pGPS4 and 5

Sequence files available at www.neb.com
See page 166 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	BsaWI	DrdI	NotI
AcuI	BsiEI	EagI	PvuII
ApoI	BsmFI	EcoRI	ScaI
AscI	Bsp1286I	FokI	SpeI
AvaII	BspCNI	HinfI	SspI
BanI	BspEI	HpyCH4V	StyI
BglII	BsrDI	I-CeuI	SwaI
Bme1580I	BssHII	I-SceI	TatI (x)
BpmI	Bsu36I	MscI	TfiI
Bpu10I	BtgI	NcoI	TspRI
BsaAI	BtsCI		

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

AcuI	BspDI	HindIII	SmaI
AscI	BspHI	I-CeuI	SmlI
AseI	BsrDI	I-SceI	SpeI
AsiSI	BsrFI	MspA1I	SspI
BanII	BssHII	NotI	StuI
BbvCI	BssSI	NruI	SwaI
BglII	Bsu36I	PaeR7I	TliI
BsaWI	ClaI	PflMI	TspMI
BsaXI	CviQI	PspXI	XhoI
BsmBI	DraIII	PvuI	XmaI
BsmFI	DrdI	RsaI	
Bsp1286I	EcoNI		

(x) = enzyme not available from NEB

pGPS4 and pGPS5 are *E. coli* plasmids used as the transposon (Transprimer) donors in the GPS-LS Linker Scanning System (NEB #E7102). 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 PmeI sites. Cleavage of transposition products with PmeI and religation removes the majority of the inserted Transprimer from the target DNA, leaving a 15 bp insertion including a unique PmeI 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 selected 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 HindIII site. This is roughly from the EcoRII to BglII sites of the R6K sequence (1).