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## **Report on the bachelor thesis of Lena Graf.**

In the thesis entitled "Transformation of marine protist *Diplonema papillatum*-characterization of selected cell lines", the bachelor candidate Lena Graf presents the results of her detailed analyses of *D. papillatum* transformed cell lines by molecular biology techniques appropriate to undergraduate students. The thesis consists of usual parts from the introduction to references on 52 pages. Additionally, there is enclosed the article already submitted to Environmental Microbiology Journal, where Lena is one of co-authors.

In the introduction of her thesis, we can learn about the position of Diplonemids within the eukaryotic tree and their vast abundance in the deep water habitats. The heterotroph *D. papillatum* shares several features with parasitic Kinetoplastids such as the gene expression machinery with RNA editing and transplicing. The previous work with experiments on the transformation of the *D. papillatum* nuclear genome is also described in this part. From them, we find that the incorporation of a foreign DNA is quite random. This problem is then tried to be solved by an article called "Targeted integration by homologous recombination enables in-situ tagging and replacement of genes in the marine microeukaryote *Diplonema papillatum*". After the successful transformation of new constructs in order to knock-out or tag Tubulin genes, 8 cell lines were selected. The aim of the thesis was to verify improved *Diplonema* transformation of selected cell lines by PCR, RT-PCR (Splice leader confirmation), immunoblotting, as well as visualization by the microscopy.

Although working with this proposed model organism was not entirely simple, the results of described molecular techniques were successful and were included in the attached article. In my opinion the presented thesis is well written without any huge mistypes. The level of used English is nicely understandable with nice coherence and flow of the text. The knowledge content in the introduction is sufficient, nor long either short. As expected, the

student had to make an effort to write a thesis where there is more information than in the scientific article itself (mostly due to the limited number of words). For example, the explanation of why the ultrastructure of cell line 9 with knock-outed  $\alpha$ -tubulin was not affected.

Overall, here are questions that comes from my curiosity:

1) In discussion of the article is mentioned a third method how to incorporate exogenous DNA: retention of extrachromosomal plastid in the cell. We can say it is opposite approach to genome integration. Try to figure out how can *D. papillatum* be transformed by recombinant DNA, not incorporated into genome.

2) Finding that *D. papillatum* is sensitive to a large number of antibiotics is a good advantage for successful transformation. Would you know what other selective markers could be theoretically used in the transformation of free- living protists?

3) How you can test if the *D. papillatum* is haploid? Suggest some ideas.

In my opinion, bachelor candidate Lena Graf presented nice work on an interesting topic. She was able to learn a variety laboratory skills during her work on the project. Her thesis surely deserves to be accepted, and I hope she found enthusiasm in the molecular biology laboratory.

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