**IGTRCN Peer-to-Peer fellowship training report**

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## Training Timeline

Training was conducted at the ITF in Rockville, MD in January and February, 2015, with follow-up sessions over email and by phone with members of David O'Brochta's lab and the ITF. A follow-up training date occurred in early May. See table 1 for a list of dates and times spent at the ITF (and associated travel costs).

## Description of Training Activities

## Injection training

The first proposed training objective was for me to learn the basic mechanics of injecting embryos: preparation of eggs, use of the injection apparatus, preparation of needles and injection solutions, etc. This training was undertaken with Rob Harrell at the ITF over a period of approximately 3 weeks in January of 2015.

Training included the following stages:

First week – I practiced using the injection apparatus to inject previously "lined up" mosquito eggs. Practiced pulling and loading injection needles.

Second week – I practiced collecting and injecting eggs from *Drosophila melanogaster*, including dechorionation with bleach, and lining up the eggs prior to injection. I received advice/training on timing and workflows for collecting and injecting Drosophila eggs. Attempted to inject a few *Teleopsis* eggs.

Third week – Continued practice with Drosophila. Injection of Drosophila with actual fluorescent transgenes and subsequent inspection of embryos for fluorescence and mortality. Intended to practice using *Teleopsis dalmanni* eggs, but by this point the Teleopsis culture at the ITF had stopped producing eggs.

Results: I obtained essential training and began to develop "hands" for the injection technique and got a sense of the workflow pace, especially through observing Rob performing his own experiments. I also pulled needles that I will use in my own injections at UMD.

**Teleopsis egg collecting**

Meanwhile, I also received advice and training specifically in obtaining, injecting, and rearing teleopsis eggs. I spent several hours each day consulting with Channa Aluvihare, a scientist at the ITF, and also attempted egg collection. Channa provided me with protocols and advice on rearing and collecting eggs from various insect species he has successfully worked with in the past. This work was undertaken concurrently with the injection training during three weeks in January 2015:

First week – Set up cultures in the ITF. Allowed flies to "rest" while beginning training on the injection apparatus. Flies were kept in the mosquito chamber (roughly 80 C, 90% humidity) due to worries about humidity being too low in lab.

Second week – Collected teleopsis eggs and practiced dechorionation (mechanical). Visualized embryos in a transmission dissecting microscope to observe development. Advice from Channa on the volume of eggs I would probably need to obtain, given viability and losses during injections, with real examples.

Third week – Attempted to recover flies (by this point many/all were dead), with no further egg collecting. Discussed potential issues and future plans with Channa.

Results: I learned that my flies were not happy at the ITF and laid no eggs, then began to die, after the second week. Further, larvae that did develop failed to thrive in the conditions at the ITF. I concluded that the environment was probably too extreme for our flies, which prefer more moderate temperature and humidity (typically 70-75C and ~60%). In addition, the space there was very *clean* whereas our flies typically live in and prefer dirtier spaces. I found this information very useful as I have continued to develop egg laying/rearing protocols (see Protocol for Teleopsis egg collection and injection).

**Cloning CRISPR targets for *T. dalmanni***

Training in the molecular methods required for creating CRISPR targets in *T. dalmanni* was undertaken in three stages.

First, during the three weeks in January when I was training at the ITF I had a meeting with Dr. O'Brochta and his research associate, Bill Reid, concerning the conceptual basis for using synthetic PCR techniques to produce the needed constructs, and how I would use the *T. dalmanni* genome to design appropriate constructs. At this time, I also received protocols and software tools for use in designing CRISPR targets.

Secondly, during the month April, 2015, I corresponded with Bill frequently by phone and email in the process of designing targets. It was decided Bill would order materials and I could come in for further training. This correspondence period provided significant and essential training in cloning and molecular. Ultimately, I

designed primers to produce the needed constructs to target a particular "control" gene (yellow) for ablation via CRISPR-mediated gene replacement.

Finally, in May of 2015 I traveled to the ITF again for a hands-on session using these primers. Results of initial PCR indicated that there were either multiple copies of the gene of interest, or an offtarget was being amplified. I also received an update from Bill on what had been working in his hands, which I then used to modify my own work flow for future construct. Finally, I obtained plasmids and stock primers that I could use for creating future constructs.

**Concluding comments**

The training I received through the IGTRCN at the ITF has supplied me with necessary skills and materials and has also given me a better idea of the time and effort that must be put forth to obtain a transgenic animal. Whether I personally manage to produce a transgenic stalk-eyed fly or advise a student on such a project, I now have a much better sense of the specific concerns and requirements involved.

Secondly, it was a great opportunity to forge new collaborations and meet more scientists working at the forefront of non-model system genetics.