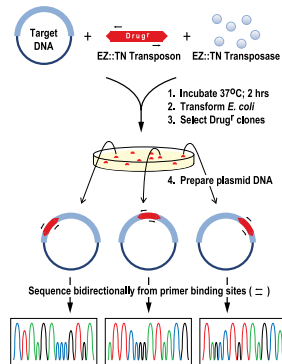


How to Make Sequencing Faster & Easier Using EZ::TN™ Transposon Tools

EPICENTRE offers EZ::TN™ Transposon Tools kits and reagents designed to make almost any DNA sequencing project faster and easier using one of 3 basic strategies.

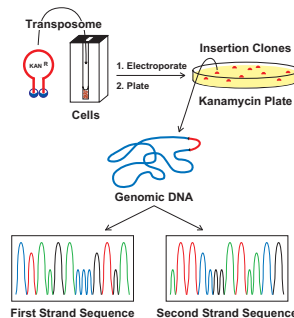


The *In Vitro* Insertion Strategy

The *In Vitro* Insertion Strategy is used if you already have DNA clones that are too big to sequence with a single set of sequencing primers (e.g. clones of >2 Kb). A simple 2-hour *in vitro* reaction randomly inserts an EZ::TN Transposon into your clone. Transform *E. coli* with an aliquot of the reaction mix and select on medium containing the transposon-encoded antibiotic. You obtain >10⁶ of independent clones - enough to completely sequence even the largest clone - each containing a single randomly inserted EZ::TN Transposon. Sequence the clones bidirectionally using a single set of primers (provided in the kits) that are homologous to the ends of the inserted EZ::TN Transposon. See the preceding article for use of the *In Vitro* Insertion Strategy in sequencing BAC clones, without sub-cloning or primer walking.

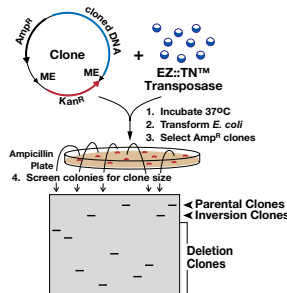
The Transposome™ Strategy

The Transposome™ Strategy is used if you're interested in finding a gene related to a specific phenotype. An EZ::TN Transposome is a stable complex formed between the EZ::TN Transposase and an EZ::TN Transposon. An EZ::TN Transposome is so stable that it can be electroporated into living cells where it is activated by intracellular Mg²⁺ and randomly inserts into the host cell's genomic DNA to create gene knockouts. Identify gene knockouts of interest and then sequence the affected gene directly, without cloning, using total bacterial genomic DNA template and primers that are homologous to the ends of the inserted EZ::TN Transposon. An EZ::TN Transposome containing an R6Kγ origin of replication, enabling rescue cloning of DNA comprising the transposon insertion site, is also available. See p. 14.



The *In Vitro* Deletion Strategy

The *In Vitro* Deletion Strategy should be considered if you need to generate a new plasmid or cosmid library for a sequencing project. First, clone your DNA into one of the specially-constructed plasmid or cosmid vectors. Incubate a single clone with EZ::TN™ Transposase to generate a complete population of random deletion subclones. Transform *E. coli* with an aliquot of the reaction mix and select deletion subclones on antibiotic plates. Size chosen deletion subclones by agarose gel and then generate the complete sequence of the original clone by choosing and sequencing a set of deletion subclones that span the entire size range of the original clone using a single primer that is provided with the kits.



Kits for the *In Vitro* Insertion Strategy

EZ::TN™ <KAN-2> Insertion Kit
EZI982K 10 Reactions

EZ::TN™ <TET-1> Insertion Kit
EZI921T 10 Reactions

EZ::TN™ <DHFR-1> Insertion Kit
EZI912D 10 Reactions

Each kit contains the specific EZ::TN Transposon, EZ::TN Transposase, Buffers and two unlabeled sequencing primers.

Kits for the Transposome™ Strategy

EZ::TN™<KAN-2>Tnp Transposome™ Kit
TSM99K2 10 Reactions

EZ::TN™<DHFR-1>Tnp Transposome™ Kit
TSM99D1 10 Reactions

EZ::TN™<R6Kγori /KAN-2>Tnp Transposome™ Kit
TSM08KR 10 Reactions

Each Transposome kit contains the specific Transposome complex and two unlabeled sequencing primers.

Kits for the *In Vitro* Deletion Strategy

EZ::TN™ Plasmid-Based Deletion Machine
DPM9401 10 Reactions

pWEB::TNC™ Cosmid Cloning Kit
WEBC931 10 Reactions

Complete kit for producing 10 unbiased cosmid libraries.

pWEB::TNC™ Deletion Cosmid Transposition Kit
WEBC942 10 Reactions

Complete kit for generating a complete population of random deletion subclones from clones produced using the pWEB::TNC Cosmid Cloning Kit.

www.epicentre.com/transposomics.htm