Respiratory Syncytial Virus (RSV) A2 Large Polymerase (L) Helper Plasmid, pA2-Lopt

Catalog No. NR-36461

For research use only. Not for human use.

Contributor:
BEI Resources

Manufacturer:
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Product Description:
NR-36461 is a component of a bacterial artificial chromosome (BAC)-based RSV rescue system that allows RSV infection to be monitored by fluorescence and is an important tool in RSV vaccine research and mutagenesis studies. Please refer to Appendix I for the manufacturer's RSV rescue protocol.

The L helper plasmid was constructed from codon-optimized RSV A2 L sequences. The codon-optimized cDNA sequences were synthesized and cloned into the pcDNA™3.1$^{(+)}$ mammalian expression plasmid (Life Technologies™ Invitrogen™).[1,2] The plasmid was produced in *Escherichia coli*, strain 10-beta (a DH10B derivative, New England BioLabs®) and extracted using a Endo-Free Plasmid Maxi Kit (Qiagen).[2] The complete sequence for pA2-Lopt is reported in Appendix II.

Material Provided:
Each vial contains 0.5 µg of plasmid DNA in RNase/DNase-free 10 mM Tris-HCl, 1 mM EDTA buffer (pH 8). The concentration is shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Packaging/Storage:
NR-36461 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Functional Activity:
Recombinant RSV was produced by co-transfection of BHK-21 clone BSR T7/5 cells[3] with pSynkRSV-I19F, a BAC plasmid containing RSV A2-line19F antigenomic DNA and the gene for the far-red fluorescent protein monomeric Katushka 2 (mKate2) to enable detection of infection through fluorescence, (NR-36460) and four helper plasmids encoding sequence-optimized genes from RSV strain A2: large polymerase (L) (NR-36461), nucleoprotein (N) (NR-36462), phosphoprotein (P) (NR-36463) and matrix 2-1 protein (M2-1) (NR-36464). RSV rescue and infection could be detected by red fluorescent syncytia.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Respiratory Syncytial Virus (RSV) A2 Large Polymerase (L) Helper Plasmid, pA2-Lopt, NR-36461."

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:
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References:
1. Hotard, A. L., et al. "A Stabilized Respiratory Syncytial Virus Reverse Genetics System Amendable to Recombination-Mediated Mutagenesis." Virology 434 (2012): 129-136. PubMed: 23062737.

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2. M. L. Moore, Personnel Communication.
3. Buchholz, U. J., S. Finke and K. -K. Conzelmann. "Generation of Bovine Respiratory Syncytial Virus (BRSV) from cDNA: BRSV NS2 Is Not Essential for Virus Replication in Tissue Culture, and Human RSV Leader Region Acts as a Functional BRSV Genome Promoter." J. Virol. 73 (1999): 251-259. PubMed: 9847328.

Support Provided by NIAID

Appendix I

Transfection Procedure for Virus Recovery of Recombinant Respiratory Syncytial Virus

Materials (Suggested suppliers and catalog numbers are indicated):

BHK-21 clone BSR T7/5 cell cultures or alternative cells [BHK21 cells (ATCC® CCL10™) transfected with phage T7 polymerase from Modified Vaccinia Ankara (MVA)] Note: This protocol is optimized for use with BHK-21 clone BSR T7/5 cells. Use of alternative cells may result in decreased recovery of RSV.
Opti-MEM (serum-free) (Gibco/Life Technologies catalog #11058-021)
GMEM [Glasgow's MEM (Gibco/Life Technologies catalog #11710-035)] + 3% FBS
MEM non-essential amino acids (NEAA) 100X solution (Gibco/Life Technologies catalog #11140-050)
G418 sulfate, 50 mg/mL solution (500X) (Agilent Technologies Genomics catalog # 200049)
Trypsin-EDTA (0.25%) (Gibco/Life Technologies catalog #25200-072)
Antibiotic-Antimycotic solution, penicillin/streptomycin/amphotericin (100X) (Corning cellgro® catalog #30-004-Cl) or equivalent
Plasmid with RSV antigenome (NR-36460) each vial contains 0.5 µg in 5 µL total volume (Note: This protocol requires 0.8 µg of pSynkRSV-I19F; thus 2 vials of NR-36460 are required per transfection.)
Helper Plasmids – (all codon optimized) each vial contains 0.5 µg in 5 µL total volume:
pA2-Lopt, L protein (NR-36461)
pA2-Nopt, N protein (NR-36462)
pA2-Popt, P protein (NR-36463)
pA2-M2-1opt, M2-1 protein (NR-36464)
Lipofectamine 2000 transfection reagent (Gibco/Life Technologies catalog #11668-019)
Phosphate buffered saline pH 7.2 (Gibco/Life Technologies catalog #20012027)
6-well tissue culture plates
25 cm^2 tissue culture flasks
Shaker/rocker plate
Tissue culture humidified incubator with 3% to 5% CO_2
Assorted sterile pipettes and tips

Procedure:

Note: This protocol assumes the user is familiar with cell culture techniques and transfection procedures.

1. Initial cell culture:
   a. For routine sub-passage of BSR T7/5 cells, prepare new 25 cm^2 cultures at a ratio of one donor culture to three new cultures, based on surface area of the culture flasks (1:3 passage ratio). Use GMEM with 3% FBS + 1X NEAA + 1X antibiotics as growth medium, 5 mL per flask. When maintaining donor cultures, add 1X G418 to the growth medium every other passage.
   b. For transfections, sub-pass BSR T7/5 cells from "donor" cultures into 6 well plates so they will be 100% confluent at time of transfection. Use one 25 cm^2 culture to prepare one 6 well plate (1:2.5 passage ratio).

2. Prepare 6 well plates for transfection from 25 cm^2 donor cultures. Determine how many plates will be required and use the corresponding number of flasks. Aspirate the growth medium from the flasks, and then add 0.25 mL of warm trypsin-EDTA per 25 cm^2 flask. Rock flasks to distribute the trypsin-EDTA and incubate at 37°C for 5 to10 minutes. When cells start to dislodge from the flask, add 12 mL of GMEM with 3% FBS to each flask and use a pipet to suspend the cells in this growth medium. Add 2 mL of the cell suspension to each well in the 6 well plates. Incubate the plates at 37°C in the tissue culture incubator until the cell sheets are confluent and ready for transfection.

3. Prepare the reagents for the transfection procedure. Transfection will be done using Lipofectamine 2000 as the transfection reagent. Additionally, it is important to include control transfections (Lipofectamine only/wild type virus for mutants etc.)
   a. Use a 3:1 ratio of Lipofectamine (µL) to plasmid/helper plasmid (µg). Dilute each component with Opti-MEM to make 100 µL of each. After dilution, allow each dilution to sit at room temperature for 5 minutes.
   b. Use the following amounts of each component per transfection:
      i. RSV antigenome (NR-36460) 0.8 µg (8 µL of 0.1 µg/µL) + 92 µL Opti-MEM (2 vials of NR-36460 are required per transfection.)
      ii. pA2-Lopt, L protein (NR-36461) 0.2 µg (2 µL of 0.1 µg/µL) + 98 µL Opti-MEM
      iii. pA2-Nopt, N protein (NR-36462) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM
      iv. pA2-Popt, P protein (NR-36463) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM
      v. pA2-M2-1opt, M2-1 protein (NR-36464) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM

vi. Lipofectamine 2000 6.6 µL + 93.4 µL Opti-MEM

Note: For multiple transfections increase the above quantities proportionally.

c. After allowing the diluted components to sit at room temperature for 5 minutes, combine all six components in one vial, mix gently and incubate the transfection mixture at room temperature for 20 minutes.
d. Transfection mixtures should be 600 µL total (Opti-MEM, Lipofectin, and DNA)
e. Aspirate the media from the BSR T7/5 cell culture plate, wash cells twice with 1 mL warm Opti-MEM for each wash, and aspirate the final wash.
f. Add 600 µL transfection mixture to each well and incubate the plate 2 hours at room temperature on a shaker/rocker plate set at low speed.
g. After 2 hours, add an additional 600 µL warm Opti-MEM per well and place plate in a 37°C tissue culture incubator overnight (8-12 hours).

4. After incubation, aspirate and discard the transfection mixture from the wells, wash each well once with 1 mL warm sterile PBS, aspirate the PBS and replace with 2 mL of warm GMEM with 3% FBS per well. Continue incubating at 37°C in the tissue culture incubator overnight.

5. Day 2 post transfection, sub-pass the cells into 25 cm^2 flasks using the trypsin-EDTA procedure described above. Pass at a 1:3 surface area ratio unless cell morphology appears weak, in which case the ratio should be decreased accordingly up to an even 1:1 ratio. (Note: surface area of each well in the 6 well plate is 10 cm^2). Cells should remain in GMEM with 3% FBS throughout the rest of recovery.

6. Monitor flasks for cytopathic effect (CPE) and sub-pass at 1:3 ratio into new 25 cm^2 flasks as needed (approximately every 48 hours). CPE shows first as mini-syncytia and then grows into rounded up clumps of cells.

7. When CPE is evident throughout the flask, scrape the cells into the growth media and aliquot into cryovials. Freeze at -80°C or colder.

Appendix 2: pA2-Lopt Sequence

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1     GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATG 60
      CTGCCTAGCCCTCTAGAGGGCTAGGGGATACCACGTGAGAGTCATGTTAGACGAGACTAC

61    CCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCG 120
      GGCGTATCAATTCGGTCATAGACGAGGGACGAACACACAACCTCCAGCGACTCATCACGC

121   CGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGC 180
      GCTCGTTTTAAATTCGATGTTGTTCCGTTCCGAACTGGCTGTTAACGTACTTCTTAGACG

181   TTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATT 240
      AATCCCAATCCGCAAAACGCGACGAAGCGCTACATGCCCGGTCTATATGCGCAACTGTAA

241   GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATA 300
      CTAATAACTGATCAATAATTATCATTAGTTAATGCCCCAGTAATCAAGTATCGGGTATAT

301   TGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACC 360
      ACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGGACCGACTGGCGGGTTGCTGG

361   CCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC 420
      GGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATCATTGCGGTTATCCCTGAAAGG

421   ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT 480
      TAACTGCAGTTACCCACCTCATAAATGCCATTTGACGGGTGAACCGTCATGTAGTTCACA

481   ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATT 540
      TAGTATACGGTTCATGCGGGGGATAACTGCAGTTACTGCCATTTACCGGGCGGACCGTAA

541   ATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA 600
      TACGGGTCATGTACTGGAATACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAGT

601   TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG 660
      AGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTTACCCGCACCTATCGCCAAAC

661   ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACC 720
      TGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTTACCCTCAAACAAAACCGTGG

721   AAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCG 780
      TTTTAGTTGCCCTGAAAGGTTTTACAGCATTGTTGAGGCGGGGTAACTGCGTTTACCCGC

781   GTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCA 840
      CATCCGCACATGCCACCCTCCAGATATATTCGTCTCGAGAGACCGATTGATCTCTTGGGT
                        T7 promoter(863,881)
                              |
841   CTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGC 900
      GACGAATGACCGAATAGCTTTAATTATGCTGAGTGATATCCCTCTGGGTTCGACCGATCG
                           KpnI   RSV large polymerase (929,7426)
                             |       |
901   GTTTAAACTTAAGCTTGGTACCGCCACCATGGACCCCATCATCAACGGCAACAGCGCCAA 960
      CAAATTTGAATTCGAACCATGGCGGTGGTACCTGGGGTAGTAGTTGCCGTTGTCGCGGTT
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961   CGTGTACCTGACCGACAGCTACCTGAAGGGCGTGATCAGCTTTAGTGAGTGCAACGCCCT 1020
      GCACATGGACTGGCTGTCGATGGACTTCCCGCACTAGTCGAAATCACTCACGTTGCGGGA

1021  GGGCAGCTACATCTTCAACGGCCCCTACCTGAAGAACGACTACACCAACCTGATCAGCCG 1080
      CCCGTCGATGTAGAAGTTGCCGGGGATGGACTTCTTGCTGATGTGGTTGGACTAGTCGGC

1081  GCAGAACCCCCTGATCGAGCACATGAACCTGAAGAAGCTGAACATCACCCAGAGCCTGAT 1140
      CGTCTTGGGGGACTAGCTCGTGTACTTGGACTTCTTCGACTTGTAGTGGGTCTCGGACTA

1141  CAGCAAGTACCACAAGGGCGAGATCAAGCTGGAAGAACCCACCTACTTCCAGAGCCTGCT 1200
      GTCGTTCATGGTGTTCCCGCTCTAGTTCGACCTTCTTGGGTGGATGAAGGTCTCGGACGA

1201  GATGACCTACAAGAGCATGACCAGCAGCGAGCAGATCGCCACCACCAACCTGCTGAAGAA 1260
      CTACTGGATGTTCTCGTACTGGTCGTCGCTCGTCTAGCGGTGGTGGTTGGACGACTTCTT

1261  GATCATCAGACGGGCCATCGAGATCAGCGACGTGAAGGTGTACGCCATCCTGAACAAGCT 1320
      CTAGTAGTCTGCCCGGTAGCTCTAGTCGCTGCACTTCCACATGCGGTAGGACTTGTTCGA

1321  GGGCCTGAAAGAGAAGGACAAGATCAAGAGCAACAACGGCCAGGACGAGGACAACAGCGT 1380
      CCCGGACTTTCTCTTCCTGTTCTAGTTCTCGTTGTTGCCGGTCCTGCTCCTGTTGTCGCA

1381  GATCACCACCATCATCAAGGACGACATCCTGAGCGCCGTGAAGGACAACCAGAGCCACCT 1440
      CTAGTGGTGGTAGTAGTTCCTGCTGTAGGACTCGCGGCACTTCCTGTTGGTCTCGGTGGA

1441  GAAGGCCGACAAGAACCACAGCACCAAGCAGAAGGACACCATCAAGACCACCCTGCTGAA 1500
      CTTCCGGCTGTTCTTGGTGTCGTGGTTCGTCTTCCTGTGGTAGTTCTGGTGGGACGACTT

1501  AAAGCTGATGTGCAGCATGCAGCACCCCCCCAGCTGGCTGATCCACTGGTTCAACCTGTA 1560
      TTTCGACTACACGTCGTACGTCGTGGGGGGGTCGACCGACTAGGTGACCAAGTTGGACAT

1561  CACCAAGCTGAACAACATCCTGACCCAGTACCGGTCCAACGAAGTGAAGAACCACGGCTT 1620
      GTGGTTCGACTTGTTGTAGGACTGGGTCATGGCCAGGTTGCTTCACTTCTTGGTGCCGAA

1621  CACCCTGATCGACAACCAGACCCTGAGCGGCTTCCAGTTCATCCTGAATCAGTACGGCTG 1680
      GTGGGACTAGCTGTTGGTCTGGGACTCGCCGAAGGTCAAGTAGGACTTAGTCATGCCGAC

1681  CATCGTGTACCACAAAGAGCTGAAGCGGATCACCGTGACCACCTACAACCAGTTTCTGAC 1740
      GTAGCACATGGTGTTTCTCGACTTCGCCTAGTGGCACTGGTGGATGTTGGTCAAAGACTG

1741  CTGGAAGGACATCAGCCTGAGCCGGCTGAACGTGTGCCTGATCACCTGGATCAGCAACTG 1800
      GACCTTCCTGTAGTCGGACTCGGCCGACTTGCACACGGACTAGTGGACCTAGTCGTTGAC

1801  CCTGAACACCCTGAACAAGAGCCTGGGACTGAGATGCGGCTTCAACAACGTGATCCTGAC 1860
      GGACTTGTGGGACTTGTTCTCGGACCCTGACTCTACGCCGAAGTTGTTGCACTAGGACTG

1861  TCAGCTGTTCCTGTACGGCGACTGCATCCTGAAGCTGTTCCACAACGAGGGCTTCTACAT 1920
      AGTCGACAAGGACATGCCGCTGACGTAGGACTTCGACAAGGTGTTGCTCCCGAAGATGTA

1921  CATCAAAGAGGTGGAAGGCTTCATCATGAGCCTGATCCTGAATATCACCGAAGAGGACCA 1980
      GTAGTTTCTCCACCTTCCGAAGTAGTACTCGGACTAGGACTTATAGTGGCTTCTCCTGGT

1981  GTTCCGGAAGCGGTTCTACAACAGCATGCTGAACAATATCACAGACGCCGCCAACAAGGC 2040
      CAAGGCCTTCGCCAAGATGTTGTCGTACGACTTGTTATAGTGTCTGCGGCGGTTGTTCCG
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2041  CCAGAAGAACCTGCTGTCCAGAGTGTGCCATACCCTGCTGGACAAGACCGTGTCCGACAA 2100
      GGTCTTCTTGGACGACAGGTCTCACACGGTATGGGACGACCTGTTCTGGCACAGGCTGTT

2101  CATCATCAATGGCCGGTGGATTATCCTGCTGTCTAAGTTCCTGAAACTGATTAAGCTGGC 2160
      GTAGTAGTTACCGGCCACCTAATAGGACGACAGATTCAAGGACTTTGACTAATTCGACCG

2161  CGGCGACAACAACCTGAACAATCTGAGCGAGCTGTACTTCCTGTTCCGGATCTTCGGCCA 2220
      GCCGCTGTTGTTGGACTTGTTAGACTCGCTCGACATGAAGGACAAGGCCTAGAAGCCGGT

2221  CCCCATGGTGGACGAGAGACAGGCCATGGACGCCGTGAAGATCAACTGCAACGAGACAAA 2280
      GGGGTACCACCTGCTCTCTGTCCGGTACCTGCGGCACTTCTAGTTGACGTTGCTCTGTTT

2281  GTTCTATCTGCTGAGTTCCCTGAGCATGCTGAGAGGCGCCTTCATCTACCGGATCATCAA 2340
      CAAGATAGACGACTCAAGGGACTCGTACGACTCTCCGCGGAAGTAGATGGCCTAGTAGTT

2341  GGGCTTCGTGAACAACTACAACCGGTGGCCCACCCTGCGGAACGCCATCGTGCTGCCCCT 2400
      CCCGAAGCACTTGTTGATGTTGGCCACCGGGTGGGACGCCTTGCGGTAGCACGACGGGGA

2401  GCGGTGGCTGACCTACTACAAGCTGAATACCTACCCCAGCCTGCTGGAACTGACCGAGCG 2460
      CGCCACCGACTGGATGATGTTCGACTTATGGATGGGGTCGGACGACCTTGACTGGCTCGC

2461  GGACCTGATCGTGCTGAGCGGCCTGAGATTCTACAGAGAGTTCCGGCTGCCCAAGAAAGT 2520
      CCTGGACTAGCACGACTCGCCGGACTCTAAGATGTCTCTCAAGGCCGACGGGTTCTTTCA

2521  GGACCTGGAAATGATCATCAACGACAAGGCCATCAGCCCCCCCAAGAACCTGATCTGGAC 2580
      CCTGGACCTTTACTAGTAGTTGCTGTTCCGGTAGTCGGGGGGGTTCTTGGACTAGACCTG

2581  CAGCTTCCCCCGGAACTACATGCCCAGCCACATCCAGAACTACATCGAGCACGAGAAGCT 2640
      GTCGAAGGGGGCCTTGATGTACGGGTCGGTGTAGGTCTTGATGTAGCTCGTGCTCTTCGA

2641  GAAGTTCAGCGAGAGCGACAAGAGCAGACGGGTGCTGGAATATTACCTGCGGGACAACAA 2700
      CTTCAAGTCGCTCTCGCTGTTCTCGTCTGCCCACGACCTTATAATGGACGCCCTGTTGTT

2701  GTTCAACGAGTGCGACCTGTACAACTGCGTGGTGAACCAGTCCTACCTGAACAACCCCAA 2760
      CAAGTTGCTCACGCTGGACATGTTGACGCACCACTTGGTCAGGATGGACTTGTTGGGGTT

2761  CCACGTGGTGTCCCTGACCGGCAAAGAACGCGAGCTGAGCGTGGGCCGGATGTTCGCTAT 2820
      GGTGCACCACAGGGACTGGCCGTTTCTTGCGCTCGACTCGCACCCGGCCTACAAGCGATA

2821  GCAGCCTGGCATGTTCAGACAGGTGCAGATCCTGGCCGAGAAGATGATCGCCGAGAACAT 2880
      CGTCGGACCGTACAAGTCTGTCCACGTCTAGGACCGGCTCTTCTACTAGCGGCTCTTGTA

2881  CCTGCAGTTCTTCCCAGAGAGCCTGACCAGATACGGCGACCTGGAACTGCAGAAGATCCT 2940
      GGACGTCAAGAAGGGTCTCTCGGACTGGTCTATGCCGCTGGACCTTGACGTCTTCTAGGA

2941  GGAACTGAAGGCTGGCATCAGCAACAAAAGCAACCGGTACAACGACAATTACAACAACTA 3000
      CCTTGACTTCCGACCGTAGTCGTTGTTTTCGTTGGCCATGTTGCTGTTAATGTTGTTGAT

3001  CATCAGCAAGTGCAGCATCATCACCGACCTGAGCAAGTTCAACCAGGCCTTCAGATACGA 3060
      GTAGTCGTTCACGTCGTAGTAGTGGCTGGACTCGTTCAAGTTGGTCCGGAAGTCTATGCT

3061  GACAAGCTGCATCTGCAGCGACGTGCTGGACGAGCTGCATGGCGTGCAGAGCCTGTTCTC 3120
      CTGTTCGACGTAGACGTCGCTGCACGACCTGCTCGACGTACCGCACGTCTCGGACAAGAG
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3121  CTGGCTGCACCTGACCATCCCCCACGTGACCATCATCTGCACCTACCGGCACGCCCCTCC 3180
      GACCGACGTGGACTGGTAGGGGGTGCACTGGTAGTAGACGTGGATGGCCGTGCGGGGAGG

3181  CTACATCGGCGACCACATCGTGGATCTGAACAACGTGGACGAGCAGAGCGGCCTGTACAG 3240
      GATGTAGCCGCTGGTGTAGCACCTAGACTTGTTGCACCTGCTCGTCTCGCCGGACATGTC

3241  ATACCACATGGGCGGCATCGAGGGCTGGTGCCAGAAGCTGTGGACCATCGAGGCCATCTC 3300
      TATGGTGTACCCGCCGTAGCTCCCGACCACGGTCTTCGACACCTGGTAGCTCCGGTAGAG

3301  CCTGCTGGATCTGATCAGCCTGAAGGGCAAGTTCTCCATCACCGCCCTGATCAACGGCGA 3360
      GGACGACCTAGACTAGTCGGACTTCCCGTTCAAGAGGTAGTGGCGGGACTAGTTGCCGCT

3361  CAACCAGTCCATCGACATCTCCAAGCCCATCCGGCTGATGGAAGGCCAGACCCACGCCCA 3420
      GTTGGTCAGGTAGCTGTAGAGGTTCGGGTAGGCCGACTACCTTCCGGTCTGGGTGCGGGT

3421  GGCCGACTACCTGCTGGCCCTGAATAGCCTGAAGCTGCTGTACAAAGAGTACGCCGGCAT 3480
      CCGGCTGATGGACGACCGGGACTTATCGGACTTCGACGACATGTTTCTCATGCGGCCGTA

3481  CGGCCACAAGCTGAAGGGCACCGAAACCTACATCAGCCGGGACATGCAGTTCATGAGCAA 3540
      GCCGGTGTTCGACTTCCCGTGGCTTTGGATGTAGTCGGCCCTGTACGTCAAGTACTCGTT

3541  GACCATCCAGCACAACGGCGTGTACTACCCCGCCAGCATCAAGAAAGTGCTGAGAGTGGG 3600
      CTGGTAGGTCGTGTTGCCGCACATGATGGGGCGGTCGTAGTTCTTTCACGACTCTCACCC

3601  CCCCTGGATCAACACCATCCTGGACGACTTCAAGGTGTCCCTGGAATCCATCGGCAGCCT 3660
      GGGGACCTAGTTGTGGTAGGACCTGCTGAAGTTCCACAGGGACCTTAGGTAGCCGTCGGA

3661  GACCCAGGAACTGGAATACCGGGGCGAGTCCCTGCTGTGCAGCCTGATCTTCCGGAACGT 3720
      CTGGGTCCTTGACCTTATGGCCCCGCTCAGGGACGACACGTCGGACTAGAAGGCCTTGCA

3721  GTGGCTGTACAACCAGATCGCCCTGCAGCTGAAGAATCACGCCCTGTGCAACAACAAGCT 3780
      CACCGACATGTTGGTCTAGCGGGACGTCGACTTCTTAGTGCGGGACACGTTGTTGTTCGA

3781  GTACCTGGACATCCTGAAGGTGCTGAAGCACCTGAAAACCTTTTTCAACCTGGACAACAT 3840
      CATGGACCTGTAGGACTTCCACGACTTCGTGGACTTTTGGAAAAAGTTGGACCTGTTGTA

3841  CGATACCGCCCTGACCCTGTACATGAATCTGCCCATGCTGTTCGGCGGAGGCGACCCCAA 3900
      GCTATGGCGGGACTGGGACATGTACTTAGACGGGTACGACAAGCCGCCTCCGCTGGGGTT

3901  TCTGCTGTACAGAAGCTTCTACCGGCGGACCCCCGACTTCCTGACCGAGGCCATCGTGCA 3960
      AGACGACATGTCTTCGAAGATGGCCGCCTGGGGGCTGAAGGACTGGCTCCGGTAGCACGT

3961  CAGCGTGTTCATCCTGTCCTACTACACCAATCACGACCTGAAGGATAAGCTGCAGGACCT 4020
      GTCGCACAAGTAGGACAGGATGATGTGGTTAGTGCTGGACTTCCTATTCGACGTCCTGGA

4021  GAGCGACGACCGGCTGAACAAGTTTCTGACATGTATCATCACCTTCGACAAGAATCCCAA 4080
      CTCGCTGCTGGCCGACTTGTTCAAAGACTGTACATAGTAGTGGAAGCTGTTCTTAGGGTT

4081  CGCCGAGTTCGTGACCCTGATGCGGGATCCTCAGGCTCTGGGCTCTGAGCGGCAGGCCAA 4140
      GCGGCTCAAGCACTGGGACTACGCCCTAGGAGTCCGAGACCCGAGACTCGCCGTCCGGTT

4141  GATCACCAGCGAGATCAACCGGCTGGCCGTGACCGAGGTGCTGTCCACCGCCCCCAACAA 4200
      CTAGTGGTCGCTCTAGTTGGCCGACCGGCACTGGCTCCACGACAGGTGGCGGGGGTTGTT
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4201  GATCTTCAGCAAGAGCGCCCAGCACTACACCACCACCGAGATCGACCTGAACGACATCAT 4260
      CTAGAAGTCGTTCTCGCGGGTCGTGATGTGGTGGTGGCTCTAGCTGGACTTGCTGTAGTA

4261  GCAGAACATCGAGCCCACCTACCCCCACGGCCTGAGAGTGGTGTATGAGAGCCTGCCCTT 4320
      CGTCTTGTAGCTCGGGTGGATGGGGGTGCCGGACTCTCACCACATACTCTCGGACGGGAA

4321  CTACAAGGCCGAGAAAATCGTGAATCTGATCTCCGGCACCAAGAGCATCACCAACATCCT 4380
      GATGTTCCGGCTCTTTTAGCACTTAGACTAGAGGCCGTGGTTCTCGTAGTGGTTGTAGGA

4381  GGAAAAGACCAGCGCCATCGACCTGACCGATATCGACCGGGCCACCGAGATGATGCGGAA 4440
      CCTTTTCTGGTCGCGGTAGCTGGACTGGCTATAGCTGGCCCGGTGGCTCTACTACGCCTT

4441  GAACATCACACTGCTGATCCGGATCCTGCCCCTGGACTGCAACCGGGACAAGCGCGAGAT 4500
      CTTGTAGTGTGACGACTAGGCCTAGGACGGGGACCTGACGTTGGCCCTGTTCGCGCTCTA

4501  CCTGAGCATGGAAAACCTGAGCATCACCGAGCTGTCCAAATACGTGCGCGAGCGGAGCTG 4560
      GGACTCGTACCTTTTGGACTCGTAGTGGCTCGACAGGTTTATGCACGCGCTCGCCTCGAC

4561  GTCCCTGAGCAACATCGTGGGCGTGACCAGCCCCAGCATCATGTACACCATGGACATCAA 4620
      CAGGGACTCGTTGTAGCACCCGCACTGGTCGGGGTCGTAGTACATGTGGTACCTGTAGTT

4621  GTACACCACCAGCACCATCAGCAGCGGCATCATCATCGAGAAGTACAACGTGAACTCCCT 4680
      CATGTGGTGGTCGTGGTAGTCGTCGCCGTAGTAGTAGCTCTTCATGTTGCACTTGAGGGA

4681  GACCAGAGGCGAGAGAGGCCCCACCAAGCCCTGGGTGGGAAGCAGCACCCAGGAAAAGAA 4740
      CTGGTCTCCGCTCTCTCCGGGGTGGTTCGGGACCCACCCTTCGTCGTGGGTCCTTTTCTT

4741  AACCATGCCCGTGTACAATCGCCAGGTGCTGACCAAGAAGCAGCGGGACCAGATTGATCT 4800
      TTGGTACGGGCACATGTTAGCGGTCCACGACTGGTTCTTCGTCGCCCTGGTCTAACTAGA

4801  GCTGGCCAAGCTGGACTGGGTGTACGCCTCCATCGACAACAAGGACGAGTTCATGGAAGA 4860
      CGACCGGTTCGACCTGACCCACATGCGGAGGTAGCTGTTGTTCCTGCTCAAGTACCTTCT

4861  ACTGTCTATCGGCACCCTGGGCCTGACCTACGAGAAGGCCAAGAAGCTGTTTCCCCAGTA 4920
      TGACAGATAGCCGTGGGACCCGGACTGGATGCTCTTCCGGTTCTTCGACAAAGGGGTCAT

4921  CCTGAGCGTGAACTACCTGCACCGGCTGACCGTGTCCAGCCGGCCTTGCGAGTTCCCTGC 4980
      GGACTCGCACTTGATGGACGTGGCCGACTGGCACAGGTCGGCCGGAACGCTCAAGGGACG

4981  CAGCATCCCCGCCTACCGGACCACCAACTACCACTTCGACACCAGCCCCATCAACCGGAT 5040
      GTCGTAGGGGCGGATGGCCTGGTGGTTGATGGTGAAGCTGTGGTCGGGGTAGTTGGCCTA

5041  CCTGACAGAGAAGTACGGCGACGAGGACATCGACATCGTGTTCCAGAACTGCATCAGCTT 5100
      GGACTGTCTCTTCATGCCGCTGCTCCTGTAGCTGTAGCACAAGGTCTTGACGTAGTCGAA

5101  CGGCCTGAGCCTGATGAGCGTGGTGGAACAGTTCACCAACGTGTGCCCCAACAGAATCAT 5160
      GCCGGACTCGGACTACTCGCACCACCTTGTCAAGTGGTTGCACACGGGGTTGTCTTAGTA

5161  CCTGATCCCCAAGCTGAATGAGATCCACCTGATGAAGCCCCCCATCTTCACCGGGGACGT 5220
      GGACTAGGGGTTCGACTTACTCTAGGTGGACTACTTCGGGGGGTAGAAGTGGCCCCTGCA

5221  GGACATCCACAAACTGAAACAGGTGATCCAGAAACAGCACATGTTTCTGCCCGACAAGAT 5280
      CCTGTAGGTGTTTGACTTTGTCCACTAGGTCTTTGTCGTGTACAAAGACGGGCTGTTCTA
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5281  CTCCCTGACACAGTACGTGGAACTGTTCCTGTCCAACAAGACCCTGAAGTCCGGCAGCCA 5340
      GAGGGACTGTGTCATGCACCTTGACAAGGACAGGTTGTTCTGGGACTTCAGGCCGTCGGT

5341  CGTGAACAGCAACCTGATCCTGGCCCACAAGATCAGCGATTACTTCCACAACACCTACAT 5400
      GCACTTGTCGTTGGACTAGGACCGGGTGTTCTAGTCGCTAATGAAGGTGTTGTGGATGTA

5401  CCTGTCCACCAATCTGGCTGGACACTGGATCCTGATCATTCAGCTGATGAAGGACAGCAA 5460
      GGACAGGTGGTTAGACCGACCTGTGACCTAGGACTAGTAAGTCGACTACTTCCTGTCGTT

5461  GGGCATCTTCGAGAAGGACTGGGGCGAGGGCTACATCACCGATCACATGTTCATCAACCT 5520
      CCCGTAGAAGCTCTTCCTGACCCCGCTCCCGATGTAGTGGCTAGTGTACAAGTAGTTGGA

5521  GAAGGTGTTCTTCAACGCCTACAAGACATACCTGCTGTGCTTCCACAAGGGCTACGGCAA 5580
      CTTCCACAAGAAGTTGCGGATGTTCTGTATGGACGACACGAAGGTGTTCCCGATGCCGTT

5581  GGCCAAACTGGAATGCGACATGAACACCAGCGATCTGCTGTGCGTGCTGGAACTGATCGA 5640
      CCGGTTTGACCTTACGCTGTACTTGTGGTCGCTAGACGACACGCACGACCTTGACTAGCT

5641  CAGCAGCTACTGGAAGTCTATGAGCAAAGTGTTCCTGGAACAGAAAGTGATCAAGTATAT 5700
      GTCGTCGATGACCTTCAGATACTCGTTTCACAAGGACCTTGTCTTTCACTAGTTCATATA

5701  CCTGAGCCAGGACGCCAGCCTGCACAGAGTGAAGGGCTGCCACTCCTTCAAGCTGTGGTT 5760
      GGACTCGGTCCTGCGGTCGGACGTGTCTCACTTCCCGACGGTGAGGAAGTTCGACACCAA

5761  CCTGAAGAGACTGAACGTGGCCGAGTTCACCGTGTGCCCTTGGGTGGTGAACATCGACTA 5820
      GGACTTCTCTGACTTGCACCGGCTCAAGTGGCACACGGGAACCCACCACTTGTAGCTGAT

5821  CCACCCCACCCACATGAAGGCCATCCTGACCTACATCGACCTGGTGCGCATGGGCCTGAT 5880
      GGTGGGGGTGGGTGTACTTCCGGTAGGACTGGATGTAGCTGGACCACGCGTACCCGGACTA

5881  CAACATCGACCGGATCCACATCAAGAACAAGCACAAGTTCAATGACGAGTTCTACACCAG 5940
      GTTGTAGCTGGCCTAGGTGTAGTTCTTGTTCGTGTTCAAGTTACTGCTCAAGATGTGGTC

5941  CAACCTGTTCTACATCAACTACAACTTCAGCGACAACACCCATCTGCTGACAAAGCACAT 6000
      GTTGGACAAGATGTAGTTGATGTTGAAGTCGCTGTTGTGGGTAGACGACTGTTTCGTGTA

6001  CCGGATCGCCAACAGCGAGCTGGAAAACAACTATAACAAACTGTACCACCCTACCCCCGA 6060
      GGCCTAGCGGTTGTCGCTCGACCTTTTGTTGATATTGTTTGACATGGTGGGATGGGGGCT

6061  GACACTGGAAAACATCCTGGCCAACCCCATCAAGTCCAACGACAAGAAAACCCTGAACGA 6120
      CTGTGACCTTTTGTAGGACCGGTTGGGGTAGTTCAGGTTGCTGTTCTTTTGGGACTTGCT

6121  CTACTGCATCGGCAAGAACGTGGACAGCATCATGCTGCCTCTGCTGAGCAACAAGAAGCT 6180
      GATGACGTAGCCGTTCTTGCACCTGTCGTAGTACGACGGAGACGACTCGTTGTTCTTCGA

6181  GATCAAGTCCAGCGCCATGATCCGGACCAACTACAGCAAGCAGGATCTGTACAACCTGTT 6240
      CTAGTTCAGGTCGCGGTACTAGGCCTGGTTGATGTCGTTCGTCCTAGACATGTTGGACAA

6241  CCCTATGGTGGTGATCGACAGGATCATCGACCACAGCGGCAATACCGCCAAGTCCAACCA 6300
      GGGATACCACCACTAGCTGTCCTAGTAGCTGGTGTCGCCGTTATGGCGGTTCAGGTTGGT

6301  GCTGTACACCACAACCAGCCACCAGATCAGCCTGGTGCACAACAGCACCAGCCTGTACTG 6360
      CGACATGTGGTGTTGGTCGGTGGTCTAGTCGGACCACGTGTTGTCGTGGTCGGACATGAC
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6361  CATGCTGCCCTGGCACCACATCAACCGGTTCAACTTCGTGTTCAGCAGCACCGGCTGCAA 6420
      GTACGACGGGACCGTGGTGTAGTTGGCCAAGTTGAAGCACAAGTCGTCGTGGCCGACGTT

6421  GATCAGCATCGAGTACATTCTGAAGGACCTGAAGATCAAGGACCCCAACTGTATCGCCTT 6480
      CTAGTCGTAGCTCATGTAAGACTTCCTGGACTTCTAGTTCCTGGGGTTGACATAGCGGAA

6481  CATCGGCGAGGGCGCTGGCAACCTGCTGCTGCGGACAGTGGTGGAACTGCACCCCGACAT 6540
      GTAGCCGCTCCCGCGACCGTTGGACGACGACGCCTGTCACCACCTTGACGTGGGGCTGTA

6541  CCGGTACATCTACAGAAGCCTGAAGGACTGCAACGACCACAGCCTGCCTATCGAGTTCCT 6600
      GGCCATGTAGATGTCTTCGGACTTCCTGACGTTGCTGGTGTCGGACGGATAGCTCAAGGA

6601  GAGACTGTACAACGGCCACATCAATATCGACTACGGCGAGAACCTGACAATCCCCGCCAC 6660
      CTCTGACATGTTGCCGGTGTAGTTATAGCTGATGCCGCTCTTGGACTGTTAGGGGCGGTG

6661  CGACGCCACCAACAACATCCACTGGTCTTACCTGCACATCAAGTTCGCCGAGCCCATCAG 6720
      GCTGCGGTGGTTGTTGTAGGTGACCAGAATGGACGTGTAGTTCAAGCGGCTCGGGTAGTC

6721  CCTGTTCGTGTGCGACGCCGAGCTGTCTGTGACCGTGAACTGGTCCAAGATCATCATTGA 6780
      GGACAAGCACACGCTGCGGCTCGACAGACACTGGCACTTGACCAGGTTCTAGTAGTAACT

6781  GTGGTCCAAGCACGTGCGGAAGTGCAAGTACTGCAGCAGCGTGAACAAGTGCATGCTGAT 6840
      CACCAGGTTCGTGCACGCCTTCACGTTCATGACGTCGTCGCACTTGTTCACGTACGACTA

6841  CGTGAAGTACCATGCCCAGGACGATATCGACTTCAAGCTGGATAACATCACCATCCTGAA 6900
      GCACTTCATGGTACGGGTCCTGCTATAGCTGAAGTTCGACCTATTGTAGTGGTAGGACTT

6901  AACATACGTGTGTCTGGGCAGCAAGCTGAAAGGCAGCGAGGTGTACCTGGTGCTGACAAT 6960
      TTGTATGCACACAGACCCGTCGTTCGACTTTCCGTCGCTCCACATGGACCACGACTGTTA

6961  CGGCCCTGCCAACATCTTCCCCGTGTTCAACGTGGTGCAGAACGCCAAGCTGATCCTGTC 7020
      GCCGGGACGGTTGTAGAAGGGGCACAAGTTGCACCACGTCTTGCGGTTCGACTAGGACAG

7021  CCGGACCAAGAACTTCATCATGCCCAAGAAGGCTGACAAAGAGAGCATCGACGCCAATAT 7080
      GGCCTGGTTCTTGAAGTAGTACGGGTTCTTCCGACTGTTTCTCTCGTAGCTGCGGTTATA

7081  CAAGTCCCTGATCCCCTTCCTGTGCTACCCCATCACCAAGAAGGGCATCAACACCGCCCT 7140
      GTTCAGGGACTAGGGGAAGGACACGATGGGGTAGTGGTTCTTCCCGTAGTTGTGGCGGGA

7141  GTCCAAGCTGAAGTCCGTGGTGTCCGGCGACATCCTGTCTTACTCTATCGCCGGCAGAAA 7200
      CAGGTTCGACTTCAGGCACCACAGGCCGCTGTAGGACAGAATGAGATAGCGGCCGTCTTT

7201  CGAGGTGTTCAGCAACAAACTGATCAACCACAAGCACATGAATATCCTGAAGTGGTTCAA 7260
      GCTCCACAAGTCGTTGTTTGACTAGTTGGTGTTCGTGTACTTATAGGACTTCACCAAGTT

7261  CCACGTGCTGAACTTCCGGTCCACCGAGCTGAACTACAATCATCTGTACATGGTGGAAAG 7320
      GGTGCACGACTTGAAGGCCAGGTGGCTCGACTTGATGTTAGTAGACATGTACCACCTTTC

7321  CACCTACCCCTACCTGTCCGAGCTGCTGAACAGCCTGACCACCAATGAGCTGAAGAAACT 7380
      GTGGATGGGGATGGACAGGCTCGACGACTTGTCGGACTGGTGGTTACTCGACTTCTTTGA
                                                      XhoI
                                                       |
7381  GATCAAGATCACCGGCAGCCTGCTGTATAACTTCCACAATGAGTGACTCGAGTCTAGAGG 7440
      CTAGTTCTAGTGGCCGTCGGACGACATATTGAAGGTGTTACTCACTGAGCTCAGATCTCC
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7441  GCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGT 7500
      CGGGCAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACGGTCGGTAGACAACA

7501  TTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTA 7560
      AACGGGGAGGGGGCACGGAAGGAACTGGGACCTTCCACGGTGAGGGTGACAGGAAAGGAT

7561  ATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGG 7620
      TATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGATAAGACCCCCCACC

7621  GGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC 7680
      CCACCCCGTCCTGTCGTTCCCCCTCCTAACCCTTCTGTTATCGTCCGTACGACCCCTACG

7681  GGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCA 7740
      CCACCCGAGATACCGAAGACTCCGCCTTTCTTGGTCGACCCCGAGATCCCCCATAGGGGT
                    f1 origin(7757,8063)
                       |
7741  CGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGC 7800
      GCGCGGGACATCGCCGCGTAATTCGCGCCGCCCACACCACCAATGCGCGTCGCACTGGCG

7801  TACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCAC 7860
      ATGTGAACGGTCGCGGGATCGCGGGCGAGGAAAGCGAAAGAAGGGAAGGAAAGAGCGGTG

7861  GTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAG 7920
      CAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGCCCCCGAGGGAAATCCCAAGGCTAAATC

7921  TGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCC 7980
      ACGAAATGCCGTGGAGCTGGGGTTTTTTGAACTAATCCCACTACCAAGTGCATCACCCGG

7981  ATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGG 8040
      TAGCGGGACTATCTGCCAAAAAGCGGGAAACTGCAACCTCAGGTGCAAGAAATTATCACC

8041  ACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATA 8100
      TGAGAACAAGGTTTGACCTTGTTGTGAGTTGGGATAGAGCCAGATAAGAAAACTAAATAT

8101  AGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAA 8160
      TCCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAAATTGTTTTTAAATT
                                  SV40 promoter (8195,8463)
                                        |
8161  CGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCA 8220
      GCGCTTAATTAAGACACCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGT

8221  GCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCC 8280
      CGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGG

8281  CCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATA 8340
      GGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTAT
                        SV40 origin(8362,8439)
                              |
8341  GTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCG 8400
      CAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGC

8401  CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAG 8460
      GGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGACGGAGACTC
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8461  CTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCCCG 8520
      GATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAACGTTTTTCGAGGGC
                                                  Neomycin^R(8578,9372)
                                                                  |
8521  GGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATG 8580
      CCTCGAACATATAGGTAAAAGCCTAGACTAGTTCTCTGTCCTACTCCTAGCAAAGCGTAC

8581  ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGC 8640
      TAACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCGATAAGCCG

8641  TATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCG 8700
      ATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGC

8701  CAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAG 8760
      GTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTC

8761  GACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTC 8820
      CTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAG

8821  GACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT 8880
      CTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTA

8881  CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGG 8940
      GAGGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTACGTTACGCC

8941  CGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATC 9000
      GCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAG

9001  GAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAG 9060
      CTCGCTCGTGCATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTC

9061  CATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC 9120
      GTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCGCGTACGGGCTGCCG

9121  GAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGC 9180
      CTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCG

9181  CGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATA 9240
      GCGAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAGTCCTGTAT

9241  GCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTC 9300
      CGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAG

9301  GTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGAC 9360
      CACGAAATGCCATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTG

9361  GAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGC 9420
      CTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACG

9421  CATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTT 9480
      GTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAA
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9481  TCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCC 9540
      AGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGCGGG

9541  ACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATT 9600
      TGGGGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAA

9601  TCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG 9660
      AGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTAC

9661  TATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCAT 9720
      ATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCAGTA
                                                lac promoter(9765,9794)
                                                        |
9721  AGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA 9780
      TCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGCCTT

9781  GCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGC 9840
      CGTATTTCACATTTCGGACCCCACGGATTACTCACTCGATTGAGTGTAATTAACGCAACG

9841  GCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCC 9900
      CGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTCGACGTAATTACTTAGCCGG

9901  AACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACT 9960
      TTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGACTGA

9961  CGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATAC 10020
      GCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTCCGCCATTATG

10021 GGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA 10080
      CCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCGTTT
                           pBR322 origin(10103,10719)
                              |
10081 AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTG 10140
      TCCGGTCCTTGGCATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGAC

10141 ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAA 10200
      TGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATATTT

10201 GATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGC 10260
      CTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCG

10261 TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAC 10320
      AATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTG

10321 GCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAAC 10380
      CGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTCGACCCGACACACGTGCTTG

10381 CCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG 10440
      GGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGGCC

10441 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT 10500
      ATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCTCCA
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10501 ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA 10560
      TACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCTT

10561 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT 10620
      GTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCATCGA

10621 CTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTTTTTTTGTTTGCAAGCAGCAGATTA 10680
      GAACTAGGCCGTTTGTTTGGTGGCGACCATCGCCAAAAAAACAAACGTTCGTCGTCTAAT

10681 CGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTC 10740
      GCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAG

10741 AGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCA 10800
      TCACCTTGCTTTTGAGTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCTAGAAGT

10801 CCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA 10860
      GGATCTAGGAAAATTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATATACTCATTT
                ampicillin^R(10874,11734)
                    |
10861 CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTAT 10920
      GAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGACAGATA

10921 TTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCT 10980
      AAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCCCTCCCGA

10981 TACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT 11040
      ATGGTAGACCGGGGTCACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAGGTCTAA

11041 TATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTAT 11100
      ATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGCTCGCGTCTTCACCAGGACGTTGAAATA

11101 CCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTA 11160
      GGCGGAGGTAGGTCAGATAATTAACAACGGCCCTTCGATCTCATTCATCAAGCGGTCAAT

11161 ATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTG 11220
      TATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACCACAGTGCGAGCAGCAAAC

11221 GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGT 11280
      CATACCGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGGGGTACA

11281 TGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCG 11340
      ACACGTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCAACCGGC

11341 CAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCG 11400
      GTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACGGTAGGC

11401 TAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGC 11460
      ATTCTACGAAAAGACACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCACATACG

11461 GGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAA 11520
      CCGCTGGCTCAACGAGAACGGGCCGCAGTTATGCCCTATTATGGCGCGGTGTATCGTCTT
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11521 CTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTAC 11580
      GAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCTAGAATG

11581 CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTT 11640
      GCGACAACTCTAGGTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTCGTAGAA

11641 TTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGG 11700
      AATGAAAGTGGTCGCAAAGACCCACTCGTTTTTGTCCTTCCGTTTTACGGCGTTTTTTCC

11701 GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAA 11760
      CTTATTCCCGCTGTGCCTTTACAACTTATGAGTATGAGAAGGAAAAAGTTATAATAACTT

11761 GCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATA 11820
      CGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCTTTTTAT

11821 AACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTC 11870
      TTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAG
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