

Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pCN49, Recombinant in *Escherichia coli

Catalog No. NR-46145

Product Description: NR-46145 is a culture of *Escherichia coli* (*E. coli*) DH5α (RN9609, NRS609) containing the *E. coli*-staphylococcal shuttle vector pCN49. Vector pCN49 contains the *E. coli* ColE1 replication origin, the thermosensitive *Staphylococcus aureus* (*S. aureus*) pT181 *cop-634ts-rep4C* replicon and the *blaZ* transcriptional terminator. Vector pCN49 was deposited as resistant to ampicillin and erythromycin in *E. coli* and resistant to erythromycin in *S. aureus*.

Lot¹: 63025316

Manufacturing Date: 31OCT2014

| TEST | SPECIFICATIONS | RESULTS |
|--|---|---|
| Phenotypic Analysis Cellular morphology Colony morphology ² Motility (wet mount) | Gram-negative rods Report results Report results | Gram-negative rods Circular, low convex, entire and cream (Figure 1) Motile |
| Confirmation of pCN49 Sequence Illumina [®] MiSeq [®] sequence (Figure 2, Table 1) | Report results | Consistent with pCN49 vector description ^{3,4} |
| Antibiotic Resistance Erythromycin (encoded by the adenine methylase gene <i>ermC</i>) Ampicillin (encoded by the beta-lactamase gene <i>amp</i>) | <i>ermC</i> sequence present <i>amp</i> sequence present | <i>ermC</i> sequence confirmed <i>amp</i> sequence confirmed |
| Antibiotic Resistance Ampicillin (100 µg/mL) ² | Resistant (growth) | Growth observed |
| Purity (post-freeze)⁵ | Growth consistent with <i>E. coli</i> | Growth consistent with <i>E. coli</i> |
| Viability (post-freeze)² | Growth | Growth |

¹NR-46145 was produced by inoculation of the deposited material in Luria-Bertani (LB) broth with 100 µg/mL ampicillin and incubated for 21 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm. Broth inoculum was passaged once in LB broth with 100 µg/mL ampicillin for 19 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm to produce this lot.

²23 hours at 37°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin

³Illumina[®] MiSeq[®] sequence was analyzed with CLC Genomics Workbench Version 7.0.2.

⁴pCN49 was sequenced and annotated by BEI Resources and is consistent with the vector described in Charpentier, E., et al. "Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria." *Appl. Environ. Microbiol.* 70 (2004): 6076-6085. PubMed: 15466553. The BEI Resources vector sequence was deposited into GenBank as NR-46145 (GenBank: KR781467).

⁵Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere

Figure 1: Colony Morphology



Date: 27 JUL 2016

Signature:

A handwritten signature in black ink, appearing to read "David C. Archer". The signature is written in a cursive style.

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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Figure 2: Shuttle Vector pCN49

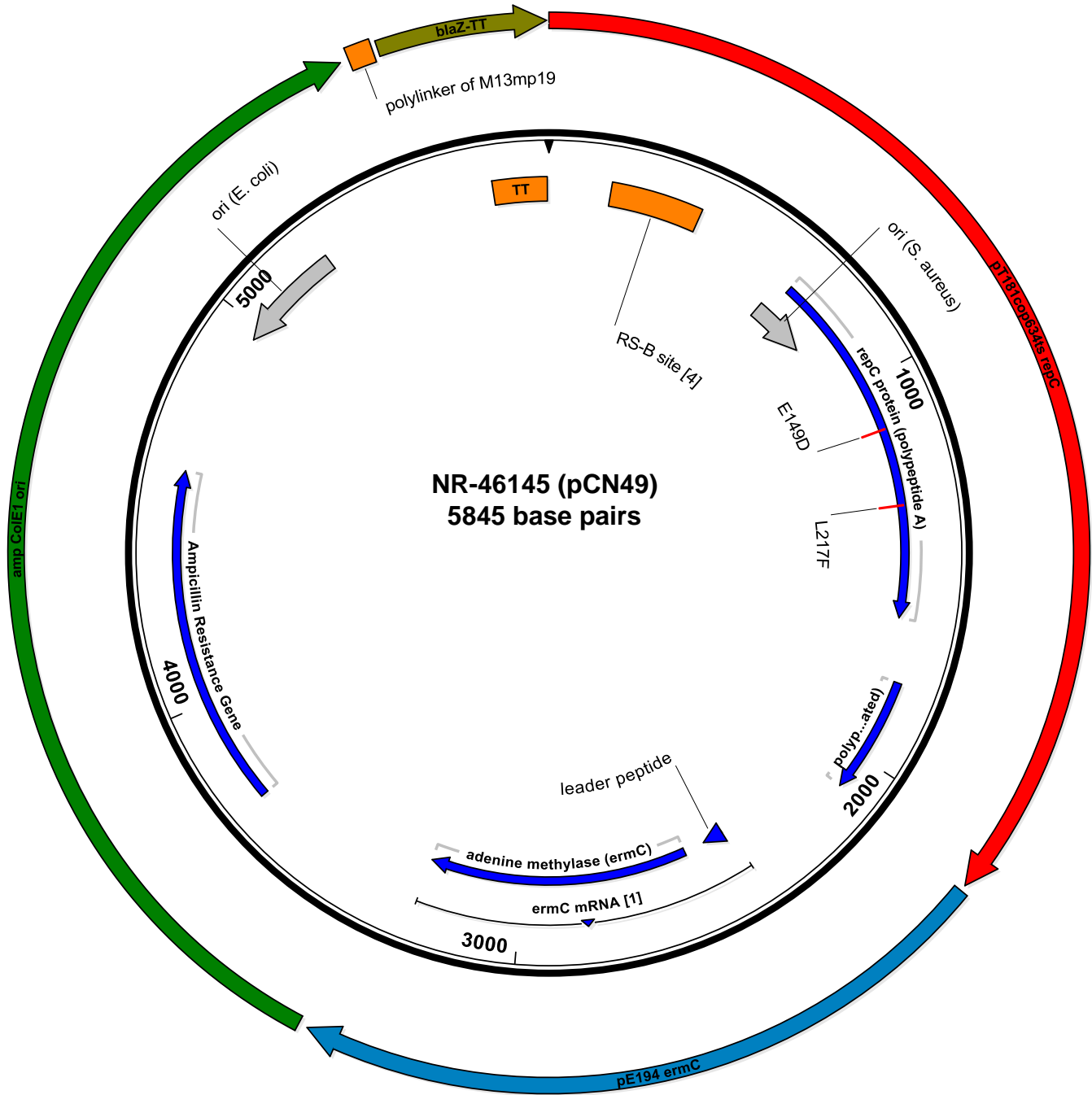


Table 1: Sequence of shuttle vector pCN49

| | | | | | | |
|------|------------|-------------|-------------|-------------|------------|------|
| 1 | CCTTTGCGGA | AAGAGTTAAT | AAGTTAACAG | AAGATGAACC | AAAACTAAAT | 50 |
| 51 | GGTTTAGCAG | GAAACTTAGA | TAAAAAATG | AATCCAGAAT | TATATTCAGA | 100 |
| 101 | ACAGGAACAG | CAACAAGAAC | AACAAAAGAA | TCAAAAACGA | GATAGAGGTA | 150 |
| 151 | TGCACTTATA | GAACATGCAT | TTATGCCGAG | AAAACTTATT | GGTTGGAATG | 200 |
| 201 | GGCTATGTGT | TAGCTAACTT | GTTAGCGAGT | TGGTTGGACT | TGAATTGGGA | 250 |
| 251 | TTAATCCCAA | GAAAGTACCA | ACTCAACAAC | ACATAAAGCC | CTGTAGGTTT | 300 |
| 301 | CGACCAATAA | GGAAATTGGA | ATAAAGCAAT | AAAAGGAGTT | GAAGAAATGA | 350 |
| 351 | AATTCAGAGA | AGCCTTTGAG | AATTTTATAA | CAAGTAAGTA | TGTACTTGGT | 400 |
| 401 | GTTTTAGTAG | TTTTAACTGT | TTACCAGATA | ATACAAATGC | TTAAATAAAA | 450 |
| 451 | AAAGACTTGA | TCTGATTAGA | CCAAATCTTT | TGATAGTGTT | ATATTAATAA | 500 |
| 501 | CAAAATAAAA | AGGAGTCGCT | CACGACCTGA | CCAAAGTTTG | TGAACGACAT | 550 |
| 551 | CATTCAAAGA | AAAAAACACT | GAGTTGTTTT | TATAATCTTG | TATATTTAGA | 600 |
| 601 | TATTAACCGA | TATTTAAATA | TACATCAAGA | TATATATTTG | GGTGAGCGAT | 650 |
| 651 | TCCTTAAACG | AAATTGAGAT | TAAGGAGTCG | ATTTTTTATG | TATAAAAACA | 700 |
| 701 | ATCATGCAAA | TCATTCAAAT | CATTTGGAAA | ATCACGATTT | AGACAATTTT | 750 |
| 751 | TCTAAAACCG | GCTACTCTAA | TAGCCGGTTG | GACGCACATA | CTGTGTGCAT | 800 |
| 801 | ATCTGATCCA | AAATTAAGTT | TTGATGCAAT | GACGATCGTT | GGAAATCTCA | 850 |
| 851 | ACCGAGACAA | CGCTCAAGCC | CTTTCTAAAT | TTATGAGTGT | AGAGCCCCAA | 900 |
| 901 | ATAAGACTTT | GGGATATTCT | TCAAACAAAG | TTTAAAGCTA | AAGCACTTCA | 950 |
| 951 | AGAAAAAGTT | TATATTGAAT | ATGACAAAGT | GAAAGCAGAT | AGTTGGGATA | 1000 |
| 1001 | GACGTAATAT | GCGTATTGAA | TTTAATCCAA | ACAAACTTAC | ACGAGATGAA | 1050 |
| 1051 | ATGATTTGGT | TAAAACAAAA | TATAATAAGC | TACATGGAAG | ATGACGGTTT | 1100 |
| 1101 | TACAAGATTA | GATTTAGCCT | TTGATTTTGA | AGATGATTTG | AGTGACTACT | 1150 |
| 1151 | ATGCAATGTC | TGATAAAGCA | GTTAAGAAAA | CTATTTTTTA | TGGTCGTAAT | 1200 |
| 1201 | GGTAAGCCAG | AAACAAAATA | TTTTGGCGTG | AGAGATAGTA | ATAGATTTAT | 1250 |
| 1251 | TAGAATTTAT | AATAAAAAAGC | AAGAACGTAA | AGATAATGCA | GATGCTGAAG | 1300 |
| 1301 | TTATGTCTGA | ACATTTATGG | CGTGTAGAAA | TCGAATTTAA | AAGAGATATG | 1350 |
| 1351 | GTGGATTACT | GGAATGATTG | CTTTAGTGAT | TTACATATCT | TGCAACCAGA | 1400 |
| 1401 | TTGGAAAACT | ATCCAACGCA | CTGCGGATAG | AGCAATAGTT | TTTATGTTAT | 1450 |
| 1451 | TGAGTGATGA | AGAAGAATGG | GGAAAGCTTC | ACAGAAATTC | TAGAACAAAA | 1500 |
| 1501 | TATAAGAATT | TGATAAAAAGA | AATTTTCGCCA | GTCGATTTAA | CGGACTTAAT | 1550 |
| 1551 | GAAATCGACT | TTAAAAGCGA | ACGAAAAACA | ATTGCAAAAA | CAAATCGATT | 1600 |
| 1601 | TTTGCCAACA | TGAATTTAAA | TTTTGGAAAT | AGTGTACATA | TTAATATTAC | 1650 |
| 1651 | TGAACAAAAA | TGATATATTT | AAACTATTCT | AATTTAGGAG | GATTTTTTTA | 1700 |
| 1701 | TGAAGTGTCT | ATTTAAAAAT | TTGGGGAATT | TATATGAGGT | GAAAGAATAA | 1750 |
| 1751 | TTTACCCCTA | TAAACTTTAG | TCACCTCAAG | TAAAGAGGTA | AAATTGTTTA | 1800 |
| 1801 | GTTTATATAA | AAAATTTAAA | GGTTTGTTTT | ATAGCGTTTT | ATTTTGCTT | 1850 |
| 1851 | TGTATTCTTT | CATTTTTTAG | TGTATTAAT | GAAATGGTTT | TAAATGTTTC | 1900 |
| 1901 | TTTACCTGAT | ATTGCAAATC | ATTTTAATAC | TACTCCTGGA | ATTACAAACT | 1950 |
| 1951 | GGGTAAACAC | TGCATATATG | TTAACTTTTT | CGATAGGAAC | AGCAGTATAT | 2000 |
| 2001 | GGAAAATTAT | CTGATTATAT | AAATATAAAA | AAATTGTTAA | TTATTGGTAT | 2050 |
| 2051 | TAGTTTGAGC | TGCTTTGGTT | CATTGATTGC | TTTTATTGGG | CCCACCTAGG | 2100 |
| 2101 | AATTGAATGA | GACATGCTAC | ACCTCCGGAT | AATAAATATA | TATAAACGTA | 2150 |
| 2151 | TATAGATTTT | ATAAAGTCTA | ACACACTAGA | CTTATTTACT | TCGTAATTAA | 2200 |
| 2201 | GTCGTTAAAC | CGTGTGCTCT | ACGACCAAAA | CTATAAAAACC | TTTAAGAACT | 2250 |
| 2251 | TTCTTTTTTT | ACAAGAAAAA | AGAAATTAGA | TAAATCTCTC | ATATCTTTTA | 2300 |
| 2301 | TTCAATAATC | GCATCCGATT | GCAGTATAAA | TTTAACGATC | ACTCATCATG | 2350 |
| 2351 | TTCATATTTA | TCAGAGCTCG | TGCTATAAAT | ATACTAATTT | TATAAGGAGG | 2400 |
| 2401 | AAAAAATATG | GGCATTTTTT | GTATTTTTGT | AATCAGCACA | GTTTATTATC | 2450 |
| 2451 | AACCAAACAA | AAAATAAGTG | GTTATAATGA | ATCGTTAATA | AGCAAATTC | 2500 |
| 2501 | ATATAACCAA | ATTAAAGAGG | GTTATAATGA | ACGAGAAAAA | TATAAACAC | 2550 |
| 2551 | AGTCAAAACT | TTATTACTTC | AAAACATAAT | ATAGATAAAA | TAATGACAAA | 2600 |

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|------|-------------|-------------|-------------|-------------|-------------|------|
| 2601 | TATAAGATTA | AATGAACATG | ATAATATCTT | TGAAATCGGC | TCAGGAAAAG | 2650 |
| 2651 | GCCATTTTAC | CCTTGAATTA | GTAAAGAGGT | GTAATTTTCGT | AACTGCCATT | 2700 |
| 2701 | GAAATAGACC | ATAAATTATG | CAAAACTACA | GAAAATAAAC | TTGTTGATCA | 2750 |
| 2751 | CGATAAATTC | CAAGTTTTAA | ACAAGGATAT | ATTGCAGTTT | AAATTTCCCTA | 2800 |
| 2801 | AAAACCAATC | CTATAAAATA | TATGGTAATA | TACCTTATAA | CATAAGTACG | 2850 |
| 2851 | GATATAATAC | GCAAAAATTGT | TTTTTGATAGT | ATAGCTAATG | AGATTTATTTT | 2900 |
| 2901 | AATCGTGGAA | TACGGGTTTG | CTAAAAGATT | ATTAAATACA | AAACGCTCAT | 2950 |
| 2951 | TGGCATTACT | TTTAATGGCA | GAAGTTGATA | TTTCTATATT | AAGTATGGTT | 3000 |
| 3001 | CCAAGAGAAT | ATTTTCATCC | TAAACCTAAA | GTGAATAGCT | CACTTATCAG | 3050 |
| 3051 | ATTAAGTAGA | AAAAAATCAA | GAATATCACA | CAAAGATAAA | CAAAAGTATA | 3100 |
| 3101 | ATTATTTTCGT | TATGAAATGG | GTTAACAAAG | AATACAAGAA | AATATTTTACA | 3150 |
| 3151 | AAAAATCAAT | TTAACAATTC | CTTAAAACAT | GCAGGAATTG | ACGATTTTAAA | 3200 |
| 3201 | CAATATTAGC | TTTGAACAAT | TCTTATCTCT | TTTCAATAGC | TATAAATTAT | 3250 |
| 3251 | TTAATAAGTA | AGTTAAGGGA | TGCATAAACT | GCATCCCTTA | ACTTGTTTTTT | 3300 |
| 3301 | CGTGTGCCA | TTTTTTGTGA | ATCGATTATG | TCTTTTGC | AGTCGGCTTA | 3350 |
| 3351 | AACCAGTTTT | CCGCGGCGCT | CGAGCGGCCG | CATAGTTAAG | CCAGCCCCGA | 3400 |
| 3401 | CACCCGCCAA | CACCCGCTGA | CGCGCCCTGA | CGGGCTTGTC | TGCTCCCGGC | 3450 |
| 3451 | ATCCGCTTAC | AGACAAGCTG | TGACCGTCTC | CGGGAGCTGC | ATGTGTCAGA | 3500 |
| 3501 | GGTTTTCCAC | GTCATCACCG | AAACGCGCGA | GACGAAAGGG | CCTCGTGATA | 3550 |
| 3551 | CGCCTATTTT | TATAGCTTAA | TGTCATGATA | ATAATGGTTT | CTTAGACGTC | 3600 |
| 3601 | AGGTGGCACT | TTTCGGGGAA | ATGTGCGCGG | AACCCCTATT | TGTTTTATTTT | 3650 |
| 3651 | TCTAAATACA | TTCAAATATG | TATCCGCTCA | TGAGACAATA | ACCCTGATAA | 3700 |
| 3701 | ATGCTTCAAT | AATATTGAAA | AAGGAAGAGT | ATGAGTATTC | AACATTTCCG | 3750 |
| 3751 | TGTCGCCCTT | ATTCCCTTTT | TTGCGGCATT | TTGCCTTCCT | GTTTTTGCTC | 3800 |
| 3801 | ACCCAGAAAC | GCTGGTGAAA | GTAAAAGATG | CTGAAGATCA | GTTGGGTGCA | 3850 |
| 3851 | CGAGTGGGTT | ACATCGAACT | GGATCTCAAC | AGCGGTAAGA | TCCTTGAGAG | 3900 |
| 3901 | TTTTTCGCC | GAAGAACGTT | TTCCAATGAT | GAGCACTTTT | AAAGTTCTGC | 3950 |
| 3951 | TATGTGGCGC | GGTATTATCC | CGTATTGACG | CCGGGCAAGA | GCAACTCGGT | 4000 |
| 4001 | CGCCGCATAC | ACTATTCTCA | GAATGACTTG | GTTGAGTACT | CACCAGTCAC | 4050 |
| 4051 | AGAAAAGCAT | CTTACGGATG | GCATGACAGT | AAGAGAATTA | TGCAGTGCTG | 4100 |
| 4101 | CCATAACCAT | GAGTGATAAC | ACTGCGGCCA | ACTTACTTCT | GACAACGATC | 4150 |
| 4151 | GGAGGACCGA | AGGAGCTAAC | CGCTTTTTTTG | CACAACATGG | GGGATCATGT | 4200 |
| 4201 | AACTCGCCTT | GATCGTTGGG | AACCGGAGCT | GAATGAAGCC | ATACCAAACG | 4250 |
| 4251 | ACGAGCGTGA | CACCACGATG | CCTGTAGCAA | TGGCAACAAC | GTTGCGCAAA | 4300 |
| 4301 | CTATTAAGTG | GCGAACTACT | TACTCTAGCT | TCCCGGCAAC | AATTAATAGA | 4350 |
| 4351 | CTGGATGGAG | GCGGATAAAG | TTGCAGGACC | ACTTCTGCGC | TCGGCCCTTC | 4400 |
| 4401 | CGGCTGGCTG | GTTTATTGCT | GATAAATCTG | GAGCCGGTGA | GCGTGGGTCT | 4450 |
| 4451 | CGCGGTATCA | TTGCAGCACT | GGGCGAGAT | GGTAAGCCCT | CCCGTATCGT | 4500 |
| 4501 | AGTTATCTAC | ACGACGGGGA | GTCAGGCAAC | TATGGATGAA | CGAAATAGAC | 4550 |
| 4551 | AGATCGCTGA | GATAGGTGCC | TCACTGATTA | AGCATTGGTA | ACTGTCAGAC | 4600 |
| 4601 | CAAGTTTACT | CATATATACT | TTAGATTGAT | TTAAAACCTC | ATTTTTTAATT | 4650 |
| 4651 | TAAAAGGATC | TAGGTGAAGA | TCCTTTTTTGA | TAATCTCATG | ACCAAAAATCC | 4700 |
| 4701 | CTTAACGTGA | GTTTTTCGTTT | CACTGAGCGT | CAGACCCCGT | AGAAAAGATC | 4750 |
| 4751 | AAAGGATCTT | CTTGAGATCC | TTTTTTTTCTG | CGCGTAATCT | GCTGCTTGCA | 4800 |
| 4801 | AACAAAAAAA | CCACCCTACT | CAGCGGTGGT | TTGTTTGCCG | GATCAAGAGC | 4850 |
| 4851 | TACCAACTCT | TTTTCCGAAG | GTAACCTGGT | TCAGCAGAGC | GCAGATACCA | 4900 |
| 4901 | AATACTGTTC | TTCTAGTGTA | GCCGTAGTTA | GGCCACCACT | TCAAGAACTC | 4950 |
| 4951 | TGTAGCACCG | CCTACATACC | TCGCTCTGCT | AATCCTGTTA | CCAGTGGCTG | 5000 |
| 5001 | CTGCCAGTGG | CGATAAGTCG | TGTCTTACCG | GGTTGGACTC | AAGACGATAG | 5050 |
| 5051 | TTACCGGATA | AGGCGCAGCG | GTCGGGCTGA | ACGGGGGGTT | CGTGACACACA | 5100 |
| 5101 | GCCCAGCTTG | GAGCGAACGA | CCTACACCGA | ACTGAGATAC | CTACAGCGTG | 5150 |
| 5151 | AGCTATGAGA | AAGCGCCACG | CTTCCCAGAG | GGAGAAAGGC | GGACAGGTAT | 5200 |
| 5201 | CCGGTAAGCG | GCAGGGTCGG | AACAGGAGAG | CGCACGAGGG | AGCTTCCAGG | 5250 |

| | | | | | | |
|------|------------|------------|------------|------------|------------|------|
| 5251 | GGGAAACGCC | TGGTATCTTT | ATAGTCCTGT | CGGGTTTCGC | CACCTCTGAC | 5300 |
| 5301 | TTGAGCGTCG | ATTTTTGTGA | TGCTCGTCAG | GGGGCGGAG | CCTATGGAAA | 5350 |
| 5351 | AACGCCAGCA | ACGCGGCCTT | TTTACGGTTC | CTGGCCTTTT | GCTGGCCTTT | 5400 |
| 5401 | TGCTCACATG | TTCTTTCCTG | CGTTATCCCC | TGATTCTGTG | GATAACCGTA | 5450 |
| 5451 | TTACCGCCTT | TGAGTGAGCT | GGCGGCCGCT | GCATGCCTGC | AGGTCGACTC | 5500 |
| 5501 | TAGAGGATCC | CCGGGTACCG | AGCTCGAATT | CAGGCGCGCC | TATTCTAAAT | 5550 |
| 5551 | GCATAATAAA | TACTGATAAC | ATCTTATATT | TTGTATTATA | TTTTGTATTA | 5600 |
| 5601 | TCGTTGACAT | GTATAATTTT | GATATCAAAA | ACTGATTTTC | CCTCTATTAT | 5650 |
| 5651 | TTTCGAGATT | TATTTTCTTA | ATTCTCTTTA | ACAAACTAGA | AATATTGTAT | 5700 |
| 5701 | ATACAAAAAA | TTATAAATAA | TAGATGAATA | GTTTAATTAT | AGGTGTTTAT | 5750 |
| 5751 | CAATCGAAAA | AGCAACGTAT | CTTATTTAAA | GTGCGTTGCT | TTTTTCTCAT | 5800 |
| 5801 | TTATAAGGTT | AAATAATTCT | CATATATCAA | GCAAAGTGAC | AGGCG | 5845 |