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(A)

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A61K 39/395

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(43)

10-2004-0105815
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(87)

2003 10 09

(30) JP-P-2002-00097424 2002 03 29

(JP)

JP-P-2003-00045088 2003 02 21

(JP)

(71) 가가

가 4 1 8

(72)

, 7-C-104

가 , 2-30-2

, , 1-2-806

, 6

, 2-10-13

, 3-3-1-301

(74)

:

(54)

B (가 ,) , 가 , (,) , 가 , 가 , .

, , , , ,

가 , 가

(DDS)

가
가
가

((,)) , , , , ,

, 100% 가

가

가

DNA

, 100% 가

가

가

가
가

가

가

가

「WO01/64930」

WO01/64930(2001 9 7)

2001-316298(2001 11 13)

가

(, ,)

()

Fc

ZZ

()

ZZ

Fc

)

, 가 ,

(

()

,

(

,

가

가

(HSV1 tk)

가

, pre-S

1

20

y

B

N

, 가 , pre-S

, N

50

153

, pre-S

N

12

31

d

B

, N

61

164

, 가 , pre-S

DDS

가

가

, HBsAg

y

Pre-S1

108

,

. 1 8

d Pre-S1

119

2

HBsAg

, (a)

, (b) High-Pi

, (c) 8S5N-P400

, (d)

, (e)

, (f) HBsAg

3		HBsAg-streptag		
4		HBsAg-streptag		
5		HBsAg-streptag		
6		HBsAg-ZZtag		
7		HBsAg-ZZtag	SDS-PAGE	(Western Blotting)
8	HBsAg-scFv)	HBsAg-ZZtag	(A22 3A21
9	HBsAg-scFv)	HBsAg-ZZtag	(A22 3A21
10		HBsAg-ZZtag	,	
11	HBsAg-scFv	SDS-PAGE		
12		HBsAg		
13		PCR		
14		PCR		
15		HBsAg	,	
16(a)(b)			HBsAg	
	, 16(a)	, 16(b)		
17	16(a)(b)			
18	16(a)(b)	HBsAg	SDS-PAGE	, (a)
, (b)				
19	16(a)(b)	HBsAg		, (a)
, (b)				
20			HBsAg	,
21	, 20		HBsAg L	,
22	, 20		HBsAg L	,
23				
24				

25 .

26 .

27 , .

가 , 가 , (,) 가 , B (Hepatitis B Virus: HBV) , HBV L 20nm, 150nm , HBV L 가 , (J. Biol. Chem., Vol. 267, No. 3, 1953-1961, 1992). , HBV L , 가 (, HBV) (,) HBV (1 L). preS (preS1, preS2) , HBV가 , preS 가 , 「preS , p reS1 , preS2 , preS (d) preS1) N 3 66 (y) 14 77 (d) preS , preS B , , preS B , preS 가 . 3 66 (y) 4 77 (d y) , preS1 N , 3 66 (y) 가 , d y , N N 12 1 31 20 , N 153 , pre-S , 50 153 , 33 153 , 21 153 , 50 50 153

, d , preS (preS1 preS2) , pre-S , 가
 , N 61 164
 , N 12 31 .
 , 가 B
 , .
 가 .
 가 .
 , 가
 , (baculovirus) , , 가
 , , 가 , 가
 , , 가 , 가
 (Invitrogen) , 가 , 가
 (DNA, RNA, , ,) , 가 .
 , , B , ,
 , , 가 , ,
 WO01/64930 B B , ,
) (B , ,
 , , , ,
 , , , , 가 ,
 , , , , ()
 , ()가 , , ,
 , DNA, RNA , , , ,
 , 가 .

RNAase 1(Jinno H, Ueda M, Ozawa S, Ikeda T, Enomoto K, Psar
 ras K, Kitajima M, Yamada H, Seno M Life Sci. 1996; 58(21); 1901-8) RNAase 3(ECP: eosinophil c
 ationic protein; Mallorqui-Fernandez G, Pous J, Peracaula R, Aymami J, Maeda T, Tada H, Yamada H, Seno
 M, de Llorens R, Gomis-Ruth FX, Coll M; J Mol Biol. 2000 Ju1 28;300(5):1297-307.) .

) , RNAase 가 ()
 , ,
 , 23 26

, 가 , (p53) , , Fas ,
, ,
, ,
, 가 , 가 , RNAase , 1
가 ,
, Fc , ZZ (Fc ,
ZZ , ZZ ,
() , 가 ,
() ,
() , 가 (NHS(N-hydroxysuccini
mide) , Maleimide , Imidoester (PIERCE) 가) ,
, 가 ,

(EGF) , 가
(HSV1 tk) , 가 (ganciclovir: GCV)
, 가 ,
, 가
, 가
, HBV HBV L L preS1 , HBV L
() , HBV DDS L

, HBsAg , B (Hepatitis B virus surface Antigen)
, HBsAg S , M
, L N 3 가 , S 3 , M S
y) 55 (pre-S2 peptide)가 가 , L M N 108(
y) 119(d) (pre-S1 peptide)가 가 ,
HBsAg L Pres-S (pre-S1, pre-S2) , HBV가
. Pre-S1 , pre-S2
HBsAg , , HBsAg L

, HBsAg L . , 2 HBsAg

(A) HBsAg

J. Biol. Chem., Vol. 267, No. 3, 1953-1961, 1992 , pGLDLIIP
 39-RcT (*Saccharomyces Cerevisiae* AH22R -) , High-Pi 8S5N-P
 400 , HBsAg L (2a 2c).

(72) , Yeast Protein Extraction Reagent(Pierce Chemical C
 o.) , (whole cell extract) ,
 (SDS-PAGE) , HBsAg .
 , HBsAg 52kDa .

(B) HBsAg

(1) 8S5N-P400 (26g) (buffer) A (7.5M , 0.1M
 , pH 7.2, 15mM EDTA, 2mM PMSF, 0.1% Tween 80) 100ml
 (BEAD-BEATER) , (2d).

(2) , 0.75 33%(w/w) PEG6000 , 30 , (7000rpm, 3
 0) , 80 A .

(3) , 10 40% CsCl , 28000 rpm, 16
 12 , HBsAg (1 -HBsAg) HBs
 Ag 가 , HBsAg 80 A

(4) (3) (12ml) 5 50% , 28000 rpm, 16
 , (3) , HBsAg , HBsAg
 Tween 80 , 0.85% NaCl A ((2) (4) :
 2e).

(5) (4) , (Ultra Filter) Q 2000()
 , 4 (2f).

CsCl (3) , HBsAg 52kDa S
 , 2.5L , 26g 24mg HBsAg

SDS-PAGE 가
 , (5) HBsAg 37 12 , SDS-PAGE

HBsAg 가 ,
 pre-S1 3 77
 (Le Seyec J, Chouteau P, Cannie I, Guguen-Guillouzo C, Gripon P., *J. Virol.* 1999, Mar; 73(3): 2052
 -7).

HBsAg ,
 HBsAg , ZZ , HBsAg
 HBsAg , HBsAg

(C) HBsAg

(C-1) HBsAg-steptag

A pGLDLIIP39-RcT
 NotI (gcggccgc) 1 2
 PCR , QuichChange™ Site-Directed Mutagenesis Kit(Stratagene)
) PCR pGLDLIIP39-RcT .

, DNA , 55 1 , 68 30 Pfu DNA (Stratagene) , PCR 95 30
 , DH5 , , 30 , PCR Dpnl

pGLDLIIP39-RcT , pGLDLIIP39-RcT-Null , 3
 「Null」 , HBsAg L 「Null」 .

3 , pGLDLIIP39-RcT-Null , SacI Sall 가
 , SacI 가 3 , PCR Sall 가 4 (GLDp) (PCKt)
 Null , Null , PCR cDNA .

, pRS405+2µm ((Stratagene) AatII 2µm
) SacI Sall , Null cDNA
 pRS405+2µm , pRS405+2µm - Null .

, (strep-tag) (5 6
) , pRS405+2µm - Null NotI ,
 pRS405+2µm-strep-tag , N ,

1) , 1) SAWRHPQFGG(27) 2) WSHPQFEK(28) .
 C , 2) .

, pRS405+2µm-strep-tag (Saccharomyces Cerevisiae AH22R -)
 , B HBsAg
 , 「HBsAg-streptag」 (HBsAg L , 1.0L , 200µg HBsAg
 -streptag .

(C-2) HBsAg-streptag

가 , 가 , ,
 가 HBsAg-streptag .

4 , C-1 pGLDLIIP39-RcT-Null 7
 (Kpn I ggtacc) 8 (Sac II cgcgcg
) PCR ,
 Null .

PCR 가 , 1.3kbp ,
 -pIZT/V5-His(Invitrogen) Kpn I Sac II , TaKaRa Liga
 tion kit ver.2(TaKaRa) , pIZT-Null
 .

, C-1 , streptag (5
 6) , pIZT-Null NotI
 , pIZT-streptag .

, High Five (BTI-TN-5B1-4: Invitrogen) 1

(Ultimate Insect Serum-Free Medium: Invitrogen) , pIZT-streptag
 Insectin-Plus (Invitrogen) High Five
 27 48 zeocin(Invitrogen) 400µg/
 ml 가 가 (confluent) , 4 7 , HBsAg-strepta
 g
 1500×g, 5 , IMx () , HBsAg-streptag
 HBsAg-streptag , HBsAg-streptag 가 , 가 ,
 IMx SDS-PAGE S ()
 가 HBsAg-streptag 42kDa .
 1L (UK-200: ADVANTEC , 2
 00K) (DEAE-Toyopearl 650M,) , HBsA
 g-streptag 1mg 가 .
 (C-3) HBsAg-streptag
 5 , XhoI , pGLDLIIP39-RcT-Null XhoI
 -(PGKt) Null , pcDNA3.1(Invitrogen)
 XhoI , pcDNA3.1 , pcDNA3.1-Null .
 , C-1 , streptag (5
 6) , pcDNA3.1-Null (NotI
 pcDNA3.1-streptag pcDNA3.1-streptag .
 pcDNA3.1-streptag COS7 , (gene pulser; Bi
 o-Rad) , 10% (Dulbecco)
 g-streptag CHO-SFMII(Gibco-BRL) , 가 , 1 , HBsA
 , C-2 , HBsAg-streptag SDS-PAGE
 , S IMx 가 HBsAg-
 streptag 42kDa . IMx , pcDNA3.1 HBsAg-
 L : 8.81 () , HBsAg Null : 3.47, HBsAg-streptag : 2.41 ,
 (C-4) HBsAg-streptag
 C-1 C-3 , 가 , HBsAg-streptag . , HBs
 Ag-streptag 가 HBsAg-streptag (, 가 HBsAg-streptag
 「HBsAg-streptag-Ab」) .
) , (EGFR) EGFR 7G7B6(
 erce , NHS- (Pierce EZ-Link() NHS-Biotin) , Pi
 (Pierce ImmunoPure Avidin) , PBS 30 , HBsAg-streptag ,
) 2: 1 , HBsAg-streptag (HBsAg-streptag
 HBsAg-streptag 가 PBS 30
 가 HBsAg-streptag-Ab .
 (C-5) HBsAg-streptag-Ab
 , WO01/64930 , HBsAg-streptag-Ab
 (pEGFP-F(Clontech)) , HBsAg-streptag-Ab pE
 GFP-F , EGFR 가 , GFP 가
 HBsAg-streptag-Ab .

A431 (JCRB9009) (NUE, WiDr) 3.5cm
 WiDr A431 HBsAg-streptag - Ab 1 μ g , 4
 GFP 가 GFP (NUE , WiDr) , GFP
 A431 , GFP (NUE , WiDr) , GFP
 EGFR 가 , GFP 가 HBsAg-strep
 tag - Ab 가 , A431 HBsAg-streptag - Ab 가
 (D) ZZ HBsAg
 (D-1) HBsAg-ZZ
 , 6 , C-1 , pRS405+2 μ m - Null
 , ZZ (ZZtag) (, 「ZZ」 , 「ZZ」)
 (Staphyrococcus aureus Protein A (ZZ) , NotI 가
 9 10 PCR PCR , ZZ
 , 2 (ZZ (G Fc 29) : N , VDNKFNK
 EQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAPK VDNKFNKEQQNAFYEILHLPNLNE
 EQRNAFIQSLKDDPSQSANLLAEAKKLNDAPK).
 , pRS405+2 μ m - Null NotI ,
 , pRS405+2 μ m-ZZ
 A , pRS405+2 μ m-ZZ S.cerevisiae AH22R -
 High-Pi3m1 30 3 , 8S5N-P4
 00 3ml 30 3 , ZZ HBsAg
 , pRS405+2 μ m-ZZ (Saccharomyces Cerevisiae AH22R -)
 B HBsAg (, 「H
 BsAg-ZZtag) , 1.0L , 20
 mg HBsAg-ZZtag
 가 , HBsAg-ZZtag SDS-PAGE , S
 IMx 7 SDS-PAGE , IMx
 , pRS405+2 μ m- HBsAg L : 49.43 (, 100) , H
 BsAg-Null : 21.87, HBsAg-ZZtag : 253.64 ,
 ZZ HBsAg-ZZtag 56kDa
 (D-2) HBsAg-ZZtag
 , 8 , C-2 , pIZT - Null
 , D-1 , ZZ pIZT - Null , pl
 ZT-ZZ
 , C-2 , pIZT-ZZ , HBsAg-ZZtag
 , S (,) HBsAg-ZZtag SDS-PAGE

, ZZ HBsAg-ZZtag 56kDa .

, C-2 1L HBsAg-ZZtag 1mg .

(D-3) HBsAg-ZZtag

, 9 , C-3 , pcDNA 3.1-Null .

, D-1 , ZZ pcDNA 3.1-Null

, pcDNA3.1-ZZ .

, C-3 , pcDNA3.1-ZZ COS7 , HBsAg-ZZtag

ag .

, COS7 , S (,) HBsAg-ZZtag SDS-PAG IMx

E , IMx , pcDNA3.1 HBsAg L : 8.81 (

, HBsAg Null : 3.47, HBsAg-ZZtag : 2.41 ,

, ZZ HBsAg-ZZtag 56kDa .

(D-4) HBsAg-ZZtag

ZZ , Fc , EGF

(EGFR) 7G7B6, IL-2 (Tac) 528,

ST-421 가 가 HBs

, HBsAg-ZZtag 가 HBsAg-ZZtag 「HBsAg-ZZtag-Ab 가」)

, HBsAg-ZZtag-Ab , EGFR 7G7B6() (

EGFR 가 HBsAg-ZZtag-Ab , PBS 1 가 .

(D-5) HBsAg-ZZtag-Ab

, WO01/64930 , HBsAg-ZZtag-Ab

(pEGFP-F(Clontech)) , HBsAg-ZZtag-Ab pEGFP-F

sAg-ZZtag-Ab , EGFR 가 , GFP 가 HB

, C-5 , A431 , NUE

HuH-7(JCRB 0403), WiDr(ATCC CCL-218) , A431 NUE, HuH-7,

WiDr 3.5cm , GFP 가 HBsAg-ZZtag-Ab-

GFP 1 μ g , 4 , GFP

, A431 , GFP , NUE GFP .

, EGFR 가 , GFP 가 HBsAg-ZZtag

g-Ab , 가 A431

가 , (: BALB/c nu/nu, : SPF, : 5) ,

(A431, HuH-7, WiDr) 1 \times 10⁷ , 2cm 2

4 ,

, GFP 가 HBsAg-ZZtag-Ab , 26G

, 4 , , , , , GFP (

Technovit 7100)

2, 4% 100% EtOH, Technovit 7100 1, 70% EtOH 100% EtOH/Technovit 7100 2, 96% EtOH 2

1, 24, 1, 37

GFP, (-, A431), GFP

(HuH-7, WiDr), HBsAg-ZZtag

g-Ab, EGFR 가 A431, GFP 가 HBsAg-ZZta

(D-6) HBsAg-ZZtag-Ab

Ag-ZZtag-Ab (HSV1 tk) HBsAg-ZZtag-Ab, HSV1 tk 가 HBs

HSV1 tk 가, HSV1 tk 가 (Ganciclovir:GCV)

, HSV1 tk

vivogen, HBsAg-ZZtag-Ab HSV1 tk, HSV1 tk In

Ab -pGT65-hIFN- HSV1 tk 가 HBsAg-ZZtag-Ab HBsAg-ZZtag-
g-ZZtag-Ab L 50 μg, HBsAg-ZZtag-Ab, HBsA

220V, 950 μ F 4mm 10 μg (cuvette), PBS

(: F334/NJcl-rnu/rnu, :) W

iDr 1×10⁷, A431 2 3cm

3

L2) HSV1 tk 가 HBsAg-ZZtag-Ab 10 μg ()

(GCV) 50mg/kg/day (alzet osmotic pump; Cat 2M

14 GCV

()

(× × /2) 3

10 27

g-Ab, EGFR 가 HSV1 tk 가 HBsAg-ZZta

A431

(E) HBsAg-scFv

(E-1) HBsAg-scFv

6 PCR, D-1, ZZ

(TOTO), A22 RNase

3A21 (Mol Immunol 1997 Aug-Sep; 34(12-13): 887-90 Kat
 akura Y, Kumamoto T, Iwai Y, Kurokawa Y, Omasa T, Suga K. Mol IMMunol 1997 Jul; 34(10): 731-4, Kata
 kura Y, Kumamoto T, Iwai Y, Kurokawa Y, Omasa T, Suga K.) , PCR
 NotI 가 11 12 (A22), N
 otl 가 13 14 (3A21) PCR
 le chain antibody, scFv) , 2 (sing

PCR pRS405+2 μ m - Null , pRS405+2 μ m-A22
 pRS405+2 μ m-3A21 ,
 (, A22 3A21 HBsAg L ,
 A22 3A21 「HBsAg-scFv」)
 S , , HBsAg-scFv SDS-PAGE
 IMx IMx HBsAg L SDS-PAGE
 3 (, 100 , pRS405+2 μ m- IMx HBsAg L : 49.4
 HBsAg-scFv : 4.02 , HBsAg-Null : 21.87, A22 HBsAg-scFv : 2.41, 3A21
 HBsAg-scFv 76kDa , 3A21 HBsAg-scFv A22
 scFv C-2 , 1.0L , 200 μ g 76kDa HBsAg-

(E-2) HBsAg-scFv
 , 8 , D-2 , ZZ
 PCR , RNase 3A21 , A22,
 NotI NotI 가 11 12 (A22), PCR
 R , A22 3A21 (3A21) PC

PCR pRS405+2 μ m - Null , pIZT - A22
 pIZT - 3A21 A22 3A21 HBsAg-scFv
 S , , HBsAg-scFv SDS-PAGE
 76kDa , 3A21 HBsAg-scFv , A22 HBsAg-scFv
 , C-2 , 1L HBsAg-scFv 1mg

(E-3) HBsAg-scFv
 , 9 , D-3 , ZZ
 PCR , RNase 3A21 , A22,
 NotI NotI 가 11 12 (A22), PCR
 NotI 가 13 14 (3A21) PCR
 , A22 3A21

PCR pcDNA3.1 - Null , pcDNA3.1 - A22
 pcDNA3.1 - 3A21 A22 3A21 HBsAg-scFv COS7
 COS7 , , HBsAg-scFv SDS-PAGE
 , S , , A22 HBsAg-scFv

(E-4) HBsAg-scFv
 , HBsAg-scFv , A22 ,
 , 3A21 RNase1 , 96 , ELISA ,
 , HRP , HBsAg , HBsAg-scFv , HBsAg-scFv ,
) 50nM , HBsAg-scFv (A22) 100nM , HBsAg-scFv (3A21
) HBsAg-scFv 가
 , A22 3A21가 HBsAg-scFv , A431

(F)
 , HBsAg L Pre-S (pre-S1, pre-S2)
 , HBsAg L 가

(F-1) HBsAg L
 PCR , HBsAg L
 HBsAg L , Pre-S (pre-S1, pre-S2) (a) (e) 5
 HBsAg L , (a) pre-S N 21 153 (12
 , 21-153,), (b) pre-S N 33 153
 (33-153), (c) pre-S N 50 153 (50-153), (d) pre-S
 N 108 153 (108-153), (e) pre-S N 1
 27 153
 , (a) (e) HBsAg L , P
 CR pBO477(가 HBsAg L) . PCR
 , 15 24 , 15 24
 가 , 17 18 (b), 19 20 (c), HBsAg L ,
 HBsAg L , 15 24 , 가 (d), 23 24가 (e)
 , 가 (reverse) . 가 (forward)

PCR , 13 , DNA Pyrobest DNA polymerase(TaKaRa) (
 0.5 μ L), PCR (5 μ L×10), dNTP (10mM, 5 μ L), (Template) DNA(pBO477)(가
 HBsAg L)) (5 μ L/mL, 2 μ L), (15 2
 4) (1 μ L) , 50 μ L .

, PCR , 14 , , 98 30 , 98 30 , 55 1
 , 68 30 , PCR 30 , 4 , DNA
 , PCR *Dpn* I(10U) 가 , 37 1 , JM109

, HBsAg L Not I . 15
 HBsAg L , Oaa , HBsAg L (5') NotI
 , 25aa , 5' 25 NotI (3') , preS , S
 (5') NotI
 , NotI 가 HBsAg L , pBO477(

가 HBsAg L) , XhoI , HBsAg L

15 , 127-153 , 12 HBsAg L 127
 153 가 () . ,
 preS , pre-S (pre-S1, pre-S2) 가

(F-2) HBsAg L

F-1 (2µg) Cos7 (3 × 10⁴) (300V, 950 µF)
 , 4 37 , 5% CO₂ ,
 IMx() L (HBsAg L)
 , PBS , Lysis buffer(20mM Tris-HCl, 1mM EDT
 A, 150mM NaCl, 10mM 2- , 1%(v/v) TritonX-100) , PBS
 200

16(a)(b) 17 L () , 16(a)(b) 17
 , S/N RATE가 , 21-153, 33-153, 50
 -153, , 50-153 HBsAg L 가

18 19 , SDS-PAGE
 1 , mouse anti-S (가) , 2 anti-mouse IgG AP (Promega
) , 19 N (EndH) L Pre-S N 가 가
 , EndH , 51-66 , F-1 , 25
 26

(a) (c) HBsAg L

(F-3) (EGF) HBsAg L

(a) (c) HBsAg L (21-153, 33-153,
 50-153) Not I EGF EGF (21-153, 33-153,
 -RcT-EGF NotI , Cos7 , pGLDLIIP39
 24 L ((a) (c) , 3 HBsAg L) ,

(pEGFP-F(Clontech)) 50V, 750 µF
 , GFP GFP HepG2 HepG2 A431
 , HepG2 A431 , A431

(F-4) HBsAg L

Cos7 21-153, 33-153, 50-153((a) (c))
 XhoI , pGLDLIIP39-RcT XhoI (20
) . S. cerevisiae AH22R -
 High-Pi3 , 8S5N-P400 3 ,
 , SDS-PAGE (1 anti-S , 2 IMx()
 nti-mouse IgG) (21 22) . AP a

NotI A ZZ , EGF
 (20) . HBsAg L
 (pGLDLIIP39-RcT- 50-153, pGLDLIIP39-RcT- 33-153, pGLDLIIP39-RcT- 21-153,
 pGLDLIIP39-RcT- 50-153-ZZ, pGLDLIIP39-RcT- 33-153-ZZ, pGLDLIIP39-RcT- 21-153-ZZ, pGLDLII
 p39-RcT- 50-153-EGF, pGLDLIIP39-RcT- 33-153-EGF, pGLDLIIP39-RcT- 21-153-EGF,

pGLDLIIp39-RcT- 3-66)

S. cerevisiae AH22R -
High-Pill3 , 8S5N-P400

3 IMx() (21 22)

g L (HBsAg) (21-153., 33-153., 50-153. HBsA
22). (LAg) 가 (21
3-66. HBsAg
ZZ HBsAg (50-153 +
ZZ)

가

가

가

가

(57)

1.

가

2.

1 가

3.

1 2 가 ZZ

4.

1 2 가

5.

1 2 가

6.

1 5 가,

7.

6 가

8.

1 7 B

9.

8 , B preS

10.

8 9 , N B 1 20 y , pre-S

11.

10 , B 가 pre-S
N 50 153

12.

8 9 , N B 12 31 d , pre-S

13.

12 , B 가 pre-S
N 61 164

14.

1 13 ,

15.

14 , 가 (HSV1 tk)

16.

1 15 ,

17.

1 16

18.

, pre-S y B , N 1 20

19.

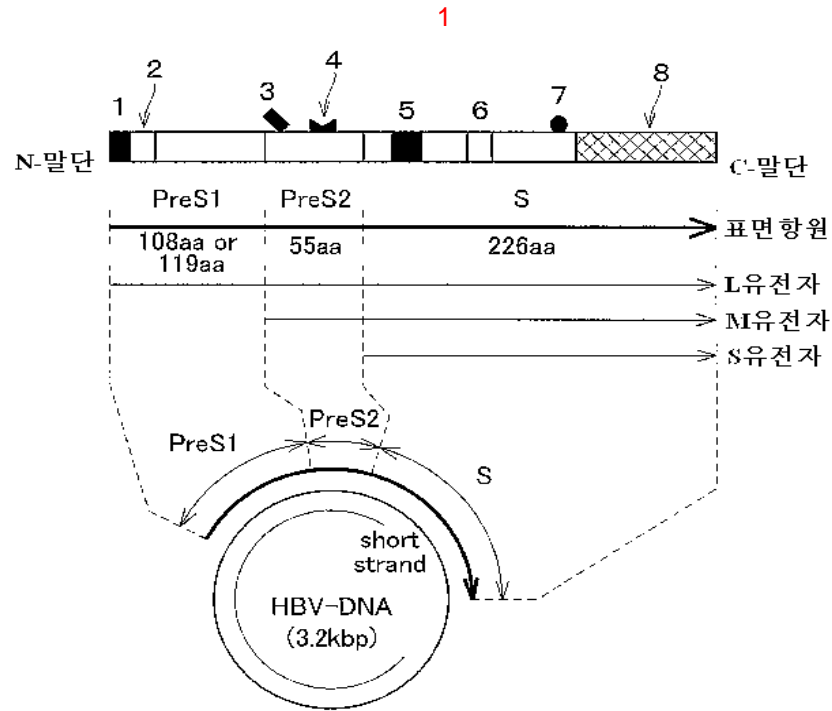
18 , B 가 pre-S
N 50 153

20.

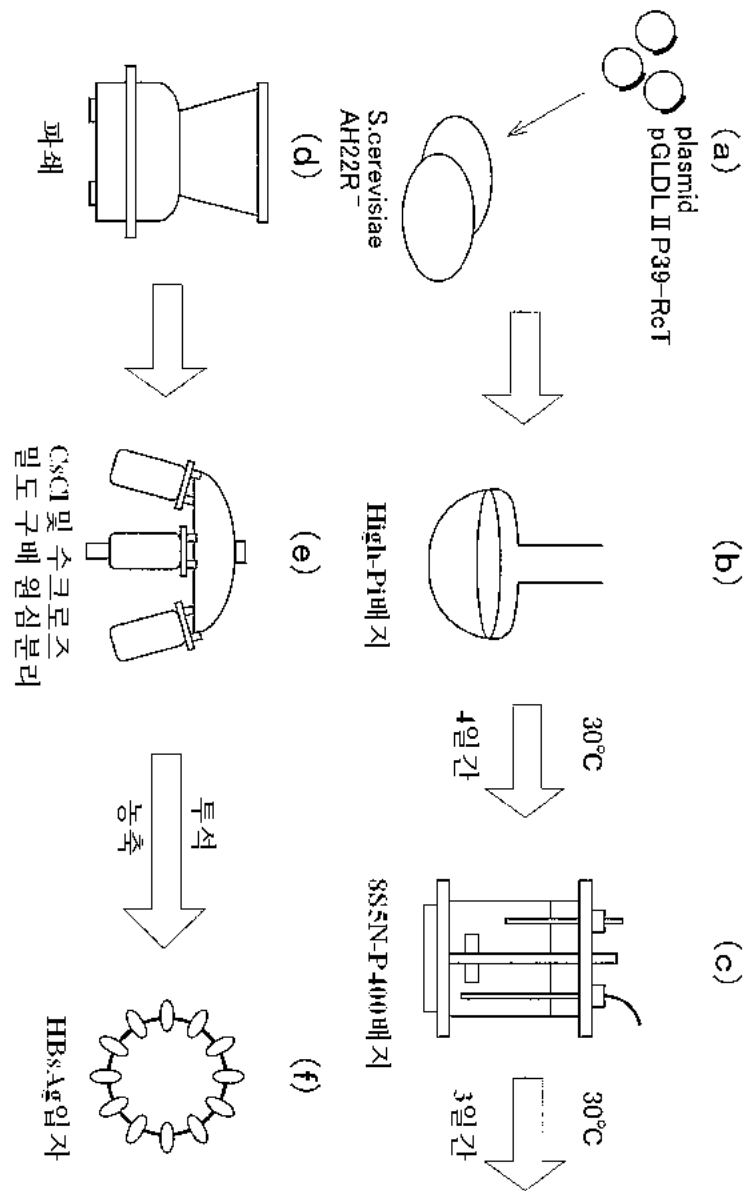
, pre-S d B , N 12 31

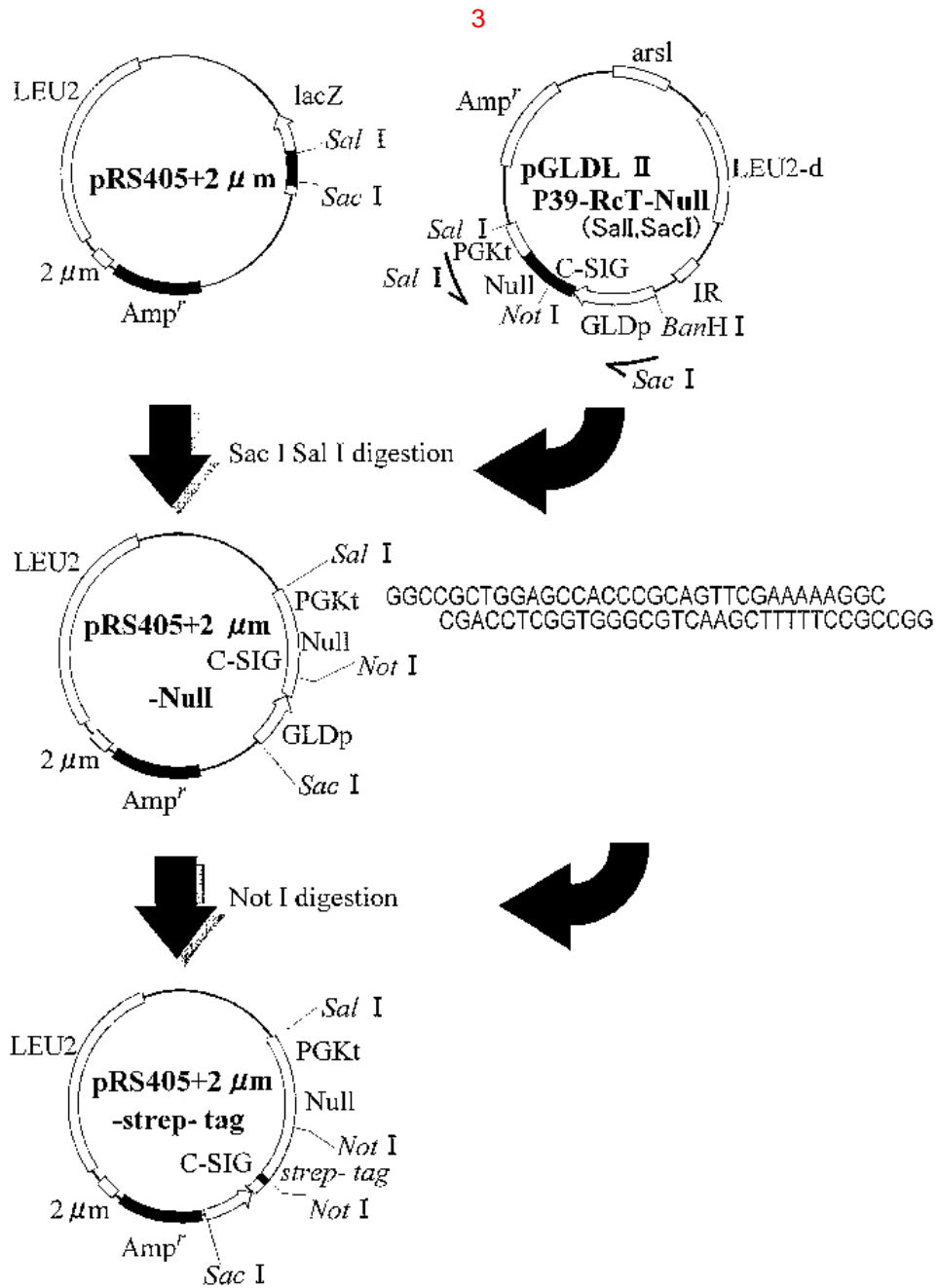
21.

20 , B 가 pre-S
N 61 164

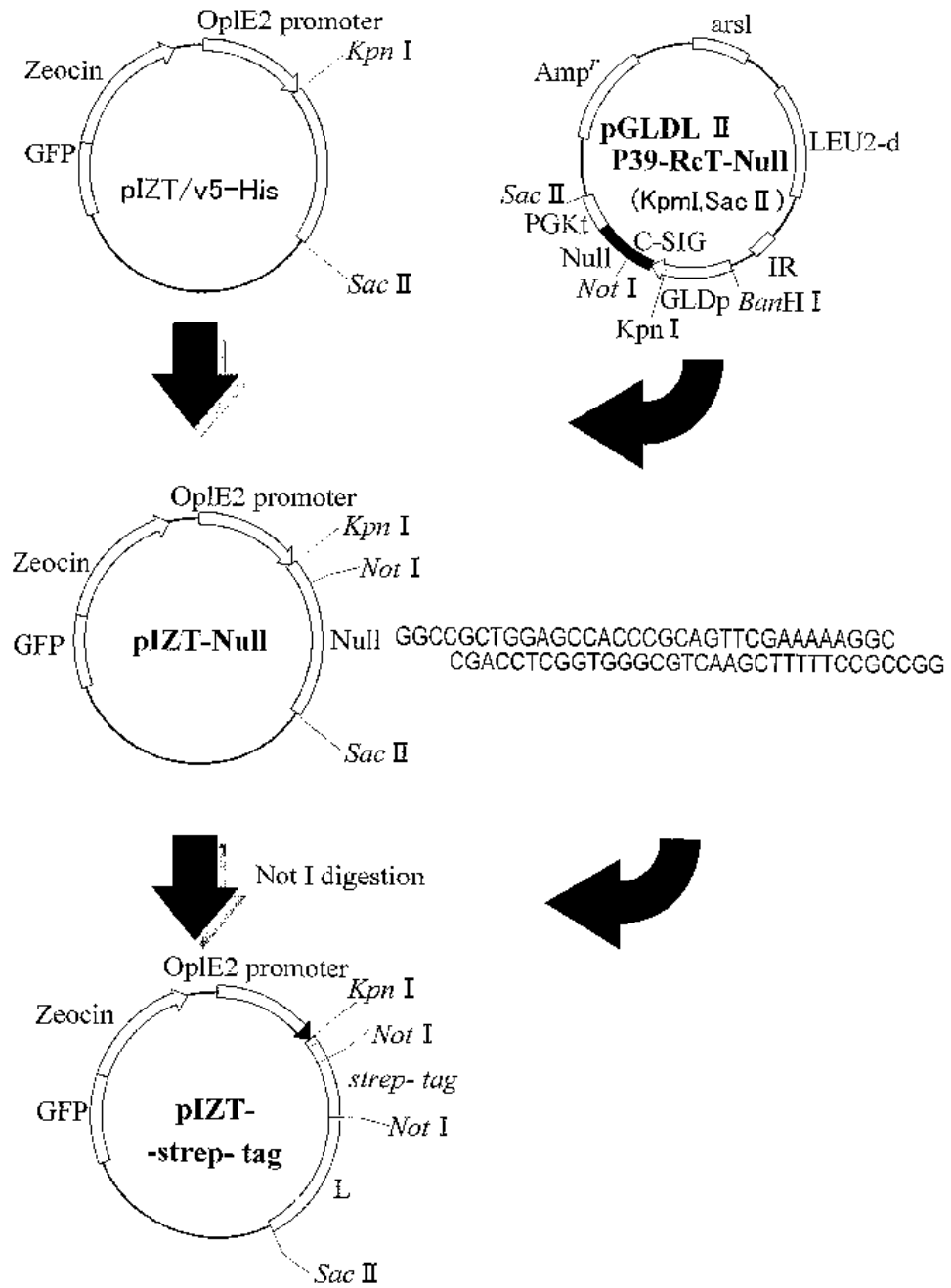


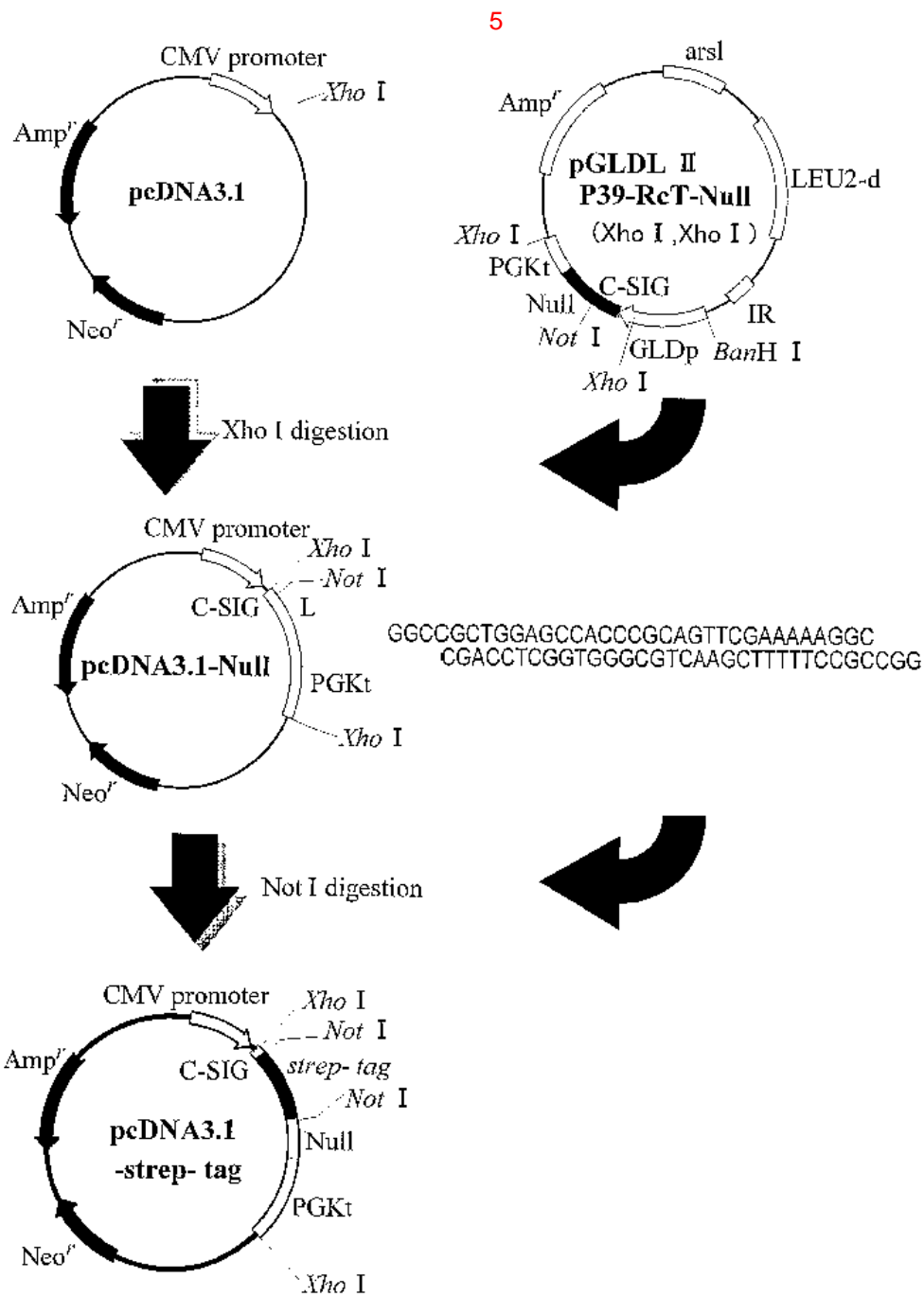
- 1 입자형성 억제 부위
- 2 직접적인 사람 간 세포 특이적 수용체
- 3 당쇄 1
- 4 간접적인 사람 간 세포 특이적 수용체 (중합 사람 혈청 알부민 수용체)
- 5 막 관통 영역 1
- 6 막 관통 영역 2
- 당쇄 2
- 8 막 관통 영역 3



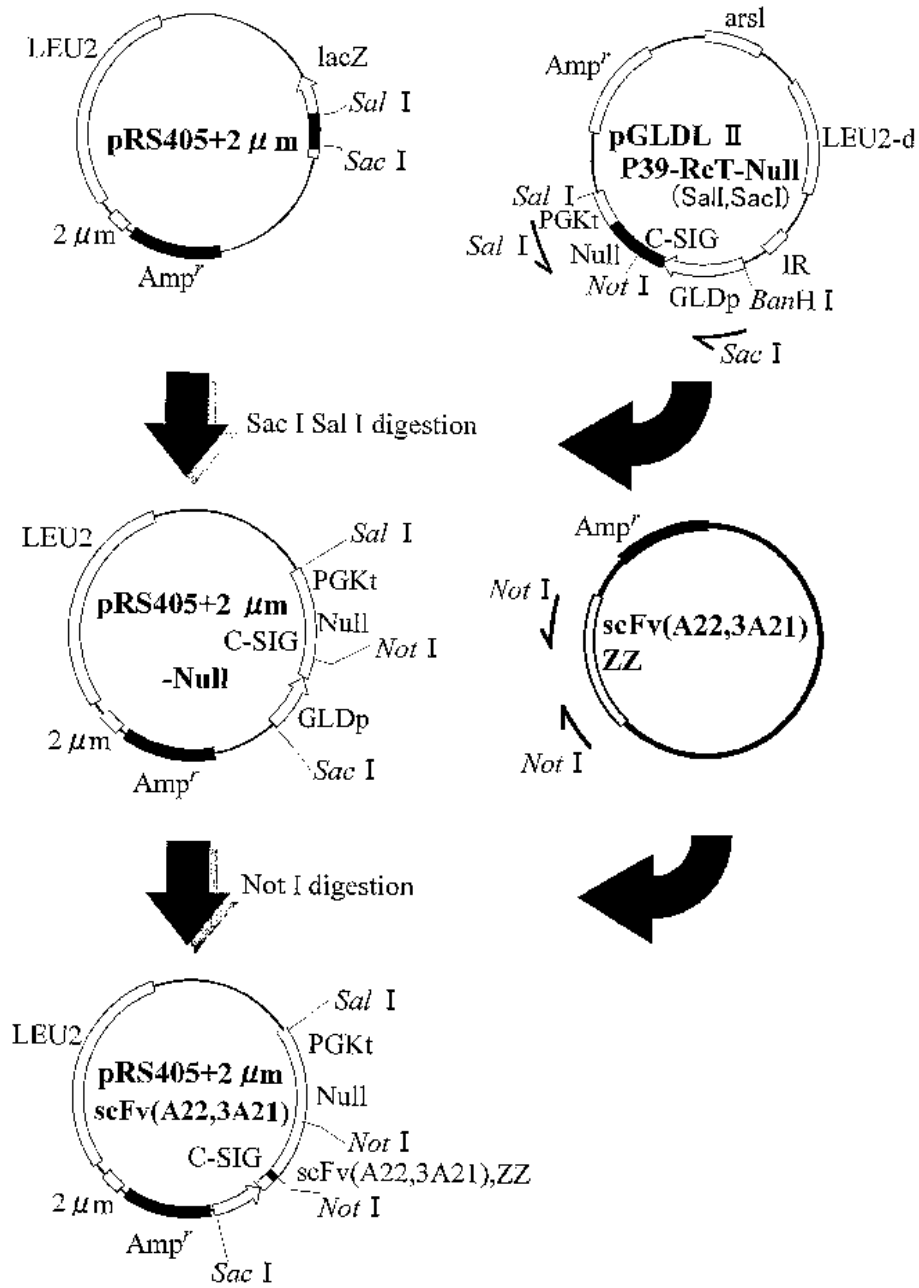


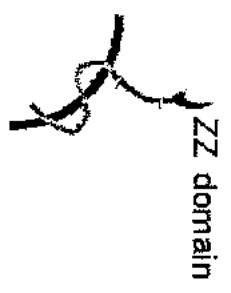
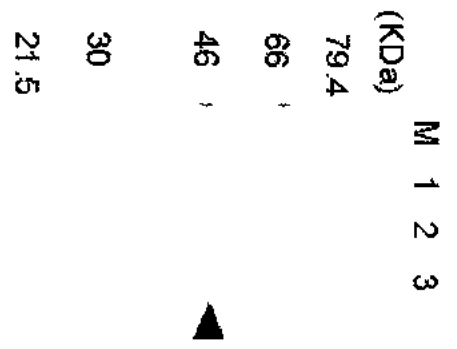
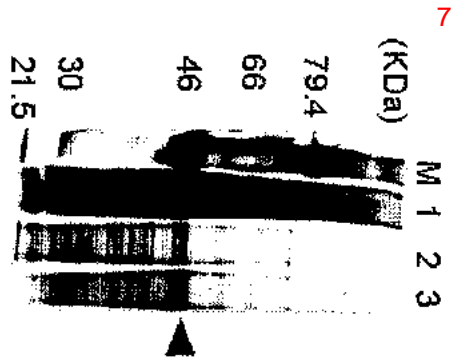
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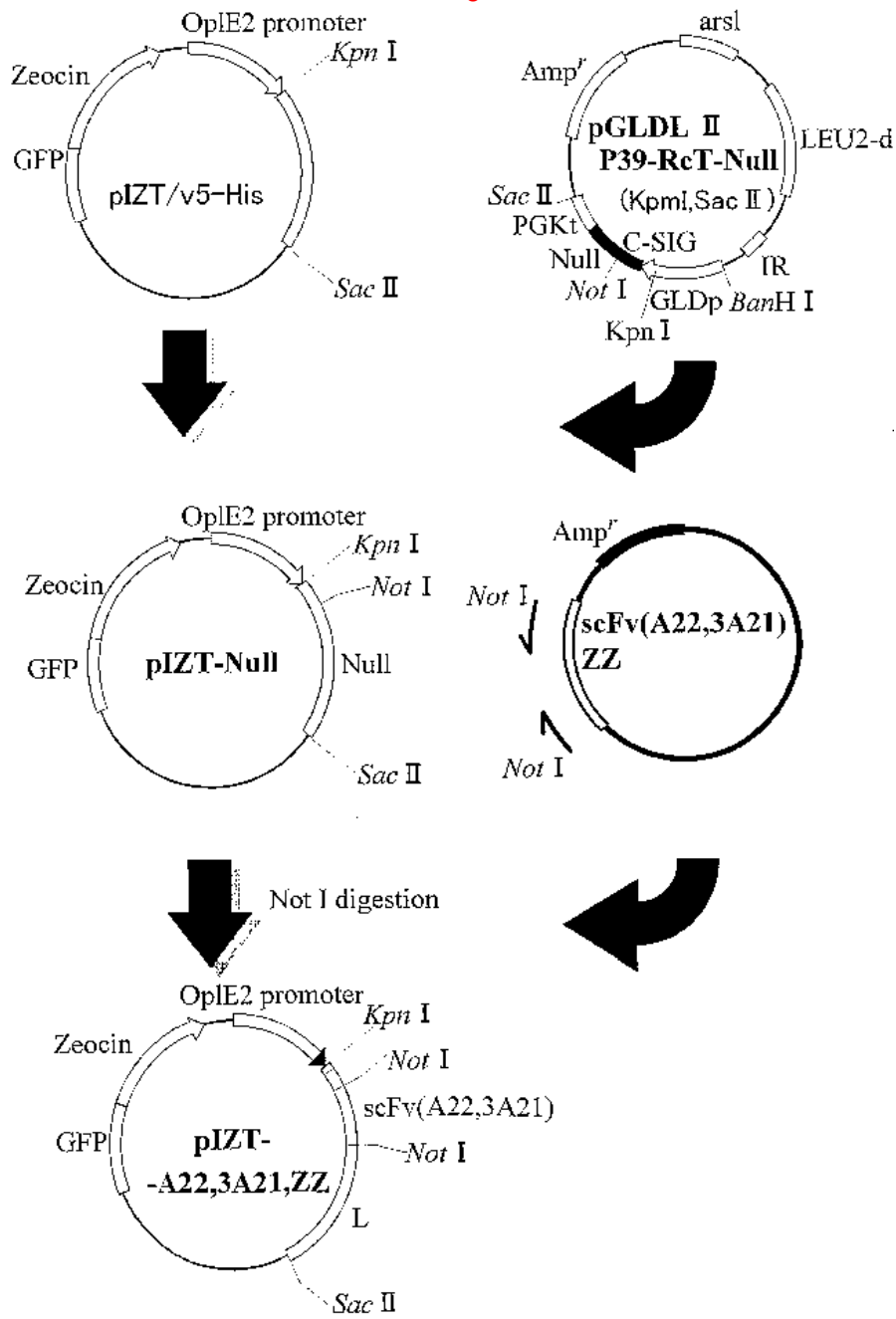
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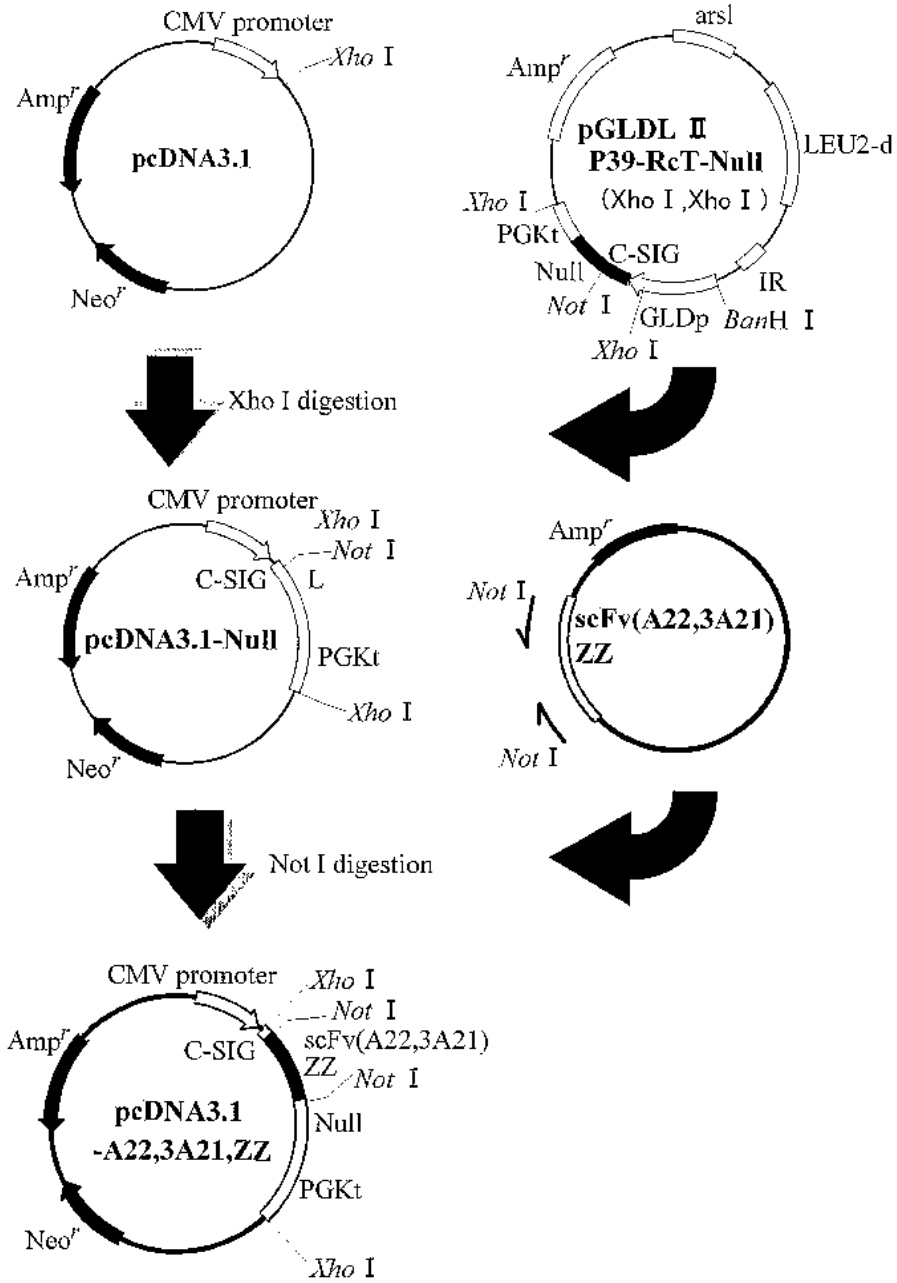


M: 마커
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2: 효모추출액
3: ZZ 도메인 제시입자(56kDa)

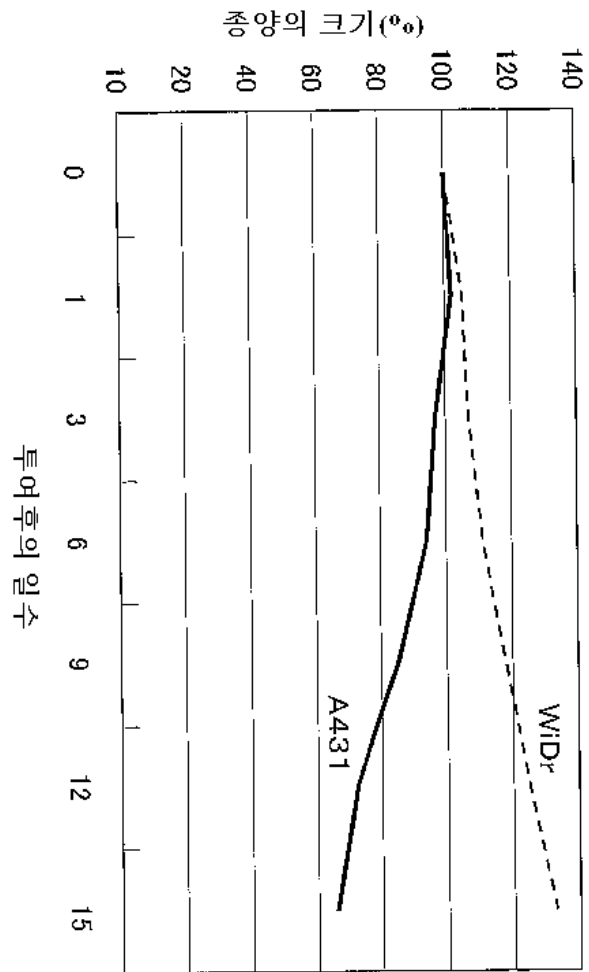
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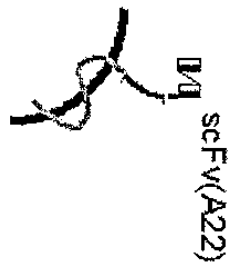
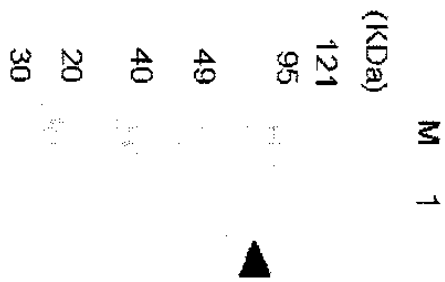
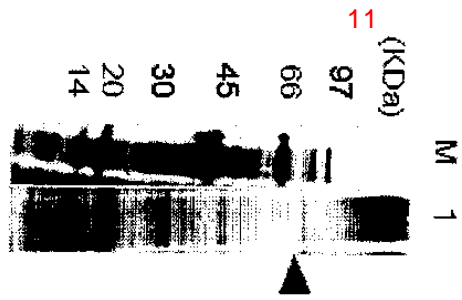


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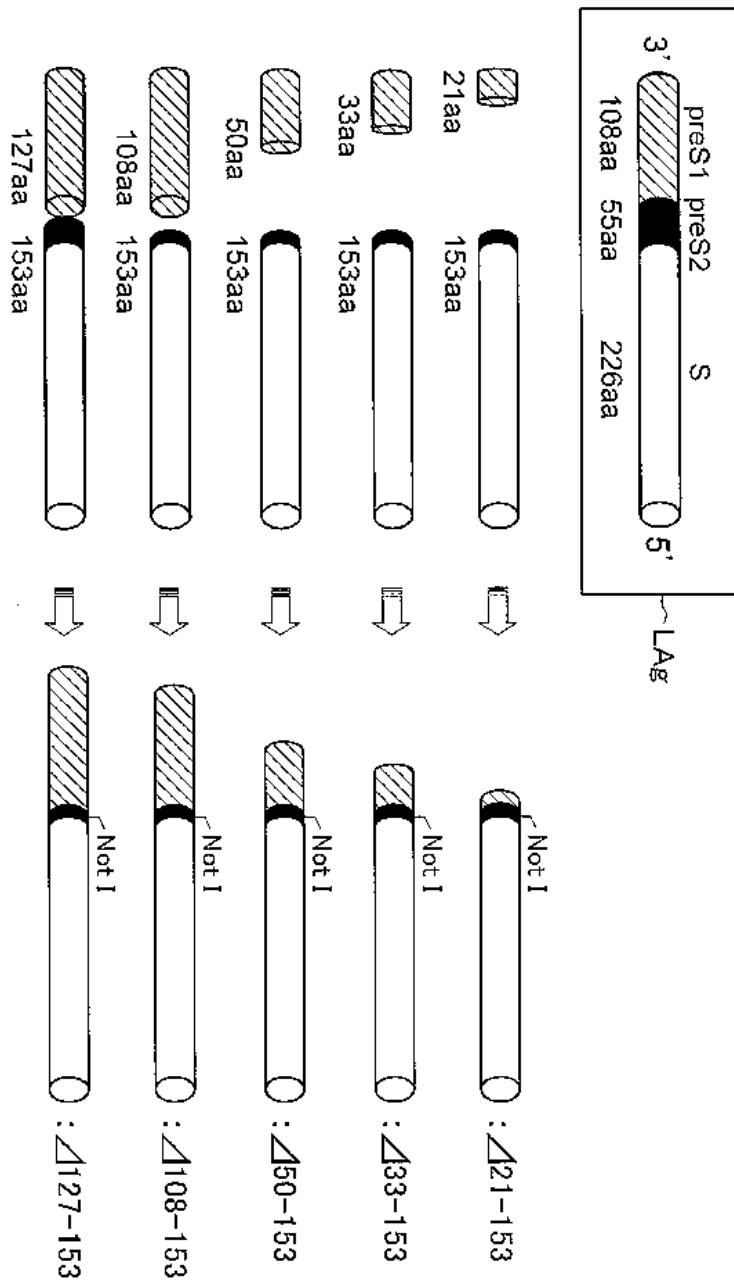
10





M: 마커
 L: A22 제시 단백질 (~60kDa)

12



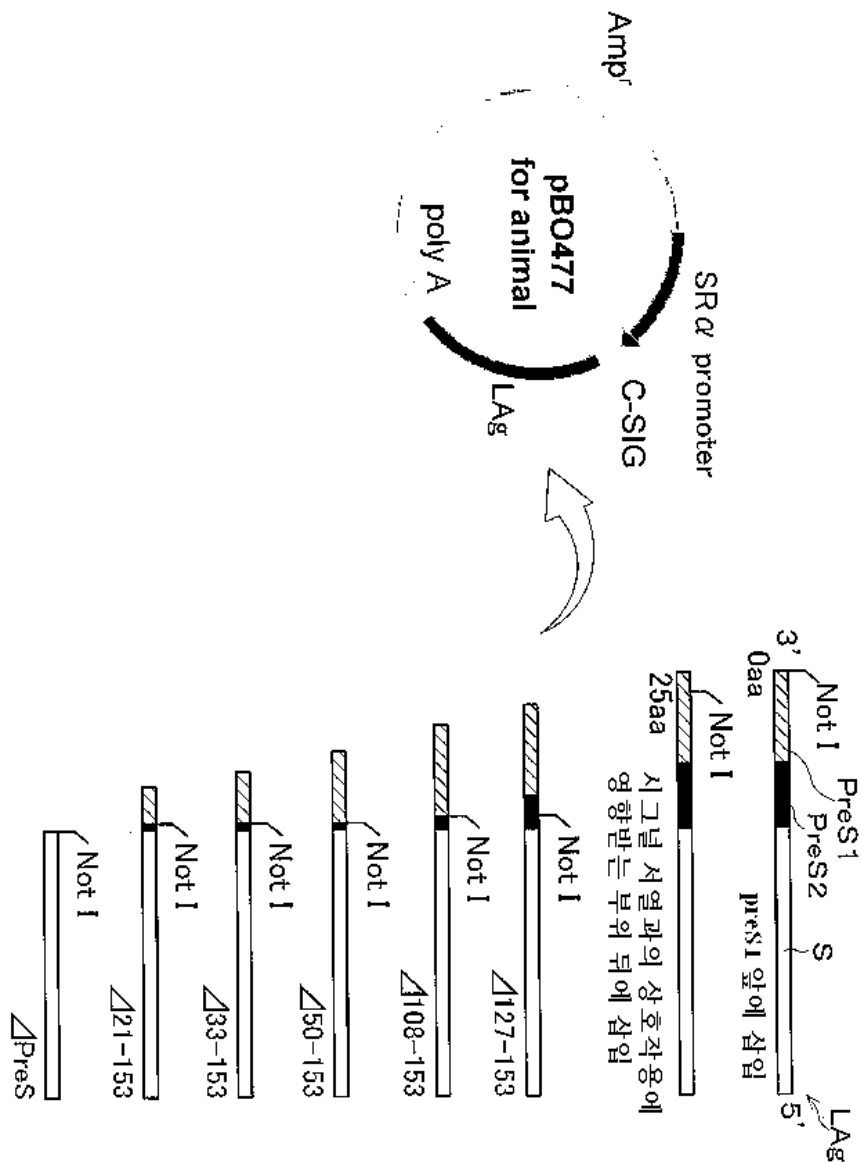
13

Pyrobest	DNA 0.5 μ l
폴리머라제 (TaKaRa)	
10 \times PCR 완충액	5 μ l
dNTP 혼합물(10mM)	5 μ l
템플레이트 DNA (5 μ g/ml)	2 μ l
프라이머 (F)(100 μ M)	1 μ l
프라이머 (R)(100 μ M)	1 μ l
증류수	35.5 μ l
전체량	50 μ l

14

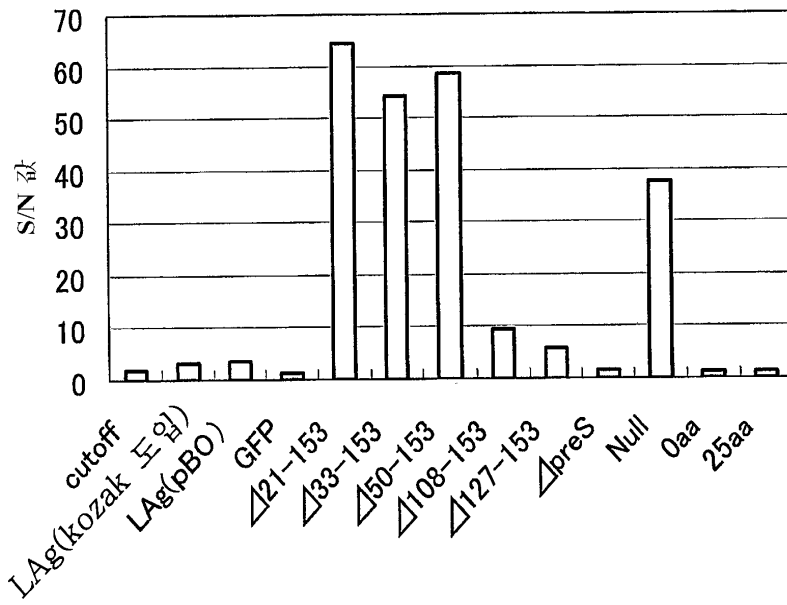
	사이클	온도	시간
1	1	98°C	30 sec.
2	30	98°C	30 sec.
		55°C	1 min.
		68°C	30 min.
3	1	4°C	∞

15



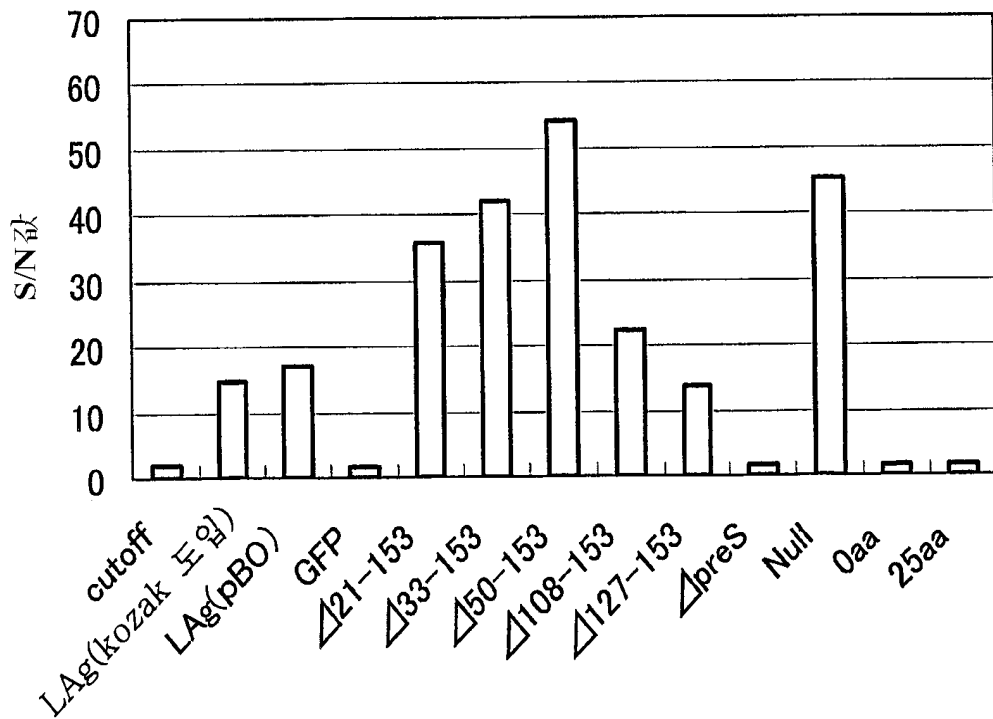
16a

배지상청(2배희석)



16b

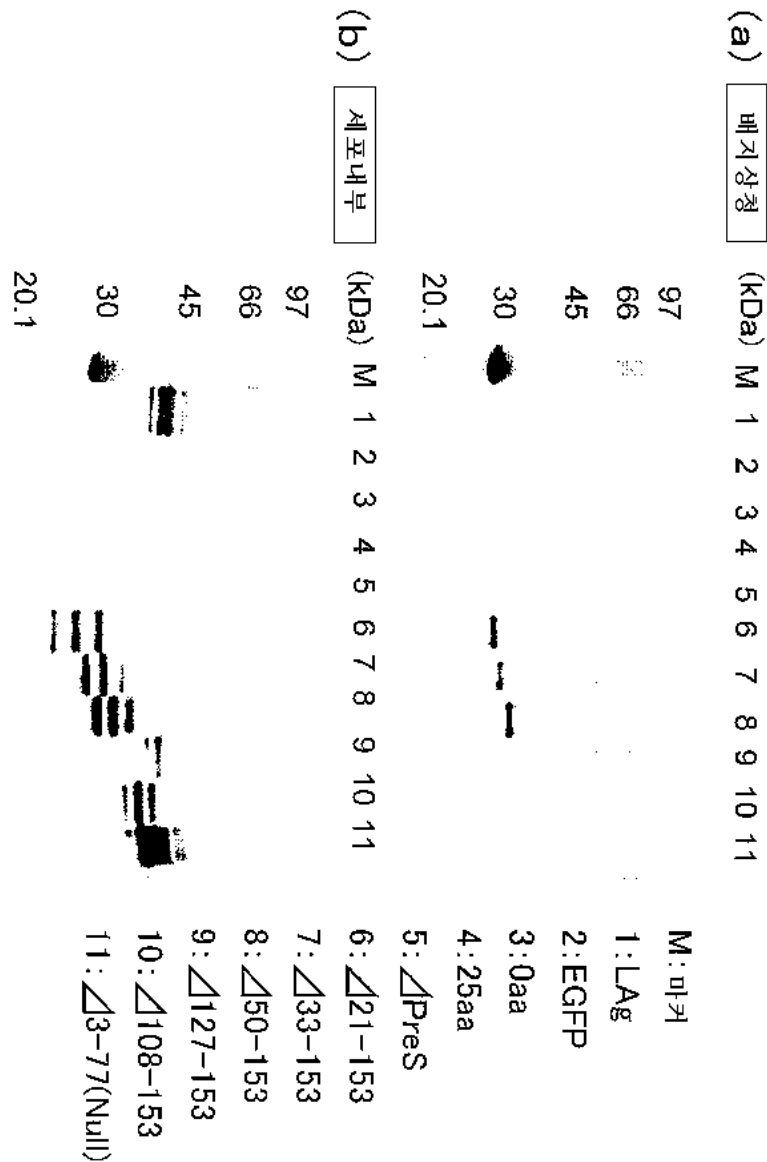
세포내부(200배희석)



17

plasmid	배지상청(2배희석)			세포내(200배희석)		
	S/N	RATE	판정	S/N	RATE	판정
LAg(kozak 도입)	3.13	26.6	R	14.74	115.0	R
LAg(pBO)	3.68	31.3	R	17.00	132.6	R
GFP	1.41	12.0		1.69	13.2	
△21-153	64.54	548.6	R	35.79	279.2	R
△33-153	54.39	462.3	R	42.03	327.8	R
△50-153	58.67	498.7	R	54.19	422.7	R
△108-153	9.58	81.4	R	22.32	174.1	R
△127-153	5.93	50.4	R	13.73	107.1	R
△preS	1.52	12.9		1.59	12.4	
Null	37.73	320.7	R	45.41	354.2	R
Oaa	1.42	12.1		1.65	12.9	
25aa	1.46	12.4		1.6	12.5	

18



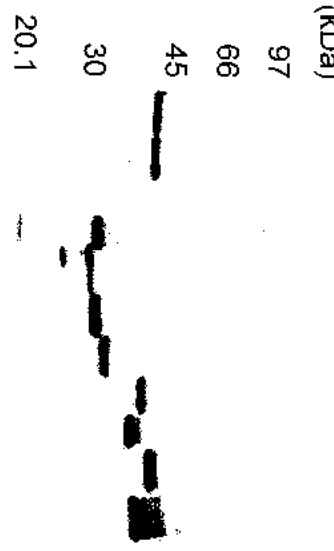
(a) 배지상청
(kDa) 1 2 3 M 4 5 6 7 8 9 10



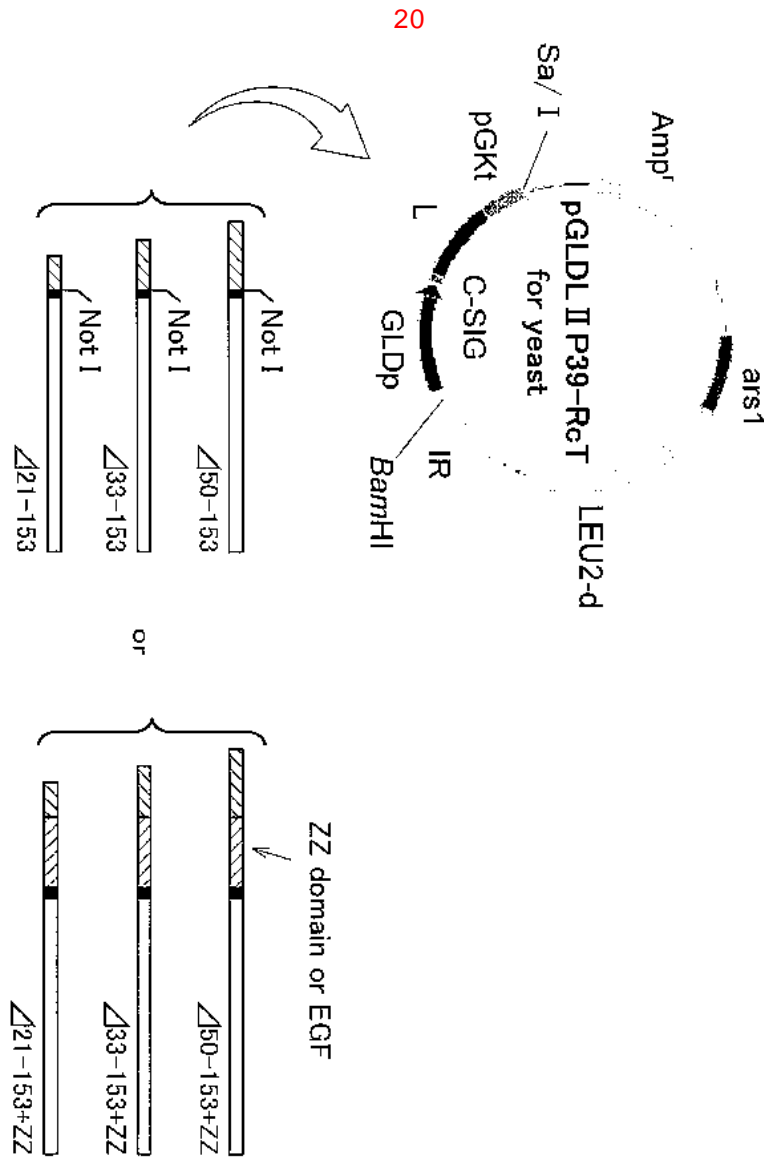
M: 마커
1: LAg(kozak)
2: LAg 42.9kDa
3: EGFP
4: Δ21-153 29.4kDa
5: Δ33-153 30.5kDa
6: Δ50-153 32.9kDa
7: Δ127-153 41.2kDa
8: Δ108-153 38.5kDa
9: Δ51-66 43.8kDa
10: Δ3-66(Null) 40.1kDa

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(b) 세포대부
(kDa) 1 2 3 M 4 5 6 7 8 9 10



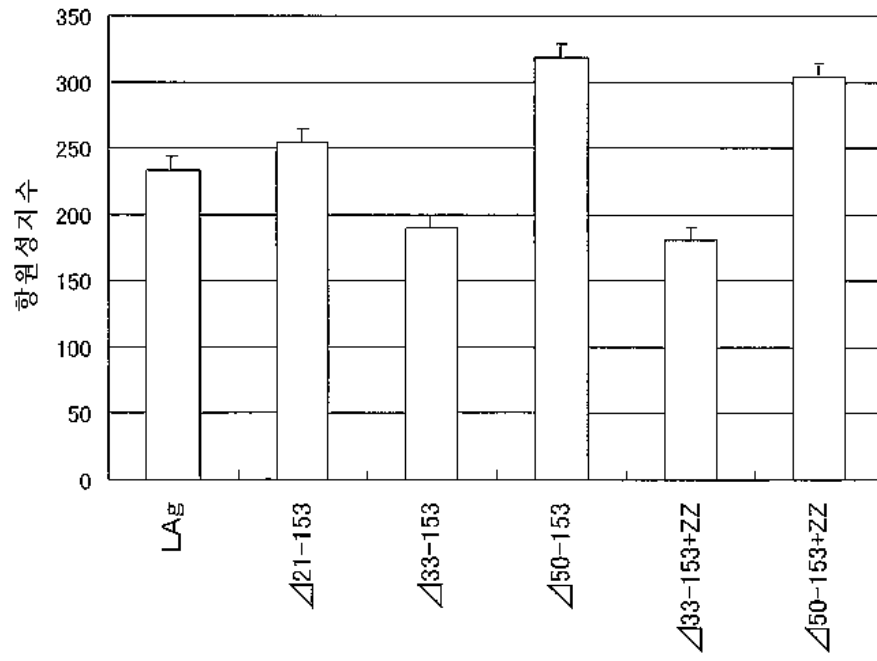
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1: LAg(kozak)
2: LAg 42.9kDa
3: EGFP
4: Δ21-153 29.4kDa
5: Δ33-153 30.5kDa
6: Δ50-153 32.9kDa
7: Δ127-153 41.2kDa
8: Δ108-153 38.5kDa
9: Δ51-66 43.8kDa
10: Δ3-66(Null) 40.1kDa



21

균주	S/N
LAg	233
Δ21-153	255.03
Δ33-153	189.22
Δ50-153	318.45
Δ21-153+ZZ	...
Δ33-153+ZZ	280.18
Δ50-153+ZZ	304

22



RNase 등의 세포질 RNA를 공격하는 단백질	Pancreatic type Rnases from vertebrates
	RNase 1 or Bovine RNase A
	Eosinophil derived neurotoxin
	Eosinophil cationic protein
	Liver RNase (RNase 4)
	Angiogenin
	Bovine seminal RNase
	Frog Rnases (Onconase etc.)
막투과를 방해하는 단백질	Streptolysin(Streptococcus pyogenes)
	Cholesterol binding toxins (Streptococcus, Bacillus, Clostridium, Listeria)
	alpha-Toxin (Staphylococcus aureus)
	Delta-Toxin (Staphylococcus aureus) and melittin (Apis mellifera)
	Aerotysin (Aeromonas hydrophila)
	Escherichia coli hemolysin
시그널 전달을 방해하는 단백질	Cholera toxin (Vibrio cholerae)
	Heat-labile enterotoxins (Escherichia ColiD)
	Pertussis toxin (Bordetella pertussis)
	Exoenzyme C3 (Clostridium botulinum)
	Adenylate cyclase toxin (Bordetella sp.)
단백질 합성을 방해하는 단백질	Anthrax edema factor (Bacillus anthracis)
	Diphtheria toxin (Corynebacterium diphtheriae)
	Pseudomonas aeruginosa exotoxin A
	Shiga toxins (Shigella dysenteriae serotype I, Escherichia Coli)
	Ricin (Ricinus communis)
	Ribosome-inactivating proteins
세포 골격을 교란하는 단백질	alpha-Sarcin and related toxins (Aspergillus)
	C2 toxin (Clostridium botulinum type C and D)
	Cytotoxic necrotizing factors (Escherichia coli)
	Enterotoxin A and cytotoxin B (Clostridium difficile)
	ActA (Listeria monocytogenes)
	IcsA (Shigella flexneri)
Zonula occludens toxin (Vibrio cholerae)	

면역 또는 염증 반응을 억제하는 단백질	Pyrogenic exotoxins (superantigens) (<i>Staphylococcus aureus</i> and <i>Streptococcus pyogenes</i>)
	Anthrax lethal toxin (<i>Bacillus anthracis</i>)
	Leukocidins and gamma lysins (<i>Staphylococcus</i> sp.)
막 운송을 교란하는 단백질	Tetanus neurotoxin (<i>Clostridium tetani</i>)
	VAMP-specific botulinum neurotoxins
	Botulinum neurotoxins type A and E (<i>Clostridium botulinum</i>)
	Botulinum neurotoxin type C (<i>Clostridium botulinum</i>)
나트륨 채널을 교란 단백질	Vacuolating cytotoxin (<i>Helicobacter pylori</i>)
	alpha-Scorpion toxins
	beta-Scorpion toxins
	Excitatory insect selective neurotoxins from scorpion venoms
	Depressant insect selective neurotoxins from scorpion venoms
	mu-Conotoxins (<i>Conus geographus</i>)
	mu-Agatoxins (<i>Agelenopsis aperta</i>)
	Anthopleurin-A, -B, and -C (anemone toxin)
	Anemone toxins (type II)
Calitoxins	
칼륨 채널 교란 단백질	Kaliotoxin
	Scyllatoxin (<i>Leiurus quinquestriatus hebraeus</i>)
	Apamin (honey bee <i>Apis mellifera</i>)
	MCD peptide (honey bee <i>Apis mellifera</i>)
	Charybdotoxin and iberiotoxin (<i>Leiurus quinquestriatus</i> var. <i>hebraeus</i> and <i>Buthus tamulus</i>)
	Margatoxin, noxiustoxin, and kaliotoxin (<i>Centruroides margaritatus</i> , <i>Centruroides noxius</i> , <i>Androctonus mauretanicus</i>)
	Dendrotoxins (<i>Dendroaspis</i> species)
	Sea anemone potassium channel toxins

25

칼슘 채널 교란 단백질	Omega-Conotoxins (<i>Conus</i> spp.)
	Omega-Agatoxins (<i>Agelenopsis aperta</i>)
	Omega-Grammotoxin SIA (<i>Grammostola spatulata</i> Chilean pink tarantula)
	Hololena toxin (<i>Hololena curta</i>)
	PLTXII (<i>Plectreurys tristis</i>)
	Calciseptine (<i>Dendroaspis polylepis</i>)
	Calcicludeine (<i>Dendroaspis angusticeps</i>)
	beta-Leptinotarsin-h
	Taicatoxin (<i>Oxyuranus scutellatus scutellatus</i>)
아세틸콜린 수용체 교란 단백질	alpha-Bungarotoxin (<i>Bungarus multicinctus</i>)
	alpha-Cobratoxin (<i>Naja kaouthia</i>)
	Erabutoxins (<i>Laticauda semifasciata</i>)
	Toxin alpha (' <i>Naja nigricollis</i> ')
	kappa-Bungarotoxin (<i>Bungarus multicinctus</i>)
	alpha-Conotoxins (<i>Conus</i> spp.)
	Snake toxins against muscarinic acetylcholine receptors
	Muscarinic toxin-1~5, -7, m1-toxin from green mamba (<i>Dendroaspis angusticeps</i>)
Muscarinic toxin-alpha, -beta from black mamba (<i>Dendroaspis polylepis</i>)	
리아노딘 수용체 칼슘 이온 채널 교란 단백질	Helothermine (<i>Heloderma horridum horridum</i>)

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시냅스전 교란 단백질	beta-Bungarotoxin (<i>Bungarus multicinctus</i>)
	Rattlesnake venom neurotoxins: crotoxin-related proteins
	Ammodytotoxins (<i>Vipera ammodytes ammodytes</i>)
	Notexins (<i>Notechis scutatus scutatus</i>)
	Textilotoxin (<i>Pseudonaja textilis textilis</i>)
	Tai poxin
	alpha-Latrotoxin (black widow spider)
	alpha-Latroinsectotoxin (<i>Latrodectus mactans tred ecimguttatus</i>)
	Pardaxin (<i>Pardachirus marmoratus</i>)
	Palytoxin (Corals of the spp. <i>Palythoa</i>)
	Equinatoxins (<i>Actinia equina</i> L., sea anemone)
글루타민산 수용체 교란 단백질	Conantokins (<i>Conus</i> spp.)

27

		G C V 투여후의 일수						
		0	1	3	6	9	1 2	1 5
종양 크기 (%)	A 4 3 1	100	102	97	94	85	72	66
	W i D r	100	105	107	111	118	125	133

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<151> 2002-3-29

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Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala

35

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45

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105

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