Complete Nucleotide Sequence of Two Generations of a Bacterial Artificial Chromosome Cloning Vector

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Recent large-scale mapping and sequencing efforts are primarily dependent on the ability to precisely clone and stably maintain large pieces of DNA. Several cloning methods, including bacteriophage P1 (7) and P1-de-

rived artificial chromosome (PAC) (3), yeast artificial chromosome (YAC) (1) and bacterial artificial chromosome (BAC) (6) based systems have been developed to accept large DNA inserts. The BAC cloning system has advantages over other methods because of its high cloning efficiency, stability of clones and smaller size of cloning vector (6). However, the average size of cloned DNA inserts in BACs is smaller than in YACs. Several BAC libraries have been constructed that include human (6,8), mouse, bovine (2), rice (4) and *Sorghum bicolor* (9). These libraries are now being applied in several mapping and sequencing projects.

We have used BAC clones to map and sequence the human and mouse T-cell receptor loci. To facilitate the mapping and sequencing processes, we have sequenced the BAC cloning vectors pBAC108L and pBeloBAC11. These two vectors share the same DNA backbone and contain 6941 and 7507 bp, respectively (Figure 1). The vectors include the chloramphenicol acetyltransferase (*cat*) gene, and thus the BAC clones are selected and maintained by their resistance to chloramphenicol. The vectors also contain F-factor replication origin (*oriS*) and all the necessary genes (*parA*, *parB*, *parC* and *repE*) to stably maintain the single-copy BAC DNA (6).

Figure 1. The BAC cloning vectors pBAC108L and pBeloBAC11.

Benchmarks

In addition, *cosN* and *loxP* sequences are contained in the vectors to take advantage of the lambda terminase and P1 phage *cre/lox* (7) mapping system to generate a restriction map for inserted DNA. The pBeloBAC11 vector has been constructed by replacing the 68-bp DNA fragment between the *NotI* sites in the pBAC108L vector with a 634-bp DNA from the pGEM[®]-3Z plasmid vector (Promega, Madison, WI, USA). The 634-bp DNA fragment contains the *lacZ* gene and polylinker region from the pGEM-3Z vector. Therefore, the pBeloBAC11 vector will allow the simple 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal)/isopropyl- β -D-thiogalactopyranoside (IPTG)-based color selection to be used during the library construction process. Restriction sites *Bam*HI, *Sph*I and *Hind*III are the only usable unique sites in the polylinker region of pBeloBAC11 for cloning. Flanking the cloning sites (*Bam*HI and *Hind*III in pBAC108L and the polylinker region in pBeloBAC11) are SP6 and T7 promoter sequences that can be used to generate end-region riboprobes for chromosomal walking. Commercial SP6 and T7 primers can also be used to obtain sequences for sequence-tagged site (STS) mapping (5) from the end regions.

The nucleotide sequence data reported in this paper have been submitted to the GenBank[®] nucleotide sequence database and have been assigned the accession numbers U51113 and U51114.

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ERRATUM

In the October 1997 article by A. Zhou et al., "Improved alkaline lysis method for rapid isolation of plasmid DNA", *BioTechniques* 23:592-594, the amount of glacial acetic acid given in Table 1, step 4, should be 11.5 mL rather than 11.5 μ L.