

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.

Feature	Coordinates	Source
<i>bla</i> (Ap ^R)	134-994	<i>Tn3</i>
origin	1165-1753	pUC19
<i>cos</i> site	2024-2422	lambda
<i>loxP</i> site	2440-2473	P1
pT7 promoter	2489-2506	T7
pSP6 promoter	2539-2523	SP6
<i>cat</i> (Cm ^R)	3338-2679	<i>Tn9</i>
Ori2 (OriS)	4280-4346	F
<i>repE</i> (<i>repA</i>)	4675-5430	F
<i>sopA</i>	6009-7184	F
<i>sopB</i>	7184-8155	F
<i>sopC</i>	8228-8701	F
<i>cos</i> site	8960-9360	lambda

ori = origin of replication
Ap = ampicillin, Cm = chloramphenicol

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.

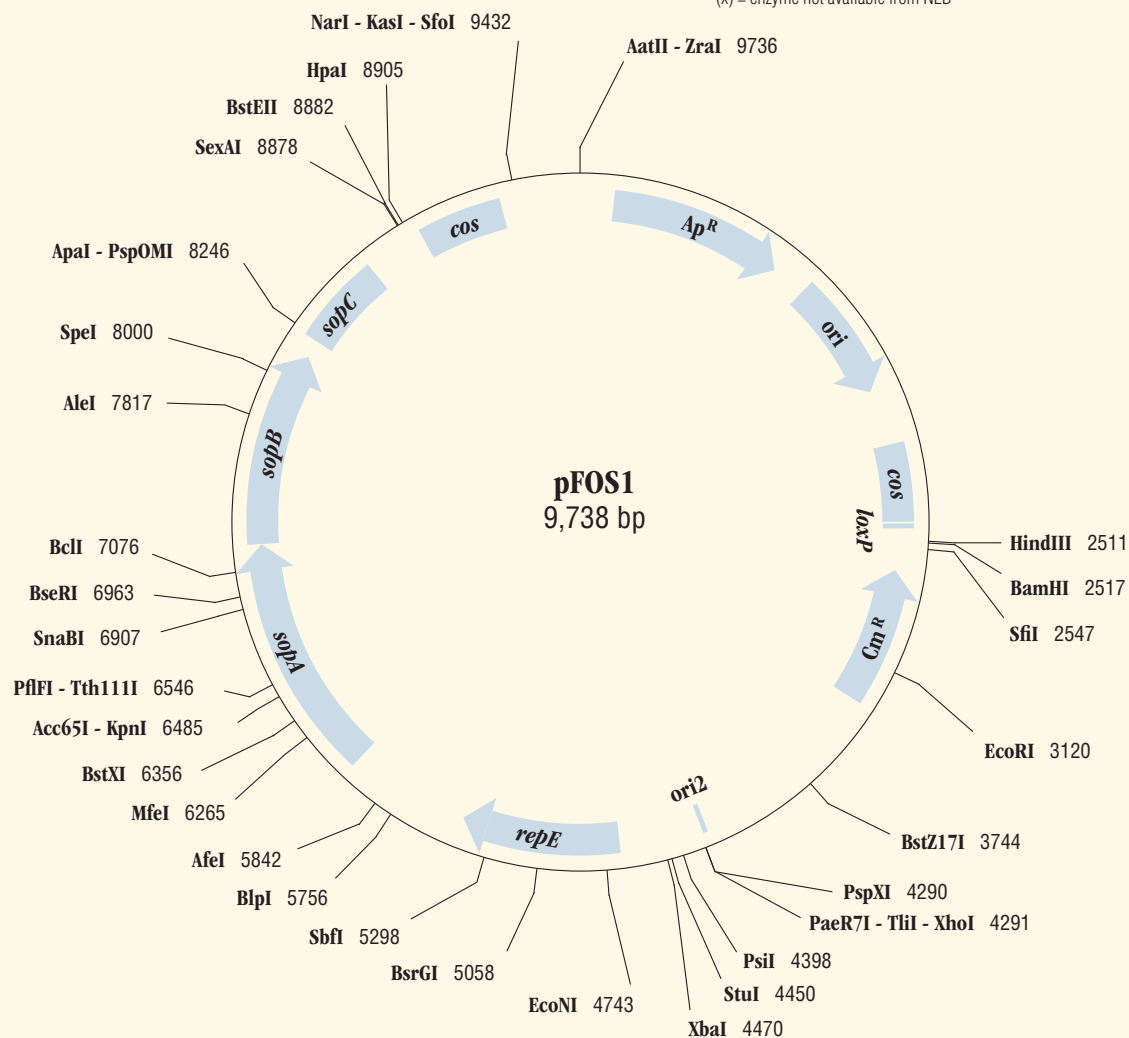
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5'-most base on the top strand in each recognition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

There are no restriction sites for the following enzymes: AarI (x), AscI, AsiSI, AvrII, BbvCI, BmtI, BsiWI, BspDI, BstBI, Bsu36I, ClaI, FseI, I-CeuI, I-SceI, MluI, NheI, NsiI, PI-PspI, PI-SceI, PacI, PmeI, PmlI, RsrII, SacII, SanDI (x), SphI, SwaI, XcmI.

(x) = enzyme not available from NEB



References

- Shizuya, H. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 8794–8797.
- Mori, H. et al. (1986) *J. Mol. Biol.* 192, 1–15.
- Imber, R., Low, R.L. and Ray, D.S. (1983) *Proc. Natl. Acad. Sci. USA* 80, 7132–7136.
- Disque-Kochem, C. et al. (1986) *Mol. Gen. Genet.* 202, 132–135.
- Komori, H. et al. (1999) *EMBO J.* 18, 4597–4607.
- Dunn, J. and Studier, F.W., personal communications.