

6

- 1 pT7IL - 2 ,
- 2 pT1GIL - 2 ,
- 3 pT2GIL - 2 ,
- 4 pG1IL - 2 ,
- 5 pG2IL - 2 ,
- 6 pG3IL - 2 ,

7 (expression level, %)

8 (, %)

9 (%)

10 (Western blot) , SDS - PAGE ,

A) 1:

2: pT7IL - 2

B) 1:

2: pT1GIL - 2

3: pT2GIL - 2

4: pG1IL - 2

5: pG2IL - 2

6: pG3IL - 2

11 (Western blot) , SDS - PAGE ,

가 XG_G - L - Y

XG_G: ()

L: ()

Y:

가

가 가

가 , N -
(immunogenicity)

(methionine) 가
N - 가
10,000

가
가
(antibacterial peptide)

가

N -

a), - - (- galactosidase), (maltose binding protein, 45 kD)
(glutathione - S - transferase, 26 kDa)
(affinity chromatography)

가

가

(inclusion bodies)
 (Ulman, A. 1984, Gene 29, 27 - 31; Nilsson, B. et al. 1985, EMBO J. 4, 107
 5 - 1080; Di Guan et al. 1988, Gene 67, 21 - 30; La Vallie, E.R. et al. 1993, Bio/Technology 11, 187 - 193).

(peptide) (folding) 가 (in
 termolecular disulfide bond) (hydrophobic interaction)
 ((chaperon), (ribosome),)
 가 (Anna Mituraki et al., 1989, Bio/Technol.7, 690 - 697).

(guanidine hydrochloride), (urea) (denaturant)
 (refolding)
 (reducing agent)
 (Marston, F. A. O. 1986, Biochem. J. 240, 1 - 12; Mitraki, A. and King,
 J. 1989, Bio/Technology 7, 690 - 697).

(glucagon) (- cells of the islets of Langerhans) X
 , 29
 가 (S
 hin et al. 1998, Appl. Microbiol. Biotechnol. 49, 364 - 370). (3.5 kDa)
 X - ray - (helix)
 (oligomer) (Sasaki, K. et al. 1975 Nature 257, 751 - 757).
 가 (hydrophobic self - association)

가 N - 가

(human tumor necrosis factor)

(異種)

1 3

(inclusion body)

, 가

가 가

pH

3.5 kDa

가

29

가

가

1

1

(G)

/

1) N - , [N - 1 57 (T1)] - [G -] - [(His6)] (T 1G)

2) N - , [N - 8 12 (T2)] - [(His10)] - [G] (T2G)

3) N - , [G] - [(His6)] (G1)

4) N - , [G 가 2 (G2)] - [(His6)] (G2)

5) N - , [G 가 3 (G3)] - [(His6)] (G3)

pH

(metal chelating resin),

- 2(interleukine - 2)

5가

- 2

1) (Polymerase Chain Reaction, PCR)

2) DNA T7 pT7 - 7 - 2 p
T7IL - 2

3) 5가 (T1G, T2G, G1, G2, G3) C - (enterokinase)
Asp Asp Asp Asp Lys(DDDDK, D4K)가 가 PCR

4) DNA pT7IL - 2 - 2 5' - -
2가 D4K (pT1GIL - 2, pT2GIL - 2, pGIL - 2, pG2IL - 2, pG3IL - 2).

NA , lac, trp, tac, pL, T3, T7, SP6, SV40, (pL/pR) C - 가 가 D
DNA (- D4K) DNA ,

DNA 3' DNA

- 2 (Hanahan, D. 1985, D
NA Cloning 1, 109 - 135, IRS press)

pT1GIL - 2, pT2GIL - 2, pG1IL - 2, pG2IL - 2, pG3IL - 2 BL21(DE3)
1998 11 13
(: KCTC 0555BP, KCTC 0556BP, KCTC 0552BP, KCTC 0553BP, KCTC 0
554BP).

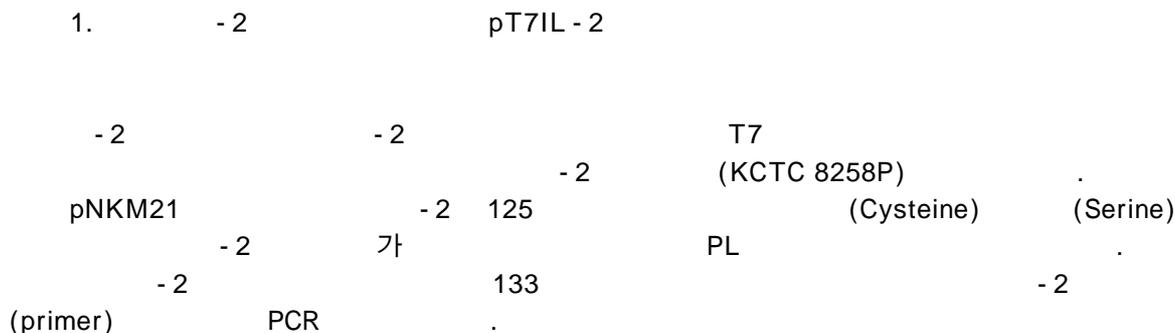
(lysozyme digestion), - 2 (French p
ress)

- 2

80%) (- 2 50 60%) - 2 (

(refolding)

N -



1) GCA CAT ATG GCA CCT ACT TCA AGT TCT (pNIL)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (pHIL)

PCR DNA (0.25mM dNTPs; 50mM KCl; 10mM (NH₄)₂SO₄; 20mM Tris - HCl(pH8.8); 2mM MgSO₄; 0.1% Triton X - 100) DNA 100ng, 50pmol Taq DNA

ngation) 30 (PCR)

PCR DNA 1% 가 (agarose gel) DNA

(Qiagen Gel extraction kit) NdeI HindIII 5'

NdeI 3' HindIII pT7 - 7 NdeI HindII

I pT7IL - 2 (1).

2. -2

가. pT1GIL - 2

U937 cDNA (Clontech) PCR

T1 (N - 1 57

) DNA (Qiagen Gel extraction kit) DNA B

amHI NdeI 5' NdeI 3' BamHI p

T7 - 7 NdeI BamHI pT7 - T1

DNA (DNA synthesizer) PCR

1(fragment - 1)

1) GCA GGA TCC GAC GAC GAC GAC AAA CAC TCT CAG GGT (BGD4K)

2) TTT GTC GTC GTC GTC CTC GAG AGT GTT CAT CAG CCA (XGD4K)

PCR DNA 1% 가 DNA (Qiagen G
 el extraction kit) .
 , pT7IL - 2 - 2 PCR
 2(fragment - 2) .

1) GAC GAC GAC GAC AAA GCA CCT ACT TCA AGT TCT (XID4K)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR DNA 1% 가 DNA (Qiagen G
 el extraction kit) .
 1 2 PCR .

1) GCA GGA TCC GAC GAC GAC GAC AAA CAC TCT CAG GGT (BGD4K)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR DNA 1% 가 DNA DNA
 . DNA BamHI HindIII 5' BamHI 3' HindIII
 I pT7 - T1GIL - 2 pT7 - T1 BamHI HindIII .
 pT7 - T1GIL - 2 PCR XH6IL - 2

1) GCA CTC GAG CAT CAT CAT CAT CAT AGC AGC GAC GAC GAC GAC AAA GCA (XH6)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR DNA 1% 가 DNA DNA
 . DNA XhoI HindIII 5' XhoI 3' HindIII
 pT1GIL - 2 pT7 - T1GIL - 2 XhoI HindIII .
 (2).
 . pT2GIL - 2

Pro - Ser - Asp - Lys - Pro(N - 8 12
) 5' NdeI 3' (His10) BamHI
 가 pT7 - 7 NdeI BamHI . pT7 - T
 2 .
 pT7 - T1GIL - 2 - 2
 PCR GIL - 2(BH) .

1) GCA GGA TCC CAC TCT CAG GGT ACT TTC (GBAM)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR
 DNA 1% 가
 DNA BamHI HindIII 5' BamHI 3' HindII
 I pT7 - T2 BamHI HindIII
 pT2GIL - 2 (3).
 . pG1IL - 2
 pT1GIL - 2 - 2
 PCR GH6IL - 2(NH)

1) GCA CAT ATG CAC TCT CAG GGT ACT (GNDE)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR
 DNA 1% 가
 DNA NdeI HindIII 5' NdeI 3' HindIII
 pT7 - 7 NdeI HindIII
 pG1IL - 2 (4).
 . pG2IL - 2
 pGIL - 2 - 2
 PCR GH6IL - 2(BH)

1) GCA GGA TCC CAC TCT CAG GGT ACT TTC (GBAM)

2) GCA AAG CTT CTA TTA AGT TAG TGT TGA GAT GAT (HIL)

PCR
 DNA 1% 가
 DNA BamHI HindIII 5' BamHI 3' HindII
 I pT7 - 7 BamHI HindIII
 pGIL - 2(BH)
 DNA PCR G(NB)

1) GCA CAT ATG CAC TCT CAG GGT ACT (GNDE)

2) GCA GGA TCC AGT GTT CAT CAG CCA (GBAM - 3)

PCR
 DNA 1.5% 가
 DNA NdeI BamHI 5' NdeI 3' BamH
 I pGIL - 2(BH) NdeI BamHI
 pG2IL - 2 (5).
 . pG3IL - 2
 pGIL - 2 PCR G(
 EB)

- 1) GCA GAA TTC CAC TCT CAG GGT ACT (GECO)
- 2) GCA GGA TCC AGT GTT CAT CAG CCA (GBAM - 3)

PCR DNA 1.5% 가 DNA
 DNA EcoRI BamHI 5' EcoRI 3' Bam
 HI pGIL - 2(BH) EcoRI BamHI
 pG2IL - 2(EH)
 pGIL - 2 PCR
 G(NE)

- 1) GCA CAT ATG CAC TCT CAG GGT ACT (GNDE)
- 2) GCA GAA TTC AGT GTT CAT CAG CCA (GECO - 3)

PCR DNA 1.5% 가 DNA
 DNA NdeI EcoRI 5' NdeI 3' EcoRI
 pG3IL - 2 pG2IL - 2(EH) NdeI EcoRI
 (6).
 3.

(Hanahan D. 1985, DNA Cloning 1, 109 - 135, IRS press) pT7IL -
 2, pT1GIL - 2, pT2GIL - 2, pG1IL - 2, pG2IL - 2, pG3IL - 2 BL21(DE3) (Studier, F. A. and Mof
 fatt, B. A. 1986, 189, 113 - 130) , (amphicillin)

1998 11 23
 (: pT7IL - 2 KCTC 0555BP, pT2GIL - 2 KCTC 0556BP, pG1IL - 2 KCTC 0552BP, pG2IL
 - 2 KCTC 0553BP, pG3IL KCTC 0554BP).

4.

(LB , +100mg/L) (1%) 37
 200rpm OD₆₀₀ 가 0.4 IPTG(isopropylthio - - D - galactoside)
 가 (0.5 mM) . IPTG 가 3 - 4

5.

BL21(DE3) (plate) 10
 LB (+100mg/L) 2ml (1%) 37
 200rpm OD₆₀₀ 가 0.4 IPTG 가(0.5mM)
 3 - 4 6000rpm
 (Branson Sonifier)
 SDS - PAGE(sodium dodecyl sulfate - polyacrylamide gel electrophoresis) (14% Tris -
 Glycine gel) , (Densitometer, Bio - Rad)
 LB 15% 가 - 70. C
 - 2
 , T2G G3 가 G3
 60% (7).

6

6000rpm
(Branson Sonifier) 5000g 10
SDS - PAGE (14% Tris - Glycine gel) 0.5% TritonX - 100
2

7. -2

(⁶Phe - ⁶Lys; ¹⁰Tyr - ¹⁰Lys; ¹³Tyr - ¹³Lys)
가 IL - 2

가 IL - 2

E GXHO 2 3 PCR (GK3IL - 2) GK3 - 1 GK3 - 2 GND

1) GCA CAT ATG CAC TCT CAG GGT ACT (GNDE)

2) GAC CTC GAG AGT GTT CAT CAG CCA (GXHO)

PCR DNA 2% 가 DNA DNA NdeI XhoI 5' NdeI 3' Xh
ol pIL - 2(NX) NdeI XhoI pG3IL - 2 NdeI XhoI G3
pGK3IL - 2 pIL - 2(NX) pG3IL - 2 NdeI XhoI G3

G1IL - 2 IL - 2 (GK3IL - 2) 1

[1]

| | | |
|-----|---------|-----------|
| | GIL - 2 | GK3IL - 2 |
| (%) | 25.02 | 32.89 |

1.

5 100ml LB (+100 mg/l)
OD₆₀₀ 0.4 IPTG 가 (0.5mM)
3 - 4 6
0.5% X - 100(TritonX - 100) 2 SDS - PAGE (14% T
ris - Glycine gel) (Bio - Rad)
-2가 가 40% 5 가
가 80% 가 가 가 가 (8). G3

2. (solubilization)

가 50 mg/ml , pH 12 2 pH 12
 (Bradford) (5000 g, 10)
 pH 12

$$\text{용해도(\%)} = \frac{\text{상등액단백질총량}(\mu\text{g}) \times 100}{\text{상등액단백질총량}(\mu\text{g}) + \text{침전물단백질총량}(\mu\text{g})}$$

9 -2
 100% 가 G3 8 g/L pH
 pH 12 pH 8

3.

(
 (Western blot)
 SDS - PAGE (14% Tris - Glycine gel)
 -2 IgG1 , 2
 IgG가 10 , -2 (alkaline phosphatase) 가
 (multimer) -2가 -2 (direct expressio

(Bio - Rad) (hydrophobically - bound hom
 ologous multimer)가 11
 가 32% ,
 60% 90%

(57)

1.

, 가

XG_G - L - Y

XG_G: (1 ,)

L: ()

Y:

2.

3.

1 , ⁶Phe ⁶Lys, ¹⁰Tyr ¹⁰Lys, ¹³Tyr ¹³Lys

4.

1 , ,

5.

1 , 가 Asp Asp Asp Asp Lys (DDDDK, D4K)

6.

1 , ,

7.

1 , - 2

8.

7 , - 2 pH

9.

8 , - 2

10.

1 , , pT7 - 7

11.

10 , - 2 .

12.

T1 가 N - 1 57 (T1G
IL - 2) - [G -] - [(His6)
pT1GIL - 2 (KCTC 0555BP.),

(T2 가 N - 8 12 T2G
IL - 2) - [(His10)] - [G
pT2GIL - 2 (KCTC 0556BP.),

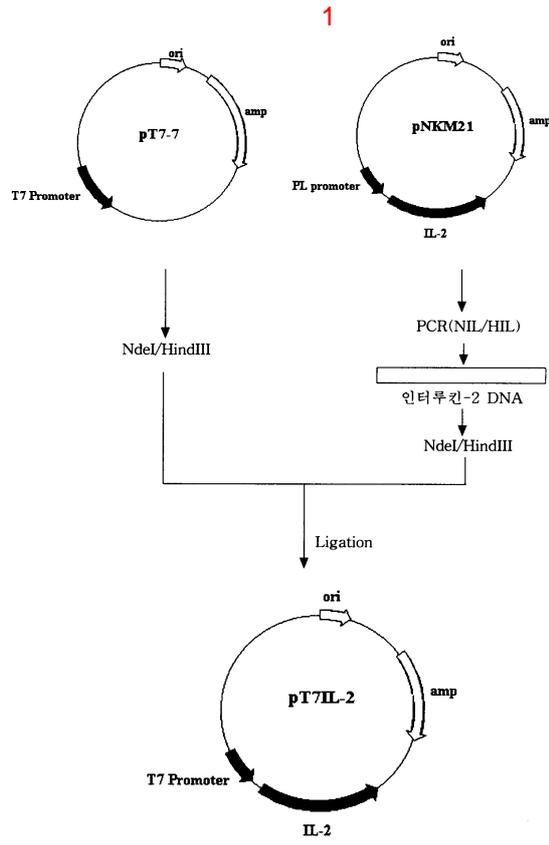
가 1 [G] - [(His6)] G1
IL - 2 pG1IL - 2 (KCTC 0552BP),

(His6)] 가 2 [G 가 2 (G2)] - [KCT
C 0553BP), G2 IL - 2 pG2IL - 2 (

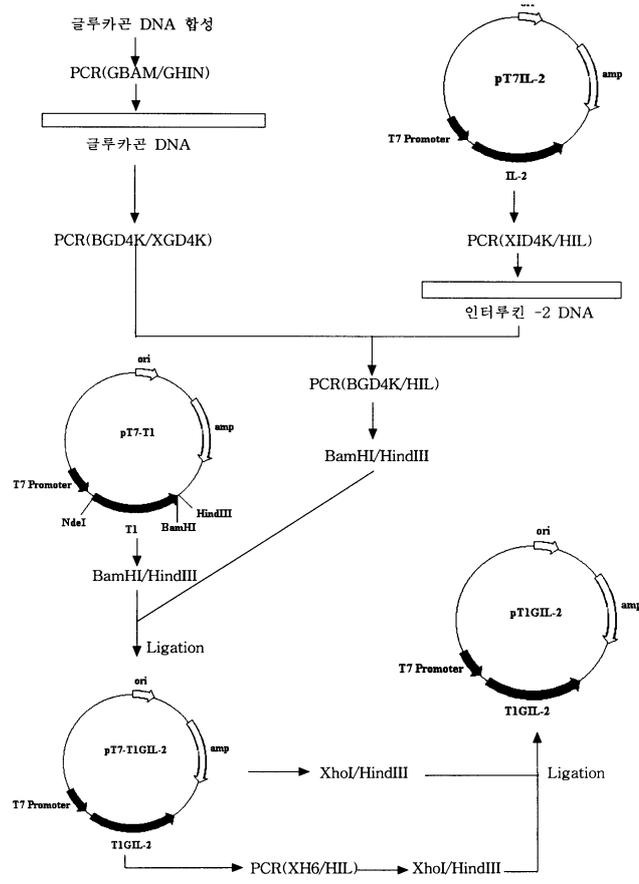
(His6)] 가 3 [G 가 3 (G3)] - [KCT
C 0554BP), G3 IL - 2 pG3IL - 2 (

가 GK3 IL - 2 pGK3IL - 2 (KCTC 05
82BP)

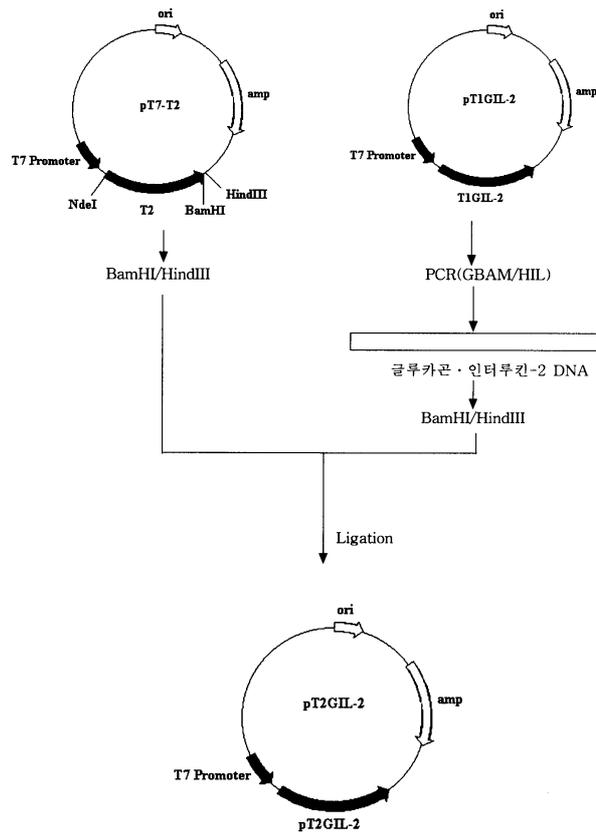
13.



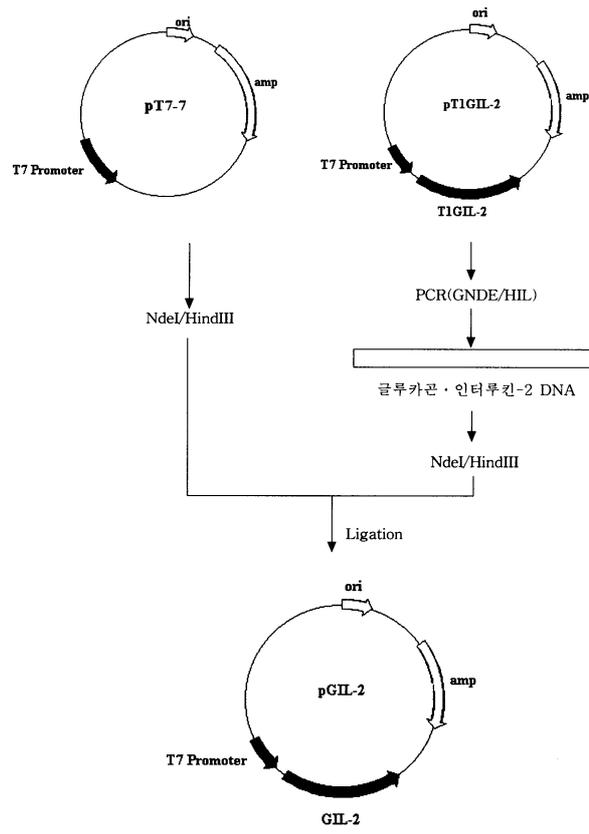
2



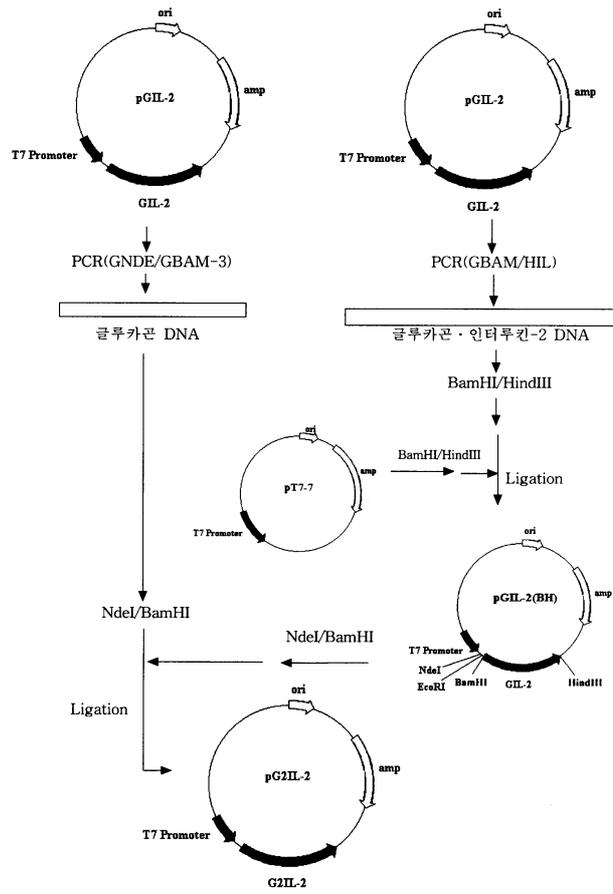
3



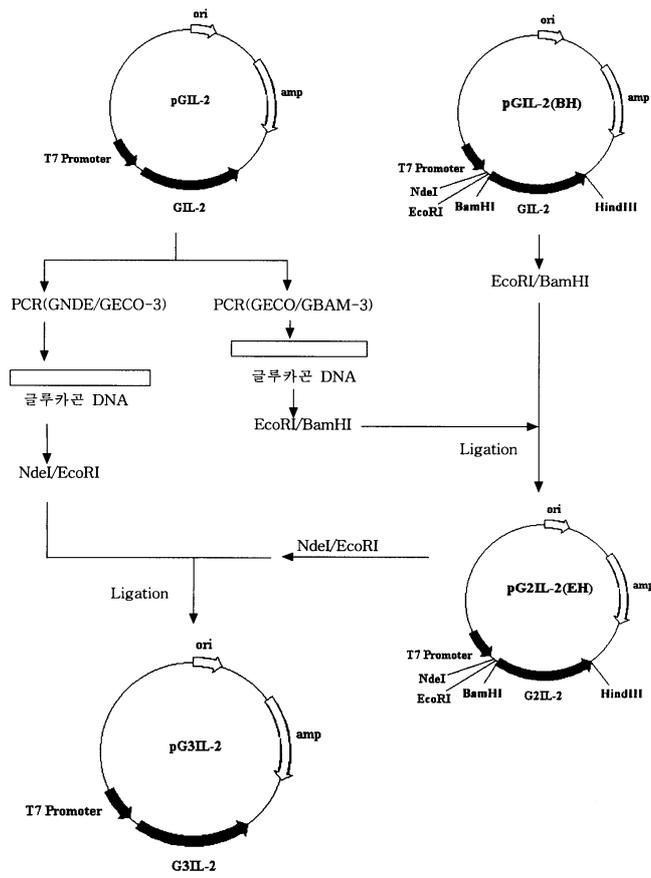
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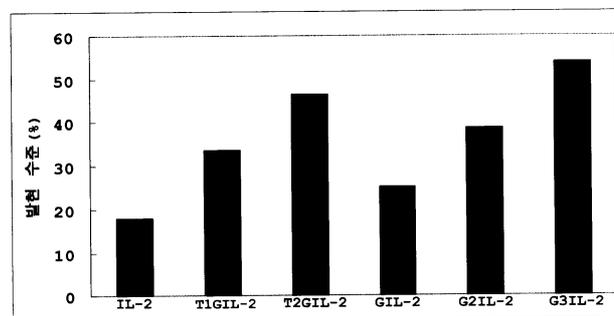
5



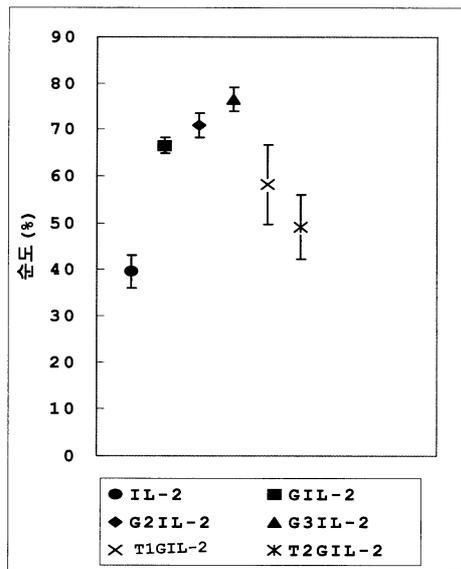
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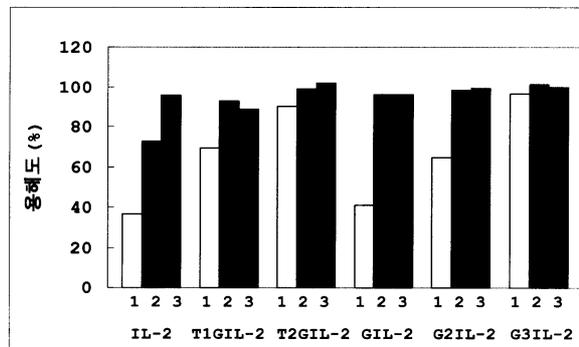
7



8

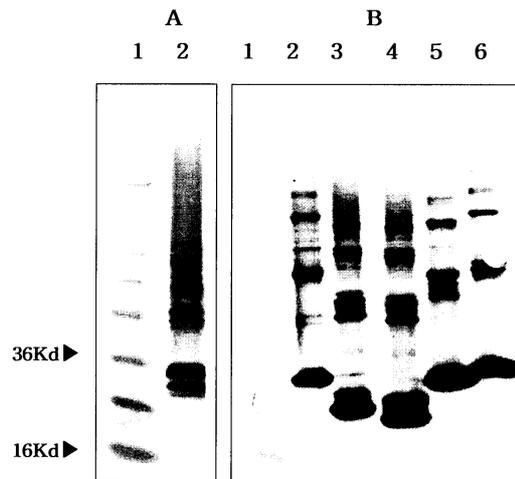


9

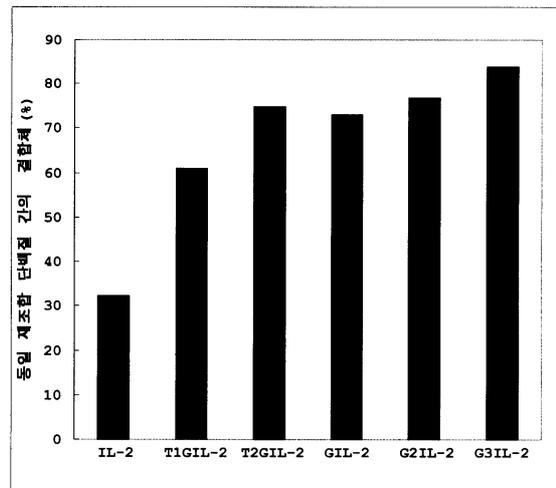


| | Column number(#) | | |
|-----|------------------|-----|-----|
| | 1 | 2 | 3 |
| g/L | 8.3 | 5.6 | 5.0 |

10



11



<110> Korea Institute of Science and Technology <120> Preparation of Peptides by Use
 f Human Glucagon Sequence as a Fusion Expression Partner <130> 8p-12-13 <160> 3 &
 lt;170> KOPATIN 1.0 <210> 1 <211> 29 <212> PRT <213> Homo s
 apiens <400> 1 His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Ser 1
 5 Met Asn Thr 10 Arg Arg Ala Gln Asp Phe Val Gln Trp Leu
 15 20

25 <210> 2 <211> 51 <212> DNA <213> Artificial Sequence
 <220> <223> modified DNA sequence for human glucagon <400> 2 cactctcagg gtact
 aaaac ttctgacaaa tctaaaaaac tggactctcg t 51 <210> 3 <211> 51 <212>

DNA <213> Artificial Sequence <220> <223> modified DNA sequence for human gl
 ucagon <400> 3 agtgttcatc agccactgaa cgaagtctcg agcagcagca gagtccagtt t
 51