



Title	Mannose-recognition mutant of the galactose/N-acetylgalactosamine-specific C-type lectin CEL-I engineered by site-directed mutagenesis
Author(s)	Moriuchi, Hiromi; Unno, Hideaki; Goda, Shuichiro; Tateno, Hiroaki; Hirabayashi, Jun; Hatakeyama, Tomomitsu
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CEL-I-F

CGCAT 5  
NdeI

ATGAACCAGTGCCCGACCGATTGGGAAGCGGAAGGCGATCATTGCTATCGCTTTTTTAAC 65  
M N Q C P T D W E A E G D H C Y R F F N 19  
-1 1

ACCCTGACCACCTGGGAAAACGCGCATCATGAATGCGTGAGCTATAGCTGCAGCACCCCTG 125  
T L T T W E N A H H E C V S Y S C S T L 39

AACGTGCGCAGCGATCTGGTGAGCGTGCATAGCGCGGCAGAACAGGCGTATGTGTTTAAC 185  
N V R S D L V S V H S A A E Q A Y V F N 59

TATTGGCGTGGTATTGATAGCCAGGCTGGCCAGCTGTGGATTGGTCTGTATGATAAATAT 245  
Y W R G I D S Q A G Q L W I G L Y D K Y 79

AACGAAGGCGATTTTATTTGGACCGATGGCAGCAAAGTGGGCTATACCAAATGGGCGGGC 305  
N E G D F I W T D G S K V G Y T K W A G 99

W105H-F

GGCGAACCGAACAACCATAACAACGCGGAAGATTATGGCCAGTTTCGCCATACCGAAGGC 365  
G E P N N H N N A E D Y G Q F R H T E G 119  
W105H-R

GGCGCGTGGAACGATAACTCCGCCGAGCGCAAGCGAAATATATGTGCAAACCTGACCTTT 425  
G A W N D N S A A A Q A K Y M C K L T F 139

BamHI  
GAATAAGGATCCCGGGATCC 445  
E \* CEL-I-R 140

Supplementary figure. The nucleotide and amino acid sequences of EPNH-CEL-I. Oligonucleotide primers used for synthesizing the 5'-terminal and 3'-terminal DNA fragments of the EPNH-CEL-I gene are indicated by arrows. The EPNH-CEL-I gene was amplified by PCR using the EPN-CEL-I gene as a template. The mutation sites ("EPN" and "H") and restriction sites (*NdeI* and *BamHI*) are enclosed within boxes. The amino acid residues are numbered according to that of native CEL-I without an initiator methionine residue.

Supplementary table

PCR primers for amplification of the CEL-I mutant genes

Primer	Nucleotide sequence
CEL-I-F	5'-CGCATATGAACCAGTGCCCGACCGATTGGG-3'
CEL-I-R	5'-GGATCCCGGGATCCTTATTCAAAGGTCAGT-3'
W105H-F	5'-CGAACCGAACAACCATAACAACGCGGAAGA-3'
W105H-R	5'-TCTTCCGCGTTGTTATGGTTGTTTCGGTTCG-3'
W105Y-F	5'-CGAACCGAACAAC TATAACAACGCGGAAGA-3'
W105Y-R	5'-TCTTCCGCGTTGTTATAGTTGTTTCGGTTCG-3'
W105A-F	5'-CGAACCGAACAACGCCAACAACGCGGAAGA-3'
W105A-R	5'-TCTTCCGCGTTGTTGGCGTTGTTTCGGTTCG-3'