pGPS4 and 5

pGPS4 and pGPS5 are *E. coli* plasmids used as the transposon (Transprimer) donors in the GPS-LS Linker Scanning System (NEB #E7102S). TnsABC transposase removes the Transprimer element from this plasmid and inserts it randomly into a target DNA molecule *in vitro*.

pGPS4 and pGPS5 have identical backbones but different Transprimers: pGPS4 contains Transprimer-4 (encoding chloramphenicol resistance), while pGPS5 contains Transprimer-5 (encoding kanamycin resistance).

Transprimer-4 and Transprimer-5 are flanked by Pme I sites. Cleavage of transposition products with Pme I and religation removes the majority of the inserted Transprimer from the target DNA, leaving a 15 bp insertion including a unique Pme I site. If this insertion is within an expressed gene, the result is an insertion of 5 amino acids in the protein product in 4 of 6 reading frames.

The backbone of both plasmids encodes tetracycline resistance and contains the R6K-γ origin of replication core region. This high-copy origin requires a replication initiation protein (the π protein, encoded by the *pir* gene) not normally present in laboratory strains of *E. coli*; therefore, after transformation of the GPS reaction, unreacted pGPS4 and pGPS5 are not recovered.

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

R6K-γ origin coordinates include nucleotides -37 to +274, numbered from the G of the Hind III site. This is roughly from the EcoR II to Bgl II sites of the R6K sequence (1).

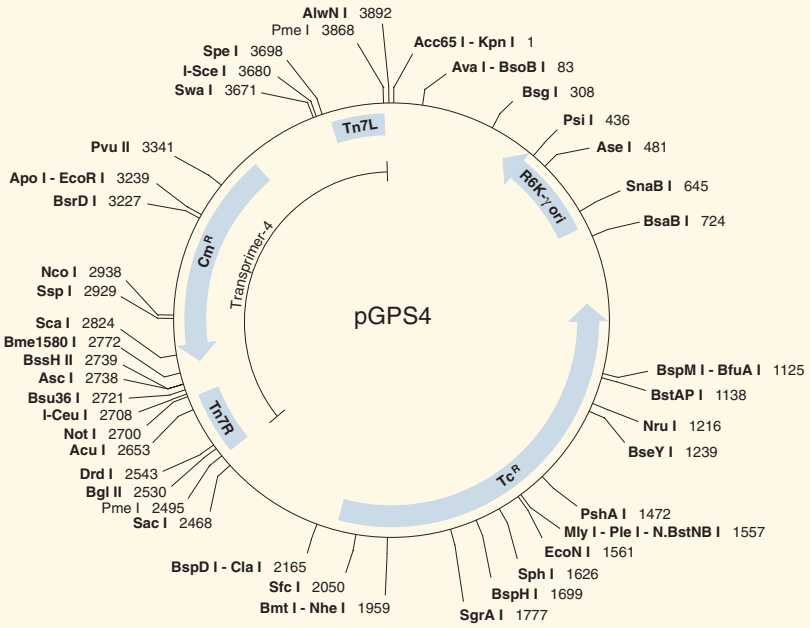
pGPS4: 3,899 base pairs
pGPS5: 4,223 base pairs

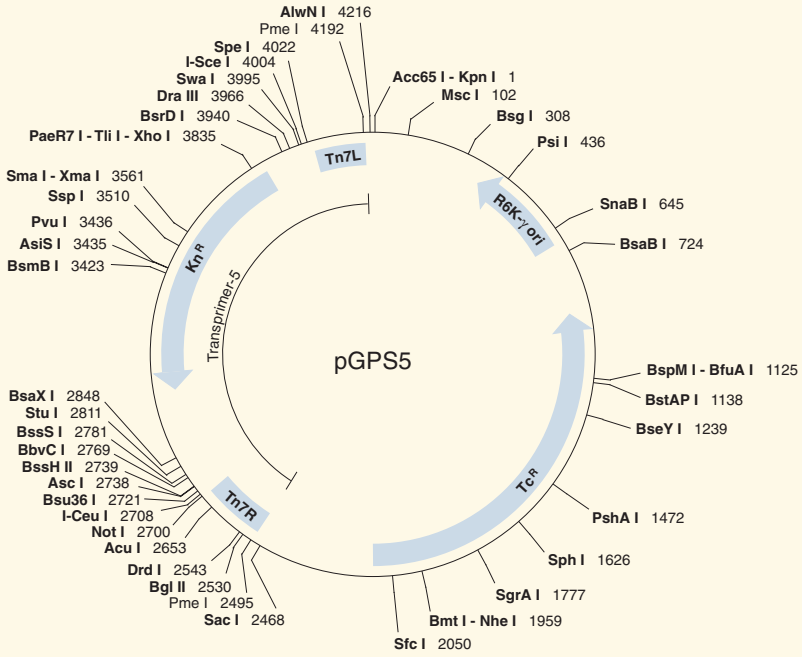
Sequence files available at www.neb.com
See page 138 for ordering information.

Feature pGPS4	**Coordinates**	**Source**
origin	678-369	R6K
tet (Tc^R)	2107-917	pSC101
Tn7R	2494-2692	*Tn7* (mutant)
cat (Cm^R)	3457-2798	*Tn9*
Tn7L	3710-3876	*Tn7* (mutant)
Transprimer-4	2494-3876	–

Feature pGPS5	**Coordinates**	**Source**
origin	678-369	R6K
tet (Tc^R)	2107-917	pSC101
Tn7R	2494-2692	*Tn7* (mutant)
aph(3´)-Ia (Kn^R)	3869-3054	*Tn903*
Tn7L	4034-4200	*Tn7* (mutant)
Transprimer-5	2494-4200	–

ori = origin of replication
Cm = chloramphenicol, Kn = kanamycin
Tc = tetracycline





Enzymes that cut **once** in Transprimer-4 (pGPS4):

AclI	BsaW I	Drd I	Not I
Acu I	BsiE I	Eag I	Pvu II
Apo I	BsmF I	EcoR I	Sca I
Asc I	Bsp1286 I	Fok I	Spe I
Ava II	BspCN I	Hinf I	Ssp I
Ban I	BspE I	HpyCH4 V	Sty I
Bgl II	BsrD I	I-Ceu I	Swa I
Bme1580 I	BssH II	I-Sce I	Tat I (x)
Bpm I	BstF5 I	Msc I	Tfi I
Bpu10 I	Bsu36 I	Nco I	TspR I
BsaA I	Btg I		

Enzymes that cut **once** in Transprimer-5 (pGPS5):

Acu I	Bsp1286 I	EcoN I	Sma I
Asc I	BspD I	Hind III	Sml I
Ase I	BspH I	I-Ceu I	Spe I
AsiS I	BsrD I	I-Sce I	Ssp I
Ban II	BsrF I	MspA1 I	Stu I
BbvC I	BssH II	Not I	Swa I
Bgl II	BssS I	Nru I	Tli I
BsaW I	Bsu36 I	PaeR7 I	Xho I
BsaX I	Cla I	PflM I	Xma I
BsmB I	Dra III	Pvu I	
BsmF I	Drd I	Rsa I	

(x) = enzyme not available from NEB