



GPS[®]-M GPS-Mutagenesis System

A Tool for Generating Insertion Mutations
at Random Locations *in vitro* with a Custom Transposon

I n s t r u c t i o n M a n u a l

Catalog #E7101S
Store at -20°C



Version 1.6
1/08

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Notice to Buyer/User: While the Transprimer donors pGPS3 may be used for all described purposes, both research and commercial, these donors may not be resold in their original or modified form without the written permission of New England Biolabs, Inc.

Kit Components:

Each kit contains sufficient reagents for 10 reactions.

■ 10X GPS Buffer – (Reagent 1)	0.5 ml
■ pGPS3 (Replicatable Transprimer-1 Donor Plasmid) – (Reagent 2)	50 μ l (200 μ g/ml)
■ TnsABC* Transposase (10 reactions) – (Reagent 3)	10 μ l
■ Start Solution – (Reagent 4)	200 μ l
■ Control Target Plasmid (LITMUS 28) – (Reagent 5)	10 μ l (80 μ g/ml)
■ PI-SceI	100 μ l
■ 10X PI-SceI Buffer	1.0 ml
■ BSA	0.5 ml

Sequencing Primers

- **Primer S for the Left (SpeI) End of the Transprimer** – (Reagent 6)
100 μ l (3.2 pmol/ μ l)
- **Primer N for the Right (NotI) End of the Transprimer** – (Reagent 7)
100 μ l (3.2 pmol/ μ l)
- **Instruction Manual**

Customer Supplied Materials:

Target DNA, 0.08 µg per reaction, (> 5 µg/ml)

Target organism competent for DNA uptake

Other equipment and materials needed are standard laboratory supplies. Particular considerations apply to:

- Water bath or heat block, 75°C, for killing the reaction. **Note: not 65°C.**

Introduction:

The GPS-M Mutagenesis System enables you to insert a transposable element of your own design into your target DNA by *in vitro* transposition. It uses TnsABC* Transposase to insert a Tn7-based transposon randomly into the DNA target (Figure 1) (1–3). Target DNA may be a plasmid, cosmid, BAC or purified chromosomal DNA, depending on your interests (3–5). If the insertion site is within a translated gene segment, this will normally result in a null (loss of function) mutation. There is minimal site preference for insertion, so disruption of any open reading frame should be possible. Due to target immunity, only one insertion occurs per DNA molecule. Target immunity acts *in vivo* over a distance of ~190 kb (6). Therefore, the *in vitro* reaction produces a population of target DNA molecules each containing the transposable element at a different position.

To facilitate adaptation of the transposable element to your particular mutagenesis application, the transposon donor (pGPS3) can be customized by adding to or replacing the kanamycin resistance marker in the Transprimer (Figure 2). For ease of manipulation by the user, the donor plasmid can be grown easily in standard laboratory *E. coli* strains; the vector backbone carries Amp^r and a pUC origin of replication. To destroy unreacted donor molecules and avoid undesirable reaction products, the donor can be destroyed by digestion with the rare-cutting enzyme P1-Sce I (VDE) (Figure 1). For applications in which the mutagenized DNA will be transformed into naturally-competent organisms (which take up single DNA strands), a protocol is supplied for filling-in and ligating the gaps left by the insertion transposition reaction.

Notes: The Kan^r marker on the Transprimer is not designed to express in Gram positive organisms or eukaryotic cells. Transposon ends have stop codons in all frames, so translational fusions are not practical.

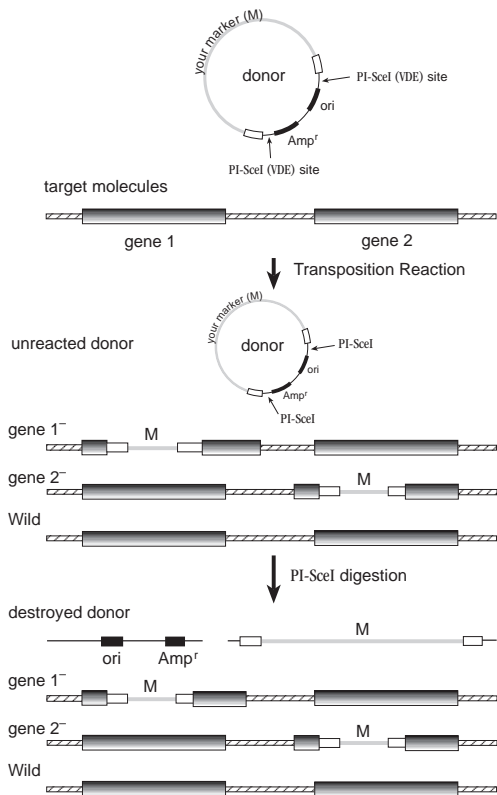
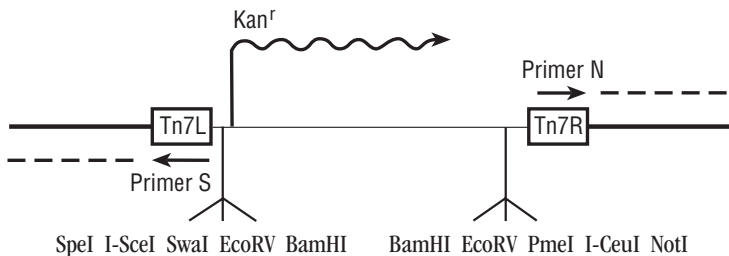


Figure 1: Overview of GPS-M: GPS Mutagenesis System



Left Transprimer end (Tn7L) and Primer S (SpeI end)

```

< TGTGGGCGGACAAAATAGTTGGGAAGCTGGGAGGGGTGGAAATGGAGTTTTTAAGGATTATTTAGGGAAGA . .
ACACCCGCCTGTTTTATCAACCTTGACCCCTCCCACCTTTACCTCAAAAATTCCCTAATAATCCCTTCT . .
-TCCCACCTTTACCTCAAAAATTCCCTAATA-5' . . .Primer S

```

Right Transprimer end (Tn7R) and Primer N (NotI end)

```

Primer N . . . 5' -ACTTTATTGTCATAGTTTAGATCTATTTTG-
. . TCTAGTTTAAGACTTTATTGTCATAGTTTAGATCTATTTTGTTTCAGTTTAAGACTTTATTGTCGCCCCACA >
. . AGATCAAATTCTGAAATAACAGTATCAAATCTAGATAAAACAAGTCAAATTTCTGAAATAACAGGCGGGTGT >

```

Figure 2, Transprimer Schematic: *Transprimer™-1* and locations of sequencing primers (Primer N and Primer S).

Advantages of the GPS Method:

- Transprimer insertion into the coding region of a gene will ordinarily produce a null mutation.
- The Transprimer is inserted randomly into the target DNA. There are no “hot spots” for insertion (3).
- The Transprimer donor carries kanamycin resistance for easy scoring.
- Standard subcloning sites (BamH I, EcoR V) flank the Kan^r fragment so that this fragment can be replaced by the marker of your choice.
- Unique rare-cutting restriction enzyme sites (Not I, Pme I, I-Ceu I, Swa I, Spe I) in the non-essential region of the Transprimer facilitate manipulation and insert mapping.
- pGPS3 and customized donors can be grown in ordinary lab strains of *E.coli*.
- The entire protocol, including digestion to destroy aberrant side products, takes less than 3 hours.
- Sequencing primer binding sites in the Transprimer ends enable rapid location of the exact position of your insertion mutation.

GPS-M Reaction Protocol:

Note: If using pGPS3 as supplied, dilute 1:10 in TE (1 μ l pGPS3:9 μ l TE)

1. Mix the following reagents (per 20 μ l reaction):

2 μ l	10X GPS Buffer (Reagent 1)
1 μ l	pGPS3, or your <u>supercoiled</u> custom donor (0.02 μ g) (Reagent 2)
variable vol	0.08 μ g Target DNA
variable vol	dH ₂ O
<hr/>	
18 μ l	Total Volume

Mix well by pipetting up and down a few times.

2. Add 1 μ l TnsABC* Transposase (Reagent 3) to each tube. Mix again.
3. Incubate for 10 minutes at 37°C. This is the assembly reaction.
4. Add 1 μ l Start Solution (Reagent 4) to each tube. Mix well by pipetting up and down a few times.
5. Incubate for 1 hour at 37°C. This is the strand transfer reaction.
6. Heat inactivate at 75°C for 10 minutes. Note: **65°C is not adequate.**

[7. Optional gap repair (see page 9)]

8. Add 5 μ l 10X PI-Sce I Buffer
0.5 μ l BSA
18.5 μ l dH₂O
6 μ l PI-Sce I (VDE) (6 units)
9. Incubate for 1 hour at 37°C.
(For Amp^r targets, incubate 2 hours, see page 11 “Selecting Insertions”)
10. Incubate for 10 minutes at 75°C.
11. Transform; if using *E. coli* as host and pGPS3 as donor we recommend:
For chemical transformation with subcloning efficiency cells (10⁷ per microgram of pUC), transform 1 μ l and 10 μ l of undiluted reaction.
For electroporation (>10⁹ per microgram of pUC), dilute 10-fold in dH₂O and transform 1 μ l and 10 μ l.
To outgrow, dilute the transformation mixture into 1 ml LB or as directed by the manufacturer, and **incubate for 1 hour at 37°C** with aeration. This period without selection is necessary for expression of drug resistance, especially kanamycin.

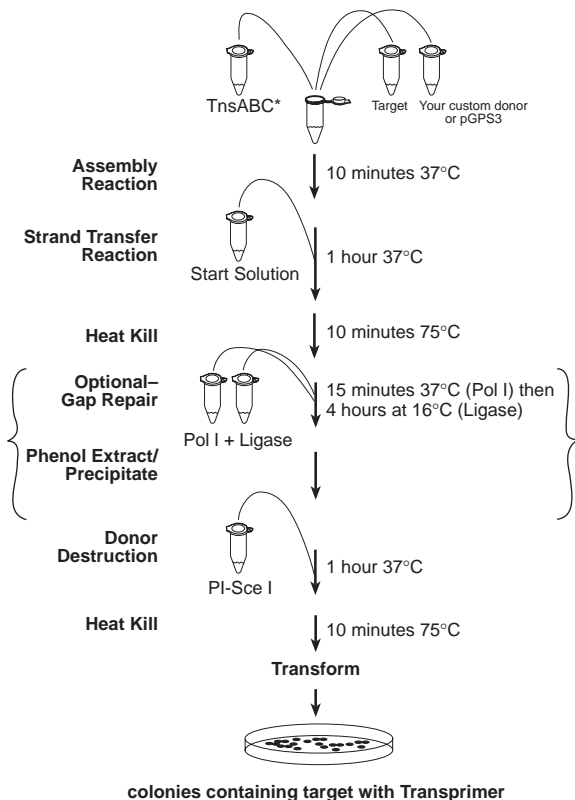


Figure 3: Reaction Overview

Protocol Tips

Amount of target: The recommended mass of target DNA (0.08 μg per reaction) works well for plasmid targets. For cosmids and BACs, a molar ratio of around 2:1 (donor to target) works well. Increasing the ratio to 4:1 decreases the efficiency slightly.

Donor:target ratio: The recommended donor:target mass ratio (1:4, 0.08 μg target per 20 μl reaction) is optimal. Small deviations produce only small changes in the number of recovered products. However, saturating amounts of donor will inhibit the reaction and may lead to accumulation of double insertions.

Order of addition: Water, target DNA, GPS Buffer (Reagent 1) and Donor Plasmid (Reagent 2) should be added first, followed by TnsABC* Transposase (Reagent 3). Start Solution (Reagent 4) should be added only after the assembly reaction (Step 3).

Assembly reaction: If this step is omitted, the proportion of complicated products will be increased.

Time of incubation: The reaction is linear at 37°C for at least one hour. Extremely long incubation times may lead to accumulation of double insertions.

Temperature of incubation: The reaction proceeds, but more slowly, at room temperature and at 30°C. For reactions with BACs, 30°C is recommended.

Heat killing: Heating at 75°C for 10 minutes effectively disrupts the reaction complexes. Few or no transformants will be obtained if this step is omitted. Heating at 65°C for 20 minutes **is not** adequate. Phenol/chloroform extraction followed by alcohol precipitation is also effective.

Scaling the procedure: Increase or reduce the final volume and the volume of all components by the same percentage; the relative concentrations of the two DNA species and the proteins are very important, as are the buffer conditions.

Enzyme names: PI-Sce I (VDE) is not the same as I-Sce I. Use PI-Sce I (VDE) to digest the donor and I-Sce I for mapping insertions obtained (Figures 2 and 6).

Selecting insertions: In experiments with a Cam^r target and pGPS3 as donor, at least 90% of Kan^r transformants were Amp-sensitive, and therefore were not uncut donor. If your target carries Amp^r , we recommend lengthening the PI-Sce I digestion time to 2 hours.

Gap repair: This step is not required for transformation into *E.coli* and is necessary only when the desired application involves transformation into naturally competent bacteria. Naturally competent bacteria include members of the genera *Neisseria*, *Haemophilus*, *Bacillus*, *Pneumococcus*, *Staphylococcus*, and *Streptococcus*. DNA uptake into these organisms involves degradation of one strand, concomitant with internalization of the other strand. Without gap repair, the 5-base gaps at the transposon insertion site (Figure 5) will unlink the transposon insertion from flanking DNA on one side or the other. Organisms in which competence is induced chemically or by electroporation (e.g., *E. coli* and other enteric bacteria, tissue culture cells, etc.) take up both DNA strands. Gaps at the insertion site are efficiently repaired by the cellular machinery.

The protocol on page 12 has been used successfully to introduce mutations into naturally competent organisms. Add these steps following Step 6 of the reaction protocol on page 8.

GAP Repair Protocol

7. Phenol/chloroform extract (50 μ l).
8. Ethanol precipitate:
6 μ l 3M NaAcetate
100 μ l EtOH
Incubate for 20 minutes at -20°C
Centrifuge for 10 minutes in a microfuge
9. Resuspend in 15 μ l TE.
10. 1 μ l DNA Polymerase I (*E.coli*) (10 units) (NEB #M0209)
3 μ l 10X EcoPol Buffer
9 μ l dNTP (at 100 μM each nucleotide; final concentration 33 μM each)
11. Incubate for 15 minutes at room temperature.
12. Add 1 μ l T4 DNA Ligase. (400 units) and ATP to a final concentration of 1 mM.
13. Incubate for 4 hours at 16°C .
14. Phenol/chloroform extract.
15. Alcohol precipitate.
16. Resuspend in 20 μ l TE.
17. Add 5 μ l 10X *Pi-Sce I* Buffer
0.5 μ l BSA
18.5 μ l dH_2O
6 μ l *Pi-Sce I* (VDE) (6 units)
18. Incubate for 1 hour at 37°C .
(For Amp^{r} targets, incubate 2 hours, see page 11 "Selecting Insertions")
19. Incubate for 10 minutes at 75°C .
20. Transform according to the appropriate method.

Tips on Donor Manipulation:

The GPS-M System includes a Transprimer-1 donor, pGPS3, that can be easily manipulated *in vitro* to enable you to add or substitute markers of interest.

Features of the transposition system that need to be kept in mind are:

1. The transposon donor must be supercoiled. The efficiency of reaction using a relaxed or linear donor will be reduced about 100-fold. The donor prep should be good quality, but CsCl-purification is not necessary.
2. For transposition purposes, all of the material between the Spe I and Not I sites of Transprimer-1 is dispensable (see Figures 2 and 6).
3. The boxes drawn on Figure 2 represent essential recognition elements for the transposase and are not dispensable. There are stop codons in all frames reading into the transposon. Transcription can proceed into the dispensable region from outside without difficulty.
4. Deletion of the Kan^r fragment of Transprimer-1 is easily accomplished by digestion with BamH I or EcoR V, followed by religation. This will leave the rare-cutting sites, useful for mapping purposes, in the transposon derivative that you make.
5. Transposition efficiency may decline somewhat as the transposon becomes longer. This is seen for IS1, IS10 and Mu-mediated transposition (7–9). However, insertion of an additional 2.8 kb into Transprimer-1 did not reduce transposition efficiency. Additionally, wild type Tn7 is 14 kb and transposes very efficiently *in vivo*.

6. For best results, ensure that your transposon donor plasmid is monomeric. Problems can arise when:

A. The donor molecule is dimeric or a higher multimer
AND

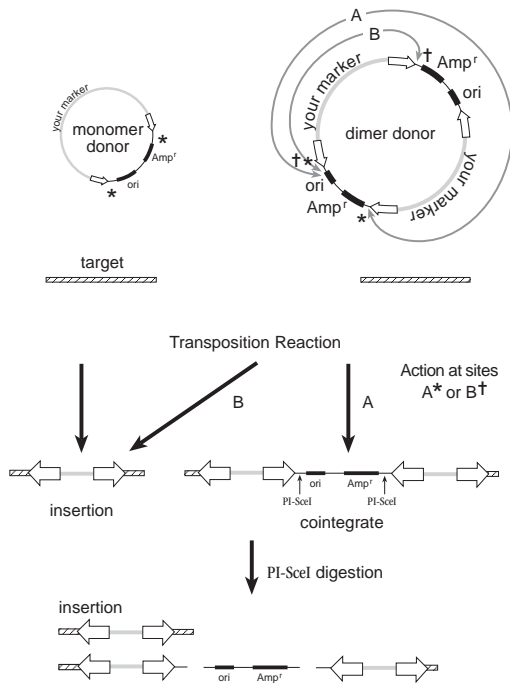
B. The donor backbone is smaller than the material carried by the transposon (Figure 4). Transprimer-1 is 1699 bp. The pGPS3 backbone is 2594 bp.

Briefly, the frequency of cointegrate products (insertion of two copies of the transposon plus the donor backbone) increases when both of these is true (Figure 4).

Note that cointegrate products as well as unreacted donor molecules will be destroyed by digestion with PI-Sce I. This enzyme has a very large recognition site, and should not cut your target DNA. We recommend including the digestion step even if you anticipate that the donor plasmid will not replicate in your organism of interest, in order to avoid cointegrate products. These cointegrate insertions will effectively disrupt gene function but sequencing from transposon primers will give two superimposed sequences.

7. You may omit the PI-Sce I digestion step if you ensure that your donor preparation is monomeric and supercoiled and if the donor molecules will not replicate in your organism.

Figure 4: Alternative Products that may result when the Transposase donor is multimeric. *TnsABC** Transposase will act at appropriately oriented transposon ends (*,†). On dimeric molecules (right side), transposase has a choice: either it can act on two ends from the same transposon (arc B), or it can act on one end each from the two transposon copies (arc A on the dimeric molecule). If the transposon is longer than the vector backbone, the arc A is chosen frequently. This results in a cointegrate structure, which can be destroyed by *PI-Sce I* digestion. Note: Target DNA is shown here as a linear molecule, but can also be circular.



Appendix I: Kit Component Composition

Stock Concentration

- 10X GPS Buffer (Reagent 1)
 - Tris-HCl 250 mM (pH 8.0)
 - DTT 20 mM
 - ATP 20 mM

- pGPS3 (Transprimer-1 Donor plasmid) (Reagent 2) 200 µg/ml
For a restriction map, see Figure 6, Appendix VII. Sequence is available from our web site www.neb.com or upon request. pGPS3 contains a pUC19 origin.

- TnsABC* Transposase (Reagent 3):
 - TnsA 7 µg/ml
 - TnsB 10 µg/ml
 - TnsC* 20 µg/mlSupplied as a mix in 50% glycerol storage buffer.
When not at -20°C, TnsABC* Transposase should be kept on ice.

- Start Solution (Reagent 4): magnesium acetate 300 mM

- Sequencing Primers 3.2 pmol/µl in dH₂O
 - Primer N Anneals to the top strand of Transprimer as in Figure 2
 - Primer S Anneals to the bottom strand of Transprimer as in Figure 2

- Control Target (Reagent 5): LITMUS 28 80 µg/ml

- PI-Sce I (VDE I): 1,000 units/ml

- 10X PI-Sce I Buffer: 100 mM Tris-HCl (pH 8.6)
 - 1 M KCl
 - 100 mM MgCl₂
 - 10 mM DTT

- BSA 10 mg/ml

Appendix II: Target DNA Requirements

Target DNA attributes:

- A. Plasmid targets for sequencing should be in circular form to facilitate recovery. Linear (e.g., chromosomal) DNA is an efficient substrate. A repair and ligation step is required before transformation, when using naturally transformable organisms (Figure 5 and Gap Repair Protocol, page 12).
- B. Large plasmids, such as cosmids and BACs, should be usable targets. We have successfully obtained sequence from cosmids and BACs. See Appendix VI for more information.

Target DNA concentration:

Target DNA must be at least 5 $\mu\text{g/ml}$ in a no-salt buffer such as 1X TE. Concentration can be estimated by comparison of agarose gel band intensity with a DNA of known concentration or by absorbance at 260.

Appendix III: Theory of the Transposition Reaction

■ **Functions of the transposase proteins**

TnsB specifically recognizes the Transprimer ends which are derived from transposon Tn7. TnsC binds to target DNA and interacts with TnsB. TnsA binds to DNA:TnsB. When all three proteins are suitably bound in a three-protein, two-DNA complex, TnsA and TnsB together carry out the strand transfer reaction. For a full review of this topic, see reference 1. The reaction used here is enabled by a mutation in TnsC (TnsC*) that bypasses the usual requirement for TnsD or TnsE (2).

■ **Tn7 Target immunity and its consequences**

The TnsABC* Transposase will not efficiently use a target molecule already containing Tn7 ends; as a result, double insertions are minimized. *In vivo*, target immunity operates at distances of at least 190 kb. At present, the model for this immunity invokes an activity of TnsC, mediated by its ATPase activity and its specific recognition of DNA:TnsB complexes. TnsC* binds nonspecifically to DNA and surveys it. If TnsB is encountered (i.e., if a Tn7 end is already present), the incipient strand transfer complex is disrupted by an ATP-mediated activity of TnsC. For more extensive discussion of this property, see reference 10.

■ **Strand transfer** (Figure 5, page 19)

Transposition proceeds via two DNA scissions and two transesterification reactions. For simplicity of representation, this is presented as six cleavages and two ligations. Donor DNA is cleaved three bases 5' to the transposon end in one strand and precisely at the transposon 3' end in the other strand (Figure 5); this occurs on each side of the transposon. A five-base staggered cleavage is made in the target. The 3' hydroxyls of the transposon are ligated to the 5' phosphates of target. The net result is excision of the transposon from the donor, together with three-base 5' extensions on each end and addition of the transposon to the target at the opening. *In vivo*, the three-base flap of donor origin is resected, and the resulting gap filled in and ligated. A five-base duplication of target sequence results from this process. The covalent linkages labeled with a star are those involved in or produced by transesterification reactions between the 3' transposon end and the 5' end of the target.

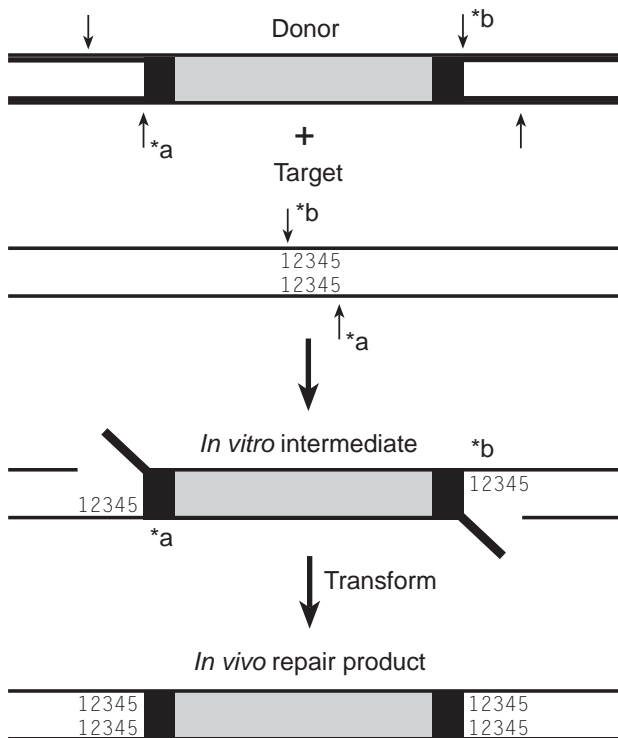


Figure 5: Strand Transfer Reaction. Note that 5 bp of target sequence will be duplicated at the insertion site: the same 5 bp will appear to the left of Tn7L and to the right of Tn7R (see Appendix III).

Appendix IV: Properties of Reaction Products

■ **Random insertion:**

In trials at New England Biolabs, 63 sequenced insertions were found at 62 separate sites. The distribution of insertions in 100 bp intervals was statistically indistinguishable from a random (Poisson) distribution (3). Physical detection methods suggest that insertion can be obtained at every internucleotide linkage. For mutagenesis purposes, this means that saturation should be achievable on a Poisson basis.

■ **Simple insertion:**

Single simple insertions are the principal products of the TnsABC* transposition reaction. Of 74 insertions examined by restriction digest, 73 were single simple insertions.

Other products, which are produced at a significant level by some other transposition systems, give unreadable sequence. These more complicated products include double insertions and cointegrates between the donor and target replicons.

Few double insertions are seen with TnsABC* Transposase presumably because of target immunity (see page 18) (10). Cointegrates are rare in reactions that employ the supplied donor and include the assembly reaction (Step 3) in the GPS-M Protocol. However, a high fraction of products were cointegrates when the assembly reaction (Step 3) was omitted (i.e., when Mg^{++} is included in the starting buffer or is added before the TnsABC* Transposase). Cointegrates also are obtained under some conditions of donor state (see Tips on Donor Manipulation, page 13).

■ **Target site duplication:**

The reaction process duplicates a five base pair sequence at the site of insertion (see Figure 5). We have not seen insertions without this duplication. This is useful to recall when assembling the sequence.

Appendix V: Recipes

■ **Luria Bertani Broth, per liter** (for outgrowth following transformation)

Tryptone (Difco)	10 g
Yeast Extract (Difco)	5 g
NaCl	5 g
NaOH (1 N)	2 ml
autoclave	

■ **Luria Bertani Agar with drug, per liter** (for plating transformation outgrowth cultures)

Tryptone (Difco)	10 g
Yeast Extract (Difco)	5 g
NaCl (Baker)	5 g
NaOH (1 N)	2 ml
Agar (Difco)	15 g
autoclave	

■ **0.85% saline, per liter** (for diluting cells after transformation)

NaCl	8.5 g
autoclave	

■ **1X TE, per liter** (for diluting DNA)

1 M Tris-HCl pH 8.0	10 ml
0.5 M EDTA pH 8.0	2 ml

Appendix VI: Frequently Asked Questions

■ *Why would I want to use the GPS-M System?*

This kit is designed for use by researchers who want to customize the transposable element for their own purposes. For example, a drug resistance gene that expresses in Gram-positive organisms or tissue culture cells might be added or substituted for kanamycin resistance, or a promoterless reporter of transcription entering from the ends might be added. The most common use may be to mutagenize plasmid or chromosomal DNA and study its behavior in the organism of interest.

■ *What is the difference between the Transprimer on pGPS3 (supplied with this system) and the one on pGPS1.1 (supplied with our GPS-1 Genome Priming System)?*

There is no difference. The Transprimer-1 transposable element is the same.

■ *What is the difference between pGPS3 and pGPS1.1?*

The origin of replication of pGPS3 is the same as that of pUC19 and will replicate in all standard laboratory strains of *E. coli*. The vector backbone of pGPS3 also carries the Amp^r gene of pUC19. The origin of replication of pGPS1.1 is derived from the conjugal plasmid R6K, and will not replicate in *E. coli* unless a plasmid-derived initiation protein is present.

■ *Can I use the GPS-1 System for mutagenesis?*

Yes, if the gene is to be studied in *E. coli* or other enteric organisms. The Kan^r and Cam^r genes in Transprimer-1 and Transprimer-2 are not arranged to express in nonenteric organisms.

■ *Where will the insertions be?*

Insertions will not be found in any target DNA that has been selected for or is essential. They will be approximately randomly located within all the rest of the DNA.

■ *How do I find the molecules with insertions?*

If using pGPS3 directly as a donor, plate on media with kanamycin (20 µg/ml) after transformation and a period of outgrowth to allow expression of drug resistance. If using a construct of your own design, be sure to include a suitable selectable marker between the transposon ends.

■ *Will all of my reaction products be simple insertions?*

Multiple transformants are seen occasionally: ~5% of transformants contain both mutagenized target and an unaltered target molecule when chemically-competent cells are used with pGPS3 and a standard target plasmid. In mutagenesis applications, this may be undesirable, since the unaltered target will express the gene of interest, even though the mutagenized target does not. The solution to this problem is to transform with a lower ratio of target plasmid molecules to cell number, e.g., by using electroporation instead of chemical competence.

If you are using a construct of your own design, be aware that multimeric plasmid donors can give rise to a cointegrate structure. See page 13 (Tips on donor manipulation) for further discussion.

■ *When do I need to carry out the gap repair step?*

Only when you will carry out steps that convert the *in vitro* product to single strands, for example by transformation into naturally competent organisms (see page 11).

■ *What happens if I heat kill at 65°C instead of 75°C?*

In *E. coli* the number of colonies will be reduced by at least 100-fold.

- *Can I use T4 DNA Ligase Buffer instead of GPS Buffer? Its composition looks very similar.*

Ligase Buffer should not be used. Since Mg^{++} is present from the beginning of the reaction, the assembly reaction (page 8) does not occur. We also find a high fraction of cointegrate molecules (replicon fusions) when Ligase Buffer is used.

- *Can I use a different Mg^{++} salt instead of magnesium acetate for the Start Solution?*

Yes. $MgSO_4$ and $MgCl_2$ have been tested and are acceptable.

- *What *E. coli* strain should I use for transformation of the GPS reaction mix?*

Any standard *E. coli* strain can be used as long as it does not contain the same antibiotic-resistance as the Transprimer in the GPS reaction being transformed. A general recommendation is to use whichever strain you would normally use for making minipreps.

- *What conditions are recommended for sequencing reactions using Primer N and Primer S?*

Standard sequencing reaction conditions work well using these primers. For example, a 50°C annealing temperature is used with these primers with an ABI Sequencer.

- *Can GPS be used with BACs?*

Yes. Recommendations for use of GPS with BACs are similar to those for cosmid targets. A donor:target molar ratio of 2:1 is effective. For a standard reaction using 0.02 μg donor, we recommend using for example: 0.1 μg of a 50 kb cosmid and 0.3 μg of a 150 kb BAC. Electroporation should always be used with such large molecules since using chemically-competent cells will tend to favor deletions. If there is any concern as to the purity of the BAC prep, the GPS reaction can be done at 30°C and is as effective as 37°C. It is useful to spread a small amount of the transformation mix onto a plate selecting only for the BAC target DNA. This ensures that the BAC prep is good and that transformation was efficient.

■ *Can I easily sequence cosmids and BACs containing Transprimer insertions?*

Yes. We have obtained excellent sequencing results by following these general recommendations:

Cosmids

per 20 μ l sequencing reaction:

500 ng–1 μ g DNA

4 μ l Primer N or Primer S at 3.2 pmol/ μ l.

Thermal cycling:

5 minutes at 96°C

25–50 cycles of:

30 seconds at 96°C

15 seconds at 50°C

3 minutes at 60°C

BACs

Protocol from Boysen, C., Simon, M. & Hood, L. (1997) *Biotechniques* 23, 978–982.

Per 40 μ l sequencing reaction:

1–2 μ g DNA

50 pmol Primer N or Primer S

Double amount of other components

Thermal Cycling:

5 minutes at 96°C

25–50 cycles of:

10 seconds at 96°C

5 seconds at 50°C

4 minutes at 60°C

■ *Can I store unused GPS reaction mix?*

Yes. After the heat-kill step the reaction mix can be stored at –20°C and transformed later.

■ *Can I use linear DNA as target DNA?*

Yes. Linear DNA is an efficient substrate for the GPS reaction. However, the reaction products must be ligated to DNA containing an origin of replication (e.g., vector DNA) in order to form a circular plasmid capable of forming colonies after transformation into *E. coli*.

■ *Can I reduce the number of colonies formed by Transprimer insertions into the vector backbone of the target DNA?*

Yes. Selecting for markers, such as antibiotic-resistance, in the vector backbone of the target will prevent colony formation by Transprimer insertions within these markers. Also, the GPS reaction can be performed using linear target DNA such as a PCR product or an insert fragment. Products of this GPS reaction can then be ligated to vector DNA, transformed and plated on media selecting for the antibiotic resistance encoded by the Transprimer.

■ *What are the melting temperatures for Primer N and Primer S?*

Primer S: 82°C

Primer N: 74°C

■ *Where can I find the entire sequences of the Transprimers and the donor plasmids?*

These are all available from our website at www.neb.com or upon request.

■ *Does Transprimer-1 encode neomycin-resistance?*

Yes. The *npt* gene in Transprimer-1 encodes neomycin phosphotransferase, which confers resistance to neomycin and kanamycin.

■ *Does the kanamycin-resistance gene of pGPS3 express in gram-positive organisms?*

No. The drug resistances in the GPS donor plasmids are not designed for expression in *B. subtilis* or other gram-positive bacteria.

■ *Can GPS be used with very GC-rich, highly-repetitive target DNA?*

The GPS System may discriminate against regions with very high G + C (> 65%). In this situation we recommend digesting minipreps before sequencing to determine whether Transprimer insertions are within the target's vector backbone DNA or the DNA of interest. If the numbers of insertions within the vector are disproportionately high, it is possible to do the GPS reaction on linear DNA of interest and then ligate this to the vector, transform and plate on media selecting for the antibiotic-resistance of the Transprimer. This ensures that all Transprimer insertions will be within the DNA of interest and not the target's vector backbone. This technique can be applied to all types of target DNA.

■ *Can I map Transprimer insertions using PCR?*

Yes. DNA from colonies can be analyzed using PCR. Since the Transprimer can insert in either orientation, PCR reactions can be performed using one primer complementary to the target DNA, and *both* GPS primers. Alternatively, two PCR reactions can be set up for each DNA, one with Primer N and one with Primer S.

Appendix VII: Additional Information

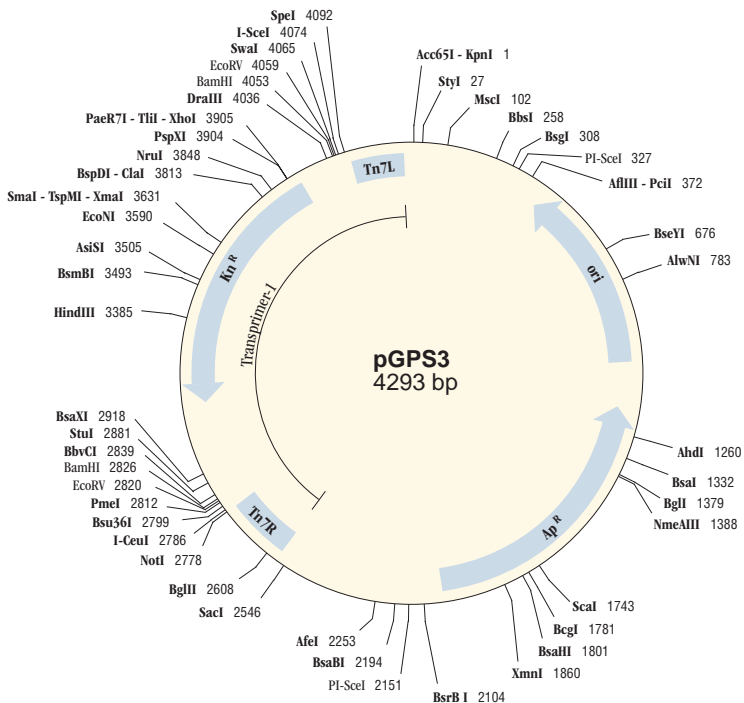
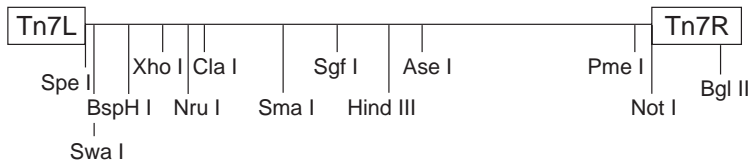


Figure 6: *pGPS3*. Note: *PI-Sce I* (VDE) is not the same as *I-Sce I*

■ **Map of Transprimer-1 (Kan^r): 1699 bp**

(Unique palindromic restriction sites)



■ **Sites in the Transprimer-1 element:**

Enzymes that cut once:

AseI, BanII, BbvCI, BglII, BsaWI, BsmBI, BsmFI, Bsp1286I, BspDI, BspHI, BsrDI, BsrFI, BssSI, Bsu36I, ClaI, DraIII, Eco57I, EcoNI, HindIII, I-CeuI, I-SceI, MspAII, NotI, NruI, PaeR7I, PflMI, PmeI, PvuI, RsaI, SgfI, SmaI, SmlI, SpeI, SspI, StuI, SwaI, XhoI, XmaI

Enzymes that do not cut:

AatII, Acc65I, AclI, AfeI, AflII, AflIII, AgeI, AhdI, AlwNI, ApaI, ApaLI, AscI, AvrII, BanI, BbsI, BbvI, BcgI, BciVI, BclI, BglI, BlpI, BpmI, BsaI, BsaAI, BsaBI, BsaHI, BseRI, BsgI, BsiHKAI, BsiWI, BspE I, BspLU11 I, BspM I, BsrBI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, DrdII, Eco47III, EcoO109I, EcoRI, FauI, FseI, FspI, HaeII, HpaI, KasI, KpnI, MfeI, MluI, MscI, MslI, NaeI, NarI, NcoI, NdeI, NgoAIV, NgoMIV, NheI, NspI, PacI, PflFI, PI-PspI, PI-SceI, PmlI, PpuMI, PshAI, PstI, PvuII, RsrII, SacI, SacII, SanDI, SapI, SbfI, ScaI, SexAI, SfcI, Sfi I, SfoI, SgrAI, SnaBI, SphI, Srf I, StyI, TatI, TseI, Tth111I, XbaI, XcmI, XmnI

Enzymes that cut once in pGPS3 but do not cut in Transprimer-1 element:

Acc65I, AfeI, AflIII, AhdI, AlwNI, BbsI, BcgI, BglI, BsaI, BsaBI, BsaHI, BsgI, BspLU11I, BsrBI, KpnI, MscI, PciI, SacI, ScaI, StyI, XmnI

■ ***Approximate sizes of selectable marker genes and origins of replication***

Ampicillin resistance (β -lactamase, *bla*; including promoter): 950 bp

Tetracycline resistance (Tet, Tc): 1,200 bp

Neomycin, kanamycin resistance (neomycin phosphotransferase, *npt*, Kan, Neo) from Tn5: 1,000 bp (found in cosmids); from Tn903: 850 bp (found in pACYC184 and the Transprimer)

Chloramphenicol resistance (chloramphenicol transacetylase, *cat*, Cam): 700 bp
Note: we have observed an insertion in the penultimate codon of cat that retained drug resistance.

ColEI origin of replication: 600 bp

p15A origin of replication: 810 bp

References

1. Craig, N.L. (1996) *Curr. Top. Microbiol. Immunol.* 204, 27–48.
2. Stellwagen, A.E. and Craig, N.L. (1997) *Genetics* 145, 573–585.
3. Biery, M.C., Stewart, F.J., Stellwagen, A.E., Raleigh, E.A., and Craig, N.L. (2000) *Nucl. Acids Res.* 28, 1067–1077.
4. Gwinn, M.L., Stellwagen, A.E., Craig, N.L., Tomb, J.F. and Smith, H.O. (1997) *J. Bacteriol.* 179, 7315–7320.
5. Xu, Y., Murray, B.E. and Weinstock, G.M. (1998) *Infect. Immun.* 66, 4313–4323.
6. DeBoy, R. and Craig N.L. (1996) *J. Bacteriol* 178, 6184–6191.
7. Chandler, M., Clerget, M. and Galas, D.J. (1982) *J. Mol. Biol.* 154, 229–243.
8. Morisato, D., Way, J.C., Kim, H.J. and Kleckner, N. (1983) *Cell* 32, 799–807.
9. Faelen, M., Toussaint, A., Waggoner, B., Desmet, L. and Pato, M. (1986) *Virology* 153, 70–79.
10. Stellwagen, A.E. and Craig, N.L. (1997) *EMBO J.* 16, 6823–6834.

Kit Components Sold Separately

pGPS3 #N7130S	10 µg
TnsABC* Transposase #P7190S	10 µl (10 reactions)
Primer N #S1267S	0.5 A ₂₆₀ units
Primer S #S1266S	0.5 A ₂₆₀ units
10X GPS Buffer and Start Solution #B7100S	
LITMUS 28 #N3628S	20 µg
PI-SceI #R0696S #R0696L	100 units 500 units
NEBuffer PI-SceI #B0696S	0.5 ml

Companion Products

1 kb DNA Ladder #N3232S #N3232L	100 µg 500 µg
I-CeuI #R0699S #R0699L	250 units 1,250 units
I-SceI #R0694S #R0694L	250 units 1,250 units
NotI #R0189S #R0189L	500 units 2,500 units
PmeI #R0560S #R0560L	500 units 2,500 units
SpeI #R0133S #R0133L	500 units 2,500 units
SwaI #R0604S #R0604L	2,000 units 10,000 units



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