Transgene integration into the ovalbumin locus of chicken cells using CRISPR/Cas9 system for transgenic chicken bioreactors

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論 文 名　：Transgene integration into the ovalbumin locus of chicken cells using CRISPR/Cas9 system for transgenic chicken bioreactors
（トランスジェニックニワトリバイオリアクターのための CRISPR/Cas9 システムを用いたオボアルブミン遺伝子座への遺伝子組込みに関する研究）

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論　文　内　容　の　要　旨

Transgenic chickens have been expected to be used as living bioreactors for the production of biopharmaceutical proteins. Development of a tissue-specific expression system of exogenous genes is a major concern in the construction of transgenic chicken bioreactors. For this purpose, it is important to develop a transgene integration method with high efficiency and specificity of expression. In recent years, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system has attracted much attention as a versatile genome editing tool because of its ease of handling and simplicity. In this thesis study, the CRISPR/Cas9 system was applied to targeted knock-in of transgene into the ovalbumin (*OVA*) locus of chicken cells. The guide RNA sequences (gRNAs) of the CRISPR/Cas9 system against the *OVA* prompter were designed and evaluated for oviduct-specific expression of exogenous gene. A reporter gene expression cassette was integrated into the *OVA* locus of chicken cells using CRISPR/Cas9 system mediated by homology-independent targeted integration (HITI). For the knock-in cells, the transgene expression was successfully induced by activation of the endogenous *OVA* promoter using the CRISPR transactivation system. These results would contribute to studies in generating transgenic chicken bioreactors and activation of tissue-specific promoters for the production of pharmaceutical proteins.

Chapter 1 introduces general information on the recombination protein expression system, including the most common expression systems of *E. coli*, *Pichia pastoris*, baculovirus/insect cell, mammalian cells, and transgenic chicken oviduct bioreactor. Additionally, the difficulty in the generation of transgenic chicken is introduced. Furthermore, the mechanisms of gene editing methods are also discussed.

In Chapter 2, the techniques and mechanisms related to the study in this thesis are reviewed.

In Chapter 3, the selection of high efficiency gRNAs for the ovalbumin and lysozyme genes in the CEF cells is discussed. The HITI pathway was used to evaluate the knock-in efficiency of an exogenous gene. We obtained gRNA sequences in chicken ovalbumin, which are 31.1%. We utilized the gRNA locus in ovalbumin gene to knock-in EGFP cassette into DF-1

cells via homology-independent targeted integration. In total, 20 clones in 1×10^5 cells contained the knock-in gene, which was confirmed by genome PCR test and FACS.

In Chapter 4, dCas9 system with 5 gRNA sequences around the ovalbumin gene promoter (TATA box) were co-cultured into CEF and DF-1 cells to initiate ovalbumin protein expression. RT-PCR, qRT-PCR, and WB analyses revealed that the dCas9 system could activate ovalbumin gene expression with high efficiency.

In Chapter 5, An *EGFP* gene expression cassette was introduced into the *OVA* locus of chicken DF-1 using the CRISPR/Cas9 system via homology-independent targeted integration. *EGFP* expression was successfully induced in the knock-in cells by activating the endogenous *OVA* promoter using the dCas9-VPR transactivation system. The combination of gRNAs designed around the *OVA* TATA box was important to induce the endogenous *OVA* gene expression with high efficiency

In Chapter 6, the contents of this study are summarized.