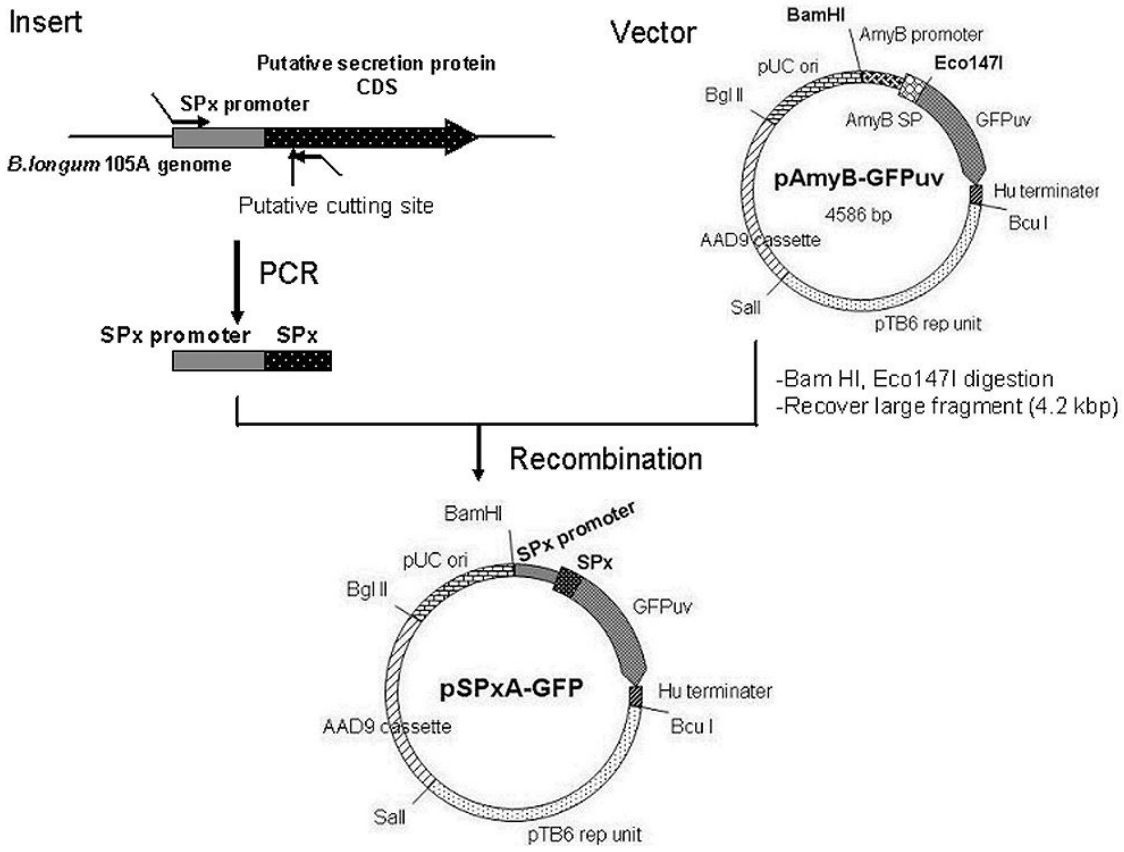
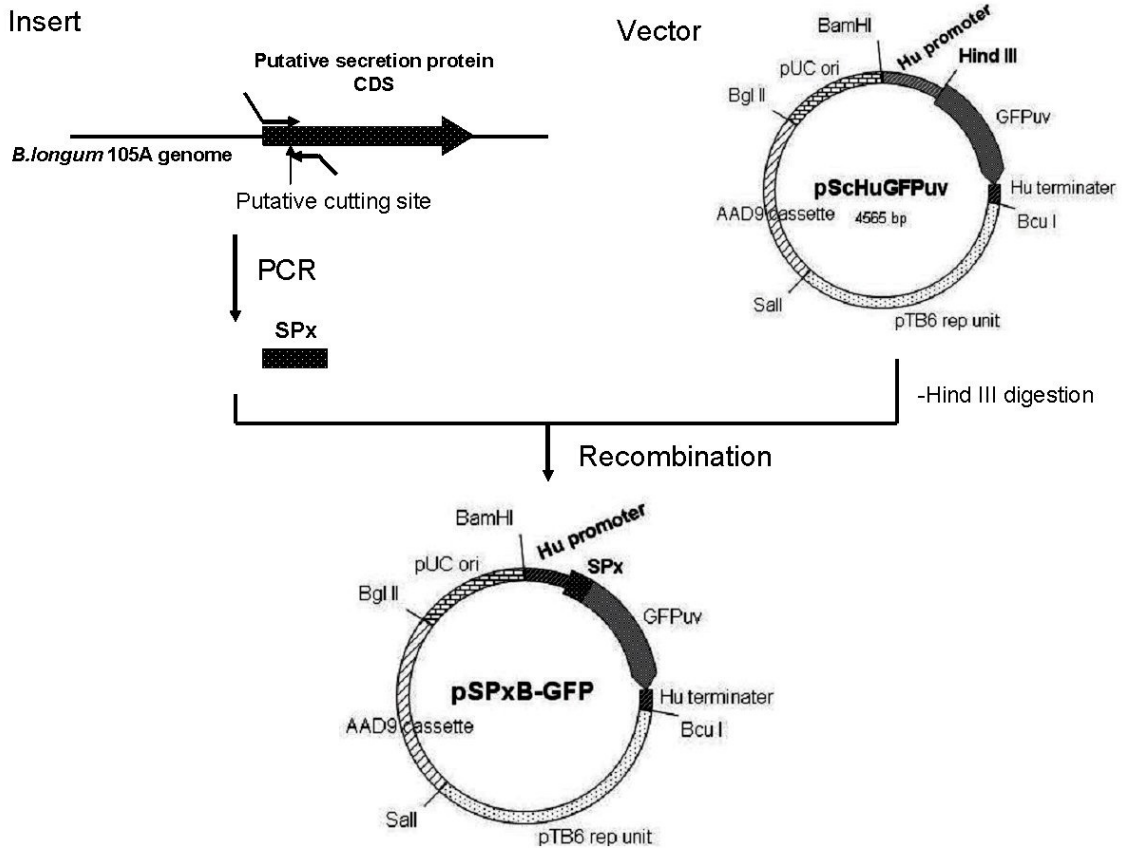
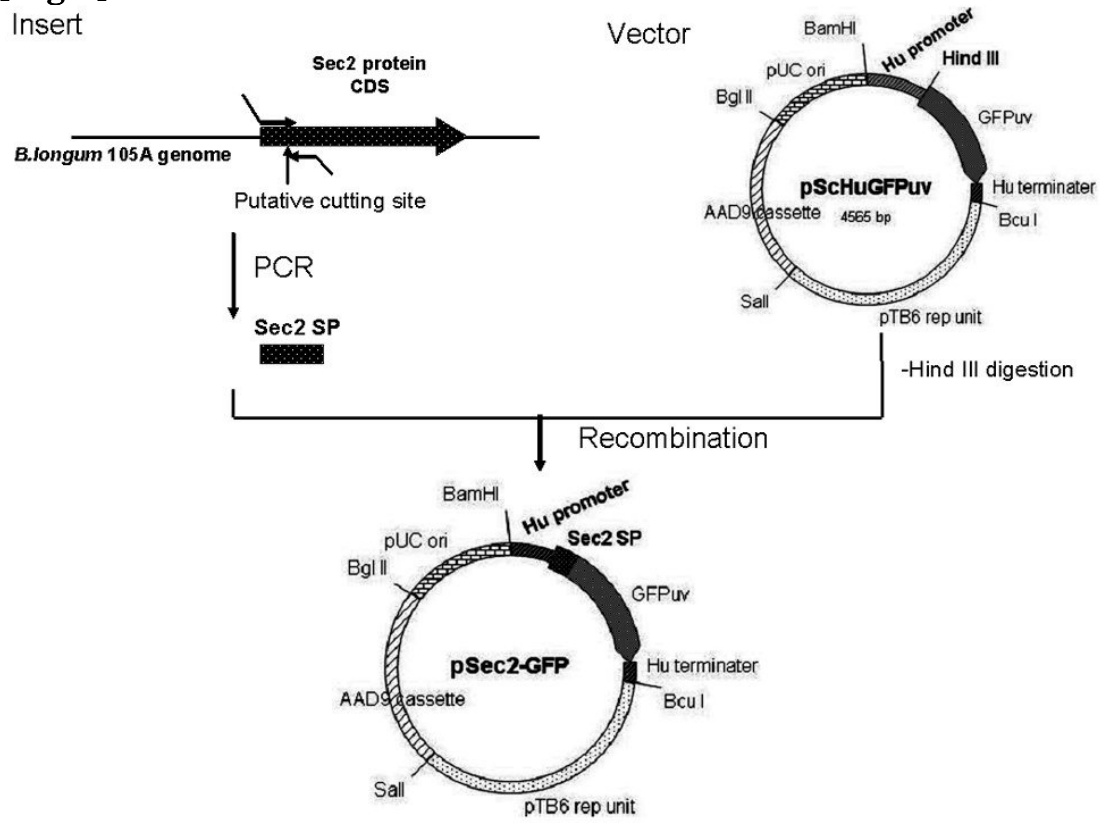
[Fig.1]


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 terminater
Bcu I
AAD9 cassette
SalI
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 terminater
AAD9 cassette
Bcu I
SalI
pTB6 rep unit

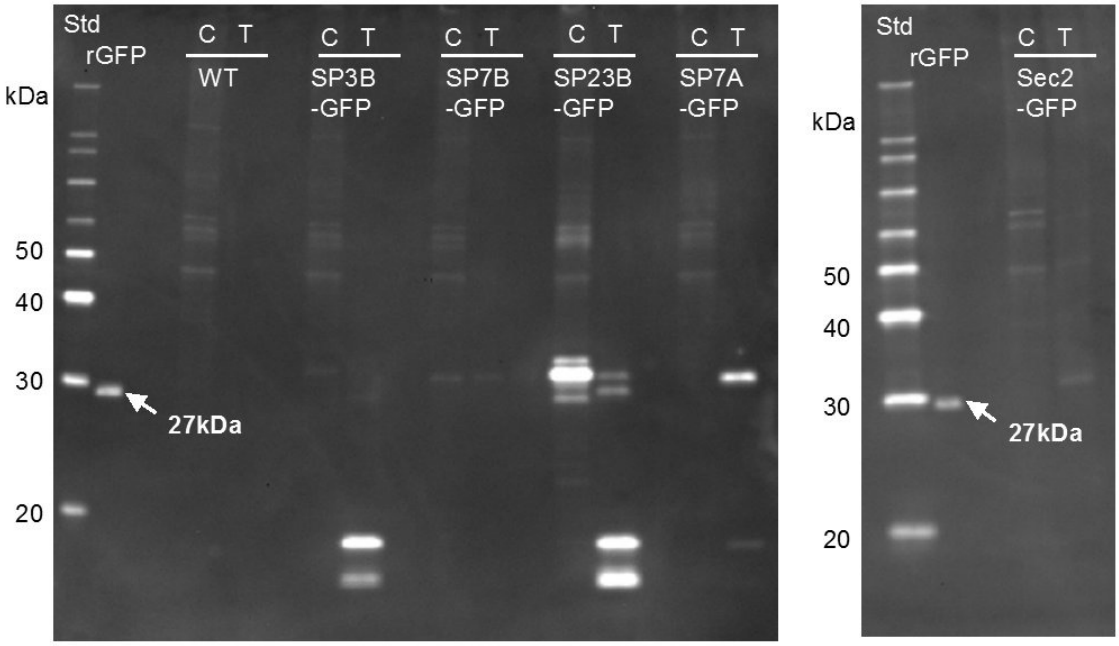
[Fig.2]


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

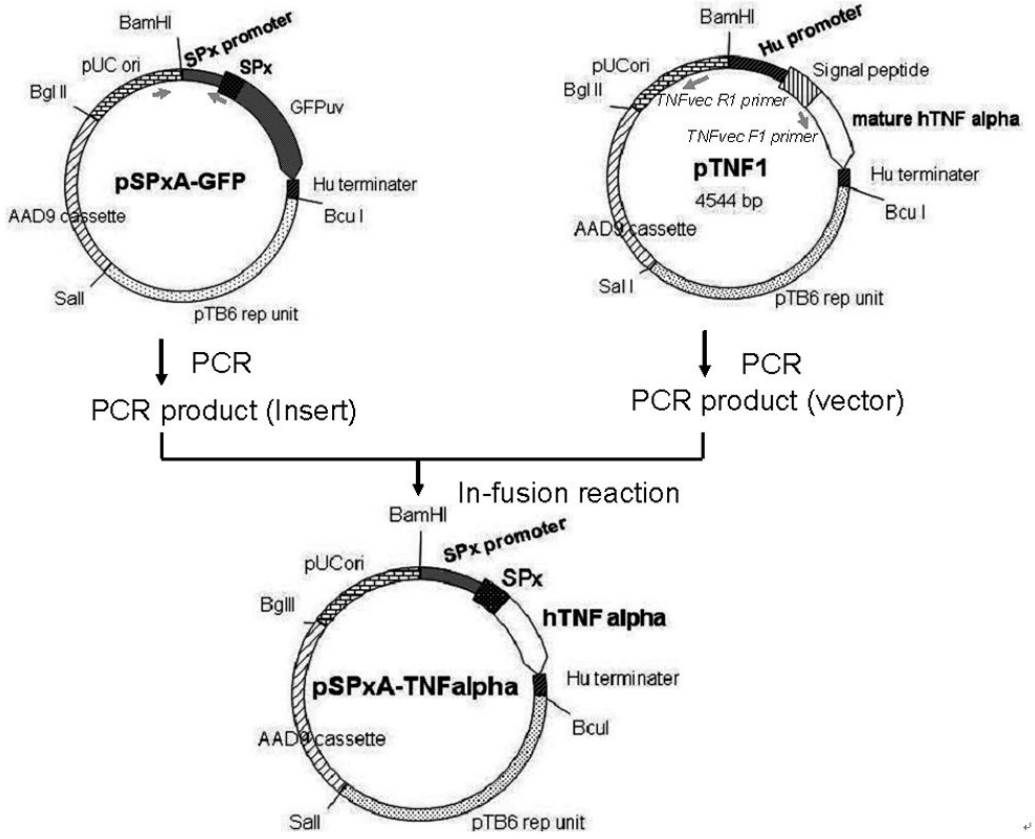
[Fig.3]


Insert
Sec2 protein CDS
B.longum 105A genome
Putative cutting site
PCR
Sec2 SP
Vector
BamHI
Hu promoter
Hind III
pUC ori
Bgl II
GFPuv
pScHuGFPuv
Hu terminater
AAD9 cassette
4565 bp
Bcu I
SalI
pTB6 rep unit
-Hind III digestion
Recombination
BamHI
Hu promoter
Sec2 SP
pUC ori
Bgl II
GFPuv
pSec2-GFP
Hu terminater
AAD9 cassette
Bcu I
SalI
pTB6 rep unit

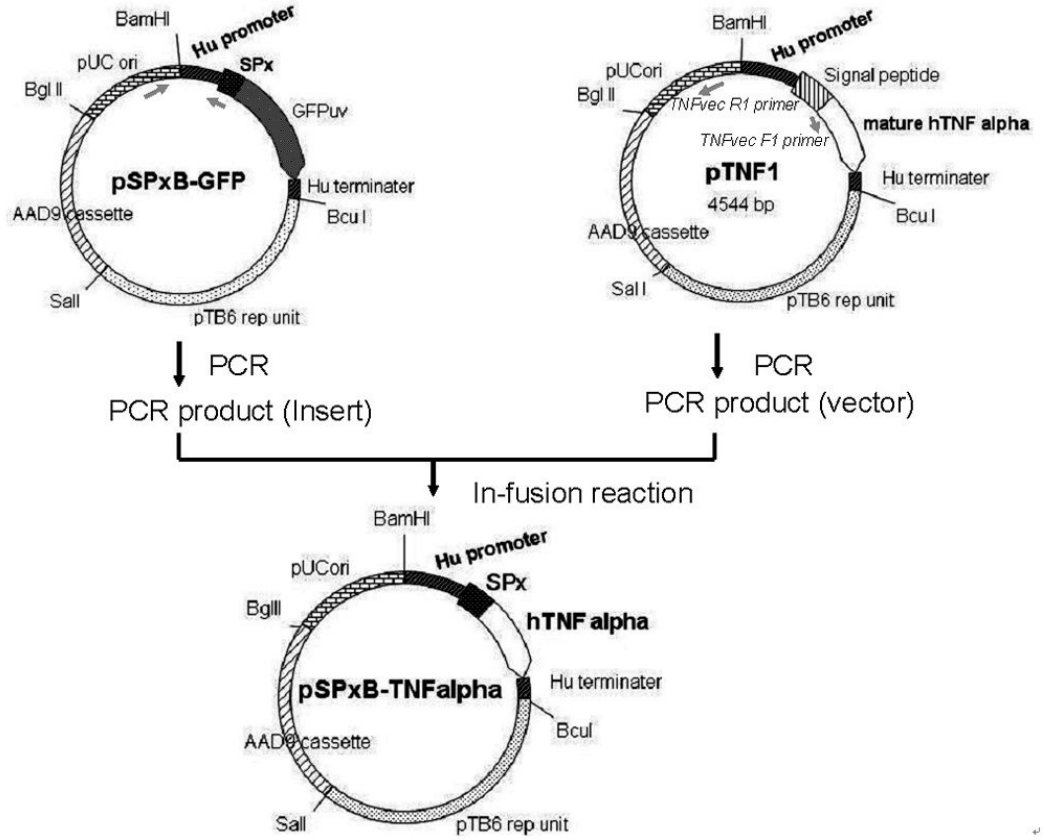
[Fig.4]


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

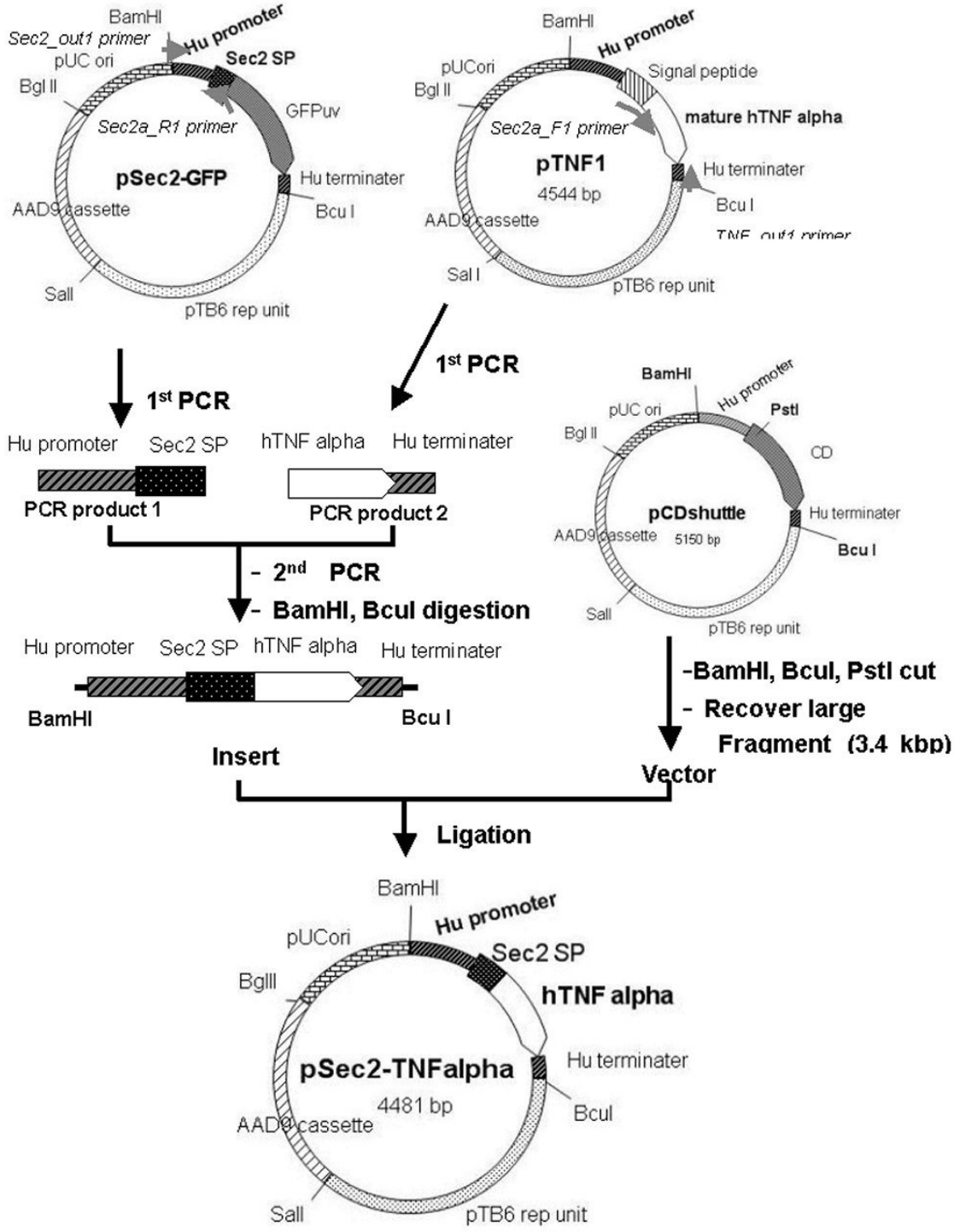
[Fig.5]


BamHI
SPx promoter
pUC ori
SPx
Bgl II
GFPuv
pSPxA-GFP
Hu terminater
Bcu I
AAD9 cassette
SalI
pTB6 rep unit
BamHI
Hu promoter
pUCori
Signal peptide
Bgl II
TNFvec R1 primer
mature hTNF alpha
TNFvec F1 primer
pTNF1
4544 bp
Hu terminater
Bcu I
AAD9 cassette
Sal I
pTB6 rep unit
PCR
PCR product (Insert)
PCR
PCR product (vector)
In-fusion reaction
BamHI
SPx promoter
pUCori
SPx
BglII
hTNF alpha
pSPxA-TNFalpha
Hu terminater
BcuI
AAD9 cassette
SalI
pTB6 rep unit

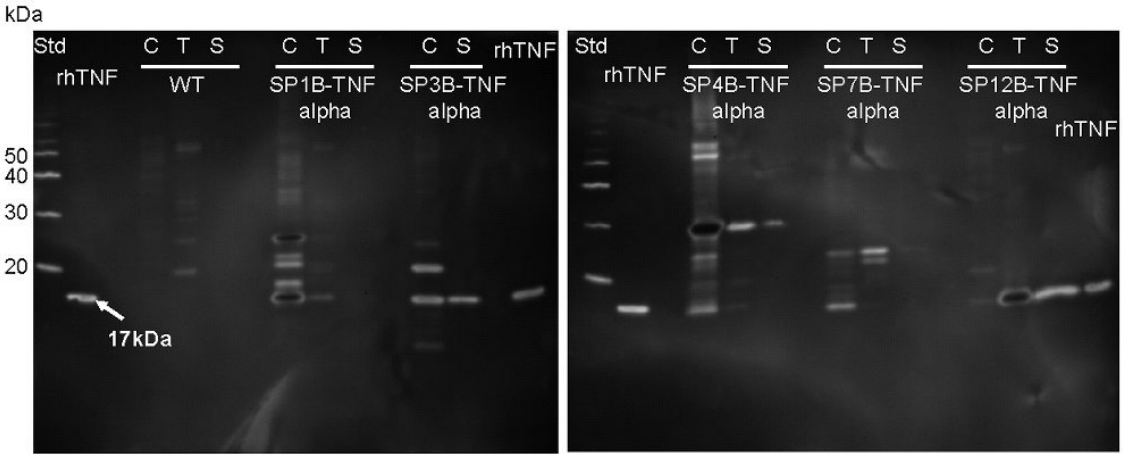
[Fig.6]


BamHI
Hu promoter
pUC ori
SPx
Bgl II
GFPuv
pSPxB-GFP
Hu terminater
Bcu I
AAD9 cassette
SalI
pTB6 rep unit
BamHI
Hu promoter
pUCori
Signal peptide
Bgl II
TNFvec R1 primer
mature hTNF alpha
TNFvec F1 primer
pTNF1
4544 bp
Hu terminater
Bcu I
AAD9 cassette
Sal I
pTB6 rep unit
PCR
PCR product (Insert)
PCR
PCR product (vector)
In-fusion reaction
BamHI
Hu promoter
pUCori
SPx
BglII
hTNF alpha
pSPxB-TNFalpha
Hu terminater
BcuI
AAD9 cassette
SalI
pTB6 rep unit

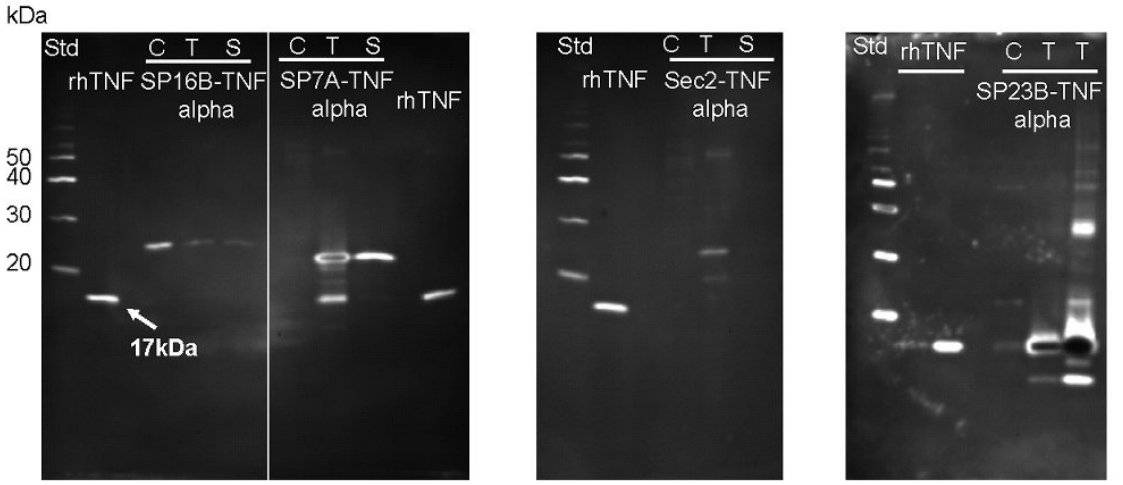
[Fig.7]


BamHI
Hu promoter
Sec2_out1 primer
pUC ori
Sec2 SP
Bgl II
GFPuv
Sec2a_R1 primer
pSec2-GFP
Hu terminater
AAD9 cassette
Bcu I
SalI
pTB6 rep unit
BamHI
Hu promoter
pUCori
Signal peptide
Bgl II
mature hTNF alpha
Sec2a_F1 primer
pTNF1
4544 bp
Hu terminater
AAD9 cassette
Bcu I
Sal I
pTB6 rep unit
1st PCR
1st PCR
Hu promoter
Sec2 SP
hTNF alpha
Hu terminater
PCR product 1
PCR product 2
- 2nd PCR
- BamHI, BcuI digestion
Hu promoter
Sec2 SP
hTNF alpha
Hu terminater
BamHI
Bcu I
BamHI
Hu promoter
pUC ori
PstI
Bgl II
CD
pCDshuttle
Hu terminater
AAD9 cassette
5150 bp
Bcu I
SalI
pTB6 rep unit
-BamHI, BcuI, PstI cut
- Recover large
Fragment (3.4 kbp)
Insert
Vector
Ligation
BamHI
Hu promoter
pUCori
Sec2 SP
BglII
hTNF alpha
pSec2-TNFalpha
Hu terminater
4481 bp
BcuI
AAD9 cassette
SalI
pTB6 rep unit

[Fig.8]

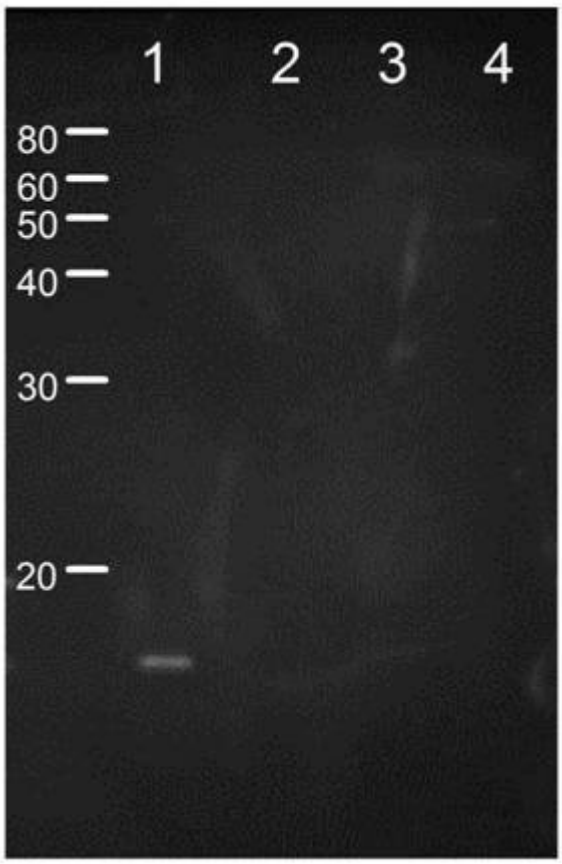


C: Intracellular protein
T: Concentrated sup by TCA precipitation
S: Supernatant



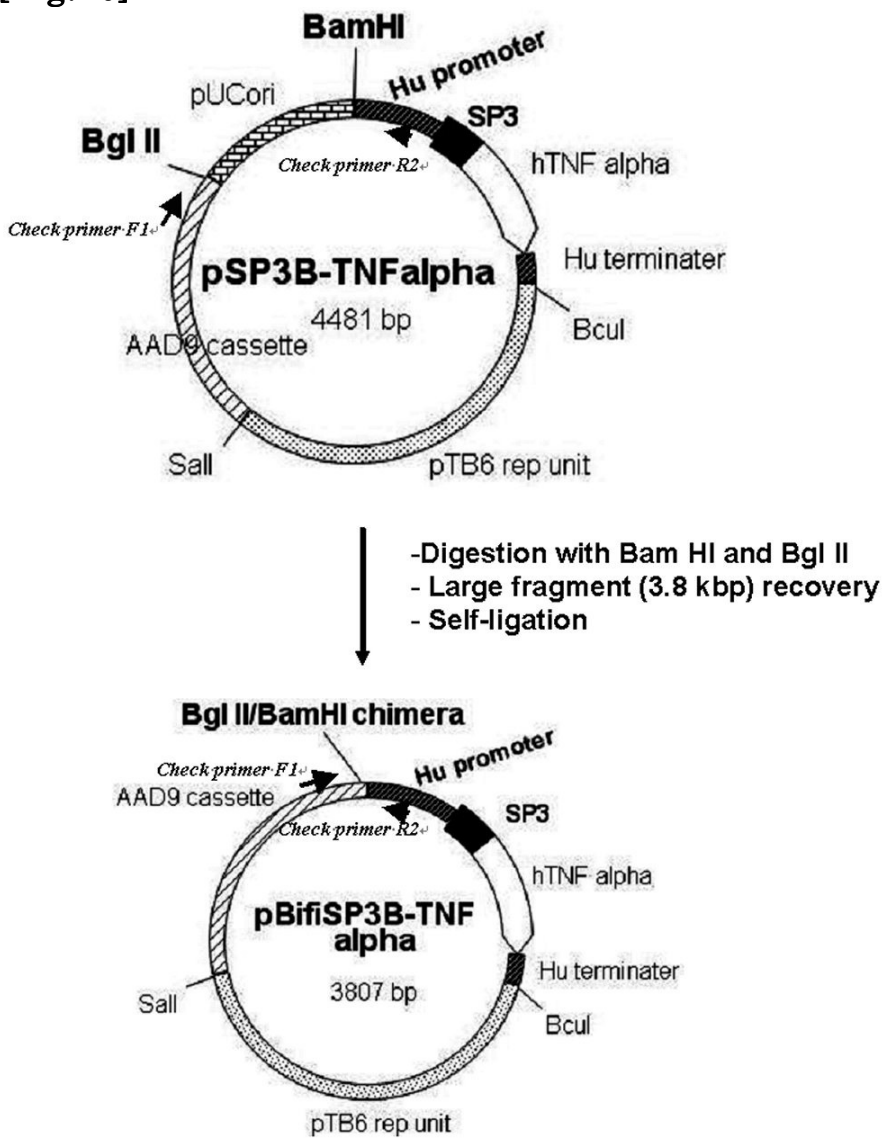
C: Intracellular protein
T: Concentrated sup by TCA precipitation
S: Supernatant

[Fig.9]

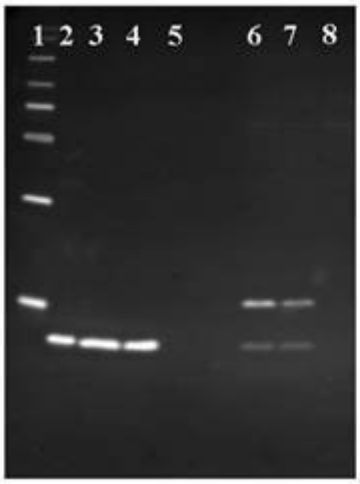


Lane 1: *B. longum* 105A/pTNF3 intracellular extract
Lane 2: *B. longum* 105A intracellular extract
Lane 3: *B. longum* 105A/pTNF3 culture supernatant
Lane 4: *B. longum* 105A culture supernatant

[Fig.10]

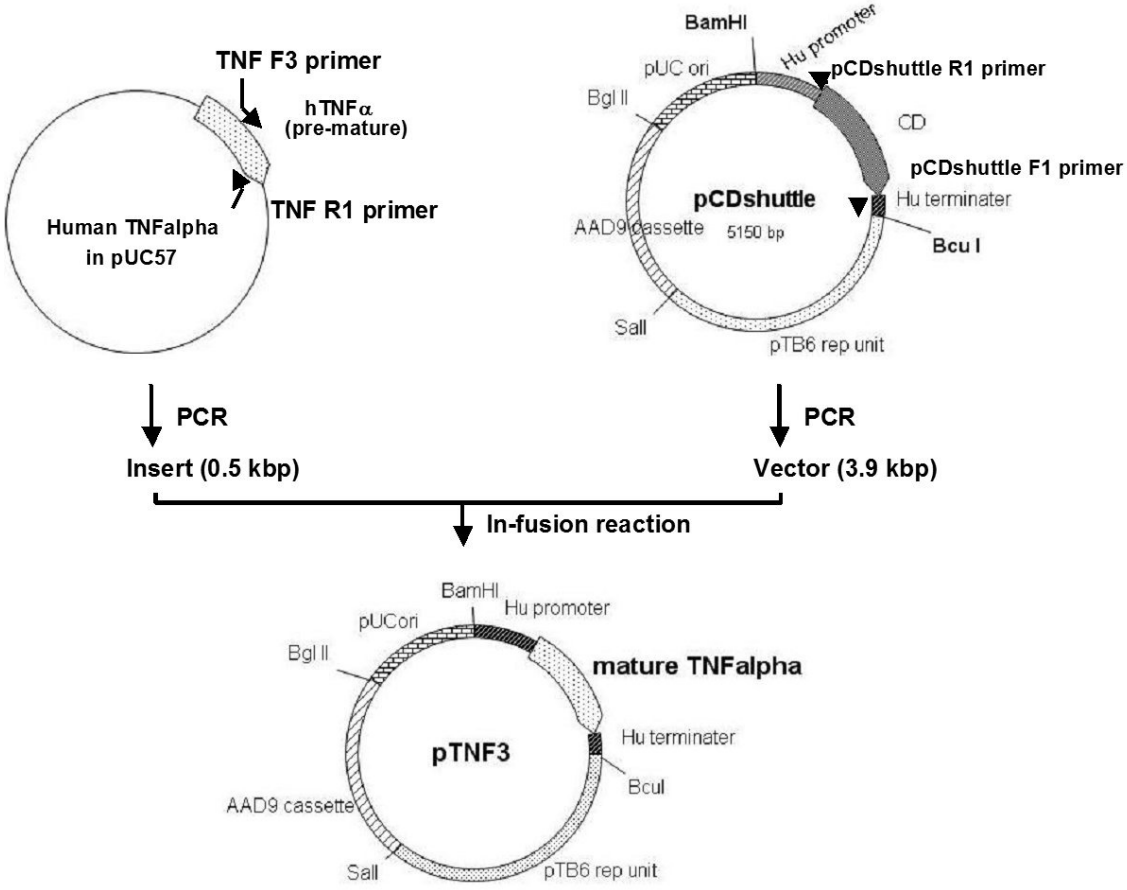


[Fig.11]

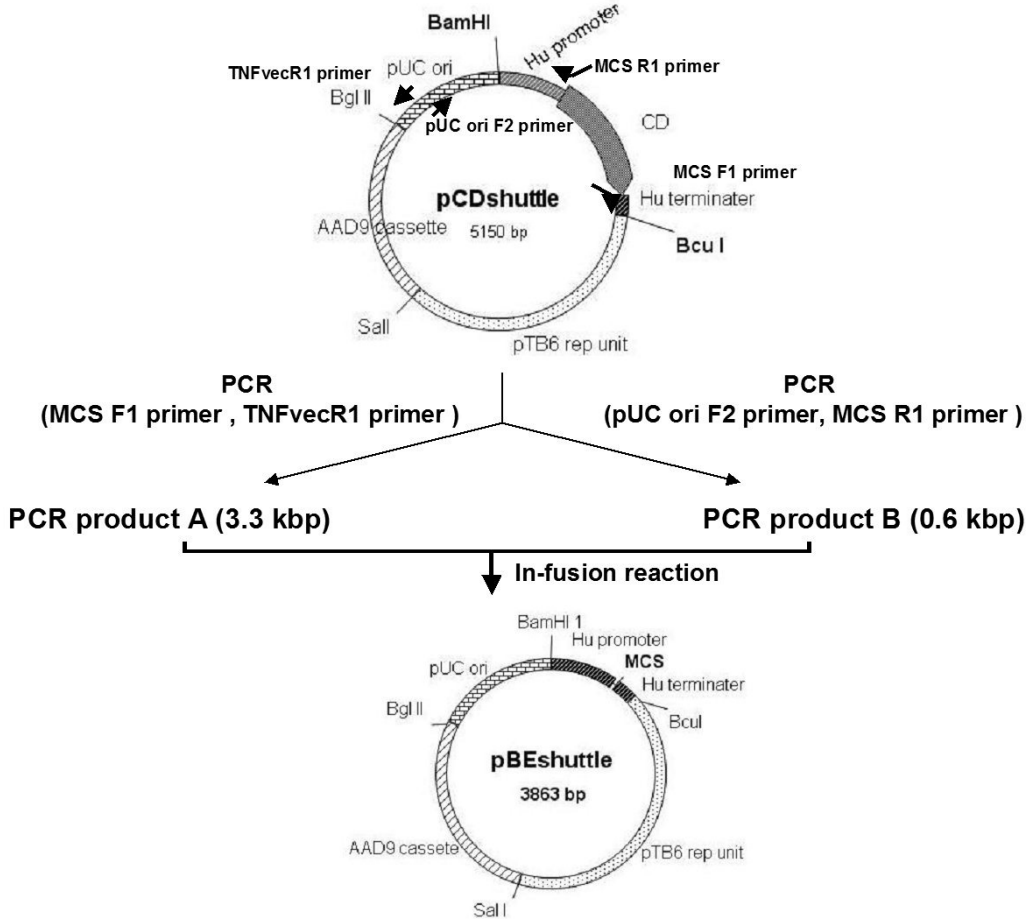


Lane 1: Molecular weight marker
Lane 2: Human recombinant TNF-alpha (positive control)
Lane 3: *B. longum* 105A/pBifiSP3B-TNF alpha culture supernatant
Lane 4: *B. longum* 105A/pSP3B-TNF alpha culture supernatant
Lane 5: *B. longum* 105A culture supernatant
Lane 6: *B. longum* 105A/pBifiSP3B-TNF alpha intracellular extract
Lane 7: *B. longum* 105A/pSP3B-TNF alpha intracellular extract
Lane 8: *B. longum* 105A intracellular extract

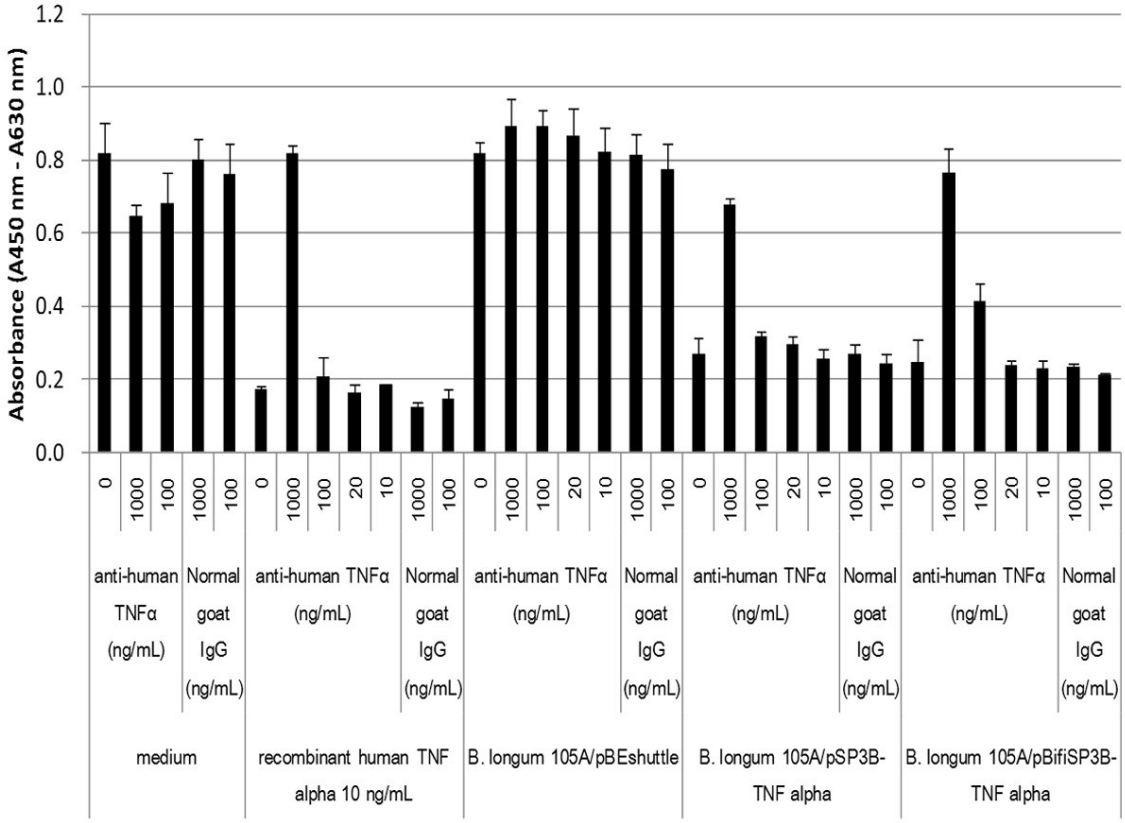
[Fig.12]


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 terminater
AAD9 cassette
5150 bp
Bcu I
Sall
pTB6 rep unit
PCR
PCR
Insert (0.5 kbp)
Vector (3.9 kbp)
In-fusion reaction
BamHI
Hu promoter
pUCori
Bgl II
mature TNFalpha
pTNF3
Hu terminater
BcuI
AAD9 cassette
Sall
pTB6 rep unit

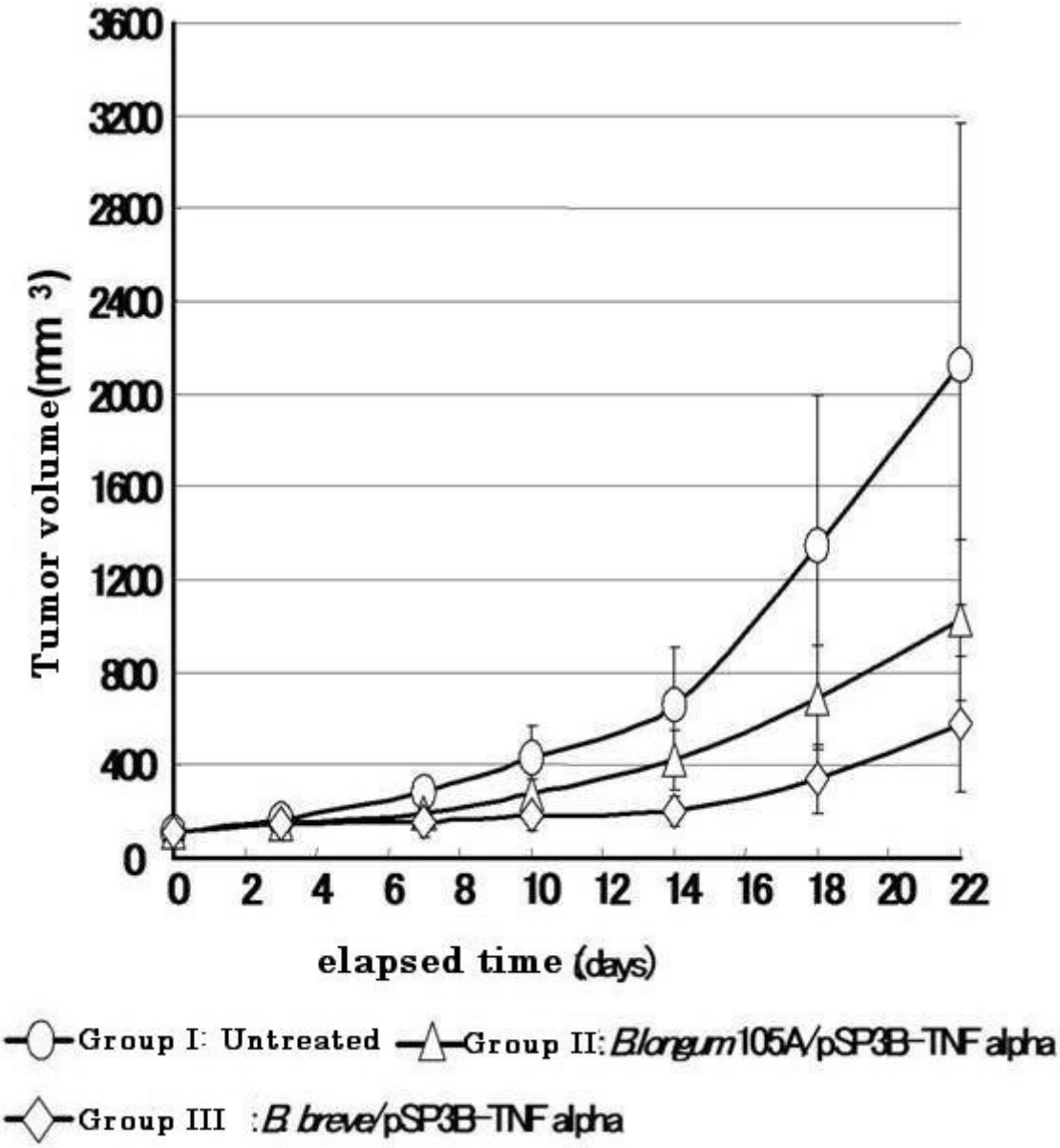
[Fig.13]


BamHI
Hu promoter
TNFvecR1 primer
pUC ori
MCS R1 primer
BgI II
pUC ori F2 primer
CD
MCS F1 primer
pCDshuttle
Hu terminater
AAD9 cassette
5150 bp
Bcu I
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 1
Hu promoter
pUC ori
MCS
Hu terminater
BgI II
BcuI
pBEshuttle
3863 bp
AAD9 cassete
pTB6 rep unit
Sal I

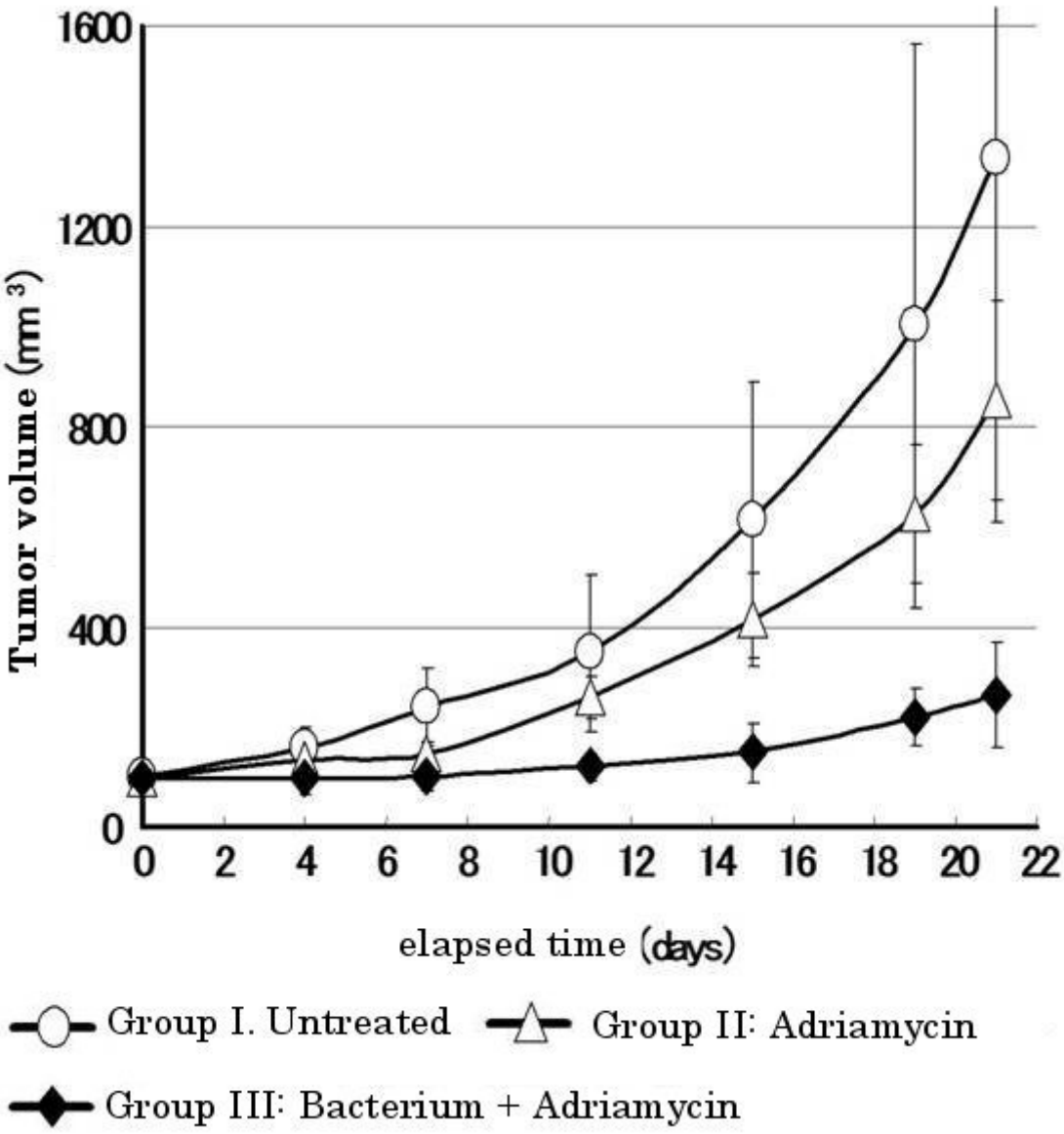
[Fig.14]


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/pSP3B-TNF alpha
B. longum 105A/pBifiSP3B-TNF alpha

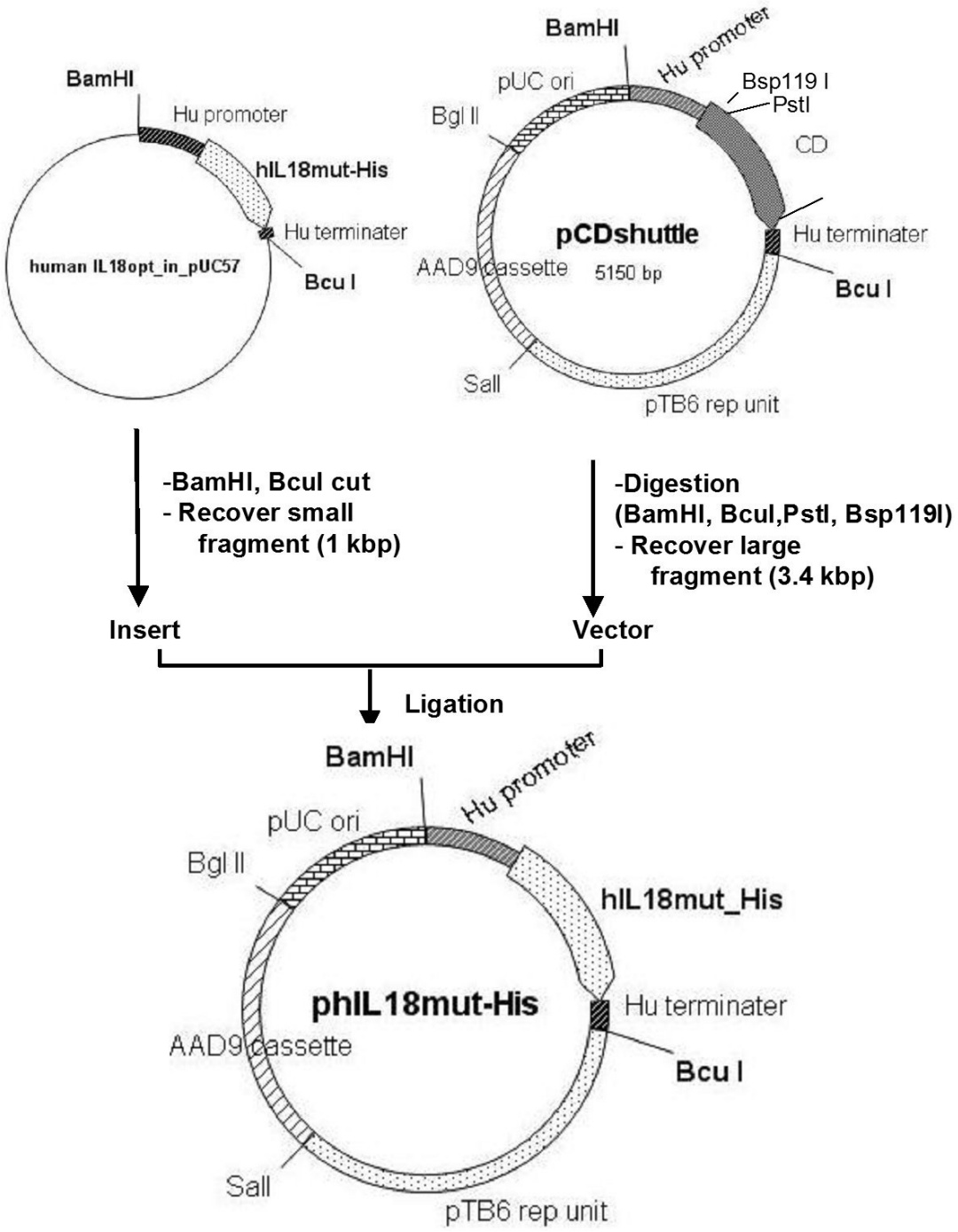
[Fig.15]


3600
3200
2800
2400
2000
1600
1200
800
400
0
Tumor volume(mm 3)
0 2 4 6 8 10 12 14 16 18 20 22
elapsed time (days)
Group I: Untreated
Group II: B.longum 105A/pSP3B-TNF alpha
Group III : B. breve/pSP3B-TNF alpha

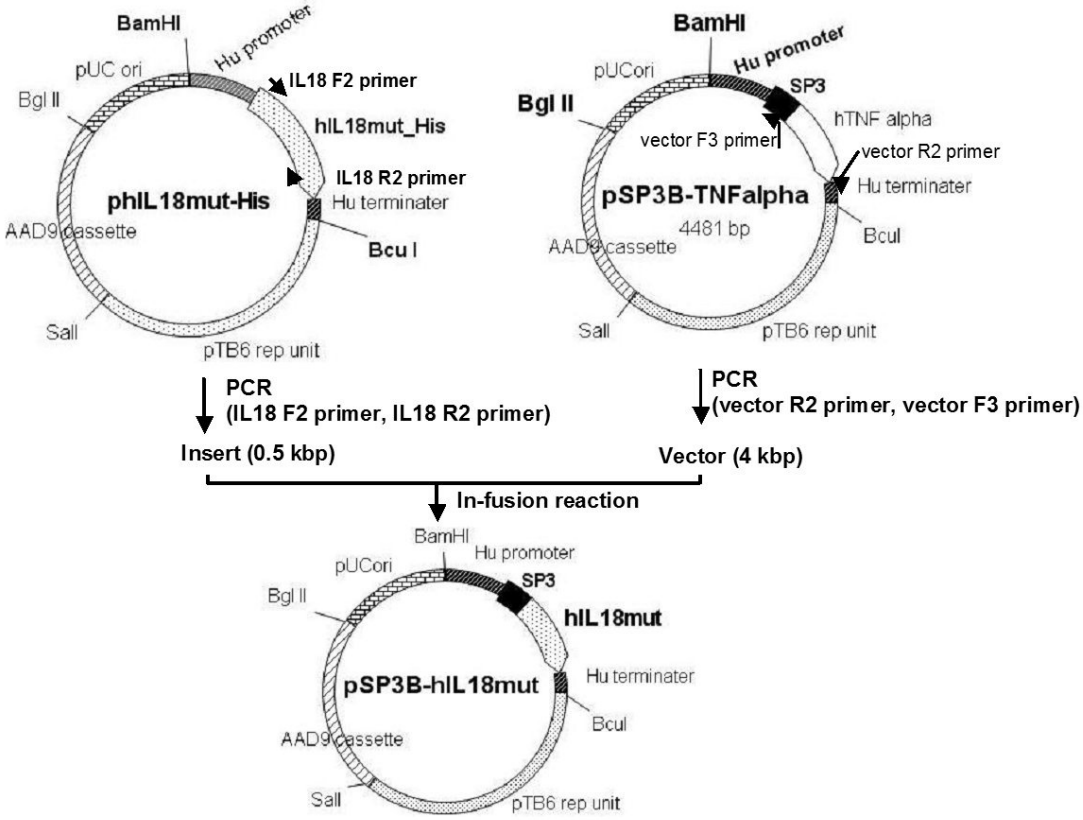
[Fig.16]


1600
1200
800
400
0
Tumor volume (mm 3)
0
2
4
6
8
10
12
14
16
18
20
22
elapsed time (days)
Group I. Untreated
Group II: Adriamycin
Group III: Bacterium + Adriamycin

[Fig.17]


BamHI
Hu promoter
hIL18mut-His
Hu terminater
human IL18opt_in_pUC57
Bcu I
BamHI
Hu promoter
pUC ori
Bsp119 I
PstI
Bgl II
CD
pCDshuttle
Hu terminater
AAD9 cassette
5150 bp
Bcu I
Sall
pTB6 rep unit
-BamHI, Bcul cut
- Recover small
fragment (1 kbp)
-Digestion
(BamHI, Bcul,PstI, Bsp119I)
- Recover large
fragment (3.4 kbp)
Insert
Vector
Ligation
BamHI
Hu promoter
pUC ori
Bgl II
hIL18mut_His
phIL18mut-His
Hu terminater
AAD9 cassette
Bcu I
Sall
pTB6 rep unit

[Fig.18]


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