# pGPS1.1 and 2.1

pGPS1.1 and pGPS2.1 are *E. coli* plasmids used as the transposon (Transprimer) donors in the GPS-1 Genome Priming System (NEB #E7100). TnsABC transposase removes the Transprimer element from this plasmid and inserts it randomly into a target DNA molecule *in vitro*.

pGPS1.1 and pGPS2.1 have identical backbones but different Transprimers: pGPS1.1 contains Transprimer-1 (encoding kanamycin resistance), while pGPS2.1 contains Transprimer-2 (encoding chloramphenicol resistance).

The backbone of both plasmids encodes tetracycline resistance and contains two tandem copies of 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 pGPS1.1 and pGPS2.1 are not recovered.

Enzymes with unique restriction sites are shown in **bold** 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.

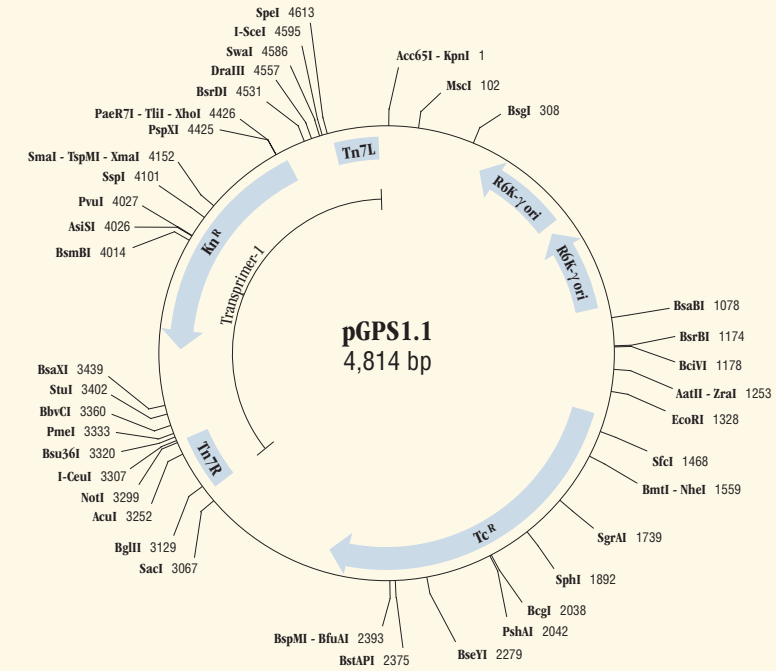
R6K-γ origin coordinates include nucleotides -37 to +274, numbered from the G of the HindIII site. This is roughly from the EcoRII to BglII sites of the R6K sequence (1).

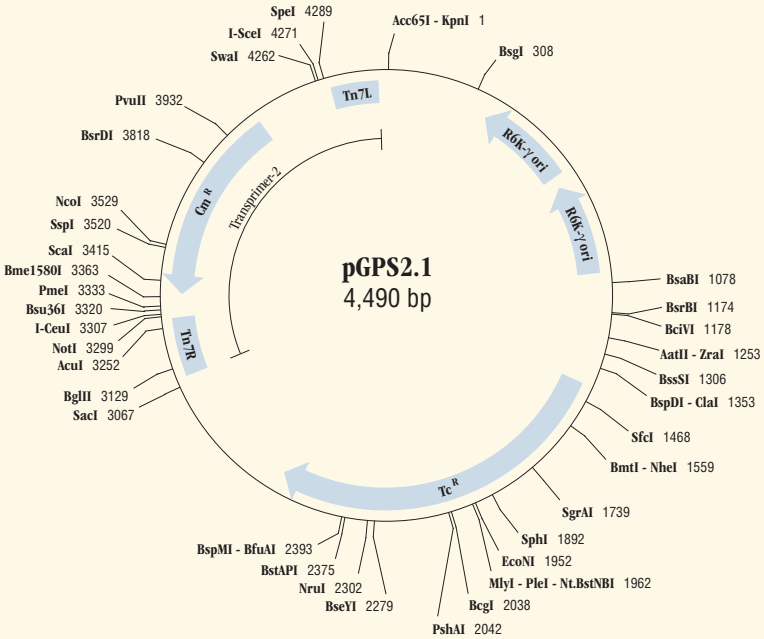
Sequence files available at www.neb.com
See page 166 for ordering information.

| **Feature** pGPS1.1 | **Coordinates** | **Source** |
|---|---|---|
| origin | 678-369 | R6K |
| origin | 1032-723 | R6K |
| *tet* (Tc$^R$) | 1416-2606 | pSC101 |
| Tn7R | 3093-3290 | *Tn7* |
| *aph(3´)-Ia* (Kn$^R$) | 4460-3645 | *Tn903* |
| Tn7L | 4625-4791 | *Tn7* |
| Transprimer-1 | 3093-4791 | – |

| **Feature** pGPS2.1 | **Coordinates** | **Source** |
|---|---|---|
| origin | 678-369 | R6K |
| origin | 1032-723 | R6K |
| *tet* (Tc$^R$) | 1416-2606 | pSC101 |
| Tn7R | 3093-3290 | *Tn7* |
| *cat* (Cm$^R$) | 4048-3389 | *Tn9* |
| Tn7L | 4301-4467 | *Tn7* |
| Transprimer-2 | 3093-4467 | – |

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




Enzymes that cut **once** in Transprimer-1 (pGPS1.1):

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

Enzymes that cut **once** in Transprimer-2 (pGPS2.1):

| | | | |
|---|---|---|---|
| AclI | BsiEI | EagI | PmeI |
| AcuI | BsmFI | EcoRI | PvuII |
| ApoI | Bsp1286I | FokI | ScaI |
| AvaII | BspCNI | HinfI | SpeI |
| BanI | BspEI | HpyCH4V | SspI |
| BglII | BsrDI | I-CeuI | StyI |
| Bme1580I | BstF5I | I-SceI | SwaI |
| BpmI | BstUI | MscI | TatI(x) |
| Bpu10I | Bsu36I | NcoI | TfiI |
| BsaAI | BtgI | NotI | TspRI |
| BsaWI | BtsCI | | |

(x) = enzyme not available from NEB

**References**

(1) Stalker, D.M., Kolter, R. and Helinski, D.R. (1979) *Proc. Natl. Acad. Sci. USA,* 76, 1150–1154.