pFOS1

9,738 base pairs
Sequence file available at www.neb.com

pFOS1 is available as a transformant of POP2136 (#E4153S) at no charge when shipped with an order or for the cost of shipping if ordered separately.

pFOS1 is an E. coli plasmid vector designed for the cloning of large DNA fragments (up to 40 kb). It is maintained in single copy, which permits the stable maintenance of such large inserts (1).

Based on the Ori2 (OriS) replicon of the F (fertility) factor of E. coli, the vector encodes the SopAB functions for active partitioning (2). These functions act at SopC to ensure that each daughter cell gets a copy of the plasmid. Initiation factor RepE (also known as RepA) mediates assembly of a replication complex at Ori2 (3-5).

This vector includes the large PvuII fragment of pUC19, including the ampicillin resistance gene (bla) and origin of replication, positioned between two lambda cos sites (6).

The high copy number origin in the vector facilitates DNA preparation, but because of the directionality of the packaging process, the pUC19 segment is deleted in the final clone, which is maintained in single copy. (Note: pFOS1 is not stably maintained in most E. coli strains due to the presence of the two cos sites.)

pFOS1 also includes the following features: unique cloning sites BamHI and HindIII; T7 and SP6 phage promoters reading into these cloning sites for generation of RNA probes for blot procedures; several GC-rich restriction sites flanking the cloning segment for removal of the cloned insert; a chloramphenicol selectable marker; and a loxP site for specific cleavage by Cre recombinase in the presence of loxP oligonucleotide.

Enzymes with unique restriction sites are shown in bold type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5'-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop", numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Lambda cos site coordinates are the boundaries of the HincII fragment surrounding the annealed 12 base overhangs.

bla (ApR) gene coordinates include the signal sequence.

Feature	 Coordinates	 Source
bla (ApR) 134-994 Tn3
origin 1165-1753 pUC19
cos site 2024-2422 lambda
pT7 promoter 2498-2506 P1
pSP6 promoter 2539-2523 SP6
cat (CmR) 3338-2679 Tn9
ori2 (OriS) 4280-4346 F
repE (repA) 6009-7184 F
sopA 6009-7184 F
sopB 7184-8155 F
sopC 8228-8701 F
cos site 8900-9360 lambda
ori = origin of replication
Ap = ampicillin, Cm = chloramphenicol

There are no restriction sites for the following enzymes: AarI(x), AscI, AsiSI, AvrII, BbvCI, BmtI, BspDI, BstBI, Bsu36I, ClaI, FseI, I-CeuI, I-SceI, MluI, NheI, NsiI, PI-PspI, PI-SceI, PacI, PmeI, PmlI, PstI, Pspl, PspI, Pst-SceI, PvuI, PstI, Pspl, PvuI, Rsfl, SacI, SamII(x), Sphi, SwaI, XcmI.

(x) = enzyme not available from NEB

References
6. Dunn, J. and Studier, F.W., personal communications.

Avaluatation: 11 stars