DOMUS
BIOTECHNOLOGIES

PRODUCT INFORMATION

Genomic Integration Kit (Gram-positive, KmR)

Cat. No. D070401
Kit size: 10 reactions

DESCRIPTION

Genomic Integration Kit (Gram-positive, KmR) has been designed for insertion mutagenesis of Gram-positive bacteria. Genomic integration is mediated by Mu DNA Transposition Complexes (KmR), which are stable protein-DNA complexes formed between v.3 MuA Transposase (in vivo Integrator) and Km-Mu Transposon (KmR). These complexes remain inactive in the absence of divalent metal ions. Mu DNA Transposition Complexes (KmR) 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 Km-Mu Transposon (KmR) into the host chromosome (Pajunen et al. 2005). Km-Mu Transposon (KmR) contains the marker gene *aphA3*, which confers resistance to kanamycin. The kit also contains Km-Mu Transposon -specific forward and reverse primers, which can be used to determine the transposon integration sites. Suitable methodologies for individual clones include direct PCR-based sequencing from microbial genomic DNA (Heiner et al. 1998, Hoffman et al. 2000), initial cloning of transposon-containing fragments (Lamberg et al. 2002), ligation-mediated PCR (Prud'hom et al. 1998), and inverse PCR (Rich and Willis 1990, Martin and Mohn 1999). For high-throughput mapping of transposon insertion sites across an entire mutant library, we recommend the Tn-seq method described by Song et al. (2025).

KIT COMPONENTS

Product No.	Product	Volume
DP10	Mu DNA Transposition Complexes (KmR) (0.1 pmol/µl)	10 µl
DP11	Km-Mu Forward Primer (50 µM) (KmMu-SeqF-1)	20 µl
DP12	Km-Mu Reverse Primer (50 µM) (KmMu-SeqR-2)	20 µl

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

Storage buffer
Mu DNA Transposition Complexes (KmR) are supplied in a solution containing 50 % glycerol (vol/vol), 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).

APPLICATIONS

Genomic Integration Kit (Gram-positive, KmR) has been designed for efficient mutagenesis of Gram-positive bacteria, and it can be used for a wide array of applications, for example:

- Insert kanamycin-resistance marker gene into the genome of Gram positive 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 (KmR) into Gram-positive bacterial cells

Electroporate Mu DNA Transposition Complexes (KmR) into electrocompetent bacterial cells using an electroporation protocol optimized for the recipient strain. Use 1 µl of the transposition complexes per electroporation as an initial recommendation.

1. Thaw competent bacterial 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 (KmR)** into 50 µl of electrocompetent cells in a cold tube using pre-chilled pipette tips. **See Note 2 for diluting the transposition complexes.**

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

4. Electroporate **IMMEDIATELY** using conditions optimized for the recipient strain. For Streptococcus pyogenes and Streptococcus suis use the following conditions: 0.1 cm electrode spacing, 1.8 kV, 200 Ω and 15 µF (Bio-Rad Gene Pulser II) (Pajunen et al. 2005). Optimal pulse parameters may differ between bacterial strains and electroporation equipment.

5. Immediately add an appropriate recovery medium. For Streptococcus pyogenes and Streptococcus suis add 1 ml of pre-warmed THY medium supplemented with 0.3 M sucrose, followed by incubation at 37 °C for 2 h without agitation (Pajunen et al. 2005).

6. Plate the recovered cells on appropriate selective agar plates containing kanamycin. For Streptococcus pyogenes and Streptococcus suis use THY agar supplemented with 500 µg/ml kanamycin (Pajunen et al. 2005). For scoring of the genomic integrants, the optimal kanamycin concentration should be determined

for each recipient strain. As genomic transposon integrations are single-copy, use the lowest concentration of kanamycin inhibiting the growth of your kanamycin sensitive target cells.

See Note 3 for the expected efficiency of integration.

Notes

1. Electroporation of Km-Mu DNA transposition complexes has been successfully demonstrated in Gram-positive bacteria, including Streptococcus pyogenes and Streptococcus suis (Pajunen et al. 2005). Electroporation parameters should be optimized for each recipient species and strain.
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. Thus, it is advised to initially optimize the protocol for the production of high-competency-status cells by the use of a replicative plasmid. In general, the efficiency of transposon integration is approx. two to 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 approx. 10^4 to 10^5 cfu per µg of transposon DNA.

PRIMERS

KmMu-SeqF-1

Product No. DP11

GGAATTTGTATCGTCGATCC

Tm 53°C (nearest neighbor method)

KmMu-SeqR-2

Product No. DP12

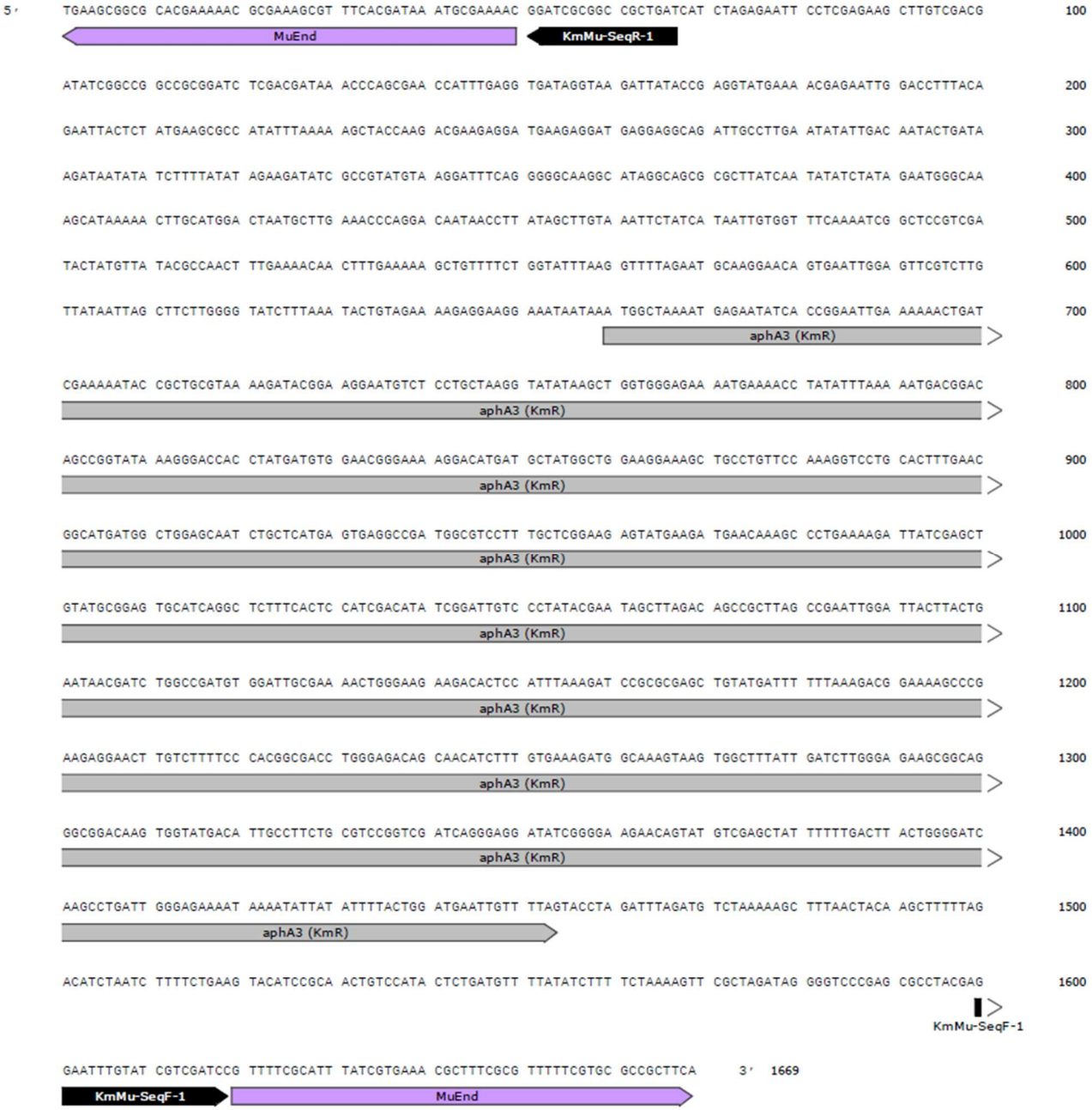
ATCAGCGGCCGCGATCC

Tm 62°C (nearest neighbor method)

SEQUENCE OF KM-MU TRANSPOSON (KmR)

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

Sequence of Km-Mu Transposon (KmR), 1 669 bp. The sequence shown represents the double-stranded region of the transposon. Additional 4-nt single-stranded 5’ overhangs (5’-GATC) are present at both ends but are not included in the sequence shown.



REFERENCES

Haapa-Paananen S, Savilahti H. 2018. Applications of the bacteriophage Mu in vitro transposition reaction and genome manipulation via electroporation of DNA transposition complexes. Methods Mol Biol. 1681:279–286.

Heiner CR et al. 1998. Sequencing multimegabase-template DNA with BigDye terminator chemistry. Genome Res. 8:557.

Hoffman LM, Jendrisak JJ, Meis RJ, Goryshin IY, Reznikoff WS. 2000. Transposome insertional mutagenesis and direct sequencing of microbial genomes. Genetica. 108(1):19–24.

Lamberg A, Nieminen S, Qiao M, Savilahti H. 2002. Efficient insertion mutagenesis strategy for bacterial genomes involving electroporation of in vitro-assembled DNA transposition complexes of bacteriophage Mu. Appl Environ Microbiol. 68:705–712.

Martin VJJ, Mohn WW. 1999. An alternative inverse PCR (IPCR) method to amplify DNA sequences flanking Tn5 transposon insertions. J Microbiol Methods. 35(2):163–166.

Pajunen MI, Pulliainen AT, Finne J, Savilahti H. 2005. Generation of transposon insertion mutant libraries for Gram-positive bacteria by electroporation of phage Mu DNA transposition complexes. Microbiology. 151:1209–1218.

Prud'hom G, Lagier B, Pelicic V, Hance AI, Gicquel B, Guilhot C. 1998. A reliable amplification technique for the characterization of genomic DNA sequences flanking insertion sequences. FEMS Microbiol Lett. 158:75–81.

Rich JJ, Willis DK. 1990. A single oligonucleotide can be used to rapidly isolate DNA sequences flanking a transposon Tn5 insertion by the polymerase chain reaction. Nucleic Acids Res. 18:6673–6676.

Song Y, Liu Y, Li Q, Chen L, Sun Z, Zhang X. 2025. A Tn5 transposase-based system for high-efficiency genome-wide gene activation in Escherichia coli. ACS Synth Biol. 14:2753–2763.**Citation**

Please cite the product in the following manner:

Genomic Integration Kit (Gram-positive, KmR) (Domus Biotechnologies)
To acknowledge the original research enabling the kit please cite Pajunen et al. (2005) paper.

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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