Table 1. Antibiotic Resistance Cassettes Useful for Gene Replacement in *Escherichia coli*

Antibiotic	Gene(s)	Priming Sequences[a]	Antibiotic Conc. (μg/mL)[b]	Cassette Length (bp)[c]
Ampicillin	Tn*3 bla*	5′-CGCGGAACCCCTATTTGTTT-3′ 5′-GGTCTGACAGTTACCAATGC-3′	50	957
Chloramphenicol	Tn*9 cat*	5′-ATGAGACGTTGATCGGCACG-3′ 5′-ATTCAGGCGTAGCACCAGGC-3′	10	824
Kanamycin	Tn*903 aph*	5′-CACGTTGTGTCTCAAAATCTC-3′ 5′-TACAACCAATTAACCAATTCTG-3′	20	944
Tetracycline	Tn*10 tetRA*	5′-CTCGACATCTTGGTTACCGT-3′ 5′-CGCGGAATAACATCATTTGG-3′	15	1996
Spectinomycin	Tn*21 aadA1*	5′-AAACGGATGAAGGCACGAA-3′ 5′-TTATTTGCCGACTACCTTGG-3′	20[d]	1080
Gentamicin	Tn*1696 aacC1*	5′-CGAATCCATGTGGGAGTTTA-3′ 5′-TTAGGTGGCGGTACTTGGGT-3′	10	616

[a]Primers with 5′ attachments targeting the recipient chromosome can be used to amplify the cassette from any strain containing the transposon, or a cloned segment, except in the case of the gentamicin cassette, which is described in this paper. Common cloning vector plasmids can be used as templates for making most of the natural cassettes. For example, we have used the priming sequences indicated here to amplify the Tn*3 bla* gene from pBR322 and the Tn*21 aadA1* gene from pGB2 (7). The indicated primers should work with the Tn*9 cat* gene in pACYC184 and the Tn*903 aph* gene in pACYC177.
[b]Antibiotics are added to LB agar (per liter: 10 g tryptone, 5 g yeast extract, 5 g NaCl, 1 mL 1 M NaOH, and 15 g agar). The concentrations given are for freshly poured plates.
[c]Length of the PCR product, exclusive of targeting flanks.
[d]The *aadA* cassette also confers resistance to 20 μg/mL streptomycin, but can be selected with spectinomycin in a strain bearing a strongly streptomycin-resistant chromosomal *rpsL* allele.