Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Sebastian Vranjes

Thesis title: In silico modelling of the effect of bacterial DNA fragmentation on gene counts using digital droplet PCR

Supervisor: Angel Roey, Dr. rer. Nat.

Referee: RNDr. Pavlína Věchtová, Ph.D.

Referee`s affiliation: University of South Bohemia in Ceske Budejovice, Faculty of Science

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages)**, balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length)**, logical structure of the thesis**	0-3	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2.5
Formal requirements – points in total		**17.5**
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	3

* Choose one

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	1.5
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2.5
Practical requirements – points in total		**22**
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(39.5)[2]**

Comments of the reviewer on the student and the thesis:

Presented bachelor thesis by Sebastian Vranjes deals with optimization of ddPCR as a tool for measurement of microbial population size. ddPCR is a cutting edge technique developed as an interesting and useful alternative to qPCR for genes and transcript quantification. As qPCR, also ddPCR presents specific advantages and limitations which have not been sufficiently addressed to date. This work aims at implementation of correction factor which should account for miscounted PCR hits caused by fragmented template DNA, which could occur during sample collection and template DNA extraction pipeline. Correcting for this limitation in this relatively new technique makes ddPCR very useful and promising tool not only for microbial community quantification. This work was done under the supervision of dr. Angel Roey. The thesis ranges 22 pages and thus complies with the formal requirements for bachelor thesis at the Faculty of Science JU.

The aim of the thesis is clearly defined, the objectives are given in three clear points. The introduction is four pages long and briefly describes the Prokaryotic group of organisms, the 16S rRNA marker gene and the principles of qPCR and ddPCR.
The Method chapter was replaced with a “Code” chapter and describes the flow of the code used to model entire process of sample acquisition, ddPCR and results collection and processing, all done in silico.
The Result chapter presents example output produced by 100 independent rounds of code executions summarized on 4 pages.
Discussion chapter along with the study limitations are written on two pages, followed by short conclusion and the list of 39 references.

The thesis is written very clearly using an excellent English. I did not find any factual or methodological problems and below I highlight a few formal flaws.

Chapter Introduction
As this thesis is purely methodological and done entirely in silico, It would be useful to introduce all methods used in the pipeline including in silico PCR and in silico genome fragmentation. These methods are not widely used and contains lots of variables that can influence their outcome.

[2] Enter the number of points awarded.

Chapter Code
Similarly to the Introduction chapter, also the Code chapter could include details about parameters used for in silico PCR, such as option for degenerated primer usage, number and position of mismatches allowed, etc. The same applies for the parameters of the SIPSim program.

Chapter results
As traditional PCR, also in silico PCR deserves some basic description, such as primer sequence, amplicon length, PCR program parameters, etc. As stated in the "Limitations" sub-chapter, the amplicon length and primers used are parameters affecting deficit in the number of hits in ddPCR results and hence they deserve to be introduced properly.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1. Please, introduce briefly principles and performance of in silico PCR program used in your code and the SIPSim program.

2. Your code takes into consideration only certain range of genome fragmentation, which represents an idealized model used for correction factor establishment. In real life, the degree of fragmentation can differ substantially and luckily, it can be estimated by simple agarose gel electrophoresis or using more sophisticated method such as microfluidic electrophoresis providing a DNA Integrity Number (DIN). Would it be possible to include the information about this specific range of genome fragmentation of particular sample into your pipeline and generate more accurate correction factor customized for each sample?

3. Could you, please, describe the importance of GC content for bacteria population characterization?

4. Do you know about studies investigating the effect of different DNA extraction techniques on the degree of genome fragmentation? If so, could you give a list of these techniques and order them from "harshest" to "mildest"?

Conclusion:

In conclusion, I find the thesis topic interesting and useful, the amount and the quality of the work is good. I would appreciate more elaborate introduction and more details in both Code and Result chapters. I again appreciate excellent English without any grammar errors and with minimum of typos. Therefore I

recommend / ~~do not recommend*~~

the thesis for the defense and I suggest the grade 1.[3]

[3] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).

In **České Budějovice** date **12. 9. 2022**

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