

plnvRecA Vector Technical Specifications

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500 µl glycerol stock of plasmid in *E. coli*® 10G cells
Catalog No. 42205-1

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Not for Drug or Diagnostic use. Not for use in humans or animals.

plnvRecA Vector for generalized P1 transduction

DNA transduction using the *E. coli* bacteriophage P1 is highly useful in generating mutant strains for microbial genomic and proteomic studies, and for moving genes from one strain to another. However, phage P1-mediated transduction can be used only with wild-type (*RecA*⁺) *E. coli* strains. This limits the applicability of this procedure, as most *E. coli* cloning strains, including Lucigen's *E. coli*® 10G cells, are *RecA*⁻ to provide stability of large inserts.

The plnvRecA vector enables phage P1 transduction to be performed in any *E. coli* strain.¹ This vector transiently converts the *RecA*⁻ to the *RecA*⁺ phenotype, allowing P1 transduction. The promoterless and inverted *RecA* gene is located between two convergently oriented Flippase (Flp) recognition target sequences (FRT) and four strong transcription terminators (*rrnBT1*) (Figure 1). This vector design prevents leaky transcription of *RecA*. The *RecA*⁺ function can then be induced with heat-inactivated chlortetracycline (cTc). cTc activates the *tetA* promoter, inducing synthesis of the Flp yeast recombinase, which then inverts the *recA* gene, resulting in *RecA* protein production. The conversion to *RecA*⁺ enables homologous recombination after phage P1 infection (Figure 1).

Following standard P1 transduction at 37°C, the *RecA*⁻ phenotype is completely restored by growing cells at 42°C. plnvRecA contains the selectable *ts*-replicon from pSC101 that allows for replication in *E. coli* at 30°C but not at temperatures above 37°C. The higher temperature incubation completely eliminates the plnvRecA vector from host cells.

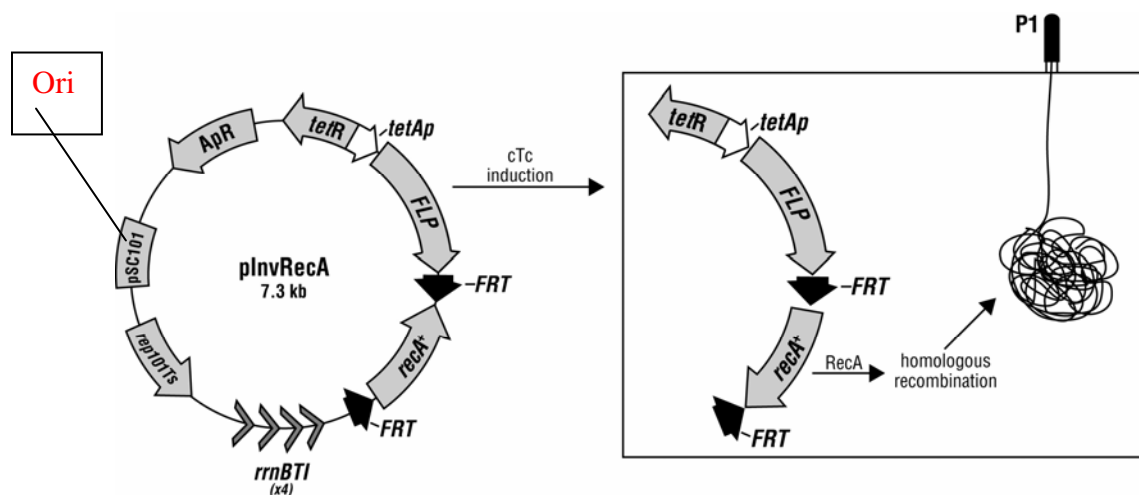


Figure 1. Schematic diagram of the plnvRecA vector and its use. *recA*⁺, promoterless *recA* gene; FRT, Flp yeast recombinase recognition sequence; FLP, Flp yeast recombinase gene; *tetAp*, tetracycline resistance gene; *ApR*, ampicillin resistance gene; Ori, pSC101 origin of replication; *rep101TS*, temperature sensitive replication protein; *rrnBT1*, transcription terminators. Incubation with heat inactivated chlortetracycline (cTc) activates the *tetA* promoter, resulting in Flp/FRT-mediated inversion of the *recA* gene. *RecA* protein is produced from the inverted gene, enabling homologous recombination of DNA injected by P1 transducing phage.

References

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