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A lambda/plasmid Cre/lox hybrid vector for large genomic (18kb) fragment insertions and fungal genomic library construction

Abstract

We have previously constructed lambda/plasmid hybrid vectors designed for both fungal cDNA and genomic library construction (Brunelli and Pall, 1994 Fungal Genet. Newslet. **41**:63-65). The genomic library inserts, however, were limited to about 11 kb in size due to the size limitations of lambda packaging. We have constructed a similar vector that has three advantages over these earlier hybrid vectors, as discussed further below. The plasmid pBARGEM7-2 (Pall and Brunelli, 1993. Fungal Genet. Newslet. **40**:59-61) was modified by inserting a stuffer sequence into the *Bam*HI site of the polylinker. The stuffer sequence was about 6 kb and can be cut out with *Bam*HI, yielding two BamHI fragments of about 4.5 kb and 1.5 kb. The 4.5 kb fragment contains the lacZ gene, producing very blue colonies (or plaques in lambda) on Xgal medium.

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A lambda/plasmid Cre/*lox* hybrid vector for large genomic (18kb) fragment insertions and fungal genomic library construction

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We have previously constructed lambda/plasmid hybrid vectors designed for both fungal cDNA and genomic library construction (Brunelli and Pall, 1994 Fungal Genet. Newslet. **41**:63-65). The genomic library inserts, however, were limited to about 11 kb in size due to the size limitations of lambda packaging. We have constructed a similar vector that has three advantages over these earlier hybrid vectors, as discussed further below.

The plasmid pBARGEM7-2 (Pall and Brunelli, 1993. Fungal Genet. Newslet. **40**:59-61) was modified by inserting a stuffer sequence into the *Bam*HI site of the polylinker. The stuffer sequence was about 6 kb and can be cut out with *Bam*HI, yielding two BamHI fragments of about 4.5 kb and 1.5 kb. The 4.5 kb fragment contains the lacZ gene, producing very blue colonies (or plaques in lambda) on Xgal medium.

This plasmid was introduced into the lambda arms previously described by Holt and May (1993, Gene 133: 95-97), using the plasmid switching procedure previously described by this laboratory (Brunelli and Pall, 1994 BioTechniques **16**:1060-1064). In this procedure, the Cre/*lox* recombination system is used to recombine out a plasmid insert in a parental lambda/plasmid hybrid, followed by insertion of a new *lox*-containing plasmid by Cre/*lox*-mediated recombination. The new lambda vector was screened for by the intensely blue plaques it produces on Xgal plates. The new vector was designated lambda IIBARGEM7B (with *Bam*HI stuffer) and is pictured in Fig. 1. It is available from the Fungal Genetics Stock Center.

lambda IIBARGEM7B shares with the earlier lambda BARGEM7 vector the fact that plasmids can be easily excised from lambda by Cre/*lox*- mediated recombination (automatic subcloning, see earlier 1994 references) and plasmids can then be used to directly transform fungi, selecting for transformants using the *bar* selection (Pall, 1993. Fungal Genet. Newslet. **40**:58). In addition, new vectors can be used to make libraries with inserts of over 18 kb and these inserts can be phosphatased before ligation, helping prevent insertion of two inserts into the same clone. The phosphatasing of inserts is possible because the vector arms, without stuffer fragment, are too small to package, preventing formation of large numbers of clones without inserts. The lambda IIBARGEM7B vector can be used to construct libraries of *Sau*3AI partial digest fragments into the *Bam*HI site of the polylinker (Fig. 1) because the lambda arms lack any *Bam*HI sites. This research was supported by funds from the College of Sciences, Washington State University.

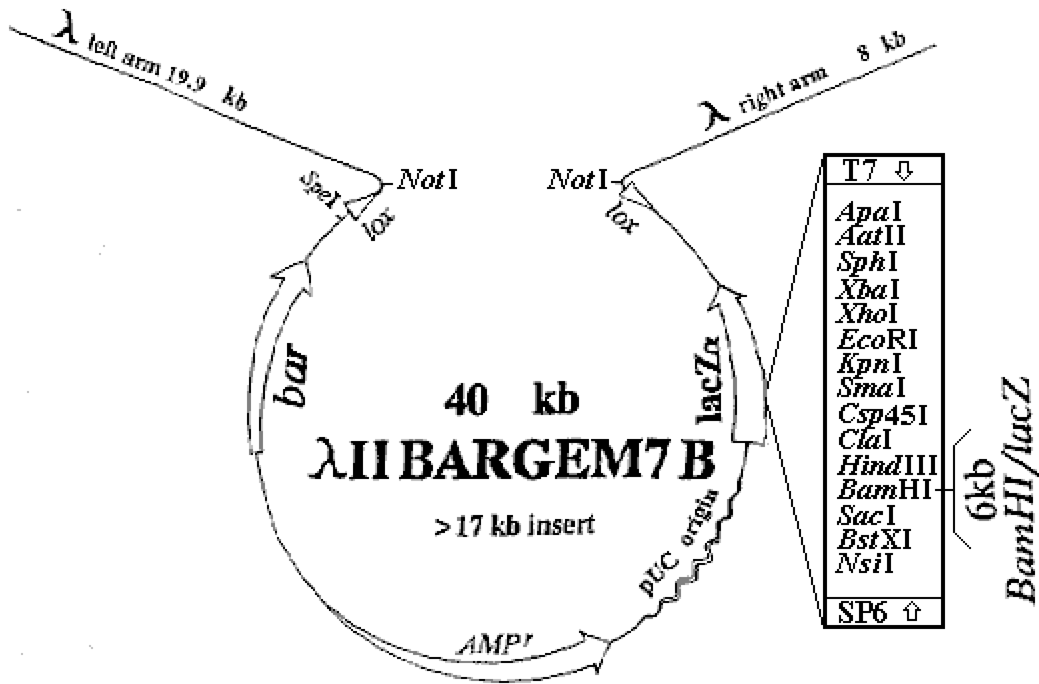

Fig 1. Restriction map of the lambda/plasmid Cre/*lox* hybrid vector, lambda IIBARGEM7B

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