

pMK3 and pMK4 Revisited:

A Closer Look at Two First Generation Shuttle Vectors*

Introduction. During the Recombinant DNA Era of *Bacillus* genetics, powerful new techniques for cloning, sequencing, mutating, and analyzing the expression of *Bacillus* genes provided deep insights into the physiology, developmental biology, and commercial potential of these bacteria. We can define this era as beginning in 1977 with the first reports of cloned *B. subtilis* genes (49, 82) and ending in 1997 with the publication of the *B. subtilis* genome sequence (43). Throughout this period, one primary emphasis was the construction of cloning vectors. Most “first generation” vectors have been superseded by increasingly sophisticated tools developed for ever more specialized purposes. It is easy, then, to overlook a group of vectors that date back to the early years of recombinant DNA technology yet continue to serve a useful function today—the general purpose shuttle vectors. These simple chimeric plasmids, capable of replication in both *Escherichia coli* and gram-positive bacteria, remain an important tool in the Genomics Era.

Two of the most useful shuttle vectors dating from this first generation are pMK3 and pMK4, developed in 1984 by Mark Sullivan, Ron Yasbin, and Frank Young at the University of Rochester (93). Their goal was to create plasmids that facilitated the rapid detection of cloned inserts in *E. coli* by means of the “blue-white” alpha complementation system and also supported replication in *B. subtilis* and other gram-positives. For an *E. coli* replicon, the Rochester group chose pUC9, which had been described two years earlier by Vieira and Messing (105). To supply replication functions and antibiotic resistance genes that function in gram-positives, the group chose two *Staphylococcus aureus* plasmids that had previously been introduced into *B. subtilis*, pUB110 and pC194 (33). The construction strategy was simple: partial Sau3AI digests of pUC9 were ligated with either a partial Sau3AI digest of pC194 or with the single BamHI fragment of pUB110. The fusion of pUC9 with pUB110 yielded pMK3, a 7.2 kb vector that specified ampicillin resistance in *E. coli* and kanamycin resistance (30 µg/ml) in *B. subtilis*. The fusion of pUC9 with pC194 yielded pMK4, a 5.6 kb vector that likewise specified ampicillin resistance in *E. coli* but chloramphenicol resistance (10 µg/ml) in *B. subtilis*. Both plasmids allowed for rapid detection of inserts in *E. coli* by a simple colony color screen on X-gal plates or MacConkey agar. In the absence of antibiotic selection in *B. subtilis*, pMK4 showed a modest structural instability (10-25% loss in ten generations), while pMK3 was completely stable.

DNA Sequences. To gain more insight into these vectors, we determined their DNA sequences. The annotated data have been accessioned in GenBank (EU549779 for pMK3 and EU549779 for pMK4). A physical and restriction map for each plasmid is given in Figure 1 below. These sequences essentially confirm the restriction analysis found in the original publication (93), but include a few unexpected results as well.

Sullivan *et al.* correctly surmised that pMK3 is a “simple fusion of pUC9 and pUB110” with the “fusion point in the pUC9 replicon located...in the *lac* region” (93). What they could not

* Daniel R. Zeigler, Ph.D., Bacillus Genetic Stock Center, Columbus, OH. zeigler.1@osu.edu (17 March 2008)

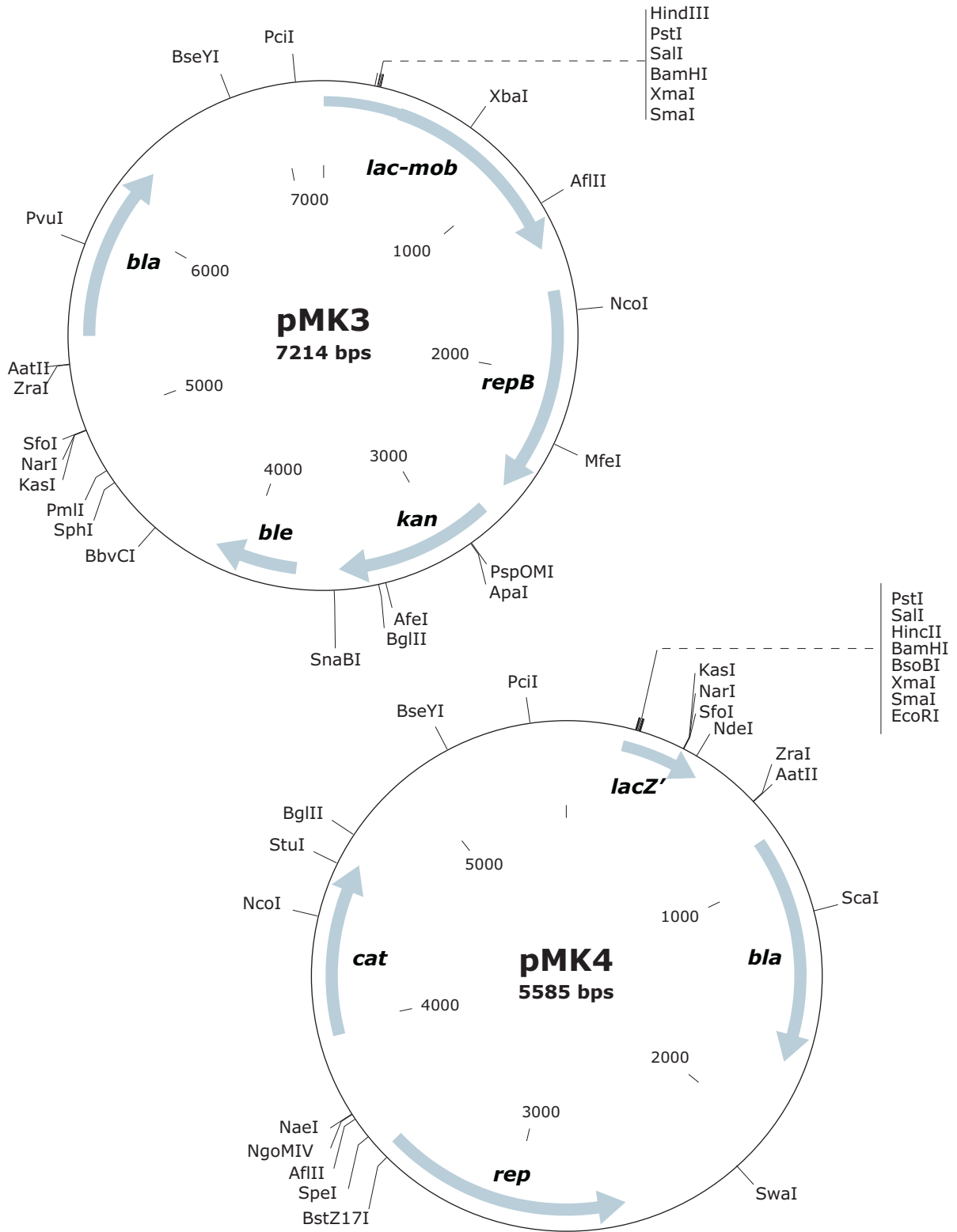


Figure 1. Genetic and physical maps of pMK3 (GenBank EU549779) and pMK4 (GenBank EU549778). Unique restriction enzyme sites are indicated. Gray arrows show location, length, and direction of transcription for plasmid reading frames. For further details, see the text and the GenBank entries cited.

have anticipated, however, was that this fusion joined a truncated version of *lacZ* α in frame with a truncated form of the pUB110 *mob* gene (see Figure 1). Interestingly, this fusion gene expresses a protein with enough activity to provide alpha-complementation for β -lactamase. (It is not known whether Mob protein activity is likewise retained.) Our sequence for pMK3 includes a stretch of seven "A" residues (positions 4843-4849) where the database sequence for pUB110 shows only six ([M19465](#)). This discrepancy may simply be due to a minor error in the published sequence, which dates from two decades ago (57). There are no conflicts between our pMK3 sequence and the database sequence for pUC9 ([L09128](#)).

For pMK4, Sullivan *et al.* were unable to determine the exact fusion point for the pUC9 moiety, due to presence of five closely spaced Sau3AI sites in the parent plasmid. Our results pin down the fusion point precisely at the Sau3AI site at bases 1801/1805 of pUC9. Interestingly, a tiny 17-bp restriction fragment from pUC9 has been duplicated in pMK4, with one copy on each end of the pUC9 moiety in the construct (1790-1806 and 4710-4726). There are several conflicts between our pMK4 sequence and the database sequence for pC194 (GenBank [L08860](#)). We are confident in our sequence, since it includes multiple reads on both strands using multiple primers at every position. Supporting our sequence is the observation that two other *Staphylococcus aureus* chloramphenicol resistance plasmids, pKH13 ([EU170347](#)) and pKH15 ([EU170348](#)), agree with our data against the published pC194 sequence at each point of conflict (not shown).

A History of Success. From today's perspective, the construction of pMK3 and pMK4 appears to be at least a minor milestone in the development of recombinant DNA methods for the gram-positive bacteria. According to the ISI Web of Knowledge, Sullivan *et al.* has been cited over 140 times since publication, including 40 times in the past five years (Thomson Scientific). A close inspection of the literature shows that both pMK3 and pMK4 have been successfully employed in cloning genes and expressing foreign proteins in *B. subtilis* and in several other intensively studied members of the species, including *B. anthracis*, *B. cereus*, *B. licheniformis*, *B. megaterium*, and *B. thuringiensis* (see Table 1). Use of these plasmids has spread into several other gram-positive research communities as well. Interestingly, pMK4 has become one of the most commonly used vectors for *Listeria monocytogenes* and *S. aureus* (see Table 1).

This record of success comes despite the fact that both vectors are based on gram-positive replicons that replicate via a rolling circle mechanism, which has been generally linked to stability problems in cloning vectors (10). What is more, the minus origin of replication in pC194 is nonfunctional in *B. subtilis* (32), which should cause high molecular-weight, single-stranded intermediates to accumulate during pMK4 replication. In recent years, small gram-positive plasmids, such as pUB110 and especially pC194, have been considered unsuitable building material for constructing cloning vectors (10). Nevertheless, the research literature contains abundant proof that pMK3 and pMK4 are highly successful tools.

Availability. The Bacillus Genetic Stock Center is pleased to offer both vectors, either in *E. coli* (ECE15 for pMK3, ECE16 for pMK4) or in *B. subtilis* (1E38 for pMK3, 1E39 for pMK4) or as purified plasmid DNA (ECE15P for pMK3, ECE16P for pMK4). Please consult our website (www.bgsc.org) to learn about our user fee policy and about the other services we offer.

Table 1. Uses of shuttle vectors pMK3 and pMK4 in bacterial systems

Host	Use	References	
		pMK3	pMK4
<i>Bacillus anthracis</i>	Expression of <i>B. anthracis</i> genes		42
	Expression of foreign genes		35
<i>Bacillus cereus</i>	Expression of foreign genes	98	
	Transformation assay		23
<i>Bacillus licheniformis</i>	Expression of <i>B. licheniformis</i> genes	26	
<i>Bacillus megaterium</i>	Expression of <i>B. megaterium</i> genes		86
<i>Bacillus subtilis</i>	Cloning system development		72
	Expression of <i>B. subtilis</i> genes	7, 8, 18, 24, 34, 40, 55, 63, 71, 84, 85, 95, 111, 112, 118	3, 14, 27, 41, 44, 47, 54, 61, 62, 64, 69, 79, 83, 92, 101, 108, 117
	Expression of foreign genes	26, 68, 107, 114	1, 6, 19, 25, 45, 96, 99, 115, 116
	Expression of phage genes	12, 51, 77	
	Expression of reporter genes	113	
	Expression of tagged proteins	70	
	Gene library construction		78
	Gene integration into chromosome	94	
	Promoter analysis		52
	Transformation assay		48, 51, 58, 109
<i>Bacillus thuringiensis</i>	Expression of <i>B. thuringiensis</i> genes	97	
<i>Escherichia coli</i>	Expression of anti-sense RNA		60
	Expression of foreign genes	8, 71, 106	5, 6, 11, 20, 53, 67, 96, 99, 102
	Expression of phage genes	12	
	Gene library construction	56	
	Subcloning for sequencing		75
<i>Leuconostoc mesenteroides</i>	Expression of <i>L. mesenteroides</i> genes		4, 59
<i>Listeria monocytogenes</i>	Cloning system development		72
	Expression of <i>L. monocytogenes</i> genes		2, 17, 21, 22, 45, 50, 65, 66, 80, 103, 104
	Expression of reporter gene		74
	Expression of viral protein		31
	Transformation assay		9
<i>Lysinibacillus sphaericus</i>	Expression of foreign genes	97	
<i>Staphylococcus aureus</i>	Cloning system development		72
	Expression of foreign genes		28, 73, 81, 100, 110
	Expression of <i>S. aureus</i> genes		15, 29, 30, 36-39, 46, 67, 68, 87-89
	Expression of tagged <i>S. aureus</i> genes		16, 90
<i>Staphylococcus carnosus</i>	Expression of foreign genes		91
<i>Streptococcus mutans</i>	Expression of <i>S. mutans</i> genes		76
	Gene library construction	13	

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