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(54) **Title**: LENTIVIRAL VECTORS CONTAINING AN MHC CLASS I, MHC CLASS II, OR BETA-2 MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE

(57) **Abstract**: The present invention relates to the insertion of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector to increase viral titers. The invention encompasses these vectors, methods of making the vectors, and methods of using them, including medicinal uses.

LENTIVIRAL VECTORS CONTAINING AN MHC CLASS I, MHC CLASS II, OR β2 MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE

TECHNICAL FIELD

The present invention is in the field of recombinant vaccine technology and relates to improvements of lentiviral vectors, which can be used as therapeutic and prophylactic vaccines. The vectors containing MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequences provide improved characteristics over other vectors.

BACKGROUND

Recombinant vaccines have been developed with the progress of recombinant DNA technology, allowing the modification of viral genomes to produce modified viruses. In this manner, it has been possible to introduce genetic sequences into non-pathogenic viruses, so that they encode immunogenic proteins to be expressed in target cells upon infection, in order to develop a specific immune response in their host.

Such vaccines constitute a major advance in vaccine technology (Kutzler et al., *Nat Rev Genet,* 9(10): 776-788, 2008). In particular, they have the advantage over traditional vaccines of avoiding live (attenuated) virus and eliminating risks associated with the manufacture of inactivated vaccines.

Gene delivery using modified retroviruses (retroviral vectors) was introduced in the early 1980s by Mann et al. (*Cell,* 33(1):153-9, 1983). The most commonly used oncogenic retroviral vectors are based on the Moloney murine leukemia virus (MLV). They have a simple genome from which the polyproteins Gag, Pol and Env are produced and are required in *trans* for viral replication (Breckpot *et al*., 2007, *Gene Ther,* 14(11):847-62; He *et al*. 2007, *Expert Rev vaccines,* 6(6):913-24). Sequences generally required in *cis* are the long terminal repeats (LTRs) and its vicinity: the inverted repeats (IR or att sites) required for integration, the packaging sequence Ψ, the transport RNA-binding site (primer binding site, PBS), and some additional sequences involved in reverse transcription (the repeat R within the LTRs, and the polypurine tracts, PPT, necessary for plus strand initiation). To generate replication-defective retroviral vectors, the *gag*, *pol*, and *env* genes are generally entirely deleted and replaced with an expression cassette.

Retroviral vectors deriving from lentivirus genomes (i.e. lentiviral vectors) have emerged as promising tools for both gene therapy and immunotherapy purposes, because they exhibit several advantages over other viral systems. In particular, lentiviral vectors themselves are not toxic and, unlike other retroviruses, lentiviruses are capable of transducing non-dividing cells, in particular dendritic cells (He *et al*. 2007, *Expert Rev vaccines,* 6(6):913-24), allowing antigen presentation through the endogenous pathway.

Lentiviruses are linked by similarities in genetic composition, molecular mechanisms of replication and biological interactions with their hosts. They are best known as agents of slow disease syndromes that begin insidiously after prolonged periods of subclinical infection and progress slowly; thus, they are referred to as the "slow" viruses (Narayan et al., 1989, *J Gen Virol,* 70(7):1617-39). They have the same basic organization as all retroviruses, but are more complex due to the presence of accessory genes (e.g., *vif*, *vpr*, *vpu*, *nef*, *tat*, and *rev*), which play key roles in lentiviral replication *in vivo*.

Lentiviruses represent a genus of slow viruses of the Retroviridae family, which includes the human immunodeficiency viruses (HIV), the simian immunodeficiency virus (SIV), the equine infectious encephalitis virus (EIAV), the caprine arthritis encephalitis virus (CAEV), the bovine immunodeficiency virus (BIV) and the feline immunodeficiency virus (FIV). Lentiviruses can persist indefinitely in their hosts and replicate continuously at variable rates during the course of the lifelong infection. Persistent replication of the viruses in their hosts depends on their ability to circumvent host defenses.

The design of recombinant integrative lentiviral vectors is based on the separation of the *cis*- and *trans*-acting sequences of the lentivirus. Efficient transduction in non-dividing cells requires the presence of two *cis*-acting sequences in the lentiviral genome, the central polypurine tract (cPPT) and the central termination sequence (CTS). These lead to the formation of a triple-stranded DNA structure called the central DNA "flap", which maximizes the efficiency of gene import into the nuclei of non-dividing cells, including dendritic cells (DCs) (Zennou *et al.,* 2000, Cell, 101(2) 173-85; Arhel *et al.,* 2007, *EMBO J,* 26(12):3025-37).

Dendritic cells are of primary importance for antigen presentation because they constitute the main class of antigen presenting cells (APCs) whose primary function is to present antigens and initiate an immune response.

To generate an immune response, antigenic proteins must be processed by cells into peptides that are displayed on the cell surface by major histocompatibility complex proteins (MHCs). Circulating APCs present the peptide-MHC complexes to T cells in the draining lymph nodes, where they interact with T cell receptors, and, in conjunction with co-stimulatory signals, activate the T cells.

A variety of studies have shown that inoculation with lentiviral vectors leads to antigen presentation by DCs and strong activation of antigen specific cytotoxic T lymphocytes (CTLs; $CD8^+$ T cells). Therefore, lentiviral vectors have been engineered for gene transfer and immunotherapy applications.

Lentiviral vectors have been improved in their safety by removal of the LTR U3 sequence, resulting in "*self-inactivating*" vectors that are entirely devoid of viral promoter and enhancer sequences originally present within the LTRs.

The lentiviral particles, which contain lentiviral vectors, can be produced by recombinant technology upon transient transfection of HEK 293T human cultured cells by different DNA plasmids:

(i) a packaging plasmid, which expresses at least the Gag, Pol Rev, Tat and, in some cases, structural and enzymatic proteins necessary for the packaging of the transfer construct;

(ii) a transfer plasmid, containing an expression cassette and HIV cis-acting factors necessary for packaging, reverse transcription, and integration; and

(iii) an envelope-encoding plasmid, in most cases the glycoprotein of vesicular stomatitis virus (VSV.G), a protein that allows the formation of mixed particles (pseudotypes) that can target a wide variety of cells, especially major histocompatibility (MHC) antigen-presenting cells (APCs), including DCs.

This procedure allows obtaining transient production of lentiviral particle vectors by the transfected cells. However, the lentiviral particle vectors may also be continuously produced by cells by stably inserting the packaging genes, the proviral coding DNA, and the envelope gene into the cellular genome. This allows the continuous production of lentiviral particle vectors by the cells without the need for transient transfection. Of course, a combination of these procedures can be used, with some of the DNAs/plasmids integrated into the cellular genome and others provided by transient transfection.

Non-integrative lentiviral vectors have designed in an attempt to mitigate the risks of potential oncogenesis linked to insertional mutagenesis events, particularly for vaccination purposes.

In vaccination based on direct injection of antigen-encoding integrative lentiviral vectors, transduced cells expressing the relevant antigen become targets of the elicited immune response and are eliminated within a few days or weeks from the vaccinated organism.

In addition, deletion in the U3 region of the 3' LTR of the viral promoter and enhancer sequences in self-inactivating lentiviral vectors limits the likelihood of endogenous promoter activation. This deletion directly addresses the experiences gained from the SCID-X1 gene therapy trial carried out in 1998-1999, performed with Moloney virus-based retroviral vectors on children suffering from a rare form of X-linked (SCID-X1 gene) severe immunodeficiency disease (Cavazzana-Calvo *et al.*, 2000, *Science*., 288(5466):669-72). During this trial, four of nine children developed leukemia as a result of the integration of the Moloney-derived retroviral vector at close proximity to the human LM02 proto-oncogene (Hacein-Bey-Abina et *al.*, 2008, *J.Clin.Invest.*, 118(9):3132-3142). It was demonstrated that malignancy was the consequence of the proximity of the viral U3 promoter/enhancer to the LM02 proto-oncogene.

Enhancers are cis-acting sequences, which can act as transcriptional activators at a distance. They have been widely employed in viral derived vectors because they appear to be the most efficient for obtaining transgene strong expression in a variety of cell types, in particular DCs (Chinnasamy, Chinnasamy *et al.*, 2000, Hum Gene Ther 11(13):1901-9; Rouas *et al.*, 2008, *Cancer Gene Ther* 9(9):715-24; Kimura *et al.*, 2007, *Mol Ther* 15(7):1390-9; Gruh *et al.*, 2008, *J Gene Med* 10(1) 21-32). However, given the safety issue of insertional mutagenesis, such transcriptional enhancer sequences should be deleted from the lentiviral vector constructs to abolish the risk of insertional mutagenesis by enhancer proximity effect. This enhancer proximity effect is by far the most frequent mechanism of insertional mutagenesis and is the only effect described in human or animal cases of tumorigenic events after gene transfer.

Thus, there is a need to develop retroviral, particularly lentiviral vectors, which do not include viral enhancers and which allow sufficient expression of transgenes encoding immunogenic peptides, if possible as much expression as that observed when using the CMV promoter. Particularly, there is a need for vectors with improved titers.

A study has reported on the replacement of viral promoters by DC-specific promoters deriving from major histocompatibility complex class II genes (MHC class II) (Kimura *et al.*, 2007, *Mol Ther* 15(7):1390-9) and dectin-2 genes (Lopes *et al.,* 2008, *J Virol* 82(1):86-95). The dectin-2 gene promoter used in Lopes et al. contains a putative enhancer and an adenoviral conserved sequence (inverted terminal repeats in adenovirus promoter) (Bonkabara *et al.*, 2001, *J. Immunology*, 167:6893-6900). The MHC class II gene promoter used by Kimura *et al.* does not contain any known enhancer.

Yet, without an enhancer, the MHC class II promoter was found not to provide sufficient transgene expression in DCs. In particular, lentiviral vectors including MHC class II promoters did not provoke an immune reaction in immunocompetent C57BL/6 mice, in contrast to the immune responses observed with CMV promoters/enhancers. Although integration and persistent transgene expression were observed after injection in mice, the lentiviral vectors transcribed through MHC class II promoters failed to stimulate an antigen-specific CD8+ cytotoxic T-lymphocyte response, even after vaccination boost. The authors of these studies therefore concluded that the use of MHC class II promoters was of interest only for applications where persistence of expression is sought as in gene replacement therapy, but not in the context of immunotherapy.

Thus, the MHC class II promoter is not an adequate promoter for lentiviral vectors for induction of an immune response against an antigen. Moreover, the dectin-2 promoter is dendritic cell specific, which does not allow elimination of vectors that are integrated into other non-expressing cell types. Moreover, the dectin-2 promoter appears to contain an enhancer. Thus, the dectin-2 promoter is not a good promoter for lentiviral vectors for safety reasons.

Preferably, in immunotherapy, lentiviral vectors provide effective expression of the transgene that elicits a desired specific immune response. This requires that the expression is at a high level in APCs, such as dendritic cells.

It is also preferable that the cells transduced by the lentiviral vectors are eliminated by the immune response to provide a higher degree of safety. That is, the immune response generated against the transgene can elicit an immune response in the host sufficient to eliminate the cells that are transduced by the lentiviral vectors. The elimination of transduced cells eliminates the persistence of the lentiviral vector in the host, and possible secondary effects of the vector. In order for the transduced cells to be eliminated, expression is required in non-dendritic cells at a level that allows elimination by the immune response.

At the same time, the promoter should maximize immune stimulation through the key cells (i.e., dendritic cells) involved in the activation of naïve and memory T cells, and should minimize the risk of insertional mutagenesis and genotoxicity in stem cells, leading to malignancies. Thus, the promoter should have sufficiently high activity in dendritic and other cells, but not contain an enhancer. Based on these criteria, viral promoters, such as the CMV promoter, are not ideal because of the presence of strong enhancers. These criteria are summarized as follows:

1. high expression in dendritic cells to induce maximal immune responses;
2. expression in other transduced cell types sufficient for elimination by the induced immune response; and
3. lack of an enhancer element to avoid insertional effects.

The vector should be capable of being generated at high titers to maximize delivery and expression, while minimizing contaminants.

Thus, a need exists in the art for improved vectors. The present invention fulfils these needs in the art.

SUMMARY OF THE INVENTION

The invention encompasses compositions comprising lentiviral vectors and methods of making and using the vectors. In one embodiment, the invention encompasses a lentiviral vector comprising an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence, preferably further comprising an MHC class I or β2 microglobulin promoter.

The invention encompasses methods for producing a lentiviral vector comprising inserting at least 300 nucleotides, preferably 300-400, 300-600 or 300-1100 nucleotides, of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector.

Preferably, the upstream promoter sequence is inserted upstream of an MHC class I or β2 microglobulin promoter. Most preferably, the upstream promoter sequence is inserted in the same orientation as the MHC class I or β2 microglobulin promoter.

In some embodiments, the upstream promoter sequence is an MHC class I upstream promoter sequence. In some embodiments, the upstream promoter sequence is a β2 microglobulin upstream promoter sequence, preferably comprising SEQ ID NO:1 or SEQ ID NO:27.

In some embodiments, the promoter is an MHC class I promoter. In some embodiments, the promoter is a β2 microglobulin promoter.

The invention encompasses a lentiviral vector comprising at least 300 nucleotides, preferably 300-400, 300-600 or 300-1100 nucleotides, of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence.

Preferably, the upstream promoter sequence is upstream of an MHC class I or β2 microglobulin promoter. Most preferably, the upstream promoter sequence is in the same orientation as the MHC class I or β2 microglobulin promoter.

In some embodiments, the upstream promoter sequence is an MHC class I upstream promoter sequence. In some embodiments, the upstream promoter sequence is a β2 microglobulin upstream promoter sequence, preferably comprising SEQ ID NO:1 or SEQ ID NO:27.In some embodiments, the promoter is an MHC class I promoter. In some embodiments, the promoter is a β2 microglobulin promoter.

Preferably, the MHC class I promoter is an HLA-A2 promoter, HLA-B7 promoter, or an HLA-E promoter.

Preferably, the upstream promoter sequence is an HLA-A2, HLA-B7, or an HLA-E, or HLA-DRα upstream promoter sequence.

Preferably, the lentiviral vector comprises a lentiviral cPPT/CTS sequence. Preferably, the lentiviral vector comprises a lentiviral Ψ (psi) sequence.

The invention encompasses an isolated host cell comprising a lentiviral vector of the invention. The invention encompasses a lentiviral vector of the invention for use as a medicament or vaccine, particularly for gene therapy.

In preferred embodiments, the upstream promoter sequence comprises the nucleotide sequence of any one of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

BRIEF DESRIPTION OF THE DRAWINGS

Figure 1 A, B, and C depict schematic representations of (A) the β2m promoter (promoter region and upstream chromosomal region), (B) the MHC class I promoter, and (C) the MHC class II promoter.

Figure 2 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned upstream of various promoters. The β2m upstream promoter sequence was cloned upstream of various promoters by fusion PCR. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 3 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned upstream of various promoters, in direct or reverse orientation. The β2m upstream promoter sequence was cloned upstream of various promoters by fusion PCR, either in direct (5'-3') or reverse (3'-5') orientation. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 4 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned downstream of the transgene (GFP), in direct or reverse orientation. The β2m upstream promoter sequence was cloned downstream of the transgene (GFP) of lentiviral constructs harboring various promoters, either in direct (5'-3') or reverse (3'-5') orientation.

The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 5 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned outside of the proviral sequence (into the plasmidic backbone), in direct or reverse orientation. The β2m upstream promoter sequence was cloned outside the proviral sequence (inside the plasmid backbone) of constructs harboring various promoters, either in direct (5'-3') or reverse (3'-5') orientation. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 6 depicts production yields of various lentiviral constructs, with or without the HLA-E upstream promoter sequence cloned upstream of various promoters, in direct or reverse orientation. The HLA-E upstream promoter sequence was cloned upstream of various promoters by fusion PCR, either in direct (5'-3') or reverse (3'-5') orientation. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by FACS analysis.

Figure 7 depicts the nucleotide sequences of β2-microglobulin (SEQ ID NO:1) and MHC Class I (SEQ ID NOs:2-7) upstream promoter sequences. A consensus sequence is shown (SEQ ID NO:8).

Figure 8A-B depicts the nucleotide sequences of β2-microglobulin (SEQ ID NO:40), MHC Class I (SEQ ID NOs:37-39), and MHC Class II (SEQ ID NO:41) promoters and short upstream promoter sequences. The locations of the κB, ISRE, and SXY module are indicated.

Figure 9A-C depicts the nucleotide sequences of β2-microglobulin (SEQ ID NO:45), MHC Class I (SEQ ID NOs:42-44), and MHC Class II (SEQ ID NO:46) promoters and long upstream promoter sequences. The locations of the κB, ISRE, and SXY module are indicated.

Figure 10 depicts production yields of various lentiviral constructs, with short or long β2-m, HLA-A2, HLA-B7, HLA-E, or HLA-DRα upstream promoter sequences upstream of their natural promoters, in direct orientation.

DETAILED DESCRIPTION OF THE INVENTION

The effect of MHC class I, MHC class II, or β2 microglobulin upstream promoter sequences on lentiviral vector titers was examined. The upstream promoter sequences are located upstream of the Ets/ISRE and NF-Kb binding sites found in the β2 microglobulin and MHC class I promoters (Fig. 1). The upstream promoter sequences are located upstream of the SXY module found in MHC class II promoters (Fig. 1). The human β2-microglobulin (β2m) promoter shows some similarity to the MHC Class I promoters, but it contains the ISRE upstream of a single NF-Kb binding site.

The upstream promoter sequences of β2m and MHC Class I promoters show some similarity at the nucleotide level (Fig. 7). Two upstream promoter sequences were selected for analysis, β2m and HLA-E.

First, an upstream promoter sequence of β2m was inserted into a lentiviral vector upstream of and in the same orientation as the β2m and MHCI promoters HLA-A2, HLA-B7, and HLA-E. For comparison, the upstream promoter sequence of β2m was inserted into lentiviral vectors upstream of and in the same orientation as the Ubiquitin (UBC) gene promoter, the CMV promoter, or an MHCII promoter (HLA-DRα). In these vectors, the promoters drive expression of green fluorescent protein (GFP).

To look for expression, the vectors were packaged by cotransfection in HEK-293T cells with an encapsidation plasmid and a plasmid providing VSV.G envelope, essentially as described in Naldini *et al*, 1996, *Science* 272:263-7. HEK-293T cells were then transduced with particles of the different vectors. Expression was detected in the cells with all vectors.

The addition of the β2m upstream promoter sequence into lentiviral vectors with a β2m, HLA-A2, HLA-B7, or HLA-E promoter resulted in an approximately 2-5 fold increase in viral titers. In contrast, addition of the β2m upstream promoter sequence into lentiviral vectors with a CMV promoter, an UBC promoter, or an HLA-A2 promoter demonstrated little effect on the titers (Fig. 2). Thus, the β2m upstream promoter sequence could increase titers from a lentiviral vector containing a β2m or MHCI promoter.

Next, the upstream promoter sequence of β2m was inserted into a lentiviral vector upstream of and in the reverse orientation as the β2m and MHCI promoters

HLA-A2, HLA-B7, and HLA-E. For comparison, the upstream promoter sequence of β2m was inserted into lentiviral vectors upstream of and in the reverse orientation as the Ubiquitin (UBC) genes promoter, the CMV promoter, or an MHCII promoter (HLA-DRα). The addition of the β2m upstream promoter sequence into lentiviral vectors upstream of and in the reverse orientation as the HLA-A2, HLA-B7, or HLA-E promoter did not result in an increase in viral titers (Fig. 3). In fact, several of the lentiviral vectors with the upstream promoter sequence of β2m inserted in the reverse orientation showed a decrease in titers. Thus, the increase in titers from a lentiviral vector caused by the β2m upstream promoter sequence was orientation dependent.

Next, the upstream promoter sequence of β2m was inserted into a lentiviral vector downstream of the transgene and in the same or reverse orientation as the β2m and MHCI promoters HLA-A2, HLA-B7, and HLA-E. For comparison, the upstream promoter sequence of β2m was inserted into lentiviral vectors downstream of the transgene and in the same or reverse orientation as the Ubiquitin (UBC) genes promoter, the CMV promoter, or an MHCII promoter (HLA-DRα). The addition of the β2m upstream promoter sequence downstream of the transgene and in the same orientation as the β2m, HLA-A2, HLA-B7, or HLA-E promoter resulted in only a small increase in viral titers (Fig. 4). Thus, the increase in titers from a lentiviral vector caused by the β2m upstream promoter sequence was position dependent.

Next, the upstream promoter sequence of β2m was inserted into a lentiviral vector outside of the LTR-LTR region in the same or reverse orientation as the β2m and MHCI promoters HLA-A2, HLA-B7, and HLA-E. For comparison, the upstream promoter sequence of β2m was inserted into lentiviral vectors outside of the LTR-LTR region in the same or reverse orientation as the Ubiquitin (UBC) genes promoter, the CMV promoter, or an MHCII promoter (HLA-DRα). The addition of the β2m upstream promoter sequence outside of the LTR-LTR region in the same or reverse orientation resulted in no apparent difference in viral titers (Fig. 5). Thus, the increase in titers from a lentiviral vector caused by the β2m upstream promoter sequence was dependent on its presence between the LTRs in the vector.

Next, an upstream promoter sequence of HLA-E was inserted into a lentiviral vector upstream of and in the same or reverse orientation as the β2m and MHCI promoters HLA-A2, HLA-B7, and HLA-E. For comparison, the upstream promoter sequence of HLA-E was inserted into lentiviral vectors upstream of and in the same or reverse orientation as the Ubiquitin (UBC) genes promoter, the CMV promoter, or an MHCII promoter (HLA-DRα).

The addition of the HLA-E upstream promoter sequence into lentiviral vectors upstream of the β2m, HLA-A2, HLA-B7, or HLA-E promoter resulted in an approximately 2-4 fold increase in viral titers (Fig. 6). In most constructs, the HLA-E upstream promoter sequence worked similarly in both orientations. The addition of the HLA-E upstream promoter sequence into lentiviral vectors with a CMV promoter or a UBC promoter demonstrated an approximately 2 fold increase in the titers (Fig. 6). However, the addition of the HLA-E upstream promoter sequence into a lentiviral vector with an HLA-DRα promoter demonstrated little or no effect on the titers. Thus, the HLA-E upstream promoter sequence could increase titers from a lentiviral vector containing a β2m, MHCI, CMV, or UBC promoter.

An upstream promoter sequence of HLA-A2 was inserted into a lentiviral vector upstream of and in the same or reverse orientation as the β2m promoter. The addition of the HLA-A2 upstream promoter sequence into lentiviral vectors upstream of the β2m promoter resulted in an approximately 3-4 fold increase in viral titers in both orientations.

An upstream promoter sequence of HLA-B7 was inserted into a lentiviral vector upstream of and in the same or reverse orientation as the β2m promoter. The addition of the HLA-B7 upstream promoter sequence into lentiviral vectors upstream of the β2m promoter resulted in an approximately 10 fold increase in viral titers in the same orientation and an approximately 4 fold increase in viral titers in the reverse orientation.

An upstream promoter sequence of HLA-DRα was inserted into a lentiviral vector upstream of and in the same or reverse orientation as the β2m promoter. The addition of the HLA-DRα upstream promoter sequence into lentiviral vectors upstream of the β2m promoter resulted in an approximately 6 fold increase in viral titers in the same orientation and an approximately 4 fold increase in viral titers in the reverse orientation.

Since the upstream sequences were all of about 300-400 nt in size, the effect of larger upstream sequences (500-1100nt) was investigated. A larger upstream promoter sequence of β2m (1058bp) was inserted into a lentiviral vector upstream of and in the same orientation as the β2m promoter. The larger upstream promoter sequence did not further increase viral titers, but did retain most of the increased viral titers, as compared to the smaller (330bp) upstream sequence (Fig. 10).

A larger upstream promoter sequence of HLA-A2 (531bp) was inserted into a lentiviral vector upstream of and in the same orientation as the HLA-A2 promoter. In this case, the larger HLA-A2 upstream promoter sequence increased viral titers 3 fold; whereas, the smaller (322bp) HLA-A2 upstream sequence had a negative effect on the HLA-A2 promoter (Fig. 10).

A larger upstream promoter sequence of HLA-B7 (511bp) was inserted into a lentiviral vector upstream of and in the same orientation as the HLA-B7 promoter. In this case, the larger HLA-B7 upstream promoter sequence further increased viral titers 2-3 fold as compared to the smaller (352bp) HLA-B7 upstream sequence (Fig. 10).A larger upstream promoter sequence of HLA-E (1047bp) was inserted into a lentiviral vector upstream of and in the same orientation as the HLA-E promoter. The larger upstream promoter sequence eliminated the increase in viral titers, as compared to the smaller (328bp) upstream sequence (Fig. 10).

A larger upstream promoter sequence of HLA-DRα (522bp) was inserted into a lentiviral vector upstream of and in the same orientation as the HLA-DRα promoter. In this case, the larger HLA-DRα upstream promoter sequence had the same effect on the viral titers as compared to the smaller (356bp) HLA-DRα upstream sequence (Fig. 10).

The effect of the insertion of multiple upstream promoter sequences was investigated. The HLA-E upstream promoter sequence was inserted into a lentiviral vector upstream, downstream, and both upstream and downstream of the β2m promoter. While insertion upstream resulted in a 3-4 fold increase in titers, insertion downstream had no effect on titers and insertion both upstream and downstream resulted in decrease in viral titers.

The present invention has thus, as a main object, a lentiviral vector comprising a β2m or MHCI or MHCII upstream promoter sequence, and methods for making and using such a vector.

LENTIVIRAL VECTOR

Within the context of this invention, a “lentiviral vector” means a non-replicating vector for the transduction of a host cell with a transgene comprising *cis*-acting lentiviral RNA or DNA sequences, and requiring lentiviral proteins (e.g., Gag, Pol, and/or Env) that are provided *in trans*. The lentiviral vector contains cis-acting packaging sequences, but lacks expression of functional Gag, Pol, and Env proteins. The lentiviral vector may be present in the form of an RNA or DNA molecule, depending on the stage of production or development of said retroviral vectors.

The lentiviral vector can be in the form of a recombinant DNA molecule, such as a plasmid. The lentiviral vector can be in the form of a lentiviral particle vector, such as an RNA molecule(s) within a complex of lentiviral and other proteins. Typically, lentiviral particle vectors, which correspond to modified or recombinant lentivirus particles, comprise a genome which is composed of two copies of single-stranded RNA. These RNA sequences can be obtained by transcription from a double-stranded DNA sequence inserted into a host cell genome (proviral vector DNA) or can be obtained from the transient expression of plasmid DNA (plasmid vector DNA) in a transformed host cell.

Lentiviral vectors derive from lentiviruses, in particular human immunodeficiency virus (HIV-1 or HIV-2), simian immunodeficiency virus (SIV), equine infectious encephalitis virus (EIAV), caprine arthritis encephalitis virus (CAEV), bovine immunodeficiency virus (BIV) and feline immunodeficiency virus (FIV), which are modified to remove genetic determinants involved in pathogenicity and introduce new determinants useful for obtaining therapeutic effects.

Such vectors are based on the separation of the *cis*- and *trans*-acting sequences. In order to generate replication-defective vectors, the trans-acting sequences (e.g., *gag*, *pol*, *tat, rev,* and *env* genes) can be deleted and replaced by an expression cassette encoding a transgene.

Efficient integration and replication in non-dividing cells generally requires the presence of two *cis*-acting sequences at the center of the lentiviral genome, the central polypurine tract (cPPT) and the central termination sequence (CTS). These lead to the formation of a triple-stranded DNA structure called the central DNA "flap", which acts as a signal for uncoating of the pre-integration complex at the nuclear pore and efficient importation of the expression cassette into the nucleus of non-dividing cells, such as dendritic cells.

In one embodiment, the invention encompasses a lentiviral vector comprising a central polypurine tract and central termination sequence referred to as cPPT/CTS sequence as described, in particular, in the European patent application EP 2 169 073.

Further sequences are usually present in *cis,* such as the long terminal repeats (LTRs) that are involved in integration of the vector proviral DNA sequence into a host cell genome. Vectors may be obtained by mutating the LTR sequences, for instance, in domain U3 of said LTR (ΔU3) (Miyoshi H *et al*, 1998, *J Virol.* 72(10):8150-7; Zufferey *et al.,* 1998, *J Virol* 72(12):9873-80).

In one embodiment, the invention encompasses a lentiviral vector comprising LTR sequences, preferably with a mutated U3 region (ΔU3) removing promoter and enhancer sequences in the 3' LTR.

The packaging sequence Ψ (psi) is incorporated to support encapsidation of the polynucleotide sequence into the vector particles (Kessler *et al*., 2007, *Leukemia*, 21(9):1859-74; Paschen *et al.*, 2004, *Cancer Immunol Immunother* 12(6):196-203).

In one embodiment, the invention encompasses a lentiviral vector comprising a lentiviral packaging sequence Ψ (psi) and an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

Further additional functional sequences, such as a transport RNA-binding site or primer binding site (PBS) or a Woodchuck PostRegulation Element (WPRE), can also be advantageously included in the lentiviral vector polynucleotide sequence of the present invention, to obtain a more stable expression of the transgene in vivo.

In one embodiment, the invention encompasses a lentiviral vector comprising a PBS. In one embodiment, the invention encompasses a lentiviral vector comprising a WPRE and/or an IRES.

Thus, in a preferred embodiment, the lentiviral vector comprises an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence, at least one cPPT/CTS sequence, one Ψ sequence, one (preferably 2) LTR sequence, and an expression cassette including a transgene under the transcriptional control of a promoter, particularly an MHC class I or β2 microglobulin promoter.

TRANSGENE

The invention encompasses a lentiviral vector containing a transgene. Within the context of this invention, a "transgene" is a nucleic acid sequence within a lentiviral vector that is not normally present in a cell to be transduced with the lentiviral vector. The lentiviral vector serves to introduce this sequence into the transduced cell. The term "transgene" does not include those sequences of the vector that facilitate transduction of the transgene. The transgene may be a nucleic acid sequence from another organism. Alternatively, the transgene may be a nucleic acid sequence from the same organism, but having different regulatory sequences controlling its expression. The transgene may be a sense or antisense nucleic acid molecule. According to a preferred embodiment of the invention, the transgene sequence encodes an immunogenic polypeptide.

Preferably, the immunogenic polypeptide is viral, parasitic, bacterial, or fungal. In one embodiment, the immunogenic polypeptide is a tumor antigen.

This immunogenic polypeptide preferably comprises one or several epitope(s) from agents of infectious diseases, for example antigen(s) from Gag, Pol, and/or Nef proteins of HIV.

Several epitopes forming a polyepitope may also be encoded by the transgene of the invention.

In a particular embodiment, such epitope is derived from target antigens identified in tumors, and can be chosen in such a way that a cell-mediated immune response is obtained against it. Target antigens are well documented in the art, which can be selected with respect to several types of tumors and in particular in melanomas or in carcinomas, including renal carcinomas, bladder carcinomas, colon carcinomas, lung carcinomas, breast cancers, leukemias, and lymphomas.

B2M AND MHCI PROMOTERS

The invention encompasses the insertion of a β2m or MHC Class I (MHCI) promoter into a lentiviral vector. As used herein, an “MHC Class I (MHCI) promoter” includes a naturally occurring or synthetic MHC Class I promoter. The term “MHC Class I promoter” does not include a β2m promoter.

Naturally Occurring MHCI and a β2m Promoters

Examples of naturally occurring MHCI promoters are the HLA-A2, HLA-B7, HLA-Cw5, HLA-E, HLA-G gene promoters. These naturally occurring MHCI promoters are generally cloned or reproduced from the promoter region of a gene encoding the MHC class I protein, or referred to as putatively encoding such proteins in genome databases (ex: NCBI polynucleotide database http://www.ncbi.nlm.nih.gov/guide/dna-rna). Both β2m and class I MHC proteins enter the Major Histocompatibility Complex (MHC). Preferred promoters are set forth in U.S. Patent Publn. 2014/0120132-A1, which are hereby incorporated by reference.

The proteins encoded by these genes are found in almost all cell types. MHCI proteins are generally present at the surface of the membrane of leucocytes, where they are associated with the β2-microglobulin (β2m) protein. The role of these associated proteins is to present peptides from endogenous sources to CD8+ T cells. They thus play a central role to the generation of the antigen-specific immune response. Because β2m and MHC proteins have been widely studied and described for many years, their genes are well characterized and detectable using sequence comparison tools, such as the BLAST method (Altschul, S.F. et *al*. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215(3):403–410).

β2m and MHC class I promoters share the ability to be strongly activated in dendritic cells, as well as, to lower intensity, in the majority of the other human body tissues.

The β2m and MHC class I promoters of the invention can contain further regulatory elements, such as one or more Sp1 and ETs binding sites. In a preferred embodiment, the MHC class I promoter contains 2 Sp1 binding sites and

1 Ets binding site. In other embodiments, Ap1 and/or Ap2 sites are further contained in the MHC class I promoter.

Preferred MHC class I promoters are human HLA-A2, HLA-B7, HLA-Cw5, HLA-E, HLA-F, and HLA-G promoters.

Synthetic β2m and MHC Class I Promoters

β2m and MHC class I promoters can also be synthetic. Synthetic β2m and MHC class I promoters include promoters that are synthesized using molecular biological techniques to assemble the individual components of an β2m and MHC class I promoter or that are derived from naturally occurring β2m and MHC class I promoters using molecular biological techniques.

ISRE

The transcription of β2m and MHC class genes is usually mediated by two major regulatory elements: Interferon stimulated response element (ISRE) and the SXY module (encompassing the W/S, X1X2/Site α and Y/enhancer B regulatory elements) (see figure 1). *See also* Van den Elsen, Immunogenetics (1998) 48:208-211.

These regulatory promoter elements are localized in a region extending approximately from nucleotides -220 to -95 upstream of the transcription initiation site. They mediate tissue-specific and cytokine-induced transcription of β2m and MHC class I genes.

The ISRE of β2m and MHC class I gene promoters generally contains binding sites for interferon regulatory factor (IRF) family members. It is thus a property of MHC class I promoters to bind to interferon regulatory factor (IRF) family members. This may be verified, for example, by gel shift assays.

NF-κB Binding Site

Another regulatory element, the enhancer A (containing binding sites for nuclear transcription factor κB (NF-κB)) is present in most cases. It is thus a property of β2m and MHC class I promoters to bind to nuclear transcription factor κB (NF-κB). This may be verified, for example, by gel shift assays.

SXY Module

In addition to ISRE, β2m and MHC class I promoters generally share another set of conserved upstream sequence motifs, consisting of four regulatory elements: the S or W box, the X1/CREX2 boxes or site α, and the Y box or enhancer B, which together are termed the SXY module. This SXY module is generally cooperatively bound by a multiprotein complex containing regulatory factor X (RFX; consisting of RFX5, RFXB/ANK and RFXAP), cAMP response element binding protein (CREB)/activating transcription factor (ATF), and nuclear factor Y (NFY), which acts as an enhanceosome driving transactivation of these genes. It is thus a property of β2m and MHC class I promoters to bind to these factors. This may be verified, for example, by gel shift assays.

In contrast, MHC class II promoters do not display enhancer A, nor ISRE, elements (Van den Elsen, P.J. *et al,* 1998, *Immunogenetics*. 48:208-221). Furthermore, RFX and CIITA in MHC class II gene regulation have been found of crucial importance as illustrated by studies with cell lines established from patients with the bare lymphocyte syndrome (BLS), a severe combined immunodeficiency due to mutations in one of the RFX subunits or CIITA (DeSandro, A. et *al*., 1999, *Am J Hum Genet*, 65:279-286). Also, lack of either CIITA or one of the RFX subunits affects the functioning and assembly of the MHC enhanceosome, respectively, leading to a lack of MHC class II and reduced levels of MHC class I transcription (Van den Elsen, P.J. *et al*. 2004, *Current Opinion in Immunology*, 16:67-75).

β2M AND MHCI AND MHCII UPSTREAM PROMOTER SEQUENCES

The invention encompasses the insertion of a β2m, MHC Class I (MHCI), or MHC Class II (MHCII) upstream promoter sequence into a lentiviral vector. As used herein, a "β2m upstream promoter sequence" refers to 1100 base pairs or less of the sequences found immediately upstream of the Ets/ISRE binding site in the naturally occurring β2 microglobulin promoter, as illustrated in Figs. 8A-B and Fig. 9A-C. *See also,* Figure 1 of Van den Elsen et al., Current Opinion in Immunology 2004, 16:67-75, which is hereby incorporated by reference. As used herein, a "MHC Class I (MHCI) upstream promoter sequence" refers to 1100 base pairs or less of the sequences found immediately upstream of the NF-Kb binding site in the naturally occurring MHC Class I promoters, as illustrated in Figs. 8A-B and Fig. 9A-C. Examples of β2m and MHC Class I (MHCI) upstream promoter sequences are shown in Figure 7. As used herein, a "MHC Class II (MHCII) upstream promoter sequence" refers to 1100 base pairs or less of the sequences found immediately upstream of the SXY module in the naturally occurring MHC class II promoter, as illustrated in Figs. 8A-B and Fig. 9A-C.

In various embodiments, the upstream promoter sequence comprises less than 1100, 1000, 900, 800, 700, 600, 550, 500, 450, 400, or 350 nucleotides of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence

In various embodiments, the upstream promoter sequence comprises at least 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 500, 600, 700, 800, 900, or 1000 nucleotides of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence.

In various embodiments, the upstream promoter sequence comprises 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, or 500 to 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 550, 600, 700, 800, 900, 1000, or 1100 nucleotides (in all possible combinations of ranges) of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence. Preferably, the upstream promoter sequence comprises 300-400, 300-500, 300-600, 300-700, or 300-1100 nucleotides of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence. Most preferably, the upstream promoter sequence comprises 300-335 nucleotides of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence.

Preferably, the B2M upstream promoter sequence comprises the nucleotide sequence:

AGAAGTTCTCCTTCTGCTAGGTAGCATTCAAAGATCTTAATCTTCTGGG
TTTCCGTTTTCTCGAATGAAAAATGCAGGTCCGAGCAGTTAACTGGCGGGGG
CACCATTAGCAAGTCACTTAGCATCTCTGGGGCCAGTCTGCAAAGCGAGGG
GGCAGCCTTAATGTGCCTCCAGCCTGAAGTCCTAGAATGAGCGCCCGGTGT
CCCAAGCTGGGGCGCGCACCCCAGATCGGAGGGCGCCGATGTACAGACAG
CAAACTCACCCAGTCTAGTGCATGCCTTCTTAAACATCACGAGACTC
(SEQ ID NO:1). Preferably, the B2M upstream promoter sequence comprises the nucleotide sequence SEQ ID NO:27 or 28.

Preferably, the MHC Class I upstream promoter sequence comprises an HLA-A2, HLA-B7, HLA-Cw5, HLA-E, or HLA-G upstream promoter sequence.

Preferably, the MHC Class I upstream promoter sequence comprises the nucleotide sequence of any of SEQ ID NOs:2-7 or SEQ ID NO:29-34.

Preferably, the MHC Class I upstream promoter sequence comprises the nucleotide sequence:

CTGGAGGGCAATGGCACGATCTTGGCTCACCGCAACCTCCTCCTCCT
GGGTTCAAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCCAGGATTACAGCC
ATGCGCCACCACGCCGGCTAATTTTTTGGACTTTTAGTAGAGACAGGGTTTCT
CCATATTGGTCGGGCTGGTCTCGAACTCCCAACCTCAGGTGATCAGCCCGC
CTTGGCCTCCCAAAGTGCTGAGATTACAGGCGTGAGCCACCGCGCCCAGCC
AGGACTAATTTCTAAGAGTGTGCAGAGATACCGAAACCTAAAAGTT
(SEQ ID NO:2).

Preferred upstream promoter sequences include the following:

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Upstream β2m (330bp):
GAGAAACCCTGCAGGGAATTCCCCAGCTGTAGTTATAAACAGAAGTTCTCCTTCTGCTAG
GTAGCATTCAAAGATCTTAATCTTCTGGGTTTCCGTTTTCTCGAATGAAAAATGCAGGTC
CGAGCAGTTAACTGGCGGGGGCACCATTAGCAAGTCACTTAGCATCTCTGGGGCCAGTCT
GCAAAGCGAGGGGGCAGCCTTAATGTGCCTCCAGCCTGAAGTCCTAGAATGAGCGCCCGG
TGTCCCAAGCTGGGGCGCGCACCCCAGATCGGAGGGCGCCGATGTACAGACAGCAAACTC
ACCCAGTCTAGTGCATGCCTTCTTAAACAT  (SEQ ID NO :27)

Upstream β2m (1058bp):
CTTCCAAGATCTCTGCCCCTCCCCATCGCCATGGTCCACTTCCTCTTCTCACTGTTCCTC
TTAGAAAAGATCTGTGGACTCCACCACCACGAAATGGCGGCACCTTATTTATGGTCACTT
TAGAGGGTAGGTTTTCTTAATGGGTCTGCCTGTCATGTTTAACGTCCTTGGCTGGGTCCA
AGGCAGATGCAGTCCAAACTCTCACTAAAATTGCCGAGCCCTTTGTCTTCCAGTGTCTAA
AATATTAATGTCAATGGAATCAGGCCAGAGTTTGAATTCTAGTCTCTTAGCCTTTGTTTC
CCCTGTCCATAAAATGAATGGGGGTAATTCTTTCCTCCTACAGTTTATTTATATATTCAC
TAATTCATTCATTCATCCATCCATTCGTTCATTCGGTTTACTGAGTACCTACTATGTGCC
AGCCCCTGTTCTAGGGTGGAAACTAAGAGAATGATGTACCTAGAGGGCGCTGGAAGCTCT
AAAGCCCTAGCAGTTACTGCTTTTACTATTAGTGGTCGTTTTTTTCTCCCCCCCGCCCCC
CGACAAATCAACAGAACAAAGAAAATTACCTAAACAGCAAGGACATAGGGAGGAACTTCT
TGGCACAGAACTTTCCAAACACTTTTTCCTGAAGGGATACAAGAAGCAAGAAAGGTACTC
TTTCACTAGGACCTTCTCTGAGCTGTCCTCAGGATGCTTTTGGGACTATTTTTCTTACCC
AGAGAATGGAGAAACCCTGCAGGGAATTCCCAAGCTGTAGTTATAAACAGAAGTTCTCCT
TCTGCTAGGTAGCATTCAAAGATCTTAATCTTCTGGGTTTCCGTTTTCTCGAATGAAAAA
TGCAGGTCCGAGCAGTTAACTGGCTGGGGCACCATTAGCAAGTCACTTAGCATCTCTGGG
GCCAGTCTGCAAAGCGAGGGGGCAGCCTTAATGTGCCTCCAGCCTGAAGTCCTAGAATGA
GCGCCCGGTGTCCCAAGCTGGGGCGCGCACCCCAGATCGGAGGGCGCCGATGTACAGACA
GCAAACTCACCCAGTCTAGTGCATGCCTTCTTAAACAT  (SEQ ID NO :28)

Upstream HLA-A2 (322bp) :
TACACCTCCATTCCCAGAGCAAGCTTACTCTCTGGCACCAAACTCCATGGGATGATTTTT
CTTCTAGAAGAGTCCAGGTGGACAGGTAAGGAGTGGGAGTCAGGGAGTCCAGTTCCAGGG
ACAGAGATTACGGGATAAAAAGTGAAAGGAGAGGGACGGGGCCCATGCCGAGGGTTTCTC
CCTTGTTTCTCAGACAGCTCTTGGGCCAAGACTCAGGGAGACATTGAGACAGAGCGCTTG
GCACAGAAGCAGAGGGGTCAGGGCGAAGTCCAGGGCCCCAGGCGTTGGCTCTCAGGGTCT
CAGGCCCCGAAGGCGGTGTATG  (SEQ ID NO :29)
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Upstream HLA-A2 (531bp) :
GAGTCCTGTTGTAATGCTTTTGGACACATTTATACATTAAGGGGCCAAAGTCACATTTTT
TACCTATTAGATTCCTGATCATTCAGGGGTTACCAAGATTCTGCTACCCACTGTAGTTAA
TAAACAAAGAGCAAATTGGTCTCTATTCTGTCTCATGCACTCAGGCACAACTTTTCCGGA
TTAAAAACAAAAACAACAACAACAAAAATCTACACCTCCATTCCCAGATCAAGCTTACTC
TCTGGCACCAAACTCCATGGGGTGATTTTTCTTCTAGAAGAGTCCAGGTGGACAGGTAAG
GAGTGGGAGTCAGGGAGTCCAGTTCAGGGACAGAGATAATGGATGAAAAGTGAAAGGAG
AGGGACGGGGCCCATGCCGAGGGTTTCTCCCTTGTTTCTCAGACAGCTCCTGGGCCAAGA
CTCAGGGAGACATTGAGACAGAGCGCTTCGCACAGGAGCAGAGGGGTCAGGGCGAAGTCC
CAGGGCCCCAGGCGTGGCTCTCAGAGTCTCAGGCCCCGAAGGCGGTGTATG
(SEQ ID NO :30)

Upstream HLA-B7 (352bp) :
AGGTTTAAAGAGAAAACCCCTGTCTCTACACCTCCATTCCCAGGGCGAGCTCACTCTCTG
GCATCAAGTTCCCCGTGCTCAGTTTCCCTACACAAGAGTCCAAGAGGAGAGGTAAGGAGT
GGGAGGCAGGGAGTCCAGTTCAGGGACAGGGATTCCAGGACGAGAAGTGAAGGGGAAGGG
GCTGGGCGCAGCCTGGGGGTCTCTCCCTGGTTTCCACAGACAGATCCTTGTCCAGGACTC
AGGCAGACAGTGTGACAAAGAGGCTTGGTGTAGGAGAAGAGGGATCAGGACGAAGTCCCA
GGTCCCGGACGGGGCTCTCAGGGTCTCAGGCTCCGAGGGCCGCGTCTGCAAT
(SEQ ID NO :31)

Upstream HLA-B7 (511bp)
GAGTTTAATTGTAATGCTGTTTTGACACAGGTCTTTTACAAATTGGAATTCTAATCATTC
AGGGATTACCAATATTGTGCTACCTACTGTATTAACAAACAAAAAGGAAACTGGTCTCTA
TGAGAATCCCTATGCGGTGCCTTCAGAGAAAACTTCACCAGGTTTAAAGAGAAAACCCCT
GTCTCTACACCTCCATTCCCAGGGCGAGCTCACTCTCTGGCATCAAGTTCCCCGTGCTCA
GTTTCCCTACACAAGAGTCCAAGAGGAGAGGTAAGGAGTGGGAGGCAGGGAGTCCAGTTC
AGGGACAGGGATTCCAGGACGAGAAGTGAAGGGGAAGGGGCTGGGCGCAGCCTGGGGGTC
TCTCCCTGGTTTCCACAGACAGATCCTTGTCCAGGACTCAGGCAGACAGTGTGACAAAGA
GGCTTGGTGTAGGAGAAGAGGGATCAGGACGAAGTCCCAGGTCCCGGACGGGGCTCTCAG
GGTCTCAGGCTCCGAGGGCCGCGTCTGCAAT (SEQ ID NO :32)

Upstream HLA-E (328bp) :
ACTAATTTCTTTTTTCTTGTTGCCCAGGCTGGAGGGCAATGGCACGATCTTGGCTCACCG
CAACCTCCTCCTCCTGGGTTCAAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCCAGGAT
TACAGCCATGCGCCACCACGCCGGCTAATTTTTTGGACTTTTAGTAGAGACAGGGTTTCT
CCATATTGGTCGGGCTGGTCTCGAACTCCCAACCTCAGGTGATCAGCCCGCCTTGGCCTC
CCAAAGTGCTGAGATTACAGGCGTGAGCCACCGCGCCCAGCCAGGACTAATTTCTAAGAG
TGTGCAGAGATACCGAAACCTAAAAGTT (SEQ ID NO :33)

Upstream HLA-E (1047bp) :
TTTTTTCCCCCTAGACATCTCACTCTGTCGCCCAGGCTGGAGTGCAGTGGTGTGATCTCG
GCTCACTGCAACCACCACCTCTCGGGTTCAAGCAATTCTCCTATCTCAGCCTCCAGAGTT
GCTGGAATTACAGGCGCGCACCACCACACCCGGCTAATTTTTGTATTGTTAGTAGAGACA
GGGTTTCATCATGTTGGCCAGGTTAGTCTTGAACTCCTGACCTCGTGATCTGCCTGCCTC
GGCCTACCAAAATGCTGCGATTACAGGCGTGAGCCACCGTTCCCGGCCTATACGTTGTTT
ATTTTGGAAAAATTAAAAATTAAGTTTTTTTTCATTAAAGATATGTTATTTCCGATCAAG
AGATCAAGACCATCCTGGCCAACATGGTGAAACCCCGTCTCTACTAAAAACACAAAAATT
AGCTGGGTGTGGTGGCACACGCCTGTAGTTCCAGTTACTGGGGAGGCTGAGGCAGGAGAA

TCGCTTGAACCCGGGAGAAGGAGGTTGCAGTGAGCCGAGATCATGCCACTGCACTCCAGC
CTGGGGACAGAGCAAGACTCTGACTCAAAAAAAAAAAAAAGTTGTTTCTATTAACATGTAA
TGGGTTATTAATATTCTCTTAAATGAATTAATATTTTTAATATTTTGTTTTAATATCTTT
TAATTTATATATGATAAAAATTGATACAATCCACAGAAACAAAATTTATTTGGGTCCTCA
CTAATTTCTTTTTTCTTGTTGCCCAGGCTGGAGGGCAATGGCACGATCTTGGCTCACCGC
AACCTCCTCCTCCTGGGTTCAAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCCAGGATT
ACAGCCATGCGCCACCACGCCGGCTAATTTTTTGGACTTTTAGTAGAGACAGGGTTTCTC
CATATTGGTCGGGCTGGTCTCGAACTCCCAACCTCAGGTGATCAGCCCGCCTTGGCCTCC
CAAAGTGCTGAGATTACAGGCGTGAGCCACCGCGCCCAGCCAGGACTAATTTCTAAGAGT
GTGCAGAGATACCGAAACCTAAAAGTT (SEQ ID NO :34)

Upstream HLA-DRα (356bp) :
ATACAGCCTTTCATCCTTCTCCAGTGTTGAGAGTGTTGAACCTCAGAGTTTCTCCTCTCA
TTTTCTCTAAATGAGATACAATGCCAGCCATCCCAAGCTCTTGGCCTGAGTTGATCATCT
TGAAGTCTAGGACTCCAAGAAGCATGAAAGAGCTTCTTTAGTGAAGCTATGTCCTCAGTA
CTGCCAAAATTCAGACAATCTCCATGGCCTGACAATTTACCTTCTATTTGGGTAATTTAT
TGTCCCTTACGCAAACTCTCCAACTGTCATTGCACAGACATATGATCTGTATTTAGCTCT
CACTTTAGGTGTTTCCATTGATTCTATTCTCACTAATGTGCTTCAGGTATATCCCT
(SEQ ID NO :35)

Upstream HLA-DRα (522bp) :
TAGGCTTTGCCCATTATACTCTCTCATATTCATTGACCTGAATCCTCAAATGAGGTGTGT
CCATTAGTCAACTCCAATCTCTTGTCATATATAAGATGGTAGAGATGAGAAGAAGGTAGC
TCCTTTACAGCCCACTATTTCCACTAACTACTACCTGTGTTTCAAGATACAGCCTTTCAT
CCTTCTCCAGTGTTGAGAGTGTTGAACCTCAGAGTTTCTCCTCTCATTTTCTCTAAATGA
GATACAATGCCAGCCATCCCAAGCTCTTGGCCTGAGTTGTTCATCTTGAAGTCTAGGACT
CCAAGAAGCATGAAAGAGCTTCTTTAGTGAAGCTATGTCCTCAGTACTGCCAAAATTCAG
ACAATCTCCATGGCCTGACAATTTACCTTCTATTTGGGTAATTTATTGTCCCTTACGCAA
ACTCTCCAGCTGTCATGGCACAGACATATGATCTGTATTTAGCTCTCACTTTAGGTGTTT
CCATTGATTCTATTCTCACTAATGTGCTTCAGGTATATCCCT (SEQ ID NO :36)

In some embodiments, the vectors comprise any of SEQ ID Nos. 37-46.

PRODUCTION OF LENTIVIRAL VECTORS

In one embodiment, the invention encompasses a method comprising inserting an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector. The method can further comprise inserting any of the other nucleic acid elements mentioned herein, such as a DNA flap sequence.

The invention encompasses methods for producing a lentiviral vector comprising inserting at least 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 500, 600, 700, 800, 900, or 1000 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector.

In various embodiments, the upstream promoter sequence comprises less than 1100, 1000, 900, 800, 700, 600, 550, 500, 450, 400, or 350 nucleotides of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence.

The invention encompasses methods for producing a lentiviral vector comprising inserting 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, or 500 to 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 550, 600, 700, 800, 900, 1000, or 1100 nucleotides (in all possible combinations of ranges) of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector. Preferably, the upstream promoter sequence comprises 300-400, 300-500, 300-600, 300-700, or 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

Most preferably, the upstream promoter sequence comprises 300-335 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

Preferably, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence is inserted into a lentiviral vector comprising a MHC class I or β2 microglobulin promoter. The upstream promoter sequence can be in the same or reverse orientation as the promoter.

The invention encompasses methods for producing a lentiviral vector comprising inserting at least 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 500, 600, 700, 800, 900, or 1000 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence and an MHC class I or β2 microglobulin promoter into a lentiviral vector.

In various embodiments, the upstream promoter sequence comprises less than 1100, 1000, 900, 800, 700, 600, 550, 500, 450, 400, or 350 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

The invention encompasses methods for producing a lentiviral vector comprising inserting 300, 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, or 500 to 305, 310, 315, 320, 325, 330, 335, 350, 357, 400, 450, 550, 600, 700, 800, 900, 1000, or 1100 nucleotides (in all possible combinations of ranges) of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence and an MHC class I or β2 microglobulin promoter into a lentiviral vector. Preferably, the upstream promoter sequence comprises 300-400, 300-500, 300-600, 300-700, or 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence. Most preferably, the upstream promoter sequence comprises 300-335 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

In one embodiment, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence is inserted into the lentiviral vector prior to insertion of a MHC class I or β2 microglobulin promoter.

In one embodiment, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence is inserted into the lentiviral vector after insertion of a MHC class I or β2 microglobulin promoter.

In one embodiment, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence and an MHC class I or β2 microglobulin promoter are inserted together into the lentiviral vector.

In one embodiment, a β2 microglobulin upstream promoter sequence is inserted upstream in the same orientation as an MHC class I or β2 microglobulin promoter.

In one embodiment, an MHC class I or MHC Class II upstream promoter sequence is inserted upstream in the same orientation as an MHC class I or β2 microglobulin promoter.

Preferably, the upstream promoter sequence comprises an β2 microglobulin, HLA-A2, HLA-B7, HLA-Cw5, HLA-E, or HLA-G upstream promoter sequence and the promoter is a β2 microglobulin, HLA-A2, HLA-B7, HLA-Cw5, HLA-E, or HLA-G promoter. All combinations individually are considered part of the invention.

Preferably the upstream promoter sequence comprises a nucleotide sequence comprising any of SEQ ID NO:1-SEQ ID NO:7 or SEQ ID NO:27-SEQ ID NO:36.

PRODUCTION OF LENTIVIRAL PARTICLE VECTOR

The present invention provides a method for producing a lentiviral particle vector, which contains an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence. Thus, the invention encompasses a lentiviral particle vector comprising an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence. A lentiviral particle vector (or lentiviral vector particle) comprises a lentiviral vector in association with viral proteins.

The insertion of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence can increase the titer of the vector.

According to one embodiment of this method, the particle vector is obtained in a host cell transformed with a DNA plasmid.

Such a DNA plasmid can comprise:

- bacterial origin of replication (ex: pUC ori);

- antibiotic resistance gene (ex: KanR) for selection; and more particularly:

- a lentiviral vector comprising an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

The invention allows the production of recombinant vector particles, comprising the following steps of:

i) transfecting or transducing a suitable host cell with a lentiviral vector;

ii) transfecting or transducing said host cell with a packaging plasmid vector, containing viral DNA sequences encoding at least structural and integrase proteins of a retrovirus (preferably lentivirus); Such packaging plasmids are described in the art (Dull *et al.*, 1998, *J Virol*, 72(11):8463-71; Zufferey *et al.,* 1998, *J Virol* 72(12):9873-80).

iii) culturing said transfected host cell in order to obtain expression and packaging of said lentiviral vector into lentiviral vector particles; and

iv) harvesting the lentiviral vector particles resulting from the expression and packaging of step iii) in said cultured host cells.

The host cell transfected or transduced with a packaging plasmid may be a stable packaging cell line. Thus, the method can comprise:

i) transfecting or transducing a packaging cell line with a lentiviral vector;

ii) culturing the cell line in order to obtain expression and packaging of said lentiviral vector into lentiviral vector particles; and

iii) harvesting the lentiviral vector particles resulting from the expression and packaging of step ii) in the cultured cell line.

For different reasons, it may be helpful to pseudotype the obtained retroviral particles, i.e. to add or replace specific particle envelope proteins. For instance, this may be advantageous to have different envelope proteins in order to distinguish the recombinant particle from natural particles or from other recombinant particles. In matter of vaccination strategy, pseudotyped particle vectors are more likely to escape the immune system, when a patient has already developed immunity against lentiviruses. This is particularly helpful when successive injections of similar particle vectors are required for immunizing a patient against a disease.

In order to pseudotype the retroviral particles of the invention, the host cell can be further transfected with one or several envelope DNA plasmid(s) encoding viral envelope protein(s), preferably a VSV-G envelope protein.

An appropriate host cell is preferably a human cultured cell line as, for example, a HEK cell line.

The method for producing the vector particle is carried out in a host cell, which genome has been stably transformed with one or more of the following components: a lentiviral vector DNA sequence, the packaging genes, and the envelope gene. Such a DNA sequence may be regarded as being similar to a proviral vector according to the invention, comprising an additional promoter to allow the transcription of the vector sequence and improve the particle production rate.

In a preferred embodiment, the host cell is further modified to be able to produce viral particle in a culture medium in a continuous manner, without the entire cells swelling or dying. One may refer to Strang *et al.*, 2005, *J Virol* 79(3):1165-71; Relander *et al.*, 2005, *Mol Ther* 11(3):452-9; Stewart *et al.*, 2009, *Gene Ther*, 16(6):805-14; and Stuart *et al.,* 2011, *Hum gene Ther* (in press), with respect to such techniques for producing viral particles.

An object of the present invention consists of a host cell transformed with a lentiviral particle vector.

The lentiviral particle vectors can comprise the following elements, as previously defined:

- cPPT/CTS polynucleotide sequence; and
- a transgene sequence under control of a promoter,
- an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence,

and optionally one of the additional elements described above.

METHODS FOR EXPRESSING A TRANSGENE IN A CELL

The present invention encompasses methods for expressing a transgene in a cell, preferably a non-dividing cell. The method comprises transducing a cell with a lentiviral vector or lentiviral particle vector of the invention under conditions that allow the expression of the transgene.

The cells are preferably mammalian cells, particularly human cells. Particularly preferred are human non-dividing cells.

The transgene preferably encodes an immunogenic polypeptide. The method can further comprise harvesting or isolating the polypeptide.

The lentiviral vector or lentiviral particle vector preferably comprises an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence. Preferably the vector further comprises an MHC class I or β2 microglobulin promoter.

In one embodiment, the invention encompasses a method for expressing a transgene comprising inserting an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector and transducing a cell with the vector containing the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

THERAPEUTIC USE OF LENTIVIRAL VECTORS

The present invention further relates to the use of the lentiviral vectors according to the invention, especially in the form of lentiviral particle vectors, for the preparation of therapeutic compositions or vaccines which are capable of inducing or contributing to the occurrence or improvement of an immunogical reaction against epitopes, more particularly those encoded by the transgene present in the vectors containing an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence.

The present invention thus provides vectors that are useful as a medicament or vaccine, particularly for gene therapy.

These vectors are preferentially used for the treatment or prophylaxis of infectious diseases, especially diseases associated with virus infection and more particularly, with retrovirus infection, such as AIDS and other immunodeficiencies.

The invention can also be used in treatment protocols against tumors and cancers and especially could be used in protocols for immunotherapy or vaccination therapy against tumors.

As the vectors of the invention more specifically target dendritic cells to obtain a cell-mediated immune response and especially the CTL response associated with the antigen expressed by the transgene in these cells, they are particularly useful as vaccines targeting slow or endogenous pathogenic microorganisms such as Mycobacteria or HIV virus.

Accordingly, the invention relates to an immunogenic composition comprising a lentiviral vector as previously defined.

The immunogenic compositions of the invention preferably contain cPPT and CTS sequences in the vector and vector particles to induce or to stimulate the nuclear import of the vector genome in the target cells.

During reverse transcription, cPPT and CTS sequences induce the formation of a three stranded DNA structure referred as DNA triplex, which stimulates the nuclear import of DNA vector sequence. Preferably, the vector comprises a transgene and regulatory signals of retrotranscription, expression and encapsidation of retroviral or retroviral-like origin, wherein the composition is capable of inducing or of stimulating a CTL (Cytotoxic T Lymphocytes) or a CD4 response against one or several epitopes encoded by the transgene sequence present in the vector.

The titer of the lentiviral vector is improved by inclusion of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence in the vector.

Thus, the lentiviral vectors according to the invention have the ability to induce, improve, or in general be associated with the occurrence of a memory CTL response. In other words, they can be used for the preparation of therapeutic composition for the treatment of tumor diseases or infectious diseases, by induction of, stimulation of, or participation in the occurrence of a cell-mediated immune response, especially a CTL response or a memory response.

The lentiviral vectors of the invention can be used in methods of treatment and methods of inducing an immune response comprising administering the lentiviral vector to a host and generating a specific immune response against the transgene in the host. The cells and antibodies generated in these hosts can be used as diagnostic reagents.

The lentiviral vectors according to the invention can be directly administered to a patient through known routes of administration, including systemic, local, or cutaneous, intramuscular, intradermal, for instance intratumoral, administration routes. Ex vivo administration, for instance ex vivo transduction of target cells followed by administration of the treated cells to the patient to be treated, is also encompassed by the invention.

In a particular embodiment, the immunogenic composition according to the invention can be directly administered to the patient, in such a way that it will induce, improve, or participate in vivo in the occurrence of a cell-mediated immune response, especially a CTL-mediated immune response.

In another embodiment, the immunogenic compositions are used once or upon repeated administrations so that they can enable the occurrence of a long-term memory cell mediated response.

The immunogenic compositions of the invention can be used to elicit or stimulate a cell-mediated immune response against multiple epitopes encoded by the nucleotides sequences of interest or transgene present in the vector or vector particles, and they can also be used to elicit or stimulate a cell-mediated immune response against the product of the entire sequence of a gene, for instance a gene of a pathogenic agent or fragments of said gene capable to encode at least 8 to 15 amino acids preferably 9 to 12 amino acids.

The invention also encompasses a lentiviral vector comprising a nucleotide sequence encoding a multiple repeat (at least 2 identical sequences) of said amino acid sequence inducing a cellular response and/or an amino acid sequence containing at least 2 different sequences corresponding to 2 epitopes of different pathogens or tumoral antigens.

As a result, the invention encompasses a composition that could be used in prophylactic and/or therapeutic vaccination protocols, for the treatment of tumors and especially as anti-cancer or anti-infectious diseases treatment.

In particular, it can be used in combination with adjuvants, other immunogenic compositions, chemotherapy, or any other therapeutic treatment.

Having thus described different embodiments of the present invention, it should be noted by those skilled in the art that the disclosures herein are exemplary only and that various other alternatives, adaptations, and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein.

EXAMPLES

Example 1. Cell lines

HEK 293T (human embryonic kidney cell line, ATCC CRL-11268, (Graham et al. 1977)) cells were maintained in Dubelcco's modified Eagle's medium (DMEM/ High modified, Hyclone) supplemented with 10% fetal bovine serum (FBS, PAA), 1% L-Glutamine (Eurobio), 1% Penicillin-Streptomycin (Gibco by Life technologies) and 1% Sodium Pyruvate (Gibco by Life technologies).). The cell line was kept in an incubator with humidified atmosphere of 5% CO_2 at 37°C.

Example 2. Plasmids construction

The promoters are cloned between the MluI and BamHI sites of the pFLAP-GFP proviral plasmid.

β2m_Upstream sequence (β2m_US) cloned upstream of the promoters:

HLA-B7 and HLA-E promoters were purchased from GeneArt (Lifetechnologies), and they were designed to encompass the β2m upstream promoter sequence (β2m_US) upstream the 5'end of the original promoter sequence. To generate the HLA-B7 and HLA-E provirus plasmids, PCR reactions were performed to only amplify the wild type promoter sequences, which were cloned between the MluI and BamHI sites of the pFlap-GFP plasmid.

To add the β2m-upstream sequence (β2m_US) in the 5' end of the HLA-A2, HLA-DRα, CMV and UBC promoters, we performed fusion PCR reactions. Briefly, three separate PCR reactions were performed: the first PCR amplify the β2m_US, the second PCR amplify the promoter, including a 25bp overhang homologous to the end of the β2m_US. The PCR 1 and 2 products are then purified in an agarose gel (QIAquick gel extraction kit, QIAGEN) and used as matrices for the third PCR that will generate the final DNA product (β2m_US-Promoter). Primers used for the three PCR of each promoter are resumed in table 1. The PCR 3 product are gel purified and cloned in pCR®2.1-TOPO® (Life Technologies), sequenced, digested by MluI and BamHI restriction enzymes and cloned into the pFlap-GFP.

β2m_Upstream sequence cloned upstream the promoters, in reverse orientation:

The β2m upstream promoter sequence in reverse orientation (β2m_USR) was cloned upstream each promoter using fusion PCR as described above. All the USR_promoters were amplified between AscI and BamHI sites and then cloned into the pflap-GFP using MluI and BamHI sites. Primers used for the three PCR of each promoter are listed in table 2.

β2m_Upstream sequence cloning downstream the GFP in direct orientation:

β2m_US was cloned downstream the GFP reporting gene using XhoI and KpnI restriction sites. First, a PCR was performed to add the XhoI and KpnI sites in 5' and 3' of the β2m_US respectively. Primers used for the PCR are: forward: 5'- CTCGAGGAGAAACCCTGCAGGGAATTC-3' (SEQ ID NO:9), reverse: 5'-GGTACCGAGTCTCGTGATGTTTAAGAAGGCA-3' (SEQ ID NO:10). The PCR products were gel purified, cloned in pCR®2.1-TOPO® (LifeTechnologies), sequenced, digested by XhoI and KpnI restriction enzymes and cloned between the XhoI and KpnI sites of the pFlap-GFP.

β2m_Upstream sequence cloned downstream the GFP in reverse orientation:

The β2m upstream promoter sequence in reverse orientation (β2m_USR) was cloned downstream the GFP reporting gene using XhoI and KpnI restriction sites. First, a PCR was realized to add the XhoI and KpnI sites in 5' and 3' of the β2m_USR respectively. Primers used for the PCR are: forward: 5'-GGTACCGAGAAACCCTGCAGGGAATTCCCCAG-3' (SEQ ID NO:11) reverse: 5'-CTCGAGGAGTCTCGTGATGTTTAAGAAGGCA-3' (SEQ ID NO:12). The PCR product is then gel purified, cloned in pCR®2.1-TOPO® (LifeTechnologies), sequenced, digested by XhoI and KpnI restriction enzymes and cloned between the XhoI and KpnI of the pFlap.

β2m_Upstream sequence cloned between pUCori et KanR:

As the number of restrictions sites present between the pUCori and KanR sequences is restrained, we chose to clone the β2m_US into the PmlI sites. As a

PmlI site is also present in the 5'LTR of the pFlap backbone, we first added the β2m_US upstream the pUCori using fusion PCR and then cloned the whole fragment between the two PmlI sites. Primers used for PCR1 (amplification of β2m_US) are: F1_5'-CACGTGGAGAAACCCTGCAGGGAATTCCCCAG-3' (SEQ ID NO:13) and R1_5'-GAGTCTCGTGATGTTTAAGAAGGCA-3' (SEQ ID NO:14). Primers used for PCR2 (amplification of pUCori and SV40) are: F2_5-**TGCCTTCTTAAACATCACGAGACTC**CTAAAACTTCATTTTTAATTT-3' (SEQ ID NO:15), containing an overhang homologous to the end of β2m_US (in bold) and R2_5'-CACGTGATGAAATGCTAGGCGGCTGTC-3' (SEQ ID NO:16). PCR 1 and 2 products were purified on an agarose gel and used as matrices for the third PCR, and the F1 and R2 primers were used for the amplification. The PCR 3 product are gel purified and cloned in pCR®2.1-TOPO® (Life Technologies), sequenced, digested by PmlI and cloned between the same sites in the pFlap-GFP. Cloning orientation was controlled by enzymatic digestion.

β2m_Upstream sequence cloning between pUCori et KanR, in reverse orientation:

β2m_USR was cloned between the PmlI sites as described above. Primers used for PCR1 (amplification of β2m_USR) are: F1_5'-CACGTGGAGTCTCGTGATGTTTAAGAAGGCATG-3' (SEQ ID NO:17) and R1_5'- GAGAAACCCTGCAGGGAATTCCCCAG -3'(SEQ ID NO:18). Primers used for PCR2 (amplification of pUCori and SV40) are: F2_5-**TGGGGAATTCCCTGCAGGGTTTCTC**CTAAAACTTCATTTTTAATTT-3' (SEQ ID NO:19) containing an overhang homologous to the end of β2m_USR (in bold) and R2_5'- CACGTGATGAAATGCTAGGCGGCTGTC-3' (SEQ ID NO:20). PCR 1 and 2 products were gel purified and used as matrice for the third PCR, and the F1 and R2 primers were used for the amplification. The PCR 3 product are gel purified and cloned in pCR®2.1-TOPO® (LifeTechnologies), sequenced, digested by PmlI and cloned between the same sites in the pFlap-GFP. Cloning orientation was controlled by enzymatic digestion.

The promoters were cloned into the pFlap-GFP plasmid using the MluI and BamHI sites. As β2m and HLA-B7 promoters contain a MluI site in their sequence, an AscI site (compatible with MluI site) is used as replacement, which makes the MluI site disappear.

Short Upstream sequences cloned upstream of the promoters

HLA-A2, HLA-E and HLA-DRα short upstream sequences were purchased from GeneArt and cloned upstream their respective promoters using MluI restriction site. Orientation of the inserted sequences was controlled by sequencing. The HLA-B7 short upstream sequence was added upstream of the HLA-B7 promoter using fusion PCR Primers used for PCR1 (amplification of HLA-B7_US) are: F1_5'- GGCGCGCCCAGGTTTAAAGAGAAAACCCCTG-3' (SEQ ID NO:17) and R1_5'- ATTGCAGACGCGGCCCTCGGAGCCTGAGA-3'(SEQ ID NO:18). Primers used for PCR2 (amplification of HLA-B7 promoter) are: F2_5-**AGGCTCCGAGGGCCGCGTCTGCAAT**GGGGAGGCGCACGTTGGGGATTC-3' (SEQ ID NO:19) containing an overhang homologous to the end of HLA-B7_US (in bold) and R2_5'- CGGAAGGAAAGTGACGGGCGAA -3' (SEQ ID NO:20). PCR 1 and 2 products were gel purified and used as matrice for the third PCR, and the F1 and R2 primers were used for the amplification. The PCR 3 product are gel purified and cloned in pCR®2.1-TOPO® (Life Technologies), sequenced, digested by MluI and BamHI restriction enzymes and cloned into the pFlap-GFP.

Long Upstream sequences cloned upstream of the promoters

HLA-A2, HLA-E and HLA-DRα long upstream sequences were purchased from GeneArt and cloned upstream their respective promoters using MluI restriction site. Orientation of the inserted sequences was controlled by sequencing.

B2m_Up and HLA-B7_Up blocks (promoters + long upstream sequences) were purchased from GeneArt and cloned in the pFlap-GFP using the MluI/BamHI restrictions sites.

Double Upstream Sequences

HLA-E_US was cloned in the pFlap-ΔU3-β2m_E_US-GFP upstream of the GFP gene using XhoI and KpnI restriction sites. First, a PCR was realized to add the XhoI and KpnI sites in 5' and 3' of the HLA-E_US respectively. Primers used for the PCR are: forward: 5'-CTCGAGACTAATTTCTTTTTTCTTGTTGCC-3' and reverse: 5'-GGTACCAACTTTTAGGTTTCGGTATCTCTGCACA-3. The PCR product is then gel purified, cloned in pCR®2.1-TOPO® (Life Technologies), sequenced, digested by XhoI and KpnI restriction enzymes and cloned between the same sites in the pFlap-ΔU3-β2m_E_US-GFP, allowing the obtaining of the pFlap-ΔU3-β2m_E_US-GFP_E_US.

Example 3. Lentiviral production

The lentiviral vectors were produced by transient transfection of HEK 293T cells using a standard calcium phosphate precipitation protocol. HEK 293T cells were seeded at $7x10^6$ cells in $10cm^2$ Tissue Culture Dish (BD Falcon) in 10mL of complete culture medium and maintained 24h in an incubator with humidified atmosphere of 5% CO_2 at 37°C to adhere. For each vector produced, one tissue culture dish is transfected as following: the lentiviral backbone plasmid pFlap (10µg), the pThV-Env1 encoding envelope plasmid (2µg), and the pThV-GP packaging plasmid (10µg) were mixed with 353µL of sterile distilled water (Gibco by Life Technologies) and 125µL of $CaCl_2$ (Fluka). The DNA mix is then added drop to drop to 500µL of 37°C prewarmed HBS 2X pH=7,3 and the 1mL of precipitate obtained was added to the culture medium of the cells. The transfected cells were then incubated at 37°C, 5% CO_2. The medium was replaced 24h after transfection by 7mL of harvest medium without serum and the viral supernatant was harvested after an additional 24h, clarified by centrifugation 5min. at 2500rpm and stored à -20°C.

Example 4. Quantification of lentiviral vectors by Flow cytometry

For the quantification of infective particles, HEK 293T cells were seeded in 24-well plates (BD Falcon) at a density of 1×10^5 cells per well in complete medium containing 10% FBS and incubated for 4 h to adhere. The cells were transduced by replacing the medium with 300 µl of dilutions 1/100, 1/300 and 1/900 of viral samples in complete medium, followed by incubation at 37 °C, 5%CO_2 for 2h. After adsorption, 1mL of complete medium was added to each well. At 72h posttransduction, the cells were trypsinized and resuspended in 300µL of complete medium, and the percentage of cells expressing GFP was determined with a FACScalibur flow cytometer (BD Biosciences), using the FL1 channel. Two sets of three dilutions were performed for each sample tested. The values corresponding to a percentage of transduced cells less than 30% were used to calculate the approximate number of transducing units (TU) present in the viral suspension.

$$Titer(TU/mL) = \frac{(\%transduced\,cells \times 1.10^5)}{100} \times \frac{1000 \times dilution\ \ factor}{300}$$

Example 5. Quantification of total produced particles by ELISA p24

The quantification of total particles was performed on dilutions 10^{-5}, 10^{-6} and 10^{-7} of each production supernatant, using a commercial kit (Perkin Elmer), following the manufacturer's recommendations.

Example 6: Quantification of efficient produced particles by qPCR

HEK 293T cells were seeded in 6-well plates (BD Falcon) in culture medium and incubated for 4 h at 37°C, 5% CO2 in moist atmosphere. Cells were transduced with 3 successive dilutions of lentiviral vector (1/5, 1/10 and 1/20). 72h post-incubation, cells were harvested and transduced HEK 293T cell pellets were realized. After intermediate storage at -20°C, total genomic DNA from transduced cell-pellets was extracted using a method based on QIAGEN QIAamp DNA mini kit handbook using single columns and a microcentrifuge. Extracted DNA was stored at -20°C till used in qPCR. Quantification of the proviral DNA integrated in the host genome was performed on extracted DNA using an optimized Taqman qPCR, based on the exonuclease activity of the 5'-3' Taq polymerase.

The probe is an oligonucleotide specific to the backbone of our lentiviral vector' sequence. The amplification is performed with a polymerase Master Mix (Fermentas Thermo Scientific) and using Flap A primer (CCCAAGAACCCAAGGAACA) (SEQ ID NO:21), Flap S primer (AGACAA GATAGAGGAAGAGCAAAAC) (SEQ ID NO:22), and Lenti TM probe (6FAM-AACCATTAGGAGTAGCACCCACCAAGG -BBQ) (SEQ ID NO:23). In order to normalize the number of integrations to the number of cells harvested a specific amplification of cellular ACTIN gene is applied in parallel using the same Master Mix and Actine A primer (CGGTGAGGATCTTCATGAGGTAGT) (SEQ ID NO:24), Actine S primer (AACACCCCAGCCATGTACGT) (SEQ ID NO:25) and Humura ACT TM probe (6FAM- CCAGCCAGGTCCAGACGCAGGA -BBQ) (SEQ ID NO:26). Both reactions are achieved on MasterCycler Ep Realplex S (Eppendorf) following the thermal program (2 min at 50°C, 10 min at 95°C and 40 cycles of 15 seconds at 95°C and 1 min at 63°C). The analysis is performed on MasterCycler Ep Realplex Software.

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CLAIMS

The claims defining the invention are:

1. A lentiviral expression vector comprising:

(i) 300-1100 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence, and

(ii) a transgene sequence under control of an MHC class I or β2 microglobulin promoter,

wherein the MHC class I upstream promoter sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33, and

wherein the upstream promoter sequence is in the same orientation as the MHC class I or β2 microglobulin promoter,

the upstream promoter sequence being inserted inside of the LTR-LTR region of the lentiviral vector.

2. The lentiviral vector of claim 1, comprising 300-600 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence.

3. The lentiviral vector of claim 1, comprising 300-400 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence.

4. The lentiviral vector of claim 1, wherein the upstream promoter sequence is a β2 microglobulin upstream promoter sequence.

5. The lentiviral vector of claim 4, wherein the upstream promoter sequence comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:28.

6. The lentiviral vector of claim 1, wherein the promoter is an MHC class I promoter.

7. The lentiviral vector of claim 1, wherein the promoter is a β2 microglobulin promoter.

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8. An isolated host cell comprising the lentiviral vector of claim 1.

9. A method for producing the lentiviral expression vector of claim 1 comprising inserting 300-1100 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence into a lentiviral vector, wherein the MHC class I upstream promoter sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33, and wherein the upstream promoter sequence is in the same orientation as the MHC class I or β2 microglobulin promoter, and is inserted inside of the LTR-LTR region of the lentiviral vector, and wherein the lentiviral vector comprises a transgene sequence under control of an MHC class I or β2 microglobulin promoter.

10. The method of claim 9, comprising inserting 300-600 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence into a lentiviral vector.

11. The method of claim 9, comprising inserting 300-400 nucleotides of an MHC class I or β2 microglobulin upstream promoter sequence into a lentiviral vector.

12. The method of claim 9, wherein the upstream promoter sequence is a β2 microglobulin upstream promoter sequence.

13. The method of claim 12, wherein the upstream promoter sequence comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:28.

14. The method of claim 9, wherein the promoter is an MHC class I promoter.

15. The method of claim 9, wherein the promoter is a β2 microglobulin promoter.

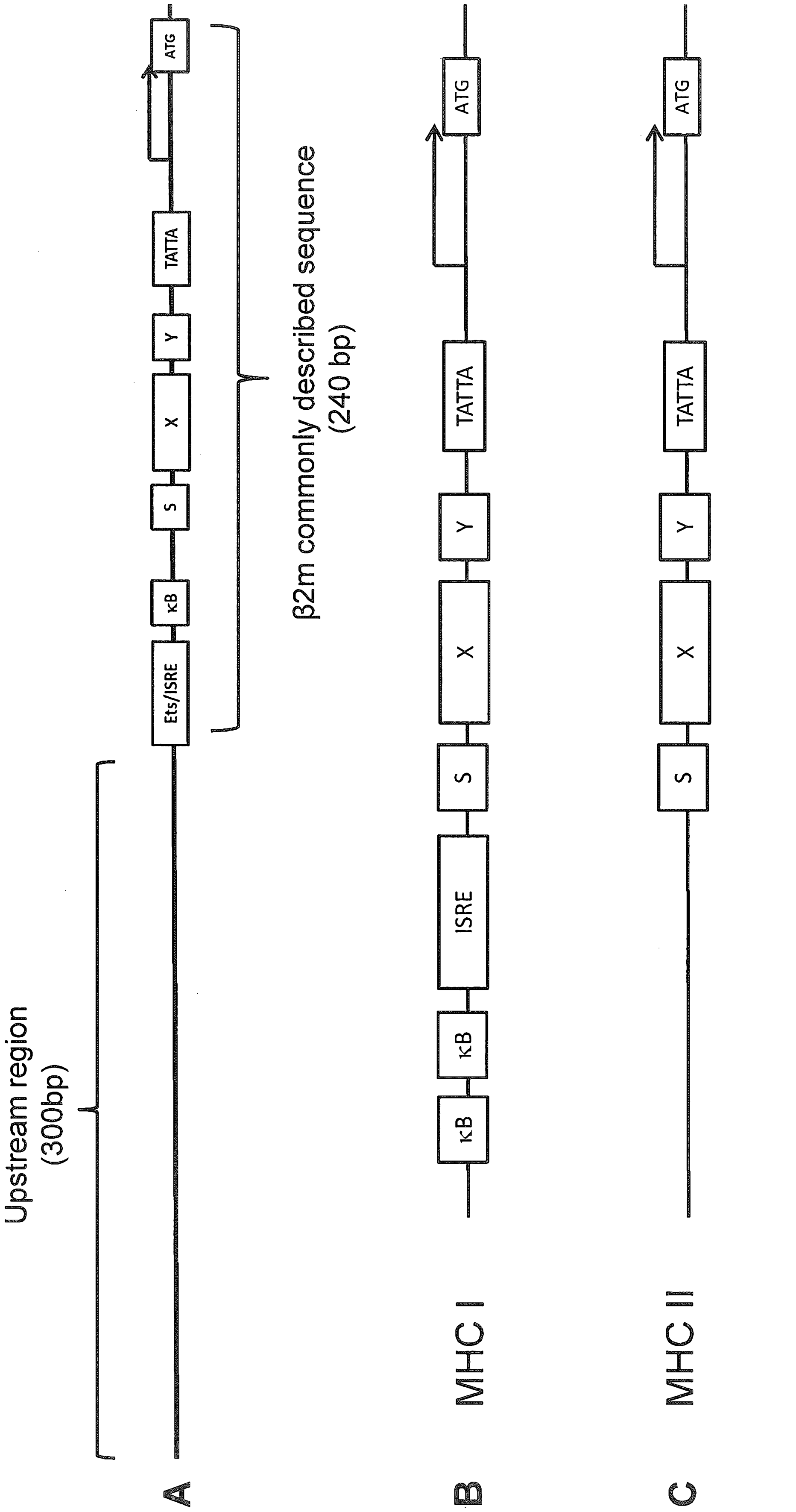
A
Upstream region
(300bp)
β2m commonly described sequence
(240 bp)
Ets/ISRE
κB
S
X
Y
TATTA
ATG
B
MHC I
κB
κB
ISRE
S
X
Y
TATTA
ATG
C
MHC II
S
X
Y
TATTA
ATG

Figure 1

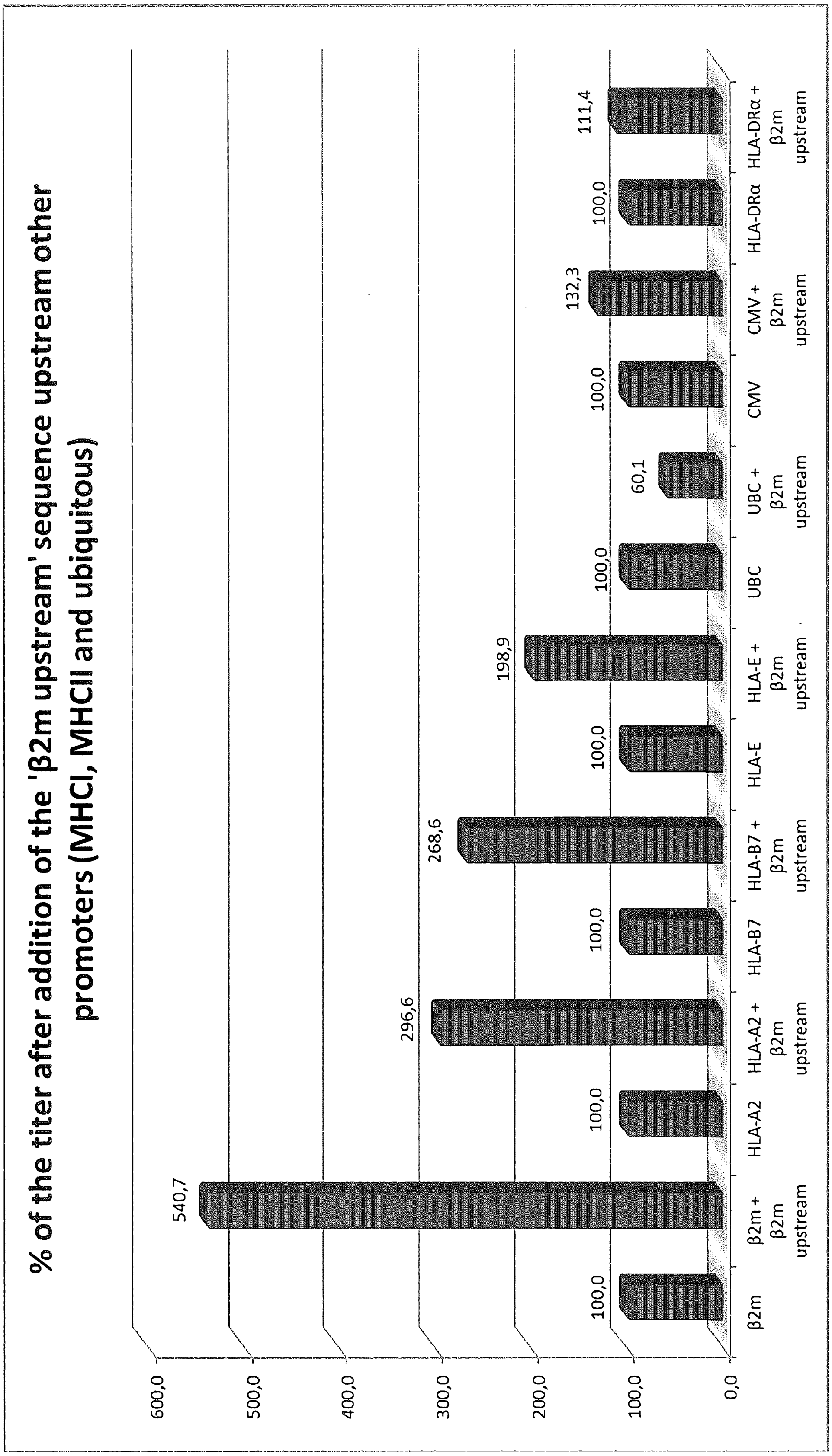
% of the titer after addition of the 'β2m upstream' sequence upstream other promoters (MHCI, MHCII and ubiquitous)
600,0
500,0
400,0
300,0
200,0
100,0
0,0
100,0
540,7
100,0
296,6
100,0
268,6
100,0
198,9
100,0
60,1
100,0
132,3
100,0
111,4
β2m
β2m + β2m upstream
HLA-A2
HLA-A2 + β2m upstream
HLA-B7
HLA-B7 + β2m upstream
HLA-E
HLA-E + β2m upstream
UBC
UBC + β2m upstream
CMV
CMV + β2m upstream
HLA-DRα
HLA-DRα + β2m upstream

Figure 2

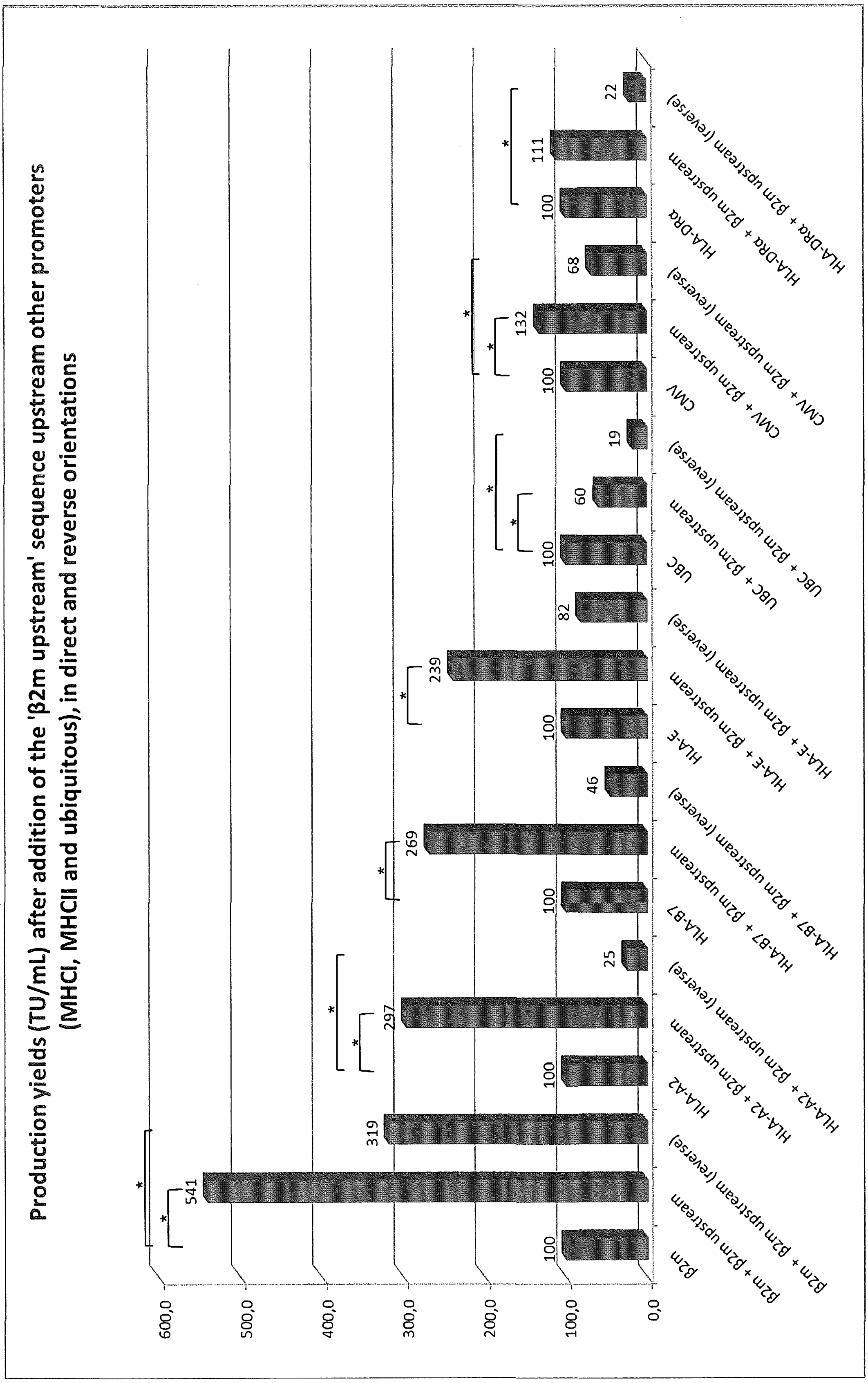
Production yields (TU/mL) after addition of the 'β2m upstream' sequence upstream other promoters (MHCI, MHCII and ubiquitous), in direct and reverse orientations
600,0
500,0
400,0
300,0
200,0
100,0
0,0
100
541
319
100
297
25
100
269
46
100
239
82
100
60
19
100
132
68
100
111
22
*
β2m
β2m + β2m upstream
β2m + β2m upstream (reverse)
HLA-A2
HLA-A2 + β2m upstream
HLA-A2 + β2m upstream (reverse)
HLA-B7
HLA-B7 + β2m upstream
HLA-B7 + β2m upstream (reverse)
HLA-E
HLA-E + β2m upstream
HLA-E + β2m upstream (reverse)
UBC
UBC + β2m upstream
UBC + β2m upstream (reverse)
CMV
CMV + β2m upstream
CMV + β2m upstream (reverse)
HLA-DRα
HLA-DRα + β2m upstream
HLA-DRα + β2m upstream (reverse)

Figure 3

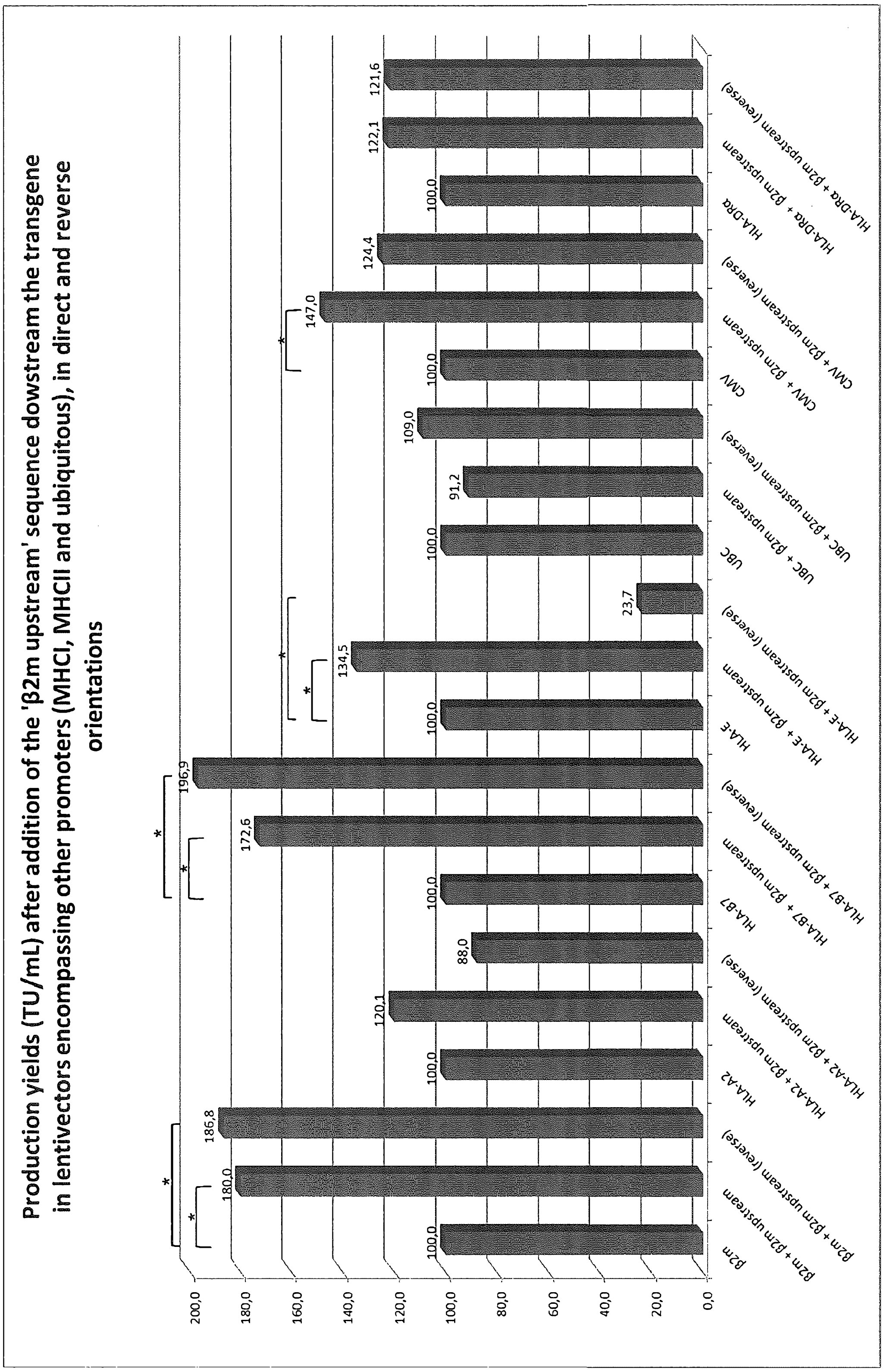
Production yields (TU/mL) after addition of the 'β2m upstream' sequence dowstream the transgene in lentivectors encompassing other promoters (MHCI, MHCII and ubiquitous), in direct and reverse orientations
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
100,0
180,0
186,8
100,0
120,1
88,0
100,0
172,6
196,9
100,0
134,5
23,7
100,0
91,2
109,0
100,0
147,0
124,4
100,0
122,1
121,6
*
*
*
*
*
*
*
β2m
β2m + β2m upstream
β2m + β2m upstream (reverse)
HLA-A2
HLA-A2 + β2m upstream
HLA-A2 + β2m upstream (reverse)
HLA-B7
HLA-B7 + β2m upstream
HLA-B7 + β2m upstream (reverse)
HLA-E
HLA-E + β2m upstream
HLA-E + β2m upstream (reverse)
UBC
UBC + β2m upstream
UBC + β2m upstream (reverse)
CMV
CMV + β2m upstream
CMV + β2m upstream (reverse)
HLA-DRα
HLA-DRα + β2m upstream
HLA-DRα + β2m upstream (reverse)

Figure 4

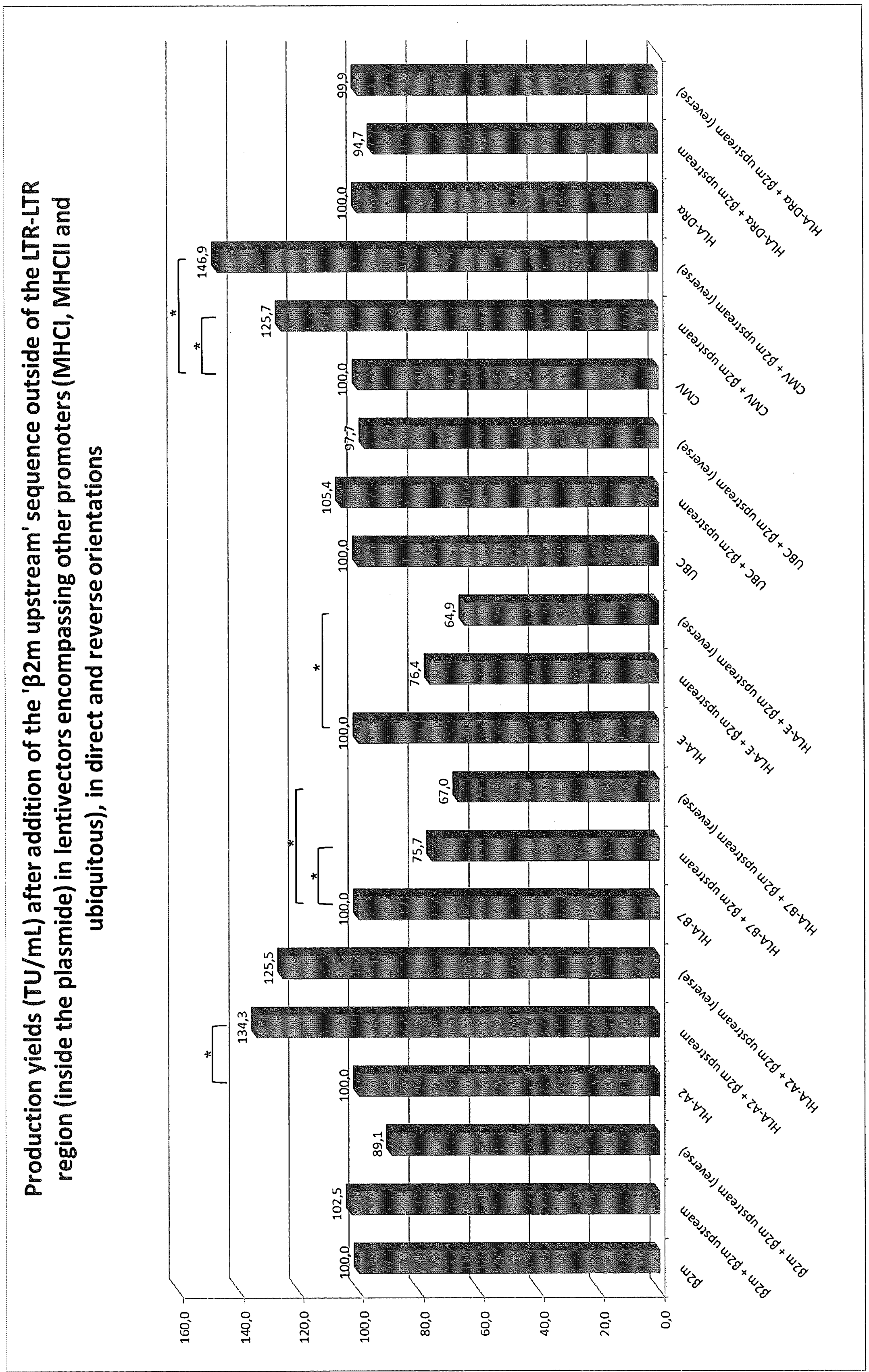
Production yields (TU/mL) after addition of the 'β2m upstream' sequence outside of the LTR-LTR region (inside the plasmide) in lentivectors encompassing other promoters (MHCI, MHCII and ubiquitous), in direct and reverse orientations
0,0
20,0
40,0
60,0
80,0
100,0
120,0
140,0
160,0
100,0
102,5
89,1
100,0
134,3
125,5
100,0
75,7
67,0
100,0
76,4
64,9
100,0
105,4
97,7
100,0
125,7
146,9
100,0
94,7
99,9
*
β2m
β2m + β2m upstream
β2m + β2m upstream (reverse)
HLA-A2
HLA-A2 + β2m upstream
HLA-A2 + β2m upstream (reverse)
HLA-B7
HLA-B7 + β2m upstream
HLA-B7 + β2m upstream (reverse)
HLA-E
HLA-E + β2m upstream
HLA-E + β2m upstream (reverse)
UBC
UBC + β2m upstream
UBC + β2m upstream (reverse)
CMV
CMV + β2m upstream
CMV + β2m upstream (reverse)
HLA-DRα
HLA-DRα + β2m upstream
HLA-DRα + β2m upstream (reverse)

Figure 5

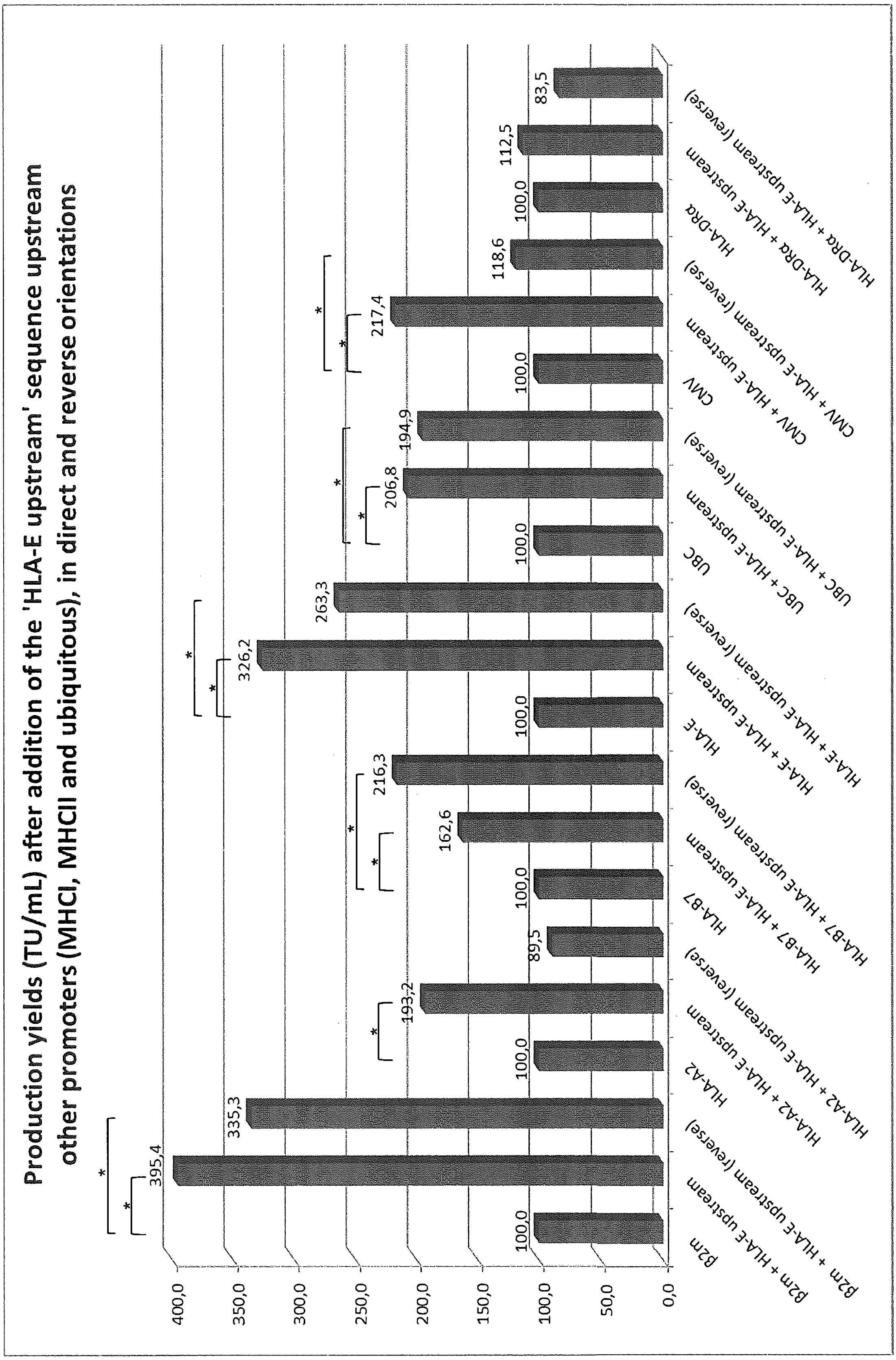
Production yields (TU/mL) after addition of the 'HLA-E upstream' sequence upstream other promoters (MHCI, MHCII and ubiquitous), in direct and reverse orientations
400,0
350,0
300,0
250,0
200,0
150,0
100,0
50,0
0,0
100,0
395,4
335,3
100,0
193,2
89,5
100,0
162,6
216,3
100,0
326,2
263,3
100,0
206,8
194,9
100,0
217,4
118,6
100,0
112,5
83,5
*
β2m
β2m + HLA-E upstream
β2m + HLA-E upstream (reverse)
HLA-A2
HLA-A2 + HLA-E upstream
HLA-A2 + HLA-E upstream (reverse)
HLA-B7
HLA-B7 + HLA-E upstream
HLA-B7 + HLA-E upstream (reverse)
HLA-E
HLA-E + HLA-E upstream
HLA-E + HLA-E upstream (reverse)
UBC
UBC + HLA-E upstream
UBC + HLA-E upstream (reverse)
CMV
CMV + HLA-E upstream
CMV + HLA-E upstream (reverse)
HLA-DRα
HLA-DRα + HLA-E upstream
HLA-DRα + HLA-E upstream (reverse)

Figure 6

```
                          1                                                                                                  100
   Upstream B2m      (1) AGAAGTTCTCCTTCTGCTAGGTAGCATTCAAAGATCTTAATCTTCTGGGTTTCCGTTTTCTCGAATGAAAAATGCAGGTCCGAGCAGTTAACTGGCGGGG
 Upstream HLA-E      (1) ------------------------CTGGAGGGCAATGGCACGATCTTGGCTCACCGCAACCTCCTCCTCCTGGGTTCAAGTGATTCTCCTGC-CTCAGCC
Upstream HLA-B7      (1) -------------------ACTCTCTGGCATCAAGTTCCCCGTGCTCAGTTTCCCTACACAAGAGTCCAAG-AGGAGAGGTAAGGAGTGGGA-GGCAGGG
 Upstream HLA-C      (1) ---------CAAGGGGAGAGGTAAGTGTCCTTTATTTTGCTGGATGTAGTTTAATATTACCTGAGGT---A-AGGTAAGGCAAAGAGTGGGA-GGCAGGG
 Upstream HLA-F      (1) ----------------TTCATTGTCTGGCACCAAGCTCCTTGGGGTGAATTTTCTTCCAAAAGAGTCCGGGGAGTCCAGGTATGGAATGGGA-GGCAGAA
 Upstream HLA-G      (1) ---------------GCTCACTCTCTGGCAACAAGCTCCCTGGGGTGATTTTTCTTCTAGAAGAGTACAGG-AGGACAGGCAAGGAGTGGGA-GGCAGGG
Upstream HLA-A2      (1) ---------------GCTTACTCTCTGGCACCAAACTCCATGGGATGATTTTTCTTCTAGAAGAGTCCAGG-TGGACAGGTAAGGAGTGGGA-GTCAGGG
      Consensus      (1)                G T A T TCTGGCA CAA CTCC TGGG TGAGTTT CTTCTACAAGAGTCCA G AGGACAGGTAAGGAGTGGGA GGCAGGG

                          101                                                                                                200
   Upstream B2m    (101) GCACCATTAGCAAGTCACTTAGCATCTCTGGGGCCAGTCTGCAAAGCGAGGGGGCAG---CCTTAATGTGCCTCCAGCCTGAAGTCCTAGAATGAGCGCC
 Upstream HLA-E     (76) TCCCAAGTAGCCAGGATTACAGCCATGCGCCACCACGCCGGCTAATTTTTTGGACTTTTAGTAGAGACAGGGTTTCTCCATA--TTGGTCGGGCTGGTCT
Upstream HLA-B7     (80) AGTCCAGTT-C-AGGG--ACAGGGATTCCAGGACGAGAAGTGAAGGGGAAGGGGCTGGGCGCAGCCTGGGGGTCTCTCCCTGGTTTCCACAGACAG--AT
 Upstream HLA-C     (87) AGTCCAGTT-C-AGGG--ACGGGGATTCCAGGA---GAAGTGAAGGGGAAGGGGCTGGGCGCAGCCTGGGGGTCTCTCCCTGGTTTCCACAGACAG--AT
 Upstream HLA-F     (84) AGTTCAAT--CAAGGG--ACTGGGATTTCGGAATGAATAATGAAGGGAGATGGACTGGGTCCATGCCGAAGGTTTCTCCCTGGTTTCT-CAGCC------
 Upstream HLA-G     (84) AGTCCAGTT-C-AGGG--ACAGGGATTCCGGGATGAAAAGTGAAGGGAGAGGGCCAGGGACCTTGCCGAGGGTTTCTCCCTGGTTTCT-CAGACAG--CT
Upstream HLA-A2     (84) AGTCCAGTT-CCAGGG--ACAGAGATTACGGGATAAAAAGTGAAAGGAGAGGGACGGGGCCCATGCCGAGGGTTTCTCCCTTGTTTCT-CAGACAG--CT
      Consensus    (101) AGTCCAGTT C AGGG  ACAGGGATTCCGGGA  AGAAGTGAAGGG  AGGG CTGGG CCAT C GAGGGTTTCTCCCTGGTTTCT CAGACAG  CT

                          201                                                                                                300
   Upstream B2m    (198) CGGTGTCCCAAGCTGGGGCGCGCACCC---CAGATC---GGAGGGCGCCGA-TGTACAGA---CAGCAAACTCACCCAGT--CTAGTGCATGCCTTCTTA
 Upstream HLA-E    (174) CGAACTCCCAACCTCAGGTGATCAGCCCGCCTTGGCCTCCCAAAGTGCTGAGATTACAGGCGTGAGCCACCGCGCCCAG---C-CAGGACTAATTTCTAA
Upstream HLA-B7    (174) CCTTGTCCAGGACTCAGGCAGACAGTGTGACAAAGA-G-GCTTGGTGTAGG-AGAAGAGGGATCAGGACGAAGTCCCAGGTCC-CGGACGG-GGCTCTCA
 Upstream HLA-C    (178) CCTTGGCCAGGACTCAGGCACACAGTGTGACAAAGA-T-GCTTGGTGTAGG-AGAAGAGGGATCAGGACGAAGTCCCAGGTCC-CGGGCGG-GGTTCTCA
 Upstream HLA-F    (173) CCCGGGCGAAGACTCAGGGAGACATTGAGACACACCCT-GCACAGGAGGGGGAGGGGGAGGGGGAGGGCAAAGTCCCAGGGCCCCAGGAGT-GGCTCTCA
 Upstream HLA-G    (177) CCTGGGCCAAGACTCAGGGAGACACTGAGACAGAAC---GCTTGGCACAAG-AGTAGCGGGGTCAGGGCGAAGTCCCAGGGCCTCAAGCGT-GGCTCTCA
Upstream HLA-A2    (178) CTTGGGCCAAGACTCAGGGAGACATTGAGACAGAGC---GCTTGGCACAGA-AGCAGAGGGGTCAGGGCGAAGTCC-AGGGCCCCAGGCGTTGGCTCTCA
      Consensus    (201) CCT GGCCAAGACTCAGG AGACA TG GACA AGC   GCTTGG GCAGG AG AGAGGGGTCAGG CGAAGTCCCAGG CC CAGGCGT GGCTCTCA

                          301                             335
   Upstream B2m    (286) AACA-TCACGAGACTC-------------------  (SEQ ID NO:1)
 Upstream HLA-E    (270) GAGTGTGCAGAGATACCGAAACCTAAAAGTT----  (SEQ ID NO:2)
Upstream HLA-B7    (269) GGGTCTC---AGGCTCCGAGGGCCGCGTCTGCAAT  (SEQ ID NO:3)
 Upstream HLA-C    (273) GGGTCTC---AGGCTCCAAGGGCCGTGTCTG----  (SEQ ID NO:4)
 Upstream HLA-F    (271) AGGGCTC---AGGCCCCG-AGGCGGTGTCTGGGG-  (SEQ ID NO:5)
 Upstream HLA-G    (272) GGGTCTC---AGGCCCCACAGGCGGTGTATGG---  (SEQ ID NO:6)
Upstream HLA-A2    (273) GGGTCTC---AGGCCCCGAAGGCGGTGTATG----  (SEQ ID NO:7)
      Consensus    (301) GGGTCTC   AGGC CCGAAGGC GTGT TG      (SEQ ID NO:8)
```

Figure 7

```
HLA-A2+Short     -------------------------------------------------------------------------------------------TACACCTCCATTCCCAGAGCAAGCT-
HLA-B7+Short     ----------------------------------------------------------------------CAGGTTTAAAGAGAAAACCCCTGTCTCTACACCTCCATTCCCAGGGCGAGCT-
HLA-E+short      -----------------------------------------------------------------------------------------ACTAATTTCTTTTTTCTTGTTGCCCAGGCTGGA-

B2m+Short        --------------------------------------------------------------------------------GAGAAACCCTGCAGGGAATTCCCCAGCTGTAGTTATAAACAGAA-

HLA-DRa+Short    ATACAGCCTTTCATCCTTCTCCAGTGTTGAGAGTGTTGAACCTCAGAGTTTCTCCTCTCATTTTCTCTAAATGAGATACAATGCCAGCCATCCCAAGCTCTTGGCCTGAGTTGATCATCT


TACTCTCTGGCACCAAACTCCATGGGATGATTTTTCTTCTAGAAGAGTCCAGGTGGACAGGTAAGGAGTGGGAG-TCAGGGAGTCCAGTTCCAGGGACAGAGATTACGGGATAAAAAGTGAAAGGAGAGGGACGGGGCC
CACTCTCTGGCATCAAGTTCCCCGTGCTCAGTTTCCCTACACAAGAGTCCAAGAGGAGAGGTAAGGAGTGGGAG-GCAGGGAGTCCAGTTC-AGGGACAGGGATTCCAGGACGAGAAGTGAAGGGGAAGGGGCTGGGCG
--GGGCAATGGCA-CGATCTTGGCTCACCGCAACCTCCTCCTCCTGGGTTCAAGTGATTCTCCTGCCTCAGCCTC-CCAAGTAGCCAGGATT-ACAGCCATGCGCCAC-CACGCCGGCTAATTTTTTGGACTTTTAGTA

GTTCTCCTTCTGCTAGGTAGCATTCAAAGATCTTAATCTTCTGGGTTTCCGTTTTCTCGAATGAAAAATGCAGGTCCGAGCAGTTAACTGGCGGGGGCACCATTAGCAAGTCACTTAGCATCTCTGGGGCCAGTCTGCA

TGAAGTCTAGGACTCCAAGAAGCATGAAAGAGCTTCTTTAGTGAAGCTATGTCCTCAGTACTGCCAAAATTCAGACAATCTCCATGGCCTGACAATTTACCTTCTATTTGGGTAATTTATTGTCCCTTACGCAAACTCT


CATGCCGAG---GGTTTCTCCCTTGTTTCT-CAGACAGCTCTTGG--GCCAAGACT-CAGGGAGACATTGAGACAGAGCG-CTTGGCACAGAAG---CAGAGGGGTCAGGGCGAAGTCCAGGGCCCCAGGCGTT-GGCT
CAGCCTGGG---GGTCTCTCCCTGGTTTCCACAGACAGATCCTTG--TCCAGGACT-CAGGCAGACAGTGTGACAAAGAGGCTTGGTGTAGGAG---AAGAGGGATCAGGACGAAGTCCCAGGTCCCGGACGG--GGCT
GAGAC--AG---GGTTTCTCCATA--TTGGTCGGGCTGGTCTCGAACTCCCAACCT-CAGGTGATCAGCCCGCCTTGGCCTCCCA----AAGTGCTGAGATTACAGGCGTGAGCCACCGCGCCCAGCCAGGACTAATTT

AAGCGAGGG---GGCAGCCTTAATGTGCCTCCAGCCTGAAGTCCTAGAATGAGCGC-CCGGTGTCC--CAAGCTGGGGCG-CGCACCCCAGATC---GGAGGGCGCCGATGTACAGACAGCAAACTCACCCAG---TCT

CCAACTGTCATTGCACAGACATATGATCTGTATTTAGCTCTCACTTTAGGTGTTTCCATTGATTCTATTCTCACTAATGTGCTTCAGGTATATCCCTGTCTAGAAGTCAGATTGGGGTTAAAGAGTCTGTCCGTGATTG

                                     κB2             κB1                  ISRE                  S                         X1  X2
CTCAGGGTCTCAGGCCCCGAAGGCGGTGTATGGATTGGGGAGTCCCAGCCTTGGGGATTCCCC----AACTCCGCAGTTTCTTTTCTCCCTCTCCCAACCTA-TGTAGGGTCCTTCTTCCTGGATACTCACGACGCGGAC
CTCAGGGTCTCAGGCTCCGAGGGCCGCGTCTGCAATGGGGAGGCGCAGCGTTGGGGATTCCCC---ACTCCCCTGAGTTTCACTTCT---TCTCCCAACTTG-TGTCGGGTCCTTCTTCCAGGATACTCGTGACGCGTCC
CTAAGAGTGTGCAGAGATACCGAAACCTAAAAGTTTAAGAACTGCTGATTGCTGGGAAACTCT----------GCAGTTTCCCGTTC--CTCTCGTAACCTGGTCATGTGTCCTTCTTCCTGGATACTCATGACGCAGAC

-----AGTGCATGCCTTCTTAAA--------CATCACGAGACTCTAAGAAAAGGAAACTG------AAAACGGGAAAGT------CCCTCTCTC-TAACCTGGCACTGCGTCGCTGG-CTTGGAGACAGGTGACGGTCCC
                                  E box         Ets/ISRE                 κB
ACTAACAGTCTTAAATACTTGATTTGTTGTTGTTGTTGTCCTGTTTGTTTAAGAACTTTACTTCTTTATCCAATGAACGGAGTATCTTGTGTCCTGGACCCTTTGCAAGAACCCTTCCCCTAGCAACAGATGCGTCA-TC
```

Figure 8A

```
               Y
CCAGTTCTCACTCCC-ATTGGGTGTCGGGTTTCCAGAGAAGCCAATCAGTGTCGTCGCGG-TCGCGGTTCTAAAGTCCGCACGCACCCACCGGGACTCA-GATTCTCCCCAGACGCCGAGG SEQ ID NO:37
CCACTTCCCACTCCC-ATTGGGTATTGGATATCTAGAGAAGCCAATCAGCGTCGCCGCGG-TCCCAGTTCTAAAGTCCCCACGCACCCACCCGGACTCA-GAG                   SEQ ID NO:38
TCAGTTCTCATTCCC-AATGGGTGTCGGGTTTCTAGAGAAGCCAATCAGCGTCGCCACGACTCCCGACTATAAAGTCC--------CCATCCGGACTCAAGAAGTTCTCAGGACTCAGAGG SEQ ID NO:39

TGCGGGCCTTGTCCTGATTGGCTGGGCACGCGTTTAATATAAGTGGAGGCGTCGCGCTGGCGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAG                          SEQ ID NO:40

TCAAAATATTTTTCTGATTGGCCAAAGAGTAATTGATTTGCATTTTAATGGTCAGACTCTATTACACCCCACATTCTCTTTTCTTTTATTCTTGTCTGTTCTGCCTCACTCCCGAGCT    SEQ ID NO:41
```

Figure 8B

```
HLA-A2+Long     ----------------------------------------------------------------------------------------------------
HLA-B7+Long     ----------------------------------------------------------------------------------------------------
HLA-E+Long      TTTTTTCCCCCTAGACATCTCACTCTGTCGCCCAGGCTGGAGTGCAGTGGTGTGATCTCGGCTCACTGCAACCACCACCTCTCGGGTTCAAGCAATTCTCCTATCTCAGCC

B2m+Long        CTTCCAAGATCTCTGCCCCTCCCCATCGCCATGGTCCACTTCCTCTTCTCACTGTTCCTCTT-AGAAAAGATCTGTGGACTCCACCACCACGAAATGGCGGCACCTTATTT

HLA-DRa+Long    ----------------------------------------------------------------------------------------------------


----------------------------------------------------------------------------------------------------
----------------------------------------------------------------------------------------------------
TCCAGAGTTGCTGGAATTACAGGCGCGCACCACCACACCCGGCTAATTTTTGTATTGTTAGTAGAGACAGGGTTTCATCATGTTGGCCAGGTTAGTCTTGAACTCCTGACCTCGTGATCTGCCTGCCTC

ATGGTCACTTTAGAGGGTAGGTTTTCTTAATGGGTCTGCCTGTCATGTTTAACGTCCTTGGCTGGGTCCAAGGCAGATGCAGTCCAAACTCTCACTAAAATTGCCGAGCCCTTTGTCTTCCAGTGTCTA

----------------------------------------------------------------------------------------------------


----------------------------------------------------------------------------------------------------
----------------------------------------------------------------------------------------------------
G-GCCTACCAAAATGCTGCGATTACAGGCGTGAGCCACCGTTCCCGGCCTATACGTTGTTTATTTTGGAAAAATTAAAAATTAAGTTTTTTTCATTAAAGATATGTTATTTCCGATCAAGAGATCAAG

AAATATTAATGTCAATGGAATCAGGCCAGAGTTTGAATTCTAGTCTCTTAGCCTTTGTTTC-CCCTGTCCATAAAATGAATGGGGGTAATTCTTTCCTCCTACAGTTTATTTATATATTCACTAATTC

----------------------------------------------------------------------------------------------------


----------------------------------------------------------------------------------------------------
----------------------------------------------------------------------------------------------------
ACCATCCTGGCCAACATGGTGAAACCCCGTCTCTACTAAAAACACAAAAATTAGCTGGGTGTGGTGGCACACGCCTGTAGTTCCAGTTACTGGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGAG

ATTCATT-CATCCATCCATTCGTTCATTCGGTTTACTGAGTACCTACTATGTGCCAGCCCCTGTTCTAGGGTGGAAACTAAGAGAATGATGTACCTAGAGGGCGCTGG-AAGCTCTAAAGCCCTAGCAG

----------------------------------------------------------------TAGGCTTTGCCCATTATACTCTCTCATATTCATTGA
```

Figure 9A

```
--------------GAGTCCTGTTGTAATGCTTTTGGACACATTTATACATTAAGG--GGCCAAAGT-CACATTTTTTACCTATTAGATTCCTGATCATTCAG-GGGTTACCAAGATTCTGCTACCCAC
--------------------------------------GAGTTTAATTGTAATGC--TGTTTTGACACAGGTCTTTTACAAATTGGAATTCTAATCATTCAG-GGATTACCAATATTGTGCTACCTAC
AAGGAGGTTGCAGTGAGCCGAGATCATGCCACTGCACTCCAGCCTGGGGACAGAGCAAGACTCTGACTCAAAAAAAAAAAAAAGTTGTTTCTATTAACATGTAATGGGTTATTAATATTCTCTTAAATGA

TTACTGCTTTTACTATTAGTGGTCGTTTTTTTCTCCCCCCCGCCCCCCGACAAATCAACAGAACAAAGAAAATTACCTAA-ACAGCAAGGACATAGGGAGGAACTTCTTGGCACAGAACTTTCCAAACA

CCTGAATCCTCAA-ATGAGGTGTGTCCATTAGTCAACTCCAATCTCTTGTCATATA--TAAGATGGTAGAGATGAG-AAGAAGGTAGCTCCTTTACAGCCCACTATTTCCACTAACTACTACCTGTGTT


TGTAGTTAATAAACAAAGAGCAAATTGGTCTCTAT-------TCTGTCTCATGCACTCAGGCACAACTTTTCCGGATTAAAAACAAAAACAACAACAACAAAAATCTACACCT--CCATTC----CCAG
TGTA-TTAACAAACAAAAAGGAAACTGGTCTCTATGAGAATCCCTATGCGGTGCCTTCAGAGAAAACTTCACCAGGTTTAAAGAGAAAACCCCTGTC--------TCTACACCT--CCATTC----CCAG
ATTA-ATATTTTTAATATTTTGTTTTAATATCTTTTA--ATTTATATATGATAAAAATTGATACAA-TCCACAGAAACAAAATTTATTTGGGTCCTCACTAATTTCTTTTTTC--TTGTTG----CCCA

CTTTTTCCTGAAGGGATACAAGAAGCAAGAAAGGTACTCTT-TCACTAGGACCTTCTCTGAGCTGTCC----TCAGGATGCTTTTGGGACTATTTTTCTTA---CCCAGAGAATGGAGAAACCCTGCAG

TCAAGATACAGCCTTTCATCCTTCTCCAGTGTTGAGAGTGTTGAACCTCAGAGTTTCTCCTCTCATTTTCTCTAAATGAGATACAATGCCAGCCATCCCAAGCTCTTGGCCTGAGTTGTTCATCTTGAA


ATCAAGCTTACTCTCTGGCACCAAA---CTCCATGGGGTGATTTTTCTTCTAGAAGAGTCC--AGGTGGACAGGTAAGGAGTGGGAGTCAGGGAGTCCAGTTCAGGGACAGAGATAATGGGATGAAAAG
GGCGAGCTCACTCTCTGGCATCAAG---TTCCCCGTGCTCAGTTTCCCTACACAAGAGTCC--AAGAGGAGAGGTAAGGAGTGGGAGGCAGGGAGTCCAGTTCAGGGACAGGGATTCCAGGACGAGAAG
GGCTGGAGGGCAAT--GGCA-CGAT---CTTGGCTCACCGCAACCTCCTCCTCCTGGGTTC--AAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCCAGGATTACAGCCATG—CGCCACCACGCCGGC

GGAATTCCCAAGCTGTAGTTATAAACAGAAGTTCTCCTTCTG-CTAGGTAGCATTCAAAGATCTTAATCTTCTGGG TTTCCGTTTTCTCGAATGAAAAATGC--AGGTCCGAGCAGTTA-ACTGGCTG

GTCTAGGACTCCAAGAAGCATGAAAGAGCTTCTTTAGTGAAGCTATGTCCTCAGTACTGCCAAAATTCAGACAATCTCCATGGCCTGACAATTTACCTTCTATTTGGGTAATTTATTGTCCCTTACGCA


TGAAAGGAGAGGGACGGGGCCCATGCCGAGGGTTTCTCCCTTG-TTTCT-CAGACAGCTCCTGG--GCCAAGACTCAGGGAGACATTGAGACAGAGCG-CTTCGCACAGGAGCAGAGGGGTCAGGGCGA
TGAAGGGGAAGGGGCTGGGCGCAGCCTGGGGGTCTCTCCCTGG-TTTCCACAGACAGATCCTTG--TCCAGGACTCAGGCAGACAGTGTGACAAAGAGGCTTGGTGTAGGAGAAGAGGGATCAGGACGA
TAATTTTTTGGACTTTTAGTAGAGAC--AGGGTTTCTCCATA---TTGGTCGGGCTGGTCTCGAACTCCCAACCTCAGGTGATCAGCCCGCCTTGGCCTCCCA-AAGTGCTGAGATTACAGGCGTGAGC

GGGCACCATTAG----CAAGTCACTTAGCATCTCTGGGGCCAGTCTGCAAAGCGAGGGGGCAGCCTTAATGTGCCTCCAGCCTGAAGTCCTAG AATGAGCGCCCGGTGTCCCAAGCTGGGGCGCGCAC

AACTCTCCAGCTGTCATGGCACAGACATATGATCTGTATTTAGCTCTCACTTTAGGTGTTTCCA---TTGATTCTATTCTCACTAATGTGCTTCAGGTATATCCCTGTCTAGAAGTCAGATTGGGGTTA
```

Figure 9B

```
κB2  κB1  ISRE  S
AGTCCCAGGGCCCCAGGCGTGGCTCTCAGAGTCTCAGGCCCCGAAGGCGGT-GTATGGATTGGGGAGTCCCAGCCTTGGGGATTCCCC-AACTCCGCAGTTTCTTTTCTCCCTCTCCCAACCTA-TGTA
AGTCCCAGG-TCCCGGACGGGGCTCTCAGGGTCTCAGGCTCCGAGGGCCGC-GTCTGCAATGGGGAGGCGCA-CGTTGGGGATTCCCCACTCCCCTGAGTTTCACTTCT---TCTCCCAACTTG-TGTC
CACCGCGCCCAGCCAGGACTAATTTCTAAGAGTGTGCAGAGATACCGAAACCTAAAAGTTTAAGAACTGCTGATTGCTGGGAAACTCT-------GCAGTTTCCCGTTC--CTCTCGTAACCTGGTCAT

CCCAGATCGGAGGGCGCCGATGTACAGACAGCAA-ACTCACCCAGTCTAGTGCATGCCTTCTTAAACATCACGAGACTCTAAGAAAAGGAAACTGAAAACGGGAAAGTCCCTCTCTCTAACCTGGCACT
E box  Ets/ISRE  κB
AAGAGTCTG--TCCGTGATTGACTAACAGTCTTAAATACTTGATTTGTTGTTGTTGTCCTGTTTGTTTAAGAACTTTACTTCTTT-ATCCAATGAACGGAGTATCTTGTGTCCTGGACCCTTTGCA

X1  X2  Y
GGGTCCTTCTTCCTGGATACTCACGACGCGGACCCAGTTCTCACTCCC-ATTGGGTGTCGGGTTTCCAG---AGAAGCCAATCAGT-GTCGTCGCGG--TCGCGGTTCTAAAGTCCGCACGCACCCACC
GGGTCCTTCTTCCAGGATACTCGTGACGCGTCCCCACTTCCCACTCCC-ATTGGGTATTGGATATCTAG---AGAAGCCAATCAGC-GTCGCCGCGG--TCCCAGTTCTAAAGTCCCCACGCACCCACC
GTGTCCTTCTTCCTGGATACTCATGACGCAGACTCAGTTCTCATTCCC-AATGGGTGTCGGGTTTCTAG---AGAAGCCAATCAGC-GTCGCCACGAC-TCCCGACTATAAAGTCCCCATCCGGACTCA

GCGTCGCT-GGCTTGGAGACAGGTGACGGTCCCTGCGGGCCTTGTCCTGATTGGCTGGGCACGCGTTTAATATAAGTGGAGGCGTCGCGCTGGCGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAG-

AGAACCCTTCCCCTAGCAACAGATGCGTCA-TCTCAAAATATTTTTCTGATTGGCCAAAGAGTAATTGATTTGCATTTTAATGGTCAGACTCTATTACACCCCACATTCTCTTTTCTTTTATTCTTGTC

GGGA---CTCAGATTCTCCCCAGACGCCGAGG          SEQ ID NO:42
CGGA---CTCAGAG------------------          SEQ ID NO:43
AGAAGTTCTCAGGACTCA--------------          SEQ ID NO:44

--------------------------------          SEQ ID NO:45

TGTTCTGCCTCACTCCCGAGCTC---------          SEQ ID NO:46
```

Figure 9C

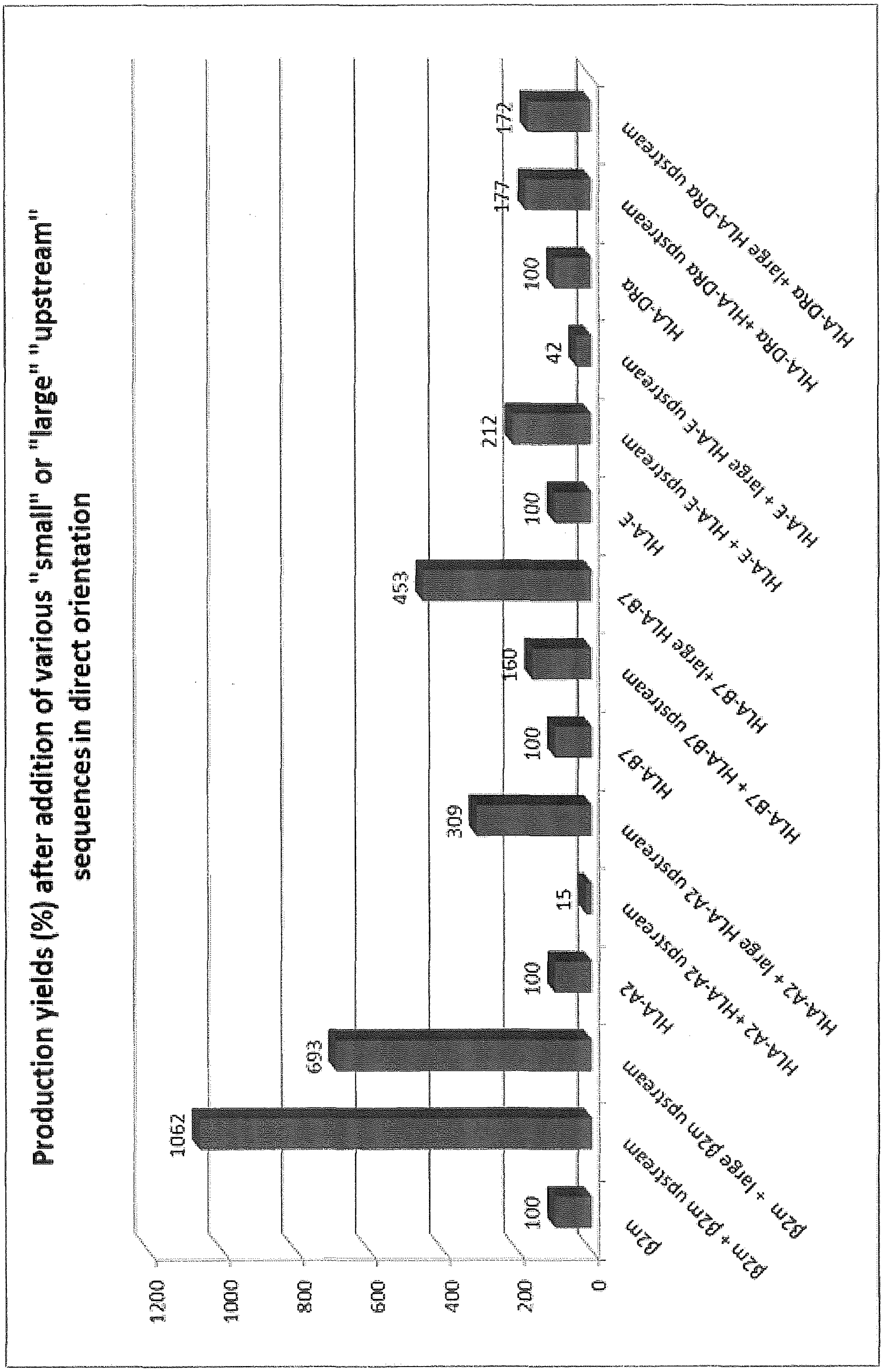
Production yields (%) after addition of various "small" or "large" "upstream" sequences in direct orientation
0
200
400
600
800
1000
1200
100
1062
693
100
15
309
100
160
453
100
212
42
100
177
172
β2m
β2m + β2m upstream
β2m + large β2m upstream
HLA-A2
HLA-A2 +HLA-A2 upstream
HLA-A2 + large HLA-A2 upstream
HLA-B7
HLA-B7 + HLA-B7 upstream
HLA-B7 +large HLA-B7
HLA-E
HLA-E + HLA-E upstream
HLA-E + large HLA-E upstream
HLA-DRα
HLA-DRα +HLA-DRα upstream
HLA-DRα +large HLA-DRα upstream

Figure 10

SEQUENCE LISTING

<110> THERAVECTYS

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       MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE

<130> P57997WO

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<151> 2013-06-03

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<170> PatentIn version 3.5

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<213> Homo sapiens

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agcatctctg gggccagtct gcaaagcgag gggcagcct taatgtgcct ccagcctgaa   180

gtcctagaat gagcgcccgg tgtcccaagc tggggcgcgc accccagatc ggagggcgcc   240

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<213> Homo sapiens

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ttttttggac ttttagtaga gacagggttt ctccatattg gtcgggctgg tctcgaactc   180

ccaacctcag gtgatcagcc cgccttggcc tcccaaagtg ctgagattac aggcgtgagc   240

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<213> Homo sapiens

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gggaaggggc tgggcgcagc ctgggggtct ctccctggtt tccacagaca gatccttgtc   180

caggactcag gcagacagtg tgacaaagag gcttggtgta ggagaagagg gatcaggacg   240

aagtcccagg tcccggacgg ggctctcagg gtctcaggct ccgagggccg cgtctgcaat   300

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gaaggggaag gggctgggcg cagcctgggg gtctctccct ggtttccaca gacagatcct 180

tggccaggac tcaggcacac agtgtgacaa agatgcttgg tgtaggagaa gagggatcag 240

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<213> Homo sapiens

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caggtatgga atgggaggca gaaagttcaa tcaagggact gggatttcgg aatgaataat 120

gaagggagat ggactgggtc catgccgaag gtttctccct ggtttctcag cccccgggcg 180

aagactcagg gagacattga gacacaccct gcacaggagg gggaggggga gggggagggc 240

aaagtcccag ggccccagga gtggctctca agggctcagg ccccgaggcg gtgtctgggg 300

<210> 6
<211> 300
<212> DNA
<213> Homo sapiens

<400> 6
gctcactctc tggcaacaag ctccctgggg tgatttttct tctagaagag tacaggagga 60

caggcaagga gtgggaggca gggagtccag ttcagggaca gggattccgg gatgaaaagt 120

gaagggagag ggccagggac cttgccgagg gtttctccct ggtttctcag acagctcctg 180

ggccaagact cagggagaca ctgagacaga acgcttggca caagagtagc ggggtcaggg 240

cgaagtccca gggcctcaag cgtggctctc agggtctcag gccccacagg cggtgtatgg 300

<210> 7
<211> 300
<212> DNA
<213> Homo sapiens

<400> 7
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caggtaagga gtgggagtca gggagtccag ttccagggac agagattacg ggataaaaag 120

tgaaaggaga gggacggggc ccatgccgag ggtttctccc ttgtttctca gacagctctt 180

gggccaagac tcagggagac attgagacag agcgcttggc acagaagcag aggggtcagg 240

gcgaagtcca gggccccagg cgttggctct cagggtctca ggccccgaag gcggtgtatg 300

<210> 8

<211> 316
<212> DNA
<213> Artificial Sequence

<220>
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<222> (269)..(269)
<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<222> (300)..(300)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (309)..(309)
<223> n is a, c, g, or t

<220>

<221> misc_feature
<222> (314)..(314)
<223> n is a, c, g, or t

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acaggtaagg agtgggangg cagggagtcc agttncnagg gnnacaggga ttccgggann 120

agaagtgaag ggnnagggnc tgggnccatn cngagggttt ctccctggtt tctncagaca 180

gnnctcctng gccaagactc aggnagacan tgngacanag cnnngcttgg ngcaggnagn 240

agaggggtca ggncgaagtc ccaggnccnc aggcgtnggc tctcagggtc tcnnnaggcn 300

ccgaaggcng tgtntg 316

<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 9
ctcgaggaga aaccctgcag ggaattc 27

<210> 10
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 10
ggtaccgagt ctcgtgatgt ttaagaaggc a 31

<210> 11
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 11
ggtaccgaga aaccctgcag ggaattcccc ag 32

<210> 12
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 12
ctcgaggagt ctcgtgatgt ttaagaaggc a 31

<210> 13
<211> 32

<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 13
cacgtggaga aaccctgcag ggaattcccc ag   32

<210> 14
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 14
gagtctcgtg atgtttaaga aggca   25

<210> 15
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 15
tgccttctta aacatcacga gactcctaaa acttcatttt taattt   46

<210> 16
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 16
cacgtgatga aatgctaggc ggctgtc   27

<210> 17
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 17
cacgtggagt ctcgtgatgt ttaagaaggc atg   33

<210> 18
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 18
gagaaaccct gcagggaatt ccccag   26

<210> 19
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 19
tggggaattc cctgcagggt ttctcctaaa acttcatttt taattt 46

<210> 20
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 20
cacgtgatga aatgctaggc ggctgtc 27

<210> 21
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 21
cccaagaacc caaggaaca 19

<210> 22
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 22
gatagaggaa gagcaaaac 19

<210> 23
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Lenti TM probe

<400> 23
aaccattagg agtagcaccc accaagg 27

<210> 24
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> Actine A primer

<400> 24
cggtgaggat cttcatgagg tagt   24

<210> 25
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Actine S primer

<400> 25
aacaccccag ccatgtacgt   20

<210> 26
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Humura ACT TM probe

<400> 26
ccagccaggt ccagacgcag ga   22

<210> 27
<211> 330
<212> DNA
<213> Homo sapiens

<400> 27
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gtagcattca aagatcttaa tcttctgggt ttccgttttc tcgaatgaaa aatgcaggtc   120

cgagcagtta actggcgggg gcaccattag caagtcactt agcatctctg gggccagtct   180

gcaaagcgag ggggcagcct taatgtgcct ccagcctgaa gtcctagaat gagcgcccgg   240

tgtcccaagc tggggcgcgc accccagatc ggagggcgcc gatgtacaga cagcaaactc   300

acccagtcta gtgcatgcct tcttaaacat   330

<210> 28
<211> 1058
<212> DNA
<213> Homo sapiens

<400> 28
cttccaagat ctctgcccct ccccatcgcc atggtccact tcctcttctc actgttcctc   60

ttagaaaaga tctgtggact ccaccaccac gaaatggcgg caccttattt atggtcactt   120

tagagggtag gttttcttaa tgggtctgcc tgtcatgttt aacgtccttg gctgggtcca   180

aggcagatgc agtccaaact ctcactaaaa ttgccgagcc ctttgtcttc cagtgtctaa   240

aatattaatg tcaatggaat caggccagag tttgaattct agtctcttag cctttgtttc   300

ccctgtccat aaaatgaatg ggggtaattc tttcctccta cagtttattt atatattcac   360

taattcattc attcatccat ccattcgttc attcggttta ctgagtacct actatgtgcc   420 agcccctgtt ctagggtgga aactaagaga atgatgtacc tagagggcgc tggaagctct 480

aaagccctag cagttactgc ttttactatt agtggtcgtt tttttctccc ccccgccccc 540

cgacaaatca acagaacaaa gaaaattacc taaacagcaa ggacataggg aggaacttct 600

tggcacagaa ctttccaaac actttttcct gaagggatac aagaagcaag aaaggtactc 660

tttcactagg accttctctg agctgtcctc aggatgcttt tgggactatt tttcttaccc 720

agagaatgga gaaaccctgc agggaattcc caagctgtag ttataaacag aagttctcct 780

tctgctaggt agcattcaaa gatcttaatc ttctgggttt ccgttttctc gaatgaaaaa 840

tgcaggtccg agcagttaac tggctggggc accattagca agtcacttag catctctggg 900

gccagtctgc aaagcgaggg ggcagcctta atgtgcctcc agcctgaagt cctagaatga 960

gcgcccggtg tcccaagctg gggcgcgcac cccagatcgg agggcgccga tgtacagaca 1020

gcaaactcac ccagtctagt gcatgccttc ttaaacat 1058

<210> 29
<211> 322
<212> DNA
<213> Homo sapiens

<400> 29
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cttctagaag agtccaggtg gacaggtaag gagtgggagt cagggagtcc agttccaggg 120

acagagatta cgggataaaa agtgaaagga gagggacggg gcccatgccg agggtttctc 180

ccttgtttct cagacagctc ttgggccaag actcagggag acattgagac agagcgcttg 240

gcacagaagc agaggggtca gggcgaagtc cagggcccca ggcgttggct ctcagggtct 300

caggccccga aggcggtgta tg 322

<210> 30
<211> 531
<212> DNA
<213> Homo sapiens

<400> 30
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tacctattag attcctgatc attcaggggt taccaagatt ctgctaccca ctgtagttaa 120

taaacaaaga gcaaattggt ctctattctg tctcatgcac tcaggcacaa cttttccgga 180

ttaaaaacaa aaacaacaac aacaaaaatc tacacctcca ttcccagatc aagcttactc 240

tctggcacca aactccatgg ggtgattttt cttctagaag agtccaggtg gacaggtaag 300

gagtgggagt cagggagtcc agttcaggga cagagataat gggatgaaaa gtgaaaggag 360

agggacgggg cccatgccga gggtttctcc cttgtttctc agacagctcc tgggccaaga 420

ctcagggaga cattgagaca gagcgcttcg cacaggagca gaggggtcag ggcgaagtcc 480

cagggcccca ggcgtggctc tcagagtctc aggccccgaa ggcggtgtat g 531

<210> 31
<211> 352
<212> DNA
<213> Homo sapiens

<400> 31
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gcatcaagtt ccccgtgctc agtttcccta cacaagagtc caagaggaga ggtaaggagt 120

gggaggcagg gagtccagtt cagggacagg gattccagga cgagaagtga aggggaaggg 180

gctgggcgca gcctgggggt ctctccctgg tttccacaga cagatccttg tccaggactc 240

aggcagacag tgtgacaaag aggcttggtg taggagaaga gggatcagga cgaagtccca 300

ggtcccggac ggggctctca gggtctcagg ctccgagggc cgcgtctgca at 352

<210> 32
<211> 511
<212> DNA
<213> Homo sapiens

<400> 32
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agggattacc aatattgtgc tacctactgt attaacaaac aaaaaggaaa ctggtctcta 120

tgagaatccc tatgcggtgc cttcagagaa aacttcacca ggtttaaaga gaaaacccct 180

gtctctacac ctccattccc agggcgagct cactctctgg catcaagttc cccgtgctca 240

gtttccctac acaagagtcc aagaggagag gtaaggagtg ggaggcaggg agtccagttc 300

agggacaggg attccaggac gagaagtgaa ggggaagggg ctgggcgcag cctgggggtc 360

tctccctggt ttccacagac agatccttgt ccaggactca ggcagacagt gtgacaaaga 420

ggcttggtgt aggagaagag ggatcaggac gaagtcccag gtcccggacg gggctctcag 480

ggtctcaggc tccgagggcc gcgtctgcaa t 511

<210> 33
<211> 328
<212> DNA
<213> Homo sapiens

<400> 33
actaatttct tttttcttgt tgcccaggct ggagggcaat ggcacgatct tggctcaccg 60

caacctcctc ctcctgggtt caagtgattc tcctgcctca gcctcccaag tagccaggat 120

tacagccatg cgccaccacg ccggctaatt ttttggactt ttagtagaga cagggtttct 180

ccatattggt cgggctggtc tcgaactccc aacctcaggt gatcagcccg ccttggcctc 240

ccaaagtgct gagattacag gcgtgagcca ccgcgcccag ccaggactaa tttctaagag 300

tgtgcagaga taccgaaacc taaaagtt 328

<210> 34
<211> 1047
<212> DNA
<213> Homo sapiens

<400> 34
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gctcactgca accaccacct ctcgggttca agcaattctc ctatctcagc ctccagagtt 120

gctggaatta caggcgcgca ccaccacacc cggctaattt ttgtattgtt agtagagaca 180

gggtttcatc atgttggcca ggttagtctt gaactcctga cctcgtgatc tgcctgcctc 240

ggcctaccaa aatgctgcga ttacaggcgt gagccaccgt tcccggccta tacgttgttt 300

attttggaaa aattaaaaat taagtttttt ttcattaaag atatgttatt tccgatcaag 360

agatcaagac catcctggcc aacatggtga aaccccgtct ctactaaaaa cacaaaaatt 420

agctgggtgt ggtggcacac gcctgtagtt ccagttactg gggaggctga ggcaggagaa 480

tcgcttgaac ccgggagaag gaggttgcag tgagccgaga tcatgccact gcactccagc 540

ctggggacag agcaagactc tgactcaaaa aaaaaaaaag ttgtttctat taacatgtaa 600

tgggttatta atattctctt aaatgaatta atatttttaa tattttgttt taatatcttt 660

taatttatat atgataaaaa ttgatacaat ccacagaaac aaaatttatt tgggtcctca 720

ctaatttctt ttttcttgtt gcccaggctg gagggcaatg gcacgatctt ggctcaccgc 780

aacctcctcc tcctgggttc aagtgattct cctgcctcag cctcccaagt agccaggatt 840

acagccatgc gccaccacgc cggctaattt tttggacttt tagtagagac agggtttctc 900

catattggtc gggctggtct cgaactccca acctcaggtg atcagcccgc cttggcctcc 960

caaagtgctg agattacagg cgtgagccac cgcgcccagc caggactaat ttctaagagt 1020

gtgcagagat accgaaacct aaaagtt 1047

<210> 35
<211> 356
<212> DNA
<213> Homo sapiens

<400> 35
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ttttctctaa atgagataca atgccagcca tcccaagctc ttggcctgag ttgatcatct 120

tgaagtctag gactccaaga agcatgaaag agcttcttta gtgaagctat gtcctcagta 180

ctgccaaaat tcagacaatc tccatggcct gacaatttac cttctatttg ggtaatttat 240

tgtcccttac gcaaactctc caactgtcat tgcacagaca tatgatctgt atttagctct 300

cactttaggt gtttccattg attctattct cactaatgtg cttcaggtat atccct 356

<210> 36
<211> 522
<212> DNA
<213> Homo sapiens

<400> 36
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ccattagtca actccaatct cttgtcatat ataagatggt agagatgaga agaaggtagc 120

tcctttacag cccactattt ccactaacta ctacctgtgt ttcaagatac agcctttcat 180 ccttctccag tgttgagagt gttgaacctc agagtttctc ctctcatttt ctctaaatga 240

gatacaatgc cagccatccc aagctcttgg cctgagttgt tcatcttgaa gtctaggact 300

ccaagaagca tgaaagagct tctttagtga agctatgtcc tcagtactgc caaaattcag 360

acaatctcca tggcctgaca atttaccttc tatttgggta atttattgtc ccttacgcaa 420

actctccagc tgtcatggca cagacatatg atctgtattt agctctcact ttaggtgttt 480

ccattgattc tattctcact aatgtgcttc aggtatatcc ct 522

<210> 37
<211> 543
<212> DNA
<213> Homo sapiens

<400> 37
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cttctagaag agtccaggtg gacaggtaag gagtgggagt cagggagtcc agttccaggg 120

acagagatta cgggataaaa agtgaaagga gagggacggg gcccatgccg agggtttctc 180

ccttgtttct cagacagctc ttgggccaag actcagggag acattgagac agagcgcttg 240

gcacagaagc agaggggtca gggcgaagtc cagggcccca ggcgttggct ctcagggtct 300

caggccccga aggcggtgta tggattgggg agtcccagcc ttggggattc cccaactccg 360

cagtttcttt tctccctctc ccaacctatg tagggtcctt cttcctggat actcacgacg 420

cggacccagt tctcactccc attgggtgtc gggtttccag agaagccaat cagtgtcgtc 480

gcggtcgcgg ttctaaagtc cgcacgcacc caccgggact cagattctcc ccagacgccg 540

agg 543

<210> 38
<211> 550
<212> DNA
<213> Homo sapiens

<400> 38
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ggcatcaagt tccccgtgct cagtttccct acacaagagt ccaagaggag aggtaaggag 120

tgggaggcag ggagtccagt tcagggacag ggattccagg acgagaagtg aaggggaagg 180

ggctgggcgc agcctggggg tctctccctg gtttccacag acagatcctt gtccaggact 240

caggcagaca gtgtgacaaa gaggcttggt gtaggagaag agggatcagg acgaagtccc 300

aggtcccgga cggggctctc agggtctcag gctccgaggg ccgcgtctgc aatggggagg 360

cgcagcgttg gggattcccc actcccctga gtttcacttc ttctcccaac ttgtgtcggg 420

tccttcttcc aggatactcg tgacgcgtcc ccacttccca ctcccattgg gtattggata 480

tctagagaag ccaatcagcg tcgccgcggt cccagttcta aagtccccac gcacccaccc 540

ggactcagag 550

<210> 39
<211> 533
<212> DNA
<213> Homo sapiens

<400> 39
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caacctcctc ctcctgggtt caagtgattc tcctgcctca gcctcccaag tagccaggat   120

tacagccatg cgccaccacg ccggctaatt ttttggactt ttagtagaga cagggtttct   180

ccatattggt cgggctggtc tcgaactccc aacctcaggt gatcagcccg ccttggcctc   240

ccaaagtgct gagattacag gcgtgagcca ccgcgcccag ccaggactaa tttctaagag   300

tgtgcagaga taccgaaacc taaaagttta agaactgctg attgctggga aactctgcag   360

tttcccgttc ctctcgtaac ctggtcatgt gtccttcttc ctggatactc atgacgcaga   420

ctcagttctc attcccaatg ggtgtcgggt ttctagagaa gccaatcagc gtcgccacga   480

ctcccgacta taaagtcccc atccggactc aagaagttct caggactcag agg   533

<210> 40
<211> 517
<212> DNA
<213> Homo sapiens

<400> 40
gagaaaccct gcagggaatt ccccagctgt agttataaac agaagttctc cttctgctag   60

gtagcattca aagatcttaa tcttctgggt ttccgttttc tcgaatgaaa aatgcaggtc   120

cgagcagtta actggcgggg gcaccattag caagtcactt agcatctctg gggccagtct   180

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<213> Homo sapiens

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<212> DNA
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<211> 1248
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<211> 1245
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tcctttacag cccactattt ccactaacta ctacctgtgt ttcaagatac agcctttcat 180

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ccaagaagca tgaaagagct tctttagtga agctatgtcc tcagtactgc caaaattcag 360

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ggttaaagag tctgtccgtg attgactaac agtcttaaat acttgatttg ttgttgttgt 600

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