

Vaccine

5 The present invention relates to methods and compositions useful in the treatment and prevention of human papilloma virus infections. In particular the invention relates to nucleic acid molecules typically encoding a polyprotein based on Early antigens from different HPV strains, and vectors suitable for DNA vaccine delivery, and pharmaceutical compositions containing them. Methods for manufacturing said molecules, vectors and composition are also contemplated, as are their use in medicine.

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Background to the Invention

15 The papillomavirus virus is highly tissue and species specific. It infects basal epithelial cells and replicates and completes its full life cycle within the cell nucleus. Viral gene expression is tightly linked to epithelial cell differentiation and capsid assembly and maturation only occurs in fully differentiated epithelial cells in the upper epithelial cell layers.

20 The infecting human papillomavirus genotypes present in genital warts are known to be either genotype 6b or genotype 11. The majority (~90%) of genital warts are infected with HPV6b, whilst approximately 10% are infected with HPV-11. The primary infecting genotypes present in infections relating to cervical carcinoma are HPV16 and 18.

25 Human genital warts may develop at the site of infection and they may become chronic, persisting for extended periods of time or, alternatively they may regress spontaneously resolving completely without scarring. The factors that trigger this regression are undefined but it is postulated that cellular response may be involved in the disease resolution process.

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Papillomaviruses are not naturally very immunogenic and during the course of natural infection antibodies may only occur very late (during or after resolution), and in a fraction of patients whilst some patients may resolve disease without developing detectable antibody at all.

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Vaccination using papillomavirus early antigens has been widely studied in several different animal model systems. However there are only a few reports studying therapeutic immunisation. For example, cattle immunised therapeutically with a cocktail of proteins comprising bovine papillomavirus (BPV) proteins E1, E2, E4 and E7 showed a reduced papilloma disease burden in a proportion of animals compared to controls.

Papilloma virus infections have been observed in a variety of species, including sheep, dogs, rabbits, monkeys, cattle and humans. Human papilloma viruses (HPV) have been classified into more than 80 types [Epidemiology and Biology of Cervical Cancer Seminars in Surgical Oncology 1999 16:203-211. Wolfgang MJ, Schoell MD, Janicek MF and Mirhashemi R.], some of which are further divided into sub-types (e.g. type 6a and 6b), based on the extent of DNA sequence homology. Papilloma viruses generally infect epithelia, but the different HPV types cause distinct diseases. For example, types 1-4, 7, 10 and 26-29 cause benign warts, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 are associated with cervical cancers and types 6 and 11 are implicated in genital warts (non-malignant condylomata of the genital tract).

HPV has proven difficult to grow in tissue culture, so there is no traditional live or attenuated viral vaccine. Development of an HPV vaccine has also been slowed by the lack of a suitable animal model in which the human virus can be studied. This is because the viruses are highly species specific, so it is very difficult to infect an animal with a papilloma virus from a host of a different species, as would be required for safety testing before a vaccine was first tried in humans.

Papilloma viruses have a DNA genome which encodes "early" and "late" genes designated E1 to E7, L1 and L2. The early gene sequences have been shown to have functions relating to viral DNA replication and transcription, evasion of host immunity, and alteration of the normal host cell cycle and other processes. For example the E1 protein is an ATP-dependent DNA helicase and is involved in initiation of the viral DNA replication process whilst E2 is a regulatory protein controlling both viral gene expression and DNA replication. Through its ability to bind to both E1 and the viral origin of replication, E2 brings about a local concentration of E1 at the origin, thus stimulating the initiation of viral DNA

replication. The E4 protein appears to have a number of poorly defined functions but amongst these may be binding to the host cell cytoskeleton, whilst E5 appears to delay acidification of endosomes resulting in increased expression of EGF receptor at the cell surface and both E6 and E7 are known to bind cell proteins p53 and pRB respectively. The E6 and E7 proteins form HPV types associated with cervical cancer are known oncogenes. L1 and L2 encode the two viral structural (capsid) proteins.

Historically, vaccines have been seen as a way to prevent infection by a pathogen, priming the immune system to recognise the pathogen and neutralise it should an infection occur. The vaccine includes one or more antigens from the pathogen, commonly the entire organism, either killed or in a weakened (attenuated) form, or selected antigenic peptides from the organism. When the immune system is exposed to the antigen(s), cells are generated which retain an immunological "memory" of it for the lifetime of the individual. Subsequent exposure to the same antigen (e.g. upon infection by the pathogen) stimulates a specific immune response which results in elimination or inactivation of the infectious agent.

There are two arms to the immune response: a humoral (antibody) response and a cell-mediated response. Protein antigens derived from pathogens that replicate intracellularly (viruses and some bacteria) are processed within the infected host cell releasing short peptides which are subsequently displayed on the infected cell surface in association with class I major histocompatibility (MHC I) molecules. When this associated complex of MHC I and peptide is contacted by antigen-specific CD8+ T-cells the T-cell is activated, acquiring cytotoxic activity. These cytotoxic T-cells (CTLs) can lyse infected host cells, so limiting the replication and spread of the infecting pathogen. Another important arm of the immune response is controlled by CD4+ T-cells. When antigen derived from pathogens is released into the extracellular milieu they may be taken up by specialised antigen-presenting cells (APCs) and displayed upon the surface of these cells in association with MHC II molecules. Recognition of antigen in this complex stimulates CD4+ T-cells to secrete soluble factors (cytokines) which regulate the effector mechanisms of other T-cells. Antibody is produced by B-cells. Binding of antigen to secreted antibody may neutralise the infectivity of a

pathogen and binding of antigen to membrane-bound antibody on the surface of B-cells stimulates division of the B-cell so amplifying the B-cell response. In general, good antibody responses are required to control bacterial infections and both antibody and cell-mediated immune responses (CD8+ and CD4+) are required to control infections by viruses.

It is believed that it may be possible to harness the immune system by vaccination, even after infection by a pathogen, to control or resolve the infection by inactivation or elimination of the pathogen. Such "therapeutic" vaccines would require a cell-mediated response to be effective, and would ideally invoke both humoral and cell-mediated immune responses.

It has been demonstrated (Benvenisty, N and Reshaf, L. PNAS 83 9551-9555) that inoculation of mice with calcium phosphate precipitated DNA results in expression of the peptides encoded by the DNA. Subsequently, intramuscular injection into mice of plasmid DNA which had not been precipitated was shown to result in uptake of the DNA into the muscle cells and expression of the encoded protein. Because expression of the DNA results in production of the encoded pathogen proteins within the host's cells, as in a natural infection, this mechanism can stimulate the cell-mediated immune response required for therapeutic vaccination. DNA vaccines are described in WO90/11092 (Vical, Inc.).

DNA vaccination may be delivered by mechanisms other than intra-muscular injection. For example, delivery into the skin takes advantage of the fact that immune mechanisms are highly active in tissues that are barriers to infection such as skin and mucous membranes. Delivery into skin could be via injection, via jet injector (which forces a liquid into the skin under pressure) or via particle bombardment, in which the DNA may be coated onto particles of sufficient density to penetrate the epithelium (US Patent No. 5371015). Projection of these particles into the skin results in direct transfection of both epidermal cells and epidermal Langerhan cells. Langerhan cells are antigen presenting cells (APC) which take up the DNA, express the encoded peptides, and process these for display on cell surface MHC proteins. Transfected Langerhan cells migrate to the lymph nodes where they present the displayed antigen fragments

to lymphocytes, invoking an immune response. Very small amounts of DNA (0.5-1 µg) are required to induce an immune response via particle delivery into skin and this contrasts with the milligram quantities of DNA known to be required to generate immune responses subsequent to direct intramuscular injection.

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It has been reported, for example in studies using virus like particles formed from the L1 and L2 capsid proteins or using these proteins alone (1), that HPV is poorly immunogenic. Furthermore, HPV genes have proven difficult to express in human or other mammalian cells, leading difficulties in developing protein subunit vaccines. Monocystronic E1 has proven particularly resistant to expression from heterologous promoters in mammalian cells (J.Virology 1999 73, 3062-3070. Remm M, Remm A and Mart Ustav. Human papilloma virus type 18 E1 is translated from polycistronic mRNA by a discontinuous scanning mechanism). Expression of E1 is most often detected using *in vitro* DNA replication of an HPV origin containing plasmid as a surrogate (Lu, JZJ, Sun et al J.Virol 1993 67, 7131-7139 and Del Vecchio AM et al J.Virol 1992 66, 5949-5958).

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International patent application WO 02/08435 provides HPV polynucleotide wherein the sequence has been optimised to resemble the usage patterns of a highly expressed human gene. In particular codon optimised HPV6bE1, and HPV 11 E2 are disclosed.

Brief Description of the Invention

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The present invention provides novel nucleic acid constructs which are useful in the prophylaxis and more particularly in the treatment of the human papillomaviral induced genital warts, or other HPV induced sequelae.

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According to a first aspect of the present invention there is provided a nucleic acid construct encoding a polyprotein containing epitopes from at least two distinct Early antigens. Preferably the present invention provides a nucleic acid construct encoding a polyprotein comprising epitopes from three distinct Early antigens. Such construct have been shown by the present inventors to be more efficacious in animal models than the single protein approach.

Detailed Description

Preferred constructs include nucleic acids coding for E2 from two different HPV genotypes such as HPV6b and E2 from HPV - 11. Additionally it is preferred if an E1 encoding sequence is present. Preferably the E1 is from HPV 6 or 11.

Preferred construct include a nucleic acid molecule having the following arrangement:

- 1) HPV6bE1 – HPV6bE2 – HPV11E2
- 2) HPV6bE2 – HPV6bE1 – HPV11E2
- 3) HPV6bE2 – HPV11E2 – HPV6bE1

Most preferably all the nucleic acid sequence of the above polyprotein has been codon optimised to resemble the codon usage of a highly expressed human gene. Preferably the E1 and E2 genes are substantially full length or more preferably full length. By substantially full length means at least 85% preferably 90% of the E1 and E2 polypeptide is encoded. Surprisingly, such constructs, express to the equivalent expression levels as codon optimised individual proteins, and have the advantage that a single plasmid encoding the polyproteins is cheaper and easier to manufacture than three individual plasmids.

It is preferred that these genes are codon optimised such that the codon usage pattern resembles that of actin, a highly expressed human gene product.

The polynucleotide sequence may be a DNA sequence, for example a double stranded DNA sequence. Preferably the polynucleotide sequence encodes a HPV polypeptide of HPV type 6, 11, 16, 18, 33 or 45, most preferably type 11, sub-type 6a or sub-type 6b. In certain embodiments the encoded amino acid sequence is a wild-type HPV amino acid sequence. In alternative embodiments, the encoded amino acid sequence is a mutated HPV amino acid sequence comprising the wild-type sequence with amino acid changes, for example amino acid point mutations, sufficient to reduce or inactivate one or more of the natural

biological functions of the polypeptide. The mutated amino acid sequence will desirably retain the immunogenicity of the wild-type polypeptide.

Proteins encoded by the polynucleotides of the invention also form an aspect of the present invention.

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In the case of E1, the primary biological role is to initiate virus specific DNA replication in infected cells. It is preferred that E1 is mutated to inactivate its replication potential.

10 The preferred mutations are: G 482 D
 K 83 G
 R 84 G

Preferably two or more mutations are included.

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Most preferably 3 mutations are included.

In the case of E2, this is a site specific binding nuclear protein functioning as the primary replication origin recognition protein and assists in the assembly of the pre-initiation replication complex. It is preferred that the E2 protein is inactivated. A preferred mutation to achieve this objective is K111 A.

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According to one aspect of 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 in humans. 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 frequently. A polynucleotide of the present invention will generally have a codon usage coefficient for highly expressed human genes of greater than 0.3, preferably greater than 0.4, most preferably greater than 0.5. According to a second 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 invention, said polynucleotide encoding a polypeptide having epitopes from two or more Early antigens. The vector may be suitable for driving expression of

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heterologous DNA in bacterial insect or mammalian cells, particularly human cells. In one embodiment, the expression vector is p7313PLc.

5 In a further aspect, the present invention provides a vaccine composition comprising a protein, or vector, or polynucleotide sequence of the invention. Preferably the vaccine composition comprises a DNA vector according to the present invention. In preferred embodiments the vaccine composition comprises a plurality of particles, preferably gold particles, coated with DNA comprising a vector containing a polynucleotide sequence which encodes a polypeptide
10 having epitopes from two or more Early antigens. In alternative embodiments, the vaccine composition comprises a pharmaceutically acceptable excipient and a DNA vector according to the second aspect of the present invention. The vaccine composition may also include an adjuvant.

15 In a further aspect, the present invention provides a method of making a vaccine composition including constructing a polynucleotide that encodes a polypeptide that has epitopes from two or more Early antigens and formulating with a pharmaceutically acceptable excipient.

20 Also provided are the use of a polynucleotide or a vector according to the invention, in the treatment or prophylaxis of an HPV infection, preferably an infection of HPV type 6, 11, 16 or 18. The invention also provides the use of a polynucleotide, a vector according to the invention, in the treatment or prophylaxis of cutaneous (skin) warts, genital warts, atypical squamous cells of
25 undetermined significance (ASCUS), cervical dysplasia, cervical intraepithelial neoplasia (CIN) or cervical cancer. Accordingly, the present invention also provides the use of a polynucleotide or of a vector according to the invention in making a vaccine for the treatment or prophylaxis of an HPV infection or any symptoms or disease associated therewith.

30 The present invention also provides methods of treating or preventing HPV infections or any symptoms or diseases associated therewith comprising administering an effective amount of a protein, polynucleotide or a vector or a vaccine according to the invention. Administration of a vaccine may take the
35 form of one or more individual doses, for example in a "prime-boost" regime. In

certain cases the "prime" vaccination may be via DNA vaccine delivery, in particular via particle mediated DNA delivery of a polynucleotide according to the present invention, preferably incorporated into a plasmid-derived vector and the "boost" by administration of a recombinant viral vector comprising the same polynucleotide sequence. Alternatively, a protein adjuvant approach may act as part of the priming or boosting approach, with DNA delivered as the other arm of the prime-boost regime (the protein being the same as the protein encoded by the DNA).

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The term "variant" refers to a polynucleotide which encodes the same amino acid sequence as another polynucleotide of the present invention but which, through the redundancy of the genetic code, has a different nucleotide sequence whilst maintaining the same codon usage pattern, for example having the same codon usage coefficient or a codon usage coefficient within 0.1, preferably within 0.05 of that of the other polynucleotide.

The term "codon usage pattern" refers to the average frequencies for all codons in the nucleotide sequence, gene or class of genes under discussion (e.g. highly expressed mammalian genes). Codon usage patterns for mammals, including humans can be found in the literature (see e.g. Nakamura et.al. Nucleic Acids Research 1996, 24:214-215).

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

5 of the selected class of genes) are 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 lower for the highly expressed genes of the target species. In order

10 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 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

15 between zero and 1 and the higher the coefficient the more codons in the 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.

20 Shorter polynucleotide sequences are within the scope of the invention. For example, a polynucleotide of the invention may encode a fragment of a HPV protein. A polynucleotide which encodes a fragment of at least 8, for example 1-10 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 the invention as long as the

25 polynucleotide encodes a polypeptide that demonstrates HPV antigenicity. In particular, but not exclusively, this aspect of the invention encompasses the situation when the polynucleotide encodes a fragment of a complete HPV protein sequence and may represent one or more discrete epitopes of that protein.

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 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 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).

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 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 expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

The vectors may be for example, plasmid, artificial chromosome, virus or phage vectors provided with a origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin or kanomycin resistance gene in the case of 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. 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 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 readily available in the art.

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 or in bacteria may be employed in order to produce quantities of the HPV protein encoded by the polynucleotides of the present invention, for example for use as subunit vaccines. Preferred viral vectors are those derived from non-human primate adenovirus such as C68 chimp adenovirus (US 6, 083, 716) other wise known as Pan 9.

Where the polynucleotides of the present invention find use as therapeutic agents, e.g. in DNA vaccination, the nucleic acid will be administered to the mammal e.g. human to be vaccinated. The nucleic acid, such as RNA or DNA, preferably DNA, is provided in the form of a vector, such as those described above, which may be expressed in the cells of the mammal. The polynucleotides

may be administered by any available technique. For example, the nucleic acid may be introduced by needle injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the nucleic acid may be delivered directly across the skin using a nucleic acid delivery device such as particle-mediated DNA delivery (PMDD). In this method, inert particles (such as gold beads) are coated with a nucleic acid, and are accelerated at speeds sufficient to enable them to penetrate a surface of a recipient (e.g. skin), for example by means of discharge under high pressure from a projecting device. (Particles coated with a nucleic acid molecule of the present invention are within the scope of the present invention, as are devices loaded with such particles).

Suitable techniques for introducing the naked polynucleotide or vector into a patient 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, 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 addition of facilitating agents such as bupivacaine to the composition. 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.

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, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectam and transfectam. The dosage of the nucleic acid to be administered can be altered. Typically the nucleic acid is administered in an amount in the range of 1pg to 1mg, preferably to 1pg to 10µg nucleic acid for particle mediated gene delivery and 10µg to 1mg for other routes.

5 A nucleic acid sequence of the present invention may also 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 systems can be used. Viral based systems include retroviral, lentiviral, adenoviral, adeno-associated viral, herpes viral, Canarypox and vaccinia-viral based systems. Non-viral based systems include direct administration of nucleic acids and liposome-based systems.

10 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 cells can later be returned to the subject. The polynucleotide of the invention may integrate into nucleic acid
15 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 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.)

20 The vaccine compositions of the present invention may include adjuvant compounds which may serve to increase the immune response induced by the protein itself or which is encoded by the plasmid DNA. Alteration of the codon bias to suit the vaccinated species is proposed herein as a means of increasing
25 expression and thereby boosting the immune response, but an adjuvant may never-the-less be desirable because, while DNA vaccines tend to work well in mice models, there is evidence of a somewhat weaker potency in larger species such as non-human primates which is thought to be predictive of the likely potency in humans.

30 The vaccine composition of the invention may also comprise an adjuvant, such as, for example, in an embodiment, imiquimod, tucareol or alum.

Preferably the adjuvant is administered at the same time as of the invention and in preferred embodiments are formulated together. Such adjuvant agents contemplated by the invention include, but this list is by no means exhaustive and does not preclude other agents: synthetic imidazoquinolines such as

5 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 activates of immune response modifier R-848: Comparison with CpG ODN', Cellular immunology 204: 64-74 (2000).), Schiff bases of carbonyls

10 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, Th1 inducers such as interferon gamma, IL-2, IL-12, IL-15 and IL-18, Th2 inducers such as IL-

15 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

20 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,

25 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

30 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; 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 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 Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins.

In an embodiment, the adjuvant comprises an immunostimulatory CpG oligonucleotide, such as disclosed in (WO96102555). Typical immunostimulatory oligonucleotides will be between 8-100 bases in length and comprises the general formula $X_1 \text{ CpGX}_2$ where X_1 and X_2 are nucleotide bases, and the C and G are unmethylated.

The preferred oligonucleotides for use in adjuvants or vaccines of the present invention preferably contain two or more dinucleotide CpG motifs preferably separated by at least three, more preferably at least six or more nucleotides.

The oligonucleotides of the present invention are typically deoxynucleotides. In a preferred embodiment the internucleotide in the oligonucleotide is phosphorodithioate, or more preferably a phosphorothioate bond, although phosphodiester and other internucleotide bonds are within the scope of the invention including oligonucleotides with mixed internucleotide linkages. e.g. mixed phosphorothioate/phosphodiesters. Other internucleotide bonds which stabilise the oligonucleotide may be used. Methods for producing phosphorothioate oligonucleotides or phosphorodithioate are described in US5,666,153, US5,278,302 and WO95/26204.

Examples of preferred oligonucleotides have the following sequences. The sequences preferably contain phosphorothioate modified internucleotide linkages.

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OLIGO 1: TCC ATG ACG TTC CTG ACG TT (CpG 1826) (SEQ ID NO 24)

OLIGO 2: TCT CCC AGC GTG CGC CAT (CpG 1758) (SEQ ID NO 25)

OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG (SEQ ID NO 26)

OLIGO 4: TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006) (SEQ ID NO 27)

10 OLIGO 5: TCC ATG ACG TTC CTG ATG CT (CpG 1668) (SEQ ID NO 28)

Alternative CpG oligonucleotides may comprise the preferred sequences above in that they have inconsequential deletions or additions thereto.

15 The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides may be synthesized utilising an automated synthesizer. An adjuvant formulation containing CpG oligonucleotide can be purchased from Qiagen under the trade name "ImmunEasy".

The following Examples serve to further illustrate the invention, with reference to the accompanying drawings, in which:

5 Fig 1 is a schematic view of HPV Immunotherapeutic vaccine construct of the invention.

Fig 2 is a plasmid map of P70776be2 – encoding HPV 6b E2 that has been codon optimised and mutated.

10 Fig 3 is a plasmid map of p73p1c6be1 – encoding HPV 6b E1 that has been codon optimised and mutated.

Fig 4 is a plasmid map of p707711e2 – encoding HPV 11 E2 that has been codon optimised and mutated.

15 Fig 5 is a plasmid map of HPV 102 – encoding HPV 11 E2 in p7313 background.

Fig 6 is a plasmid map of HPV 104 – fusion of E2 from HPV 6b and E2 from HPV 11 in p7313 background.

20 Fig 7 is a plasmid map of HPV 105 – fusion of codon optimised, mutated HPV 6b E2 and E2 from HPV 11.

25 Fig 8 is a plasmid map of HPV 108 – HPV 6b E1 codon optimised, mutated in p7313 background.

Fig 9 is a plasmid map of HPV 110 – HPV 6b E2 codon optimised, mutated in p7313 background.

30 Fig 10 is a plasmid map of HPV 116 – HPV 6b E1, HPV 6b E2, HPV 11 E2.

Fig 11 is a plasmid map of HPV 117 – HPV 6b E2, HPV 11 E2, HPV 6b E1.

Fig 12 is a plasmid map of HPV 118 – HPV 6b E2, HPV 11 E2, HPV 6b E1.

Fig 13 is a western blot analysis of three polyprotein constructs of the invention in 293 T cells.

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Fig 14 shows the incapacity of KIIIA mutated E2 in an invitro CAT transcriptional reporter assay.

Fig 15 shows cellular immune response in mice to E1

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Fig 16 shows cellular immune response to E2

Fig 17 - CTL assay data with HPV 118 after PMID

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Fig 18 shows reduction of warts after administration of E1/E2 in the COPV model.

1. Plasmid: pWRG7077 6be2 c/o mutated

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Gene of interest:

The HPV6be2 gene is approximately 1.1Kb in size and a codon optimised sequence (for human expression) was created using a visual basic programme called Syngene. In addition the sequence included a codon change at amino acid position 111, whereby a lysine residue (AAG) in the wild type was changed to an alanine residue (GCA) creating a mutated gene. This change inactivates the transcriptional activity of 6be2. Overlapping primers incorporating the whole gene with selected restriction sites at both the 5' and 3' ends were designed accordingly.

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Cloning:

The 1.1kb PCR fragment was gel purified and digested with restriction enzymes Not I and Bam HI for ligation into vector pWRG7077 (Powderject). The gene is

35

under control of the full immediate early CMV promoter and have a bovine growth hormone poly A tail.

Clones were sequenced indicated a number of base errors. A number of suitable clones were identified to enable construction of the correct gene sequence by using restriction digests. From re-cloning, one clone C7 was found to have only one base error at position 497 (T to C). Other clones were o.k. in this area and a simple fragment swap was just needed to correct the error. The final clone C7a was confirmed to be codon optimised mutated 6be2. (See Fig. 2)

6be2 sequence in pWRG7077 (Sequence ID No. 1)

ATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGTACGA
GGAAAACAGCACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCATGCGCCACGAGT
CAGTGCTCCTGTACAAGGCCAAGCAGATGGGGCTGTCCCACATCGGGATGCAGGTCGTG
CCCCCGCTGAAGGTGAGCGAAGCCAAGGGCCACAACGCTATCGAGATGCAGATGCACCT
GGAGAGCCTGCTGCGGACCGAATAACAGCATGGAGCCCTGGACTCTCCAGGAGACGTCCT
ACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCGCAAAGCGCGGCAAGACAGTTGAG
GTGAAATTCGATGGGTGCGCAAACAACACGATGGACTACGTGGTGTGGACCGATGTCTA
CGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAGGGCATCT
ATTACACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAG
TATGGTTCCACCAAGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCGC
CTCCGTGTCGTCCACCACCCAGGAAGTGAGCATTCGGGAGAGCACCACATACACCCCGG
CCCAAACGAGCACGCTCGTCAGCAGCAGCACCAGGAGGACGCGTCCAGACGCCCCC
CGGAAGAGGGCCCGGGGGGTCCAGCAGTCTCCCTGCAATGCCCTGTGCGTTGCTCACAT
CGGCCCTGTGATTCTGGGAACCACAATCTCATCAGAACCAACGACCAGCACCAAA
GGCGCAACAACCTCTAACAGCTCCGCAACTCCAATAGTGCAGTTCAGGGGGAGTCCAAC
TGCCTCAAGTGTTTCCGCTACCGCTCAACGACCGCCACCGCCACCTGTTGACTTGAT
CAGTTCACGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAAACACGCTATCGTGACGG
TGACCTACGACTCCGAGGAGCAGAGGCAGCAGTTCCTGGACGTCGTGAAGATTCTCCG
ACAATCAGCCACAAGCTTGGCTTCATGTCCCTGCACCTGCTGTGA

Amino acid sequence (Seq. ID No. 2)

MEAIKRLDA CQEQLLELYE ENSTD LHKHV LHWKMRHES VILLYKAKQMG LSHIGMQVVP

5 PLKVSEAKGH NAIEMQMHL E SLLRTEYSME PWTLOETSYE MWQTPPKRCF AKRGKTVEVK
 FDGCANNTMD YVWTDVYVQ DNDTWVKVHS MVDAGGIYYT CGQFKTYIVN FVKEAEKYGS
 TKHWEVCYGS TVICSPASVS STTQEVSIPE STTYTPAOTS TLVSSSTKED AVQTPPRKRA
 RGVQQSPCNA LCVAHIGPVD SGNHNLITNN HDQHQRNNS NSSATPIVQF QGESNCLKCF
 RYRLNDRHRH LFDLISSTWH WASSKAPHKH AIVTVTYDSE EQRQQFLDVV KIPPTISHKL
 GFMSLHLL

2. Plasmid: p7313plc 6be1 c/o mut

10 Gene of interest:

15 The HPV6be1 gene is approximately 2Kb in size and a codon optimised wild
 type (wt) sequence (for E.coli and human expression) was created using a
 statistical visual basic programme called Syngene. Overlapping primers
 incorporating the whole gene with selected restriction sites at both the 5' and 3'
 ends were designed accordingly. The synthesised gene was then digested with
 Bam HI and Not I restriction enzymes for ligation into vector pCIN4. From the
 sequencing data for a number of selected clones, numerous base errors were
 discovered. A correct clone was generated by combining a correct Pst I-Bam HI
 20 fragment from clone #24 and a Not I-Pst I fragment from clone #21 into p7313-
 plc. A correct clone (#1) was confirmed by sequencing. For mutagenesis
 primers were designed to change the following amino acids; lysine (AAA) to
 glycine (GGA) at position 83, arginine (CGC) to glycine (GGC) at position 84 and
 glycine (GGC) to asparagine(GAC) at position 482.

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6be1 codon optimised mutated sequence (Seq ID No. 3)

30 ATGGCAGACGATTCCGGTACTGAGAACGAAGGTTCTGGTTGTACCGGTTGGTTCATGGT
 TGAAGCAATCGTTCAGCATCCGACTGGTACCCAGATCTCCGATGACGAAGACGAAGAAG
 TTGAAGATTCTGGTTACGACATGGTTGACTTCATCGATGACTCCAACATCACTCATAAC
 TCTCTGGAAGCACAGGCTCTGTTTAAACCGCCAGGAAGCTGATACCCATTACGCTACTGT
 TCAGGACCTGGGAGGCAAATATCTGGGCTCTCCGTACGTTTCCCCGATCAACACTATCG
 35 CAGAAGCAGTTGAGTCTGAAATCTCCCCGCGCCTGGACGCTATCAAAGTACTCGTCAG
 CCGAAGAAGGTTAAACGTCGTCTGTTCCAGACTCGTGAAGTACCGACTCCGGTTACGG

TTATAGCGAAGTTGAGGCTGGCACC GG CACCCAGGTTGAAAAACACGGTGTACCGGAAA
 ACGGCGGCGACGGTCAGGAAAAGGACACCGGCCGCGACATCGAGGGTGAGGAACACACC
 GAAGCTGAAGCTCCGACTAACTCTGTTCTGTGAACACGCAGGTACTGCGGGTATCCTGGA
 ACTGCTGAAATGCAAAGACCTGCGCGCGGCTCTGCTGGGCAAATTCAAAGAATGCTTCG
 5 GCCTGTCTTTTCAATTGACCTGATCCGTCCGTTTAAGTCTGACAAAACCTACCTGTCTGGAC
 TGGGTGTAGCAGGCTTCGGCATCCACCACTCTATCTCTGAAGCATTCAGAAAACCTGAT
 CGAGCCGCTGTCTCTGTACGCGCACATCCAGTGGCTGACTAACGCTTGGGGTATGGTTC
 TGCTGGTACTGCTGCGCTTTAAAGTAAACAAATCTCGTTCCACTGTTGCTCGTACTCTG
 GCTACCCCTGCTGAACATCCCGGAGAACCAGATGCTGATCGAACCGCCGAAAATCCAGTC
 10 TGGTGTAGCTGCACTGTACTGGTTTCGTACTGGCATCTCTAACGCTAGCACTGTTATCG
 GTGAAGCACCGGAATGGATCACTCGTCAGACCGTTATCGAACACGGTCTGGCAGATTCT
 CAGTTCAAACCTGACTGAAATGGTTTCAGTGGGCATACGACAACGACATCTGCGAGGAATC
 TGAAATTGCGTTTGAATACGCTCAGCGTGGCGACTTCGACTCCAACGCTCGTGCTTTCC
 TGAACAGCAACATGCAGGCTAAATACGTAAAGACTGCGCTACCATGTGCCGCTCACTAC
 15 AAACACGCGGAAATGCGTAAAATGTCTATCAAACAGTGGATCAAGCACCGCGGTTCTAA
 AATCGAAGGTACCGGTAACTGGAAACCGATCGTTTCAGTTCTGCGCCATCAGAACATCG
 AATTCATCCCGTTCTTGACCAAATTCAGCTGTGGCTGCACGGTACCCCGAAAAAAAC
 TGCATCGCTATCGTAGGTCCACCGGACACTGACAAGTCTTACTTCTGTATGTCCCTGAT
 CTCTTTTCTGGGCGGCACTGTAATCTCTCACGTTAACTCTTCTCCCATTTCTGGCTGC
 20 AGCCACTGGTAGACGCGAAAGTAGCTCTGCTGGACGACGCGACCCAGCCGTGCTGGATC
 TACATGGATACTTACATGCGCAACCTGCTGGACGGTAACCCGATGTCTATCGACCGTAA
 ACACAAAGCGCTGACTCTGATCAAGTGCCCGCCGCTGCTGGTAACCTTCTAACATCGACA
 TCACCAAGGAAGATAAATACAAGTACCTGCATACCCGTGTTACTACCTTTACTTTCCCG
 AACCCGTTCCCGTTTGATCGTAACGGTAACGCTGTTTACGAACTGTCCAACACTAACTG
 25 GAAATGCTTCTTCGAGCGTCTGTCTTCTCCCTGGACATCCAGGACTCTGAAGATGAAG
 AAGATGGTTCTAACTCTCAGGCTTTCCGTTGTGTTCCGGGTACTGTTGTTCTGACTCTG
 TGA

Amino acid sequence (Seq. ID No. 4)

30 MADDSGTENE GSGCTGWF MV EAIVQHPTGT QISDDEDEEV EDSGYDMVDF
 IDDSNITHNS LEAQALFN RQ EADTHYATVQ DLGGKYL GSP YVSPINTIAE
 AVESEISPRL DAIKLTRQPK KVKRRLFQTR ELTDSGYGYS EVEAGTGTQV
 EKHGVPENGG DGQEKDTGRD IEGEEHTEAE APTNSVREHA GTAGILELLK
 35 CKDLRAALLG KFKECFGLSF IDLIRPFKSD KTTCLDWVVA GFGIHHSISE

AFQKLIEPLS LYAHIQWLTN AWGMVLLVLL RFKVNKSRST VARTLATLLN
 IPENQMLIEP PKIQSGVAAL YWFRTGISNA STVIGEAPew ITRQTVIEHG
 LADSQFKLTE MVQWAYDNDI CEESEIAFEY AQRGDFDSNA RAFLNSNMQA
 KYVKDCATMC RHYKHAEMRK MSIKQWIKHR GSKIEGTGNW KPIVQFLRHQ
 5 NIEFIPFLTK FKLWLHGTPK KNCIAIVGPP DTDKSYFCMS LISFLGGTVI
 SHVNSSSHFW LQPLVDAKVA LLDDATQPCW IYMDTYMRNL LDGNPMsIDR
 KHKALTLIKc PPLLVTsNID ITKEDKYKYL HTRVTTFTFP NPFPPDRNGN
 AVYELsNTNW KCFFERLSSS LDIQDSEDEE DGSNSQAFRC VPGTVVRTL

10 3. Plasmid: WRG7077 11e2 c/o mut

Gene of interest:

15 The HPV11e2 gene is approximately 1.1Kb in size and a codon optimised
 sequence (for human expression) was created using a visual basic programme
 called Syngene. In addition the sequence included a codon change at amino
 acid position 111, whereby a lysine residue (AAG) in the wild type was changed
 to an alanine residue (GCC) creating a mutated gene. This change has been
 20 shown in the literature to inactivate the transcriptional activity of the E2 protein.
 Overlapping primers incorporating the whole gene with selected restriction sites
 at both the 5' and 3' ends were designed accordingly, and were used to
 assemble the synthetic codon optimised mutant 11e2.

Cloning:

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The 1.2kb PCR fragment was gel purified and digested with restriction enzymes
 Not I and Bam HI for ligation into vector pWRG7077 (Powderject). The gene is
 under control of the full immediately early CMV promoter and has a bovine
 growth hormone poly A tail.

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Clones that were sequenced had indicated a number of base errors, these were
 subsequently corrected. A final clone F1 was found to be codon optimised
 mutated 11E2.

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11e2 sequence in pWRG7077 (Seq. ID No. 5)

ATGGAAGCCATCGCGAAGAGGCTCGACGCCTGCCAGGACCAGCTGCTCGAGCTGTACGA
 GGAGAACAGCATTGACATCCATAAGCACATCATGCACTGGAAGTGCATTGCGCTGGAGA
 GCGTGCTGTTGCACAAGGCCAAGCAGATGGGCCTGTCCACATAGGCCTTCAGGTGGTC
 5 CCCCCCTCTGACCGTGTGAGAGACAAAGGGCCATAACGCAATCGAGATGCAGATGCACCT
 CGAGTCGCTGGCGAAAACACAGTACGGCGTGGAGCCATGGACCTGTCAGGACACCTCGT
 ACGAAATGTGGCTGACCCACCTAAGCGATGCTTCGCCAAACAGGGCAACACAGTGGAG
 GTGAAGTTCGACGGCTGTGAGGATAACGTTATGGAGTATGTCGTGTGGACGCACATCTA
 TCTGCAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACGCGAAGGGCATCT
 10 ACTATACCTGTGGGCAGTTTAAACCTACTATGTGAACCTCAACAAAGAGGCCCAAAG
 TATGGCTCCACCAACCCTGAGGTCTGCTATGGGAGCACGGTGATTTGCTCTCCCGC
 CAGCGTGTCTAGCACTGTGCGCGAGGTGAGCATTGCCGAGCCGACCACGTACACCCCTG
 CCCAGACGACCGCTCCGACCGTGTCTGCTTGTACTACCGAGGACGGCGTGAGCGCTCCA
 CCCAGGAAGCGTGCGAGGGGGCCCAAGCACCAACAACACCCCTCTGTGTGGCGAACATTG
 15 CAGCGTCGACAGTACCATCAATAACATCGTGACGGATAACTATAACAAGCACCAGAGGC
 GTAACAACTGTCACTCTGCCGCAACCCCCATCGTGCAGCTCCAGGGAGACAGCAATTGC
 CTTAAGTGCTTCCGCTATCGCCTCAACGACAAGTACAAGCACCTCTTTGAGCTCGCCTC
 GTCGACGTGGCACTGGGCCTCACCCGAGGCACCTCACAAGAAGCCATCGTCACTCTCA
 CTTACTCCAGTGAGGAGCAGAGACAGCAGTTTCTGAACAGCGTGAAGATCCCACCGACG
 20 ATCCGTCATAAGGTGCGGCTTCATGTCACTGCATCTCCTGTGA

Amino acid sequence (Seq. ID No. 6)

MEAIAKRLDA CQDQLELYE ENSIDIHKHI MHWKCIRLES VLLHKAKQMG LSHIGLQVVP
 25 PLTVSETKGH NAIEMQMHL E SLAKTQYGV E PWTLQDTSY E MWLTPPKRCF AKQGNTEVEK
 FDGCEDNVME YVWTHIYLQ DND SWVKVTS SVD AKGIYTT CGQFKTY YVN FNKEAQKYGS
 TNHWEVCYGS TVICSPASVS STVREVSIAE PTTYTPAQT APTVSACTT DGV SAPPRKR
 ARG PSTNNTL CVANIRSVDS TINNI VTDNY NKHQR RNCH SAATPIVQLQ GDSNCLKCFR
 YRLNDKYKHL FELASSTWHW ASPEAPHKNA IVTLTYSSEE QRQQLNSVK IPPTIRHKVG
 30 FMSLHLL

4. Plasmid: HPV102 (p7313me 11e2 c/o mut)

Gene of interest:

Codon optimised mutated 11e2 was transferred from pWRG7077 11e2 c/o mut into another expression vector p7313me.

Cloning:

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The 11e2 c/o mut fragment was cut out of pWRG7077 11e2 vector by Bam HI and Not I restriction enzymes. This fragment was then ligated into p7313me vector using these sites.

10 11e2 sequence in HPV102 (Seq. ID No. 7)

ATGGAAGCCATCGCGAAGAGGCTCGACGCCTGCCAGGACCAGCTGCTCGAGCTGTACGA
GGAGAACAGCATTGACATCCATAAGCACATCATGCACTGGAAGTGCATTGCGCTGGAGA
GCGTGCTGTTGCACAAGGCCAAGCAGATGGGCCTGTCCACATAGGCCTTCAGGTGGTC
15 CCCCCCTCTGACCGTGTCTCAGAGACAAAGGGCCATAACGCAATCGAGATGCAGATGCACCT
CGAGTCGCTGGCGAAAACACAGTACGGCGTGGAGCCATGGACCCCTGCAGGACACCTCGT
ACGAAATGTGGCTGACCCACCTAAGCGATGCTTCGCCAAACAGGGCAACACAGTGGAG
GTGAAGTTCGACGGCTGTGAGGATAACGTTATGGAGTATGTCGTGTGGACGCACATCTA
TCTGCAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACGCGAAGGGCATCT
20 ACTATACCTGTGGGCAGTTTAAAACCTACTATGTGAACCTTCAACAAAGAGGCCCAAAG
TATGGCTCCACCAACCACTGGGAGGTCTGCTATGGGAGCACGGTGATTTGCTCTCCCGC
CAGCGTGTCTAGCACTGTGCGCGAGGTGAGCATTGCCGAGCCGACCACGTACACCCCTG
CCCAGACGACCGCTCCGACCGTGTCTGCTTGTACTACCGAGGACGGCGTGAGCGCTCCA
CCCAGGAAGCGTGCAGGGGGCCCAAGCACCAACAACACCCTCTGTGTGGCGAACATTGC
25 CAGCGTCGACAGTACCATCAATAACATCGTGACGGATAACTATAACAAGCACCAGAGGC
GTAACAACCTGTCACTCTGCGCAACCCCCATCGTGCAGCTCCAGGGAGACAGCAATTGC
CTTAAGTGCTTCCGCTATCGCCTCAACGACAAGTACAAGCACCTCTTTGAGCTCGCCTC
GTCGACGTGGCACTGGGCCTCACCCGAGGCACCTCACAAGAACGCCATCGTCACTCTCA
CTTACTCCAGTGAGGAGCAGAGACAGCAGTTTCTGAACAGCGTGAAGATCCACCGACG
30 ATCCGTCATAAGGTCCGCTTCATGTCACTGCATCTCCTGTGA

Amino acid sequence (Seq. ID No. 8)

35 MEAIKRLDA CQDQLELYE ENSIDIHKHI MHWKCIRLES VLLHKAKQMG LSHIGLQVVP
PLTVSETKGH NAIEMQMHLE SLAKTQYGVF PWTLDQTSYE MWLTTPPKRCF AKQGNTEVK

FDGCEENVME YVWTHIYLQ DNDSWVKVTS SVDKGIYYT CGQFKTYVFN FNKEAQKYGS
 TNHWEVCYGS TVICSPASVS STVREVSIAE PTTYTPAQT APTVSACTTE DGVSAPPRKR
 ARGPSTNNTL CVANIRSVDS TINNIIVTDNY NKHQRNNCH SAATPIVQLQ GDSNCLKCFR
 YRLNDKYKHL FELASSTWHW ASPEAPHKNA IVTLTYSSEE QRQOFLNSVK IPPTIRHKVG
 5 FMSLHLL

5. Plasmid: HPV104 (p7313me 6b/11e2 c/o mut)

Gene of interest:

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A fusion protein of 6be2 and 11e2 was constructed using 2x PCR with HPV102 and HPV110 as templates and appropriate designed primers. The fusion fragment ~2.2kb was cloned into p7313me expression vector with the 6be2 at the beginning of the fusion protein.

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Cloning:

The 2.2kb fusion was digested with Bam HI and Not I restriction enzymes and ligated into p7313me expression vector. Isolated clones were checked by
 20 sequencing and indicated no errors had been incorporated

6b/11e2 fusion sequence in HPV104 (Seq. ID No. 9)

ATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGTACGAGGAAAACAG
 25 CACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCATGCGCCACGAGTCAGTGCTCCTGTACAAGG
 CCAAGCAGATGGGGCTGTCCCACATCGGGATGCAGGTCGTGCCCCGCTGAAGGTGAGCGAAGCCAAG
 GGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCGGACCGAATACAGCATGGAGCC
 CTGGACTCTCCAGGAGACGTCCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCGCAAAGCGCG
 GCAAGACAGTTGAGGTGAAATTCGATGGGTGCGCAAACAACACGATGGACTACGTGGTGTGGACCGAT
 30 GTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAGGGCATCTATTA
 CACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAGTATGGTTCCACCA
 AGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCGCTCCGTGTCGTCCACCACCCAG
 GAAGTGAGCATTCCGGAGAGCACCATACACCCCGGCCCAAACGAGCAGCTCGTCAGCAGCAGCAC
 CAAGGAGGACGCCGTCCAGACGCCCCCCCCGGAAGAGGGCCCGGGGGTCCAGCAGTCTCCCTGCAATG
 35 CCCTGTGCGTTGCTCACATCGGCCCTGTGATTCTGGGAACCACAATCTCATCAGAACCAACCACGAC

CAGCACCAAAGGCGCAACAACTCTAACAGCTCCGCAACTCCAATAGTGCAGTTCCAGGGGGAGTCCAA
 CTGCCTCAAGTGTTCCTCGCTACCGCCTCAACGACCGCCACCGCCACCTGTTGACTTGATCAGTTCCA
 CGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAAACACGCTATCGTGACGGTGACCTACGACTCCGAG
 GAGCAGAGGCAGCAGTTCTGGACGTCGTGAAGATTCTCCGACAATCAGCCACAAGCTTGGCTTCAT
 5 GTCCCTGCACCTGCTGATGGAAGCCATCGCGAAGAGGCTCGACGCCTGCCAGGACCAGCTGCTCGAGC
 TGTACGAGGAGAACAGCATTGACATCCATAAGCACATCATGCACTGGAAGTGCATTGCGCTGGAGAGC
 GTGCTGTTGCACAAGGCCAAGCAGATGGGCCTGTCCCACATAGGCCTTCAGGTGGTCCCCCTCTGAC
 CGTGTGAGAGACAAAGGGCCATAACGCAATCGAGATGCAGATGCACCTCGAGTCGCTGGCGAAAACAC
 AGTACGGCGTGGAGCCATGGACCCTGCAGGACACCTCGTACGAAATGTGGCTGACCCACCTAAGCGA
 10 TGCTTCGCCAAACAGGGCAACACAGTGGAGGTGAAGTTCGACGGCTGTGAGGATAACGTTATGGAGTA
 TGTCTGTGAGCAGCACATCTATCTGCAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACG
 CGAAGGGCATCTACTATACCTGTGGGCAGTTTAAACCTACTATGTGAACCTCAACAAAGAGGGCCAA
 AAGTATGGCTCCACCAACCACTGGGAGGTCTGCTATGGGAGCACGGTGATTTGCTCTCCCGCCAGCGT
 GTCTAGCACTGTGCGCGAGGTGAGCATTGCCGAGCCGACCACGTACACCCCTGCCAGACGACCGCTC
 15 CGACCGTGTCTGCTTGTACTACCGAGGACGGCGTGAGCGCTCCACCCAGGAAGCGTGCGAGGGGCCCA
 AGCACCAACAACACCCCTCTGTGTGGCGAACATTGCGAGCGTCGACAGTACCATCAATAACATCGTGAC
 GGATAACTATAACAAGCACCAGAGGCGTAACAACTGTCACTCTGCCGCAACCCCCATCGTGACGCTCC
 AGGGAGACAGCAATTGCCTTAAGTGCTTCCGCTATCGCCTCAACGACAAGTACAAGCACCTCTTTGAG
 CTCGCCTCGTCGAGTGGCACTGGGCCTCACCGAGGCACCTCACAAGAACGCCATCGTCACTCTCAC
 20 TTAATCCAGTGAGGAGCAGAGACAGCAGTTTCTGAACAGCGTGAAGATCCCACCGACGATCCGTCATA
 AGGTGGGCTTCATGTCACTGCATCTCCTGA

Amino acid sequence (Seq. ID No. 10)

25 MEAIAKRLDA CQEQLLELYE ENSTD LHKHV LHWKCMRHS VLLYKAKQMG LSHIGMQVVP
 PLKVSEAKGH NAIEMQMHL E SLLRTEYSME PWT LQETSYE MWQTPPKRCF AKRGKTVEVK
 FDGCANN TMD YVWTDVYVQ DNDTWVKVHS MVDAGGIYTT CGQFKTYVFN FVKEAEKYGS
 TKHWEVCYGS TVICSPASVS STTQEVS IPE STTYTPAQTS TLVSSSTKED AVQT PPRKRA
 RGVQQSPCNA LCVAHIGPVD SGNHN LITNN HDQHQRNNNS NSSATPIVQF QGESNCLKCF
 30 RYRLNDRHRH LFDLISSTWH WASSKAPHKH AIVTVTYDSE EQRQQFLDVV KIPPTISHKL
 GFMSLHLLME AIAKRLDACQ DQLELYEEN SIDIHKHIMH WK CIRLESVL LHKAKQMGLS
 HIGLQVVPPL TVSETKGHNA IEMQMHL E SL AKTYQGVPEW TLQDTSYEMW LTPPKRCFAK
 QGNTVEVKFD GCEDNVMEYV WTHIYLQDN DSWVKVTSSV DAKGIYYTCG QFKTYVNFN
 KEAQKYGSTN HWEVCYGSTV ICSPASVSST VREVSIAEPT TYTPAQTTAP TVSACTTEDG
 35 VSAPPRKRAR GPSTNNTLCV ANIRSV DSTI NNIVTDN YNK HQRRNNCHSA ATPIVQLQGD

SNCLKCFRYR LNDKYKHLFE LASSTWHWAS PEAPHKNAIV TLTYSSSEQR QOFLNSVKIP
PTIRHKVGFM SLHLL

5 6. Plasmid: HPV105 (p7313me 11/6be2 c/o mut)

Gene of interest:

10 A fusion protein of 6be2 and 11e2 was constructed using 2x PCR with HPV102 and HPV110 as templates and appropriate designed primers. The fusion fragment ~2.2kb was cloned into p7313me expression vector and with the 11e2 at the beginning of the fusion protein.

Cloning:

15 The 2.2kb fusion was digested with Bam HI and Not I restriction enzymes and ligated into p7313me expression vector. Isolated clones were checked by sequencing and indicated no errors had been incorporated.

11/6bE2 fusion sequence in HPV105 (Seq. ID No. 11)

20
ATGGAAGCCATCGCGAAGAGGCTCGACGCCTGCCAGGACCAGCTGCTCGAGCTGTACGAGGAGAACAG
CATTGACATCCATAAGCACATCATGCACTGGAAGTGCATTTCGCCTGGAGAGCGTGCTGTTGCACAAGG
CCAAGCAGATGGGCCTGTCCACATAGGCCTTCAGGTGGTCCCCCTCTGACCGTGTGAGAGACAAAG
GGCCATAACGCAATCGAGATGCAGATGCACCTCGAGTCGCTGGCGAAAACACAGTACGGCGTGGAGCC
25 ATGGACCTGCAGGACACCTCGTACGAAATGTGGCTGACCCACCTAAGCGATGCTTCGCCAAACAGG
GCAACACAGTGGAGGTGAAGTTCGACGGCTGTGAGGATAACGTTATGGAGTATGTGCTGTGGACGCAC
ATCTATCTGCAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACGCGAAGGGCATCTACTA
TACCTGTGGGCAGTTTAAACCTACTATGTGAACCTCAACAAAGAGGCCCAAAAGTATGGCTCCACCA
ACCACTGGGAGGTCTGCTATGGGAGCACGGTGATTGTCTCTCCCGCCAGCGTGTCTAGCACTGTGCGC
30 GAGGTGAGCATTGCCGAGCCGACCAGTACACCCCTGCCAGACGACCGCTCCGACCGTGTCTGCTTG
TACTACCGAGGACGGCGTGAGCGCTCCACCCAGGAAGCGTGCAGGGGGCCCAAGCACCAACAACACCC
TCTGTGTGGCGAACATTTCGACGCGTCGACAGTACCATCAATAACATCGTGACGGATAACTATAACAAG
CACCAGAGGGCGTAACAACCTGTCACTCTGCCGCAACCCCCATCGTGACGCTCCAGGGAGACAGCAATTG
CCTTAAGTGCTTCCGCTATCGCCTCAACGACAAGTACAAGCACCTCTTTGAGCTCGCCTCGTGCAGCT
35 GGCACCTGGGCCTCACCGAGGCACCTCACAAGAACGCCATCGTCACTCTCACTTACTCCAGTGAGGAG
CAGAGACAGCAGTTTCTGAACAGCGTGAAGATCCCACCGACGATCCGTCATAAGGTGGGCTTCATGTC

ACTGCATCTCCTGATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGT
 ACGAGGAAAAACAGCACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCATGCGCCACGAGTCAGTG
 CTCCTGTACAAGGCCAAGCAGATGGGGCTGTCCACATCGGGATGCAGGTCGTGCCCCCGCTGAAGGT
 GAGCGAAGCCAAGGGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCGGACCGAAT
 5 ACAGCATGGAGCCCTGGACTCTCCAGGAGACGTCCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGT
 TTCGCAAAGCGCGGCAAGACAGTTGAGGTGAAATTCGATGGGTGCGCAAACAACACGATGGACTACGT
 GGTGTGGACCGATGTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCA
 AGGGCATCTATTACACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAG
 TATGGTTCCACCAAGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCCGCTCCGTGTC
 10 GTCCACCACCCAGGAAGTGAGCATTCGGGAGAGCACCACATACACCCCGGCCCAAACGAGCACGCTCG
 TCAGCAGCAGCACCAAGGAGGACGCCGTCCAGACGCCCCCCCCGGAAGAGGGCCCGGGGGTCCAGCAG
 TCTCCCTGCAATGCCCTGTGCGTTGCTCACATCGGCCCTGTGATTCCTGGGAACCACAATCTCATCAC
 GAACAACCACGACCAGCACCAAGGCGCAACAACCTCTAACAGCTCCGCAACTCCAATAGTGCAGTTCC
 AGGGGGAGTCCAACCTGCCTCAAGTGTTCGCTACCGCCTCAACGACCGCCACCGCCACCTGTTGAC
 15 TTGATCAGTTCCACGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAAACACGCTATCGTGACGGTGAC
 CTACGACTCCGAGGAGCAGAGGCAGCAGTTCTTGGACGTGCTGAAGATTCTCCGACAATCAGCCACA
 AGCTTGGCTTCATGTCCCTGCACCTGCTGA

Amino acid sequence (Seq. ID No. 12)

20 MEAIAKRLDA CQDQLLELYE ENSIDIHKHI MHWKCIRLES VLLHKAKQMG LSHIGLQVVP
 PLTVSETKGH NAIEMQMHLE SLAKTQYQVE PWTLQDTSYE MWLTPPKRCF AKQGNITVEVK
 FDGCEDNME YVWVTHIYLQ DNDSWVKVTS SVDAGIYYT CGQFKTYIVN FNKEAQKYGS
 TNHWEVCYGS TVICSPASVS STVREVSIAE PTITYTPAQT APTVSACTTE DGVSAAPPRKR
 25 ARGPSTNNTL CVANIRSVDS TINNIIVTDNY NKHQRRNNCH SAATPIVQLQ GDSNCLKCFR
 YRLNDKYKHL FELASSTWHW ASPEAPHKNA IVTLTYSSEE QRQOFLNSVK IPPTIRHKVG
 FMSLHLIMEA IAKRLDACQE QLLELYEENS TDLHKHVLHW KCMRHESVLL YKAKQMGLSH
 IGMQVVPPLK VSEAKGHNAI EMQMHLESLL RTEYSMEPWT LQETSYEMWQ TPPKRCFAKR
 GKTVEVKFDG CANNTMDYVV WTDVYVQDND TWVKVHSMVD AKGIYYTCGQ FKTYIVNFEVK
 30 EAEKYGSTKH WEVCYGSTVI CSPASVSSTT QEVSIPBSTT YTPAQTSTLV SSSTKEDAVQ
 TPPRKRARGV QQSPCNALCV AHIGPVDNHN HNLITNNHDQ HQRRNNSNSS ATPIVQFQGE
 SNCLKCFRYR LNDRHRHLFD LISSTWHWAS SKAPHKHAIV TVTYDSEEQR QQFLDVVKIP
 PTISHKLGFM SLHL

35 7. Plasmid: HPV108 (p7313ie 6be1 c/o mut)

Gene of interest:

5 Codon optimised mutated 6be1 was transferred from p7313plc 6be1 c/o mut clone N into vector p7313ie.

Cloning:

10 The 6be1 c/o mut fragment was cut out of the p7313plc 6be2 clone by Not I and Bam HI restriction digests. This fragment was then ligated into p7313ie vector using these sites. The gene is under the control of the ie promoter (immediate early cmv + exon1) and followed by a rabbit *b*-globin poly-adenylation signal.

15

6be1 sequence in p7313ie (Seq. ID No. 13)

ATGGCAGACGATTCCGGTACTGAGAACGAAGGTTCTGGTTGTACCGGTTGGTTCATGGTTGAAGCAAT
CGTTCAGCATCCGACTGGTACCCAGATCTCCGATGACGAAGACGAAGAAGTTGAAGATTCTGGTTACG
20 ACATGGTTGACTTCATCGATGACTCCAACATCACTCATAACTCTCTGGAAGCACAGGCTCTGTTTAAC
CGCCAGGAAGCTGATACCCATTACGCTACTGTTTCAGGACCTGGGAGGCAAATATCTGGGCTCTCCGTA
CGTTTCCCGATCAACACTATCGCAGAAGCAGTTGAGTCTGAAATCTCCCGCGCCTGGACGCTATCA
AACTGACTCGTCAGCCGAAGAAGGTTAAACGTCGTCTGTTCCAGACTCGTGAACTGACCGACTCCGGT
TACGGTTATAGCGAAGTTGAGGCTGGCACC CGCACCCAGGTTGAAAAACACGGTGTACCGGAAAAACGG
25 CGCGCAGGTCAGGAAAAGGACACCGGCCGACATCGAGGGTGAGGAACACACCGAAGCTGAAGCTC
CGACTAACTCTGTTCTGTAACACGCAGGTA CTGCGGGTATCCTGGAAGTGTGAAATGCAAAGACCTG
CGCGCGGCTCTGCTGGGCAAATTCAAAGAATGCTTCGGCCTGTCTTTCA TTGACCTGATCCGTCCGTT
TAAGTCTGACAAAACTACCTGTCTGGACTGGGTTGTAGCAGGCTTCGGCATCCACCACTCTATCTGT
AAGCATTCAGAAACTGATCGAGCCGCTGTCTCTGTACGCGCACATCCAGTGGCTGACTAACGCTTGG
30 GGTATGGTTCTGCTGGTACTGCTGCGCTTTAAAGTAAACAAATCTCGTTCCACTGTTGCTCGTACTCT
GGCTACCCCTGTGTAACATCCCGGAGAACAGATGCTGATCGAACCGCCGAAATCCAGTCTGGTGTAG
CTGCACTGTACTGGTTTTCGTACTGGCATCTCTAACGCTAGCACTGTTATCGGTGAAGCACCGGAATGG
ATCACTCGTCAGACCGTTATCGAACACGGTCTGGCAGATTCTCAGTTCAAAC TGAAGTGAATGGTTCA
GTGGGCATACGACAACGACATCTGCGAGGAATCTGAAATTGCGTT CGAATACGCTCAGCGTGGCGACT
35 TCGACTCCAACGCTCGTGCTTTCTGTAACAGCAACATGCAGGCTAAATACGTAAAAGACTGCGCTACC

ATGTGCCGTCACTACAAACACGCGGAAATGCGTAAAATGTCTATCAAACAGTGGATCAAGCACCGCGG
 TTCTAAAATCGAAGGTACCGGTAACCTGGAAACCGATCGTTCAGTTCCTGCGCCATCAGAACATCGAAT
 TCATCCCGTTCCTGACCAAATTCAAGCTGTGGCTGCACGGTACCCCGAAAAAACTGCATCGCTATC
 GTAGGTCCACCGGACACTGACAAGTCTTACTTCTGTATGTCCCTGATCTCTTTCTGGGCGGCACTGT
 5 AATCTCTCACGTAACTCTTCTCCCATTTCTGGCTGCAGCCACTGGTAGACGCGAAAGTAGCTCTGC
 TGGACGACGCGACCCAGCCGTGCTGGATCTACATGGATACTTACATGCGCAACCTGCTGGACGGTAAC
 CCGATGTCTATCGACCGTAAACACAAAGCGCTGACTCTGATCAAGTGCCCGCCGCTGCTGGTAACTTC
 TAACATCGACATCACCAAGGAAGATAAATACAAGTACCTGCATACCCGTGTTACTACCTTTACTTTCC
 CGAACCCGTTCCCGTTTGATCGTAACGGTAACGCTGTTTACGAACTGTCCAACACTAACTGGAAATGC
 10 TTCTTCGAGCGTCTGTCTTCTCCCTGGACATCCAGGACTCTGAAGATGAAGAAGATGGTTCTAACTC
 TCAGGCTTTCCGTTGTGTTCCGGGTACTGTTGTTTCGTACTCTGTGA

Amino acid sequence (Seq. ID No. 14)

15 MADDSGTENE GSGCTGWFMV RAIVQHPTGT QISDDEDEEV EDSGYDMVDF IDDSNITHNS
 LEAQALFNRO EADTHYATVQ DLGGKYLGSF YVSPINTIAE AVESEISPRL DAIKLTRQPK
 KVKRRLFQTR ELTDSGYGYS EVEAGTGTQV EKHGVPENGG DGQEKDTGRD IEGEEHTEAE
 APTNSVREHA GTAGILELLK CKDLRAALLG KFKECFGLSF IDLIRPFKSD KTTCLDWVVA
 GFGIHHSISE AFQKLIEPLS LYAHIQWLTN AWGMVLLVLL RPKVNRKRST VARTLATLIN
 20 IPENQMLIEP PKIQSGVAAL YWFRGTGISA STVIGEAPFW ITRQTVIEHG LADSQFKLTE
 MVQWAYDNDI CESEIAFEY AQRGDFDSNA RAFLNSNMQA KYVKDCATMC RHYKHAEMRK
 MSIKQWIKHR GSKIEGTGNW KPIVQFLRHQ NIEFIPFLTK FKLWLHGTPK KNCIAIVGPP
 DTDKSYFCMS LISFLGGTVI SHVNSSSHFW LQPLVDKVA LLDDATQPCW IYMDTYMRNL
 LDGNPMSIDR KHKALTLIKC PPLLVTSNID ITKEDKYKYL HTRVTTFTFP NPFFPDRNGN
 25 AVYELSNNTW KCFFERLSSS LDIQDSEDEE DGSNSQAFRC VPGTVVRTL

8. Plasmid: HPV110 (p7313ie 6be2 c/o mut)

Gene of interest:

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Codon optimised mutated 6be2 was transferred from pWRG7077 6be2 into vector p7313ie.

Cloning:

35

The 6be2 c/o mut fragment was cut out of pWRG7077 6be2 clone by Not I and Bam HI restriction digests. This fragment was then ligated into p7313ie vector using these sites. The gene is under the control of the ie promoter (immediate early cmv + exon1) and followed by a rabbit *b*-globin poly-adenylation signal.

5

6be2 sequence in p7313ie (Seq. ID No. 15)

ATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGTACGAGGAAAACAG
CACAGACCTCCACAAGCACGCTGCTGCACTGGAAGTGCATGCCGCCACGAGTCAGTGCTCCTGTACAAGG
10 CCAAGCAGATGGGGCTGTCCACATCGGGATGCAGGTCGTGCCCCCGCTGAAGGTGAGCGAAGCCAAG
GGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCGGACCGAATACAGCATGGAGCC
CTGGACTCTCCAGGAGACGTCCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCGCAAAGCGCG
GCAAGACAGTTGAGGTGAAATTCGATGGGTGCGCAAACAACACGATGGACTACGTGGTGTGGACCGAT
GTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAGGGCATCTATTA
15 CACCTGCGGGCAGTTCAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAGTATGGTTCCACCA
AGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCCGCCTCCGTGTGCTCCACCACCCAG
GAAGTGAGCATTCCGGAGAGCACCACATACCCCCGGCCCAAACGAGCAGCTCGTCAGCAGCAGCAC
CAAGGAGGACGCGCTCCAGACGCCCCCGGAAGAGGGCCCGGGGGTCCAGCAGTCTCCCTGCAATG
CCCTGTGCGTTGCTCACATCGGCCCTGTGATTCGTGGGAACCACAATCTCATCACGAACAACCACGAC
20 CAGCACCAAAGGCGCAACAACCTCTAACAGCTCCGCAACTCCAATAGTGCAAGTTCAGGGGGGAGTCCAA
CTGCCTCAAGTGTTCGCTACCGCCTCAACGACCGCCACCGCCACCTGTTGACTTGATCAGTTCCA
CGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAAACACGCTATCGTGACGGTGACCTACGACTCCGAG
GAGCAGAGGCAGCAGTTCCTGGACGTGCTGAAGATTCTCCGACAATCAGCCACAAGCTTGGCTTCAT
GTCCCTGCACCTGCTGTGA

25

Amino acid sequence (Seq. ID No. 16)

MEAIKRLDA CQEQLLELYE ENSTD LHKHV LHWKCMRHES VLLYKAKQMG LSHIGMQVVP
PLKVSEAKGH NAIEMQMHL E SLLRTEYSME PWT LQETSYE MWQTPPKRCF AKRGKTVEVK
30 FDGCANNIMD YVWTDVYVQ DNDTWVKVHS MVDAGGIYTT CGQFKTYIVN FVKEAEKYGS
TKHWEVCYGS TVICSPASVS STTQEVSIPE STTYTPAOTS TLVSSSTKED AVQTPPRKRA
RGVQQSPCNA LCVAHIGPVD SGNHNLITNN HDQHQRNNNS NSSATPIVQF QGESNCLKCF
RYRLNDRHRH LFDLISSTWH WASSKAPHKH AIVTVTYDSE EQRQQFLDVV KIPPTISHKL
GFMSLHLL

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9. Plasmid: HPV116 (p7313ie 6be1.6be2.11e2)

Gene of Interest:

- 5 The gene for the polyprotein in construct HPV116 is a triple fusion protein comprised in order of 6be1, 6be2, 11e2 all codon optimised and mutated. The polyprotein gene was assembled by PCR from using 2 previous PCR fragments; 6be1 and 6b/11e2. The size of the gene is ~4.1kb, producing a polyprotein of ~170kD, observed by PAGE and Western blot.

10

Cloning:

- 15 The polyprotein gene was digested with Bam HI + Not I restriction enzymes and ligated into p7313ie vector. Sequencing analysis of selected clones had indicated the 'odd' base change, but this was overcome by various fragment swapping. A resulting clone hpv116 #1 was found to have no errors.

20

Polyprotein sequence in HPV116 (Seq. ID No. 17)

ATGGCAGACGATTCCGGTACTGAGAACGAAGGTTCTGGTTGTACCGGTTGGTTCATGGTTGAAGCAA
 TCGTTCAGCATCCGACTGGTACCCAGATCTCCGATGACGAAGACGAAGAAGTTGAAGATTCTGGTTA
 25 CGACATGGTTGACTTCATCGATGACTCCAACATCACTCATAACTCTCTGGAAGCACAGGCTCTGTTT
 AACCGCCAGGAAGCTGATACCCATTACGCTACTGTTTCAGGACCTGGGAGGCAAATATCTGGGCTCTC
 CGTACGTTTTCCCCGATCAACACTATCGCAGAAGCAGTTGAGTCTGAAATCTCCCCGCGCCTGGACGC
 TATCAAACCTGACTCGTCAGCCGAAGAAGGTTAAACGTCGTCTGTTCCAGACTCGTGAACCTGACCGAC
 TCCGGTTACGGTTATAGCGAAGTTGAGGCTGGCACCAGGTTGAAAAACACGGTGTACCGG
 30 AAAACGGCGGCGACGGTCAGGAAAAGGACACCGGCCGCGACATCGAGGGTGAGGAACACACCGAAGC
 TGAAGCTCCGACTAACTCTGTTCTGTAACACGCAGGTACTGCGGGTATCCTGGAACCTGCTGAAATGC
 AAAGACCTGCGCGCGGCTCTGCTGGGCAAATTCAAAGAATGCTTCGGCCTGTCTTTCATTGACCTGA
 TCCGTCCGTTTAAAGTCTGACAAAACCTACCTGTCTGGACTGGGTTGTAGCAGGCTTCGGCATCCACCA
 CTCTATCTCTGAAGCATTCCAGAACTGATCGAGCCGCTGTCTCTGTACGCGCACATCCAGTGGCTG
 35 ACTAACGCTTGGGGTATGGTTCTGCTGGTACTGCTGCGCTTTAAAGTAAACAAATCTCGTTCCACTG

TTGCTCGTACTCTGGCTACCCCTGCTGAACATCCCGGAGAACCCAGATGCTGATCGAACCGCCGAAAAT
 CCAGTCTGGTGTAGCTGCACGTACTGGTTTCGTACTGGCATCTCTAACGCTAGCACTGTTATCGGT
 GAAGCACCGGAATGGATCACTCGTCAGACCGTTATCGAACACGGTCTGGCAGATTCTCAGTTCAAAC
 TGA CTGAAATGGTT CAGTGGGCATACGACAACGACATCTGCGAGGAATCTGAAATTGCGTTCGAATA
 5 CGCTCAGCGTGGCGACTTCGACTCCAACGCTCGTGCTTTCTGAAACAGCAACATGCAGGCTAAATAC
 GTAAAAGACTGCGCTACCATGTGCCGTCCTACAAACACGCGGAAATGCGTAAAATGTCTATCAAAC
 AGTGGATCAAGCACCGCGGTTCTAAAATCGAAGGTACCGGTAACGGAAACCGATCGTT CAGTT CCT
 GCGCCATCAGAACATCGAATTCATCCCGTTCTTGACCAAATTCAGCTGTGGCTGCACGGTACCCCG
 AAAAAAACTGCATCGCTATCGTAGGTCCACCGGACACTGACAAAGTCTTACTTCTGTATGTCCCTGA
 10 TCTCTTTCTGGGGCGGCACTGTAATCTCTCAGTTAACTCTTCTCCATTTCTGGCTGCAGCCACT
 GGTAGACGCGAAAGTAGCTCTGCTGGACGACGCGACCCAGCCGTGCTGGATCTACATGGATACTTAC
 ATGCGCAACCTGCTGGACGGTAACCCGATGTCTATCGACCGTAAACACAAAGCGCTGACTCTGATCA
 AGTGCCCGCGCTGCTGGTAACTTCTAACATCGACATCACCAAGGAAGATAAATACAAGTACCTGCA
 TACCCGTGTTACTACCTTTACTTTCCCGAACCCGTTCCCGTTTGATCGTAACGGTAACGCTGTTTAC
 15 GAACTGTCCAACACTAACTGGAAATGCTTCTTCGAGCGTCTGTCTTCTCCCTGGACATCCAGGACT
 CTGAAGATGAAGAAGATGGTTCTAACTCTCAGGCTTTCCGTTGTGTTCCGGGTACTGTTGTTCTGAC
 TCTGATGGAAGCTATTGCCAAGCGACTGGACGCGCTGCCAGGAGCAGCTGCTGGAGCTGTACGAGGAA
 AACAGCACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCATGCGCCACGAGTCAGTGCTCCTGT
 ACAAGGCCAAGCAGATGGGGCTGTCCACATCGGGATGCAGGTCTGTGCCCCCGCTGAAGGTGAGCGA
 20 AGCCAAGGGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCGGACCGAATACAGC
 ATGGAGCCCTGGACTCTCCAGGAGACGTCCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCG
 CAAAGCGCGGCAAGACAGTTGAGGTGAAATTCGATGGGTGCGCAAACAACAGATGGACTACGTGGT
 GTGGACCGATGTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAG
 GGCATCTATTACACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAGT
 25 ATGGTTCCACCAAGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCGCTCCGTGTC
 GTCCACCACCCAGGAAGTGAGCATTCCGGAGAGACCACATACACCCCGGCCCAAACGAGCACGCTCG
 TCAGCAGCAGCACCAAGGAGGACGCCGTCCAGACGCCCCCCCCGGAAGAGGGCCCGGGGGTCCAGCA
 GTCTCCCTGCAATGCCCTGTGCGTTGCTCACATCGGCCCCGTGTCGATTCTGGGAACCACAATCTCATC
 ACGAACAACCACGACCAGCACCAAGGCGCAACAACCTCTAACAGCTCCGCAACTCCAATAGTGCAGT
 30 TCCAGGGGGAGTCCAACCTGCCTCAAGTGTTTCCGCTACCGCCTCAACGACCGCCACCGCCACCTGTT
 CGACTTGATCAGTTCCACGTGGCACTGGGGCCAGCAGCAAGGCGCCCCACAAACAGCTATCGTGACG
 GTGACCTACGACTCCGAGGAGCAGAGGCAGCAGTTCTTGACGTGCGTGAAGATTCTCCGACAATCA
 GCCACAAGCTTGGCTTCATGTCCCTGCACCTGCTGATGGAAGCCATCGCGAAGAGGCTCGACGCCTG
 CCAGGACCAGCTGCTCGAGCTGTACGAGGAGAACAGCATTGACATCCATAAGCACATCATGCACTGG
 35 AAGTGCAATTCGCTGAGAGCGTGCTGTTGCACAAGGCCAAGCAGATGGGCTGTCCACATAGGCC

TTCAGGTGGTCCCCCTCTGACCGTGTGACAGACAAAGGGCCATAACGCAATCGAGATGCAGATGCA
 CCTCGAGTCGCTGGCGAAAACACAGTACGGCGTGGAGCCATGGACCCCTGCAGGACACCTCGTACGAA
 ATGTGGCTGACCCACCTAAGCGATGCTTCGCCAAACAGGGCAACACAGTGGAGGTGAAGTTTCGACG
 GCTGTGAGGATAACGTTATGGAGTATGTCGTGTGGACGCACATCTATCTGCAGGACAACGACAGTTG
 5 GGTGAAGGTGACCAGCTCCGTGGACGCGAAGGGCATCTACTATACCTGTGGGCAGTTTAAACCTAC
 TATGTGAACCTCAACAAAGAGGCCCAAAAGTATGGCTCCACCAACCACTGGGAGGTCTGCTATGGGA
 GCACGGTGATTTGCTCTCCCGCCAGCGTGTCTAGCACTGTGCGCGAGGTGAGCATTGCCGAGCCGAC
 CACGTACACCCCTGCCAGACGACCGCTCCGACCGTGTCTGCTTGTACTACCGAGGACGGCGTGAGC
 GCTCCACCCAGGAAGCGTGCGAGGGGCCAAAGCACCAACAACACCCCTCTGTGTGGCGAACATTGCA
 10 GCGTCGACAGTACCATCAATAACATCGTGACGGATAACTATAACAAGCACCAGAGGCGTAACAACCTG
 TCACTCTGCCGCAACCCCCATCGTGACGCTCCAGGGAGACAGCAATTGCCTTAAGTGCTTCCGCTAT
 CGCCTCAACGACAAGTACAAGCACCTCTTTGAGCTCGCCTCGTTCGACGTGGCACTGGGCCTCACCCG
 AGGCACCTCACAAGAACGCCATCGTCACTCTCACTTACTCCAGTGAGGAGCAGAGACAGCAGTTTCT
 GAACAGCGTGAAGATCCCAACGACGATCCGTCATAAGGTCCGGCTTCATGTCACTGCATCTCCTGTGA
 15

Amino acid sequence (Seq. ID No. 18)

MADDSGTENE GSGCTGWMV EAIHQHTGT QISDDEDEEV EDSGYDMVDF IDDSNITHNS
 LEAQALFNRO EADTHYATVQ DLGGKYLGSF YVSPINTIAE AVESEISPRL DAIKLTRQPK
 20 KVKRRLEFQR ELTDSGYGYS EVEAGTGTQV EKHGVPENGG DGQEKDTRD IEGEEHTEAE
 APTNSVREHA GTAGILELLK CKDLRAALLG KFKECFGLSF IDLIRPFKSD KTTCLDWVVA
 GFGIHHSISE AFQKLIEPLS LYAHIQWLTN AWGMVLLVLL RFKVNKSRST VARTLATLLN
 IPENQMLIEP PKIQSGVAAL YWFRTGISNA STVIGEAPFW ITROTVEIHG LADSQFKLTE
 MVQWAYDNDI CEESEIAFEY AQRGDFDSNA RAFLNSNMQA KYVKDCATMC RHYKHAEMRK
 25 MSIKQWIKHR GSKIEGTGNW KPIVQFLRHQ NIEFIPFLTK FKLWLHGTPK KNCIAIVGPP
 DTDKSYFCMS LISFLGGTVI SHVNSSSHFW LQPLVDAKVA LLDDATQPCW IYMDTYMRNL
 LDGNPMSIDR KHKALTLIK PPLLVTSNID ITKEDKYKYL HTRVTTFTFP NPPFPDRNGN
 AVYELSNNTW KCFFERLSSS LDIQDSEDEE DGSNSQAFRC VPGTVVRTLM EAIKRLDAC
 QEQLLELYEE NSTDLHKHVL HWKCMRHESV LLYKAKQMGL SHIGMQVPP LKVSEAKGHN
 30 AIEMQMHLBS LLRTEYSMEP WTLQETSYEM WQTPPKRCFA KRGKTVEVKF DGCANNMTDY
 VVWTDVYVQD NDTWVKVHSM VDAKGIYYTC GQFKTYVNF VKEAEKYGST KHWEVCYGST
 VICSPASVSS TTQEVSIPE TTYTPAQTST LVSSSTKEDA VQTPPRKRAR GVQQSPCNAL
 CVAHIGPVDS GNHNLTNNH DQHQRNNSN SSATPIVQFQ GESNCLKCFR YRLNDRHRHL
 FDLISSTWHW ASSKAPHKHA IVTVTYDSEE QRQQFLDVVK IPPTISHKLG FMSLHLLMEA
 35 IAKRLDACQD QLELYEENS IDIHKHIMHW KCIRLESVLL HKAKQMGLSH IGLQVVPPLT

VSETKGHNAI EMQMHLESIA KTQYGVPEWT LQDTSYEMWL TPPKRCFAKQ GNTVEVKFDG
 CEDNVMEYVV WTHIYLQDND SWVKVTSSVD AKGIYYTCGQ FKTYVNFNK EAQKYGSTNH
 WEVCYGSTVI CSPASVSSTV REVSIAEPTT YTPAQTTAPT VSACTTEDGV SAPPRKRARG
 PSTNNTLCVA NIRSVDSTIN NIVTDNYNKH QRRNNCHSAA TPIVQLQGDS NCLKCFRYRL
 5 NDKYKHLFEL ASSTWHWASP EAPHKNAIVT LTYSSSEQRQ QFLNSVKIPP TIRHKVGFMS
 LHLL

10. Plasmid: HPV117 (p7313ie 6be2.6be1.11e2)

10 Gene of interest:

The gene for the polyprotein in construct HPV117 is a triple fusion protein
 comprised in order of 6be2, 6be1, 11e2 all codon optimised and mutated. The
 polyprotein gene was assembled by PCR from using 3 previous PCR fragments;
 15 6be1 and 6be2 and 11e2. The size of the gene is ~4.1kb, producing a polyprotein
 of ~170kD, observed by PAGE and Western blot.

Cloning:

20 The polyprotein gene was digested with Bam HI + Not I restriction enzymes and
 ligated into p7313ie vector. Sequencing analysis of selected clones had
 indicated the 'odd' base change, but this was overcome by various fragment
 swapping. A resulting clone hpv117 #6 was found to have no errors.

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Polyprotein sequence in HPV117 (Seq. ID No. 19)

ATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGTACGAGGAAACAG
 30 CACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCAATGCCCCACGAGTCAGTGCTCCTGTACAAGG
 CCAAGCAGATGGGGCTGTCCACATCGGGATGCAGGTCGTGCCCCCGCTGAAGGTGAGCGAAGCCAAAG
 GGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCCGACCGAATACAGCATGGAGCC
 CTGGACTCTCCAGGAGACGTCCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCGCAAAGCGCG
 GCAAGACAGTTGAGGTGAAATTGATGGGTGCGCAAACAACACGATGGACTACGTGGTGTGGACCGAT
 35 GTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAGGGCATCTATTA

CACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAAGTATGGTTCCACCA
 AGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCGCCTCCGTGTCTCCACCACCCAG
 GAAGTGAGCATTCCGGAGAGCACCACATACACCCCGGCCAAACGAGCACGCTCGTCAGCAGCAGCAC
 CAAGGAGGACGCCGTCCAGACGCCCCCCCCGGAAGAGGGCCCCGGGGGTCCAGCAGTCTCCCTGCAATG
 5 CCCTGTGCGTTGCTCACATCGGCCCTGTGATTCCTGGGAACCACAATCTCATCACGAACAACCACGAC
 CAGCACCAAGGCGCAACAACTCTAACAGCTCCGCAACTCCAATAGTGACGTTCCAGGGGGAGTCCAA
 CTGCCTCAAGTGTTCCTCGCTACCGCCTCAACGACCGCCACCGCCACCTGTTGACTTGATCAGTTCCA
 CGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAAACACGCTATCGTGACGGTGACCTACGACTCCGAG
 GAGCAGAGGCAGCAGTTCTCGGACGTCGTGAAGATTCTCCGACAATCAGCCACAAGCTTGCGTTTCAT
 10 GTCCCTGCACCTGCTGATGGCAGACGATTCCGGTACTGAGAACGAAGGTTCTGGTTGTACCGGTTGGT
 TCATGGTTGAAGCAATCGTTCAGCATCCGACTGGTACCCAGATCTCCGATGACGAAGACGAAGAAGTT
 GAAGATTCTGGTTACGACATGGTTGACTTCATCGATGACTCCAACATCACTCATAACTCTCTGGAAGC
 ACAGGCTCTGTTTAACCGCCAGGAAGCTGATACCCATTACGCTACTGTTCAGGACCTGGGAGGCAAAT
 ATCTGGGCTCTCCGTACGTTTCCCCGATCAACACTATCGCAGAAGCAGTTGAGTCTGAAATCTCCCCG
 15 CGCCTGGACGCTATCAAACGACTCGTCAGCCGAAGAAGGTTAAACGTCGTCTGTTCCAGACTCGTGA
 ACTGACCGACTCCCGTTACGGTTATAGCGAAGTTGAGGCTGGCACCGGCACCCAGGTTGAAAAACACG
 GTGTACCGGAAAAACGGCGCGGACGGTCAGGAAAAGGACACCGGCCGCGACATCGAGGGTGAGGAACAC
 ACCGAAGCTGAAGCTCCGACTAACTCTGTTTCGTGAACACGCAGGTACTGCGGGTATCCTGGAACCTGCT
 GAAATGCAAAGACCTGCGCGCGGCTCTGCTGGGCAAATTCAAAGAATGCTTCGGCCTGTCTTTCATG
 20 ACCTGATCCGTCCGTTTAAGTCTGACAAAACCTGCTGCTGGACTGGGTTGTAGCAGGCTTCGGCATC
 CACCACTCTATCTCTGAAGCATTCCAGAACTGATCGAGCCGCTGTCTCTGTACGCGCACATCCAGTG
 GCTGACTAACGCTTGGGGTATGTTCTGCTGGTACTGCTGCGCTTTAAAGTAAACAAATCTCGTTCCA
 CTGTTGCTCGTACTCTGGCTACCCTGCTGAACATCCCGGAGAACCAGATGCTGATCGAACCGCCGAAA
 ATCCAGTCTGGTGTAGCTGCACTGTACTGGTTTCGTACTGGCATCTCTAACGCTAGCACTGTTATCGG
 25 TGAAGCACCGGAATGGATCACTCGTCAGACCGTTATCGAACACCGGTCGCGCAGATTCTCAGTTCAAAC
 TGAATGAAATGGTTCACTGGGCATACGACAACGACATCTGCGAGGAATCTGAAATGCGTTGGAATAC
 GCTCAGCGTGGCGACTTCGACTCCAACGCTCGTGCTTTCTGAAACAGCAACATGCAAGGCTAAATACGT
 AAAAGACTGCGCTACCATGTGCCGCTCACTACAAACACGCGGAAATGCGTAAATGTCTATCAAACAGT
 GGATCAAGCACCGCGGTTCTAAATCGAAGGTACCGGTAACTGGAAACCGATCGTTCACTTCCTGCGC
 30 CATCAGAACATCGAATTCATCCCGTTCTGACCAAATTCAGCTGTGGCTGCACGGTACCCCGAAAAA
 AAACCTGCATCGCTATCGTAGGTCCACCGGACACTGACAAGTCTTACTTCTGTATGTCCCTGATCTCTT
 TCCTGGGCGGCACTGTAATCTCTCACGTTAACTCTTCTCCCATTTCTGGCTGCAGCCACTGGTAGAC
 GCGAAAGTAGCTCTGCTGGACGACGCGACCCAGCCGTGCTGGATCTACATGGATACTTACATGCGCAA
 CCTGCTGGACGGTAACCCGATGTCTATCGACCGTAAACACAAAGCGCTGACTCTGATCAAGTGCCCGC
 35 CGCTGCTGGTAACTTCTAACATCGACATCACCAAGGAAGATAAATACAAGTACCTGCATACCCGTGTT

ACTACCTTTACTTTCCCGAACCCGTTCCCGTTTGATCGTAACGGTAACGCTGTTTACGAACTGTCCAA
 CACTAACTGGAAATGCTTCTTCGAGCGTCTGTCTTCTCCCTGGACATCCAGGACTCTGAAGATGAAG
 AAGATGGTTCTAACTCTCAGGCTTTCCGTTGTGTTCCGGGTACTGTTGTTCTGACTCTGATGGAAGCC
 ATCGCGAAGAGGCTCGACGCCCTGCCAGGACCAGCTGCTCGAGCTGTACGAGGAGAACAGCATTGACAT
 5 CCATAAGCACATCATGCACCTGGAAGTGCAATTCGCCTGGAGAGCGTGTGTTGCACAAGGCCAAGCAGA
 TGGGCCTGTCCACATAGGCCTTCAGGTGGTCCCCCTCTGACCGTGTCTAGAGACAAAGGGCCATAAC
 GCAATCGAGATGCAGATGCACCTCGAGTCGCTGGCGAAAACACAGTACGGCGTGGAGCCATGGACCCCT
 GCAGGACACCTCGTACGAAATGTGGCTGACCCACCTAAGCGATGCTTCGCCAAACAGGGCAACACAG
 TGGAGGTGAAGTTTCGACGGCTGTGAGGATAACGTTATGGAGTATGTCGTGTGGACGCACATCTATCTG
 10 CAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACGCGAAGGGCATCTACTATACTGTGG
 GCAGTTTAAAACCTACTATGTGAACCTCAACAAAGAGGCCCAAAAGTATGGCTCCACCAACCACTGGG
 AGGTCTGCTATGGGAGCACGGTGATTGCTCTCCCGCCAGCGTGTCTAGCACTGTGCGCGAGGTGAGC
 ATTGCCGAGCCGACCACGTACACCCCTGCCAGACGACCGCTCCGACCGTGTCTGCTTGTACTACCGA
 GGACGGCGTGAGCGCTCCACCCAGGAAGCGTGCAGAGGGGCCCAAGCACCAACAACACCCCTCTGTGTGG
 15 CGAACATTTCGAGCGTCGACAGTACCATCAATAACATCGTGACGGATAACTATAACAAGCACCAGAGG
 CGTAACAACCTGTCACTCTGCGCAACCCCCATCGTGACGCTCCAGGGAGACAGCAATTGCCTTAAGTG
 CTTCCGCTATCGCCTCAACGACAAGTACAAGCACCTCTTTGAGCTCGCCTCGTTCGACGTGGCACTGGG
 CCTCACCCGAGGCACCTCACAAGAACGCCATCGTCACTCTCACTTACTCCAGTGAGGAGCAGAGACAG
 CAGTTTCTGAACAGCGTGAAGATCCACCGACGATCCGTCATAAGGTTCGGCTTCATGTCACTGCATCT
 20 CCTGTGA

Amino acid sequence (Seq. ID No. 20)

25 MEAIKRDLDA CQEQLLELYE ENSTDHLHKHV LHWKCMRHES VLLYKAKQMG LSHIGMQVVP
 PLKVSEAKGH NAIEMQMHLE SLLRTEYSME PWTLOETSYE MWQTPPKRCF AKRGKTVEVK
 FDGCANNTMD YVWTDVYVQ DNDTWVKVHS MVDAGKIYYT CGQFKTYYN FVKEAEKYGS
 TKHWEVCYGS TVICSPASVS STTQEVSIPE STTYTPAOTS TLVSSSTKED AVQTPPRKRA
 RGVQQSPCNA LCVAHIGPVD SGNHNLITNN HDQHQRNNS NSSATPIVQF QGESNCLKCF
 30 RYRLNDRHRH LFDLISSTWH WASSKAPHKH AIVTVTYDSE EQRQOFLDVV KIPPTISHKL
 GFMSLHLLMA DDSGTENEGS GCTGWFMVEA IVQHPTGTQI SDDDEEVED SGYDMVDFID
 DSNITHNSLE AQALFNRQEA DTHYATVQDL GSKYLGSFYV SPINTIAEAV ESEISPRLDA
 IKLTRQPKKV KRRLFQTRRL TDSGYGYSEV EAGTGTQVEK HGVPEGGDG QEKDTGRDIE
 GEEHTEAEAP TNSVREHAGT AGILELLKCK DLRAALLGKF KECFGLSFID LIRPFKSDKT
 35 TCLDWVVAGF GIHHSISEAF OKLIEPLSLY AHIQWLTNAW GMVLLVLLRF KVNKSIRSTVA

RTLATLLNIP ENQMLIEPPK IQSGVAALYW FRTGISNAST VIGEAPWIT RQTVIEHGLA
 DSQFKLTEMV QWAYDNDICE ESEIAFEYQA RGDFDSNARA FLNSNMQAKY VKDCATMCRH
 YKHAEMRKMS IKQWIKHRGS KIEGTGNWKP IVQFLRHQNI EFIPFLTKEK LWLHGTPKKN
 CIAIVGPPDT DKSIFYCMSLI SFLGGTVISH VNSSSHFWLQ PLVDAKVALL DDATQPCWIY
 5 MDTYMRNLLD GNPMSIDRKH KALTLIKCPP LLVTSNIDIT KEDKYKYLHT RVTTFTFPNP
 FPFDRNGNAV YELSNTINWKC FFERLSSSLD IQDSEDEEDG SNSQAFRCVP GTVVRTLMEA
 IAKRLDACQD QLLELYEENS IDIHKHIMHW KCIRLESVLL HKAKQMGLSH IGLQVVPPLT
 VSETKGHNAI EMQMHLESLA KTQYGVPEWT LQDTSYEMWL TPPKRCFAKQ GNTVEVKFDG
 10 CEDNMEYVV WTHIYLQDND SWVKVTSSVD AKGIYYTCGQ FKTYVNFNK EAQKYGSTNH
 WEVCYGSTVI CSPASVSSTV REVSIAEPTT YTPAQTTAPT VSACTTEDGV SAPPRKRARG
 PSTNNTLCVA NIRSVDSTIN NIVTDNYNKH QRRNNCHSAA TPIVQLQGDS NCLKCFRYRL
 NDKYKHLFEL ASSTWHWASP EAPHKNAIVT LTYSSSEQRQ QFLNSVKIPP TIRHKVGFMS
 LHLL

15

11. Plasmid: HPV118 (p7313ie 6be2.11e2.6be1)

Gene of interest:

20 The gene for the polyprotein in construct HPV118 is a triple fusion protein
 comprised in order of 6be2, 11e2, 6be1 all codon optimised and mutated. The
 polyprotein gene was assembled by PCR from using 2 previous PCR fragments;
 6be1 and 11/6be2. The size of the gene is ~4.1kb, producing a polyprotein of
 ~170kD, observed by PAGE and Western blot.

25

Cloning:

30 The polyprotein gene was digested with Bam HI + Not I restriction enzymes and
 ligated into p7313ie vector. Sequencing analysis of selected clones had
 indicated the 'odd' base change, but this was overcome by various fragment
 swapping. A resulting clone hpv118 #3 was found to have no errors.

Polyprotein sequence in HPV118 (Seq. ID No. 21)

ATGGAAGCTATTGCCAAGCGACTGGACGCCTGCCAGGAGCAGCTGCTGGAGCTGTACGAGGAAAAACAG
 CACAGACCTCCACAAGCACGTGCTGCACTGGAAGTGCATGCGCCACGAGTCAGTGCTCCTGTACAAGG
 CCAAGCAGATGGGGCTGTCCACATCGGGATGCAGGTCTGTGCCCCGCTGAAGGTGAGCGAAGCCAAAG
 5 GGCCACAACGCTATCGAGATGCAGATGCACCTGGAGAGCCTGCTGCGGACCGAATACAGCATGGAGCC
 CTGGACTCTCCAGGAGACGTCTACGAAATGTGGCAGACTCCTCCGAAGCGCTGTTTCGCAAAGCGCG
 GCAAGACAGTTGAGGTGAAATTCGATGGGTGCGCAAACAACACGATGGACTACGTGGTGTGGACCGAT
 GTCTACGTGCAGGACAATGACACCTGGGTGAAGGTACATAGTATGGTGGATGCCAAGGGCATCTATTA
 CACCTGCGGGCAGTTCAAGACGTACTACGTCAACTTCGTCAAGGAAGCCGAAAAGTATGGTTCCACCA
 AGCACTGGGAGGTGTGTTACGGGAGTACTGTGATCTGCAGCCCCGCTCCGTGTCTCCACCACCCAG
 10 GAAGTGAGCATTCCGGAGAGCACCACATACACCCCGGCCCAAACGAGCACGCTCGTCAGCAGCAGCAC
 CAAGGAGGACGCCGTCAGACGCCCCCGGAAGAGGGCCCGGGGGTCCAGCAGTCTCCCTGCAATG
 CCCTGTGCGTTGCTCACATCGGCCCTGTGATTCGTGGGAACCAATCTCATCACGAACAACCACGAC
 CAGCACCAAAGGCGCAACAACCTTAACAGCTCCGCAACTCCAATAGTGCAGTTCCAGGGGGAGTCCAA
 CTGCCTCAAGTGTTTCCGCTACCGCTCAACGACCGCCACCGCCACCTGTTGACTTGATCAGTTCCA
 15 CGTGGCACTGGGCCAGCAGCAAGGCGCCCCACAACACGCTATCGTGACGGTGACCTACGACTCCGAG
 GAGCAGAGGCAGCAGTTCTCGACGTCTGGAAGATTCTCCGACAATCAGCCACAAGCTTGGCTTCAT
 GTCCCTGCACCTGCTGATGGAAGCCATCGGAAGAGGCTCGACGCTGCCAGGACCAGCTGCTCGAGC
 TGTACGAGGAGAACAGCATTGACATCCATAAGCACATCATGCACTGGAAGTGCAATTCGCCTGGAGAGC
 GTGCTGTTGCACAAGGCCAAGCAGATGGGCCTGTCCACATAGGCCTTCAGGTGGTCCCCCTCTGAC
 20 CGTGTFCAGAGACAAAGGGCCATAACGCAATCGAGATGCAGATGCACCTCGAGTCTGGCGAAAACAC
 AGTACGGCGTGAGCCATGGACCTGCAGGACACCTCGTACGAAATGTGGCTGACCCACCTAAGCGA
 TGCTTCGCCAAACAGGGCAACACAGTGGAGGTGAAGTTGACGGCTGTGAGGATAACGTTATGGAGTA
 TGTCTGTGGACGCACATCTATCTGCAGGACAACGACAGTTGGGTGAAGGTGACCAGCTCCGTGGACG
 CGAAGGGCATCTACTATACCTGTGGGCAGTTTAAACCTACTATGTGAACCTCAACAAAGAGGGCCAA
 25 AAGTATGGCTCCACCAACCACTGGGAGGTCTGCTATGGGAGCACGGTGATTTGCTCTCCCGCCAGCGT
 GTCTAGCACTGTGCGCGAGGTGAGCATTGCCGAGCCGACCACGTACACCCCTGCCAGACGACCGCTC
 CGACCGTGTCTGCTTGTACTACCGAGGACGGCGTGAGCGCTCCACCCAGGAAGCGTGGAGGGGGCCCA
 AGCACCAACAACACCCTCTGTGTGGCGAACATTGCGAGCGTCGACAGTACCATCAATAACATCGTGAC
 GGATAACTATAACAAGCACCAGAGGCGTAACAACGTCACTCTGCCGCAACCCCCATCGTGACGCTCC
 30 AGGGAGACAGCAATTGCCTTAAGTGCTTCCGCTATCGCTCAACGACAAGTACAAGCACCTCTTTGAG
 CTCGCCTCGTCGACGTGGCACTGGGCCTCACCCGAGGCACCTCACAAGAAGCCATCGTCACTCTCAC
 TTACTCCAGTGAGGAGCAGAGACAGCAGTTTCTGAACAGCGTGAAGATCCCAACGACGATCCGTGATA
 AGGTGCGCTTCATGTCACTGCATCTCCTGATGGCAGACGATTCCGGTACTGAGAAAGGTTCTGGT
 TGTACCGGTTGGTTTCATGGTTGAAGCAATCGTTCAGCATCCGACTGGTACCCAGATCTCCGATGACGA
 35 AGACGAAGAAGTTGAAGATTCTGGTTACGACATGGTTGACTTCATCGATGACTCCAACATCACTCATA

ACTCTCTGGAAGCACAGGCTCTGTTTAACCGCCAGGAAGCTGATACCCATTACGCTACTGTTTCAGGAC
 CTGGGAGGCAAATATCTGGGCTCTCCGTACGTTTCCCCGATCAACACTATCGCAGAAGCAGTTGAGTC
 TGAAATCTCCCCCGCCTGGACGCTATCAAAC'TGACTCGTCAGCCGAAGAAGGTTAAACGTCGTCTGT
 TCCAGACTCGTGAAC'TGACCGACTCCGGTTACGGTTATAGCGAAGTTGAGGCTGGCACCCGGCACCCAG
 5 GTTGAAAAACACGGTGTACCGGAAACCGGCGGCGACGGTCAGGAAAAGACACCCGGCCGCGACATCGA
 GGGTGAGGAACACACCGAAGCTGAAGCTCCGACTAACTCTGTTCTGTGAACACGCAGGTA'CTGCGGGTA
 TCCTGGAAC'TGCTGAAATGCAAAGACCTGCGCGCGGCTCTGCTGGGCAAATTCAAAGAATGCTTCGGC
 CTGCTCTTTCA'ATTGACCTGATCCGTCCGTTTAAGTCTGACAAA'ACTACCTGTCTGGACTGGGTTGTAGC
 AGGCTTCGGCATCCACCACTCTATCTCTGAAGCATTCCAGAAACTGATCGAGCCGCTGTCTCTGTACG
 10 CGCACATCCAGTGGCTGACTAACGCTTG'GGGTATGGTTCTGCTGGTACTGCTGCGCTTTAAAGTAAAC
 AAATCTCGTTC'CACTGTTGCTCGTACTCTGGCTACCCCTGCTGAACATCCCGGAGAACCAGATGCTGAT
 CGAACCGCCGAAAATCCAGTCTGGTGTAGCTGCACTGTACTGGTTTCGTACTGGCATCTCTAACGCTA
 GCACTGTTATCGGTGAAGCACCCGGAATGGATCACTCGTCAGACCGTTATCGAACACGGTCTGGCAGAT
 TCTCAGTTCAA'ACTGACTGAAATGGTT'CAGTGGGCATACGACAACGACATCTGCGAGGAATCTGAAAT
 15 TGCGTT'CGAATACGCTCAGCGTGGCGACTTCGACTCCAACGCTCGTGCTTTCTGAACAGCAACATGC
 AGGCTAAATACGTAAAGACTGCGCTACCATGTGCCGTCACTACAAACACGCGGAAATGCGTAAATG
 TCTATCAAACAGTGGATCAAGCACCGCGGTTCTAA'AAATCGAAGGTACCGGTA'ACTGGAAACCGATCGT
 TCAGTTCTCGCCATCAGAACATCGAATT'CATCCCGTTCTGACCAAATTCAAGCTGTGGCTGCACG
 GTACCCCGAAAAAA'AACTGCATCGCTATCGTAGGTCCACCGGACACTGACAAGTCTTACTTCTGTATG
 20 TCCCTGATCTCTTTCTGGGCGGCACTGTAATCTCTCAGGTTAACTCTTCCTCCCATTTCTGGCTGCA
 GCCACTGGTAGACGCGAAAGTAGCTCTGCTGGACGACGCGACCCAGCCGTGCTGGATCTACATGGATA
 CTTACATGCGCAACCTGCTGGACGGTAACCCGATGTCTATCGACCGTAAACACAAAGCGCTGACTCTG
 ATCAAGTGCCCGCGCTGCTGGTAACTTCTAACATCGACATC'ACCAAGGAAGATAAATACAAGTACCT
 GCATACCCGTGTTACTACCTTTACTTTCCCGAACCCGTTCCCGTTTGATCGTAACGGTAACGCTGTTT
 25 ACGAACTGTCCAACACTAACTGGA'AAATGCTTCTTCGAGCGTCTGTCTTCCTCCCTGGACATCCAGGAC
 TCTGAAGATGAAGAAGATGGTTCTAACTCTCAGGCTTCCGTTGTGTTCCGGGTACTGTGTGTTCGTAC
 TCTGTGA

Amino acid sequence (Seq. ID No. 22)

30 MEALAKRLDA CQEQLLELYE ENSTD LHKHV LHWKCMRHES VLLYKAKQMG LSHIGMQVVP
 PLKVSEAKGH NAIEMQMHL E SLLRTEYSME PWT LQETSYE MWQTPPKRCF AKRGKTVEVK
 FDGCANNTMD YVWTDVYVQ DNDTWVKVHS MVD AKGIYYT CGQFKTY YVN FVKEAEKYGS
 TKHWEVCYGS TVICSPASVS STTQEVSIP E STTYTPAQTS TLVSSSTKED AVQTPPKRA
 35 RGVOQSPCNA LCVAHIGPVD SGNHNLITNN HDQHQRNNS NSSATPIVQF QGESNCLKCF

RYRLNDRHRH LFDLISSTWH WASSKAPHKH AIVTVTYDSE EQRQQFLDVV KIPPTISHKL
 GFMSLHLLME AIAKRLDACQ DQLELYEEN SIDIHKHIMH WKCIRLESVL LHKAKQMGLS
 HIGLQVVPPL TVSETKGHNA IEMQMHLESL AKTOYGVEPW TLQDTSYEMW LTPPKRCFAK
 QGNTVEVKFD GCEDNVMEYV VWTHIYLQDN DSWVKVTSSV DAKGIYYTCG QFKTYIVNFN
 5 KEAQKYGSTN HWEVCYGSTV ICSPASVSST VREVSIAEPT TYTPAQTTAP TVSACTTEDG
 VSAPPRKRAR GPSTNNTLCV ANIRSV DSTI NNIVTDNYNK HQRRNNCHSA ATPIVQLQGD
 SNCLKCFRFR LNDKYKHLFE LASSTWHWAS PEAPHKNAIV TLTYSSSEEQR QQFLNSVKIP
 PTIRHKVGFM SLHLIMADDS GTENEGSGCT GWFVVEAIVQ HPTGTQISDD EDEEVEDSGY
 DMVDFIDDSN ITHNSLEAQA LFNREQEADTH YATVQDLGGK YLGSPYVSPI NTIAEAVESE
 10 ISPRLDAIKL TRQPKVKRR LFQTRELTDS GYGYSEVEAG TGTQVEKHGV PENGGDGQEK
 DTGRDIEGEE HTEAEAPTNS VREHAGTAGI LELLKCKDLR AALLGKFKEC FGLSFIDLIR
 PFKSDKTTCL DWVVAGFGIH HSISEAFQKL IEPLSLYAH I QWLTNAWGMV LLVLLRKFVN
 KSRSTVARTL ATLLNIPENQ MLIEPPKIQS GVAALYWFRT GISNASTVIG EAPWITRQT
 VIEHGLADSQ FKLTEMVQWA YDNDICEESE IAFEYAQRGD FDSNARAFIN SNMQAKYVKD
 15 CATMCRHYKH AEMRKMSIKQ WIKHRGSKIE GTGNWKPIVQ FLRHQNI EFI PFLT KFKLWL
 HGTPKKNCIA IVGPPD TDKS YFCMSLISFL GGTVISHVNS SSHFWLQPLV DAKVALLDDA
 TQPCWIYMDT YMRNLLDGNP MSIDRKHKAL TLIKCPPLL V TSNIDITKED KYKYLHTRVT
 TETFPNPF PF DRNGNAVYEL SNTNWKCF FE RLSSSLDIQD SEDEEDGSNS QAFRCVPGTV
 20 VRTL

The ColE1 cer sequence was obtained from a subclone from plasmid pDAH212
 from David Hodgeson (Warwick University) and amplified by PCR using primers
 to place EcoRI restriction sites at the ends of the sequence. The cer sequence
 25 was then inserted into the EcoRI site of p7313-PL to produce plasmid p7313-
 PLc. The sequence of the amplified cer was verified against the Genbank entry
 M11411.

Example 2 - Expression in mammalian 293T cells

30 Mammalian 293T cells were grown at log phase at a final concentration of 2×10^5 cells per 6 well Corning Costar™ (Corning Science Products, 10 The
 Valley Centre, Gordon Road, High Wycombe, Bucks, UK) tissue culture plate
 overnight at 37°C in 5% CO₂. The following transfection mix was prepared and
 35 complexed for 25 minutes:

	DNA of Interest	2µg
		2µg
	Made up with sterile double distilled water	16µl
5	OPTI-mem™ (Gibco BRL, Paisley, Scotland)	8µl
	Lipofectamine™ (GibcoBRL)	6µl.

Each cell monolayer in a well was washed carefully twice with OPTI-mem™. 800µl of OPTI-mem™ was added to each well. 200µl of OPTI-mem™ was added to each transfection mix, mixed and added gently to a cell monolayer. The plate was incubated for 5 hours at 37°C in 5% CO₂ after which the transfection mix and OPTI-mem™ were discarded. The cell monolayers were washed gently with cell growth medium twice and finally transfected cells were incubated for 24 hours in Dulbecco's Modified Eagle Medium containing 10% foetal calf serum and 29.2mg/ml of L-glutamine at 37°C in 5% CO₂. The cells were scraped off into microtubes, washed twice with PBS, spun down and the cell pellet was resuspended in SDS Page Laemmli dye. The cell pellets were boiled and loaded onto a 10%SDS Page gel, electrophoresed in 1X Tris Glycine SDS buffer. After electrophoresis, the gel was blotted onto Nitrocellulose membrane (Amersham) and Western Blotted. The nitrocellulose membrane was blocked with 5% Marvel™ (Premier Beverages, Knighton, Adbaston, Stafford, UK) in PBS for 30 min at room temperature and washed twice with PBS and 0.1% Tween 20. A polyclonal antibody raised against the C terminal protein sequence of HPV6bE1 (protein sequence: CSSSLDIQDSEDEEDGSNSQAFR Seq. ID No. 23) in rabbits, was diluted in 5% Marvel™ in PBS and added to the nitrocellulose membrane. This was incubated at room temperature for 1 hour with gentle agitation. A polyclonal antibody against HPV11E1 was also used to check cross reactivity. The diluted antibody was removed and the membrane washed three times with PBS and 0.1% Tween 20. A secondary conjugate, Swine anti-rabbit horseradish peroxidase (HRP) (DAKO), was diluted 1:20000 in PBS and 0.1% Tween 20. This was added to the washed membrane and incubated with gentle agitation at room temperature for 1 hour. The membrane was then washed thoroughly with PBS and 0.1% Tween20. A Chemiluminescent HRP kit (Amersham) was used to detect the transferred proteins on the membrane.

Results:

The results (Fig. 13) show a correct protein size expressed by each of HPV 116, 117, 118 containing the codon optimised HPV polyproteins.

5

HEK293T cells were transfected with ~0.5ug DNA of the respective constructs and the cells harvested 24hrs later. These samples were then analysed by first polyacrylamide electrophoresis and then Western blotting. Two peptide antibodies were used to detect for polyprotein expression (~180kd); Anti-6bE1(no.1097) and anti-6bE2 (no.1101).

10

Example 3**15 E1 antigen inactivation and experimental confirmation**

The HPV E1 protein is a well conserved nuclear protein with non-specific DNA binding, ATPase and helicase activities. E1 also binds to host cellular DNA polymerase- α primase and, to the HPV E2 protein which then 'recruits' E1 into the pre-initiation viral DNA replication complex. The primary role of E1 is to initiate virus specific DNA replication in infected cells.

20

The DNA replication functions of E1 (and E2) are relatively non-specific and many studies have now shown that the E1 and E2 proteins from one genotype can drive the origin specific DNA replication of a plasmid carrying the replication origin sequence from a different genotype. Studies have also shown that the introduction of highly expressed E1 and E2 into cells already harbouring low copy number HPV plasmid can result in a significant amplification of that plasmid. This promiscuity carries with it a small potential safety risk which the project sought to eliminate. Consequently, mutations in E1 (and E2) which inactivate their replication potential were sought.

25

30

The E1 mutation G482D occurs in a highly conserved ATP binding consensus sequence and E1 protein carrying this mutation has been shown to have multiple functional deficits. Other mutations, towards the N-terminus of the protein

35

(K83G, R84G) have been shown to abrogate nuclear localisation of E1. Failure to locate to the nuclear compartment would also serve to separate E1 from host replication proteins and viral DNA, providing an additional level of incapacity and safety. These mutations (G428D, K83G, R84G) were selected and incorporated into E1 as part of the HPV DNA immunotherapeutic E1 vector.

An *in vitro* HPV DNA replication assay was used to confirm disablement of the DNA replication functions of E1 (as a corollary the mutational inactivation of the replication enhancing activity of E2 could also be confirmed in this same assay). Briefly, both E1 and E2 co-operatively activate the HPV origin of replication and the E1 and E2 proteins from HPV 6b were known to activate and drive *de novo* DNA replication from the HPV-11 origin. Plasmids encoding our codon-optimised E1 and E2 sequences were co-transfected into 293 cells with a plasmid carrying the HPV-11 origin of replication (ori plasmid). E1 and E2 dependent replication of the input ori plasmid is measured by harvesting DNA from cells 48 hours after co-transfection (Hirt lysis). Extracted DNA is restriction enzyme digested first with Hind III and then Dpn I which digests unmethylated unreplicated DNA. DNA's are then southern blotted and hybridised with ori plasmid DNA as probe. Bands with a size equivalent to ori plasmid after DpnI digestion are markers for *de novo in vitro* replicated plasmid DNA.

Wild type E1 and E2 (HPV 119 + HPV 120) show a strong band indicative of replicated input plasmid DNA. Each of the three lead constructs are negative, (HPV116, HPV117 and HPV118) showing results; no replication.

Conclusion: The lead constructs HPV 116, HPV 117 and HPV 118 have no DNA replication activity.

Example 4

The E2 protein of papillomaviruses is a site-specific DNA binding nuclear protein functioning as the primary replication origin recognition protein and assists in the assembly of the pre-initiation DNA replication complex. Full length E2 protein can also act as either a repressor or activator of viral transcription depending upon the position (relative to other transcription factor sites), and the affinity of

the protein for its cognate binding site. E2 is also known to influence the transcription of several host cellular promoters. The mutational inactivation of E2 has been studied extensively and one point mutation in particular Lys 111 → Ala (K111A) has been shown to inactivate both the transcriptional and replication functions of E2. This mutation may also have the addition benefit of preventing nuclear translocation of the protein. This mutation (K111A) was incorporated into each E2 antigen as part of the HPV DNA immunotherapeutic.

We set out to confirm the incapacity of K111A mutated E2 and each polyprotein construct in an *in vitro* CAT transcriptional reporter assay. We used two positive controls (sources of active E2 protein). These were a construct expressing unmutated (active) HPV-11 E2 protein, and a second vector expressing BPV E2 protein, a strong transcriptional transactivator. These data are shown in figure 14.

Conclusion: These data show that protein expressed from the native (unmutated) HPV 6b E2 vector is transcriptionally active, whilst mutated (K111A) E2 is inactive, as are each of the polyprotein vectors HPV 116, 117 and 118.

Example 5

Expression of and Comparison with Individual Gene Constructs HPV 116, HPV 117 and HPV 118.

Gene expression studies comparing the leads constructs HPV 116, HPV 117 and HPV 118 failed to identify any clear differences in *in vitro* gene expression. In addition, expression of the polyprotein was equivalent to expression of the individual (unfused) antigen in a single plasmid (HPV 110). Equally important, the introduction of the point mutations did not impact on gene expression (HPV 108 and HPV 110).

Example 6

In Vivo Immunogenicity Studies in Mice

In order to compare the immunogenicity of the three different constructs HPV 116, HPV 117 and HPV 118 *in vivo*, mice were immunised using PMID.

5 Each immunisation comprised two shots of 0.5 µg DNA fired into the shaved abdomen of Balb/c (H-2K^d) or C57 BL6 (H-2K^b) mice. Animals were primed with 1µg DNA, boosted 21 days later with an equivalent dose and culled 5-7 days post boost. Sera and spleens were taken for analysis of the humoral and cellular immune response generated following PMID.

10 Humoral Assays

Antibodies raised in PMID immunised mice were evaluated using standard ELISA methods and recombinant E1 and E2 protein as capture antigen. Antibody responses could not be reliably detected except after extended
15 immunisation schedules in E2 immunised mice. We did not confirm detection of antibody to the E1 antigen in mice. These weak/undetectable antibody responses are in keeping with the published literature.

20 Cellular Assays

ELISPOT assays were used to study cellular immune responses in mice. This technique is suitable for assessing the frequency of cells within a culture of known density that are capable of secreting cytokines specifically in response to antigen presented in the context of syngeneic MHC molecules.

25 Briefly, a single cell suspension of splenocytes isolated from immunised animals is added to specialised microtitre plates coated with anti-cytokine capture antibody and incubated overnight in the presence of antigen presented by suitable target cells. Cytokine is captured by antibody bound to the plate in the area directly around the cell and this remains bound when cells are lysed and
30 washed away. Detection is achieved by use of a biotinylated secondary anti-cytokine antibody and a streptavidin alkaline phosphatase conjugate. The action of this enzyme on a chromophoric substrate allows visualisation of the frequency of cytokine producing cells.

35

Vaccinia ELISPOT Assays and Data

Due to the absence of defined murine T cell epitopes, antigen was provided in the form of recombinant vaccinia viruses engineered to express target antigens.
5 Such viruses were used to infect appropriate target cells for the presentation of antigen to effector cells in ELISPOT assays.

Responses to HPV 6bE1 were detected following PMID of the three candidate constructs to C57BL/6 mice. The results of 2 separate experiments were
10 analysed statistically. The results of a representative experiment are shown in the figure 15 and 16.

Illustrative immunogenicity data using lead constructs and PMID in mice:

15 CTL Assays and Data

Activated CD8+ T cells are able to lyse cells in response to specific peptide presented in the context of syngeneic MHC I molecules. This function can be determined by Eu3+ release bioassay, a non-radioactive modification of the
20 traditional chromium release assay.

Use of this assay for these purposes required the identification of a CD8+ T cell epitope derived from the primary sequence of the HPV 6bE1 protein. This was achieved by screening a peptide library consisting of 15-mers overlapping by 11
25 using cytokine ELISPOT. Responding populations were identified as CD4+ or CD8+ T cells by standard flow techniques.

The basis of this technique involves lysis of Eu3+ labeled target cells pulsed with cognate peptide. During the course of a two hour incubation, Eu3+ is released
30 into the culture supernatant upon lysis of target cells by cytolytic T cells. This is detected by time-resolved fluorimetry. Specific lysis is expressed as a percentage of the total amount of lysis detected when target cells are lysed by chemical means.

Assessment of cellular immunology data

5 The immunologic evaluation of HPV 116, HPV 117 and HPV 118, comprised repeat PMID immunisation studies in mice with Vaccinia ELISPOT and CTL assay analysis as immunologic outputs. All candidates raised a strong immune response to each antigen.

10 Collectively, the vaccinia ELISPOT data show that responses to E1 are not compromised by mutation or by fusion to the E2 antigen components. When comparing E1 responses between HPV-108 (single 6b E1 construct), HPV 116, HPV 117 and HPV 118 the responses are not statistically different. Vaccinia ELISPOT data do however reveal a difference in responses to the HPV-11 E2 antigen component. E2 antigen specific responses are significantly greater in mice immunised with HPV 118 than in mice immunised with HPV 116 or HPV 117. On this basis alone HPV 118 appears to be a superior immunogen than HPV 116 or HPV 117.

20 The analysis of E1 antigen specific CTL lysis also revealed a trend in potency. The percentage specific lysis was higher using T-cells from HPV 118 immunised mice than with either of HPV 116 or HPV 117. This observation is reproducible.

Taken together, and on the basis of both vaccinia ELISOT and CTL lysis data, HPV 118 is the stronger immunogen.

25 **Conclusion.** On purely immunological criteria construct HPV 118 is the most immunogenic of the polypeptides.

Example 7

30 **PMID delivery of codon-optimised COPV E1/E2 fusion protein is more effective in protecting against canine oral papillomavirus disease than either codon-optimised E1 or codon-optimised E2 alone.**

Introduction

The canine oral papillomavirus (COPV) animal model is a good mimic of mucosal human papillomavirus disease. The features of disease caused in dogs by COPV are very similar to that which occurs in humans (Nicholls et al Virology 2001, 283(1) 31-39). Importantly it is a mucosal papillomavirus disease model. The COPV virus infects the canine mucosal epithelia and, after a lag period of a few weeks warts appear which then regress spontaneously after an additional period of some weeks.. The COPV virus encodes homologues of each of the human papillomavirus genes (E1, E2, E4, E6, E7, L1 and L2).

The dog COPV mucosal disease model has previously been used as a key model in developing the rationale for human virus-like-particle (VLP) papillomavirus vaccines (Ghim et al, Vaccines 1995 25, 375-379, Suzich et al, PNAS 1995, 92 11553-11557). Human papillomavirus VLP vaccines are now in development, and early stage clinical trials have recently been completed in humans.

We show that plasmid DNA encoding a codon-optimised fusion of E1 and E2 genes when administered by PMID reduces disease burden more effectively than either a plasmid encoding codon-optimised E1 or codon-optimise E2 alone.

Methods

Construction of the codon-optimised E2/E1 fusion vector

A synthetic gene encoding a codon-optimised COPV E2 sequence was generated using methods described previously. This was fused to the synthetic codon-optimised COPV E1 gene recovered from clone pCOPVE1 c/o and inserted into vector WRG7077 to generate a new clone which was designated pCOPVE2/E1 c/o. This clone expresses a polyprotein comprising a fusion of COPV E2 (N terminal) and COPV E1 (C terminal). The polyprotein is of the expected size as determined by western blotting.

Immunisation of Beagle Dogs with pCOPVE1 c/o, pCOPVE2 c/o, and pCOPVE2/E1 c/o

5 Beagle dogs were immunised by PMID with each of three purified plasmids pCOPVE1 c/o, pCOPVE2 c/o and, pCOPV E2/E1 c/o. Animal were immunised at 12 cutaneous sites, 6 non-overlapping sites on each side of the abdominal midline. All vaccinations were performed under general anesthesia. There were five animals in each group. Six weeks after the first vaccination, a boosting vaccination was undertaken in an identical manner, using the same procedure.

10 Immunised animals were challenged with infectious COPV virus 2 weeks after the final boosting immunisation. The mucosa of the upper lip of each animal was lightly scarified. 10µl of purified COPV virus preparation was applied to each of ten sites (five on each side of the upper lip) and allowed to absorb for a few minutes. The isolation and purification of infectious COPV virus has been described (Virology 1999, 265 (2) 365-374).

15 After challenge with COPV virus the sites of mucosal challenge were examined weekly. The time (after challenge) of wart (papilloma) appearance, and wart size (mm) was measured.

20 In animals immunised with pCOPVE1 c/o papillomas developed at the mucosal challenge sites beginning at week 7 after challenge. Papillomas continued to grow in size reaching a mean size of >3.5 mm by week 11. In animals immunised with pCOPV E2 c/o papilloma's first appeared at week 8 but and the mean papilloma size reached 1.5 mm at week 11. In animals immunised with pCOPVE2/E1 c/o whilst the first signs of disease are co-incident with that of the other groups the overall disease burden is significantly reduced. One animal (of five) in the pCOPVE2/E1 c/o group was fully protected from disease development whilst all other animals in the group developed only very small papilloma's which regressed in a short period (1-2 weeks).

Plasmid DNA encoding a fusion of COPV E1 and COPV E2 are more effective than either of COPV E1 or COPV E2 in preventing disease development in this animal model of papillomavirus infection. (Figure 18)

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Claims

1. A polynucleotide sequence encoding a Human Papillomavirus (HPV) polypeptide having epitopes from at least three Early antigens or fragments thereof from at least two different HPV strains and wherein the polynucleotide has a codon usage coefficient for human genes of greater than 0.4 and less than 1.0
2. A polynucleotide as claimed in claim 1 wherein at least one antigen is from HPV E1 or fragment thereof.
3. A polynucleotide as claimed in claim 2 wherein at least one antigen is from HPV E2.
4. A polynucleotide sequence according to any of claims 1 to 3 which is a DNA sequence.
5. A polynucleotide sequence according to any of claims 1 to 4 which encodes a HPV polypeptide of a HPV type or sub-type associated with cervical cancer, benign cutaneous warts or genital warts.
6. A polynucleotide sequence according to any of claims 1 to 5 which encodes a HPV polypeptide of one of types 1-4, 6, 7, 10, 11, 16, 18, 26-29, 31, 33, 35, 39, 49, 51, 52, 56, 58, 59 and 68.
7. A polynucleotide sequence according to claim 6 which encodes a HPV polypeptide of an HPV type or sub-type which is associated with cervical cancer or genital warts.
8. A polynucleotide sequence according to claim 4 or 5 which encodes a HPV polypeptide of one of types 6, 11, 16, 18, 33 or 45.
9. A polynucleotide sequence according to claim 5 which encodes a HPV polypeptide of a HPV type or sub-type selected from HPV 11, 6a or 6b.
10. A polynucleotide sequence according to any preceding claim in which encodes a mutated HPV polypeptide having reduced biological function.

11. A polynucleotide sequence according to any of claims 1 to 10 which encodes a mutated HPV polypeptide comprising one or more point mutations by which one or more of the polypeptide's natural biological functions is inactivated.
12. A polynucleotide sequence according to claim 1 comprising an epitope from E1 antigen of HPV 6b an epitope from HPV 6b E2, and an epitope from HPV 11 E2.
13. A polynucleotide sequence according to claim 1 to 12 having a codon usage coefficient for human genes of greater than 0.5 but less than 1.
14. An expression vector comprising a polynucleotide sequence according to any preceding claim operably linked to a control sequence which is capable of providing for the expression of the polynucleotide sequence by a host cell.
15. An expression vector according to claim 14 which is p7313PLc.
16. A pharmaceutical composition comprising a polynucleotide sequence according to any one of claims 1-13.
17. A pharmaceutical composition comprising a vector according to any one of claims 14-15.
18. A pharmaceutical composition according to claim 16 or claim 17 comprising a plurality, gold particles, coated with DNA.
19. A pharmaceutical composition according to any one of claims 16, 17 or 18 further comprising an adjuvant.
20. A pharmaceutical composition according to claim 19 in which the adjuvant is encoded as a fusion with the HPV polypeptide encoded by the polynucleotide.
21. The use of a polynucleotide according to any one of claims 1-13 in the treatment or prophylaxis of an HPV infection.

22. The use of a vector according to any one of claims 14-15 in the treatment or prophylaxis of a HPV infection.
23. The use of a composition according to any one of claims 18-20 in the treatment or prophylaxis of an HPV infection.
24. The use of a polynucleotide according to any one of claims 1-13, a vector according to any one of claims 14-15 or a pharmaceutical composition according to any one of claims 16-20 in the treatment or prophylaxis of cutaneous (skin) warts, genital warts, atypical squamous cells of undetermined significance (ASCUS), cervical dysplasia, cervical intraepithelial neoplasia (CIN) or cervical cancer.
25. The use of an effective amount of a polynucleotide according to any one of claims 1-13, a vector according to any one of claims 14 or 15 or a pharmaceutical composition according to any one of claims 16-20 in the treatment or prophylaxis of HPV infections or any symptoms or diseases associated therewith.
26. The use a pharmaceutical composition according to any one of claims 16-20 comprising a polynucleotide according to any one of claims 1-13 in a prime-boost dosage regime with a recombinant viral vector or non-viral based system in the treatment or prophylaxis of HPV infections or any symptoms or diseases associated therewith.