Ceske Budejovice, 10th June 2011

Review of the bachelor thesis of Marion Sieber: Promoter analysis of lin-3::GFP transgene in Caenorhabditis elegans

The aim of Marion's bachelor thesis was to clarify the identity of a *C. elegans* transgenic strain that was poorly described in the previous literature. She needed to amplify various parts of the lin-3::GFP transgene by PCR, sequence them and use a DNA analysis and alignment software to describe the precise sequence composition of an enhancer/promoter fragment cloned into the transgenic construct.

It is clear that Marion took this task seriously, she understood the problem as well as the background of *C.elegans* genetics and she did decent amount of work. The thesis is well written, however, there are a few technical issues that make the reading difficult in places:

1. The description of the constructs from the *Hwang and Stenberg* paper in Fig.4, Fig.7 and Fig.14 does not match the description in the text. In the figures it looks like construct 2 is shorter than construct 3 but in the text it says that construct 2 is 4kb and construct 3 is 3kb. Can you please clarify that?
2. There is not a single picture where the position of ALL the primers used to amplify the lin-3 transgene would be shown at the same time in the context of a DNA map. Instead, some primers are shown in Fig. 7, some in Fig.5 and Fig.9. This makes it difficult for a reader to follow. Perhaps it would have been good if such a picture was present straight after Table 1 that lists all the primers used.
3. Some of the bands expected in the PCR reactions were rather big, between 5-15kb. However, according to the description in the 'Methods' the extension time in the PCR reactions was only 3 minutes in all cases. Would you be able to amplify longer fragment with the polymerase you used?
4. Legend to Fig.9 says 'Primer positions in the construct'. It should be more specific: you probably mean your construct number 2.
5. Fig.7 and Fig.14 are exactly the same. Fig.14 could have been skipped and a reference made to Fig.7.
6. In the result section the full alignment of all fragments sequenced should be presented. As this is missing, the reader of the thesis can only rely on the interpretation Marion provides in the text and can not make his/her own conclusion.

Point for discussion:
Marion found out that the transgenic construct does not contain the second lin-3 promoter, a result in contradiction to the published data. Could the data presented in the *Hwang and Stenberg* paper be interpreted differently in the light of these new observations? Where is the construct expressed and where would you expect it to be expressed if it contained also promoter 2?

I suggest to pass this bachelor thesis with the grade excellent.

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