IGTRCN Peer-to-Peer Training Fellowship Report

Project title: Advancing pest control strategies with CRISPR/Cas genome editing technology in *Bactrocera tryoni* (Queensland fruit fly)

Trainee: Amanda Choo, University of Adelaide, Australia

Host: Associate Professor Zachary Adelman, Virginia Tech, United States

Visit: 25 April 2016 – 6 May 2016

Objectives of visit

The main objective of the visit to the Adelman laboratory in Virginia Tech, USA was to gain expertise and current knowledge of the CRISPR/Cas genome editing technology in *Aedes Aegypti* for application in the non-model dipteran insect, *Bactrocera tryoni* (Queensland fruit fly). Whilst I had previous experience with CRISPR/Cas mutagenesis in *Drosophila melanogaster*, I had not worked with non-model organisms, in which there are limited genetic tools available. Hence the goals of this training visit were to discuss and learn new strategies for improving the efficiency of CRISPR/Cas mutagenesis as well as to learn methods of large-scale handling and genotyping to identify germline mutants.

Training activities and accomplishments

Insect handling

During my visit, I worked closely with three different members of the Adelman laboratory and was shown how they reared and handled *Aedes Aegypti* at the different life stages in their facility. It was useful to see how the multi-user facility was run and the regulations in place to avoid cross-contamination between strains and to prevent the escape of any mosquitoes from the facility. I was shown the different cages/containers that they had for rearing and mating of the mosquitoes, which was extremely handy as I'll be looking to make and use different size cages for rearing and mating of *Bactrocera tryoni.* I was also shown how to make a transfer cage, which is used to minimise escape of mosquitoes when handling large populations of adults.

I was able to observe and assist in how they set up their mating crosses, did the blood-feeding, transfer of females from a large cage into a tube for egg laying by the use of an aspirator, collected embryos for microinjections, sorted the females and males at the pupae stage with a pupal sorter as well as stored and hatched eggs of different genotypes. In addition, I was also shown how to remove a leg of the

mosquito for genotyping purposes without having to sacrifice the insect. This exposure to their handling techniques will be helpful in improving our *Bactrocera tryoni* rearing and handling methods.

CRISPR/Cas mutagenesis strategies

In regards to CRISPR/Cas mutagenesis, we went through the process of designing guide RNAs and primers for detecting mutations, evaluating potential off-target effects and discussed strategies of improving the efficiency of mutagenesis for different types of mutations. These strategies included multiplexing guide RNAs, potential use of different enzymes, such as the high fidelity Cas9 variant, SpCas9-HF1 (Kleinstiver *et al*., 2016) and Cpf1 (Zetsche *et al*., 2015) as well as targeting components of the DNA repair pathway (Basu *et al*., 2015; Overcash *et al*., 2015). I was also able to compare protocols for generating guide RNAs and Cas9 mRNA (both based on Bassett *et al*., 2013) and to verify that I was using the optimal conditions for generating and introducing the CRISPR/Cas components into the embryos. As there are regularly new advances in the CRISPR/Cas technology, it was a real advantage to be able to have discussions with researchers working at the forefront of this technology.

Embryo microinjections

Over the course of my visit, I undertook training for *Aedes aegypti* embryo microinjections (protocol detailed in Aryan *et al.,* 2013) to gain experience with a different microinjection setup and doing microinjections in a different system. This was particularly useful as I was able to obtain beneficial advice and tips for handling embryos and improving the survival of injected embryos. Although *Aedes aegypti* embryos are different from *Bactrocera tryoni* embryos and potentially not all steps of the protocol will be transferable, nevertheless it was helpful to be exposed to a different method of microinjections other than for *Drosophila melanogaster* embryos. I also gained a better understanding of the specific conditions required for each step of microinjections, which will assist greatly in optimising *Bactrocera tryoni* embryos microinjections in the future.

I was shown how to bevel needles, which I had not done before and will now be able to consider doing for my microinjection needles. I was also able to obtain a specific double-sided tape (for sticking down the embryos) to bring back to Australia as we previously had issues with the effectiveness and toxicity of the tape and embryo glue that we were using. During the training, I injected a line of embryos with an injection mix containing guide RNAs and Cas9 mRNA for an embryo assay (to assess the efficiency of the guide RNAs).

Genotyping by High Resolution Melting Assay (HRMA)

A substantial portion of the visit was spent being trained on the mating scheme and genotyping of adults to detect germline mutations post-injections using the High Resolution Melting Assay (HRMA) **(**protocol detailed in Basu *et al.,* 2015). This was extremely useful as this technique enables us to determine if CRISPR/Cas mutagenesis has been successful and to identify germline mutants through large-scale screening in a short period of time. I was shown how to perform embryo assays (to firstly determine the efficiency of the CRISPR/Cas guide RNAs) as well as germline assays (to detect and identify individuals with the desired mutations). This involved DNA extraction (from either pooled embryos or individual adult legs), specific PCRs to amplify the region where the guide RNA was designed to target and analyses of thermal melt profiles using a LightScanner instrument to identify any variations from wildtype samples, indicative of a difference in the genomic DNA sequence at that region. Results from an embryo assay performed on the line of embryos that I injected showed that two different guide RNAs injected were efficient in inducing a double strand break at the desired site. With the knowledge gained from this training, I will be setting up a similar assay for verifying CRISPR/Cas mutagenesis efficiency and screening of germline mutants in *Bactrocera tryoni.*

Acknowledgements

I would like to acknowledge and thank IGTRCN for providing me with this peer-to-peer training fellowship, which allowed me this wonderful opportunity to receive training from the Adelman laboratory. It was an invaluable learning experience, which will undoubtedly assist me in making significant progress with my project.

I would also like to thank Associate Professor Zachary Adelman and his laboratory members, Dr. Azadeh Aryan, Michelle Anderson and Justin Overcash for graciously hosting me in their lab, sharing with me all their knowledge and for all the training, help and advice provided. I am extremely appreciative to have had this opportunity to engage with such excellent researchers working in a similar field and build a working relationship that would hopefully lead to future collaborations.

References

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