Cloning with NEB Instant Sticky-end Ligase Master Mix (M0370)

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Abstract

Taken directly from NEB's website, this is the protocol for using their optimized sticky-end ligation and transformation. For more information, see https://www.neb.com/products/m0370-instant-sticky-end-ligase-master-mix.

Instant Sticky-end Ligase Master Mix is a ready-to-use 2X solution of T4 DNA ligase and a proprietary ligation enhancer in an optimized reaction buffer. Specifically formulated to rapidly ligate cohesive-end (2–4 bp) substrates and improve transformation, the mix simplifies reaction set-up and provides an optimized ratio of enzyme and buffer components. No thawing of the master mix is required as it maintains a liquid state during storage at -20°C* and no incubation time is necessary to achieve ligation efficiencies sufficient for successful sub-cloning of sticky-end substrates. Just add the master mix to DNA with compatible ends, mix and transform; thereby reducing the valuable time needed for routine ligations. Ligations for subcloning can be carried out in small volumes with low concentrations, allowing users to conserve precious DNA samples, and be used directly to transform many strains of chemically competent *E. coli* without dilution.

* Freezers vary in their actual internal temperature. Our testing demonstrates that the master mix is liquid at -20°C. Freeze-thaw testing at -70°C has confirmed that the performance after 20 freeze/thaw cycles is close to the original mix.

Product Source

Purified from an *E. coli* strain containing a recombinant gene encoding T4 DNA Ligase.

Reaction Volume Definition

1X Instant Sticky-end Ligase Master Mix with DNA substrates in a 10 µl reaction volume. A 10 µl reaction contains 1800 cohesive end units of T4 DNA Ligase.

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Guidelines

Once, completed, ligation products can be used immediately for transformation or stored at -20C until ready to use. In-house testing has demonstrated that maximal transformation efficiency is achieved using between 20–100 ng of vector (sticky) and a corresponding 3-fold molar excess of the insert to be ligated into the vector.

Chemically competent strains of *E. coli* (commercially available or prepared by user) can be transformed by ligation products prepared using the Instant Sticky-end Ligase Master Mix. Electrocompetent cells are not compatible. Users of competent cells from other vendors may need to dilute ligation reactions 4-fold, prior to transformation, in order to achieve maximum transformation efficiency. Not all cells from other vendors will benefit from this additional step. The following protocol is recommended by NEB. Other protocols can be used but the volume of ligation reaction used should not exceed 5 µl reaction per 50 µl cells.

Transformation efficiencies around 2 x 10^6 cfu/µg are typically achieved for recombinant cohesive end substrates (vector + insert), using cells with a 7 x 10^8 calculated efficiency with uncut DNA. This corresponds to several hundred colonies on a plate when 100 µl of a 1 ml outgrowth is plated at a 1:5 dilution. As with all ligation and transformation protocols, many factors affect the calculated transformation efficiency, including purity and integrity of DNA ends, competence of the cells being transformed, media choices, incubation temperatures and times and biological effects (intact ORF in high-copy vector, toxic genes, etc.).

Materials

Instant Sticky-end Ligase Master Mix - 50 rxns M0370S by New England Biolabs

Protocol

Preparation

Step 1.

Place master mix tube on ice and flick a few times to mix.

REAGENTS

Instant Sticky-end Ligase Master Mix - 50 rxns M0370S by New England Biolabs

Ligation

Step 2.

Combine 20–100 ng of vector with a 3-fold molar excess of insert and q.s. to 5µl with dH_2O.

AMOUNT

5 µl Additional info:

Ligation

Step 3.

Add 5 µl of Instant Sticky-end Ligase Master Mix, mix thoroughly by pipetting up and down 7-10 times, and place on ice. The sample is now ready to be used for transformation.

AMOUNT

5 µl Additional info:

REAGENTS

Instant Sticky-end Ligase Master Mix - 50 rxns M0370S by New England Biolabs

Transformation

Step 4.

Thaw competent cells on ice.

Transformation

Step 5.
Aliquot 50 µl of cells into a 1.5 ml microcentrifuge tube.

AMOUNT
50 µl Additional info:

Transformation

Step 6.
Add 2 µl of the ligation reaction to the cells and mix by finger-flicking. Do not vortex the tube.

AMOUNT
2 µl Additional info:

Transformation

Step 7.

- Incubate the tube on ice for 30 minutes. Do not mix.

DURATION
00:30:00

Transformation

Step 8.
Heat shock at 42°C for 30 seconds

DURATION
00:00:30

Transformation

Step 9.
Return tube to ice for 2 minutes.

DURATION
00:02:00

Transformation

Step 10.
Add 950 µl recovery media (e.g. SOC) to the tube

AMOUNT
950 µl Additional info:

Transformation

Step 11.
Incubate for one hour at 37°C with rotation or shaking (200–250 rpm).

DURATION
01:00:00

Transformation

Step 12.
Spread 100 µl of the outgrowth (undiluted or diluted 1:5 with recovery media) onto appropriate antibiotic selection plates and incubate overnight at 37°C.

AMOUNT
100 µl Additional info:

Warnings

Do not heat inactivate.

Heat inactivation dramatically reduces transformation efficiency.