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Review Bachelor's Thesis Joan Elorm Abla Ahiable

The bachelor's thesis by Joan Elorm Abla Ahiable, entitled "Cloning Candidate Novel Cell-Fate Genes (Pre-Implantation Mouse Development)", describes her efforts in cloning several cDNAs and gene fragments from putative cell-fate genes for future use in mouse embryos. The project involved basic molecular methods such as PCR, DNA isolation, restriction digestion, plasmid construction via ligation of vector and insert DNA fragments and miniprep DNA preparations, among other techniques. It also concludes with a description of her success in generating RNAs from the described constructs and PCR templates by *in vitro* transcription to generate HA-tagged ORFs or dsRNAs for eventual injection into embryos, which is I consider to be a rather sophisticated technique. I think the scope of the project is realistic for a year-long bachelor's thesis in the Biological Chemistry, and Ms. Ahiable has met the aims of the project, even when four out of the seven genes were not successfully cloned/brought to the stage of *in vitro* translation.

In the thesis, she more or less conveys an adequate understanding of the techniques and the underlying biology of early mouse embryo development. However, at 66 pages, 43 representing the main text, the thesis is too long in my opinion. There is a lot of needless repetition, exemplified by the 4-5 times phenol/chloroform extractions are described. Also, the seven putative genes are discussed at length, including their advantageous properties for the employed cloning strategy, while only three of the genes successfully submitted to the workflow from PCR to RNA product. I would have only discussed the genes that worked in length. Also, the information in the introduction is presented in a rather confusing way, at least to a non-specialist like me. Genes known to have a role in cell-fate determination are dealt with rather haphazardly, and no connection between this information, the early stages of embryo development and the seven genes that are the topic of the thesis is made. A discussion about different models of cell fate determination also seemed a bit extraneous. In short, streamling of the text would have yielded a clearer and more concise thesis.

Other major comments on the text (an annotated copy pointing out mistakes is also submitted with this report):

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- While there are very little incidents of typos, there are countless places where spaces between words are missing, which was very distracting to the reader (these are pointed out in annotated copy).
- In the introduction, many abbreviated gene names are used without defining the abbreviations first. Proper practice is to use the full name of gene/compound/etc. and then define the abbreviation in parenthesis.
- A table of the discussed genes would have been very beneficial for the reader, and a easier way for the author to convey information.
- Speaking of parenthesis, these are used to much in the thesis for asides that are either not important, or are as important as the content of the enclosing sentence. Parenthetic statements break up the flow of the text and should be used sparingly.
- Ms. Ahiable often uses "that" and "which" improperly. This is not unusual for novice writers and a web site briefly explaining how these words should be used is provided in the annotated copy.
- There are often very convoluted sentences (e.g. "The double stranded DNA T7-linked PCR products that were generated and purified were sequence specific to the candidate cell-fate related genes whose expression we want to knock-down by RNAi in the embryo." p. 30) in which it is clear that Ms. Ahiable tries to clarify the subject and processes for the reader, but in this way makes it more confusing. But, I admit, writing about molecular biology is hard and needs practice.
- There are many run-on sentences.
- Nanodrop data describing nucleic acid yield should be in the Results section and not Materials and Methods
- In the introduction, the seven candidate genes are described at length with bullet points of what I presume to be advantages each sequence has for cloning. This is not explicitly stated by the author and also this information is not appropriate in this section, as it pertains to the cloning described in Material and Methods.
- Figures 4.1-4.6 should have been edited in a program like Photoshop or GIMP to crop, rotate, adjust contrast/brightness of images. The labeling of lanes should be more self-explanatory for the reader.

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- Figure 4.2 contains information on the top that should be have been cropped out (see questions below).

- Please look over the annotated copy for a listing of other mistakes, suggestions for better usage, etc.

I conclude this report with some questions for Ms. Ahiable to clarify some points and dig deeper into what she learned in this project:

- In section 1.3, a screen was mentioned that identified the seven putative cell-fate genes for the presented study. Can the author elaborate on how the screen was performed and what were the main criteria for such genes, as it is poorly described in the text.
- On page 19, the author states: "The addition of this HA-tag was designed to allow the discrimination of endogenous and recombinant protein expressions after successful microinjection." How can recombinant and endogenous proteins discriminated via the HA tag?
- Page 23 states a PCR annealing time of 38 sec. Why was such an unusual time used for this step? I am sued to round numbers.
- The author reports the A260/A280 values from the UV spectroscopy. What is the relevance of this number?
- On page 25, the author states that the T3 oligo was used for sequencing of the pRN3P plasmid and refers to figure 2.1. It is not clear though why this oligo was used. Please elaborate.
- Why was the alkaline phosphatase heat inactivated? (p. 26)
- Why where the pRN3P-based constructs isolated by MIDIpreps, which yeialds a lot of DNA. Only 2 ug was used in the subsequent IVT (p. 29)
- The IVT products were treated with an RNase cocktail to degrade potentially remaining ssRNAs (p. 32). How can an RNase discriminate between and ssRNA and dsRNA?
- In the description of *E. coli* transformation, what are the purposes of the heatshock and subsequent ice incubation steps (p. 34)?

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- You say (p. 35) that you had to repeat the PCR to amplify the Soc3 product. What did you do differently (if anything) to get the reaction to work the seond time?
- Figure 4.2 is very confusing. Why are you showing the top bands, which appear to have run through the wells if the second tier of the gel? Again, I would have cropped this part out.

- On page 38, you mention that HA-tagged *Zbtb32*had a 2 bp deletion and explained that it was most likely due to a faulty primer. Why do you think this is the reason?
- In Fig 4.3, there are 2 bands in all the "-" lanes? What are the identities of the 2 bands? Does one represent the supercoiled plasmid DNA? How does the mobility of these bands compare to the linearized plasmid? Why?
- In Figure 4.4, what do you think are the smears under the full length ssRNA bands?
- In Figure 4.5 B you show what I guess are the dsRNAs compared with dsDNA markers. Is this a fair comparison of mobility? Also, there are smears above the major bands (dsRNA?). What do you think these are?
- In Future Experiments (p. 42), you mention that you would alter PCR conditions to amplify the four other candidate genes. Can you please make some more concrete suggestions as how you would solve this problem.

In conclusion, I think that Ms. Ahiable adequately fulfiulled the requirements of a bachelor's thesis. She achieved the aims and conveyed a basic underdstanding of her project and technqiues employed. I would award the written thesis with the grade 2 to acknowledge her solid effort, but also places for improvement.

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