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Mentor's assessment of thesis by František Kitzberger: Interaction of proteins Methoprene tolerant and Taiman – Preparation of constructs for expression in S2 cells

František started to work in our lab nearly 2 years ago. This indicates that he did not leave his decision to the last moment. (Indeed, František was extremely motivated and attended lectures on advanced methods of molecular biology, among other advanced courses). Thematically, his project follows up our genetic evidence of interaction between and among several transcription factors. These factors include classical circadian proteins, but also recently identified receptor of Juvenile hormone Met and its partner Tai. The actual physical interaction is elusive, at the same time important for our further research and really interesting for this chronobiology field. There are no good antibodies for these proteins, and our insect model species is beautiful for its biology, but it is not user friendly model. Therefore we need to use different system, such as yeast, *Drosophila* cell lines, and perhaps even mammalian systems. Since some of the proteins in questions are quite long (around 1400 AA for at least for two of them), some reliable and effective subcloning assay was needed. So Frantisek served as our experimental guinea pig to test if the Gateway cloning approach from Invitrogen is really student-proof.

The dream (and completely unrealistic) version of the project was following: Generate clones for two bHLH-PAS proteins, test localization of resulting recombinant proteins in *Drosophila* S2 cells, and also elucidate the impact of JH (using its mimics) on these localizations. Given the relatively short amount of time (Frantisek has to spend half of the time in Linz), the optimal version simply is not possible.

František managed to subclone open reading frames (orf) of two proteins into different destination vectors. All these resulting plasmids were verified and are suitable for further experiments. So we learned that (i) the Gateway approach works, that (ii) the destination vectors obtained from colleagues are compatible with Gateway technology, that (iii) František is compatible with Gateway technology, too, and (iv) as a bonus we have obtained some constructs ready.

In case of František I would particularly highlight his enthusiasm. Perhaps, writing is not his strongest skill. But, (at least) sometimes, it would be nice to do things with a slight advance (= not at the very last moment...). Altogether I think that the thesis completely fulfills criteria for Master thesis and I recommend it for defense.

Last, but not least I would like to specifically thank Olina Bazalova for co-supervising František during all experiments, troubleshooting and time devoted to the project. I would like to wish František good luck in his future scientific, chemical and personal life.

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