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HyperMu™ Transposon Tools

HyperMu™ <CHL-1> Insertion Kit

Cat. No. HMI039C

Table of Contents

1. Introduction 3

2. Materials
- a. Kit Contents 4
- b. Storage 4
- c. Performance Specifications and Quality Control 4-5

3. Transposon Insertion Reaction
- a. Target DNA Preparation 5
- b. *In Vitro* Transposon Insertion Reaction 5

4. Selection of Transposon Insertion Clones
- a. Transformation and Recovery 6
- b. Plating and Selecting of Transformants 6

5. DNA Sequencing of Transposon Insertion Clones
- a. Transposon Insertion Mapping 7
- b. Primer Considerations 7
- c. Target Site Duplication 7
- d. Distinguishing Transposon Sequence from Insert Sequence 7-8

6. Troubleshooting 9

7. Related Products 10

8. Appendix
- a. Primer Data 10
- b. HyperMu <CHL-1> Transposon Schematic Map 11
- c. HyperMu <CHL-1> Transposon Restriction Data 12-13
- d. HyperMu <CHL-1> Transposon Sequence 14

9. References 15

1. Introduction

The HyperMu™ <CHL-1> Insertion Kit simplifies and speeds up complete sequencing of any cloned DNA that is too large to sequence with a single set of sequencing reactions. Epicentre's Mu-based transposition system uses a unique HyperMu MuA Transposase that retains the highly random insertion characteristics of MuA transposase[1] but is at least 50 times more active *in vitro* than the MuA transposase from other suppliers. The higher activity of HyperMu MuA Transposase results in much higher transposition efficiencies which are critical for obtaining a sufficient number of transposon insertions to completely sequence a clone, especially those with large inserts.

The process for sequencing even the largest BAC clone without the time and expense of subcloning or primer walking is shown in Fig. 1. First, the HyperMu <CHL-1> Transposon is randomly inserted into the target DNA using a simple, 2-hour *in vitro* reaction catalyzed by HyperMu MuA Transposase. Then, *E. coli* cells are transformed with an aliquot of the reaction and plated on medium containing chloramphenicol. Up to a million independent insertion clones are recovered, each with a transposon at a different site.

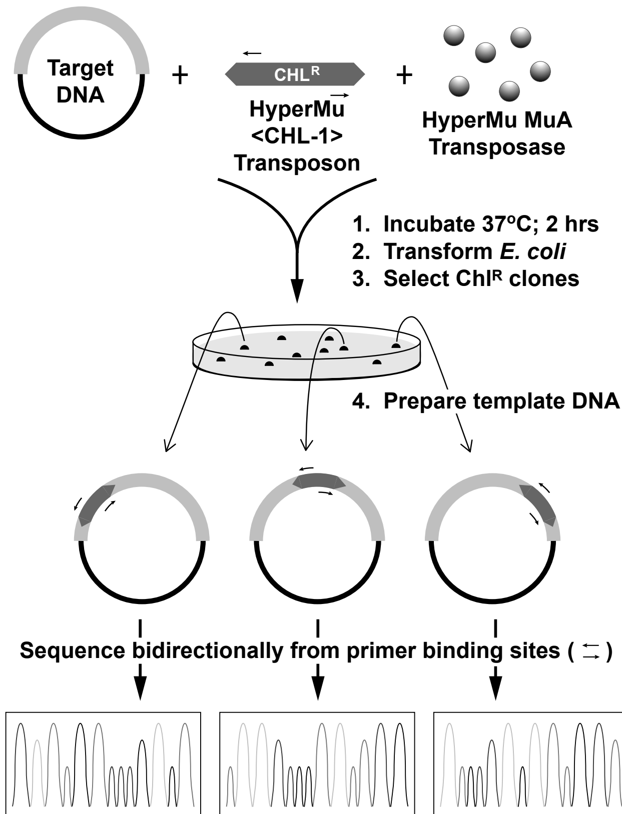


Figure 1.The process for complete sequencing of target DNA using the HyperMu™ <CHL-1> Insertion Kit.

The complete sequence of the target DNA is easily obtained by sequencing selected insertion clones bidirectionally using the two primers in the kit that are homologous to the ends of the inserted HyperMu Transposon. Details of this reaction are shown in Figs. 2 and 3.

2. Materials

2-a. Kit Contents

Desc.	Concentration	Quantity
Reagents included in the kit are sufficient for 10 *In Vitro* Transposon Insertion Reactions.		
HyperMu™ MuA Transposase	10 U @ 1 U/µl; 250 ng/µl	10 µl
in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol and 0.1% Triton®X-100.		
HyperMu™ <CHL-1> Transposon	25 ng/µl	10 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
HyperMu™ 10X Reaction Buffer		100 µl
0.50 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate and 40 mM spermidine.		
HyperMu™ 10X Stop Solution	100 µl	
1% SDS solution.		
MUCHL-1 FP-1 Forward Primer	1 nmol @ 50 µM	20 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
MUCHL-1 RP-1 Reverse Primer: 1 nmol	@ 50 µM	20 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
pUC19/3.4 Control Target DNA	1 µg @ 0.1 µg/µl	10 µl
A 3.4 kb *Hpa* II fragment of bacteriophage DNA cloned into the *Acc* I site of pUC19.		
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
Sterile Water		1 ml

2-b. Storage

Store the HyperMu <CHL-1> Insertion Kit only at –20°C in a freezer without a defrost cycle. The enzyme solution will not freeze. Other component solutions will freeze. After thawing, be sure to mix the contents of each tube thoroughly before using. After use, return all kit components to –20°C for storage.

2-c. Performance Specifications and Quality Control

The HyperMu <CHL-1> Insertion Kit is function-tested using the pUC19/3.4 Control Target DNA followed by electroporation into a *rec*A^{-} *E. coli* host strain having a transformation efficiency of $>10^9$ cfu/µg DNA. Transposition frequency, defined as the ratio of the number of Chl^{R} clones divided by the number of transformants resistant to the antibiotic marker of the target vector, (Chl^{R} colonies/Amp^{R} colonies; for the control DNA) must be >0.5% (commonly 2 to 3%) and transposition efficiency must be $>10^6$ Chl^{R} colonies/µg target DNA. Primers are function-tested in a DNA cycle sequencing reaction using the SequiTherm EXCEL™ II DNA Sequencing Kit and in a PCR reaction using a plasmid containing an HyperMu <CHL-1> Transposon as template. All components of the HyperMu <CHL-1> Insertion 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 HyperMu MuA Transposase.

3.Transposon Insertion Reaction

3-a. Target DNA Preparation

The target DNA must not contain a chloramphenicol resistance gene. The transposon insertion reaction is not significantly inhibited by high levels of RNA contamination in target DNA preparations. However, if the target DNA is heavily contaminated with chromosomal DNA, which is a direct competitor for target transposition, the number of clones will be greatly reduced.

Plasmid and cosmid clones can be purified by standard minilysate procedures and used as target DNA in the insertion reaction without further clean-up. Low copy-number vectors, for example BAC or fosmid clones, are often contaminated with a higher molar proportion of *E. coli* chromosomal DNA thus reducing the transposon insertion frequency. Therefore, BAC and fosmid DNA should be purified, to remove the chromosomal DNA prior to the insertion reaction.

3-b. *In Vitro* Transposon Insertion Reaction

Reaction conditions have been optimized to maximize the efficiency of the HyperMu Transposon insertion while minimizing multiple insertion events.

Amount of Target DNA: Use 300 ng of DNA if the size of the target is 10 kb or less. If the target DNA is larger, increase the amount of DNA present in the reaction in proportion to the size. For example, if the target DNA is 20 kb, use up to 600 ng per reaction; if the target DNA is 40 kb, use up to 1,200 ng per reaction. For targets larger than 40 kb, use up to 1,200 ng per reaction.

1. Prepare the Transposon Insertion Reaction mixture by adding in the following order:

2 µl	HyperMu 10X Reaction Buffer
x	ng target DNA (see above; 3 µl of the Control Target DNA [0.1 µg/µl])
1 µl	HyperMu <CHL-1> Transposon (25 ng/µl)
x µl	sterile water to a reaction volume of 19 µl
1 µl	HyperMu MuA Transposase (1 U/µl)
20 µl	Total reaction volume

2. Incubate the reaction mixture for 2 hours at 37°C.
3. Stop the reaction by adding 2 µl HyperMu 10X Stop Solution.
4. Mix and heat for 10 minutes at 70°C.

   Proceed to 4-a Transformation and Recovery or store the reaction mixture at –20°C.

4. Selection of Transposon Insertion Clones

4-a. Transformation and Recovery

The number of HyperMu Transposon insertion clones obtained per reaction depends on, among other factors, the transformation efficiency of the competent cells used. The greater the transformation efficiency of the competent cells, the greater the number of insertion clones obtained. We recommend using electrocompetent or chemically competent *rec*A⁻ *E. coli* with a transformation efficiency of $>10^8$ cfu/µg of DNA. A *rec*A⁻

strain of *E. coli* is recommended to eliminate the possibility of generating multimeric forms of the vector. Finally, the host strain used must not have a chloramphenicol resistance marker when used with the HyperMu <CHL-1> Transposon. Epicentre's TransforMax™ EC100™ Electrocompetent *E. coli* or Phage T1-Resistant TransforMax EC100-T1^R Electrocompetent *E. coli* (available separately) are ideal for this application.

1) Using 1 µl of the insertion reaction mixture, transform *rec*A$^-$ *E. coli*. If electrocompetent cells are used, perform electroporation according to the equipment manufacturer's recommended conditions. Use of >1 µl of the Transposon Insertion Reaction for transformation may result in arcing. The unused portion of the Transposon Insertion Reaction can be stored at –20°C for future use.
2) 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.

4-b. Plating and Selecting Transformants

1) If transformation was done using cells with an efficiency of >5 x 10^8 cfu/µg DNA, it may be necessary to dilute the cells 1:10 or 1:100 prior to plating. Plate portions (e.g., 100 µl) of cells onto LB plates containing 12.5 µg/ml chloramphenicol. Store the unused portion of the recovered cells at 4°C for up to 2 days in the event additional plates need to be prepared.
2) (Optional) To determine the transposon insertion frequency, plate dilutions of the transformation reaction on a second plate containing an antibiotic specific for selecting target DNA. Dilutions are generally 1:10^2 to 1:10^3.

   For transpositions performed with the Control Target DNA, plate cells on media containing 100 µg/ml ampicillin. The transposition frequency is given by the ratio of Chl^R/Amp^R clones.
3) Grow plates overnight at 37°C. If too few (or too many) colonies appear, replate the transformed cells at a lower (or higher) dilution.

   For transpositions performed with the Control Target DNA, one should see 100-500 Chl^R clones for the 1:10^2 dilution or >10^6 transposition clones per microgram of the Control DNA. The transposition frequency is >0.5%, commonly 2-3%.

The actual number of HyperMu <CHL-1> Transposon insertion clones obtained will vary depending on factors such as target DNA size and the transformation efficiency of the competent cells used to recover the transposon insertion clones.

5.DNA Sequencing of Transposon Insertion Clones

5-a. Transposon Insertion Mapping (optional)

HyperMu Transposon insertion clones can be sequenced bidirectionally using the unlabeled forward and reverse transposon-specific primers provided in the kit. The insertion site of each clone can also be mapped prior to sequencing, depending on the cost-effectiveness and experience of the user.

HyperMu <CHL-1> Transposon insertion sites can be mapped by size analysis of PCR products using colony minilysate DNA as a template. To map the insertion sites, use the MUCHL-1 FP-1 Forward or MUCHL-1 RP-1 Reverse Primers provided with the kit and vector-specific flanking primers (not provided).

Alternatively, insertion sites can be mapped by restriction endonuclease digest(s). Use the nucleotide sequence and restriction information of the HyperMu <CHL-1> Transposon provided in the Appendix, sections 9-c and 9-d for reference.

5-b. Primer Considerations

The MUCHL-1 FP-1 Forward and MUCHL-1 RP-1 Reverse Primers supplied with the kit have been constructed to minimize homology to commonly used cloning vectors. However, the sequence of each primer should be compared to that of the user's specific cloning vector to ensure minimal sequence homology to the vector. The sequence and theoretical melting temperatures for each primer are presented in the Appendix, section 9-a.

Note: *Occasionally a clone will yield the sequence of the cloning vector. This occurs when the HyperMu Transposon randomly inserts into a non-essential region of the vector rather than into the DNA insert. The frequency of this occurrence is dependent on the size of the DNA insert relative to the size of non-essential regions of vector. The larger the DNA insert, the less frequently an insertion will occur into the vector.*

5-c. Target Site Duplication

HyperMu MuA Transposase-catalyzed transposon insertion results 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 the nucleotide sequence of a recombinant clone insert. The process of transposon insertion site duplication is depicted in Fig. 2.

5-d. Distinguishing Transposon Sequence from Insert Sequence

The primers provided in the HyperMu <CHL-1> Insertion Kit anneal to a region near the ends of the transposon. Hence, the first sequence data obtained from each sequencing reaction is that of HyperMu <CHL-1> Transposon DNA and will include the HyperMu MuA Transposase recognition sequences R1 and R2. These recognition sequences are useful landmarks to distinguish HyperMu Transposon DNA sequence from target DNA sequence (Fig. 2).

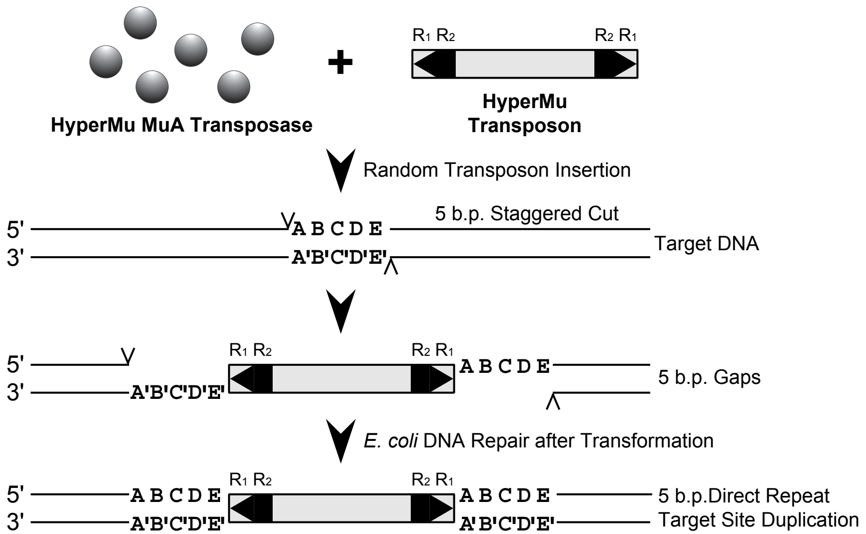
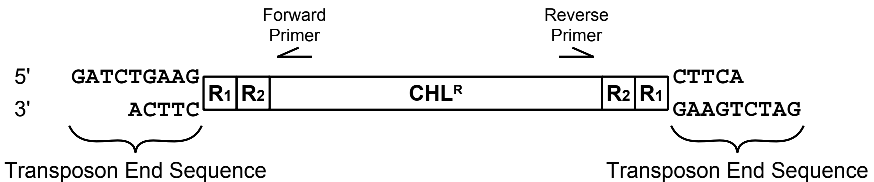


Figure 2. **The process for HyperMu™ <CHL-1> Transposon insertion site duplication.**



R1 R2 = HyperMu MuA Transposase Recognition Sequence

Figure 3. **Schematic of the HyperMu™ <CHL-1> Transposon.**

6. Troubleshooting

Arcing during transformation by electroporation

1) Excessive salt in target DNA preparation. Use less sample for electroporation. Ethanol precipitate and 70% ethanol wash, or drop dialyze DNA.

   Arcing inevitably results in failed transformation. Discard the electroporation reaction and try again with 0.5 µl of the transposition reaction. With the control DNA, no arcing is observed when up to 2 µl of transposition reaction is used for electroporation of 50 µl of electrocompetent *rec*A⁻ *E. coli* in a 0.2 cm width electroporation cuvette and using an Eppendorf Multiporator at 2500 V. A voltage gradient of approximately 12,500 V/cm is fairly standard for *E. coli*.

No, or few transposition clones on selective plates

1) Transformation reaction was unsuccessful; low competence. Test by plating outgrowth using drug resistance marker on target DNA to distinguish between transformation or transposon insertion failure.

   If competent cells have a transformation efficiency $<10^8$ cfu/µg DNA, one may not obtain sufficient clones on a plate. For example, transforming into cells with transformation efficiency $<10^5$ cfu/µg DNA results in as few as 2 insertion clones on a plate. Use cells with a transformation efficiency $>10^8$ cfu/µg DNA.

2) Transposon Insertion Reaction was unsuccessful. Inhibitor contamination in target DNA. Purify target DNA further. Perform procedure with control plasmid provided with kit to assure system components are functional.

DNA sequencing results are ambiguous

1) Two or more transposon insertions into target clone. Discard the clone. Choose other clones to sequence.

   The protocol was designed to minimize multiple transposon insertion events. Even so, about 1% of the transposition clones may contain >1 transposon. One can verify single insertion clones by agarose gel electrophoresis of colony minilysates prior to sequencing. A single insertion should be about 1.2 kb larger than the parental target DNA. A double insertion will increase the size by 2.4 kb and also result in "double sequence".

2) The sequencing primer used has significant homology to the cloning vector or to the DNA being sequenced. Check homology of primer against the vector. Alter primer annealing conditions or synthesize a new primer with less homology.

3) Components of the DNA sequencing kit and/or of the electrophoresis step are compromised. Reverify the integrity of the components of the kit and/or electrophoresis step with appropriate controls. Use a new kit and/or new reagents.

Confluent plates following transformation

1) Target DNA or host cells have the same selective marker (antibiotic resistance) as the transposon used. Use a different host for transformation and retransform with a portion of your remaining reaction.

   Some host cells carry Chl^R transposons. Confirm that the genotype of the host strain chosen for the transformation is not Chl^R.

7. Related Products

The following products are also available:

– HyperMu™ MuA Transposase
– HyperMu™ <R6Kγ*ori*/KAN-1>Tnp Transposome™ Kit
– HyperMu™ <KAN-1> Insertion Kit
– TransforMax™ EC100™ Electrocompetent *E. coli*
– Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli*
– Colony Fast-Screen™ Kits

Visit our website for more information on our wide variety of Tn5-based EZ-Tn5™ Transposon Tools for genetic analysis and screening.

8.Appendix

8-a. Primer Data

MUCHL-1 FP-1 Forward Primer

5′ - CACAGGTATTTATTCGGTCGA - 3′

Length: 21 nucleotides

G+C content: 9

Molecular Weight: 6,436 daltons

Temperatures of Dissociation & Melting:

T_d: 62°C (nearest neighbor method)
T_m: 67°C (% G+C method)
T_m: 60°C ([2 (A+T) + 4 (G+C)] method)
T_m: 59°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

MUCHL-1 RP-1 Reverse Primer

5′ - TGGAGGTAATAATTGACGATA - 3′

Length: 21 nucleotides

G+C content: 7

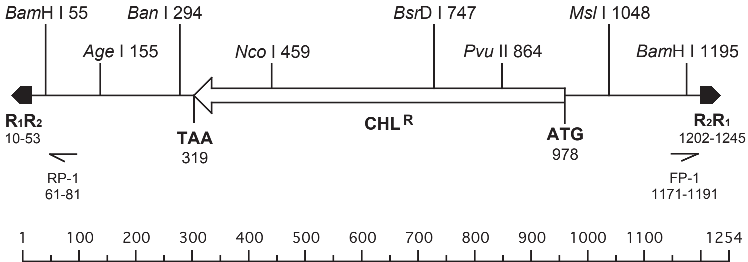
Molecular Weight: 6,533 daltons

Temperatures of Dissociation & Melting:

T_d: 57°C (nearest neighbor method)
T_m: 63°C (% G+C method)
T_m: 56°C ([2 (A+T) + 4 (G+C)] method)
T_m: 55°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

8-b. HyperMu <CHL-1> Transposon Schematic Map

HyperMu™ <CHL-1> Transposon
(1,254 bp)



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

FP-1 = MUCHL-1 FP-1 Forward Primer 5' CACAGGTATTTATTCGGTCGA 3'
RP-1 = MUCHL-1 RP-1 Reverse Primer 5' TGGAGGTAATAATTGACGATA 3'
R_1 R_2 = HyperMu MuA Transposase Recognition Sequence

Figure 4. **HyperMu™ <CHL-1> Transposon.**

8-c. HyperMu <CHL-1> Transposon Restriction Data

Restriction Enzymes that cut the HyperMu <CHL-1> Transposon one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Aci I	3	250, 326, 1243	Eae I	1	495
Acl I	1	675	Fau I	2	333, 777
Age I	1	155	Hae I	3	104, 497, 584
Apo I	2	221, 233	Hha I	2	14, 1242
BamH I	2	55, 1195	Hinf I	1	411
Ban I	1	294	HinP I	2	12, 1240
Bme1580 I	1	297	Hpy188 I	2	5, 1250
Bpu10 I	2	92, 988	Hpy99 I	2	212, 649
BsaA I	2	758, 1071	Mbo II	2	490, 1155
BsaW I	3	140, 155, 764	Msc I	1	497
BsiE I	2	214, 1188	Msl I	1	1048
Bsm I	2	366, 773	MspA1 I	2	420, 864
BsmA I	2	536, 1089	Mwo I	2	426, 989
BsmB I	2	536, 1089	Nci I	3	215, 1026, 1118
Bsp1286 I	1	297	Nco I	1	459
BspE I	1	764	PflF I	1	215
Bsr I	3	105, 519, 959	PflM I	2	535, 1102
BsrD I	1	747	Pvu II	1	864
BsrF I	1	155	Rsa I	2	347, 885
BstB I	1	231	Sau96 I	1	1105
BstDS I	2	83, 463	Sca I	1	347
BstF5 I	2	246, 773	SfaN I	2	432, 917
BstU I	2	25, 1229	Sim I	2	192, 217
BstY I	3	55, 1195, 1250	SnaB I	1	758
Bsu36 I	1	106	Ssp I	1	452
Btg I	2	79, 459	Sty I	1	459
Bts I	1	132	Tat I	1	345
Cac8 I	2	418, 786	Tfi I	1	411
Dra I	2	505, 844	Tsp45 I	2	128, 1168
Drd I	1	215	TspR I	2	144, 966
Dsa I	2	79, 459	Tth111 I	1	215

Restriction Enzymes that cut the HyperMu <CHL-1> Transposon four or more times:

Alu I
Alw I
BsaJ I
Bsl I
BssK I
BstN I
CviJ I
Dde I
Dpn I
Fnu4H I
Hae III
Hpa II
Hph I
Hpy188 III
HpyCH4 III
HpyCH4 IV
Mae II
Mae III
Mbo I
Mnl I
Mse I
Msp I
Nla III
Nla IV
PspG I
Sau3A I
ScrF I
Taq I
Tsp4C I
Tsp509 I

Restriction Enzymes that do not cut the HyperMu <CHL-1> Transposon:

Aat II
Acc65 I
Acc I
Afe I
Afl II
Afl III
Ahd I
Ale I
AlwN I
Apa I
ApaB I
ApaL I
Asc I
Ase I
AsiS I
Ava I
Ava II
Avr II
Ban II
Bbs I
BbvC I
BciV I
Bcl I
Bfa I
BfrB I
BfuA I
Bgl I
Bgl II
Blp I
BmgB I
Bmr I
Bsa I
BsaB I
BsaH I
BseY I
BsiHKA I
BsiW I
BspD I
BspH I
BspLU11 I
BspM I
BsrB I
BsrG I
BssH II
BssS I
BstAP I
BstE II
BstX I
BstZ17 I
Cla I
Dra III
Eag I
Ear I
Eco47 III
EcoN I
EcoO109 I
EcoR I
EcoR V
Fse I
Fsp I
Gdi II
Hae II
Hinc II
Hind III
Hpa I
HpyCH4 V
Kpn I
Mfe I
Mlu I
Mly I
Nae I
Nar I
Nde I
NgoM IV
Nhe I
Not I
Nru I
Nsi I
Nsp I
Pac I
PaeR7 I
Pci I
Ple I
Pme I
Pml I
PpuM I
PshA I
Psi I
PspOM I
Pst I
Pvu I
Rsr II
Sac I
Sac II
Sal I
SanD I
Sap I
Sbf I
SexA I
Sfc I
Sfi I
Sfo I
SgrA I
Sma I
Sml I
Spe I
Sph I
Srf I
Sse8647 I
Stu I
Swa I
Tli I
Tse I
Xba I
Xcm I
Xho I
Xma I
Xmn I

8-d. HyperMu <CHL-1> Transposon Sequence

HyperMu™ <CHL-1> Transposon 1,254 bp.

```
                             R1                           R2           BamH I
   1 GATCTGAAGC GGCGCACGAA AAACGCGAAA GCGTTTCACG ATAAATGCGA AAACGGATCC
     <-------------------------------- MUCHL-1 RP-1
  61 TATCGTCAAT TATTACCTCC ACGGGGAGAG CCTGAGCAAA CTGGCCTCAG GCATTTGAGA
                                           Age I
 121 AGCACACGGT CACACTGCTT CCGGTAGTCA ATAAACCGGT AAACCAGCAA TAGACATAAG

 181 CGGCTATTTA ACGACCCTGC CCTGAACCGA CGACCGGGTC GAATTTGCTT TCGAATTTCT
                                                                Ban I
 241 GCCATTCATC CGCTTATTAT CACTTATTCA GGCGTAGCAA CCAGGCGTTT AAGGGCACCA
                                 <-- ChlR translational stop
 301 ATAACTGCCT TAAAAAAATT ACGCCCCGCC CTGCCACTCA TCGCAGTACT GTTGTAATTC

 361 ATTAAGCATT CTGCCGACAT GGAAGCCATC ACAAACGGCA TGATGAACCT GAATCGCCAG
                                                Nco I
 421 CGGCATCAGC ACCTTGTCGC CTTGCGTATA ATATTTGCCC ATGGTGAAAA CGGGGGCGAA

 481 GAAGTTGTCC ATATTGGCCA CGTTTAAATC AAAACTGGTG AAACTCACCC AGGGATTGGC

 541 TGAGACGAAA AACATATTCT CAATAAACCC TTTAGGGAAA TAGGCCAGGT TTTCACCGTA

 601 ACACGCCACA TCTTGCGAAT ATATGTGTAG AAACTGCCGG AAATCGTCGT GGTATTCACT

 661 CCAGAGCGAT GAAAACGTTT CAGTTTGCTC ATGGAAAACG GTGTAACAAG GGTGAACACT
                                  BsrD I
 721 ATCCCATATC ACCAGCTCAC CGTCTTTCAT TGCCATACGT AATTCCGGAT GAGCATTCAT

 781 CAGGCGGGCA AGAATGTGAA TAAAGGCCGG ATAAAACTTG TGCTTATTTT TCTTTACGGT
                                 Pvu II
 841 CTTTAAAAAG GCCGTAATAT CCAGCTGAAC GGTCTGGTTA TAGGTACATT GAGCAACTGA

 901 CTGAAATGCC TCAAAATGTT CTTTACGATG CCATTGGGAT ATATCAACGG TGGTATATCC
   ChlR translational start <--
 961 AGTGATTTTT TTCTCCATTT TAGCTTCCTT AGCTCCTGAA AATCTCGACA ACTCAAAAAA
                                     Msl I
1021 TACGCCCGGT AGTGATCTTA TTTCATTATG GTGAAAGTTG GAACCTCTTA CGTGCCGATC

1081 AACGTCTCAT TTTCGCCAAA AGTTGGCCCA GGGCTTCCCG GTATCAACAG GGACACCAGG
                              MUCHL-1 FP-1 -------------------> BamH I
1141 ATTTATTTAT TCTGCGAAGT GATCTTCCGT CACAGGTATT TATTCGGTCG AAAAGGATCC
                    R2                     R1
1201 GTTTTCGCAT TTATCGTGAA ACGCTTTCGC GTTTTTCGTG CGCCGCTTCA GATC
```

The transposon sequence can be downloaded at the URL: http://www.epibio.com/sequences

9. References

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