, 2012). These figures will more than double over the next few decades as our population ages, particularly if research provides no new meaningful treatments. Current research into late onset neurodegenerative disease can be modelled *in vitro*, and in smaller organisms such as the mouse, however these systems have limitations. *In vitro* cultures lack the complexity of multiple interacting cellular populations, and whilst mouse models of late onset neurodegenerative disease have provided a valuable experimental system, they still do not recapitulate key features of human disease.

Recent attempts to produce mouse models of Alzheimer's disease have resulted in knock-in mouse lines with high construct validity. Mice with humanised endogenous amyloid precursor protein genetic sequence and dominantly inherited Alzheimer's disease mutations (knock-in mice) produce amyloid plaques and show memory deficit (Saito T et al., Nat Neurosci. 2014 17(5):661-3) but still do not show other neuropathological hallmarks such as tauopathy. Although these animal models are still valuable for research, it shows we need to look to other animal models to develop experimental platforms that recapitulate a more complete pathogenesis.

Sheep are likely to provide a more promising animal model for several reasons. Sheep brains are anatomically much more similar to human brains than what rodent brains are. For example, sheep brains have a convoluted cerebral cortex whereas the mouse is lissencephalic. Changes in brain volume also impact neuronal physiology. Due to dramatically increased brain volume, human neurons suffer higher physiological load than rodent neurons (Hunn et al., Trends Neurosci. 2015 38(3):178-88) that will undoubtedly lead to higher disease susceptibility (Braak H & Del Trecidi K. Adv Anat Embryol Cell Biol. 2015;215:1-162). The sheep brain represents a more acceptable intermediate.

Time is also another factor that renders mouse models less suited to research of late onset neurodegenerative disease. The mouse's lifespan of approximately two years will poorly model a disease that takes decades to develop even before dementia presents itself. At ages up to 15 years, sheep are again a more acceptable intermediate between the mouse and human and will therefore be expected to model neuropathological features more faithfully. Of note, tau neurofibrillary tangles were detected within degenerating neurons of the aged sheep cerebral

cortex using antibodies such as Alz-50 and PHF-1, indicating this model is physiologically well suited to modelling Alzheimer's disease (Nelson PT et al, Neurosci Lett. 1994 28:170(1):187-90). These factors, in combination with the efficiency of CRISPR-Cas9 genome engineering and knock-in strategies that have been validated in the mouse, will produce neurodegenerative disease models in the sheep that will yield unprecedented platforms for translating research into therapy.

The need for a better translational model of Alzheimer's disease is perhaps best represented by the overwhelming failure of clinical trials for Alzheimer's disease. In the decade to 2012, over 400 drug trials had failed to find a treatment that could slow or stop the progression of Alzheimer's disease (Cummings JL et al, Alzheimers Res Ther. 2014 3;6(4):37). This amounts to tremendous expenditure by pharmaceutical companies on programmes that have ended with few results. We anticipate a sheep model that better models aspects of Alzheimer's disease, such as age, neuroanatomy and biochemistry, will provide a valuable preclinical testing ground for a disease that is an increasing strain on our society and health system, and is attracting increasingly large amounts of research funding.

Large animal models for cardiology research

Cardiovascular diseases are collectively the leading cause of death in Australia and other developed countries worldwide. Over the last fifty years, cardiovascular research has contributed to an unprecedented ten year increase in life expectancy, largely by reductions in mortality from heart disease and stroke. This impressive extension of human life has been underpinned by fundamental discoveries, the majority of which came about by investigations performed in various small and large animal models, ranging from mice to pigs. The ability to study cardiovascular pathophysiology in clinically relevant animal platforms continues to contribute crucial mechanistic insights that enable the pursuit of new and improved therapies, the refinement of these treatments and ultimately their translation to the clinic. Large animal models, including those in pigs and sheep are particularly favoured at the translational stage of investigation, as they are anatomically and physiologically more proximal to humans; they can therefore (1) undergo clinically relevant diagnostic and interventional procedures/surgeries (e.g. imaging of coronary atherosclerosis and myocardial function; valve repair and replacement; arrhythmia ablation), (2) be implanted with clinical devices (e.g. pacemakers, stents) and (3) be administered human-like doses of drugs, biological (e.g. stem cells, gene therapy) and other therapeutic agents.

To this point, the use of gene edited large animals in cardiovascular research has been very limited. However, there are numerous potential applications of transgenic models in pigs and sheep, especially spanning research into dilated and hypertrophic cardiomyopathies, ventricular and atrial arrhythmias, atherosclerosis and its risk factors (hypertension, hypercholesterolaemia, diabetes, and obesity). Researchers in the School of Medicine, University of Adelaide and SAHMRI have been prolific in the fields of atrial fibrillation and stem cell treatment of cardiomyopathy, using non-transgenic sheep models. Although highly successful, some of these studies have required extremely laborious and expensive surgeries/treatments to induce the disease process. One notable example was the administration of repeated intracoronary doses of the anthracycline chemotherapeutic drug, doxorubicin, to induce nonischaemic, dilated cardiomyopathy in sheep (Psaltis, J Card Fail 2008). Although very challenging (five invasive drug treatments requiring general anaesthesia; 16 week time frame to create disease; 25% premature attrition rate), this novel model enabled a world-first study of catheter-based, transendocardial stem cell delivery in the setting of nonischaemic cardiomyopathy, as well as several additional studies into omega-3/fish oil therapy for cardiomyopathy and nonischaemic atrial fibrillation (Psaltis, JACC CV Interv 2010; Psaltis, Int J CV Imaging 2011; Lau, Heart Rhythm 2011;

Carbone, J Card Fail 2012). Access to CRISPR genetic engineering technology in large animals will allow us to use more practical and reproducible models of nonischaemic cardiomyopathy and arrhythmia for our studies, especially as the basis for these conditions is often genetic in human patients (Kang, Prog Biophys Mol Biol 2016;121(2):97-109).

Atherosclerosis is the principle pathology responsible for myocardial infarction and stroke. It is a chronic inflammatory disease of blood vessels, heralded by the build-up of cholesterol-rich lesions, called plaques in the artery wall. These plaques firstly cause arteries to narrow, restricting luminal blood flow and causing tissue ischaemia. Of more concern, they become prone to destabilise if their inflammatory basis is uncontrolled, leading to plaque erosion or rupture, thrombotic occlusion and acute tissue infarction. Despite advancements in revascularisation techniques (e.g. stent and scaffolds) and the widespread use of cholesterol-lowering statins and antiplatelet blood thinners to prevent these complications, patients with atherosclerosis have an unacceptably high residual risk of recurrent adverse cardiac events leading to high death rates. Animal models of atherosclerosis have greatly increased our understanding of the systemic and local regulators of plaque formation and progression, and provide critical platforms to evaluate an enormous array of new therapeutic options.

Currently the mouse is the most frequently employed species for atherosclerosis studies, with focus on plaque in the aorta, brachiocephalic and carotid arteries. While these murine models are of unquestionable value to atherosclerosis research, their ability to accurately model human disease has important limitations. Among these are: (1) a lack of unstable plaque formation with overlying thrombus and acute cardiovascular complications; (2) the low rate of development of plaque in coronary arteries; (3) variable and often underwhelming development of plaque vasa vasorum, an important correlate of human plaque burden and instability; (4) different lipid profiles, most notably of HDL subsets; and (5) the small mouse size which complicates analysis and precludes use of clinical catheter imaging systems, stents and other devices.

The lipoprotein profile and coronary anatomy of pigs closely resembles humans. Although pigs do spontaneously develop coronary atherosclerosis, this can often be a long and variable process. The use of familial hypercholesterolaemic pigs with mutant ApoB alleles may accelerate the development of atherosclerotic coronary lesions, although existing models also have limitations (Prescott Am J Path 1991;139). Local expertise and access to a large animal gene editing facility would provide unprecedented opportunities to develop, validate and study novel models of coronary atherosclerosis in pigs that are more time-friendly and practical, not to mention more clinically relevant in containing multivessel coronary plaques that are complex, unstable and prone to rupture and thrombotic events. Quite feasibly, this could be achieved by the use of constitutional, cell-specific (e.g. endothelium, smooth muscle, macrophages) and inducible gene editing technology, to over- or under-express key genes known to be implicated in atherogenesis. This would be of great human relevance given increasing evidence for the genetic basis of atherosclerosis, and particularly the considerable prevalence of familial hypercholesterolaemia which leads to early onset, aggressive coronary artery disease in our society. Moreover, gene edited pig models of coronary atherosclerosis would provide direct opportunities to evaluate the following in the setting of stable and unstable lesions: (1) biomarkers from peripheral blood and coronary sinus sampling; (2) catheter-based and non-invasive plaque imaging techniques; (3) new stents and scaffolds; (4) lipid-lowering, anti-inflammatory and blood-thinning pharmacotherapies; (5) novel biological therapies (e.g. stem cells, genes, nanoparticles). The use of serial coronary plaque imaging in these pig models would provide clinically relevant insights by tracking the natural history of plaque progression and complication, along with their responsiveness to novel treatments (Andrews Cardiovasc Diagn Ther 2016).

Porcine gene edited models of atherosclerosis and other vascular diseases (e.g. aneurysms) would be of inestimable value to the work conducted at the Vascular Research Centre, Heart Health Theme, SAHMRI (and University of Adelaide) led by Prof Stephen Nicholls and Dr Peter Psaltis, who are both practising Cardiologists and internationally renowned Clinician Scientists with bench-to-bed expertise in numerous aspects of atherobiology. The incorporation of gene edited large animal models of atherosclerosis would add enormously to this large, dynamic and already highly translational atherobiology program.

Large animal models of cancer

Animal models have enabled the discovery and validation of many key molecular drivers of cancer development, growth and spread (Hanahan D, & Weinberg RA, Cell,144:646–674, 2011). They provide a preclinical opportunity to screen for new therapies, new screening tests, new diagnostic approaches and new procedures (Hruban, RH, et al, Amer Assoc Can Res, 95–106, 2006; Martin et al, Clin. Cancer Res. 19:2929–2940, 2013; Tuveson & Jacks, Oncogene 18:5318–5324, 1999). Mouse models of cancer, whether genetically-engineered mouse models (GEMMS), carcinogen-induced models or xenograft approaches have been the mainstay of preclinical *in vivo* cancer research. Mice are a convenient intermediary between the lab and the clinic, but for many applications our reliance on mice has been incredibly limiting (Watson et al, Front Genet 7:398–416, 2016). For instance, human organs are many thousand times larger than mice requiring many more stem cell divisions to reach adult dimensions. Stem cell division is a critical determinant of cancer, it is the opportunity for molecular error and is many orders of magnitude different between mice and humans (Tomasetti C & Vogelstein B, Science 347:78–81, 2015). Furthermore, mouse and human organs are biologically divergent. In colorectal cancer research this is seen most readily in genetic mutation of the tumour suppressor gene *APC.* In humans, *APC* loss causes a classical inherited bowel cancer syndrome, familial adenomatous polyposis with many hundred colorectal adenomas and colorectal cancer. In mice, however, loss of *APC* largely results in benign adenomas within the small intestine. Finally, except in some rare examples, murine cancer models seldom metastasize. Given that metastasis is the defining cause of death in the majority of solid organ cancers, therapies to preserve human life are greatly curtailed by our sole reliance in mouse work. We need an animal system in which the dimensions, the biology and the genetics are more closely aligned with our own.

In contrast to mice, the structure, function and genetics of pigs are very similar to humans. The example of clinical xenotransplantation belies the extraordinary similarity between our tissues. The swine genome is considerably more similar to ours than mouse, with considerable shared organization of genetic loci within given chromosomes allowing, amongst other things, genetic instability to be reliably modelled (Watson et al, Front Genet 7:398–416, 2016).

A large animal model, such as the pig, is attractive in cancer science, to allow us not only to model the biology, but to do so within a system of sufficient physical size. Much of medicine is defined by the biology and the context, only provided by studying cancer in larger animal models. For instance, in cancer science, mice have been limited as a practical tool for developing new clinical tests and procedures, radiological, endoscopic, biochemical and genetic. One example is in colorectal cancer screening. Orthotopic colorectal cancer experiments must be terminated rapidly in mice due to the small colonic lumen in mice as they succumb from local rather than metastatic disease. Larger models, like the pig, are needed to develop new tests, new imaging, and new therapeutic modalities up scaling from murine experiments, to bring transformational discoveries and approaches more rapidly into human practice.

There is currently a great opportunity to combine the biological and physical advantages of swine models with the versatility of genetically modified mice to drive cancer research in this country. Experiments proposed here include the development of genetically-engineered swine models of cancer (GESMs), the development of a Cas9 transgenic swine to allow rapid and specific post-natal genetic manipulation, the development of sleeping beauty transposon systems in swine to identify new cancer drivers and targets and finally bringing orthotopic organoid approaches to a model of comparable dimensions to humans. These would have a profound opportunity to help Australians with cancer and to drive entrepreneurship in translational cancer science in Australia and beyond.

Large animal models of cystic fibrosis

One example of a large-animal capability that we do not have is the ability to create and maintain disease-specific animals for use in better understanding disease natural histories, and to enable testing of potential treatments for the disease that arise from the basic, applied and clinical research sectors.

One example is the disease cystic fibrosis (CF). There are no large animal models of CF disease available in Australia. Existing models in the USA and Europe (e.g. of CF pigs) cannot be imported due to Biosecurity and quarantine regulations designed to protect Australia's commercial herds. CF-diseased pigs could be created housed and used in Australia, and could also be made available in various forms worldwide, for testing the large number of emerging CF therapeutics.

Projects can use CRISPR-Cas9 gene editing technology to establish several lines of CF heterozygote pigs that are then cross-bred to produce new-mutation homozygote or compound-heterozygote mild-disease CF pigs. This gene editing capacity is well established in Adelaide (Dr Paul Thomas, University of Adelaide). Available husbandry, biomedical / analytical equipment, general resources, and specific needs (e.g. early surgery to reduce gut obstruction, continued antibiotic cover) must be commensurate with the disease-model phenotype needs.

Pigs as models of disease have a long history and high expertise level in Adelaide, led by Professor Mark Nottle at the University of Adelaide, so are a rational initial choice. Furthermore, pig lungs are perhaps the most desirable species for studying CF lung disease, as they are well established as a very good mimic of the physiology and anatomy of human lungs generally.

Data from CF animal models in the northern hemisphere have provided otherwise unattainable data on CF pathogenesis, potential treatment effects, and with the acceleration of animal model choices from the gene editing technology the options for treatments-testing are advancing.

Gene edited sheep for immunology and therapeutic purposes

The Immunology theme can be used as a demonstration of the way the Centre may commercialise the scientific outputs. The ability to gene edit the cassette of genes which code for certain elements of the immunoglobulin molecule (IgG) will allow the creation of new BioPharma enterprises in Australia. The vision is to generate sheep which produce humanised polyclonal antibody. This will create a whole new approach to immunotherapy that will be available to Australian enterprises.

Amongst the proponents of the NLABC there is direct experience in the establishment of a human immunotherapy enterprise in Australia. The activity continues in the hands of BTG (Australia) and is an apposite example of expertise and people moving from a tertiary research environment to a

commercial one. The product range of BTG in Australia includes snake antivenom and immunotherapy directed against drugs for which there is no practical chemical antagonist.

A project is in place in South Australia to produce antibodies in sheep, as a part of a project where those antibodies are directed against all epitopes of certain bacterial toxins and against Ebola virus surface antigens. This project brings Australia into the internationally critical sphere of Counter Measures and Bioterrorism.

The Sponsors of this project have recently shown that only whole molecule polyclonal antibody (raised in sheep in the UK) protects guinea pigs and non-human primates against 3X LD_{50} dose of Ebola virus. This has shown that for therapeutic purposes, where rendering a molecule or a virus particle biologically inactive is required, polyclonal antibodies, not monoclonal antibodies are necessary. Several large pharmaceutical companies have scaled back their R&D which aimed to produce monoclonal antibodies against many epitopes on the surface of viruses, particularly the Filoviridae viruses, of which Ebola is one. Vaccines are proving to be highly effective against these diseases, but once contracted there is no readily available way to curtail the development of full blown Ebola virus infections. It is not possible for a mixture of monoclonal antibodies directed against Ebola surface antigens to replicate the success of polyclonal antibodies generated against the same antigens. It is now becoming clear that in order to 'salvage' Ebola infected and unwell civilians, military or NGO personnel, only polyclonal antibodies will provide the commercially viable answer.

Importantly, while Ebola is presented as a pertinent example of the application of this technology it is anticipated that there are many other possibilities which include but are not limited to passive protection of the immunocompromised, influenza pandemics and bacterial resistance.

Therefore, by developing the technology which causes sheep to generate antibody which is 'human like' in an antigenicity sense, and therefore much safer especially upon re-treatment where this might be necessary, this will be entirely disruptive to the previously ascribed 'immunological safety' of humanised monoclonal antibody.

Market advantage achieved by the NLABC

There are two reasons why Australia will be at the forefront of developments of new therapeutic technologies.

The first will be the scientific and technological capability developed in gene editing of livestock species which will become most effectively developed in Australia when sufficient investment in material and human capital is made in a timely manner. Australia will develop the technology first and will hold the subsequent IP and proprietary knowledge in the normal commercial way.

The second element, which Australia must exploit in the most effective ways, is the freedom from prion diseases in all sheep in Australia. Developing a BioPharma industry in this clean environment gives us an enormous advantage.

Freedom from prion disease means that sheep antibody produced here is acceptable to all human markets in the world without extensive 'proof' that the antibody has no prion associated with it. It was the sheep prion disease called Scrapie which jumped from sheep to cattle to produce Mad Cow Disease in the UK, and then which jumped to human to produce Variant CJD. This experience makes the creation of a BioPharma enterprise in Australia most attractive, provided we have a technological edge as well. Human safety regulators are very keen to avoid any risk from a potential animal-based therapy. Australia is not the only Scrapie free country, so

we need another material edge to secure the future of these developments. Gene edited sheep which produce antibody which mimics human antibody in an immunogenicity sense, will be the edge which will be of critical importance.

The market for various sheep antibody products has been conservatively estimated, by commercial consultants to be in the order of US $5Billion over 3 years. When Australia produces humanised sheep polyclonal antibodies, which are immediately acceptable in a microbiological and allergenic sense, new application for the new therapies will be huge.

This makes the service function of the NLABC a critical contingency, where the product of the fee for service provided can be sent anywhere in the world, no matter where the client comes from. On a full cost recovery basis the Centre will create gene edited livestock models of disease ' to order' and there will be no impediment to their distribution under licence anywhere in the world.

Gene editing in agriculture

Understanding the genetic control of traits that are of economic importance has been a subject of molecular research over the past century, and the genes controlling some simple monogenetic traits were identified several decades ago. However, many traits are under complex control involving the interaction among several genes each with potentially small effect, so call quantitative trait loci. Although the chromosomal location of loci involved in the control of quantitative traits (QTL) are readily localised by linkage mapping and more recently genome-wide association studies, the identification of causal variation, and proving that the variation does indeed affect the phenotype has been much more difficult.

An extreme phenotype in some breeds of beef cattle is double muscling, which is the result of extreme muscle hypertrophy and hyperplasia. The gene responsible was located on chromosome 1 by genetic mapping, but identification of the gene involved and likely mutation, an 11bp in the growth differentiation factor *(GDF8)* was only identified following the creation of a GDF8 knock out mouse, which displayed the same phenotypic effects. Several loss of function mutations have been described in GDF8 in several species, including man, and are associated with increased muscling. However, conclusive proof that GDF8 causes double muscling, and the opportunity to study the biology of the trait was only provided by gene editing GDF8 and showing loss of function variations were associated with the phenotype.

Many variations that control strong phenotypes have now been found in non-coding regions. These include variations in non-coding regulatory regions, such as an intronic variation in insulin like growth factor 2 which has a major effect on muscling in pigs (van Laere et al 2003, Nature 425, 832-336) or in regions where no regulatory elements have been described, such as the polled locus in cattle, which controls the development of horns. The polled locus is on chromosome 1 and is a complex deletion and insertion in regions where no regulatory elements have been identified. Proof that this variation is indeed responsible for the polled phenotype was achieved by editing animals that genetically were horned to carry the putative polled variation and demonstrating that resultant animals were polled.

Gene editing, therefore has two important applications for agriculture:

1) Creating animals edited to create variations believed to have an effect on a phenotype allows the effect of the mutation to be tested to confirm or refute the role of the variation in controlling the phenotype. Having proved the role of the variation editing then facilitates the testing of the biological effects of the variation against the same genetic background as the non-edited “wild type” version. This allows the biology of traits to be dissected and interactions among genes to be explored.

2) Animals edited for known variations are informative for the effect of the variation in a new genetic background. For example, editing Merino sheep, which are selected for wool production, for the GDF8 variant from the Texel sheep, which are meat sheep, allows the effects of the variation to be tested, prior to introgressing the variation by cross breeding. Introgression takes a long time, is expensive and is associated with a loss of genetic progress. Therefore, prior knowledge of the outcome introgression is highly valuable

Collectively the information gained will enable more efficient and strategic breeding selection that has great potential to advance agricultural research and the livestock industry.

Business proposition for a gene editing centre

The establishment of a gene editing facility will promote research and development activities for biomedical research as well as applications in agriculture and expanding basic biological knowledge. In addition to the editing facility the core infrastructure will include animal breeding facilities and animal phenotyping capability to measure phenotypes such as behavioural characteristics and physiological responses. The gene editing centre will create opportunities for cutting edge research with the associated emerging IP. The physical infrastructure and associated scientific capability will attract companies to co-locate with the internationally competitive research capability. The companies that will be attracted initially are likely to have diverse capability and customers centred on animal models. These companies will expand interactive capacity with additional physical infrastructure and intellectual capability. The core infrastructure will then become self-sustaining, attracting additional business to take advantage of opportunities as providers to the established companies or as their customers.

Initial commercial interest spans both biomedical applications and agriculture. Companies that would be attracted in the biomedical space include pharma companies wishing to create animal models for specific diseases to identify new drug targets, for drug development and testing; specialist contract research organisations (CROs) that are service providers that will add specialist skills, for the characterisation of the animal models; diagnostics companies wishing to use edited animal models as resources for development platforms for new diagnostics. These companies will attract and provide business opportunities, beyond the capability of the editing centre itself;

In the agriculture area, companies active in the gene editing space will be attracted to the well set up infrastructure to provide sub-contracted services to clients e.g. to test specific genetic variants for potentially creating edited livestock to suit specific production systems, to improve environmental adaptation or confer resistance to specific diseases. Initially these may be proof of principle studies. In the longer term such companies may carry out gene variant discovery for genetic improvement in farm species to create livestock for commercial production.

The business models will include rental of space in multi-user buildings to the companies, offering land to construct bespoke facilities as well as co-investment in the expansion of capability and capacity of the Centre. Projects in the Centre may be funded by companies as confidential research, be join ventures between local companies and with external clients. In addition, companies may support grant applications for research project by matching funding, with in kind contributions or by providing essential know-how. Joint funding by these companies will demonstrate the commercial viability of projects and provide a route to exploitation.

The Centre will be administered by a Board appointed according to the requirements of NRIC. The Board will establish the cost recovery methods to be applied to projects initiated by Australian researchers, to overseas researchers and to local or obsess companies. Within the proponents of

this Centre is extensive experience of fee-for-service activity, both to researchers as well as for companies requiring GLP-recognised studies to be conducted.

In summary, creation of an internationally competitive infrastructure for gene editing will not only create an infrastructure to compete with the global scientific community, but the infrastructure will in itself be a catalyst for inward commercial investment to expand intellectual capability and physical capacity.

Quality systems

The proponents of the Centre are fully aware of the value, indeed the imperative, of Quality Systems to be applied to the operation of the Centre. We have current working knowledge and compliance with both ISO and GLP recognition systems. The Centre will need to have sufficient allocations within its organisational structure for Quality Managers, and appropriate QMS software will need to be used at all levels of the operation of the Centre.

Large animal preclinical studies

The proponents of the NLABC have collaborative arrangements with members of what is currently the NCRIS funded Therapeutic Innovation Australia capability and are partners in the Australian Therapeutic Pipeline. Whatever is the longer term mechanism by which Industry and Pharma engagement is facilitated in Australia the NLABC will be a direct and enthusiastic participant.

It is quite clear that in Australia there is a paucity of infrastructure to conduct world standard preclinical studies in livestock species under PC-2/QC-2 containment conditions. Fine facilities exist at the UQ Gatton campus to enable certain studies to be performed and close cooperation with that unit will be pivotal to the development of a comprehensive capacity in Australia. The NCRIS/ National Imaging Facility supported Large Animal Research and Imaging Facility (LARIF) at SAHMRI has an important array of imaging modalities and has the dual purpose of being a one-stop-shop for experimental surgery/physiology and preclinical studies. It is used by interstate and overseas researchers, and by medical devices and drug companies local and overseas, because of it range of imaging equipment and professional/scientific expertise. We know from recent discussions with US based Pharma companies that Australia would be an even more attractive place for the conduct of preclinical studies if these studies could be conducted under both AQIS (DAWR) and OGTR regulatory controls and constraints. Such facilities do not exist to the required scale and within an environment where fee-for-service and proof of concept studies can be conducted in a timely manner and in conformity with QMS principle. We believe at an investment in large animal preclinical study capability will dovetail in an essential way with the primary CRISPR-Cas thrust of the Centre.

New large animal facilities are needed to explore the phenotype, both physical and behavioural, of animal models created by the Centre by the way of state of the art imaging equipment, experimental physiology techniques, and tailored behavioural assays.

The development of therapies for neurodegenerative diseases, for example, requires studies under PC-2 containment conditions as well as being in close proximity to cutting edge imaging equipment.

As previously noted in this proposal, there is also a critical need for germ-free (very high health status) pigs in Australia, to enable gene-edited pigs to be rederived into such a facility and for their organs be immediately available for use in humans you way of xenotransplantation. A further direct benefit of such a germ-free pig facility associated with the Centre is its immediate use by

microbiome researchers who need to extend their microbiome mouse studies into a large omnivore such as the pig. This will enable a substantial addition to the insights into the role gut bacteria have on physical and mental health which has already been elucidated from mouse studies. Microbiome research is a key emerging discipline around the world, and indeed the NIH has recently called for EOIs to establish research centres where the use of livestock species will be developed to enable the study of the influence of the microbiome on human and animal health. This call from the NIH is an endorsement of the suggestion that whilst mouse studies have informed enormously on the mechanism by which the microbiome can influence the whole animal, taking the next translational step will require the use of germ-free pigs. A major consortium of microbiome researchers throughout Australia will have access to the resource managed by the Centre. Having gene-edited pigs available to microbiome researchers, as well as to the xenotransplantation theme of the Centre, makes for a powerful justification to make the significant investment required to create and maintain germ-free pigs. Additionally, it is possible to gene-edit porcine retroviruses and so render them non-active in pig embryos, to further modify the extraneous load within xenotransplantable organs.

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