EXPRESSION VECTORS COMPRISING CHIMERIC CYTOMEGALOVIRUS PROMOTER AND ENHANCER SEQUENCES

FIELD OF THE INVENTION

The present invention relates to mammalian expression systems, and in particular to expression constructs comprising chimeric promoter regulatory sequences for the heterologous expression of a nucleic acid sequence of interest in mammalian cells. The chimeric promoter regulatory sequences are composed of a promoter sequence derived from murine or from human cytomegalovirus and an upstream region and/or enhancer sequence derived from human and/or simian cytomegalovirus provided that the sequence elements are derived from at least two different cytomegalovirus species.

BACKGROUND

Recombinant (poly)peptides and proteins for applications in basic research, diagnostics, and therapy, such as antibody molecules, vaccines, hormones, and growth factors, are produced using a wide variety of genetically engineered organisms that include both prokaryotic and eukaryotic cells. However, the vast majority of recombinant peptides or proteins include post-translational modifications that cannot be mimicked or re-produced when using prokaryotic host cells. For this reason, mammalian gene expression systems have turned out to represent a preferred choice.

Mammalian expression systems based on Chinese Hamster ovary (CHO) cells are widely used in production of recombinant protein. Apart from lymphoid cell lines, CHO cells represent one of the few cell types allowing for simple and efficient high-density suspension batch culture of animal cells. Furthermore, the use of CHO cells results in high product yields, while lymphoid cells are more difficult to culture at an industrial scale. In view of considerable costs for recombinant production of polypeptides and proteins, it is also of utmost importance to maximize the yield of recombinant protein per bioreactor run. Process

parameters that have considerable impact on product yield include *inter alia* the cell culture conditions, the copy number of the nucleic acids (genes) to be expressed, the efficiency with which these genes are transcribed and the corresponding mRNAs are translated, the stability of the mRNA, and the like.

Accordingly, improvements of the strength or transcriptional activity of the regulatory genetic elements controlling gene expression constitute a particularly critical factor in order to augment the yield of recombinant protein produced. Even small incremental increases in transcriptional activity at the single cell level will finally translate into considerable improvements in product yield in high-density industry-scale batch cultures.

The vast majority of mammalian gene expression systems employ expression vectors encoding the heterologous nucleic acid sequences to be expressed under the control of promoter regulatory sequences derived from viruses. Two of the most frequently used viral regulatory elements in these expression cassettes are those of the human cytomegalovirus (hCMV) immediate early genes 1 and 2 (IE1 and IE2). However, a disadvantage associated with the use of hCMV IE1 and IE2 regulatory elements is their pronounced species specificity.

U.S. patent 5,866,359 discloses that gene expression from such hCMV promoter can be improved by co-expressing adenoviral EIA protein under the control of a weak promoter. EIA is a multifunctional transcription factor which may act on cell cycle regulation and has both independent transcriptional activating and repressing functional domains. Fine tuning of EIA expression is crucial to achieve the ideal balance between gene transactivation and any negative impact on cell cycle progression. However, overexpression of EIA expression could reduce the capacity of the cell to synthesize the recombinant protein of interest.

U.S. patent 5,591,639 describes vectors comprising, upstream (5') of a heterologous nucleic acid sequence to be expressed, the enhancer, promoter, and complete 5'-untranslated region of the major immediate early gene of the human cytomegalovirus (hCMV-MIE) including intron A (i.e. the first natural intron). However, if the first 400 bp (5'-end) of this sequence (total length of about 2100 bp) were present, poor gene expression rates were

observed in both COS7 and CHO cells (Chapman, B.S. et al. (1991) *Nucl. Acids Res.* **19**, 3979-3986).

The transcriptional activity of the regulatory elements of the immediate early genes of the murine cytomegalovirus (mCMV) is higher than that of the hCMV counterparts without exhibiting the pronounced species preference observed for the human sequences (Addison, C.L. et al. (1987) *J. Gen. Virol.* **78**, 1653-1661).

However, attempts to enhance the activity of the mCMV IE promoter regulatory elements, analogously to the hCMV counterparts, by insertion of the natural first intron of the murine major immediate early gene downstream (3') of the mCMV IE promoter failed (cf. *inter alia* EP patent 1 525 320 B1). However, the generation of expression vectors comprising a chimeric cassette composed of the regulatory elements of mCMV IE1 and the natural first intron of the human major immediate early gene resulted in product yields comparable to the use of the fully human sequences (cf., e.g., WO 2006/111387 A2). Similar gene expression rates were also obtained for expression vectors comprising the mCMV IE2 regulatory sequences (cf. *inter alia* EP patent 1 601 776 B1).

Thus, there still remains a need for improved mammalian gene expression systems resulting in high yields of the recombinant polypeptides or proteins produced. In particular, there is a need for mammalian gene expression systems that overcome the above-mentioned limitations, that is, expression systems based on the mCMV or the hCMV promoter sequences but achieving higher expression rates (and thus, product yields) than with the available system

Accordingly, it is an object of the present invention to provide such gene expression systems, primarily suitable expression constructs and corresponding mammalian host cells.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to an expression vector for the heterologous expression of a nucleic acid sequence of interest in mammalian cells, the vector comprising

a first chimeric promoter regulatory sequence being operably linked to a first nucleic acid sequence to be expressed, wherein the chimeric promoter regulatory sequence comprises:

(i) a promoter sequence being derived from murine cytomegalovirus or from human cytomegalovirus and being operably linked to the transcriptional start site of the nucleic acid sequence to be expressed; and

(ii) an upstream region and/or enhancer sequence being derived from human and/or simian cytomegalovirus, wherein the upstream region and/or enhancer sequence is located 5' of and operably linked to the murine or the human promoter sequence; and

wherein the chimeric promoter regulatory sequence comprises sequence elements being derived from at least two of the group consisting of murine cytomegalovirus, human cytomegalovirus and simian cytomegalovirus.

In particular embodiments, the promoter sequence is derived from the murine cytomegalovirus IE1 promoter; and/or the upstream region and/or enhancer sequence is derived from the human and/or simian cytomegalovirus IE1 enhancer.

In preferred embodiments, the murine cytomegalovirus IE1 promoter sequence has the nucleotide sequence of SEQ ID NO: 4.

In other particular embodiments, the promoter sequence is derived from the human cytomegalovirus IE1 promoter; and/or the upstream region and/or enhancer sequence is derived from the human and/or simian cytomegalovirus IE1 enhancer.

In preferred embodiments, the human cytomegalovirus IE1 promoter sequence has the nucleotide sequence of SEQ ID NO: 5.

In other preferred embodiments, the upstream region and/or enhancer sequence comprises the nucleotide sequence of SEQ ID NO: 6 being derived from the simian cytomegalovirus IE1 region.

In particularly preferred embodiments, the chimeric promoter regulatory sequence comprises a nucleotide sequence being selected from the group consisting of SEQ ID NO: 7, SEQ ID

NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

In other particular embodiments, the expression vector further comprises a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is identical to the first chimeric promoter regulatory sequence.

In alternative particular embodiments, the expression vector further comprises a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is different from the first chimeric promoter regulatory sequence.

Preferably, the first and second nucleic acid sequences to be expressed encode different polypeptides. In specific embodiments, the different polypeptides represent subunits of a dimeric or multimeric protein. Particularly preferably, the dimeric or multimeric protein is an antibody molecule.

In another aspect, the present invention relates to a mammalian host cell transfected with an expression vector as defined herein above. Preferably, the host cell is a CHO cell.

In yet another aspect, the present invention relates to a method for heterologous expression of a nucleic acid sequence of interest in a mammalian host cell, comprising:

(i) transfecting the mammalian host cell with an expression vector as defined herein above; and

(ii) culturing the transfected mammalian host cell under conditions allowing the expression of the nucleic acid sequence of interest.

In preferred embodiments, the transfection is stable transfection.

In a further aspect, the present invention relates to the use of an expression vector as defined herein above for the heterologous expression of a nucleic acid sequence of interest in a mammalian host cell.

Other embodiments of the present invention will become apparent from the detailed description hereinafter.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates expression vector pRY42 (SEQ ID NO: 1) used as "parent vector" for generating the mammalian expression vectors as defined herein. pRY42 encompasses two regulatory cassettes for driving heterologous gene expression (between "mCMV" and "pA", respectively): Multiple cloning sites located 3' (i.e. "downstream") of the "Ex2" regions for insertion of heterologous nucleic acid sequences to be expressed. The regulatory cassettes are flanked by mutant ("F5-mFRT") and wild type ("wFRT") flippase recognition target sites. An in-frame initiation methionine codon has been added to the 5'-end of the wFRT site ("ATG+F") An SV40 early promoter ("SV") is located 5' ("upstream") of ATG+F. Transcription of heterologous nucleic acid sequences is driven by the promoter of the murine cytomegalovirus IE1 gene ("mCMV") which is followed by the 5'UTR, where exon 1 ("Ex1") is a hybrid of murine ("m") and human ("h") CMV derived sequences, and where exon 2 ("Ex2") and the intron A sequence ("Int A") are derived from the hCMV sequence. The β-lactamase selection marker gene is denoted as "bla". pRY42 is used as a target vector for cloning the different chimeric promoter regulatory sequences as defined herein.

Figure 2 illustrates expression vector pRY57 (SEQ ID NO: 2) encoding the light ("LC") and heavy ("HC") chains of the mouse-human chimeric monoclonal antibody (mAb) cB72.3 (Whittle, N. et al. (1987) *Protein Eng.* **1**, 499-505), each located between the cloning sites 3' of the "Ex2" regions and the polyadenylation sites ("pA"), respectively. Otherwise, pRY57 is identical to pRY42. The LC and HC nucleic acid sequences of pRY57 were removed and cloned into pRY42 variants in which original the mCMV promoter sequence was replaced with the different chimeric promoter regulatory sequences as defined herein.

Figure 3 schematically depicts the original mCMV promoter sequence (SEQ ID NO: 3) encompassed in pRY42 (top) as well as five different chimeric promoter regulatory sequences (constructs "1 to 5"), as defined herein (SEQ ID NO: 7 to SEQ ID NO: 11, respectively), which comprise murine CMV (mCMV) promoter sequences located 3' of upstream region and/or enhancer elements being derived from simian (sCMV) and/or human (hCMV) CMV. Furthermore, two additional chimeric promoter regulatory sequences (constructs "6 and 7"), as defined herein (SEQ ID NO: 12 and SEQ ID NO: 13) are shown, which comprise hCMV promoter sequences located 3' of upstream region and/or enhancer elements being derived from sCMV and/or human hCMV. All mCMV and hCMV promoter sequences specifically employed herein include at their 3'-ends an additional guanosine ("G") nucleotide, which represents the transcriptional start site.

Figure 4 shows a comparison of the concentrations of mAb cB72.3 produced in stable CHO lines where gene expression of the mAb sequences was under the control of chimeric constructs 1 to 5, as illustrated in Figure 3. Determination was performed by Protein A HPLC after 15 days growth in 50 ml growth medium in E250 shake flask culture using a fed batch overgrow (FOG) protocol. For each of chimeric constructs, n = 4, representing duplicate fed batch analyses for duplicate transfections, with the exception of construct 4, where n = 6 (duplicate FOG analyses for triplicate transfections) and pRY57 (original mCMV), where n = 8, with data points from experiments 1 and 2 being combined.

Figure 5 shows a comparison of the concentrations of mAb cB72.3 produced in stable CHO lines where gene expression of the mAb sequences was under the control of chimeric constructs 6 and 7, as illustrated in Figure 3. Determination was performed by Protein A HPLC after 7 days growth in 30 ml growth medium in E125 shake flask culture using a batch overgrow (BOG) protocol. For mCMV and construct 7, n = 6 (duplicate batch analysis for triplicate transfections), and for construct 6, n = 4 (duplicate batch analyses for duplicate transfections).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the unexpected finding that mammalian expression vectors comprising chimeric (i.e. hybrid) promoter regulatory sequences being composed of a mCMV or a hCMV promoter sequence (in particular, a mCMV or a hCMV IE1 promoter sequence) in operable linkage to the transcriptional start site of the nucleic acid sequence to be expressed and an hCMV and/or sCMV upstream region and/or enhancer sequence (in particular, a hCMV IE1 and/or sCMV IE1 enhancer sequence) being located 5' of and operably linked to the mCMV or the hCMV promoter sequence resulted in significantly improved gene expression rates as compared to existing expression systems only based on mCMV promoter sequences, and thus also in much higher yields (up to an almost 3-fold increase) of the recombinant proteins produced.

Accordingly, the mammalian expression vectors as defined herein represent superior molecular tools for the production of recombinant proteins, particularly in an industry-scale.

The present invention illustratively described in the following may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein.

Where the term "comprising" is used in the present description and the claims, it does not exclude other elements or steps. For the purposes of the present invention, the term "consisting of" is considered to be a preferred embodiment of the term "comprising". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also to be understood to disclose a group, which preferably consists only of these embodiments.

Where an indefinite or definite article is used when referring to a singular noun, e.g., "a", "an" or "the", this includes a plural of that noun unless specifically stated otherwise.

In case, numerical values are indicated in the context of the present invention the skilled person will understand that the technical effect of the feature in question is ensured within an

interval of accuracy, which typically encompasses a deviation of the numerical value given of ± 10%, and preferably of ± 5%.

Furthermore, the terms first, second, third, (a), (b), (c), and the like, in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

Further definitions of term will be given in the following in the context of which the terms are used. The following terms or definitions are provided solely to aid in the understanding of the invention. These definitions should not be construed to have a scope less than understood by a person of ordinary skill in the art.

In one aspect, the present invention relates to an expression vector for the heterologous expression of a nucleic acid sequence of interest in mammalian cells, the vector comprising a first chimeric promoter regulatory sequence being operably linked to a first nucleic acid sequence to be expressed, wherein the chimeric promoter regulatory sequence comprises:

(i) a promoter sequence being derived from murine cytomegalovirus or from human cytomegalovirus and being operably linked to the transcriptional start site of the nucleic acid sequence to be expressed; and

(ii) an upstream region and/or enhancer sequence being derived from human and/or simian cytomegalovirus, wherein the upstream region and/or enhancer sequence is located 5' of and operably linked to the murine or the human promoter sequence, and

wherein the chimeric promoter regulatory sequence comprises sequence elements being derived from at least two of the group consisting of murine cytomegalovirus, human cytomegalovirus and simian cytomegalovirus.

In other words, the provision that the chimeric promoter regulatory sequence, as defined herein, comprises sequence elements being derived from at least two of the group consisting of murine cytomegalovirus, human cytomegalovirus and simian cytomegalovirus ensures that

the claimed subject matter does not include any constructs only derived from human cytomegalovirus.

The term "expression vector", as used herein, denotes a nucleic acid vehicle (plasmid) that is characterized by the presence of at least one "expression cassette". The term "expression cassette", as used herein, refers to a genetic construct that is capable to allow gene expression of a nucleic acid sequence of interest (i.e. a "heterologous" nucleic acid sequence). This requires that such expression cassette comprises regulatory sequence elements which contain information regarding to transcriptional and/or translational regulation, and that such regulatory sequences are "operably linked" to the nucleic acid sequence of interest. An operable linkage is a linkage in which the regulatory sequence elements and the nucleic acid sequence to be expressed are connected in a way that enables gene expression.

The precise nature of the regulatory regions of an "expression cassette" that are necessary for controlling and driving gene expression may vary among species, but in general these regions comprise promoter regulatory sequences (i.e. a sequence region located 5' ("upstream") of the nucleic acid sequence of interest) and 3'-untranslated regulatory sequences (i.e. a sequence region located 3' ("downstream") of the nucleic acid sequence of interest).

The term "promoter", (also referred to as “core promoter") as used herein, denotes sequence elements that *per se* direct the initiation of transcription (e.g., binding sites for transcription factors and for DNA-dependent RNA-polymerase, TATA box, CAAT sequences, and 5'-capping elements). As long as this functionality of promoting transcription initiation is retained or substantially retained (e.g., at least 70%, at least 80%, at least 90% or at least 95% of wild-type activity, that is, activity of a full-length sequence), any truncated, mutated or otherwise modified variants of a (naturally occurring) wild-type promoter sequence are also within the above definition. As used herein, the term "core promoter" refers to a sequence of minimal length that retains promoter activity. As used herein, the promoter sequence is operably linked to the transcriptional start site of the nucleic acid sequence to be expressed.

In particular embodiments, the expression vectors of the present invention comprise (as part of an expression cassette) a first (chimeric) promoter regulatory sequence (i.e. at least one such sequence), which, in turn, encompasses a (core) promoter sequence being derived from murine cytomegalovirus (mCMV). This mCMV promoter sequence is operably linked to the transcriptional start site of a first nucleic acid sequence to be expressed. Generally, any mCMV promoter sequence can be employed. Preferably, promoter sequences of the mCMV immediate early (IE) genes, such as mCMV IE1 and mCMV IE2 (Dorsch-Hasler, K. et al. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8325-8329; Messerle, M. et al. (1991) *J. Virol.* **65**, 1638-1643), are employed, with the mCMV IE1 promoter being particularly preferred.

These and additional mCMV promoters are well known in the art and can be easily derived from the mCMV genome deposited in the NCBI Viral Genomes database under accession no. U68299.1 (http://www.ncbi.nlm.nih.gov/genomes/GenomesHome; Bao, Y. et al. (2004) *J. Virol.* **78**, 7291-7298).

In a specific embodiment of the present invention, the mCMV IE1 promoter sequence comprised in the expression vector has a nucleic acid sequence of 492 bp in length as shown in SEQ ID NO: 3:

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  1 tactgagtca ttagggactt tccaatgggt tttgcccagt acataaggtc
 51 aatagggggtg aatcaacagg aaagtcccat tggagccaag tacactgagt
101 caatagggac tttccattgg gttttgccca gtacaaaagg tcaatagggg
151 gtgagtcaat gggtttttcc cattattggc acgtacataa ggtcaatagg
201 ggtgagtcat tgggtttttc cagccaattt aattaaaacg ccatgtactt
251 tcccaccatt gacgtcaatg ggctattgaa actaatgcaa cgtgaccttt
301 aaacggtact ttcccatagc tgattaatgg gaaagtaccg ttctcgagcc
351 aatacacgtc aatgggaagt gaaagggcag ccaaaacgta acaccgcccc
401 ggttttcccc tggaaattcc atattggcac gcattctatt ggctgagctg
451 cgttctacgt gggtataaga ggcgcgacca gcgtcggtac cg
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In a preferred embodiment, the mCMV IE1 promoter sequence comprised in the expression vector has a nucleic acid sequence of 102 bp in length (also referred to as "core promoter") as shown in SEQ ID NO: 4:

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  1 acaccgcccc ggttttcccc tggaaattcc atattggcac gcattctatt
 51 ggctgagctg cgttctacgt gggtataaga ggcgcgacca gcgtcggtac
101 cg
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Both SEQ ID NO: 3 and SEQ ID NO: 4 include at their respective 3'-ends an additional guanosine ("G") nucleotide, which represents the transcriptional start site.

In other particular embodiments, the expression vectors of the present invention comprise (as part of an expression cassette) a first (chimeric) promoter regulatory sequence (i.e. at least one such sequence), which, in turn, encompasses a (core) promoter sequence being derived from human cytomegalovirus (hCMV). This hCMV promoter sequence is operably linked to the transcriptional start site of a first nucleic acid sequence to be expressed. Generally, any hCMV promoter sequence can be employed. Preferably, promoter sequences of the hCMV immediate early (IE) genes, such as hCMV IE1 and hCMV IE2 (You, C.Y. et al. (1992) *Intervirology* **34**, 94-104; Klucher, K.M. et al. (1993) *Mol. Cell. Biol.* **13**, 1238-1250), are employed, with the hCMV IE1 promoter being particularly preferred.

These and additional hCMV promoters are well known in the art and can be easily derived from the hCMV genome deposited in the NCBI Viral Genomes database under accession no. NC_006273 (http://www.ncbi.nlm.nih.gov/genomes/GenomesHome; *supra*).

In a preferred embodiment, the hCMV IE1 promoter sequence comprised in the expression vector has a nucleic acid sequence of 117 bp in length (also referred to as "core promoter") as shown in SEQ ID NO: 5:

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  1 gcaccaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat
 51 tgacgcaaat gggcggtagg cgtgtacggt gggaggtcta tataagcaga
101 gctcgtttag tgaaccg
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SEQ ID NO: 5 includes at its 3'-end an additional guanosine ("G") nucleotide, which represents the transcriptional start site.

Furthermore, the promoter regulatory sequences of an expression cassette usually comprise an "enhancer" sequence. The term "enhancer", as used herein, denotes sequence elements that augment, improve or ameliorate transcription of a nucleic acid sequence irrespective of its location and orientation in relation to the nucleic acid sequence to be expressed. An enhancer may enhance transcription from a single promoter or simultaneously from more than one promoter. As long as this functionality of improving transcription is retained or substantially retained (e.g., at least 70%, at least 80%, at least 90% or at least 95% of wild-type activity, that is, activity of a full-length sequence), any truncated, mutated or otherwise modified variants of a (naturally occurring) wild-type enhancer sequence are also within the above definition.

The expression vectors of the present invention comprise (as part of an expression cassette) in their respective first (chimeric) promoter regulatory sequence an upstream region (sequence) and/or enhancer sequence being derived from human cytomegalovirus (hCMV) and/or simian cytomegalovirus (sCMV). In other words, the promoter regulatory sequence may comprise an upstream region and/or enhancer sequence being solely derived from hCMV or an upstream region and/or enhancer sequence being solely derived from sCMV or a chimeric upstream region and/or enhancer sequence composed of sequences derived from hCMV and sCMV. Within the promoter regulatory sequence, the upstream region and/or enhancer sequences are located 5' (i.e. "upstream") of the mCMV or the hCMV (core) promoter sequences and are in operable linkage to the murine or the human promoter sequence. Typically, the enhancer sequences are arranged in the same orientation as the promoter sequences. However, in specific embodiments, the upstream region and/or enhancer sequences are arranged in reverse orientation in relation to the promoter sequences.

Generally, any hCMV and/or sCMV sequences can be employed as upstream region and/or enhancer sequence. Preferably, sequences of the hCMV and/or sCMV immediate early (IE) genes, such as hCMV IE1, hCMV IE2, sCMV IE1, and sCMV IE2 (Meier, J.L. and Stinski,

M.F. (1996) *Intervirology* **39**, 331-342; Kim, G.Y. et al. (2011) *Biotechnol. Lett.* **33**, 1319-1326), are employed, with the hCMV and/or sCMV IE1 enhancer sequences being particularly preferred.

Hence, the expression vectors of the present invention comprise a first promoter regulatory sequence which is chimeric in that it comprises mCMV promoter sequences or hCMV promoter sequences in combination with hCMV and/or sCMV upstream region and/or enhancer sequences. In particular embodiments, the promoter sequence is derived from the mCMV IE1 promoter; and/or the upstream region and/or enhancer sequence is derived from the hCMV and/or sCMV IE1 enhancer. In other particular embodiments, the promoter sequence is derived from the mCMV IE1 promoter; and/or the upstream region and/or enhancer sequence is derived from the hCMV and/or sCMV IE2 enhancer. In yet other particular embodiments, the promoter sequence is derived from the hCMV IE1 promoter; and/or the upstream region and/or enhancer sequence is derived from the hCMV and/or sCMV IE1 enhancer. In yet other particular embodiments, the promoter sequence is derived from the hCMV IE2 promoter; and/or the upstream region and/or enhancer sequence is derived from the hCMV and/or sCMV IE2 enhancer.

These and additional hCMV and/or sCMV sequences are well known in the art and can be easily derived from the hCMV and sCMV genomes deposited in the NCBI Viral Genomes database under accession nos. X17403.1 and U38308.1, respectively

In further preferred embodiments, the sequence of the upstream region comprises the nucleotide sequence of 452 bp in length, as shown in SEQ ID NO: 6, being derived from the sCMV IE1 enhancer region.

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  1 gaccatagcc aattcaatat ggcgtatatg gactcatgcc aattcaatat
 51 ggtggatctg gacctgtgcc aattcaatat ggcgtatatg gactcgtgcc
101 aattcaatat ggtggatctg gaccccagcc aattcaatat ggcggacttg
151 gcaccatgcc aattcaatat ggcggacctg gcactgtgcc aactggggag
201 gggtctactt ggcacggtgc caagtttgag gaggggtctt ggccctgtgc
251 caagtccgcc atattgaatt ggcatggtgc caataatggc ggccatattg
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301 gctatatgcc aggatcaata tataggcaat atccaatatg gccctatgcc
351 aatatggcta ttggccaggt tcaatactat gtattggccc tatgccatat
401 agtattccat atatgggttt tcctattgac gtagatagcc cctcccaatg
451 gg
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In some of these preferred embodiments, the nucleic acid sequence of SEQ ID NO: 6 is an integral part of a longer sequence element being derived from the sCMV IE1 region (cf., e.g., SEQ ID NO: 11). In some other of these preferred embodiments, the sequence element being derived from the sCMV IE1 region has the sequence of SEQ ID NO: 6, which is present in combination with a further sequence element being derived from the hCMV IE1 region (cf., e.g., SEQ ID NO: 10).

In particularly preferred embodiments, the chimeric promoter regulatory sequence comprises a nucleotide sequence being selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

The chimeric promoter regulatory sequence according to SEQ ID NO: 7 (herein also referred to as "construct 1") has a total length of 1074 bp and is composed of a 582 bp hCMV IE1 upstream and mCMV IE1 enhancer sequence (shown in italics) and a 492 bp mCMV IE1 promoter sequence (also shown as SEQ ID NO: 3).

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   1 ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gttttgagat
  51 ttctgtcgcc gactaaattc atgtcgcgcg atagtggtgt ttatcgccga
 101 tagagatggc gatattggaa aaatcgatat ttgaaaatat ggcatattga
 151 aaatgtcgcc gatgtgagtt tctgtgtaac tgatatcgcc atttttccaa
 201 aagtgatttt tgggcatacg cgatatctgg cgatagcgct tatatcgttt
 251 acgggggatg gcgatagacg actttggtga cttgggcgat tctgtgtgtc
 301 gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg
 351 ccgatagagg cgacatcaag ctggcacatg gccaatgcat atcgatctat
 401 acattgaatc aatattggcc attagccata ttattcattg gttatatagc
 451 ataaatcaat attggctatt ggccattgca tacgttgtat ccatatcata
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501 *atatgtacat ttatattggc tcatgtccaa cattaccgcc atgttgacat*
551 *tgattattga ctagttatta atagtaatca attactgagt* cattagggac
601 tttccaatgg gttttgccca gtacataagg tcaatagggg tgaatcaaca
651 ggaaagtccc attggagcca agtacactga gtcaataggg actttccatt
701 gggttttgcc cagtacaaaa ggtcaatagg gggtgagtca atgggttttt
751 cccattattg gcacgtacat aaggtcaata ggggtgagtc attgggtttt
801 tccagccaat ttaattaaaa cgccatgtac tttcccacca ttgacgtcaa
851 tgggctattg aaactaatgc aacgtgacct ttaaacggta ctttcccata
901 gctgattaat gggaaagtac cgttctcgag ccaatacacg tcaatgggaa
951 gtgaaagggc agccaaaacg taacaccgcc ccggttttcc cctggaaatt
1001 ccatattggc acgcattcta ttggctgagc tgcgttctac gtgggtataa
1051 gaggcgcgac cagcgtcggt accg

The chimeric promoter regulatory sequence according to SEQ ID NO: 8 (herein also referred to as "construct 2") has a total length of 1128 pb and is composed of a 1026 bp sequence including the hCMV IE1 upstream sequence and the hCMV IE1 enhancer sequence (shown in italics) and a 102 bp mCMV IE1 "core" promoter sequence (also shown as SEQ ID NO: 4)

1 *ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gttttgagat*
51 *ttctgtcgcc gactaaattc atgtcgcgcg atagtggtgt ttatcgccga*
101 *tagagatggc gatattggaa aaatcgatat ttgaaaatat ggcatattga*
151 *aaatgtcgcc gatgtgagtt tctgtgtaac tgatatcgcc atttttccaa*
201 *aagtgatttt tgggcatacg cgatatctgg cgatagcgct tatatcgttt*
251 *acgggggatg gcgatagacg actttggtga cttgggcgat tctgtgtgtc*
301 *gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg*
351 *ccgatagagg cgacatcaag ctggcacatg gccaatgcat atcgatctat*
401 *acattgaatc aatattggcc attagccata ttattcattg gttatatagc*
451 *ataaatcaat attggctatt ggccattgca tacgttgtat ccatatcata*
501 *atatgtacat ttatattggc tcatgtccaa cattaccgcc atgttgacat*
551 *tgattattga ctagttatta atagtaatca attacggggt cattagttca*
601 *tagcccatat atggagttcc gcgttacata acttacggta aatggcccgc*
651 *ctggctgacc gcccaacgac ccccgcccat tgacgtcaat aatgacgtat*

701 *gttcccatag taacgccaat agggactttc cattgacgtc aatgggtgga*
751 *gtatttacgg taaactgccc acttggcagt acatcaagtg tatcatatgc*
801 *caagtacgcc ccctattgac gtcaatgacg gtaaatggcc cgcctggcat*
851 *tatgcccagt acatgacctt atgggacttt cctacttggc agtacatcta*
901 *cgtattagtc atcgctatta ccatggtgat gcggttttgg cagtacatca*
951 *atgggcgtgg atagcggttt gactcacggg gatttccaag tctccacccc*
1001 *attgacgtca atgggagttt gttttgacac* cgccccggtt ttcccctgga
1051 aattccatat tggcacgcat tctattggct gagctgcgtt ctacgtgggt
1101 ataagaggcg cgaccagcgt cggtaccg

The chimeric promoter regulatory sequence according to SEQ ID NO: 9 (herein also referred to as "construct 3") has a total length of 509 pb and is composed of a 407 bp hCMV IE1 enhancer sequence (shown in italics) and a 102 bp mCMV IE1 "core" promoter sequence (also shown as SEQ ID NO: 4).

1 *cgcgttacat aacttacggt aaatggcccg cctggctgac cgcccaacga*
51 *cccccgccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa*
101 *tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc*
151 *cacttggcag tacatcaagt gtatcatatg ccaagtacgc cccctattga*
201 *cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag tacatgacct*
251 *tatgggactt tcctacttgg cagtacatct acgtattagt catcgctatt*
301 *accatggtga tgcggttttg gcagtacatc aatgggcgtg gatagcggtt*
351 *tgactcacgg ggatttccaa gtctccaccc cattgacgtc aatgggagtt*
401 *tgttttgaca* ccgccccggt tttcccctgg aaattccata ttggcacgca
451 ttctattggc tgagctgcgt tctacgtggg tataagaggc gcgaccagcg
501 tcggtaccg

The chimeric promoter regulatory sequence according to SEQ ID NO: 10 (herein also referred to as "construct 4") has a total length of 961 pb and is composed of a 452 bp sCMV IE1 upstream sequence (shown in bold; SEQ ID NO: 6), a 407 bp hCMV IE1 enhancer sequence (shown in italics) and a 102 bp mCMV IE1 "core" promoter sequence (also shown as SEQ ID NO: 4).

1 **gaccatagcc aattcaatat ggcgtatatg gactcatgcc aattcaatat**
51 **ggtggatctg gacctgtgcc aattcaatat ggcgtatatg gactcgtgcc**
101 **aattcaatat ggtggatctg gaccccagcc aattcaatat ggcggacttg**
151 **gcaccatgcc aattcaatat ggcggacctg gcactgtgcc aactggggag**
201 **gggtctactt ggcacggtgc caagtttgag gaggggtctt ggccctgtgc**
251 **caagtccgcc atattgaatt ggcatggtgc caataatggc ggccatattg**
301 **gctatatgcc aggatcaata tataggcaat atccaatatg gccctatgcc**
351 **aatatggcta ttggccaggt tcaatactat gtattggccc tatgccatat**
401 **agtattccat atatgggttt tcctattgac gtagatagcc cctcccaatg**
451 **gg***cgcgttac ataacttacg gtaaatggcc cgcctggctg accgcccaac*
501 *gacccccgcc cattgacgtc aataatgacg tatgttccca tagtaacgcc*
551 *aatagggact ttccattgac gtcaatgggt ggagtattta cggtaaactg*
601 *cccacttggc agtacatcaa gtgtatcata tgccaagtac gccccctatt*
651 *gacgtcaatg acggtaaatg gcccgcctgg cattatgccc agtacatgac*
701 *cttatgggac tttcctactt ggcagtacat ctacgtatta gtcatcgcta*
751 *ttaccatggt gatgcggttt tggcagtaca tcaatgggcg tggatagcgg*
801 *tttgactcac ggggatttcc aagtctccac cccattgacg tcaatgggag*
851 *tttgttttga* caccgccccg gttttccct ggaaattcca tattggcacg
901 cattctattg gctgagctgc gttctacgtg ggtataagag gcgcgaccag
951 cgtcggtacc g

The chimeric promoter regulatory sequence according to SEQ ID NO: 11 (herein also referred to as "construct 5") has a total length of 909 bp and is composed of a 807 bp sequence including elements of the sCMV IE1 upstream region and the sCMV IE1 enhancer sequence (shown in bold; SEQ ID NO: 6 being underlined) and a 102 bp mCMV IE1 "core" promoter sequence (shown as SEQ ID NO: 4).

1 **gaccatagcc aattcaatat ggcgtatatg gactcatgcc aattcaatat**
51 **ggtggatctg gacctgtgcc aattcaatat ggcgtatatg gactcgtgcc**
101 **aattcaatat ggtggatctg gaccccagcc aattcaatat ggcggacttg**
151 **gcaccatgcc aattcaatat ggcggacctg gcactgtgcc aactggggag**
201 **gggtctactt ggcacggtgc caagtttgag gaggggtctt ggccctgtgc**

251 **caagtccgcc atattgaatt ggcatggtgc caataatggc ggccatattg**
301 **gctatatgcc aggatcaata tataggcaat atccaatatg gccctatgcc**
351 **aatatggcta ttggccaggt tcaatactat gtattggccc tatgccatat**
401 **agtattccat atatgggttt tcctattgac gtagatagcc cctcccaatg**
451 **ggcggtccca tataccatat atggggcttc ctaataccgc ccatagccac**
501 **tcccccattg acgtcaatgg tctctatata tggtctttcc tattgacgtc**
551 **atatgggcgg tcctattgac gtatatggcg cctcccccat tgacgtcaat**
601 **tacggtaaat ggcccgcctg gctcaatgcc cattgacgtc aataggacca**
651 **cccaccattg acgtcaatgg gatggctcat tgcccattca tatccgttct**
701 **cacgcccccc attgacgtca atgacggtaa atggcccact tggcagtaca**
751 **tcaatatcta ttaatagtaa cttggcaagt acattactat tggaagtacg**
801 **ccagggt**aca ccgccccggt tttcccctgg aaattccata ttggcacgca
851 ttctattggc tgagctgcgt tctacgtggg tataagaggc gcgaccagcg
901 tcggtaccg

The chimeric promoter regulatory sequence according to SEQ ID NO: 12 (herein also referred to as "construct 6") has a total length of 976 bp and is composed of a 452 bp sCMV IE1 upstream region (shown in bold), a 407 bp hCMV IE1 enhancer sequence (shown in italics), and a 117 bp hCMV "core" promoter sequence (shown as SEQ ID NO: 5).

1 **gaccatagcc aattcaatat ggcgtatatg gactcatgcc aattcaatat**
51 **ggtggatctg gacctgtgcc aattcaatat ggcgtatatg gactcgtgcc**
101 **aattcaatat ggtggatctg gaccccagcc aattcaatat ggcggacttg**
151 **gcaccatgcc aattcaatat ggcggacctg gcactgtgcc aactggggag**
201 **gggtctactt ggcacggtgc caagtttgag gaggggtctt ggccctgtgc**
251 **caagtccgcc atattgaatt ggcatggtgc caataatggc ggccatattg**
301 **gctatatgcc aggatcaata tataggcaat atccaatatg gccctatgcc**
351 **aatatggcta ttggccaggt tcaatactat gtattggccc tatgccatat**
401 **agtattccat atatgggttt tcctattgac gtagatagcc cctcccaatg**
451 **gg***cgcgttac ataacttacg gtaaatggcc cgcctggctg accgcccaac*
501 *gacccccgcc cattgacgtc aataatgacg tatgttccca tagtaacgcc*
551 *aatagggact ttccattgac gtcaatgggt ggagtattta cggtaaactg*

601 *cccacttggc agtacatcaa gtgtatcata tgccaagtac gccccctatt*
651 *gacgtcaatg acggtaaatg gcccgcctgg cattatgccc agtacatgac*
701 *cttatgggac tttcctactt ggcagtacat ctacgtatta gtcatcgcta*
751 *ttaccatggt gatgcggttt tggcagtaca tcaatgggcg tggatagcgg*
801 *tttgactcac ggggatttcc aagtctccac cccattgacg tcaatgggag*
851 *tttgttttgg* caccaaaatc aacgggactt tccaaaatgt cgtaacaact
901 ccgccccatt gacgcaaatg ggcggtaggc gtgtacggtg ggaggtctat
951 ataagcagag ctcgtttagt gaaccg

The chimeric promoter regulatory sequence according to SEQ ID NO: 13 (herein also referred to as "construct 7") has a total length of 924 bp and is composed of a 807 bp sCMV IE1 upstream region and enhancer sequence (shown in bold; the portion also included in "construct 4" being underlined) and a 117 bp hCMV "core" promoter (shown as SEQ ID NO: 5).

1 **gaccatagcc aattcaatat ggcgtatatg gactcatgcc aattcaatat**
51 **ggtggatctg gacctgtgcc aattcaatat ggcgtatatg gactcgtgcc**
101 **aattcaatat ggtggatctg gaccccagcc aattcaatat ggcggacttg**
151 **gcaccatgcc aattcaatat ggcggacctg gcactgtgcc aactggggag**
201 **gggtctactt ggcacggtgc caagtttgag gaggggtctt ggccctgtgc**
251 **caagtccgcc atattgaatt ggcatggtgc caataatggc ggccatattg**
301 **gctatatgcc aggatcaata tataggcaat atccaatatg gccctatgcc**
351 **aatatggcta ttggccaggt tcaatactat gtattggccc tatgccatat**
401 **agtattccat atatgggttt tcctattgac gtagatagcc cctcccaatg**
451 **ggcggtccca tataccatat atggggcttc ctaataccgc ccatagccac**
501 **tcccccattg acgtcaatgg tctctatata tggtctttcc tattgacgtc**
551 **atatgggcgg tcctattgac gtatatggcg cctcccccat tgacgtcaat**
601 **tacggtaaat ggcccgcctg gctcaatgcc cattgacgtc aataggacca**
651 **cccaccattg acgtcaatgg gatggctcat tgcccattca tatccgttct**
701 **cacgcccccc attgacgtca atgacggtaa atggcccact tggcagtaca**
751 **tcaatatcta ttaatagtaa cttggcaagt acattactat tggaagtacg**
801 **ccagggt**gca ccaaaatcaa cgggactttc caaaatgtcg taacaactcc

851 gccccattga cgcaaatggg cggtaggcgt gtacggtggg aggtctatat

901 aagcagagct cgtttagtga accg

In specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE2 upstream region and/or enhancer sequence and a mCMV IE2 promoter sequence. In other specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE2 upstream region and/or enhancer sequence and a hCMV IE2 promoter sequence In yet other specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE1 upstream region and/or enhancer sequence and a mCMV IE2 promoter sequence. In yet other specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE1 upstream region and/or enhancer sequence and a hCMV IE2 promoter sequence. In yet other specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE2 upstream region and/or enhancer sequence and a mCMV IE1 promoter sequence. In yet other specific embodiments, the chimeric promoter regulatory sequence is composed of a hCMV and/or sCMV IE2 upstream region and/or enhancer sequence and a hCMV IE1 promoter sequence.

The 3'-regulatory sequences of an "expression cassette" as defined herein typically encompass regulatory elements involved in transcriptional termination, polyadenylation, or the like. If, however, these termination sequences are not satisfactory functional in a particular host cell, then they may be substituted with signals functional in that cell.

Further regulatory elements comprised in such an "expression cassette" include *inter alia* *i*nternal ribosome entry sites (IRES; allowing for the expression of "polycistronic" nucleic acid sequences) as well as translated signal sequences for targeting the native polypeptide to a specific compartment of a host cell. Exemplary signal sequences suitable for CHO cells are disclosed, for example, in WO 2008/148519 A2. The skilled person is also well aware of all these regulatory elements and how to select such elements suitable for the expression of a nucleic acid sequence in a particular cellular setting.

The expression vector of the present invention may be, e.g., a plasmid, cosmid, phagemid, artificial chromosome, or another vehicle commonly used in genetic engineering.

Such expression vectors typically include, aside from one or more "expression cassettes" encompassing the regulatory sequences described above, one or more multiple cloning sites in order to facilitate insertion and/or removal of nucleic acid sequences. The multiple cloning sites may be located upstream and downstream of the expression cassettes described above, thus allowing replacement of the entire cassette. Multiple cloning sites may also be located within such an expression cassette downstream of the promoter regulatory sequences and upstream of the 3'-regulatory sequences, thus allowing insertion or replacement of a nucleic acid sequence to be expressed. For stable transfections (cf. below), the expression vectors may further comprise recognition sequences for site-specific integrases or recombinases in order to facilitate recombination and stable integration in the genome of the host cell.

In addition, the expression vectors as defined herein typically comprise at least one origin of replication as well as control sequences derived from a species compatible with the host cell employed in order to ensure autonomous replication/episomal maintenance of the expression vector (in particular, for use in transient transfections; cf. below). Exemplary origins of replication in mammalian include the SV40 or and the EBV origin of replication. Specifically designed expression vectors (i.e. shuttle vectors) comprising more than one origin of replication allow the shuttling between different hosts, such as between bacterial and animal cells. Suitable origins of replication for prokaryotic cells include, for example, the ColE1 and M13 origins of replication.

Furthermore, an expression vector as defined herein may comprise one or more selection markers conferring a selectable phenotype on transfected cells. Suitable selection markers include *inter alia* the hygromycin B phosphatase gene, the thymidine kinase gene, the ornithine decarboxylase gene, the dihydrofolate reductase gene, and the glutamine synthase gene. Preferably, the glutamine synthase (GS) gene is employed (Cockett, D.K. et al. (1990) *Bio/Technology* **8**, 662-667; Bebbington, C.R. et al. (1992) *Bio/Technology* **10**:169-175;).

Numerous methods that can be used to design and/or modify recombinant expression vectors are well established in the art (see, e.g., Sambrook, J., and Russel, D.W. (2001), *Molecular cloning: A laboratory manual (3rd Ed.)* Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press; Ausubel, F.M. et al. (2001) *Current Protocols in Molecular Biology*, Wiley & Sons, Hoboken, NJ, USA). Large numbers of suitable mammalian expression vectors are also commercially available and well known to the skilled person who is also able to determine which vectors are suitable for expressing a nucleic acid molecule of interest in a given setting. Examples of such vectors include *inter alia* pcDNA3, pFRT, pTARGET, pSV2-dhfr as well as derivatives of the vectors pRY42 (SEQ ID NO: 1) and pRY57 (SEQ ID NO: 2) described herein below.

The nucleic acid sequences to be expressed by employing the expression vectors of the invention may be monocistronic (i.e. encode a single polypeptide or protein including fusion proteins) or polycistronic (i.e. encode two or more individual polypeptides or proteins).

In particular embodiments, the expression vector comprises a single (i.e. first) chimeric promoter regulatory sequence being operably linked to a (first) nucleic acid sequence to be expressed,

In other particular embodiments, the expression vector further comprises a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is identical to the first chimeric promoter regulatory sequence. For example, the first and second chimeric promoter regulatory sequences are composed of an hCMV/sCMV IE1 upstream region and/or enhancer sequence in combination with a mCMV IE1 or hCMV IE1 promoter sequence.

In alternative particular embodiments, the expression vector further comprises a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is different from the first chimeric promoter regulatory sequence. For example, the first chimeric promoter regulatory sequence is composed of an hCMV and/or sCMV IE2 upstream region and/or enhancer sequence and a mCMV IE2 or a hCMV IE2 promoter sequence, whereas

the second chimeric promoter regulatory sequence is composed of an hCMV and/or sCMV IE1 upstream region and/or enhancer sequence and a mCMV IE1 or a hCMV IE1 promoter sequence.

In specific embodiments, the expression vector comprises a third chimeric promoter regulatory sequence being operably linked to a third nucleic acid sequence to be expressed, wherein the third chimeric promoter regulatory sequence may be identical to the first and/or second promoter regulatory sequence or may be different from both the first and second chimeric promoter regulatory sequences. In further specific embodiments, the expression vector comprises more than three chimeric promoter regulatory sequences.

The nucleic acid sequences to be expressed by employing the expression vectors of the present invention may encode any polypeptides or proteins of interest, in particular polypeptides or proteins having diagnostic or therapeutic applicability, such as *inter alia* growth factors, cytokines (interferons, interleukins), hormones, tyrosine kinases, receptors (GPCRs), integrins, transcription factors, blood clotting factors, single-chain antibodies, antibody fragments or antibody-like molecules (anticalins), and the like.

In case of expression vectors as defined herein comprising two chimeric promoter regulatory sequences (as part of expression cassettes), the first and second nucleic acid sequences to be expressed encode different polypeptides (or proteins). In specific embodiments, the different polypeptides represent subunits of a dimeric or multimeric protein, such as *inter alia* homomeric or heteromeric receptor molecules, peptide hormones, DNA/RNA polymerases, hemoglobins, vaccines, and the like.

In particularly preferable embodiments, the dimeric or multimeric protein is a "classical" antibody molecule comprising the light chain as the first subunit and the heavy chain as the second subunit. The antibody molecule may be a naturally occurring or a genetically engineered antibody, either a full-length antibody or a truncated variant thereof (such as Fab fragments, Fab' fragments, $F(ab')_2$ fragments). IgG immunoglobulin antibodies are particularly preferred. Depending on the specific application, the antibody molecules may be chimeric (e.g., murine/human), humanized or fully human

In other embodiments employing expression vectors comprising two chimeric promoter regulatory sequences, the first and second nucleic acid sequence to be expressed encode a "target protein" to be analyzed and a corresponding reporter protein (such as green fluorescent protein, luciferase, alkaline phosphatase, β-galactosidase, and horseradish peroxidase) for monitoring, e.g., cellular localization or functional activity of the target protein.

When using two (or more) chimeric promoter regulatory sequences exhibiting different rates of gene expression it may be possible to produce certain molar ratios of the corresponding proteins of interest.

In another aspect, the present invention relates to a mammalian host cell transfected with an expression vector as defined herein above.

Suitable host cells include any type of mammalian cells, the cells being of human or non-human origin. Mammalian cells of non-human origin include *inter alia* cells derived from mouse, rat, hamster, rabbit, cat, dog, pig, cow, horse or monkey.

Suitable mammalian host cells include immortalized cell lines such as human Hela, MRC5 fibroblasts, 983M melanoma, HEK293, H9, MCF7, and Jurkat cells; MDCK canine kidney cells; RF cultured rat lung fibroblasts isolated from Sprague-Dawley rats; murine NIH3T3, C127, P815 mastocytoma, MT1A2 mammary adenocarcinoma, and L cells; simian COS1 and COS7 cells, quail QC1-3 cells; and Chinese hamster ovary (CHO) cells or cell lines.

In preferred embodiments, the host cells employed are CHO cells or CHO cell lines.

Suitable CHO cell lines include *inter alia* CHO KI (Tjio, J.T. and Puck, T.T. (1958) *J. Exp. Med.***108**, 945-955), CHO pro3-, CHO DG44, CHO P12, dhfr-negative DUK-B11 (Urlaub, G. and Chasin L.A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4216-4220), and particularly CHOK1SV (Lonza Ltd. Basel, Switzerland). CHOK1SV is a suspension, protein-free adapted CHOK1 derivative utilizing the glutamine synthetase (GS) gene expression system: positive transfectants were obtained under dual selection of methionine sulfoximine and glutamine-free media.

All these host cells or cell lines may be obtained from depositories such as the American Type Culture Collection (Manassas, VA, USA) or the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) as well as from various commercial suppliers. Also within the present invention are primary mammalian cells, that is, cells directly obtained from an organism (at any developmental stage including *inter alia* blastocytes, embryos, larval stages, and adults). Examples of suitable primary cells comprise cardiomyocytes, primary hepatocytes, fibroblasts, neuronal cells, as well as stem cells. Also within the present invention are immortalized stable cell lines derived from primary cells.

In some embodiments, the host cell of the present invention constitutes a part of a multi-cellular organism. In other words, the invention also relates to transgenic mammalian organisms comprising at least one host cell as defined herein.

Within the present invention, the expression vector introduced may be propagated and maintained in the host cell as an independent genetic unit (i.e. episomally) (herein also referred to as "transient transfection") or vector fragments may become stably integrated into the host cell's genome (herein also referred to herein as "stable transfection"). Such recombination may either occur at random positions of the genome by non-homologous recombination or at specific positions of the genome by homologous recombination or via site-specific integrases. Preferably, the vector fragments (including the heterologous nucleic acid sequences to be expressed) become integrated in the host cell's genome as a single copy.

For introducing the expression vectors as defined herein into a mammalian host cell any transfection technique may be employed that is appropriate for the particular cell type employed. Numerous transfection methods are well established in the art including *inter alia* electroporation, calcium phosphate co-precipitation, chemical transfection (e.g., cyclodextrin, DEAE-dextran, polyethylenimine), lipofection, magnetofection, and "gene gun" (see, e.g., Sambrook, J., and Russel, D.W. (2001), *supra*; Ausubel, F.M. et al. (2001), *supra*).

In yet another aspect, the present invention relates to a method for heterologous expression of a nucleic acid sequence of interest in a mammalian host cell, comprising:

(i) transfecting the mammalian host cell with an expression vector as defined herein above; and

(ii) culturing the transfected mammalian host cell under conditions allowing the expression of the nucleic acid sequence of interest.

In other words, the present invention is also directed to a process for the recombinant (i.e. heterologous) production of polypeptides or proteins of interest in mammalian host cells being transfected with an expression vector as defined herein that comprise the corresponding nucleic acid sequences encoding said polypeptides or proteins. Transfection can be performed with a single expression vector or, as a co-transfection, with two or more different expression vectors.

As already outlined above, numerous methods are available for the transient or stable transfection of mammalian host cell. In preferred embodiments, the transfection is stable transfection in order to establish cells or cell lines the continuously express the heterologous nucleic acid sequences encoding the polypeptides or proteins of interest.

In some embodiments, the method further comprises the step of harvesting (and optionally purifying) the recombinant polypeptides or proteins produced. Depending on the nature of said polypeptides or protein they may become secreted into the cell culture supernatant, integrated in membrane of the host cell, or remain in an intracellular compartment.

Typically, if a unicellular mammalian host cell is employed the person skilled in the art can revert to a variety of cell culture conditions which allow the expression of the nucleic acid sequence of interest. Conveniently, the polypeptides or proteins produced are harvested (and optionally purified) from the culture medium, lysates or extracts of the cultured cells or from isolated (biological) membranes by established techniques, such as *inter alia* fractionated precipitation with salts or organic solvents, ion exchange chromatography, gel chromatography, size exclusion chromatography, HPLC, affinity chromatography (see, e.g., Sambrook, J., and Russel, D.W. (2001), *supra*). In case, the host cell is part of a multi-cellular organism, a fraction of these cells may serve as source for isolating the peptide of the invention.

Appropriate culture media and conditions for the above-described host cells are well known in the art (cf., e.g., Fresney, R.I. (2000) *Culture of Animal cells. A manual* (*4th Ed.)* Wiley-Liss, New York). Depending on the specific growth requirements of the host cell employed, mammalian cell culture can be performed, e.g., in RPMI 1640 medium, Ham's F12 medium or DMEM (Dulbecco's Modified Eagle Medium). Alternatively, a growth medium with a reduced serum concentration, such as OptiMEM, may be used. The media may optionally be supplemented with 10% (v/v) FCS (fetal calf serum), various growth factors, amino acids, antibiotics, and other additives Cell culture media specially adapted for CHO cells are described in, e.g., EP 0 481 791 B1 and EP 1 525 320 B1. The transfected mammalian host cells may be incubated at 37°C in a 5% CO_2, water saturated atmosphere. The respective growth media, kits, and reagents are commercially available from various suppliers.

In a further aspect, the present invention relates to the use of an expression vector as defined herein above for the heterologous expression of a nucleic acid sequence of interest in a mammalian host cell. Preferably, the nucleic acid sequence of interest may encode a polypeptide or protein intended for diagnostic or therapeutic applications.

In preferred embodiments, the expression vector is used for the concomitant expression of two or more nucleic acid sequences of interest that are inserted into the expression vector under the control of separate chimeric promoter regulatory sequences. For example, an expression vector may be used for the expression of a gene of interest along with a reporter gene for monitoring cellular targeting and/or functionality of the gene of interest.

Particularly preferably, the expression vector is used for the concomitant expression of two or more nucleic acids sequences of interest that encode subunits of a dimeric or multimeric protein, for example, light and heavy chains of an antibody molecule or subunits of a vaccine. By employing different chimeric promoter regulatory sequences resulting in different gene expression rates an expression vector as defined herein may be used for the expression of two or more nucleic acid sequences of interest in a particular (molar) ratio.

In a specific embodiment, the expression vectors as defined herein are used as medicaments (or as parts of a medicament or kit-of-parts) for gene therapy.

The invention is further described by the figures and the following examples, which are solely for the purpose of illustrating specific embodiments of this invention, and are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

Rationale:

Seven different chimeric promoter regulatory sequences, as specified in the claims, were generated based on sequences from the murine, human and simian cytomegalovirus (mCMV, hCMV, and sCMV) genomes (see **Table 1**, **Figure 3**). These constructs were analyzed for their efficacy in controlling heterologous gene expression of the light and heavy chains (LC and HC) of a monoclonal antibody in Chinese hamster ovary (CHO) cells.

Example 1: Vector construction

Gene synthesis was used to generate the seven different chimeric promoter regulatory constructs employed herein (i.e. constructs "1-7"). The constructs were provided ready for cloning into the "empty" targeting vector pRY42 (cf. **Figure 1**, SEQ ID NO: 1), in order to replace the original murine cytomegalovirus (mCMV) promoters (SEQ ID NO: 3) contained therein. Parent vector pRY42 comprises two expression cassettes, each under the control of a promoter regulatory sequence (originally derived from mCMV).

The chimeric constructs (cf. **Table 1**, **Figure 3**, SEQ ID NO: 7 to SEQ ID NO: 13) were synthesized to include additional DNA sequences at the 5' and 3' ends flanking the promoter regulatory sequences, thus allowing for the incorporation of endonuclease restriction sites (i.e. also present in pRY42) in order to facilitate exchange of nucleic acid fragments.

Table 1 illustrates the various sequence elements comprised in the chimeric promoter regulatory constructs 1-7 employed herein. Shown are the respective lengths and genetic locations of the individual regulatory sequences, as indicated in the NCBI Viral Genomes database (http://www.ncbi.nlm.nih.gov/genomes/GenomesHome; Bao, Y. et al. (2004) *J.*

Virol. **78**, 7291-7298). Notably, all mCMV or hCMV promoter sequences employed herein include at their 3'-end an additional guanosine ("G") nucleotide, which represents the transcriptional start site.

Construct	sCMV length	NCBI viral genome accession no.	Coordinates	hCMV length	NCBI viral genome accession no.	Coordinates
1	-	-	-	582	X17403.1	174292-174873
2	-	-	-	1026	X17403.1	173848-174873
3	-	-	-	407	X17403.1	173848-174254
4	452	U38308.1	3873-4324	407	X17403.1	173848-174254
5	807	U38308.1	3873-4679	-	-	-
6	452	U38308.1	3873-4324	524	X17403.1	173731-174254
7	807	U38308.1	3873-4679	117	X17403.1	173731-173847

Construct	mCMV length	NCBI viral genome accession no.	Coordinates
1	492	U68299.1	182895-183386
2	102	U68299.1	182895-182996
3	102	U68299.1	182895-182996
4	102	U68299.1	182895-182996
5	102	U68299.1	182895-182996

The new vectors comprising chimeric constructs 1-7 were each constructed by a four way ligation reaction according to the scheme illustrated in **Table 2**. The chimeric promoter regulatory sequence for expression of the antibody light chain nucleic acid sequence was inserted 3' (i.e. "downstream") of the mutated FRT (Flippase Recognition Target) site (F5-mFRT) of pRY42, followed by an inter-chain fragment and the chimeric promoter regulatory sequence for expression of the antibody heavy chain nucleic acid sequence.

Ligation reactions were performed using the Rapid DNA Ligation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The resulting plasmid vectors were transformed into DH5α *E. coli* competent cells (Invitrogen/Life

Technologies GmbH; Darmstadt, Germany). Samples were plated onto Luria-Bertani (LB) agar supplemented with 50 µg/ml ampicillin and incubated over-night at 37°C. Single colonies were used to inoculate 500 ml shake flasks containing 200 ml LB liquid media plus 50 µg/ml ampicillin. Flasks were incubated over-night in a shaking incubator at 37°C, 200 rpm. Resultant cultures were used for preparation of plasmid DNA using a Nucleobond Maxi Kit (Macherey-Nagel GmbH, Düren, Germany) according to the manufacturer's instructions. Plasmids produced were verified by diagnostic restriction digest.

Table 2 shows the cloning scheme for generating chimeric promoter regulatory sequence constructs 1-5.

Construct	LC regulatory element	Inter-chain fragment (derived from pRY42)	HC regulatory element	Vector backbone (derived from pRY42)
1	AatII-PacI	PacI-EcoRI	EcoRI-XhoI	XhoI-AatII
2	PvuI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-PvuI
3	SspI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-SspI
4	SspI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-SspI
5	SspI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-SspI
6	SspI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-SspI
7	SspI-SacII	SacII-EcoRI	EcoRI-Bsu36I	Bsu36I-SspI

In a subsequent step, the LC and HC of IgG4 monoclonal antibody cB72.3 (Whittle, N. et al. (1987) *Protein Eng.* **1**, 499-505) were cloned into each of the seven vectors comprising chimeric constructs 1-7. The nucleic acid sequences encoding the antibody chains were derived from vector pRY57 (**Figure 2**, SEQ ID NO: 2) as EcoRI/HindIII (LC) and BamHI/NruI (HC) restriction fragments and cloned sequentially into the vectors for each promoter construct, using the same restriction endonucleases. Methods used were as described above. The resulting plasmid vectors were verified by diagnostic restriction digest and DNA sequencing of promoter regions, respectively.

Example 2: Transfection and cell line construction

A cell line derived from suspension adapted Chinese hamster ovary cell line CHOK1SV was used for all experiments (Lonza Ltd., Basel, Switzerland). In this cell line, nucleic acid expression constructs are inserted at a specific genomic locus of the host cell and in defined copy number by means of a site-specific integration (SSI) system.

Positive selection for integration is accomplished by functional restoration of a hygromycin B resistance cassette present at the SSI site. Integration of the expression construct into the host genome also removes a copy of the thymidine kinase gene, which converts the pro-drug ganciclovir into a toxic, phosphorylated nucleotide analogue. Thus, addition of hygromycin B and ganciclovir during cell line construction provides positive and negative selection pressures, respectively, for integration at the target genomic locus.

The host cell line was revived from cryopreserved vials and sub-cultured. All cell culture was performed in CD-CHO medium (Invitrogen/Life Technologies GmbH; Darmstadt, Germany). 48 h prior to transfection, cells were seeded in 30 ml CD-CHO medium, at a final concentration of 0.3 x 10^6 cells/ml.

On the day of transfection, 1.2 x 10^7 cells were pelleted and re-suspended in 1 ml CD-CHO before co-transfection with 45 µg of pOG44 plasmid (Invitrogen/Life Technologies GmbH; Darmstadt, Germany) and 5 µg of each targeting vector (comprising chimeric constructs 1-7, respectively) was performed using electroporation in a 0.4 cm Bio-Rad GenePulser Xcell cuvette (single pulse 300 V / 900 µF, time constant 12-16 msec). The pOG44 plasmid encompasses an expression cassette for the yeast FLP recombinase (flippase) required to facilitate recombination at the FRT sites present at the target locus. All transfection experiments were performed at least in duplicate.

Each electroporation sample was transferred to 20 ml of CD-CHO medium in a T75 flask (BD Biosciences, Heidelberg, Germany) and incubated in static mode at 36.5°C, in a humidified incubator (5% (v/v) CO_2 in air). 48 h post-transfection cells were pelleted (150 x g, 5 min) and re-suspended in 20 ml of CD-CHO medium containing 200 µg/ml hygromycin B (positive

selection). The culture was maintained in static mode and after 72 h the medium exchanged with fresh CD-CHO containing 200 µg/ml hygromycin B.

Subsequently, every 72 h the viable cell concentration was determined and the medium exchanged with 20 ml fresh CD-CHO containing 200 µg/ml hygromycin B and 3 µM ganciclovir (negative selection). Once the culture in the T75 flask reached a total cell concentration of 9 x 10^6 cells/ml, the culture was adjusted to a final volume of 30 ml CD-CHO containing 200 µg/ml hygromycin B and 3 µM ganciclovir. Each diluted culture was then transferred to an E125 shake flask.

Example 3: **Fed batch overgrow (FOG) suspension culture for determining the concentration of monoclonal antibody produced by using "promoter constructs 1-5"**

Fed batch overgrow (FOG) shake flask analysis was performed as described in international patent publication WO 2008/148519 A2. All FOG experiments for a given transfected cell suspension culture were performed at least in duplicate.

In brief, transfected cells were seeded at a concentration of 2 x 10^5 cells/ml in 250 ml shake flasks, each containing 50 ml of CM42/SPE growth medium (Lonza Ltd., Basel, Switzerland) and incubated at 37°C in a humidified orbital shaking incubator (5% (v/v) CO_2 in air) at 140 rpm. Cells were fed, starting on day 3 of the culture, with a feed consisting of mixture of amino acids and trace elements. Daily viabilities and viable cell concentrations were determined using a Cedex Automated Cell Viability Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Antibody concentration in the medium was determined by Protein A-HPLC on day 15 of culture (harvest of the "overgrown" cultures).

Example 4: **Determination of the concentration of monoclonal antibody produced by means of Protein A-HPLC (using "promoter constructs 1-5")**

The concentrations of the cB72.3 IgG4 monoclonal antibody (mAb) produced by the respective cell lines harboring the LC/HC gene expression cassettes under the control of the

different chimeric constructs 1-5 and secreted to the cell culture medium were determined by Protein A-high performance liquid chromatography (HPLC). Cell-free supernatants (passed through a 0.22 µm filter unit) were loaded onto a POROS Protein A Immunodetection Column (applied Biosystems Inc., Foster City, Ca, USA), connected to an Agilent 1200 HPLC. The column was washed and bound mAb was eluted by lowering the pH of the solvent.

The concentration of the mAb was determined by comparison to a standard curve generated with serial dilutions of MabSelect SuRe-purified (GE Healthcare GmbH, Freiburg, Germany) cB72.3 lgG4 (range of standard curve: 1025 ng/µl to 200 ng/µl).

The results of the above experiments are summarized in **Table 3** and **Figure 4**, respectively: for each of chimeric constructs employed herein, n = 4, representing duplicate FOG analyses for duplicate transfections, with the exception of construct 4, where n = 6 (duplicate FOG analyses for triplicate transfections) and pRY57 (original mCMV), where n = 8, with data points from experiments 1 and 2 being combined. The calculations of cell culture parameters were performed as described previously (Porter et al. (2010) *Biotechnol. Progr.* **26**, 1446-1454).

From **Figure 4**, it is evident that at the day of culture harvest (i.e. day 15), the use of any one of chimeric constructs no. 2-5 resulted in the production of higher antibody concentrations than with the use of the original mCMV promoter sequence (i.e. vector pRY57). The use of chimeric construct no. 1 also resulted in a higher antibody concentration than with the mCMV promoter (a factor of 1.31), even though the result does not reach statistical significance.

The best results were obtained with chimeric construct no.4 resulting in an about 2.88 times higher gene expression as compared to the mCMV promoter, followed by (in descending order) chimeric construct no. 5 (factor of about 2.54), chimeric construct no. 2 (factor of about 1.80), and chimeric construct no. 3 (factor of about 1.45).

Table 3 illustrates the growth rates and the amounts of mAb produced by the different transfected host cell lines employed herein (comprising chimeric promoter regulatory sequences/constructs 1-5).

Transfectant pool / FOG		Max. (10^6 cells/ml)	μ (1/h)	IVC (10^6 cells.h/ml)	ρ_P (pg/cell.h)	[mAb] (mg/l)
Experiment 1	mCMV	9.36 ± 1.02	0.0183 ± 0.0018	1523.35 ± 164.78	0.29 ± 0.02	439.74 ± 23.51
	Construct 1	8.5 ± 0.75	0.0206 ± 0.0013	1445.11 ± 70.55	0.46 ± 0.07	638.87 ± 110.18
	Construct 2	7.97 ± 0.41	0.0198 ± 0.0008	1409.29 ± 87.92	0.71 ± 0.06	883.10 ± 109.78
	Construct 3	11.17 ± 1.02	0.0191 ±0.0009	1873.58 ± 250.14	0.40 ± 0.03	707.98 ± 80.28
Experiment 2	mCMV	9.29 ± 0.82	0.0143 ± 0.0013	1790.34 ± 104.97	0.29 ± 0.05	539.31 ± 76.24
	Construct 4	10.39 ± 0.90	0.0165 ± 0.0023	1848.05 ± 306.29	0.73 ± 0.22	1408.44 ± 337.47
	Construct 5	11.58 ± 0.46	0.0139 ± 0.0028	2261.28 ± 50.84	0.46 ± 0.03	1244.15 ± 74.90

Legend: Max. – maximal viable cell concentration (10^6 cells/ml); μ – specific growth rate (1/h); IVC – time integral of viable cell concentration (10^6 cells·h/ml); ρ_P – specific production rate of the mAb (pg/cell·h);and [mAb] – concentration of the mAb produced at harvest (mg/l)

The above data show that the use of chimeric promoter regulatory sequences comprising sCMV and/or hCMV upstream region and/or enhancer elements in combination with mCMV promoter elements represent superior genetic tools for obtaining highly efficient heterologous gene expression systems for mammalian cells.

Example 5: **Batch overgrow (BOG) suspension culture for determining the concentration of monoclonal antibody produced by using "promoter constructs 6-7"**

Batch cultures were performed in vented E125 flasks containing 30 mL CD-CHO in suspension mode (Kühner 4 tier incubator, 36.5°C, 5% CO_2 in air (v/v) and 85% humidity (v/v)). In brief, transfected cells were seeded at a concentration of 2 x 10^5 cells/ml and viable cell concentrations were monitored throughout culture. Medium samples were taken at day seven of culture (high culture viability) for determination of the concentration of cB72.3 in medium using Protein A HPLC. All BOG experiments for a given transfected cell suspension culture were performed at least in duplicate.

Example 6: **Determination of the concentration of monoclonal antibody produced by means of Protein A-HPLC (using "promoter constructs 6-7")**

The concentrations of the cB72.3 IgG4 monoclonal antibody (mAb) produced by the respective cell lines harboring the LC/HC gene expression cassettes under the control of the different chimeric constructs 6-7 and secreted to the cell culture medium were determined by Protein A-high performance liquid chromatography (HPLC). Cell-free supernatants (passed through a 0.22 µm filter unit) were loaded onto a POROS Protein A Immunodetection Column (applied Biosystems Inc., Foster City, Ca, USA), connected to an Agilent 1100 HPLC. The column was washed and bound mAb was eluted by lowering the pH of the solvent.

The concentration of the mAb was determined by comparison to a standard curve generated with serial dilutions of MabSelect SuRe-purified (GE Healthcare GmbH, Freiburg, Germany) cB72.3 IgG4 (range of standard curve: 1025 ng/µl to 64 ng/µl).

The results of the above experiments are summarized in **Figure 5**: For mCMV and construct 7, n = 6 (duplicate batch analysis for triplicate transfections), and for construct 6, n = 4 (duplicate batch analysis for duplicate transfections).

From **Figure 5**, it is evident that at day 7 of culture the use of any one of chimeric constructs 6 and 7 resulted in the production of higher antibody concentrations than with the use of the original mCMV promoter sequence (i.e. vector pRY57).

The best results were obtained with chimeric promoter construct 6 resulting in about 2.27 times higher mAb productivities as compared to the mCMV promoter.

The above data show that the use of chimeric promoter regulatory sequences comprising sCMV and/or hCMV upstream region and/or enhancer elements in combination with hCMV core promoter elements represent superior genetic tools for obtaining highly efficient heterologous gene expression systems for mammalian cells.

The present invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments and optional features, modifications and variations of the inventions embodied therein may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

1. An expression vector for the heterologous expression of a nucleic acid sequence of interest in mammalian cells, the vector comprising a first chimeric promoter regulatory sequence being operably linked to a first nucleic acid sequence to be expressed, wherein the chimeric promoter regulatory sequence comprises:
   (i) a promoter sequence being derived from murine cytomegalovirus or from human cytomegalovirus and being operably linked to the transcriptional start site of the nucleic acid sequence to be expressed; and
   (ii) an upstream region and/or enhancer sequence being derived from human and/or simian cytomegalovirus, the upstream region and/or enhancer sequence being located 5' of and operably linked to the murine or the human promoter sequence, and

   wherein the chimeric promoter regulatory sequence comprises sequence elements being derived from at least two of the group consisting of murine cytomegalovirus, human cytomegalovirus and simian cytomegalovirus.

2. The expression vector of claim 1, wherein the promoter sequence is derived from the murine cytomegalovirus IE1 promoter; and/or wherein the upstream region and/or enhancer sequence is derived from the human and/or simian cytomegalovirus IE1 region.

3. The expression vector of claim 2, wherein the murine cytomegalovirus IE1 promoter sequence has the nucleotide sequence of SEQ ID NO: 4.

4. The expression vector of claim 1, wherein the promoter sequence is derived from the human cytomegalovirus IE1 promoter; and/or wherein the upstream region and/or enhancer sequence is derived from the human and/or simian cytomegalovirus IE1 region.

5. The expression vector of claim 4, wherein the murine cytomegalovirus IE1 promoter sequence has the nucleotide sequence of SEQ ID NO: 5.

6. The expression vector of any one of claims 1 to 5, wherein the upstream region and/or enhancer sequence comprises the nucleotide sequence of SEQ ID NO: 6 being derived from the simian cytomegalovirus IE1 region.

7. The expression vector of any one of claims 1 to 4, wherein chimeric promoter regulatory sequence comprises a nucleotide sequence being selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

8. The expression vector of any one of claims 1 to 7, further comprising a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is identical to the first chimeric promoter regulatory sequence.

9. The expression vector of any one of claims 1 to 7, further comprising a second chimeric promoter regulatory sequence being operably linked to a second nucleic acid sequence to be expressed, wherein the second chimeric promoter regulatory sequence is different from the first chimeric promoter regulatory sequence.

10. The expression vector of claim 8 or 9, wherein the first and second nucleic acid sequences to be expressed encode different polypeptides.

11. The expression vector of claim 10, wherein the different polypeptides represent subunits of a dimeric or multimeric protein.

12. The expression vector of claim 11, wherein dimeric or multimeric protein is an antibody molecule.

13. A mammalian host cell transfected with an expression vector as defined in any one of claims 1 to 12.

14. The mammalian host cell of claim 13, wherein the host cell is a CHO cell.

15. A method for heterologous expression of a nucleic acid sequence of interest in a mammalian host cell, comprising:
    (i) transfecting the mammalian host cell with an expression vector as defined in any one of claims 1 to 12; and
    (ii) culturing the transfected mammalian host cell under conditions allowing the expression of the nucleic acid sequence of interest.

16. The method of claim 15, wherein the transfection is stable transfection.

17. Use of an expression vector as defined in any one of claims 1 to 12 for the heterologous expression of a nucleic acid sequence of interest in a mammalian host cell.