

Ligation Protocol for Cloning with Blunt/TA Ligase Master Mix (M0367)

Overview

Protocol

1. Transfer master mix to ice prior to reaction set up. Mix tube by finger flicking before use.
2. Combine 20–100 ng of vector* with a 3-fold molar excess of insert and adjust volume to 5 μ l with dH₂O.
3. Add 5 μ l of Blunt/TA Ligase Master Mix and mix thoroughly by pipetting up and down 7-10 times or by finger-flicking.
4. Incubate at room temperature (25°C) for 15 min, place on ice.
5. Use for transformation or store at -20°C.
6. **Do not heat inactivate.**

Heat inactivation dramatically reduces transformation efficiency.

* In-house testing has demonstrated that maximal transformation efficiency is achieved using between 20–100 ng of vector (blunt or sticky, including T-vectors) and a corresponding 3-fold molar excess of the insert to be ligated into the vector.