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PRODUCT INFORMATION & PROTOCOL

v.2 MuA 15 bp Insertion Mutagenesis Kit

Cat. No. D070701

Kit size: 10 reactions

Generate random, defined 15 bp insertions in cloned target DNA using the hyperactive v.2 MuA Transposase.

DESCRIPTION

The v.2 MuA 15 bp Insertion Mutagenesis Kit builds on our original research demonstrating the use of MuA transposition for generating random, defined 15 bp insertions in target DNA (Poussu et al., 2004).

The kit is designed for the construction of random insertion libraries for the functional analysis of proteins, regulatory DNA, and cloned viral genomes. The system combines the hyperactive v.2 MuA Transposase (in vitro Manipulator) to be used either with the Cat-Mu(NotI) Transposon or Kan-Mu(NotI) Transposon.

MuA-mediated in vitro transposition inserts the selectable Cat-Mu(NotI) Transposon or Kan-Mu(NotI) Transposon at random positions in the target DNA. Following transformation and proper selection, DNA is isolated from the pool of insertion-containing clones. The transposon body is removed by NotI digestion, and the target DNA is self-ligated to generate a defined 15 bp insertion at each of the original MuA integration sites.

MuA transposition generates a 5 bp target-site duplication. Together with the remnant transposon-end-derived sequences, retained following the NotI digestion and re-ligation, the final 15 bp insertion is produced. In a protein-coding region, the 15 bp insertion maintains the downstream reading frame and corresponds to five additional amino acids. The insertion sequence does not introduce a stop codon in any of the three possible reading frames.

KIT COMPONENTS

Component	Amount
v.2 MuA Transposase (in vitro Manipulator)	10 µl
Cat-Mu(NotI) Transposon	10 µl
Kan-Mu(NotI) Transposon	10 µl
2 x MuA Reaction Buffer	200 µl
Insertion Mapping Primer	20 µl

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STORAGE

Store all kit components at -20°C.

APPLICATIONS

- Protein engineering and functional mapping - generate random five-amino-acid insertion libraries to identify functionally important and insertion-tolerant regions.
- Promoter and regulatory-region mutagenesis - introduce short insertions throughout cloned non-coding DNA to identify regions involved in gene regulation.
- Viral genome mutagenesis - generate random 15 bp insertions in cloned viral DNA for functional mapping and downstream engineering.
- Insertion-tolerance mapping - identify positions that tolerate sequence additions and thus reveal sites that potentially may be useful for subsequent engineering.
- Random NotI site insertion - introduce NotI sites at random positions in cloned DNA constructs to allow straightforward further manipulation possibilities.
- Downstream applications - use NotI sites for the straightforward introduction of extra domains into your target protein.

WORKFLOW

1. Random MuA transposition - v.2 MuA Transposase inserts Cat-Mu(NotI) Transposon or Kan-Mu(NotI) Transposon at random positions in the target DNA.

2. Selection of the primary insertion library - transform the reaction into *E. coli* and select transposon-containing clones with chloramphenicol or kanamycin.

3. Pool the insertion library - collect independent insertion clones and prepare pooled plasmid DNA.

Optional: Transfer the mutagenized target region to an intact vector – if insertions in the vector backbone are undesirable, excise the mutagenized DNA region with suitable restriction enzymes, purify it and transfer it into an intact vector backbone before transposon removal.

4. Remove the transposon - digest the pooled library with NotI to remove the transposon body.

5. Generate the 15 bp insertion library - self-ligate the digested target DNA and transform the re-ligated DNA.

6. Verify, map, and analyze the library - analyze the final 15 bp insertion library e.g. by restriction analysis, insertion mapping, next-generation sequencing and/or functional screening.

USER-SUPPLIED MATERIALS

- Purified target DNA in water or a low-salt buffer.
- High-efficiency competent *E. coli* cells for transformation or electroporation. The latter option is recommended when maximum library diversity is required.
- SOC or other suitable recovery medium and LB agar plates.
- Chloramphenicol or kanamycin and the antibiotic used to select the target plasmid.
- Plasmid DNA purification reagents.
- NotI restriction endonuclease and the manufacturer's recommended buffer.
- DNA ligase and the manufacturer's recommended ligation buffer.
- Agarose gel electrophoresis reagents.
- For DNA purification: DNA purification reagents or an appropriate electroelution device
- For insertion mapping: a suitable PCR polymerase and target/vector-specific forward and reverse primers.

BEFORE YOU START

- The target DNA should be available as a cloned DNA construct suitable for propagation and selection in E. coli.
- Confirm that the target DNA does not contain a NotI site that would interfere with the transposon-removal step.
- The target DNA should not carry chloramphenicol resistance when chloramphenicol is used to select Cat-Mu(NotI) insertion clones or kanamycin resistance when kanamycin is used to select Kan-Mu(NotI) insertion clones.
- MuA insertions can occur in both the DNA sequence of interest and the vector backbone.
- Use high-efficiency transformation or electroporation to maximize the number of independent clones for exhaustive mutagenesis and sizeable library diversity.

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PROTOCOL

1. MuA IN VITRO TRANSPOSITION

Assemble the reaction on ice. Add v.2 MuA Transposase last.

Reagent	Volume
2 x MuA Reaction Buffer	12.5 µl
Target DNA (typically 50-500 ng)	typically 1-2 µl
Cat-Mu(NotI) Transposon or Kan-Mu(NotI) Transposon, 0.5 pmol/µl	1 µl
H_2O	up to 24 µl
v.2 MuA Transposase (220 ng/µl)	1 µl
Total	25 µl

1. Assemble all reaction components except v.2 MuA Transposase on ice.
2. Add 1 µl v.2 MuA Transposase to start the reaction.
3. Incubate at 30°C for 10 minutes.
4. Stop the reaction by adding 25 µl of 1 % SDS and incubate at room temperature for 30 minutes.
5. Add 50 µl water to reduce the salt concentration prior to electroporation.

Optional: Purification and Concentration of Reaction Products

If concentrated reaction products are required, several transposition reactions can be pooled and purified by phenol extraction, followed by chloroform extraction and ethanol precipitation. Resuspend the purified DNA in an appropriate volume of suitable buffer or water for further processing (Haapa-Paananen and Savilahti, 2018).

2. TRANSFORMATION AND SELECTION

Use 1 µl aliquots for transformation or electroporation and plate the cells on appropriate selective media containing antibiotics for both the transposon and the target plasmid. High transformation efficiency is essential to maximize the number of independent clones and library diversity. For electrocompetent E. coli, a transformation efficiency of $>10^8$ CFU/µg pUC19 is recommended.

3. POOL THE PRIMARY INSERTION LIBRARY

Following colony growth, collect the transformants from the selection plates and pool the bacteria. Prepare plasmid DNA from the pooled culture using a standard plasmid purification method.

Optional: if insertions in the vector backbone are undesirable, the mutagenized DNA region can be excised with suitable restriction enzymes, purified and transferred into a fresh vector before the NotI-removal step.

4. NOTI DIGESTION AND TRANSPOSON REMOVAL

Digest the pooled plasmid library with NotI using the restriction enzyme manufacturer's recommended reaction conditions. NotI cleavage removes the body of the NotI-containing transposon.

Separate the digestion products by agarose gel electrophoresis and purify the fragment corresponding to the DNA sequence of interest containing the transposon insertion. Use a suitable DNA gel purification kit, or alternatively, an appropriate electroelution device.

5. RE-LIGATION

Self-ligate the NotI-digested target DNA using a suitable DNA ligase. Follow the ligase manufacturer's recommendations for buffer, enzyme amount, temperature and incubation time. Preferably use a low DNA concentration in the ligation mixture (e.g., 1 ng/µl)

6. TRANSFORMATION OF THE FINAL 15 BP LIBRARY

Transform the re-ligation mixture into competent E. coli cells using a standard cloning transformation or electroporation protocol. Plate the cells on selective media containing only the antibiotic required for the vector plasmid. Do not use chloramphenicol or kanamycin for selection following transposon removal. Pool transformants for library-scale applications or pick individual colonies for clone-level analysis.

INSERTION MAPPING BY COLONY PCR (OPTIONAL)

The supplied Insertion Mapping Primer anneals to the sequence retained at the final 15 bp insertion. Use it together with vector-specific primers flanking the DNA region of interest.

For positional mapping of an individual clone, two PCR reactions can be performed: one with the Insertion Mapping Primer plus a forward flanking primer, and one with the Insertion Mapping Primer plus a reverse flanking primer. PCR chemistry and cycling conditions should be adjusted for the polymerase and flanking primers used.

Because the short insertion-mapping primer can anneal to both strands at the insertion site, non-specific products may occur. Confirm insertion positions by sequencing when required.

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FINAL LIBRARY AND DOWNSTREAM USE

The final library contains clones carrying 15 bp insertions at different positions. In protein-coding regions, the insertion maintains the downstream reading frame and introduces five additional amino acids. The library can be subjected to functional screening, selection, sequencing, or other downstream analyses. The 15 bp insertion contains a NotI site, into which it is straightforward to clone additional DNA (e.g. coding for an extra protein domain).

TROUBLESHOOTING AND PRACTICAL NOTES

Observation	Points to consider
Low number of primary insertion clones	Check competent-cell efficiency, target DNA quality, reaction setup, and selection conditions.
Low library diversity	Increase transformation efficiency and the number of recovered transformants.
Unexpected NotI fragments	Re-check the target construct for endogenous NotI sites and confirm complete digestion.
Poor recovery after re-ligation	Confirm DNA integrity and ligase activity.
Vector-backbone insertions	If not desired, transfer the mutagenized DNA region into a fresh vector backbone prior to NotI-mediated transposon removal.
Ambiguous colony-PCR products	Optimize flanking primers and PCR conditions and confirm candidate clones by sequencing.

REFERENCES AND CITATION

Please cite the product as: V.2 MuA 15 bp Insertion Mutagenesis Kit (Domus Biotechnologies) as well as the original strategy paper (Poussu et al. 2004).

Haapa, S., Taira, S., Heikkinen, E. and Savilahti, H. (1999). An efficient and accurate integration of mini-Mu transposons in vitro: a general methodology for functional genetic analysis and molecular biology applications. Nucleic Acids Research 27, 2777–2784.

Kekarainen, T., Savilahti, H. and Valkonen, J.P.T. (2002). Functional Genomics on Potato Virus A: Virus Genome-Wide Map of Sites Essential for Virus Propagation. Genome Research 12, 584–594.

Poussu, E., Vihinen, M., Paulin, L. and Savilahti, H. (2004). Probing the alpha-complementing domain of E. coli beta-galactosidase with use of an insertional pentapeptide mutagenesis strategy based on Mu in vitro DNA transposition. Proteins 54, 681–692.

Haapa-Paananen, S. and Savilahti, H. (2018). Applications of the Bacteriophage Mu In Vitro Transposition Reaction and Genome Manipulation via Electroporation of DNA Transposition Complexes. Methods in Molecular Biology 1681, 279–286.

RESEARCH USE AND PATENT

For research use only. Not for diagnostic or therapeutic use.

v.2 MuA Transposase (in vitro Manipulator) is covered by International Patent No. WO 2014/013127 A1.

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Appendix I: Descriptions of kit components

Cat-Mu(NotI) Transposon

10 µL 0.5 pmol/µL in TE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 7.5)

Kan-Mu(NotI) Transposon

10 µL 0.5 pmol/µL in TE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 7.5)

Cat-Mu(NotI) Transposon and **Kan-Mu(NotI) Transposon** are selectable Mu transposons designed for 15 bp insertion mutagenesis. Each transposon carries modified Mu end sequences containing *NotI* restriction sites and an antibiotic resistance marker for selection of insertion-containing clones. Cat-Mu(NotI) carries the ***cat*** gene for chloramphenicol resistance (CamR), whereas Kan-Mu(NotI) carries the ***npt*** gene for kanamycin resistance (KanR).

Following *NotI* digestion, the selectable transposon sequence can be removed, leaving a defined 15 bp insertion at the original integration site.

Complete transposon sequences are shown in **Appendix II**.

v.2 MuA Transposase

10 µL 0.22 µg/µL in Storage Buffer.

Storage buffer:

25 mM Hepes, pH7.6, 0.1 mM EDTA, 1 mM DTT, 50% (w/v) glycerol, 500 mM KCl

2X MuA Reaction Buffer

200 µL

50mM Tris pH 8.0, BSA 200µg/ml, 30% (w/v) glycerol, 0.1 % Triton X-100, 200 mM NaCl, 20 mM MgCl2

Insertion Mapping Primer

20 µL, 25 µM in TE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 7.5)

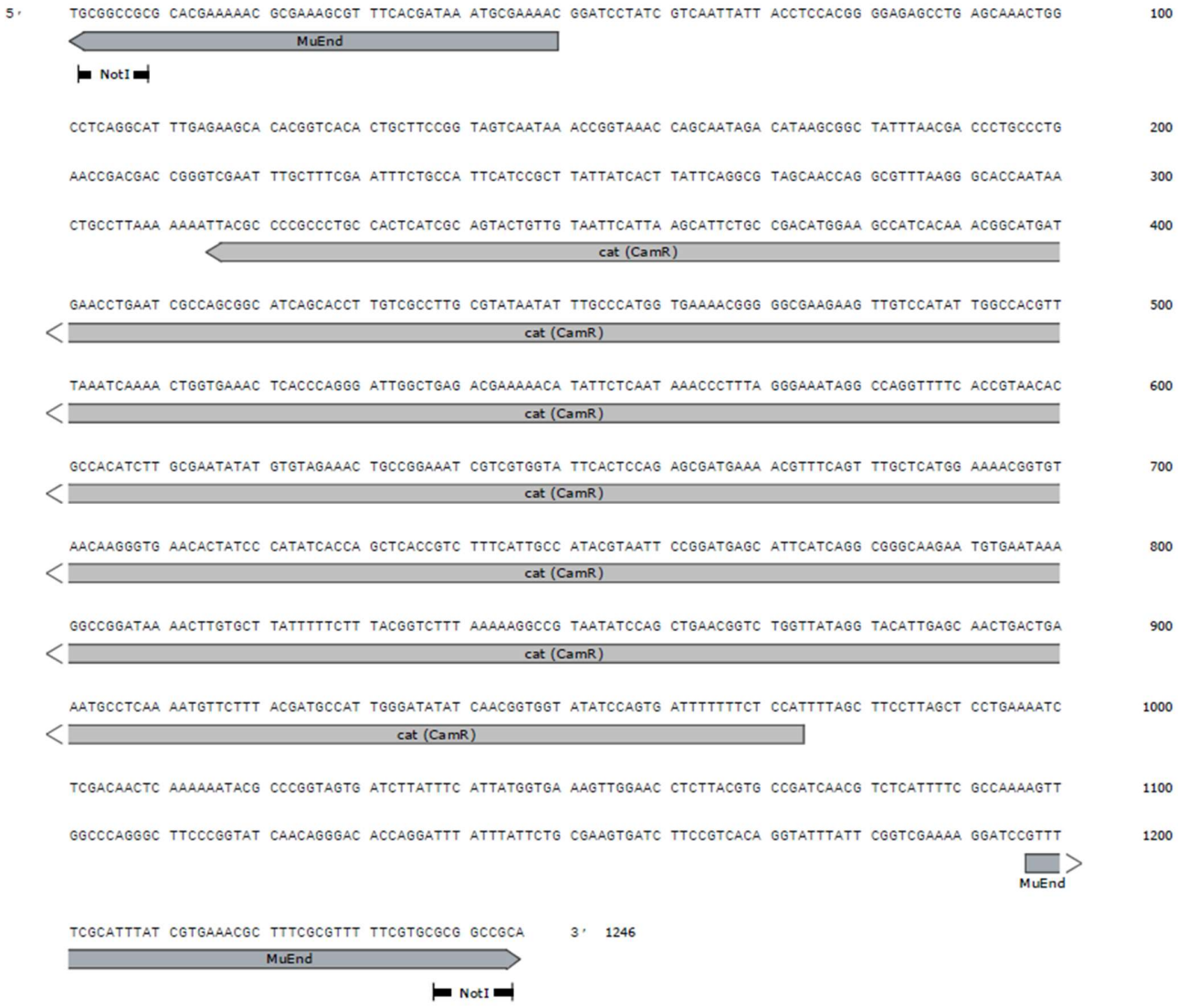
5’-TGCGGCCGCA-3’ Tm 58°C

The Insertion Mapping Primer can anneal to the 15 bp insertion sequence on either DNA strand, enabling PCR-based analysis of insertion sites. Due to its short length, additional non-specific products may occasionally be observed in colony PCR.

Appendix II: Maps of Transposons

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

Sequence of Cat-Mu(NotI) Transposon, 1 246 bp. The sequence shown represents the double-stranded region of the transposon. Additional 4-nt single-stranded 5' overhangs (GATC) are present at both ends but are not included in the sequence shown.



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Sequence of Kan-Mu(NotI) Transposon (1131 bp). The sequence shown represents the double-stranded region of the transposon. Additional 4-nt single-stranded 5’ overhangs (GATC) are present at both ends but are not included in the sequence shown.

