

Resource Summary Report

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

DOI:10.17504/protocols.io.d659g5

Type: Protocol

Proper Citation

Harold Bien 2015. Cloning with NEB Instant Sticky-end Ligase Master Mix (M0370). protocols.io dx.doi.org/10.17504/protocols.io.d659g5

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.d659g5>

Authors: Harold Bien

Summary: 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 subcloning 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. Purified from an E. coli strain containing a recombinant gene encoding T4 DNA Ligase. 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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External URL: <https://www.neb.com/protocols/2012/08/27/protocol-transfer-master-mix-to-ice-prior-to-reaction-set-up-mix-tube-by-finger-flicking-before-u>

Version: 1

Publication Date: 2015

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