

RIKEN Clone ID : IRAK004N05

Vector : pCMV-SPORT6

Gene	MLL5		
Accession No.	BC001296.2	1978 bp	1..>1978
	<i>CDS</i>	1482 bp	497..>1978

● Plasmid DNA purification

Date : 121025

Culture : LB (100 ug/ml Ampicillin) 5 ml -> at 37 deg C over night

Date : 121026

Purification : QIAGEN Miniprep kit -> dH₂O 100 ul

● Digestion by restriction enzyme/Concentration calibration

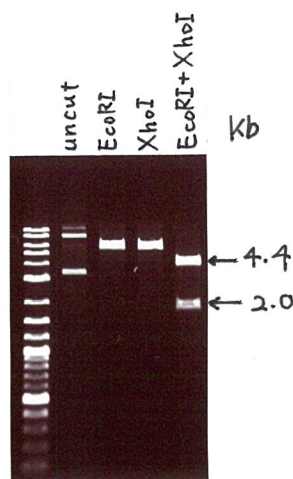
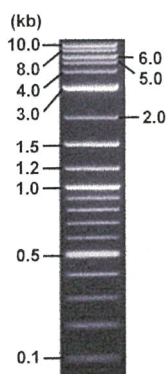
Date : 121026

DNA concentration (O.D.): 38 ng/ul

DNA	1	ul
Enzyme (EcoRI + XhoI)	0.5 + 0.5	ul
Buffer H	1	ul
dH ₂ O	7	ul
Total	10	ul

Electrophoresis : 1% agarose gel, 1x TAE Buffer

Marker : 2-Log DNA Ladder (NEB#N3200L)



<Expected size of fragment from BC001296.2 >

4339, 2021 bp

● Confirmation of the insertion sequence

Date : 121101

Primer A	Reverse2
Primer B	M13
Primer C	-
List of Sequencing Primers	http://dna.brc.riken.jp/en/GNPclone3en.html

● Adjustment of DNA concentration

Shipping amount : 40 ul

Concentration at the time of preparation of plasmid DNA : 25 ng/ul

Date : 121105

DNA (<u>38</u> ng/ul)	75.0	ul
10x TE	11.4	ul
dH ₂ O	27.6	ul
Total	114.0	ul

APPROVED BY :



Primer B : M13

5' GTTTTCCCAAGTCACGACGTTTGTA 3'

CGGC FG T C AGC TTATA TAC GACTCAC TATA GGG ACCACTT TGTACAA GAAA GCTGGG TACGGCTAAGCT TGGGGCCCTCGAGG GFTACTCTAGAGCGGCCGCC

1 11 21 31 41 51 61 71 81 91 101

attB2 MluI ApsI XbaI NotI

111 121 131 141 151 161 171 181 191 201 211

TTTTTTTTTTTCCCGTCTGGTCTCAATAACCACCTTGTATTTTCCATGGATTCA GAACCTAAGCTTTTAAACAAGGGCAGCTGGTTTCTTTGAGGCATGCACAGTCC

0 510 1020

GTTACTTACAATTTCCATAGTCAAAATCA AAGGCAATAGTAATTTCA GTTCCCTTTG GAATACTGTGTAAGAATTAATAAGATGTATGGTTCCATCTGAAATTTCA

231 241 251 261 271 281 291 301 311 321 331

0 510 1020

CACCTCTGCATTGGGTTGTAACA GACCGCCTGATGAATCGAAGCCTCATTTCCCAAAGTCTTG CATCAACA CAATTTCTAAGCCCATGAAATTTAGAGTAATAACA

341 351 361 371 381 391 401 411 421 431 441

0 510 1020

GGGTATGCTCTTTTAAAGAAATACCCTTTGCTTCAAACTGTTCTCTCAAGCATTAACCTCCCTCTGTATTC AATGATAAGTGCA TCA GGAAGCCAAATCTTTTGCA GAT

451 461 471 481 491 501 511 521 531 541 551

0 510 1020

AATTTTCTTATTTCTTTGTAATATGGCTCTCTACAGGAGGTTTGAAGAGCAAAATGTTGGTATTC A AATCGGATCTCTTTTGTCA TTTCCATTGCGCCTA

571 581 591 601 611 621 631 641 651 661

0 510 1020

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