

(54)

가 200

RNA

가

RNA

RNA

RNA

1A

RNA

, DNA

가 /

가

ncing) DNA (sile
 RNA,
 (Drosophila)
 [: L. Timmons and A. Fire, Nature, 395: 354 (Oct. 29, 1998); A. Fire et al., Nature, 391: 806 - 810 (Feb. 19, 1998); and R. Jorgensen et al., Science, 279: 1486 - 1487 (March 6, 1998)].
 DNA
 [: Bahramian and Zarbl, Mol. Cell. Biol., 19(1): 274 - 283 (Jan. 1999)].

, 2 RNA / RNase W
 WO98/05770 (1998. 2. 12.)
 O99/53050 (1999. 10. 21.) RNA

가 200
 RNA

RNA
 RNA
 RNA

RNA

2

가

가

/

2

가

가

/

2

가

가

2

가

/

2

가

/

2

가

2

, " (silence)" .

" " " " 가
, 가 , , 가 , ,

A. A.

RNA , " RNA" ,
, RNA - DNA , R
RNA 2' - OH 가 200
NA

, RNA ,
RNA ,
RNA ,
RNA ,

" RNA " 가 100 10,000 RNA
, 가 200 8000 , 가 200 ,
, RNA 가 7500
A , RNA 가 1000 , RNA 가 2000 , RN
RNA 가 750 , RN

RNA 가 , - 9.2kcal/mol ΔG
, 가 11 30 , 가 , ΔG
[: Jaeger et al., Proc. Natl. Aca
d. Sci., USA, 20: 7706 - 7710(1989); and Soler and Jankowski, Math. Biosci., 2: 167 - 190(1991)].

RNA 10% ,
RNA 30% ,
RNA 50% , 70% ,
, 90% ,
, 가 , 가
A 가 , RNA ,
2 , RNA DNA , 가 RNA/DN
RNA 가 RNA DNA

RNA

, 2

2

[: COMPUTATION

AL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, (1988); BIOCOMPUTING : INFORMATICS AND GENOME PROJECT, Smith, D.W., ed., Academic Press, New York, (1993); COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, (1994); SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, (1987) ; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J. eds., M Stockton Press, New York, (1991)].

[: H. Carillo and D. Lipton,SIAM J.

Applied Math., 48: 1073(1988)].

[: Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and H. Carillo and D. Lipton,SIAM J. Applied Math., 48: 1073(1988)]

가

GCG

BESTFIT[: J. Devereux et al.,Nucl. Acids Re

s., 12(1): 387(1984)], MACVECTOR (Oxford) FASTA(Pearson)

RNA / RNA

가

, RNA

, 10%

, 37

MACVECTOR

30

50%

30

() , RNA 30 , 30%

30

50%

, RNA

50%

50%

30

30

, RNA

, 70%

30

50%

, RNA

30

90%

30

50%

30

, RNA

, 10%

30

70%

30

, 10%

, RNA

30

30

90%

30

()

RNA(RNA/DNA) , (ariat)(
) / / 가 가 (,
 (tandem)) mRNA 가
 , " " 가 3'-5' 2'
 -5' 가 -
 5' () (loop back) 2'-OH 가
 10
 2'-5' /

mRNA RNA 2'-OH [: Re
 DNA , 2'-5' RNA 2'-OH
 es C and Song Q.Nucl. Acid Res., 27,2672 - 2681(1999); Dame E et al.,Biochemistry, 38, 3157 - 3167, 199
 9; Clement J. Q. et al.,RNA, 5, 206 - 220, 1999; Block T and Hill J.J. Neurovirol., 3, 313 - 321, 1997; Schi
 ndewolf CA and Domdey H.,Nucl. Acid Res., 23,1133 - 1139 (1995)].

RNA(RNA - DNA)가 2'-5' 3'-5'
 (splinter) RNA 가
 , 가
 가
 RNA RNA(RNA
), RNA 2

RNA(RNA - DNA) (mRNA 가
 RNA) RNA - DNA , mRNA
 DNA (가)
 가

Schindewolf CA and Domdey H.,Nucl. Acid Res., 23,1133 - 1139 (1995); Rees C and Song Q.Nucl. Acid Re
 s., 27,2672 - 2681(1999); Block T and Hill J.J. Neurovirol., 3, 313 - 321, 1997].

가 , (capped) RNA . dsRNA가
 A 가 , RN

RNA가 RNA RNA RNA RNA polIII
 가 RNA RNA - DNA 가 가 가

RNA [: Promega Protocols and Applications Guide, (3rd ed. 1996), eds. D
 oyle, ISBN No. 1 - 882274 - 57 - 1] T7, T3
 SP6 RNA RNA
 RNA RNA/DNA

[: Q. Xu et al., Nucl. Acid
 s Res., 24(18):3643 - 4(Sept. 1996); N. Naryshkin et al., Bioorg. Khim., 22(9):691 - 8(Sept. 1996); J. A.
 Grasby et al., Nucl. Acids Res., 21(19):4444 - 50(Sept. 1993); C. Chaix et al., Nucl. Acids Res., 17(18):73
 81 - 93(1989); S.H. Chou et al., Biochem., 28(6):2422 - 35(Mar. 1989); O. Odai et al., Nucl. Acids Symp. S
 er., 21:105 - 6(1989); N.A. Naryshkin et al., Bioorg. Khim. 22(9):691 - 8(Sept. 1996); S. Sun et al., RNA,
 3(11):1352 - 1363(Nov. 1997); X. Zhang et al., Nucl. Acids Res., 25(20):3980 - 3(Oct. 1997); S.M. Grava
 znov et al., Nucl. Acids Res., 26(18):4160 - 7(Sept. 1998); M. Kadokura et al., Nucl. Acids Symp. Ser., 37
 :77 - 8(1997); A. Davison et al., Biomed. Pept. Proteins Nucl. Acids, 2(1):1 - 6(1996); and A. V. Mudrakov
 skaia et al., Bioorg. Khim., 17(6):819 - 22(Jun. 1991)].

RNA
 [: Sambrook et al, MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.;
 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989] 5,
 824,538 ; 5,877,159 65,643,771 ()

RNA
가
RNA (, 2) 가 RNA RNA RNA RNA
가 (fold back) 가 RNA RNA
RNA RNA 가
(rod) RNA [: K - S. Wang et al.,Nature, 323
:508 - 514(1986)], [: Wang et al.,Nucl. Acids Res., 22(12):2326 - 33(J

une 1994); Y. Matsumoto et al.,Proc. Natl. Acad. Sci., USA, 87(19):7628 - 32(Oct. 1990);Proc. Natl. Aca
d. Sci., USA, 91(8):3117 - 21(Apr. 1994); M Tsagris et al.,Nucl. Acids Res., 19(7):1605 - 12(Apr. 1991)
; S. Braun et al.,Nucl. Acids Res., 24(21):4152 - 7(Nov. 1996); Z. Pasman et al., RNA, 2(6):603 - 10(Jun.
1996); P. G. Zaphiropoulos,Proc. Natl. Acad. Sci., USA, 93(13):6536 - 41(Jun. 1996); D. Beaudry et al.,N
ucl. Acids Res., 23(15):3064 - 6(Aug. 1995):

RNA
DNA DNA RNA가 RNA DNA RNA DNA
가 가 RNA DNA RNA DNA
RNA/DNA RNA DNA DNA (Sambrook) 가(Prom
ega)

RNA DNA
RNA , RNA polII, RNA polIII RNA polIII RNA RNA
RNA (가
RNA)
RNA polII, RNA polIII RNA polIII RNA
A polI, II polIII, DNA , RN
가 RNA polII , RNA

300

가

가 RNA DNA DNA RNA DNA

1 DNA 가 DNA RNA RNA 2 DNA RNA 가 ()

ak) 가 (Koz

dsRNA dsRNA dsRNA dsRNA

RNA RNA RNA 5,877,159 ; 65,643,771 5,824,538 ; (Salmonella typhimurium), (Escherichia coli), (Bacillus subtilis), (Staphylococcus), (Pseudomonas), (Streptomyces)

RNA RNA RNA RNA (: SV40), RNA [

: M. Di Nocola et al., Cancer Gene Ther., 5(6):350 - 6(1998)]

RNA
RNA DNA
, C127, 3T3, CHO, HeLa, 293, BHK COS - 7
[Emerich et al., J. Neurosci., 16:5168 - 81(1996)]
[; D.B. Kohn et al., Nature Med., 4(7):775 - 80(1998)]
(adoptive)

B. () B. ()
RNA RNA 가
RNA
/ 가

RNA 가 , DNA ,
" (naked)"
" , () , 가 (:) ,
3 가 (:) ,
가 5,593,972 ; 5,703,055 ; 5,739,118 ; 5,837,533 ;
WO 96/10038(1996. 4. 4.) WO 94/16737(1994. 8. 8.) ()

01 가 0.1 1.0 % 가 0.03 % PCT/US98/22841 0.0
(가).

가
) 가
 가 (adjuvant), 가
 , N-Z 가
 가 (Ribi), (Amphigen), (Aviridine), L121/ , D- -
 / (Quil A)가 (pluronic ployois), (Bordetella),
 / /
 , G-CSF, GM-CSF, (TNF), (EGF), (PDGF),
 , IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 IL-12가 가 ,
 (ISCOMS), , LPS (A(MPL)),

가
 가
 가

[: Remington: T
 he Science and Practice of Pharmacy, Vol. 2, 19th edition (1995), e.g., Chapter 95 Aerosols;
 PCT/US99/05547; 가].

(RNA , DNA)
 1ng 20mg , 10n
 g 10mg , 500µg
 1 350µg
 25 250µg
 가 100µg .

가 , 가 , 1 - 10⁷ / 1 x 10² pfu 1 x 10¹² pf

가 ,

가 ,

C.

C.

; 가

RNA

RNA

RNA

()

가 ,

DNA

DNA

(Herpesvirus),

(Papillomavirus)

(Hepadenovirus),

(Papovavirus)

(Foxvirus),

(Retrovirus), (Parvovirus),
가 , HIV, HBV, HSV, CMV, HPV, HTLV E

BV가

RNA

, HPV11 L1 E2 , HPV16 E6 E7 , HPV18 E6 E7 , HIV gag, env pol , HPV6 L1 E2
, HBV , HSV gD , HSV vp16 , HSV gC, gH, gL gB , HBV , HBV
6 , (Varicella zoster) gB, gC GH BCR - abl , HSV ICP0, ICP4 ICP
, HBV , HSV vp16 , HSV - ICP0 , HSV - ICP4, ICP6 gD

RNA 가 , RNA 가 (,) , 가 RNA 가
 RNA 가 RNA 가
 RNA
 , HIV gag , /
 , HIV (Varicella Zoster)
 , ,
 , 가 , HSV - 1 HSV - 2 , HPV , , , HBV
 , /
 ,
 ,
 NA 가 , 가 , 가 R
 ,
 " - " HPV E6/E7 - , HTLV , EBV ,
 (Burkitts lymphoma) 가
 , HPV 16 E6 E
 7 HPV 18 E6 E7 ,

(Leishmania), (Brucella), (Mycobacteria), (Listeria), (Chlamydia),
 ia), (P. falciparum), (Shigella), (Plasmod
 Illia) / (Gonorrhoeae) (Borre

RNA

2

가

가

[: 5,593,972 ()]

가

(

)

가

2

(CML)

(ALL)

2

, bcr - abl .

WO94/13793(1994. 6. 23.)

, CML

가

, CML

bcr - abl

bcr abl .

2

RNA

RNA 가

D.

D.

, RNA

nock - out)"

RNA

(k

가

HIV gD2 RNA HIV gag

가

1: HIV p24

HIV DNA HIV 가 , HIV

HIVgpt HIVgpt HIV p24 (envelope) (MPA) (RD) COS7 HIV MPA MPA MPA HIV gag

p24 ELISA (Coulter Corporation) 가 p24 p24(HIV gag

2 RD 2 COS7 HIV p24 p24

t RNA PCR (Genbank K03455) HIV HXB2 gag RNA(dsRNA), 600 (nt) RNA 600n T7 HIV(HXB2) [: L. Ratner et al., AIDS Res. Hum. Retr oviruses, 3(1):57 - 69(1987)]. gag 901 - 924 T7 gag T7 gag 1476 - 1500 , T7 gag

가 RNA (T7) , T7 PCR 5' DNA (2)

5'GTAATACGACTCACTATAGGGCGGCAGGGAGCTAGAACGATTCGCAG 3'(1);

5'CTGCTATGTCACTTCCCCTTGGTTC 3'(2).

가 RNA T7 gag (3) gag PCR (4) :

5'GTAATACGACTCACTATAGGGCGCTGCTATGTCACTTCCCCTTGGTTC 3'(3);

5'GCAGGGAGCTAGAACGATTCGCAG 3'(4).

PCR, 가 RNA RNA RNA T7
 RNA, RNA dsRNA gD
 PCR T7 . HSV gD PCR Ge
 nbank K01408 HSV gD2 . gD 313 - 336 Ge
 T7 gD T7 . gD 849 - 87
 2 , T7 gD T7 .
 :

T7 gD (5):

5'GTAATACGACTCACTATAGGGCGGTTCGCGGTGGGACTCCGCGTCGTC 3';

gD (6): 5'GTCGCGGTGGGACTCCGCGTCGTC 3';

T7 gD (7):

5'GTAATACGACTCACTATAGGGCGGTGATCTCCGTCCAGTCGTTTATC 3';

gD (8): 5'GTGATCTCCGTCCAGTCGTTTATC 3'.

RNA RD COS7 : 6
 5 6 x 10⁵ / 80 90% (confluence) , 10μl
 (Gibco - BRL) , RNA 2 3μg .
 1 17 , RNA 1 500μg
 A , 0.5 2 , gag RN
 , gD RNA , ds gag RNA
 , ds gD RNA , 가 gD RNA 가

37 p24 . p24 ELISA (Coulter Corp.)
 - IgG(Sigma) p24 - p24 (Intracell Corp.) FITC -
 3 , RNA 2

RNA가 , gD RNA p24 , RNA ,
 , ds gag RNA p24 , p24 ()

2: p24

/ (, p24)

A. COS7 RD

p24 1 RNA p24
 p24
 0⁵ 1/1000 1 COS 7 1/10(COS 7/RD) 6 p24 6 7 x 1
 4 1 3 3 2 RD RD p2

p24 가
 , p24 ELISA (Coulter) p24 , FITC -
 IgG p24 (Intracell Corp.) , p24 . COS
 7 RD , RD COS 7
 . COS 7 T , RD , r - (PE) -
 IgG SV40 T (Pharmagen Corp.) , T
 Ag . COS 7

RD p24 , RD FITC , RD
 . FITC PE , RD , p24
 p24 1 RNA (ds RNA)

B. RD RD
 , p24 1 RD ,
 10(RD/ RD) , 6 6 7 x 10⁵ 1/1000 1/
 RD p24 RD : RD (5 6 x 10⁵) 6
 80 90% (TK)
 pCEP4(Invitrogen Corp.) Nru 1 - Sal 1 2.5µg
 (Gibco BRL) 2 , 400µg/ml
 1 1 400µg/ml
 RD RD
 ELISA , p24 1

, p24

3: - 12

- A. RNA
- RNA 가 600nt , RNA IL - 12 p40 RNA
 - A ; RNA
 RNA 가 :
- (1) IL - 12 p40 RNA(mRNA) (ss) RNA ;
 - (2) IL - 12 p40 mRNA ss RNA ;
 - (3) p40 IL - 12 mRNA (ds) RNA ;
 - (4) IL - 12 p40 RNA(hnRNA) ss RNA ;
 - (5) IL - 12 p40 hnRNA ss RNA ;
 - (6) IL - 12 p40 hnRNA ds RNA ;
 - (7) IL - 12 p40 ss RNA ;
 - (8) IL - 12 p40 ss RNA ;
 - (9) IL - 12 p40 RNA ds RNA .
- , 1 HSV2 gD , ds RNA
 RNA가 .

1 , (1) (9) RNA T7 PCR
 T7 RNA , RNA가 , T7 PCR PCR
 5' dsRNA가 , PCR T7 5'
 RNA RNA .

RNA PCR 5' 3' , T7 가

IL - 12 (hnRNA)(9): 5'TCAGCAAGCACTTGCCAAACTCCTG3'

IL - 12 (hnRNA)(10): 5'GAGACAAGGTCTCTGGATGTTATTG 3';

T7 IL - 12 (hnRNA)(11):

5'GTAATACGACTCACTATAGGGTCAGCAAGCACTTGCCAAACTCCTG 3'

T7 IL - 12 (hnRNA)(12):
 5'GTAATACGACTCACTATAGGGGAGACAAGGTCTCTGGATGTTATTG 3';

T7 IL - 12 (13):
 5'GTAATACGACTCACTATAGGGCCTATAAGCATAAGAGACGCCCTC 3'

IL - 12 (14):
 5'CCTATAAGCATAAGAGACGCCCTC 3';

IL - 12 (15):
 5'GGCTGCTCCTGGTGCTTATATAC 3'

T7 IL - 12 (16):
 5'GTAATACGACTCACTATAGGGGGCTGCTCCTGGTGCTTATATAC 3';

T7 IL - 12 cDNA(mRNA)(17):
 5'GTAATACGACTCACTATAGGGTGTGTCCTCAGAAGCTAACCATC 3'

IL - 12 cDNA(mRNA)(18):
 5'TGTGTCCTCAGAAGCTAACCATC 3';

IL - 12 cDNA(mRNA)(19):
 5'GCAGGTGACATCCTCCTGGCAGGA 3'

T7 IL - 12 cDNA(mRNA)(20):
 5'GTAATACGACTCACTATAGGGGCAGGTGACATCCTCCTGGCAGGA 3'.

PCR [: Tone et al.,Eur. J. Immunol., 26:1222 - 1227(1996)]
 IL - 12 8301 - 8325 IL - 12
 8889 - 8913 IL - 12 83 - 106 IL - 12
 659 - 682 cDNA PCR Genbank M86671
 IL - 12 cDNA 36 - 58 IL - 12 cDNA
 659 - 682

B.

Balb/c (5) , IL - 12 p40 RNA 1
 0 500µg . 3 4 ,
 (Quantikine) M - IL - 12 p40 ELISA (Genzyme) IL - 12 p40

IL - 12 mRNA, IL - 12 hnRNA ds RNA ds
 RNA 가 IL - 12 RNA 가
 가 . HSV gD RNA ()
 IL - 12

4:

A.

20 30% (Vero) / BHK , 10% FBS DMEM 37
 6 가 80 90% RNA 2 3µg (Gibco -
 BRL) 1 HIV gag - HSV gD - RNA 5 100µg
 RNA RNA가

/ BHK , HCMV SV40 A HSV2 gD
 DNA 24(
 5,851,804) 2 3µg

10% FBS DMEM 37 1, 2, 4 7
 , 250µl DMEM 0.1 (MOI) HSV2 1
 , DMEM(10% FBS) 2ml 가 4 7
 6 ,

36 48 ,
 가 [: Clinical Virology Manual, 2d edit., eds. S. Specter and G. Lancz, pp. 473 - 94(19
 92)]. , ds DNA APL - 400 - 024 , gD2
 ds RNA HSV2 HSV2 HSV2

B.

HIV gag RNA HSV gD , 1
 HSV - gD RNA HSV gD RNA HSV (challe
 nge) 가 .

Balb/c (5) 10 500µg RNA RNA
 . RNA 1, 2, 4 7 , HSV - 2(30µl 10⁵pfu)
 . HSV - 2 , 0 4 . 0
 가 , 1 , 2 , 3 ,
 , 4 .

, HIV gag , HSV gD RNA 가
 , HIV gag , RNA 가
 , RNA
 , dsRNA 가
 , dsRNA HSV gD
 , ss RNA 가
 ,

(57)

1.

- , 가 200
 RNA

2.

1 , RNA 11 가,
 - 9.2kcal/mol ΔG

3.

2 , RNA

4.

1 , RNA
 12 16 , RNA
 10%

5.

4 , 50%

6.

1 , 가 RNA

7.

1 , 가
 RNA

8.

1 , 가 RNA .

9.

1 , 가 RNA .

10.

1 , 가 RNA .

11.

10 , RNA 가 , .

12.

10 , RNA 가, 가 (fold back) 가 .

13.

1 , 가 RNA .

14.

13 , RNA 가 , .

15.

13 , RNA 가, 가 가 .

16.

1 , 가, RNA , RNA
가 .

17.

1 , 가 (rod) RNA .

18.

8 , 가 RNA DNA .

19.

- 18 , DNA가 RNA .
20.
- 18 , DNA가 RNA .
21.
- 20 , DNA가, , RN
A .
22.
- 20 , DNA가, 가 가 RNA .
23.
- 18 , DNA가 RNA .
24.
- 23 , DNA가, , RN
A .
25.
- 23 , DNA가, 가 가 RNA .
26.
- 18 , DNA가, 가 RNA .
27.
- 18 , DNA가, RNA .
28.
- 1 , 가 .
29.
- 1 , 가, RNA 2 DNA , 1 DNA RNA RNA
가 .
30.

- 28 . , 가 .
- 31.
- 1 . , 가 .
- 32.
- 1 . , 가 .
- 33.
- 1 . , 가 2 32 .
- 34.
- 30 32 , 가 , .
- 35.
- 1 . , .
- 36.
- 1 , RNA 가 .
- 37.
- 1 , (Kozak) .
- 38.
- 1 . , .
- 39.
- 1 , RNA (cap) 가 .
- 40.
- 1 . , .
- 41.
- 1 . , .
- 42.

1 , , /

43.

42 , 가 DNA , DNA

44.

43 , 가 (Retrovirus), (Herpesvirus), (H
epadenovirus), (Poxvirus), (Parvovirus), (Papillomavirus)
(Papovavirus)

45.

44 , 가 HIV, HBV, HSV, CMV, HPV, HTLV EBV

46.

1 , , -

47.

46 , HPV E6/E7 - , HTLV - EBV -

48.

1 , , /

49.

1 , , 가
- ,

50.

49 , 2

51.

50 , 가

52.

2, 1 51 ,

53.

52 , 2 가 , , (, ,), 가 ,
3 가 , , , , , , .

54.

53 , 2 가 .

55.

2 1
, , 가 /

56.

2 1
, 가 , 가 /

57.

2 1
, , 가 , ,

58.

1 2
1 , 가 / ,

59.

가
가
2
1

60.

가
1

61.

/ 가
2 1

62.

61

63.

61 가

64.

가
2 1

65.

,

가 / 1
 , 2 , .

66.

가 , 가
 가 ,
 1 2 , .

67.

,
 ,
 1 .

1A

T 7 F gag

R gag



T 7 전사

센스 gag RNA

1B

F gag**T 7 R gag****T 7 전사****안티센스 gag RNA**

<110> American Home Products Corporation
 <120> Methods and Compositions for Inhibiting the Function of
 Polynucleotide Sequences
 <130> AHP28APCT
 <150> US 60/130,377
 <151> 1999-04-21
 <160> 20
 <170> KopatentIn 1.71
 <210> 1
 <211> 47
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 forward
 gag primer
 <400> 1
 gtaatacgac tcactatagg gcggcagga gctagaacga ttcgcag
 <210> 2
 <211> 25
 <212> DNA
 <213> Artificial Sequence
 <220>

47

<223> Description of Artificial Sequence: reverse gag
 primer
 <400> 2
 ctgctatgtc acttcccctt ggttc 25
 <210> 3
 <211> 48
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 reverse
 gag primer
 <400> 3
 gtaatacgac tcactatagg gcgctgctat gtcacttccc cttggttc 48
 <210> 4
 <211> 24
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: forward gag
 primer
 <400> 4
 gcagggagct agaacgattc gcag 24
 <210> 5
 <211> 47
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 forward gD
 primer
 <400> 5
 gtaatacgac tcactatagg gcggtcgagg tgggactccg cgtcgtc 47
 <210> 6
 <211> 24
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: forward gD
 primer
 <400> 6
 gtcgagggtg gactccgagt cgtc 24
 <210> 7
 <211> 47
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 reverse gD
 primer
 <400> 7
 gtaatacgac tcactatagg gcggtgatct ccgtccagtc gtttacc 47
 <210> 8

<211> 24
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: reverse gD
 primer
 <400> 8
 gtgatctccg tccagtcggt tatc 24
 <210> 9
 <211> 25
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: forward IL-12
 genomic
 <400> 9
 tcagcaagca cttgccaaac tcctg 25
 <210> 10
 <211> 25
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: reverse IL-12
 genomic
 <400> 10
 gagacaaggt ctctggatgt tattg 25
 <210> 11
 <211> 46
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 forward
 IL-12 genomic
 <400> 11
 gtaatacgac tcactatagg gtcagcaagc acttgccaaa ctctg 46
 <210> 12
 <211> 46
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 reverse
 IL-12 genomic
 <400> 12
 gtaatacgac tcactatagg ggagacaagg tctctggatg ttattg 46
 <210> 13
 <211> 45
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: T7 forward

IL-12 primer

<400> 13
gtaatacgac tcactatagg gcctataagc ataagagacg ccctc 45
<210> 14
<211> 24
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: forward IL-12
promoter

<400> 14
cctataagca taagagacgc cctc 24
<210> 15
<211> 23
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: reverse IL-12
promoter

<400> 15
ggctgctcct ggtgcttata tac 23
<210> 16
<211> 44
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: T7 reverse
IL-12 promoter

<400> 16
gtaatacgac tcactatagg gggctgctcc tggctgcttat atac 44
<210> 17
<211> 44
<212> DNA
<213> Artificial Sequence
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<223> Description of Artificial Sequence: T7 forward
IL-12 cDNA

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<210> 18
<211> 23
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<213> Artificial Sequence
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<223> Description of Artificial Sequence: forward IL-12
cDNA

<400> 18
tgtgtcctca gaagctaacc atc 23
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<211> 24

<212> DNA
<213> Artificial Sequence
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<223> Description of Artificial Sequence: reverse IL-12
cDNA
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IL-12 cDNA
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