The Lambda EMBL3 Polylinker and Surrounding Region for PCR Primers

Since its construction (5), the lambda EMBL3 bacteriophage has become a very common vector for the generation of genomic libraries. Much modified from wild-type lambda, the sequence of the vector itself has never been published, although an extra segment, a construction artifact, was reported in the right arm’s polylinker (7).

In order to design primer pairs of balanced Tm for PCR amplification of insert fragments from lambda EMBL3 clones or libraries (4), we first determined the sequence of the polylinkers and immediate surrounding regions. BamHI-digested EMBL3 (Stratagene, La Jolla, CA) was cut with Smal. The 550-bp and 3-kb BamHI-Smal fragments bordering the polylinker in the left and right arms, respectively, were subcloned into pGEM®-Zf (Promega, Madison, WI) and sequenced from the plasmids as double-stranded DNA by the Sanger dideoxy method (8) with Sequenase® Version 2.0 (United States Biochemical, Cleveland, OH). Both strands were sequenced for the region reported here.

The EMBL3 left arm is completely homologous to the sequence available for wild-type and c857s7 strains, at least between bases 19399-19787 and then 27724-27976 in the EMBL database (2) accession (Figure 1, 1-237), at which point the polylinker begins. The region 27740-27977 includes part of the integrase (int) gene (1) required during lysogenic DNA integration and also the int binding site (27788-278113; Figure 1, 49-74) on the complementary strand. Bases 19788-27723 in the wild type therefore define the b189 deletion (5) in EMBL3, damaging the att site necessary for lysogenic integration. The left arm also contains the extra 12 bp and PstI site in the polylinker (Figure 1, 233-260) previously reported (7).

The region sequenced from the right arm is identical to that in the database (2). The polylinker (Figure 2, 1-56), however, contains an additional 35 bp including a PstI site and two extra Sall/AccI sites. The remaining sequence reported here for the right arm covers the 5’ end of the gat gene (6) involved in the lytic pathway [34500-34686 in the database (2)].

The actual EMBL3 polylinkers, presented here, include dissimilar cloning artifacts and extra restriction sites not included in available EMBL3 maps. Since a pair of PstI sites are therefore present in EMBL3, the extra sites should not interfere with use of the vector. Knowledge of the correct sequences, however, is important in the design of oligonucleotide primers for PCR amplification or sequencing of cloning joints. These sequences have been deposited in the EMBL database (3).

REFERENCES

2. EMBL accession J02459 M17233.
3. EMBL accessions X58667 (right arm) and X58668 (left arm).

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Figure 1. Nucleotide sequence of the lambda EMBL3 left arm, 238 bases nearest the polylinker. Restriction enzymes indicated at base just 3’ to cleavage site: A, AccI; B, BamHI; P, PstI; S, Sall. EcoRI site not shown because it was used to subclone the arms into the plasmid vector. Polylinker is underlined, additional bases in boldface.

Figure 2. Nucleotide sequence of the lambda EMBL3 right arm, 260 bases nearest the polylinker. Restriction sites and legend as in Figure 1.