

**RIKEN Clone ID : W01A054M07**

Vector : pENTR/D-TOPO

Gene	PITX1		
Accession No.	BC003685.1	1299 bp	1..1299
	<i>CDS</i>	945 bp	135..1079

● Plasmid DNA purification

Date : 130306

Culture : LB (25 ug/ml Kanamycin) 5 ml -> at 37 deg C over night

Date : 130307

Purification : QIAGEN Miniprep kit -> dH<sub>2</sub>O 100 ul

● Digestion by restriction enzyme/Concentration calibration

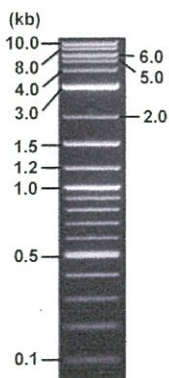
Date : 130307

DNA concentration (O.D.): 121 ng/ul

DNA	0.2	ul
Enzyme (NotI + BssHII)	0.5 + 0.5	ul
Buffer H	1	ul
dH <sub>2</sub> O	7.8	ul
Total	10	ul

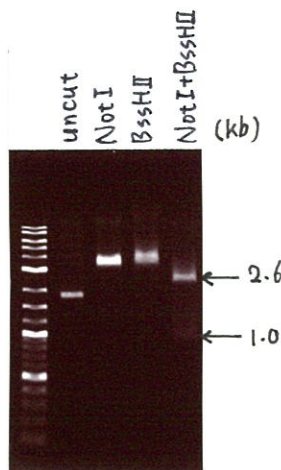
Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



<Expected size of fragment from BC003685.1 CDS>

2553, 1045 bp



● Confirmation of the insertion sequence

Date : 130313

Primer A	M13
Primer B	T7 long
Primer C	-
List of Sequencing Primers	<a href="http://dna.brc.riken.jp/en/GNPclone3en.html">http://dna.brc.riken.jp/en/GNPclone3en.html</a>

● Adjustment of DNA concentration

Shipping amount : 40 ul

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

Date : 130314

DNA ( <u>121</u> ng/ul)	92	ul
10x TE	44.5	ul
dH <sub>2</sub> O	308.8	ul
Total	445.3	ul



APPROVED BY :

<http://dna.brc.riken.jp/index.html>

