Respiratory Syncytial Virus (RSV) A2 Matrix 2-1 (M2-1) Helper Plasmid, pA2-M2-1opt

Catalog No. NR-36464

For research use only. Not for human use.

Contributor:
BEI Resources

Manufacturer:
Martin L. Moore, Assistant Professor, Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia, USA

Product Description:
NR-36464 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 M2-1 helper plasmid was constructed from codon-optimized RSV A2 M2-1 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-M2-1opt 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-36464 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 Matrix 2-1 (M2-1) Helper Plasmid, pA2-M2-1opt, NR-36464."

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.

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

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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-M2-1opt 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 matrix 2-1 (929,1513)
                             |       |
901   GTTTAAACTTAAGCTTGGTACCGCCACCATGAGCCGGCGGAACCCCTGCAAGTTCGAGAT 960
      CAAATTTGAATTCGAACCATGGCGGTGGTACTCGGCCGCCTTGGGGACGTTCAAGCTCTA
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961   CCGGGGCCACTGCCTGAACGGCAAGCGGTGCCACTTCAGCCACAACTACTTCGAGTGGCC 1020
      GGCCCCGGTGACGGACTTGCCGTTCGCCACGGTGAAGTCGGTGTTGATGAAGCTCACCGG

1021  CCCTCACGCCCTGCTGGTGCGCCAGAACTTCATGCTGAACCGGATCCTGAAGTCCATGGA 1080
      GGGAGTGCGGGACGACCACGCGGTCTTGAAGTACGACTTGGCCTAGGACTTCAGGTACCT

1081  CAAGAGCATCGACACCCTGAGCGAGATCAGCGGAGCTGCCGAGCTGGACCGGACCGAGGA 1140
      GTTCTCGTAGCTGTGGGACTCGCTCTAGTCGCCTCGACGGCTCGACCTGGCCTGGCTCCT

1141  ATATGCCCTGGGCGTGGTGGGAGTGCTGGAAAGCTACATCGGCAGCATCAACAACATCAC 1200
      TATACGGGACCCGCACCACCCTCACGACCTTTCGATGTAGCCGTCGTAGTTGTTGTAGTG

1201  CAAGCAGAGCGCCTGCGTGGCCATGAGCAAGCTGCTGACCGAGCTGAACAGCGACGACAT 1260
      GTTCGTCTCGCGGACGCACCGGTACTCGTTCGACGACTGGCTCGACTTGTCGCTGCTGTA

1261  CAAGAAGCTGCGGGACAACGAGGAACTGAACAGCCCCAAGATCCGGGTGTACAACACCGT 1320
      GTTCTTCGACGCCCTGTTGCTCCTTGACTTGTCGGGGTTCTAGGCCCACATGTTGTGGCA

1321  GATCAGCTACATCGAGAGCAACCGGAAGAACAACAAGCAGACCATCCATCTGCTGAAGCG 1380
      CTAGTCGATGTAGCTCTCGTTGGCCTTCTTGTTGTTCGTCTGGTAGGTAGACGACTTCGC

1381  GCTGCCCGCCGACGTGCTGAAGAAAACCATCAAGAACACCCTGGACATCCACAAGTCCAT 1440
      CGACGGGCGGCTGCACGACTTCTTTTGGTAGTTCTTGTGGGACCTGTAGGTGTTCAGGTA

1441  CACCATCAACAACCCCAAAGAAAGCACCGTGTCCGACACCAACGACCACGCCAAGAACAA 1500
      GTGGTAGTTGTTGGGGTTTCTTTCGTGGCACAGGCTGTGGTTGCTGGTGCGGTTCTTGTT
                  XhoI
                   |
1501  CGACACCACCTGACTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGT 1560
      GCTGTGGTGGACTGAGCTCAGATCTCCCGGGCAAATTTGGGCGACTAGTCGGAGCTGACA

1561  GCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA 1620
      CGGAAGATCAACGGTCGGTAGACAACAAACGGGGAGGGGGCACGGAAGGAACTGGGACCT

1621  AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG 1680
      TCCACGGTGAGGGTGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTC

1681  TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA 1740
      ATCCACAGTAAGATAAGACCCCCCACCCCACCCCGTCCTGTCGTTCCCCCTCCTAACCCT

1741  AGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAAC 1800
      TCTGTTATCGTCCGTACGACCCCTACGCCACCCGAGATACCGAAGACTCCGCCTTTCTTG
                                                 f1 origin(1844,2150)
                                                    |
1801  CAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGG 1860
      GTCGACCCCGAGATCCCCCATAGGGGTGCGCGGGACATCGCCGCGTAATTCGCGCCGCCC

1861  TGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT 1920
      ACACCACCAATGCGCGTCGCACTGGCGATGTGAACGGTCGCGGGATCGCGGGCGAGGAAA

1921  CGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCG 1980
      GCGAAAGAAGGGAAGGAAAGAGCGGTGCAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGC
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1981  GGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGA 2040
      CCCCGAGGGAAATCCCAAGGCTAAATCACGAAATGCCGTGGAGCTGGGGTTTTTTGAACT

2041  TTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGAC 2100
      AATCCCACTACCAAGTGCATCACCCGGTAGCGGGACTATCTGCCAAAAAGCGGGAAACTG

2101  GTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCC 2160
      CAACCTCAGGTGCAAGAAATTATCACCTGAGAACAAGGTTTGACCTTGTTGTGAGTTGGG

2161  TATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAA 2220
      ATAGAGCCAGATAAGAAAACTAAATATTCCCTAAAACGGCTAAAGCCGGATAACCAATTT

2221  AAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTA 2280
      TTTACTCGACTAAATTGTTTTTAAATTGCGCTTAATTAAGACACCTTACACACAGTCAAT
      SV40 promoter (2282,2603)
        |
2281  GGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAAT 2340
      CCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTA

2341  TAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGC 2400
      ATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCG
                                          SV40 origin (2449,2526)
                                                    |
2401  ATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTA 2460
      TACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGAT

2461  ACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCA 2520
      TGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGT

2521  GAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGA 2580
      CTCCGGCTCCGGCGGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCT

2581  GGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAA 2640
      CCGGATCCGAAAACGTTTTTCGAGGGCCCTCGAACATATAGGTAAAAGCCTAGACTAGTT
                              Neomycin^R (2665,3459)
                                    |
2641  GAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCG 2700
      CTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGC

2701  GCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCT 2760
      CGGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGA

2761  GATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGAC 2820
      CTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTG

2821  CTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG 2880
      GACAGGCCACGGGACTTACTTGACGTCCTGCTCCGTCGCGCCGATAGCACCGACCGGTGC

2881  ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTG 2940
      TGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGAC
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2941  CTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAA 3000
      GATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTT

3001  GTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCA 3060
      CATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGT

3061  TTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTT 3120
      AAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAA

3121  GTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCC 3180
      CAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGG

3181  AGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGC 3240
      TCCGAGTTCCGCGCGTACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACG

3241  TTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTG 3300
      AACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGAC

3301  GGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTT 3360
      CCACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAA

3361  GGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAG 3420
      CCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTC

3421  CGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAA 3480
      GCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTT

3481  TGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCT 3540
      ACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGA

3541  ATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCG 3600
      TACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGC

3601  GGGATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTT 3660
      CCCTAGAGTACGACCTCAAGAAGCGGGTGGGGTTGAACAAATAACGTCGAATATTACCAA

3661  ACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTA 3720
      TGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGAT

3721  GTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTA 3780
      CAACACCAAACAGGTTTGAGTAGTTACATAGAATAGTACAGACATATGGCAGCTGGAGAT

3781  GCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCA 3840
      CGATCTCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAATAGGCGAGT
               lac promoter (3852,3881)
                  |
3841  CAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAG 3900
      GTTAAGGTGTGTTGTATGCTCGGCCTTCGTATTTCACATTTCGGACCCCACGGATTACTC

3901  TGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGT 3960
      ACTCGATTGAGTGTAATTAACGCAACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACA
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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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3961  CGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGC 4020
      GCACGGTCGACGTAATTACTTAGCCGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCG

4021  GCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGG 4080
      CGAGAAGGCGAAGGAGCGAGTGACTGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCC

4081  TATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA 4140
      ATAGTCGAGTGAGTTTCCGCCATTATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTT
                                                pBR322 origin (4190,4806)
                                                        |
4141  AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGG 4200
      TCTTGTACACTCGTTTTCCGGTCGTTTTCCGGTCCTTGGCATTTTTCCGGCGCAACGACC

4201  CGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA 4260
      GCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCT

4261  GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG 4320
      CCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGC

4321  TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGG 4380
      ACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCC

4381  GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTC 4440
      CTTCGCACCGCGAAAGAGTATCGAGTGCGACATCCATAGAGTCAAGCCACATCCAGCAAG

4441  GCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCG 4500
      CGAGGTTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGC

4501  GTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCA 4560
      CATTGATAGCAGAACTCAGGTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGT

4561  CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGT 4620
      GACCATTGTCCTAATCGTCTCGCTCCATACATCCGCCACGATGTCTCAAGAACTTCACCA

4621  GGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG 4680
      CCGGATTGATGCCGATGTGATCTTCTTGTCATAAACCATAGACGCGAGACGACTTCGGTC

4681  TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCG 4740
      AATGGAAGCCTTTTTCTCAACCATCGAGAACTAGGCCGTTTGTTTGGTGGCGACCATCGC

4741  GTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTT 4800
      CAAAAAAACAAACGTTCGTCGTCTAATGCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAA

4801  TGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGG 4860
      ACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGAGTGCAATTCCCTAAAACC

4861  TCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTA 4920
      AGTACTCTAATAGTTTTTCCTAGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAAAT
                                          Ampicilliln^R (4961,5821)
                                                  |
4921  AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTG 4980
      TTAGTTAGATTTCATATATACTCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCAC
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4981  AGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG 5040
      TCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGC

5041  TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGC 5100
      ACATCTATTGATGCTATGCCCTCCCGAATGGTAGACCGGGGTCACGACGTTACTATGGCG

5101  GAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCG 5160
      CTCTGGGTGCGAGTGGCCGAGGTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGC

5161  AGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGG 5220
      TCGCGTCTTCACCAGGACGTTGAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCCC

5221  AAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAG 5280
      TTCGATCTCATTCATCAAGCGGTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTC

5281  GCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGAT 5340
      CGTAGCACCACAGTGCGAGCAGCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCTA

5341  CAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTC 5400
      GTTCCGCTCAATGTACTAGGGGGTACAACACGTTTTTTCGCCAATCGAGGAAGCCAGGAG

5401  CGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC 5460
      GCTAGCAACAGTCTTCATTCAACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACG

5461  ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAA 5520
      TATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAGACACTGACCACTCATGAGTT

5521  CCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATAC 5580
      GGTTCAGTAAGACTCTTATCACATACGCCGCTGGCTCAACGAGAACGGGCCGCAGTTATG

5581  GGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTT 5640
      CCCTATTATGGCGCGGTGTATCGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAA

5641  CGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTC 5700
      GCCCCGCTTTTGAGAGTTCCTAGAATGGCGACAACTCTAGGTCAAGCTACATTGGGTGAG

5701  GTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAA 5760
      CACGTGGGTTGACTAGAAGTCGTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTT

5761  CAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCA 5820
      GTCCTTCCGTTTTACGGCGTTTTTTCCCTTATTCCCGCTGTGCCTTTACAACTTATGAGT

5821  TACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT 5880
      ATGAGAAGGAAAAAGTTATAATAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTA

5881  ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAA 5940
      TGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTT

5941  AAGTGCCACCTGACGTC 5957
      TTCACGGTGGACTGCAG
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