Manual

EZ-Tn5 Transposase

For Research Use Only. Not for use in diagnostic procedures.

EZ-Tn5™ Transposase is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

EZ-Tn5 Transposase

Contents

Manual

EZ-Tn5 Transposase

1. Introduction

EZ-Tn5 Transposase is a hyperactive, mutated form of Tn5 transposase and a highly efficient enzyme for insertion of an EZ-Tn5 Transposon into any target DNA, *in vitro*.[1] In addition to EZ-Tn5 Transposase, efficient transposition requires that each EZ-Tn5 Transposon have a specific 19-bp transposase recognition sequence (Mosaic End or ME sequence) at each of its ends. Mutations engineered into both the 19-bp MEs and the EZ-Tn5 Transposase result in an *in vitro* transposition frequency that is 1000-fold greater than wild type.

EZ-Tn5 Transposase catalyses a multi-step "cut and paste" transposition reaction. Initially, the enzyme binds the 19-bp ME of the transposon to form a Transposome™ complex (synaptic complex). The Transposome then randomly attacks and cleaves the phosphodiester backbone of the target DNA. Finally, the EZ-Tn5 Transposase catalyses the covalent linkage of the 3′-OH ends of the transposon to the exposed 5′-phosphorylated ends of the target DNA. Transposition creates a 9-bp sequence duplication immediately flanking the transposon insertion site.

EZ-Tn5 Transposase can be used to:

1. Insert a marker, T7 promoter or R6Kγ origin of replication flanked by the 19-bp MEs of LGC, Biosearch Technologies' EZ-Tn5 standard Transposons into any target DNA.
2. Insert any custom DNA sequence flanked by the 19-bp MEs of an EZ-Tn5 Transposon into any target DNA.
3. Prepare Transposomes, in the absence of Mg^{2+}, for electroporation into living bacteria and subsequent random insertion of the transposon into the bacterial chromosome.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Volume
EZ-Tn5 Transposase	10 units	TNP92110	EZ-Tn5 Transposase (1 U/µL)	10 µL
			EZ-Tn5 10X Reaction Buffer	100 µL
			EZ-Tn5 10X Stop Solution	100 µL
			Sterile Water	1 mL

3. Product specifications

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

Storage buffer: EZ-Tn5 Transposase 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 (Rohm & Haas) and 1 mM dithiothreitol.

Unit definition: One unit of EZ-Tn5 Transposase catalyses the release of the donor backbone fragment from 1 µg of transposed DNA in 1 hour at 37 °C, as determined by agarose gel electrophoresis.

Enzyme structure: Single polypeptide of 55 Kd.
Contaminating activity assays: All components of the EZ-Tn5 Transposase 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-Tn5 Transposase.
EZ-Tn5 10X Reaction Buffer: 0.5 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate and 40 mM spermidine.
Note: *This buffer contains Mg^{2+}. Do not use for the production of Transposomes.*
EZ-Tn5 10X Stop Solution: 1% SDS.
Note: *This product is not compatible with Nextera™ (Illumina, Inc.) sequencing.*

4. Applications

1. *In vitro* transposon insertion reaction

This reaction inserts an EZ-Tn5 Transposon into target DNA, *in vitro*. The target DNA should be free of contaminating chromosomal DNA which is a direct competitor of the target DNA for insertion. Reaction conditions given have been optimised to maximise transposition frequency while minimising multiple insertion events. Be sure to calculate the moles of target DNA used in the reaction and add an equimolar amount of the EZ-Tn5 Transposon.

1. Prepare the transposon insertion reaction mixture by adding in the following order:

1	µL EZ-Tn5 10X Reaction Buffer
0.2	µg target DNA**
x	µL molar equivalent EZ-Tn5 Transposon
x	µL sterile water to a reaction volume of 9 µL
1	µL EZ-Tn5 Transposase
10	µL Total reaction volume

2. Incubate the reaction mixture for 2 hours at 37 °C.
3. Stop the reaction by adding 1 µL EZ-Tn5 10X Stop Solution. Mix and heat for 10 minutes at 70 °C.
4. Use 1 µL for electroporation into a competent bacterial strain and plate on selective media as dictated by the transposon insert. Use of a recA⁻, endA⁻ strain is preferable, for target stability and subsequent purification steps (e.g. Biosearch Technologies' TransforMax™ EC100™ Electrocompetent *E. coli*), but not absolutely necessary. Store unused reaction mixture at -20 °C.

** Calculation of µmol target DNA:
µmol target DNA = µg target DNA / [(# base pairs in target DNA) x 660]
For example: 0.2 µg of a 6,100 bp target clone
= 0.2 µg / [6,100 bp x 660] = 0.05 ×10-6 µmol = 0.05 pmol

2. Creating a custom EZ-Tn5 Transposon with any DNA insert

A custom EZ-Tn5 Transposon consisting of any DNA sequence flanked by the 19-bp (ME) EZ-Tn5 Transposase recognition sequences can be generated by PCR using primers with the ME ends.

The DNA of interest, which can be anything - a resistance marker, a gene, a control element, etc. - must be amplified by PCR using primers that, in addition to having 3′-sequences homologous to the template, also have non-homologous tails with 19-base ME sequences at their 5′-ends. PCR amplification of the template using these ME-Tailed primers produces an EZ-Tn5 Transposon that can be used directly, without further purification, for *in vitro* insertion into any DNA target.

Design your forward and reverse primers as shown in Figure 1 below, adding the ME sequence to the 5′ end of **both** the forward and reverse primer.

ME sequence: 5′ - CTG TCT CTT ATA CAC ATC T - 3′

For optimum transposition efficiency, add a 5′ phosphate to both PCR primers.

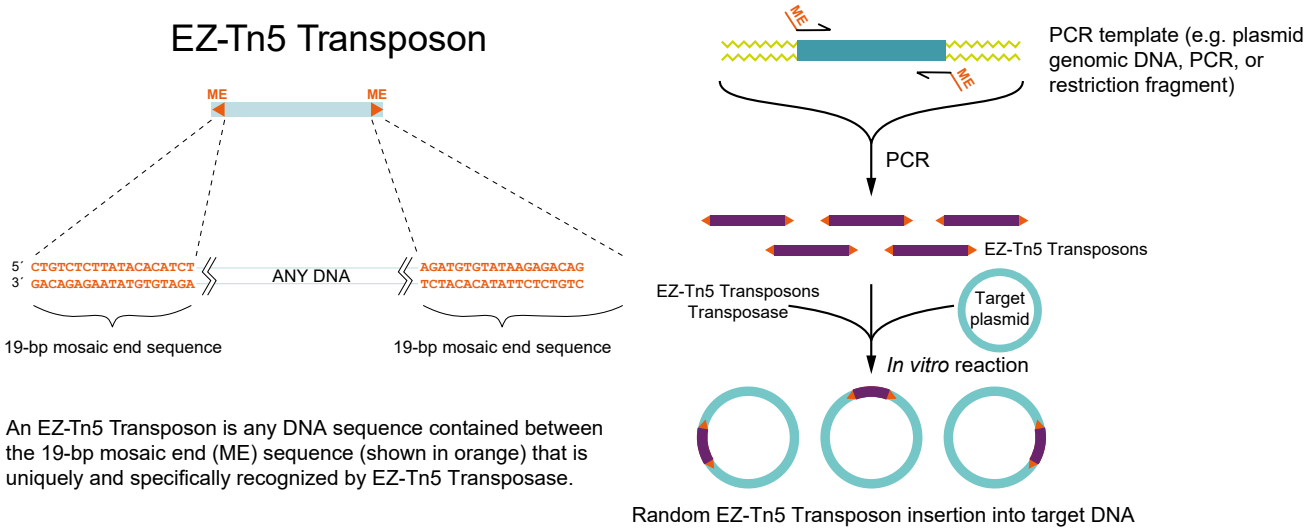

Figure 1.

5. Production of EZ-Tn5 Transposomes

Production of stable EZ-Tn5 Transposomes can only be accomplished in the absence of Mg^{2+}.

Do not use the EZ-Tn5 10X Reaction Buffer provided with the EZ-Tn5 Transposase to prepare EZ-Tn5 Transposomes.

1. Prepare the Transposome reaction mixture by adding in the following order: ‡

2 µL	EZ-Tn5 Transposon DNA (100 µg/ml in TE Buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA])
4 µL	EZ-Tn5 Transposase
2 µL	100% glycerol
8 µL	Total reaction volume

2. Mix by vortexing. Incubate for 30 minutes at room temperature.
3. Store the solution at -20 °C.
   The solution will not freeze stored at -20 °C and is stable for at least one year.
4. Use 1 µL of the EZ-Tn5 Transposome for electroporation into a competent bacterial strain and plate on selective media as dictated by the transposon insert.

7. References

Cited:

1. Goryshin IY and Reznikoff WS. (1998) *J. Biol. Chem.* 273, 7367.

Related:

1. York D *et al*., (1998) *Nucl. Acids Res*. 26, 1927.

8. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

‡ The EZ-Tn5 Transposome production protocol can be scaled up or scaled down as needed.

@LGCBiosearch | biosearchtech.com

GEN/906/EK/1122

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC