

### A correction in the nucleotide sequence of the Tn903 kanamycin resistance determinant in pUC4K

Laura A. Taylor and Ronald E. Rose

Calgene Inc., 1920 Fifth St., Davis, CA 95616, USA  
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pUC4K is a vector that carries the kanamycin resistance determinant from Tn903 (1) and has been used not only in the construction of new vectors but also as a restriction mobilization element to create codon insertions of varying length and location in cloned genes (1). pUC4K was originally constructed by cloning a 1.4 kb *Hae*III fragment from Tn903 into the *Pst*I site of pUC7 by G-C tailing (1). The kanamycin resistance gene contained within the *Hae*III fragment should be flanked by 226 bp inverted repeats (2), but in the process of constructing pUC kanamycin derivatives we have discovered that one of these inverted repeats is missing. By sequencing through each of the G-C tails into the kanamycin resistance determinant we found that the inverted repeat at the 5' end of the gene had been entirely deleted except for 6 bp. Initially we thought this deletion was unique to our isolate of pUC4K, but pUC4K from other sources (Pharmacia, colleagues) contained the same deletion. Thus, the Tn903 fragment contained in pUC4K extends from bases 1052 to 2264 instead of bases 831 to 2264 as originally suggested (2). The deletion appears to have occurred during the initial construction of pUC4K, perhaps by tailing at a nick at base 1052.

		EcoRI	BamHI	Sali	PstI	G-TAIL	
Universal Primer	---->	GGCCA	GTGAATTC	GGGATCCG	GACCTGCAGG	GGGGGGGGG	433
<u>CGCTGAGGTC</u>	<u>TGCCTCGTGA</u>	<u>AGAAGGTGTT</u>	<u>GCTGACTCAT</u>	<u>ACCAGGCCCTG</u>	<u>AATCGCCCCA</u>	<u>TCATCCAGCC</u>	503
<u>AGAAAGTGTG</u>	<u>GGAGCCACGG</u>	<u>TTGATGAGAG</u>	<u>CTTGTGTTGA</u>	<u>GGTGGACCAG</u>	<u>TTGGTGATTT</u>	<u>TGAACTTTTG</u>	573
CTTTGCCACG	GAACGGTCTG	CGTTGTCCGG	AAGATGCGTG	ATCTGATCCT	TCAACTCAGC	AAAAGTTCGA	643
TTTATTCAAC	AAAGCCGCCG	TCCCCTCAAG	TCAGCGTAAT	GCTCTGCCAG	TGTTACAACC	AATTAACCAA	713
TTCTGATTAG	AAAAACTCAT	CGAGCATCAA	ATGAACTGC	AATTTATTCA	TATCAGGATT	ATCAATACCA	783
TATTTTTGAA	AAAGCCGTTT	CTGTAATGAA	GGAGAAACT	CACCGAGGCA	GTTCCATAGG	ATGGCAAGAT	853
CCTGGTATCG	GTCGTGCGATT	CCGACTCGTC	CAACATCAAT	ACAACCTATT	AATTTCCCTT	CGTCAAAAAT	923
AAGGTTATCA	AGTGAGAAAT	CACCATGAGT	GACGACTGAA	TCCGGTGA	ATGGCAAAAG	CTTATGCATT	993
TCTTTCCAGA	CTTGTTCAC	AGGCCAGCCA	TTACGTCGT	CATCAAAATC	ACTCGCATCA	ACCAAACCGT	1063
TATTCATTCG	TGATTGCGCC	TGAGCGAGAC	GAAATACCGC	ATCGCTGTTA	AAAGGACAAT	TACAACACGG	1133
AATCGAATGC	AACCGGCCA	GGAACTCTGC	CAGCGCATCA	ACAATATTTT	CACCTGAATC	AGGATATTC	1203
TCTAATACCT	GGAATGCTGT	TTTCCCAGGG	ATCGCAGTGG	TGAGTAACCA	TGCATCATCA	GGAGTACGGA	1273
TAAATGCTT	GATGGTCGGA	AGAGGCATAA	ATTCCGTGAG	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	1343
ATCATTGGCA	ACGCTACCTT	TGCCATGTTT	CAGAAACAAC	TCTGGCGCAT	CGGGCTTCCC	ATACAATCGA	1413
TAGATTGTGC	CACCTGATTG	CCCAGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA	GCATCCATGT	1483
<u>TGGAAATTTAA</u>	<u>TCCGGGCTC</u>	<u>GAGCAAGAGG</u>	<u>TTTCCGTTG</u>	<u>AATATGGCTC</u>	<u>ATAACACCCC</u>	<u>TTGTATTACT</u>	1553
<u>GTTTATGTAA</u>	<u>GCAGACAGTT</u>	<u>TTATTGTTCA</u>	<u>TGATGATATA</u>	<u>TTTTTATCTT</u>	<u>GTGCAATGTA</u>	<u>ACATCAGAGA</u>	1623
<u>TTTTGAGACA</u>	<u>CAACGTGGCT</u>	<u>TTCCCCCCC</u>	<u>CCCCTGCAGG</u>	<u>TCCAGGATCC</u>	<u>GGGGAATTC</u>	<u>GTAATCA&lt;---B-GAL</u>	
		C-TAIL	PstI	Sali	BamHI	EcoRI	

The sequence above is a portion of pUC4K comprising the linker and the kanamycin region. We sequenced from nucleotide 400 to 572 and from 1477 to 1671 (underlined); the rest of the sequence was taken from the published sequence of the Tn903 kanamycin gene (2) and from the published sequence of pUC19 (3). Bases 434 and 1645 of pUC4K correspond to bases 1052 and 2264 of the published Tn903 sequence respectively (2). Bases 395 and 1683 of the pUC4K linker region correspond to bases 395 and 455 of the published pUC19 sequence respectively (3).

#### REFERENCES

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