

(19) AUSTRALIAN PATENT OFFICE

(54) Title
HIV-gag codon-optimised DNA vaccines

(51)⁶ International Patent Classification(s)
C07K 14/16 (2006.01) 20060101AFI2006010
C07K 14/16 1BHAU
PCT/EP02/10592

(21) Application No: 2002362368 (22) Application Date: 2002 .09 .18

(87) WIPO No: W003/025003

(30) Priority Data

(31) Number	(32) Date	(33) Country
0129604.5	2001 .12 .11	GB
PCT/GB01/04207	2001 .09 .20	GB
0206462.4	2002 .03 .19	GB

(43) Publication Date : 2003 .04 .01

(43) Publication Journal Date : 2003 .06 .05

(71) Applicant(s)
Glaxo Group Limited

(72) Inventor(s)
Beaton, Andrew; Van Wely, Catherine Ann; Lear, Andrew; Gough, Gerald Wayne; Ertl, Peter Franz; Tite, John Philip

(74) Agent/Attorney
Phillips Ormonde & Fitzpatrick, Level 22 367 Collins Street, Melbourne, VIC, 3000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 March 2003 (27.03.2003)

PCT

(10) International Publication Number
WO 03/025003 A3

- (51) International Patent Classification⁷: C12N 15/48, 15/09, 15/49, A61P 31/18, C12N 15/869
- (21) International Application Number: PCT/EP02/10592
- (22) International Filing Date:
18 September 2002 (18.09.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PCT/GB01/04207
20 September 2001 (20.09.2001) GB
0129604.5 11 December 2001 (11.12.2001) GB
0206462.4 19 March 2002 (19.03.2002) GB
- (71) Applicant (for all designated States except US): **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **BEATON, Andrew** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **ERTL, Peter, Franz** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **GOUGH, Gerald, Wayne** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **LEAR, Andrew** [—/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **TITE, John, Philip** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **VAN WELY, Catherine, Ann** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).
- (74) Agent: **GIDDINGS, Peter, John**; GlaxoSmithKline, 980 Great West Road, Brentford, Middlesex TW8 9GS (GB).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GI, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
with international search report
- (88) Date of publication of the international search report:
4 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/025003 A3

(54) Title: HIV-GAG CODON-OPTIMISED DNA VACCINES

(57) Abstract: The invention provides a nucleotide sequence that encodes an HIV-1 gag protein or fragment thereof containing a gag epitope and a second HIV antigen or a fragment encoding an epitope of said second HIV antigen, operably linked to a heterologous promoter. Preferred polynucleotide sequences further encode nef or a fragment thereof and RT or a fragment thereof.

Vaccines

Field of the Invention

5 The present invention relates to nucleic acid constructs, host cells comprising such constructs and their use in nucleic acid vaccines. The invention further relates to vaccine formulations comprising such constructs and the use of such formulations in medicine. The invention in particular relates to DNA vaccines that are useful in the prophylaxis and treatment of HIV infections, more particularly when administered by particle mediated delivery.

15 Background to the Invention

HIV-1 is the primary cause of the acquired immune deficiency syndrome (AIDS) which is regarded as one of the world's major health problems. Although extensive research throughout the world has been conducted to produce a vaccine, such efforts thus far have not been successful.

Non-envelope proteins of HIV-1 have been described and include for example internal structure proteins such as the products of the *gag* and *pol* genes and, other non-structural proteins such as Rev, Nef, Vif and Tat (Green et al., New England J. Med, **324**, 5, 308 et seq (1991) and Bryant et al. (Ed. Pizzo), *Pediatr. Infect. Dis. J.*, **11**, 5, 390 et seq (1992)).

The *Gag* gene is translated from the full-length RNA to yield a precursor polyprotein which is subsequently cleaved into 3 - 5 capsid proteins; the matrix protein, capsid protein and nucleic acid binding protein and

protease. (1. Fundamental Virology, Fields BN, Knipe DM and Howley M 1996 2. Fields Virology vol 2 1996).

The gag gene gives rise to the 55-kilodalton (kD) Gag precursor protein, also called p55, which is expressed from the unspliced viral mRNA. During translation, the N terminus of p55 is myristoylated, triggering its association with the cytoplasmic aspect of cell membranes. The membrane-associated Gag polyprotein recruits two copies of the viral genomic RNA along with other viral and cellular proteins that triggers the budding of the viral particle from the surface of an infected cell. After budding, p55 is cleaved by the virally encoded protease (a product of the pol gene) during the process of viral maturation into four smaller proteins designated MA (matrix [p17]), CA (capsid [p24]), NC (nucleocapsid [p9]), and p6.(4)

In addition to the 3 major Gag protein, all Gag precursors contain several other regions, which are cleaved out and remain in the virion as peptides of various sizes. These proteins have different roles e.g. the p2 protein has a proposed role in regulating activity of the protease and contributes to the correct timing of proteolytic processing.

The MA polypeptide is derived from the N-terminal, myristoylated end of p55. Most MA molecules remain attached to the inner surface of the virion lipid bilayer, stabilizing the particle. A subset of MA is recruited inside the deeper layers of the virion where it becomes part of the complex which escorts the viral DNA to the nucleus.(5) These MA molecules facilitate the nuclear transport of the viral genome because a karyophilic signal on MA is recognized by the cellular

nuclear import machinery. This phenomenon allows HIV to infect nondividing cells, an unusual property for a retrovirus.

- 5 The p24 (CA) protein forms the conical core of viral particles. Cyclophilin A has been demonstrated to interact with the p24 region of p55 leading to its incorporation into HIV particles. The interaction between Gag and cyclophilin A is essential because the
10 disruption of this interaction by cyclosporine A inhibits viral replication.

- The NC region of Gag is responsible for specifically recognizing the so-called packaging signal of HIV. The
15 packaging signal consists of four stem loop structures located near the 5' end of the viral RNA, and is sufficient to mediate the incorporation of a heterologous RNA into HIV-1 virions. NC binds to the packaging signal through interactions mediated by two zinc-finger motifs.
20 NC also facilitates reverse transcription.

- The p6 polypeptide region mediates interactions between p55 Gag and the accessory protein Vpr, leading to the incorporation of Vpr into assembling virions. The p6
25 region also contains a so-called late domain which is required for the efficient release of budding virions from an infected cell

- The Pol gene encodes two proteins containing the two
30 activities needed by the virus in early infection, the RT and the integrase protein needed for integration of viral DNA into cell DNA. The primary product of Pol is cleaved by the virion protease to yield the amino terminal RT peptide which contains activities necessary for DNA
35 synthesis (RNA and DNA directed DNA polymerase, ribonuclease H) and carboxy terminal integrase protein.

HIV RT is a heterodimer of full-length RT (p66) and a cleavage product (p51) lacking the carboxy terminal Rnase integrase domain.

5 RT is one of the most highly conserved proteins encoded by the retroviral genome. Two major activities of RT are the DNA Pol and Ribonuclease H. The DNA Pol activity of RT uses RNA and DNA as templates interchangeably and like all DNA polymerases known is unable to initiate DNA
10 synthesis de novo, but requires a pre existing molecule to serve as a primer (RNA).

The Rnase H activity inherent in all RT proteins plays the essential role early in replication of removing the
15 RNA genome as DNA synthesis proceeds. It selectively degrades the RNA from all RNA - DNA hybrid molecules. Structurally the polymerase and ribo H occupy separate, non-overlapping domains with the Pol covering the amino two thirds of the Pol.

20 The p66 catalytic subunit is folded into 5 distinct subdomains. The amino terminal 23 of these have the portion with RT activity. Carboxy term to these is the Rnase H Domain.

25 After infection of the host cell, the retroviral RNA genome is copied into linear ds DNA by the reverse transcriptase that is present in the infecting particle. The integrase (reviewed in Skalka AM '99 Adv in Virus Res
30 52 271-273) recognises the ends of the viral DNA, trims them and accompanies the viral DNA to a host chromosomal site to catalyse integration. Many sites in the host DNA can be targets for integration. Although the integrase is sufficient to catalyse integration in vitro, it is not
35 the only protein associated with the viral DNA in vivo -

the large protein - viral DNA complex isolated from the infected cells has been denoted the pre integration complex. This facilitates the acquisition of the host cell genes by progeny viral genomes.

5

The integrase is made up of 3 distinct domains, the N terminal domain, the catalytic core and the c terminal domain. The catalytic core domain contains all of the requirements for the chemistry of polynucleotidyl

10 transfer.

The Nef protein is known to cause the removal of CD4, the HIV receptor, from the cell surface, but the biological importance of this function is debated. Additionally Nef
15 interacts with the signal pathway of T cells and induces an active state, which in turn may promote more efficient gene expression. Some HIV isolates have mutations in this region, which cause them not to encode functional protein and are severely compromised in their replication
20 and pathogenesis in vivo.

DNA vaccines usually consist of a bacterial plasmid vector into which is inserted a strong promoter, the gene of interest which encodes for an antigenic peptide and a
25 polyadenylation/transcriptional termination sequences. The gene of interest may encode a full protein or simply an antigenic peptide sequence relating to the pathogen, tumour or other agent which is intended to be protected against. The plasmid can be grown in bacteria, such as
30 for example E.coli and then isolated and prepared in an appropriate medium, depending upon the intended route of administration, before being administered to the host. Following administration the plasmid is taken up by cells of the host where the encoded peptide is produced. The
35 plasmid vector will preferably be made without an origin

2002362368 16 Aug 2006

of replication which is functional in eukaryotic cells, in order to prevent plasmid replication in the mammalian host and integration within chromosomal DNA of the animal concerned.

5

There are a number of advantages of DNA vaccination relative to traditional vaccination techniques. First, it is predicted that because of the proteins which are encoded by the DNA sequence are synthesised in the host, the structure or conformation of the protein will be similar to the native protein associated with the disease state. It is also likely that DNA vaccination will offer protection against different strains of a virus, by generating cytotoxic T lymphocyte response that recognise epitopes from

10

15

conserved proteins. Furthermore, because the plasmids are taken up by the host cells where antigenic protein can be produced, a long-lasting immune response will be elicited.

The technology also offers the possibility of combining diverse immunogens into a single preparation to facilitate

20

simultaneous immunisation in relation to a number of disease states.

Helpful background information in relation to DNA vaccination is provided in Donnelly et al "DNA vaccines"

25

Ann. Rev Immunol. 1997 15: 617-648, the disclosure of which is included herein in its entirety by way of reference.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was, in Australia, known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

30

Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

5

Summary of the Invention

The present invention provides novel constructs for use in nucleic acid vaccines for the prophylaxis and treatment of HIV infections and AIDS.

10

Accordingly, in one aspect, there is provided a nucleic acid molecule comprising a nucleotide sequence encoding HIV gag protein or fragment thereof linked to a nucleotide sequence encoding a further HIV antigen or fragment thereof and operably linked to a heterologous promoter. The fragment of said nucleotide sequence will encode an HIV epitope and typically encode a peptide of at least 8 amino acids.

15

20

In another aspect, the present invention provides a nucleotide sequence that encodes an HIV-1 gag protein or fragment containing a gag epitope thereof, an HIV-1 Nef protein or a fragment thereof containing a nef epitope, and an RT protein or a fragment containing an RT epitope, operably linked to a heterologous promoter.

25

The nucleotide sequence is preferably a DNA sequence and is preferably contained within a plasmid without an origin of replication. Such nucleic acid molecules are formulated with pharmaceutically acceptable excipient, carriers, diluents or adjuvants to produce pharmaceutical composition suitable for the treatment and/or prophylaxis of HIV infection and AIDS.

30

2002362368 16 Aug 2006

5 In a preferred embodiment the DNA sequence is formulated onto the surface of inert particles or beads suitable for particle mediated drug delivery. Preferably the beads are gold.

10 In a preferred embodiment of the invention there is provided a DNA sequence that highly expressed codes for gag protein which sequence is optimised to resemble the codon usage of genes in mammalian cells. In particular, the gag protein is optimised to resemble that of highly expressed human genes.

15 The DNA code has 4 letters (A, T, C and G) and uses these to spell three letter "codons" which represent the amino acids the proteins encoded in an organism's genes. The linear sequence of codons along the DNA molecule is translated into the linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly
20 degenerate, with 61 codons coding for the 20 natural

amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons.

5

Where more than one codon is available to code for a given amino acid, it has been observed that the codon usage patterns of organisms are highly non-random.

Different species show a different bias in their codon

- 10 selection and, furthermore, utilisation of codons may be markedly different in a single species between genes which are expressed at high and low levels. This bias is different in viruses, plants, bacteria and mammalian cells, and some species show a stronger bias away from a
- 15 random codon selection than others. For example, humans and other mammals are less strongly biased than certain bacteria or viruses. For these reasons, there is a significant probability that a mammalian gene expressed in E.coli or a foreign or recombinant gene expressed in
- 20 mammalian cells will have an inappropriate distribution of codons for efficient expression. It is believed that the presence in a heterologous DNA sequence of clusters of codons or an abundance of codons which are rarely observed in the host in which expression is to occur, is
- 25 predictive of low heterologous expression levels in that host.

In an embodiment of the present invention provides a gag polynucleotide sequence which encodes an amino acid

- 30 sequence, wherein the codon usage pattern of the polynucleotide sequence resembles that of highly expressed mammalian genes. Preferably the polynucleotide sequence is a DNA sequence. Desirably the codon usage pattern of the polynucleotide sequence is typical of
- 35 highly expressed human genes.

In the polynucleotides of the present invention, the codon usage pattern is altered from that typical of human immunodeficiency viruses to more closely represent the

5 codon bias of the target organism, e.g. a mammal, especially a human. The "codon usage coefficient" is a measure of how closely the codon pattern of a given polynucleotide sequence resembles that of a target species. Codon frequencies can be derived from literature

10 sources for the highly expressed genes of many species (see e.g. Nakamura et.al. Nucleic Acids Research 1996, 24:214-215). The codon frequencies for each of the 61 codons (expressed as the number of occurrences occurrence per 1000 codons of the selected class of genes) are

15 normalised for each of the twenty natural amino acids, so that the value for the most frequently used codon for each amino acid is set to 1 and the frequencies for the less common codons are scaled to lie between zero and 1. Thus each of the 61 codons is assigned a value of 1 or

20 lower for the highly expressed genes of the target species. In order to calculate a codon usage coefficient for a specific polynucleotide, relative to the highly expressed genes of that species, the scaled value for each codon of the specific polynucleotide are noted and

25 the geometric mean of all these values is taken (by dividing the sum of the natural logs of these values by the total number of codons and take the anti-log). The coefficient will have a value between zero and 1 and the higher the coefficient the more codons in the

30 polynucleotide are frequently used codons. If a polynucleotide sequence has a codon usage coefficient of 1, all of the codons are "most frequent" codons for highly expressed genes of the target species.

According to the present invention, the codon usage pattern of the polynucleotide will preferably exclude codons with an RSCU value of less than 0.2 in highly expressed genes of the target organism. Alternatively,

5 the codon usage pattern will exclude codons representing <10% of the codons used for a particular amino acid. A relative synonymous codon usage (RSCU) value is the observed number of codons divided by the number expected if all codons for that amino acid were used equally

10 frequently. A polynucleotide of the present invention will generally have a codon usage coefficient (or RSCU) for highly expressed human genes of greater than 0.3, preferably greater than 0.4, most preferably greater than 0.5 Codon usage tables for human can also be found in

15 Genebank.

In comparison, a highly expressed beta actin gene has a RSCU of 0.747. The codon usage table for a homo sapiens is set out below:

20

Codon Usage Table 1:

Homo sapiens [gbpri]: 27143 CDS's (12816923 codons)

25 fields: [triplet] [frequency: per thousand] ([number])

UUU 17.0(217684)	UCU 14.8(189419)	UAU 12.1(155645)	UGU 10.0(127719)
UUC 20.5(262753)	UCC 17.5(224470)	UAC 15.8(202481)	UGC 12.3(157257)
UUA 7.3(93924)	UCA 11.9(152074)	UAA 0.7(9195)	UGA 1.3(16025)
30 UUG 12.5(159611)	UCG 4.5(57572)	UAG 0.5(6789)	UGG 12.9(165930)
CUU 12.8(163707)	CCU 17.3(222146)	CAU 10.5(134186)	CGU 4.6(59454)
CUC 19.3(247391)	CCC 20.0(256235)	CAC 14.9(190928)	CGC 10.8(137865)
CUA 7.0(89078)	CCA 16.7(214583)	CAA 12.0(153590)	CGA 6.3(80709)
35 CUG 39.7(509096)	CCG 7.0(89619)	CAG 34.5(441727)	CGG 11.6(148666)
AUU 15.8(202844)	ACU 12.9(165392)	AUA 17.0(218508)	AGU 12.0(154442)
AUC 21.6(277066)	ACC 19.3(247805)	AAC 19.8(253475)	AGC 19.3(247583)
AUA 7.2(92133)	ACA 14.9(191518)	AAA 24.0(308123)	AGA 11.5(147264)
40 AUG 22.3(285776)	ACG 6.3(80369)	AAG 32.6(418141)	AGG 11.3(145276)

- 10 -

GUU 10.9(139611) GCU 18.5(236639) GAU 22.4(286742) GGU 10.8(138606)
 GUC 14.6(187333) GCC 28.3(362086) GAC 26.1(334158) GGC 22.7(290904)
 GUA 7.0(89644) GCA 15.9(203310) GAA 29.1(373151) GGA 16.4(210643)
 5 GUG 28.8(369006) GCG 7.5(96455) GAG 40.2(515485) GGG 16.4(209907)

Coding GC 52.51% 1st letter GC 56.04% 2nd letter GC
 42.35% 3rd letter GC 59.13%

10 Codon Usage Table 2 (preferred):

Codon usage for human (highly expressed) genes 1/24/91
 (human_high.cod)

	AmAcid	Codon	Number	/1000	Fraction	..
15	Gly	GGG	905.00	18.76	0.24	
	Gly	GGA	525.00	10.88	0.14	
	Gly	GGT	441.00	9.14	0.12	
	Gly	GGC	1867.00	38.70	0.50	
20	Glu	GAG	2420.00	50.16	0.75	
	Glu	GAA	792.00	16.42	0.25	
	Asp	GAT	592.00	12.27	0.25	
	Asp	GAC	1821.00	37.75	0.75	
25	Val	GTG	1866.00	38.68	0.64	
	Val	GTA	134.00	2.78	0.05	
	Val	GTT	198.00	4.10	0.07	
	Val	GTC	728.00	15.09	0.25	
30	Ala	GCG	652.00	13.51	0.17	
	Ala	GCA	488.00	10.12	0.13	
	Ala	GCT	654.00	13.56	0.17	
	Ala	GCC	2057.00	42.64	0.53	
35	Arg	AGG	512.00	10.61	0.18	
	Arg	AGA	298.00	6.18	0.10	
	Ser	AGT	354.00	7.34	0.10	
	Ser	AGC	1171.00	24.27	0.34	
40	Lys	AAG	2117.00	43.88	0.82	
	Lys	AAA	471.00	9.76	0.18	
	Asn	AAT	314.00	6.51	0.22	
	Asn	AAC	1120.00	23.22	0.78	
45	Met	ATG	1077.00	22.32	1.00	
	Ile	ATA	88.00	1.82	0.05	
	Ile	ATT	315.00	6.53	0.18	
	Ile	ATC	1369.00	28.38	0.77	
50	Thr	ACG	405.00	8.40	0.15	

- 11 -

	Thr	ACA	373.00	7.73	0.14
	Thr	ACT	358.00	7.42	0.14
	Thr	ACC	1502.00	31.13	0.57
5	Trp	TGG	652.00	13.51	1.00
	End	TGA	109.00	2.26	0.55
	Cys	TGT	325.00	6.74	0.32
	Cys	TGC	706.00	14.63	0.68
10	End	TAG	42.00	0.87	0.21
	End	TAA	46.00	0.95	0.23
	Tyr	TAT	360.00	7.46	0.26
	Tyr	TAC	1042.00	21.60	0.74
15	Leu	TTG	313.00	6.49	0.06
	Leu	TTA	76.00	1.58	0.02
	Phe	TTT	336.00	6.96	0.20
	Phe	TTC	1377.00	28.54	0.80
20	Ser	TCG	325.00	6.74	0.09
	Ser	TCA	165.00	3.42	0.05
	Ser	TCT	450.00	9.33	0.13
	Ser	TCC	958.00	19.86	0.28
25	Arg	CGG	611.00	12.67	0.21
	Arg	CGA	183.00	3.79	0.06
	Arg	CGT	210.00	4.35	0.07
	Arg	CGC	1086.00	22.51	0.37
30	Gln	CAG	2020.00	41.87	0.88
	Gln	CAA	283.00	5.87	0.12
	His	CAT	234.00	4.85	0.21
	His	CAC	870.00	18.03	0.79
35	Leu	CTG	2884.00	59.78	0.58
	Leu	CTA	166.00	3.44	0.03
	Leu	CTT	238.00	4.93	0.05
	Leu	CTC	1276.00	26.45	0.26
40	Pro	CCG	482.00	9.99	0.17
	Pro	CCA	456.00	9.45	0.16
	Pro	CCT	568.00	11.77	0.19
	Pro	CCC	1410.00	29.23	0.48

- 45 According to a further aspect of the invention, an expression vector is provided which comprises and is capable of directing the expression of a polynucleotide sequence according to the first aspect of the invention, in particular the codon usage pattern of the gag

polynucleotide sequence is typical of highly expressed mammalian genes, preferably highly expressed human genes. The vector may be suitable for driving expression of heterologous DNA in bacterial insect or mammalian cells,
5 particularly human cells. In one embodiment, the expression vector is p7313 (see figure 1).

In a third embodiment there is provided a gag gene under the control of a heterologous promoter fused to a DNA
10 sequence encoding NEF, a fragment thereof, or HIV Reverse Transcriptase (RT) or fragment thereof. The gag portion of the gene may be either the N or C terminal portion of the fusion.

15 In a preferred embodiment, the gag gene does not encode the gag p6 peptide. Preferably the NEF gene is truncated to remove the sequence encoding the N terminal region i.e. removal of 30-85, preferably 60-85, typically about 81, preferably the N terminal 65 amino acids.

20 In a further embodiment the RT gene is also optimised to resemble a highly expressed human gene. The RT preferably encodes a mutation to substantially inactivate any reverse transcriptase activity. A preferred
25 inactivation mutation involves the substitution of W tryptophan 229 for K (lysine).

According to a further aspect of the invention, a host cell comprising a polynucleotide sequence according to
30 the invention, or an expression vector according to the invention is provided. The host cell may be bacterial, e.g. E.coli, mammalian, e.g. human, or may be an insect cell. Mammalian cells comprising a vector according to the present invention may be cultured cells transfected

in vitro or may be transfected in vivo by administration of the vector to the mammal.

The present invention further provides a pharmaceutical composition comprising a polynucleotide sequence according to the invention. Preferably the composition comprises a DNA vector. In preferred embodiments the composition comprises a plurality of particles, preferably gold particles, coated with DNA comprising a vector encoding a polynucleotide sequence of the invention. Preferably the sequence encodes an HIV gag amino acid sequence, wherein the codon usage pattern of the polynucleotide sequence is typical of highly expressed mammalian genes, particularly human genes. In alternative embodiments, the composition comprises a pharmaceutically acceptable excipient and a DNA vector according to the second aspect of the present invention. The composition may also include an adjuvant.

Thus it is an embodiment of the invention that the vectors of the invention be utilised with immunostimulatory agents. Preferably the immunostimulatory agent are administered at the same time as the nucleic acid vector of the invention and in preferred embodiments are formulated together. Such immunostimulatory agents include, but this list is by no means exhaustive and does not preclude other agents: synthetic imidazoquinolines such as imiquimod [S-26308, R-837], (Harrison, et al. 'Reduction of recurrent HSV disease using imiquimod alone or combined with a glycoprotein vaccine', Vaccine 19: 1820-1826, (2001)); and resiquimod [S-28463, R-848] (Vasilakos, et al. 'Adjuvant activities of immune response modifier R-848: Comparison with CpG ODN', Cellular immunology 204: 64-74 (2000).), Schiff bases of carbonyls and amines that are

constitutively expressed on antigen presenting cell and T-cell surfaces, such as tucaresol (Rhodes, J. et al. 'Therapeutic potentiation of the immune system by costimulatory Schiff-base-forming drugs', Nature 377: 71-75 (1995)), cytokine, chemokine and co-stimulatory molecules as either protein or peptide, this would include pro-inflammatory cytokines such as GM-CSF, IL-1 alpha, IL-1 beta, TGF- alpha and TGF - beta, Th1 inducers such as interferon gamma, IL-2, IL-12, IL-15 and IL-18, Th2 inducers such as IL-4, IL-5, IL-6, IL-10 and IL-13 and other chemokine and co-stimulatory genes such as MCP-1, MIP-1 alpha, MIP-1 beta, RANTES, TCA-3, CD80, CD86 and CD40L, , other immunostimulatory targeting ligands such as CTLA-4 and L-selectin, apoptosis stimulating proteins and peptides such as Fas, (49), synthetic lipid based adjuvants, such as vaxfectin, (Reyes et al., 'Vaxfectin enhances antigen specific antibody titres and maintains Th1 type immune responses to plasmid DNA immunization', Vaccine 19: 3778-3786) squalene, alpha- tocopherol, polysorbate 80, DOPC and cholesterol, endotoxin, [LPS], Beutler, B., 'Endotoxin, 'Toll-like receptor 4, and the afferent limb of innate immunity', Current Opinion in Microbiology 3: 23-30 (2000)) ; CpG oligo- and di-nucleotides, Sato, Y. et al., 'Immunostimulatory DNA sequences necessary for effective intradermal gene immunization', Science 273 (5273): 352-354 (1996). Hemmi, H. et al., 'A Toll-like receptor recognizes bacterial DNA', Nature 408: 740-745, (2000) and other potential ligands that trigger Toll receptors to produce Th1-inducing cytokines, such as synthetic Mycobacterial lipoproteins, Mycobacterial protein p19, peptidoglycan, teichoic acid and lipid A.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a Lipid A

derivative such as monophosphoryl lipid A, or preferably 3-de-O-acylated monophosphoryl lipid A. MPL[®] adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 5 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent 10 Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila 15 Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins.

Also provided are the use of a polynucleotide according to the invention, or of a vector according to the 20 invention, in the treatment or prophylaxis of an HIV infection.

The present invention also provides methods of treating or preventing HIV infections, any symptoms or diseases 25 associated therewith, comprising administering an effective amount of a polynucleotide, a vector or a pharmaceutical composition according to the invention. Administration of a pharmaceutical composition may take the form of one or more individual doses, for example as 30 repeat doses of the same DNA plasmid, or in a "prime-boost" therapeutic vaccination regime. In certain cases the "prime" vaccination may be via particle mediated DNA delivery of a polynucleotide according to the present invention, preferably incorporated into a plasmid-derived 35 vector and the "boost" by administration of a recombinant

viral vector comprising the same polynucleotide sequence,
or boosting with the protein in adjuvant. Conversely the
priming may be with the viral vector or with a protein
formulation typically a protein formulated in adjuvant
5 and the boost a DNA vaccine of the present invention.
Multiple doses of prime and/or boost may be employed.

In embodiments of the invention fragments of gag, nef or
RT proteins are contemplated. For example, a
10 polynucleotide of the invention may encode a fragment of
an HIV gag, nef or RT protein. A polynucleotide which
encodes a fragment of at least 8, for example 8-10 amino
acids or up to 20, 50, 60, 70, 80, 100, 150 or 200 amino
acids in length is considered to fall within the scope of
15 the invention as long as the encoded oligo or polypeptide
demonstrates HIV antigenicity. In particular, but not
exclusively, this aspect of the invention encompasses the
situation when the polynucleotide encodes a fragment of a
complete HIV protein sequence and may represent one or
20 more discrete epitopes of that protein. Such fragments
may be codon optimised such that the fragment has a codon
usage pattern which resembles that of a highly expressed
mammalian gene.

25 Preferred constructs according to the present invention
include:

1. p17, p24, fused to truncated NEF (devoid of
nucleotides encoding terminal amino-acids 1-65)
30
2. p17, p24, RT, truncated NEF (devoid of nucleotides
encoding terminal amino-acids 1-65)
3. p17, p24 (optimised gag) truncated NEF (devoid of
35 nucleotides encoding terminal amino-acids 1-65)

4. p17, p24 (optimised gag) RT (optimised) truncated
NEF (devoid of nucleotides encoding terminal amino-
acids 1-85)
- 5
5. p17, p24, RT (optimised) truncated NEF (devoid of
nucleotides encoding terminal amino-acids 1-65)
6. Truncated NEF - (devoid of nucleotide 1-65) fused to
10 optimised p17, p24 gag.
7. Particularly preferred constructs of the invention
include triple fusions RT-NEF-Gag, and RT-Gag-Nef
particularly:
- 15
8. Optimised RT, truncated NEF and optimised P17, p24
(gag) (RNG)
and
9. Optimised RT, optimised p17, 24 (gag), Nef truncate
20 (devoid of aa 1-65)RGN

It is preferred that the HIV constructs are derived from
an HIV Clade B or Clade C, particularly clade B.

- 25 As discussed above, the present invention includes
expression vectors that comprise the nucleotide sequences
of the invention. Such expression vectors are routinely
constructed in the art of molecular biology and may for
example involve the use of plasmid DNA and appropriate
30 initiators, promoters, enhancers and other elements, such
as for example polyadenylation signals which may be
necessary, and which are positioned in the correct
orientation, in order to allow for protein expression.
Other suitable vectors would be apparent to persons
35 skilled in the art. By way of further example in this

regard we refer to Sambrook et al. Molecular Cloning: a Laboratory Manual. 2nd Edition. CSH Laboratory Press. (1989).

- 5 Preferably, a polynucleotide of the invention, or for use in the invention in a vector, is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably
10 linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in such a way that
15 expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

- The vectors may be, for example, plasmids, artificial chromosomes (e.g. BAC, PAC, YAC), virus or phage vectors
20 provided with a origin of replication, optionally a promoter for the expression of the polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin or kanamycin resistance gene in the case of
25 a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell e.g. for the production of protein encoded by the vector.
30 The vectors may also be adapted to be used *in vivo*, for example in a method of DNA vaccination or of gene therapy.

- Promoters and other expression regulation signals may be
35 selected to be compatible with the host cell for which

expression is designed. For example, mammalian promoters include the metallothionein promoter, which can be induced in response to heavy metals such as cadmium, and the β -actin promoter. Viral promoters such as the SV40 large T antigen promoter, human cytomegalovirus (CMV) immediate early (IE) promoter, rous sarcoma virus LTR promoter, adenovirus promoter, or a HPV promoter, particularly the HPV upstream regulatory region (URR) may also be used. All these promoters are well described and readily available in the art.

A preferred promoter element is the CMV immediate early promoter, devoid of intron A but including exon 1. The promoter element may be the minimal promoter element or the enhanced promoter, the enhanced promoter being preferred. Accordingly there is provided a vector comprising a polynucleotide of the invention under the control of HCMV IE early promoter.

Examples of suitable viral vectors include herpes simplex viral vectors, vaccinia or alpha-virus vectors and retroviruses, including lentiviruses, adenoviruses and adeno-associated viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide of the invention into the host genome, although such recombination is not preferred. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression. Vectors capable of driving expression in insect cells (for example baculovirus vectors), in human cells, in yeast or in bacteria may be employed in order to produce quantities of the HIV protein encoded by the polynucleotides of the present invention, for example for use as subunit vaccines or in immunoassays.

The polynucleotides according to the invention have utility in the production by expression of the encoded proteins, which expression may take place *in vitro*, *in vivo* or *ex vivo*. The nucleotides may therefore be involved in recombinant protein synthesis, for example to increase yields, or indeed may find use as therapeutic agents in their own right, utilised in DNA vaccination techniques. Where the polynucleotides of the present invention are used in the production of the encoded proteins *in vitro* or *ex vivo*, cells, for example in cell culture, will be modified to include the polynucleotide to be expressed. Such cells include transient, or preferably stable mammalian cell lines. Particular examples of cells which may be modified by insertion of vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa, 293 and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of a polypeptide. Expression may be achieved in transformed oocytes. A polypeptide may be expressed from a polynucleotide of the present invention, in cells of a transgenic non-human animal, preferably a mouse. A transgenic non-human animal expressing a polypeptide from a polynucleotide of the invention is included within the scope of the invention.

The invention further provides a method of vaccinating a mammalian subject which comprises administering thereto an effective amount of such a vaccine or vaccine composition. Most preferably, expression vectors for use in DNA vaccines, vaccine compositions and immunotherapeutics will be plasmid vectors.

35

- DNA vaccines may be administered in the form of "naked DNA", for example in a liquid formulation administered using a syringe or high pressure jet, or DNA formulated with liposomes or an irritant transfection enhancer, or
- 5 by particle mediated DNA delivery (PMDD). All of these delivery systems are well known in the art. The vector may be introduced to a mammal for example by means of a viral vector delivery system.
- 10 The compositions of the present invention can be delivered by a number of routes such as intramuscularly, subcutaneously, intraperitoneally, intravenously or mucosally.
- 15 In a preferred embodiment, the composition is delivered intradermally. In particular, the composition is delivered by means of a gene gun particularly particle bombardment administration techniques which involve coating the vector on to a bead (eg gold) which are then
- 20 administered under high pressure into the epidermis; such as, for example, as described in Haynes et al, J Biotechnology 44: 37-42 (1996).
- In one illustrative example, gas-driven particle
- 25 acceleration can be achieved with devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent
- 30 No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide, are accelerated to high speed within a helium gas jet generated by a hand held device, propelling the particles
- 35 into a target tissue of interest, typically the skin.

The particles are preferably gold beads of a 0.4 - 4.0 μm , more preferably 0.6 - 2.0 μm diameter and the DNA conjugate coated onto these and then encased in a cartridge or cassette for placing into the "gene gun".

5

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, OR), some examples

10 of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

The vectors which comprise the nucleotide sequences

15 encoding antigenic peptides are administered in such amount as will be prophylactically or therapeutically effective. The quantity to be administered, is generally in the range of one picogram to 1 milligram, preferably 1 picogram to 10 micrograms for particle-mediated delivery, 20 and 100 nanograms to 1 milligram, preferably 10 micrograms to 1 milligram, for other routes, of nucleotide per dose. The exact quantity may vary considerably depending on the weight of the patient being immunised and the route of administration,

25

It is possible for the immunogen component comprising the nucleotide sequence encoding the antigenic peptide, to be administered on a once off basis or to be administered repeatedly, for example, between 1 and 7 times,

30 preferably between 1 and 4 times, at intervals between about 1 day and about 18 months. However, this treatment regime will be significantly varied depending upon the size the patient concerned, the amount of nucleotide sequence administered, the route of administration, and 35 other factors which would be apparent to a skilled

veterinary or medical practitioner. The patient may receive one or more other anti HIV retroviral drugs as part of their overall treatment regime. Additionally the nucleic acid immunogen may be administered with an
5 adjuvant.

The adjuvant component specified herein can similarly be administered via a variety of different administration routes, such as for example, via the oral, nasal,
10 pulmonary, intramuscular, subcutaneous, intradermal or topical routes. Preferably, the adjuvant component is administered via the intradermal or topical routes. Most preferably by the topical route. This administration may take place between about 14 days prior to and about 14
15 days post administration of the nucleotide sequence, preferably between about 1 day prior to and about 3 days post administration of the nucleotide sequence. The adjuvant component is, in an embodiment, administered substantially simultaneously with the administration of
20 the nucleotide sequence. By "substantially simultaneous" what is meant is that administration of the adjuvant component is preferably at the same time as administration of the nucleotide sequence, or if not, at least within a few hours either side of nucleotide
25 sequence administration. In the most preferred treatment protocol, the adjuvant component will be administered substantially simultaneously to administration of the nucleotide sequence. Obviously, this protocol can be varied as necessary, in accordance with the type of
30 variables referred to above. It is preferred that the adjuvant is a 1H - imidazo [4,5c] quinoline - 4 - amine derivative such as imiquimod. Typically imiquimod will be presented as a topical cream formulation and will be administered according to the above protocol.

35

Once again, depending upon such variables, the dose of administration of the derivative will also vary, but may, for example, range between about 0.1mg per kg to about 100mg per kg, where "per kg" refers to the body weight of the mammal concerned. This administration of the, 1H-imidazo[4,5-c]quinolin-4-amine derivative would preferably be repeated with each subsequent or booster administration of the nucleotide sequence. Most preferably, the administration dose will be between about 1mg per kg to about 50mg per kg. In the case of a "prime-boost" scheme as described herein, the imiquimod or other 1H-imidazo[4,5-c]quinolin-4-amine derivative may be administered with either the prime or the boost or with both the prime and the boost.

While it is possible for the adjuvant component to comprise only 1H-imidazo[4,5-c]quinolin-4-amine derivatives to be administered in the raw chemical state, it is preferable for administration to be in the form of a pharmaceutical formulation. That is, the adjuvant component will preferably comprise the 1H-imidazo[4,5-c]quinolin-4-amine combined with one or more pharmaceutically acceptable carriers, and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with other ingredients within the formulation, and not deleterious to the recipient thereof. The nature of the formulations will naturally vary according to the intended administration route, and may be prepared by methods well known in the pharmaceutical art. All methods include the step of bringing into association a 1H-imidazo[4,5-c]quinolin-4-amine derivative with an appropriate carrier or carriers. In general, the formulations are prepared by uniformly and intimately bringing into association the derivative with liquid carriers or finely divided solid

carriers, or both, and then, if necessary, shaping the product into the desired formulation. Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, 5 cachets or tablets each containing a pre-determined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion. The active ingredient may 10 also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a 15 suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the 20 powdered compound moistened with an inert liquid diluent.

The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient.

25 Formulations for injection via, for example, the intramuscular, intraperitoneal, or subcutaneous administration routes include aqueous and non-aqueous sterile injection solutions which may contain 30 antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in 35 unit-dose or multi-dose containers, for example, sealed

ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous

5 injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Formulations suitable for pulmonary administration via the buccal or nasal cavity are presented such that particles containing the active

10 ingredient, desirably having a diameter in the range of 0.5 to 7 microns, are delivered into the bronchial tree of the recipient. Possibilities for such formulations are that they are in the form of finely comminuted powders which may conveniently be presented either in a

15 piercable capsule, suitably of, for example, gelatine, for use in an inhalation device, or alternatively, as a self-propelling formulation comprising active ingredient, a suitable liquid propellant and optionally, other ingredients such as surfactant and/or a solid diluent.

20 Self-propelling formulations may also be employed wherein the active ingredient is dispensed in the form of droplets of a solution or suspension. Such self-propelling formulations are analogous to those known in the art and may be prepared by established procedures.

25 They are suitably provided with either a manually-operable or automatically functioning valve having the desired spray characteristics; advantageously the valve is of a metered type delivering a fixed volume, for example, 50 to 100 μ L, upon each operation thereof.

30

In a further possibility, the adjuvant component may be in the form of a solution for use in an atomiser or nebuliser whereby an accelerated airstream or ultrasonic agitation is employed to produce a fine droplet mist for

35 inhalation.

Formulations suitable for intranasal administration generally include presentations similar to those described above for pulmonary administration, although it is preferred for such formulations to have a particle diameter in the range of about 10 to about 200 microns, to enable retention within the nasal cavity. This may be achieved by, as appropriate, use of a powder of a suitable particle size, or choice of an appropriate valve. Other suitable formulations include coarse powders having a particle diameter in the range of about 20 to about 500 microns, for administration by rapid inhalation through the nasal passage from a container held close up to the nose, and nasal drops comprising about 0.2 to 5% w/w of the active ingredient in aqueous or oily solutions. In one embodiment of the invention, it is possible for the vector which comprises the nucleotide sequence encoding the antigenic peptide to be administered within the same formulation as the 1H-imidazo[4,5-c]quinolin-4-amine derivative. Hence in this embodiment, the immunogenic and the adjuvant component are found within the same formulation.

In an embodiment the adjuvant component is prepared in a form suitable for gene-gun administration, and is administered via that route substantially simultaneous to administration of the nucleotide sequence. For preparation of formulations suitable for use in this manner, it may be necessary for the 1H-imidazo[4,5-c]quinolin-4-amine derivative to be lyophilised and adhered onto, for example, gold beads which are suited for gene-gun administration.

In an alternative embodiment, the adjuvant component may be administered as a dry powder, via high pressure gas propulsion.

- 5 Even if not formulated together, it may be appropriate for the adjuvant component to be administered at or about the same administration site as the nucleotide sequence.

- 10 Other details of pharmaceutical preparations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania (1985), the disclosure of which is included herein in its entirety, by way of reference.

- 15 Suitable techniques for introducing the naked polynucleotide or vector into a patient also include topical application with an appropriate vehicle. The nucleic acid may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, 20 intravaginal or intrarectal administration. The naked polynucleotide or vector may be present together with a pharmaceutically acceptable excipient, such as phosphate buffered saline (PBS). DNA uptake may be further facilitated by use of facilitating agents such as 25 bupivacaine, either separately or included in the DNA formulation. Other methods of administering the nucleic acid directly to a recipient include ultrasound, electrical stimulation, electroporation and microseeding which is described in US-5,697,901.

- 30 Uptake of nucleic acid constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, 35 calcium phosphate and DEAE-Dextran and lipofectants, for

example, lipofectam and transfectam. The dosage of the nucleic acid to be administered can be altered.

- A nucleic acid sequence of the present invention may also
- 5 be administered by means of specialised delivery vectors useful in gene therapy. Gene therapy approaches are discussed for example by Verme *et al*, Nature 1997, 389:239-242. Both viral and non-viral vector systems can be used. Viral based systems include retroviral,
- 10 lentiviral, adenoviral, adeno-associated viral, herpes viral, Canarypox and vaccinia-viral based systems. Non-viral based systems include direct administration of nucleic acids, microsphere encapsulation technology (poly(lactide-co-glycolide) and, liposome-based systems.
- 15 Viral and non-viral delivery systems may be combined where it is desirable to provide booster injections after an initial vaccination, for example an initial "prime" DNA vaccination using a non-viral vector such as a plasmid followed by one or more "boost" vaccinations
- 20 using a viral vector or non-viral based system. Similarly the invention contemplates prime boost systems with the polynucleotide of the invention, followed by boosting with protein in adjuvant or vice versa.
- 25 A nucleic acid sequence of the present invention may also be administered by means of transformed cells. Such cells include cells harvested from a subject. The naked polynucleotide or vector of the present invention can be introduced into such cells *in vitro* and the transformed
- 30 cells can later be returned to the subject. The polynucleotide of the invention may integrate into nucleic acid already present in a cell by homologous recombination events. A transformed cell may, if desired, be grown up *in vitro* and one or more of the
- 35 resultant cells may be used in the present invention.

Cells can be provided at an appropriate site in a patient by known surgical or microsurgical techniques (e.g. grafting, micro-injection, etc.)

- 5 The pharmaceutical compositions of the present invention may include adjuvant compounds, as detailed above, or other substances which may serve to increase the immune response induced by the protein which is encoded by the DNA. These may be encoded by the DNA, either separately
10 from or as a fusion with the antigen, or may be included as non-DNA elements of the formulation. Examples of adjuvant-type substances which may be included in the formulations of the present invention include ubiquitin, lysosomal associated membrane protein (LAMP), hepatitis B
15 virus core antigen, FLT3-ligand (a cytokine important in the generation of professional antigen presenting cells, particularly dendritic cells) and other cytokines such as IFN- γ and GM-CSF. Other preferred adjuvants include Imiquimod and Resiquimod and Tucarasol. Imiquimod being
20 particularly preferred.

- The present invention in a preferred embodiment of the invention provides the use of a nucleic acid molecule as herein described for the treatment or prophylaxis of HIV
25 infection. The nucleic acid molecule is preferably administered with Imiquimod. The Imiquimod is preferably administered topically, whereas the nucleic acid molecule is preferably administered by means of the particle mediated delivery.
30 Accordingly the present invention provides a method of treating a subject suffering from or susceptible to HIV infection, comprising administering a nucleic acid molecule as herein described and Imiquimod.

35

The present invention will now be described by reference to the following examples:

EXAMPLES

5

Example 1: Optimisation of p55 gag (p17, p24, p13) to resemble codon usage of highly expressed human genes.

Gene of interest

10

A synthetic gene coding for the p55gag antigen of the HIV-1 clade B strain HXB2 (GenBank entry K03455), optimised for expression in mammalian cells was assembled from overlapping oligonucleotides by PCR.

15

Optimisation involved changing the codon usage pattern of the viral gene to give a codon frequency closer to that found in highly expressed human genes. Codons were assigned using a statistical Visual Basic program called

20

Syngene (an updated version of Calcgene, written by R.S. Hale and G. Thompson, Protein Expression and Purification Vol. 12 pp 185-188, 1998)

Cloning:

25

The 1528bp gag PCR product was gel purified, cut with restriction endonucleases NotI and Bam HI and ligated into NotI/BamHI cut vector WRG7077. This places the gene between the CMV promoter/intron A and the Bovine growth hormone polyadenylation signal.

30

Clones were sequenced and checked for errors. No single clone was 100% correct. Regions of correct sequence from two clones were therefore combined by overlapping PCR

using appropriate combinations of the optimisation oligo set to give a full length codon optimised gag gene. This final clone was subsequently found to contain a single nucleotide deletion which resulted in a frame shift and premature termination of translation. The deletion was repaired by cutting out the region of the gene containing the incorrect sequence and cloning in the correct sequence from the equivalent region of another clone. This gave the final codon optimised p55 gag clones:

10 Gagotrpr2. (See figure 2)

Example 2: Production of a p17/p24 truncated Nef fusion gene

15 Gene of interest

The p17 and p24 portions of the p55gag gene derived from the HIV-1 clade B strain HXB2 was PCR amplified from the plasmid pHXB?Pr (E.Maschera, E Furfine and E.D. Blair 1995 J.Virol **69** 5431-5436). pHXB?Pr. 426bp from the 3' end of the HXB2 nef gene were amplified from the same plasmid. Since the HXB2 nef gene contains a premature termination codon two overlapping PCRs were used to repair the codon (TGA [stop] to TGG [Trp])

20

25 The p17/p24linker and trNEFlinker PCR products were joined to form the p17p24trNEF fusion gene (figure 3) in a PCR reaction (**antisense**)

30 The 1542bp product was gel purified, cut with restriction endonucleases NotI and BamHI and cloned into the NotI BamHI sites of vector WRG7077. This places the gene between the CMV promoter/intron A and the Bovine growth hormone polyadenylation signal.

Example 3: Production of an Gag p17/24opt/trNef1 ('Gagopt/Nef') fusion gene.

5

Gene of interest

The p17/p24 portion of the codon optimised p55gag gene derived from the HIV-1 clade B strain HXB2 was PCR amplified from the plasmid pGagOPTTrpr2. The truncated HXB2 Nef gene with the premature termination codon repaired (TGA [stop] to TGG [Trp]) was amplified by PCR from the plasmid 7077trNef20. The two PCR products were designed to have overlapping ends so that the two genes could be joined in a second PCR.

The 1544bp product was gel purified, cut with restriction endonucleases NotI and BamHI and cloned (see figures) into the NotI BamHI sites of vector WRG7077. This places the gene between the CMV promoter/intron A and the Bovine growth hormone polyadenylation signal.

Example 4: Plasmid: p7077-RT3 Clone #A

25 **Gene of interest:**

A synthetic gene coding for the RT portion of the pol gene of HIV-1 clade B strain HXB2, optimised for expression in mammalian cells assembled from overlapping oligonucleotides by PCR. The sequence cloned is equivalent to positions 2550-4222 of the HXB2 reference sequence (GenBank entry K03455). To ensure expression the cloned sequence has two additional codons at the 5' end not present in the original gene - AUG GGC (Met Gly).

- Optimisation involved changing the codon usage pattern of the viral gene to give a codon frequency closer to that found in highly expressed human genes, but excluding rarely used codons. Codons were assigned using a
- 5 statistical Visual Basic program called Syngene (an updated version of Calcgene, written by R.S. Hale and G. Thompson, Protein Expression and Purification Vol. 12 pp 185-188, 1998)
- 10 The final clone was constructed from two intermediate clones, # 16 and #21.

Cloning:

- 15 The 1.7kb PCR products were gel purified, cut with NotI and BamHI and PCR cleaned, before being ligated with NotI / BamHI cut pWRG7077. This places the gene between the CMV promoter and bovine growth hormone polyadenylation signal. Clones were sequenced. No clone was 100% correct,
- 20 but clone #16 was corrected by replacing the 403bp KpnI-BamHI fragment containing 3 errors with a correct KpnI-BamHI fragment from clone#21. The final clone was verified by sequencing. (see figure 5)

25

Example 5: Optimised RT**Gene of interest**

- The synthetic gene coding for the RT portion of the pol
- 30 gene of HIV-1 clade B strain HXB2, optimised for expression in mammalian cells was excised from plasmid p7077-RT3 as a 1697bp NotI / BamHI fragment, gel purified, and cloned into the NotI & BamHI sites of p7313-ie (derived from pspC31) to place the gene

downstream of an Iowa length HCMV promoter + exon1, and
upstream of a rabbit globin poly-adenylation signal.
(R7004 p27) (figure 6)

5 Example 6

Plasmid: 7077trNef20

Gene of interest

The insert comprises part of the Nef gene from the HIV-1
clade B strain HXB2. 195bp are deleted from the 5' end of
the gene removing the codons for the first 65 amino acids
of Nef. In addition the premature termination codon in
the published HXB2 nef sequence has been repaired (TAG to
TGG [Trp]) as has been described for plasmid
p17/24trNEF1. The truncated nef sequence was PCR
amplified from the plasmid p17/24trNef1. The sequence
cloned is equivalent to positions 8992-9417 of the HXB2
reference sequence (GenBank entry K03455). To ensure
expression the cloned sequence has an additional codon at
the 5' end not present in the original gene - AUG (Met).

Primers:

StrNef (sense) ATAAGAATGCGGCCGCCATGGTGGGTTTTCCAGTCACACCTT
[SEQ ID NO: 1]

AStrNef (antisense)

CGCGGATCCTCAGCAGTTCTTGAAGTACTCC [SEQ ID NO: 2]

PCR: 94° C 2min, then 25 cycles: 94° C 30sec, 50° C
30sec, 72° C 2min, ending 72° C 5min

30 Cloning:

The 455bp RT PCR product was gel purified, cut with
restriction endonucleases NotI and Bam HI and ligated
into NotI/BamHI cut vector WRG7077. This places the gene

between the CMV promoter/intron A and the Bovine growth hormone polyadenylation signal.

5 **Example 7**

Plasmid: 7077RT 8

Gene of interest

The RT portion of the pol gene was derived from the HIV-1
clade B strain HXB2. It was PCR amplified from the
10 plasmid p7077Pol14.

The sequence cloned is equivalent to positions 2550-4234
of the HXB2 reference sequence (GenBank entry K03455). To
ensure expression the cloned sequence has two additional
15 codons at the 5' end not present in the original gene -
AUG GGC (Met Gly).

Primers:

SRT (sense) ATAAGAATGCGGCCGCCATGGGCCCCATTAGCCCTATTGAGACT
20 [SEQ ID NO: 3]
ASRT (antisense)
CGCGGATCCTTAATCTAAAAATAGTACTTTCCTGATT [SEQ ID NO: 4]

PCR: 94° C 2min, then 25 cycles: 94° C 30sec, 50° C
25 30sec, 72° C 4min, ending 72° C 5min

Cloning:

The 1720bp RT PCR product was gel purified, cut with
restriction endonucleases NotI and Bam HI and ligated
30 into NotI/BamHI cut vector WRG7077. This places the gene
between the CMV promoter/intron A and the Bovine growth
hormone polyadenylation signal.

Example 8**p17/24opt/RT/trNef13 ('Gagopt/RT/Nef')**

This construct contains a PCR that causes an R to H amino
 5 acid change.

Gene of interest:

The p17/p24 portion of the codon optimised p55gag gene
 derived from the HIV-1 clade B strain HXB2 was PCR
 amplified from the plasmid pGagOPTtrpr2. The RT coding
 10 sequence was PCR amplified from the plasmid 7077RT 8. The
 truncated HXB2 Nef gene with the premature termination
 codon repaired (TGA [stop] to TGG [Trp]) was amplified by
 PCR from the plasmid 7077trNef20. The three PCR products
 were designed to have overlapping ends so that the three
 15 genes could be joined in a second PCR.

Primers:

(P17/24)

20 Sp17p24opt (sense)

ATAAGAATGCGGCCCGCCATGGGTGCCCCGAGCTTCGGT [SEQ ID NO: 5]

ASp17p24optRTlinker (antisense)

TGGGGCCCATCAACACTCTGGCTTTGTGTC [SEQ ID NO: 6]

25 PCR: 94° C 1min, then 20 cycles: 94° C 30sec, 50° C
 30sec, 72° C 2min, ending 72° C 4min

The 1114bp p17/24opt product was gel purified.

30 (RT)

Sp17p24optRTlinker (sense)

CAGAGTGTGATGGGCCCCATTAGCCCTAT [SEQ ID NO: 7]

ASRTtrNeflinker (antisense)

AACCCACCATATCTAAAAATAGTACTTTCC [SEQ ID NO: 8]

PCR: as above

- 5 The 1711bp RT PCR product was gel purified

(5' truncated nef)

SRTtrNef linker (sense)

- 10 CTATTTTATAGATATGGTGGGTTTCCAGTCAC [SEQ ID NO: 9]

AStrNef (antisense)

CGCGGATCCTCAGCAGTTCTTGAAGTACTCC [SEQ ID NO: 10]

PCR as above.

- 15 The 448bp product was gel purified.

The three PCR products were then stitched together in a second PCR with primers Sp17/24opt and AStrNef.

- 20 PCR: 94° C 1min, then 30 cycles: 94° C 30sec, 50° C 30sec, 72° C 4min, ending 72° C 4min

The 3253bp product was gel purified, cut with restriction endonucleases NotI and BamHI and cloned into the NotI

- 25 BamHI sites of vector WRG7077. This places the gene between the CMV promoter/intron A and the Bovine growth hormone polyadenylation signal.

Example 9

- 30 Plasmid: pGRN#16 (p17/p24opt corr /RT/trNef.)

Gene of interest:

The polypeptide generated by p17/24opt/RT/trNef13

('Gagopt/RT/Nef') was observed to express a truncated

- 35 product of ~30kDa due to a cluster of unfavourable codons

within p24 around aminoacid 270. These were replaced with optimal codons by PCR stitching mutagenesis. p17/24opt/RT/trNef13 was used as a template to amplify the portion of Gag 5' to the mutation with primers
 5 Sp17/p24opt and GTR-A, and the portion of Gag 3' to the mutation with primers GTR-S and Asp17/p24optRTlinker. The overlap of the products contained the codon changes, and the gel purified products were stitched together using the Sp17/p24opt and Asp17/p24optRTlinker primers. The
 10 product was cut with NotI and AgeI and inserted into similarly cut p17/24opt/RT/trNef13, to generate pGRN. Clone #16 was verified and progressed.

Primers:

15

5' PCR:

Sp17p24opt (sense)

ATAAGAAATGCGCCGCCATGGGTGCCCCGAGCTTCGGT [SEQ ID NO: 11]

20

GTR-A (Antisense)

GCGCACGATCTTGTTCAGGCCCAGGATGATCCACCGTTTATAGATTTCTCC [SEQ ID NO: 12]

25 3' PCR

Sense: GTR-S (Sense)

ATCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCC [SEQ ID NO: 13]

30 ASp17p24optRTlinker (antisense)

TGGGGCCCATCAACACTCTGGCTTTGTGTC [SEQ ID NO: 14]

PCR conditions for individual products and stitch, using PWO DNA polymerase (Roche):

35

95°C 1min, then 20 cycles 95°C 30s, 55°C 30s, 72°C 180s, ending 72°C 120s and 4°C hold.

- The 1114bp product was gel purified and cut with NotI and
 5 AgeI to release a 6647bp fragment which was gel purified and ligated into NotI / AgeI cut gel purified p17/24opt/RT/trNef13 to generate pGRN#16.

Example 10:

- 10 Plasmid: p73i-GRN2 Clone #19
 (p17/p24(opt)/RT(opt)trNef) - repaired

Gene of interest:

- The p17/p24 portion of the codon optimised gag, codon
 15 optimised RT and truncated Nef gene from the HIV-1 clade B strain HXB2 downstream of an Iowa length HCMV promoter + exon1, and upstream of a rabbit β -globin polyadenylation signal.
- 20 Plasmids containing the trNef gene derived from plasmid p17/24trNef1 contain a PCR error that gives an R to H amino acid change 19 amino acids from the end of nef. This was corrected by PCR mutagenesis, the corrected nef PCR stitched to codon optimised RT from p7077-RT3, and
 25 the stitched fragment cut with ApaI and BamHI, and cloned into ApaI/BamHI cut p73i-GRN.

Primers:

- PCR coRT from p7077-RT3 using primers:
 30 (Polymerase = PWO (Roche) throughout.

Sense: U1

GAATTCGCGGCCGCGATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTG
 AAACCCGGGAT [SEQ ID NO: 15]

AScoRT-Nef

GGTGTGACTGGAAAACCCACCATCAGCACCTTTCTAATCCCCGC [SEQ ID NO:
16]

- 5 Cycle: 95°C(30s) then 20 cycles 95°C(30s), 55°C(30s),
72°C(180s), then 72°C(120s) and hold at 4°C
The 1.7kb PCR product was gel purified.

PCR 5' Nef from p17/24trNef1 using primers:

10

Sense: S-Nef

ATGGTGGGTTTTCAGTCACACC [SEQ ID NO: 17]

Antisense: ASNef-G:

GATGAAATGCTAGGCGGCTGTCAAACCTC [SEQ ID NO: 18]

- 15 Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(120s) and hold at 4°C

PCR 3' Nef from p17/24trNef1 using primers:

- 20 Sense: SNEF-G

GAGGTTTGACAGCCGCCTAGCATTTTCATC [SEQ ID NO: 19]

Antisense:

AStrNef (antisense)

CGCGGATCCTCAGCAGTTCTTGAAGTACTCC [SEQ ID NO: 20]

- 25 Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(120s) and hold at 4°C

The PCR products were gel purified. Initially the two Nef
products were stitched using the 5' (S-Nef) and 3'

- 30 (AStrNef) primers.

Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(180s) and hold at 4°C .

The PCR product was PCR cleaned, and stitched to the RT
product using the U1 and AStrNef primers:

Cycle: 95°C(30s) then 20 cycles 95°C(30s), 55°C(30s),
72°C(180s), then 72°C(180s) and hold at 4°C

The 2.1kb product was gel purified, and cut with ApaI and
5 BamHI. The plasmid p73I-GRN was also cut with ApaI and
BamHI gel purified and ligated with the ApaI-Bam RT3trNef
to regenerate the p17/p24(opt)/RT(opt)trNef gene.

Example 11

10 p73i-GN2 Clone #2 (p17/p24opt/trNef) - repaired

Gene of interest:

The p17/p24 portion of the codon optimised gag and
truncated Nef genes from the HIV-1 clade B strain HXB2
15 downstream of an Iowa length HCMV promoter + exon1, and
upstream of a rabbit β -globin poly-adenylation signal.

Plasmids containing the trNef gene derived from plasmid
p17/24trNef1 contain a PCR error that gives an R to H
20 amino acid change 19 amino acids from the end of Nef.
This was corrected by PCR mutagenesis and the corrected
fragment cut with BglII and BamHI, and cloned into
BglII/BamHI cut p73I-GN. (Figure 12) regenerate the
corrected p17/p24opt/trNef fusion gene downstream of the
25 Iowa length HCMV promoter + exon1, and upstream of the
rabbit β -globin polyadenylation signal.

PCR 5' Nef from p17/24trNef1 using primers:

Polymerase = PWO (Roche) throughout.

30

Sense: S-Nef

ATGGTGGGTTTCCAGTCACACC [SEQ ID NO: 21]

Antisense: ASNef-G:

GATGAAATGCTAGGCGGCTGTCAAACCTC [SEQ ID NO: 22]

35

Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(120s) and hold at 4°C

PCR 3' Nef from p17/24trNef1 using primers:

5 Sense: SNEF-G

GAGGTTTGACAGCGCCTAGCATTTCATC [SEQ ID NO: 23]

Antisense: AStrNef

CGCGGATCCTCAGCAGTTCTTGAAGTACTCC [SEQ ID NO: 24]

- 10 Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(120s) and hold at 4°C

The PCR products were gel purified, and stitched using
the 5' (S-Nef) and 3' (AStrNef) primers.

- 15 Cycle: 95°C(30s) then 15 cycles 95°C(30s), 55°C(30s),
72°C(60s), then 72°C(180s) and hold at 4°C .

The PCR product was PCR cleaned, cut with BglIII / BamHI ,
and the 367bp fragment gel purified and cloned into
BglIII/BamHI cut gel purified p73i-GN.

20

Example 12

Plasmid: p73I-RT w229k (Inactivated RT)

Gene of Interest:

- 25 Generation of an inactivated RT gene downstream of an
Iowa length HCMV promoter + exon 1, and upstream of a
rabbit β -globin poly-adenylation signal.

- 30 Due to concerns over the use of an active HIV RT species
in a therapeutic vaccine inactivation of the gene was
desirable. This was achieved by PCR mutagenesis of the
RT (derived from P73I-GRN2) amino acid position 229 from
Trp to Lys (R7271 p1-28).

Primers:

PCR 5' RT + mutation using primers:
(polymerase = PWO (Roche) throughout)

5 Sense : RT3-u:1
GAATTTCGGCGCCGCGATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTG
AAACCCGGGAT [SEQ ID NO: 25]

Antisense: AScoRT-Trp229Lys

10 GGAGCTCGTAGCCCATCTTCAGGAATGGCGGCTCCTTCT [SEQ ID NO: 26]

Cycle:

1 x [94°C (30s)]
15 x [94°C (30s)/55°C (30s)/72°C (60s)]

15 1 x [72°C (180s)]
PCR gel purify

PCR 3' RT + mutation using primers:

20 Antiense: RT3- l:1
GAATTTCGGATCCTTACAGCACCTTTCTAATCCCCGCACTCACCAGCTTGTGACCTG
CTCGTTGCCGC [SEQ ID NO: 27]

Sense: ScoRT-Trp229Lys

25 CCTGAAGATGGGCTACGAGCTCCATG [SEQ ID NO: 28]

Cycle:

1 x [94°C (30s)]
15 x [94°C (30s)/55°C (30s)/72°C (60s)]

30 1 x [72°C (180s)]
PCR gel purify

The PCR products were gel purified and the 5' and 3' ends
of RT were stitched using the 5' (RT3-U1) and 3' (RT3-L1)

35 primers.

Cycle:

1 x [94°C (30s)]
15 x [94°C (30s)/55°C (30s)/72°C (120s)]
1 x [72°C (180s)]

5

The PCR product was gel purified, and cloned into p73i3ie, utilising NotI and BamHI restriction sites, to generate p73I-RT w229k. (See figure 13)

10

Example 13:**Plasmid: p73i-Tgrn (#3)****Gene of interest:**

15 The p17/p24 portion of the codon optimised gag, codon optimised RT and truncated Nef gene from the HIV-1 clade B strain HXB2 downstream of an Iowa length HCMV promoter + exon1, and upstream of a rabbit β -globin polyadenylation signal.

20

Triple fusion constructs which contain an active form of RT, may not be acceptable to regulatory authorities for human use thus inactivation of RT was achieved by Insertion of a NheI and ApaI cut fragment from p73i-RT w229k, into NheI/ApaI cut p73i-GRN2#19 (Figure 14). This results in a W $\rightarrow$ K change at position 229 in RT.

Example 1430 **p73I-Tnrg (#16)****Gene of interest:**

The truncated Nef, inactivated codon optimised RT and p17/p24 portion of the codon optimised gag gene from the

HIV-1 clade B strain HXB2 downstream of an Iowa length HCMV promoter + exon1, and upstream of a rabbit β -globin poly-adenylation signal.

- 5 The order of the genes in the polyprotein encoded by p73i-Tgrn were rearranged by PCR and PCR stitching to generate p73I-Tnrg (Figure 15). Each gene was PCR amplified and gel purified prior to PCR stitching of the genes to form a single polyprotein. The product was gel
 10 purified, NotI/BamHI digested and ligated into NotI/BamHI cut p73I3ie.

Primers:

trNef PCR

15

S-Nef (Not I)

CATTAGACGGCCGCGATGGTGGGTTTCCAC [SEQ ID NO: 29]

AS-Nef-coRT linker

- 20 GATGGGACTGATGGGGCCCATGCAGTTCTTGAATACTCCGG [SEQ ID NO: 30]

RTw229k PCR

25

S-coRT

ATGGGCCCCATCAGTCCCATCGAG [SEQ ID NO: 31]

AS-coRT-p17p24 linker

- 30 CAGTACCGAAGCTCGGGCACCCATCAGCACCTTTCTAATCCCCGC [SEQ ID NO: 32]

p17p24opt PCR

S-p17p24opt

- 35 ATGGGTGCCCCGAGCTTCGGTACTG [SEQ ID NO: 33]

AS-p17p24opt (BamHI)

GATGGGGGATCCTCACAACACTCTGGCTTTGTGTCC [SEQ ID NO: 34]

- 5 PCR conditions for individual products and stitching
using VENT DNA polymerase (NEB):

1 x [94°C (30s)]

25 x [94°C (30s)/55°C (30s)/72°C (120s [p17p24 or RT] or

10 60s [trNef])]

1 x [72°C (240s)]

The PCR products were gel purified and used in a PCR

stitching utilising the primers S-trNef (NotI) and AS-

15 p17p24opt (BamHI):

1 x [94°C (30s)]

25 x [94°C (30s)/55°C (30s)/72°C (210s)]

1 x [72°C (240s)]

20

The 3000bp product was gel purified and cut with NotI and

BamHI which was PCR cleaned and ligated into NotI/BamHI

digested gel purified p7313ie to generate p73i-Tnrg.

25 **Example 15:**

1. Plasmid: P73i-Tnrg (#3)

Gene of Interest:

The truncated Nef , p17/p24 portion of the codon

30 optimised gag and inactivated codon optimised RT gene

from the HIV-1 clade B strain HXB2 downstream of an Iowa

length HCMV promoter + exon1, and upstream of a rabbit β -

globin poly-adenylation signal.

The order of the genes in the polyprotein encoded by p73i-Tgrn were rearranged by PCR to generate p73I-Tngr (Figure 16). Codon optimised p17/p24 and RT were generated as a single product, and PCR stitched to amplified trNef. The product was gel purified, NotI/BamHI digested and ligated into NotI/BamHI cut p7313ie.

Primers:

10 P17/p24 - RT 3' PCR:

Sp17p24opt (sense)

ATGGGTGCCCCGAGCTTCGGTACTG [SEQ ID NO: 35]

15 RT3 1:1 (antisense)

GAATTCGGATCCTTACAGCACCTTTCTAATCCCCGCACTCACCAGCTTGTCGACCTG

CTCGTTGCCGC [SEQ ID NO: 36]

TrNef 5' PCR

20

S-Nef (NotI)

CATTAGAGCGCGCGATGGTGGGTTTCCAC [SEQ ID NO: 37]

AS-Nef-p17p24

25 CAGTACCGAAGCTCGGGCACCCATGCAGTTCTTGAAGTACTCCGG [SEQ ID NO: 38]

PCR conditions for individual products and stitching using VENT DNA polymerase (NEB):

30

1 x [94°C (30s)]

25 x [94°C (30s)/55°C (30s)/72°C (180s [p17p24+RT] or 60s [trNef] or 210s [stitching])]

1 x [72°C (240s)]

35

The 3000bp product was gel purified and cut with NotI and BamHI which was PCR cleaned and ligated into NotI/BamHI digested gel purified p7313ie to generate p73i-Trgr.

5 Example 16:

Plasmid: p73I-Trgn (#6)

Gene of interest:

- The inactivated codon optimised RT, p17/p24 portion of
- 10 the codon optimised gag and truncated Nef gene from the HIV-1 clade B strain HXB2 downstream of an Iowa length HCMV promoter + exon1, and upstream of a rabbit β -globin poly-adenylation signal.
- 15 The order of the genes within the construct was achieved by PCR amplification of p17p24-trNef and RTw229k from the plasmids p73I-GN2 and p73I-RTw229k respectively. PCR stitching was performed and the product gel purified and NotI/BamHI cut prior to ligation with NotI/BamHI digested
- 20 p7313ie. Sequencing revealed that p17p24 was not fully optimised a 700bp fragment was then AgeI/MunI cut from the coding region and replaced with MunI/Age fragment from p73i-Trgr#3 containing the correct coding sequence. (See figure 17).

25

Primers:

p17p24-trNef PCR

S-p17p24opt

- 30 ATGGGTGCCCCGAGCTTCGGTACTG [SEQ ID NO: 39]

AstrNef (BamHI)

RTw229k

RT3-U:1

GAATTCGGCGCCGCGATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTG

5 AAACCCGGGAT [SEQ ID NO: 40]

AS-coRT-p17p24opt linker

CAGTACCGAAGCTCGGGCACCCATCAGCACCTTTCTAATCCCCGC [SEQ ID NO:
41]

10

PCR conditions for individual products and stitching
using VENT DNA polymerase (NEB):

1 x [94°C (30s)]

15 25 x [94°C (30s)/55°C (30s)/72°C (120s (PCR) or 180s
(stitching)]

1 x [72°C (240s)]

20 The 3000bp product from the PCR stitch was gel purified
and cut with NotI and BamHI which was PCR cleaned and
ligated into NotI/BamHI digested gel purified p73I3ie to
generate p73i-Tngr. Sequence analysis showed that p17p24
sequence obtained from p73I-GN2 was not fully codon
optimised and that this had been carried over into the
25 new plasmid. This was rectified by cutting a 700bp
fragment from p73i-Tngr cut with MunI and AgeI, and
replacing it by ligation with a 700bp MunI/AgeI digested
product from p73i-Tgrn to generate the construct p73I-
Tngr#6.

30

Example 17:**Plasmid: p73i-Trng (#11)****Gene of Interest:**

The inactivated codon optimised RT, truncated Nef and p17/p24 portion of the codon optimised gag gene from the HIV-1 clade B strain HXB2 downstream of an Iowa length HCMV promoter + exon1, and upstream of a rabbit β -globin poly-adenylation signal.

The order of the genes within the construct was achieved by PCR amplification of the RT-trNef and p17p24 genes from p73i-Trn. PCR stitching of the two DNA fragments was performed and the 3kb product gel purified and NotI/BamHI cut prior to ligation with NotI/BamHI digested p73i3ie, and yielded p73I Trng (#11).

Primers:

15 RTw229k-trNef

RT3-u:1

GAATTTCGCGGCGCGATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTG
AAACCCGGGAT [SEQ ID NO: 42]

20

AS-Nef-p17p24opt linker

CAGTACCGAAGCTCGGGCACCCATGCAGTTCTTGAAGTACTCCGG [SEQ ID NO: 43]

25

P17p24

S-p17p24opt

ATGGGTGCCCCGAGCTTCGGTACTG [SEQ ID NO: 44]

30

AS-p17p24opt (BamHI)

GATGGGGGATCCTCACAACACTCTGGCTTTGTGTCC [SEQ ID NO: 45]

PCR conditions for individual products and stitching using VENT DNA polymerase (NEB):

- 1 x [94°C (30s)]
 25 x [94°C (30s)/55°C (30s)/72°C (120s (PCR of genes) or
 180s (stitching)
 5 1 x [72°C (240s)]

The 3000bp product from the PCR stitch was gel purified
 and cut with NotI and BamHI which was PCR cleaned and
 ligated into NotI/BamHI digested gel purified p7313ie to
 10 generate p73i-Tngr.

Example 18:

p73i-Tngr (#f1)

15 Gene of interest:

The p17/p24 portion of the codon optimised gag, truncated
 Nef and codon optimised inactivated RT gene from the
 HIV-1 clade B strain HXB2 downstream of an Iowa length
 HCMV promoter + exon1, and upstream of a rabbit β -globin
 20 poly-adenylation signal.

The order of the genes within the construct was achieved
 by PCR amplification of p17p24-trNef and RTw229k from the
 plasmids p73I-GN2 and p73I-RTw229k respectively. PCR
 25 stitching was performed and the product gel purified and
 NotI/BamHI cut prior to ligation with NotI/BamHI digested
 p7313ie. Two sequence errors were spotted in the sequence
 (p17p24 and RT) which were subsequently repaired by
 replacement with correct portions of the genes utilising
 30 restriction sites within the polyprotein. (See figure
 19).

Primers:

p17p24-trNef PCR

S-p17p24opt

ATGGGTGCCCCGAGCTTCGGTACTG [SEQ ID NO: 46]

5

AS-Nef-coRTlinker

GATGGGACTGATGGGGCCCATGCAGTTCTTGAAGTACTCCGG [SEQ ID NO:
47]10 RTw229k

S-coRT

ATGGGGCCCATCAGTCCCATCGAG [SEQ ID NO: 48]

15 RT3-1:1

GAATTCGGATCCTTACAGCACCTTTCTAATCCCCGCACTCACCAGCTTGTGACCTG
CTCGTTGCCGC [SEQ ID NO: 49]

PCR conditions for individual products and stitching

20 using VENT DNA polymerase (NEB):

1 x [94°C (30s)]

25 x [94°C (30s)/55°C (30s)/72°C (120s (PCR) or 180s
(stitching)]

25 1 x [72°C (240s)]

The 3000bp product was gel purified and cut with NotI and
BamHI which was PCR cleaned and ligated into NotI/BamHI
digested gel purified p73i3ie to generate p73i-Tngr.30 Sequencing revealed that p17p24 was not fully optimised a
700bp fragment was subsequently AgeI/MunI cut from the
coding region and replaced with MunI/AgeI fragment from
p73i-Tgrn#3 containing the correct coding sequence. The
polyprotein also contained a single point mutation
35 (G2609A) resulting in an amino acid substitution of Thr

to Ala in the RT portion of the polyprotein. The mutation was corrected by ApaI/BamHI digestion of the construct and PCR clean up to remove the mutated sequence, which was replaced by ligation with an
5 ApaI/BamHI digested portion of RT from p73i-Tgnr.

Example 19:

Preparation of plasmid-coated 'gold slurry' for 'gene gun' DNA cartridges

10 Plasmid DNA (approximately 1µg/µl), eg. 100 ug, and 2µm gold particles, eg. 50 mg, (PowderJect), were suspended in 0.05M spermidine, eg. 100 ul, (Sigma). The DNA was precipitated on to the gold particles by addition of 1M
15 CaCl₂, eg. 100ul (American Pharmaceutical Partners, Inc., USA). The DNA/gold complex was incubated for 10 minutes at room temperature, washed 3 times in absolute ethanol, eg. 3 x 1 ml, (previously dried on molecular sieve 3A (BDH)). Samples were resuspended in absolute ethanol
20 containing 0.05mg/ml of polyvinylpyrrolidone (PVP, Sigma), and split into three equal aliquots in 1.5 ml microfuge tubes, (Eppendorf). The aliquots were for analysis of (a) 'gold slurry', (b) eluate- plasmid eluted from (a) and (c) for preparation of gold/ plasmid
25 coated Tefzel cartridges for the 'gene gun', (see Example 3 below). For preparation of samples (a) and (b), the tubes containing plasmid DNA / 'gold slurry' in ethanol / PVP were spun for 2 minutes at top speed in an Eppendorf 5418 microfuge, the supernatant was removed and the 'gold
30 slurry' dried for 10 minutes at room temperature. Sample (a) was resuspended to 0.5 - 1.0 ug / ul of plasmid DNA in TE pH 8.0, assuming approx. 50 % coating. For elution, sample (b) was resuspended to 0.5 - 1.0 ug / ul of plasmid DNA in TE pH 8.0 and incubated at 37°C for 30
35 minutes, shaking vigorously, and then spun for 2 minutes

at top speed in an Eppendorf 5418 microfuge and the supernatant, eluate, was removed and stored at -20°C. The exact DNA concentration eluted was determined by spectrophotometric quantitation using a Genequant II (Pharmacia Biotech).

Example 20:**Preparations of Cartridges for DNA immunisation**

- 10 Preparation of cartridges for the Accell gene transfer device was as previously described (Eisenbraun et al DNA and Cell Biology, 1993 Vol 12 No 9 pp 791-797; Pertner et al). Briefly, plasmid DNA was coated onto 2 µm gold particles (DeGussa Corp., South Plainfield, N.J., USA)
- 15 and loaded into Tefzel tubing, which was subsequently cut into 1.27 cm lengths to serve as cartridges and stored desiccated at 4°C until use. In a typical vaccination, each cartridge contained 0.5 mg gold coated with a total of 0.5 µg DNA/cartridge.

20

Example 21:**Immune Response to HIV antigens following DNA vaccination utilising the gene gun.**

- 25 Mice (n=3/group) were vaccinated with antigens encoded by nucleic acid and located in two vectors. P7077 utilises the HCMV IE promoter including Intron A and exon 1 (fcmv promoter). P73I delivers the same antigen, but contains the HCMV IE promoter (icmv promoter) that is devoid of
- 30 Intron A, but includes exon 1.

Plasmid was delivered to the shaved target site of abdominal skin of F1 (C3H x Balb/c) mice. Mice were given a primary immunisation of 2 x 0.5 µg DNA on day 0,

35 boosted with 2 x 0.5 µg DNA on day 35 and cellular

response were detected on day 40 using IFN - gamma
Elispot.

- P73I - empty vector
- 5 • P7077 - empty vector
- P7077 GRN - (f CMV promoter) Gag, RT, Nef
- P73I GRN - (i CMV promoter) Gag, RT, Nef
- P73I GR3N - (CMV promoter) Optimised Gag,
Optimised RT, Nef
- 10 • P7077 GN - (f CMV promoter)Gag, Nef
- P73I GN - (i CMV promoter) Gag, Nef

Cytotoxic T Cell Responses

- 15 The cytotoxic T cell response was assessed by CD8+ T cell-restricted IFN- γ ELISPOT assay of splenocytes collected 5 days later. Mice were killed by cervical dislocation and spleens were collected into ice-cold PBS. Splenocytes were teased out into phosphate buffered
- 20 saline (PBS) followed by lysis of red blood cells (1 minute in buffer consisting of 155mM NH_4Cl , 10 mM KHCO_3 , 0.1mM EDTA). After two washes in PBS to remove particulate matter the single cell suspension was aliquoted into ELISPOT plates previously coated with
- 25 capture IFN- γ antibody and stimulated with CD8-restricted cognate peptide (Gag, Nef or RT). After overnight culture, IFN- γ producing cells were visualised by application of anti-murine IFN- γ -biotin labelled antibody (Pharmingen) followed by streptavidin -conjugated
- 30 alkaline phosphatase and quantitated using image analysis.

The result of this experiment are shown in figures 20, 21, and 22.

Example 22:**Immunogenicity of Vaccine Constructs****5 1. Cellular Assays**

The cellular immune response comprises cytotoxic CD8 cells and helper CD4 cells. A sensitive method to detect specific CD8 and CD4 cells is the ELISpot assay which can be used to quantify the number of cells capable of secreting interferon- γ or IL-2. The ELISpot assay relies on the capture of cytokines secreted from individual cells. Briefly, specialised microtitre plates are coated with anti-cytokine antibodies. Splenocytes isolated from immunised animals are incubated overnight in the presence of specific peptides representing known epitopes (CD8) or proteins (CD4). If cells are stimulated to release cytokines they will bind to the antibodies on the surface of the plate surrounding the locality of the individual producing cells. Cytokines remain attached to the coating antibody after the cells have been lysed and plates washed. The assay is developed in a similar way to an ELISA assay using a biotin/avidin amplification system. The number of spots equates to the number of cytokine producing cells.

25 CD8 responses to the following K2^d-restricted murine epitopes: Gag (AMQMLKETI), Nef (MTYKAAVDL) and RT (YYDPSKDLI) and CD4 responses to Gag and RT proteins were recorded for all 6 constructs. The results of these assays were analysed statistically and constructs were ranked according to their immunogenicity. The result is shown in figure 23 of the figures.

2. Humoral Assays

35

Blood samples were collected for antibody analysis at 7 and 14 days post-boost from two experiments. Serum was separated and stored frozen until antibody titres could be measured using specific ELISA assays. All samples were
5 tested for antibodies to Gag, Nef and RT. Briefly, ELISA plates were coated with the relevant protein. Excess protein was washed off before diluted serum samples were incubated in the wells. The serum samples were washed off and anti-mouse antiserum conjugated to an appropriate tag
10 was added. The plate was developed and read on a plate reader. The results are shown in figure 24.

3. Antibody Data

15 Antibody titres were measured for all six constructs in four experiments. Construct p73i-GNR consistently generated no antibody responses to Gag and limited antibody responses to Nef. The reason for this is unclear, as T-cell responses were observed from
20 splenocytes isolated from the same mice, indicating that the Gag protein was being expressed *in vivo*.

The ranking for the generation of Gag specific antibodies was:

25
RNG>GRN>NRG>RGN>NGR>GNR

Analysis cellular immunology data

30 The objective was to rank the 6 constructs on the basis of spot count data from 3 immunology experiments. Three sets of responses were assessed:

CD8 responses to Gag, Nef and RT at Day 7 (7 days post
35 primary),

CD4 responses to Gag and RT at Day 35 (7 days post boost),
CD8 responses to Gag, Nef and RT at Day 35 (7 days post boost).

5

Each response (e.g. CD8 response to Gag) was modeled using a linear mixed effect model in SAS version 8. The model included fixed effects of construct, whether the particular antigen (Gag, Nef or RT) was present or absent, and whether IL-2 was present or absent. In addition, for CD8 responses, where data were available from each individual mouse, subject was included as a random effect in the model. The model included interaction terms to allow for a different effect of construct for each combination of the antigen (present/absent) and IL-2 (present/absent).

10

15

From the model, the difference in adjusted mean response between each construct and p7313 (the control group) was estimated separately for each combination of antigen (present/absent) and IL-2 (present/absent), together with a p-value indicating whether the difference was statistically significant. Based on the differences and p-values in the presence of the antigen and the absence of IL-2, constructs were ranked, by assigning a score of 6 to the construct with the largest difference, 5 to the next largest, etc, but 0 to any constructs where the difference was not statistically significant at the 5% level.

25
30

The assumptions of the model - that the residuals were normally distributed with constant variance, were assessed using graphical methods and sensitivity analyses, where first a log and second a square root transformation of the response was modeled. The ranking

35

of the constructs was not sensitive to departures from the assumptions of the model.

Having calculated the ranks for each response in each experiment separately, total ranks for the 3 sets of responses were calculated across all 3 experiments. The following table shows the total rankings across the 3 experiments.

10 *Total rankings of constructs for each of 3 sets of responses, combined across 3 immunology experiments.*

Construct	Day 7 (7 days post primary)	Day 35 (7 days post boost)	
	CD8	CD4	CD8
GRN	5	18	3
GNR	17	24	28
RGN	28	23	33
RNG	25	27	37
NRG	25	19	0
NGR	4	14	10

RNG has the highest ranking for both sets of responses at Day 35, and the second highest ranking behind RGN at Day

15 7. RGN also receives high rankings for both sets of responses at Day 35.

The Claims Defining The Invention Are As Follows:

1. A nucleotide sequence that encodes an HIV-1 gag protein or fragment containing a gag epitope thereof, an
5 HIV-1 Nef protein or a fragment thereof containing a nef epitope, and an RT protein or a fragment containing an RT epitope, operably linked to a heterologous promoter.
2. A nucleotide sequence as claimed in claim 1 wherein
10 the gag protein comprises p17.
3. A nucleotide sequence as claimed in claim 2 wherein the gag protein additionally comprises p24.
- 15 4. A nucleotide sequence as claimed in any one of claims 1 to 3 wherein the gag sequence is codon optimised to resemble the codon usage in a highly expressed human gene having an RSCU value of 0.5.
- 20 5. A nucleotide sequence as claimed in any one of claims 1 to 4 wherein the RT sequence or fragment thereof is codon optimised to resemble a highly expressed human gene.
6. A nucleotide sequence as claimed in any one of claims
25 1 to 5 wherein the order of the sequence is RT, gag, Nef or RT Nef gag.
7. A nucleotide sequence as claimed in any one of claims 1 to 6 selected from the group consisting of:
30
 - Gag (p17, p24), codon optimised, RT codon optimised, Nef truncate
 - RT (codon optimised), Gag (p17, p24) codon optimised, Nef truncate

- RT (codon optimised), Nef truncate, gag p17, p24
codon optimised
- 8. A nucleotide sequence as claimed in any one of claims
5 1 to 7 wherein the heterologous promoter is the promoter
from HCMV IE gene.
- 9. A nucleotide sequence as claimed in claim 8 wherein
the 5' of the promoter comprises exon 1.
- 10 10. A nucleotide sequence as claimed in any one of claims
1 to 9 wherein the RT encodes a mutation to substantially
inactivate any reverse transcriptase activity.
- 15 11. A nucleotide sequence as claimed in claim 10 which the
RT is mutated by substituting tryptophan 229 for Lysine.
- 12. A vector comprising a nucleotide sequence as claimed
in any one of claims 1 to 11.
- 20 13. A vector as claimed in claim 12, which is a viral
vector.
- 14. A viral vector as claimed in claim 13 which is a
25 replication defective adenovirus.
- 15. A vector as claimed in claim 12 which is a double
stranded DNA plasmid.
- 30 16. A protein encoded by a nucleotide sequence as claimed
in any one of claims 1 to 11.
- 17. A pharmaceutical composition comprising a nucleotide
sequence of any one of claims 1 to 11 or a vector of any

2002362368 16 Aug 2006

one of claims 13, 14, 15 or 16 and a pharmaceutically acceptable excipient, diluent, carrier or adjuvant.

18. A pharmaceutical composition as claimed in claim 17
5 adapted for intra-muscular or intra-dermal delivery.

19. A pharmaceutical composition as claimed in claim 17 or 18 wherein the carrier is a gold bead.

10 20. An intra-dermal delivery device comprising a pharmaceutical composition of any one of claims 17, 18 or 19.

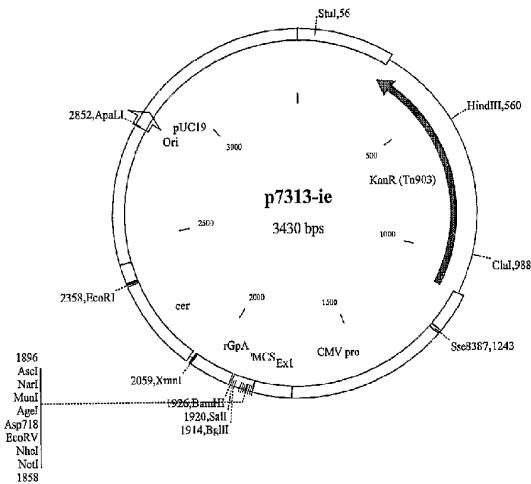
21. A nucleotide sequence of any one of claims 1 to 11, or
15 a composition of any one of claims 18 to 20, for use in medicine.

22. A process for the production of a nucleotide as claimed in any one of claims 1 to 11 comprising operably
20 linking a nucleotide sequence encoding an HIV-1 gag protein or fragment thereof and a second HIV-1 Nef protein or fragment thereof to a heterologous promoter sequence.

23. A nucleotide prepared by the process of claim 22.
25

24. A nucleotide sequence according to claim 1 substantially as hereinbefore described with reference to the Examples and Figures.

Figure 1



5

10

15

20

Figure 2

Sequence of p55 gag insert in pGagOptpr2

```

5  ATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
   AGGCTGCCCGGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCG
   AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGT
   CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGG
   TCCTTGTATAACACAGTGGCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAAG
10  GATACCAAGGAGGCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAG
   GCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCT
   ATTCTCCAAAACAATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTC
   AATGCCTGGGTGAAGGTTGTTCGAAGGAAGGCCTTTTCTCCTGAGGTTATCCCCATG
   TTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCCTTAATACC
15  GTGGCCGGCCATCAGCCGCGCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
   GCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGGACAGATG
   CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATC
   GGATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATC
   ATTCTCGTCTCAATAAAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATT
20  AGACAGGGACCCAAAGAGCCTTTIAGGGATTACGTCGACCGGTTTTATAAGACCTG
   CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGGATGACGGAGACACTCCTGGTA
   CAGAACGCTAACCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACC
   CTGGAAGAGATGATGACCGCTGTGAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
   GTGTTGGCCGAAGCCATGAGCCAGGTGACGAACTCCGCAACCATCATGATGCAGAGA
25  GGGAACTTCCGCAATCAGCGGAAGATCGTGAAGTGTTCATTTGCGGCAAGGAGGGT
   CATACCGCCCGCAACTGTGCGGGCCCTAGGAAGAAGGGTGTGGAAGTGCGGCAAG
   GAGGGACACCAGATGAAAGACTGTACAGAACGACAGGCCAATTTTCTTGAAAGATT
   TGGCCGAGCTACAAGGGGAGACCTGGTAATTTCTTGCAAAGCAGGCCCGAGCCACC
   GCCCCCCCTGAGGAATCCTTCAGGTCCGGAGTGGAGACCACAACGCTCCCCAAAAA
30  CAGGAACCAATCGACAAGGAGCTGTACCCCTTAACTTCTCTGCGTTCTCTTTTGGC
   AACGACCCGTCGTCTCAATAA [SEQ ID NO: 50]

```

```

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
LETSEGRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
35  LDKIEEEQNK SKKKAQAAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAI SPR
   TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAQM
   LKETINEEAA EWDVRVHPVHA GPIAPGQMR PRGSDIAGTT STLQEQIGWM
   TNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGP K EPFRDYVDRF
   YKTLRAEQAS QEVKNWMTET LLVQANPDC KTLKALGPA ATLEENMTAC
40  QGVGGPGHKA RVLAEAMSQV TNSATIMMOR GNFRNQRKIV KCFNCGKEGH
   TARNCRAPRK KGCWKCGKEG HQMKDCTERQ ANFLGKIWPS YKGRPGNFLQ
   SRPEPTAPPE ESFRSGVETT TPPQKQEPID KELYPLTSLR SLFGNDPSSQ
   *

```

[SEQ ID NO: 51]

Figure 3

Sequence of the p17/24trNEF insert in p17/24trNEF1

```

5  ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAATTAGATCCATGGGAAAAAAT'
   CGGTAAAGGCCAGGGGGAAGAAAAAATATAAATTAAACATATAGTATGGGCAAGC
   AGGGAGCTAGAACGATTTCGCA GTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGT
   AGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGA
   TCATTATATAATACAGTAGCAACCTCTATTTGTGTGCATCAAAGGATAGAGATAAAA
   GACACCAAGGAAGCTTTAGACAAGATAGAGGAAGACAAAACAAAAGTAAGAAAAAA
10  GCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCTT
   ATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTA
   AATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATG
   TTTTCAGCATTATCAGAAGGAGCCACCCCAAGATTTAAACACCATGCTAAACACA
   GTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCT
15  GCAGAATGGGATAGAGTGCATCCAGTGCATGCAGGCCCTATTGCACCCAGGCCAGATG
   AGAGAACCAAGGGGAAGTGACATAGCAGGAACCTACTAGTACCTTCAGGAACAAATA
   GGATGGATGACAAATAATCCACCTATCCCAGTAGGAGAAATTTATAAAAGATGGATA
   ATCCTGGGATTTAAATAAAATAGTAAGAATGTATAGCCCTACCAGCATTTCTGGACATA
   AGACAAGGACCAAAAGAACCCTTTAGAGACTATGTAGACCGGTTCTATAAACTCTA
20  AGAGCCGAGCAAGCTTCACAGGAGGTAAAAAATTGGATGACAGAAACCTTGTTGGTC
   CAAAATGCGAACCAGATTGTAAGACTATTTTAAAAGCATTGGGACCAGCGGCTACA
   CTAGAAGAAATGATGACAGCATGTGAGGGAGTAGGAGGACCCGGCCATAAGGCAAGA
   GTTTTGTGGGTTTTCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
   GCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATT
25  CACTCCCAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
   TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGTCAGATATCCACTGACCTTT
   GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
   GGAGAGAACACCAGCTTGTTACACCTGTGAGCCTGCATGGGATGGATGACCCGGAG
   AGAGAAGTGTAGAGTGGAGGTTTGACAGCCACCTAGCATTTTCATCACGTGGCCCGA
30  GAGCTGCATCCGAGTACTTCAAGAACTGCTGA [SEQ ID NO: 52]

MGARASVLSG GELDRWEKIR LRPGGKKKKYK LKHIVWASRE LERFAVNPGL
LETSEGCQRQI LQQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
LDKIEEEQNK SKKKAQQA AAA DTGHSNQVSQ NYPIVQNIQG QMVHQAI SPR
35  TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNMTLNT VGGHQAAMQM
   LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQIGWM
   TNNPPIPVGE IYKRWIILGL NKIVRMYSTP SILDIRQGPB EPFRDYVDRF
   YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMMTAC
   QGVGGPGHKA RVLVGFVPTP QVPLRPMTYK AAVDLSHFLK EKGGLEGLIH
40  SQRRQDILDL WIYHTQGYFP DWQNYTPGPG VRYPLTFGWC YKLVPVEPDK
   VEEANKGENT SLLHPVSLHG MDDPEREVL E WRFDSHLAFH HVARELHPEY
   FKNC*
   [SEQ ID NO: 53]

```

Figure 4

Sequence of the p17/24opt/trNef insert in
p17/24opt/trNef1

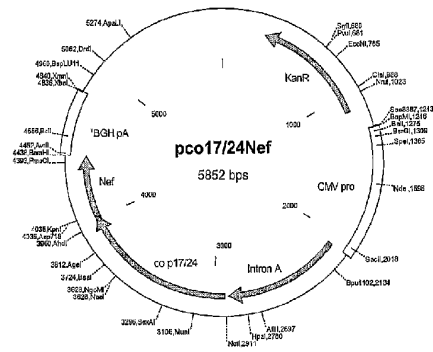
```

5  ATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
   AGGCTGCGCCCGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCG
   AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAACATCTGAGGGATGT
   CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAACAGCTGAGG
10  TCCTTGTTATAACACAGTGGCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAG
   GATACCAAGGAGGCCTTGGACAAAATTGAGGAGGAGCAAAACAGAGCAAGAAGAAG
   GCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCT
   ATTGTCCAAAACATTGAGGGCCAGATGGTTCATCAGGCCATCAGCCCCGGACGCTC
   AATGCCCTGGGTGAAGTTGTGGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATG
   TTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACC
15  GTGGGCGGCCATCAGGCCGCCATGCAAATGTTGAAGGAGACTATCAACGAGGAGGCA
   GCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGACAGATG
   CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATC
   GGATGGATGACCAACAATCCTCCCATCCAGTTGGAGAAATCTATAAACGGTGGATC
   ATTCTCGTCTCAATAAAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATT
20  AGACAGGACCCAAAGAGCCTTTTAGGGATTACGTGACCGGTTTATAAGACCCTG
   CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTA
   CAGAACGCTAACCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACC
   CTGGAAGAGATGATGACCGCTGTGAGGGAGTAGCGGACCCGGACACAAAGCCAGA
   GTGTTGATGTTGGGTTTTCAGTCAACCTCAGGTACCTTTAAGACCAATGACTTAC
25  AAGGCAGCTGTAGATCTTAGCCACTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTA
   ATTCACTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGC
   TACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACC
   TTTGGATGCTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAAT
   AAAGGAGAGAACACCAGCTTGTACACCTGTGAGCCTGCATGGGATGGATGACCCG
30  GAGAGAGAAGTGTAGAGTGAGGTTTGACAGCCACCTAGCATTTTCATCACGTGGCC
   CGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGA [SEQ ID NO: 54]

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
LETSEGRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
35  LDKIEEEQNK SKKKAQQAAD DTGHSNQVSQ NYPIVQNIQG QMVHQAI SPR
   TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNLTMLNT VGGHQAAQM
   LKETINEEAA EWDRVHPVHA GPIAPGQMRB PRGSDIAGTT STLQRQIGWM
   TNNPPIPVGE IYKRWIILGL NKIVRMYSPS SILDIRQGPB EPFRDYVDRF
   YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTIKALGPA ATLEEMMTAC
40  QGVGGPGHKA RVLVVGFPVT PQVPLRPMTY KAAVDLSHFL KEKGGLEGLI
   HSQRRQDILD LWIYHTQGYF PDWQNYTPGP GVRYPITFGW CYKLVPEVPD
   KVEEANKGEN TSLLHPVSLH GMDDPEREVL EWRFDShLAF HHVARELHPE
   YPKNC*
   [SEQ ID NO: 55]

```

45



5

10

15

20

25

30

Figure 5

Sequence of RT insert of p7077-RT3:

5 ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGAC
 GGCCCCAAGGTCAAGCAGTGGCCACTCACCAGGAGAAGATCAAGGCCCTGGTGGAG
 ATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCTGAGAACCCA
 TACAACACCCCGTGTTGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTG
 GTGGATTTCCGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGGC
 10 ATCCCCCATCCGGCCCGCCTGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGC
 GACGCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACC
 ATCCCATCTATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGTCTCCCC
 CAGGGCTGCAAGGGCTCTCCCGCCATTTCCAGAGCTCCATGACCAAGATCCTGGAG
 CCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGTAC
 15 GTGGGCTCTGACCTGGAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAG
 CATCTGCTGAGATGGGGCCTGACCACTCCGACACAAGAAGCATCAGAAGGAGCCGCCA
 TTCTGTGGATGGGTACGAGCTCCATCCCGACAAAGTGGACCGTGACGCTATCGTC
 CTCCCGAGAAGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTC
 AACTGGGCTAGCCAGATCTATCCCGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTG
 20 CGCGGCACCAAGGCCCTGACCGAGGTGATTCCCCTCACGGAGGAAGCCGAGCTCGAG
 CTGGCTGAGAACCGGGAGATCCTGAAGGAGCCCGTGACGCGGTGTACTATGACCC
 TCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAGGGGAGTGGACATACCAG
 ATTTACCAGGACCTTTCAAGAACCTCAAGACCGCAAGTACGCGCGCATGAGGGGC
 GCCCACACCAACGATGTCAAGCAGCTGACCGAGGCGGTCCAGAAGATCACGACCGAG
 25 TCCATCGTGATCTGGGGGAAGACACCCAAGTCAAGCTGCCTATCCAGAAGGAGACC
 TGGGAGACGTGGTGGACCGAATATTGGCAGGCCACCTGGATTCCCAGTGGGAGTTC
 GTGAATACACCTCCTCTGGTGAAGCTGTGTGTTACAGCTCGAGAAGGAGCCCATCTGT
 GCGCGGAGACATTCTACGTGGACGCGCGGCCAACCGCGAAACAAAGCTCGGGAAAG
 GCCGGGTACGTACCAACCGGGGCCGCCAGAAGTCTGTCACCCCTGACCGACACCACC
 30 AACCCAGAAGACGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAG
 GTGAACATCGTGACCGACAGCCAGTACGCGCTGGGCATTATTACGGCCAGCCGGAC
 CAGTCCGAGAGCGAACTGGTGAACAGATTATCGAGCAGCTGATCAAGAAAGAGAAG
 GTCTACCTCGCCTGGGTCCCGGCCATAAGGGCATTGGCGGCAACGAGCAGGTCCGAC
 AAGCTGGTGAAGTCCGGGATTAGAAAGGTGCTGTAA [SEQ ID NO: 56]
 35
 MGPISPIETV SVKLKPGMDG PKVKQWPLTE EKI KALVEIC TEMEKEGKIS
 KIGPENPYNT PVFAIKKKDS TKWRKLVDFR ELNKRQTDFW BVQLGIPHPA
 GLKKKKS VTV LDVGDYFVS PLDEDFRKYT AFTIPSINNE TPGIRYQYNV
 LPQGWKGSFA IFQSSMTKIL EPFRKQNPDI VIYQYMDLY VGS DLEIGQH
 40 RTKIEELRQH LLRWGLTTPD KKHQKEPPFL WMGYELHPDK WTVQPIVLPE
 KDSWTVNDIQ KLVGKLNWAS QIYPGIKVRQ LCKLLRGTKA LTEVIPLTEE
 AELELAENRE ILKEPVHGVY YDPSKDLIAE IQKQGGQWT YQIYQEPFKN
 LKTGKYARMR GAHTNDVKQL TEAVQKITTE SIVIWGKTPK FKLPIQKETW
 ETWWTEYWQA TWIPEWEFVN TPPLVKLWYQ LEKEPIVGAE TFYVDGAANR
 45 ETKLGKAGYV TNRGRQKVVT LTDITNQKTE LQAIYLALQD SGLEVNIVTD
 SQYALGIIQA QPDQSESELV NQIEQLIKK EKVYLAWVPA HKGIGGNEQV

5

10

15

20



Figure 6

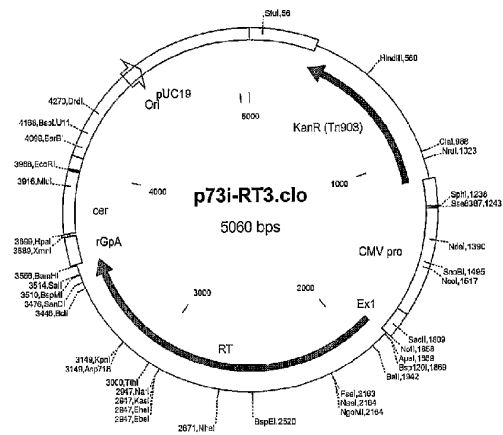
Sequence of the coding insert in p73i-RT3:

```

5  ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGAC
   GGCCCCAAGGTCAAGCAGTGGCCACTCACCAGGAGAAGATCAAGGCCCTGGTGGAG
   ATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCTCAGAACCCA
   TACAACACCCCCGTGTTTGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTG
   GTGGATTTCGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGGC
10 ATCCCCCATCCGGCCCGCCTGAAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGC
   GACGCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACC
   ATCCCATCTATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGCTCCTCCCC
   CAGGGCTGGAAGGGCTCTCCCGCCATTTTCCAGAGCTCCATGACCAAGATCCTGGAG
   CCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGTAC
15 GTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAG
   CATCTGCTGAGATGGGGCCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGCCA
   TTCTGTGGATGGGCTACGAGCTCCATCCCGACAAGTGGACCGTGACGCTATCGTC
   CTCCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGCAAGCTC
   AACTGGGCTAGCCAGATCTATCCCGGGATCAAGGTCCGCCAGCTCTGCAAGCTGCTG
20 CGCGGCACCAAGGCCCTGACCGAGGTGATTTCCCTCACCAGGGAAGCCGAGCTCGAG
   CTGGCTGAGAACCGGGAGATCCTGAAGGAGCCCGTGACGGCGTGACTATGACCCC
   TCCAAGGACCTGATCGCCGAATCCAGAAGCAGGGCCAGGGGCAGTGGACATACCAG
   ATTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAGTACGCCCGCATGAGGGGC
   GCCCACACCAACGATGTCAAGCAGCTGACCGAGGCCGTCCAGAAGATCACGACCGAG
25 TCCATCGTGATCTGGGGGAAGACACCCAAGTTCAGCTGCCTATCCAGAAGGAGACC
   TGGGAGACGTGGTGGACCGAATATTGGCAGGCCACTGGATTCCCGAGTGGGAGTTC
   GTGAATACACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCCATCGTG
   GGC CGGAGACATTCTACGTGGACGGCGCGGCCAACCGGAAACAAAGCTCGGGAA
   GGCCGGGTACGTACCAACCGGGCGGCCAGAAGGTGCTCACCTTGACCGACACCCAC
30 CAACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGA
   GGTGAACATCGTGACGGACAGCCAGTACGCGCTGGGCATTATTAGGCCAGCCGGA
   CCAGTCCGAGAGCGAAGTGGTGAACCAGATTATCGAGCAGCTGATCAAGAAAGAGAA
   GGTCTACCTCGCCTGGGTCCCGGCCATAAGGGCATTTGGCGGCAACGAGCAGGTCCA
   CAAGCTGGTGAGTCCGGGGATTAGAAAGGTGCTGTAA [SEQ ID NO: 58]
35
   MGPISPIETV SVKLKPGMDG PKVKQWPLTE EKI KALVEIC TEMEKEGKIS
   KIGPENPYNT PVFAIKKKDS TKWRKLVDFR ELNKRTQDFW EVQLGIPHPA
   GLKKKKSVTV LDVGDAYFSV PLDEDFRKYT AFTIPSINNE TPGIRYQYNV
   LPQGWKGSFA IFQSSMTKIL EFRKQNPDI VIYQYMDLY VGS DLEIGQH
40 RTKIEELRQH LLRWGLTPD KKHQKEPPFL WMGYELHPDK WTVQPIVLPE
   KDSWTVNDIQ KLVGKLNWAS QIYPGIKVRQ LCKLLRGTKA LTVIPLTEE
   AELELAENRE ILKEPVHGVY YDPSKDLIAE IQKQGQGWY YQIYQEPFKN
   LKTGKYARMR GAHTNDVKQL TEAVQKITTE SIVIWGKTPK FKLPIQKETW
   ETWWTEYWQA TWIPEWEPVN TPPLVKLWYQ LEKEPIVGAE TFYVDGAANR
45 BTKLGKAGYV TNRGRQKVVT LTDTTNQKTE LQAIYLALQD SGLEVNIVTD
   SQYALGIIQA QPDQSESELV NQIIEQLIKK EKVYLAWVPA HKGIGGNEQV

```

DKLVSAGIRK VL*
[SEQ ID NO: 59]



5

10

15

Figure 7**Sequence of Nef insert in 7077trNef20**

```
ATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCA
5 GCTGTAGATCTTAGCCACTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCAC
TCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTC
CCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGA
TGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGA
GAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATGACCCGAGAGA
10 GAAGTGTTAGAGTGGAGGTTTGACAGCCACCTAGCATTTTCATCACGTGGCCCGAGAG
CTGCATCCGGAGTACTTCAAGAACTGCTGA [SEQ ID NO: 60]

MVGFPVTPQV PLRPMTYKAA VDLSHFLKEK GGLEGLIHSQ RRQDILDLWI
YHTQGYFPDW QNYTPGPGVR YPLTFGWCYK LVPVEPKVE EANKGENTSL
15 LHPVSLHGMD DPEREVLEWR FDSVLAFFHV ARELHPEYFK NC*
[SEQ ID NO: 61]
```

20

25

30

Figure 8

Sequence of RT insert in 7077RT8

ATGGGCCCCATTAGCCCTATTGAGACTGTGTCTAGTAAAAATTAAAGCCAGGAATGGAT
 GGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATTAGTAGAA
 ATTTGTACAGAGATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAAATCCA
 5 TACAATACTCCAGTATTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAAATTA
 GTAGATTTTCAGAGAACCTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGA
 ATACCACATCCCGCAGGGTTAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGT
 GATGCATATTTTTCAGTTCCTTAGATGAAGACTTCAGGAAATATACCTGCATTTACC
 ATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCA
 10 CAGGGATGGAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAG
 CCTTTTAGAAAAACAAATCCAGACATAGTTATCTATCAATACATGGATGATTTGTAT
 GTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAGCTGAGACAA
 CATCTGTTGAGGTGGGGACTTACCACACCAGACAAAAACATCAGAAAGAACCTCCA
 TTCCTTTGGATGGGTATGAACCTCCATCTGATAAATGGACAGTACAGCCTATAGTG
 15 CTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGTGGGGAATTG
 AATTGGGCAAGTCAGATTTACCCAGGGATTAAAGTAAGGCAATTATGTAACTCCTT
 AGAGGAACCAAGCACTAACAGAAGTAATACCACTAACAGAAGAGCAGAGCTAGAA
 CTGGCAGAAAAAGAGAGATTCTAAAAGAACAGTACATGGAGTGTATTATGACCCA
 TCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAA
 20 ATTTATCAAGAGCCATTTAAAAATCTGAAAAACAGGAAAAATGCAAGAATGAGGGGT
 GCCCACACTAATCATGTAAAACAATTAACAGAGGCAGTGCAAAAAATAACCACAGAA
 AGCATAGTAATATGGGGAAAGACTCCTAAATTTAACTGCCCATACAAAAGGAAACA
 TGGGAAACATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTT
 GTTAATACCCCTCCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCATAGTA
 25 GGAGCAGAAACCTTCTATGTAGATGGGGCAGCTAACAGGGAGACTAAATTAGGAAAA
 GCAGGATATGTACTAATAGAGGAAGACAAAAAGTTGTACCCCTAACTGACACAACA
 AATCAGAACTGAGTTACAAGCAATTTATCTAGCTTTGCAGGATTTCGGGATTAGAA
 GTAAACATAGTAACAGACTCACAATATGCATTAGGAATCATTCAAGCACAACCAGAT
 CAAAGTGAATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAG
 30 GTCTATCTGGCATGGGTACCAGCACACAAAGCAATTGGAGGAAATGAACAAGTAGAT
 AAATTAGTCAGTGCTGGAATCAGGAAAGTACTATTTTATAGATTAA
 [SEQ ID NO: 62]

MGPISPIETV SVKLKPGMDG PKVKQWPLTE EKIKALVEIC TEMEKEBKIS
 35 KIGPENPYNT PVFAIKKKDS TKWRKLVDFR ELNKRQTQDFW BVQLGIPHPA
 GLKKKKSVTV LDVGDAYFSV PLDEDFRKYT APTIPSINNE TPGIRYQYNV
 LPQGWKGSPA IFQSSMTKIL EPFRKQNPDI VIYQYMDLQY VGSDEIGQH
 RTKIEELRQH LLRWGLTTPD KKHQKEPPFL WMGYELHDPK WTVQPIVLPE
 KDSWTVNDIQ KLVGKLNWAS QIYPGIKVRQ LCKLLRGTKA LTEVIPLTBE
 40 AELELAENRE ILKEPVHGVY YDPSKDLIAE IQKQGQGWQ YQIYQEPFKN
 LKTGKYARMR GAHTNDVKQL TEAVQKITTE SIVIWGKTPK FKLPIQKETW
 ETWWTYEQQA TWIPEWEFVN TPPLVKLWYQ LEKEPIVGAE TFYVDGAANR
 ETKLGKAGYV TNRGRQKVVT LTDITNQKTE LQAIYLALQD SGLEVNIVTD
 SQYALGIIQA QPDQSESELV NQIIEQLIKK EKVYLAWVPA HKGIGGNEQV
 45 DKLVSAIRK VLFLD*

WO 03/025003

PCT/EP02/10592

12/50

[SEQ ID NO: 63]

5

10

15

20

25

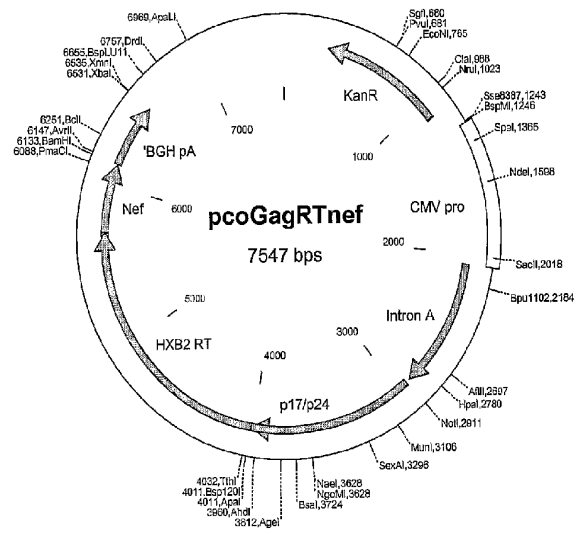
Figure 9

Sequence of the p17/24opt/RT/trNef insert in p17/24opt/RT/trNef13

5 ATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
 AGGCTGCGCCCGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCTCG
 AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGT
 CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGAGTGAAGAGCTGAGG
 TCCTTGTATAACACAGTGGCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAG
 10 GATACCAAGGAGGCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAG
 GCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCT
 ATTGTCCAAAACATTCAGGGCCAGATGGTTCATCAGGCCATCAGCCCCGGACGCTC
 AATGGCTGGGTGAAGGTTGTGGAAGAGAAGGCCCTTTTCTCCTGAGGTTATCCCCATG
 TTCTCCGCTTTGAGTGAGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACC
 GTGGGCGGCCATCAGGCGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
 15 GCGGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGGACAGATG
 CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCCCTCTACACTGCAAGAGCAAAATC
 GGATGGATGACCAACAATCTCCCATCCAGTTGGAGAAATCTATAAACGGTGGATC
 ATTCTCGGTCTCAATAAAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATT
 AGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTGACCGGTTTATAAGACCCTG
 20 CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTA
 CAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCCTAGGCCCGGCTGCCACC
 CTGGAAGAGATGATGACCGCCTGTGAGGGAGTAGCGGACCCGGACACAAAGCCAGA
 GTGTTGATGGGCCCCATTAGCCCTATTGAGACTGTGTGAGTAAATTAAGCCAGGA
 ATGGATGGCCCCAAAGTTAAACAATGGCCATTGACAGAAGAAAAATTAAGCATTAA
 25 GTAGAAATTTGTACAGAGATGGAAGGAAGGGAATAATTTCAAAAATTGGGCTGAA
 AATCCATACAACTACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGA
 AAATTAGTAGATTTCAGAGAACTTAATAAGAGAACTCAACACTTCTGGGAAGTTCAA
 TTAGGAATACCACATCCCGCAGGCTTAAAAAGAAAAATCAGTAACAGTACTGGAT
 GTGGGTGATGCATATTTTTCAGTTCCCTTAGATGAAGACTTCAGGAAATATACTGCA
 30 TTTACCATACCTAGTATAAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTG
 CTTCCACAGGGATGGAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATC
 TTGATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAGCTG
 AGACAACATCTGTGAGGTGGGACTTACCACACCAGACAAAAACATCAGAAAGAA
 35 CCTCCATTCTTTGGATGGGTTATGAACTCCATCCCTGATAAATGGACAGTACAGCCT
 ATAGTGCTGCCAGAAAAAGACAGCTGGACTGCAATGACATACAGAAGTTAGTGGGG
 AAATTGAATTGGGCAAGTCAGATTTACCCAGGGATTAAAGTAAGGCAATTATGTAAA
 CTCCTTAGAGGAACCAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAG
 CTAGAAGTGGCAGAAAACAGAGAGATTCTAAAAGAACAGTACATGGAGTGTATTAT
 40 GACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACA
 TATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAAAGGAAAAATGCAAGAATG
 AGGGGTGCCACACTAATGATGTAAACAATTAACAGAGGCAGTGCAAAAAATAACC
 ACAGAAAGCATAGTAATATGGGGAAGACTCCTAAATTTAACTGCCCATACAAAAG
 GAAACATGGGAACATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGG

GAGTTTGTAAATACCCCTCCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCC
 ATAGTAGGAGCAGAAACCTTCTATGTAGATGGGGCAGCTAACAGGGAGACTAAATTA
 GGAAAAGCAGGATATGTTACTAATAGAGGAAGACAAAAGTTGTCACCCCTAACTGAC
 ACAACAAATCAGAAGACTGAGTTACAAGCAATTTATCTAGCTTTGCAGGATTCGGGA
 5 TTAGAAGTAAACATAGTAACAGACTCACAATATGCATTAGGAATCATTCAGCACAA
 CCAGATCAAAGTGAATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAG
 GAAAAGGTCTATCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAA
 GTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTATTTTATAGATATGGTGGGT
 TTTCAGTCAACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGAT
 10 CTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCCTCCCAAAGA
 AGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGG
 CAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTAC
 AAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAAGAGGCCAATAAAGGAGAGAACACC
 AGCTTGTACACCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTTA
 15 GAGTGGAGGTTTGACAGCCACCTAGCATTTCATCAGTGGCCCGAGAGCTGCATCCG
 GAGTACTTCAAGAACTGCTGA [SEQ ID NO: 64]

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
 LETSEGRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIBIKDTKEA
 20 LDKIEEBQNK SKKKAQOAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAI SPR
 TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNMLNT VGGHQAAMQM
 LKETINEEAA EWDVRVHPVHA GPIAPGQMR PRGSDIAGTT STLQEIQI GWM
 TNNPPIPVGE IYKRWIILGL NKIVRMYSP SILDIRQGP EPFRDYVDRF
 YKTLRAEQAS QEVKNWMTET LLVQANPDC KTILKALGPA ATLEEMMTAC
 25 QGVGGPGHKA RVLMPISPI BTVSVKLKPG MDGPKVKQWP LTEEKIKALV
 EICTEMEKEG KISKIGPENP YNTPVFAIK KDSTKWRKLV DFRELNKRTQ
 DFWEVQLGIP HPAGLKKKKS VTVLDVGDAY FSVPLDEDPR KYTAFTIPSI
 NNETPGIRYQ YNVLPGQWKG SPAIFQSSMT KILEPFRKQN PDIVIQYMD
 DLYVGSDEI QHRTKIEEL RQHLLRWGLT TPDKKHQKEP PFLWMGYELH
 30 PDKWTVQPIV LPEKDSWTVN DIQKLVGKLN WASQIYPGIK VRQLCKLLRG
 TKALTEVIPL TEEAELELAE NREILKEPVH GVEYDPSKDL IAEIQKQGGG
 QWTYQIYQEP FKNLKTGKYA RMRGAHTNDV KQLTEAVQKI TTESIVIWGK
 TPKFKLPIQK ETWETWWTEY WQATWIPWEV FVNTPLPLVKL WYQLEKEPIV
 GAETFFYDGA ANRETKLGKA GYVTNRGRQK VVTLTDTTNQ KTELQAIYLA
 35 LQDSGLEVNI VTD SQYALGI IQAQPDQSES ELVNQIEQL IKKEKVYLA
 VPAHKGIGGN EQVDKLVSA IRKVLFLDMV GFPVTPQVPL RPMTYKAAVD
 LSHFLKEKGG LEGLIHSQRR QDILDLIWYH TQGYFPDWQN YTPGPGVRYP
 LTFGWCYKLV PVEPKVVEA NKGENTSLLH PVSLHGMDDP EREVLEWREF
 SHLAFHHVAR BLHPEYFKNC *
 40 [SEQ ID NO: 65]



5

10

15

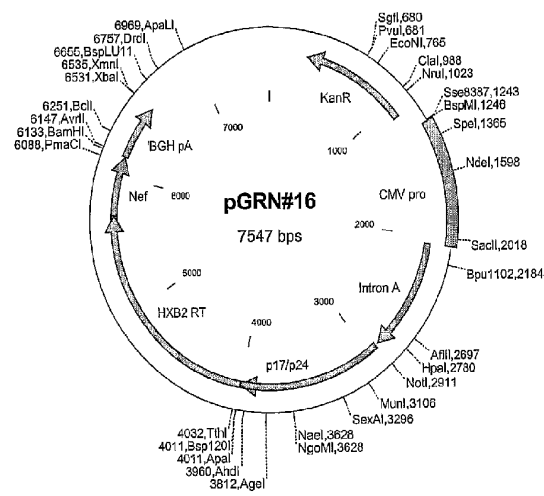
Figure 10

Sequence of the p17/p24opt(cor)/RT/trNef coding insert in WRG7077:

```
ATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
5 AGGCTGCGCCCGGAGGCAAAAAGAAATACAGCTCAAGCATATCGTGTGGCCCTCG
AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCCTGCTGGAAACATCTGAGGGATGT
CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGG
TCCTTGTATAACACAGTGGCTACCCCTCTACTGCGTACACCAGAGGATCGAGATTAAG
GATACCAAGGAGGCCCTTGACAAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAG
10 GCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAGAACTATCCT
ATTGTCCAAAACATTGAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGACGCTC
AATGCCTGGGTGAAGGTTGTGCAAGAGAAGGCCCTTTCTCCTGAGGTTATCCCCATG
TTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACC
GTGGGCGGCCATCAGGCCGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
15 GCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGACAGATG
CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAAATC
GGATGGATGACCAACAATCCTCCCATCCAGTTGGAGAAATCTATAACGGTGGATC
ATCCTGGGCTTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTTGACATT
AGACAGGGACCCAAAGAGCCCTTTAGGGATTACGTCGACCGGTTTATATAAGACCCCTG
20 CGAGCAGAGCAGGCCCTCTCAGGAGGTCAAAAACCTGATGACGGAGACACTCCTGGTA
CAGAACGCTAACCCGACTGCAAAACAATCTGAAGGCACTAGGCCCGGCTGCCACC
CTGGAAGAGATGATGACCGCCTGTGAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
GTGTTGATGGGCCCCATTAAGCCCTATTGAGACTGTGTGAGTAAATTAAGCCAGGA
ATGGATGGCCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATTA
25 GTAGAAATTTGTACAGAGATGAAAAGGAAGGGAAAATTTCAAAAATTTGGCCCTGAA
AATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGA
AAATTAGTAGATTTGAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAA
TTAGGAATACCATATCCCGCAGGGTTAAAAAAGAAAAATCAGTAACAGTACTGGAT
GTGGGTGATGCATATTTTTCAGTTCCCTTAGATGAAGACTTCAGGAAATATACTGCA
30 TTTACCATACCTAGTATAAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTG
CTTCCACAGGGATGGAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAATC
TTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGAT
TTGTATGATAGGATCTGACTTAGAAAATAGGGCAGCATAGAACAAAAATAGAGGAGCTG
AGACAACATCTGTTGAGGTGGGGACTTACCACACCAGACAAAAAACATCAGAAAGAA
35 CTCCATTCTTTGGATGGGTATGAACTCCATCCTGATAAATGGACAGTACAGCCT
ATAGTGTGCGCAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGTGGGG
AAATTGAATTGGGCAAGTCAGATTTACCCAGGGATTAAAGTAAGGCAATTATGTAAA
CTCCTTAGAGGAACCAAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAG
CTAGAAGTGGCAGAAAACAGAGAGATTCTAAAAGAACCAGTACATGGAGTGTATTAT
40 GACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACA
TATCAAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAAATATGCAAGAATG
AGGGGTGCCCACTAATGATGTAAACAAATTAACAGAGGCAGTGCAAAAAATAACC
ACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAACTGCCCATACAAAAG
GAAACATGGGAAACATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTTGAGTGG
45 GAGTTTGTAAATACCCCTCCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCC
```

ATAGTAGGAGCAGAAACCTTCTATGTAGATGGGGCAGCTAACAGGGAGACTAAATTA
 GGAAAAGCAGGATATGTTACTAATAGAGGAAGACAAAAAGTTGTCACCCTAACGTGAC
 ACAACAAATCAGAAGACTGAGTTACAAGCAATTTATCTAGCTTTGCAGGATTCGGGA
 TTAGAAGTAAACATAGTAACAGACTCACAATATGCATTAGGAATCATTCAAGCACAA
 5 CCAGATCAAAGTGAATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAG
 GAAAAGGTCTATCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAA
 GTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTATTTTATAGATATGGTGGGT
 TTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGAT
 CTTAGCCACTTTTAAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGA
 10 AGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGG
 CAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTAC
 AAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAAACACC
 AGCTTGTACACCCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTTA
 GAGTGGAGGTTTGACAGCCACCTAGCATTTCATCACGTGGCCCCGAGAGCTGCATCCG
 15 GAGTACTTCAAGAACTGCTGA [SEQ ID NO: 66]

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
 LETSEGRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
 20 LDKIEEBQNK SKKKAQQAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAISPR
 TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNMTLNT VGGHQAAMQM
 LKETINEEAA EWDVRHVPVHA GPIAPGQMR PRGSDIAGTT STLQEQIGWM
 TNNPPIPVGE IYKRWIILGL NKIVRMYST SILDIRQGPB EPFRDYVDRF
 YKTLRAEQAS QEVKNWMTET LLVQNPANPD KTLKALGPA ATLEEMMTAC
 25 QGVGGPGHKA RVLMPISPI ETVSVKLKPG MDGPKVKQWP LTEEKIKALV
 EICTEMEKEG KISKIGPENP YNTPVFAIKK KDSTKWRKLV DFRELNRKTQ
 DFWEVQLGIP HPAGLKKKKS VTVLDVGDAY FSVPLDEDFR KYTAPTIPSI
 NNETPGIRYQ YNVLPQGWKG SPAIFQSSMT KILEPFRKQN PDIVYQYMD
 DLYVGSDEI GQHRTKIEEL RQHLLRWGLT TPDKKHQKEP PFLWMGYELH
 30 PDKWTVQPIV LPEKDSWTVN DIQKLVGKLN WASQIYPGIK VRQLCKLLRG
 TKALTEVIPL TEEAELELAE NREILKEPVH GVYDPSKDL IAEIQKQGGG
 QWTYQIYQEP FKNLTKGYA RMGAHTNDV KQLTEAVQKI TTESIVIWGK
 TPKFKLPIQK ETWETWTEY WQATWIPEWE FVNTPLVLKL WYQLEKEPIV
 GAETFFYDGA ANRETKLGKA GYVTNRGRQK VVTLTDTTNQ KTELQAIYLA
 35 LQDSGLEVNI VTDQYALGI IQAQPQSES ELVNQIEQL IKKEKVYLAW
 VPAHKGIGGN EQVDKLVSAQ IRKVLFLDMV GFPVTPQVPL RPMTYKAAVD
 LSHFLKEKGG LEGLIHSQRR QDILDWLYH TQGYFPDWQN YTPGPGVRYF
 LTFGCWYKLV PVEPDKVEEA NKGENTSLLH PVSLHGMDDP EREVLEWRFD
 SHLAFHHVAR BLHPEYFKNC *
 40 [SEQ ID NO: 67]



5

10

15

Figure 11

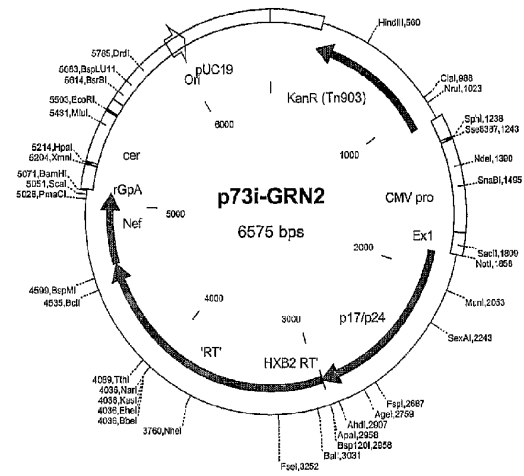
Sequence of the p17/p24(opt)/RT(opt)trNef insert in p73i-GRN2:

ATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGA
5 GAAAATTAGGCTGCGCCCGGAGGCAAAAAGAAATACAAGCTCAAGCAT
ATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCT
GCTGGAAACATCTGAGGGATGTCGCCAGATCCTGGGGCAATTGCAGCCAT
CCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTGGCT
ACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGC
10 CTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAGGCCAG
CAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCC
TATTGTCCAAAACATTTCAGGGCCAGATGGTTCATCAGGCCATCAGCCCC
GGACGCTCAATGCCTGGGTGAAGGTTGTCTGAAGAGAAGGCCTTTCTCCT
GAGGTTATCCCCATGTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGAC
15 CTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAT
GTTGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCAT
CCCGTCCACGCTGGCCCAATCGCGCCCGGACAGATGCGGGAGCCTCGCGG
CTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAAATCGGATGGA
TGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATC
20 ATCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTT
GACATTAGACAGGGACCCAAAGAGCCTTTAGGGATTACGTCGACCGGTT
TTATAAGACCCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGA
TGACGGAGACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATC
TTGAAGGCACTAGGCCCGGCTGCCACCCTGGAAGAGATGATGACCGCCTG
25 TCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGCCCC
ATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGACGG
CCCCAAGGTCAAGCAGTGGCCACTCACCGAGGAGAAGATCAAGGCCCTG
GTGGAGATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCG
GGCCTGAGAACCACATAACAACCCCGTGTTTGCCATCAAGAAGAAGGAC
30 AGCACCAAGTGGCGCAAGCTGGTGGATTTCGGGAGCTGAATAAGCGGAC
CCAGGATTTCTGGGAGGTCCAGCTGGGCATCCCCATCCGGCCGGCCTGA
AGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCAGC
GTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACCATCCCATCT

ATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGTCCTCCCCCA
GGGCTGGAAGGGCTCTCCCGCCATTTCCAGAGCTCCATGACCAAGATCC
TGGAGCCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATG
GACGACCTGTACGTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAA
5 GATTGAGGAGCTGAGGCAGCATCTGCTGAGATGGGGCCTGACCACTCCGG
ACAAGAAGCATCAGAAGGAGCCGCCATTCTGTGGATGGGCTACGAGCTC
CATCCCGACAAGTGGACCGTGCAGCCTATCGTCCTCCCGAGAAGGACAG
CTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTCAACTGGGCTA
GCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTGCGC
10 GGCACCAAGGCCCTGACCGAGGTGATTCCCCTCACGGAGGAAGCCGAGCT
CGAGCTGGCTGAGAACC GGGAGATCCTGAAGGAGCCCGTGACCGCGTGT
ACTATGACCCCTCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAG
GGGCAGTGGACATAACGATTTACCAGGAGCCTTTCAAGAACCTCAAGAC
CGGCAAGTACGCCC GCATGAGGGGCGCCACACCAACGATGTCAAGCAG
15 CTGACCGAGGCCGTCCAGAAGATCACGACCGAGTCCATCGTGATCTGGGG
GAAGACACCCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGT
GGTGGACCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTCTGTG
AATACACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCCAT
CGTGGGCGCGGAGACATTCTACGTGGACGGCGCGGCCAACCGCGAAACA
20 AAGCTCGGGAAGGCCGGGTACGTCACCAACCGGGGCCCGCCAGAAGGTCG
TCACCTGACCGACACCACCAACCAGAAGACGGAGCTGCAGGCCATCTAT
CTCGCTCTCCAGGACTCCGGCCTGGAGGTGAACATCGTGACGGACAGCCA
GTACGCGCTGGGCATTATTTCAGGCCCAGCCGACCAAGTCCGAGAGCGAAC
TGGTGAACCAGATTATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTC
25 GCCTGGGTCCCGGCCCATAGGGCATTGGCGGCAACGAGCAGGTCGACAA
GCTGGTGAGTGCGGGGATTAGAAAGGTGCTGATGGTGGGTTTTCCAGTCA
CACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTT
AGCCACTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACCTCCA
AAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACT
30 TCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTG
ACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGA
AGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGC

ATGGGATGGATGACCCGGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAG
CCG CCTAGCATTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCA
AGAACTGCTGA [SEQ ID NO: 68]

5 MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
LETSEGCQRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
LDKIEEEQNK SKKKAQQAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAISPR
TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNMTLNT VGGHQAAMQM
LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQIGWM
10 TNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPB EPFRDYVDRF
YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMMTAC
QGVGGPGHKA RVLMPISPI ETVSVKLKPG MDGPKVKQWP LTEEKIKALV
EICTEMEKEG KISKIGPENP YNTPVFAIKK KDSTKWRKLV DFRBLNKRITQ
DFWEVQLGIP HPAGLKKKKS VTVLVGDVAY FSVPLDEDFR KYTAFITPSI
15 NNETPGIRYQ YNVLPGQWKG SPAIFQSSMT KILEPFRKQN PDIVYQYMD
DLYVGSDEI GQHRTKIEL RQHLLRWGLT TPDKKHQKEP PFLWMGYELH
PDKWTVQPIV LPEKDSWTVN DIQKLVGKLN WASQIYPGIK VRQLCKLLRG
TKALTEVIPL TEEAELELAE NREILKEPVH GYYDPSKDL IAEIQKQGGG
QWTYQIYQEP FKNLKTGKYA RMRGAHTNDV KQLTEAVQKI TTESIVIWVK
20 TPKFKLPIQK ETWETWWTBY WQATWIPWE FVNTPLVLKL WYQLEKEPIV
GAETFYVDGA ANRETCLGKA GYVTNRGRQK VVTLTDTTNQ KTELQAIYLA
LQDSGLEVNI VTDSQYALGI IQAQPDSSES ELVNQIIEQL IKKEKVYLA
VPAHKGIGGN EQVDKLVSAK IRKVLVVGFP VTPQVPLRPM TYKAAVDLSH
FLKEKGLEG LIHSQRRQDI LDLWIYHTQG YFPDWQNYTP GPGVRYPLTF
25 GWCYKLPVE PDKVEEANKG ENTSLLEHVS LHGMDDPERE VLEWRFDSE
AFHHVARELH PEYFKNC*
[SEQ ID NO: 69]



5

10

15

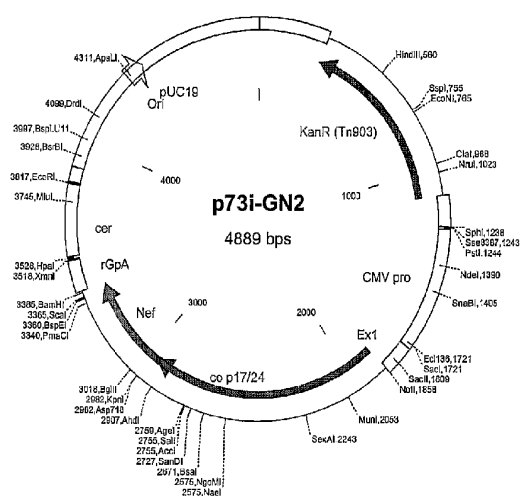
Figure 12**Sequence of the p17/p24opt/trNef insert in p73i-GN2:**

```

ATGGGTGCCCGAGCTTCGGTACTGTCCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
5  AGGCTGCGCCCGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCG
   AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGT
   CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGG
   TCCTTGTATAACACAGTGGCTACCCCTCTACTGCGTACACCAGAGGATCGAGATTAAG
   GATACCAAGGAGGCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAG
10  GCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCT
   ATTTCCAAAACATTAGGGCCAGATGGTTCATCAGGCCATCAGCCCCCGGACGCTC
   AATGCCTGGGTGAAGGTTGTGCGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATG
   TTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACC
   GTGGCGGGCATCAGGCCGCGCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
15  GCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGGACAGATG
   CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAAATC
   GGATGGATGACCAACAATCCTCCATCCCAGTTGGAGAAATCTATAAACCGTGGATC
   ATTTCCGGTCTCAATAAAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATT
   AGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTCGACCGGTTTTATAAGACCTTG
20  CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAACCTGGATGACGGAGACACTCCTGGTA
   CAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACC
   CTGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
   GTGTTGATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTAC
   AAGGAGCTGTAGATCTTAGCCACTTTTAAAGAAAGGGGGGACTGGAAGGGCTA
25  ATTTACTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGC
   TACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACC
   TTTGGATGGTGTCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAAT
   AAAGGAGAGAACCACAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATGACCCG
   GAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCTTAGCATTTTCATCACGTGGCC
30  CGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGA [SEQ ID NO: 70]

MGARASVLSG  GELDRWEKIR  LRPGGKKKYK  LKHIVWASRE  LERFAVNPGL
LETSBEGCRQI  LGQLQPSLQT  GSEELRSLYN  TVATLYCVHQ  RIEIKDTKEA
35  LDKIEEEQNK  SKKKAQQAAA  DTGHSNQVSQ  NYPIVQNIQG  QMVHQAI SPR
   TLNAWVKVVE  EKAFSPEVIP  MFSALSEGAT  PQDLNTMLNT  VGGHQAAMQM
   LKETINEEAA  EWDRVHPVHA  GPIAPGQMRE  PRGSDIAGTT  STLQEQIGWM
   TNNPPIPVGE  IYKRWIILGL  NKIVRMYSPT  SILDIRQGPK  EPFRD YVDRF
   YKTLRAEQAS  QEVKNWMTET  LLVQANPDC  KTI LKALGPA  ATLEEMMTAC
40  QGVGGPGHKA  RVL MVGFPVT  PQVPLRPMTY  KAAVDLSHFL  KEKGGLEGLI
   HSQRRQDILD  LWIYHTQGYF  PDWQNYTPGP  GVRYP LTFGW  CYKLVPVEPD
   KVEEANKGEN  TSL LHPVSLH  GMDDPEREVL  EWRFD SRLAF  HHVARELHPE
   YFKNC* [SEQ ID NO: 71]

```



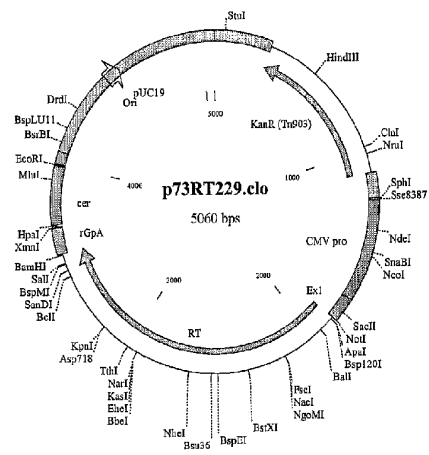
5

10

Figure 13**Sequence of RT w229k:**

ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGACGGCCC
5 CAAGGTCAAGCAGTGGCCACTCACCAGGAGAAGATCAAGGCCCTGGTGGAGATCTGCACCG
AGATGGAGAAAAGAGGGCAAGATCAGCAAGATCGGGCTGAGAACCCATACAACACCCCGTG
TTTGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGATTTCGGGAGCTGAA
TAAGCGGACCCAGGATTCTGGGAGGTCCAGCTGGGCATCCCCATCCGGCCGGCTGAAGA
AGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCAGCGTCCCTCTGGACGAG
10 GACTTTAGAAAGTACACCGCCTTTACCATCCCATCTATCAACAACGAGACCCCTGGCATCAG
ATATCAGTACAACGTCTCCCCAGGGCTGGAAGGGCTCTCCCGCATTTCAGAGCTCCA
TGACCAAGATCCTGGAGCCGTTTCGGAAGCAGAACCCGATATCGTCATCTACCACTACATG
GACGACCTGTACGTGGGCTCTGACCTGGAATCGGGCAGCATCGCACGAAGATTGAGGAGCT
GAGGCAGCATCTGCTGAGATGGGGCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGC
15 CATTCCTgaagATGGGCTACGAGCTCCATCCGACAAGTGGACCGTGCAGCCTATCGTCCTC
CCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTCAACTGGGC
TAGCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTGCGCGGCACCAAGG
CCCTGACCGAGGTGATTCCCCTCACGGAGGAAGCCGAGCTCGAGCTGGCTGAGAACCGGGAG
ATCCTGAAGGAGCCCGTGCACGGCGTGTAATGACCCCTCCAAGGACCTGATCGCCGAAAT
20 CCAGAAGCAGGGCCAGGGCAGTGGACATACAGATTTACCAGGAGCCTTTCAAGAACCTCA
AGACCGGCAAGTACGCCCGCATGAGGGCGGCCACACCAACGATGTCAAGCAGCTGACCGAG
GCCGTCCAGAAGATCACGACCGAGTCCATCGTGATCTGGGGGAAGACACCAAGTTCAAGCT
GCCTATCCAGAAGGAGACCTGGGAGACGTGGTGGACCGAATATTGGCAGGCCACCTGGATTTC
CCGAGTGGGAGTTCTGTGAATACACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAG
25 CCCATCGTGGGCGCGGAGACATTTACGTGGACGGCGCGGCCAACCGGAAACAAAGCTCGG
GAAGGCCGGGTACGTACCAACCGGGCCGCCAGAAGTCTGTACCCCTGACCGACACCACCA
ACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAGGTGAAC
ATCGTGACGGACAGCCAGTACGCGCTGGGCATTATTAGGGCCAGCCGACAGTCCGAGAG
CGAACTGGTGAACCAGATTATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTCGCCTGGG

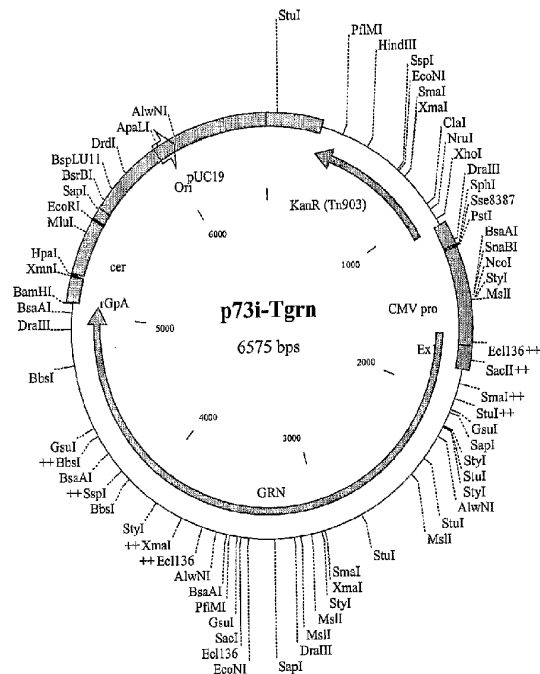
TCCCGGCCCATAGGGCATTGGCGGCAACGAGCAGGTCGACAAGCTGGTGAGTGCGGGGATT
AGAAAGGTGCTGTAA [SEQ ID NO: 72]



5

10

15



5 ATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAAATT
AGGCTGCGCCCCGGGAGGCCAAAAAGAAATAACAAGCTCAAGCATATCGTGTGGCCCTCG
AGGGAGCTTGACCGGTTTTCCGTTGAACCCAGGCCCTCTGGAAACATCTGAGGGATGT
CGCCAGATCTTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGG
10 TCCTTTGTATAACACAGTGGCTACCTCTACTCGTTACCCAGAGGATCGAGATTAAAG
GATACCAAGGAGGCCCTTGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAAG
GCCACGACGGCAGCTGTGACACTGGGCATAGCAACCGATTACAGAACTATCTCT
ATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCCTCAGCCCCGGACGCTC
AATGCTTGGGTGAAGGTTGTCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATG
15 TTCTCCGCTTTGAGTGAAGGGGCCACTCTCAGGACCTCAATACAAATGCTTAATACC
GTGGGCGGCCATCAGGCCGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
GCCGAGTGGGACAGAGTGACATCCGTCACGCTGGCCCAATCGCGCCGACAGATG
CGGGAGCCTCGCGCTCTGACATTGTCGCGGACCACTCTACACTGCAAGAGCAAACT
GGATGGATGACCAACAATCTCCCATCCAGTTGGAGAAATCTATAAACGGTGGATC

ATCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTTGACATT
AGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTGACCGGTTTATAAGACCCTG
CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTA
CAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACC
5 CTGGAAGAGATGATGACCGCCTGTGAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
GTGTTGATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGG
ATGGACGGCCCCAAGGTCAAGCAGTGGCCACTCACCAGGAGAGAAGATCAAGGCCCTG
GTGGAGATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCTGAG
AAGCCATACAACACCCCGTGTGTTGCCATCAAGAAGAAGGACAGCACCAGTGGCGC
10 AAGCTGGTGGATTTCGCGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAG
CTGGGCATCCCCATCCGGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGTGAC
GTGGCGACGCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCC
TTTACCATCCCATCTATCAACAACGAGACCCCTGCGATCAGATATCAGTACAACGTC
CTCCCCAGGGCTGGAAGGGCTCTCCCGCCATTTTCAGAGCTCCATGACCAAGATC
15 CTGGAGCCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACAGTACATGGACGAC
CTGTACGTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAAGATTGAGGAGCTG
AGGCAGCATCTGCTGAGATGGGGCCTGACCACTCCGGACAAGAAGCATCAGAAGGAG
CCGCCATTCTGaaGATGGGCTACGAGCTCCATCCCGACAAGTGGACCGTGCAGCCT
ATCGTCTCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGC
20 AAGCTCAACTGGGCTAGCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAG
CTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGATTCCCTCAGCGAGGAAGCCGAG
CTCGAGCTGGCTGAGAACCGGAGATCCTGAAGGAGCCCGTGACGGCGTGTACTAT
GACCCCTCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAGGGGCACTGGACA
TACCAGATTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAGTACGCCCGCATG
25 AGGGGCGCCACACCAACGATGTCAAGCAGCTGACCGAGGCCGTCAGAAAGATCACG
ACCGAGTCCATCGTGATCTGGGGGAAGACACCCAAGTTCAAGCTGCCTATCCAGAA
GAGACCTGGGAGACGTGCTGACCGAATATTTGGCAGGCCACCTGGATTCCCGAGTGG
GAGTTCTGTAATACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCC
ATCGTGGGCGCGGAGACATTCTACGTGGACGGCGCGGCCAACCGCAAAACAAGCTC
30 GGGAAGGCCGGGTACGTACCAACCGGGGCGCCAGAAGGTCTGTCACCTGACCGAC
ACCAACCAACGAGACCGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGC
CTGGAGGTGAACATCGTGACGGACAGCCAGTACCGCTGGGCAITATTCAGGCCAG
CCGGACCACTCCGAGAGCGAAGTGGTGAACAGATTATCGAGCAGCTGATCAAGAAA
GAGAAGGTCTACCTCGCTGGGTCCCGGCCATAAGGGCATTTGGCGCAACGAGCAG
35 GTCGACAAGCTGGTGAGTGGGGGATTAGAAAGTGCTGATGCTGGGTTTTCAGTC
ACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGAGCTGTAGATCTTAGCCAC
TTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGACAGAT
ATCCTTGATCTGTGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTAC
ACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGTACAAGCTAGTA
40 CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTA
CACCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTAGAGTGGAGG
TTTGACAGCCGCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTC
AAGAACTGCTGA [SEQ ID NO: 73]

45

5 MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
 LETSEGCRQI LGQLQPSLOT GSEELRSLYN TVATLYCVHQ RIBIKDTKEA
 LDKIEEEQNK SKKKAQAAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAI SPR
 TLNAWVKVVE BKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAQM
 LKETINBEAA BWDRVHEVHA GPIAPQMRE PRGSDIAGTT STLQEQIGWM
 TNNPPIPVGE IYKRWII LGL NKIVRMYSP T SILDIRQGPK EPFRDYVDRF
 YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTLKALGPA ATLEEMMTAC
 QGVGGPGHKA RVLMPISPI ETVSVKLKPG MDGPKVKQWP LTEEKIKALV
 10 EICTEMBEKEG KISKIGPENP YNTPVFAIKK KDSTKWRKLV DFRELNKRTQ
 DFWEVQLGIP HPAGLKKKKS VTVLDVGDAY FSVPLDEDFR KYTAFTIPSI
 NNETPGIRYQ YNVLPQGKKG SPAIFQSSMT KILEPFRKQN PDIVIQYMD
 DLYVGSLEI GQHRTKIEEL RQHLLRWGLT TPDKKHQKEP PFLWMGYELH
 PDKWTVPPIV LPEKDSWTVN DIQKLVGKLN WASQIYPGIK VRQLCKLLRG
 15 TKALTEVIPL TBEABELEAE NREILKEPVH GVVYDPSKDL IAEIQKQCG
 QWTYQIYQEP FKNLKTGKYA RMRGAHTNDV KQLTEAVQKI TTESIWIWVK
 TPKFKLPIQK ETWETWWTEY WQATWIPBWE FVNTPLVKL WYQLEKEPIV
 GAETFYVDGA ANRETKLGKA GYVTNRGRQK VVTLTDTTNQ KTELQAIYLA
 LQDSGLEVNI VIDSQYALGI IQAQPQSES ELVNQIIEQL IKKEKVYLA
 20 VPAHKGIGGN EQVDKLVSA G IRKVLVGVFP VTPQVPLRPM TYKAAVDLSH
 FLKEKGGLEG LIHSQRRQDI LDLWIYHTQG YFPDWQNYTP GPGVRYPLTF
 GWCYKLVPE PDKVEEANKG ENTSLHPVS LHGMDDPERE VLEWRFDSRL
 AFHHVARELH PEYFKNC*
 [SEQ ID NO: 74]

25

30

35

Figure 15**Sequence of Tnrg:**

ATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCA
 5 GCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCAC
 TCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTC
 CCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGA
 TGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGA
 GAGAACACCAGCTGTACACCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGA
 10 GAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCACGTGGCCCGAGAG
 CTGCATCCGGAGTACTTCAAGAACTGCATGGGCCCCATCAGTCCCATCGAGACCGTG
 CCGGTGAAGCTGAAACCCGGGATGGACGGCCCCAAGGTCAAGCAGTGCCCACTCACC
 GAGGAGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAAGAGGGCAAG
 ATCAGCAAGATCGGGCCTGAGAACCCATACAACACCCCGTGTGTGCCATCAAGAAG
 15 AAGGACAGCCCAAGTGGCGCAAGCTGGTGGATTTCGGGAGCTGAATAAGCGGACC
 CAGGATTTCTGGGAGGTCCAGCTGGGCATCCCCCATCCGGCCGGCCTGAAGAAGAAG
 AAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCAGCGTCCCTCTGGACGAG
 GACTTTAGAAAGTACACCGCCTTTACCATCCCATCTATCAACAACGAGACCCCTGGC
 ATCAGATATCAGTACACGTCTCCCCAGGGCTGGAAGGGCTCTCCCGCCATTTTC
 20 CAGAGCTCCATGACCAAGATCCTGGAGCCGTTTCGGAAGCAGAACCCCGATATCGTC
 ATCTACCAGTACATGGACGACCTGTACGTGGGCTCTGACCTGGAATCGGGCAGCAT
 CGCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGATGGGGCCTGACCACTCCG
 GACAAGAAGCATCAGAAGGAGCCGCCATTCTGaaGATGGGCTACGAGCTCCATCCC
 GACAAGTGGACCGTGACGCTATCGTCTCCCCGAGAAGGACAGCTGGACCGTGAAC
 25 GACATCCAGAAGCTGGTGGCAAGCTCAACTGGGCTAGCCAGATCTATCCCGGATC
 AAGGTGCGCCAGCTCTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGATT
 CCCCTCACGGAGGAAGCCGAGCTCGAGCTGGCTGAGAACCGGGAGATCTTGAAGCAG
 CCCGTGCACGGCGTGTACTATGACCCCTCCAAGGACCTGATCGCCGAAATCCAGAAG
 CAGGGCCAGGGGAGTGGACATACCAGATTTACCAGGAGCCTTTCAAGAACCTCAAG
 30 ACCGGCAAGTACGCCCGCATGAGGGGCGCCACACCAACGATGTCAAGCAGCTGACC
 GAGGCCGTCCAGAAGATCACGACCGAGTCCATCGTGATCTGGGGGAAGACCCCAAG
 TTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGTGGTGGACCGAATATTGGCAG
 GCCACCTGGATTCCCGAGTGGGAGTTCTGTGAATACACCTCCTCTGGTGAAGCTGTGG
 TACCAGCTCGAGAAGGAGCCCATCGTGGGCGCGGAGACATTCTACGTGGACGGCGCG
 35 GCCAACCGCGAAACAAAGCTCGGGAAGGCCGGGTACGTCAACCAACGGGGCCGCGCAG
 AAGGTCTGTACCCCTGACCGACACCAACCAACCAGAAGACGGAGCTGCAGGCCATCTAT
 CTGCTCTCCAGGACTCCGGCCTGGAGGTGAACATCGTGACGGACAGCCAGTACGCG
 CTGGGCATTATTAGGCCACGCGGACAGTCCGAGAGCGAACTGGTGAACAGATT
 ATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTCGCCTGGGTCCCGGCCCATAG
 40 GGCATTGGCGGCAACGAGCAGGTTCGACAAGCTGGTGGTGGAGAGCTGGACAGATGGGAGAAA
 ATTAGGCTGCGCCCGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCC
 TCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAACATCTGAGGGA
 TGTGCGCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGAGTGAAGAGCTG
 45 AGGTCTTGTATAACACAGTGGCTACCTCTACTGCGTACACCAGAGGATCGAGATT

AAGGATACCAAGGAGGCCTTGGACAAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAG
 AAGGCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTAT
 CCTATTGTCCAAAACATTTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCGGACG
 CTCAATGCCTGGGTGAAGTTGTGGAAGAGAAGGCCTTTTCTCCTGAGGTATATCCCC
 5 ATGTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAAT
 ACCGTGGGCGGCCATCAGGCCGCCATGCAAATGTTGAAGGAGACTATCAACGAGGAG
 GCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGGACAG
 ATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAA
 ATCGGATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGG
 10 ATCATCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTTGAC
 ATTAGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTCGACCGGTTTTATAAGACC
 CTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTG
 GTACAGAACGCTAACCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCC
 ACCCTGGAAGAGATGATGACCGCCTGTGAGGGAGTAGGCGGACCCGGACACAAAGCC
 15 AGAGTGTGTGA [SEQ ID NO: 75]

MVGFPPVTPQV PLRPMTYKAA VDLSHFLKEK GGLEGLIHSQ RRQDILDWI
 YHTQGYFPDW QNYTPGPGVR YPLTFGWYK LVPVEPDKVE EANKGENTSL
 20 LHPVSLHGMD DPEREVLEWR FDSRLAFHHV ARELHPEYFK NCMGPISPIE
 TVSVKLKPGM DGPVKQWPL TEEKIKALVE ICTEMEKEGK ISKIGPENPY
 NTPVFAIKKK DSTKWRKLVD FRELNKRTQD FWEVQLGIPH PAGLKKKKS
 TVLDVGDAYF SVPLDEDFRK YTAFTIPSIN NETPGIRYQY NVLPQGWKGS
 PAIFQSSMTK ILEPFRKQNP DIVIYQYMD LYVGSDEIG QHRTKIEELR
 25 QHLLRWGLTT PDKKHQKEPP FLWMGYELHP DKWTVQPIVL PEKDSWTVND
 IQKLVGKLNW ASQIYPGIKV RQLCKLLRGT KALTEVIPLT BEAELELAEN
 REILKEPVHG VYYDPSKDLI AEIQKQGQGG WTYQIYQEPF KNLKTGKYAR
 MRGAHTNDVK QLTEAVQKIT TESIVIWGKT PKFKLPIQKE TWETWWTEYW
 QATWIPEWEF VNTPLVLKLW YQLEKEPIVG AETFYVDGAA NRETKLGKAG
 30 YVTNRGRQKV VILTDITNOK TELQAIYLAL QDSGLEVNIV TDSQYALGII
 QAQPDQSESE LVNQIIEQLI KKEKVYLAWV PAHKGIGGNE QVDKLVSAGI
 RKVLMGARAS VLSGGELDRW EKIRLRPGGK KKYKLKHIVW ASRELERFAV
 NPGLLETSEG CRQILGQLQP SLQTGSEELR SLYNTVATLY CVHQRIEIKD
 TKEALDKIEE EQNKSKKKAQ QAAADTGHSN QVSQNYPIVQ NIQGMVHQA
 35 ISPRTLNAWV KVVBEKAFSP EVIPMFSALS EGATPQDLNT MLNTVGGHQA
 AMQMLKETIN BEAAEWDVRH PVHAGPIAPG QMREPRGSDI AGTTSTLQEQ
 IGWMTNNPPI PVGEIYKRWI ILGLNKIVRM YSPTSILDIR QGPKBPFRDY
 VDRFYKTLRA EQASQEVKNW MTEFLLVQNA NPDCKTILKA LGPAATLEEM

MTACQGVGGP GHKARVL* [SEQ ID NO: 76]

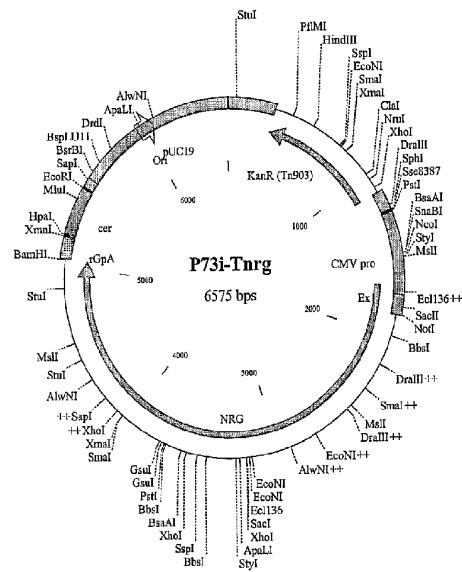


Figure 16

Sequence of the Tngr insert in p7313ie:

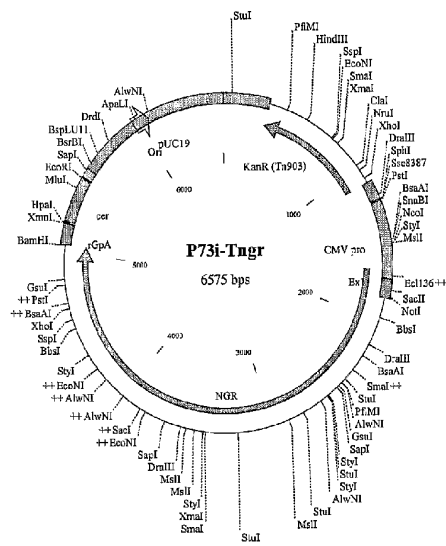
```

5  ATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGT
   AGATCTTAGCCACTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAA
   GACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATGGCAGAAC
   TACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGTCTACAAGCTAGTACC
   AGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTACACCCGTG
   TGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGC
10  CTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCATGGGTGC
   CCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGG
   GAGGCAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTT
   GCCGTGAACCCAGGCTGTGGAACATCTGAGGGATGTGCGCAGATCCTGGGGCAATTGCA
   GCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGTCTCTTGTATAACACAGTGGCTACCCCTCT
15  ACTGCGTACACCAGAGGATCGAGATTAAAGGATACCAAGGAGGCCCTTGGACAAAATTGAGGAG
   GAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCA
   GGTATCACAGAACTATCCTATTGTCCAAACATTGAGGCCAGATCGTTTCATCAGGCCATCA
   GCCCCCGGACGCTCAATGCCCTGGGTGAAGGTTGTGGAAGAGAAGGCCCTTTCTCCTGAGGTT
   ATCCCATGTTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAA
20  TACCGTGGGCGGCCATCAGGCGGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCAG
   CCGAGTGGGACAGAGTGCATCCCGTCCACGCTGCCCCAATCGCGCCCGGACAGATGCGGGAG
   CCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAAATCGGATGGATGAC
   CAACAATCCTCCCATCCAGTTGGAGAAATCTATAAACGGTGGATCATCCTGGGCCTGAACA
   AGATCGTGGCATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCT
25  TTTAGGGATTACGTGACCGGCTTTTATAAGACCCGTGCGAGCAGAGCAGGCCCTCTCAGGAGGT
   CAAAACTGGATGACGAGACACTCCTGGTACAGAACGCTAACCCGACTGCAAAACAATCT
   TGAAGGCATAGGCCCGGCTGCCACCTGGAAGAGATGATGACCGCTGTGTCAGGGAGTAGGC
   GGACCCGGACACAAAGCCAGAGTGTGTGATGGGCCCATCAGTCCCATCGAGACCGTGCCGCT
   GAAGCTGAACCCGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCACTCACCGAGGAGAAGA
30  TCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGG
   CCTGAGAACCATAACAACCCCCGTTTGGCCATCAAGAAGAAGACAGCACCAAGTGGCG
   CAAGCTGGTGGATTTCGGGAGCTGAATAAGCGGACCCAGGATTCTGGGAGGTCCAGCTGG
   GCATCCCCCATCGGCGCGCTGAAGAAGAAGAAGAGCGTGACCGTGTCTGGACGTGGGCGAC
   GCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACCATCCCATC
35  TATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGTCTCCCCAGGGCTGGAAGG
   GCTCTCCCGCCATTTTCCAGAGCTCCATGACCAAGATCCTGGAGCCGTTTCGGAAGCAGAAC
   CCCGATATCGTCATCTACCAGTACATGGACGACCTGTACGTGGGCTCTGACCTGGAAATCGG
   GCAGCATCGCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGATGGGGCTGACCACTC
   CGGACAAGAAGCATCAGAAGGAGCCGCCATTCTTgaaGATGGGCTACGAGCTCCATCCCGAC
40  AAGTGGACCGTGCAGCCTATCGTCTCCCCGAGAAGGACAGCTGGACCGTGAAACGACATCCA
   GAAGCTGGTGGGCAAGCTCAACTGGGCTAGCCAGATCTATCCGGGATCAAGGTGCGCCAGC
   TCTGCAAGCTGCTGCGGGCACCAAGGCCCTGACCGAGGTGATTCCCTCACGGAGGAAGCC
   GAGCTCGAGCTGGCTGAGAACCGGGAGATCCTGAAGGAGCCGTCACGGCGTGTACTATGA
   CCCCTCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAGGGGCAGTGGACATACCAGA
45  TTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAGTACGCCCGCATGAGGGCGCCAC

```

ACCAACGATGTCAAGCAGCTGACCGAGGCCGTCAGAGATCAGACCGAGTCCATCGTGAT
 CTGGGGGAAGACACCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGTGGTGGA
 CCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTCGTGAATACACCTCCTCTGGTG
 AAGCTGTGGTACCAGCTCGAGAAGGAGCCCATCGTGGGCGCGGAGACATTCTACGTGGACGG
 5 CGCGGCCAACCGCGAAACAAAGCTCGGGAAGGCCGGGTACGTACCAACCGGGGCCCGCAGA
 AGGTGCTCACCTGACCGACACCACCAACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCT
 CTCCAGGACTCCGGCCTGGAGGTGAACATCGTGACGACAGCCAGTACGCGCTGGGCATTAT
 TCAGGCCAGCCGACAGTCCGAGAGCGAACTGGTGAACCAGATTATCGAGCAGCTGATCA
 AGAAAGAGAAGGTCTACCTCGCTGGGTCCCGGCCATAAGGGCATTGGCGGCAACGAGCAG
 10 GTCGACAAGCTGGTGAGTGCGGGGATTAGAAAGGTGCTGTAA [SEQ ID NO: 77]

MVGFVPTPV PLRPMTYKAA VDLSHFLKEK GGLEGLIHSQ RRQDILDWI
 YHTQGYFPDW QNYTPGPGVR YPLTFGWYK LVPVEPKVE EANKGENTS
 15 LHPVSLHGMD DPEREVLEWR FDSRLAFHHV ARELHPEYFK NCMGARASVL
 SGGELDRWEK IRLRPGGKKK YKLKHIVWAS RELERFAVNP GLEETSEGR
 QILGQLQPSL QTGSEELRSL YNTVATLYCV HQRIEIKDTK EALDKIEEQ
 NKSKKKAQQA AADTGHSNQV SQNYPIVQNI QGQMVHQAIS PRTLNAWVKV
 VEEKAFSPEV IPMFSALSEG ATPQDLNML NTVGGHQAAM QMLKETINEE
 20 AAEWDRVHPV HAGPIAPGQM REPRGSDIAG TTSTLQEQIG WMTNNPPIPV
 GEIYKRWIL GLNKIVRMY SPTSILDIRG PKEPFRDYVD RFYKTLRAEQ
 ASQEVKNWMT ETLLVQNANP DCKTILKALG PAATLEEMMT ACQGVGGPGH
 KARVLMGPIS PIETVSVKLK PGMDGPKVKQ WPLTEEKIKA LVEICTEMEK
 EGKISKIGPE NPYNTPVFAI KKKDSTKWRK LVDPRELNKR TQDFWEVQLG
 25 IHPAGLKKK KSVTVLDVGD AYFSVPLDED FRKYTAFTIP SINNETPGIR
 YQYNVLPQGW KGSPAIFQSS MTKILEPFRK QNPDIVIYQY MDDLIVGSDL
 EIGQHRKIE ELROHLLRWG LTPDKKHQK EPPFLWMGYE LHPDKWTVQP
 IVLPEKDSWT VNDIQKLVGK LNWASQIYPG IKVRQLCKLL RGTALTEVI
 PLTEEALEL AENREILKEP VHGVYDPSK DLIAETQKQG QGQWYQIYQ
 30 EPPKLNLTGK YARMRGAHTN DVKQLTEAVQ KITTESIVIW GKTPEFKLPI
 QKETWETWWT EYQATWIPE WEFVNTPLV KLWYQLEKEP IVGAETFYVD
 GAANRETKLG KAGYVTNRGR QKVVTLDTT NQKTELQAIY LALQDSGLEV
 NIVTDSQYAL GIIQAQPDQS ESELVNQIE QLIKKEKVYL AWVFAHKGIG
 GNEQVDKLVS AGIRKVL*
 35 [SEQ ID NO: 78]



5

10

15

Figure 17

Sequence of insert Trgn #6

5 ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGAC
 GGCCCCAAGGTCAAGCAGTGGCCACTCACCGAGGAGAAGATCAAGGCCCTGGTGGAG
 ATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCTGAGAACCCA
 TACAACACCCCCGTGTTTGCATCAAGAAGAAGACAGCACCAAGTGGCGCAAGCTG
 GTGGATTTCGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGGC
 10 ATCCCCCATCCGGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGTGGACGTGGGC
 GACGCTTACTTCAGCGTCCCTCTGGACGAGCACTTTAGAAAGTACACGCGCTTTACC
 ATCCCCTCTATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGCTCCTCCCC
 CAGGGCTGGAAGGGCTCTCCGCGCATTTTCCAGAGCTCCATGACCAAGATCCTGGAG
 CCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGTAC
 15 GTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAG
 CATCTGCTGAGATGGGGCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGCCA
 TTCCCTgaagATGCGCTACGAGCTCCATCCCGACAAGTGGACCGTGCAGCCTATCGTC
 CTCCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTC
 AACTGGGCTAGCCAGATCTATCCCGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTG
 20 CGCGGCACCAAGGCCCTGACCGAGGTGATTCCCCCTCACGGAGGAAGCCGAGCTCGAG
 CTGGCTGAGAACCGGGAGATCCTGAAGGAGCCCGTGACGGCGTGTAATATGACCCC
 TCCAAGGACCTGATCGCCGAATCCAGAAGCAGGGCCAGGGGAGTGGACATACCAG
 ATTTACCAGGAGCCTTTCAAGAACCCTCAAGACCGGCAAGTACGCCCCGATGAGGGGC
 GCCCACACCAACGATGTCAAGCAGCTGACCGAGGCCGTCCAGAAGATCACGACCGAG
 25 TCCATCGTGATCTGGGGGAAGACACCAAGTTCAAGCTGCCTATCCAGAAGGAGACC
 TGGGAGACGTGGTGGACCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTT
 GTGAATACACCTTCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCCATCGTG
 GCGCGGAGACATTCTACGTGGACGGCGCGGCCAACCGCAAACAAAGCTCGGGAAG
 GCCGGGTACGTACCAACCGGGGCCGCCAGAAGGTGCTCACCCCTGACCGACACCAAC
 30 AACCAAGACCGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAG
 GTGAACATCGTGACGGACAGCCAGTACGCGCTGGGCATTATTAGGCCCAGCCGGAC
 CAGTCCGAGAGCGAAGTGGTGAACAGATTATCGAGCAGCTGATCAAGAAAGAGAAG
 GTCTACCTCGCCTGGGTCCCGGCCATAAGGGCATTGGCGGCAACGAGCAGGTCCAC
 AAGCTGGTGAGTGGGGGATTAGAAAGGTGCTGATGGGTGCCCCGAGCTTCGGTACTG
 35 TCTGGTGGAGAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGAGGCAAAAAG
 AAATACAAGCTCAAGCATATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTG
 AACCCAGGCCTGCTGGAACATCTGAGGGATGTCGCCAGATCCTGGGGCAATTGCAG
 CCATCCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCTTGTATAACACAGTGGCTACC
 CTCTACTGCGTACACAGAGGATCGAGATTAAGGATACCAAGGAGGCCCTTGGACAAA
 40 ATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACT
 GGGCATAGCAACCAGGTATCACAGAACTATCCTATTGTCCAAAACATTGAGGGCCAG
 ATGGTTCATCAGGCCATCAGCCCCGGACGCTCAATGCCTGGGTGAAGGTTGTCCAA
 GAGAAGGCCTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGAGGGGGCC
 ACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATG

CAAATGTTGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCC
 GTCCACGCTGGCCCAATCGCGCCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATT
 GCCGGCACCACCTCTACACTGCAAGAGCAAATCGGATGGATGACCAACAATCCTCCC
 ATCCCAGTTGGAGAAATCTATAAACGGTGGATCATCCTGGGCCTGAACAAGATCGTG
 5 CGCATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTT
 AGGGATTACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAG
 GTCAAAAACCTGGATGACGGAGACACTCCTGGTACAGAACGCTAACCCCGACTGCAAA
 ACAATCTTGAAGGCACTAGGCCCGGCTGCCACCCTGGAAGAGATGATGACCGCCTGT
 CAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGTGGGTTTTCCAGTC
 10 ACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCAC
 TTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCCTCCCAAAGAAGACAAGAT
 ATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTAC
 ACACCGAGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGTCTACAAGCTAGTA
 CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTA
 15 CACCCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTAGAGTGGAGG
 TTTGACAGCCGCTAGCATTTCATCACGTGGCCCCGAGAGCTGCATCCGGAGTACTTC
 AAGAAGTGTCTGA [SEQ ID NO: 79]

20 MGPISPIETV SVKCLKPGMDG PKVKQWPLTE EKIKALVEIC TEMEKEGKIS
 KIGPENPYNT PVFAIKKDS TKWRKLVDFR ELNKRQDFW EVQLGIPHPA
 GLKKKKSVTV LDVGDAYFSV PLDEDFRKYT AFTIPSINNE TPGIRYQYNV
 LPQGWKGSFA IFQSSMTKIL EPFRKQNPDI VIYQYMDLTY VGSLEIGQH
 RTKIEELRQH LLRWGLTTPD KKHQKEPPFL WMGYELHPDK WTVQPIVLPE
 25 KDSWTVNDIQ KLVGKLNWAS QIYPGIKVRQ LCKLLRGTKA LTEVIPLTEE
 AELELAENRE ILKEPVHGVY YDPSKDLIAE IQKQGGQWT YQIYQEPFKN
 LKTGKYARMR GAHTNDVKQL TEAVQKITTE SIVIWGKTPK FKLPIQKETW
 ETWWTEYWQA TWIPEWEFVN TPPLVKLWYQ LEKEPIVGAE TFYVDGAANR
 ETKLGKAGYV TNRGRQKVVT LTDITNQKTE LQAIYLLALQD SGLEVNIVTD
 30 SQYALGIIQA QPDQSESELV NQIIEQLIKK EKVYLAWVPA HKGIGGNEQV
 DKLVSAIRK VLMGARASVL SGGELDRWEK IRLRPGGKKK YKLKHIVWAS
 RELERFAVNP GILETSECCR QILGQLQPSL QTGSEELRSL YNTVATLYCV
 HQRIEIKDTK EALDKIEBEEQ NKSCKKAQQA AADTGHSNQV SQNYPIVQNI
 QGQMVHQAIS PRTLNAWVKV VEEKAFSPEV IPMFSALSEG ATPQDLNMTL
 35 NTVGGHQAAM QMLKETINEE AAEDWRVHPV HAGPIAPGQM REPRGSDIAG
 TTSTLQEQIG WMTNPPPIPV GEIYKRWIL GLNKIVRMYS PTSILDIRQG
 PKBEFRDYVD RPYKTLRAEQ ASQEVKNWMT ETLLVQANANP DCKTILKALG
 PAATLEEMMT ACQGVGGPGH KARVLMVGFP VTPQVPLRPM TYKAAVDLSH
 FLKEKGGLBG LIHSQRRQDI LDLWIYHTQG YFPDWNQNYTP GPGVRYPLTF

GWCYKLVPE PDKVEEANKG ENTSLHPVS LHGMDDPERE VLEWRPDSRL
 AFHHVARELH PEYFKNC * [SEQ ID NO: 80]

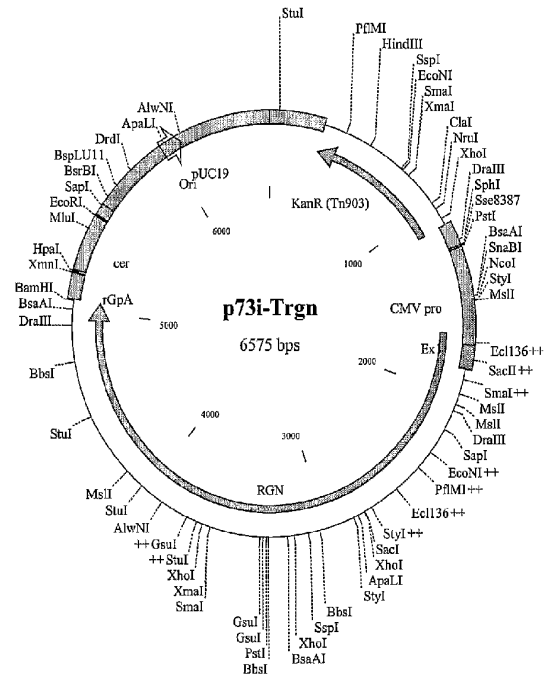


Figure 18

Sequence of insert of Trng #11

```

5  ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAAACCCGGGATGGAC
   GGCCCCAAGGTCAAGCAGTGGCCACTCACCGAGGAGAAGATCAAGGCCCTGGTGGAG
   ATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCGGAGAACCCA
   TACAACACCCCGTGTGTTGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTG
   GTGGATTTCGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGGC
   ATCCCCCATCCGGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGC
10  GACGCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACC
   ATCCCATCTATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGTCCTCCCC
   CAGGGCTGGAAGGGCTCTCCCGCCATTTCCAGAGCTCCATGACCAAGATCCTGGAG
   CCGTTTCGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGTAC
   GTGGGCTCTGACCTGGAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAG
15  CATCTGCTGAGATGGGGCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGCCA
   TTCTGaaGATGGGCTACGAGCTCCATCCCGACAAGTGGACCGTGACGCTATCGTC
   CTCCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGTTGGGCAAGCTC
   AACTGGGCTAGCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTG
   CGCGGCACCAAGGCCCTGACCGAGGTGATTTCCCTCACGGAGGAAGCCGAGCTCGAG
20  CTGGCTGAGAACCGGGAGATCCTGAAGGAGCCCGTGACCGCGTGTACTATGACCCC
   TCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAGGGGCACTGGACATACCAG
   ATTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAGTACGCCCGCATGAGGGC
   GCCACACCAACGATGTCAAGCAGCTGACCGAGGCCGTCCAGAAGATCACGACCGAG
   TCCATCGTGATCTGGGGGAAGACACCCAAGTTCAAGCTGCCTATCCAGAAGGAGACC
25  TGGGAGACGTGGTGACCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTC
   GTGAATACACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCCATCGTG
   GGC CGGAGACATTCTACGTGGACGGCGCGGCCAACCGCGAAACAAAGCTCGGGGAG
   GCCGGGTACGTACCAACCGGGGCCGCCAGAAGGTTCGTACCCTGACCGACACCAAC
   AACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAG
30  GTGAACATCGTGACGGACAGCCAGTACGCGCTGGGCATTATTCAGGCCCAGCCGGAC
   CAGTCCGAGAGCGAACTGGTGAACAGATTATCGAGCAGCTGATCAAGAAGAGAAG
   GTCCTACCTCGCCTGGGTCCCGGCCATAAGGGCATTGGCGGCAACGAGCAGGTGAC
   AAGCTGGTGAGTGGGGGATTAGAAAGGTGCTGATGGTGGGTTTTCCAGTCACACCT
   CAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTA
35  AAAGAAAAGGGGGGACTGGAAGGCTAATTCACCTCCCAAAGAAGACAAGATATCCTT
   GATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCA
   GGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTT
   GAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTACACCTT
   GTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTAGAGTGGAGGTTTGAC
40  AGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAC
   TGTGAATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAG
   AAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGG
   GCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCCTGCTGGAAACATCTGAG
   GGATCTCGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAG
45  CTGAGGTCTTGTATAACACAGTGGCTACCTCTACTGCGTACACCAGAGGATCGAG

```

ATTAAGGATACCAAGGAGGCCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAG
AAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAAGAAC
TATCCTATTGTCCAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCGG
ACGCTCAATGCCTGGGTGAAGGTTGTGGAAGAGAAGGCCCTTTCTCCTGAGGTTATC
5 CCCATGTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTT
AATACCGTGGGCGGCCATCAGGCCGCCATGCAAAATGTTGAAGGAGACTATCAACGAG
GAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGGA
CAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAG
CAATCCGATGGATGACCAACAATCCTCCCATCCAGTTGGAGAAATCTATAAACGG
10 TGGATCATCTGGGCCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTT
GACATTAGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTCGACCGGTTTTATAAG
ACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTC
CTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCT
GCCACCCTGGAAGAGATGATGACCGCTGTGACGGAGTAGGCGGACCCGGACACAAA
15 GCCAGAGTGTTG [SEQ ID NO: 81]

MGPISPIETV SVKLKPGMDG PKVKQWPLTE BKIKALVEIC TEMEKEGKIS
KIGPENPYNT PVFAIKKKDS TKWRKLVDFR ELNKRQDFW EVQLGIPHPA
GLKKKKSVTV LDVGDAYFSV PLDEDFRKYT AFTIPSINNE TPGIRYQYNV
20 LPQGWKGSFA IFQSSMTKIL EPFRKQNPDI VIYQYMDL YVSDLEIGQH
RTKIEELRQH LLRWGLTTPD KKHQKEPPFL WMGYELHPDK WTVQPIVLPE
KDSWTVNDIQ KLVGKLNWAS QIYPGIKVRQ LCKLLRGTKA LTEVIPLTEB
AELELAENRE ILKEPVHGVY YDPSKDLIAE IQKQGQGWY YQIYQEPFKN
LKTGKYARMR GAHTNDVKQL TEAVQKITTE SIVIWKTPK FKLPIQKETW
25 ETWWTEYWQA TWIPBWEFVN TPPLVKLWYQ LEKEPIVGAE TFYVDGAANR
ETKLGKAGYV TNRGRQKVVT LTDITNQTKE LQAIYLLALQD SGLEVNIVTD
SQYALGIIQA QPDQSESELV NQIIEQLIKK EKVYLAWVPA HKGIGGNEQV
DKLVSAGIRK VLMVGFPVTP QVPLRPMTYK AAVDLSHFLK EKGGLLEGLIH
SQRQDILDL WIYHTQGYFP DWQNYTPGPG VRYPLTFGWC YKLVPVEPDK
30 VEBANKGENT SLLHPVSLHG MDDPEREVLE WRFDSRLAFH HVARELHPEY
FKNCMGARAS VLSGGELDRW EKIRLRPGGK KKYKLKHIVW ASRELERFAV
NPGLEETSEG CRQILGQLQP SLQTGSEELR SLYNTVATLY CVHQRIEIKD
TKEALDKIEE EQNKSKKKAQ QAAADTGHEN QVSQNYPIVQ NIQGMVHQA
ISPRTLNAWV KVVEEKAFSP EVIPMFSALS EGATPQDLNT MLNTVGGHQA
35 AMQMLKETIN BEAAEWDRVH PVHAGPIAPG QMREPRGSDI AGTTSTLQEQ
IGWMTNPPPI PVGEIYKRWI ILGLNKIVRM YSPTSILDIR QGPKEPFRDY
VDRFYKTLRA EQASQEVKNW MTETLLVQNA NPDCKTILKA LGPAATLEEM
MTACQGVGGP GHKARVL [SEQ ID NO: 82]

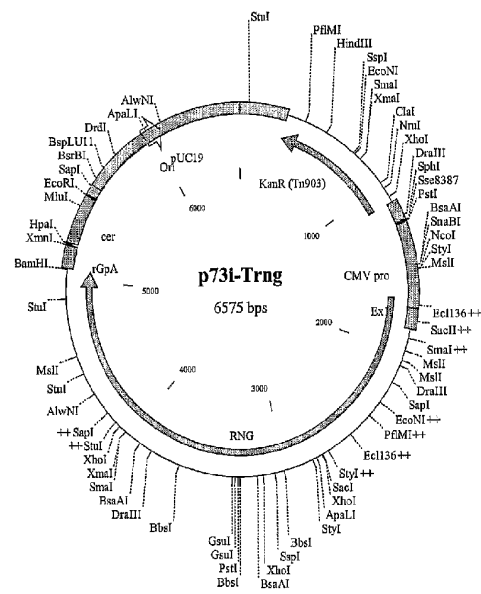


Figure 19

Sequence of TgnR (F1):

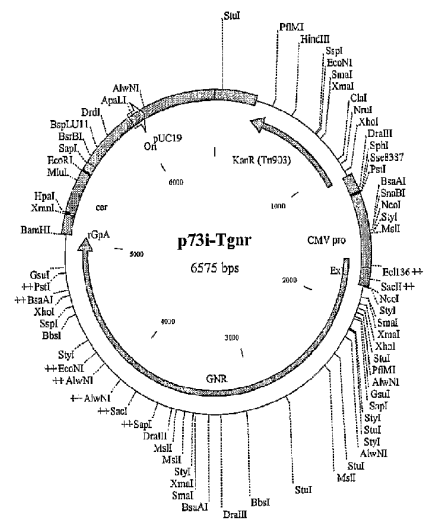
ATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATT
 5 AGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCG
 AGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCCTGCTGGAAACATCTGAGGGATGT
 CGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGG
 TCCTTGTATAACACAGTGGCTACCCCTCTACTGCGTACACCAGAGGATCGAGATTAAAG
 GATACCAAGGAGGCCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAG
 10 GCCCAGCAGGCAGCTGCTGACACTGGCCATAGCAACCAGGTATCACAGAACTATCCT
 ATTGTCCAAAACATTTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTC
 AATGCCTGGGTGAAGGTGTGCGAAGAGAAGGCCCTTTCTCCTGAGGTTATCCCCATG
 TTCTCCGCTTTGAGTGAAGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACC
 GTGGCGGCCATCAGGCGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCA
 15 GCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCGACAGATG
 CGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAAATC
 GGATGGATGACCAACAATCCTCCCATCCAGTTGGAGAAATCTATAAACGGTGGATC
 ATCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTTGACATT
 AGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTGACCGGTTTATAAGACCCTG
 20 CGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTA
 CAGAACGCTAACCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACC
 CTGGAAGACATGATGACCGCTGTGAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
 GTGTTGATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTAC
 AAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAAGGGGGGACTGGAAGGGCTA
 25 ATTCACTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGC
 TACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACC
 TTTGGATGGTGTACAAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAAT
 AAAGGAGAGAACCAGCTTGTACACCCTGTGAGCCTGCATGGGATGGATGACCCG
 GAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCTAGCATTTCATCAGTGGCC
 30 CGAGAGCTGCATCCGGAGTACTTCAAGAACTGCATGGGCCCATCAGTCCCATCGAG
 ACCGTGCCGTGAAGCTGAAACCCGGATGGACGGCCCAAGGTCAAGCAGTGGCCA
 CTCACCGAGGAGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAAGAG
 GGCAAGATCAGCAAGATCGGGCCTGAGAACCACATAACAACCCCCGTGTTTGCCATC
 AAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGATTTCCGGGAGCTGAATAAG
 35 CGGACCCAGGATTTCTGGGAGGTCCAGCTGGGCATCCCCATCCGCGCGGCTGAAG
 AAGAAGAAGAGCGTGACCGTGTGGACGTGGGCGACGCTTACTTCAGCGTCCCTCTG
 GACGAGGACTTTAGAAAGTACACCGCCTTTACCATCCCATCTATCAACAACGAGACC
 CCTGGCATCAGATATCAGTACAACGTCTCCCCAGGGCTGGAAGGGCTCTCCCGCC
 ATTTTCCAGAGCTCCATGACCAAGATCCTGGAGCCGTTTCGGAAGCAGAACCCCGAT
 40 ATCGTCATCTACCAGTACATGGACGACCTGTACGTGGGCTCTGACCTGGAAATCGGG
 CAGCATCGCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGATGGGGCTGACC
 ACTCCGGACAAGAAGCATCAGAAGGAGCCGCCATTCTTgaaGATGGGCTACGAGCTC
 CATCCCGACAAGTGGACCGTGCAGCCTATCGTCCTCCCCGAGAAGCACAGCTGGACC
 GTGAACGACATCCAGAAGCTGCTGGCAAGCTCAACTGGGCTAGCCAGATCTATCCC
 45 GGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCCGAG

GTGATTCCCCTCACGGAGGAAGCCGAGCTCCAGCTGGCTGAGAACCGGGAGATCCTG
AAGGAGCCCGTGACGGCGTGACTATGACCCCTCCAAGGACCTGATCGCCGAAATC
CAGAAGCAGGGCCAGGGGCAGTGGACATACCAGATTTACCAGGAGCCTTTCAAGAAC
CTCAAGACCGGCAAGTACGCCCGCATGAGGGCGCCACACCAACGATGTCAAGCAG
5 CTGACCGAGGCGCTCCAGAAGATCACGACCGAGTCCATCGTGATCTGGGGGAAGACA
CCCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGTGGTGGACCGAATAT
TGGCAGGCCACCTGGATTCCCAGTGGGAGTTCGTGAATACACCTCCTCTGGTGAAG
CTGTGGTACCAGCTCGAGAAGGAGCCCATCGTGGGCGCGGAGACATTCTACGTGGAC
GGCGCGGCCAACCGCGAAACAAAGCTCGGGAAGGCCGGGTACGTACCAACCGGGGC
10 CGCCAGAAGGTCTGACCCCTGACCGACACCAACCAAGACGGAGCTGCAGGCC
ATCTATCTCGCTCTCCAGGACTCCGGCTGGAGGTGAACATCGTGACGACAGCCAG
TACGCGCTGGGCATTATTTCAGGCCAGCCGACCAAGTCCGAGAGCGAACTGGTGAAC
CAGATTATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTCGCTGGTCCCGGCC
CATAAGGCCATTGGCGCAACGAGCAGGTGACAAAGCTGGTGAGTGCGGGGATTAGA
15 AAGGTGCTGTAA [SEQ ID NO: 83]

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
LETSEGCRI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
LDKIEEEQNK SKKKAQAAA DTGHSNQVSQ NYPIVQNIQG QMVHQAISPR
20 TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNMTLNT VGGHQAAMQM
LKETINEEAA EWDRVHPVHA GPIAPGQMR PRGSDIAGTT STLQEQIGWM
TNNPPIPVGE IYKRWIILGL NKIVRMYSP SILDIRQGP EPFRDYVDRF
YKTLRAEQAS QEVKNWMTET LLVQMANPDC KTIKALGPA ATLEEMMTAC
QGVGGPGHKA RVLVGVFPVT PQVPLRPMTY KAAVDLSHFL KEKGGLEGLI
25 HSQRRQDILD LWIYHTQGYF PDWQNYTPGP GVRYPITFGW CYKLVPVEPD
KVEEANKGEN TSLHHPVSLH GMDDPEREVL EWRFDSEALF HHVARELHPE
YFKNCMPIS PIETVSVKLK PGMDGPKVKQ WPLTEEKIKA LVEICTEMEK
EGKISKIGPE NPYNTPVFAI KKDSTKWRK LVDFRELNRK TQDFWEVQLG
IPHPAGLKKK KSVTVLDVGD AYFSVPLDED FRKYTAFTIP SINNETPGIR
30 YQYNVLPQGW KGSPAIFQSS MTKILEPFRK QNPDIVIYQY MDDLQVGS DL
RIGQHRKTIE ELRQHLLRWG LTPDKKHQK EPPFLWMGYE LHPDKWTVQP
IVLPEKDSWT VNDIQKLVGK LNWASQIYPG IKVRQLCKLL RGTALTEVI
PLTEBAELEL AENREILKEP VHGVYDPSK DLIAEIQKQG QGQWTVQIYQ
EPFKNLKTGK YARMGAHTN DVKQLTEAVQ KITTESIVIW GKTPKFKLPI
35 QKETWETWWT EYWQATWIPE WEFVNTPLV KLWYQLEKEP IVGAETFYVD
GAANRETKLG KAGYVTNRGR QKVVTLDTT NQKTELQAIY LALQDSGLEV
NIVTDSQYAL GIIQAQPDQS ESELVNQIIE QLIKKEKVYL AWVPAHKGIG
GNEQVDKLVS AGIRKVL*
[SEQ ID NO: 84]

40

45



5

10

Figure 20.
Responses to Gag peptide measured using IFN-gamma ELISpot at 5 days post-boost

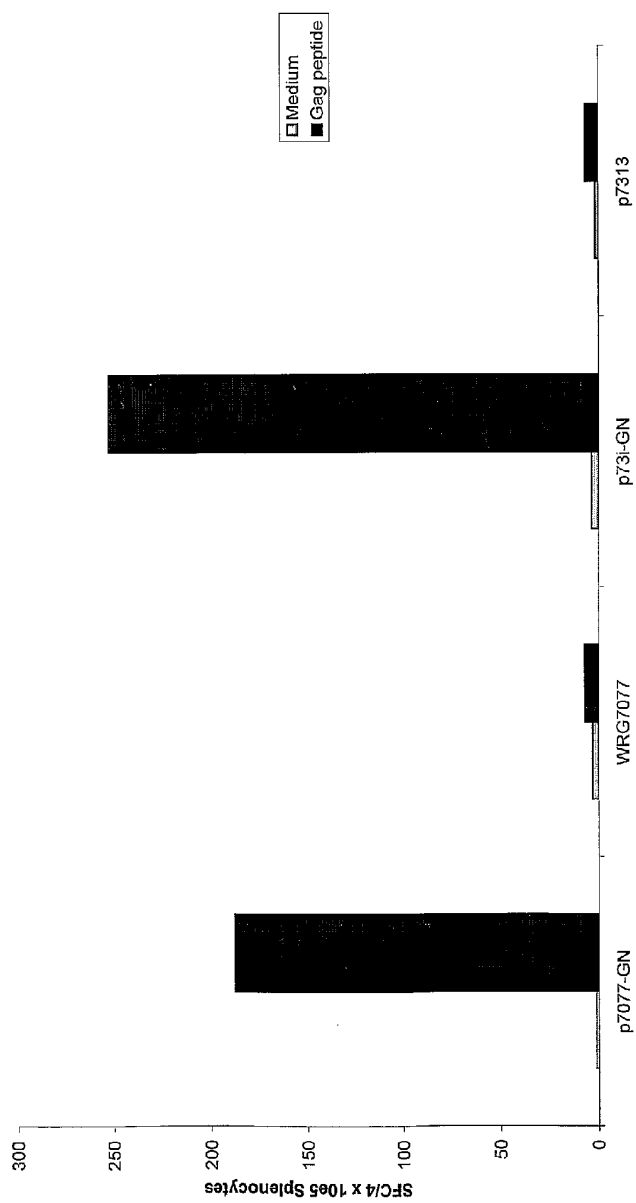
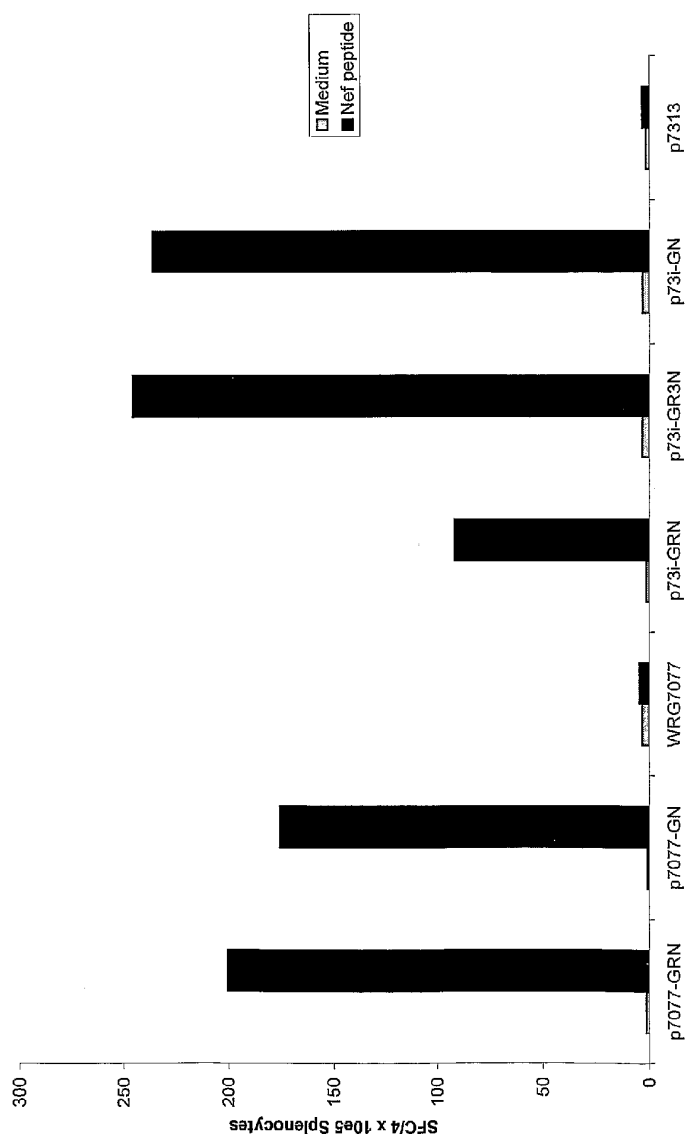


Figure 21.
Responses to Nef peptide using IFN-gamma ELISPOT at 5 days post-boost



PG4653

Figure 22.
Responses to Rt peptide by IFN-gamma ELISPOT at 5 days post-boost

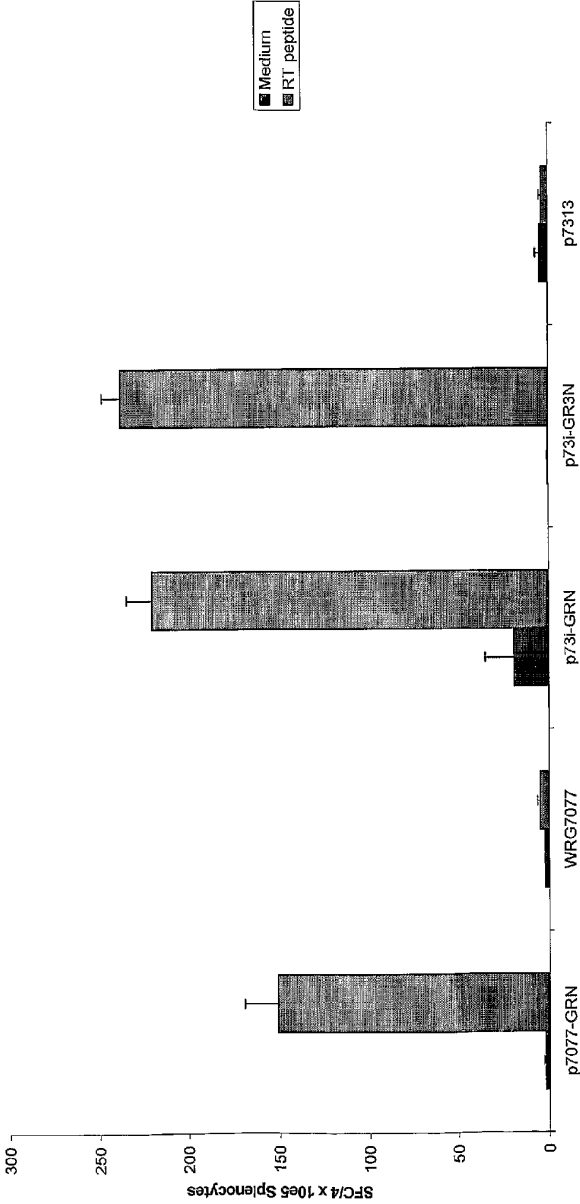
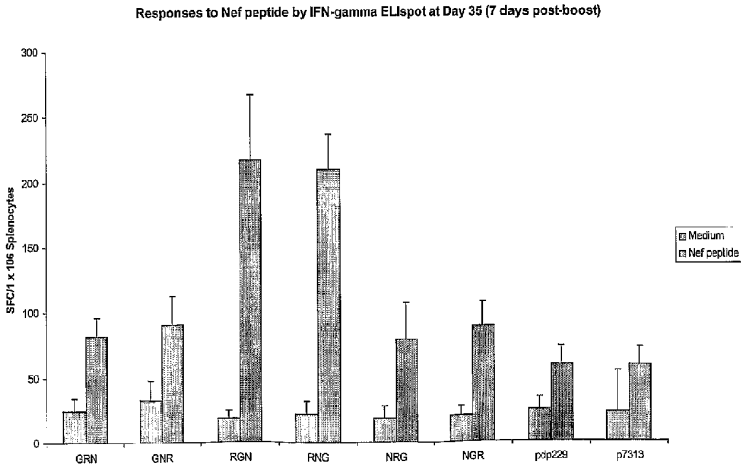
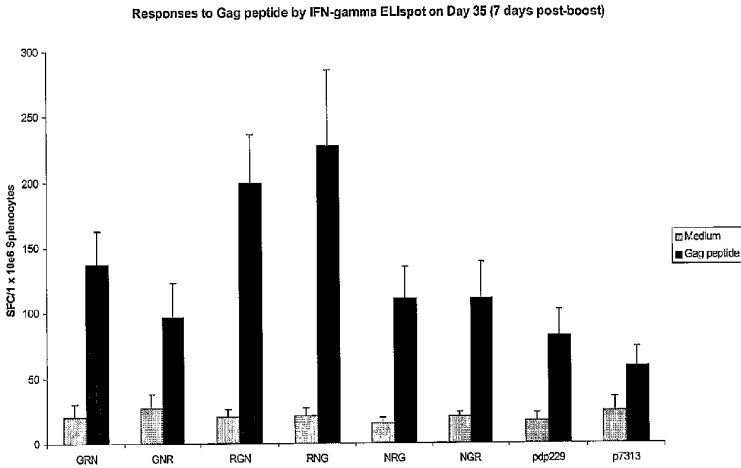
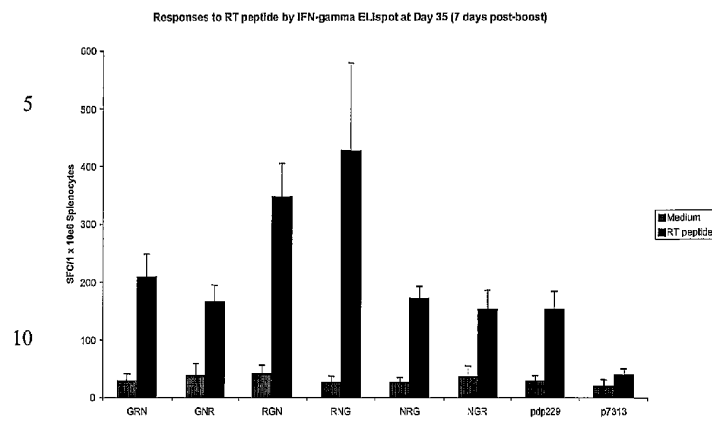


Figure 23

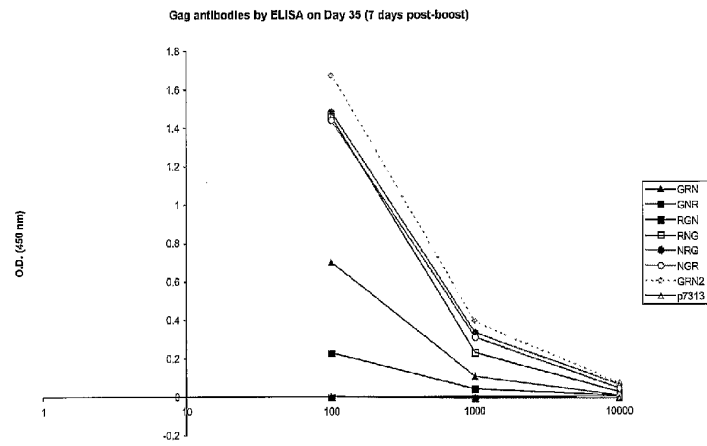
5 Example of the CD8 immune response that is detected in the mouse model following immunisation with the reformat constructs. The constructs are comprised Gag, RT and Nef as a triple fusion. G is Gag, R is RT and N is Nef.





50/50

Figure 24



5