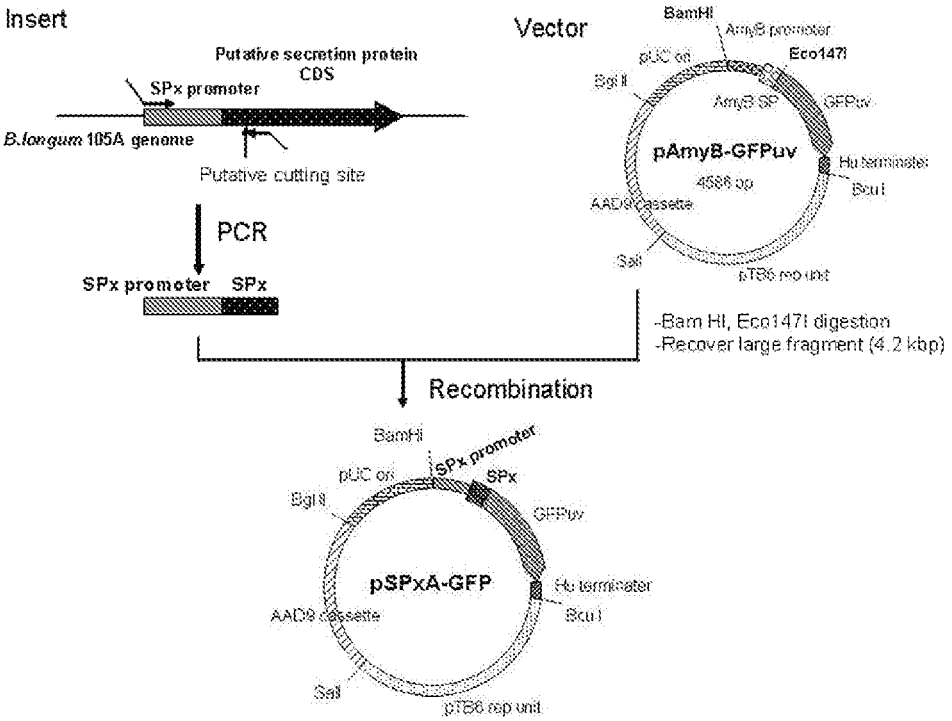
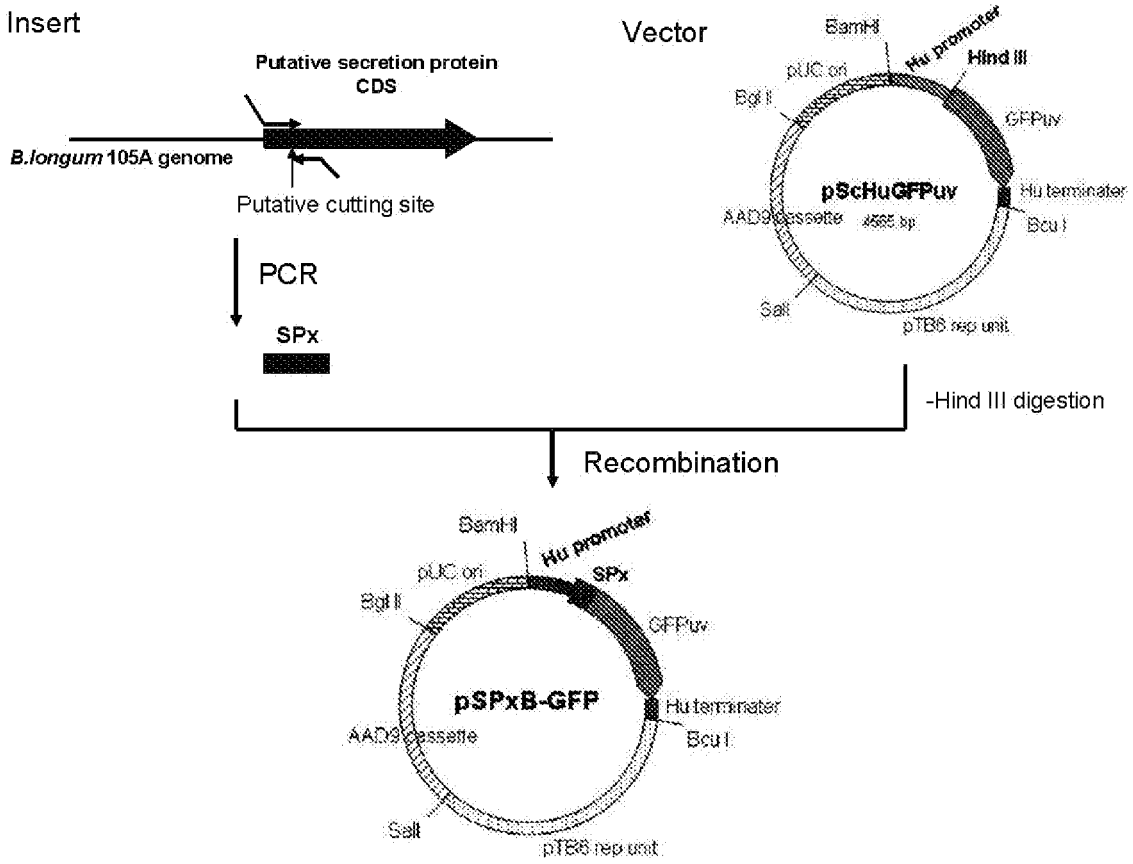
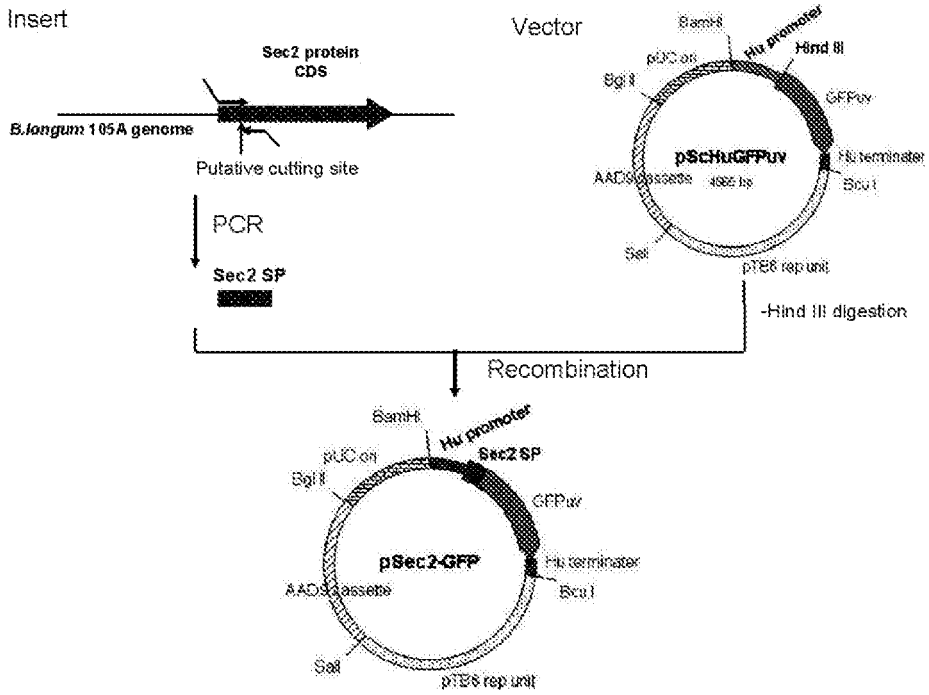
[図1]


Insert
Putative secretion protein
CDS
SPx promoter
B.longum 105A genome
Putative cutting site
PCR
SPx promoter SPx
Vector
BamHI
AmyB promoter
pUC ori
Eco147I
Bgl II
AmyB SP
GFPuv
pAmyB-GFPuv
4586 bp
Hu terminator
Bcu I
AAD9 cassette
Sal I
pTB6 rep unit
-Bam HI, Eco147I digestion
-Recover large fragment (4.2 kbp)
Recombination
BamHI
SPx promoter
pUC ori
SPx
Bgl II
GFPuv
pSPxA-GFP
Hu terminator
AAD9 cassette
Bcu I
Sal I
pTB6 rep unit

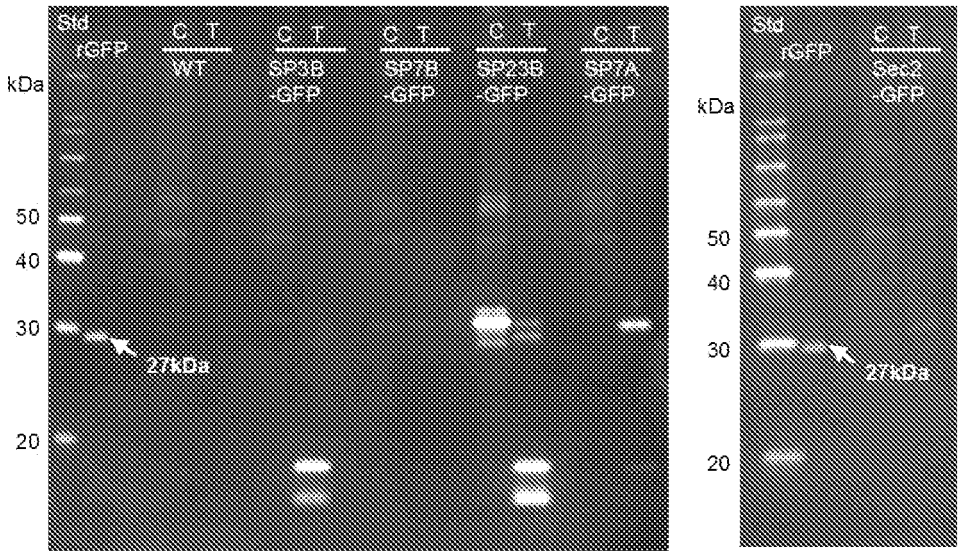
[図2]


Insert
Putative secretion protein
CDS
B.longum 105A genome
Putative cutting site
PCR
SPx
Vector
BamHI
Hu promoter
pUC ori
Hind III
Bgl II
GFPuv
pScHuGFPuv
Hu terminator
AAD9 cassette
4565 bp
Bcu I
Sal I
pTB6 rep unit
-Hind III digestion
Recombination
BamHI
Hu promoter
pUC ori
SPx
Bgl II
GFPuv
pSPxB-GFP
Hu terminator
AAD9 cassette
Bcu I
Sal I
pTB6 rep unit

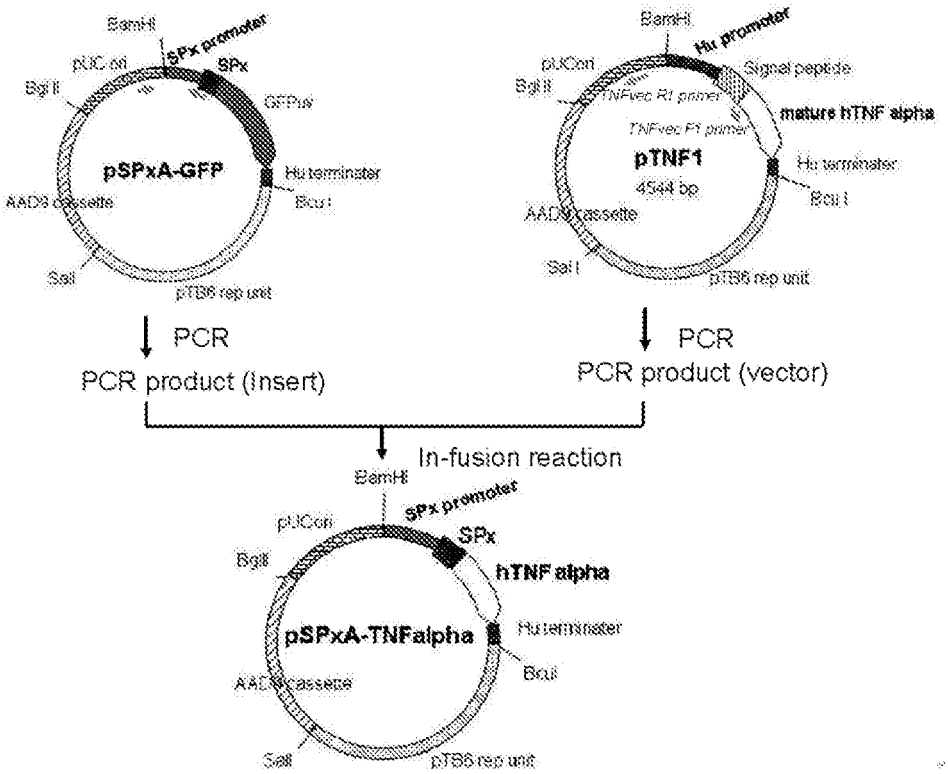
[図3]


Insert
Sec2 protein CDS
B.longum 105A genome
Putative cutting site
PCR
Sec2 SP
Vector
BamHI
Hu promoter
pUC ori
Hind III
Bgl II
GFPuv
pScHuGFPuv
Hu terminator
AADS cassette
4865 bp
BcuI
SalI
pTB6 rep unit
-Hind III digestion
Recombination
BamHI
Hu promoter
pUC ori
Sec2 SP
Bgl II
GFPuv
pSec2-GFP
Hu terminator
AADS cassette
BcuI
SalI
pTB6 rep unit

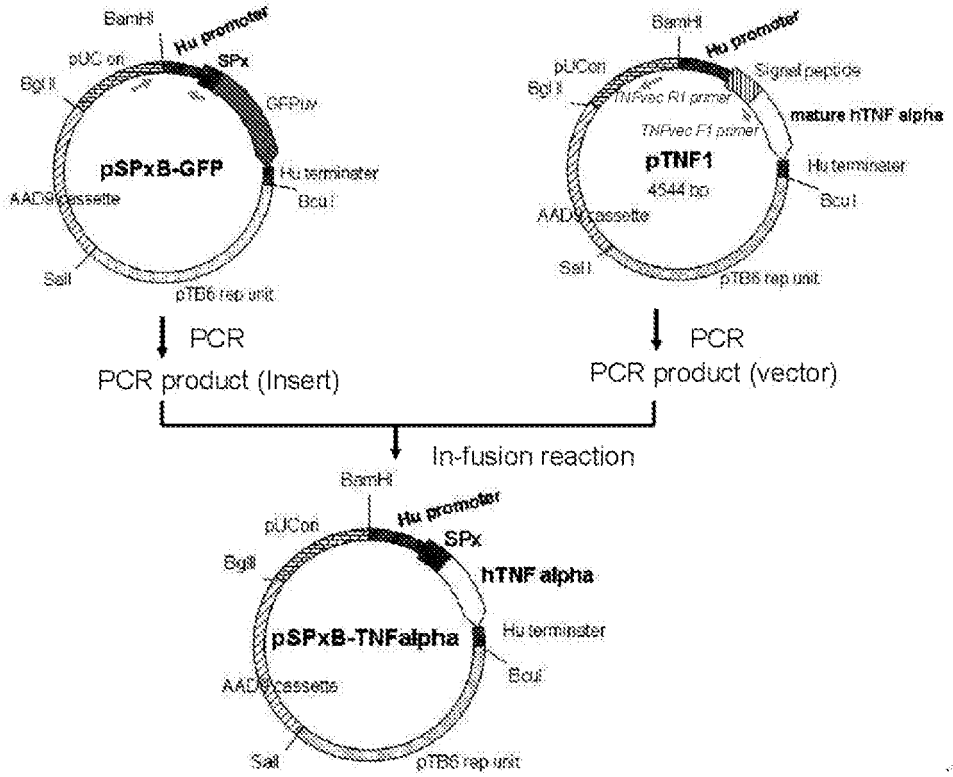
[図4]


Std
rGFP
C T
WT
SP3B -GFP
SP7B -GFP
SP23B -GFP
SP7A -GFP
kDa
50
40
30
20
27kDa
Std
rGFP
C T
Sec2 -GFP
kDa
50
40
30
20
27kDa

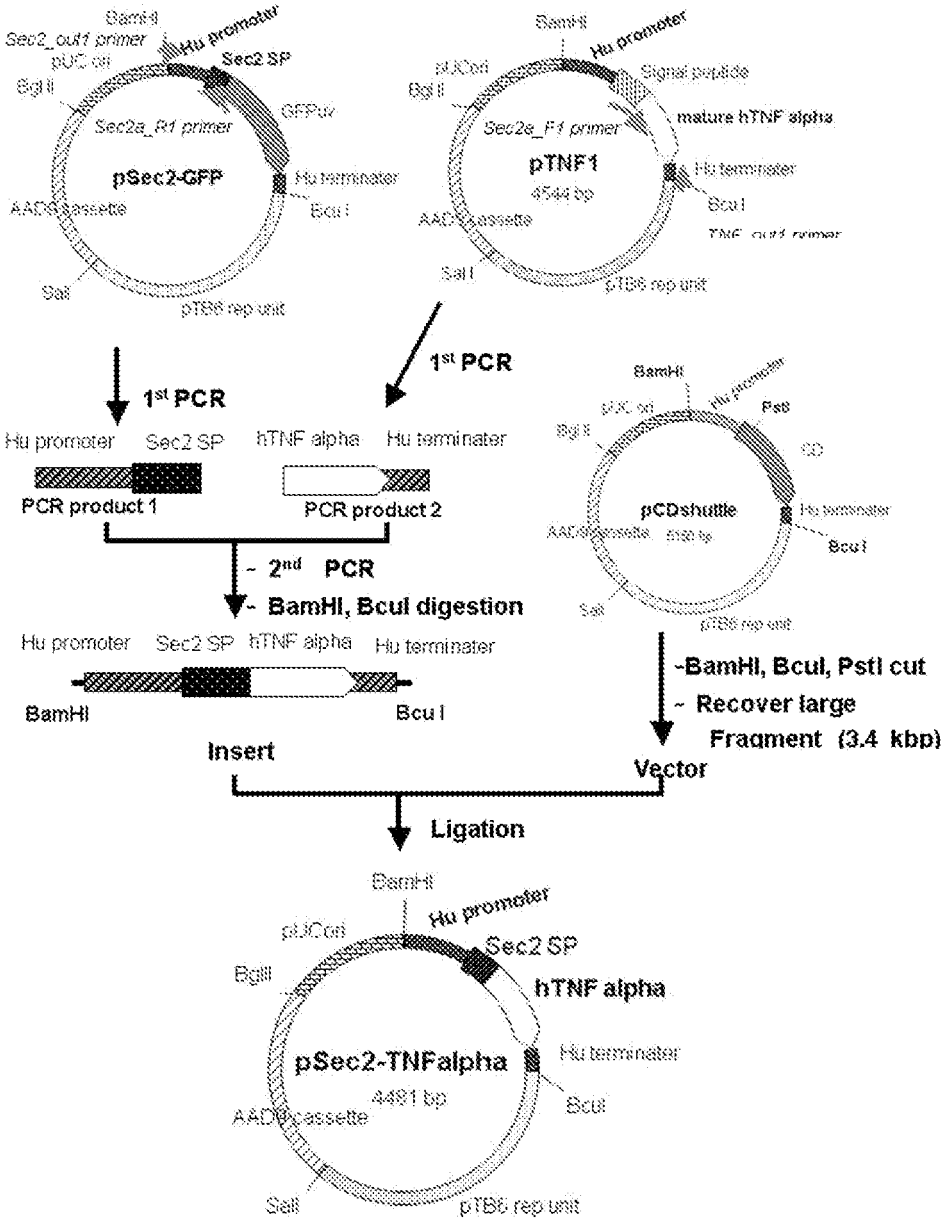
[図5]


BamHI
SPx promoter
pUC ori
SPx
BglII
GFPuv
pSPxA-GFP
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit
PCR
PCR product (insert)
BamHI
Hu promoter
pUCori
Signal peptide
BglII
TNFvec R1 primer
mature hTNF alpha
TNFvec F1 primer
pTNF1
4544 bp
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit
PCR
PCR product (vector)
In-fusion reaction
BamHI
SPx promoter
pUCori
SPx
BglII
hTNF alpha
pSPxA-TNFalpha
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit

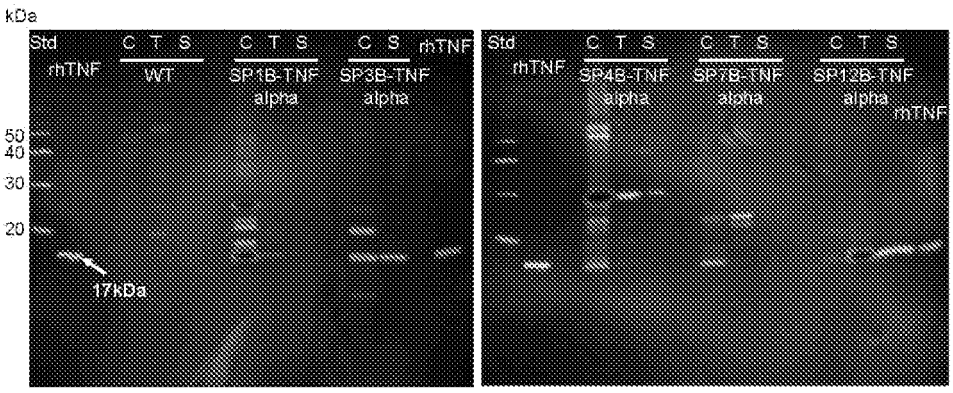
[図6]


BamHI
Hu promoter
pUC ori
SPx
BglII
GFPuv
pSPxB-GFP
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit
PCR
PCR product (Insert)
BamHI
Hu promoter
pUCori
Signal peptide
BglII
TNFvec R1 primer
mature hTNF alpha
TNFvec F1 primer
pTNF1
4544 bp
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit
PCR
PCR product (vector)
In-fusion reaction
BamHI
Hu promoter
pUCori
SPx
BglII
hTNF alpha
pSPxB-TNFalpha
Hu terminator
AADA cassette
BcuI
SalI
pTB6 rep unit

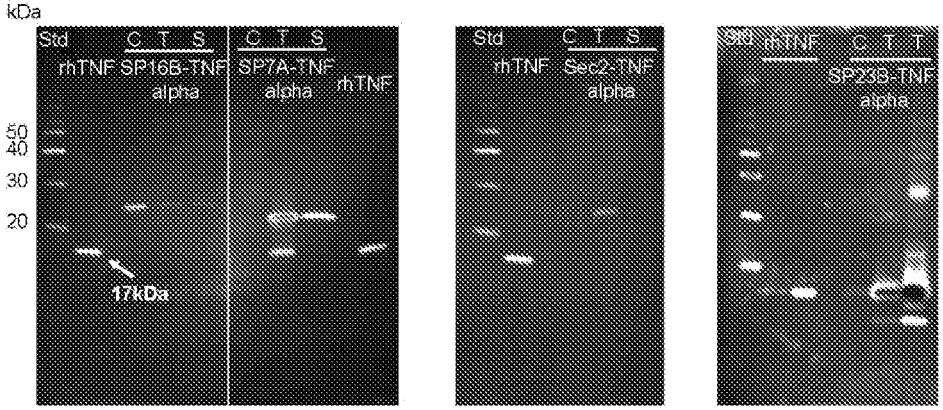
[図7]


BamHI
Hu promoter
Sec2_out1 primer
pUC ori
Sec2 SP
Bgl II
GFPuv
Sec2a_R1 primer
pSec2-GFP
Hu terminater
AAD1 cassette
Bcu I
Sal I
pTB6 rep unit
Signal peptide
mature hTNF alpha
Sec2e_F1 primer
pTNF1
4544 bp
1st PCR
Hu promoter
Sec2 SP
hTNF alpha
Hu terminater
PCR product 1
PCR product 2
- 2nd PCR
- BamHI, BcuI digestion
pCDshuttle
PstI
CD
-BamHI, BcuI, PstI cut
- Recover large
Fragment (3.4 kbp)
Insert
Vector
Ligation
pSec2-TNFalpha
4491 bp

[図8]

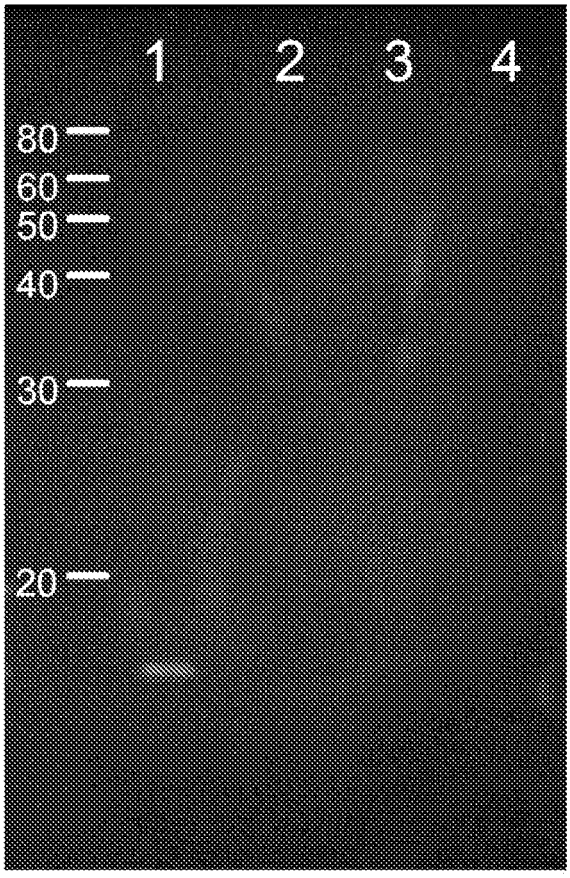


C: Intracellular protein
T: Concentrated sup by TCA precipitation
S: Supernatant



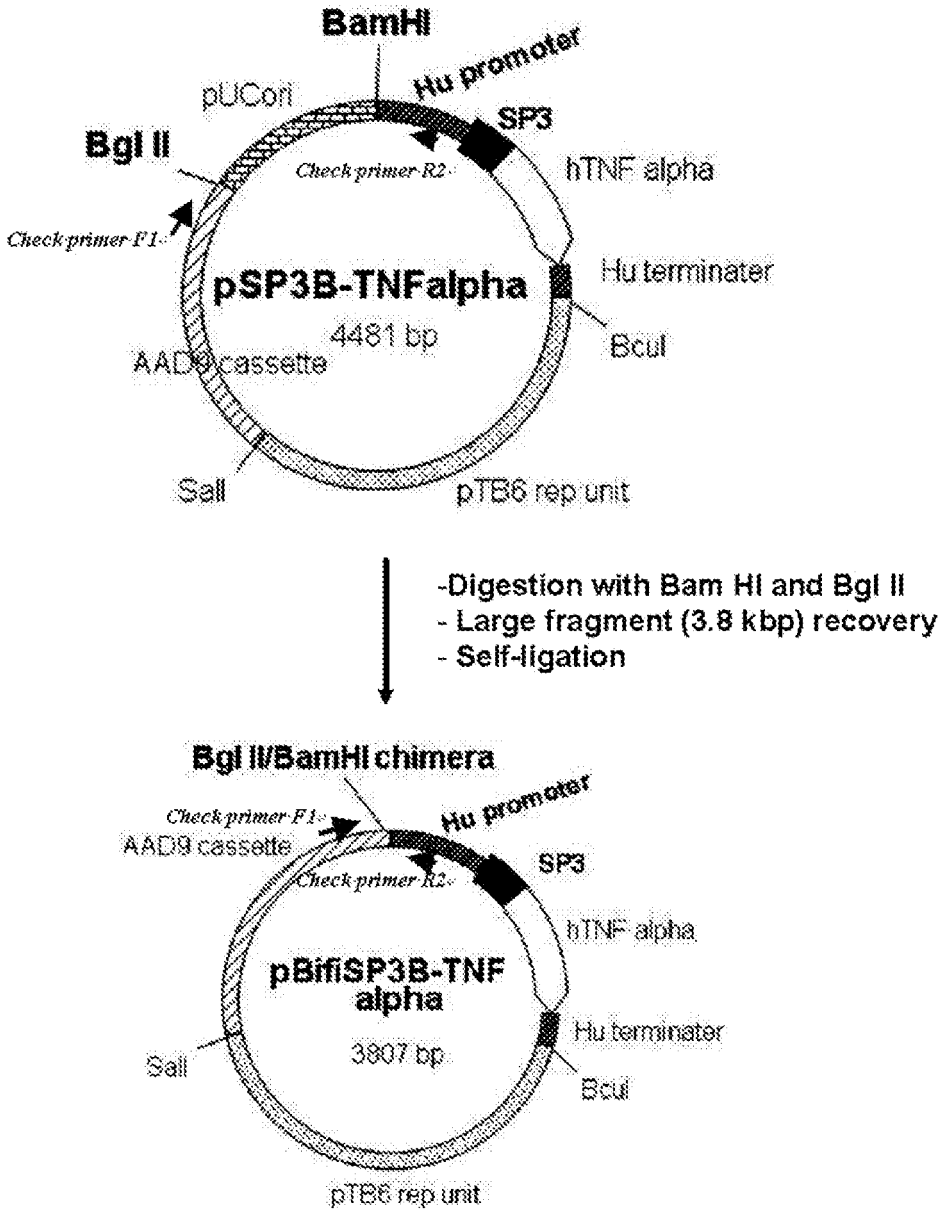
C: Intracellular protein
T: Concentrated sup by TCA precipitation
S: Supernatant

[図9]

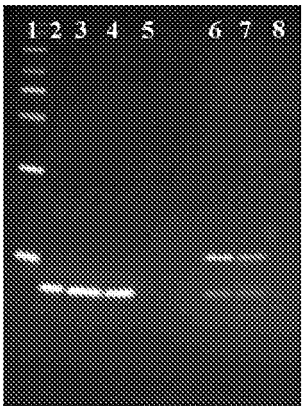


レーン1:B. longum 105A/pTNF3 細胞内抽出物
レーン2:B. longum 105A 細胞内抽出物
レーン3:B. longum 105A/pTNF3 培養上清
レーン4:B. longum 105A 培養上清

[図10]

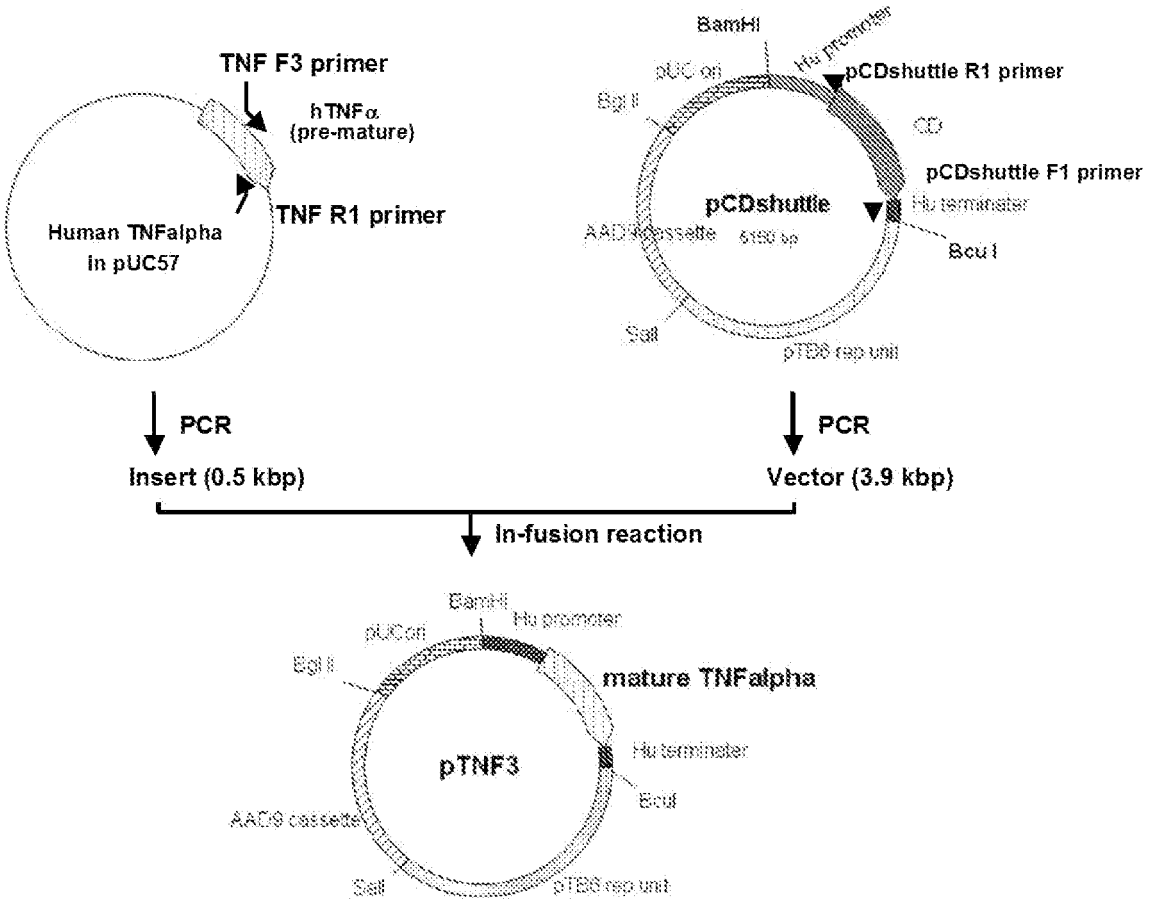


[図11]

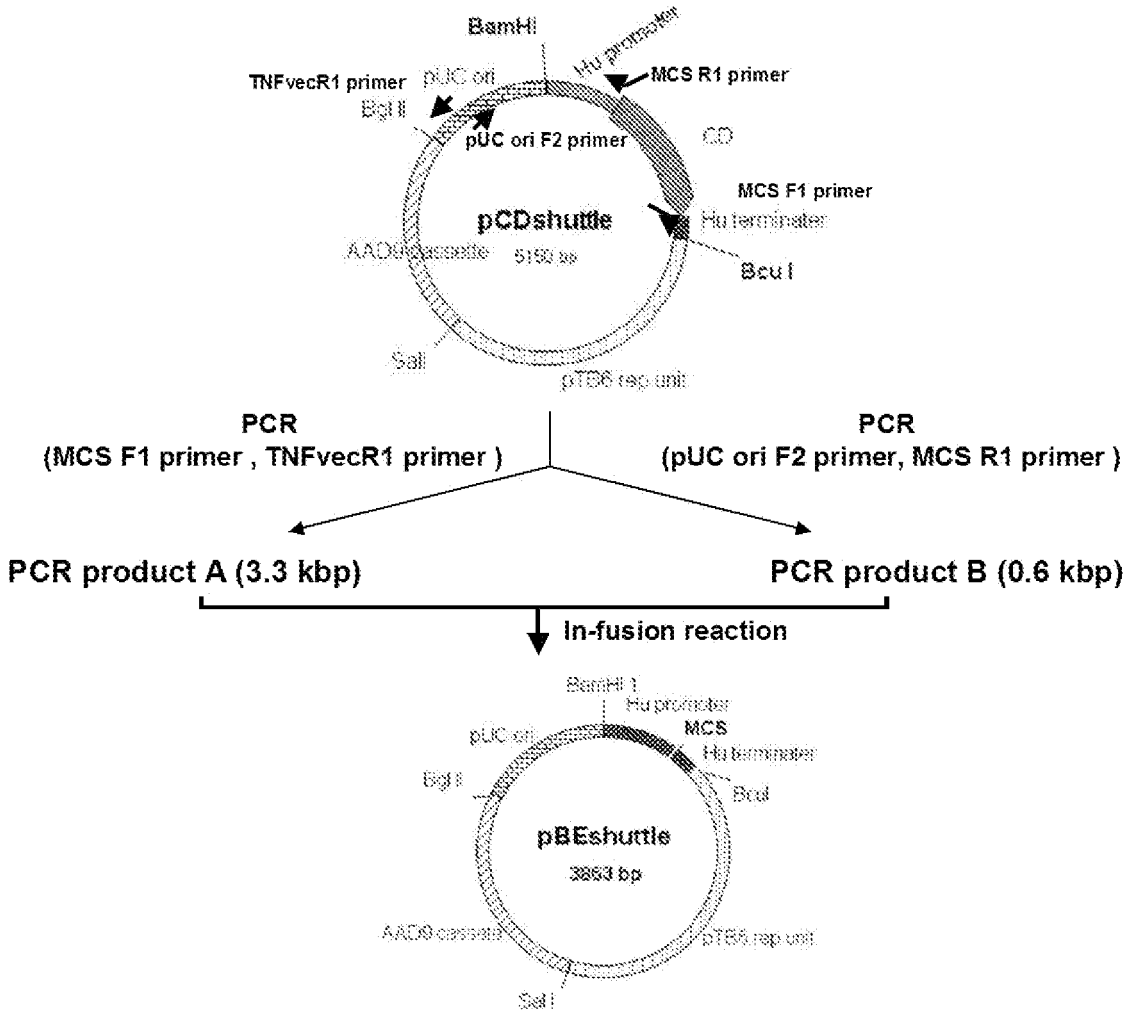


レーン 1：分子量マーカー
レーン 2：ヒト組換え TNF-alpha (陽性コントロール)
レーン 3：*B.longum* 105A/pBifiSP3B-TNF alpha 培養上清
レーン 4：*B.longum* 105A/pSP3B-TNF alpha 培養上清
レーン 5：*B.longum* 105A 培養上清
レーン 6：*B.longum* 105A/pBifiSP3B-TNF alpha 細胞内抽出物
レーン 7：*B.longum* 105A/pSP3B-TNF alpha 細胞内抽出物
レーン 8：*B.longum* 105A 細胞内抽出物

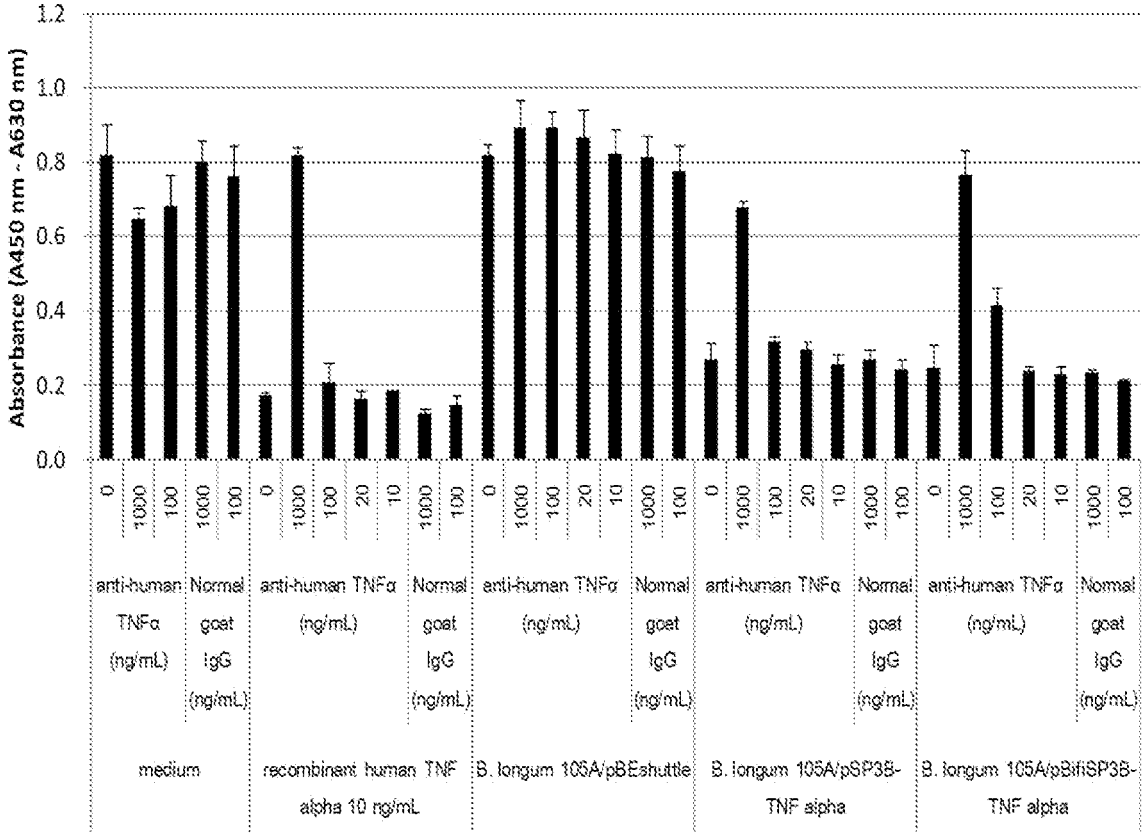
[図12]


TNF F3 primer
hTNFα
(pre-mature)
TNF R1 primer
Human TNFalpha
in pUC57
BamHI
Hu Promoter
pUC ori
pCDshuttle R1 primer
Bgl II
CD
pCDshuttle F1 primer
pCDshuttle
Hu terminator
AAD9 cassette
Bcu I
Sal I
pTB9 rep unit
PCR
Insert (0.5 kbp)
PCR
Vector (3.9 kbp)
In-fusion reaction
BamHI
Hu promoter
pUC ori
Bgl II
mature TNFalpha
pTNF3
Hu terminator
Bcu I
AAD9 cassette
Sal I
pTB9 rep unit

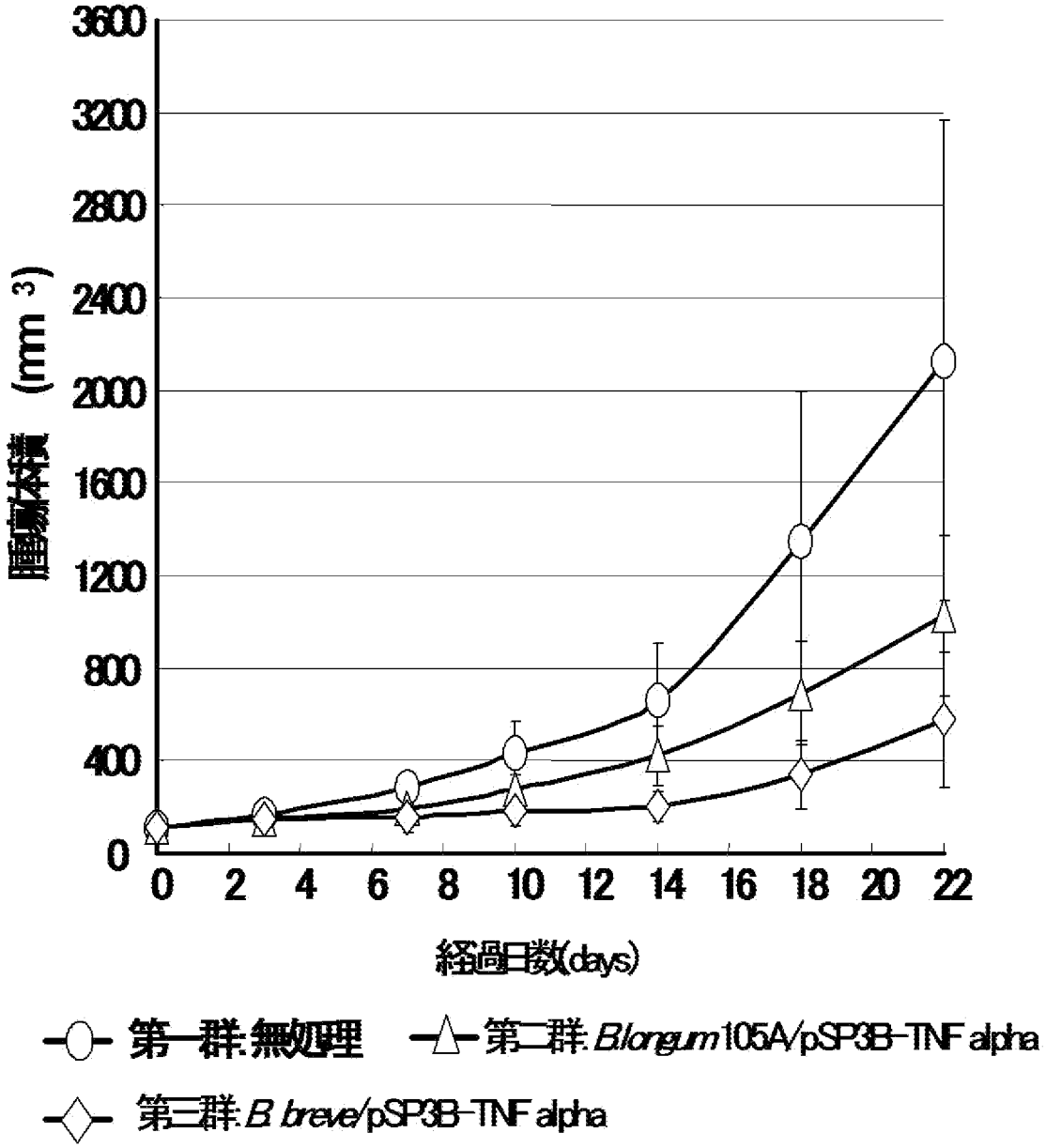
[図13]


BamHI
Hu promoter
TNFvecR1 primer
pUC ori
MCS R1 primer
BglII
pUC ori F2 primer
CD
MCS F1 primer
pCDshuttle
Hu terminator
AAD9 cassette
5150 bp
BcuI
SalI
pTB6 rep unit
PCR
(MCS F1 primer , TNFvecR1 primer)
PCR
(pUC ori F2 primer, MCS R1 primer)
PCR product A (3.3 kbp)
PCR product B (0.6 kbp)
In-fusion reaction
BamHI
Hu promoter
MCS
pUC ori
Hu terminator
BglI
BcuI
pBEshuttle
3263 bp
AAD9 cassette
pTB6 rep unit
SalI

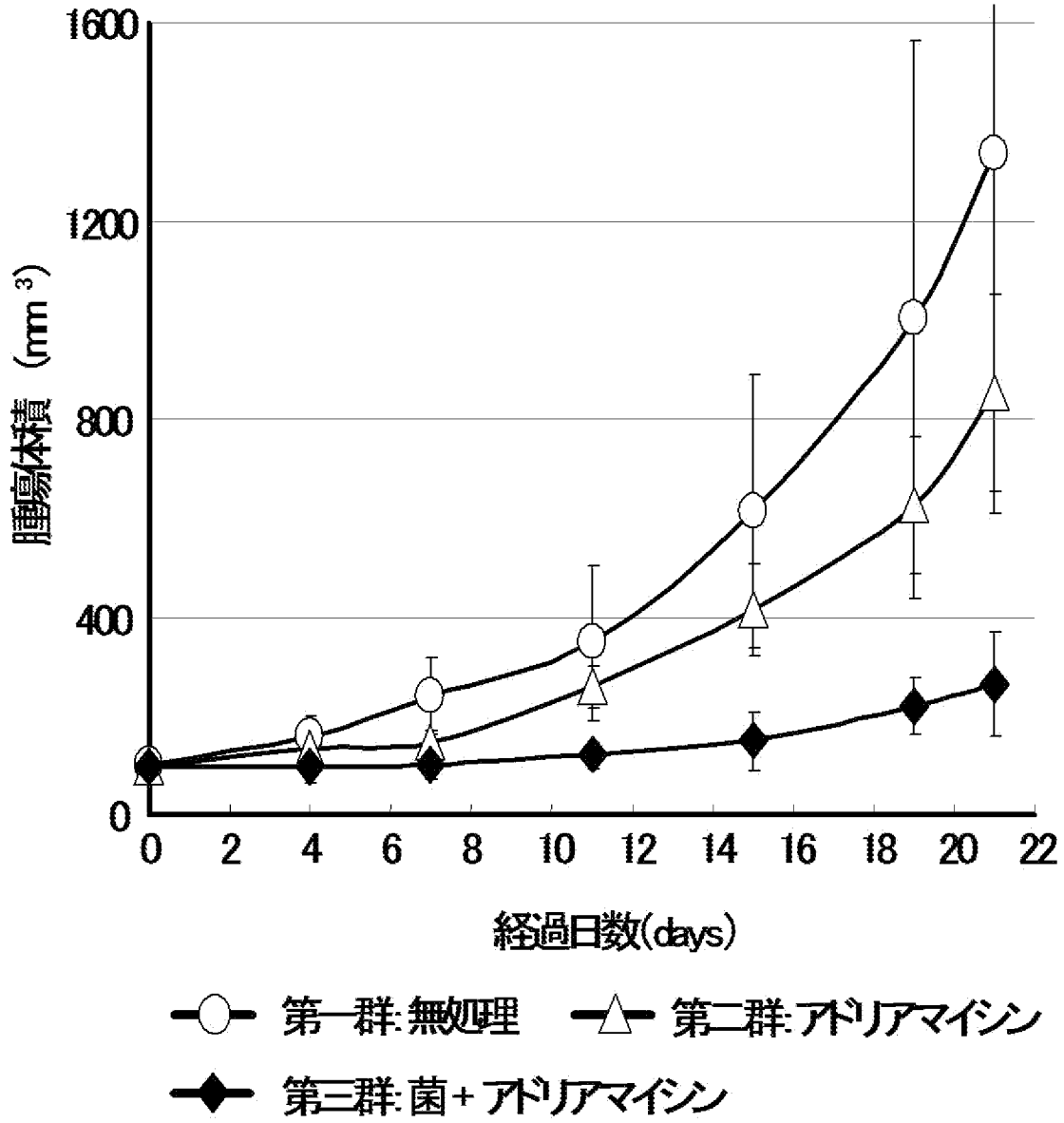
[図14]


Absorbance (A450 nm - A630 nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
100
20
10
anti-human TNFα (ng/mL)
Normal goat IgG (ng/mL)
medium
recombinant human TNF alpha 10 ng/mL
B. longum 105A/pBEshuttle
B. longum 105A/pSP38-TNF alpha
B. longum 105A/pBifiSP38-TNF alpha

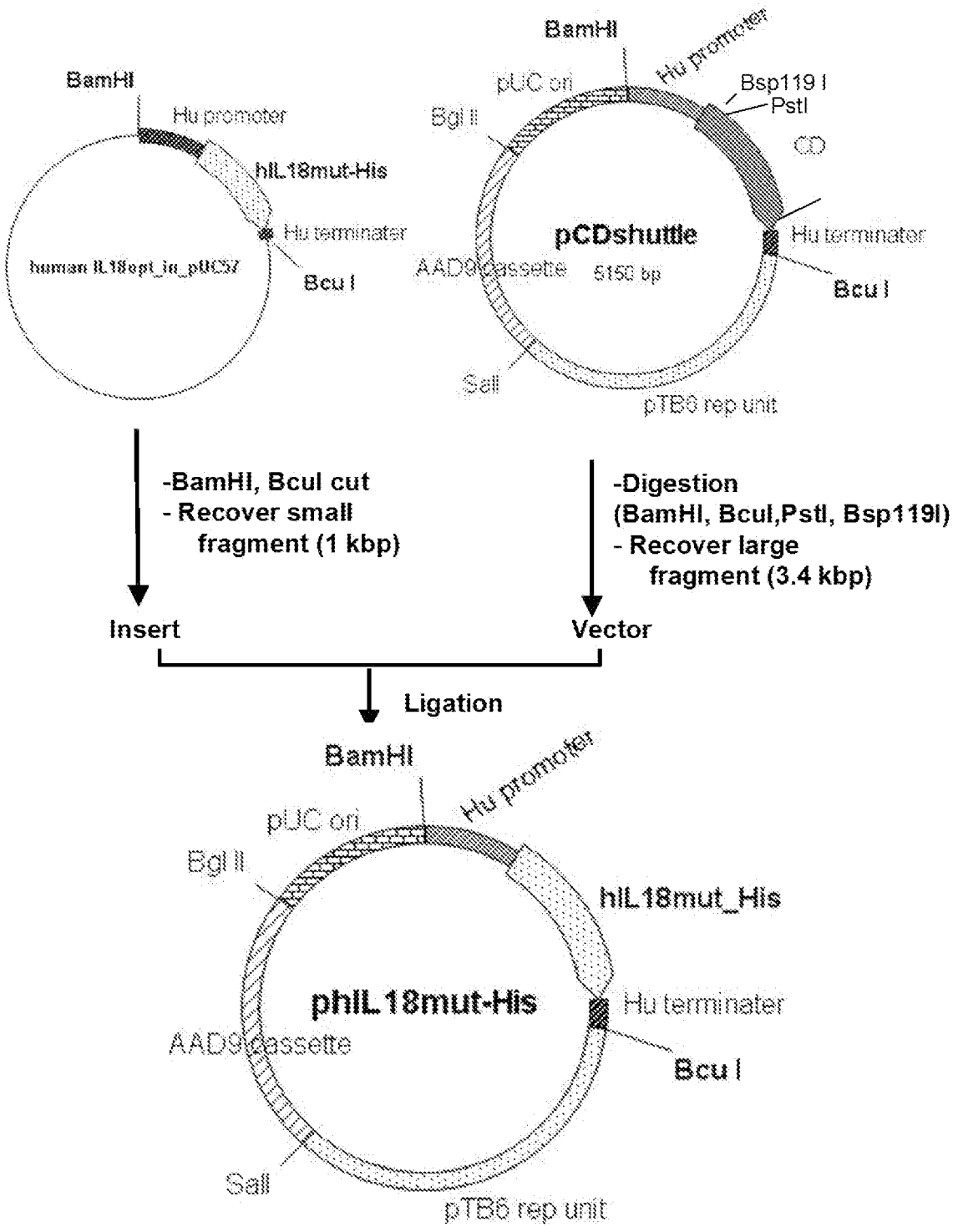
[図15]


3600
3200
2800
2400
2000
1600
1200
800
400
0
腫瘍体積 (mm 3)
0 2 4 6 8 10 12 14 16 18 20 22
経過日数(days)
第一群:無処理
第二群:B.longum 105A/pSP3B-TNF alpha
第三群:B.breve/pSP3B-TNF alpha

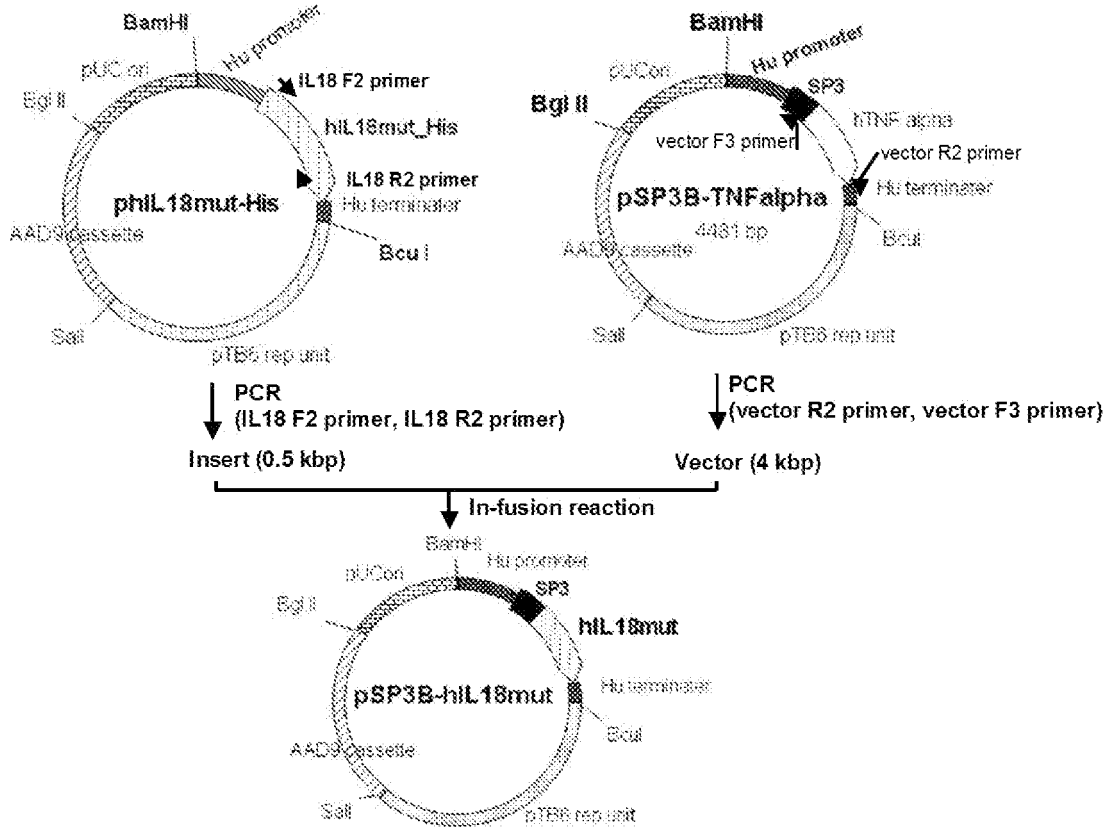
[図16]


1600
1200
800
400
0
腫瘍体積 (mm 3)
0 2 4 6 8 10 12 14 16 18 20 22
経過日数(days)
第一群:無処理
第二群:アドリアマイシン
第三群:菌+アドリアマイシン

[図17]


BamHI
Hu promoter
hIL18mut-His
Hu terminater
human IL18opt_in_pUC57
Bcu I
BamHI
pUC ori
Hu promoter
Bsp119 I
PstI
Sgl II
CD
pCDshuttle
Hu terminater
AAD9cassette
5150 bp
Bcu I
Sall
pTB6 rep unit
-BamHI, BcuI cut
- Recover small
fragment (1 kbp)
-Digestion
(BamHI, BcuI,PstI, Bsp119I)
- Recover large
fragment (3.4 kbp)
Insert
Vector
Ligation
BamHI
Hu promoter
pUC ori
Bgl II
hIL18mut_His
phIL18mut-His
Hu terminater
AAD9cassette
Bcu I
Sall
pTB6 rep unit

[図18]


BamHI
Hu promoter
pUC ori
IL18 F2 primer
Bgl II
hIL18mut_His
IL18 R2 primer
phIL18mut-His
Hu terminator
AAD9 cassette
BcuI
SalI
pTB6 rep unit
PCR
(IL18 F2 primer, IL18 R2 primer)
Insert (0.5 kbp)
BamHI
Hu promoter
Bgl II
SP3
vector F3 primer
hTNF alpha
vector R2 primer
pSP3B-TNFalpha
4481 bp
Hu terminator
BcuI
SalI
pTB6 rep unit
PCR
(vector R2 primer, vector F3 primer)
Vector (4 kbp)
In-fusion reaction
BamHI
Hu promoter
pUCori
SP3
Bgl II
hIL18mut
pSP3B-hIL18mut
Hu terminator
BcuI
AAD9 cassette
SalI
pTB6 rep unit