

EXPRESSION SYSTEM WITH SAR ELEMENT FROM IFN α 2Cross-reference to Related Applications

This application claims the benefit of United States Provisional Patent Application
5 USSN 61/474,879 filed April 13, 2011, the entire contents of which is herein incorporated
by reference.

Field of the Invention

The present invention relates to polynucleotides for enhancing protein expression
and to expression systems comprising the polynucleotides.

Background of the Invention

10 High level and stable recombinant protein (r-protein) production in mammalian
cells is important for cost-effective biotherapeutic manufacturing. S/MARs (Scaffold/Matrix
Attachment Regions) are 70% AT-rich sequences, which are believed to play many
important roles in chromatin function. In addition to their structural function, S/MARs play
important roles in temporal and spatial organization of gene expression (Alvarez 2000;
15 Liu 1997). The inclusion of an S/MAR sequence in an expression vector can thus help
increase the level of expression and prevent silencing of the transgene (Phi-Van 1990;
Jenke 2004; Zahn-Zabal 2001; Kim 2004). MAR elements have been shown to work
either after integration into the host genome or as part of episomal vectors (Halweg 2005;
Girod 2005).

20 Genomic elements such as UCOE (from Millipore) or MAR (from Selexis) are
available and have proven to enhance expression level and stability when provided in *cis*
or in *trans* in expression vectors used to generate stable cell lines. There are many
S/MARs in the human genome that can be incorporated into expression vectors for
enhancing productivity and stability of clonal cell lines (Girod 2007). However, not all of
25 these sequences show beneficial effects (Sass 2005) and these sequences are very
often very large (1.5 to 4 kb) and as such not practical to incorporate in expression
vectors. There is a need to identify S/MAR sequences that are short (<1 kb) while still
efficient, but the only way to achieve this is through a trial-and-error approach.

Summary of the Invention

30 It has now been found that a short human genomic nucleotide sequence from the
SAR3 region of the human interferon α 2 gene permits enhanced expression stability in

the absence of drug selection and permits generation of stable clones or stable pools of cells for producing recombinant proteins. Although stable clones may be generated, the ability to generate stable pools reduces the burden of generating stable clones.

Thus, in one aspect of the present invention there is provided an isolated
5 polynucleotide comprising no more than 755 nucleotides and comprising at least 500 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.

In another aspect of the present invention there is provided an expression system for producing recombinant protein in a host cell comprising: a gene for encoding a protein of interest; nucleotide sequences for operation of the expression system; and, an
10 expression enhancer incorporated in *cis* in the expression system for enhancing expression of the gene, the expression enhancer comprising a nucleotide sequence from human interferon alpha2 upstream scaffold associated region 3 (SAR3) comprising no more than 755 nucleotides and comprising at least 500 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.

15 In another aspect of the present invention, there is provided a host cell comprising the expression system of the present invention.

In another aspect of the present invention, there is provided a method of producing recombinant protein comprising: transfecting a host cell with an expression system of the present invention; growing the host cells under conditions suitable for
20 expression of the gene to produce the protein of interest; and, recovering the protein of interest.

In another aspect of the present invention, there is provided a use of the polynucleotide of the present invention as an expression enhancer in *cis* in an expression system for producing recombinant protein.

25 The isolated polynucleotide comprises no more than 755 nucleotides and comprises at least 500 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1. Preferably, the isolated polynucleotide comprises at least 550, 600, 650, 700 or 750 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1. The isolated polynucleotide may be incorporated in *cis* in the expression
30 system for enhancing r-protein expression in host cells transfected with the expression system. Advantageously, the expression enhancement is realized even in the absence of drug selection.

The expression system comprises a gene for encoding a protein of interest. Some particular proteins of interest include, for example, monoclonal antibodies (e.g. trastuzumab), erythropoietins, interferons, vascular endothelial growth factors, stem cell growth factors, growth hormones, insulin-like growth factor binding proteins, regulatory proteins (e.g. cumate operator, tetracycline repressor, steroid hormone receptors, transmembrane receptors), etc. The amino acid sequences of proteins of interest and the nucleotide sequences of the genes that encode such proteins are generally known in the art.

The expression system may comprise any suitable vector, for example plasmid vectors, episomal vectors (e.g. oriP/EBV vectors), viral vectors (e.g. Bacman) and cosmid vectors. The type of vector utilized in the expression system will depend on the intended host cell, among other factors, which can be readily determined by one skilled in the art. The vector comprises various nucleotide sequences for operation of the expression system. Such nucleotide sequences include, for example, promoters, origins of replication (e.g. bacterial origin of replication (pMB1ori), Epstein-Barr Virus origin of replication (oriP)), leaders (e.g. adenovirus tripartite leader (TPL)), splice donors (SD), introns possibly including other enhancers (e.g. adenovirus major late promoter enhancer (Enh MLP)), splice acceptors (SA), selectable markers (e.g. antibiotic resistance genes), cloning sites (preferably multiple cloning sites) with restriction enzyme consensus sites (preferably multiple restriction enzyme consensus sites, e.g. EcoRV, Cla1, SfoI), and polyadenylation signals (e.g. rabbit beta-globin polyadenylation signal (pA)), among others. The expression system preferably comprises a plasmid vector.

Promoters are useful for controlling expression of various protein-encoding polynucleotides in the expression system. Strong or weak promoters may be used. Some promoters include, for example, cytomegalovirus (CMV) promoters, simian virus 40 promoter (SV40p), Elongation Factor 1 alpha-HTLV (EF1 α -HTLV) hybrid promoter and Rous sarcoma virus (RSV) promoter. Selectable markers allow the selection of positively transfected cells. Common selectable markers include, for example, antibiotic resistance genes such as puromycin and hygromycin B for eukaryotic cells, and ampicillin and kanamycin for prokaryotic cells.

The expression system of the present invention may be transfected into a host cell by any suitable method. Such methods are generally known in the art (Kim 2010). The host cell is preferably a eukaryotic cell, more preferably a mammalian cell, for example, a Human Embryonic Kidney 293 (HEK293) cell, a Chinese Hamster Ovary (CHO) cell, a

Baby Hamster Kidney (BHK21) cell, a PerC6 cell or a COS7 cell. Human Embryonic Kidney 293 (HEK293) and Chinese Hamster Ovary (CHO) cells are particularly preferred.

Transfected host cells are grown under conditions suitable to permit expression of the polynucleotide for encoding the protein of interest. Such conditions are generally well known for known host cells, for example mammalian cells such as HEK and CHO cells. Growth of cells is typically done in a culture medium and recovering the protein of interest from the cultured cells may also be conveniently done by known methods (Hauser 1997).

Advantageously, the isolated polynucleotide of the present invention can enhance stable expression in host cells when incorporated in *cis* in an expression system, permits enhanced expression stability in the absence of drug selection, can be combined with an episomal expression system (e.g. an oriP/EBNA1 system) for enhancing expression stability in the absence of drug selection even though pools generated with an episomal expression system (e.g. an oriP/EBNA1 system) are not stable without selection, and permits generation of stable pools for producing r-proteins without the burden of generating stable clones.

Further features of the invention will be described or will become apparent in the course of the following detailed description.

Brief Description of the Drawings

In order that the invention may be more clearly understood, embodiments thereof will now be described in detail by way of example, with reference to the accompanying drawings, in which:

Fig. 1A depicts a restriction enzyme map taken from the prior art (Fig. 1a in Strissel 1998) of the human interferon $\alpha 2$ gene showing the location of various scaffold associated regions, including the upstream scaffold associated region 3 (SAR3). The two EcoRI sites are indicated by "E" and are circled. A 0.7 kb SAR identified by Strissel in the SAR3 region is indicated by "j, R=70" and is also circled.

Fig. 1B depicts a restriction enzyme map taken from the prior art (Fig. 1c in Strissel 1998) showing the SAR3 region of human interferon $\alpha 2$ gene, and showing where the SAR of the present invention (SAR-BRI) is located on the map.

Fig. 1C depicts a map of a 3483 bp upstream region of SAR IFN α showing the location of the SAR-BRI in relation to restriction sites in the region.

Fig. 2 depicts a restriction map of a SAR of the present invention showing the location of EcoRV and SfoI restriction sites.

Fig. 3A depicts a vector map of pTT54-EPO plasmid containing SAR-BRI.

Fig. 3B depicts a vector map of pTT55-EPO plasmid not containing SAR-BRI.

5 Fig. 3C depicts western blots of CHO-DG44 pools transfected with pTT54-EPO and pTT55-EPO and expressing EPO, 20 days post-transfection (left) and 60 days post-transfection (right).

10 Fig. 3D depicts a western blot of a pTT54-EPO clone (+S/MAR element) maintained for 50 days without selection (right lane) compared to a control (Ctrl) batch of freshly thawed pTT54-EPO clone (+S/MAR element) (left lane).

Fig. 3E depicts dotblots of the supernatant from cell cultures of cells transfected with pTT54-EPO (upper) and pTT55-EPO (lower), in which the presence of EPO in the supernatant is detected using an anti-EPO antibody.

Fig. 4A depicts a vector map of pTT54-TZMHc plasmid containing SAR-BRI.

15 Fig. 4B depicts vector map of pTT55-TZMHc plasmid not containing SAR-BRI.

Fig. 4C depicts vector map of pTT52-TZMLc plasmid not containing SAR-BRI.

Fig. 4D depicts western blots comparing of CHO-DG44 pools transfected with pTT54-TZMHc+pTT52-TZMLc and pTT55-TZMHc+pTT52-TZMLc expressing Herceptin™, 6 days post-transfection (left) and 25 days post-transfection (right).

20 Fig. 5A depicts a vector map of pTT44-EG2Fc plasmid containing SAR-BRI.

Fig. 5B depicts a maintenance schedule for CHO-DG44-EG2Fc (clone 1A7) cells over 41 days without selection.

25 Fig. 5C depicts a graph showing cell density and viability of CHO cells maintained as in Fig. 5B and then transferred at day 4 (Batch 1) into a new culture flask and cultured for another 8 days.

Fig. 5D depicts a graph showing cell density and viability of CHO cells maintained as in Fig. 5B and then transferred at day 36 (Batch 2) into a new culture flask and cultured for another 8 days.

Fig. 5E depicts SDS-PAGE with Coomassie staining for determining EG2Fc titers in Batch 1 and Batch 2 cultures after 4 days and 8 days.

Description of Preferred Embodiments

Example 1: Synthesis of SAR-BRI

5 An example of a SAR of the present invention (SAR-BRI) is set forth in SEQ ID NO: 1, which is a 751 nucleotide sequence corresponding to bp numbers 1000-1750 of the human interferon $\alpha 2$ upstream scaffold associated region 3 (SAR3) sequence (gi|1791229|gb|U82705.1|HSU82705). SEQ ID NO: 1, which is 73.4% AT-rich (38.9% A, 34.5% T, 12.2% C, 14.4% G), was identified as a potential expression insulator and
10 Geneart was contracted to synthesize it using generally known methods. The sequence possesses two base mutations that were generated to destroy endogenous EcoRI and EcoRV restriction sites (C1639→G and G1695→T, respectively). The sequence was also flanked by EcoRV and SfoI restriction sites on the 5' and 3' ends, respectively, for cloning purposes, as shown in Fig. 2.

15 Previously, a 0.7 kb "strong" SAR element from the SAR3 region of IFN- $\alpha 2$ was identified (Strissel 1998). As shown in Fig. 1A, this 0.7 kb SAR (j, R=70) is located between two EcoRI sites (circled E's). In contrast, the SAR of the present invention (SAR-BRI) is located upstream of the second EcoRI site and ends just after the first EcoRI site as shown in Fig. 1B. The location of SAR-BRI in SAR3 encompassing bps 1000-1750 of
20 the 3483 bp SAR IFN α sequence is further depicted in Fig. 1C. It is clear from Fig. 1 that the SAR of the present invention (SAR-BRI) does not correspond to the 0.7 kb SAR identified by Strissel (j, R=70).

 Further, the strength of the SARs disclosed in Strissel 1998 is based on repartition of the DNA fragment between the pellet (nuclei) fraction and the supernatant, as indicated
25 in the Strissel reference:

 "The relative strength (R) of each individual DNA fragment to partition in the pellet fraction was estimated as the band intensity in the pellet divided by the sum of intensities of pellet plus supernatant ($R = IP/IP + IS$). R values were determined on previously published SAR and non-SAR DNA fragments, all of which were
30 hybridized to the same Southern blots simultaneously to set standards for binding affinity (see Table 1 in Strissel *et al.* 1996). R values set for strong SAR DNA fragments represent 70% ($R > 70$) enrichment into the pellet fraction, for example, the B1 5* SAR (detected with the.. "

Therefore, Strissel does not provide functional *in vivo* activity of the 0.7 kb SAR per se, meaning that the *in vivo* strength of the SAR identified in Strissel 1998 is unknown.

Example 2: Erythropoietin (EPO) expression plasmids

5 The SAR-BRI sequence synthesized in Example 1 was inserted in a pTT55-EPO vector (Fig. 3B) at the EcoRV restriction site lying between the puromycin resistance cassette and the prokaryotic origin of replication (pMB1) to yield pTT54-EPO plasmid (Fig. 3B). The pTT55-EPO plasmid encodes a codon-optimized human erythropoietin cDNA (Geneart) under the control of the CMV5 promoter (Durocher 2002; Massie 1998).

10 The pTT55-EPO and pTT54-EPO plasmids were transfected into Chinese Hamster Ovary (CHO) cells and the cells were cultured under puromycin selection to form stable CHO-DG44 EPO pools. To generate the CHO-DG44 pools expressing EPO, cells grown in CHO CD-DG44 medium (Invitrogen) were transfected with PEI_{max} (Polysciences) using 1 µg/ml of supercoiled plasmid DNA at a DNA:PEI ratio of 1:5 (w:w). Puromycin was then added 24 hours post-transfection (hpt) at a concentration of 10
15 µg/ml. The culture medium was regularly replaced with fresh medium containing puromycin for over 60 days.

After 20 and 60 days post-transfection, EPO expression between pools generated with pTT54-EPO and pTT55-EPO vectors was compared (Fig. 3C). To do so, cells were transferred in a new flask at a density of 0.2×10^6 cells/ml in fresh medium and the culture
20 was maintained for 5-6 days. A sample of the culture medium was then analyzed by western blot using an anti-EPO antibody (Fig. 3C). Due to its extensive and heterogeneous glycosylation, EPO migrated as multiple bands (smear) on SDS-PAGE.

It is evident from Fig. 3C that CHO cells transfected with pTT54-EPO containing the SAR-BRI of the present invention are capable of expressing r-protein at high levels
25 over a longer period of time. While both the pTT54-EPO plasmid and pTT55-EPO plasmid provide good expression 20 days post-transfection (left blot), only pTT54-EPO having the SAR-BRI provided significant r-protein expression 60 days post-transfection (right blot). This demonstrates that use of a SAR of the present invention permits generation of stable pools for producing r-proteins without the need for generating stable
30 clones.

Further, an EPO clone (+S/MAR element) maintained for 50 days without selection was compared in parallel to a freshly thawed batch of EPO clone (+S/MAR

element) as a control (Ctrl). The western blot (Fig. 3D) shows similar productivity (140 mg/L) for both indicating that the clone is very stable.

Furthermore, cells from the pools obtained with pTT54-EPO and pTT55-EPO vectors were plated at low cell density in semi-solid medium (Caron 2009) in the absence of selection. Once the colonies reached 4-10 cells, these were randomly picked and transferred into 96-well plates. After one week in culture, an aliquot of the supernatant from each well was spotted on a nitrocellulose membrane and the presence of EPO detected using an anti-EPO antibody. It is clear from Fig. 3E that the number of positive clones is higher with cells transfected with pTT54-EPO containing the SAR-BRI (upper dotblot). Also most of the clones obtained with the SAR-BRI express more EPO than without the SAR. EPO standards comprising the same quantity of EPO deposited on all membranes are shown in grid location H12 on each dotblot. Longer exposure was needed in the pTT55 not containing the SAR-BRI.

These results demonstrate that use of a SAR of the present invention permits generation of stable clones for producing r-protein at high levels.

Example 3: Herceptin™ expression plasmids

Herceptin™ is a trade name for the monoclonal antibody trastuzumab. Codon-optimized Herceptin™ heavy chain cDNA (Geneart) was cloned into pTT54 or pTT55 vectors to yield pTT54-TZMHc plasmid (Fig. 4A) and pTT55-TZMHc plasmid (Fig. 4B). The Herceptin™ light chain was cloned into pTT52 vector to yield pTT52-TZMLc plasmid (Fig. 4C). The pTT52 vector is the same as the pTT55 vector except that the puromycin resistance gene was replaced by a glutamine synthase gene (hGS). The pTT54-TZMHc plasmid contains the SAR-BRI sequence while pTT55-TZMHc and pTT52-TZMLc do not.

To generate stable CHO-DG44 pools expressing Herceptin™, cells were transfected as described for EPO in Example 2 except that two vectors were co-transfected at a 1:1 (w:w) ratio (pTT54-TZMHc + pTT52-TZMLc or pTT55-TZMHc + pTT52-TZMLc). Selection was done as for EPO. Expression of Herceptin™ by the resulting pools was compared 6 days or 25 days post-transfection by western blot under reducing or non-reducing condition using an anti-hFc antibody (Fig. 4D). The presence of partially disassembled antibody species on SDS-PAGE may be due to disulphide bond reduction during the culture and has been already described (Trexler-Schmidt 2010).

It is evident from Fig. 4D that CHO cells transfected with pTT54-TZMHc containing the SAR-BRI of the present invention are capable of expressing r-protein at high levels

over a longer period of time. While both the pTT54-TZMHc plasmid and pTT55-TZMHc plasmid provide good expression 6 days post-transfection (left blot), only pTT54-TZMHc having the SAR-BRI provided significant r-protein expression 25 days post-transfection (right blot). This further demonstrates that the use of a SAR of the present invention permits generation of stable pools for producing r-proteins without the need for generating stable clones. Since EPO pools generated with the SAR-BRI sequence (Example 2, Fig. 3E) provide higher frequency of high-expressing EPO clones, it is expected that higher frequency of high-expressing Herceptin™ clones would also be observed in the presence of the SAR-BRI element.

10 *Example 4: EG2 cHCAb expression plasmids*

The chimeric heavy chain antibody EG2 (Zhang 2009) was cloned into pTT44 vector to yield pT44-EG2Fc plasmid (Fig. 5A). The pT44 vector is identical to the pTT54 vector except for the puromycin polyadenylation signal which is from the bovine growth hormone instead of the rabbit beta-globin.

15 To generate CHO-DG44 clones expressing EG2Fc, cells were transfected as described for EPO in Example 2 except that the plasmid was linearized by digestion with PvuI enzyme. Following transfection, cells were selected in the presence of 10 µg/ml of puromycin for eight days. Then, puromycin resistant cells were plated at a density of 250 cells/ml in a semi-solid medium without puromycin selection as previously described (Caron 2009). The presence of EG2Fc was monitored by inclusion of fluorescent-labeled anti-IgG antibodies in the semi-solid medium and clones expressing high levels of the cHCAb were identified by fluorescence microscopy and transferred in 96 well-plates using a CellCelector™ clone picker (Caron 2009).

25 As depicted in Fig. 5B, one clone (1A7) was selected for high productivity, further expanded and a Master Cell Bank (MCB) was made. Following thawing of one vial from the MCB, productivity was assessed after extensive culture of this clone in suspension without puromycin selection. To do so, cells were transferred at day 4 (Batch 1) and day 36 (Batch 2) post-thawing to a new flask and cultured for 8 days. Figs. 5C and 5D demonstrate that the new cultures for both Batch 1 (Fig. 5C) and Batch 2 (Fig. 5D) were still very similar in terms of viability and total cell density.

30 EG2Fc titers in Batch 1 and Batch 2 cultures after 4 days and 8 days were compared by SDS-PAGE and Coomassie staining (Fig. 5E). A purified EG2Fc control (Lane 2 in Fig. 5E) at 100 mg/L was loaded in parallel for comparison purpose. It is

evident from Fig. 5E that after 4 days of culturing the cells without selection pressure (Lanes 3 and 4), r-protein expression was evident and that after 8 days (Lanes 5 and 6), r-protein expression levels were very high. As productivity was very similar whether cells were taken from an early (day 4) or late (day 36) passage in the absence of selection, this demonstrates that the use of a SAR of the present invention leads to robust expression stability in clones even in the absence of drug selection pressure.

Example 5: SAR-BRI in an Epstein-Barr Virus (EBV) episome

It is well known that EBV's episomes are lost at a rate of 1-4% per generation in the absence of selection pressure (Lindner 2007). To enhance retention of EBV episomes, attempts to combine a S/MAR element to an oriP plasmid have hitherto failed (Giannakopoulos 2009).

Pools of HEK293-EBNA1 cells transfected with the oriP-containing pTT22-GFP vector lose GFP expression when selection pressure is removed after 72 days in culture. However, in preliminary unpublished studies, the inclusion of one or two S/MAR sequences from human beta-globin and/or beta-interferon in the pTT22-GFP vector enhances episome stability upon removal of selection pressure as significantly higher percentage of GFP-positive cells can be observed after 125 days (53 days without selection). Likewise, it is expected that the inclusion of SAR-BRI in an episomal vector will also enhance episome stability upon removal of selection pressure.

Free listing of sequences:

SEQ ID NO: 1 - 751 nucleotides corresponding to bp 1000 to 1750 of gb#U82705.1

AATCAGAGAAACAAAATGTTAGAAAATTCTTGCAGGTGATTTTCAATATTGTTTTATTTGTGC
 AAAAAATAATTAACCTTTTAGAAGGTCCCAGAGTATTAGAAGCCCAAACCTCTGGAATATTCTCA
 ATTTTAGTTGAGCTTTTCAGTTATTATAATATTGTATAGCTACTCATATAATTAGTAATACAAAAG
 ATTCTGAGTCTTATTTGTAAATAAAGTTCAAATAAGTCATATGTTATATGTTATAGGTAATCAT
 GTTAGATATAAACCACTATTGAAAAAGATATAAAAAACAAAATAACTTTATTTTTGTTATCATATA
 TATAGTCCATTATGTTACATTTAGAATGATGTTAACAATTGTTTTGACATTTTTAAATGAAAA
 ACTCATATATCTAGTTCATGAAATTGTTAATAAACTAGAAAATATATGGACATAAAAATGACATT
 AGCCAAGATATGATAATGAGGCAATGGTGGCAGGGTACACAAGGCATAAAAGCCATTATTTCC
 CACCCAAAATGTTATGTCACATTTGTGCCTTACTCAGCTATAATTTATGTAAAAATCTGATTTGT
 GAATTAAGATAACTTTTTAAAGATTGTACAAAGGTATATCTACATTTTTGAATTGAAGTAGAGA
 TGGGAATTATCATGTCGTATTAACCACTACATTAACCACTTAAGTATATCTAGGGCATAAAA
 ATAAAAATCGATGTAATGGCACTTAAGATATGTATTA

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Other advantages that are inherent to the structure are obvious to one skilled in the art. The embodiments are described herein illustratively and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments will be
10 evident to a person of ordinary skill and are intended by the inventor to be encompassed by the following claims.

Claims:

1. An isolated polynucleotide comprising no more than 755 nucleotides and comprising at least 500 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.
- 5 2. The isolated polynucleotide according to claim 1, comprising at least 600 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.
3. The isolated polynucleotide according to claim 1, comprising at least 700 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.
4. The isolated polynucleotide according to claim 1, comprising at least 750
10 contiguous nucleotides from the nucleotide sequence as set forth in SEQ ID NO: 1.
5. An expression system for producing recombinant protein in a host cell comprising: a gene for encoding a protein of interest; nucleotide sequences for operation of the expression system; and, an expression enhancer incorporated in *cis* in the expression system for enhancing expression of the gene, the expression enhancer comprising a
15 polynucleotide as defined in any one of claims 1 to 4.
6. The expression system according to claim 5 comprising a plasmid vector containing the gene and the expression enhancer.
7. The expression system according to claim 5 comprising an episomal vector containing the gene and the expression enhancer.
- 20 8. The expression system according to any one of claims 5 to 7, wherein the gene encodes a monoclonal antibody, an erythropoietin, an interferon, a vascular endothelial growth factor, a stem cell growth factor, a growth hormone or an insulin-like growth factor binding protein.
9. The expression system according to any one of claims 5 to 8, wherein the
25 nucleotide sequences for operation of the expression system comprise one or more of a promoter, an origin of replication, a leader, a splice donor, an intron, a splice acceptor, a selectable marker, a cloning site, a restriction enzyme consensus site and a polyadenylation signal.
10. A host cell comprising the expression system as defined in any one of claims 5 to
30 9.

11. The host cell according to claim 10 which is mammalian cell.

12. The host cell according to claim 11, wherein the mammalian cell comprises a Human Embryonic Kidney 293 (HEK293) cell, a Chinese Hamster Ovary (CHO) cell, a Baby Hamster Kidney (BHK21) cell, a PerC6 cell or a COS7 cell.

5 13. The host cell according to claim 11, wherein the mammalian cell comprises a Human Embryonic Kidney 293 (HEK293) or a Chinese Hamster Ovary (CHO) cell.

14. A method of producing recombinant protein comprising: transfecting a host cell with an expression system as defined in any one of claims 5 to 9; growing the host cells under conditions suitable for expression of the gene to produce the protein of interest;
10 and, recovering the protein of interest.

15. The method according to claim 14, wherein the host cell comprises a mammalian cell.

16. The method according to claim 14, wherein the host cell comprises a Human Embryonic Kidney 293 (HEK293) cell, a Chinese Hamster Ovary (CHO) cell, a Baby
15 Hamster Kidney (BHK21) cell, a PerC6 cell or a COS7 cell.

17. The method according to claim 14, wherein the host cell comprises a Human Embryonic Kidney 293 (HEK293) or a Chinese Hamster Ovary (CHO) cell.

18. Use of a polynucleotide as defined in any one of claims 1 to 4 as an expression enhancer in *cis* in an expression system for producing recombinant protein.