

HyperMu™ <R6KΔpri/KAN-1>Tnp Transposome™ Kit

Cat. No. MTS32RK

The HyperMu™ <R6KΔpri/KAN-1>Tnp Transposome™* is the stable complex formed between the HyperMu Transposase enzyme and the HyperMu <R6KΔpri/KAN-1> Transposon. The HyperMu <R6KΔpri/KAN-1> Transposon contains an R6KΔ conditional origin of replication (R6KΔpri) and the Tn903 kanamycin resistance gene (Kan^R) that is functional in *E. coli*, flanked by R1 and R2¹ HyperMu Transposase recognition sequences. The HyperMu Transposome can be electroporated into living cells where the HyperMu Transposase is activated by Mg²⁺ in the host's cellular environment resulting in random insertion of the HyperMu Transposon into the genomic DNA of the host.

The R6KΔpri makes this transposon useful for "rescue cloning" the region of genomic DNA into which the transposon has been randomly inserted. An overview of the rescue cloning process is presented in Figure 1, page 2. Genomic DNA transposed with the HyperMu <R6KΔpri/KAN-1> Transposon is first purified and then fragmented, self-ligated and finally transformed into an *E. coli* host that expresses the *pir* gene product which is required for replication from the R6KΔ origin.² When selected on kanamycin-containing plates, only the cells propagating the HyperMu <R6KΔpri/KAN-1> Transposon as a plasmid will grow.

Unlabeled forward and reverse transposon-specific primers are supplied in the kit. These primers can be used for bidirectional DNA sequencing or mapping of transposon insertion sites in target genomic DNAs or rescue clones.

Note: EPICENTRE recommends that researchers consider using an EZ::TN™ Transposome Kit in lieu of or in addition to a HyperMu Transposome Kit. An EZ::TN Transposome typically generates 10-100 times more *in vivo* insertions than a HyperMu Transposome in *E. coli*. Since *in vitro* transposition efficiencies are comparable for the two complexes, we believe that the difference in transposition efficiencies is related to a difference in electroporation efficiencies for the two complexes in *E. coli*. Given that this difference could vary with other organisms, we offer the HyperMu Transposome Kit for those who wish to explore its potential.

HyperMu™ <R6KΔpri/KAN-1>Tnp Transposome™ Kit Contents

HyperMu™ <R6KΔpri/KAN-1>Tnp Transposome™ (25 ng/μl of Transposon DNA) ..	10 μl
MUKAN-1 FP-1 Forward Primer @ 50 μM	20 μl
MUKAN-1 RP-1 Reverse Primer @ 50 μM	20 μl
Sterile Water	1 ml

Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: The HyperMu <R6KΔpri/KAN-1>-Tnp Transposome is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton[®]X-100 and 1 mM dithiothreitol. The MUKAN-1 FP-1 Forward and MUKAN-1 RP-1 Reverse Primers are supplied in 10 mM Tris-HCl, (pH 7.5), 1 mM EDTA.

Size: Reagents included in the kit are sufficient for 10 *in vivo* transposon insertion reactions.

Quality Control: HyperMu <R6KΔpri/KAN-1>Tnp Transposome activity is assayed by electroporation into a *recA*⁻ *E. coli* host strain having a transformation efficiency of >10⁹ cfu/μg DNA. Assays must yield >10⁴ Kan^R colonies/μg of DNA or >2.5 x 10² Kan^R colonies/μl of transposome respectively. Primers are function-tested via PCR and in a DNA cycle sequencing reaction using the SequiTherm EXCEL™ II DNA Sequencing Kit and a plasmid containing a HyperMu <R6KΔpri/KAN-1> Transposon as template.

Contaminating Activity Assays: All components of the HyperMu <R6KΔpri/KAN-1>Tnp Transposome Kit are free of detectable DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays with the exception of the inherent endonucleolytic function of the EZ::TN Transposase.

Related Products: The following products are also available:

- TransforMax™ EC100D™ *pir*+ and *pir*-116 Electrocompetent *E. coli*
- HyperMu™ <KAN-1> Insertion Kit
- HyperMu™ Transposase
- End-It™ DNA End-Repair Kit
- Fast-Link™ DNA Ligation Kits
- MasterPure™ DNA Purification Kits

References:

1. Savilahti, H. *et al.*, (1995) *EMBO J.* **14**, 4893.
2. Metcalf, W.W. *et al.*, (1994) *Gene* **138**, 1.

*The use of Transposome™ complexes for *in vivo* insertion of a transposon, including, but not limited to HyperMu™ and EZ::TN™ Transposome complexes, is covered by U.S. Patent No. 6,159,736 and related patent applications, exclusively licensed to EPICENTRE.

Visit our website at <http://www.epicentre.com/transposomics.asp> for more information on our wide variety of Tn5-based EZ::TN Transposon Tools for genetic analysis and sequencing.

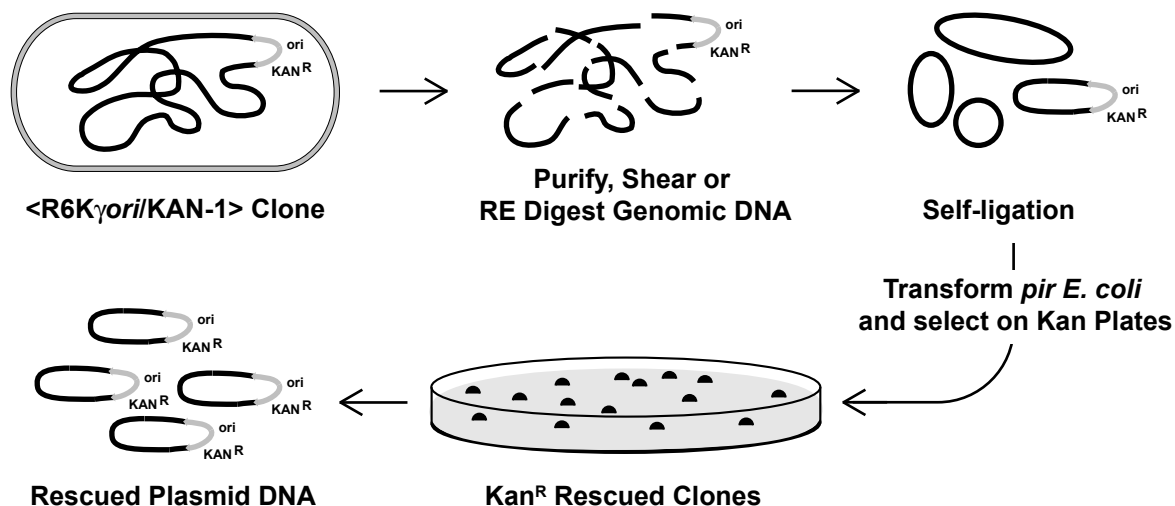
Triton is a registered trademark of Rohm & Haas, Philadelphia, Pennsylvania.

HyperMu, *Transposome*, *EZ::TN*, *SequiTherm EXCEL*, *TransforMax*, *EC100D*, *End-It*, *Fast-Link* and *MasterPure* are trademarks of EPICENTRE, Madison, Wisconsin.

Rescue Cloning of HyperMu <R6K γ ori/KAN-1> Transposed Genomic DNA

The protocol for rescue cloning the HyperMu <R6K γ ori/KAN-1> Transposon insertion site in genomic DNA is described on page3.

Figure 1. Rescue Cloning Overview.



Protocol

1. **Electroporation of Host Cells with the HyperMu <R6K[*pri*/KAN-1>Tnp Transposome and Selection of Transposition Clones:** Electroporate electrocompetent cells using 1 μ l of the HyperMu <R6K[*pri*/KAN-1>Tnp Transposome. The electrocompetent cells should have a transformation efficiency of $>10^7$ cfu/ μ g of DNA, but use cells of the highest transformation efficiency possible to maximize the number of transposon insertion clones. Perform electroporation according to the equipment manufacturer's recommendations.

Immediately recover the electroporated cells after electroporation. Even slight delays in initiating the cell recovery process will result in a reduced number of transposition clones. For *E. coli*, add SOC medium to the electroporation cuvette to 1 ml final volume **immediately** after electroporation. Pipette the medium/cells gently to mix. Transfer to a tube and incubate on a 37°C shaker for 30-60 minutes to facilitate cell outgrowth.

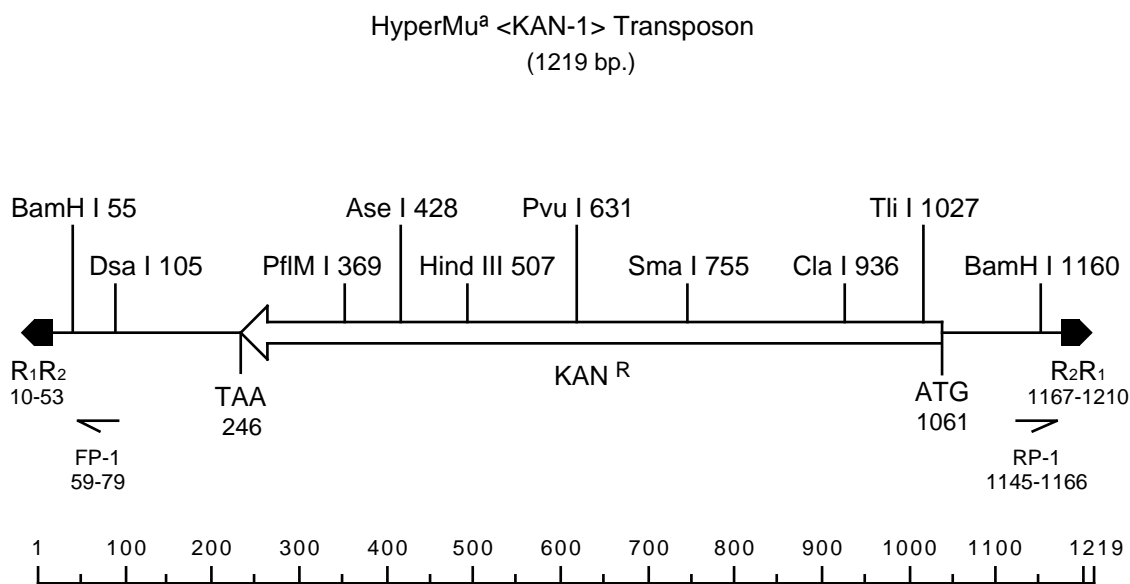
If working with *E. coli*, dilute aliquots of the recovered cells (e.g. 1:10 and 1:100). Plate 100 μ l of undiluted cells and each cell dilution separately on plates containing 50 μ g/ml kanamycin. Other species may require plating of undiluted cells on plates containing 25-50 μ g/ml kanamycin. Store the unused portion of the electroporated cells at +4°C for up to 2 days in the event that additional plates need to be prepared. The number of Kan^R colonies/ μ l of HyperMu <R6K[*pri*/KAN-1>-Tnp Transposome will be dependent on the transformation efficiency of the cells used and the level of expression of the Tn903 kanamycin resistance marker in that species. Select transposition clones for "rescue cloning" by any of a number methods including observing a desired phenotypic change, Southern Blot analysis or selecting for a desired "gene knockout".

2. **Preparation of Transposed Genomic DNA from Host Cells:** Prepare genomic DNA from chosen clones using, for example, the MasterPure DNA Purification Kit. Fragment 1 μ g of the genomic DNA by random shearing or by restriction endonuclease digestion(s) (See pages 4 and 5 for the restriction sites in the HyperMu <R6K[*pri*/KAN-1> Transposon in order to avoid restriction within the transposon). If desired, size-select the fragmented genomic DNA (e.g. by low-melting point agarose gel electrophoresis). Genomic DNA that has been fragmented by random shearing or by digestion with two different restriction endonucleases must be end-repaired (made blunt-ended) and 5'-phosphorylated in order to be self-ligated. End-repair and 5'-phosphorylate the DNA as necessary (e.g. by using the End-It DNA End-Repair Kit).
3. **Ligation of Fragmented Genomic DNA:** Self-ligate 0.1-1 μ g of DNA using 2 U of T4 DNA Ligase in 10-20 μ l total volume for 1 hour at room temperature. The extent of ligation can be quickly monitored by running aliquots of the reaction before and after addition of the T4 DNA Ligase addition, on an agarose gel. Terminate the reaction and inactivate the T4 DNA Ligase by heating at 70°C for 10 minutes.
4. **Transformation and Selection of Rescue Clones:** Electroporate electrocompetent *pir E. coli*, e.g. TransforMax EC100D *pir*⁺ or TransforMax EC100D *pir*-116 Electrocompetent *E. coli*, using 1-2 μ l of the ligation mix. The *pir* gene encodes the π protein which is required for replication from the R6K[*ori*] origin. Recover the electroporated cells by adding SOC medium to the electroporation cuvette to 1 ml final volume **immediately** after electroporation. Pipette the medium/cells gently to mix. Transfer to a tube and incubate on a 37°C shaker for 30-60 minutes to facilitate cell outgrowth. Plate cells on LB agar containing 50 μ g/ml of kanamycin. Select Kan^R colonies overnight.
5. **DNA Sequencing of Transposon Insertion Sites:** DNA flanking a HyperMu <R6K[*pri*/KAN-1> Transposon insertion can be sequenced bidirectionally using the unlabeled forward and reverse transposon-specific primers provided in the kit. *Note:* Transposon insertions catalyzed by HyperMu Transposase result in the generation of a 5-bp target site sequence duplication where one copy immediately flanks each side of the inserted transposon. This is important to consider when assembling nucleotide sequence.

Primer Information

<p>MUKAN-1 FP-1 Forward Primer</p> <p>5' - CTGGTCCACCTACAACAAAGG</p> <p><u>Length</u> : 21 nucleotides</p> <p><u>G+C content</u> : 11</p> <p><u>Molecular Weight</u> : 6384 daltons</p> <p><u>Temperatures of Dissociation & Melting</u> :</p> <p>T_d: 64°C (nearest neighbor method)</p> <p>T_m: 71°C (% G+C method)</p> <p>T_m: 64°C ([2 (A+T) + 4 (G+C)] method)</p> <p>T_m: 63°C ((81.5 + 16.6 (log [Na⁺])) + ([41 (#G+C) - 500] / length) method) where [Na⁺] = 0.1 M</p>	<p>MUKAN-1 RP-1 Reverse Primer</p> <p>5' - AGAGATTTTGAGACAGGATCCG</p> <p><u>Length</u> : 22 nucleotides</p> <p><u>G+C content</u> : 10</p> <p><u>Molecular Weight</u> : 6824 daltons</p> <p><u>Temperatures of Dissociation & Melting</u> :</p> <p>T_d: 64°C (nearest neighbor method)</p> <p>T_m: 70°C (% G+C method)</p> <p>T_m: 64°C ([2 (A+T) + 4 (G+C)] method)</p> <p>T_m: 61°C ((81.5 + 16.6 (log [Na⁺])) + ([41 (#G+C) - 500] / length) method) where [Na⁺] = 0.1 M</p>
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Figure 2. HyperMu <R6K_gpri/KAN-2> Transposon.



Note: Not all restriction enzymes that cut only once are indicated above.
See the following page for further information.
BamH I cuts twice in the map above.
Primers are not drawn to scale.

FP-1 = MUKAN-1 FP-1 Forward Primer 5' CTGGTCCACCTACAACAAAGG 3'
 RP-1 = MUKAN-1 RP-1 Reverse Primer 5' AGAGATTTTGAGACAGGATCCG 3'
 R1 R2 = HyperMu Transposase Recognition Sequence

