5min Universal Ligation Mix

C311

Version 22.1

Introduction

5min Universal Ligation Mix is a ready-to-use 2 × premix solution, containing T4 DNA ligase that catalyzes the formation of a phosphodiester bond between adjacent 5'-phosphate and 3'-hydroxyl termini in double-strand DNA or RNA. This kit is suitable for sticky end ligation, blunt-end ligation and TA ligation. Buffer with optimized ligation enhancer makes the reaction more efficient and convenient. Ligation can be completed in 5 min at 25℃. The product can be used directly to transform many chemically competent cells.

Components

Components	C311-01 (50 rxns)	C311-02 (100 rxns)
2 × Universal Ligation Mix*	250 μl	2 × 250 μl

*It contains enzyme and buffer.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

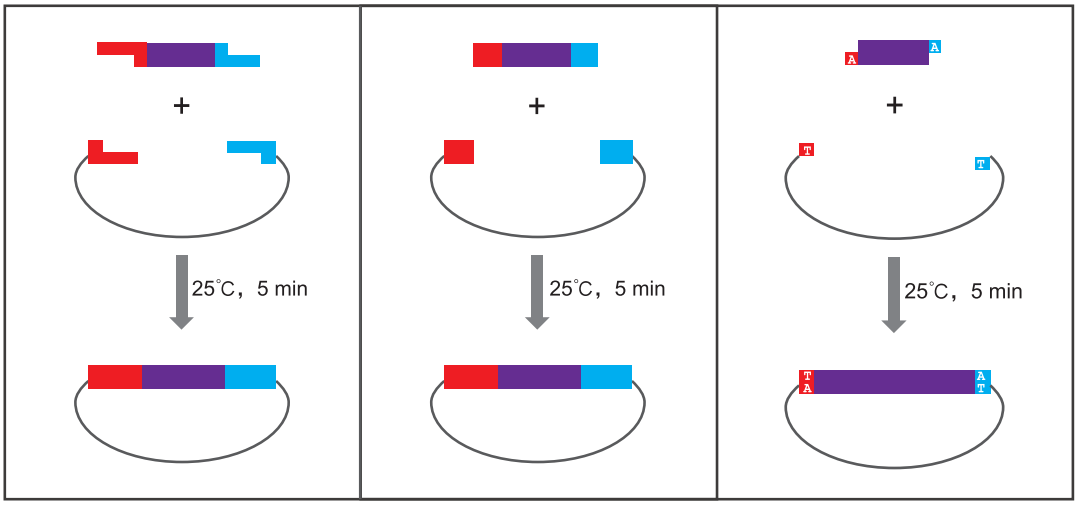
Applications

- ◇ Sticky end ligation
- ◇ Blunt-end ligation
- ◇ TA ligation
- ◇ Linker or Adapter ligation

Notes

For research use only. Not for use in diagnostic procedures.

Mechanism & Workflow



A: Sticky end ligation B: Blunt-end ligation C: TA ligation

Fig 1. Workflow of 5min Universal Ligation Mix

1. Ligation Reaction

a. Prepare the following reaction on ice:

Components	Volume
Vector	0.03 pmol
Insert	0.03 - 0.3 pmol
2 × Universal Ligation Mix	5 μl
ddH_2O	to 10 μl

The optimal mass of vector required (0.03 pmol) = [0.02 × number of base pairs] ng

The optimal mass of insert required (0.09 pmol) = [0.06 × number of base pairs] ng

For example, when cloning an insert of 1 kb to a vector of 4 kb, the optimal mass of vector is 0.02 × 4,000 = 80 ng, and that of insert is 0.06 × 1,000 = 60 ng.

① The molar ratio of vector: fragment ranged from 1:1 to 1:10, and the optimal molar ratio is 1:3.

② The amount of linearized vectors should be between 20 - 100 ng. When the optimal amount calculated using the above formula is beyond these ranges, just choose the maximum or minimum amount for recombination.

③ Please dephosphorylate the vector before ligating with the insert to prevent self-ligation of the vector.

④ Nanodrop, Onedrop and Qubit are recommended for concentration measurement.

b. Mix thoroughly by pipetting up and down several times(**DO NOT VORTEX!**), and centrifuge briefly to collect the reaction liquid.

c. Incubate 5 min at 25℃; Cool down to 4℃ or place on ice immediately.

① Other conditions for ligation: overnight at 4℃ or 16℃.

② When the total volume of vector and fragment is more than 5 μl, the reaction system can be enlarged to 20 μl. For blunt-end ligation or TA ligation, the reaction time can be prolonged to 2 h to improve the efficiency.

③ The ligation product needs to be purified by column or ethanol precipitation before electroporation transformation.

④ The ligation product can be stored at -20℃ for one week and then thawed before use.

2. Transformation

a. Thaw the competent cells on ice (e.g., Fast-T1 Competent Cell, Vazyme #C505).

b. Pipette 5 - 10 μl of the recombination products to 100 μl competent cells, flick the tube wall to mix thoroughly (**DO NOT VORTEX!**), and then place the tube on ice for 30 min.

▲ The volume of recombination products should be ≤1/10 of the volume of competent cells.

c. Heat shock at 42℃ water bath for 30 sec and then immediately place on ice for 2 - 3 min.

d. Add 900 μl of SOC or LB liquid medium (without antibiotics). Then, shake at 37℃ for 1 h at 200 - 250 rpm.

e. Preheat the LB plate which contains appropriate selection antibiotic at 37℃.

f. Centrifuge at 5,000 rpm (2,400 × g) for 5 min, discard 900 μl of supernatant. Then, use the remaining medium to suspend the bacteria and use a sterile spreading rod to gently spread on the plate which contains appropriate selection antibiotic.

g. Incubate at 37℃ for 12 - 16 h.

3. Identification of positive clones

Colony PCR identification; Digestion identification; Plasmid identification; DNA sequencing.