論文 / 著書情報
Article / Book Information

題目(和文)	枯草菌ゲノムベクターシステムを用いたクラスⅠ嗅覚受容体遺伝子の発現制御領域の解析
Title(English)	Studies on the cis-element for mouse class I odorant receptor genes using the Bacillus subtilis genome vector system
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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	生物プロセス　専攻	申請学位（専攻分野）： Academic Degree Requested	博士　（　工学　） Doctor of
学生氏名： Student's Name	岩田　哲郎	指導教員（主）： Academic Advisor(main)	廣田　順二
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Technological developments in chromosome engineering are essential for the manipulation and functional analysis of genomic DNA fragments. Artificial chromosomes, such as bacterial and yeast artificial chromosomes (BACs and YACs), have been used for these purposes in combination with transgenesis. However, there exist several technological limitations in cloning size, genetic modification and insert stability. The *Bacillus subtilis* genome (BGM) vector is a novel cloning system for large DNA fragments, in which the entire 4.2 Mb genome of *B. subtilis* functions as a vector. The BGM vector system has several attractive properties, such as a large cloning capacity of over 3 Mb, stable propagation of cloned DNA and various modification strategies using RecA-mediated homologous recombination. However, genetic modifications using the BGM vector system have not been fully established, and this system has not been applied to transgenesis. To explore the potential of the BGM vector, I focused on the mouse class I odorant receptor (class I OR / fish-like OR) family, which consists of 158 genes and forms a single gene cluster in the genome. Although a *cis*-acting locus control region is expected to activate singular OR gene expression, such element has not been experimentally identified yet. In this study, I provided a complete genetic modification method of cloned DNA fragments, including insertion, deletion, inversion and fusion to elongate a DNA fragment. I further demonstrated that the modified, enlarged genomic DNA fragments could be used to generate transgenic mice.

First, I developed a complete genetic manipulation method for the BGM vector system by demonstration of a target insertion and an inversion modification. Using two contiguous BAC clones containing several class I OR genes, I constructed two transgenes in the BGM vector by inserting a reporter gene cassette *IRES-tauEGFP* into one class I OR gene, *MOR42-3*. Because they were oriented in opposite directions, I aligned their orientation by inversion modification, and reconstructed a ~252 kb transgene of a part of class I OR gene cluster for transgenic reporter assay. The DNA sequencing analysis of the constructed transgenes revealed that no mutations occurred during gene manipulation and all targeted modifications were precisely conducted.

Second, I applied the BGM vector system to mouse transgenesis (BGM transgenesis). I established a new protocol to purify BGM transgenes from the BGM vector. I succeeded to establish several transgenic lines by the pronuclear-microinjection of the purified transgenes into mouse eggs. Transgenic mice carrying the enlarged transgene recapitulated the expression and axonal projection patterns of the target class I OR gene in the main olfactory system, indicating that a *cis*-acting element for the class I OR gene is present in the extended region from the original transgene.

Third, to identify the *cis*-acting element for the class I OR gene, I performed the deletion mutagenesis of the BGM transgene and the bioinformatic analyses. I successfully shortened the putative *cis*-acting element by several transgenic analyses. Functional analyses revealed that the identified sequence was necessary for the transgene expression and was capable of recapitulating the expression pattern of class I OR genes in the main olfactory epithelium.

Through this study, I demonstrated the utility of the BGM vector for engineering large DNA fragments to modify and reconstruct genomic structure and to generate transgenic mice. Using the BGM vector system, I provided the first experimental evidence of a *cis*-acting element for class I OR gene expression. Since this system can be applied to any BAC and other library resources of any species, the BGM vector system provides a novel tool for recapitulation of genomic structure and functional studies of genomic DNA.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).