

***Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pCN54, Recombinant in *Escherichia coli***

**Catalog No. NR-46154**

**Product Description:** NR-46154 is a culture of *Escherichia coli* (*E. coli*) DH5α (RN9619, NRS619) containing the *E. coli*-staphylococcal shuttle vector pCN54. Vector pCN54 contains the *E. coli* ColE1 replication origin and the *Staphylococcus aureus* (*S. aureus*) pT181 *cop-wt-repC* replicon, the cadmium-inducible promoter  $P_{cad-cadC}$ , the GFP reporter gene *gfpmut2* and the *blaZ* transcriptional terminator. Vector pCN54 was deposited as resistant to ampicillin and erythromycin in *E. coli* and resistant to erythromycin in *S. aureus*.

**Lot<sup>1</sup>: 63381374**

**Manufacturing Date: 19MAR2015**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis</b> Cellular morphology Colony morphology <sup>2</sup>  Motility (wet mount)	Gram-negative rods Report results  Report results	Gram-negative rods Circular, slight peaked, entire and cream (Figure 1) Motile
<b>Confirmation of pCN54 Sequence</b> Illumina <sup>®</sup> MiSeq <sup>®</sup> sequence (Figure 2, Table 1)	Report results	Consistent with pCN54 vector description <sup>3,4</sup>
<b>Antibiotic Resistance</b> 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
<b>Antibiotic Resistance</b> Ampicillin (100 µg/mL) <sup>2</sup>	Resistant (growth)	Growth observed
<b>Purity (post-freeze)<sup>5</sup></b>	Growth consistent with <i>E. coli</i>	Growth consistent with <i>E. coli</i>
<b>Viability (post-freeze)<sup>2</sup></b>	Growth	Growth

<sup>1</sup>NR-46154 was produced by inoculation of the deposited material in Luria-Bertani (LB) broth with 100 µg/mL ampicillin and incubated for 20 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 22 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm to produce this lot.

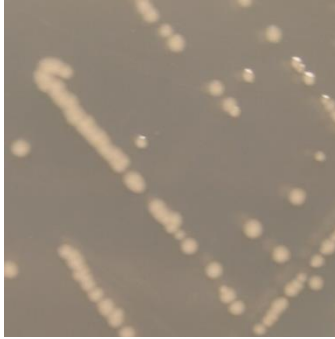
<sup>2</sup>23 hours at 37°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin

<sup>3</sup>Illumina<sup>®</sup> MiSeq<sup>®</sup> sequence was analyzed with CLC Genomics Workbench Version 7.0.2.

<sup>4</sup>pCN54 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-46154 (GenBank: KR781470).

<sup>5</sup>Purity 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: 28 JUL 2016

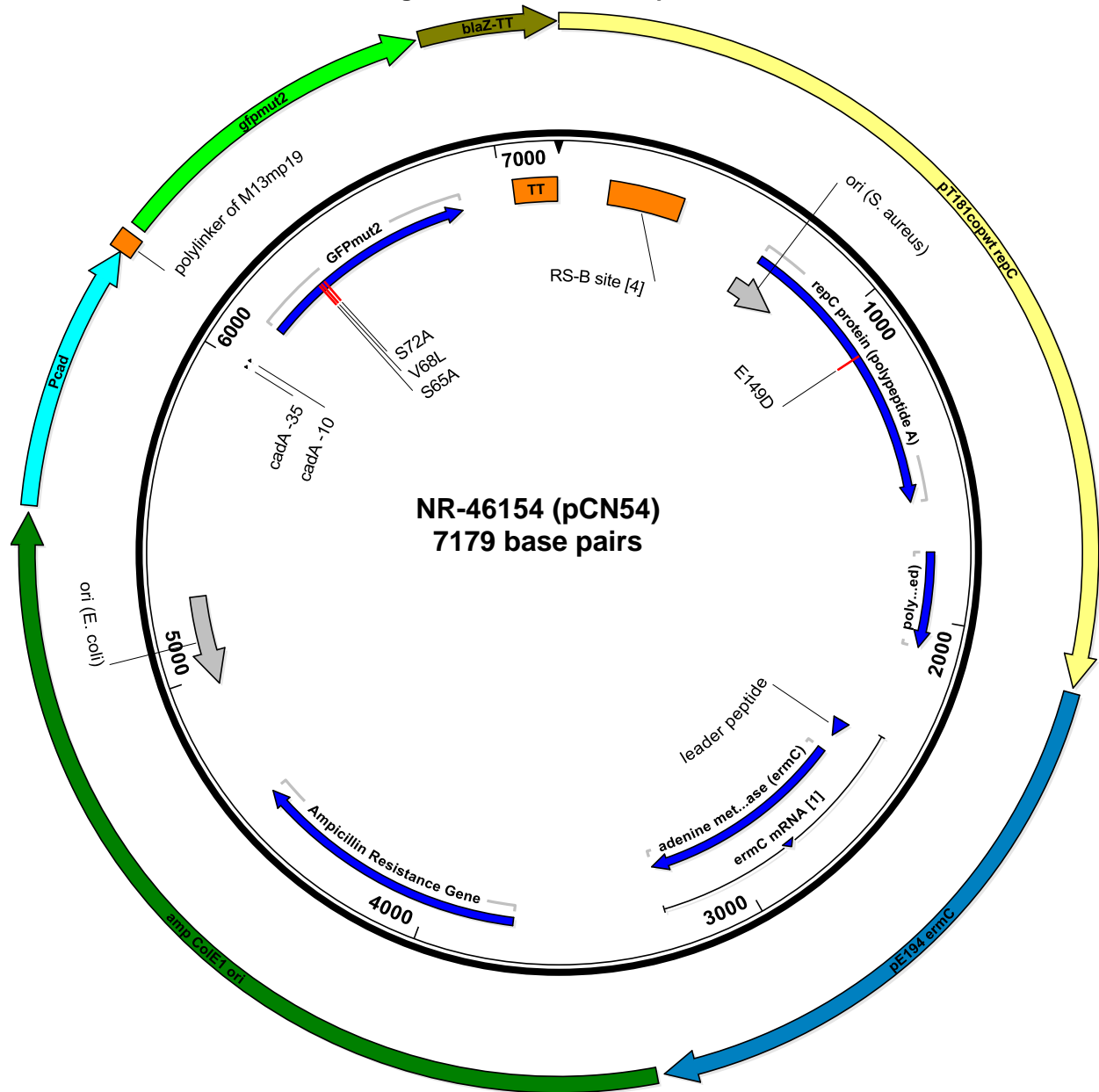
Signature:   
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Figure 2: Shuttle Vector pCN54



**Table 1: Sequence of shuttle vector pCN54**

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	TGTACTIONGGT	400
401	GTTTTAGTAG	TTTTAACTGT	TTACCAGATA	ATACAAATGC	TTAAATAAAAA	450
451	AAAGACTTGA	TCTGATTAGA	CCAAATCTTT	TGATAGTGTT	ATATTAATAA	500
501	CAAAATAAAA	AGGAGTCGCT	CACGCCCTGA	CCAAAGTTTG	TGAACGACAT	550
551	CATTCAAAGA	AAAAAACACT	GAGTTGTTTT	TATAATCTTG	TATATTTAGA	600
601	TATTAACCGA	TATTTAAATA	TACATCAAGA	TATATATTTG	GGTGAGCGAT	650
651	TCCTTAAACG	AAATTGAGAT	TAAGGAGTCG	ATTTTTTATG	TATAAAAAACA	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	TTTAAATCCAA	ACAAACTTAC	ACGAGATGAA	1050
1051	ATGATTTGGT	TAAAACAAAA	TATAATAAGC	TACATGGAAG	ATGACGGTTT	1100
1101	TACAAGATTA	GATTTAGCCT	TTGATTTTGA	AGATGATTTG	AGTGACTACT	1150
1151	ATGCAATGTC	TGATAAAGCA	GTTAAGAAAA	CTATTTTTTTA	TGGTCGTAAT	1200
1201	GGTAAGCCAG	AAACAAAATA	TTTTGGCGTG	AGAGATAGTA	ATAGATTTAT	1250
1251	TAGAATTTAT	AATAAAAAAGC	AAGAACGTAA	AGATAATGCA	GATGCTGAAG	1300
1301	TTATGTCTGA	ACATTTATGG	CGTGTAGAAA	TCGAACTTAA	AAGAGATATG	1350
1351	GTGGATTACT	GGAATGATTG	CTTTAGTGAT	TTACATATCT	TGCAACCAGA	1400
1401	TTGGAAAAC	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	GATTTTTTTTA	1700
1701	TGAAGTGTCT	ATTTAAAAAAT	TTGGGGAAAT	TATATGAGGT	GAAAGAATAA	1750
1751	TTTACCCCTA	TAAACTTTAG	TCACCTCAAG	TAAAGAGGTA	AAATTGTTTA	1800
1801	GTTTATATAA	AAAATTTAAA	GGTTTGTTTT	ATAGCGTTTT	ATTTTGGCTT	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	TTTAAACGATC	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

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	TTTTGATAGT	ATAGCTAATG	AGATTTATTT	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	AATATTTACA	3150
3151	AAAAATCAAT	TTAACAATTC	CTTAAAACAT	GCAGGAATTG	ACGATTTAAA	3200
3201	CAATATTAGC	TTTGAACAAT	TCTTATCTCT	TTTCAATAGC	TATAAATTAT	3250
3251	TTAATAAGTA	AGTTAAGGGA	TGCATAAACT	GCATCCCTTA	ACTTGTTTTT	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
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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
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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	GGGCGCAGAT	GGTAAGCCCT	CCCGTATCGT	4500
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4651	TAAAAGGATC	TAGGTGAAGA	TCCTTTTTTGA	TAATCTCATG	ACCAAAATCC	4700
4701	CTTAACGTGA	GTTTTCGTTC	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
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5351	AACGCCAGCA	ACGCGGCCTT	TTTACGGTTC	CTGGCCTTTT	GCTGGCCTTT	5400
5401	TGCTCACATG	TTCTTTCCTG	CGTTATCCCC	TGATTCTGTG	GATAACCGTA	5450
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5501	GTATTTTTTA	ATAAATTATT	TTACTTATTG	AAATGTATTA	TTTTCTAATG	5550
5551	TCATACCCTG	GTCAAAACCG	TTCGTTTTTG	AGACTAGAAT	TTTATGCCCT	5600
5601	ACTTACTTCT	TTTATTTTCA	TTCAAATATT	TGCTTGCATG	ATGAGTCGAA	5650
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5701	TATGAAAAAG	AAAGATACTT	GTGAAATTTT	TTGTTATGAC	GAAGAAAAGG	5750
5751	TTAATCGAAT	ACAAGGGGAT	TTACAAACAG	TTGATATTTT	TGGTGTTAGC	5800
5801	CAAATTTTAA	AGGCTATTGC	CGATGAAAAT	AGAGCAAAAA	TTACTTACGC	5850
5851	TCTGTGTCAG	GATGAAGAGT	TGTGTGTTTG	TGATATAGCA	AATATCTTAG	5900
5901	GTGTTACGAT	AGCAAATGCA	TCTCATCATT	TACGTACGCT	TTATAAGCAA	5950
5951	GGGGTGGTCA	ACTTTAGAAA	AGAAGGAAAA	CTAGCTTTAT	ATTCTTTAGG	6000
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6051	AAGTGAAGGT	CAATGTCTGA	ACCTGCAGGT	CGACTCTAGA	GGATCCCCGG	6100
6101	GTACCGAGCT	CGAATTCGTT	AACTAATTAA	TTTAAGAAGG	AGATATACAT	6150
6151	ATGAGTAAAG	GAGAAGAACT	TTTCACTGGA	GTTGTCCCAA	TTCTTGTGTA	6200
6201	ATTAGATGGT	GATGTTAATG	GGCACAAATT	TTCTGTCACT	GGAGAGGGTG	6250
6251	AAGGTGATGC	AACATACGGA	AAACTTACCC	TTAAATTTAT	TTGCACTACT	6300
6301	GGAAAACACT	CTGTTCCATG	GCCAACACTT	GTCACTACTT	TCGCGTATGG	6350
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6801	CATGGTCCTT	CTTGAGTTTG	TAACAGCTGC	TGGGATTACA	CATGGCATGG	6850
6851	ATGAACTATA	CAAATAAGGC	GCGCCTATTC	TAATGCATAA	TAAATACTGA	6900
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7051	ATAATAGATG	AATAGTTTAA	TTATAGGTGT	TCATCAATCG	AAAAAGCAAC	7100
7101	GTATCTTATT	TAAAGTGCCT	TGCTTTTTTC	TCATTTATAA	GGTTAAATAA	7150
7151	TTCTCATATA	TCAAGCAAAG	TGACAGGCG			7179