ELK Biotechnology
For research use only.

# Limitless™ ELZ Fusion kit

## Kit Components

| Components | Usage count | Volume |
|---|---|---|
| 5× Limitless™ ELZ Fusion Mix | 25 tests | 100 µl |

## Storage

Store at -20°C. Blue ice transport.

## Introduction

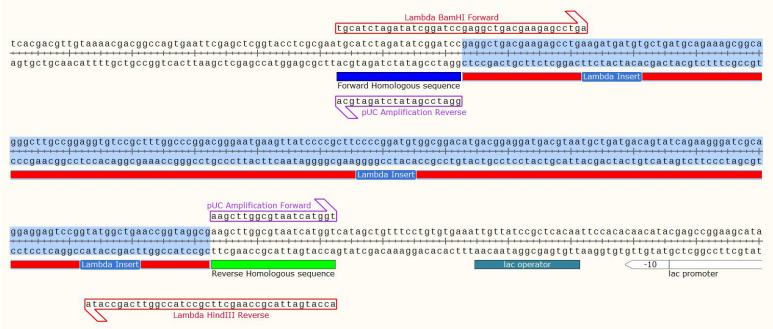
Limitless™ ELZ Fusion Seamless Cloning Kit is a new, fast and efficient Gibson Assembly DNA directed cloning technology that allows multiple insertions of target genes at any position in any vector, eliminating the need for cloning. Dots and multiple cloning sites. The Limitless™ ELZ Fusion Seamless Cloning Kit is extremely simple to operate, requiring only linearization of the vector, mixing with PCR-amplified fragments that are completely homologous to the vector (15-40 bp) and reacting at 37℃. In hours, without enzymes, direct transformation can complete the cloning. The positive rate is over 95%.

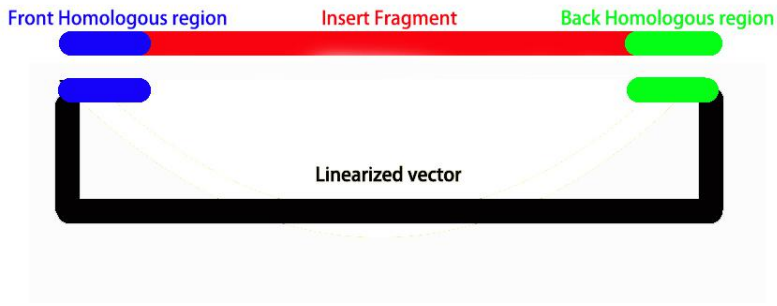
## Experimental principle

Using pUC57 as a vector, insert a random sequence as an example.

The pUC57 vector was digested with BamHI and HindIII or amplified using the pUC Amplification primer (purple font primer) to obtain a linearized pUC57 vector, which was purified to obtain a linearized vector.

The Lambda amplification primers (red font primers) were used to amplify the inserts containing the same homology to the vector at both ends. After mixing, the homologous recombination was carried out in the Limitless™ ELZ Fusion system, and the recombinant product was transformed into the host strain. After lag, the recombinant cloning vector can be extracted.




## Operation steps

Specific steps of the operation:

1. Designing an amplification primer, amplifying and recovering an insert containing a homologous sequence with the vector;

2. Restriction endonuclease digestion vector or design amplification

primer amplification vector, and recover linearized vector fragment;

3. Add the fragment and vector sequence according to the molar ratio of fragment to vector = 2:1~5:1. The dosage is as follows:

| | |
|---|---|
| Linearization vector | 1 copy |
| Insert fragment | 2 copy |
| 5× Limitless™ ELZ Fusion Mix | 4μl |
| $H_2O$ | Up to 20μl |

4. 37℃ reaction for 30 min;

5. Ice bath 5 min;

6. 10μl of the transformation experiment was carried out, and another 10 μl was stored at 4℃ or -20℃.

## Operation diagram