FELLOWSHIP SUMMARY REPORTS

- Please submit this Summary Report in Word, in Times New Roman, font size 11, using UK English spellings.

Cover page – which should include:

- Your name
  Thomas Lubberstedt

- The subject title and theme number of your research fellowship
  Accelerating Plant Breeding; Theme III: Transformational Technologies & Innovation

- Your host institution
  AgriBio at La Trobe University

- The name of your host collaborator
  German Spangenberg

- The dates of your fellowship
  12.12.2017 to 19.4.2018

- Your consent to your report being posted on the Co-operative Research Programme's website, or alternatively, a short paragraph about your fellowship which could be used anonymously.
  I consent with my report being posted

1. What were the objectives of the research project? Why is the research project important?

Progress in plant breeding depends on the ability to master two major tasks. One is the ability to predict performance of offspring with as few resources as possible. This is successfully addressed by the recent advent of genomic selection (GS) strategies. GS benefits from low cost sequencing and DNA marker technologies and enables to evaluate offspring at the DNA level only, reducing expensive field testing. The other major task is time, i.e., the time needed to develop new varieties, or parents of varieties, such as inbred lines. The period of time needed has been shortened by using off-season nurseries, allowing 2-4 generations per year for major crop species. For developing inbred lines more efficiently, doubled haploid (DH) technology is meanwhile routine for major crops and breeding companies. Using DH technology, fully inbred lines can be obtained in 2 rather than 5-8 generations, as in traditional programs. The overall limitation still is, however, the time needed to produce seed. While this can be manipulated to a limited extent, it constrains the number of generations possible per year. Moreover, for several important crop species such as soybean and sorghum, no DH system is currently available.

The **quantum leap** would be implementation of **the in vitro nursery concept.** Therein, selected genotypes are maintained at minimal space in a lab setting as cell culture. New genotypes are formed by in vitro production and fusion of gametes, a cycle that can be repeated multiple times, before mature plants are regenerated. Generation time is limited by how quickly new gametes can be formed and fused. This in vitro system has the potential to immediately induce gametes for new crosses, or for genome doubling to produce homozygous cell lines. This process needs to be coupled with genomic selection. Selection within the in vitro nursery would be accomplished by genotyping gametes. This allows for targeted mating, resulting in optimal genotypes with minimal resources and time.

While respective research on gamete formation is ongoing in my laboratory, the specific objectives for my research visit at La Trobe University were, to 1) establish a protocol for successful whole genome amplification and generation of genotype data from as few cells as possible from gamete-based cell lines, and 2) to develop a statistical method to identify the most promising gamete pairs for formation of superior genotypes in vitro.

2. Were the objectives of the fellowship achieved?

Or are they on the way to being achieved?

If not, for what reasons? (The data or research is still ongoing or being analysed; technical reasons (e.g. equipment not working, adverse weather conditions, unexpected results, etc.; other reasons?)

My host German Spangenberg assigned four experts in his team to work with me: Dr. Matthew Hayden, Research Director Plant Sciences at Agriculture Victoria Research, and Associate Professor, School of Applied Systems Biology, La Trobe University; Dr. Noel Cogan, Senior Research Scientist Molecular Genetics; Dr. Hans Daetwyler, Research Leader Computational Biology; and Dr. Sareena Sahab, Senior Research Scientist Biosciences Research. Dr. Hayden has been my primary contact, Drs. Cogan, Daetwyler and Sahab are domain experts with in-depth understanding of their respective areas. Their expertises perfectly matched my research objectives on genotyping (Drs. Hayden, Cogan) of single cells (Dr. Sahab), and predicting best matching cells for mating to form the next generation (Dr. Daetwyler).

The key question during an initial meeting with all four experts December 18, 2017 was, how relevant the specific objectives still were, since the OECD proposal was submitted summer 2016: 1.5 years are a long time in the rapidly evolving areas on sequencing and genotyping technology, as well as genomic prediction. The goal was to focus my research activities on the most relevant task(s) pertaining to the above-mentioned specific objectives.

Literature research revealed, that a recent method (iDOP-PCR) published by Blagodatskikh et al. (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0184507) in September 2017, appeared to be a good solution for fingerprinting DNA from single or few cells at low cost. In other words, this problem appeared to be solved, additional experiments did not appear to be necessary. Similarly, recent research published by Dr. Daetwyler (Daetwyler et al. 2015, Genetics, DOI: 10.1534/genetics.115.178038) along with ongoing research by his team building on this publication or by other researchers (e.g., Cameron et al. 2017, Genetics, DOI: 10.1534/genetics.116.197095) appeared to have addressed the problem of finding optimal gamete pairs sufficiently, that dedicated research on this topic did not seem justified.

In contrast, capturing single haploid cells, and developing cell lines after a limited number of mitotic divisions seemed to be a major challenge, in particular if this needed to be done for large numbers of cells in parallel. For this reason, experimental research was focussed on the question, whether it is possible to obtain mitotically

dividing cells in liquid culture when starting from reduced canola protoplast numbers compared to the routine protocol applied at AgriBio in the context of plant transformation.
During my visit, I did have several meetings with those four scientists to discuss my research topic, and also with various other scientists at AgriBio. These discussions about the current status and feasibility of in vitro nurseries is currently being extended into a review article titled "Challenges and opportunities of *in vitro* nurseries" with coauthors from both AgriBio and my team at ISU, targeted for Plant Biotech Journal.

3. What were the major achievements of the fellowship? (up to three)

(i) Joint publication "Challenges and opportunities of *in vitro* nurseries" in a peer-reviewed journal.
(ii) Experiments with canola protoplasts showing that mitotically dividing cells can be obtained in 100-fold lower concentrated protoplast populations, after being encapsulated in alginate; while the experimental work was too limited to be published, it became apparent that the host team has the expertise and also dedicated equipment (flow-cytometer) to establish a protocol for single-cell derived cell lines, as needed in the in vitro nursery concept. Thus the host institution is an obvious partner for future collaboration on this topic.
(iii) Learning about history and organisation of research at Agribio (including visits of facilities, participation in research seminars), and more generally Australia by conversations in particular with my host German Spangenberg.

4. Will there be any follow-up work?

- Is a publication envisaged? Will this be in a journal or a publication? When will it appear?
  - Yes, see above. I expect submission of the review article fall 2018.
- Is your fellowship likely to be the start of collaboration between your home institution and your host?
  - Yes, see above. The most critical step of the in vitro nursery concept, not addressed during my visit, is the ability to initiate gamete formation starting from undifferentiated diploid cells in liquid cell culture. If this problem can be solved, then the next major challenge will be development of single cell-derived lines, for which I would aim for collaboration with the host team.
- Is your research likely to result in protected intellectual property, novel products or processes?
  - Not the research conducted during my sabbatical.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

The research conducted at AgriBio contributed to realization of the in vitro nursery concept, which is a long-term research effort. If successful, then the process of plant and animal breeding can be increased substantially. This would lead to significant increase of genetic gain for any plant / animal and trait of interest, and thus help to develop varieties in shorter time, that help to address needs relating to environmental (e.g., drought tolerance), food security (e.g., grain yield), economic (lower breeding costs), and health (e.g., resistance breeding) considerations.

6. How was this research relevant to:

- The objectives of the CRP?
  - The in vitro nursery concept, if established, would represent a "Novel and innovative technologies that achieve a step change", which is the objective of the 3rd CRP theme, "Transformational Technologies and Innovation".
- The CRP research theme?
  - Within the 3rd CRP theme, the in vitro nursery concept is closely aligned with the research topic "Advanced breeding tools/Genetic and genomic technologies", as it combines novel biotechnology with genomic selection to speed up the breeding process.

7. Satisfaction

- Did your fellowship conform to your expectations?
  - Yes
- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.
  - No. This was not my expectation, as I am already advanced in my career (Full Professor, Director of RF Baker Center for Plant Breeding)
- Did you encounter any practical problems?
  - No
- Please suggest any improvements in the Fellowship Programme.
  - Flexibility with starting time. I did have to start before Christmas to use 2017 funding. However, this is (like everywhere) holiday period – which I usually like to spend with my family, and which is a "slow period" in most research institutes. Ability to start just 3 weeks later would have been good.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?
  - Internet
- What would you suggest to make it more "visible"?
  - Ask key administrative units of major universities to distribute information. At ISU, this is the Vice president for research (VPR). Once VPR office circulates it ISU-wide, then other lower level administrative units re-distribute it. There will be similar units at other university.
- Are there any issues you would like to record?
  - No