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(12) (A)

(51) . Int. Cl.⁷
C07K 14/56

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(43)

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2004 03 11

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(30)	60/311,866	2001 08 12	(US)
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(71)	94502	1265
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(72)	94501	2930
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94502	14
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92064	16515
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(74)

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(54)	/	,
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C-

C-

가

C-

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(IFN)
1957, IFN

(Walter, et al., 1998).

IFN I () 1 IFN IFN- () II ,

[1]

인터페론의 개요

양상	유형 I	유형 I	유형 I	유형 II
타입	α & ω	β	τ	γ
생성의 유래 :	백혈구	섬유아세포	영양아종	림프구
형-바이러스	+	+	+	+
형-증식	+	+	+	+
임신 신호	-	-	+	-

IFN- 2 14 IF
N- , 14 12 IFN- 14 IF
80%

-D(IFN D) -1 (IFN 1) IFN D I (Noisakra

n and Carr, 2000; Noisakran, et al., 1999). IFN D
가

1982 IFN D (rHuIFN D) lac *Methylophilus methylotrophus*
E. coli (De Maeyer, 1982). 1984 ntech -
IFN D 가 가 IFN D *Saccharo*
myces cerevisiae (Singh, 1984). 1989 *E. coli* *Bacillus subtilis* (*B. subtil*
is) - (Breitling,
1989). , *E. coli* *B. subtilis* rHuIFN D
. *E. coli* 1990
, 11 IFN D *E. coli* rHuIFN D
가

IFN- 18-19 kDa IFN- (OvIFN-). 가 가 ()
) (Martal, et al., 1979). , (Godkin, et al., 1982).
OvIFN-
-one (oTP-1) OvIFN- (Bazer and Johnson, 1991).

, N-
cDNA (Imakawa, et al., 1987) , , , IFN- 45-55% IFN- IFN-
N- II 70% , , , IFN- 45-55% IFN- IFN-
ewart, et al., 1989; Klemann, et al., 1990; and Charlier, M., et al., 1991). 23 (St
72 585 가 1 kb 1
4 IFN- II IFN (Jarpe, et al., 199
4).
IFN- I IFN (1), II IFN . IFN-

al., 1982). dsRNA . , (Roberts, et al., 1992). IFN- . IFN- (Godkin, et al., 1991). IFN- 300 IFN- 가 (Cross and Roberts, 1991).

IFN-
JEN- . , IFN-
JAR IFN-
IFN-

IFN-₁ (126)) IFN-₂ IFN-₃
 ; -126 -450 IFN-₂ IFN-₃
 . (Cross and Roberts, 1991). . | IFN-₂ IFN-₃

IFN- γ . . . , IFN- γ (Godkin, et al., 1982), IFN- γ (Whaley, et al., 1994).

et al., 2000). , IFN- 1991 1992 C 가 (, (Saito, e 1×10⁶ (Tyring, et al., 1992).

$5-10 \times 10^6$ / . IFN- FN- , , (Dieperink, et al., 2000). 가 , | (Saito, et al., 2000) IFN- 가 (Jimenez-Saenz, et al., 2000)

가 , - C- C- 가 . , C- 1-30 , C- , . C- . C- 163-166
4 가 D C-

163-172 C-
D D C-

163-166

C-

163-172

가

가

 1×10^8
MDBK/VSV

/mg

MDBK/VSV

 2×10^8

가

/mg

가

;

P. pastoris

가

가

가

. 1 HVV 1

;

. 2 HVV 2

;

. 3 HVV 3

;

. 4 HVV 4

;

. 5A-5C HVV-pPICZ-

;

. 6A-6B HVV

;

. 7 1% 72 *P. pastoris* HVV

SDS-PAGE

;

. 8 *P. pastoris* HVV

SDS-PAGE

9

PBMC

10

1. —

1989, Ausubel F M et al., 1993,
가

Sambrook et al.,

가
()

가
/DNA

가
DNA

가
DNA, DNA,

가
가 (ds) DNA

가
가
가

가
()

가
()
가
가
가
가
()

가
가
가
DNA

가
DNA
가
DNA

The diagram illustrates a gene structure. At the top, the word 'DNA' is written vertically. Below it, the word '가' (ga) is repeated four times. The bottom part of the diagram shows a horizontal line with three main segments: '5' UTR' on the far left, 'DNA' in the middle, and '3' UTR' on the far right. The 'DNA' segment is flanked by two '가' characters, and the '3' UTR' segment is also flanked by two '가' characters. The '5' UTR' segment is preceded by a single '가' character.

(DNA), , mRNA) (

가

N-

E. coli / (), , , , ,

C HCV' - A - B (NANBH)
 RNA 10⁻³ 10⁻⁴ HCV RNA
 RNA HCV , , , , 가 HCV
 . HCV , , , , 가

'OAS' '2',5'-(OAS)

(Endomycetales), , (Blastomycetes)

Spermophthoraceae Saccharomycetaceae

4

Schizosaccharomycoideae (*Schizosaccharomyces*), Nadsonioideae, Lipomycoideae, Saccharomycoideae (*Pichia*, *Kllyveromyces*, *Saccharomyces*) Leucosporidium, Rhodosporidium, Sporibolus, Filobasidium, Filobasidiella

Sporobolomycetaceae (, Sporoholomyces, Bullera) Cryptococcaceae (, Candida)
Pichia, Kluyveromyces, *Saccharomyces*, *Schizosaccharomyces*, *Candida*
Pichia *P. pastoris* ,

ose and Harrison: Strathern et al.; Skinner et al. , 가 , , Bacila et al.; R

가

가 가
가 , 2 가

가, , , , .

가 . 가 , (a)
, , , , (b)
) , , , , (c) , *Bacilli* D -

'IFN-
N- . 10 IFN- IFN- 70% , 80% ,
90% , 95% 가
가 ALIGN .

FASTA 1.7 (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, Va.).

, 'IFN', IFN, , RNA, mRNA, , IFN, ,
IFN mRNA (RT -PCR)

, IFN
가

() C- 가 / ; N- /

III.

A. *P. pastoris* _____

/

<i>oris</i>	<i>Pichia</i> X-33	<i>Pichia</i> <i>P. pastoris</i>
-------------	-----------------------	----------------------------------

P. pastoris (Cregg, 1993).
P. pastoris 가

P. pastoris (Mut) AOX

(Brierley, 1998; and White et al., 1995).

, Yeast Genetic Stock Center, Department of Biophysics and Medical Physics, University of California (Berkeley, Calif.); the American Type Culture Collection (Manassas, Va.); Northern Regional Research Laboratories (Peoria, Ill.); Invitrogen (San Diego, Calif.)

B.

가 ()

가 DNA

. 5A - 5C pPICZ - HVV

IFN

i. amp;

가

가

			(3')	RNA
	가			
가				

a. AOX1

Pichia 가 , AOX1 AOX2 , AOX1
AOX1 . (Tschopp, *et al.*, 1987; Ellis, *et al.*, 1985; and Cregg, *et al.*, 1989). AOX1
A 5%가 AOX1 ; , AOX1 , poly(A) RN
(Cregg, *et al.*, 1988). AOX1 가 : *S. cerevisiae* GA
L1 / ,

GAL1, AOX1 가
. (Tschopp, et al., 1987).

b. GAP

P AOX1 가 가 *P. pastoris* GA
AP 가 (Waterham, et al., 1997). 3 2 3 1 GAP
 GAP

c. FLD1

FLD1 () .(Shen, *et al.*, 1998). FLD1 PFLD1 , AOX1 FLD1

d. PEX8 YPT1

P. pastoris , AOX1, GAP, FLD1

YPT1, P. pastoris PEX8 (Li, et al., 1995). YPT1, GTPase (Sears, et al., 1998).

e. _____

가

RNA

ereau-Puigalon et al. (1980); Panthier et al. (1980); Henikoff et al. (1981); and Hollenberg et al. (1981) Cohen et al. (1980); Merc

ii. _____

가 ,

가

IFN

N-

(ER)

FR

가

ER 가

Lys-Lys, Lys-Arg, Arg-Lys,

Arg-Arg, 가

Lys-Arg

). KEX2

P. pastoris KEX2
가

(Julius et al. 1983)

(*...*, Sambrook et al. (1989)).

가

3'

ER

가

a. *S. cerevisiae* -

4); Smith et al. (1985). Singh et al. (1983) , Elliott et al. (1983); Bitter et al. (198

가 11 -

가 11 -

, Kex2 pro Arg-Lys , pre , Ste13
 Glu-Ala (Brake, et al, 1984).

1. 가

b.

PHA-E (*Phaseolus vulgaris* (Cereghino and Clegg, 2000; and Raemaekers, et al., 1999). 가 ,
cDNA (Sambrook et al. (1989)). (PHO1)

IFN D

iii. IFN

1-4 IFN 2-HVV . 1

가 가

mRNA

iv.

mRNA

AOX1 Mat- (-)

v. 가

가 *P. pastoris* *S. cerevisiae*, HIS4, *S. cerevisiae* ARG4,
- *Streptoalloteichus hindustanus* Sh ble (Cregg
et al., 1985; Cregg and Madden, 1989; and Higgins, et al., 1998)

P. pastoris ADE1 (PR-, URA3 (5'-)), ARG4 (),

vi. _____

IFN

1989) Sambrook et al. (1989) 가

가 DNA

(

가 , 가 .
(Clare, et al., 1991).

B. _____

. (Cregg et al., 1985; Liu et al., 1992; Waterham et al., 1996; and Cregg and Russell, 1998).

C. IFN

DNA

가	3	IFN	, HVV	2	HVV
		<i>P. pastoris</i>		4	
			IFN		2.75×10^{-8} U/mg

D. IFN D

Pichia pastoris

rHuIFN- β

Pichia 가
Pichia
rHuIFEN D 가

er, 1990 and Scopes, 1982

Deutsch

IV.

HCV 1 7 가 10% (Cuthbert, et al., 1994; Mansell, et al., 1995). The Centers for Control and Prevention HCV 가 160,000 - HCV 가

HCV 가 Flaviviridae - 가 , - RNA (Choo, et al., 1989). B HCV DNA 가 . (Berenguer, et al., 1996). HCV , (Trepo, 2000). HCV , 85% . (Trepo, 2000).

HCV 가

V. _____

(1 - -D- - 1,2,4- - 3-) mRNA

(Fernandez, et al., 1986; Balzarini, et al., 1991).
 92). , C HCV RNA 가 (Di Bisceglie, et al., 19
 , IFN- (Clarke, 2000; Koskinas, et al., 1995)
 , IFN- (Cotler, et al., 2000). , IF

[2]

본 발명에 따라 제공된 서열

설명	SEQ. ID NO.
<u>HVV</u> 아미노산 서열	
CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEDGN QFQKAPAIASVLHELIQQIFNLFTTDSSAAWDEDLLDKFCTELYQQLN DLEACVMQEERVGETPLMNADSDILAVKKYFRRITLYLTEKKYSPCAWE VVRAEIMRSLSLSTNLQERLTKMGGDLNSP	
<u>HVV</u> 핵산 서열	2
TGTGATTTGCCAGAGACTCACTCTTGGACAACAGAAGAACATTGA TGCTTTGGCCCAAATGCTAGAATCTCCATCCTCTGTTGAT GGATAGACACGATTCGGTTCCCAAGAAGAATTGACGGTAA CCAATTCCAAAAGGCTCTGCTATTTCTGTTTGACGAGTTGATT CAACAAATTTCACCTGTTACCACATAAGGACTCTCTGCTGCCT GGGACGAAGACTTGGACAAGTTCTGACTGAGCTTACCAAC AATTGAACGACTTGGAGGGCTGTATTGCAAGAGGGAGAGACTG GTGAGACCCATTGATGAACGCTGATTCCATCTGGCTGCAAGA AGTACTTCAGAAGAATTACCTTGTACTTGACCGAAAAGAAGTACTC CCCATGTGCCTGGGAAGTCGTTAGAGCCGAAATCATGAGATCTT GTCCTTGCCACTAAGTCAAGAGAGACTTACCAAGATGGGTGG	

AGACTTGAACCTCTCCATAA

VII.

1

HVV

HVV C- 4 IFN- 10 6 C-
ulFN D . , *Pichia pastoris* H
가 DNA . (가 150

; . 1-4) G2
Technologies, Alameda, Calif.).

DNA

(Operon

(가 40 bp) 4 150 5' 3' (HVV 1-4) .

A. #1

:G2

:XbaI BamHI

: 1 (. 1)

:G2HVV1F1

B. #2

:G2HVV1F1

:EcoRI BamHI

: 2 (. 2)

:G2HVV1F2

C. #3

:G2HVV1F2

:Sac I BamHI

: 3 (. 3)

:G2HVV1F3

D. #4

:G2HVV1F3

:EcoRI BamHI

: 4 (. 4)

: G2HVV1F4

E.

: Sac II

XbaI NotI HVV G2HVV1F4 pPICZ- (In
vitrogen, San Diego) . HVV-pPICZ- . 5A-5. .

2

P. pastoris HVV _____

2 *P.pastoris* HVV BMGY 24 , BMMY
 OD₆₀₀ 72 , 48 , 10% 1Mℓ 가
 . 14% Tris-glycine SDS-PAGE . pPICZ Colloidal Coomassie (Novex, San Diego, Calif
 . 7 . HVV-16 HVV 가
 . 3

HVV

. 8 *P.pastoris* HVV (20Mℓ) 10 Tris,
 NaCl 150 HiPrep Sephacryl 26/60 S-100 - 2
 Mℓ (pre- 120 Mℓ 1 Mℓ/min 1
 . 20 Mℓ 2, 3, 4 2, 3 4 1 Mℓ 1
 4% SDS-PAGE Novex colloidal blue

4

HVV -

3 HVV 4 HVV 16(. 7) *P.past*
 oris (IDs: S-0018; S-0019; S-0
 063; S-0064) 3(. 8) 1 Mℓ -
 5 3

[3]

HVV-16 항-바이러스 특이적 활성

표본 ID	단백질 농도 (MG/ML)	항-바이러스 단위 (AVU/ML)	항-바이러스 특이적 활성 (AVU/MG)	평균 AVU/MG ± STD (N)
S-0018	0.751	2.22×10^8	2.96×10^8	$2.86 \times 10^8 \pm 0.09$ (N = 4)
S-0019	0.696	1.92×10^8	2.76×10^8	
S-0063	1.102	3.11×10^8	2.82×10^8	
S-0064	1.422	4.10×10^8	2.88×10^8	

5

5

Madin Darby bovine kidney(MDBK) 96- 10% fetal serum(FBS)
 Eagle's MEM . 가 , 2%FBS
 Eagle's MEM (16-24) MDBK 100% CPE 96-
 VSV 4 VSV 50μℓ/ 10⁻¹ 10⁻⁶
 200μℓ/ , TCID₅₀ , ,
 10^{6.5} TCID₅₀ /Mℓ 가 10^{3.5} TCID
 50 /
 , 가 PBS MDBK

100
 μl 10 (10⁻¹ 10⁻¹⁰) 3 가 18
37 HuIFN A(Biosource Intl., Camarillo, CA)
PBS , @ 10^{3.5} TCID₅₀ / 100 μl 가
37 100 μl 0.2% neutral red solution (Gibco-BRL) 100 μl
37 1 100 μl (50% , 1%) 가
가 PBS 2 A₅₅₀ Bio-Kinetics Reader (Bio-Tek In
struments, Winooski Vt.).

$$\text{보호퍼센트} = \frac{AVG(A550시험 웨일) - AVG(A550바이러스대조구웨일)}{AVG(A550비처리된세포대조구웨일)} \times 100$$

6

IFN

(PBMC) HuIFN α , OvIFN β , IFN
PBS 1:4 Nycoprep 1.077 (Nycomed Pharma, Oslo, Norway). 20 20 600×g PBMC
PBS 96- 5×10 6 / 3
IFN α , IFN β , IFN 2000 U/ml 128,000 U/ml 12
, V 1 (Pharmingen).

V

V

PBMC 3 . Pharmingen . Becton dickinson FACScan . Quest Software
 . PBMC (. V .) , . V , . V ()
 (. V , .)
 . , 10,000 (. V + . +) .

$$\text{특이적 세포 죽음 \%} = \frac{\text{처리되지 않은 웰유래의 아넥신 } V^* \text{ 양성 세포 \%}}{\text{처리된 웰유래의 아넥신 } V \text{ 양성 세포 \%}} \times 100$$

* . . . % √

7

1.5×10^5 - U/Mℓ rHuIFN roIFN PBMC (5×10^5 /) 12
 V-FITC : [(% AV+ PI+) / (% AV+ PI+) $\times 100$].

[4]

애렉신 V 양성 세포에 근거한 특이적 세포 죽음의 퍼센트

	HVV-16	rHuIFNαD (Biosource)	rHuIFNαD (Pepgen)	BSA 대조구
AVG ± STD	12.88 ± 15.44	65.00 ± 22.49	45.46 ± 23.64	7.67 ± 14.57
중앙값	4.88	65.78	43.95	0.00
N	6	10	10	10

[5]

프로피디움 아이오다이드 양성 세포에 근거한 특이적 세포 죽음의 퍼센트

	HVV-16	rHuIFNαD (Biosource)	rHuIFNαD (Pepgen)	BSA 대조구
AVG ± STD	2.08 ± 3.85	32.88 ± 15.69	19.32 ± 12.55	3.78 ± 8.19
중앙값	0.56	34.59	15.27	0.00
N	6	10	9	10

8

9×10^5 - U/Mℓ rHuIFN roIFN PBMC (5×10^5 /) 9, 10, 12 1.5
 PI+) / (% AV+ PI+) $\times 100$].
 V-FITC : [(% AV+ V) / (% AV+ V) $\times 100$].

(57)

1.

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18 , *P.pastoris*

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HVV 단편 1

합성된 올리고의 목록:

HVV1F1T1 5'-PCTAGGCTCGAGAA-3'
 HVV1F1T2 5'-PGAGATGTGATTTGCCAGAGACTCACTCTT-3'
 HVV1F1T3 5'-PTGAGACAACAGAAGAACTTGTGATGCTTT-3'
 HVV1F1T4 5'-PGGCCAAATGTCAGAACATCTCCATCCCTC-3'
 HVV1F1T5 5'-PTTGTGATGGATAGAACAGATTGCGTTT-3'
 HVV1F1T6 5'-PCCACAAAGAAGAATTCCCTGCTCGCG-3'
 HVV1F1B1 5'-PGATCCGGAGCA-3'
 HVV1F1B2 5'-PGGAATTCTCTTGTGGAAACCGAAATCGT-3'
 HVV1F1B3 5'-PGTCTATCCATCAAACAAGAGGATGGAGA-3'
 HVV1F1B4 5'-PGATTCTAGACATTGGGCCAAAGCATCA-3'
 HVV1F1B5 5'-PAAGTCTCTGTTGCTCAAAGACTGAGTCT-3'
 HVV1F1B6 5'-PTGGCAAATCACATCTCTCGAGC-3'

최종 연결된 산물:

[HVV1F1T1] [HVV1F1T2] [HVV1F1T3]
 5'-CTAGGCTCGAGAAAGAGATGTGATTTGCCAGAGACTCACTCTTGGACAACAGAAGAACTT
 3'-CGAGCTCTCTACACTAACGGTCTGTGAGTGGAAACCTGTTGTCTCTTGAA
 [HVV1F1B6] [HVV1F1B5]

[HVV1F1T4] [HVV1F1T5]
 TTTGGCCAAATGTCAGAACATCTCCATCTCTGTGATGGATAGAACAGATTTCGTTT
 AAACCGGGTTACAGATCTTAGAGAGGTTAGGAGAACAAACTACCTATCTGTGCTAAAGCCAA
 F1B4 [HVV1F1B3] [HVV1F1B2]

1T6 [GAAGAATTCCCTGCTCGCG-3'
 CTTCTTAAGGGACGAGCGCTAG-5'
] [HVV1F1B1]

HVV 단편 2

합성된 올리고의 서열:

HVV1F2T1 5'-PAATTGACGGTAAC-3'
 HVV1F2T2 5'-PCAATTCCAAAAGGCTCCTGCTATTCTGT-3'
 HVV1F2T3 5'-PTTGACGAGTTGATTCAACAAATT-3'
 HVV1F2T4 5'-PTCAACTTGTCAACCAAGGACTCTT-3'
 HVV1F2T5 5'-PCTGCTGCCTGGGACGAAGACTTGTGGAC-3'
 HVV1F2T6 5'-PAAGTTCTGTACTGAGCTCAGCGCGAATG-3'
 HVV1F2B1 5'-PGATCCATTGCGCT-3'
 HVV1F2B2 5'-PGAGCTCAGTACAGAACTTGTCCAACAA-3'
 HVV1F2B3 5'-PGTCTTCGTCCAGGCAGCAAGAGTCCT-3'
 HVV1F2B4 5'-PTAGTGTGAAAGTTGAAAGATTTGTTG-3'
 HVV1F2B5 5'-PAATCAACTCGTCAAAACAGAAATA-3'
 HVV1F2B6 5'-PGCAGGAGCCTTGGAAATTGGTTACCGTCA-3'

최종 연결된 산물:

[HVV1F2T1] [HVV1F2T2] [HVV1F2T3]
 5'-AATTGACGGTAACCAATTCCAAAAGGCTCCTGCTATTCTGTGCAAGAGTTGAT-
 3'-ACTGCCATTGGTTAAGGTTTCCGAGGACGATAAGAACAAACGTCACACTA
 [HVV1F2B6] [HVV1F2B5]

[HVV1F2T4] [HVV1F2T5] [HVV1F2T6]
 TTCAACTTGTCAACCAAGGACTCTCTGCTGCCCTGGGACGAAGACTTGTGGACAG
 AAAGTTGAAACAAGTGGTGATTCCTGAGAACAGACGACGGACCCCTGCTGAACAAACCTGTC
 2B4 [HVV1F2B3] [HVV1F2B2]

[GAGCTCAGCGCGAATG-3'
 CTCGAGTCGCCTAACCTAG-5'
] [HVV1F2B1]

HVV 단편3

합성된 올리고의 목록:

HVV1F3T1 5' - PTTACCAACAAATT-3'
 HVV1F3T2 5' - PGAACGACTTGAGGCTTGTGTTATGC-3'
 HVV1F3T3 5' - PAAGAGGAGAGACTCGGTGAGACCCATTGATG-3'
 HVV1F3T4 5' - PAACGCTGATTCCATCTGGCTGTCAAGAACT-3'
 HVV1F3T5 5' - PACTTCAGAAGAAATTCAAGCTCATTTG-3'
 HVV1F3B1 5' - PGATCCAATGAGC-3'
 HVV1F3B2 5' - PTGAATTCTCTGAAGTACTCTTGACAGCCAA-3'
 HVV1F3B3 5' - PGATGGAATCAGCGTTCATCAATGGGGT-3'
 HVV1F3B4 5' - PCTCAACGACTCTCCCTTGCAATAACACA-3'
 HVV1F3B5 5' - PAGCCTCCAAGTCGTTCAATTGTTGGTAAAGCT-3'

최종 연결된 산물:

[HVV1F3T1] [HVV1F3T2] [HVV1F3T3]
 5' - TTACCAACAAATTGAACGACTTGAGGCTTGTGTTATGCAGAGGAGAGACTCGGTG/
 3' - TCGAAATGGTTAACTTGCTGAACCTCGAACATAAGCTCTCCCTCTCAGCCACT/
 [HVV1F3B5] [HVV1F3B4]
 [HVV1F3T4] [HVV1F3T5]
 ATTGATGAACGCTGATTCCATCTGGCTGTCAAGAAGTACTCAGAAGAAATTCAAGCTCATTTG-
 TAACTACTTGCAGACTAAGGTAGAACCGACAGTTCTCATGAAGTCTTCTTAAGTCGAGTAAAC(/
 V1F3B3 [HVV1F3B2] [HVV1F3B1]

합성된 올리고의 목록:

HVV1F4T1 5' - PAATTACCTTGTACT-3'
 HVV1F4T2 5' - PTGACCGAAAAGAAGTACTCCCCATGTGCC-3'
 HVV1F4T3 5' - PTGGGAAGTCGTTAGAGGCCAAATCAT-3'
 HVV1F4T4 5' - PGAGATCTTGTCCCTGTCCACTAACCTT-3'
 HVV1F4T5 5' - PGCAAGAGAGACTTACCAAGATGGGTGGAG-3'
 HVV1F4T6 5' - PACTTGAACTCTCATAAGCGGCCGCG-3'
 HVV1F4B1 5' - PGATCCGCGGCCGCT-3'
 HVV1F4B2 5' - PTATGGAGAGTTCAAGTCTCCACCCAT-3'
 HVV1F4B3 5' - PCTTGGTAAGTCTCTTGCAAGTTAGTGG-3'
 HVV1F4B4 5' - PACAAGGACAAAGATCTCATGATTTCGGC-3'
 HVV1F4B5 5' - PTCTAACGACTTCCCAGGCACATGGGGAGTA-3'
 HVV1F4B6 5' - PCTTCTTTCGGTCAAGTACAAAGGT-3'

최종 연결된 산물:

[HVV1F4T1] [HVV1F4T2] [HVV1F4T3]
 5' - AATTACCTTGTACTGACCGAAAAGAAGTACTCCCCATGTGCCCTGGGAAGTCGTTAGAGC/
 3' - TGGAAACATGAACTGGCTTTCTCATGAGGGTACACGGACCCCTCAGCAAATCTCG/
 [HVV1F4B6] [HVV1F4B5] [HVV1F4B4]
 [HVV1F4T4] [HVV1F4T5] [HVV1F4T6]
 ATGAGATCTTGTCCCTGTCCACTAACATTGCAAGAGAGACTTACCAAGATGGGTGGAGACTTC/
 TACTCTAGAAACAGGAACAGGTGATTGAACGTTCTCTGAATGGTTCTACCCACCTCTGAA(/
 B4 [HVV1F4B3] [HVV1F4B2]
 [HVV1F4B1]
 CATAAGCGGCCGCG-3'
 GTATTGCGCCGGCGCTAG-5'
 [HVV1F4B1]

5A

Bgl II

CGAAATCATGAGATCTTGTCCCTGCCCCACTAACTTCAAGAGAGAATTCAGATGGGAGACTTGAACCTCCATAACGGGGCCAGCTT 1728
GCTTAGTACTCTAGAACAGGAACAGGTGATTGAGCTCTCTGAATGGCTACCCACCTCTGAACCTTGAGAGGTATTCGCCCCGTCGAA ~

E I M R S L S L S T N L D E R L T K M G G D L N S P A A A S F

Xba I Mbo II

TETAGAACAAAAACTATCTAGAACAGGAGCTGAATAGCGCCGTCGACCATCATCATCACTACATCACTTCAGTTAGCTTACAGATCTGTT 1824
AGATCTCTGTTTGTAGTAGACTCTCTCTAGACTTATCGGGCACTGGTAGTAGTAGTAGTAACCTAAACATCGGAATCTGFACTGACAAG

L E O K L I S E E D L N S A Y D H H H H H H V C S L R H D C S

Mbo II

CTCAGTTCAGTTGGGCACTAACAGAACCGGCTCTGCTAGATTCTAAAGAGGTGTCAGAATGCCATTGGCTGAGAGATGAGGCTTC 1920
GAGTCAGTCAACCGTGAATGCTCTCTGGCAGAACGATCTAAGATTAGTCTCTACAGCTTACGGTAAACCGACTCTACGTCGAAGT

S V O V G H L R E D R S C . I L I K R M S E C H L P E R C R L H

Mbo II

TTTTGATACTTTTATGTAACCTATATAGTATAGGATTTTGTGATTTGTTCTCTCGTACGAGCTTGCTCTGATCAGGCTATCTC 2016
AAAAACTATGAAAAAAATAACATTGGATATACTCATCTCTAAACAGTAAACAAAGAAGACATGCTCGAACGAGGACTAGTCGGATAGAG

F . Y F F I C N L Y S I G F F L S F C F F S Y E L A P D O P I S

Tse I Mbo II

GCAGGETGATAATCTGTGGTAGGGGTTGGGAAATCATTCGAGTTCTGTTCTGGTATTTCCACTCTCTCAGACTACAGAAGAT 2112
CTCGACTACTTATAGAACACCATCCCAAACCTTTAGTAAGCTAACTACAAAAGACCTAAAGGGTAGGGAGACTCTCATGCTCTA

Q L M N I L W . G F G K I I R V . C F S W Y F P L L F R Y O K I

Mbo II BamHI Mbo II

TAAGTGACCCCTTGTGGCGATCCCCACACACCATAGCTCAAAATGTTCTACTCTCTTACTCTCCAGATTTCTCGGACTCCGG 2206
ATTCACTCTGGAGCAAACACGCTAGGGGTGTCGGTATCGAAGTTTACAAAGATGAGGAAAGATGAGAGCTAAAGACCTGAGGCG

K . D L R L C G S P T H H S F K M F L L L F Y S S R F S R T P R

Mbo II

CATCGCCGTAACCACTCAAAACACCCAGCACGCACTAAATTCTCCCTCTGGCTGCTGTTAAATTACCCGACTAAAGCTTG 2304
GTAGCGGCATGGTGAAGTTGTGGCTGGTCTGCTGCTGTTAAAGGAGAAAGAAGGAGATCCCACCGCAATTATGGCATGATTCTAAC

I A V P L O N T O A O H T K F S L F L P L G C R . L P V L K V W

Mbo II

GAAAAAGAAAAAGAGACGGCTGGTTCTTCTCTGGCTGAAAAAGGCAATAAAATTTATCACGTTCTTTCTTGAAATTTTTTTTA 240
CTTTCTTTCTCTGGCGAGCAAGAAAAGAAGGAGCTTCTGGTTATTTAAAGTAGTGCAGAAAGAAAAGACTTTAAAAAAAT

K R K K R P P R F F F V E K G N K N F Y H V S F S . N F F F

5B

Mbo II

|||||||CAGTGAACCTCCATTGATATTAAAGTTAATAAAGCTCAAGTTCAAGTTCAATTTCATTTCTGTTCTATTACAA
|AAAAAAAGAGAAAGTCACTGGAGGTAACATAAATTCATTGGCCAGAAGTTAAAGAGTCAAAAGTCAAAGTAAAAAGAACAAAGATAATGTT 2496

F F S L S V T S I O I . V N K R S S I S O V S V S F F L F Y Y N

|||||||ACTTCTTGTTCATTGAAAGAAAGCATGCAATCTAATCTAACCGCCGCTGGTACAACATTAATCACTGGCATAGTATACGGCATAGT
|AAAAAAATGAAGAACAAAGTAACTTCTTCTGATTCCTAGATTAGATCCTCCACAACTGTTAATTGACCGTATCATATAGCCGATATCA 2592

F F Y F L F I R K K A O S N L R G G V D N . S S A Y I G I V

ATAATACGACAAGGTGAGGAACAAACCATGGCAAGTGGCCAGCTGGCTCCGGTCTACCCGGCGGACCTCGCCGGACGGTCAGITCTG
TATTATCGCTGTTCCACTCTTGATTTGGTACCGTTCACTGGTACCGCAAGGCAAGACTGGCGCGEGTGCAGGGCTCGCCAGCTCAAGAC 2688

. Y D K V R N . T M A K L T S A V P V L T A R D V A G A V E F W

Sma I

GACCGACCGGCTGGGTCTCCGGGACTTCTGGGAAAGGAGACTTCGGGCGGCTGGGCGGCTGGGACGACGACTGGGACGGCTGGGACGGCTCCAGGA
CTGGCTGGCGAGGCCAACAGGGCCCTGAAGCACCTCTGCTGAAGCGGCCACACCGGGCTGGTCACTGGGACAGTAGTGTGCGCCAGGTCT 2784

T O R L G F S R D F V E D D F A G V Y R D D V T L F I S A V O O

CCAGGTGGTGGCGGACAACACCCCTGGGCTGGGTGCGGCTGGGACGAGCTGACGGCTGGTCAAGGGAGTGGTCGGGAGGTCGTGTCACCGAACCTCCG
GGTCCACACCGCCCTGTGTTGGGACCCACCCACCGCCGGGACCTGCTGACATGGGCTCACCGCCCTCACACAGGTGCTGAAGGC 2880

Q Y V P D N T L A W V W V R G L D E L Y A E W S E V V S T N F R

Hga I Tse I

GGACGCTCCGGGCGGCCATGACCGAGATCGCCAGCAACCGCTGGGGGGACTTCGGGCTGGGCGGAACTGGCTGCACCTCGT
CCTGGGGAGGGCCGGCCGGTACTGGCTCTAGGGCTCGTGGCACCCGGCTCAAGCGGGACGGCTGGGCGGGCTGGCACGTGAAGCA 2974

D A S G P A M T E I G E O P W G R E F A L R D P A G N C V H F V

GGCGGAGGAGGAGGACTGACAGTCCGACGGGCCACGGGCTCCAGGGCTCGGAGATCGCCCTGGGAGGCTGGGAGGCTCTAGGCAAGGAAACAGCTATAGTACATTAATC
CGGGCTCTGCTCTGACTGTGCGAGCTGCGCCGGTGGGCTGGGAGGCTCTAGGCAAGGAAACAGCTATAGTACATTAATC 3074

A E E Q D . H Y R R R P T G P R P R R S V P L F L C R Y H V I S

TTATGTACCGCTTACATTCCACCCCTCCCCACATCCCTTAACCGAAAGGAGCTAGACACCTGAAGTCAAGCTTCTGATATCATGTAATTAG
AATACAGTGCCATGAACTGGGAGGGGGTGTAGGGAGATGGCTTCCCTCAACTGTTGACTTCAGATCCAGGGATAATAAAA 316

Y V T L T F T P S P H I R S N R K G R S . T T . S L G P Y L F F

MuI Hga I

TTATAGTTATGTTAGTAAAGAAGCTTATATTCAAATTCTTCTTCTGTCAGACGCGCTGTACGCTGTAACATTAACTGAAAA
AATATCAATAACATATAATCTTCAATAAATAAAGTTAAAAGAAAAAAAGACATGTCTGGCACATGGTACATTGTAATATGACTTT 326

Y S Y V S I K N V I Y I S N F S F F S V Q T R Y R M . H Y T E N

5C

Friday, July 6, 2001 5:20 PM
 HVV+pPICZ-alphaB Map (1 > 4051) 부위와 서열

Hga I

CCTTGCTTGAAGCTTGGGACCCCTGAAAGCTTAAATTGCAAGCTGGAGACCAACATGTGACCAAAAGGCCAGCAAAAGGCCAGGAACCGTA 3360
 GGAACGAACCTCTTCAAAACCCCTGCGAGCTCGAAATTAAACGTTGACCTCTGGTGTACACTGTTTCCGGTCTTCCGGTCTGGCAT

L A . E G F G T L E G F N L O A G D O O H Y S K R P A K G O E P .

Hga I

AAAAGGCCGCGTTGCTGGCGTTTCCATAGGCTCCGCCCTGACGACCATCACAAATGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG 3456
 TTTCCGGCGCAACGACCGCAAAAGGTATCCGAGGCGGGGGACTGCTGTAGTTAGCTGCGAGTYCAGTCTCACCGCTTGGCTGTC

K G R V A C V F P . A P P P . R A S O K S T L K S E V A K P D R

GAATATAAAAGATAACCAAGCGCTTCCCCCTGAAACCTCCCTGTCGGCTCTCTCTGTTCCGACCTGCCCTTACCGGATACTGTCGGCTTCTCC 3552
 CTGATATTCATGGTCCGCAAAGGGGACCTCGAGGGAGACCGGAGAGGACAAGGCTGGGACGGCAATGGCTATGGACAGCGGAAAGAGG

T I K I P G V S P W K L P R A L S C S D P A A Y R I P V R L S P

CTTCGGGAAGCGTGGCCCTTCTCAATGCTCACGCTCTAGGTATCTCAGTTGGTGTAGGTCTGCTCCAAAGCTGGCTGTGTGCACGAACCC 3648
 GAAGCCCTTCCACCCGAAAGAGTTACAGATGCGACATCCATAGAGTCAAGCCACATCCAGGAACGGAGGTTGACCCGACACGTTGGGG

F G K R G A F S M L T L . V S O F G V G R S L O A G L C A R T P

Tse I Tse I

CCGTTCAAGCCGACCGCTGGCCCTATCCGTAACTATCGCTTGAAGTCAACCCGGTAAGACACGACTTATGCCACTGGCAGCGCCACTGGTA 3744
 GCGCAAGTCGGCTGGCGACCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGCCATTCTGCTCAATACCCGTACCCGTGTCGGTACCAT

R S A R P L R L I R . L S S . V O P G K T R L I A T G S S H W .

ACAGGATTACACAGCCAGGTATGAGCGCTCACAGAGTTCTGAAAGTGGCTGCTAACTACGGCTACTAGAAGGACAGTATTTGGTATCT 3840
 TGTCTTAATGCTCTCGCTCCATACATCCGACGATGCTCAAGAAACTCACCACGGATTGATGCCATGTTCTGCTCAATACCCGTACCCGTGTCGGTACCATAGA

O O . O S E V C R R C Y R V L E V V A . L R L H . K D S I W Y L

GCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTGATCCGGCAAACAAACCCACCGCTGGTAGGGTGGTTTTTGCTGCA 3936
 CGGAGACGACITCGGTCAATGGAAGCTTTCTCAACCACGAGAACTAGGGCTTGTGTTGGTGGCGACCATGCCACCAAAAAAACAAACGT

R S A E A S Y L R K K S W . L L I R O T N H R W . R W F F C L O

Tse I Mbo II Hga I

ACGAGCAGATTAACCCGACAAAAAAAGGATCTCAAGAACATCTTGTATTTCTACGGGTCTGACGCTCAGTGGAAACGAAACTCACCTTAAG 4032
 TGTCGTCTAATGCCGCTCTTTCTAGAGTTCTAGGAAACTAGAAAAGATCCCCAGACTCGAGTCACCTGCTTGTGAACTTC

A A O Y A O K K R I S R R S F D L F Y G V . R S V E R K L T L R

GGATTTGGTCATGAGATC 4051
 CCTAAAAACCACTCTAG

D F G H E I

6A

HVV 단백질 정보

HVV 아미노산 서열

CDLPETHSLDNRRRLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAIISVLHELI
 QIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDEACVMQEERVGETPLMNADSLAVKI
 YFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLTKMGGDLNSP.

단백질 정보

분자량 19816.67 달톤
 172 아미노산

18 강한 양기성(+) 아미노산(K, R)
 26 강한 산성(-) 아미노산(D,E)
 56 소수성 아미노산 (A, I, L, F,W,V)
 50 극성 아미노산(N,C,Q,S,T,Y)
 4.800 등전점
 PH 7.0 에서 7.714 전하

번역된 열기의 전체 수는 5190이다.

% A = 27.36 [142]
 % G = 21.97 [114]
 % T = 28.90 [150]
 % C = 21.77 [113]
 분명하지 않은 % = 0.00 [0]

% A+T = 56.26 [292]
 % C+G = 43.74 [227]

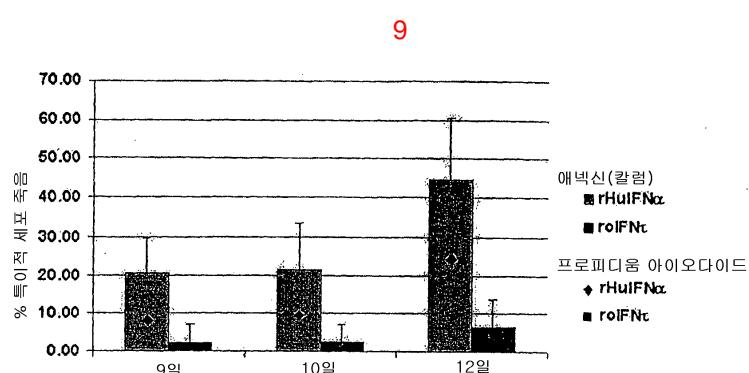
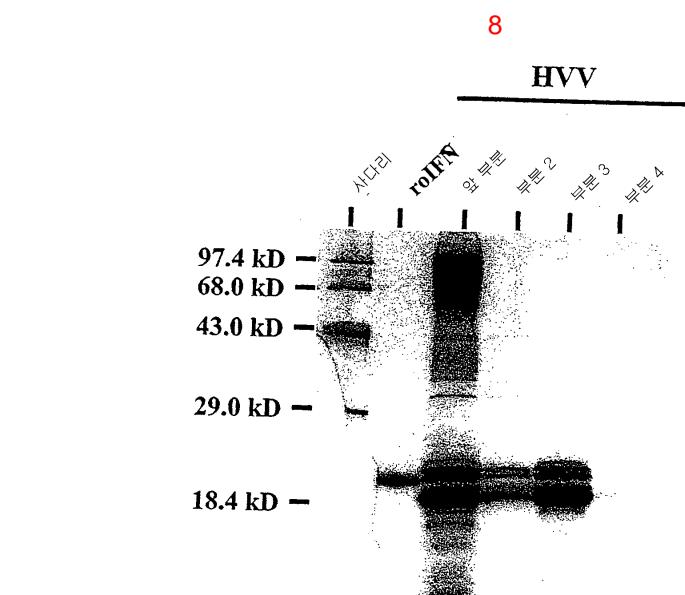
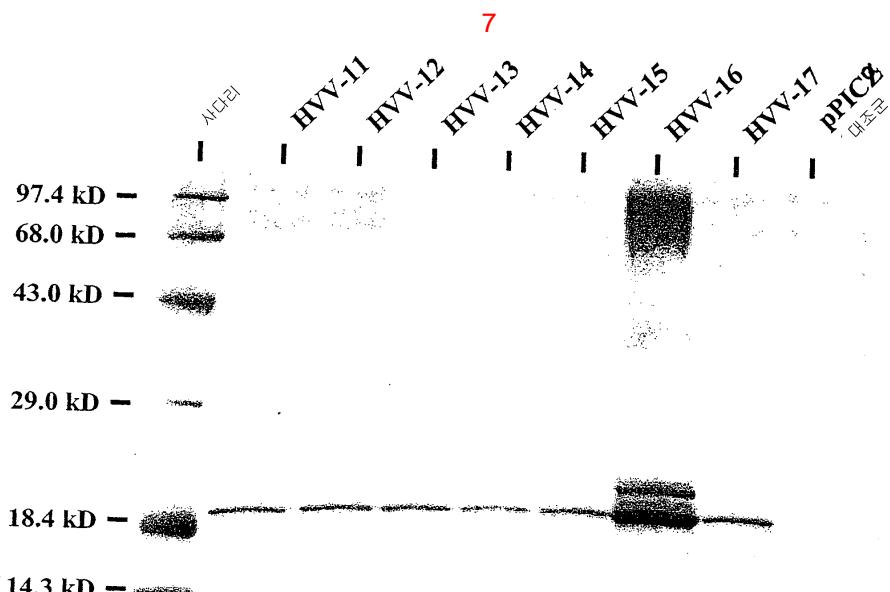
Davis, Botstein, Roth 녹는점 C. 81.87
 Wallace Temp C 1718.00

코돈 사용

gca	Ala (A)	0	# cag	Gln (Q)	0	# uug	Leu (L)	20	# uaa	Ter(.)	1
gcc	Ala (A)	4	# ---	Gln (Q)	10	# ---	Leu (L)	23	# uag	Ter(.)	0
gct	Ala (A)	0	# gaa	Glu (E)	6	# aaa	Lys (K)	0	# uga	Ter(.)	0
gcu	Ala (A)	6	# gag	Glu (E)	8	# aag	Lys (K)	8	# ---	Ter(.)	1
---	Ala (A)	10	# ---	Glu (E)	14	# ---	Lys (K)	8	# aca	Thr (T)	0
aga	Arg (R)	10	# gga	Gly (G)	1	# aug	Met (M)	7	# acc	Thr (T)	5
agg	Arg (R)	0	# ggc	Gly (G)	0	# ---	Met (M)	7	# acg	Thr (T)	0
cga	Arg (R)	0	# ggg	Gly (G)	0	# uuc	Phe (F)	7	# acu	Thr (T)	5
cgc	Arg (R)	0	# ggu	Gly (G)	4	# uuu	Phe (F)	1	# ---	Thr (T)	10
cgg	Arg (R)	0	# ---	Gly (G)	5	# ---	Phe (F)	8	# ugg	Trp (W)	2
cgu	Arg (R)	0	# cac	His (H)	3	# cca	Pro (P)	6	# ---	Trp (W)	2
---	Arg (R)	10	# cau	His (H)	0	# ccc	Pro (P)	0	# uac	Tyr (Y)	4
aac	Asn (N)	7	# ---	His (H)	3	# ccg	Pro (P)	0	# uau	Tyr (Y)	0
aau	Asn (N)	0	# aua	Ile (I)	0	# ccu	Pro (P)	1	# ---	Tyr (Y)	4

6B

---	Asn (N)	7	# auc	Ile (I)	3	# ---	Pro (P)	7	# gua	Val (V)	0
gac	Asp (D)	8	# auu	Ile (I)	4	# agc	Ser (S)	0	# guc	Val (V)	3
gau	Asp (D)	4	# ---	Ile (I)	7	# agu	Ser (S)	0	# gug	Val (V)	0
---	Asp (D)	12	# cua	Leu (L)	0	# uca	Ser (S)	0	# guu	Val (V)	3
ugc	Cys (C)	0	# cuc	Leu (L)	0	# ucc	Ser (S)	5	# ---	Val (V)	6
ugu	Cys (C)	5	# cug	Leu (L)	0	# ucg	Ser (S)	0	# nnn	???(X)	0
---	Cys (C)	5	# cuu	Leu (L)	3	# ucu	Ser (S)	9	# TOTAL		173
caa	Gln (Q)	10	# uua	Leu (L)	0	# ---	Ser (S)	14	#		



Alignment Report of NLVHVVTau.seqal using Clustal method with PAM250 residue weight table.
Tuesday, May 29, 2001 4:03 PM

C Y L S E T L M L D A R E N L K L L D R M N R L S P H S C L												Majority
	10	20	30									
1	C Y L S E R I M L D A R E N L K I L D R M N R L S P H S C L											IFN-T.OTT.AASEQ.ER
1	C Y L S R K I M L D A R E N L K I L D R M N R L S P H S C L											IFP.Y00287.AA
1	C D L P B T H S L H N R T L M I L D A Q M S H I S P S C L											IFNalphaD.AA
1	C D I P C T H S T I D N R T L M I L D A Q M S H I S P S C L											HVV.AA
1	C Y L S R K I M L D A R E N L K I L D R M N R L S P H S C L											NLV.AA
M D R H D F G F P Q E E F D G N Q F Q K A P A I S V L H E L												Majority
	40	50	60									
31	Q D R K D P T G L P Q E M V E G D Q I L O K D Q A F P V I Y G M											IFN-T.OTT.AASEQ.ER
31	Q D R K D P G L P Q E M V E G D Q I L O K D Q A F P V I Y G M											IFP.Y00287.AA
31	M D R H D P G M P Q E E F D G N Q P K A P A I S V L H E L											IFNalphaD.AA
31	M D R H D P G M P Q E E F D G N Q P K A P A I S V L H E L											HVV.AA
31	M D R H D P G M P Q E E F D G N Q P K A P A I S V L H E L											NLV.AA
I Q Q I F N L F T T K D S S A A W D E D L L D K F C T E L Y												Majority
	70	80	90									
61	L Q Q S P F I S T Y T E H S S A A W D E D L L D K F C T E L Y											IFN-T.OTT.AASEQ.ER
61	L Q Q S P F I S T Y T E H S S A A W D E D L L D K F C T E L Y											IFP.Y00287.AA
61	I Q Q I F N L F T T K D S S A A W D E D L L D K F C T E L Y											IFNalphaD.AA
61	I Q Q I F N L F T T K D S S A A W D E D L L D K F C T E L Y											HVV.AA
61	I Q Q I F N L F T T K D S S A A W D E D L L D K F C T E L Y											NLV.AA
Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V												Majority
	100	110	120									
91	Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V											IFN-T.OTT.AASEQ.ER
91	Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V											IFP.Y00287.AA
91	Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V											IFNalphaD.AA
91	Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V											HVV.AA
91	Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V											NLV.AA
K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R												Majority
	130	140	150									
121	K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R											IFN-T.OTT.AASEQ.ER
121	K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R											IFP.Y00287.AA
121	K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R											IFNalphaD.AA
121	K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R											HVV.AA
121	K K Y F R R I T I L Y L T E K K Y S P C A W E V V R A E I M R											NLV.AA
S L S L S T N L Q E R L T K M G G D L N S P -												Majority
	160	170										
151	A I T V S I T T Q Q K R I T T M G G D L N S P -											IFN-T.OTT.AASEQ.ER
151	A I T V S I T T Q Q K R I T T M G G D L N S P -											IFP.Y00287.AA
151	S I I S S T N I Q C P R I R R K E .											IFNalphaD.AA
151	S I I S S T N I Q C P R I R R K E .											HVV.AA
151	S I I S S T N I Q C P R I R R K E .											NLV.AA

Decoration 'Decoration #1': Shade (with bright turquoise at 40% fill) residues that match the Consensus exactly.

<110> PEPGEN CORPORATION

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35 40 45

Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln Ile
50 55 60

Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp
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Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
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Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
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Asn Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
115 120 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val

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Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu

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Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro

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gaagaatttg acggtaacca attccaaaag gtcctgcta tttctgtttt gcacgagttg 180

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aagaagtact tcagaagaat taccttgtac ttgaccgaaa agaagtactc cccatgtgcc 420

tgggaagtgc ttagagccga aatcatgaga tctttgcct tgtccactaa cttgcaagag 480

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