Expression Vectors for Enhanced Transient Gene Expression and Mammalian Cells expressing them

FIELD OF THE INVENTION

This invention relates to new mammalian cells and cell lines, especially CHO and 293 cell lines, which comprise expression vectors encoding truncated EBNA1 genes which enhance transient gene expression. The invention also relates to expression cassettes which include such truncated genes.

BACKGROUND OF THE INVENTION

Mammalian cells are an established expression system in the biotechnology industry for the production of recombinant proteins (r-proteins). In contrast to lower eukaryotes or prokaryotes, mammalian cells provide active r-proteins that possess relevant post-translational modifications. However, in order to obtain sufficient amount of protein for structure/activity analyses or high-throughput screenings, one needs to go through the long and tedious process of stable clone isolation and characterization. Protein production by large-scale transfection is an interesting alternative to the generation of stable clones as it allows the very fast generation of mg to gram quantities of r-protein within few days.

The use of vectors containing the Epstein-Barr virus (EBV) oriP in cell lines stably expressing EBV's EBNA1 protein, such as the HEK293-EBNA1 (293E) cell line (ATCC#CRL-10852) significantly increases protein yield (Durocher et al., 2002). EBNA1 is a multi-functional protein that have been shown to positively regulate many viral promoters present on plasmid DNA when the oriP is present in *cis* (Reisman and Sugden, 1986).

The production of secreted r-protein often needs to be performed in serum-free medium in order to facilitate their purification. Adaptation of the 293E cell line to serum-free medium formulations is not straightforward and is rarely successful. To circumvent this problem, the generation of new 293-EBNA1 cell line from a serum-free medium adapted 293 cell line is preferable (Pham et al., 2003;Pham et al., 2005). However, these new cell lines do not always show optimal growth properties or high transfectabilities in serum-free medium. Also, the isolation of new clones stably expressing full-length EBNA1 is difficult as this protein seems to be cytotoxic to the cells.

Preliminary transient gene expression studies with the commercially available 293F cells adapted to the FreeStyle™ medium showed that this cell line has a good potential for the large-scale r-protein production in serum-free medium. Improvement of this cell line by stably expressing a less cytotoxic but functional EBNA1 protein is needed.

Kennedy, G. and Sugden, B. (2003) *EBNA-1, a Bifunctional Transcriptional Activator* Molecular and Cellular Biology, **23**: 6901-6908 disclose that the ability of EBNA1 to activate transcription from both integrated and transfected templates can be inhibited by a derivative of EBNA1 lacking the amino acids required for activation from integrated templates (aa 65-89). We have found, against previous expectations, that truncations of these amino acids from EBNA1-coding nucleotide sequences can enhance transient gene expression in HEK293 cells to a level similar to EBNA1.

SUMMARY OF THE INVENTION

This invention relates to the unexpected discovery that nucleotide coding sequences coding for a truncated Epstein Barr Nuclear Antigen 1 (e.g. EBNA1t) protein (lacking the Gly-Gly-Ala domain), when in cells of mammalian origin, are associated with increased transient gene expression when compared with control cells. In addition, expression of this truncated EBNA1 gene is more stable and expressed at higher levels than expression of the full-length EBNA1 gene. This results in cell lines with better growth properties and with enhanced transient gene expression. Mammalian cell lines in general are contemplated and human embryonic kidney 293 cells, CHO cells and PER-C6™ cells are of particular interest. This invention also relates to a mammalian cell line such as a 293 cell line stably expressing a processed version of EBNA1t (e.g. 293-6E cells) also showing enhanced transient gene expression compared to EBNA1t, EBNA1 and control cell lines.

Preferably the transfected gene expression is performed in a cell line stably expressing truncated EBNA1. Alternatively, the transfected gene expression is associated with a transiently transfected EBNA1 gene. Also, preferably the EBNA1 nucleotide sequence is truncated to lack most of (i.e. more than 50%, preferably nmore than 75% and, in some embodiments, all) the Gly-Gly-Ala domain. Preferably the nucleotide sequence is less than 70% of a complete EBNA1 coding sequence, especially less than 50% of the complete EBNA1 coding sequence. Alternatively, or as well, one or more of the DNA linking regions LR1 and LR2 can be absent from the truncated sequence. One of the

truncated sequences we have used lacks LR1 and we expect that an equivalent sequence lacking LR2 (with or without LR1 present) to serve a similar purpose. The nucleotide sequence can be included in an expression vector, such as a pTT vector or any other vectors containing a complete or partial Epstein Barr Virus (EBV) oriP sequence, allowing expression of the gene.

Stable cell lines including such expression vectors with truncated EBNA1 nucleotide coding sequences comprise an aspect of the invention.

According to one aspect of the invention, we provide new stable serum-free 293F-EBNA1 cell lines, including full-length of truncated versions of EBNA1.

The use of EBNA1t reduces the difficulty of obtaining stable clones (apparent deleterious effects of over-expressing the full-length EBNA1 protein). To our knowledge, no reports describing stable 293-EBNA1t cell lines exist. Also, by isolating and characterizing a stable 293F-EBNA1t cell line (clones 6E), we observed another new further truncated and functional form of EBNA1, of even shorter amino acid sequence length than EBNA1t (location of truncation not yet identified).

According to another aspect of the invention we provide a series of new truncated EBNA1t expressed proteins (including EBNA1c).

The following aspects of the invention are described in detail below.

1. The new 293FEt cell line, where Et is a truncated version of the EBNA1 protein e.g. EBNA1t described below and in the figures.
2. The new 293-6E cell line expressing a processed form of EBNA1t protein
3. The new truncated EBNA1 protein, EBNA1c consisting of LR2+NLS+DBD domains
4. Using transient EBNA1 (full-length or truncated) expression in *trans* to increase protein production in EBNA1 (full-length or truncated) and non-EBNA1 cell lines
5. The use of an EBNA1t or EBNA1c expression cassette in the pTT vector or other oriP-containing vectors (expression in *cis*) to increase protein production in EBNA1 and non-EBNA1 cells.
6. New truncated EBNA1 protein consisting of LR1+NLS+DBD domains.

The invention further relates to a process for *in vitro* production of a protein which process comprises:

(a) transfecting a mammalian cell with an expression vector coding for said protein, said mammalian cell having been transfected with a truncated EBNA1 expression vector of the invention;

(b) culturing a transfected cell resulting from (a) to yield said protein.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate the invention, Figure 1 shows transient SEAP expression in 293F cells following co-transfection of various amounts of pTT/EBNA1t vector. Figure 2 shows stable or transient EBNA1 constructs expression in 293 cells. Figure 3 shows transient GFP expression in various 293F-EBNA1 clones or pools. Figure 4 shows a Western Blot analysis of EBNA1 expression in various 293F clones. Figure 5 shows transient human placental secreted alkaline phosphatase (SEAP) expression in various EBNA1 clones. Figure 6 shows the growth of various 293F-EBNA1 clones following transfection (hpt = hours post-transfection). Figure 7 shows the growth curve of 293-6E cells compared to 293F cell in 125 ml shaker flasks. Figure 8 shows the amino acid sequence of EBNA1 (SEQ ID NO: 1) with various parts of the sequence identified in the Figure. Figure 9 shows the amino acid sequence of full-length EBNA1 protein (SEQ ID NO: 1) and EBNA1t (underline) (SEQ ID NO: 2) and EBNA1c (bold) (SEQ ID NO: 3) truncated versions. The first amino acid of the new EBNA1c protein is a methionine (as indicated above the glycine residue). Figure 10 shows the schematic structure of various EBNA1 constructs. Figure 11 (A-C) shows DNA sequence of full-length EBNA1(SEQ ID NO: 4), and truncated EBNA1 (EBNA1t (SEQ ID NO: 5) and EBNA1c (SEQ ID NO: 6)). Figure 12 shows transient EBNA1t and EBNA1c expression in 293F cells compared to 293F or 293-6E cells. Figure 13 shows the effect of co-expressing EBNA1t or EBNA1c on transient SEAP expression in 293F cells. Figure 14 shows examples of proteins transiently expressed in 293-6E cells.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to nucleotide coding sequences coding for a truncated Epstein Barr Nuclear Antigen 1 (EBNA1) protein which, when in cells of a mammalian cell line, are associated with increased transfected gene expression when compared with cells of a control cell line comprising a complete EBNA1 coding sequence. By "truncated" we mean a sequence which is less than the full EBNA1 nucleotide sequence. As shown in Figures 8 and 10 there are identified components of the full EBNA1 sequence. These

include DNA Linking Regions 1 and 2, a Transcription activation domain, a Nuclear Localization Signal and a DNA Binding and Dimerization region. Truncated sequences of the invention preferably contain the DNA Binding and Dimerization region along with the Nuclear Localization Signal and one or more DNA Linking Regions. Figure 1 shows that transient SEAP expression can be increased significantly by co-expression of EBNA1t protein. Similar increase can be observed using full length EBNA1 protein (not shown). This Figure also shows that transient SEAP expression does increase by augmenting EBNA1t expression. However, it seems that over expressing full-length EBNA1 is difficult to achieve in mammalian cells. This is illustrated in Figure 2 where stable expression of full-length EBNA1 in the commercially available cell line HEK293-EBNA1 (formerly available at Invitrogen or available at ATCC #CRL-10852) or in our best SFE clone (SFE41;(Pham et al., 2003)) is significantly lower than in 293FEt bulks (lanes 6 and 7) or 293-6E cells (lanes 8 and 9). In addition, expression of full-length EBNA1 in 293F cells (bulk) in also very low (lanes 4 and 5). Note that while truncated forms of EBNA1 increases with time in these bulks (lanes 6 vs 7 and lanes 8 vs 9), expression of full length EBNA1 drops with time (lanes 4 vs 5), indicating that overexpression of full-length EBNA1 may have negative effects on cell physiology. Unexpectedly, a major and smaller form of EBNA1t was observed in clone 6E (lanes 8 and 9).

EBNA1t was amplified by PCR using forward (ACGGAATTCGCCGCCACCATGTCTGAC GAGGGGCCA) (SEQ ID NO:7) and reverse (GAGGAAGGGCAGGA GTGAGAATTCCCT) (SEQ ID NO:8) primers and cloned at the EcoRI site of pIRES-Neo vector (Clontech). We made the 293FEt cell line (including the 293-6E clone) following transfection of 293F cells with the pIRES-EBNA1t-Neo vector and selection with 25 ug/ml geneticin. Stable clones were isolated by limiting dilution and clones selected based on EBNA1 expression using the rat monoclonal antibody 1H4 (Grasser et al., 1994)

Figure 1 shows that expressing EBNA1t increases transient SEAP expression in a dose-dependent manner. 293F cells were transfected with 100% pTT-SEAP vector (CTRL) or with mixtures of 99 to 60% pTT-SEAP and 1 to 40% of pTT-EBNA1t respectively. With 60% pTT-SEAP and 40% pTT-EBNA1t, the expression level of SEAP was increased by 3-fold over control.

Figure 2 shows EBNA1 expression levels in various stable HEK293 cell lines or following transient transfection. Stable expression of full-length EBNA1 in HEK293-EBNA1 cell line (lane 1) and 293-SFE cell line (lane 2). Lane 3: 293F cells (no EBNA1 expression). Expression of full-length EBNA1 in 293F cells following transfection and G418 selection for 2 months (lane 4) and 4 months (lane 5). Note that expression of full-length EBNA1 decreases with time in this non-clonal cell population. Expression of truncated EBNA1 (EBNA1t) in 293F cells following transfection and G418 selection for 2 months (lane 6) and 4 months (lane 7). Note that expression of EBNA1t increases with time in this non-clonal cell population. Expression of the new form of EBNA1t in clone 6E derived from 293F-EBNA1t after 4 months in culture in the presence of G418 and 1% serum (lane 8) or G418 in serum-free medium (lane 9). Transient expression of full-length EBNA1 (lane 10) or EBNA1t (lane 11) in 293F cells.

The precise nature of the new EBNA1t protein remains to be solved. Detection of EBNA1 was performed using a rat monoclonal antibody (clone 1H4). The two bands seen at Mr 200 and above are not-specific.

Figure 3 shows transient GFP expression in various EBNA1 cell lines. Cells (cultured for 3 months under G418 selection following transfection) were transfected with pTT-GFP and GFP expression was measured 3 days later by flow cytometry. The 293F-EBNA1t clone 6E shows the highest GFP expression. Transfection efficiency was between 40% and 65% for all clones.

Figure 4 shows EBNA1 expression levels in various 293 clones. All clones were cultured in the presence of 25 μg/ml geneticin. Expression of EBNA1 in clone 6A can be detected with longer exposure time.

Figure 5 shows that when various 293F-EBNA1 stable clones were transfected with pTT/SEAP, the clones expressing truncated EBNA1 coding sequences showed enhanced SEAP expression when measured 5 days later (clones 6E, 11 and 13) when compared to clones expressing the full length (clones 1A and 2B) or another uncharacterized truncated form of EBNA1 (clone 6A). In the context of this invention, SEAP is an example of a recombinant protein. Genetic material coding for a protein or polypeptide of choice can be used in place of SEAP coding sequences and, indeed, this is an aim of this invention (see Figure 14 for additional examples).

Figure 6 shows that cell growth and viability does not appear to be affected when truncated EBNA1 nucleotide sequences are stably overexpressed. Cells were fed with 0.5% TN1 24 hpt (Pham et al., 2005) and counted 6 days after transfection.

Figure 7 shows the growth characteristic of the 293FEt-clone 6E (lower panel) compared to the parental 293F cell line (upper panel). Maximum viable cell density is about $3.5x10^6$ cells/ml for the clone 6E compared to $4.2x10^6$ cells/ml for the 293F cell line.

Figure 8, Figure 9, Figure 10 and Figure 11 are best reviewed together. They show the amino acid sequence and schematic structure of EBNA1 constructs and the relationship to the EBNA1 DNA sequences (Figures 11 A-C).

Figure 8 shows the EBNA1 full length protein (641 aa, 56.4 kDa) (Accession number: NC_001345) with its main features. Figure 9 highlights the differences between EBNA1, EBNA1t and EBNA1c and the amino acid level. EBNA1t truncated protein (underline: 417 aa, 42.5 kDa) and EBNA1c further truncated protein (bold: 306 aa, 32.5 kDa). The first amino acid of the new EBNA1c protein is a Methionine (as indicated above the Glycine residue).

Figure 12 contrasts transient expression of two truncated EBNA1 constructs with 293F and 293-6E cells. Cells were transfected with pTT/EBNA1t or pTT/EBNA1c vectors and EBNA1 expression was detected 3 days later by Western blot. Non-transfected 293F cells and 293-6E cells are also shown as controls.

Figure 13 shows 293F cells co-transfected with pTT/SEAP and pTT/EBNA1 constructs. 293F cells were co-transfected with a mixture of 50% pTT-SEAP vector with pTT/EBNA1t, 50% pTT/EBNA1c, or 50% salmon sperm DNA (stuffer DNA). SEAP expression was measured 5 days later.

Figure 14 shows examples of proteins transiently expressed in 293-6E cells. 293-6E cells were transfected with pTT vectors encoding various secreted proteins and culture medium (20 microliters) was harvested 5 days after transfection and analyzed by SDS-PAGE and Coomassie staining.

Reference List

Durocher, Y., Perret, S., and Kamen, A., 2002. High-level and high-throughput recombinant protein production by transient transfection of suspension-growing human 293-EBNA1 cells. Nucleic Acids Res. 30, E9.

Grasser, F.A., Murray, P.G., Kremmer, E., Klein, K., Remberger, K., Feiden, W., Reynolds, G., Niedobitek, G., Young, L.S., and Mueller-Lantzsch, N., 1994. Monoclonal antibodies directed against the Epstein-Barr virus-encoded nuclear antigen 1 (EBNA1): immunohistologic detection of EBNA1 in the malignant cells of Hodgkin's disease. Blood 84, 3792-3798.

Pham, P.L., Perret, S., Cass, B., Carpentier, E., St-Laurent, G., Bisson, L., Kamen, A., and Durocher, Y., 2005. Transient gene expression in HEK293 cells: peptone addition posttransfection improves recombinant protein synthesis. Biotechnol. Bioeng. 90, 332-344.

Pham, P.L., Perret, S., Doan, H.C., Cass, B., St-Laurent, G., Kamen, A., and Durocher, Y., 2003. Large-scale transient transfection of serum-free suspension-growing HEK293 EBNA1 cells: peptone additives improve cell growth and transfection efficiency. Biotechnol. Bioeng. 84, 332-342.

Reisman, D. and Sugden, B., 1986. trans activation of an Epstein-Barr viral transcriptional enhancer by the Epstein-Barr viral nuclear antigen 1. Mol. Cell Biol. 6, 3838-3846.

The above-described embodiments of the present invention are intended to be examples only. Alterations, modifications and variations may be effected to the particular embodiments by those of skill in the art without departing from the scope of the invention, which is defined solely by the claims appended hereto.

SEQUENCE LISTING

<110> National Research Council

<120> Expression Vectors for Enhanced Transient Gene Expression and Mammalian Cells expressing them

<130> PAT 3361W-90

<140> Unknown
<141> 2006-03-17

<150> US 60/662,392
<151> 2005-03-17

<160> 8

<170> PatentIn version 3.1

<210> 1
<211> 641
<212> PRT
<213> Epstein Barr Virus

<220>
<221> MISC_FEATURE
<223> EBNA1 Full Length Protein Sequence

<400> 1

Met Ser Asp Glu Gly Pro Gly Thr Gly Pro Gly Asn Gly Leu Gly Glu
1 5 10 15

Lys Gly Asp Thr Ser Gly Pro Glu Gly Ser Gly Gly Ser Gly Pro Gln
20 25 30

Arg Arg Gly Gly Asp Asn His Gly Arg Gly Arg Gly Arg Gly Arg Gly
35 40 45

Arg Gly Gly Gly Arg Pro Gly Ala Pro Gly Gly Ser Gly Ser Gly Pro
50 55 60

Arg His Arg Asp Gly Val Arg Arg Pro Gln Lys Arg Pro Ser Cys Ile
65 70 75 80

Gly Cys Lys Gly Thr His Gly Gly Thr Gly Ala Gly Ala Gly Ala Gly
85 90 95

Gly Ala Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Ala Gly
100 105 110

Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly
115 120 125

Gly Ala Gly Ala Gly Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala
130 135 140

Gly Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Gly Ala Gly Ala Gly
145 150 155 160

```
Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly
                165                 170                 175

Ala Gly Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Gly
            180                 185                 190

Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Ala Gly Gly Ala Gly
        195                 200                 205

Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala
    210                 215                 220

Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala
225                 230                 235                 240

Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly
                245                 250                 255

Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly
            260                 265                 270

Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Gly Ala Gly
        275                 280                 285

Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Ala Gly Gly Ala Gly
    290                 295                 300

Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly Gly Ala Gly Ala Gly
305                 310                 315                 320

Gly Ala Gly Ala Gly Gly Gly Gly Arg Gly Arg Gly Gly Ser Gly Gly
                325                 330                 335

Arg Gly Arg Gly Gly Ser Gly Gly Arg Gly Arg Gly Gly Ser Gly Gly
            340                 345                 350

Arg Arg Gly Arg Gly Arg Glu Arg Ala Arg Gly Gly Ser Arg Glu Arg
        355                 360                 365

Ala Arg Gly Arg Gly Arg Gly Arg Gly Glu Lys Arg Pro Arg Ser Pro
    370                 375                 380

Ser Ser Gln Ser Ser Ser Ser Gly Ser Pro Pro Arg Arg Pro Pro Pro
385                 390                 395                 400

Gly Arg Arg Pro Phe Phe His Pro Val Gly Glu Ala Asp Tyr Phe Glu
                405                 410                 415

Tyr His Gln Glu Gly Gly Pro Asp Gly Glu Pro Asp Val Pro Pro Gly
            420                 425                 430

Ala Ile Glu Gln Gly Pro Ala Asp Asp Pro Gly Glu Gly Pro Ser Thr
        435                 440                 445

Gly Pro Arg Gly Gln Gly Asp Gly Gly Arg Arg Lys Lys Gly Gly Trp
    450                 455                 460

Phe Gly Lys His Arg Gly Gln Gly Gly Ser Asn Pro Lys Phe Glu Asn
465                 470                 475                 480
```

```
Ile Ala Glu Gly Leu Arg Ala Leu Leu Ala Arg Ser His Val Glu Arg
                485                 490                 495

Thr Thr Asp Glu Gly Thr Trp Val Ala Gly Val Phe Val Tyr Gly Gly
            500                 505                 510

Ser Lys Thr Ser Leu Tyr Asn Leu Arg Arg Gly Thr Ala Leu Ala Ile
        515                 520                 525

Pro Gln Cys Arg Leu Thr Pro Leu Ser Arg Leu Pro Phe Gly Met Ala
    530                 535                 540

Pro Gly Pro Gly Pro Gln Pro Gly Pro Leu Arg Glu Ser Ile Val Cys
545                 550                 555                 560

Tyr Phe Met Val Phe Leu Gln Thr His Ile Phe Ala Glu Val Leu Lys
                565                 570                 575

Asp Ala Ile Lys Asp Leu Val Met Thr Lys Pro Ala Pro Thr Cys Asn
            580                 585                 590

Ile Arg Val Thr Val Cys Ser Phe Asp Asp Gly Val Asp Leu Pro Pro
        595                 600                 605

Trp Phe Pro Pro Met Val Glu Gly Ala Ala Ala Glu Gly Asp Asp Gly
    610                 615                 620

Asp Asp Gly Asp Glu Gly Gly Asp Gly Asp Glu Gly Glu Glu Gly Gln
625                 630                 635                 640

Glu
```

<210> 2
<211> 417
<212> PRT
<213> Epstein Barr Virus

<220>
<221> MISC_FEATURE
<223> EBNA1t Truncated Protein Sequence

<400> 2

```
Met Ser Asp Glu Gly Pro Gly Thr Gly Pro Gly Asn Gly Leu Gly Glu
1               5                   10                  15

Lys Gly Asp Thr Ser Gly Pro Glu Gly Ser Gly Gly Ser Gly Pro Gln
            20                  25                  30

Arg Arg Gly Gly Asp Asn His Gly Arg Gly Arg Gly Arg Gly Arg Gly
        35                  40                  45

Arg Gly Gly Gly Arg Pro Gly Ala Pro Gly Gly Ser Gly Ser Gly Pro
    50                  55                  60

Arg His Arg Asp Gly Val Arg Arg Pro Gln Lys Arg Pro Ser Cys Ile
65                  70                  75                  80
```

```
Gly Cys Lys Gly Thr His Gly Gly Thr Gly Ala Gly Ala Gly Ala Gly
                85                  90                  95

Gly Ala Gly Ala Gly Gly Gly Gly Arg Gly Arg Gly Gly Ser Gly Gly
            100                 105                 110

Arg Gly Arg Gly Gly Ser Gly Gly Arg Gly Arg Gly Gly Ser Gly Gly
        115                 120                 125

Arg Arg Gly Arg Gly Arg Glu Arg Ala Arg Gly Gly Ser Arg Glu Arg
    130                 135                 140

Ala Arg Gly Arg Gly Arg Gly Arg Gly Glu Lys Arg Pro Arg Ser Pro
145                 150                 155                 160

Ser Ser Gln Ser Ser Ser Ser Gly Ser Pro Pro Arg Arg Pro Pro Pro
                165                 170                 175

Gly Arg Arg Pro Phe Phe His Pro Val Gly Glu Ala Asp Tyr Phe Glu
            180                 185                 190

Tyr His Gln Glu Gly Gly Pro Asp Gly Glu Pro Asp Val Pro Pro Gly
        195                 200                 205

Ala Ile Glu Gln Gly Pro Ala Asp Asp Pro Gly Glu Gly Pro Ser Thr
    210                 215                 220

Gly Pro Arg Gly Gln Gly Asp Gly Gly Arg Arg Lys Lys Gly Gly Trp
225                 230                 235                 240

Phe Gly Lys His Arg Gly Gln Gly Gly Ser Asn Pro Lys Phe Glu Asn
                245                 250                 255

Ile Ala Glu Gly Leu Arg Ala Leu Leu Ala Arg Ser His Val Glu Arg
            260                 265                 270

Thr Thr Asp Glu Gly Thr Trp Val Ala Gly Val Phe Val Tyr Gly Gly
        275                 280                 285

Ser Lys Thr Ser Leu Tyr Asn Leu Arg Arg Gly Thr Ala Leu Ala Ile
    290                 295                 300

Pro Gln Cys Arg Leu Thr Pro Leu Ser Arg Leu Pro Phe Gly Met Ala
305                 310                 315                 320

Pro Gly Pro Gly Pro Gln Pro Gly Pro Leu Arg Glu Ser Ile Val Cys
                325                 330                 335

Tyr Phe Met Val Phe Leu Gln Thr His Ile Phe Ala Glu Val Leu Lys
            340                 345                 350

Asp Ala Ile Lys Asp Leu Val Met Thr Lys Pro Ala Pro Thr Cys Asn
        355                 360                 365

Ile Arg Val Thr Val Cys Ser Phe Asp Asp Gly Val Asp Leu Pro Pro
    370                 375                 380

Trp Phe Pro Pro Met Val Glu Gly Ala Ala Ala Glu Gly Asp Asp Gly
385                 390                 395                 400
```

Asp Asp Gly Asp Glu Gly Gly Asp Gly Asp Glu Gly Glu Glu Gly Gln
405 410 415

Glu

<210> 3
<211> 306
<212> PRT
<213> Epstein Barr Virus

<220>
<221> MISC_FEATURE
<223> EBNA1c Truncated Protein Sequence

<400> 3

Met Arg Gly Arg Gly Gly Ser Gly Gly Arg Gly Arg Gly Gly Ser Gly
1 5 10 15

Gly Arg Arg Gly Arg Gly Arg Glu Arg Ala Arg Gly Gly Ser Arg Glu
20 25 30

Arg Ala Arg Gly Arg Gly Arg Gly Arg Gly Glu Lys Arg Pro Arg Ser
35 40 45

Pro Ser Ser Gln Ser Ser Ser Ser Gly Ser Pro Pro Arg Arg Pro Pro
50 55 60

Pro Gly Arg Arg Pro Phe Phe His Pro Val Gly Glu Ala Asp Tyr Phe
65 70 75 80

Glu Tyr His Gln Glu Gly Gly Pro Asp Gly Glu Pro Asp Val Pro Pro
85 90 95

Gly Ala Ile Glu Gln Gly Pro Ala Asp Asp Pro Gly Glu Gly Pro Ser
100 105 110

Thr Gly Pro Arg Gly Gln Gly Asp Gly Gly Arg Arg Lys Lys Gly Gly
115 120 125

Trp Phe Gly Lys His Arg Gly Gln Gly Gly Ser Asn Pro Lys Phe Glu
130 135 140

Asn Ile Ala Glu Gly Leu Arg Ala Leu Leu Ala Arg Ser His Val Glu
145 150 155 160

Arg Thr Thr Asp Glu Gly Thr Trp Val Ala Gly Val Phe Val Tyr Gly
165 170 175

Gly Ser Lys Thr Ser Leu Tyr Asn Leu Arg Arg Gly Thr Ala Leu Ala
180 185 190

Ile Pro Gln Cys Arg Leu Thr Pro Leu Ser Arg Leu Pro Phe Gly Met
195 200 205

Ala Pro Gly Pro Gly Pro Gln Pro Gly Pro Leu Arg Glu Ser Ile Val
210 215 220

```
Cys Tyr Phe Met Val Phe Leu Gln Thr His Ile Phe Ala Glu Val Leu
225                 230                 235                 240

Lys Asp Ala Ile Lys Asp Leu Val Met Thr Lys Pro Ala Pro Thr Cys
                245                 250                 255

Asn Ile Arg Val Thr Val Cys Ser Phe Asp Asp Gly Val Asp Leu Pro
            260                 265                 270

Pro Trp Phe Pro Pro Met Val Glu Gly Ala Ala Ala Glu Gly Asp Asp
        275                 280                 285

Gly Asp Asp Gly Asp Glu Gly Gly Asp Gly Asp Glu Gly Glu Glu Gly
    290                 295                 300

Gln Glu
305
```

<210> 4
<211> 1926
<212> DNA
<213> Epstein Barr Virus

<220>
<221> misc_feature
<223> EBNA1 Full Length DNA Sequence

<400> 4

```
atgtctgacg aggggccagg tacaggacct ggaaatggcc taggagagaa gggagacaca     60

tctggaccag aaggctccgg cggcagtgga cctcaaagaa gaggggtga taaccatgga    120

cgaggacggg gaagaggacg aggacgagga ggcggaagac caggagcccc gggcggctca    180

ggatcagggc caagacatag agatggtgtc cggagacccc aaaaacgtcc aagttgcatt    240

ggctgcaaag ggacccacgg tggaacagga gcaggagcag gagcgggagg ggcaggagca    300

ggaggggcag gagcaggagg aggggcagga gcaggaggag gggcaggagg ggcaggaggg    360

gcaggagggg caggagcagg aggaggggca ggagcaggag gaggggcagg aggggcagga    420

ggggcaggag caggaggagg ggcaggagca ggaggagggg caggaggggc aggagcagga    480

ggaggggcag gaggggcagg aggggcagga gcaggaggag gggcaggagc aggaggaggg    540

gcaggagggg caggagcagg aggaggggca ggaggggcag gaggggcagg agcaggagga    600

ggggcaggag caggaggggc aggaggggca ggaggggcag gagcaggagg ggcaggagca    660

ggaggagggg caggaggggc aggaggggca ggagcaggag gggcaggagc aggaggggca    720

ggagcaggag gggcaggagc aggaggggca ggaggggcag gagcaggagg ggcaggaggg    780

gcaggagcag gaggggcagg aggggcagga gcaggaggag gggcaggagg ggcaggagca    840

ggaggagggg caggaggggc aggagcagga ggggcaggag gggcaggagc aggaggggca    900

ggaggggcag gagcaggagg ggcaggaggg gcaggagcag gaggaggggc aggagcagga    960
```

ggggcaggag caggaggtgg aggccggggt cgaggaggca gtggaggccg gggtcgagga 1020

ggtagtggag gccggggtcg aggaggtagt ggaggccgcc ggggtagagg acgtgaaaga 1080

gccaggggggg gaagtcgtga aagagccagg gggagaggtc gtggacgtgg agaaaagagg 1140

cccaggagtc ccagtagtca gtcatcatca tccgggtctc caccgcgcag gccccctcca 1200

ggtagaaggc catttttcca ccctgtaggg gaagccgatt attttgaata ccaccaagaa 1260

ggtggcccag atggtgagcc tgacgtgccc ccgggagcga tagagcaggg ccccgcagat 1320

gacccaggag aaggcccaag cactggaccc cggggtcagg gtgatggagg caggcgcaaa 1380

aaaggagggt ggtttggaaa gcatcgtggt caaggaggtt ccaacccgaa atttgagaac 1440

attgcagaag gtttaagagc tctcctggct aggagtcacg tagaaaggac taccgacgaa 1500

ggaacttggg tcgccggtgt gttcgtatat ggaggtagta agacctccct ttacaaccta 1560

aggcgaggaa ctgcccttgc tattccacaa tgtcgtctta caccattgag tcgtctcccc 1620

tttggaatgg cccctggacc cggcccacaa cctggcccgc taagggagtc cattgtctgt 1680

tatttcatgg tctttttaca aactcatata tttgctgagg ttttgaagga tgcgattaag 1740

gaccttgtta tgacaaagcc cgctcctacc tgcaatatca gggtgactgt gtgcagcttt 1800

gacgatggag tagatttgcc tccctggttt ccacctatgg tggaaggggc tgccgcggag 1860

ggtgatgacg gagatgacgg agatgaagga ggtgatggag atgagggtga ggaagggcag 1920

gagtga 1926

<210> 5
<211> 1254
<212> DNA
<213> Epstein Barr Virus

<220>
<221> misc_feature
<223> EBNAlt Truncated DNASequence

<400> 5

atgtctgacg aggggccagg tacaggacct ggaaatggcc taggagagaa gggagacaca 60

tctggaccag aaggctccgg cggcagtgga cctcaaagaa gaggggggtga taaccatgga 120

cgaggacggg gaagaggacg aggacgagga ggcggaagac caggagcccc gggcggctca 180

ggatcagggc caagacatag agatggtgtc cggagacccc aaaaacgtcc aagttgcatt 240

ggctgcaaag ggacccacgg tggaacagga gcaggagcag gagcgggagg ggcaggagca 300

ggaggtggag gccggggtcg aggaggcagt ggaggccggg gtcgaggagg tagtggaggc 360

cggggtcgag gaggtagtgg aggccgccgg ggtagaggac gtgaaagagc caggggggga 420

agtcgtgaaa gagccagggg gagaggtcgt ggacgtggag aaaagaggcc caggagtccc 480

agtagtcagt catcatcatc cgggtctcca ccgcgcaggc cccctccagg tagaaggcca 540

tttttccacc ctgtagggga agccgattat tttgaatacc accaagaagg tggcccagat 600

ggtgagcctg acgtgccccc gggagcgata gagcagggcc ccgcagatga cccaggagaa 660

ggcccaagca ctggaccccg gggtcagggt gatggaggca ggcgcaaaaa aggagggtgg 720

tttggaaagc atcgtggtca aggaggttcc aacccgaaat ttgagaacat tgcagaaggt 780

ttaagagctc tcctggctag gagtcacgta gaaaggacta ccgacgaagg aacttgggtc 840

gccggtgtgt tcgtatatgg aggtagtaag acctcccttt acaacctaag gcgaggaact 900

gcccttgcta ttccacaatg tcgtcttaca ccattgagtc gtctcccctt tggaatggcc 960

cctggacccg gcccacaacc tggcccgcta agggagtcca ttgtctgtta tttcatggtc 1020

tttttacaaa ctcatatatt tgctgaggtt ttgaaggatg cgattaagga ccttgttatg 1080

acaaagcccg ctcctacctg caatatcagg gtgactgtgt gcagctttga cgatggagta 1140

gatttgcctc cctggtttcc acctatggtg gaaggggctg ccgcggaggg tgatgacgga 1200

gatgacggag atgaaggagg tgatggagat gagggtgagg aagggcagga gtga 1254

<210> 6
<211> 921
<212> DNA
<213> Epstein Barr Virus

<220>
<221> misc_feature
<223> EBNA1c Truncated DNA Sequence

<400> 6

atgcggggtc gaggaggtag tggaggccgg ggtcgaggag gtagtggagg ccgccggggt 60

agaggacgtg aaagagccag ggggggaagt cgtgaaagag ccaggggggag aggtcgtgga 120

cgtggagaaa agaggcccag gagtcccagt agtcagtcat catcatccgg gtctccaccg 180

cgcaggcccc ctccaggtag aaggccattt ttccaccctg taggggaagc cgattatttt 240

gaataccacc aagaaggtgg cccagatggt gagcctgacg tgcccccggg agcgatagag 300

cagggccccg cagatgaccc aggagaaggc ccaagcactg gaccccgggg tcagggtgat 360

ggaggcaggc gcaaaaaagg agggtggttt ggaaagcatc gtggtcaagg aggttccaac 420

ccgaaatttg agaacattgc agaaggttta agagctctcc tggctaggag tcacgtagaa 480

aggactaccg acgaaggaac ttgggtcgcc ggtgtgttcg tatatggagg tagtaagacc 540

tccctttaca acctaaggcg aggaactgcc cttgctattc cacaatgtcg tcttacacca 600

ttgagtcgtc tccccttttgg aatggcccct ggacccggcc cacaacctgg cccgctaagg 660

gagtccattg tctgttattt catggtcttt ttacaaactc atatatttgc tgaggttttg 720

aaggatgcga ttaaggacct tgttatgaca aagcccgctc ctacctgcaa tatcagggtg 780

actgtgtgca gctttgacga tggagtagat ttgcctccct ggtttccacc tatggtggaa 840

ggggctgccg cggagggtga tgacggagat gacggagatg aaggaggtga tggagatgag 900

ggtgaggaag ggcaggagtg a 921

<210> 7
<211> 36
<212> DNA
<213> Artificial

<220>
<223> Forward Primer

<400> 7

acggaattcg ccgccaccat gtctgacgag gggcca 36

<210> 8
<211> 27
<212> DNA
<213> Artificial

<220>
<223> Reverse Primer

<400> 8

gaggaagggc aggagtgaga attccct 27

The invention claimed is:

1. A mammalian cell line that stably expresses a truncated Epstein Barr Nuclear Antigen 1 (EBNA1) protein, which cell line is the 293-6E cell line deposited at the International Depositary Authority of Canada under accession number 150305-01.

2. A process for transient in vitro production of a recombinant protein, which process comprises:

(a) transfecting a mammalian cell line in accordance with claim **1** with an expression vector coding for a recombinant protein; and

(b) culturing the transfected cell resulting from (a) to yield said recombinant protein.

3. A process in accordance with claim **2**, wherein said expression vector further includes a complete or partial Epstein Barr Virus (EBV) oriP sequence.