US 20080064862A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2008/0064862 A1**

Harvey et al. (43) **Pub. Date: Mar. 13, 2008**

(54) **TRANSGENE EXPRESSION IN A AVIANS**

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(21) Appl. No.: **11/978,360**

(22) Filed: **Oct. 29, 2007**

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/699,257, filed on Jan. 26, 2007.
Continuation-in-part of application No. 11/799,253, filed on May 1, 2007, which is a continuation-in-part of application No. 11/210,165, filed on Aug. 23, 2005, now abandoned.

(60) Provisional application No. 60/930,491, filed on May 16, 2007. Provisional application No. 60/994,203, filed on Sep. 18, 2007. Provisional application No. 60/640,203, filed on Dec. 29, 2004.

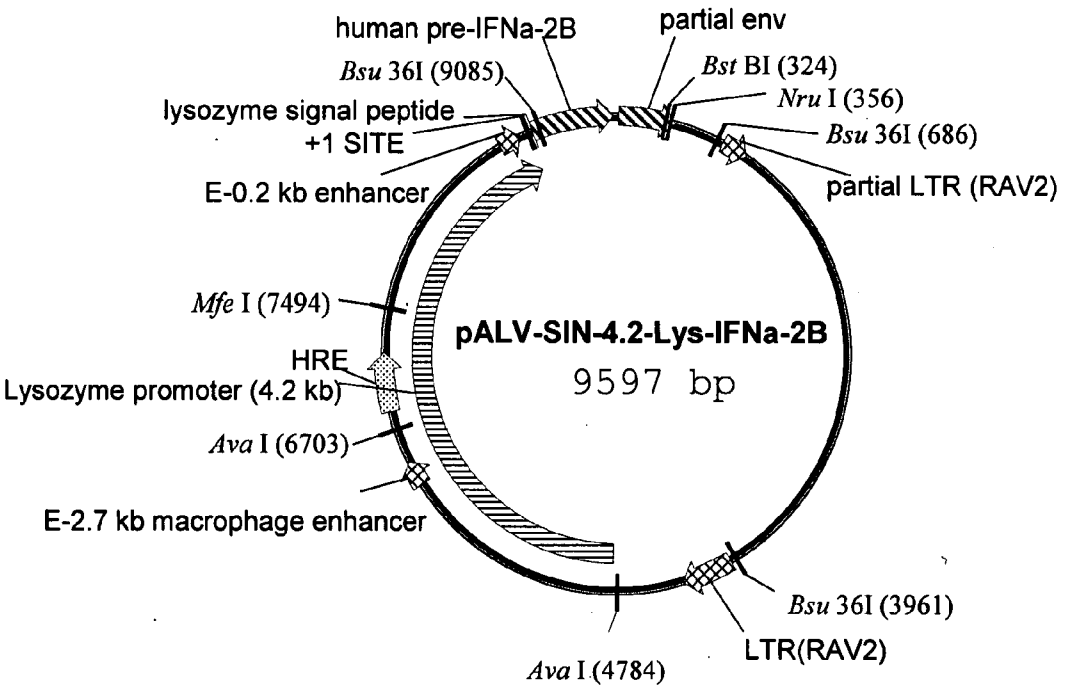
Publication Classification

(51) **Int. Cl.**
A01K 67/00 (2006.01)
C07H 21/04 (2006.01)
C12P 21/00 (2006.01)

(52) **U.S. Cl.** **536/22.1**; 800/19; 800/4

(57) **ABSTRACT**

A transgenic avian containing in its genome an exogenous nucleotide sequence which includes a promoter component and a vector with reduced promoter interference wherein the exogenous nucleotide sequence is integrated into the genome and the avian.



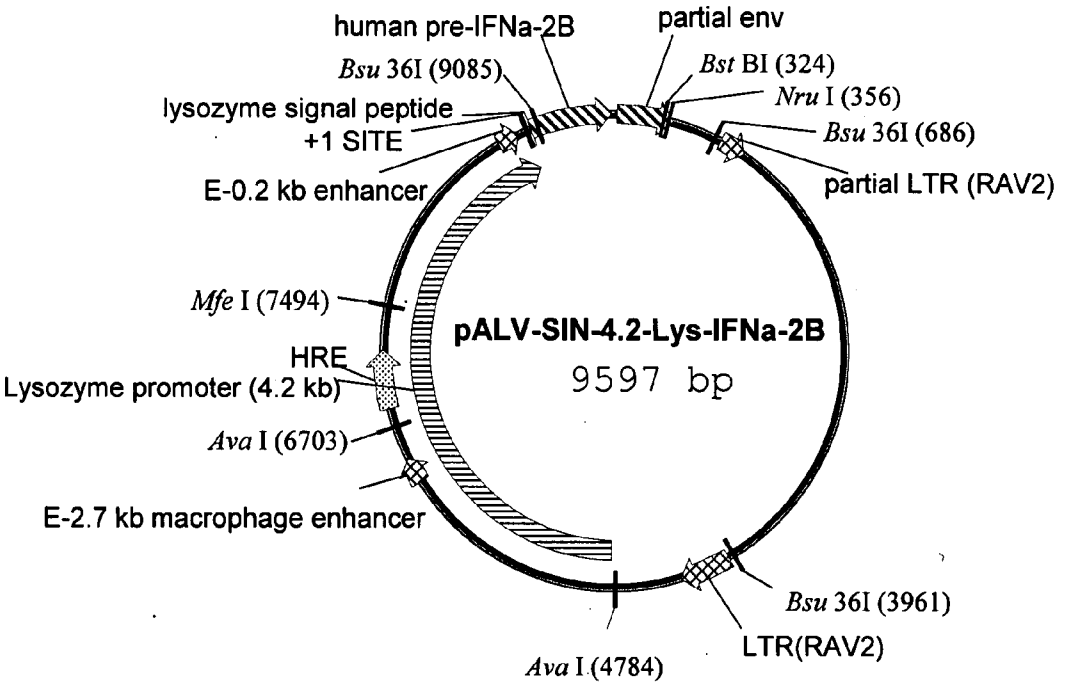

human pre-IFNa-2B
partial env
Bsu 36I (9085)
Bst BI (324)
lysozyme signal peptide
Nru I (356)
+1 SITE
Bsu 36I (686)
E-0.2 kb enhancer
partial LTR (RAV2)
Mfe I (7494)
pALV-SIN-4.2-Lys-IFNa-2B
9597 bp
HRE
Lysozyme promoter (4.2 kb)
Ava I (6703)
E-2.7 kb macrophage enhancer
Bsu 36I (3961)
LTR(RAV2)
Ava I (4784)

FIG. 1

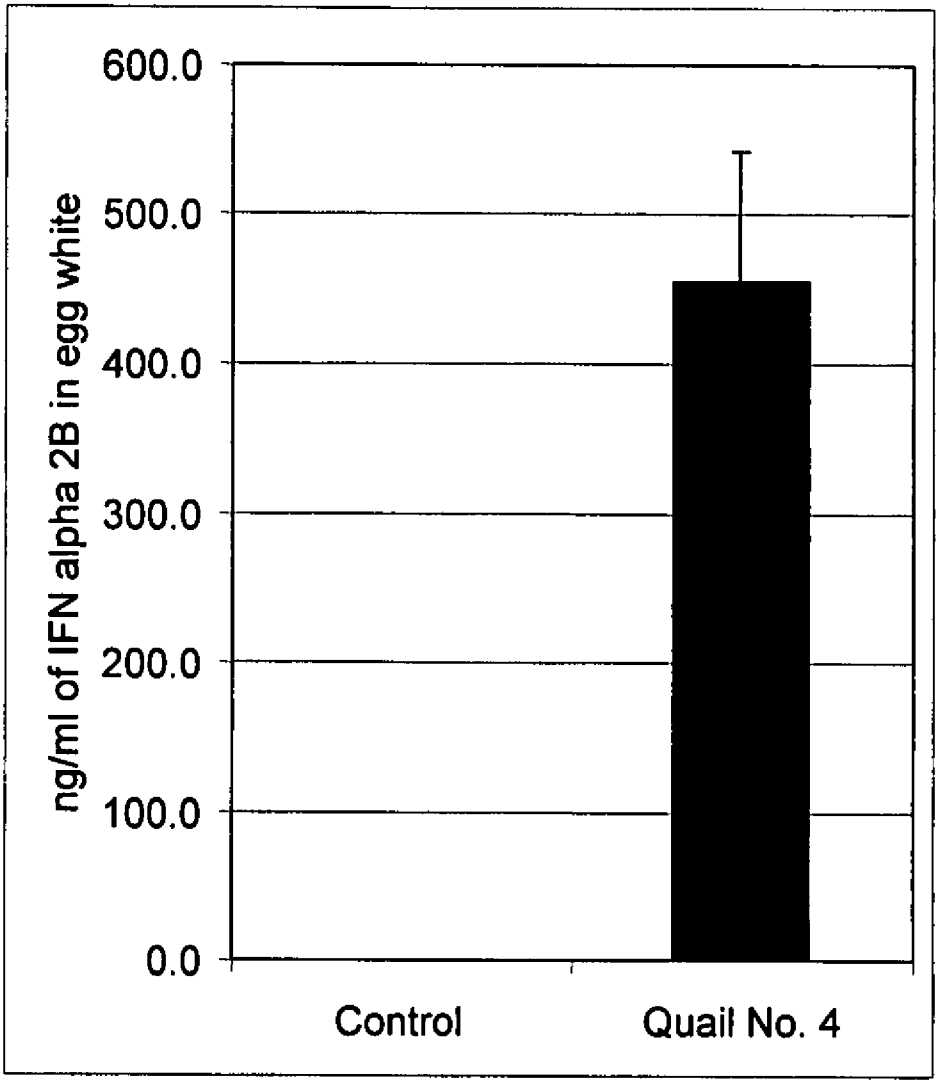

600.0
500.0
400.0
300.0
200.0
100.0
0.0
ng/ml of IFN alpha 2B in egg white
Control
Quail No. 4

FIG. 2

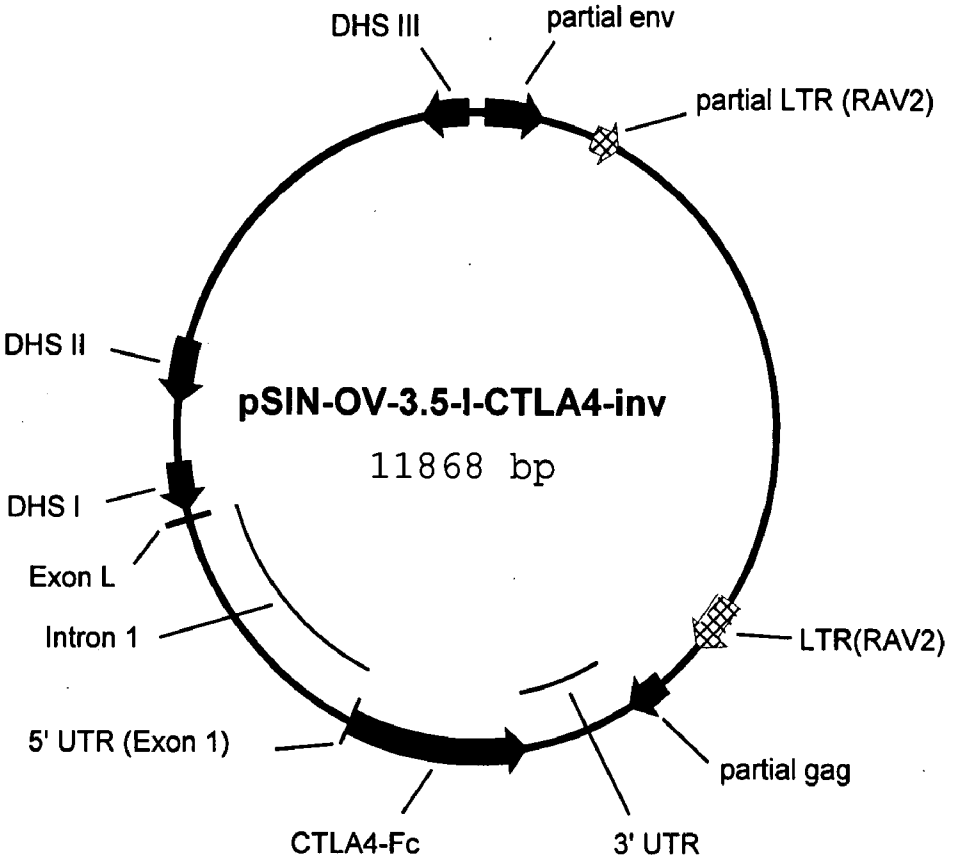

DHS III
partial env
partial LTR (RAV2)
DHS II
pSIN-OV-3.5-I-CTLA4-inv
11868 bp
DHS I
Exon L
Intron 1
LTR(RAV2)
5' UTR (Exon 1)
partial gag
CTLA4-Fc
3' UTR

FIG. 3

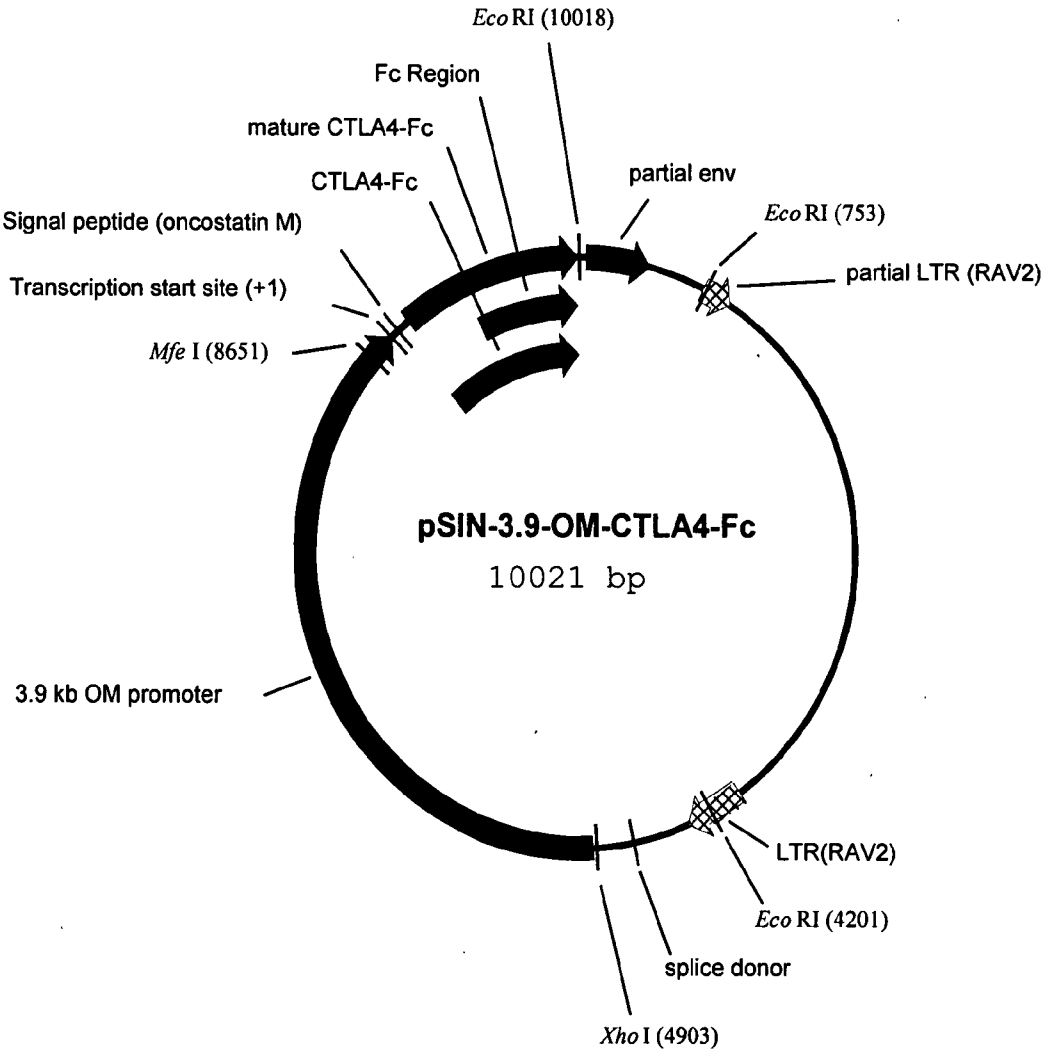

EcoRI (10018)
Fc Region
mature CTLA4-Fc
CTLA4-Fc
Signal peptide (oncostatin M)
Transcription start site (+1)
MfeI (8651)
partial env
EcoRI (753)
partial LTR (RAV2)
pSIN-3.9-OM-CTLA4-Fc
10021 bp
3.9 kb OM promoter
LTR(RAV2)
EcoRI (4201)
splice donor
XhoI (4903)

FIG. 4

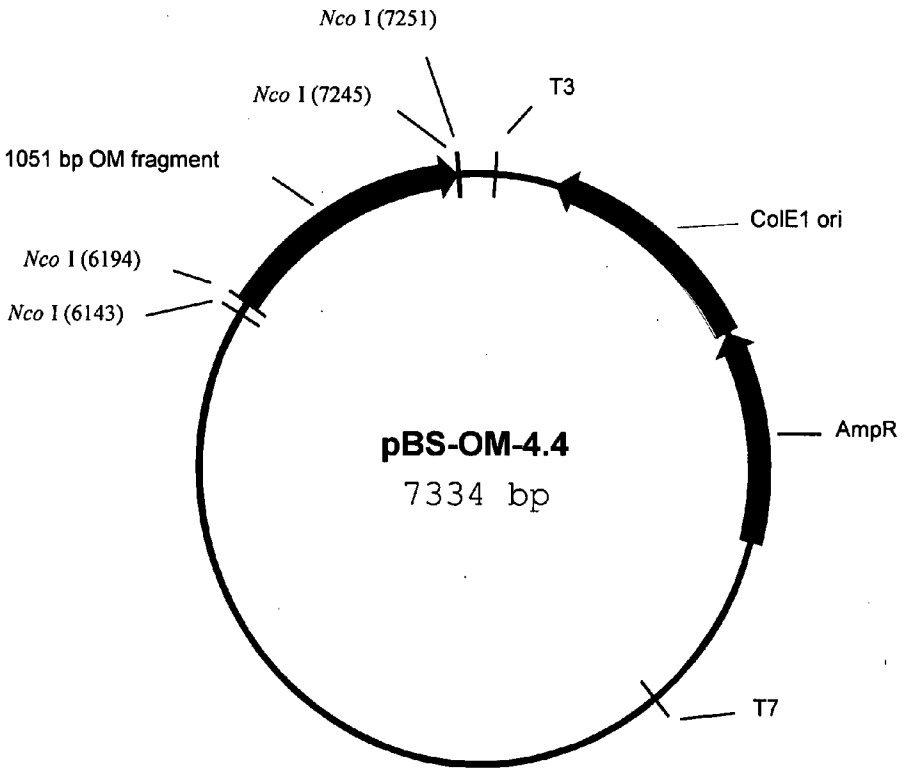

Nco I (7251)
Nco I (7245)
T3
1051 bp OM fragment
ColE1 ori
Nco I (6194)
Nco I (6143)
pBS-OM-4.4
7334 bp
AmpR
T7

FIG. 5

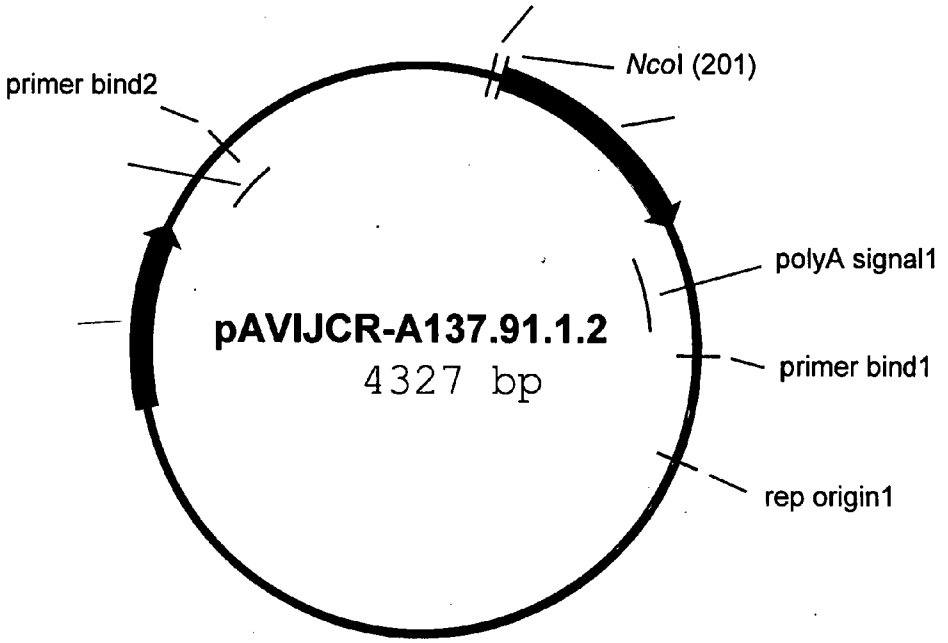

primer bind2
NcoI (201)
polyA signal1
pAVIJCR-A137.91.1.2
4327 bp
primer bind1
rep origin1

FIG. 6

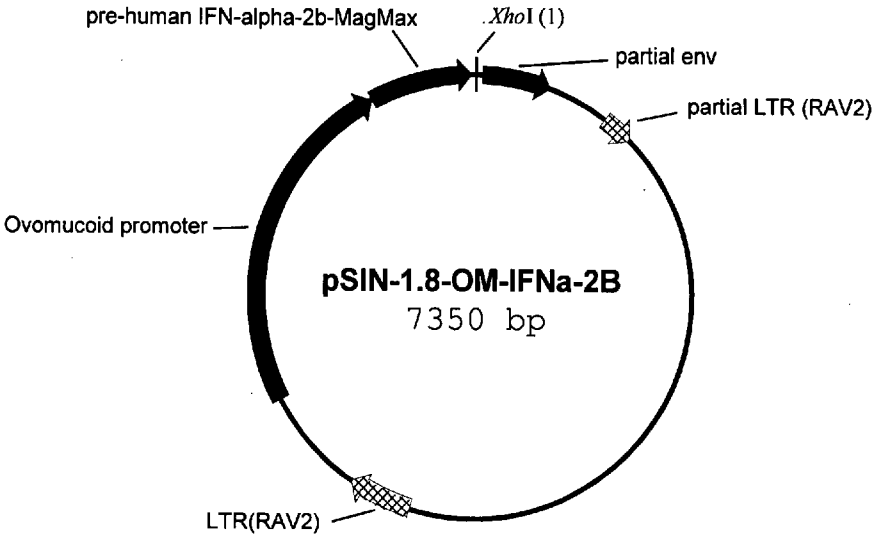

pre-human IFN-alpha-2b-MagMax
XhoI (1)
partial env
partial LTR (RAV2)
Ovomucoid promoter
pSIN-1.8-OM-IFNa-2B
7350 bp
LTR(RAV2)

FIG. 7

```
ctgcagccca ggcagcacac tagagcagag aaatctagtt agcagcaacc actggcagac      60
agaaatgatt atatagatta catactgacc ctagcctctt acactgccta ctgcatcact     120
gaaaggactg ggaagaagag agtgcaataa cgaagctgaa gctaggagga aggcaaggag     180
aactgaagct gactagggaa aaggggatt aaaggtttaa gtgtctattc catagtttgc      240
tggtttgttt tttgtcaatt cctgaatcag taatttttat gttaattagc aaaaaattac     300
aaacactccc caagtcagga ctgttaccta caacagaagc tcagatcagc tgagccttag     360
tcttttggtc cctccctagg gaatgctgta tgtgtctctc tctccaggcc tgctcaaaat     420
tgacctcaga cccaaacttt tgctgaatct ccagtaccac ctcttttgct cctaactaga     480
taacaaagcc ctgagcgctt tgctttttagc aaagctttaa gtgccattac caactgcacc     540
tggagccttt acctacccct atggacccag gctctatatt taagctctgc cctgaacctt     600
cacttctttc ctgtcctaag ttagatgtac tagtatggtg tgtactatgt ctccagttca     660
aacacagctg tgcccatacc tggccaagga ctcctagtat gacctgggct gtgccttgct     720
gctaaggacc tgctgggtga ttgctggacc tgatcctaat cctgaattaa gaaatgattt     780
cttggcttga ctggatgtgc cctgtggtat gatactgcct tatgatttgg actcttgttt     840
gcagctgtgc aaatccctaa ggagcccagt ctctggccac ctggaatctt gtcactacca     900
aacttcctga gggactggtc ttgctctggg ttctgatctc tggacagtac tcacccttta     960
ctcagcccag gctcccagtt aagccccttt ccaccctgcc aggctctccg ctccatccct    1020
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cactttgctc agaaggatgc gaggctgctt ctgagctctg agggcactga cctcccatga    1260
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cctgctgatc tcaggatgca gacttgcttc tcagcttgac ctaagcattg ccctgtcttt    1440
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tccacccaca ttactgtctg ctgactcatt ctacgtctct cccatcttgt tacaataata    1560
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ctgtgcttga taaatccatg ctggcttttc ctatcacctt atattccttc atatgccttg    1740
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ccataccaac atcagaccac atatctacag gaagctttaa ggaatcattg actgtacagt    2700
gaagggtaaa tcaaattaaa atgaatgtga ggtctgatac gagatatcct catgggaatc    2760
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ccaaggtatg caatggcaat ccattcgaca ttcatctgtg acctgagcaa aatgatttat    3060
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caatgtgtga caaaactatt tgtactgctt tgcacagctg tgctgggcag ggcaatccat    3480
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tcaagatctc atttgttcat ggacaatttt tgttgcttgc agctggtctt ccattgggaa    3660
agagtgtagt atatccttct catctgacag aaaagcagaa attctcatgc tccacactta    3720
atctacattg ttttaaacca ccagctactt cttggagagg aaaaatggct tttataagac    3780
tcacaaaaca aagctctgca agtcaaatgc atacaaaact gttctgtagg tctggaatca    3840
```

FIG. 8a

```
ggacactatg tggaagtcaa atagagaagc tttaaaaaaa cctttgggat cattctcatc    3900
ttatatttgc agcacgatac tatgacagtg ataactgaca taactgcatc aatttccttg    3960
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acaagaattc attcagtggc tctgttttat agtaaacatt gctattttat catgtctgca    7560
tttctcttct gtctgaatgt caccactaaa atttaactcc acagaaagtt tatactacag    7620
tacacatgca tatctttgag caaagcaaac catacctgaa agtgcaatag agcagaatat    7680
```

FIG. 8b

```
gaattacatg cgtgtctttc tcctagacta catgacccca tataaattac attacttatc   7740
tattctgcca tcaccaaaac aaaggtaaaa atacttttga agatctactc atagcaagta   7800
gtgtgcaaca aacagatatt tctctacatt tatttttagg gaataaaaat aagaaataaa   7860
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tatttcagct actattattt tcaattacag gttgttcgct ttgataaact tccaggattc   8760
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ccattctgta atgcaaaaac agtcagagtt ccacatgttt cactaagaaa atttcttttt   9600
ctcttgtttt tacaaatgaa agagaggaca aataacattt ctctatcacc gacctgaaac   9660
tctacagtct tcagagaatg aatggcttgc taaaagaatg tcaaatctta ctatacagct   9720
atttcatatt acactactaa atacactata aggcatagca tgtagtaata cagtgtaaaa   9780
tagcttttta cactactata ttattaatat ctgttaattc cagtcttgca tttcacattt   9840
gcaaaacgtt ttgaaattcg tatctgaaag ctgaatactc ttgctttaca ggaatacttg   9900
cagtgtgtga aggaactgta tagaggaggc ttggaaccta tcaactttca aacagctgca   9960
gatcaagcca gagagctcat caattcctgg gtagaaagtc agacaaatgg taaggtagaa  10020
catgctttgt acatagtgag agttggttca ccctaatact gagaacttgg atatagctca  10080
gccagcgtgc tttgcgttca agcttaccag agctgttgta tgcctgttaa gcagggcata  10140
cagtcatgag gctcttgaaa aatcttaaca gacaaagggc aatggaaaat cggagttaag  10200
ggatggtagg gataaaatgc atagaaagag gtaccacaat tttgattttt gccctaatgc  10260
ctctctgcgt ggttcctcaa tttttctact tcattcctca tctcctcaga gcattccttt  10320
ccctcatgct tgaaacacag atgaaagact gtgaattcta actgagatga aaacatccac  10380
aaccacacaa cctctggtgt ggagtcacat tctgtgaagg caaaaactag gccacgtaat  10440
ctatgcgtgc aagctacgcg taagctatgt gtgtgacagg acaatgtgag gaacatacta  10500
tgtgcacaag gactgcagaa taaacaggag caaagttttt gaagaaaaca gagtaaaatc  10560
ctgttttcct cttttgttac attctttaca tatatctcaa atttcctctt tggttagaag  10620
caagtaatat ttatgtttct tggtactgtt tgggttgaag accattctgg gataagagaa  10680
attccagtgg ttcttcccct aatcataaaa tgtcaggttt agtttttttg taacacagaa  10740
atctcttcat cttttatctt ttgttgtgat tcttgataga gagagaaaca agacttactg  10800
acaatagcag caagaaaatc aatcttggaa gaacaagatt gcaattgcaa aaacaaacca  10860
atgtccttgc ccctacatcc tcttccccat aaattctaca ttctctatct accttgtgct  10920
tgccaacatg atatacgtaa actctctttt cctattcatt cttaaaggaa ttatcagaaa  10980
tgtccttcag ccaagctccg tggattctca aactgcaatg gttctggtta atgccattgt  11040
cttcaaagga ctgtgggaga aagcatttaa ggatgaagac acacaagcaa tgcctttcag  11100
agtgactgag gtatatgggc ataccttaga gatgtaatct agaatttatg aagagagtag  11160
acatgttgtt atatgaacac tgcattagcg tatctgctca tttgtctgca tctctttcag  11220
acactgtgtt aaaagcaggg aattttcctt atgtctctct cgtcacaata ttcctgacat  11280
tgcaaagctc ctgagaaata acttcagatt ccacttttcc taggaaggct tctggatgag  11340
aactaatcat cttaactgta actagacatt tctgcatcca agaataatct ttgttaaaac  11400
tatattctct ctctcttttt ttttttttt tggttctcca gcaagaaagc aaacctgtgc  11460
agatgatgta ccagattggt ttatttagag tggcatcaat ggcttctgag aaaatgaaga  11520
```

FIG. 8c

```
tcctggagct tccatttgcc agtgggacaa tgagcatgtt ggtgctgttg cctgatgaag   11580
tctcaggcct tgagcaggta tggccctaga agttggcttc agaatattaa aaacacatgg   11640
aaatttagct gttgtaaagc tcttttcaac acagttatcc taaaacattt aaccagcaca   11700
aatttcatca tgattcaata tgtgattgtt gcatagaagt gtagatttgt cccactgggt   11760
cctgcaatag cccatgctga gcatggcttg ctgaaagaac tgctttagag ggtgaaaagt   11820
ttgacacagc agacaagatg attctcacct aagcagctgt tactgtagtg gcttgaactc   11880
taaaggtctt gtatctccat tcctgtgcac tgaggagctt cttggaaagt tcatataagg   11940
tttactagtt ctaactatta tctcatttgg tggcactcaa tgtgctttgt tcacgtcttc   12000
ataaattaat ctatctaaaa attggatgtg gttaaagcaa tttcagaaat aacatgtaca   12060
taatgtacaa ttattgatat gaacagaaca caggcatagc atattgtaat taggaggact   12120
gtagttattt tgaataggaa acacaatgta ataaatgaga attcattgaa atgttagtat   12180
gctaactcaa tctaaattat aaagataaag aggcatttaa tcacagctag atttccatca   12240
cttgtgacag acaggcatat gaatgattat gtacagctct aggaaaaaaa gtatgtagga   12300
aaactagtac attttgatta gaaagtctga aaatgaggtg ccttgatcaa agagaatacg   12360
tgtgtttgag aaaaaaaaag tttggataga ggtggtaaga gagaatatat tgaaatggtg   12420
tttctacaaa ctgccatggc cagatttgtg taagagacat tcagtaagta ggcaaggaaa   12480
gaaatattac taggtacaaa gcaacatcag taataccaaa agaaaccaat tattccagat   12540
gccaatctcg taatagggtt aagagatttc caccccctcta gtggtcacca gtgcaaccag   12600
taactttgct aatttacatt ttctttttt aaatggcaga tatagctttg aactgagtga   12660
tcatgaactg gtactgtgta atagatgaag acatacttga cgactaaact tctgattttt   12720
aaaaactcaa attctcttga aagatcagtt cccagtctag taacagctga tagtttaagt   12780
atcagtaatt ggctaccatt aacaactggc tcctgagagg tcttaaatgt agagacagct   12840
ttaaactcaa aagcacagag tgatttttag aatagatttc ccaagcaaag aaaataaaca   12900
gggaggagct ttaagggagt agccatctca ttattattat tatttaaaga aatggcagca   12960
agcctacaaa agaaaaataa gacagagcag agaagaaaga gtcatggtat gcttttctat   13020
cttagcaaaa ttaatctcta catgcctagg aaaaagccat gacaagagca atcagttcaa   13080
aaggtgtatg caaaaaacca cataatagta actagtactg cattgccagg aaggaagtta   13140
tgtcgccatt ccatggatct cattctcatt tccttgcagc ttgagagtat aatcaacttt   13200
gaaaaactga ctgaatggac cagttctaat gttatggaag agaggaagat caaagtgtac   13260
ttacctcgca tgaagatgga ggaaaaatac aacctcacat ctgtcttaat ggctatgggc   13320
attactgacg tgtttagctc ttcagccaat ctgtctggca tctcctcagc agagagcctg   13380
aagatatctc aagctgtcca tgcagcacat gcagaaatca atgaagcagg cagagaggtg   13440
gtagggtcag cagaggctgg agtggatgct gcaagcgtct ctgaagaatt tagggctgac   13500
catccattcc tcttctgtat caagcacatc gcaaccaacg ccgttctctt ctttggcaga   13560
tgtgtttccc cttaaaaaga agaaagctga aaaactctgt cccttccaac aagacccaga   13620
gcactgtagt atcaggggta aaatgaaaag tatgttatct gctgcatcca gacttcataa   13680
aagctggagc ttaatctaga aaaaaaatca gaaagaaatt acactgtgag aacaggtgca   13740
attcactttt cctttacaca gagtaatact ggtaactcat ggatgaaggc ttaagggaat   13800
gaaattggac tcacagtact gagtcatcac actgaaaaat gcaacctgat acatcagcag   13860
aaggtttatg ggggaaaaat gcagccttcc aattaagcca gatatctgta tgaccaagct   13920
gctccagaat tagtcactca aaatctctca gattaaatta tcaactgtca ccaaccattc   13980
ctatgctgac aaggcaattg cttgttctct gtgttcctga tactacaagg ctcttcctga   14040
cttcctaaag atgcattata aaaatcttat aattcacatt tctccctaaa ctttgactca   14100
atcatggtat gttggcaaat atggtatatt actattcaaa ttgttttcct tgtacccata   14160
tgtaatgggt cttgtgaatg tgctcttttg ttcctttaat cataataaaa acatgtttaa   14220
gcaaacactt ttcacttgta gtatttgaag tacagcaagg ttgtgtagca gggaaagaat   14280
gacatgcaga ggaataagta tggacacaca ggctagcagc gactgtagaa caagtactag   14340
tgggtgagaa gttgaacaag agtcccctac aagcaactta atctaataag ctagtggtct   14400
acatcagcta aaagagcata gtgagggatg aaattggttc tcctttctaa gcatcacctg   14460
ggacaactca tctggagcag tgtgtccaat ctgccgctgc cctgatctcg gctggggtga   14520
tgggacagac cttggctgcc actgagacat ctgagacact gagatctgtc tcaactcaga   14580
tttacccaag aacagctcat tgccaacaga acaaaatctc aaacttatgg ctagtgatga   14640
cagcagtcag ttgtcccatc tgtgacccac caaggctggc atgctggaat gagcaggctt   14700
tggtggcatg tagttactgg acagcaccac tgacatgggc aggggaaaaa ctgagcatgg   14760
tgtaaatcac tgcctcaaag ccacttctct gtgcctgcac catgcttgaa agctcttcta   14820
caggagctgg gtttgttcaa gaaagcttct gtttctccca tctgcttctt gtaccttcac   14880
agggacagag ttagaagggt acagccatgg ctggaagggg ctgactttca aatgtgccta   14940
attttccttt ggttgctgct gcagctgcag aagaaggggt tcagaagcca agagctttga   15000
gataaggatg cctaacctat gttgaagaca tttgtgctga cacctcaggc cccaggatag   15060
gacaactgct ggattgtggc taacccacta gctacagaac ctaatttata ttaccagatt   15120
aggaagagca aaagaacatg tatttataac aggaggtctt ctgtgcttct ctactaaaag   15180
gtgctgtgaa ggagcccaca gtgcagcagt gtatgaggcc tgaaagaggc cgcagcacac   15240
gaagagccct ggtaggagca gcacacagag gggcaggagg gctgggggaa ctgccaccca   15300
tggggacctg tgtgaagcag tgcactcctg aggggtggac tgcgtgggaa aggaaaagaa   15360
```

FIG. 8d

```
agcaaacaga cctgtgatga actgtcacac agactgcaga gtgacagagg agggcacgag   15420
gcagtgcgcc cactgcaggg agtggcgctc cttcctcaca gcagcgctaa cagcttggca   15480
ccaatattca gtagtctgtg gtgatacttt ttccagtttc accacacagc atttcgcttg   15540
ttctacttgt tttagctttc cccctccaca agataacaca tactttgcca gtcagtccct   15600
aagaccttaa cttaacagtt agcaaacagg atcttgcaaa agaaggaaga taacatgaca   15660
ccaccttcac tggtgtataa atagttcaaa tactttcctt cactttcccg taaattagtt   15720
gattgcaggt caggagataa caggggaact tactgcaaga gagaaaatga tgtttaatat   15780
tgtcttggac tttctggtgg tctgggcatg aaaatggggt actcaaaatc ctcgggacgt   15840
ttatttttca cctgatttat tcccaaactg cactatttct aggccattgg agttcttatc   15900
aattaaatta tactttggct ctctgctatc tcactccctt tcatcttcag catcactttc   15960
agcacaatta caggagaaga cttagactca gagctttagg actcatcata agaggctttc   16020
attgctctgt caccacaccc catatagatc t                                  16051
```

FIG. 8e

AATTGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGC
GTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGA
ACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGC
ATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCT
CCTGGTAC

FIG. 9a

AATGTGGGGAGGGCAAGGCTTGCGAATCGGGTTGTAACGGGCAAGGCTTGACTGAGGGG
ACAATAGCATGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTC
AGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAAT

FIG. 9b

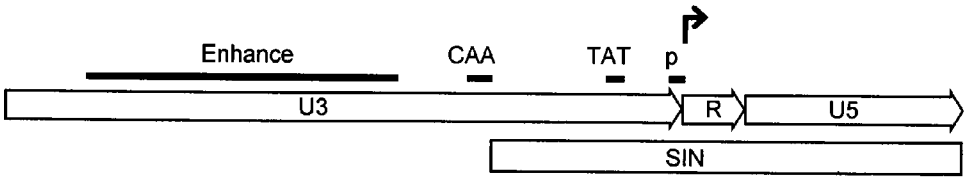

FIG. 10 a

```
AATGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGCTTATGTAACGATGAGTTAG   60
CAACATGCCTTATAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGGAGTAAGGTGG  120
TATGATCGTGGTATGATCGTGCCTTGTTAGGAAGGCAACAGACGGGTCTAACACGGATTG  180
GACGAACCACTGAATTCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAA  240
TAAACGCCATTTGACCATTCACCACATTGGTGTGCACCTGGGTTGATGGCCGGACCGTTG  300
ATTCCCTGRCGACTACGAGCACATGCATGAAGCAGAAGGCTTCATT                346
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FIG. 10 b

TRANSGENE EXPRESSION IN A AVIANS

RELATED APPLICATION INFORMATION

[0001] This application claims the benefit of U.S. provisional application Nos. 60/930,491, filed May 16, 2007 and 60/994,203, filed Sep. 18, 2007 and is a continuation-in-part of U.S. patent application Ser. No. 11/699,257, filed Jan. 26, 2007 and is also a continuation-in-part of U.S. patent application Ser. No. 11/799,253, filed May 1, 2007 which is a continuation-in-part of U.S. patent application Ser. No. 11/210,165, filed Aug. 23, 2005 which claims the benefit of U.S. provisional application No. 60/640,203, filed Dec. 29, 2004. The disclosures of each of these three U.S. patent applications and two provisional applications are incorporated in their entirety herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the use of promoters which function in cells of a transgenic avian (e.g., oviduct cells) such as a transgenic chicken and vectors which contain such promoters. More specifically, the invention relates to recombinant nucleic acids and expression vectors, transfected cells and transgenic animals, for example, transgenic avians such as transgenic chickens, that contain vectors with gene expression controlling regions operably linked to coding sequences.

BACKGROUND

[0003] The field of transgenics was initially developed to understand the action of a single gene in the context of the whole animal and the phenomena of gene activation, expression and interaction. Transgenics technology has also been used to produce models for various diseases in humans and other animals and is among the most powerful tools available for the study of genetics, and the understanding of genetic mechanisms and function. From an economic perspective, the use of transgenic technology to convert animals into "protein factories" for the production of specific proteins or other substances of pharmaceutical interest (Gordon et al., 1987, Biotechnology 5: 1183-1187; Wilmut et al., 1990, Theriogenology 33: 113-123) offers significant advantages over more conventional methods of protein production by gene expression.

[0004] One system useful for expressing foreign proteins is the avian reproductive system. The production of an avian egg begins with formation of a large yolk in the ovary of the hen. The unfertilized oocyte or ovum is positioned on top of the yolk sac. After ovulation, the ovum passes into the infundibulum of the oviduct where it is fertilized, if sperm are present, and then moves into the magnum of the oviduct, which is lined with tubular gland cells. These cells secrete the egg-white proteins, including ovalbumin, lysozyme, ovomucoid, conalbumin and ovomucin, into the lumen of the magnum where they are deposited onto the avian embryo and yolk. In the past exogenous protein production has been performed in the avian reproductive system specifically targeting the avian oviduct.

[0005] Advantages of targeting the avian oviduct for exogenous protein expression can include proper folding and post-translation modification of the target protein, the ease of product recovery, and a shorter developmental period of birds such as chickens compared to other animal species.

[0006] Directing expression of a heterologous gene product in the oviduct of a transgenic avian can be significantly advantageous over ubiquitous expression in the bird. That is, the consequences of ubiquitous expression of a bioactive gene product in a host animal may be undesirable. For example, in certain instances the ubiquitous presence of the recombinant protein may be harmful to the development of the avian which can kill the bird. Additionally, the bird's health may be negatively effected leading to reduced levels of protein production.

[0007] By weight, approximately 60% of an avian egg is composed of albumen which is composed of four major protein components; ovalbumin, ovomucoid, lysozyme and ovotransferrin with ovalbumin and ovomucoid being present in the greatest quantities.

[0008] The ovalbumin promoter, ovomucoid promoter and lysozyme promoter have been successfully employed for the production of heterologous (exogenous) protein in the oviduct of transgenic avians in the past. See, for example, U.S. Pat. Nos. 6,875,588, issued Apr. 5, 2005; U.S. Pat. No. 7,176,300, issued Feb. 13, 2007; U.S. Pat. No. 7,199,279, issued Apr. 3, 2007; and US patent publication No. 2006/0130170, published Jun. 15, 2006 (the disclosures of each of these three issued patents and one published patent application are incorporated in their entirety herein by reference) which discloses the production of exogenous protein in the avian oviduct facilitated by various avian promoters which are primarily or exclusively expressed in the oviduct. Though expression levels in avians using the promoters and fragments of the promoters disclosed in these issued patents and published application have been at useful levels, the yeilds have typically been well below 0.1 mg/ml of egg white.

[0009] What is needed is a system that will provide for high level expression of an exogenous coding sequence in the cells of a transgenic avian, in particular, in the oviduct cells (e.g., tubular gland cells) of a transgenic avian.

SUMMARY OF THE INVENTION

[0010] The present invention meets this need and more. After years of exogenous protein production in transgenic avian oviduct tissue with modest yield the inventors of the present invention have discovered that such production levels can be boosted by about 10 fold to about 100 fold and more by employing new compositions and methods as disclosed herein.

[0011] In one aspect, the invention is directed to transgenic avians (e.g., chicken, turkey, quail) containing in their genome an exogenous nucleotide sequence which includes a promoter component and a SIN vector. Typically, the promoter component is linked to a coding sequence exogenous to the avian, i.e., the coding sequence is not normally or naturally present in the avian. Typically, the exogenous nucleotide sequence is integrated into the genome of the avian. In one particularly useful embodiment, the promoter component functions or expresses primarily in the oviduct (e.g., tubular gland cells) of an avian. For example, the promoter component may be an oviduct specific promoter. For example, the promoter component may be one of an avian ovomucoid promoter component, an avian ovalbumin promoter component, an avian lysozyme promoter compo-

nent and an avian ovoinhibitor promoter component (i.e., conalbumin promoter component).

[0012] SIN vectors have been shown by the inventors to be particularly useful for increasing the quantity of exogenous protein produced in the avian oviduct. This effect can be further enhanced when the SIN vector is also an SC negative vector (i.e., a vector not containing a selectable marker cassette with a functional promoter).

[0013] The invention also includes methods of making the transgenic avians of the invention and methods of producing an exogenous protein using transgenic avians of the invention. In one embodiment, the transgenic avian has a nucleotide sequence in its genome comprising a vector which is at least one of a SIN vector and an SC negative vector. Typically, the nucleotide sequence includes a promoter component linked to an exogenous coding sequence.

[0014] In one useful embodiment, the exogenous coding sequence is expressed in avian oviduct cells and is secreted from the oviduct cells. For example, the exogenous coding sequence may be expressed in tubular gland cells. In one embodiment, the exogenous protein is deposited in a hard shell egg laid by the transgenic avian. In one embodiment, the exogenous protein is a human protein. In one embodiment, the exogenous protein is a therapeutic protein, e.g., a cytokine.

[0015] In one embodiment, the transgenic avian contains an exogenous nucleotide sequence in its genome which has a SC negative vector and a promoter component linked to an exogenous coding sequence encoding an exogenous protein. In one embodiment, the SC negative vector is also a SIN vector.

[0016] In one aspect, avian leukosis virus vector (ALV), a murine leukemia virus (MLV) retroviral vector, moloney murine leukemia Virus (MMLV) and a lentiviral vector can be used in accordance with the invention.

[0017] The invention includes chimeric transgenic avians and fully transgenic germline avians which can be obtained from germline chimeras as is understood by a practitioner of skill in the art of poultry breeding.

[0018] The invention also includes gene expression controlling regions or promoters having a nucleotide sequence (i.e., DNA sequence) similar or identical to the following sequences numbered 1 to 8. In a particularly useful embodiment of the invention, the fragments are listed top to bottom in the 5' to 3' linear order in which they are present on a single DNA molecule. For example, the 3' end of the 3.5 kb OV fragment of sequence 1 would be covalently linked to the 5' end of the 5' UTR-5' portion and the 3' end of the 5' UTR-5' portion would be covalently linked to the 5' end of 5' UTR-3' portion. However, the invention is not limited to any particular order of the fragments and intervening nucleotide sequences may be present between the fragments.

[0019] 1. 3.5 kb OV fragment (includes DHS I, II & III)

[0020] 5' UTR-5' portion (from Exon L)

[0021] 5' UTR-3' portion (from Exon 1);

[0022] 2. 3.5 kb OV fragment (includes DHS I, II & III)

[0023] 5' UTR-5' portion (from Exon L)

[0024] Intron A

[0025] 5' UTR-3' portion (from Exon 1)

[0026] 3' UTR;

[0027] 3. 3.5 kb OV fragment (includes DHS I, II & III)

[0028] 5' UTR-5' portion (from Exon L)

[0029] Intron A

[0030] 5' UTR-3' portion (from Exon 1);

[0031] 4. 3.5 kb OV fragment (includes DHS I, II & III)

[0032] 5' UTR-5' portion (from Exon L)

[0033] 5' UTR-3' portion (from Exon 1)

[0034] 3' UTR;

[0035] 5. 3.5 kb OV fragment (includes DHS I, II & III)

[0036] 5' UTR-5' portion (from Exon L)

[0037] Intron A

[0038] 5' UTR-3' portion (from Exon 1)

[0039] 3' UTR/DHS A (bp 13576 to 15163 of SEQ ID NO: 22)

[0040] 6. 3.5 kb OV fragment (includes DHS I, II & III)

[0041] 5' UTR-5' portion (from Exon L)

[0042] 5' UTR-3' portion (from Exon 1)

[0043] 3' UTR/DHS A (bp 13576 to 15163 of SEQ ID NO: 22)

[0044] 7. 3.5 kb OV fragment (includes DHS I, II & III)

[0045] 5' UTR-5' portion (from Exon L)

[0046] Intron A

[0047] 5' UTR-3' portion (from Exon 1)

[0048] partial 3' UTR

[0049] RRE (Rev response element) FIG. **9***a*

[0050] 8. ALV CTE (FIG. **9***b*) inserted 5' of 3.5 kb OV fragment

[0051] 3.5 kb OV fragment (includes DHS I, II & III)

[0052] 5' UTR-5' portion (from Exon L)

[0053] Intron A

[0054] 5' UTR-3' portion (from Exon 1)

[0055] partial 3' UTR;

[0056] Coordinates of some of the elements for specific ovalbumin constructs disclosed herein (e.g., constructs 1 to 8 described above) are shown in the 16051 bp ovalbumin DNA segment of SEQ ID NO: 22 as follows:

[0057] 3.5 kb OV fragment (includes DHS I, II & III): Start: 3199 End: 6659 of FIG. **8** (SEQ ID NO: 22);

[0058] 1.4 kb OV fragment (includes DHS I & II): Start: 5209 End: 6659 of FIG. **8** (SEQ ID NO: 22);

[0059] 3.8 kb OV fragment: Start: 2863 End: 6659 of FIG. **8** (SEQ ID NO: 22);

[0060] 5.2 kb OV fragment: Start: 1463 End: 6659 of FIG. **8** (SEQ ID NO: 22);

[0061] 5' UTR-5' portion (from Exon L): Start: 6659 End: 6705 of FIG. **8** (SEQ ID NO: 22);

[0062] 5' UTR-3' portion (from Exon 1): Start: 8295 End: 8311 of FIG. **8** (SEQ ID NO: 22);

[0063] 3' UTR: Start: 13576 End: 14209 of FIG. **8** (SEQ ID NO: 22);

[0064] partial 3' UTR: Start 13576 End: 13996 of FIG. **8** (SEQ ID NO: 22);

[0065] Intron A: Start: 6706 End: 8294 of FIG. **8** (SEQ ID NO: 22);

[0066] Intron E: Start: 10010 End: 10968 of FIG. **8** (SEQ ID NO: 22);

[0067] Exon L: Start: 6659 End: 6705 of FIG. **8** (SEQ ID NO: 22);

[0068] Exon 1: Start: 8295 End: 8478 of FIG. **8** (SEQ ID NO: 22);

[0069] Exon 2: Start: 8731 End: 8781 of FIG. **8** (SEQ ID NO: 22);

[0070] Exon 3: Start: 9363 End: 9491 of FIG. **8** (SEQ ID NO: 22);

[0071] Exon 4: Start: 9892 End: 10009 of FIG. **8** (SEQ ID NO: 22);

[0072] Exon 5: Start: 10968 End: 11110 of FIG. **8** (SEQ ID NO: 22);

[0073] Exon 6: Start: 11442 End: 11597 of FIG. **8** (SEQ ID NO: 22);

[0074] Exon 7: Start: 13180 End: 13575 of FIG. **8** (SEQ ID NO: 22);

[0075] +1 SITE: Start: 6659 End: 6659 of FIG. **8** (SEQ ID NO: 22);

[0076] ATG: Start: 8312 End: 8312 of FIG. **8** (SEQ ID NO: 22);

[0077] Poly A: Start: 14204 End: 14209 of FIG. **8** (SEQ ID NO: 22);

[0078] TATA: Start: 6627 End: 6632 of FIG. **8** (SEQ ID NO: 22);.

[0079] DHS A: Start: 13858 End: 15163 of FIG. **8** (SEQ ID NO: 22);

[0080] DHS IV: Start: 459 End: 859 of FIG. **8** (SEQ ID NO: 22);

[0081] DHS III: Start: 3253 End: 3559 of FIG. **8** (SEQ ID NO: 22);

[0082] DHS II: Start: 5629 End: 6009 of FIG. **8** (SEQ ID NO: 22); and

[0083] DHS I: Start: 6359 End: 6659 of FIG. **8** (SEQ ID NO: 22).

[0084] Promoter constructs are also contemplated that have a nucleotide sequence 80% identical and 85% identical and 90% identical and 91% identical and 92% identical and 93% identical and 94% identical and 95% identical and 96% identical and 97% identical and 98% identical and 99% identical to each of the promoter constructs disclosed herein, such as those described above (i.e., 1 to 8 above).

[0085] The invention also contemplates promoter constructs which correspond to promoter constructs 1 through 8 above in which the 3.5 kb OV fragment is replaced with the 3.8 kb OV fragment. The invention also contemplates promoter constructs which correspond to promoter constructs 1 through 8 in which the 3.5 kb OV fragment is replaced with the 5.2 kb OV fragment.

[0086] Promoter constructs are also contemplated for each of the above specified recombinant promoters (i.e., 1 to 8) in which DHS III is omitted from the construct.

[0087] Promoter constructs are contemplated corresponding to each of constructs 2, 3, 5, 7 and 8 above in which Intron A is replaced with Intron E which may lead to increased levels of exogenous protein production. Intron A and E have DNA sequences that induce alignment of histones in surrounding DNA regions. Such alignment can provide for transcriptional regulation of the OV gene. Without wishing to be bound to any particular theory or mechanism of operation, substitution of Intron E with Intron A may provide a preferential spacing of histones that result from use of Intron E (i.e., the periodicity for Intron A is 202 bp±5 bp, for Intron E is 196 bp±5 bp). For example, it is believed that the packaging of DNA by histones leads to topological alteration of DNA the manipulation of which can lead to preferential alignment of binding sites for proteins responsible for the transcription regulation (e.g., transcription factors) leading to an enhanced level of transcription.

[0088] Also included in the invention are vector constructs, and other constructs and nucleotide sequences disclosed herein, having a nucleotide sequence 80% identical and 85% identical and 90% identical and 91% identical and 92% identical and 93% identical and 94% identical and 95% identical and 96% identical and 97% identical and 98% identical and 99% identical to each vector construct and other constructs and nucleotide sequences disclosed herein.

[0089] Any useful combination of features described herein is included within the scope of the present invention provided that the features included in any such combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art Additional objects and aspects of the present invention will become more apparent upon review of the detailed description set forth below when taken in conjunction with the accompanying figures, which are briefly described as follows.

BRIEF DESCRIPTION OF THE FIGURES

[0090] FIG. **1** shows a circular map of the pALV-SIN-4.2-Lys-IFNa-2B vector. The sequence of pALV-SIN-4.2-Lys-IFNa-2B is shown in SEQ ID NO: 1.

[0091] FIG. **2** is a bar graph illustrating expression levels of IFNa in the egg white of a transgenic quail. G0 quail was produced by injection of pALV-SIN-4.0-Lys-IFNa-2B retroviral vector transduction particles into Japanese quail embryos.

[0092] FIG. **3** shows a circular map of the pSIN-OV-3.5-I-CTLA4-inv vector. The nucleotide sequence of pSIN-OV-3.5-I-CTLA4-inv is shown in SEQ ID NO: 19.

[0093] FIG. **4** shows a circular map of the pSIN-3.9-OM-CTLA4-Fc vector. The nucleotide sequence of pSIN-3.9-OM-CTLA4-Fc is shown in SEQ ID NO: 20.

[0094] FIG. **5** shows a circular map of the pBS-OM-4.4 vector. The nucleotide sequence of pBS-OM-4.4 is shown in SEQ ID NO: 23.

[0095] FIG. **6** shows a circular map of the pAVIJCR-A137.91.1.2 vector. The nucleotide sequence of pAVIJCR-A137.91.1.2 is shown in SEQ ID NO: 24.

[0096] FIG. **7** shows a circular map of the pSIN-1.8-OM-IFNa-2B plasmid vector. The nucleotide sequence of pSIN-1.8-OM-IFNa-2B is shown in SEQ ID NO: 21.

[0097] FIG. **8***a-e* (SEQ ID NO: 22) shows a segment of a chicken ovalbumin gene.

[0098] FIG. **9***a* (SEQ ID NO: 25) shows the RRE (rev responsive element) sequence of a lenti virus. FIG. **9***b* (SEQ ID NO: 26) shows the ALV CTE (constitutive transport element) sequence.

[0099] FIG. **10***a* shows a diagram of the segment deleted from an exemplary retroviral LTR (ALV) to make a SIN vector. FIG. **10***b* (SEQ ID NO: 29) shows the sequence of the LTR shown in **10***a*. The underlined sequence is the deleted sequence.

DETAILED DESCRIPTION

Definitions

[0100] The term "animal" is used herein to include all vertebrate animals, including avians and may include humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages.

[0101] The term "antibody" as used herein refers to polyclonal and monoclonal antibodies and functional fragments thereof. An antibody includes modified or derivatised antibody variants that retain the ability to specifically bind an epitope. Antibodies are capable of selectively binding to a target antigen or epitope. Antibodies may include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized and other chimeric antibodies, single chain antibodies (scFvs), Fab fragments, $F(ab')_2$ fragments and disulfide-linked Fvs (sdFv) fragments.

[0102] The term "avian" as used herein refers to any species, subspecies or strain of organism of the taxonomic class ava, such as, but not limited to, such organisms as chicken, turkey, duck, goose, quail, pheasants, parrots, finches, hawks, crows and ratites including ostrich, emu and cassowary. The term includes the various known strains of Gallus gallus, or chickens, (for example, White Leghorn, Brown Leghorn, Barred-Rock, Sussex, New Hampshire, Rhode Island, Ausstralorp, Minorca, Amrox, California Gray, Italian Partridge-colored), as well as strains of turkeys, pheasants, quails, duck, ostriches and other poultry commonly bred in commercial quantities.

[0103] The phrase "based on" or "derived from" as in a retroviral vector being based on or derived from a particular retrovirus or based on a nucleotide sequence of a particular retrovirus mean that the genome of the retroviral vector contains a substantial portion of the nucleotide sequence of the genome of the particular retrovirus. The substantial portion may be a particular gene or nucleotide sequence such as the nucleotide sequence encoding the gag, pol and/or env proteins or other structural or functional nucleotide sequence of the virus genome such as sequences encoding the LTRs or may be substantially the complete retrovirus genome, for example, most (e.g., more than 60% or more than 70% or more than 80% or more than 90%) or all of the retrovirus genome, as will be apparent from the context in the specification as the knowledge of one skilled in the art. Examples of retroviral vectors that are based on or derived from a retrovirus are the NL retroviral vectors (e.g., NLB) which are based on the ALV retrovirus as disclosed in Cosset et al, Journal of Virology (1991) vol 65, p 3388-3394.

[0104] The term "coding sequence" and "coding region" as used herein refer to nucleotide sequences and nucleic acid sequences, including both RNA and DNA, that encode genetic information for the synthesis of an RNA, a protein, or any portion of an RNA or protein. Nucleotide sequences that are not naturally part of a particular organism's genome are referred to as "foreign nucleotide sequences,""heterologous nucleotide sequences" or "exogenous nucleotide sequences". "Heterologous proteins" are proteins encoded by foreign, heterologous or exogenous nucleotide sequences and therefore are often not naturally expressed in the cell. A nucleotide sequence that has been isolated and then reintroduced into the same type (e.g., same species) of organism is not considered to be a naturally occurring part of a particular organism's genome and is therefore considered exogenous or heterologous.

[0105] The term "construct" as used herein refers to a linear or circular nucleotide sequence such as DNA that has been assembled from more than one segments of nucleotide sequence which have been isolated from a natural source or have been chemically synthesized, or combinations thereof.

[0106] The term "complementary" as used herein refers to two nucleic acid molecules that can form specific interactions with one another. In the specific interactions, an adenine base within one strand of a nucleic acid can form two hydrogen bonds with thymine within a second nucleic acid strand when the two nucleic acid strands are in opposing polarities. Also in the specific interactions, a guanine base within one strand of a nucleic acid can form three hydrogen bonds with cytosine within a second nucleic acid strand when the two nucleic acid strands are in opposing polarities. Complementary nucleic acids as referred to herein, may further comprise modified bases wherein a modified adenine may form hydrogen bonds with a thymine or modified thymine, and a modified cytosine may form hydrogen bonds with a guanine or a modified guanine.

[0107] The term "cytokine" as used herein refers to any secreted amino acid sequence that affects the functions of cells and is a molecule that modulates interactions between cells in the immune, inflammatory or hematopoietic responses. A cytokine includes, but is not limited to, monokines and lymphokines regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a mononuclear cell, such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibro-

blasts, basophils, neutrophils, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes and B-lymphocytes. Lymphokines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include, but are not limited to, Interleukin-l (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-alpha) and Tumor Necrosis Factor beta (TNF-beta).

[0108] The term "expressed" or "expression" as used herein refers to the transcription from a gene to give an RNA nucleic acid molecule at least complementary in part to a region of one of the two nucleic acid strands of the gene. The term "expressed" or "expression" as used herein can also refer to the translation of RNA to produce a protein or peptide.

[0109] The term "expression vector" as used herein refers to a nucleic acid vector that comprises a gene expression controlling region, such as a promoter or promoter component, operably linked to a nucleotide sequence coding at least one polypeptide.

[0110] The term "fragment" as used herein can refer to, for example, an at least about 10, 20, 50, 75, 100, 150, 200, 250, 300, 500, 1000, 2000, 5000, 6,000, 8,000, 10,000, 20,000, 30,000, 40,000, 50,000 or 60,000 nucleotide long portion of a nucleic acid that has been constructed artificially (e.g., by chemical synthesis) or by cleaving a natural product into multiple pieces, using restriction endonucleases or mechanical shearing, or enzymatically, for example, by PCR or any other polymerizing technique known in the art, or expressed in a host cell by recombinant nucleic acid technology known to one of skill in the art. The term "fragment" as used herein may also refer to, for example, an at least about 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 400, 500, 1000, 2000, 5000, 6,000, 8,000 or 10,000 amino acid portion of an amino acid sequence, which portion is cleaved from a naturally occurring amino acid sequence by proteolytic cleavage by at least one protease, or is a portion of the naturally occurring amino acid sequence synthesized by chemical methods or using recombinant DNA technology (e.g., expressed from a portion of the nucleotide sequence encoding the naturally occurring amino acid sequence) known to one of skill in the art. "Fragment" may also refer to a portion, for example, of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80% about 90% about 95% or about 99% of a particular nucleotide sequence or amino acid sequence.

[0111] "Functional portion" or "functional fragment" are used interchangeably and as used herein means a portion or fragment of a whole capable of performing, in whole or in part, a function of the whole. For example, a biologically functional portion of a molecule means a portion of the molecule that performs a biological function of the whole or intact molecule. For example, a functional portion of a gene expression controlling region is a fragment or portion of the specified gene expression controlling region that, in whole or in part, regulates or controls gene expression (e.g., facilitates either in whole or in part) in a biological system (e.g., a promoter). Functional portions may be of any useful size. For example, a functional fragment may range in size from about 20 bases in length to a length equal to the entire length of the specified sequence minus one nucleotide. In another example, a functional fragment may range in size from about 50 bases in length to a length equal to the entire length of the specified sequence minus one nucleotide. In another example, a functional fragment may range in size from about 50 bases in length to about 20 kb in length. In another example, a functional fragment may range in size from about 500 bases in length to about 20 kb in length. In another example, a functional fragment may range in size from about 1 kb in length to about 20 kb in length. In another example, a functional fragment may range in size from about 0.1 kb in length to about 10 kb in length. In another example, a functional fragment may range in size from about 20 bases kb in length to about 10 kb in length.

[0112] The term "gene expression controlling region" as used herein refers to nucleotide sequences that are associated with a coding sequence and which regulate, in whole or in part, expression of the coding sequence, for example, regulate, in whole or in part, the transcription of the coding sequence. Gene expression controlling regions may be isolated from a naturally occurring source or may be chemically synthesized and can be incorporated into a nucleic acid vector to enable regulated transcription in appropriate cells. The "gene expression controlling regions" may precede, but is not limited to preceding, the region of a nucleic acid sequence that is in the region 5' of the end of a coding sequence that may be transcribed into mRNA.

[0113] The terms "heterologous", "exogenous" and "foreign" are used interchangeably herein and in general refer to a biomolecule such as a nucleic acid or a protein that is not normally found in a certain organism or in a certain cell, tissue or other component contained in or produced by an organism. For example, a protein that is heterologous or exogenous to an egg is a protein that is not normally found in the egg. As used herein, the terms "heterologous", "exogenous" and "foreign" with reference to nucleic acids, such as DNA and RNA, are used interchangeably and refer to nucleic acid that does not occur naturally as part of a chromosome, a genome or cell in which it is present or which is found in a location(s) and/or in amounts that differ from the location(s) and/or amounts in which it occurs in nature. It can be nucleic acid that is not endogenous to the genome, chromosome or cell and has been exogenously introduced into the genome, chromosome or cell. Examples of heterologous DNA include, but are not limited to, a DNA comprising a gene expression control region and DNA that encodes a product or products, for example, RNA or protein product. Examples of heterologous DNA include, but are not limited to, gene expression controlling regions or promoters disclosed herein once isolated from the avian and as used thereafter, e.g., after re-introduction into an avian genome.

[0114] The term "isolated nucleic acid" as used herein covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic molecule but is not flanked by at least one of the sequences that flank that part of the molecule in the genome of the species in which it naturally occurs; (b) a nucleic acid which has been incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting vector or genomic DNA is not identical to naturally occurring DNA from which the nucleic acid was obtained; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), ligase chain reaction (LCR) or chemical synthesis, or a restriction fragment; (d) a recombinant nucleotide sequence that is part of

a hybrid gene, i.e., a gene encoding a fusion protein, and (e) a recombinant nucleotide sequence that is part of a hybrid sequence that is not naturally occurring. Isolated nucleic acid molecules of the present invention can include, for example, natural allelic variants as well as nucleic acid molecules modified by nucleotide deletions, insertions, inversions, or substitutions.

[0115] The term "nucleic acid" as used herein refers to any linear or sequential array of nucleotides and nucleosides, for example cDNA, genomic DNA, mRNA, tRNA, oligonucleotides, oligonucleosides and derivatives thereof. For ease of discussion, non-naturally occurring nucleic acids may be referred to herein as constructs. Nucleic acids can include bacterial plasmid vectors including expression, cloning, cosmid and transformation vectors such as, animal viral vectors such as, but not limited to, modified adenovirus, influenza virus, polio virus, pox virus, retroviruses such as avian leukosis virus (ALV) retroviral vector, a murine leukemia virus (MLV) retroviral vector, and a lentivirus vector, and the like and fragments thereof. In addition, the nucleic acid can be an LTR of an avian leukosis virus (ALV) retroviral vector, a murine leukemia virus (MLV) retroviral vector, or a lentivirus vector and fragments thereof. Nuclic acids can also include NL vectors such as NLB, NLD and NLA and fragments thereof and synthetic oligonucleotides such as chemically synthesized DNA or RNA. Nucleic acids can include modified or derivatised nucleotides and nucleosides such as, but not limited to, halogenated nucleotides such as, but not only, 5-bromouracil, and derivatised nucleotides such as biotin-labeled nucleotides.

[0116] The term "vector" and "nucleic acid vector" as used herein refers to a natural or synthetic single or double stranded plasmid or viral nucleic acid molecule that can be transfected or transformed into cells and replicate independently of, or within, the host cell genome. A circular double stranded vector can be linearized by treatment with an appropriate restriction enzyme based on the nucleotide sequence of the vector. A nucleic acid can be inserted into a vector by cutting the vector with restriction enzymes and ligating the desired pieces together.

[0117] The term "operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Gene expression controlling regions or promoters (e.g., promoter components) operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The controlling sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present. between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0118] The term "oviduct specific promoter" as used herein refers to promoters and promoter components which are functional, i.e., provide for transcription of a coding sequence, to a large extent, for example, primarily (i.e., more than 50% of the transcription product produced in the animal by a particular promoter type being produced in oviduct cells) or exclusively in oviduct cells of a bird. Examples of oviduct specific promoters include, ovalbumin promoter, ovomucoid promoter, ovoinhibitor promoter, lysozyme promoter and ovotransferrin promoter and functional portions of these promoters, e.g., promoter components.

[0119] The terms "percent sequence identity""percent identity" as used in, for example, "% identical" and "percent sequence homology""percent homology", as used in, for example, "% homology" and "percent sequence similarity" each refer to the degree of sequence matching between two nucleic acid sequences or two amino acid sequences as determined using the algorithm of Karlin & Attschul (1990) Proc. Natl. Acad. Sci. 87: 2264-2268, modified as in Karlin & Attschul (1993) Proc. Natl. Acad. Sci. 90: 5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Attschul et al. (1990) T. Mol. Biol. Q15: 403-410. BLAST nucleotide searches are performed with the NBLAST program,. score=100, wordlength =12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences homologous to a reference amino acid sequence. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Attschul et al. (1997) Nucl. Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g. XBLAST and NBLAST) are used. Other algorithms, programs and default settings may also be suitable such as, but not only, the GCG-Sequence Analysis Package of the U.K. Human Genome Mapping Project Resource Centre that includes programs for nucleotide or amino acid sequence comparisons.

[0120] The terms "polynucleotide,""oligonucleotide", "nucleotide sequence" and "nucleic acid sequence" can be used interchangeably herein and include, but are not limited to, coding sequences, i.e., polynucleotide(s) or nucleic acid sequence(s) which are transcribed and translated into polypeptide in vitro or in vivo when placed under the control of appropriate regulatory or control sequences; controlling sequences, e.g., translational start and stop codons, promoter sequences, ribosome binding sites, polyadenylation signals, transcription factor binding sites, transcription termination sequences, upstream and downstream regulatory domains, enhancers, silencers, DNA sequences to which a transcription factor(s) binds and alters the activity of a gene's promoter either positively (induction) or negatively (repression) and the like. No limitation as to length or to synthetic origin are suggested by the terms described herein.

[0121] As used herein the terms "polypeptide" and "protein" refer to a polymer of amino acids of three or more amino acids in a serial array, linked through peptide bonds. The term "polypeptide" includes proteins, protein fragments, protein analogues, oligopeptides and the like. The term "polypeptides" includes polypeptides as defined above that are encoded by nucleic acids, produced through recombinant technology (e.g., isolated from a transgenic bird), or synthesized. The term "polypeptides" further contemplates polypeptides as defined above that include chemically modified amino acids or amino acids covalently or noncovalently linked to labeling ligands.

[0122] The term "promoter" as used herein refers to a DNA sequence useful to initiate transcription initiation by an RNA polymerase in an avian cell. A "promoter component" is a DNA sequence that can, by itself or, in combination with

other DNA sequences effect or facilitate transcription. Specific promoter components such as ovalbumin promoter components, ovomucoid promoter components and lysozyme promoter components and other promoters and promoter components disclosed and claimed herein do not describe a specific promoter sequence. Rather, they encompass any sequence or sequence fragment of the respective promoter that is useful to effect or facilitate transcription of a coding sequence. For example, an ovomucoid promoter component includes, without limitation, the about 1.8 kb, the about 3.9 kb and the about 10 kb ovomucoid promoters disclosed in U.S. Publication No. 11/649,543, published May 17 2007, which is incorporated in its entirety herein by reference. "Promoter components" can also encompass rearranged gene expression controlling regions which function to initiate RNA transcription and hybrid DNA molecules composed of naturally occurring DNA sequences and/or synthetic DNA sequences which function to initiate RNA transcription.

[0123] The terms "recombinant nucleic acid" and "recombinant DNA" as used herein refer to combinations of at least two nucleic acid sequences that are not naturally found in a eukaryotic or prokaryotic cell. The nucleic acid sequences may include, but are not limited to, nucleic acid vectors, gene expression regulatory elements, origins of replication, suitable gene sequences that when expressed confer antibiotic resistance, protein-encoding sequences and the like. The term "recombinant polypeptide" is meant to include a polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location, purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature.

[0124] As used herein, the term "regulatory sequences" includes promoters, enhancers, and other elements that may control gene expression.

[0125] An "SC negative vector" is a vector that does not contain a selectable or screenable cassette marker having a functional promoter. The promoter may be deleted in whole or in part or may be inactivated by a nucleotide sequence insertion. Screenable cassettes include, without limitation, DNA sequences for antibiotic resistance markers such as neomycin resistance and DNA sequences for other selectable markers such as GFP or lacZ.

[0126] A "SIN vector" is a self-inactivating vector. In particular, a SIN vector is a retroviral vector having an altered genome such that upon integration into genomic DNA of the target cell (e.g., avian embryo cells) the 5' LTR of the integrated retroviral vector will not function as a promoter. For example, a portion or all of the nucleotide sequence of the retroviral vector that results in the U3 region of the 5' LTR of the retroviral vector once integrated may be deleted or altered in order to reduce or eliminate promoter activity of the 5' LTR. In certain examples, deletion of the CAAT box and/or the TAATA box from U3 of the 5' LTR can result in a SIN vector, as is understood in the art.

[0127] A "SIN/SC negative vector" is a vector, i.e., a retroviral vector, that is both a SIN vector and a SC negative vector.

[0128] The term "sense strand" as used herein refers to a single stranded DNA, molecule from a genomic DNA that may be transcribed into RNA and translated into the natural polypeptide product of the gene. The term "antisense strand" as used herein refers to the single strand DNA molecule of a genomic DNA that is complementary with the sense strand of the gene.

[0129] A "therapeutic protein" or "pharmaceutical protein" is a substance that, in whole or in part, makes up a drug. In particular, "therapeutic proteins" and "pharmaceutical proteins" include an amino acid sequence which in whole or in part makes up a drug.

[0130] The terms "transcription regulatory sequences" and "gene expression control regions" and "promoter components" as used herein refer to nucleotide sequences that are associated with a nucleic acid sequence and which regulate the transcriptional expression of a coding sequence. Exemplary transcription regulatory sequences include enhancer elements, hormone response elements, steroid response elements, negative regulatory elements, and the like. The "transcription regulatory sequences" may be isolated and incorporated into a vector nucleic acid to enable regulated transcription in appropriate cells of portions of the vector DNA. The "transcription regulatory sequence" may precede, but is not limited to, the region of a nucleic acid sequence that is in the region 5' of the end of a protein coding sequence that may be transcribed into mRNA. Transcriptional regulatory sequences may also be located within a protein coding region, in regions of a gene that are identified as "intron" regions, or may be in regions of nucleic acid sequence that are in the region of nucleic acid.

[0131] The terms "transformation" and "transfection" as used herein refer to the process of inserting a nucleic acid into a host. Many techniques are well known to those skilled in the art to facilitate transformation or transfection of a nucleic acid into a prokaryotic or eukaryotic organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt such as, but not only a calcium or magnesium salt, an electric field, detergent, or liposome mediated transfection, to render the host cell competent for the uptake of the nucleic acid molecules.

[0132] As used herein, a "transgenic animal" is any non-human animal, such as an avian species, including the chicken, in which one or more of the cells of the avian may contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques known in the art (see, for example, US patent publication No. 2007/0243165, published Oct. 18, 2007, the disclosure of which is incorporated in its entirety herein by reference) including those disclosed herein. The nucleic acid is introduced into an animal, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animal, the transgene can cause cells to express a recombinant form of the target protein or polypeptide. The terms "chimeric animal" or "mosaic animal" are used herein to refer to animals in which a transgene is found, or in which the recombinant nucleotide sequence

is expressed in some but not all cells of the animal. A germ-line chimeric animal contains a transgene in its germ cells and can give rise to a transgenic animal in which most or all cells of the offspring animal will contain the transgene.

[0133] As used herein, the term "transgene" means a nucleic acid sequence (encoding, for example, a human protein) that is partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene according to the present invention can include a vector of the invention (e.g., SIN vector) which contains sequences useful for exogenous protein production in an avian (e.g., in an avian oviduct).

[0134] Techniques useful for isolating and characterizing the nucleic acids and proteins of the present invention are well known to those of skill in the art and standard molecular biology and biochemical manuals may be consulted to select suitable protocols for use without undue experimentation. See, for example, Sambrook et al, 1989, "Molecular Cloning: A Laboratory Manual", 2nd ed., Cold Spring Harbor, the content of which is herein incorporated by reference in its entirety.

Abbreviations:

[0135] Abbreviations used herein may include the following: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to an RNA; nt, nucleotide(s); kb, 1000 base pairs; μg, microgram; ml, milliliter; ng, nanogram.

Description:

[0136] SIN vectors designed and used in accordance with the invention can reduce or eliminate promoter interference of promoters of interest which are employed in transgenic avians. In a particularly useful embodiment, the promoters (i.e., promoter components) of interest preferentially express their gene product in oviduct cells or oviduct tissue, e.g., oviduct specific promoters. Examples of such promoters (e.g., promoter components) include but are not limited to, functional portions of the ovalbumin, lysozyme, conalbumin (i.e., ovotransferrin), ovomucoid, ovomucin, and/or ovoinhibitor gene expression controlling regions or promoter regions. In one embodiment, the promoter of interest is a combination or a fusion of one or more promoters or a fusion of a fragment of one or more promoters such as ovalbumin, lysozyme, conalbumin (i.e., ovotransferrin), ovomucoid, ovomucin, and/or ovoinhibitor promoters with another promoter or promoter fragment such as a viral promoter (e.g., an LTR promoter).

[0137] SIN vectors have been shown to be particularly useful with oviduct specific promoters. Without wishing to limit the invention to any particular theory or mechanism of operation it is believed that oviduct specific promoters can be particularly susceptible to influences of a retroviral LTR promoter. As a result, SIN vectors are particularly useful when employed in combination with avian oviduct specific promoters.

[0138] In one particularly useful embodiment, a SIN vector is produced in which an interfering promoter (e.g., an LTR promoter) that can at least partially inhibit transcription of a coding sequence operably linked to an oviduct specific promoter of the invention is inactivated, for example, by a deletion, insertion or transposition of all or part of the interfering promoter sequence. For example, the vector pALV-SIN-4.2-Lys-IFNa-2B, shown in FIG. **1**, the 3' RAV2 LTR has a deletion in the enhancer such that when the retroviral region integrates, the 5' LTR is inactivated, as is understood in the art. For a detailed diagrammatic of an LTR deletion, see FIG. **10**.

[0139] In one useful embodiment of the invention, a SIN vector is employed that is also an SC negative vector to produce a SIN/SC negative vector. The combination of SC negative vector and SIN vector can result in a vector with a substantially reduced amount of promoter interference compared to a vector that is only a SIN vector or only a SC negative vector. For example, pALV-SIN-4.2-Lys-IFNa-2B as well as other SIN vectors disclosed in the Examples also lacks an antibiotic resistance marker making it both a SC negative vector and a SIN vector.

[0140] SIN vectors, SC negative vectors and SIN/SC negative vectors are contemplated for use in accordance with the invention in any useful avian such as chicken, quail and turkey to produce chimeras including germ-line chimeras and progeny birds produced using breeding techniques such as those known to practitioners of ordinary skill in the art. In addition, it is contemplated that an SC negative retroviral vector (which is a non-SIN vector) will also enhance or increase the quantity of exogenous protein produced in a transgenic avian relative to a transgenic avian produced with essentially the same retroviral vector that is not a SC negative vector.

[0141] Without wishing to limit the invention to any particular theory or mechanism of operation it is believed that the lack of a selectable marker cassette decreases the presence of promoter elements such as enhancers which would otherwise be in cis and in close proximity to the promoter employed for exogenous protein production in avian oviduct cells (e.g., oviduct specific promoters). This close proximity may allow for interference by the transcription regulating elements of the marker gene with the promoter of interest, i.e., the promoter employed for exogenous protein production. However, the invention contemplates that marker gene coding sequences, for example, and without limitation, neomycin resistance coding sequence and beta lactamase coding sequence, may be operably linked to a promoter (i.e., second promoter) which does not interfere with the promoter employed for exogenous protein production in avian oviduct cells (i.e., first promoter). For example, it is contemplated that if the marker promoter and the promoter of interest are the same or similar promoters, interference by the selectable cassette will be minimized or eliminated. For example, a second ovalbumin promoter operably linked to a marker gene coding sequence may not interfere with a first ovalbumin promoter employed for exogenous protein production in avian oviduct cells.

[0142] The invention contemplates the employment of any useful oviduct specific promoter, and oviduct specific promoter fragments, in vectors of the invention for exogenous protein expression in avians. For example, promoters and useful (e.g., functional) fragments of promoters (e.g., promoter components) disclosed in US patent publication No.

2005/0176047, filed Jan. 31, 2005, the disclosure of which is incorporated in its entirety herein by reference, and US patent publication No. 2007/0124829, filed Jan. 26, 2007, the disclosure of which is incorporated in its entirety herein by reference, and US patent publication No. 2006/0130170, filed Dec. 11, 2003, the disclosure of which is incorporated in its entirety herein by reference, are contemplated for use in conjunction with SIN vectors and SC negative vectors and SIN/SC negative vectors in accordance with the invention.

[0143] The invention also contemplates other promoters and transcriptionally functional portions thereof (e.g., promoter components) for use as promoters of interest in accordance with the invention such as a cytomegalovirus (CMV) promoter, a rous-sarcoma virus (RSV) promoter, a β-actin promoter (e.g., a chicken β-actin promoter) a murine leukemia virus (MLV) promoter, a mouse mammary tumor virus (MMTV) promoter.

[0144] The invention also includes various ovalbumin promoter components which are contemplated for use in producing exogenous proteins in transgenic avians. Each of the promoters disclosed herein are contemplated for use in vectors in accordance with the invention.

[0145] Examples of vectors of the invention which contain recombinant ovalbumin DNA are shown below. The fragments are listed top to bottom in the 5' to 3' linear order in which they are present on a single DNA molecule. For example, the 3' end of the 3.5 kb OV fragment of sequence 1 would be covalently linked to the 5' end of the 5' UTR-5' portion and the 3' end of the 5' UTR-5' portion would be covalently linked to the 5' end of 5' UTR-3' portion.

[0146] 1. pSIN-OV-3.5-CSI

[0147] 3.5 kb OV fragment (includes DHS I, II & III)

[0148] 5' UTR-5' portion (from Exon L)

[0149] 5' UTR-3' portion (from Exon 1)

[0150] 2. pSIN-OV-3.5-Int-CSI-inv

[0151] 3.5 kb OV fragment (includes DHS I, II & III)

[0152] 5' UTR-5' portion (from Exon L)

[0153] Intron A

[0154] 5' UTR-3' portion (from Exon 1)

[0155] 3' UTR

[0156] 3. pSIN-OV-3.5-Int-CSI

[0157] 3.5 kb OV fragment (includes DHS I, II & III)

[0158] 5' UTR-5' portion (from Exon L)

[0159] Intron A

[0160] 5' UTR-3' portion (from Exon 1)

[0161] 4. pSIN-OV-3.5-CSI-UTR-inv

[0162] 3.5 kb OV fragment (includes DHS I, II & III)

[0163] 5' UTR-5' portion (from Exon L)

[0164] 5' UTR-3' portion (from Exon 1)

[0165] 3' UTR

[0166] 5. pSIN-OV-3.5-Int-CSI-LUTR-inv

[0167] 3.5 kb OV fragment (includes DHS I, II & III)

[0168] 5' UTR-5' portion (from Exon L)

[0169] Intron A

[0170] 5' UTR-3' portion (from Exon 1)

[0171] 3' UTR/DHS A (bp 13576 to 15163 of FIG. **8**);

[0172] 6. pSIN-OV-3.5-CSI-LUTR-inv

[0173] 3.5 kb OV fragment (includes DHS I, II & III)

[0174] 5' UTR-5' portion (from Exon L)

[0175] 5' UTR-3' portion (from Exon 1)

[0176] 3' UTR/DHS A (bp 13576 to 15163 of FIG. **8**);

[0177] 7. pSIN-OV-3.5-Int-CSI-RRE

[0178] 3.5 kb OV fragment (includes DHS I, II & III)

[0179] 5' UTR-5' portion (from Exon L)

[0180] Intron A

[0181] 5' UTR-3' portion (from Exon 1)

[0182] partial 3' UTR

[0183] RRE (Rev response element) FIG. **9***a*

[0184] Construct 7 includes RRE to allow transport of the unspliced RNA genome to the cytoplasm and thus may enhance packaging of intact retroviral RNA. RRE is only active in presence of the Rev protein. Rev activity is provided in the form of DNA encoding the Rev, RNA encoding the Rev, and/or the Rev protein, which is well known in the art and commercially available (e.g., Invitrogen, Inc.), during the transient transfection of retroviral components. Thus the intron will be present in the transgene contained in the genome of the transgenic bird produced by the virus particles (the rev protein is not present in the cells of the transgenic bird). As a result the RNA should be spliced in the oviduct cells of a laying hen resulting in an enhanced level of protein expression compared to a same transgenic bird having the same transgene without the intron.

[0185] 8. pSIN-CTE-OV-3.5-Int-CSI

[0186] ALV CTE (FIG. **9***b*_k) inserted 5' of 3.5 kb OV fragment

[0187] 3.5 kb OV fragment (includes DHS I, II & III)

[0188] 5' UTR-5' portion (from Exon L)

[0189] Intron A

[0190] 5' UTR-3' portion (from Exon 1)

[0191] partial 3' UTR

[0192] Coordinates for some of the elements for the above eight vectors are described elsewhere in the application. For example, coordinates of sequences from the ovalbumin nucleotide sequence are described in the Summary section above. CSI means a coding sequence of interest, i.e., nucleotide sequence encoding the protein desired to be expressed in a transgenic avian oviduct.

[0193] SIN vectors, SIN/SC negative vectors and SC negative vectors for use in accordance with the invention include vectors such as Avian Leukemia/Leukosis Viruses

(ALV), for example, and without limitation, RAV-0, RAV-1, RAV-2; Avian Sarcoma Viruses (ASV); Avian Sarcoma/Acute Leukemia Viruses (ASLV) including, without limitation, Rous Sarcoma Virus (RSV); Fujinami Sarcoma Viruses (FSV); Avian Myeloblastosis Viruses (AMV); Avian Erythroblastosis Viruses (AEV); Avian Myelocytomatosis Viruses (MCV), for example, and without limitation, MC29; Reticuloendotheliosis Viruses (REV), for example, and without limitation, Spleen Necrosis Virus (SNV). The invention also contemplates other useful retroviral vector, including, without limitation, retroviral vectors based upon Murine Leukemia Viruses (MLV); Molony Murine Sarcoma Viruses (MMSV); Moloney Murine Leukemia Viruses (MMLV); and lentiviruses (e.g., human immunodeficiency virus (HIV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV) and simian immunodeficiency virus (SIV) which are altered to be SIN vectors, SIN/SC negative vectors or SC negative vectors as is understood by a practitioner of ordinary skill in the art.

[0194] In one very specific embodiment, a portion of the 5' LTR of a modified ALV vector disclosed in Cosset et al, J of Virology (1991) vol 65, no. 6, p 3388-3394, the disclosure of which is incorporated in its entirety herein by reference, is deleted to produce a SIN vector. In particular, nucleotides 1 to 173 were deleted from the ALV based vector LTR sequence shown in SEQ ID NO: 29. Specific deletions from 5' LTR sequences useful to produce SIN vectors from other vectors which can be used in avian transgenesis can be determined by a practitioner of ordinary skill in the art.

[0195] In one particularly useful embodiment, the invention is drawn to the production of therapeutic proteins which may be produced in the oviduct of a transgenic avian, such as a chicken, in accordance with the invention. Exemplary proteins for production in accordance with the invention include, without limitation, erythropoietin, GM-CSF, interferon β, fusion protein, CTLA4-Fc fusion protein, growth hormones, cytokines, structural proteins, interferon, lysozyme, β-casein, albumin, α-1 antitrypsin, antithrombin III, collagen, factors VIII, IX, X (and the like), fibrinogen, lactoferrin, protein C, tissue-type plasminogen activator (tPA), somatotropin, and chymotrypsin, immunoglobulins, antibodies, immunotoxins, factor VIII, b-domain deleted factor VIII, factor VIIa, factor IX, anticoagulants; hirudin, alteplase, tpa, reteplase, tpa, tpa—3 of 5 domains deleted, insulin, insulin lispro, insulin aspart, insulin glargine, long-acting insulin analogs, glucagons, tsh, follitropin-beta, fsh, pdgh, inf-beta, inf-alpha 1, ifn-alpha 2, inf-beta, inf-beta 1b, ifn-beta 1a, ifn-gamma, ifn-gamma 1b, il-2, il-1 1, hbsag, ospa, dornase-alpha dnase, beta glucocerebrosidase, tnf-alpha, il-2-diptheria toxin fusion protein, tnfr-lgg fragment fusion protein laronidase, dnaases, alefacept, tositumomab, murine mab, alemtuzumab, rasburicase, agalsidase beta, teriparatide, parathyroid hormone derivatives, adalimumab (lgg1), anakinra, nesiritide, human b-type natriuretic peptide (hbnp), colony stimulating factors, pegvisomant, human growth hormone receptor antagonist, recombinant activated protein c, omalizumab, immunoglobulin e (lge) blocker, lbritumomab tiuxetan, ACTH, glucagon, somatostatin, somatotropin, thymosin, parathyroid hormone, pigmentary hormones, somatomedin, luteinizing hormone, chorionic gonadotropin, hypothalmic releasing factors, etanercept, antidiuretic hormones, prolactin and thyroid stimulating hormone, an immunoglobulin polypeptide, immunoglobulin polypeptide D region, immunoglobulin polypeptide J region, immunoglobulin polypeptide C region, immunoglobulin light chain, immunoglobulin heavy chain, an immunoglobulin heavy chain variable region, an immunoglobulin light chain variable region and a linker peptide. Production of each of these, and other, proteins is contemplated using methods, vectors and promoters of the invention.

[0196] The present invention is further illustrated by the following examples, which are provided by way of illustration and should not be construed as limiting. The contents of all references, published patents and patents cited throughout the present application are hereby incorporated by reference in their entireties.

EXAMPLE 1

Production of pALV-SIN-4.2-Lys-IFNa-2B

[0197] The vector pALV-SIN-4.2-Lys-IFNa-2B (shown in FIG. **1**) was constructed and is shown in FIG. **1**. The sequence of pALV-SIN-4.2-Lys-IFNa-2B is shown in SEQ ID NO: 1. The 4.2 Kb lysozyme promoter spans from nucleotides 4810 to 9008 of SEQ ID NO: 1. The lysozyme signal peptide coding sequence spans from nucleotides 9037 to 9090 of SEQ ID NO: 1. The interferon alpha 2b coding sequence spans from nucleotides 9091 to 9585 of SEQ ID NO: 1. Other components of the sequence include LTRs spanning from nucleotides 4000 to 4345 and from nucleotides 725 to 897 of SEQ ID NO: 1.

[0198] pALV-SIN-4.2-Lys-IFNa-2B can be constructed by a variety of methods which are apparent to a practitioner of skill in the art. However, the method believed to be the most useful for making the vector is as follows: A 3427 bp region of pNLB-CMV-IFN-alpha2B (disclosed in U.S. patent application Ser. No. 11/167,052, filed Jun. 24, 2005, the disclosure of which is incorporated in its entirety herein by reference) is PCR amplified using primers ATGCGCGCATTGGTAATTGATCGGCTGG (Primer ALV-SIN-1, SEQ ID NO: 2) and ATATGCGGCCGCGGTACCGCCCGGGCATCGATATCAAGCTTACGGTTCACT A AACGAGCTCTGCTTATATAGACCTCCCA (Primer ALV-SIN-2, SEQ ID NO: 3). The product is digested with BssHII and Not I resulting in a 3428 bp fragment which can be isolated by gel purification. A 1436 bp region of pNLB-CMV-IFN-alpha2B is PCR amplified with primers ATATGCGGCCGCGTCGACGGCCGGCCAGATCTGCT-GAGCCGGTCGCTACCA TTACCAGT (Primer ALV-SIN-3, SEQ ID NO: 4) and ATACGCGTATTCCCTAACGATCACGTCG (Primer ALV-SIN-4, SEQ ID NO: 5). The resulting product is digested with Not I and Mlu I yielding a 1438 bp fragment which is isolated by gel purification. A Bluescript II SK vector containing a BssHII stuffer fragment is digested with BssHII resulting in a linearized Bluescript vector of 2788 bp which is gel purified and then ligated to the 3428 bp and 1438 bp PCR products to yield JCR.A108.49.5.24.

[0199] JCR.A108.49.5.24 is digested with Hind III and the resulting 6823 bp fragment is circularized by ligation to yield JCR.A108.76.1.1.

[0200] A 1175 bp region of JCR.A108.76.1.1 is PCR amplified with primers CTGAAGTGTAAGGAATGTAAG (Primer ALV-SIN-5, SEQ ID NO: 6) and GCGCGTCTCATCCCCCTCCCTATGCAAAAG (Primer ALV-SIN-6, SEQ ID NO: 7) and the resulting fragment is digested with

Blp I and Esp3I producing a 1030 bp fragment which is isolated by gel purification. A 660 bp region of JCR.A108.76.1.1 is PCR amplified with primers GGGCGTCTCAGGGACGGATTGGACGAAC-CACTGAATT (Primer ALV-SIN-7, SEQ ID NO: 8) and TTAGTGCTTTACGGCACCTC (Primer ALV-SIN-8, SEQ ID NO: 9) and digested with Esp3I and DraIII resulting in a 596 bp fragment which is isolated by gel purification. JCR.A108.76.1.1 is digested with DraIII and Blp I and the 5024 bp linear vector is ligated to the 1030 and 596 bp PCR fragments to produce pALV-SIN.

[0201] pALV-SIN is digested with BamHI and the 4795 bp linear vector is isolated by gel purification. A 4815 bp region of JCR.115.93.1.2 (disclosed in US patent application No. 2007/0124829, filed Jan. 26, 2007,) is PCR amplified with primers GACGGATCCGATACCGTC-CCTATTTTTGTGTTTGCTTC (Primer ALV-SIN-9, SEQ ID NO: 10) and TAACGGATCCTAGACTTTTTACTCCT-TAGA (Primer ALV-SIN-10, SEQ ID NO: 11) and is digested with BamHI. The resulting 4802 fragment is ligated to the 4795 bp linear pALV-SIN to produce pALV-SIN-4.0-Lys-IFNa-2B.

EXAMPLE 2

Production of Transgenic Ouail Using pALV-SIN-4.2-Lys-IFNa-2B

[0202] Transduction particles of the vector pALV-SIN-4.2-Lys-IFNa-2B were produced in fibroblast cells as disclosed in US patent publication No. 2007/0077650, published Apr. 5, 2007, entitled: Rapid Production of High Titer Virus, the disclosure of which is incorporated in its entirety herein by reference.

[0203] Fertilized Japanese quail eggs were windowed essentially according to the Speksnijder procedure disclosed in U.S. Pat. No. 5,897,998, the disclosure of which is incorporated in its entirety herein by reference. Eighty eggs were injected in the subgerminal cavity with about 7 microliters (approximately 7×10^4 viral particles total) of pALV-SIN-4.2-Lys-IFNa-2B transducing particles per egg. Since no selectable marker is used in pALV-SIN-4.2-Lys-IFNa-2B, the concentration of viral particles is estimated based upon past results for viral particle production where a selectable cassette or marker was used in the vector which allowed for particle quantification. Sixteen chicks hatched about 18 days after injection and human IFN levels were measured by IFN ELISA from serum samples collected from chicks 12 weeks after hatch. None were positive for the IFN protein in the serum.

[0204] In order to identify G0 quail which contained the interferon alpha 2 coding sequence containing transgene in their genome, DNA was extracted from blood of the birds and the DNA samples were subjected to Taqman® analysis on a 7700 Sequence Detector (Perkin Elmer).

[0205] Eggs from eight G0 quail were tested for the presence of the IFN protein in the egg white by ELISA. Quail No. 4 was found to have significant levels of IFN in egg white from her eggs. FIG. **2** shows a bar graph illustrating expression levels of IFN in the egg white of Quail No. 4. Quail No. 4 expressed IFN-alpha-2 at 0.45 μg/ml of egg white, which is a high level of expression for a G0 avian. There was no interferon alpha 2 detected in the blood of Quail No. 4 which is particularly significant. For example, in certain instances the recombinant protein may be harmful to the development or health of the avian when present in the blood which can kill the bird or can lead to reduced levels of protein production.

EXAMPLE 3

Production of Transgenic Ouail Using pALV-SIN-6.5-Lys-IFNa-2B

[0206] The 4.2 kb lysozyme promoter of vector pALV-SIN-4.2-Lys-IFNa-2B is removed and replaced with a 6.5 kb lysozyme promoter corresponding to about nucleotides 5363 to 11863 of SEQ ID NO: 12, using standard methodologies known to practitioners of skill in the art, resulting in pALV-SIN-6.5-Lys-IFNa-2B. Transduction particles of the new vector pALV-SIN-6.5-Lys-IFNa-2B are produced as disclosed in US patent publication No. 2007/0077650, published Apr. 5, 2007.

[0207] Fertilized chicken eggs or Japanese quail eggs are windowed and about 7×10^4 pALV-SIN-6.5-Lys-IFNa-2B transducing particles are injected into the subgerminal cavity of each egg. Eggs hatch 21 or 18 days after injection and chimeric birds are identified that contain the active transgene in their genome, as described in Example 2. Fully transgenic G1 birds which contain the transgene in their genome are produced from chimeras using methods known in the art, i.e., crossing male chimeras with non-transgenic females.

EXAMPLE 4

Production of Vector pSIN-OV-3.5-I-CTLA4-Fc-Inv

[0208] This vector includes the ovalbumin Dnase hypersensitive sites (DHS) I, II and III, the first exon (exon L), the first intron and the CTLA4-Fc fusion protein coding sequence inserted in frame with the ATG of second exon (exon 1) and with the 3' untranslated region (UTR). The expression cassette is inserted in the inverse orientation into an avian leukosis virus (ALV) vector, which was made self-inactivating (SIN) by deletion of nucleotides 1 to 173 of the ALV LTR sequence shown in SEQ ID NO: 29.

[0209] The vector was constructed as follows: pNLB-3.9-OM-CTLA4-Fc, disclosed in Example 20 of US patent publication No. 2007/0113299, published May 17, 2007, the disclosure of which is incorporated in its entirety herein by reference, was cut with Nae I and Not I. The Not I site was filled in by Klenow reaction. The resulting 8125 bp fragment was gel purified, religated, producing pOM-3.9-CTLA4-dSacl. pOM-3.9-CTLA4-dSacl was cut with EcoRI and Kpn I and the 8115 bp fragment gel purified. The 3' UTR of the chicken ovalbumin gene was PCRed from BAC 26, disclosed in US patent publication No. 2006/0130170, published Jun. 15, 2006, with the primers 5'-GCGGAAT-TCAAAGAAGAAAGCTGAAAAAC-3' (SEQ ID NO: 13) and 5'-GCGGGTACCTTCAAATACTACAAGTGAAA-3' (SEQ ID NO: 14). The 3' UTR PCR was cut with Eco RI and Kpn I and the 684 bp fragment gel purified. The 8115 bp fragment of pOM-3.9-CTLA4-dSacl was ligated to the 684 bp fragment of 3' UTR PCR, producing pOM-3.9-CTLA4-OV3' UTR.

[0210] The 3.5 kb OV promoter region, exon L, first intron and the UTR of exon 1 was PCR amplified with BAC26 as

a template and with primers 5'-GGCCTCGAGTCAAGT-TCTGAGTAGGTTTTAGTG-3' (SEQ ID NO: 15) and 5'-GCGCGTCTCTGTCTAGAGCAAACAGCA-GAACAGTGAAAATG-3' (SEQ ID NO: 16). The PCR product was cut with Xho I and Esp3I and the 5094 bp product was gel purified.

[0211] A 5' portion of the CTLA4-Fc gene was PCR amplified using pOM-3.9-CTLA4 as a template and primers 5'-GCGCGTCTCAAGACAACTCAGAGTTCAC-CATGGGTGTACTGCTCACACAG-3' (SEQ ID NO: 17) and 5'-GGCCCGGGAGTTTTGTCAGAAGATTTGGG-3' (SEQ ID NO: 18). The PCR product was cut with Esp3I and SacI and the 384 bp product gel purified.

[0212] pOM-3.9-CTLA4-OV3' UTR was cut with Sac I and Xho I, the 4473 bp product gel purified and ligated to the 5094 bp OV PCR fragment and 384 bp CTLA4-Fc fragment, producing pOV-3.5-I-CTLA4.

[0213] pALV-SIN, disclosed, for example, in Example 10 of parent case US patent publication No. 2007/0124829, published May 31, 2007, was cut with Mfe I and Xho I, filled in with Klenow and the 4911 bp fragment gel purified.

[0214] pOV-3.5-I-CTLA4 was cut with XhoI and BamHI, filled in with Klenow and the 6957 bp fragment gel purified. This fragment was ligated into the 4911 bp fragment of pAVI-SIN such that the CTLA4-Fc gene and flanking expression elements are in the opposite orientation of the ALV long terminal repeats, producing pSIN-OV-3.5-I-CTLA4-inv. See FIG. **3** and SEQ ID NO: 19. Such opposite orientation may be preferred if the coding sequence of interest (i.e., CSI) in the transgene contains one or more introns or splice sites.

EXAMPLE 5

Production Of Transgenic Ouail Using SIN-OV-3.5-I-CTLA4-inv

[0215] Retroviral particles containing the pSIN-OV-3.5-I-CTLA4-inv vector (FIG. **3**) and pseudotyped with the VSV envelope protein were produced as described in US patent publication No.2007/0077650, published Apr. 5, 2007. Virus particles were harvested at 48 hours post-transfection, concentrated and on the same day, approximately 4 microliters of the virus suspension containing about 1×10^5 particles was injected into the subgerminal cavity of stage X quail eggs. Eggs were resealed and hatched.

[0216] ALV has a CTE element in the 3' end of its genome that allows transport of unspliced retroviral RNA to the cytoplasm. In pSIN-OV-3.5-I-CTLA4-inv, due to the inverse orientation of the OV promoter relative to the LTRs, the CTE is upstream of the OV promoter such that the CTE element is only in RNAs derived from the 5' LTR promoter and not in RNAs transcribed by the OV promoter. Therefore, any RNA transcribed by the OV promoter should be spliced prior to being transported into the cytoplasm.

[0217] Egg whites from chimeric quail were assayed using an ELISA for CTLA4-Fc. One quail was found to have CTLA4-Fc in her egg white at approximately 16 μg/ml. The transgenesis level in these quail is estimated at about 5% or less. Thus the level in a GI should be substantially greater. It is expected that similar levels would be seen in a chicken and other avians, as the quail and chicken ovalbumin genes, as well as ovalbumin genes of other avians, are very similar.

EXAMPLE 6

Construction of pSIN-3.9-OM-CTLA4-Fc

[0218] The 4907 bp Mfe I/Xho I fragment of pALV-SIN (disclosed, for example, in US patent publication No. 2007/0124829, published May 31, 2007) was ligated to the 5115 XhoI/EcoRI fragment of pOM-3.9-CTLA4 (shown in FIG. **15** of US patent publication No. 2007/0113299, published May 17, 2007), producing pSIN-3.9-OM-CTLA4-Fc Shown in FIG. **4** and SEQ ID NO: 20,

EXAMPLE 7

Production Of Transgenic Chickens Using pSIN-3.9-OM-CTLA4-Fc

[0219] Retroviral particles pseudotyped with the VSV envelope protein and containing the pSIN-3.9-OM-CTLA4-Fc (FIG. **4**) vector were produced as described in US patent publication No. 2007/0077650, published Apr. 5, 2007. Virus was harvested at 48 hours post-transfection, concentrated and on the same day approximately 7 microliters injected into the subgerminal cavity of stage X eggs. Eggs were resealed and incubated until hatch.

[0220] Egg white from hens was assayed using an ELISA for CTLA4-Fc. One hen was found to have CTLA4-Fc in her egg white at approximately 0.37 μg/ml. The transgenesis level in these hens is estimated at 5% or less. Thus the levels in a G1 should be substantially greater.

[0221] Any useful coding sequence may be inserted in place of the CTLA4-Fc coding sequence for production of the corresponding product.

EXAMPLE 8

Construction of pSIN-1.8-OM-IFNa-2B

[0222] The 1051 bp Nco I-Nco I fragment from pBS-OM-4.4 (FIG. **5** SEQ ID NO: 23) was inserted into the Nco I site of pAVIJCR-A137.91.1.2 (FIG. **6** SEQ ID NO: 24), thereby inserting the 1 kb ovomucoid promoter in front of an IFN coding sequence and SV40 polyadenylation signal and producing plkb-OM-IFNMM. A 1816 bp Cla I-Sac I fragment of p1kb-OM-IFNMM was inserted into the 6245 bp Cla I-Sac I fragment of pBS-OM-4.4, thereby fusing the 4.4 kb ovomucoid fragment with the IFN coding sequence and producing p4.40M-IFNMM. The 8511 bp BamH I-Sal I fragment of pBS-OMUP-10 was ligated to the 5148 bp BamH I-Sal I fragment of p4.40M-IFN, thereby placing the 10 kb ovomucoid promoter in front of the IFN coding sequence, producing p10-OM-IFN.

[0223] Region 2487-4889 of p10.0-OM-IFN was PCR amplified with primers 5'-GGCGTCGACGGATCCGT-TAACCCTAGAACTAGTGGATCTCTGCCCTTGTGC TGAC-3' (SEQ ID NO: 27) and 5'-GGCCTCGAGCCTA-GACTTTTTACTCCTTAGA-3' (SEQ ID NO: 28). The PCR product was digested with Sal I and Xho I and the 2435 bp isolated. pALV-SIN (disclosed, for example, in US patent publication No. 2007/0124829, published May 31, 2007) was digested with Xho I and the 4915 bp fragment isolated

and ligated to the 2435 bp fragment, producing pSIN-1.8-OM-IFNa-2B, shown in FIG. **7** and SEQ ID NO: 21.

EXAMPLE 9

Production Of Transgenic Chickens Using pSIN-1.8-OM-IFNa-2B

[**0224**] Retroviral particles having the pSIN-1.8-OM-IFNa-2B transgene and pseudotyped with the VSV envelope protein were produced as described in US patent publication No. 2007/0077650, published Apr. 5, 2007. Virus was harvested at 48 hours post-transfection, concentrated and, on the same day, approximately 7 microliters injected into the subgerminal cavity of stage X eggs. Eggs were resealed and incubated until hatch.

[**0225**] Egg whites from hens were assayed using an ELISA for IFNa-2B. Hens were found to have IFNa-2B in egg white at levels that ranged from 1.5 to 865.0 ng/ml with IFNa-2B levels at least about 600 fold lower in the serum. The transgenesis level in these hens is estimated at 5% or less.

[**0226**] Five G0 sperm positive roosters were bred to non-transgenic hens. Of 1251 offspring, 30 carried the pSIN-1.8-OM-IFNa-2B transgene. Six of the 30 hens expressed human IFN-α-2B at 34.1 to 165.6 μg/ml of egg white. Each of the six hens had a single copy of the transgene. Serum levels of human IFN-α-2B were 0.3 to 9.2 ng/ml which, on average, was 30,000 fold lower than egg white levels.

EXAMPLE 10

Production Of Transgenic Chickens Using Lentivirus Vectors And Moloney Murine Leukemia Virus

[**0227**] The invention specifically contemplates the employment of other retroviral vectors that are useful in avian transgenesis to be used in accordance with the present invention. Such vectors can be employed to produce transgenic avians, for example, in the same way as ALV-SIN vectors have been used in Examples' 1 to 9 above. For example, Moloney Murine Leukemia Virus (MMLV) and Lentiviral Vectors can be used in accordance with the invention, each, for example, by deleting one or more of the CAAT box; the TAATA box; and enhancer contained in the U3 region of the upstream LTR of each virus to produce a SIN vector. Alternatively, or in addition (i.e., in conjunction with a SIN vector) no transcriptionally active markers or selectable cassettes are included in each of the retroviral vectors.

[**0228**] Although preferred embodiments of the invention have been described using specific terms, devices, and methods, such description is for illustrative purposes only. The words used are words of description rather than of limitation. It is to be understood that changes and variations may be made by those of ordinary skill in the art without departing from the spirit or the scope of the present inventions which is set forth in the following claims. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part.

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cattaagcgc ggcgggtgtg gtggttacgc gcagcgtgac cgctacactt gccagcgccc 3180

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gaacaacact caaccctatc tcggtctatt cttttgattt ataagggatt ttgccgattt 3480

cggcctattg gttaaaaaat gagctgattt aacaaaaatt taacgcgaat tttaacaaaa 3540

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aggtacggga ggtacttgga gcggccgcaa taaaatatct ttattttcat tacatctgtg 3720

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gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata 3960

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cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 4260

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<210> SEQ ID NO 25
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The RRE (rev responsive element) sequence

<400> SEQUENCE: 25

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tcaatgacgc tgacggtaca ggccagacaa ttattgtctg gtatagtgca gcagcagaac 120

aatttgctga gggctattga ggcgcaacag catctgttgc aactcacagt ctggggcatc 180

aagcagctcc aggcaagaat cctggctgtg gaaagatacc taaaggatca acagctcctg 240

gtac 244

-continued

<210> SEQ ID NO 26
<211> LENGTH: 158
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The ALV CTE (constitutive transport element) sequence

<400> SEQUENCE: 26

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caatagcatg tttaggcgaa aagcggggct tcggttgtac gcggttagga gtcccctcag 120

gatatagtag tttcgctttt gcatagggag ggggaaat 158

<210> SEQ ID NO 27
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 27

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<210> SEQ ID NO 28
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: p10.0-OM-IFN-2

<400> SEQUENCE: 28

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<210> SEQ ID NO 29
<211> LENGTH: 346
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ALV vector 5' LTR sequence

<400> SEQUENCE: 29

aatgtagtct tatgcaatac tcttgtagtc ttgcaacatg cttatgtaac gatgagttag 60

caacatgcct tataaggaga gaaaaagcac cgtgcatgcc gattggtggg agtaaggtgg 120

tatgatcgtg gtatgatcgt gccttgttag gaaggcaaca gacgggtcta acacggattg 180

gacgaaccac tgaattccgc attgcagaga tattgtattt aagtgcctag ctcgatacaa 240

taaacgccat ttgaccattc accacattgg tgtgcacctg ggttgatggc cggaccgttg 300

attccctgrc gactacgagc acatgcatga agcagaaggc ttcatt 346

What is claimed is:

1. A transgenic avian containing in its genome an exogenous nucleotide sequence comprising a promoter component and a SIN vector wherein the exogenous nucleotide sequence is integrated into the genome and the avian produces an exogenous protein which is deposited in a hard shell egg laid by the avian.

2. The transgenic avian of claim 1 wherein the promoter component is an oviduct specific promoter.

3. The transgenic avian of claim 1 wherein the avian is selected from the group consisting of a chicken, a turkey and a quail.

4. The transgenic avian of claim 1 wherein the promoter component is linked to a coding sequence exogenous to the avian.

5. The transgenic avian of claim 1 wherein the promoter component is an avian ovomucoid promoter component.

6. The transgenic avian of claim 1 wherein the promoter component is an avian ovalbumin promoter component.

7. The transgenic avian of claim 1 wherein the promoter component is an avian lysozyme promoter component.

8. The transgenic avian of claim 1 wherein the exogenous protein is a therapeutic protein.

9. The transgenic avian of claim 1 wherein the exogenous protein is a cytokine.

10. The transgenic avian of claim 1 wherein the exogenous protein is selected from the group consisting of erythropoietin, GM-CSF, interferon, fusion protein, CTLA4-Fc fusion protein, growth hormones, cytokines, structural, interferon, lysozyme, β-casein, albumin, α-1 antitrypsin, antithrombin III, collagen, factors VIII, IX, X (and the like), fibrinogen, lactoferrin, protein C, tissue-type plasminogen activator (tPA), somatotropin, and chymotryp sin, immunoglobulins, antibodies, immunotoxins, factor VIII, b-domain deleted factor VIII, factor VIIa, factor IX, anticoagulants; hirudin, alteplase, tpa, reteplase, tpa, tpa—3 of 5 domains deleted, insulin, insulin lispro, insulin aspart, insulin glargine, long-acting insulin analogs, glucagons, tsh, follitropin-beta, fsh, pdgh, inf-beta, inf-alpha 1, ifn-alpha 2, inf-beta, inf-beta 1b, ifn-beta 1a, ifn-gamma, ifn-gamma 1b, il-2, il-1 1, hbsag, ospa, dornase-alpha dnase, beta glucocerebrosidase, tnf-alpha, il-2-diptheria toxin fusion protein, tnfr-lgg fragment fusion protein laronidase, dnaases, alefacept, tositumomab, murine mab, alemtuzumab, rasburicase, agalsidase beta, teriparatide, parathyroid hormone derivatives, adalimumab (lggl), anakinra, biological modifier, nesiritide, human b-type natriuretic peptide (hbnp), colony stimulating factors, pegvisomant, human growth hormone receptor antagonist, recombinant activated protein c, omalizumab, immunoglobulin e (lge) blocker, lbritumomab tiuxetan, ACTH, glucagon, somatostatin, somatotropin, thymosin, parathyroid hormone, pigmentary hormones, somatomedin, luteinizing hormone, chorionic gonadotropin, hypothalmic releasing factors, etanercept, antidiuretic hormones, prolactin and thyroid stimulating hormone, an immunoglobulin polypeptide, immunoglobulin polypeptide D region, immunoglobulin polypeptide J region, immunoglobulin polypeptide C region, immunoglobulin light chain, immunoglobulin heavy chain, an immunoglobulin heavy chain variable region, an immunoglobulin light chain variable region and a linker peptide.

11. The transgenic avian of claim 1 wherein the retrovirus is selected from the group consisting of avian leukosis virus vector (ALV), a murine leukemia virus (MLV) retroviral vector, moloney murine leukemia Virus (MMLV) and a lentiviral vector.

12. A transgenic avian comprising an oviduct cell which contains an exogenous nucleotide sequence comprising a promoter component linked to an exogenous coding sequence contained in an integrated SIN vector wherein the exogenous coding sequence is expressed in the oviduct cell and is secreted from the oviduct cell.

13. The transgenic avian of claim 12 wherein the avian is a chicken.

14. The transgenic avian of claim 12 wherein the oviduct cell is a tubular gland cell.

15. The transgenic avian of claim 12 wherein the promoter component is an avian ovomucoid promoter component.

16. The transgenic avian of claim 12 wherein the promoter component is an avian ovalbumin promoter component.

17. The transgenic avian of claim 12 wherein the promoter component is an avian lysozyme promoter component.

18. A method of producing an exogenous protein comprising producing a transgenic avian having a nucleotide sequence in its genome comprising a vector which is at least one of a SIN vector and an SC negative vector wherein the nucleotide sequence comprises a promoter component linked to an exogenous coding sequence.

19. The method of claim 18 wherein the exogenous coding sequence encodes a human protein.

20. The method of claim 18 wherein the exogenous coding sequence encodes a therapeutic protein.

21. The method of claim 18 wherein the promoter component comprises a functional promoter sequence of a promoter selected from the group consisting of avian ovalbumin promoter component, avian ovomucoid promoter component, avian lysozyme promoter component and avian conalbumin promoter component.

22. The method of claim 18 wherein the avian is a chicken.

23. A transgenic avian containing in its genome an exogenous nucleotide sequence comprising a promoter component and a SC negative vector wherein the exogenous nucleotide sequence is integrated into the genome and the avian produces an exogenous protein.

24. The transgenic avian of claim 23 wherein the promoter component comprises a functional promoter sequence of a promoter selected from the group consisting of avian ovalbumin promoter component, avian ovomucoid promoter component and avian lysozyme promoter component.

25. A nucleic acid 90% identical to a nucleic acid molecule selected from the group consisting of nucleotide sequences that contain:

1. 3.5 kb OV fragment (includes DHS I, II & III)

   5' UTR-5' portion (from Exon L)

   5' UTR-3' portion (from Exon 1);

2. 3.5 kb OV fragment (includes DHS I, II & III)

   5' UTR-5' portion (from Exon L)

   Intron A

   5' UTR-3' portion (from Exon 1)

   3' UTR;

3. 3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   Intron A
   5' UTR-3' portion (from Exon 1);
4. 3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   5' UTR-3' portion (from Exon 1)
   3' UTR;
5. 3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   Intron A
   5' UTR-3' portion (from Exon 1)
   3' UTR/DHSA(bp 13576 to 15163 of FIG. **8**);
6. 3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   5' UTR-3' portion (from Exon 1)
   3' UTR/DHSA(bp 13576 to 15163 of FIG. **8**);
7. 3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   Intron A
   5' UTR-3' portion (from Exon 1)
   partial 3' UTR
   RRE;
8. ALV CTE
   3.5 kb OV fragment (includes DHS I, II & III)
   5' UTR-5' portion (from Exon L)
   Intron A
   5' UTR-3' portion (from Exon 1)
   partial 3' UTR;
   wherein,

3.5 kb OV fragment (includes DHS I, II & III): Start: 3199 End: 6659 of FIG. **8** (SEQ ID NO: 22);

5' UTR-5' portion (from Exon L): Start: 6659 End: 6705 of FIG. **8** (SEQ ID NO: 22);

5' UTR-3' portion (from Exon 1): Start: 8295 End: 8311 of FIG. **8** (SEQ ID NO: 22);

3' UTR: Start: 13576 End: 14209 of FIG. **8** (SEQ ID NO: 22);

partial 3' UTR: Start 13576 End: 13996 of FIG. **8** (SEQ ID NO: 22);

Intron A: Start: 6706 End: 8294 of FIG. **8** (SEQ ID NO: 22);

Exon L: Start: 6659 End: 6705 of FIG. **8** (SEQ ID NO: 22);

Exon 1: Start: 8295 End: 8478 of FIG. **8** (SEQ ID NO: 22);

DHS III: Start: 3253 End: 3559 of FIG. **8** (SEQ ID NO: 22);

DHS II: Start: 5629 End: 6009 of FIG. **8** (SEQ ID NO: 22);

DHS I: Start: 6359 End: 6659 of FIG. **8** (SEQ ID NO: 22); and

RRE: shown in FIG. **9***a* (SEQ ID NO: 25)

ALV CTE shown in FIG. **9***b* (SEQ ID NO: 26)

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