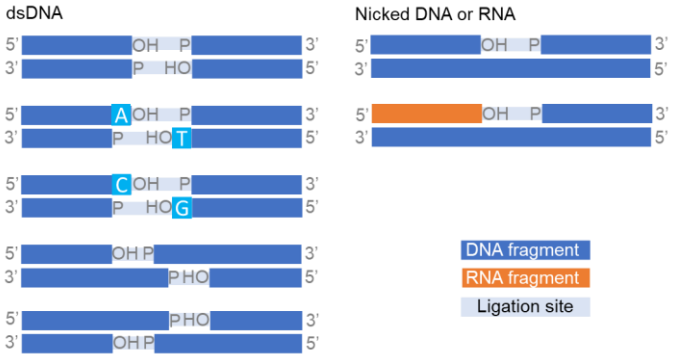
Sticky End Ligation Master Mix

Catalog No. 40103S: 50 reactions
Catalog No. 40103L: 250 reactions

Description

The **Sticky End Ligation Master Mix** is developed for DNA fragment ligation of sticky end with 2-4 bases of overhangs in just 2-4 minutes. The 2X ligation master mix contains T4 DNA Ligase and proprietary components for ligation reaction. The mixed components improve the cohesive end ligation efficiency significantly with the substrates of typical 2-4 bases of overhangs.

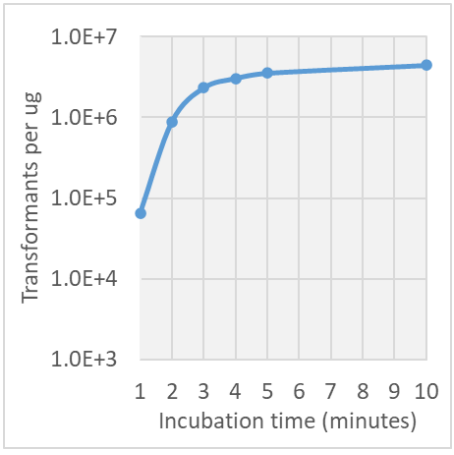
The **Sticky End Ligation Master Mix** can be used for the ligation of the following fragments:



The protocol for the **Sticky End Ligation Master Mix** is very simple: just mix the DNA fragments with the 2X Master Mix. The ligation of DNA fragments with sticky ends is fast at room temperature with high efficiency. Our modified downstream transformation protocol can save efforts and time for users.

Features

- Quick ligation in just 2-4 minutes for sticky ends (cohesive ends)
- Room temperature (20-25°C) ligation
- 2X Master Mix format made it easy to set up reaction
- Small ligation volume compatible with low DNA amount
- Modified transformation protocol with fewer steps and less time



Ligations with sticky-end DNA fragments using the **Sticky End Ligation Master Mix** between 1-10 minutes at room temperature. The ligated plasmids were transformed into DH-5α competent cells and grown on plates with LB medium at 37°C overnight.

Component

Catalog No.	40103S	40103L
Sticky End Ligation Master Mix (2X)	250 ul	1250 ul

Storage Condition

- Store at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Agar plates containing antibiotics
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g., plates, spreaders)
- Competent cells for transformation

Protocol

Note: Thaw the Master Mix on icy water if the mix is frozen. Tapping the tube to mix before use. Store the Master Mix at -20°C immediately after use.

Ligation Protocol

1) Mix 20-100 ng of vector with a 3-fold molar excess of inserts in a volume of 5 ul. Adjust the total volume with water if needed.
2) Add 5 µl of the Master Mix and mix thoroughly by pipetting up and down around 10 times or by tube tapping.
3) Incubate at room temperature (20°C-25°C) for 2-4 min.
   Note: extended incubation time may increase the efficiency slightly.
4) Continue with transformation protocol or store samples at -20°C.
   Note: Do not heat inactivate. Heat inactivation reduces transformation efficiency.

Transformation Protocol (Recommended)

Chemically competent strains of E. coli can be used. Electrocompetent cells are not compatible. If the transformation efficiency is low, dilution of the ligation reactions 4-fold may be needed prior to transformation. The volume of ligation reaction used should not exceed 10% of the competent cells.

1) Thaw competent cells on icy water.
2) Add 50 µl of competent cells into a 1.5 ml tube.
3) Add 2 µl of the ligation reaction to the tube and mix by tapping the tube gently. Do not vortex.
4) Incubate the tube on ice for 30 minutes. Do not mix.
5) Heat the tube at 42°C for 40 seconds, then place it on icy water for 2 minutes.
6) **Optional**: Add 950 µl recovery media (e.g. SOC) in the tube and incubate for one hour at 37°C with shaking at 200–250 rpm. This step can be skipped although the colony numbers maybe slightly lower.
7) Spread 50 µl of the bacteria onto plates with appropriate antibiotic selection. Incubate overnight at 37°C.

Note: Many factors can affect the transformation efficiency, including media type, integrity and purity of DNA fragments, vector type, vector size, competence of the E. coli cells used, insert DNA fragment size, incubation time and incubation temperatures, and copy numbers etc.

Troubleshooting

Problem: Few or no colonies

Possible cause	Solution
Impurity of DNA.	Purify DNA using column or beads to remove contamination.
DNA degraded or insufficient DNA.	Check DNA by gel electrophoresis. Determine DNA concentration and add the correct amount. Use the supplied positive control to test the system.
Incorrect amounts of antibiotics or wrong antibiotics was used in agar plates.	Check the correct amount of antibiotics was used in agar plates. Do not spread antibiotics onto the surface of agar plates.
The transformation efficiency of competent cells is too low.	Use competent cells with high transformation efficiency. Check the transformation efficiency of competent cells.

Problem: High background of colonies that do not contain inserts.

Possible cause	Solution
Non-specific DNA or plasmid contamination in ligation.	Gel purify DNA before ligation.
Enzyme contamination in ligation.	Purify DNA using column or beads.
Incorrect amounts of antibiotics or wrong antibiotics were used in agar plates.	Check the correct amount of antibiotics was used for agar plates. Do not spread antibiotics onto the surface of agar plates.

Quality Control

Reagent passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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BioDynami

601 Genome Way, Huntsville, Alabama 35806, USA
https://biodynami.com
support@biodynami.com

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