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(54) **METHODS AND MATERIALS FOR EXPRESSION OF A RECOMBINANT PROTEIN**

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Publication Classification

(51) **Int. Cl.**

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C12N	***15/63***	(2006.01)
C12N	***15/74***	(2006.01)
C12N	***15/00***	(2006.01)
C12N	***15/70***	(2006.01)
C12N	***15/09***	(2006.01)

(52) **U.S. Cl.** **435/320.1**; 435/6

(57) **ABSTRACT**

Recombinant expression vectors are provided comprising a 3' UTR of a light chain and an Epstein-Barr virus origin of replication. Also provided are host cells comprising such vectors and methods of producing recombinant protein with such vectors. Additional methods of producing a recombinant protein involve contacting cells with a first and second vector, each of which encode a different polypeptide chain, and wherein the second vector is present in an amount which is about 1.5 to 2.5 times as much as that of the first vector. Cells also can be transfected with a recombinant transient expression vector encoding a protein and are cultured in a medium in a membrane-enhanced culturing vessel to produce recombinant protein.

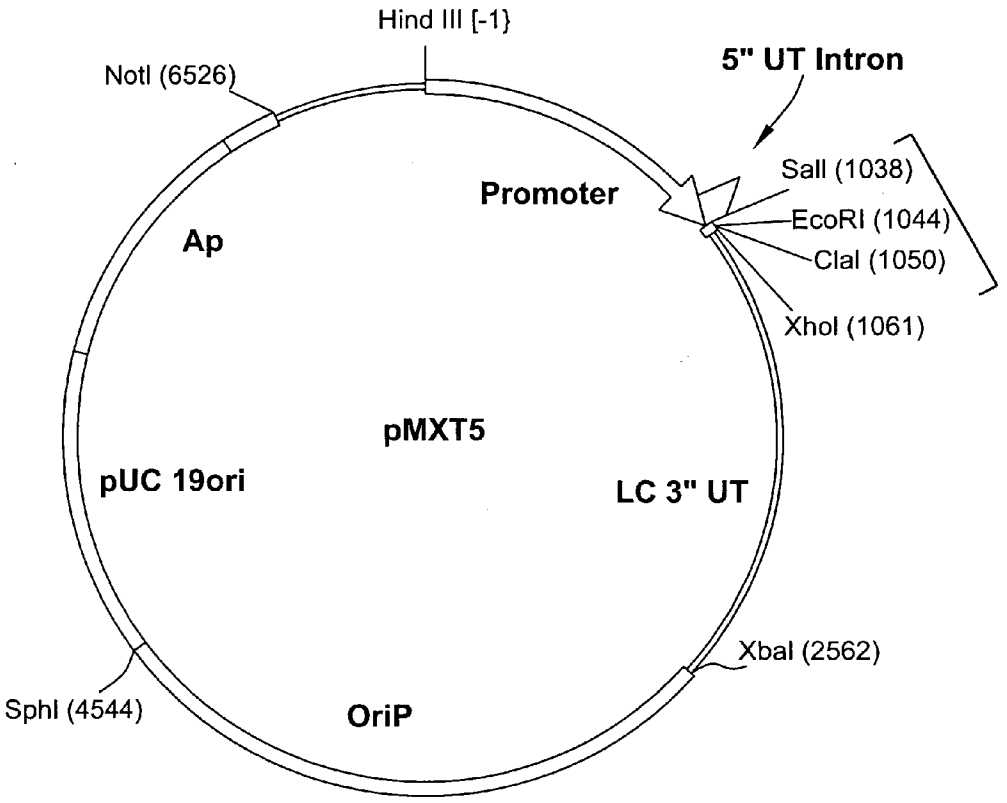

FIGURE 1A

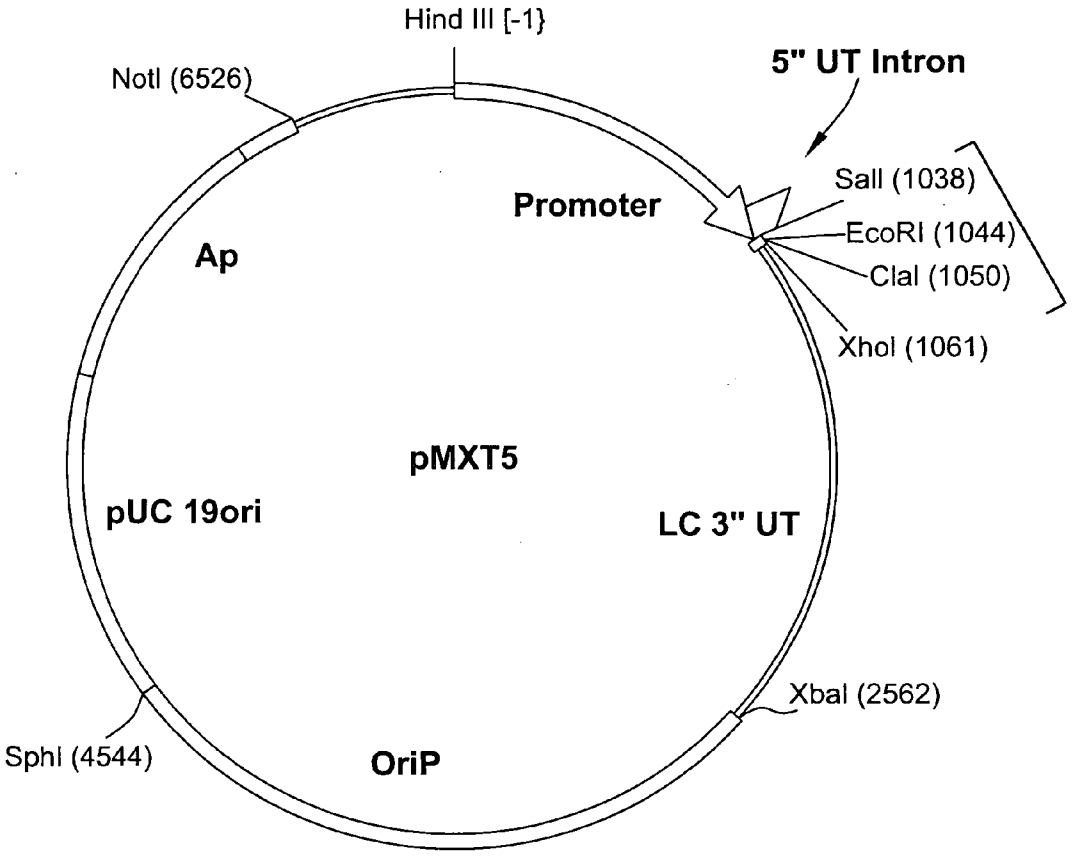
Hind III [-1]
5" UT Intron
NotI (6526)
SalI (1038)
Promoter
EcoRI (1044)
Ap
ClaI (1050)
XhoI (1061)
pMXT5
pUC 19ori
LC 3" UT
XbaI (2562)
SphI (4544)
OriP

FIGURE 1B

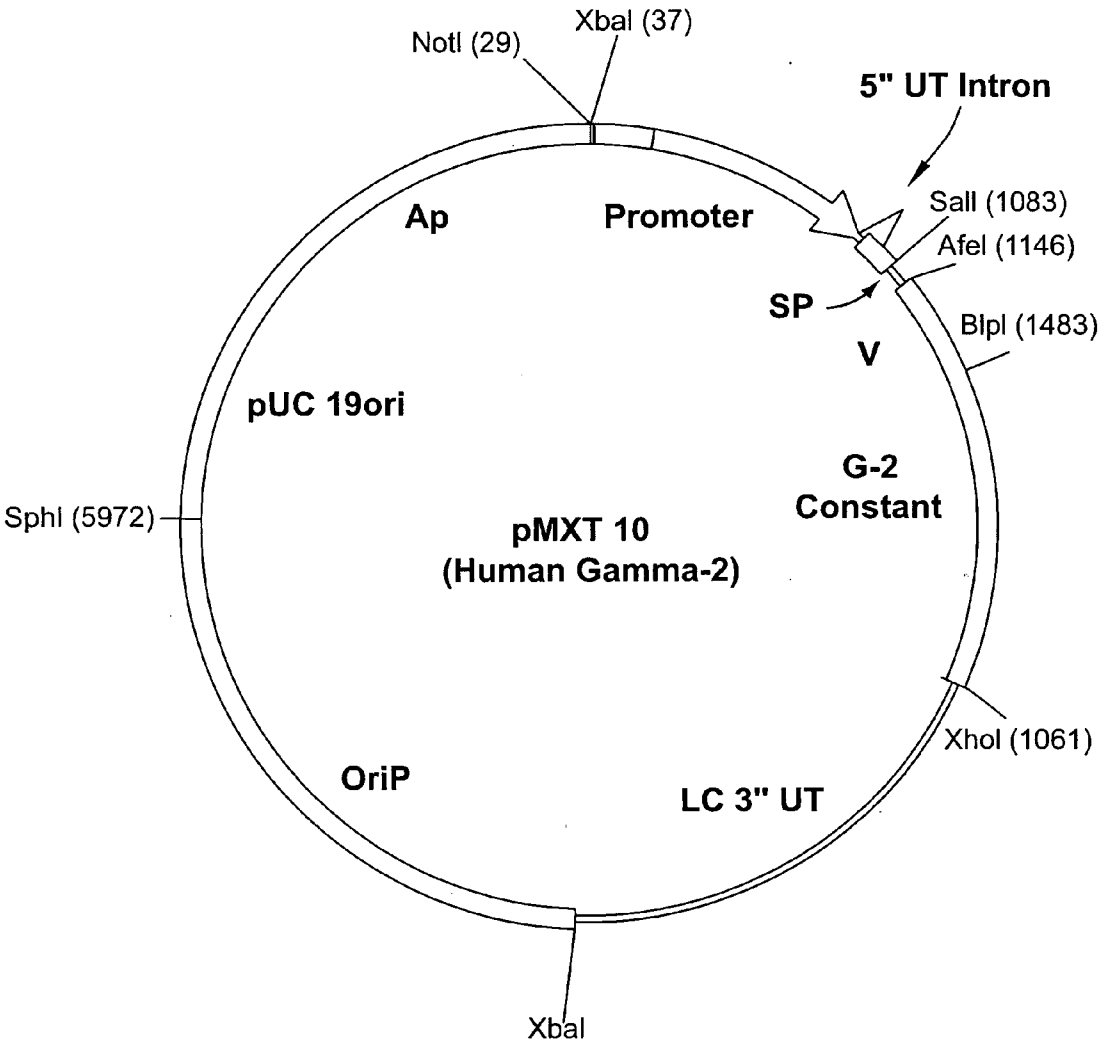
NotI (29)
XbaI (37)
5" UT Intron
SalI (1083)
AfeI (1146)
SP
V
BlpI (1483)
Ap
Promoter
pUC 19ori
G-2
Constant
SphI (5972)
pMXT 10
(Human Gamma-2)
XhoI (1061)
OriP
LC 3" UT
XbaI

FIGURE 1C

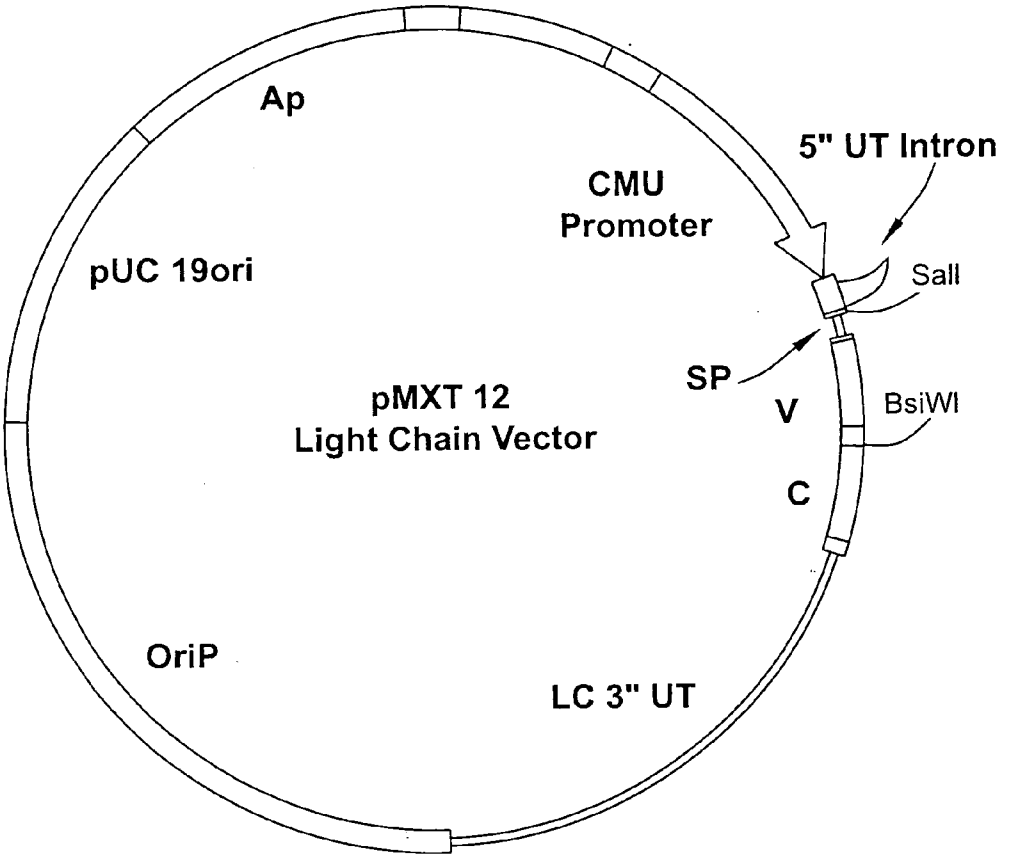
Ap
5" UT Intron
CMU
Promoter
pUC 19ori
SalI
SP
pMXT 12
Light Chain Vector
V
BsiWI
C
OriP
LC 3" UT

FIGURE 2A

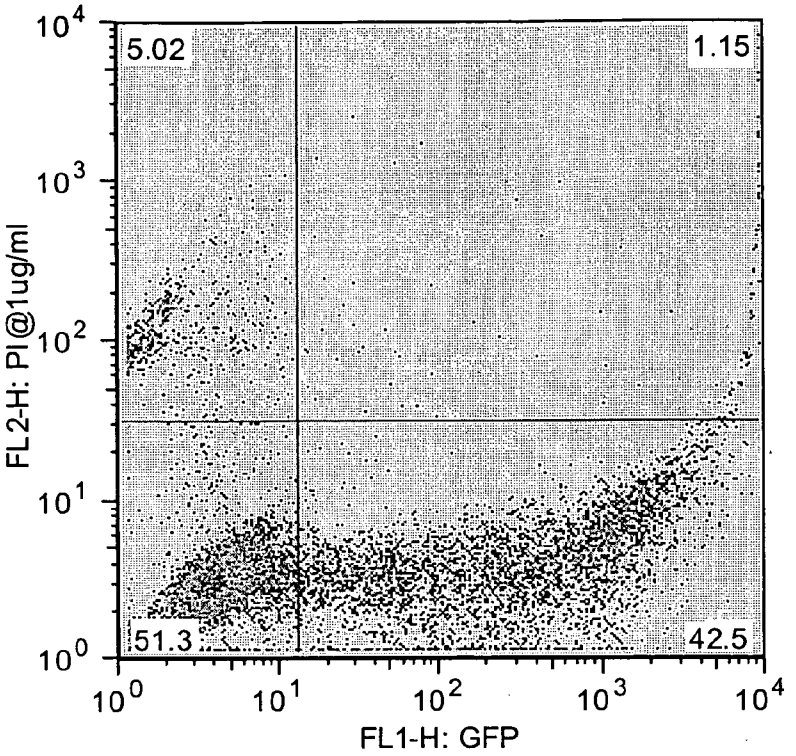

FIGURE 2B

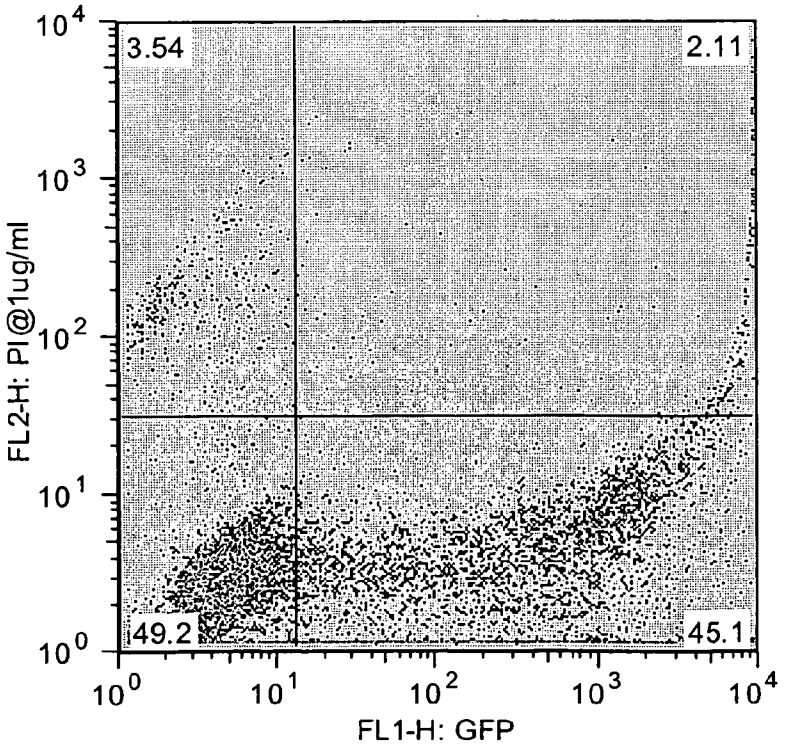

FIGURE 2C

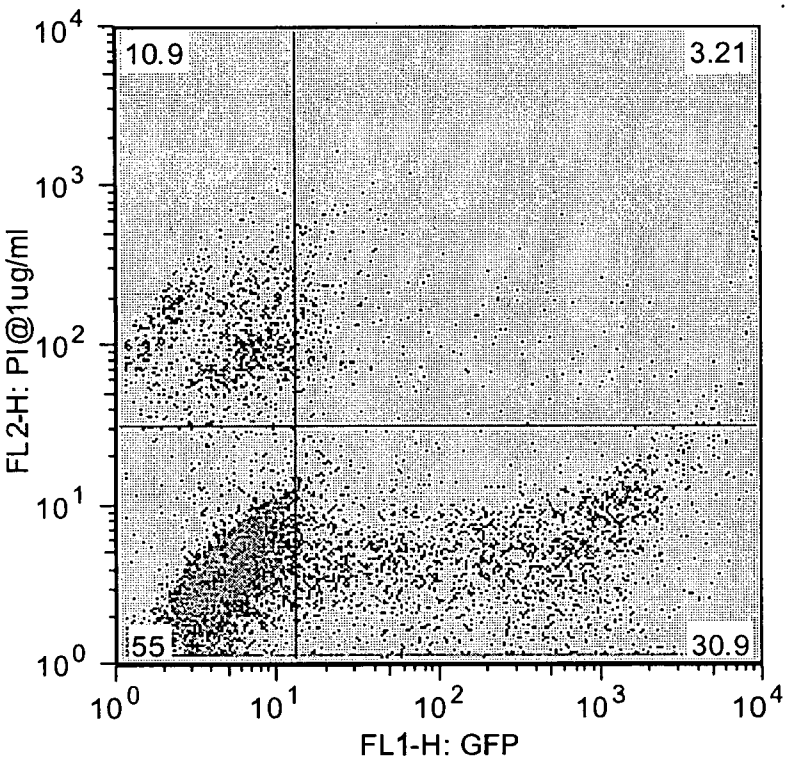

FIGURE 2D

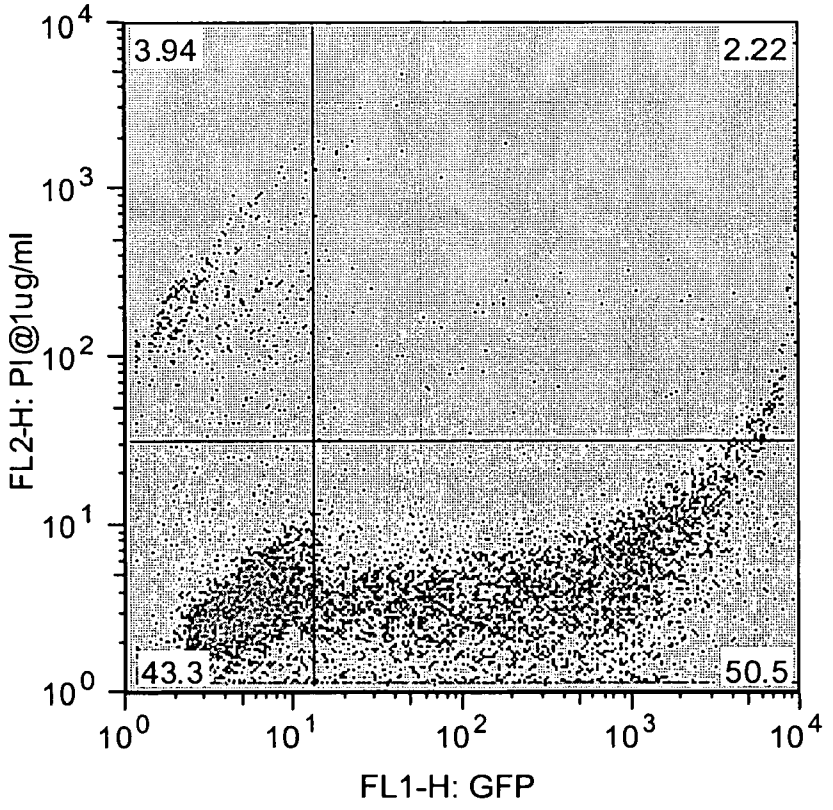

FIGURE 2E

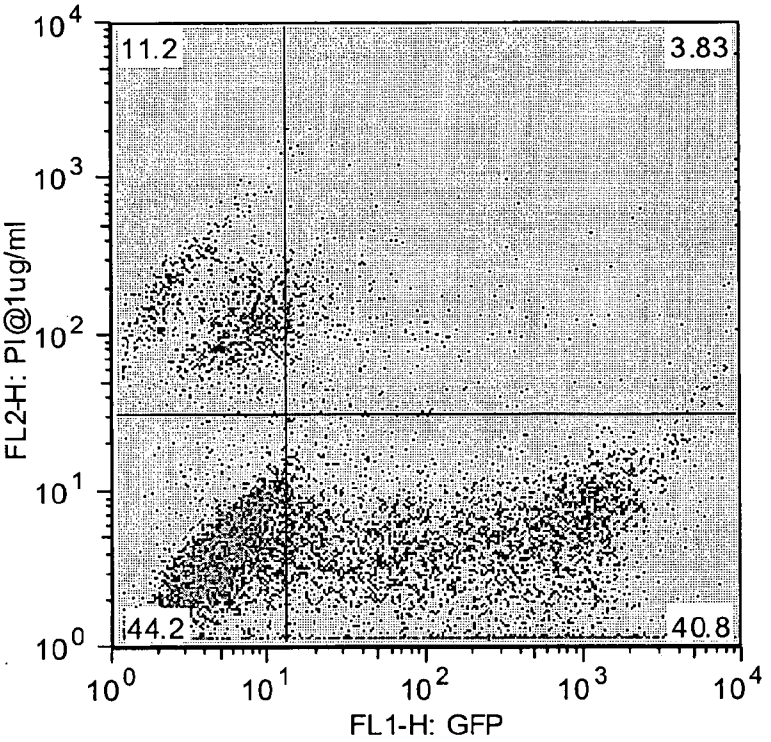

FIGURE 2F

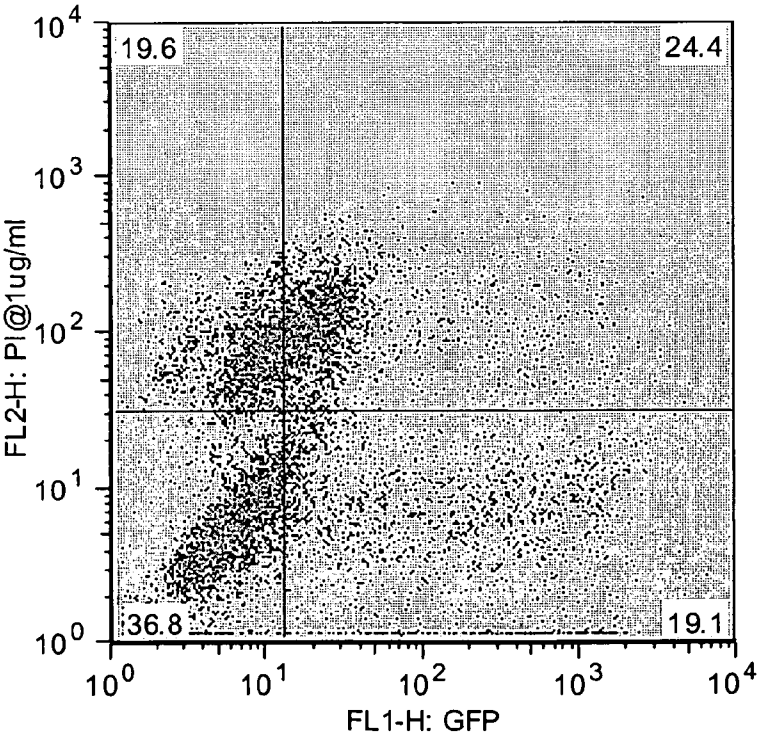

FIGURE 2G

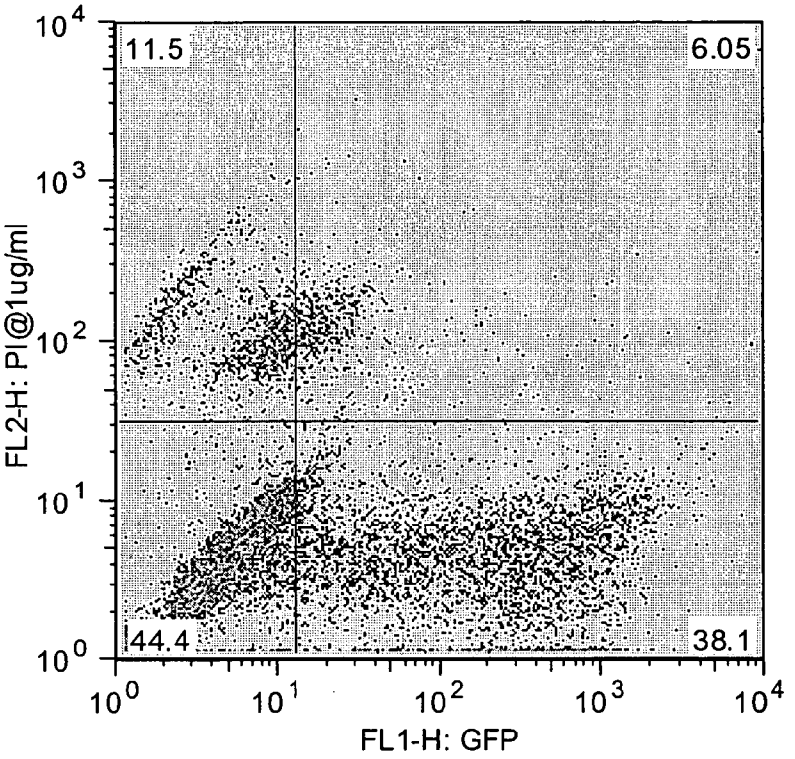

FIGURE 2H

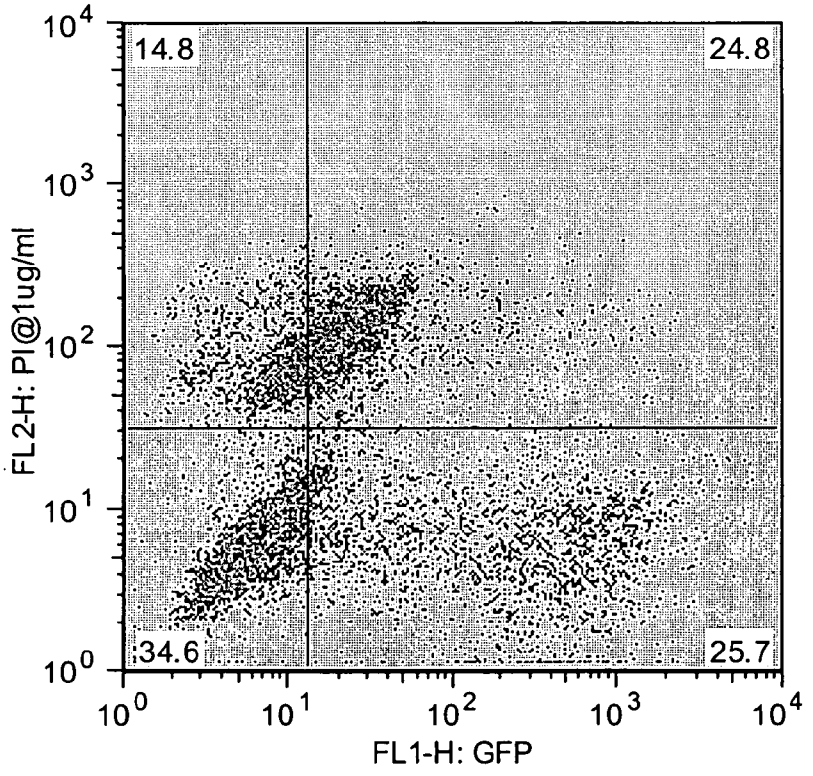

FIGURE 2I

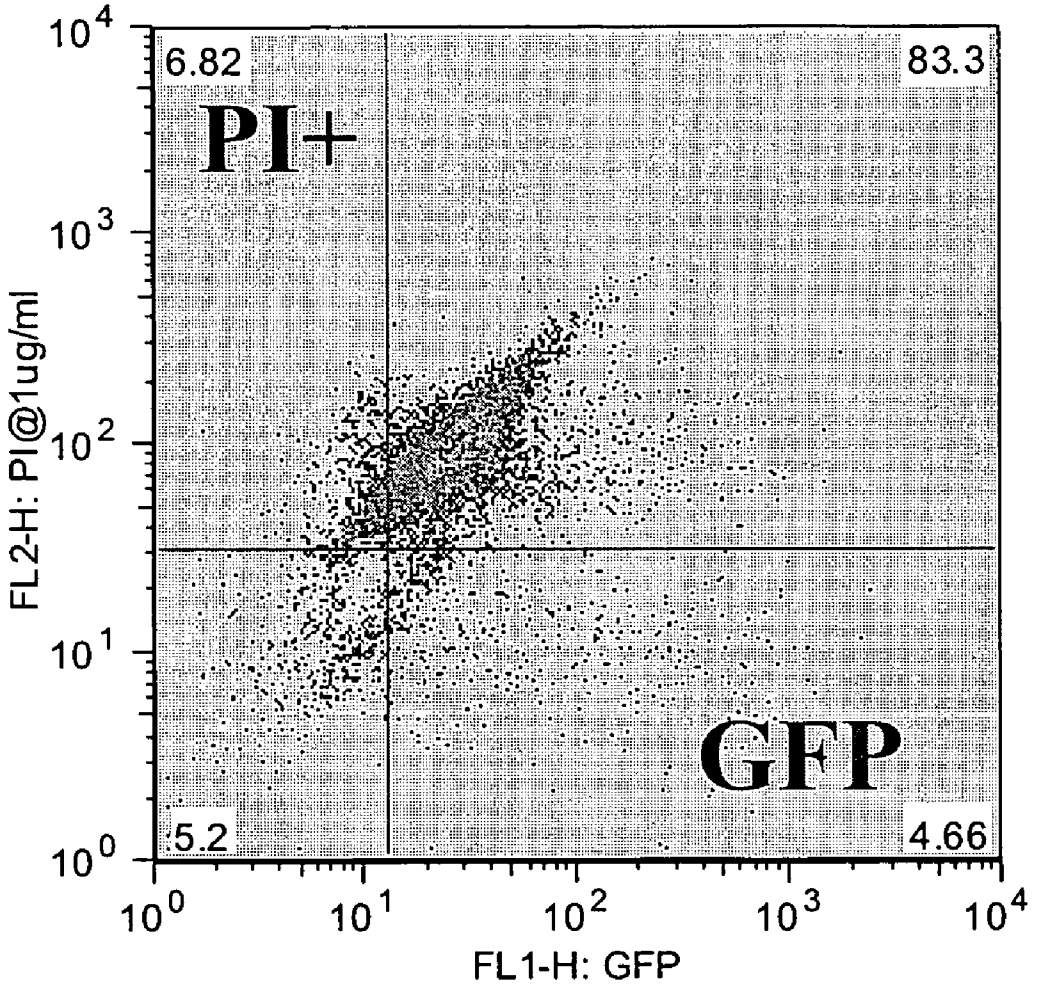

FIGURE 3

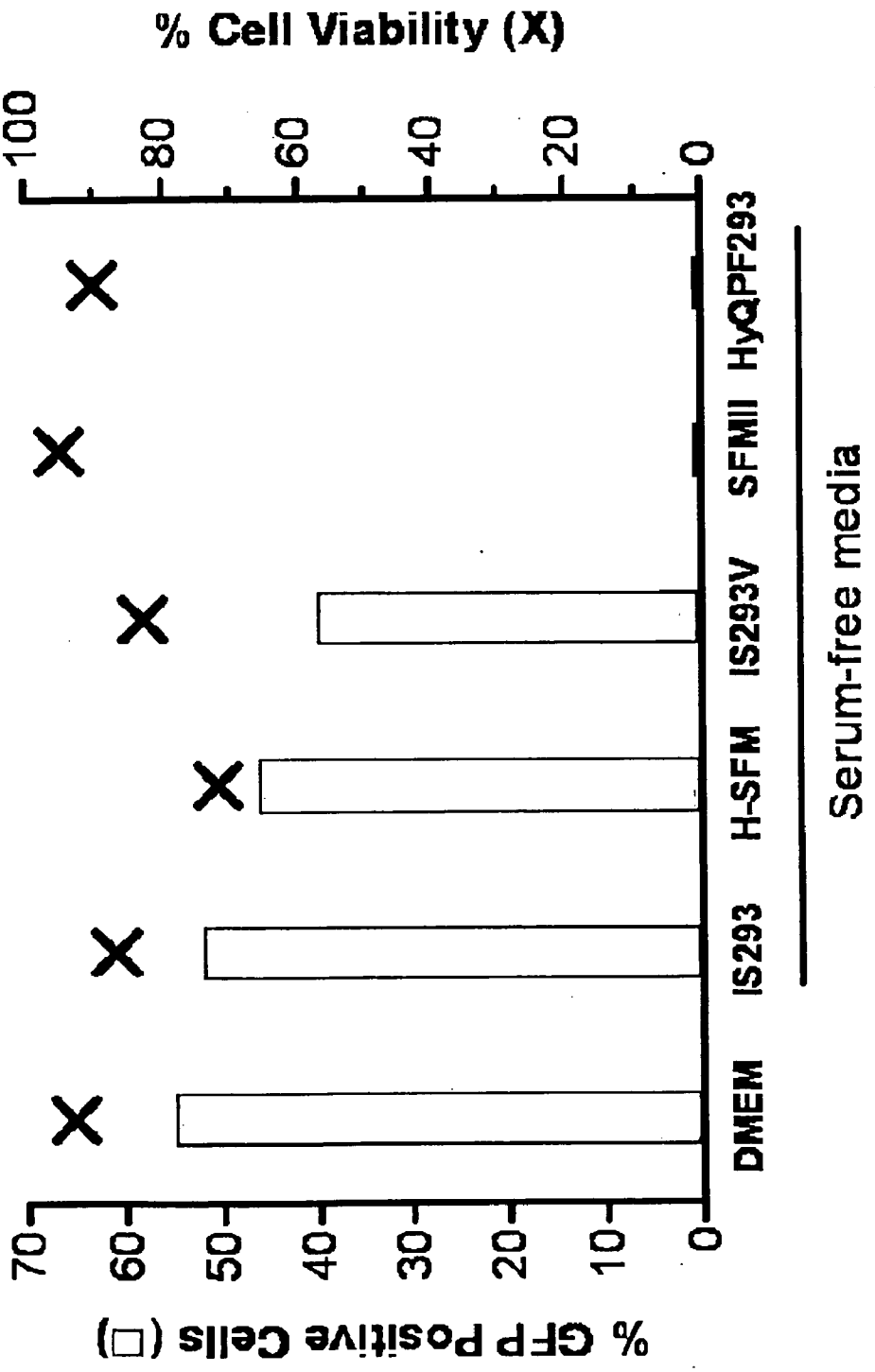
% Cell Viability (X)
100
80
60
40
20
0
% GFP Positive Cells (□)
70
60
50
40
30
20
10
0
DMEM
IS293
H-SFM
IS293V
SFMII
HyQPF293
Serum-free media

FIGURE 4

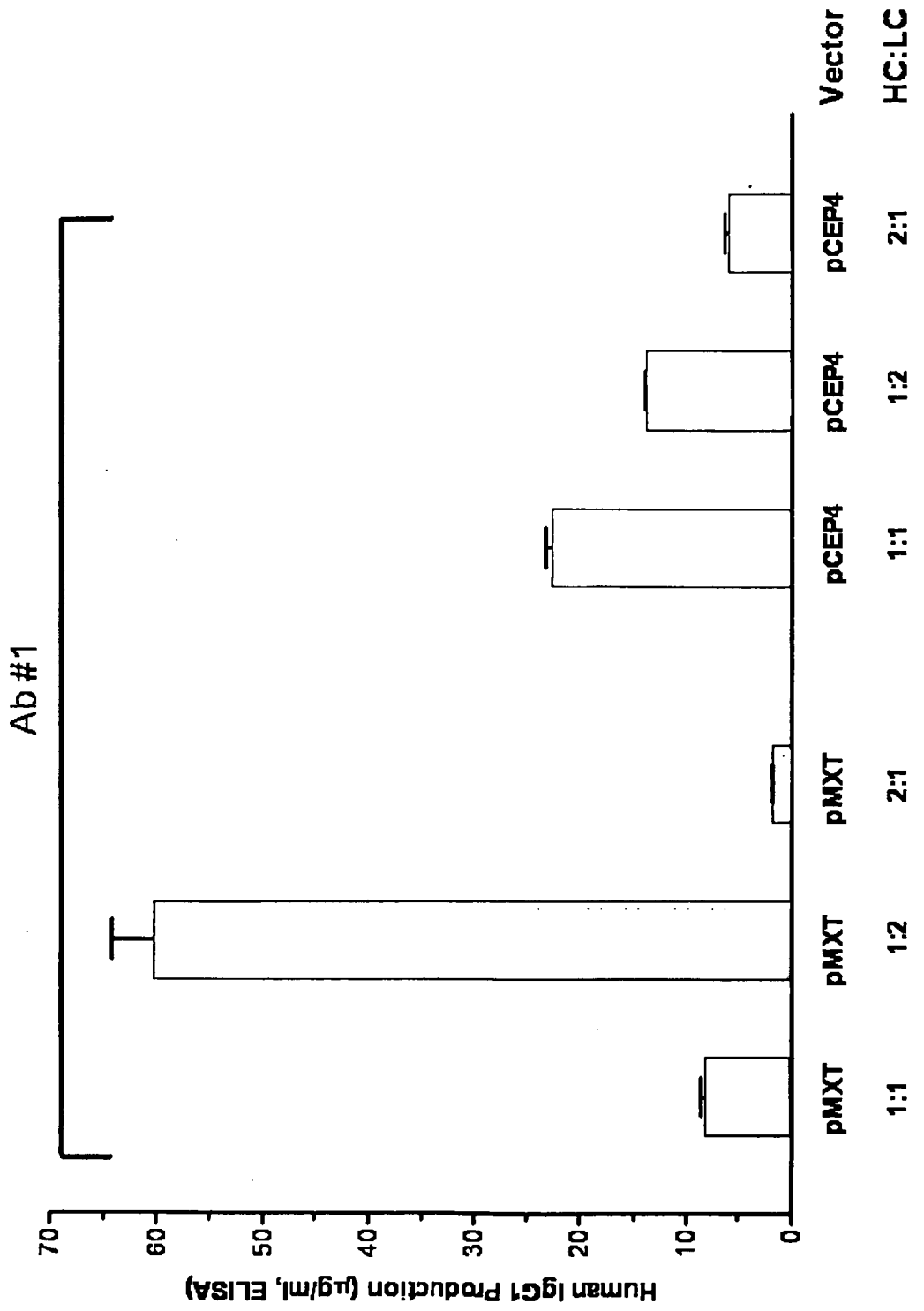
Ab #1
Human IgG1 Production (µg/ml, ELISA)
0
10
20
30
40
50
60
70
pMXT
pMXT
pMXT
pCEP4
pCEP4
pCEP4
Vector
1:1
1:2
2:1
1:1
1:2
2:1
HC:LC

FIGURE 5A

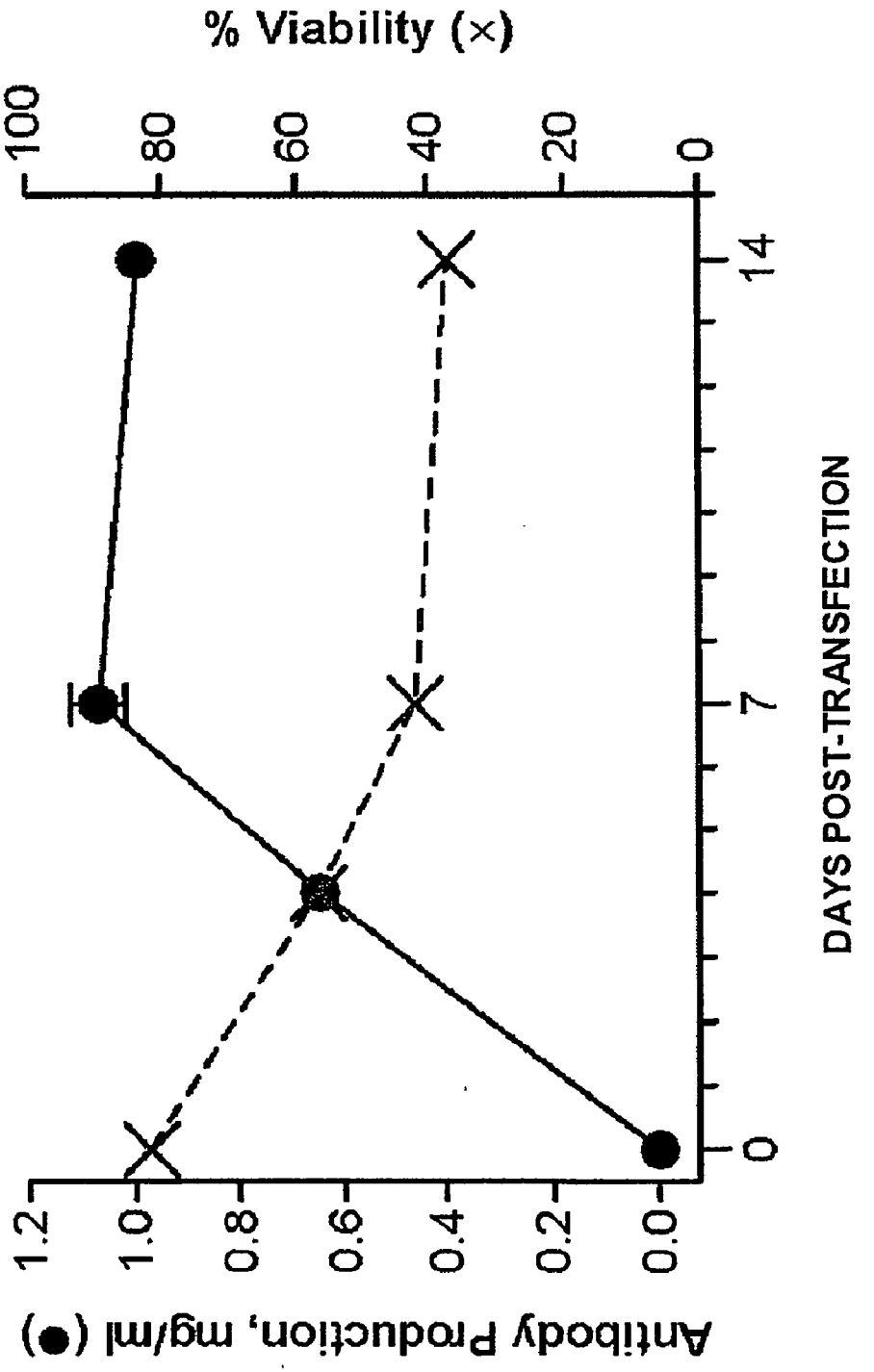
% Viability (×)
100
80
60
40
20
0
14
7
0
DAYS POST-TRANSFECTION
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antibody Production, mg/ml (●)

FIGURE 5B

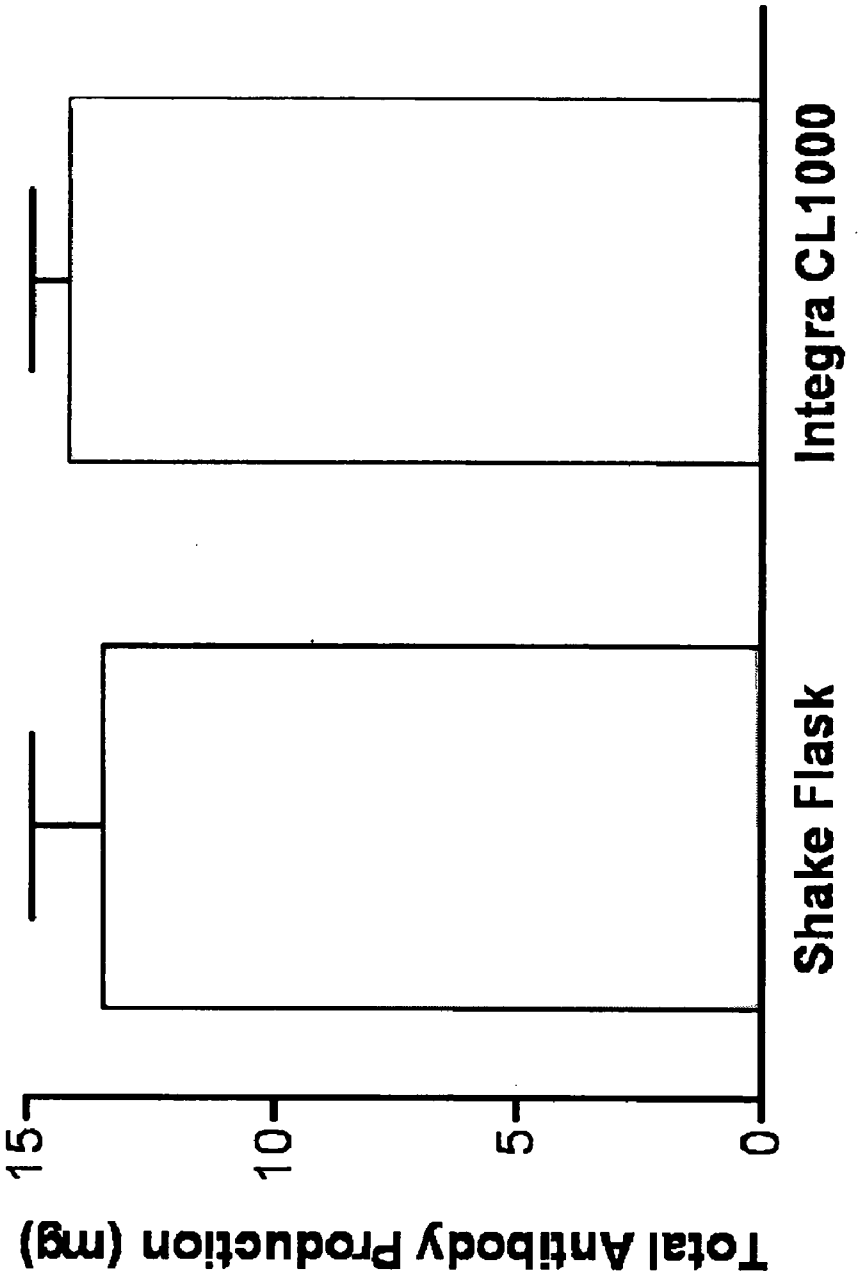
Total Antibody Production (mg)
0
5
10
15
Shake Flask
Integra CL1000

FIGURE 6A

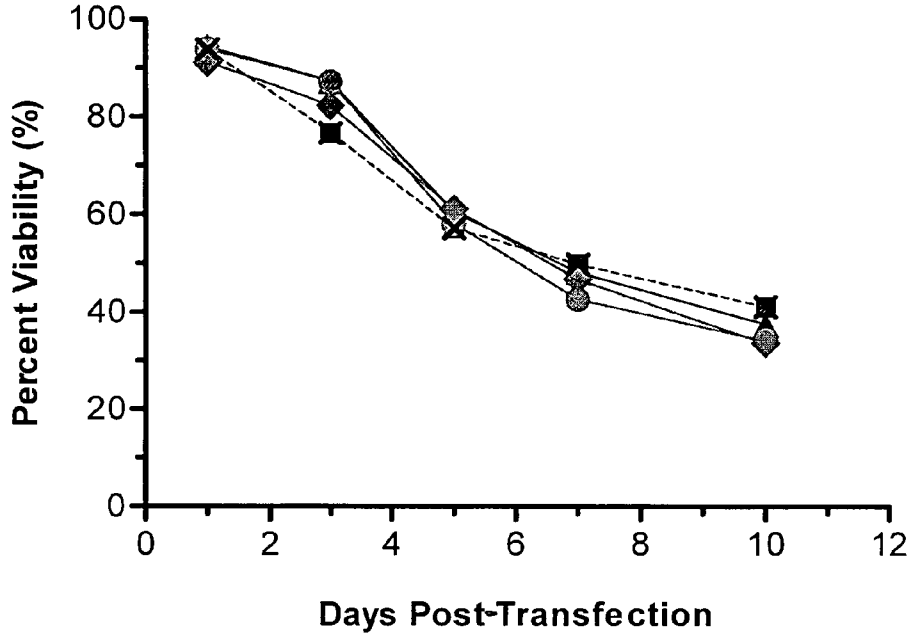

FIGURE 6B

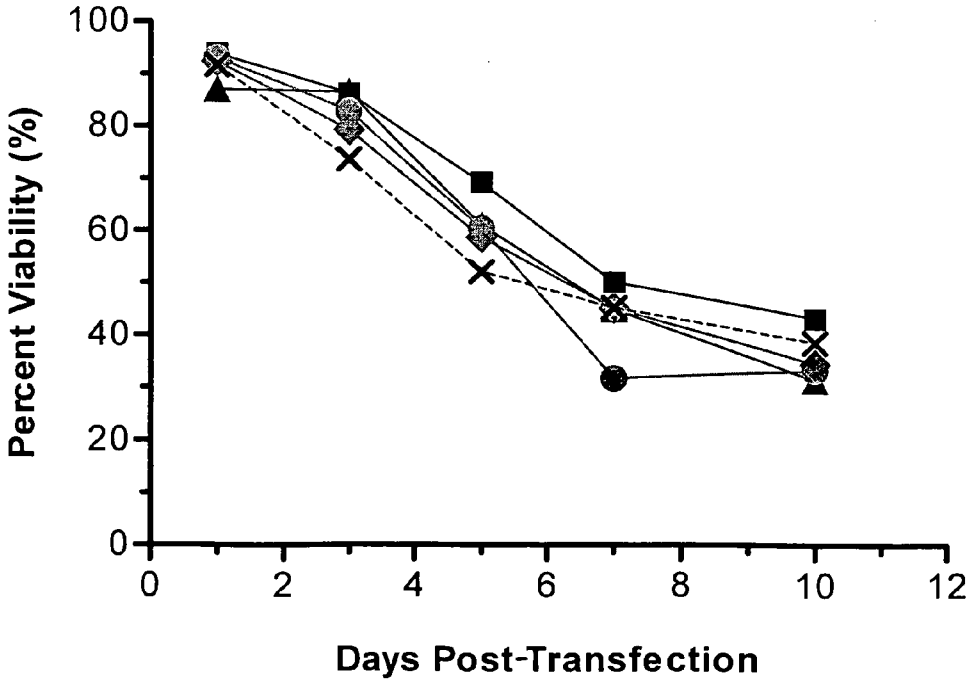

FIGURE 7A

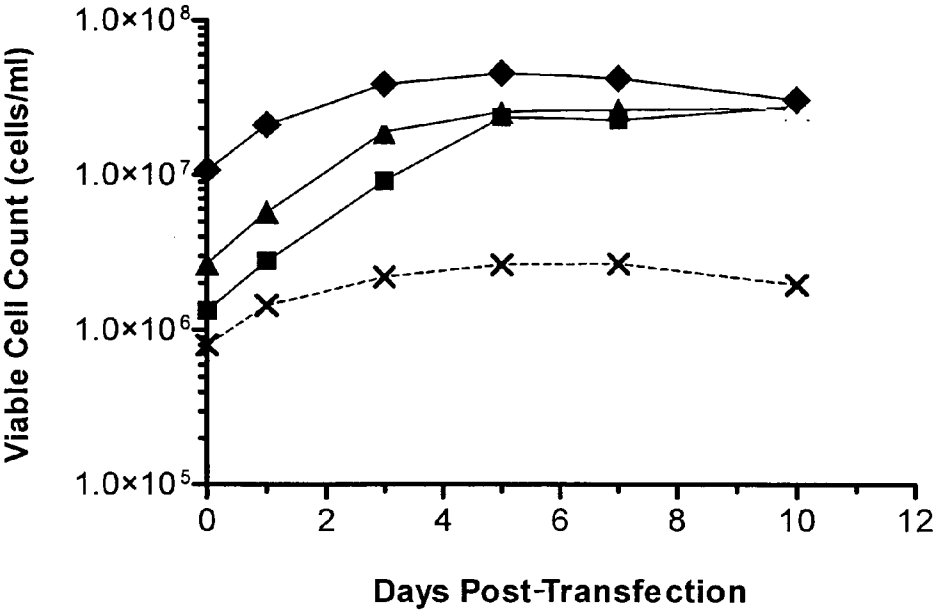

FIGURE 7B

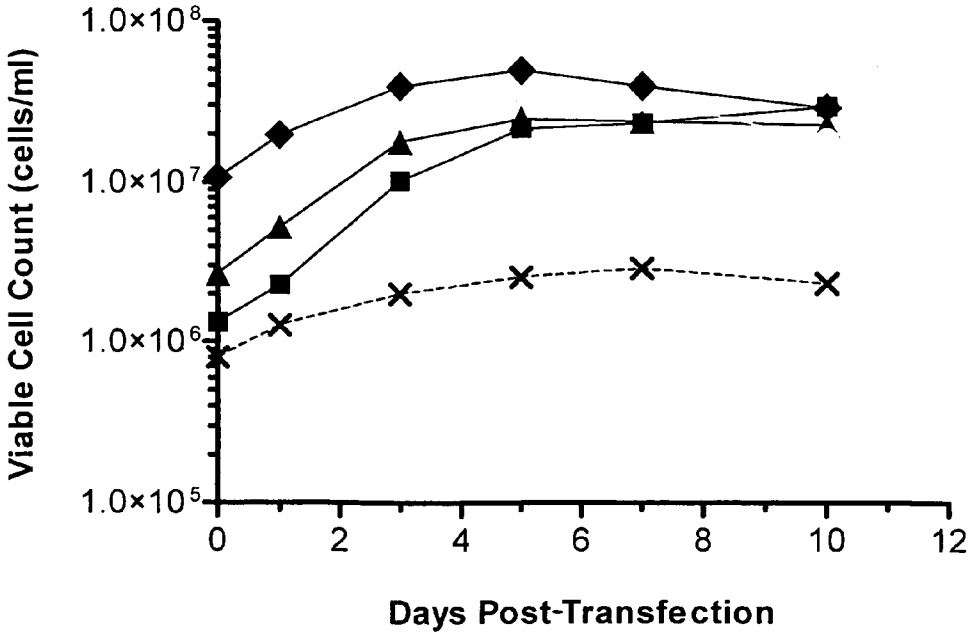

FIGURE 8A

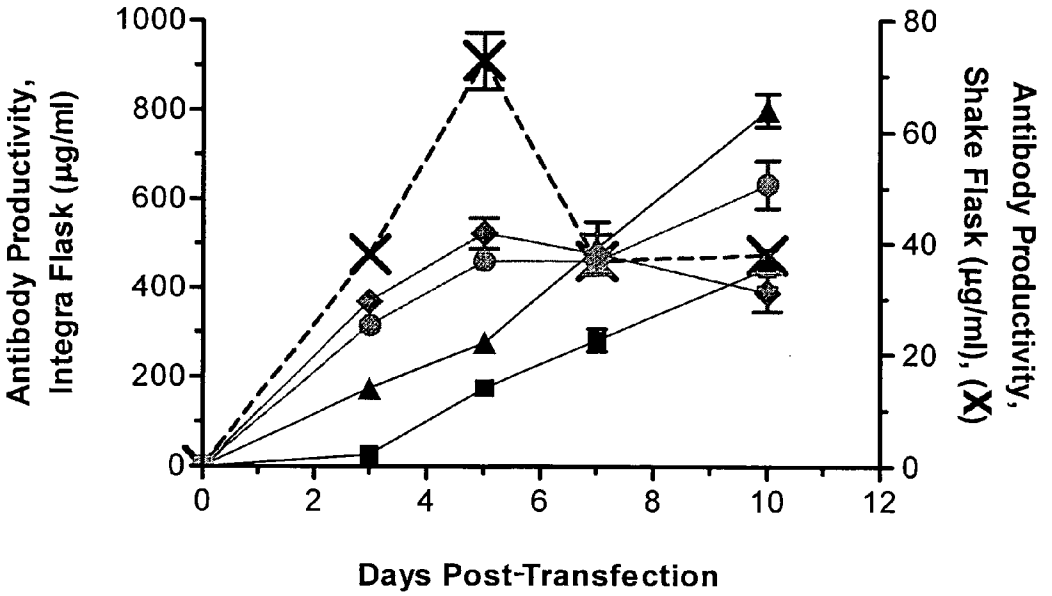

FIGURE 8B

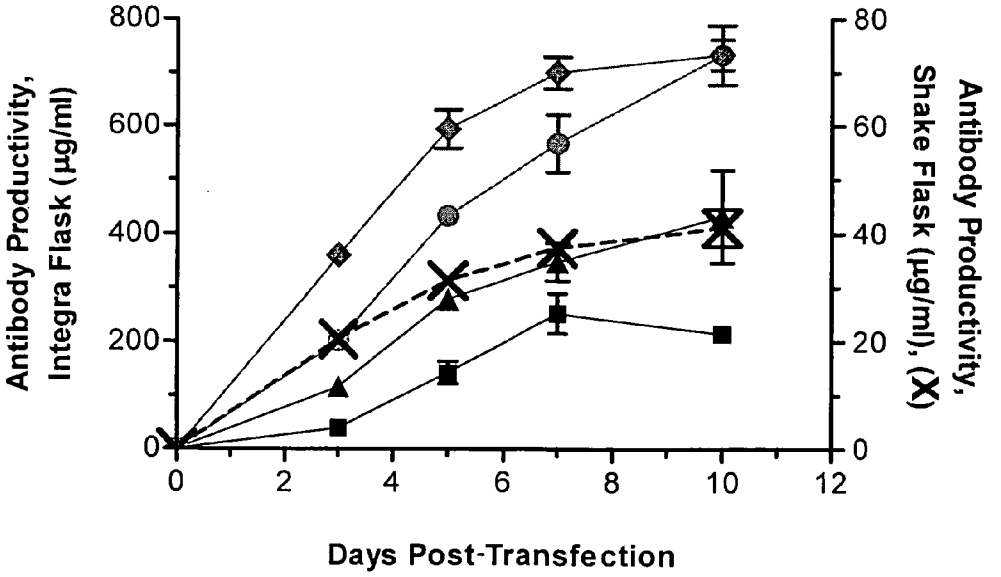

FIGURE 9A

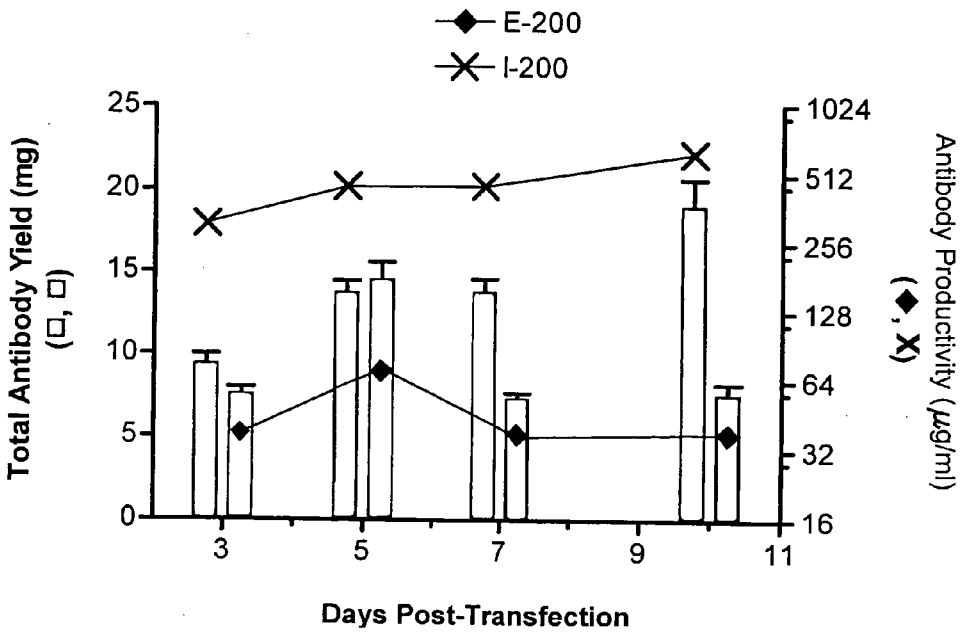

FIGURE 9B

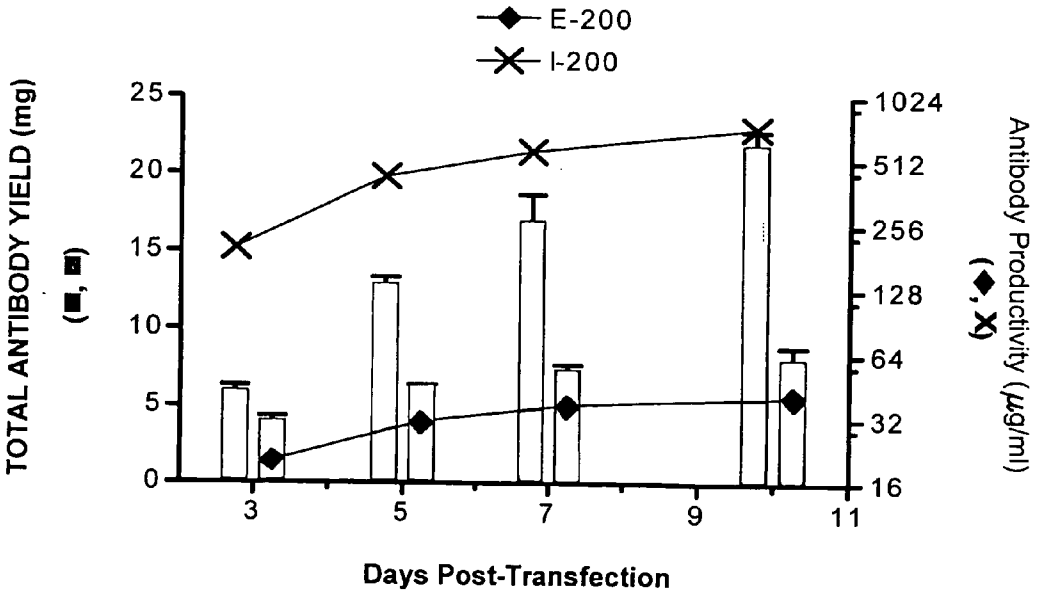

FIGURE 10

SEQ ID NO: 1

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   71 GGGCTCCCCA AAATCTGAAT TTCTGAGTAG TCTTCATCCC CTCTCCTGCT CTAAGGTCAG GTCCATCCTC
  141 TCTGGTCCTT ACCTTGATGA CAAGGATCGA CATTGATTAT TGACTAGTTA TTAATAGTAA TCAATTACGG
  211 GGTCATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG
  281 ACCGCCCAAC GACCCCCGCC CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT
  351 TTCCATTGAC GTCAATGGGT GGAGTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA
  421 TGCCAAGTAC GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC
  491 CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT GATGCGGTTT
  561 TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTCC AAGTCTCCAC CCCATTGACG
  631 TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT TCCAAAATGT CGTAACAACT CCGCCCCATT
  701 GACGCAAATG GGCGGTAGGC GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTCGTTTAGT GAACCGTCAG
  771 ATCGCCTGGA GACGCCATCC ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGCG
  841 GCCGGGAACG GTGCATTGGA ACGCGGATTC CCCGTGCCAA GAGTGACGTA AGTACCGCCT ATAGAGTCTA
  911 TAGGCCCACC CCCTTGGCTT CTTATGGATC CGGTGGTGGT GCAAATCAAA GAACTGCTCC TCAGTGGATG

                                                                    SalI   EcoRI
  981 TTGCCTTTAC TTCTAGGCCT GTACGGAAGT GTTACTTCTG CTCTAAAAGC TGCTGCAGGT CGACGAATTC

      ClaI  EcoRV XhoI
 1051 ATCGATGATA TCTCGAGCCC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG
 1111 GAGAAGTGCC CCCACCTGCT CCTCAGTTCC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCTTTTT
 1181 CCACAGGGGA CCTACCCCTA TTGCGGTCCT CCAGCTCATC TTTCACCTCA CCCCCCTCCT CCTCCTTGGC
 1251 TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTTG CACCTGTGGT TTCTCTCTTT
 1321 CCTCACTAGA GGATCTCTGT CTTTCTTACT AAATGGTAGT AATCAGTTGT TTTTCCAGTT ACCTGGGTTT
 1391 CTCTTCTAAA GAAGTTAAAT GTTTAGTTGC CCTGAAATCC ACCACACTTA AAGGATAAAT AAAACCCTCC
 1461 ACTTGCCCTG GTTGGCTGTC CACTACATGG CAGTCCTTTC TAAGGTTCAC GAGTACTATT CATGGCTTAT
 1531 TTCTCTGGGC CATGGTAGGT TTGAGGAGGC ATACTTCCTA GTTTTCTTCC CCTAAGTCGT CAAAGTCCTG
 1601 AAGGGGGACA GTCTTTACAA GCACATGTTC TGTAATCTGA TTCAACCTAC CCAGTAAACT TGGCGAAGCA
 1671 GTAGAATCAT TATCACAGGA AGCAAAGGCA ACCTAAATGT GCAAGCAATA GGAAAATGTG GAAGCCCATC
 1741 ATAGTACTTG GACTTCATCT GCTTTTGTGC CTTCACTAAG TTTTTAAACA TGAGCTGGCT CCTATCTGCC
 1811 ATTGGCAAGG CTGGGCACTA CCCACAACCT ACTTCAAGGA CCTCTATACC GTGAGATTAC ACACATACAT
 1881 CAAAATTTGG GAAAAGTTCT ACCAAGCTGA GAGCTGATCA CCCCACTCTT AGGTGCTTAT CTCTGTACAC
 1951 CAGAAACCTT AAGAAGCAAC CAGTATTGAG AGACTCATTT ATGAAAGTCT AAAACTGGAT ACAACCAAAA
 2021 TGTCCACCAA CAGTTAAATT ATGACATGTT CACAATTGAG CTATTACTTA ATAAGGAGAA TTAATAAAAT
 2091 AAAACTTAAG AGCATAGTTT AATCTCATAA ACAAGATAAT AAGCAAAACA AAACATTTTT TCATCCATGT
 2161 AAGTTTAAAA GCAGGTAAAA TTTAAAATTA AGAGAGACAT AAGTTTTGAG GTAGCAAGAT GGAAACTCTG
 2231 GGGCTTGGGG AATGTTCTGT CTCTCTGTAT GGGATGTGAA AGTTACTATT GTGGAATTGG GATCTATGTT
 2301 CTTCCTGTAT ATATTGTATA CTTCATAATA ACTTCACCTA AAGAAATATC TAATACCCAG TGCATACATA
 2371 AAAGAGGATA CAAGGAATGA ATCATACGTC AAGGCCAGAA AGACAATAAA GTAGGGGATC CAGACATGAT
 2441 AAGATACATT GATGAGTTTG GACAAACCAC AACTAGAATG CAGTGAAAAA AATGCTTTAT
 2501 TTGTGAAATT TGTGATGCTA TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAAACAAGT
        XbaI
 2561 TctctagaTG TGTAACTCTT GGCTGAAGCT CTTACACCAA TGCTGGGGGA CATGTACCTC CCAGGGGCCC
 2631 AGGAAGACTA CGGGAGGCTA CACCAACGTC AATCAGAGGG GCCTGTGTAG CTACCGATAA GCGGACCCTC
 2701 AAGAGGGCAT TAGCAATAGT GTTTATAAGG CCCCCTTGTT AACCCTAAAC GGGTAGCATA TGCTTCCCGG
 2771 GTAGTAGTAT ATACTATCCA GACTAACCCT AATTCAATAG CATATGTTAC CCAACGGGAA GCATATGCTA
 2841 TCGAATTAGG GTTAGTAAAA GGGTCCTAAG GAACAGCGAT ATCTCCCACC CCATGAGCTG TCACGGTTTT
 2911 ATTTACATGG GGTCAGGATT CCACGAGGGT AGTGAACCAT TTTAGTCACA AGGGCAGTGG CTGAAGATCA
 2981 AGGAGCGGGC AGTGAACTCT CCTGAATCTT CGCCTGCTTC TTCATTCTCC TTCGTTTAGC TAATAGAATA
 3051 ACTGCTGAGT TGTGAACAGT AAGGTGTATG TGAGGTGCTC GAAAACAAGG TTTCAGGTGA CGCCCCCAGA
 3121 ATAAAATTTG GACGGGGGGT TCAGTGGTGG CATTGTGCTA TGACACCAAT ATAACCCTCA CAAACCCCTT
 3191 GGGCAATAAA TACTAGTGTA GGAATGAAAC ATTCTGAATA TCTTTAACAA TAGAAATCCA TGGGGTGGGG
 3261 ACAAGCCGTA AAGACTGGAT GTCCATCTCA CACGAATTTA TGGCTATGGG CAACACATAA TCCTAGTGCA
 3331 ATATGATACT GGGGTTATTA AGATGTGTCC CAGGCAGGGA CCAAGACAGG TGAACCATGT TGTTACACTC
 3401 TATTTGTAAC AAGGGGAAAG AGAGTGGACG CCGACAGCAG CGGACTCCAC TGGTTGTCTC TAACACCCCC
 3471 GAAAATTAAA CGGGGCTCCA CGCCAATGGG GCCCATAAAC AAAGACAAGT GGCCACTCTT TTTTTGAAA
 3541 TTGTGGAGTG GGGGCACGCG TCAGCCCCCA CACGCCGCCC TGCGGTTTTG GACTGTAAAA TAAGGGTGTA
 3611 ATAACTTGGC TGATTGTAAC CCCGCTAACC ACTGCGGTCA AACCACTTGC CCACAAAACC ACTAATGGCA
 3681 CCCCGGGGAA TACCTGCATA AGTAGGTGGG CGGGCCAAGA TAGGGGCGCG ATTGCTGCGA TCTGGAGGAC
 3751 AAATTACACA CACTTGCGCC TGAGCGCCAA GCACAGGGTT GTTGGTCCTC ATATTCACGA GGTCGCTGAG
 3821 AGCACGGTGG GCTAATGTTG CCATGGGTAG CATATACTAC CCAAATATCT GGATAGCATA TGCTATCCTA
 3891 ATCTATATCT GGGTAGCATA GGCTATCCTA ATCTATATCT GGGTAGCATA TGCTATCCTA ATCTATATCT
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FIGURE 10 (CONTINUED)

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3961 GGGTAGTATA TGCTATCCTA ATTTATATCT GGGTAGCATA GGCTATCCTA ATCTATATCT GGGTAGCATA
4031 TGCTATCCTA ATCTATATCT GGGTAGTATA TGCTATCCTA ATCTGTATCC GGGTAGCATA TGCTATCCTA
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4241 ATCCTAATCT ATATCTGGGT AGCATATGCT ATCCTAATCT ATATCTGGGT AGTATATGCT ATCCTAATTT
4311 ATATCTGGGT AGCATAGGCT ATCCTAATCT ATATCTGGGT AGCATATGCT ATCCTAATCT
4371 ATATCTGGGT AGTATATGCT ATCCTAATCT GTATCCGGGT AGCATATGCT ATCCTCATGC ATATACAGTC
4441 AGCATATGAT ACCCAGTAGT AGAGTGGGAG TGCTATCCTT TGCATATGCC GCCACCTCCC AAGGGGCGT
                                    SphI
4511 GAATTTTCGC TGCTTGTCCT TTTCCTGCTG GTTGGCATGC CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT
4581 CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC
4651 AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG
4721 CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
4791 ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC
4861 CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
4931 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTCGCT
5001 CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT
5071 TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
5141 AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGAACAGTAT
5211 TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
5281 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA
5351 GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
5421 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA
5491 AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC
5561 TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG GAGGGCTTAC
5631 CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
5701 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT
5771 TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG
5841 GCATCGTGGT GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT
5911 TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG
5981 TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCCGTAA
6051 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG
6121 CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA
6191 AAACGTTCTT CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC
6261 GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA
6331 AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
6401 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC
                                                              NotI
6471 AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCTAA GAAACCGCGG CCGCAACAGA
6541 CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTCTC
6611 GCGCGTTTCG GTGATGACGG TGAAAACCTC TGACACATGC AGCTCCCGGA GACGGTCACA GCTTGTCTGT
6681 AAGCGGATGC CGGGAGCAGA CAAGCCCGTC AGGGCGCGTC AGCGGGTGTT GGCGGGTGTC GGGGCTGGCT
6751 TAACTATGCG GCATCAGAGC AGATTGTACT GAGAGTGCAC CATATGCGGT GTGAAATACC GCACAGATGC
6821 GTAAGGAGAA AATACCGCAT CAGGCGCCAT TCGCCATTCA GGCTGCGCAA CTGTTGGGAA GGGCGATCGG
6891 TGCGGGCCTC TTCGCTATTA CGCCAGCTGG CGAAAGGGGG ATGTGCTGCA AGGCGATTAA GTTGGGTAAC
6961 GCCAGGGTTT TCCCAGTCAC GACGTTGTAA AACGACGGCC AGTGCC
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METHODS AND MATERIALS FOR EXPRESSION OF A RECOMBINANT PROTEIN

FIELD OF THE INVENTION

[0001] This invention pertains to methods of producing a recombinant protein and recombinant expression vectors and host cells for use therein.

BACKGROUND OF THE INVENTION

[0002] Large-scale transient expression of recombinant proteins has been an area of rapid development in the past several years as an alternative or precursor to stable cell line development to generate multi-milligram quantities of protein (Wurm et al., *Curr. Opn. Biotech.* 10: 156-159 (1999)). Human embryonic kidney (HEK293) cells are one of the most widely used cell lines for transient expression and have been successfully adapted to suspension-growth to help facilitate culture scale-up. Recent reports have successfully demonstrated the usage of transiently expressing suspension-adapted HEK293 cells in 1-3 L cultures to generate recombinant proteins including soluble polypeptides, transmembrane proteins, and human antibodies (Durocher et al., *Nucleic Acids Res.* 30: 1-9 (2002); Meissner et al., *Biotechnol. Bioeng.* 75: 197-203 (2000); and Cote et al., *Biotechnol. Bioeng.* 59: 567-575 (1998)).

[0003] In particular, Durocher et al. has shown that HEK293E cells expressing the Epstein-Barr virus (EBV) nuclear antigen-1 protein (EBNA1) were able to routinely generate >10 mg/L of a number of different recombinant proteins using the cationic polymer transfection reagent, polyethyleneimine (PEI) (Boussif et al., *Proc. Natl. Acad. Sci.* 92: 7297-7301 (1995); and Mislick et al., *Proc. Natl. Acad. Sci.* 93: 12349-12354 (1996)).

[0004] Despite these advances, there is still a need in the art for improved expression systems including optimized transient transfection systems for time- and cost-efficient production of recombinant proteins. The invention provides such optimized methods of producing recombinant proteins. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

[0005] The invention provides recombinant expression vectors useful in methods of producing a recombinant protein. One of the inventive recombinant expression vectors comprises a 3' untranslated region (UTR) of a light chain gene. Another recombinant expression vector provided herein comprises a 3' UTR and an Epstein-Barr virus origin of replication. Host cells comprising any of the inventive recombinant expression vectors are also provided herein.

[0006] The invention further provides methods of producing a recombinant protein. In a first method, the recombinant protein is a heterodimeric or heteromultimeric protein, either of which comprises a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain is different from the second polypeptide chain. The method comprises contacting cells in a medium with a first vector and a second vector, wherein the first vector encodes the first polypeptide chain and the second vector encodes the second polypeptide chain, and the second vector is present in the medium in an amount which is about 1.5 to about 2.5 times as much as the amount of the first vector.

[0007] In a second method of producing a recombinant protein, the method comprises culturing cells, which have been contacted with a recombinant transient expression vector encoding the protein, in a medium in a membrane-enhanced culturing vessel, whereupon a recombinant protein is produced. Alternatively, the second method comprises culturing cells, which have been contacted with a recombinant transient expression vector encoding the protein, in a medium in a Fembach flask.

[0008] In a third method, the recombinant protein is produced upon contacting cells with at least one of the inventive recombinant expression vectors described herein. In a fourth method, the recombinant protein is produced upon culturing host cells comprising any of the inventive recombinant expression vectors described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **FIG. 1A** is an illustration of the pMXT recombinant expression vector without any recombinant protein coding sequences. **FIG. 1B** is an illustration of the pMXT vector encoding a human γ_2 heavy chain, while **FIG. 1C** is an illustration of the pMXT vector encoding a human κ light chain. The following abbreviations are used in FIGS. **1A-1C**: Ap, ampicillin resistance marker; CMV promoter, cytomegalovirus promoter; MCS, multiple cloning sequence; 5' UT intron, 5' untranslated region intron; SP, signal peptide; V, variable region; C, constant region; LC 3' UT, light chain 3' untranslated region, OriP, Epstein-Barr virus origin of replication; pUC19ori, origin of replication from the pUC19 plasmid.

[0010] **FIGS. 2A-2I** are flow cytometry data graphs depicting the levels of fluorescence of green fluorescence protein (GFP) and propidium iodide (PI) under differing transfection conditions, specifically differing DNA and polyethyleneimine (PEI) concentrations. In **FIG. 2A**, cells were transfected with 1 μg/ml DNA and 1 μg/ml PEI. In **FIG. 2B**, cells were transfected with 2 μg/ml DNA and 2 μg/ml PEI. In **FIG. 2C**, cells were transfected with 5 μg/ml DNA and 5 μg/ml PEI. In **FIG. 2D**, cells were transfected with 1 μg/ml DNA and 2 μg/ml PEI. In **FIG. 2E**, cells were transfected with 2 μg/ml DNA and 4 μg/ml PEI. In **FIG. 2F**, cells were transfected with 5 μg/ml DNA and 10 μg/ml PEI. In **FIG. 2G**, cells were transfected with 1 μg/ml DNA and 5 μg/ml PEI. In **FIG. 2H**, cells were transfected with 2 μg/ml DNA and 10 μg/ml PEI. In **FIG. 2I**, cells were transfected with 5 μg/ml DNA and 25 μg/ml PEI.

[0011] **FIG. 3** is a graph showing the % cell viability (X) and % GFP positive 293E cells (■) that were adapted to suspension growth in different serum-free media and optimized for transfection. A control set of 293E cells were grown in DMEM.

[0012] **FIG. 4** is a graph showing the antibody production by cells which were co-transfected with different heavy chain (HC):light chain (LC) ratios of different vector types.

[0013] **FIG. 5A** is a graph showing antibody production (●) and cell viability (X) of transiently transfected 293E cells in Integra flasks as a function of time post-transfection.

FIG. 5B is a graph showing the production of antibodies in shake flasks vs. Integra flasks by transfected cell cultures at day 7 post-transfection.

[0014] **FIG. 6A** is a graph of the percentage of viable cells transfected with DNA encoding Ab#1 as a function of time post-transfection. **FIG. 6B** is a graph of the percentage of viable cells transfected with DNA encoding Ab#2 as a function of time post-transfection. In both **FIGS. 6A and 6B**, ■ is I-50; ▲ is I-100; ● is I-200; ♦ is I-400; and X is E-200.

[0015] **FIG. 7A** is a graph of the number of viable cells transfected with DNA encoding Ab#1 as a function of time post-transfection. **FIG. 7B** is a graph of the number of viable cells transfected with DNA encoding Ab#2 as a function of time post-transfection. In both **FIGS. 7A and 7B**, ■ is I-50; ▲ is I-100; ● is I-200; ♦ is I-400; and X is E-200.

[0016] **FIG. 8A** is a graph of the concentration of antibody produced by cells transfected with DNA encoding Ab#1 as a function of time post transfection. **FIG. 8B** is a graph of the concentration of antibody produced by cells transfected with DNA encoding Ab#2 as a function of time post-transfection. In both **FIGS. 8A and 8B**, ■ is I-50; ▲ is I-100; ● is I-200; ♦ is I-400; and X is E-200.

[0017] **FIG. 9A** is a graph of the total antibody produced by cells tranfected with DNA encoding Ab#1 as a function of time post-transfection. **FIG. 9B** is a graph of the total yield of antibody produced by cells transfected with DNA encoding Ab#2 as a function of time post-transfection.

[0018] **FIG. 10** shows SEQ ID NO: 1, which is the nucleotide sequence of pMXT5 (**FIG. 1A**) without any coding sequences. Restriction enzyme sites are labeled with the name of the enzyme above the position of the site. CMV promoter comprises nucleotides 1-1037; 5' UTR intron comprises nucleotides 888-974; MCS comprises nucleotides 1038-1061; LC 3' UT comprises nucleotides 1062-2560; OriP comprises nucleotides 2561-4550; pUC19 ori comprises nucleotides 4551-5220; and Ap comprises nucleotides 5221-6380.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The invention provides recombinant expression vectors useful in methods of producing a recombinant protein. One of the inventive recombinant expression vectors comprises a 3' untranslated region (UTR) of a light chain gene. Another recombinant expression vector provided herein comprises a 3' UTR and an Epstein-Barr virus origin of replication (oriP). Inventive recombinant expression vectors optionally comprise a pUC19 origin of replication (pUC19ori).

[0020] For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide (i.e., polynucleotide) construct that permits the production of a protein within a cell, when the construct comprises a nucleotide sequence encoding the protein, and the construct is contacted with the cell under conditions sufficient to have the protein expressed within the cell. As the expression vector is recombinant, the vector of the invention is not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring.

[0021] The recombinant expression vector can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, which can be synthesized or obtained in part from natural sources, and which can contain natural or non-natural or altered nucleotides. Examples of non-natural or altered nucleotides that can be used to generate the recombinant expression vectors include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3) w, and 2,6-diaminopurine.

[0022] The recombinant expression vector can comprise naturally-occurring or non-naturally-occuring internucleotide linkages, or both types of linkages, such as phosphoroamidate linkages or phosphorothioate linkages, instead of the phosphodiester linkages found between the nucleotides of an unmodified oligonucleotide. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder in any way the transcription or replication of the vector.

[0023] The recombinant expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. For example, one of ordinary skill in the art appreciates that transformation or transfection is a process by which, for example, exogenous nucleic acids such as DNA are introduced into cells wherein the transformation or transfection process involves contacting the cells with the exogenous nucleic acids such as the recombinant expression vector as described herein. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNML149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121, and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). A preferred recombinant expression vector includes the pMXT vector as shown in **FIGS. 1A-1C**.

[0024] The recombinant expression vector can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *Molecular Cloning, a Laboratory Manual,* 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel

et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[0025] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0026] A construct of a recombinant expression vector, which is circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived from ColEl, 2 μplasmid, λ, SV40, bovine papilloma virus, and the like. The recombinant expression vector of the invention can comprise a replication system, which comprises an oriP. Preferably, the inventive recombinant expression vector comprises an oriP, and not an Epstein Barr virus nuclear antigen (EBVNA), which EBVNA is known to activate an oriP.

[0027] As used herein, the term "oriP" or "Epstein-Barr virus origin of replication" refers to a nucleotide sequence that is substantially identical to the Epstein-Barr virus origin of replication, which has the nucleotide sequence of nucleotides 2561-4550 of SEQ ID NO: 1. It is preferred that no insertions, deletions, inversion, and/or substitutions are present in this nucleotide sequence. However, one of ordinary skill in the art appreciates that the nucleotide sequence of nucleotides 2561-4550 of SEQ ID NO: 1 can have insertions, deletions, inversion, and/or substitutions that will not negatively affect the function of the nucleotide sequence, which is to promote high copy episomal plasmid replication. One of ordinary skill in the art further appreciates that such high copy episomal plasmid replication occurs in mammalian cells.

[0028] The recombinant expression vector also preferably comprises a pUC19 origin of replication. As used herein, the term "pUC19 origin of replication" refers to the nucleotide sequence of the origin of replication from a pUC 19 vector, which is commercially available from Fermentas Life Sciences and has the nucleotide sequence of nucleotides 4551-5220 of SEQ ID NO: 1. It is preferred that no insertions, deletions, inversion, and/or substitutions are present in this nucleotide sequence. However, one of ordinary skill in the art appreciates that nucleotides 4551-5220 of SEQ ID NO: 1 can have insertions, deletions, inversion, and/or substitutions that will not affect the function of the nucleotide sequence, which is to promote high copy episomal plasmid replication. One of ordinary skill in the art further appreciates that such high copy episomal plasmid replication occurs in bacterial cells.

[0029] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive recombinant expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0030] The recombinant expression vector can comprise a native or normative promoter operably linked to the nucleic acid encoding the protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific, and developmental-specific, is within the ordinary skill in the art. Similarly, the combining of a nucleic acid with a promoter is also within the skill in the art. The promoter can be a viral promoter or a non-viral promoter. Preferably, the promoter is a viral promoter. More preferably, the viral promoter is a strong viral promoter, such as a cytomegalovirus (CMV) promoter. The CMV promoter is known in the art and has the nucleotide sequence of nucleotides 1-1037 of SEQ ID NO: 1. It is preferred that no insertions, deletions, inversion, and/or substitutions are present in this nucleotide sequence. However, one of ordinary skill in the art appreciates that nucleotides 1-1037 of SEQ ID NO: 1 can have insertions, deletions, inversion, and/or substitutions that will not affect the function of the nucleotide sequence, which is to drive the transcription of the recombinant protein coding sequence.

[0031] The recombinant expression vector comprises a 3' UTR of a light chain gene. Preferably, the recombinant expression vector comprises a 3' UTR of a light chain gene in combination with an Epstein-Barr virus origin of replication (oriP). As used herein, the term "3' UTR" refers to a nucleotide sequence of a gene that is untranslated and is located 3' to the stop codon of the coding sequence of that gene. The phrase "light chain gene" refers to a gene encoding a light chain of an immunoglobulin. Thus, in regard to the invention, the 3' UTR of a light chain gene is a nucleotide sequence that is originally found in a light-chain gene and that is inserted into the inventive vector. The light chain gene can be a light chain gene of any mammal, such as a human, mouse, rat, goat, rabbit, horse, pig, etc. Preferably, the light chain gene is a mouse (murine) light chain gene. More preferably, the mouse light chain gene has the nucleotide sequence of nucleotides 1062-2560 of SEQ ID NO: 1. It is preferred that no insertions, deletions, inversion, and/or substitutions are present in this nucleotide sequence. However, one of ordinary skill in the art appreciates that nucleotides 1062-2560 of SEQ ID NO: 1 can have insertions, deletions, inversion, and/or substitutions that will not affect the function of the nucleotide sequence, which is to provide signals for polyadenylation. With respect to the inventive vectors, the 3' UTR of a light chain gene is preferably located immediately 3' to the stop codon of the coding sequence of the vector. If no coding sequence is present, then the 3' UTR of a light chain gene preferably is located 3' to the multiple cloning sequence and/or the CMV promoter. The recombinant expression vector can comprise a single copy of a 3' UTR or multiple copies of a 3' UTR. Preferably, the recombinant expression vector comprises a single copy of a 3' UTR.

[0032] The recombinant expression vector preferably comprises a 5' UTR intron. As used herein, the term "5' UTR intron" refers to a nucleotide sequence that is transcribed but is removed by RNA splicing and thus not retained in the final transcript. It further is not translated and, thus, is not expressed as part of the protein, polypeptide, or peptide encoded by the vector. The 5' UTR intron is preferably located after the promoter in the 5' untranslated region of the recombinant expression vector. The 5' UTR intron promotes enhanced expression. The 5' UTR intron can be from any naturally-occurring source or can be constructed from portions of different sources, e.g., constructed from splice donor and acceptor sequences from different sources. For example, the 5' UTR intron comprises a portion of a CMV intron and

a portion of a SV40 16S intron. Preferably, the splice donor for the 5' UTR intron is from the sequence downstream of the start of transcription from the viral promoter, and the splice acceptor is from the SV40 16S intron and has the nucleotide sequence of nucleotides 888-974 of SEQ ID NO: 1. It is preferred that no insertions, deletions, inversion, and/or substitutions are present in this nucleotide sequence. However, one of ordinary skill in the art appreciates that nucleotides 888-974 of SEQ ID NO: 1 can have insertions, deletions, inversion, and/or substitutions that will not affect the function of the nucleotide sequence, which is to drive the transcription of the recombinant protein coding sequence.

[0033] In a preferred embodiment, the recombinant expression vector comprises a 3' UTR, an oriP, a pUC19 origin of replication, a viral promoter, and a 5' UTR intron. Preferably, the viral promoter is a CMV promoter and the 5' UTR intron comprises a portion of a CMV intron and a portion of a SV40 16S intron, e.g., comprises nucleotides 888-974 of SEQ ID NO: 1. Most preferably, the recombinant expression vector is the vector plasmid pMXT5, which is shown pictorially in **FIG. 1A** (pMXT5), and which has the nucleotide sequence (without any coding sequences) of SEQ ID NO: 1 (**FIG. 10**). For example, as shown in **FIG. 10**, the CMV promoter comprises nucleotides 1-1037; 5' UTR intron comprises nucleotides 888-974; MCS comprises nucleotides 1038-1061; LC 3' UT comprises nucleotides 1062-2560; OriP comprises nucleotides 2561-4550; pUC19 ori comprises nucleotides 4551-5220; and Ap comprises nucleotides 5221-6380.

[0034] The recombinant expression vector can be designed for either transient expression or for stable expression. Preferably, the vector of the invention promotes transient expression, i.e., is a recombinant transient expression vector, such that the vector is one that does not integrate into the genome of a host cell. Without being bound to any particular theory, it is believed that the recombinant expression vector can be made to be a transient expression vector by incorporating into the vector an oriP, which promotes high copy episomal plasmid replication.

[0035] The recombinant expression vector can comprise a nucleic acid sequence encoding any protein, such as a hormone, growth factor, antibody, receptor, structural protein, enzyme, etc. The protein can be, for example, a therapeutic protein, and can be naturally-occurring or non-naturally occurring, e.g., a genetically engineered protein including, for example, a fusion protein, a chimeric protein, etc. Preferably, the recombinant expression vector comprises such a nucleic acid for the expression of the protein. It is to be understood that the term "protein" as used herein includes parts or fragments thereof, and thus, polypeptides and peptides of any length are included within the meaning of this term. For example, polypeptides and peptides are included wherein the polypeptides can comprise, for instance, about 50 or more amino acids and the peptides can comprise, for instance, about 8-49 amino acids. The nucleic acid sequence encoding the protein can be obtained from any source, e.g., isolated from nature, synthetically generated, isolated from a genetically-engineered organism, and the like. An ordinarily skilled artisan will appreciate that any type of nucleic acid sequence (e.g., DNA, RNA, genomic DNA, and cDNA) that can be inserted into a recombinant expression vector can be used in connection with the invention. For example, the nucleic acid sequence encoding a protein can be naturally-occurring, e.g., a gene. Alternatively, the nucleic acid sequence encoding a protein can be non-naturally occurring, e.g., non-native to any organism, e.g., mammal. For instance, the nucleic acid sequence can be a codon optimized nucleic acid sequence in which codons within the nucleic acid sequence, which codons are not generally used by the host cell translation system, termed "rare codons," are changed by in vitro mutagenesis to preferred codons without changing the amino acids of the synthesized protein (Bradel-Tretheway et al., *J. Virol. Meth.*, 111: 145-156 (2003); Ramakrishna et al., *J. Virol.* 78: 9174-9189 (2004)). In addition, the nucleic acid sequence encoding a protein can be further modified, e.g., codon optimized, to improve the folding of the RNA, such that the folding of the RNA transcript encoded by the nucleic acid sequence is minimized. Whatever type of nucleic acid sequence is used, the nucleic acid sequence preferably encodes a secreted protein. By "secreted" is meant that the protein is released from the cell into the extracellular environment, thereby facilitating the purification of the protein. In this regard, the recombinant expression vector preferably comprises a signal sequence, which causes the expressed protein to be secreted from the cell by which it was expressed.

[0036] In a preferred embodiment, the recombinant expression vector comprises a nucleic acid encoding an immunogloblin chain, e.g., light chain or heavy chain. The immunoglobulin chain can be any immunoglobulin chain derived from any source, genetically-modified, or synthesized. Preferably, the immunoglobulin chain is a human immunoglobulin chain selected from the group consisting of a γ_1 heavy chain, a γ_2 heavy chain, a γ_4 heavy chain, a κ light chain, and a λ light chain. Exemplary heavy chain constant region sequences include: a γ_1 heavy chain constant region, which is encoded by the nucleotide sequence of SEQ ID NO: 4 and comprises the amino acid sequence of SEQ ID NO: 5; a γ_2 heavy chain constant region, which is encoded by the nucleotide sequence of SEQ ID NO: 6 and comprises the amino acid sequence of SEQ ID NO: 7; and a γ_4 heavy chain constant region, which is encoded by the nucleotide sequence of SEQ ID NO: 8 and comprises the amino acid sequence of SEQ ID NO: 9. Exemplary light chain constant region sequences include: a κ light chain constant region, which is encoded by the nucleotide sequence of SEQ ID NO: 10 and comprises the amino acid sequence of SEQ ID NO: 11, and a λ light chain constant region, which is encoded by the nucleotide sequence of SEQ ID NO: 12 and comprises the amino acid sequence of SEQ ID NO: 13. Exemplary antibody heavy and light chains include: an LDP-01 heavy chain, which is encoded by the nucleotide sequence of SEQ ID NO: 14 and comprises the amino acid sequence of SEQ ID NO: 15, and an LDP-01 light chain, which is encoded by the nucleotide sequence of SEQ ID NO: 16 and comprises the amino acid sequence of SEQ ID NO: 17. The LDP-01 antibody is referred to herein as Ab#1 and has been described in WO 2004/033693 (PCT/US2003/010154) and U.S. Patent Application Publication No. 2003/0203447 A1.

[0037] In this regard, the recombinant expression vector desirably comprises an antibody signal sequence, which promotes the secretion of the antibody into the extracellular environment. Suitable antibody signal sequences are known in the art. For example, a preferred signal sequence comprises SEQ ID NO: 2 or SEQ ID NO: 3.

[0038] The recombinant expression vector can alternatively comprise a nucleic acid sequence encoding a functional fragment of a protein. The term "functional fragment" which is synonymous with "functional part" or "functional portion," when used in reference to a protein, refers to any part or fragment of the protein, which part or fragment retains a biological activity of the protein of which it is a part. Functional fragments encompass, for example, those parts of a protein (the parent protein) that retain a function of the parent protein to a similar extent, the same extent, or to a higher extent, as the parent protein. For instance, if the protein is an immunoglobulin, functional fragments thereof can include any portion of the immunoglobulin which, for example, retains the ability to bind to the antigen of the parent immunoglobulin. Also, for example, if the protein is a cell surface receptor, functional fragments thereof can include any portion of the cell surface receptor which, for instance, retains the ability to bind to the ligand of the parent cell surface receptor. In reference to the parent protein, the functional fragment can comprise, for instance, about 10%, 25%, 30%, 50%, 60%, 80%, 90%, 95%, or more of the parent protein. The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent protein. Desirably, the additional amino acids do not interfere with the biological function of the functional portion.

[0039] The invention further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The cell can be a cultured cell or a primary call, i.e., isolated directly from an organism, e.g., a human. The cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5a *E. coli* cells, Chinese hamster ovarian (CHO) cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell. More preferably, the host cell is a DH5α cell. For purposes of producing a recombinant protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the cell can be any cell of the human body, it is preferred that the cell is a human embryonic kidney cell. More preferred is that the human embryonic kidney cell expresses an Epstein Barr virus nuclear antigen-1 (EBNA-1) protein, e.g., a 293E cell.

[0040] As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Camivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0041] The invention further provides methods of producing a recombinant protein. In a first method, the recombinant protein is a heterodimeric or heteromultimeric protein comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain is different from the second polypeptide chain. The first method comprises contacting cells in a medium with a first vector and a second vector, wherein the first vector encodes the first polypeptide chain and the second vector encodes the second polypeptide chain, and the second vector is present in the medium in an amount which is about 1.5 to about 2.5 times as much as the amount of the first vector, whereupon a recombinant protein is produced. The first and second vectors can be any suitable vector and preferably are inventive recombinant expression vectors as described herein.

[0042] For purposes of the first inventive method of producing a protein, the first and second vectors can independently be any type of vector, i.e., the first and second vectors can have the same regulatory elements but differ only in the recombinant protein coding sequence contained therein. By way of example, both the first vector and second vector can be the pMXT vector as shown in **FIG. 1A**. Preferably, each of the first vector and the second vector is one of the inventive recombinant expression vectors described herein. Most preferably, the first and second vectors are pMXT vectors. For example, it is preferred that each of the first and the second vector is a recombinant transient expression vector. It is also preferred that each of the first and second vector comprises a 3' UTR of a light chain gene and an oriP. It is also preferred that each of the first and second vector comprises a viral promoter, a pUC19 origin of replication, a 5' UTR intron, or a combination of any of the foregoing. Preferably, the viral promoter is a CMV promoter, and the 5' UTR intron comprises nucleotides 888-974 of SEQ ID NO: 1. Moreover, it is preferred that each of the first and second vector comprises an antibody signal sequence.

[0043] Also, with respect to the first inventive method of producing a protein, the second vector is present in the medium in an amount which is about 1.5 to about 2.5, e.g., 1.6, 1.7, 1.75, 1.8, 1.9, 2.0, 2.125, 2.25, 2.3, 2.4, and 2.5, times as much as the amount of the first vector. Preferably, the second vector is present in the medium in an amount which is about 1.75 to about 2.25 times as much as the amount of the first vector. More preferably, the second vector is present in the medium in an amount which is about twice as much as the amount of the first vector.

[0044] The invention further provides a second method of producing a recombinant protein. The second method comprises culturing cells, which have been contacted with a recombinant transient expression vector encoding the protein, in a medium in a membrane-enhanced culturing vessel, whereupon a recombinant protein is produced. The second method can alternatively comprise culturing cells, which have been contacted with a recombinant transient expression vector encoding the recombinant protein, in a medium in a Fembach flask, whereupon a recombinant protein is produced. The recombinant transient expression vector can be any suitable such vector and preferably is an inventive recombinant expression vector as described herein.

[0045] In a third method, the recombinant protein is produced upon contacting cells with at least one of the inventive recombinant expression vectors described herein. In a fourth

method, the recombinant protein is produced upon culturing any of the inventive host cells comprising any of the inventive recombinant expression vectors described herein.

[0046] Any suitable method can be employed to contact cells with a first vector, a second vector, or a recombinant expression vector, such that the cells express the protein encoded by the vector. Methods of contacting cells, such that the cells are modified to express a particular protein, polypeptide, or peptide, are well-known in the art. See the references listed in Sambrook et al. (1989), supra. Suitable methods of contacting cells to this end include, for instance, infection with a viral vector, transfection with a lipofection reagent, cationic polymer, DEAE, or calcium phosphate, and electroporation.

[0047] The cells can be contacted with a first vector, a second vector, or a recombinant expression vector in the presence of a suitable cationic polymer. Suitable cationic polymers for transfecting cells are known in the art, and include, for example, polylysine and polyethyleneimine (PEI). In a preferred embodiment of the inventive method, the cationic polymer is PEI. PEI can be linear or branched and can vary in molecular weight, depending on the number of base units, which comprise the polymer. Preferably, the PEI is a linear PEI. More preferably, the linear PEI has a molecular weight of about 25 kDa. Although the amount of PEI used in the method can be any amount, it is preferred that the linear PEI is present in an amount that is about 1.5 to about 4.5, e.g., 1.5, 1.6, 1.75, 2.0, 2.25, 2.5, 2.6, 2.7, 2.75, 2.8, 2.9, 3.0, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.75, 3.8, 3.9, 4.0, 4.1, 4.25, 4.3, 4.4, and 4.5, times the amount of the vector(s) contacting the cells. Preferably, the PEI is present in an amount that is about 2.5 to 3.5 times the amount of the vector(s) contacting the cells. More preferably, the PEI is present in an amount that is about twice the amount of the vector(s) contacting the cells.

[0048] For purposes of the inventive method comprising contacting cells with more than one vector, e.g., a first vector and a second vector, the cells can be contacted with the first vector and second vector in a sequential fashion, e.g., first vector contacted with the cells before the second vector. Alternatively, the cells can be contacted with the first vector and second vector simultaneously. Preferably, the cells are contacted with the first vector and second vector simultaneously. For example, in a method comprising contacting cells with more than one vector, the cells can be contacted with a first vector before or simultaneously with a second or additional vector.

[0049] As used herein, the term "culturing" is synonymous with "maintaining." Methods of culturing cells are known in the art (see, e.g., Tissue Engineering Methods and Protocols, Morgan and Yarmush (eds.), Humana Press, Inc., Totowa, N.J., 1999). As one ordinarily skilled recognizes, the conditions under which cells are cultured varies depending on the cell type. The conditions include temperature of the environment, the culturing vessel containing the cells, the composition of the various gases, e.g., CO_2, which comprises the cell culture atmosphere or environment, the medium in which the cells are maintained, the components and pH of the medium, the density at which cells are maintained, the schedule by which the medium needs to be replaced with new medium, etc. These parameters are often known in the art or can be empirically determined. For example, with respect to the inventive methods, wherein cells are cultured in a medium, e.g., a first medium, a second medium, etc., any method can be employed to culture the cells in the medium, such that the cells express (and, in some instances, secrete) the protein encoded by the vector, which was contacted to the cells.

[0050] The cells are desirably cultured in a membrane-enhanced culturing vessel or a Fembach flask. For purposes herein, the term "membrane-enhanced culturing vessel" refers to a container for holding cell cultures that have been improved upon by the addition of at least one membrane. Suitable membrane-enhanced culturing vessels include membrane-based cell culture vessels, dialysis-based cell culture vessels, membrane-based high density cell culture vessels, and two-compartment vessels. The term "vessel" as used herein is synonymous with systems, reactors, bioreactors, flasks, and devices. Suitable membrane-enhanced culturing vessels include, for instance, miniPerm® flasks, OptiCell® flasks, and the CELLINE™ CL1000 (referred to herein as Integra flasks or Integra CL1000 flasks), which are commercially available from companies, such as IBS Integra Biosciences AG (Chur, Switzerland), OptiCell (Westerville, Ohio), VWR, Fisher Scientific, and Labmate (Asia). Most preferably, the membrane-enhanced culturing vessel is an Integra CL1000. For example, one of the ordinary skill in the art appreciates that a membrane-enhanced culturing vessel such as an Integra flask may comprise a nutrient chamber and a cultivation chamber, wherein nutrients from a media reservoir in the nutrient chamber pass through a semi-permeable membrane into the cultivation chamber containing cells so as to provide a continuous supply of nutrients and wherein the membrane also allows for diffusion of metabolites out of the cultivation chamber and away from contact with the cells but does not permit diffusion of a recombinant protein produced by the cells (e.g., an antibody or antibody fragment) out of the cultivation chamber, and further wherein the cells also have sufficient gas exchange such as access to oxygen and carbon dioxide through a separate silicone membrane at the bottom of the vessel.

[0051] As used herein, the term "Fembach flask" refers to a commercially available Corning® polycarbonate Erlenmeyer flask having the Fembach design. Such flasks are commercially-available from companies such as Life Sciences.

[0052] Without being bound to any particular theory, membrane-enhanced flasks (e.g., Integra CL1000, OptiCell® flasks, and miniPerm® flasks) and Fernbach flasks are particularly suitable for culturing transfected cells, for example, transiently transfected cells, as these devices permit efficient gas exchange between the cells and the environment, e.g., the incubator environment, which permits optimal cell growth and production of the recombinant protein. Under certain conditions, shake flasks can also be suitable culturing vessels in which cells can be cultured for optimal cell growth and production of the recombinant protein. It should be understood that any flask or culturing vessel that permits efficient gas exchange between the cells and the environment are included in the scope of the invention and are not limited to only the aforementioned flasks and culturing vessels.

[0053] In the inventive methods comprising culturing cells, the medium can be any suitable medium for culturing

cells known in the art. The medium can be, for example, a culture medium containing 1% low immunoglobulin (Ig) fetal bovine serum (FBS). Alternatively, the medium can be a serum-free cell culture medium, e.g., IS293™ medium. In some instances, the medium is preferably a serum-free IS293™ medium (Irvine Scientific, Irvine, Calif.).

[0054] The cell cultures of the inventive methods can be initiated or seeded at any suitable cell density. As one of ordinary skill in the art recognizes, the seeding density depends on a variety of factors, such as cell type, culturing conditions, and the day which has been selected for harvesting or purifying the recombinant protein from the cell culture. Desirably, the cell density is within the range of about 1.0×10^6 to about 2.0×10^7 (e.g., about 1.0×10^6 to about 1.5×10^7). More preferably, the initiating seeding cell density of the cell culture is about 3.0×10^6 to about 1.0×10^7. Without being bound to any particular theory, it is believed that the seeding density of cells, which have been transiently transfected with a vector encoding a protein, is a factor in obtaining efficient production of a recombinant protein.

[0055] For purposes of the inventive methods, the cells that are cultured or are contacted with a first vector, a second vector, or a recombinant expression vector can be any cell, such as those described herein as "host cells." For example, the cells that are cultured and/or contacted with one or more than one recombinant expression vector can be any host cells. Preferably, the cells are mammalian cells, and, more preferably, the cells are human cells. The cells are desirably human embryonic kidney cells. In a most preferred embodiment, the human embryonic kidney cells express Epstein-Barr virus nuclear antigen-1 protein (EBNA-1), e.g., 293E cells.

[0056] Cells, which have been contacted with a recombinant transient expression vector, can be obtained by transiently transfecting cells by any method known in the art, including those described herein. Recombinant transient expression vectors are known in the art and include, for instance, pCEP4, pcDNA3, and any of the recombinant expression vectors described herein which comprise an oriP. Preferably, the recombinant transient expression vectors are pMXT vectors. For example, the vectors can be any of the inventive recombinant expression vectors as described herein.

[0057] With respect to the first method of producing a recombinant protein (e.g., comprising contacting cells with a first vector and a second vector), the method can further comprise the second inventive method of producing a recombinant protein. That is, the method of producing a recombinant protein can further comprise the step of culturing the cells, which have been contacted with a first vector and a second vector, in a second medium in a membrane-enhanced culturing vessel (e.g., an Integra CL1000, an OptiCell® flask, a miniPerm® flask), a Fernbach flask, or like flask. In such an embodiment, the second medium can be different from the medium in which the first and second vectors are present. For purposes of the methods, which comprise culturing cells in a membrane-enhanced culturing vessel, a Fembach flask, or like flask, the suitable medium for use in such a vessel or flask can be a serum-free cell culture medium, e.g., IS293 medium. Preferably, the medium is serum-free IS293 medium (Irvine Scientific, Irvine, Calif.).

[0058] With respect to the second inventive method of producing a recombinant protein, the method can comprise the first inventive method of producing a recombinant protein. One of ordinary skill in the art recognizes that the methods described herein can be combined in such a way, such that all of the limitations of the methods are met. Such a combined method is within the scope of the invention.

[0059] With respect to any of the inventive methods comprising culturing cells, e.g., in a membrane-enhanced culturing vessel, a Fernbach flask, or like flask, the method can further comprise purifying or isolating the recombinant protein from the medium, e.g., the serum-free medium. As used herein, the terms "purifying" and "isolating" do not necessarily refer to absolute purity or isolation, as one of ordinary skill in the art appreciates that a partially purified or partially isolated protein can be useful or of value.

[0060] Methods of purifying proteins from mixtures are known in the art. Suitable purification methods include, for example, chromatography, electrophoresis, and the like. Suitable chromatographic methods of purifying polypeptides include, for example, HPLC, ion-exchange chromatography, affinity chromatography, etc. Preferably, the purifying comprises chromatographing the medium through a resin, such as a cationic resin, an anionic resin, and an affinity resin. If the polypeptide is an immunoglobulin chain, the purifying preferably comprises the use of resin comprising *Staphylococcus aureus* Protein A, which is a bacterially-produced protein that binds to the Fc regions of IgG antibodies. More preferably, the purifying comprises centrifuging the medium through a column comprising Protein A, e.g., centrifuging the medium through a Protein A spin column (which is commercially available from Pro-Chem).

[0061] The purifying can occur at any point in time after culturing the cells, which have been contacted with a vector. In some instances, it is preferable for the purifying to occur after about 3 days of culturing, e.g., after about 3, 4, 5, 6 or more days. In other instances, it is preferable for the purifying to occur after about 7 days of culturing, e.g., after about 7, 8, 9, 10, 11, 12, 13, 14, 15 or more days.

[0062] The invention provides fast and efficient methods of producing high levels of recombinant proteins. In some instances, at least 300 μg/ml recombinant protein is produced after 3 days of culturing. In other instances, at least 500 μg/ml recombinant protein is produced after 3 days of culturing. In some preferred instances, at least 700 μg/ml recombinant protein is produced after 3 days of culturing.

[0063] The term "recombinant protein" as used herein, refers to any protein or part thereof that is produced by a genetically-engineered organism. For example, the recombinant protein can be any of the proteins described herein.

[0064] For purposes of the first method of producing a recombinant protein, the recombinant protein is a heterodimeric protein or a heteromultermeric protein, such as a tetramer, which comprises two copies of two different polypeptide chains. Such proteins are known in the art, and include, for instance, hemoglobin, immunoglobulins, T cell receptors, and B cell receptors, etc. In a preferred embodiment of the first inventive method, the recombinant protein is a heterotetrameric protein. Desirably, the heterotetrameric protein is an immunoglobulin. In this instance, it is preferred

that the first vector encodes a heavy chain of an immunoglobulin, or a part thereof, and the second vector encodes a light chain of an immunoglobulin, or a part thereof. The heavy chain can be any heavy chain of any immunoglobulin, as described herein. The light chain can be any light chain of any immunoglobulin, as described herein. Exemplary antibody heavy and light chains: an LDP-01 heavy chain, which is encoded by the nucleotide sequence of SEQ ID NO: 14 and comprises the amino acid sequence of SEQ ID NO: 15, and an LDP-01 light chain, which is encoded by the nucleotide sequence of SEQ ID NO: 16 and comprises the amino acid sequence of SEQ ID NO: 17. The LDP-01 antibody is referred to herein as Ab#1 and has been described in WO 2004/033693 (PCT/US2003/010154) and U.S. Patent Application Publication No. 2003/0203447 A1.

EXAMPLES

[0065] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

Example 1

[0066] This example demonstrates the construction of recombinant expression vectors of the invention.

[0067] Transient expression vectors for expression of any gene were constructed with a multilinker site containing unique restriction sites positioned between the 3' end of the CMV promoter and the 5' end of the mouse light chain 3' untranslated region. Transient expression vectors containing cDNAs, which encode light chain κ or λ genes or heavy chain γ_1, γ_2, or γ_4 genes, under the control of a CMV promoter (Boshart et al., *Cell* 41: 521-530 (1985)) and mouse light chain 3' untranslated region (Xu et al., *J. Biol. Chem.* 261: 3838-3845 (1986)) were contructed. Unique restriction sites were positioned at the 5' end of the V region (e.g., SalI) and in the junction regions between the V and constant regions (BlpI for heavy chain, BsiWI for κ light chain and AvrII for lambda) for the cloning of any new V region adjacent to the desired cognate constant region. The vectors also contained the Epstein Barr virus oriP sequence (Reisman et al., *Mol. Cell. Biol.* 5: 1822-1832 (1985)) for episomal plasmid replication in 293E cells, the origin of replication from the vector pUC19, and the gene encoding resistance to ampicillin for selection of tranformants in *E. coli*. The transient expression vectors containing the multilinker sites, the heavy chain, and the light chain are shown in **FIGS. 1A-1C**.

Example 2

[0068] This example demonstrates a method of transiently transfecting cells for producing recombinant proteins.

[0069] 293E cells (Invitrogen, R620-07) were maintained as adherent cultures in Dulbecco's Modified Eagle Medium (DMEM) (Gibco-Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone), 2 mM glutamine, and 250 μg/ml G418 antibiotic (Gibco-Invitrogen). For growth in suspension culture, the cells were adapted to the following serum-free media formulations: IS293™ (Irvine Scientific), IS293-V™ (Irvine Scientific), 293 SFM II (Gibco-Invitrogen), H-SFM (Gibco-Invitrogen), and HYQ®PF293 (HyClone). The cells were originally supplemented with 10% low IgG FBS (HyClone) and 2 mM glutamine and gradually weaned down to 1% low IgG FBS over a period of several weeks. Once in 1% low IgG FBS, the cells were transferred to shake flasks for continued adaptation to suspension growth. Growth and viability were monitored using the VICELL™ XR Cell Viability Analyzer (Beckman-Coulter).

[0070] All plasmids were transformed into DH5a cells (Invitrogen) and purified using endotoxin-free plasmid purification kits (QIAGEN®). For transfections in 6-well plates, 2 ml of cells at 5×10^5 cells/ml were seeded per well. For transfections in shake flask cultures, cells were seeded at 8×10^5 cells/ml at the appropriate volumes prior to transfection. DNA (2 μg/ml) was pre-incubated with linear polyethyleneimine (PEI, 25 kDa MW, Polysciences) at a concentration of 4 μg/ml for 10 min at room temperature prior to addition to cells. The DNA/PEI mixture was then added to cells, and the cells with the DNA/PEI were either maintained in shake flasks or transferred to Integra flasks.

Example 3

[0071] This example demonstrates the determination of the optimal PEI:DNA ratio for transient transfections.

[0072] Adherent 293E cells grown in DMEM supplemented with 10% FBS in 6-well plates were transfected with pQBI-pGK (GFP expressing plasmid, Q-biogene) using linear polyethyleneimine (PEI) as described in Example 2. DNA (1 μg/ml, 2 μg/ml, or 5 μg/ml) was pre-incubated with linear PEI (1, 2, 4, 5, 10, or 25 μg/ml) for 10 min at room temperature prior to the addition to cells, then the PEI/DNA mixture was added to cells, and the cells were maintained in shake flasks or Integra flasks.

[0073] GFP expression was monitored 24 hours post-transfection using a Becton Dickinson FACScan flow cytometer equipped with the Cytek Automated Microsampler System (AMS) 96-well plate reader. Flow data was analyzed using FlowJo (Tree Star, Inc.). Cells also were counterstained with 1 μg/ml propidium iodide (PI) to determine cell viability. Growth and viability of the cells post-transfection were monitored using the VICELL™ XR Cell Viability Analyzer (Beckman-Coulter). The cells transfected with 1 μg/ml DNA and 1 μg/ml PEI (**FIG. 2A**); 2 μg/ml DNA and 2 μg/ml PEI (**FIG. 2B**); 5 μg/ml DNA and 5 μg/ml PEI (**FIG. 2C**); 1 μg/ml DNA and 2 μg/ml PEI (**FIG. 2D**); 2 μg/ml DNA and 4 μg/ml PEI (**FIG. 2E**); 5 μg/ml DNA and 10 μg/ml PEI (**FIG. 2F**); 1 μg/ml DNA and 5 μg/ml PEI (**FIG. 2G**); 2 μg/ml DNA and 10 μg/ml PEI (**FIG. 2H**); and 5 μg/ml DNA and 25 μg/ml PEI (**FIG. 2I**) were measured for GFP expression α-axis) and PI staining (y-axis) by flow cytometry, and the resulting data was plotted in the series of graphs of **FIGS. 2A-2I**.

[0074] As shown in **FIGS. 2A-2I**, the DNA concentration of 1 μg/ml at a PEI:DNA ratio of 2:1 gave the highest percentage of cells expressing GFP with relatively low cellular cytotoxicity 24 hours post-transfection.

[0075] The results of this example demonstrated the production of recombinant protein and confirmed that the optimal PEI:DNA ratio for transient transfection is 2:1.

Example 4

[0076] This example demonstrates the determination of the optimal medium for culturing transiently transfected cells.

[0077] 293E cells were grown and transfected in the presence of 1% low-IgG serum in 6-well plates and shake flasks, as described in Example 2. Twenty-four hours after transfection, 293E cells were adapted to suspension growth in one of 5 different serum-free media formulations (IS293™, H-SFM, IS293V™, SFMII, or HYQ®PF293) or one serum-containing media formulation (DMEM) as in Example 2.

[0078] Twenty-four to forty-eight hours later, GFP expression by transfected cells were determined as described in Example 3. Cells also were counterstained with 1 μg/ml PI to determine cell viability. Growth and viability of the cells post-transfection were monitored using the VICELL™ XR Cell Viability Analyzer (Beckman-Coulter). The resulting data from GFP expression (bars) and for PI staining (X) was plotted to form the graph of **FIG. 3**.

[0079] As shown in **FIG. 3**, IS293™ medium (Irvine Scientific) gave the highest percentage of GFP-expressing cells with minimal cytotoxicity in shake flasks; values were comparable to those obtained with adherent 293E cells cultured in DMEM supplemented with 10% FBS.

[0080] The results of this example demonstrated that IS293™ medium is the optimal serum-free medium to be used with transiently transfected cells for producing recombinant proteins.

Example 5

[0081] This example demonstrates the determination of optimum heavy and light chain plasmid ratios for maximum antibody productivity.

[0082] Various ratios of pMXT (heavy chain (HC)):pMXT (light chain (LC)) (see Example 1) or pCEP4 (HC):pCEP4 (LC) were tested for effects on antibody productivity in cells grown in IS293™ medium supplemented with 1% low-IgG serum in shake flasks. The pCEP4 vector containing the nucleotide sequence encoding the Ab# 1 heavy chain (SEQ ID NO: 14) was constructed by cloning the coding sequence into KpnI and Xho sites. The pCEP4 vector containing the nucleotide sequence encoding the Ab#1 light chain (SEQ ID NO: 16) was constructed by cloning the coding sequences into Nhe and Xho sites. The encoded heavy chain and light chain of Ab#1 is set forth as SEQ ID NOs: 15 and 17, respectively. All plasmids were amplified by transformation into DH5 α cells and purified as described in Example 2. 293E cells were transiently transfected as described in Example 2. Transfected cells were transferred to IS293™ medium in shake flasks for 7-10 days. Antibody expression by the cells transfected with a 1:1, 1:2, or 2:1 ratio of vector encoding HC:vector encoding LC, wherein the vectors were either pMXT or pCEP4 was determined by sandwich ELISA and the data analyzed in PRISM™ (GraphPad). The resulting data was plotted to form the graph of **FIG. 4**.

[0083] As shown in **FIG. 4**, a 1:2 ratio of HC:LC generated the highest antibody productivity with Ab#1 achieving levels of 60-70 μg/ml after 7-10 days. The highest productivity for Ab#1 (LDP-01) in pMXT was ~3× greater than the best output achieved using pCEP4.

[0084] The results of this example demonstrated that the pMXT vector is optimal for co-transfecting cells with vectors encoding different polypeptide chains at a ratio of 1:2.

Example 6

[0085] This example demonstrates that the level of antibody production by transiently transfected cells cultured post-transfection in membrane-enhanced culturing vessels are comparable to the level of antibody production achieved by transfected cells cultured post-transfection in shake flasks.

[0086] 293E cells were transiently transfected in shake flasks as described in Example 2. Cells were either maintained in the shake flasks or transferred to 15 ml of medium and placed in Integra CL1000 flasks. After 7-10 days, cell culture supernatant was harvested, clarified, and purified for antibodies using a standard Protein A column, if cells were cultured in shake flasks, or a Protein A spin column, if cells were cultured in Integra flasks. Cell viability and antibody production of both sets of cells 0, 4, 7, and 14 days post-transfection were assayed as described in Examples 4 and 5, respectively. For antibody expression using the Integra CL1000 flask, 200 ml of transfected 293E cells were resuspended in 15 ml of IS293™ medium supplemented with 1% low IgG FBS and 250 μg/ml G418 antibiotic and transferred into the membrane compartment. One liter of IS293™ medium was added to the upper media chamber.

[0087] The cell viability (X) and antibody production (●) of the transfected cells maintained in Integra flasks are shown in **FIG. 5A**, whereas the levels of antibody production for Ab#1 by cells maintained in either shake flasks or by Integra CL1000 flasks are shown in **FIG. 5B**.

[0088] As shown in **FIG. 5A**, antibody production of cells cultured in Integra flasks peaked at 7 days, producing over 1 mg/ml antibody. This level is comparable to the level of antibody production of transiently transfected cells cultured in shake flasks as shown in **FIG. 5B**.

[0089] The results of this example demonstrated that Integra flasks are suitable culturing vessels for maintaining small volumes of transiently transfected cells. The small volume permits the use of Protein A spin columns, which facilitates the purification of antibodies from the cell culture supernatant.

Example 7

[0090] This example demonstrates a method of producing antibodies in membrane-enhanced culturing vessels at optimized seeding densities.

[0091] Suspension-adapted HEK 293E cells were maintained in IS293™ medium (Irvine Scientific) supplemented with 1% low IgG FBS (HyClone), 2 mM glutamine (Gibco-Invitrogen), and 250 μg/ml G418 antibiotic (Gibco-Invitrogen). For transfection, cells were seeded at 8×10^5 cells/ml in shake flasks at the appropriate volumes prior to transfection. DNA encoding Ab#1 or Ab#2 (which differed from Ab#1) was pre-incubated with linear polyethyleneimine (PEI, 25 kDa MW, Polysciences) at optimized conditions (see, e.g., Example 3; see also, e.g., Handa et al., *American Society for Cell Biology*, poster presentation #1937 (2004)) prior to addition to cells. For antibody expression using the Integra CL1000 flask, cells at the following seeding densities were resuspended in 30 ml of IS293™ medium supplemented with 1% low IgG FBS, 2 mM glutamine, and 250 μg/ml G418 antibiotic and transferred into the cultivation chamber: 1.3×10^6 (I-50), 2.7×10^6 (I-100), 5.3×10^6 (I-200), and 1.1×10^7 (I-400). For comparison, 8×10^5 cells (E-200) were seeded in Erlenmeyer flasks. All flasks were incubated for 3,

5, 7, or 10 days post-transfection. One ml samples from the nutrient chambers and cultivation chambers of the Integra CL 1000 flasks were removed and analyzed at 3, 5, 7, or 10 days post-transfection.

[0092] Growth and viability were monitored using the VICELL™ XR Cell Viability Analyzer (Beckman-Coulter). The percentage of viable cells 1, 3, 5, 7, and 10 days after transfection for cells tranfected at different seeding densities is shown in **FIGS. 6A and 6B**. The viable cell count of cells 0, 1, 3, 5, 7, and 10 days after transfection for cells transfected at different seeding densities is shown in **FIGS. 7A and 7B**.

[0093] As shown in **FIGS. 6A** (Ab#1), 6B (Ab#2), **7**A (Ab#1) and 7B (Ab#2), cell viability did not vary between flasks, but viable cell growth was improved in the Integra flasks for all seeding densities tested. Maximum densities of 3-5×10^7 cells/ml were achieved for all conditions over the 10 day analysis period.

[0094] Analytes, gases, and pH of the samples were determined 3, 5, 7, and 10 days post-transfection using a BIOPROFILE™ Chemistry Analyzer (Nova Biomedical). The data for selected nutrients and metabolites of the media containing cells producing Ab#1 or the media containing no cells (Media Only) are set forth in Table 1.

TABLE 1

Nutrient	Sample	Flask Chamber	Media Only	Day 3	Day 5	Day 7	Day 10
Glucose (g/L)	I-50	cultivation	5.37	3.35	2.85	2.57	2.76
	I-50	nutrient	5.36	5.11	4.49	4.12	3.47
	I-100	cultivation	5.37	3.86	2.93	2.99	2.41
	I-100	nutrient	5.36	5.05	4.39	3.97	3.85
	I-200	cultivation	5.37	3.27	3.09	3.01	2.84
	I-200	nutrient	5.36	4.62	4.12	3.68	3.10
	I-400	cultivation	5.37	2.86	3.11	2.98	2.74
	I-400	nutrient	5.36	4.52	3.94	3.67	3.23
	E-200	N/A	5.36	4.42	2.51	2.36	2.06
Glutamine (mmol/L)	I-50	cultivation	6.96	6.07	4.77	4.80	4.32
	I-50	nutrient	6.82	6.30	5.89	5.54	4.79
	I-100	cultivation	6.96	5.84	4.57	4.65	4.03
	I-100	nutrient	6.82	6.37	5.88	5.41	5.10
	I-200	cultivation	6.96	5.36	4.64	4.50	4.68
	I-200	nutrient	6.82	6.07	5.62	5.19	4.63
	I-400	cultivation	6.96	5.47	5.25	5.02	4.21
	I-400	nutrient	6.82	6.37	5.91	5.68	5.12
	E-200	N/A	6.82	6.31	5.82	5.53	5.08
Lactate (g/L)	I-50	cultivation	0.26	1.92	2.42	3.17	2.82
	I-50	nutrient	0.34	0.74	1.36	1.82	2.27
	I-100	cultivation	0.26	1.85	2.43	2.74	2.82
	I-100	nutrient	0.34	0.99	1.62	1.94	2.52
	I-200	cultivation	0.26	2.47	2.32	2.84	2.72
	I-200	nutrient	0.34	1.25	1.86	2.09	2.33
	I-400	cultivation	0.26	2.57	2.31	2.78	2.66
	I-400	nutrient	0.34	1.63	2.17	2.35	2.38
	E-200	N/A	0.34	1.53	2.50	2.59	2.48

[0095] As shown by the resulting data, transiently transfected cells maintained in 30 ml media in the cultivation chamber of an Integra CL1000 flask can reach cell densities of up to 3-5×10^7 viable cells/ml (e.g., 4.5×10^7). Nutrients from the media reservoir in the nutrient chamber pass through a semi-permeable membrane into the cultivation chamber providing a continuous supply of essential nutrients. The membrane also allows for diffusion of metabolites out of the cultivation chamber and away from contact with cells. Cells also have efficient access to oxygen and carbon dioxide through a separate silicone membrane at the bottom of the flask.

[0096] The Integra supernatant from the cultivation chamber had higher glucose levels than shake flasks but lower glutamine levels. The levels of lactate appeared similar between the two cultures. The higher relative levels of glucose to lactate in the Integra cultures could indicate that the cells are generating more ATP by promoting efficient entry of pyruvate from glycolysis into the TCA cycle.

[0097] Antibody titers of transfected cells placed in Integra flasks or shake flasks at different seeding densities were determined using the EASY-TITER™ Human IgG Assay Kit (Pierce) 0, 3, 5, 7, and 10 days post-tranfection. The data expressed as the concentration of antibody titers (μg/ml) is shown in **FIGS. 8A** (Ab#1) and 8B (Ab#2), whereas the data expressed as the total antibody yield (mg) is shown in Table 2.

TABLE 2

	Day 3		Day 5		Day 7		Day 10	
Sample	Yield (mg)	% of E-200 Max Yield	Yield (mg)	% of E-200 Max Yield	Yield (mg)	% of E-200 Max Yield	Yield (mg)	% of E-200 Max Yield
				Ab#1				
E-200	7.6	52%	14.5	100%	7.4	51%	7.6	52%
I-50	0.8	6%	5.2	36%	8.5	59%	13.3	92%
I-100	5.2	36%	8.3	57%	14.7	101%	24.0	166%
I-200	9.4	65%	13.8	95%	13.8	95%	19.0	131%
I-400	11.00	76%	15.7	108%	14.3	99%	11.7	81%
				Ab#2				
E-200	4.1	50%	6.3	77%	7.5	92%	8.2	100%
I-50	1.2	15%	4.3	52%	7.6	93%	6.4	78%
I-100	3.5	43%	8.3	101%	10.4	127%	12.9	157%
I-200	6.1	74%	13.0	159%	17.0	207%	22.0	268%
I-400	10.8	132%	17.8	217%	21.0	256%	22.0	268%

[0098] As shown in **FIGS. 8A and 8B**, antibody productivity for the two antibodies tested, Ab#1 and Ab#2, were different in Erlenmeyer flasks. Ab#1 peaked early at day 5 (~70 μg/ml), followed by a decrease in antibody concentration. Ab#2 showed slower and steadier productivity over the full 10 days, achieving a maximal antibody output of ~40 μg/ml. For both Ab#1 and Ab#2, antibody productivity in the Integra flasks achieved steady levels of increasing Ab productivity over the 10 day period. An exception was the 1-400 sample for Ab# 1, which showed slight decreases in productivity at days 7 and 10; however, the decrease was substantially less as compared to that in the E-200 samples.

[0099] As shown in Table 2, the maximal yield for Ab#1 in the E-200 culture was ~15 mg at day 5. Comparable levels (≧90% of E-200 maximum) were obtained in the I-200 and I-400 cultures at day 5 as well, and higher total yields were obtained with I-100 and I-200 after day 10, namely 166% (24 mg) and 131% (19 mg), respectively.

[0100] As also shown in Table 2, the maximal yield for Ab#2 in the E-200 culture was ~8 mg at day 10. Comparable levels (≧90% of E-200 maximum) were obtained as early as day 3 with I-400 (~11 mg), at day 5 with I-100 and I-200 (~8 mg and ~13 mg, respectively), and day 7 with I-50 (~8 mg). Higher yields were obtained for I-100, I-200, and I-400 at day 10, 157% (13 mg), 268% (22 mg), and 268% (22 mg), respectively.

[0101] The results of this example demonstrated high levels of antibody production in Integra flasks within short periods of time. Cell densities of 1.0×10^6 and 1.5×10^7 were examples of optimal densities for producing high levels of antibodies. As shown herein, transiently transfected cells, for example, 293E cells, in membrane-enhancing culturing vessels such as Integra flasks, generated higher total antibody yields over cells cultured in Erlenmeyer flasks, irrespective of antibody productivity levels in shake cultures. Transiently expressing antibodies in membrane-enhancing culturing vessels such as Integra flasks also appeared to better retain antibody stability upon exhaustion of the culture.

[0102] As shown by the results obtained herein, total antibody yields from transiently transfected 293E cells are significantly increased when cultured in Integra flasks vs. standard Erlenmeyer flasks. Increasing the number of transfected cells seeded in the Integra flask can substantially decrease the time to reach maximum antibody yield, while decreasing the seeding density allows for multi-mg production of antibodies using a fraction of the cells under normal conditions in an Erlenmeyer flask. Generating transiently expressed antibodies in Integra flasks also better maintains the antibody titer for longer periods of time thus allowing for greater confidence to allow cultures to proceed to extinction without significant loss of antibody. Advantageously, usage of membrane-enhancing culturing vessels, such as Integra flasks, for transient protein production, such as antibody production, allows for increased total yield, faster production by using more cells, and/or conservation of cells by using fewer cells while maintaining productivity comparable to non-membrane culturing vessels, such as Erlenmeyer flasks.

[0103] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0104] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising,""having,""including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0105] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

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<400> SEQUENCE: 5

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
```

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```
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 6
<211> LENGTH: 981
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Heavy Chain Gamma 2
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(981)

<400> SEQUENCE: 6

gcc agc aca aag ggc cca tcg gtc ttc ccc ctg gcg ccc tgc tcc agg      48
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15
```

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```
agc acc tcc gag agc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac       96
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gct ctg acc agc      144
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc      192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

ctc agc agc gtg gtg acc gtg acc tcc agc aac ttc ggc acc cag acc      240
Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

tac acc tgc aac gta gat cac aag ccc agc aac acc aag gtg gac aag      288
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

aca gtt gag cgc aaa tgt tgt gtc gag tgc cca ccg tgc cca gca cca      336
Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

cct gtg gca gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac      384
Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125

acc ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac      432
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

gtg agc cac gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc      480
Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

atg gag gtg cat aat gcc aag aca aag cca cgg gag gag cag ttc aac      528
Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175

agc acg ttc cgt gtg gtc agc gtc ctc acc gtc gtg cac cag gac tgg      576
Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
            180                 185                 190

ctg aac ggc aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca      624
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205

gcc ccc atc gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa      672
Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220

cca cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac      720
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240

cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc      768
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc      816
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270

aca cct ccc atg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag      864
Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc      912
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300

tcc gtg atg cat gag gct ctg cac aac cac tac aca cag aag agc ctc      960
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320
```

-continued

```
tcc ctg tct ccg ggt aaa tga                                          981
Ser Leu Ser Pro Gly Lys
                325


<210> SEQ ID NO 7
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 7

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
            180                 185                 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320

Ser Leu Ser Pro Gly Lys
                325
```

-continued

<210> SEQ ID NO 8
<211> LENGTH: 984
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Heavy Chain Gamma 4
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(984)

<400> SEQUENCE: 8

```
gcc agc aca aag ggc cca tcc gtc ttc ccc ctg gcg ccc tgc tcc agg       48
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15

agc acc tcc gag agc aca gcc gcc ctg ggc tgc ctg gtc aag gac tac       96
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc      144
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc      192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc acg aag acc      240
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

tac acc tgc aac gta gat cac aag ccc agc aac acc aag gtg gac aag      288
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

aga gtt gag tcc aaa tat ggt ccc cca tgc cca tca tgc cca gca cct      336
Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110

gag ttc ctg ggg gga cca tca gtc ttc ctg ttc ccc cca aaa ccc aag      384
Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

gac act ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg      432
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

gac gtg agc cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat      480
Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag ttc      528
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac      576
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

tgg ctg aac ggc aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc      624
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

ccg tcc tcc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga      672
Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

gag cca cag gtg tac acc ctg ccc cca tcc cag gag gag atg acc aag      720
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac      768
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
```

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```
                245                 250                 255

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag      816
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270

acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc      864
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285

agg cta acc gtg gac aag agc agg tgg cag gag ggg aat gtc ttc tca      912
Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300

tgc tcc gtg atg cat gag gct ctg cac aac cac tac aca cag aag agc      960
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320

ctc tcc ctg tct ctg ggt aaa tga                                     984
Leu Ser Leu Ser Leu Gly Lys
                325


<210> SEQ ID NO 9
<211> LENGTH: 327
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 9

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
```

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```
               245                 250                 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320

Leu Ser Leu Ser Leu Gly Lys
               325


<210> SEQ ID NO 10
<211> LENGTH: 318
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Light Chain Kappa
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(318)

<400> SEQUENCE: 10

gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg       48
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
1               5                   10                  15

aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc       96
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
            20                  25                  30

aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt      144
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
        35                  40                  45

aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac      192
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
    50                  55                  60

agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac      240
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
65                  70                  75                  80

aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc      288
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
                85                  90                  95

aca aag agc ttc aac agg gga gag tgt tag                              318
Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210> SEQ ID NO 11
<211> LENGTH: 105
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 11

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
1               5                   10                  15

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
            20                  25                  30

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
        35                  40                  45
```

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```
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
    50                  55                  60

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
65                  70                  75                  80

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
                85                  90                  95

Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210> SEQ ID NO 12
<211> LENGTH: 312
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Light Chain Lambda
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(312)

<400> SEQUENCE: 12

aag gcc aac ccc act gtc act ctg ttc ccg ccc tcc tct gag gag ctc       48
Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
1               5                   10                  15

caa gcc aac aag gcc aca cta gtg tgt ctg atc agt gac ttc tac ccg       96
Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
            20                  25                  30

gga gct gtg aca gtg gcc tgg aag gca gat ggc agc ccc gtc aag gcg      144
Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys Ala
        35                  40                  45

gga gtg gag acc acc aaa ccc tcc aaa cag agc aac aac aag tac gcg      192
Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
    50                  55                  60

gcc agc agc tac ctg agc ctg acg ccc gag cag tgg aag tcc cac aga      240
Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
65                  70                  75                  80

agc tac agc tgc cag gtc acg cat gaa ggg agc acc gtg gag aag aca      288
Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
                85                  90                  95

gtg gcc cct aca gaa tgt tca tag                                     312
Val Ala Pro Thr Glu Cys Ser
            100


<210> SEQ ID NO 13
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 13

Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
1               5                   10                  15

Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
            20                  25                  30

Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys Ala
        35                  40                  45

Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
    50                  55                  60

Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
65                  70                  75                  80
```

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```
Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
                85                  90                  95

Val Ala Pro Thr Glu Cys Ser
            100


<210> SEQ ID NO 14
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: HomoSapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: LDP-01 Heavy Chain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: heavy chain variable region sequence comprises
      amino acids 1-120
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: heavy chain constant reqion sequences comprise
      amino acids 121-450
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: signal sequence comprises amino acids -19 to -1
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1410)

<400> SEQUENCE: 14

atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt       48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                   10                  15

gtc cac tcc cag gtc caa ctg cag gag agc ggt cca ggt ctt gtg aga       96
Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
            20                  25                  30

cct agc cag acc ctg agc ctg acc tgc acc gtg tct ggc ttc acc ttc      144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe
        35                  40                  45

acc gat tac ctt ctg cac tgg gtg aga cag cca cct gga cga ggt ctt      192
Thr Asp Tyr Leu Leu His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
    50                  55                  60

gag tgg att gga tgg att gat cct gag gat ggt gaa aca aag tat ggt      240
Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
65                  70                  75                  80

cag aag ttt caa agc aga gtg aca atg ctg gta gac acc agc aag aac      288
Gln Lys Phe Gln Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
                85                  90                  95

cag ttc agc ctg aga ctc agc agc gtg aca gcc gcc gac acc gcg gtc      336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
            100                 105                 110

tat tat tgt gca aga ggc gaa tat aga tac aac tcg tgg ttt gat tac      384
Tyr Tyr Cys Ala Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
        115                 120                 125

tgg ggt caa ggc tca cta gtc aca gtc tcc tca gcc tcc acc aag ggc      432
Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
    130                 135                 140

cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc      480
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145                 150                 155                 160

aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg      528
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                165                 170                 175
```

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```
acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc      576
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
            180                 185                 190

ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg      624
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
        195                 200                 205

acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg      672
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
    210                 215                 220

aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc aaa      720
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
225                 230                 235                 240

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc      768
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
                245                 250                 255

gcg ggg gca ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag cac acc      816
Ala Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys His Thr
            260                 265                 270

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg      864
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        275                 280                 285

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg      912
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    290                 295                 300

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc      960
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305                 310                 315                 320

acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg     1008
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                325                 330                 335

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc     1056
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            340                 345                 350

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca     1104
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        355                 360                 365

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag     1152
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
    370                 375                 380

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc     1200
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg     1248
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc     1296
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc     1344
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc     1392
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

ctg tct ccg ggt aaa tga                                            1410
Leu Ser Pro Gly Lys
465
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<210> SEQ ID NO 15
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: HomoSapiens

<400> SEQUENCE: 15

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
20 25 30

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe
35 40 45

Thr Asp Tyr Leu Leu His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
50 55 60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
65 70 75 80

Gln Lys Phe Gln Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
85 90 95

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
115 120 125

Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
130 135 140

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210 215 220

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
225 230 235 240

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245 250 255

Ala Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys His Thr
260 265 270

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275 280 285

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290 295 300

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305 310 315 320

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325 330 335

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340 345 350

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355 360 365

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Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
    370                 375                 380

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

Leu Ser Pro Gly Lys
465


<210> SEQ ID NO 16
<211> LENGTH: 702
<212> TYPE: DNA
<213> ORGANISM: HomoSapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: LDP-01 Light Chain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: light chain variable region sequence comprises
      amino acids 1-109
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: light chain constant region sequence comprises
      amino acids 110-214
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: signal sequence comprises amino acids -19 to -1
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(702)

<400> SEQUENCE: 16

atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt       48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                   10                  15

gtc cac tcc gac atc cag atg acc cag agc cca agc agc ctg agc gcc       96
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
            20                  25                  30

agc gtg ggt gac aga gtg acc atc acc tgt aaa gca agt aag agc att      144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Lys Ser Ile
        35                  40                  45

agc aat tat tta gcc tgg tac cag cag aag cca ggt aag gct cca aag      192
Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
    50                  55                  60

ctg ctg atc tac tat ggg tca act ttg cga tct ggt gtg cca agc aga      240
Leu Leu Ile Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Val Pro Ser Arg
65                  70                  75                  80

ttc agc ggt agc ggt agc ggt acc gac ttc acc ttc acc atc agc agc      288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
                85                  90                  95

ctc cag cca gag gac atc gcc acc tac tac tgc caa cag tat tat gaa      336
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Glu
            100                 105                 110

aga ccg ctc acg ttc ggc caa ggg acc aag gtg gaa atc aaa cga act      384
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Arg Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
        115                 120                 125

gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg      432
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
    130                 135                 140

aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc      480
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
145                 150                 155                 160

aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt      528
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
                165                 170                 175

aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac      576
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
            180                 185                 190

agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac      624
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
        195                 200                 205

aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc      672
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
    210                 215                 220

aca aag agc ttc aac agg gga gag tgt tag                              702
Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230


<210> SEQ ID NO 17
<211> LENGTH: 233
<212> TYPE: PRT
<213> ORGANISM: HomoSapiens

<400> SEQUENCE: 17

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                   10                  15

Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
            20                  25                  30

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Lys Ser Ile
        35                  40                  45

Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
    50                  55                  60

Leu Leu Ile Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Val Pro Ser Arg
65                  70                  75                  80

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
                85                  90                  95

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Glu
            100                 105                 110

Arg Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
        115                 120                 125

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
    130                 135                 140

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
145                 150                 155                 160

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
                165                 170                 175

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
            180                 185                 190

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
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        195                 200                 205

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
    210                 215                 220

Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230
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What is claimed is:

1. A recombinant expression vector comprising a 3' UTR of a light chain gene and an Epstein-Barr virus origin of replication (oriP).

2. The recombinant expression vector of claim 1, wherein the light chain gene is a murine light chain gene.

3. The recombinant expression vector of claim 2, wherein the 3' UTR comprises a nucleotide sequence of nucleotides 1062-2560 of SEQ ID NO: 1.

4. The recombinant expression vector of claim 1, wherein the recombinant expression vector is a recombinant transient expression vector.

5. The recombinant expression vector of claim 1 further comprising a pUC19 origin of replication, a viral promoter, a 5' UTR intron, or a combination of any of the foregoing.

6. The recombinant expression vector of claim 5 further comprising a pUC19 origin of replication, a viral promoter, and a 5' UTR intron.

7. The recombinant expression vector of claim 5, wherein the viral promoter is a CMV promoter.

8. The recombinant expression vector of claim 5, wherein the 5' UTR intron comprises nucleotides 888-974 of SEQ ID NO: 1.

9. The recombinant expression vector of claim 5, wherein the pUC19 origin of replication comprises the nucleotide sequence of nucleotides 4551-5220 of SEQ ID NO: 1.

10. The recombinant expression vector of claim 1 further comprising an antibody signal sequence.

11. The recombinant expression vector of claim 1, wherein the recombinant expression vector does not comprise an antibody signal sequence.

12. The recombinant expression vector of claim 1 further comprising a nucleotide sequence encoding a protein or a functional fragment thereof.

13. The recombinant expression vector of claim 12, wherein the nucleotide sequence encodes an immunoglobulin chain.

14. The recombinant expression vector of claim 13, wherein the immunoglobulin chain is selected from the group consisting of a γ_1 heavy chain, a γ_2 heavy chain, a γ_4 heavy chain, a κ light chain, and a λ light chain.

15. A method of producing a recombinant protein, comprising contacting a cell with the recombinant expression vector of claim 1, whereupon a recombinant protein is produced.

16. A host cell comprising the recombinant expression vector of claim 1.

17. The host cell of claim 16, wherein the host cell is a mammalian cell.

18. The host cell of claim 17, wherein the mammalian cell is a human cell.

19. The host cell of claim 18, wherein the human cell is a human embryonic kidney cell.

20. The host cell of claim 19, wherein the human embryonic kidney cell expresses an Epstein Barr virus nuclear antigen-1 (EBNA-1) protein.

21. The host cell of claim 20, wherein the host cell is a 293E cell.

22. A method of producing a recombinant protein, comprising culturing the host cell of claim 16, whereupon a recombinant protein is produced.

23. A method of producing a recombinant protein, the method comprising contacting cells in a medium with a first vector and a second vector, wherein the first vector encodes a first polypeptide chain and the second vector encodes a second polypeptide chain, wherein the first polypeptide chain is different from the second polypeptide chain, and wherein the second vector is present in the medium in an amount which is about 1.5 to 2.5 times as much as the amount of the first vector, whereupon a recombinant heterodimeric or heteromultimeric protein is produced.

24. The method of claim 23, wherein the second vector is present in the medium in an amount which is about 1.75 to 2.25 times as much as the amount of the first vector.

25. The method of claim 24, wherein the second vector is present in the medium in an amount which is twice as much as the amount of the first vector.

26. The method of claim 23, wherein the recombinant protein is a heterotetrameric protein.

27. The method of claim 26, wherein the heterotetrameric protein is an immunoglobulin.

28. The method of claim 27, wherein the first vector encodes a heavy chain of an immunoglobulin, or a functional fragment thereof, and the second vector encodes a light chain of an immunoglobulin, or a functional fragment thereof.

29. The method of claim 28, wherein the heavy chain is a human γ_1 heavy chain, human γ_2 heavy chain, or a human γ_4 heavy chain.

30. The method of claim 28, wherein the light chain is a human κ light chain or a λ light chain.

31. The method of claim 23, wherein each of the first vector and the second vector is a recombinant transient expression vector.

32. The method of claim 23, wherein each of the first vector and the second vector comprises a 3' untranslated region (UTR) of a light chain gene and an oriP.

33. The method of claim 23, wherein each of the first vector and the second vector comprises a viral promoter, a pUC19 origin of replication, a 5' UTR intron, or a combination of any of the foregoing.

34. The method of claim 33, wherein the viral promoter is a CMV promoter.

35. The method of claim 33, wherein the 5' UTR intron comprises a nucleotides 888-974 of SEQ ID NO: 1.

36. The method of claim 23, wherein each of the first vector and second vector comprises an antibody signal sequence.

37. The method of claim 23, wherein the cells are contacted with the first vector and second vector simultaneously.

38. The method of claim 23, wherein the cells are contacted with the first vector and second vector in the presence of a cationic polymer.

39. The method of claim 38, wherein the cationic polymer is polyethyleneimine (PEI).

40. The method of claim 39, wherein the PEI is a linear PEI.

41. The method of claim 40, wherein the linear PEI is present in an amount that is about 1.5 to 4.5 times the amount of the first vector and second vector.

42. The method of claim 41, wherein the linear PEI is present in an amount that is about 2.5 to 3.5 times the amount of the first vector and second vector.

43. The method of claim 42, wherein the linear PEI is present in an amount that is twice the amount of the first vector and second vector.

44. The method of claim 23, wherein the cells are mammalian cells.

45. The method of claim 44, wherein the mammalian cells are human cells.

46. The method of claim 45, wherein the cells are human embryonic kidney cells.

47. The method of claim 46, wherein the human embryonic kidney cells express Epstein-Barr virus nuclear antigen-1 protein (EBNA-1).

48. The method of claim 47, wherein the cells are 293E cells.

49. The method of claim 23 further comprising isolating the cells from the medium and culturing the cells in a second medium in a membrane-enhanced culturing vessel, wherein the second medium is different from the medium.

50. The method of claim 49, wherein the second medium is a serum-free cell culture medium.

51. The method of claim 50, wherein the second medium is IS293™ medium.

52. The method of claim 49, wherein the membrane-enhanced culturing vessel is an Integra CL1000.

53. The method of the claim 49 further comprising purifying the recombinant protein from the second medium.

54. The method of claim 53, wherein the purifying comprises centrifuging the second medium through a column comprising Protein A.

55. The method of claim 53, wherein the purifying occurs after 3 days of culturing the cells in the second medium.

56. The method of claim 53, wherein the purifying occurs after 7 days of culturing the cells in the second medium.

57. The method of claim 55, wherein at least 300 μg/ml recombinant protein is produced in the second medium.

58. The method of claim 57, wherein at least 500 μg/ml recombinant protein is produced in the second medium.

59. The method of claim 58, wherein at least 700 μg/ml recombinant protein is produced in the second medium.

60. The method of claim 49, wherein the culturing comprises seeding cells in the second medium at a cell density between about 1.0×10^6 and 2.0×10^7 cells/ml.

61. The method of claim 60, wherein the cell density is about 3.0×10^6 to about 1.0×10^7 cells/ml.

62. A method of producing a recombinant protein comprising culturing cells, which have been contacted with a recombinant transient expression vector encoding a recombinant protein, in a medium in a membrane-enhanced culturing vessel or in a Fembach flask, whereupon the recombinant protein is produced.

63. The method of claim 62, wherein the medium is a serum-free cell culture medium.

64. The method of claim 63, wherein the serum-free medium is IS293™ medium.

65. The method of claim 62, wherein the membrane-enhanced culturing vessel is an Integra CL1000.

66. The method of claim 62, wherein the method further comprises purifying the recombinant protein from the medium.

67. The method of claim 66, wherein the purifying comprises centrifuging the medium through a column comprising Protein A.

68. The method of claim 66, wherein the purifying occurs after 3 days of culturing the cells in the medium.

69. The method of claim 66, wherein the purifying occurs after 7 days of culturing the cells in the medium.

70. The method of claim 68, wherein at least 300 μg/ml recombinant protein is produced in the medium.

71. The method of claim 70, wherein at least 500 μg/ml recombinant protein is produced in the medium.

72. The method of claim 71, wherein at least 700 μg/ml recombinant protein is produced in the medium.

73. The method of claim 62, wherein the culturing comprises seeding cells in the medium at a cell density between about 1.0×10^6 and 2.0×10^7 cells/ml.

74. The method of claim 73, wherein the cell density is 3.0×10^6 to about 1.0×10^7 cells/ml.

75. The method of claim 62, wherein the recombinant transient expression vector comprises a 3' untranslated region (UTR) of a light chain gene and an oriP.

76. The method of claim 62, wherein the recombinant transient expression vector comprises a pUC19 origin of replication, a viral promoter, a 5' UTR intron, or a combination of any of the foregoing.

77. The method of claim 76, wherein the viral promoter is a CMV promoter.

78. The method of claim 76, wherein the 5' UTR intron comprises nucleotides 888-974 of SEQ ID NO: 1.

79. The method of claim 62, wherein the cells have been contacted with a recombinant transient expression vector in the presence of a cationic polymer.

80. The method of claim 79, wherein the cationic polymer is a polyethyleneimine (PEI).

81. The method of claim 80, wherein the PEI is a linear PEI.

82. The method of claim 81, wherein the PEI is present in an amount that is about 1.5 to 4.5 times the amount of the recombinant transient expression vector.

83. The method of claim 82, wherein the PEI is present in an amount that is about 2.5 to 3.5 times the amount of the recombinant transient expression vector.

84. The method of claim 83, wherein the PEI is present in an amount that is twice the amount of the recombinant transient expression vector.

85. The method of claim 62, wherein the cells are mammalian cells.

86. The method of claim 85, wherein the mammalian cells are human cells.

87. The method of claim 86, wherein the human cells are human embryonic kidney cells.

88. The method of claim 87, wherein the human embryonic kidney cells express Epstein-Barr virus nuclear antigen-1 protein (EBNA-1).

89. The method of claim 88, wherein the cells are 293E cells.

90. The method of claim 62, wherein the protein is an immunoglobulin chain or a functional fragment thereof.

91. The method of claim 90, wherein the immunoglobulin chain is a heavy chain or a light chain.

92. The method of claim 91, wherein the immunoglobulin chain is a heavy chain, and the heavy chain is a human γ_1 heavy chain, human γ_2 heavy chain, or a human γ_4 heavy chain.

93. The method of claim 91, wherein the immunoglobulin chain is a light chain, and the light chain is a human κ light chain or a λ light chain.

* * * * *