NIPPON Genetics EUROPE

Application

Insertion of mouse-derived osteoblast specific gene into expression vector

Product

FastGene® Gel/PCR Extraction Kit（FG-91302）
FastGene® Plasmid Mini Kit（FG-90502）
FastGene® Gel cutter（FG-830）

Manufacturer

NIPPON Genetics EUROPE

The following data has been posted by the courtesy of customers of K university, Japan.

Overview

For the mechanism of the development of osteoporosis and the function analysis of osteoblasts etc., elucidation of the molecular mechanism by basic research has become necessary, and research in mice and clinical research is under way now.
In this application note, samples were prepared to analyze the function of mouse osteoblast.
After identifying the expressed gene in the osteoblast by microarray and cloning into the expression vector, the gene was introduced into cultured cells and functional analysis was performed.
In this application note, we introduce actual examples where FastGene® products could be widely used in the experimental flow of general sample preparation up to the DNA sequence.

Experimental preparation

● Sample:	Osteoblasts cultured from mouse skull (primary culture cells)
E.coli strain:	DH5α
Vector:	pcDNA3.1（5.3～5.4 kb）* Not including insert (1.7 kb)
● PCR Enzyme:	KAPA HiFi HS ReadyMix（KK2601, KK2602）
	KAPA 2G Fast HotStart PCR Kit without dNTP（KK5523）
● Nucleic acid purification:	FastGene® Gel/PCR Extraction Kit (FG-91302)
	FastGene® Plasmid Mini Kit (FG-90502)
● Ladder:	1 kb DNA Ladder（MWD1）
Gel cutting:	FastGene® Gel cutter（FG-830）
● Nucleic acid stain reagent:	SAFELOOK™ pre-green nucleic acid stain (Wako Pure Chemical Industries, Ltd.)
Illuminator:	UV
● PCR apparatus:	T100™ thermal cycler（Bio-Rad）
	GeneAmp PCR system 9700（Applied Biosystems）

Protocol

1. RNA extraction
2. cDNA synthesis
3. PCR reaction —— cDNA was amplified using KAPA HiFi HS ReadyMix
4. Electrophoresis —— The gel was cut out with a Fast Gene® gel cutter. (Result 1)
5. Gel extraction —— Gel extraction was performed using FastGene® Gel/PCR Extraction Kit (elution volume 30 µL)
   (* 100 µL of isopropanol was added at the time of sample preparation in order to raise the recovery rate.)
6. Restriction enzyme treatment —— cut to the desired gene size with restriction enzymes.
7. Ligation —— The sample was ligated into the vector pcDNA 3.1 (insert size 1.7 kb).
8. Transformation into E.coli —— E.coli DH5α was transformed, plated and cultured.
9. Colony PCR —— Amplified by KAPA 2G Fast HotStart PCR Kit.
10. Size check by electrophoresis (insert check)
11. E.coli culture —— cultured in 3 ml LB medium
12. Plasmid purification —— FastGene® Plasmid Mini Kit was used to purify 2 mL of culture.
    (* 50 µL of elution buffer was used and heated to 50°C in order to raise the recovery rate)
13. Size checked by electrophoresis（plasmid check）(Result 2)
14. Sequencing —— DNA concentration and purity were measured by Nano Drop.
    and submitted for sequencing (Result 3 and 4)
15. Gene transfer into cultured cells

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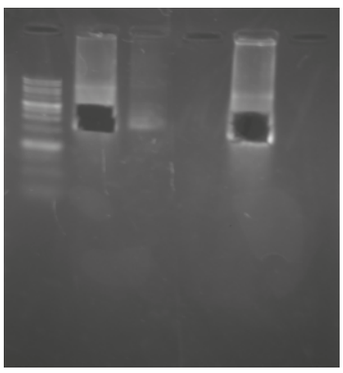
FastGene®
Plasmid Mini Kit
(FG-90502)

Result

Result 1

Cut by FastGene® gel cutter



Result 2

Electrophoresis of protocol 13

①：No cutting of sample ②
②：+*Nhe* I /*Xho* I （Sample 1）
③：+*Nhe* I /*Xho* I （Sample 2）

Result 3

DNA concentration / purity

Sample name	DNA (ng/uL)	A260/A280	A260/A230	A260	A280
Sample 1	156.492	1.895	2.304	3.13	1.651
Sample 2	195.499	1.883	2.297	3.91	2.076

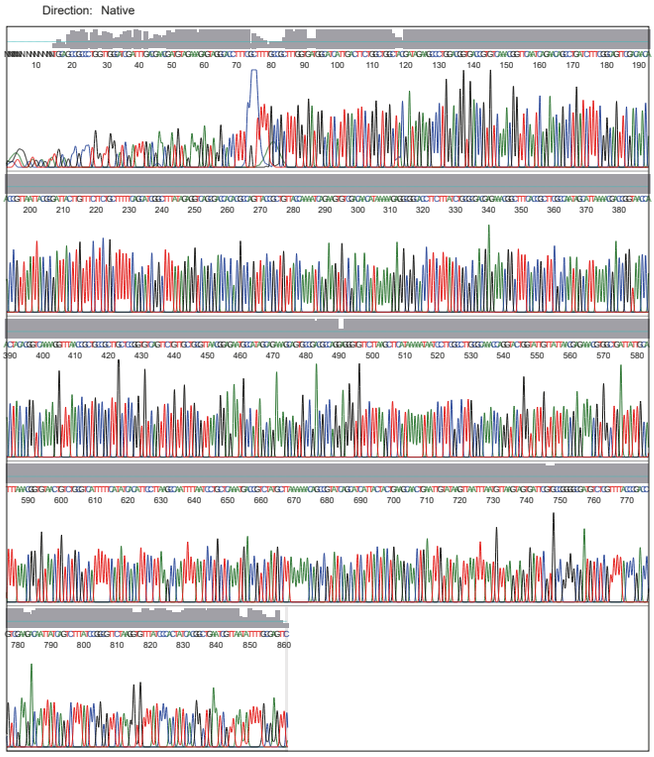
Result 4

Sequencing

Sample 1

Geospiza www.geospiza.com

Signal Strengths: A = 793, C = 739, G = 935, T = 759
Lane/Cap#: 87
Matrix: n/a
Direction: Native



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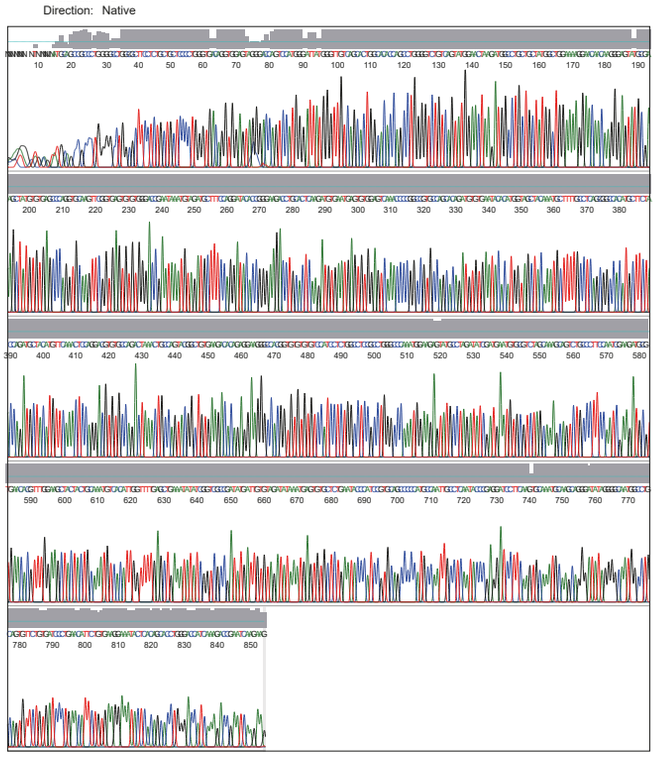
Sample 2

Geospiza www.geospiza.com

Signal Strengths: A = 717, C = 591, G = 973, T = 579
Lane/Cap#: 85
Matrix: n/a
Direction: Native



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As a result of the sequencing, it was confirmed that sample 1 is a genome derived from Eschericia coli and Sample 2 is the gene of interest.

Customer comment

I began research in the field of bone metabolism last year. We are developing research using the technology we possess. I think that your products are economically and of excellent quality.

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu
TEL +49 2421 554960 FAX +49 2421 55496 11 info@nippongenetics.eu