NRCPD-OUAVM Joint Research Report

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Project no: 2019-joint-2

1. Principal investigator

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Position: Research Scientist – Laboratory of Vector Immunology

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2. Project title:

The development of a DiCre recombinase-expressing strain of *Babesia* for the creation of conditional gene knockouts.

3. Collaborating research group members at NRCPD

Name: Prof. Shin-Ichiro Kawazu and Dr. Masahito Asada

Position: Professor and Associate Professor

4. Research period (in mm/dd/yyyy, and total number of years)

01/01/2019 -12/31/2021

3 years

5. Purposes and objectives

The major objective of this project is the development of novel functional genomic tools for tick-borne *Babesia* parasites, namely the creation of a stable transgenic DiCre recombinase-expressing strain(s) of *Babesia*. DiCre conditional recombinase system enables functional analysis of indispensable parasite genes where conventional non-inducible knock-out systems cannot be used. This technique has been previously applied to Apicomplexa model species *Toxoplasma gondii* and *Plasmodium falciparum* but *Babesia* recombinase-expressing strain has not yet been introduced.

The individual objectives of this project include (i) the design and cloning of Babesia plasmid constructs allowing for the integration of both Cre subunits into the same genomic locus of selected *Babesia* species, (ii) generation of “parental” DiCre parasite line(s) incl. optimization of transfection strategy for *Babesia*, (iii) implementation of the loxP sites into the parasite via the both episomal and intragenomic approaches to verify recombinase activity, and (iv) performance of conditional knock-out(s) of selected *Babesia* target genes.

6. Outline of research process

The first collaborative visit of two Institute of Parasitology, Biology Centre, the Czech Academy of Sciences (IoP BC CAS) research team members, Dr. Sojka and Dr. Jalovecká, to the NRCPD laboratory of prof. Kawazu took place between October 27 and November 20, 2019. The short research stay of two members was enabled by partial funding of the mobility costs from the IoP BC CAS funding resources. The research stay was primarily focused on the determination of optimal research strategies and the initial objective assessment including methodological training of techniques necessary for transgenic *Babesia* preparation. During this visit, we agreed to establish stable transgenic DiCre recombinase-expressing strain(s) of two *Babesia* species – *Babesia divergens* (Dr. Sojka and Dr. Jalovecká, Institute of IoP BC CAS, Czech Republic) and *Babesia bovis* (Prof. Kawazu and Dr. Asada, NRCPD-OUAVM, Japan). This agreement reflects the model species that have already been established in both laboratories and, thus, minimize the time that would be necessary for introduction of novel *Babesia* species.

Since the home laboratory at IoP BC CAS enables working with *ex vivo* cultures of *B. divergens*, the team of Dr. Sojka follows a pioneer mission in the development of specific transgenic tools for this particular species. In the first year of the project, the team has already identified and sequenced several *B. divergens*-specific promoters. Among selected were those that drive activity of *actin* (Bdiv_007890), *elongation factor-1α* (EF-1α, Bdiv_030590) and *hsp70* (heat shock protein, Bdiv_029570) genes as those particular promoters showed high activity in other *Babesia* species. Other newly identified promoters are those controlling for the expression of *B. divergens calmodulin* (Bdiv_005010c) and *chloroquine resistance transporter* (Bdiv_036760) because such promoters are commonly used in *Babesia*-related *Plasmodium* models. In addition, the team has also successfully amplified and sequenced *B. divergens* 3' UTRs (untranslated regions) of the above-mentioned genes. Another primary objective was testing the sensitivity of *B. divergens* to selection markers used for plasmid transfection of other Babesia species. We successfully validated the effect of Blasticidin-S-Deaminase (BSD) and WR99210 for *B. divergens*. At the moment the last step prior final plasmid construction is the determination of optimal concentrations of these selection markers for their direct use in transfected *B. divergens* bovine erythrocyte cultures. To fulfill this task, we have recently introduced and optimized the technique of rapid parasitemia levels detection by flow cytometry analysis.

The recipient locus for DiCre cassette has already been selected based on the communication with Dr. Collins (research group of Dr. Blackman, Francis Crick Institute, London, UK) who is one of the major team members behind the introduction of the DiCre system to *P. falciparum* (Collins et al. 2013 Mol Microbiol). The most recent experimental design protocol employs the putative *Bd-6cys-E* gene (Bdiv_04560c) displaying high homology with the *P. falciparum p230p* gene (PF3D7_0208900) that has been used as stable recipient locus for the DiCre cassette in this malaria parasite. Moreover, targeted disruption of *B. bovis Bbo-6cys-E* gene (BBOV_II006600), an orthologue of Bdiv_044560c and PF3D7_0208900 genes, indicated its dispensability for parasite blood stages. The design of construct for the generation of parental DiCre recombinase-expressing *B. divergens* lineage is depicted in Figure 1.

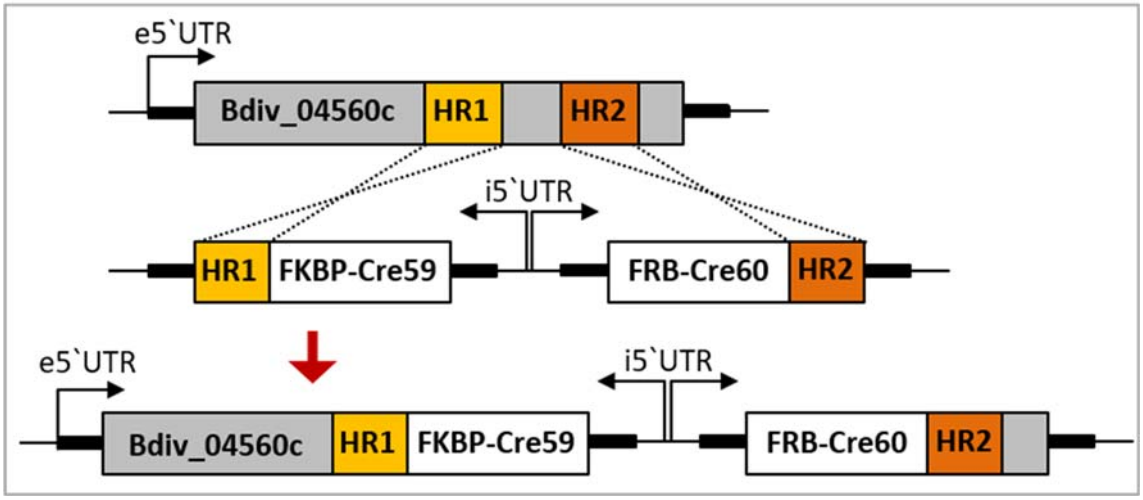

Figure 1. Establishment of the inducible knock-out DiCre system in *B. divergens*.

Schematic representation of the selected strategy to develop the parental *B. divergens* DiCre line. Bdiv_044560c gene represents the recipient locus for rapamycin-binding FKBP-Cre59 and FRB-Cre60 proteins under the control of inserted *B. divergens*-specific promoters. HR1 and HR2 represent homologous regions, i5`UTR = inserted promoter; e5`UTR = promotor of the endogenous *B. divergens* gene.

7. Outline of research achievements

- *B. divergens* selected as the model *Babesia* species
- *B. divergens*-specific promoters identified and sequenced
- *B. divergens*-specific 3' untranslated regions identified and sequenced
- Flow cytometry protocol for parasitemia determination optimized for *B. divergens*
- Blasticidin-S-Deaminase (BSD) and WR99210 selection markers validated for *B. divergens*
- Optimal selection marker concentrations determination for their use in *B. divergens* cultures in progress
- Recipient locus for DiCre cassette selected
- Final plasmid construct for the generation of parental DiCre *B. divergens* lineage designed and under construction (cloning)

8. Publication of research achievements

Publications from this project should await future collaboration between the two groups. The two team members gave two seminar talks during their visit to NRCPD, OBIHIRO: Jalovecká et al.: Establishment of Babesia laboratory model and its experimental application; Sojka et al.: Proteolytic targets in ticks and tick-borne diseases.

Attach reference materials as necessary.