

**Supervisor's report on the master's thesis of Bc. Dominika Reichensdörferová entitled:
Development of DiCre parental lineage of *Babesia divergens*.**

Dominika started in our lab as a bachelor's degree student in 2017, when she first learned about the tick-borne *Babesia* parasites. She defended her Bc. thesis dealing with the isolation and purification of *Babesia* proteasomes as a novel drug target in 2019. This topic was a part of our that time finishing GACR standard grant. Dominika decided to continue in our lab as a Ms. degree student working on a novel and long-time prepared topic of functional genomic tools for *Babesia*, namely the establishment of the yet missing platform of conditional knock-out of genes in *Babesia divergens* blood stages by creating the DiCre expressing *B. divergens* parasite lineage. I was successfully using this newly established tool in *Plasmodium falciparum* and *Toxoplasma gondii* during my last postdoc stay at prof. Soldati-Favre lab in Geneva, in 2012-13. Based on the experience and recommendations from authors of these parasite strains (team of prof. Mike Blackman and team of prof. Marcus Meissner, respectively), I have found the Cre/LoxP perfectly suitable to evaluate the role of essential genes in our *Babesia* parasites. However, ...

Although I would not highlight this in the case of some other student, in the case of Dominika I must say that she got massively unlucky, and some basic negative circumstances must be mentioned prior the evaluation of this thesis. 1. **Timing**- her time in the lab for conduct the experiments for this **thesis perfectly matches the lifespan of the COVID-19 pandemic**, when her colleagues including myself had to often stay home with kids, got multiple times quarantined etc. 2. **Her topic was supposed to be covered by two standard GACR grants and one Mobility plus grant with the Japan Society for the Promotion of Science.** The last listed was hardly affected by absolute travel restrictions to Japan in 2021. First one is the known case at BC, as it was not, by an unbelievable mistake of the BC project department, send to GACR on the final day for submission, although the email confirmation was sent out to me twice. Thus, in 2020, for one year of her Ms. program, we had to limit our experiments to some "basal metabolism". This was kindly, though only partially, enabled by the holders of another large grant at the Institute of Parasitology BC CAS. A second GACR project, supporting this topic, was achieved the year after (starting 2021), but was postponed by one year /started 2022) due to 3. **the maternity leave of her co-supervisor Dr. Jalovecká.** This was further supported by the **continuous maternity leave of the main postdoc** working in my lab, Dr. Luise Robbertse since then. 4. **Due to the missing GACR founding** we had to use **another project** to order the full Di-Cre cassette construct. Due to restrictions in the form of **public procurement** we could not use DNA construct synthesis by a well-established Germany based company and we ended up with a Canadian -Chinese company, which postponed the order (from 22 delivery days to 6 months!!!) and finally totally messed up the synthesis (I am really glad we have saved **30 kCZK** to spent another **200 kCZK** in subsequent experimental procedures due to the byrocratic requirements of the CZ Ministry of Education and sports btw.)

Despite the negative circumstances, Dominika anyway represented a valuable member in the lab. Although we initially planned to show the DiCre *Babesia divergens* strain and the excision of the episomally transfected LoxP flanked green fluorescent protein as a "cherry on the pie of this thesis", it has not been achieved. Instead, she was "pressed to sacrifice" her master thesis ambitions to reconstruct the original DiCre cassette holding vector using the delivered "Made in China" plasmids A and B with partial sequences as templates. The defended thesis is thus mostly dedicated to this "cloning & reconstruction" of the original plasmid vector. To add on, Dominika was able to assemble this thesis in English language mostly by her own which further highlights her scientific skills. To sum up, I do not wonder that Dominika has decided to not continue in her PhD program, although she belongs to the most skilled persons within our team and would be generally welcomed to stay as a PhD student in our lab. I feel sorry to see her go, and I wish her the very best for both her personal life and professional carrier, wherever she goes and whatever she will decide to do in her future.

In České Budějovice, 24. 5. 2022,

RNDr. Daniel Sojka, Ph.D.