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(54) Title: HUMANISED BACULOVIRUS

(57) Abstract: The invention relates to a genetically engineered baculovirus wherein said virus is engineered to target therapeutic agents to cells, typically cancer cells, for example prostate cancer cells.

HUMANISED BACULOVIRUS

The invention relates to a baculovirus wherein the baculovirus genome comprises a nucleic acid molecule encoding a therapeutic agent and a nucleic acid molecule
5 which encodes a polypeptide wherein said polypeptide functions to target the baculovirus to at least one cell type; methods of treatment using said baculovirus and pharmaceutical compositions comprising said baculovirus.

Gene therapy involves the transfer, and optionally the stable insertion, of new genetic
10 information into cells for the therapeutic treatment of disease. The main issues with respect to gene therapy relate to the efficient targeting of nucleic acid to cells and the establishment of high level transgene expression in selected tissues. A number of methodologies have been developed which purport to facilitate either or both of these requirements. For example, US 6043339 discloses the use of signal peptides which
15 when fused to a nucleic acid can facilitate the translocation of the linked nucleic acid across cell membranes. US 6083714 discloses a combined nucleic acid and targeting means which uses the polycation poly-lysine coupled to an integrin receptor thereby targeting cells expressing the integrin. EP1013770 discloses the use of nuclear localisation signals (NLS) coupled to oligonucleotides. The conjugate may be
20 covalently linked to vector DNA and the complex used to transfect cells. The NLS sequence serves to facilitate the passage of the vector DNA across the nuclear membrane thereby targeting gene delivery to the nucleus.

A range of viral based vectors have been used to successfully transfect mammalian
25 cell lines. These include adenovirus, adenovirus-associated virus, papovaviruses and vacciniavirus. These viral based vectors have considerable disadvantages. Adenovirus vectors are well established in gene therapy trials, although recent difficulties in the USA may restrict their use. (Wickham TJ, Gene therapy, 7: 110, 2000). The major problems appear to be non-selective cytotoxicity (particularly in the
30 liver) and pre-existing immune responses against the virus. The cytolytic T cell response induced against adenovirus capsid-derived peptides has been shown to

mediate the destruction of vector transduced cells and has been associated with localised tissue damage and inflammation. (Gilgenkrantz, H. et al. (1995) Hum Gene Ther 6, 1265-1274; Yang, Y. and Wilson, J.M. (1995) J Immunol 155, 2564-2570). The possibility of recombination with endogenous infecting adenovirus, particularly
5 at high input dose, is also a potential safety concern.

Limitations to the amount of extra genetic material inserted into recombinant viruses are imposed by the defined size of the adenovirus capsid. Adenoviruses will recombine with pre-existing material; a potential drawback where endogenous
10 adenovirus is wide spread in the human population. Similarly it has been demonstrated that adenovirus vectors have the ability to aid the replication of related endogenous human viruses.

Safety concerns are also associated with the clinical use of Herpes Simplex Virus.
15 Lytic replication of the virus in the human brain has been linked to encephalitis. (Latchman, D.S. (1994). Mol Biotechnol 2, 179-195).

Although retroviral vectors are widely used in clinical trials, a number of disadvantages are associated with these vectors. Integration into cells is random, a
20 major safety concern. Use of these vectors is limited, as they require dividing cells for infectivity.

An alternative vector, which has been shown to infect mammalian cells, is the baculovirus. Baculovirus is a rod form virus and therefore limitations to the amount
25 of genetic material inserted into recombinant baculovirus is not as limiting as those imposed by adenovirus capsid.

The baculovirus will not express its own genes from insect-specific promoters in human cells. This is an attractive feature since the baculovirus will not provoke an
30 immune response as a consequence of viral gene expression of virally encoded genes. However, insertion of a marker or therapeutic gene under control of a mammalian promoter allows high level expression of the transgene. Unlike the adenovirus vector,

baculovirus will not recombine with pre-existing material. Infection with baculovirus will not facilitate the replication of endogenous human viruses, as has been demonstrated with adenovirus vectors. In contrast to many of the other therapeutic viruses, baculoviruses can be grown in a serum free culture media in large quantities.

5 This method of production can be readily scaled up to industrial level and removes the potential hazards of serum contamination of the therapeutic agent with viral and prion agents. Most importantly, unlike all other human viral vectors, there is no pre-existing immune response against baculovirus in humans.

10 The construction of recombinant baculovirus is well documented. EP0340359, which is incorporated by reference, discloses a method of obtaining a recombinant baculovirus incorporating a foreign gene through use of a transfer vector. The novel transfer vector incorporates a restriction site a short distance downstream of the N-terminus of the polyhedrin gene body, into which a foreign gene may be cloned. The
15 natural ATG start codon for the polyhedrin gene is not provided, such that the N-terminal polyhedrin coding sequence prior to the restriction site is retained but not capable of translation. A recombinant baculovirus incorporating a foreign gene is derived from the transfer vector by co-transfecting insect cells susceptible to baculovirus infection with wild type baculovirus and the transfer vector.

20

Similarly US6126944, which is incorporated by reference, relates to the construction of baculovirus transfer vectors for efficient expression of foreign genes, and more particularly expression of glycoprotein gG1 and gG2 of the Herpes Simplex virus. The foreign gene to be expressed is juxtaposed with the baculovirus polyhedrin gene
25 at the translation initiation site, without the addition of further nucleotides to the initiation site.

US5750383, which is incorporated by reference, discloses a baculovirus cloning system. The system is a marker rescue system using an essential gene. The selected
30 essential gene is inactivated. Cloning into the baculovirus containing the null mutation is then achieved by using the virus to infect wild type host cells that are co-

infected with a plasmid containing a functional copy of the gene linked to a foreign gene under the control of a regulatable promoter. The baculovirus null mutation is “rescued” by the rescue gene linked to a foreign gene. The function of the essential gene is restored and the foreign gene is expressed. An example of an essential gene is
5 gp64 efp (envelope fusion protein), that encodes a protein essential for viral infectivity and propagation.

Although disclosing methods by which baculovirus may be manipulated the prior art is not related to the use of baculovirus as a viral vector for targeted gene therapy.

10

We have developed a recombinant baculovirus which includes targeting sequences incorporated into the baculovirus genome which facilitate the delivery of the baculovirus and thereby the therapeutic agent to a specific cell type.

15 An example of a candidate gene for targeting is the baculovirus gp64, an extensively processed type 1 integral membrane glycoprotein. The role of gp64 in baculovirus infectivity has been demonstrated by the neutralization of infectivity with antibodies specific to gp64. It has also been shown that gp64 is both necessary and sufficient for low pH activated membrane fusion activity. Although conclusive data has been
20 lacking, indirect data on the role of gp64 in the infection cycle strongly suggests that the protein is essential for infectivity of the baculovirus.

The baculovirus gp64 envelope protein has been found to be sufficiently mutable to allow rapid insertion of new and more specific attachment sequences, without
25 perturbing its function as a carrier of genetic information. The gp64 envelope protein has a long loop, which has previously been used for antigen presentation and is ideal for insertions. When modified gp64 is expressed together with wild type gp64 in a mosaic membrane viral infectivity is not grossly affected.

30 According to a first aspect of the invention there is provided a baculovirus wherein the baculovirus genome has been modified to include a nucleic acid molecule which

encodes a therapeutic agent and a nucleic acid molecule which encodes a polypeptide which functions to target said baculovirus to at least one cell type.

In a preferred embodiment said baculovirus genome is adapted for eukaryotic gene
5 expression of said nucleic acid molecules.

Typically said adaptation includes, by example and not by way of limitation, the provision of transcription control sequences (promoter sequences) which mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific,
10 inducible or constitutive.

Promoter is an art recognised term and, for the sake of clarity, includes the following features which are provided by example only, and not by way of limitation. Enhancer elements are *cis* acting nucleic acid sequences often found 5' to the transcription
15 initiation site of a gene (enhancers can also be found 3' to a gene sequence or even located in intronic sequences and is therefore position independent). Enhancers function to increase the rate of transcription of the gene to which the enhancer is linked. Enhancer activity is responsive to *trans* acting transcription factors (polypeptides) which have been shown to bind specifically to enhancer elements. The
20 binding/activity of transcription factors (please see Eukaryotic Transcription Factors, by David S Latchman, Academic Press Ltd, San Diego) is responsive to a number of environmental cues.

Promoter elements also include so called TATA box and RNA polymerase initiation
25 selection (RIS) sequences which function to select a site of transcription initiation. These sequences also bind polypeptides which function, *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers
30 and autonomous replication sequences which both facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic host.

Adaptations which facilitate the expression of baculovirus encoded genes include the provision of transcription termination/polyadenylation sequences. This also includes the provision of internal ribosome entry sites (IRES) which function to maximise expression of baculovirus encoded genes arranged in bicistronic or multi-cistronic expression cassettes.

These adaptations are well known in the art. There is a significant amount of published literature with respect to expression vector construction and recombinant DNA techniques in general. Please see, Sambrook et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) DNA Cloning Techniques: A Practical Approach Vol III IRL Press, Oxford UK; DNA Cloning: F M Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, Inc.(1994).

In a preferred embodiment of the invention said eukaryotic expression is through the provision of cancer cell specific promoter elements. Preferably, said promoters are active in prostate cancer cells.

More preferably the promoter elements are selected from the group as represented in Table 1.

In a preferred embodiment of the invention said therapeutic agent is a polypeptide.

Preferably said polypeptide is a tumour suppressor polypeptide selected from the following group represented in Table 2.

30

In a further preferred embodiment of the invention said polypeptide is an antigenic polypeptide.

5

Preferably a tumour rejection antigen precursor selected from the following families represented in Table 3.

10

In a further preferred embodiment said polypeptide is a prostate tumour rejection antigen.

15

In a further preferred embodiment of the invention said polypeptide is a cytotoxic polypeptide. For example pseudomonas exotoxin, ricin toxin, diptheria toxin (Genbank acc. #: A04646).

20

In a yet further preferred embodiment of the invention said polypeptide is a polypeptide which induces cell-cycle arrest.

Preferably said cell-cycle arrest polypeptide is selected from the group represented in Table 4.

25

In a further preferred embodiment of the invention said therapeutic polypeptide is a pharmaceutically active polypeptide. Preferably said polypeptide is a cytokine.

Preferably said cytokine is selected from the group represented in Table 5.

In a yet further preferred embodiment of the invention said polypeptide is an antibody, or active binding fragment thereof, for example a Fab fragment.

30

Antibody fragments smaller than Fab fragments which bind cellular targets are also within the scope of the invention. For example, single chain Fv molecules (scFv).

These are engineered antibody fragments composed of a variable region of the heavy chain and a variable region of the light chain which are coupled via a linker sequence, see Adams and Schier (1999) Journal of Immunological Methods 249-260.

5

In a yet still further preferred embodiment of the invention said polypeptide is a polypeptide which induces apoptosis.

Preferably said apoptosis inducing polypeptide is represented in Table 6.

10

In a yet still further preferred embodiment of the invention said polypeptide is a pro-drug activating polypeptide.

Preferably said prodrug activating polypeptide is represented in Table 7.

15

In a still further preferred embodiment of the invention said polypeptide has anti-angiogenic activity. For example angiostatin, Tie2 (Genbank acc. no: AF451865), endostatin (Genbank acc.no: NM130445) .

20 In a further preferred embodiment of the invention said therapeutic agent is an antisense nucleic acid molecule.

As used herein, the term "antisense nucleic acid molecule" or "antisense" describes a nucleic acid which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene. Those skilled in the art will recognize that the exact length of the antisense nucleic acid and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases, which comprise that sequence.

25
30

It is preferred that the antisense nucleic acid be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions.

5

Although nucleic acids may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense nucleic acid correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. The 3'- untranslated regions are known to contain *cis* acting sequences which act as binding sites for proteins involved in stabilising mRNA molecules. These *cis* acting sites often form hair-loop structures which function to bind said stabilising proteins. A well known example of this form of stability regulation is shown by histone mRNA's, the abundance of which is controlled, at least partially, post-transcriptionally.

The present invention, thus, contemplates a baculovirus genome which has been modified by incorporation of an antisense nucleic acid to a specific target sequence, for example a target sequence encoding a cell-cycle regulatory gene, (eg p21 (Genbank acc. #: NM_078467 ,c-myc (Genbank acc. #: D10493 and D90467), cyclin dependent kinase inhibitors, p16 (Genbank acc. #: NM058196), p15 (Genbank acc. #: BC002010), p18 (mouse ssequence Genbank acc. #: BC027026), or p19 (Genbank acc. #: NM_079421) and apoptosis inhibitors such as caveolin .

In a further preferred embodiment of the invention said therapeutic agent is a double stranded RNA molecule. In this embodiment the baculovirus genome would include a nucleic acid molecule under the control of a first promoter positioned upstream (ie 5' of the nucleic acid molecule) and a second promoter positioned downstream (ie 3' of the nucleic acid molecule). The orientation of the promoters being such that both sense and antisense nucleic acid molecules are produced.

A technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a
5 sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated. Alternatively said RNAi molecule is derived from intronic sequences or the 5' and/or 3' non-coding sequences which flank coding/exon sequences of genes. Recent studies suggest that RNAi
10 molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

15

The exact mechanism of RNAi action is unknown although there are theories to explain this phenomenon. For example, all organisms have evolved protective mechanisms to limit the effects of exogenous gene expression. For example, a virus often causes deleterious effects on the organism it infects. Viral gene expression
20 and/or replication therefore needs to be repressed. In addition, the rapid development of genetic transformation and the provision of transgenic plants and animals has led to the realisation that transgenes are also recognised as foreign nucleic acid and subjected to phenomena variously called quelling (Singer and Selker, Curr Top Microbiol Immunol. 1995;197:165-77), gene silencing (Matzke and Matzke, Novartis
25 Found Symp. 1998;214:168-80; discussion 181-6. Review) and co-suppression (Stam et. al., . Plant J. 2000;21(1):27-42.

In a still further preferred embodiment said therapeutic agent is a ribozyme.

A ribozyme is a catalytic RNA which is well known in the art. A ribozyme comprises a catalytic core having flanking sequences adjacent to the sequence which hybridises to the substrate RNA. The simplest catalytic core is an RNA motif known as a hammerhead. Since the discovery of catalytic RNA there has been a desire to
5 design ribozymes which have a targeted gene function such that disease gene mRNA's can be selectively ablated.

In yet a further preferred embodiment of the invention the baculovirus genome includes a nucleic acid molecule which encodes a polypeptide which binds the
10 baculovirus to the cell surface of at least one cell type.

In a preferred embodiment of the invention said nucleic acid encodes a polypeptide selected from the following group: GnRH (Genbank acc.no: L03380), fibroblast growth factors; insulin and insulin-like growth factors; neurotensin platelet derived
15 growth factor (Genbank acc.no: NM_002609 & NM_006206); somatostatin (Genbank acc.no:BC032625).

In a preferred embodiment of the invention the nucleic acid encoding said polypeptide is inserted into the baculovirus genome at a site which fuses said
20 polypeptide to a baculovirus capsid polypeptide. Preferably the capsid polypeptide is gp64.

Advantageously the fusion of the targeting polypeptide to a capsid polypeptide will result in its presentation at the baculovirus particle surface thereby presenting the
25 baculovirus to said cell type and thereby facilitating cell targeting.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising the baculovirus according to any previous aspect or embodiment of the invention. Preferably said composition is for use in the
30 manufacture of a medicament for the treatment of cancer, ideally prostate cancer.

According to a yet further aspect of the invention there is provided a method of treatment comprising the administration of a therapeutically effective amount of the baculovirus according to the invention.

- 5 In a preferred method of the invention said treatment is cancer, preferably prostate cancer.

An embodiment of this invention will now be provided by example only and with reference to the following materials, methods, vectors and figures:

10

Figure 1 illustrates reporter gene expression by baculoviral based vector AcMNPV;

Figure 2 illustrates baculoviral expression of AcMNPV in insect cells, Sf9;

- 15 Figure 3 illustrates the lack of expression of baculoviral encoded genes in mammalian cells;

Figure 4 is baculovirus vector pBA surf-1 MCS2;

- 20 Figure 5 is baculovirus vector pBA surf-1 GnRH(MKII); and

Figure 6 is baculovirus vector pBacMam2 EGFP.

Materials and Methods

25

Targeting baculoviruses are generated in two stages (i) by generation of a transfer vector in a bacterial plasmid, which is multiplied in bacteria, and whose DNA sequence is determined to verify the insertion of the recombinant DNA sequence; and (ii) recombination of the transfer vector, via homologous non essential region on either side of the gp64 recombinant, into a multiply cut Bv genome by cotransfection into recipient insect cells (sf9 or sf21).

30

An example of the experimental procedure is as follows.

The DNA sequence encoding the minimal peptide required for receptor binding for
 5 the GnRH and neurotensin receptors was determined and a DNA oligonucleotides
 for both strands were chemically synthesised, including PstI and KpnI restriction
 endonuclease sites to facilitate insertion into the pBACsurf vector (Figure 4). The
 synthesised oligonucleotides were then ligated into the pBACsurf vector via these
 restriction endonuclease sites. The sequences of the peptides and a map of the vector
 10 are shown below, see Figure 5 and Figure 6:

GnRH peptide coding sequence

CTGCAGCAACATTGGAGCTACGGCTTGCGCCCGGGCGCGGTACC

15 GnRH amino acid sequence

LeuGlnGlnHisTrpSerTyrGlyLeuArgProGlyAlaVal

Neurotensin peptide coding sequence

20 CTGCAGGAATTGTACGAAAACAAACCGCGCCGCCCCGTACATTTTGGCGGT
 ACC

Neurotensin peptide

LeuGlnGluLeuTyrGluAsnLysProArgArgProTyrIleLeuAlaVal

25 Full DNA sequence data for the constructs should be available for the final
 constructs, particularly the segment of the gp64 fusion protein.

The sequenced plasmid is then recombined into the Bacvector-1000 triple cut
 baculovirus DNA (Novagen) by cotransfection into sf21 cells. The resulting
 30 baculoviruses are only viable if recombination has occurred, and are diploid for the
 gp64 gene, as insertion does not occur in the native gp64 locus. This is essential to
 preserve high infectivity of the baculovirus, and has been observed in other systems
 eg HIV, where *env* protein modification can be carried out.

A further modification of the pBACsurf vector was carried out, in order to facilitate a single recombination step for both of the humanising sequences (ie human promoter and cell surface attachment), whereby a second multiple cloning site (MCS2) was inserted into the recombination area, which contains unique (ie single cut for the
5 plasmid) RE sites. This is shown below:

The alternative method of deriving the multiple recombinants is to co-transfect the promoter vector pBACMAM2 with the singly modified pBACsurf with the Bacvector 1000 triple cut DNA into sf21 insect cells, and to screen for double
10 recombinant viruses by polymerase chain reaction. This is the method of choice when large (>3kb) promoter fragments are inserted, as the capacity of the pBACsurf (MCS2) vector is limited. Viral DNA from the recombinant plaques therefore is characterised by a wild-type PCR product and a larger product from the insertion recombinant. The sense of the insertion is verified by direct DNA sequencing of the
15 purified PCR product.

Promoter fragments are inserted into the pBACMAM vector to replace the hybrid CAG promoter (CMV enhancer (within Genbank acc.#: AF477200)), Chicken beta actin promoter (Genbank acc.#: E02199) and rabbit beta globin terminator (Genbank
20 acc.#:AX451706). To facilitate this a general insertion construct was prepared in pT7 blue vector, such that the promoter is inserted upstream of either indicator genes (for activity in human cells such as the enhanced green fluorescent protein (EGFP) (Genbank acc.#: U57609) or a hybrid consisting of the EGFP fused to the common bacterial indicator chloramphenicol acetyl transferase or CAT gene (Genbank acc.#:
25 D14641). This construct is then excised from the pT7 blue carrier and inserted via SphI/SwaI and HindIII/BclI sites into the pBACMAM vector. The use of the multiple cloning site in the pBACMAM vector (thus retaining the BglII, StuI, Sae8387, NotI, KpnI, SmaI, Bsu36 and MacI sites) and inserting the promoter construct upstream of the Rabbit beta globin terminator (Genbank acc.#: AX451706)
30 is also possible.

Gene maps are created in Gene Construction Kit v2 (Textco Inc, USA)

EXAMPLE 1

5

The baculovirus used to infect one insect and two human cell lines (Table 2) was a recombinant AcMNPV engineered to carry the EGFP reporter gene under the control of the strong mammalian CAG promoter. The human cells were inspected 48Hrs post infection, using fluorescent microscopy, to visualise the EGFP expression (Figure 1).

10 Significantly, visual inspection suggests the infection efficiency *in vitro* for the LNCaP prostate cancer cells was equivalent to that of the 293 cells (25% by flow cytometry analysis, data not shown) - no appreciable EGFP expression can be seen from the CAG promoter in insect Sf-9 cells (data not shown). Total RNA was extracted from all cells and RT-PCR carried out using the six sets of primers
15 designed against baculoviral mRNA (Table 1), plus a G3PDH primer set as a positive control for the human cells.

In the Sf9 cells, expression of four out of the six genes examined was detected via RT-PCR in infected cells and but not in uninfected cells (Figure 2) indicating
20 baculoviral specific expression. The exceptions to this were the ubiquitin gene and the egt gene. Ubiquitin expression was detected in both infected and uninfected insect cells using the primers designed against baculovirus ubiquitin (Figure 2), although the levels in uninfected cells were much lower. This can be explained in that ubiquitin is a highly conserved molecule and there may be enough homology
25 between insect and baculoviral ubiquitin to produce an RT-PCR product of the same size. It is also possible that the baculoviral ubiquitin may actually have derived from its insect cell host during evolution. The other exception was the egt gene which was not expressed in either infected or uninfected cells (Figure 2). Egt is expressed very early in the baculoviral life cycle to prevent larval moulting and Sf9 cells are derived
30 from pupal ovarian tissue. Therefore, it can be hypothesised that there are factors in

the larval form of the host which are not found in the pupal stage and are necessary for egt expression.

EXAMPLE 2

5 For expression analysis in human cells, the 293 human embryonic kidney cell line was chosen due to its reported ease of infection with baculovirus (Condreay JP, *Proc.Natl.Acad.Sci.USA* 1999; **96**: 127-132; Boyce FM, Bucher NLR. *Proc.Natl.Acad.Sci.USA* 1996; **93**: 2348-2352) and the wide range of gene it can be induced to express. Additionally, the LNCaP prostate cancer cell line (derived from
10 lymph node metastases) was chosen as a model for prostate cancer gene therapy – especially as these cells are difficult to transduce by liposome based techniques.. The results for the RT-PCR for both of these cells lines show that none of the six baculoviral genes examined are expressed in either uninfected or infected cells in either cell line (Figure 3), despite the high levels of expression of the CAG controlled
15 EGFP. Messenger RNA for the housekeeping gene, G3PDH, was also found in uninfected and infected cells in both of the cells lines using significantly fewer PCR cycles (25 cycles compared to 35 when screening for the baculoviral mRNA). These results indicate that a representative portion of the endogenous genes found in baculovirus, most of which are highly expressed in the normal eukaryotic baculovirus
20 host environment and can interact with essential human cell processes, are not expressed when the virus is used to infect human cells. In contrast protein can be effectively synthesised from a reporter gene under the control of a mammalian promoter. This lack of baculoviral gene expression in human cells further underlines the safety of the baculovirus as a vector for gene therapy, particularly for transient
25 suicide gene protocols, since neither baculoviral gene expression or genome integration will be a likely complication.

Table 1: Promoter sequence	DNA Accession number
Prostate androgen regulated transcript 1	BC026274 or NM005551
Prostate transglutaminase,	BC007003
Prostate	XM031805
Prostate-derived Ets factor	AF071538
Prostatic acid phosphatase	X53605
Pr LeuZip	
PAGE-4	AF275258
DD3	
NKX3.1	AF247704
probasin	AX259949
prostate-specific antigen	AJ459782
prostate-specific membrane antigen	XM165392
prostate stem cell antigen	XM030742
prostate carcinoma tumour antigen-1	NM006499
AIPC	AF338650
Trp-p8	AC005538
E2F4	AF527540
Daxx	AF015956
TRPM-2	NM001831
PART-1	nm016590
TMPRSS2	
Bomesin	
Steap	Nm 012449
TARP	Af151103
PcGEM1	Af223389

Table 2: Tumour suppressor Polypeptide	DNA accession number
p53	AF136270
Retinoblastoma	
APC polypeptide	NM000038
DPC-4 polypeptide	U73825
BRCA-1 polypeptide	
BRCA-2 polypeptide	
WT-1 polypeptide	XM_034418
MMAC-1 polypeptide	XM083839
Familial polyposis coli polypeptide	NM000038

Table 3: Tumour Rejection Antigen Precursor Family	DNA Accesssion Number
MAGE	XM066465
BAGE	NM001187
GAGE	NM_003785
DAGE	Q99958

5

Table4: Cell-Cycle Arrest Polypeptide	DNA accession number
p21	NM078467
p16	NM058196
p15	BC002010
p18	BC027026
p19	NM079421
PTEN	AF143312

Table 5: Cytokine	DNA Accession number
growth hormone	
leptin	
erythropoietin	
prolactin	
IL-2	XM_035511
IL-3	U81493
IL-4	AF395008
IL-5	AF353265
IL-6	AF039224
IL-7	NM000880
IL-9	AF361105

IL-10	BC022315
IL-11	BC012506
the p35 subunit of IL-12	AF101062
IL-13	AF377331
IL-15	AF031167
G-CSF	E09569
GM-CSF	M13207
CNTF	E09734
CT-1	XM096076
LIF	XM009915
oncostatin M	NM020530
IFN α	J00207

Table6: Apoptosis inducing polypeptide	DNA Accession number
P53	AF136270
adenovirus E3.11.6K	
adenovirus E4	
adenovirus f4	
caspase	
Fas ligand	E11157
C-Cam 1	XM113980
ODC	NM052998
OAZ	XM037830
spermidine/spermine N1-acetyltransferase	BC002503
ZNF145	NM006006
PTEN phosphatase	AF143312
androgen receptor	NM_000044
Bcl2 family members.	

Table 7: Prodrug Activating polypeptide	<u>DNA Accession number</u>
cytosine deaminase	AL627278
thymidine kinase	AB078742
nitroreductase RdxA	AY063488
Cytochrome P450 CYP1A2	NM_000761
CYP2E1	AB052259
CYP3A4	AF209389

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Baculoviral Gene	Gene Function	Stage of Life Cycle Expressed	Human Homologue	Primer Name	Size of PCR Product (bp)
Proliferating cell nuclear antigen	Stimulates DNA replication and late gene expression	Early	Yes	pcna	308
DNA polymerase	DNA replication	Early	Yes	DNA-pol	247
Ubiquitin	Blocks ubiquitin dependent proteolysis	Late	Yes	ubi	142
gp37 (p34.8)	Spindle body protein	Late/Very Late	No	gp37	347
p10	Viral lysis (?)	Very Late	No	p10	202
Ecdysteroid UDP-glucosyltransferase	Blocks larval moulting	Early	No	egt	281

Table 1 – Baculoviral genes chosen for expression analysis

Cell Line	Description
Sf-9	Insect cell line, derived from pupal ovarian tissue of the fall army worm, <i>Spodoptera frugiperda</i>
293	Human cell line derived from human embryonic kidney and transformed to immortality by adenovirus 5
LNCaP	Androgen-dependent, non-metastatic, non-tumourigenic human prostate cancer cell line derived from lymph node metastasis

Table 2 – Cell lines used to investigate native baculovirus gene expression.

CLAIMS

1. A baculovirus wherein the baculovirus genome has been modified to include
5 a nucleic acid molecule which encodes a therapeutic agent and a nucleic acid molecule which encodes a polypeptide which functions to target said baculovirus to at least one cell type.
2. A baculovirus according to Claim 1 wherein said genome is adapted for
10 eukaryotic gene expression of said nucleic acid molecules.
3. A baculovirus according to Claim 2 wherein the expression of said nucleic acid molecule encoding a therapeutic agent is controlled by a cancer specific promoter.
15
4. A baculovirus according to Claim 3 wherein said cancer specific promoter is a prostate cancer cell specific promoter.
5. A baculovirus according to Claim 4 wherein said promoter is selected from
20 the group as represented in Table 1.
6. A baculovirus according to any of Claims 1-5 wherein said therapeutic agent is a polypeptide.
- 25 7. A baculovirus according to Claim 6 wherein said polypeptide is a tumour suppressor polypeptide selected from the group as represented in Table 2.
8. A baculovirus according to Claim 7 wherein said polypeptide is an antigenic polypeptide.
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9. A baculovirus according to Claim 8 wherein said antigenic polypeptide is a prostate tumour rejection antigen.
- 10 A baculovirus according to Claim 6 wherein said polypeptide is a cytotoxic
5 polypeptide.
- 11 A baculovirus according to Claim 10 wherein said cytotoxic agent is selected from the group consisting of: *pseudomonas* exotoxin; ricin toxin; diphtheria toxin (Genbank acc.#: A04646).
- 10 12 A baculovirus according to Claim 6 wherein said polypeptide is a polypeptide which induces cell-cycle arrest.
13. A baculovirus according to Claim 12 wherein said polypeptide is selected from
15 the group represented in Table 4.
14. A baculovirus according to Claim 6 wherein said therapeutic polypeptide is a pharmaceutically active polypeptide.
- 20 15. A baculovirus according to Claim 14 wherein said polypeptide is a cytokine.
16. A baculovirus according to Claim 15 wherein said cytokine is selected from the group represented in Table 5.
- 25 17 A baculovirus according to Claim 6 wherein said polypeptide is an antibody or active binding fragment thereof.
- 18 A baculovirus according to Claim 17 wherein said fragment is a Fab
fragment.
- 30 19. A baculovirus according to Claim 6 wherein said polypeptide is a polypeptide

which induces apoptosis.

20 A baculovirus according to Claim 19 wherein said polypeptide is selected from the group represented in Table 6.

5

21 A baculovirus according to Claim 6 wherein said polypeptide is a pro-drug activating polypeptide.

22 A baculovirus according to Claim 21 wherein said polypeptide is selected
10 from the group represented in Table 7.

23. A baculovirus according to Claim 22 wherein said polypeptide has anti-angiogenic activity.

15 24 A baculovirus according to Claim 23 wherein said polypeptide is selected from the group consisting of: angiostatin (Genbank acc. #: NM007037), Tie2 (Genbank acc. #: AF451865), endostatin (Genbank acc. #: NM130445).

25. A baculovirus according to any of Claims 1-5 wherein said therapeutic agent
20 is an antisense nucleic acid molecule.

26. A baculovirus according to Claim 25 wherein said antisense nucleic acid molecule binds a nucleic acid molecule encoding a cell-cycle regulatory gene.

25 27 A baculovirus according to Claim 26 wherein said cell-cycle regulatory gene is selected from the group represented in Table 4.

28 A baculovirus according to Claim 25 wherein said antisense nucleic acid molecule binds a nucleic acid molecule encoding an apoptosis inhibitor.

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29 A baculovirus according to Claim 28 wherein said apoptosis inhibitor is

caveolin (Genbank acc. #: AF095591)

30. A baculovirus according to any of Claims 1-5 wherein said therapeutic agent is a double stranded RNA molecule.

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31. A baculovirus according to any of Claims 1-5 wherein said therapeutic agent is a ribozyme.

32. A baculovirus according to any of Claims 1-31 wherein said baculovirus genome includes a nucleic acid molecule which encodes a polypeptide which binds the baculovirus to the cell surface of at least one cell type.

33. A baculovirus according to Claim 32 wherein said nucleic acid encodes a polypeptide selected from the group consisting of: GnRH (Genbank acc. #: L03380), fibroblast growth factors; insulin and insulin-like growth factors; neurotensin; platelet derived growth factor (Genbank acc. #: NM_002609 & NM_006206); somatostatin (Genbank acc. #: BC032625).

34. A baculovirus according to Claim 32 wherein said nucleic acid encoding said polypeptide is inserted into the baculovirus genome at a site which fuses said polypeptide to a baculovirus capsid polypeptide.

35. A baculovirus according to Claim 34 wherein said capsid polypeptide is gp64.

36. A pharmaceutical composition comprising the baculovirus according to any of Claims 1-35.

37. A baculovirus according to any of Claims 1-35 for use in the manufacture of a medicament for the treatment of cancer.

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38. Use according to Claim 37 wherein said cancer is prostate cancer.

39. A method of treatment comprising the administration of a therapeutically effective amount of the baculovirus according to any of Claims 1-35.

5 40. A method according to Claim 39 wherein said treatment is cancer.

41. A method according to Claim 40 wherein said cancer is prostate cancer.

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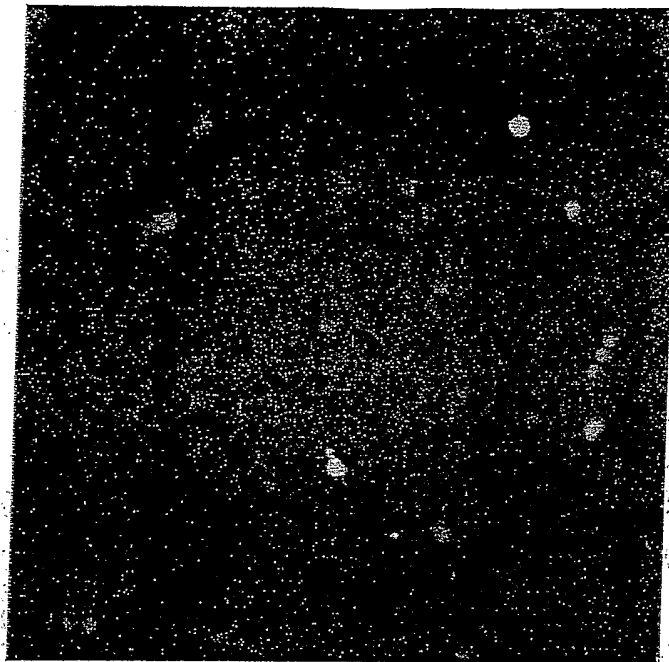
25

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A



B

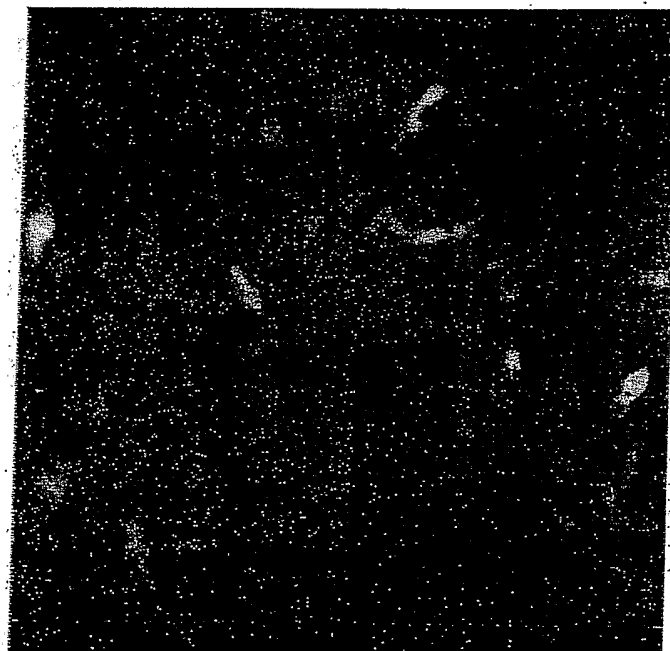


Figure 1

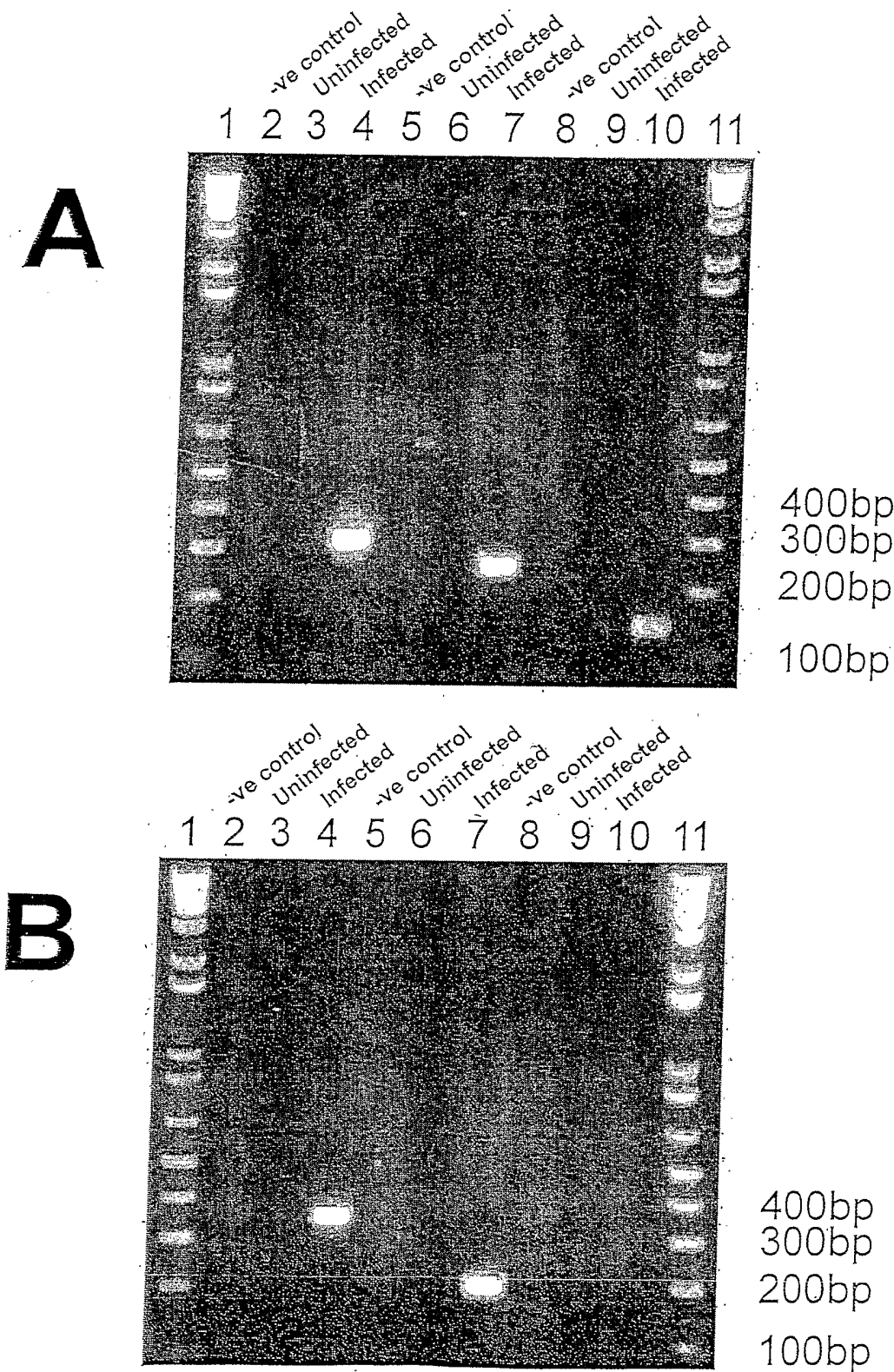


Figure 2

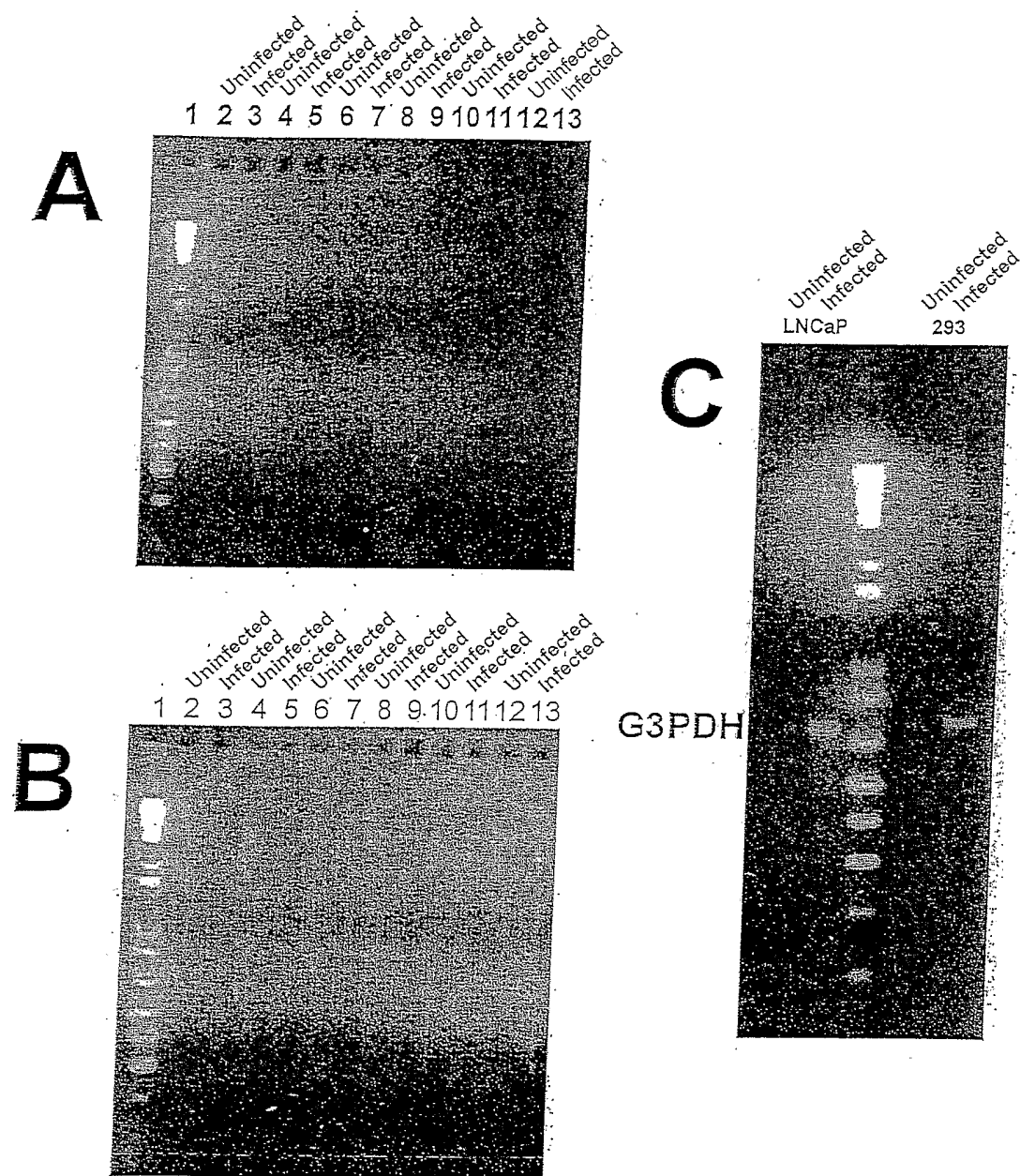


Figure 3

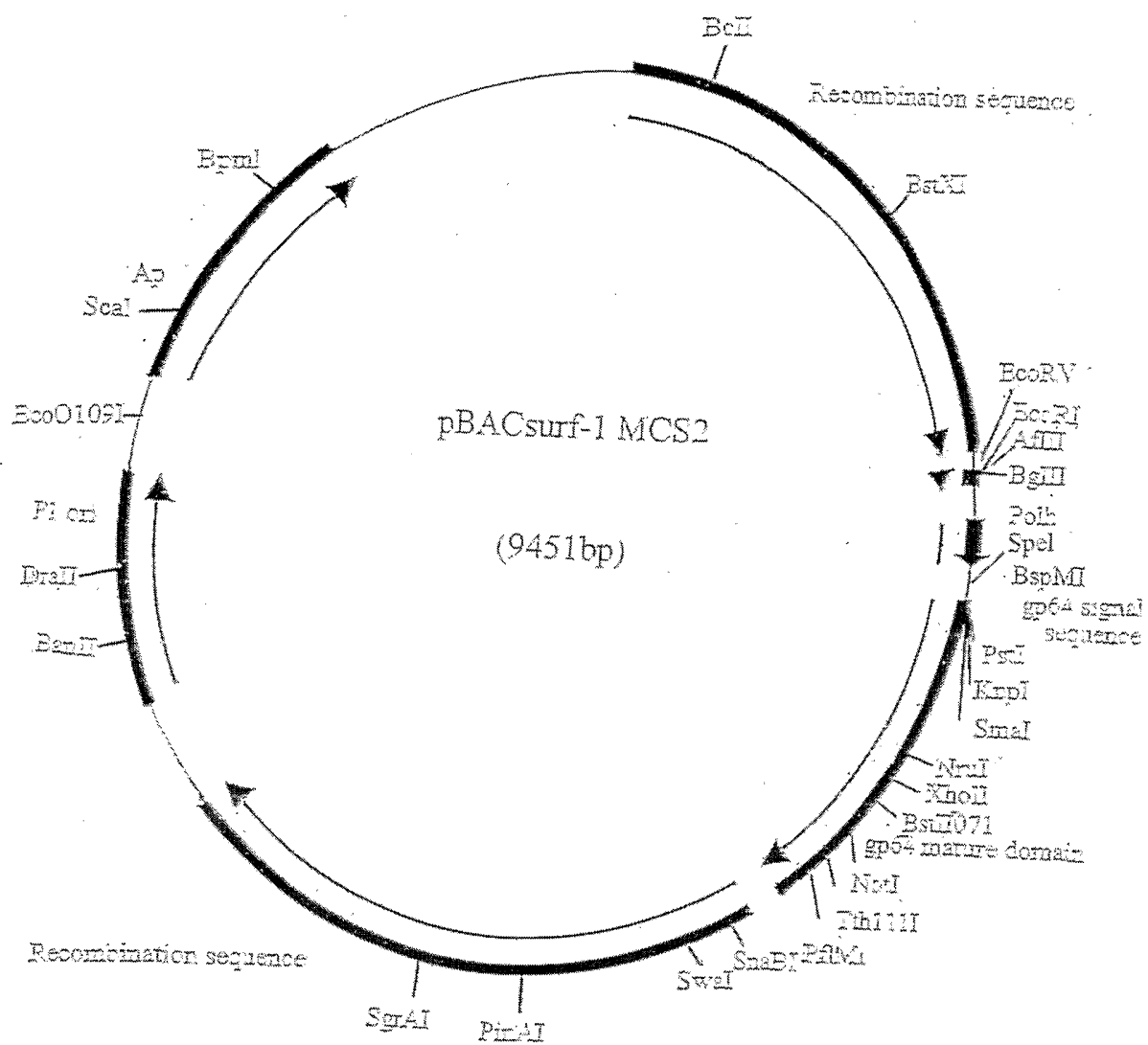


FIGURE 4

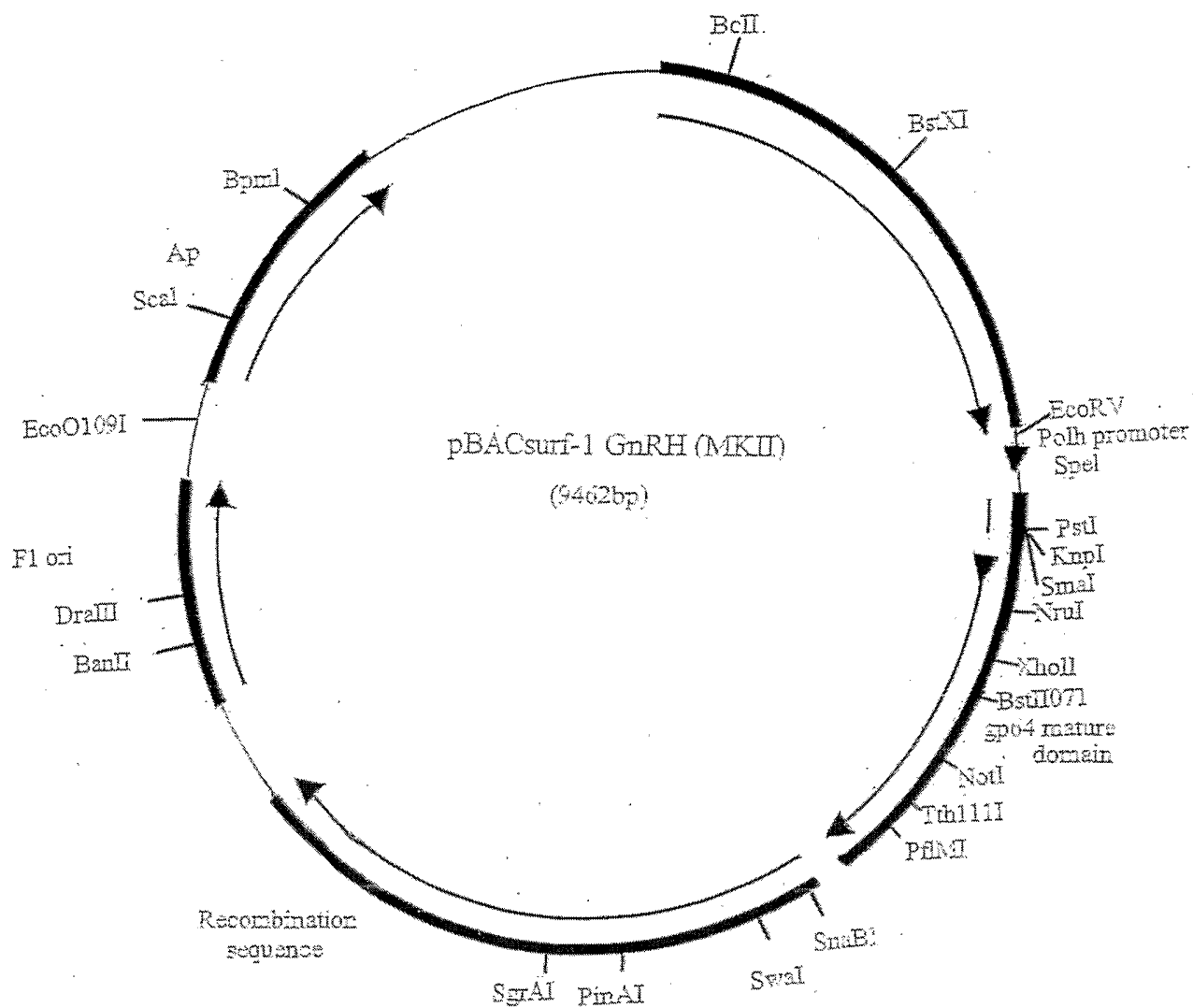


FIGURE 5

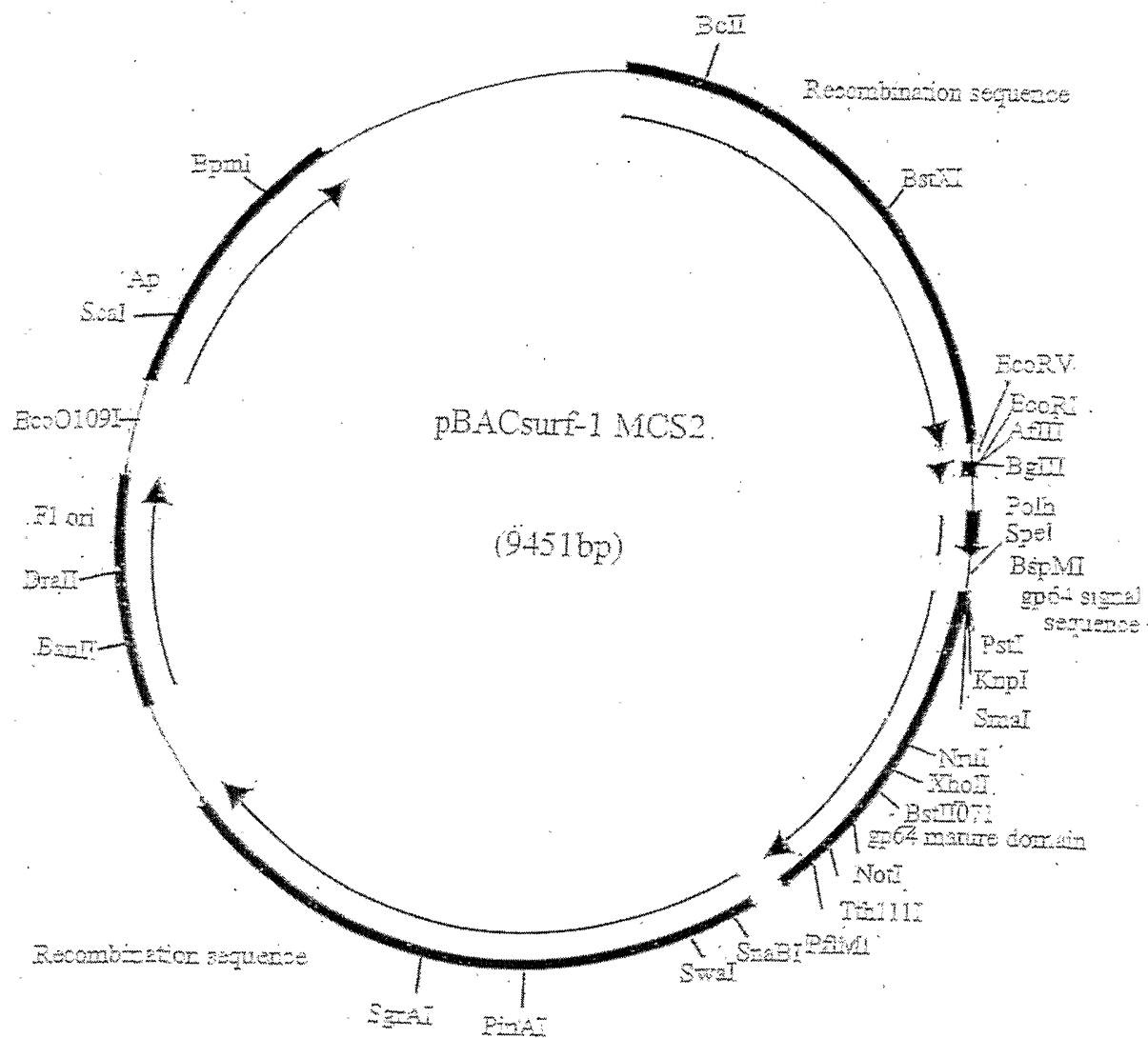


FIGURE 6