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学　位　論　文　要　旨

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論文題目：Establishment of transient and stable transfection systems for genetic manipulation of *Babesia gibsoni*（バベシア・ギブソニの遺伝子操作のための一過性及び安定したトランスフェクションシステムの確立）

要旨

Babesiosis is a tick-transmitted, zoonotic disease caused by hematotropic parasites of the genus *Babesia*. *Babesia* parasites are the second most common and widespread blood parasites in the world, after trypanosomes. Consequently these parasites have considerable medical, economic and veterinary impact. More than 100 *Babesia* species have been described which infect many types of mammalian hosts, even in several species of birds. The increasing reported cases of babesiosis may be due to the rise in actual incidence, as well as the enhanced awareness of this disease. Despite the prevention and diagnostic advances resulting from the extensive research and a greater understanding of this disease, babesiosis continues to significant economic and medical impacts.

Babesia gibsoni is a tick-transmitted intraerythrocytic protozoan parasite that causes piroplasmosis in dogs. Infected dogs display serious clinical signs, including remittent fever, hemoglobinuria, progressive anemia, marked splenomegaly and hepatomegaly, and sometimes causing mortality. *B. gibsoni* is widely distributed in Asia, including Korea, Japan, Malaysia and Taiwan. Recently, *B. gibsoni* has also been reported in Bangladesh and India. Previously, transmission of *B. gibsoni* in fighting dogs was thought to occur by biting wounds, rather than by ticks. However, one epidemiological survey of *B. gibsoni* infection indicated that tick infestation was the most dominant risk factor for *B. gibsoni* infection in non-fighting dogs. The other routes of *B. gibsoni* transmission, such as fighting and transplacental transmission appeared to be less important in ordinary dogs in Japan. With efficacious drugs and reliable rapid diagnostic kits yet to be developed, there is a need to further explore the genome of *B. gibsoni* for potential vaccine and diagnostic antigens. However, the success of such approach will depend largely on identifying immunoprotective antigens that are stable, especially against positive selection pressure.

Genetic manipulation techniques, such as transfection, have been previously reported in many protozoan parasites. In *Babesia*, stable transfection systems have only been established for bovine *Babesia* parasites. The establishment of transfection system for *B. gibsoni* is considered to be urgent in

order to improve our understanding of the basic biology of canine *Babesia* parasites for a better control of babesiosis.

The objectives of the present study can be summarized as follows: (1) to establish a useful transient transfection system of *B. gibsoni*, towards a stable transfection method of *B. gibsoni*; (2) to identify and characterize of interchangeable cross-species functional promoters between *B. gibsoni* and *B. bovis*; (3) to establish a stable transfection system for *B. gibsoni*, towards a further genome editing of *B. gibsoni*.

In chapter 1, I established the successful transient transfection of *B. gibsoni*. The plasmid containing the firefly luciferase reporter gene (pBS-ELA) was transfected into *B. gibsoni* by an AMAXA 4D Nucleofector™ device. Transfection using program FA113 and Lonza buffer SF showed the highest luciferase expression. Twenty micrograms of plasmid produced the highest relative transfection efficiency. The fluorescent protein-expressing parasites were determined by GFP-containing plasmid (pBS-EGA) at 48 and 72 hours post transfection. This finding is the first step towards a stable transfection method for *B. gibsoni*, which may contribute to a better understanding of the biology of the parasite.

In chapter 2, I investigated 6 homologous and 6 heterologous promoters for *B. gibsoni* and *B. bovis* and identified novel interchangeable cross-species functional promoters between *B. gibsoni* and *B. bovis*. Ten out of twelve promoters had heterologous promoter function. In particular, *Bg 5′-ef-1α* and *Bg 5′-actin* heterologous promoters resulted in a significantly higher luciferase activity than *Bb 5′-ef-1α* homologous promoter in *B. bovis*. The present study showed that *Bg 5′-actin* promoted the highest luciferase activity in both *B. gibsoni* and *B. bovis*. The study further indicates that heterologous promoter function widely exists between *B. gibsoni* and *B. bovis*. This finding is an important step for future stable transfection construct design and for the production of vaccines based on transfected *B. gibsoni* and *B. bovis* parasites.

In chapter 3, I generated a plasmid construct in which the 5′-intergenic (IG) region-B of the *ef-1α* gene (5′-*ef-1α*) drives the *gfp* reporter gene, and the 5′-*actin* promotes the expression of the selection marker *hdhfr*. The plasmid was designed for integration into the *ef-1α* locus of *B. gibsoni* genome by double cross-over homologous recombination. Linearized plasmid was transfected by 4D Nucleofector™ into *in vitro* cultured *B. gibsoni* and 10 nM WR99210 was added for drug selection two days after transfection. GFP-expressing parasites were observed by fluorescence microscopy as early as two weeks after drug selection, and consistently expressed GFP for more than 3 months without drug pressure. Genome integration was confirmed by PCR, sequencing and Southern blot analysis. This finding will facilitate functional analysis of *Babesia* genomes using genetic manipulation and will serve as a foundation for the development of tick-*Babesia* and host-*Babesia* infection models.

Overall, a host-*Babesia* infection model may be easier to achieve using canine *Babesia* rather than bovine *Babesia* because using dogs for animal experiments is more feasible than using cattle. The urgently needed genome-edited host-*Babesia* infection model may help us monitor transmission *in vivo*, investigate mechanisms of infection and immunity, and also improve the development of novel strategies for controlling babesiosis. Transfection systems improve our understanding of the molecular biology of parasites and pave the way for genetic manipulation. The application of transfection systems can also lead to a better understanding of the mechanisms underlying drug resistance and provide novel information for vaccine development and drug target discovery. Currently, there is a lack of genetic manipulation technique for *B. gibsoni*. In order to fill this gap, I describe herein the development of a stable transfection system for *B. gibsoni*.

In summary, I established the transient and stable transfection systems of *B. gibsoni* parasite, which successfully integrated exogenous genes into the *B. gibsoni* genome. The genetic manipulation method described in this study will serve as the knockout parasite construction, which is widely used in analyzing the gene function and drug target discovery.

備考　1　論文題目が英語の場合には，（　）書きで和訳を付す。

2　博士論文については，日本語の場合１８００～２２００字，英語の場合１０００～１４００語とする。修士論文については，それ以下でもかまわない。

3　図表は，要旨には記載しないこととする。

4　枚数は１枚を超えても差し支えない。