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PRODUCT INFORMATION

Genomic Integration Kit (KanR)

Cat. No. D070201
Kit size: 10 reactions

DESCRIPTION

Genomic Integration Kit (KanR) has been designed for efficient mutagenesis of all types of gram-negative bacterial species. Genomic integration is mediated by Mu DNA Transposition Complexes (KanR), which are stable protein-DNA complexes formed between v.3 MuA Transposase (in vivo Integrator) and Kan-Mu Transposon (KanR). These complexes remain inactive in the absence of divalent metal ions. Mu DNA Transposition Complexes (KanR) can be electroporated into cells, where the v.3 MuA Transposase is activated by host cell's Mg^{2+} ions, resulting in the random integration of Kan-Mu Transposon (KanR) into the host chromosome (Lamberg et al. 2002). Kan-Mu Transposon (KanR) contains the marker gene *kan*, which confers resistance to kanamycin, and is designed for all types of gram-negative bacteria. The kit also contains Kan-Mu Transposon -specific forward and reverse primers, which can be used for determining the transposon integration sites, e.g. by direct sequencing of microbial genomic DNA without cloning (Heiner, C.R. et al., 1998, Hoffman et al. 2000), by cloning a fragment of DNA containing the transposon (Lamberg et al. 2002), or using PCR-based protocols, such as ligation-mediated PCR (Prud'hom et al., 1998), or inverse PCR (Rich and Willis 1990, Martin et al. 1998).

KIT COMPONENTS

Product No.	Product	Volume
DP04	Mu DNA Transposition Complexes (CamR) (0.1 pmol/µl)	10 µl
DP05	Kan-Mu Forward Primer (50 µM) (KanMu-SeqF-1)	20 µl
DP06	Kan-Mu Reverse Primer (50 µM) (KanMu-SeqR-1)	20 µl

Storage conditions
Store at -20°C. Guaranteed stable for 12 months when properly stored.

Storage buffer
Mu DNA Transposition Complexes (KanR) are supplied in a solution containing 50 % glycerol, 150mM Tris (pH 6.0), 0.025% Triton™ X-100, 50 mM NaCl, 0.1 mM EDTA. Primers are supplied in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Activity of Mu DNA Transposition Complexes (KanR) was determined using electroporation of transposition complexes into *E. coli* electrocompetent cells (transformation efficiency > 10^9 cfu/µg of pUC19 DNA). As a result, up to $5x10^5$ chloramphenicol resistant colonies were recovered per µg of transposon DNA.

APPLICATIONS

Genomic Integration Kit (KanR) has been designed for efficient mutagenesis of all types of gram-negative bacterial strains, and it can be used for a wide array of applications, for example for:

- Insert kanamycin-resistance marker gene into the genome of bacterial species
- Efficiently generate gene knock-outs *in vivo*
- Generate exhaustive single-insertion transposon mutant libraries
- Map the transposon insertion sites within genomic DNA using the primers included in the kit

PROTOCOL

Electroporation of MuA Transposition complexes (KanR) into *Escherichia coli* host cells

Use 1 µl of the **Mu DNA Transposition Complexes (KanR)** for electroporation into a recipient strain (**See Note 1**) and plate on appropriate selection plates.

1. Thaw competent *E. coli* cells on ice. To maximize the number of clones with transposon insertion, use electrocompetent cells with the highest transformation efficiency possible.

2. Add 1 µl of **Mu DNA Transposition Complexes (CamR)** into 25 µl of electrocompetent cells in a cold tube using pre-chilled pipette tips. **See Note 2 for diluting the transposition complexes.**

3. **IMMEADIATELY** transfer the mixture into an ice-cold electroporation cuvette (0.1 cm electrode spacing, Bio-Rad).

4. Electroporate **IMMEDIATELY** using Genepulser II electroporation apparatus (Bio-Rad) with the following pulse settings: voltage 1.8 kV, resistance 200 Ω, and capacitance 25 µF. If other equipment is used, optimal pulse parameters may differ.

5. Add 1 ml SOC (room temperature solution), transfer to a 15 ml tube.

6. Incubate at 37 °C by shaking (220 rpm) for 40-60 min.

7. Spread the cells onto selection plates containing kanamycin (25 µg/ml). **See Note 3 for the expected efficiency of integration.**

Notes

1. Electroporation of Mu DNA transposition complexes has been used successfully with many bacterial species, including gram-negatives, such as *E. coli, Salmonella enterica, Erwinia carotovora, Yersinia enterocolitica, Erwinia carotovora, Pseudomonas aeruginosa* (Lamberg et al. 2002, Li et al. 2007).
2. With some strains diluting the transposition complexes prior to electroporation **will increase** the total number of genomic integration events. You can dilute the transposition complexes e.g. 4 or 8-fold in sterile water. Use 1 µl of the dilution per electroporation.
3. The efficiency of genomic integration is influenced mostly by the competence status of a given strain. In general, the efficiency of transposon integration is about three orders of magnitude lower than the plasmid electroporation efficiency (per µg of plasmid DNA). For example, if the plasmid electroporation efficiency of your favorite bacterial species is 10^7 cfu per µg of plasmid DNA, the expected integration efficiency will be about 10^4 cfu per µg of transposon DNA.

PRIMERS

KanMu-SeqF-1

Product No. DP05

AATCGAACTTATTCGGTCGA

Tm 53°C (nearest neighbor method)

KanMu-SeqR-1

Product No. DP06

GATGATCAGCGGCCGCGATC

Tm 62°C (nearest neighbor method)

SEQUENCE OF KAN-MU TRANSPOSON (KanR)

Transposon sequence can be downloaded at : https://www.domusbiotechnologies.com/documents

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Precautions

This product is for research use only. Not for use in diagnostic or therapeutic procedures.
Triton is a trademark of The Dow Chemical Company or an affiliated company of Dow.

Patent

v.3 MuA Transposase (in vivo Integrator) is covered by International Patent No. WO 2014/013127 Al

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