pGPS3

4,293 base pairs
Sequence file available at www.neb.com

There are no restriction sites for the following enzymes: AarI(x), AatII, AflII, AgeI, AleI, ApaI, AscI, AvrII, BaeI, BclI, BfuAI, BlpI, BmgBI, BmtI, BsaAI, BseRI, BsiWI, BspMI, BspQI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, CspCI, EcoRI, FseI, HpaI, KasI, MfeI, MluI, NaeI, NarI, NcoI, NdeI, NgoMIV, NheI, PI-PspI, PacI, PflFI, PmlI, PshAI, PsiI, PspOMI, PstI, PvuII, RsrII, SacII, SanDI(x), SapI, SbfI, SexAI, SfiI, SfoI, SgrAI, SnaBI, SphI, SrfI(x), Tth111I, XbaI, XcmI, ZraI.

(x) = enzyme not available from NEB

pGPS3 is an *E. coli* plasmid used as the transposon (Transprimer) donor in the GPS-M Mutagenesis System (NEB #E7101S). TnsABC transposase removes the Transprimer element from this plasmid and inserts it randomly into a target DNA molecule *in vitro*.

The Transprimer in pGPS3 (Transprimer-1) can be easily customized by adding to or replacing its kanamycin selectable marker (Kn^R), which is removable with BamHI, EcoRV, or any combination of unique restriction enzymes whose recognition sites flank the gene. For ease of manipulation, pGPS3 can be grown in standard laboratory *E. coli* strains, unlike other donor vectors of the pGPS series. Its backbone contains the origin of replication from pUC19, and it is maintained at a similar copy number to pUC19. The pGPS3 backbone also contains two recognition sequences for the rare-cutting enzyme PI-SceI, which can be used to selectively destroy unreacted pGPS3 molecules following the GPS reaction.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular 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.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
origin	1021-433	pUC19
bla (Ap^R)	2052-1192	*Tn3*
Tn7R	2572-2769	*Tn7*
aph(3´)-Ia (Kn^R)	3939-3124	*Tn903*
Tn7L	4104-4270	*Tn7*
Transprimer-1	2572-4270	–

ori = origin of replication
Ap = ampicillin, Kn = kanamycin