Quality Controls
The Instant Sticky-end Ligase Master Mix is tested for transformation efficiency using the following protocol.

LITMUS 28 vector is cut with HindIII (cohesive), treated with calf intestinal phosphatase and gel purified. Cohesive inserts from a HindIII digest of λ DNA are ligated into the vector at a 3:1 insert:vector ratio using the Instant Sticky-end Ligase Master Mix Protocol. Ligation products are transformed as described.

Each lot exceeds the following standards:

<table>
<thead>
<tr>
<th>Efficiency (transformants/μg)</th>
<th>Recircularization</th>
<th>Insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohesive ends</td>
<td>&gt; 1 x 10^7</td>
<td>&gt; 2.5 x 10^6</td>
</tr>
<tr>
<td>Uncut vector</td>
<td>&gt; 1 x 10^8</td>
<td></td>
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</tbody>
</table>

Protocols

Ligation Protocol for Subcloning:
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 μl with dH₂O.
3. Add 5 μl of Instant Sticky-end Ligase Master Mix thoroughly by pipetting up and down 7–10 times, and place on ice. The sample is now ready to be used for transformation.
4. Use for transformation or store at −20°C.
5. 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 (sticky) and a corresponding 3-fold molar excess of the insert to be ligated into the vector.

Time and Temperature: Ligations performed using the Instant Sticky-end Ligase Master Mix are immediately ready to be used to transform *E. coli*. If it is necessary to incubate the reaction prior to transformation, performance is generally good between 4–25°C. Incubation can be performed for up to one hour at the chosen temperature without negatively impacting the performance of the mix.

Blunt and TA ends can also be ligated by this mix but require a 5–15 minute incubation time to reach our internal performance standards. The “instant” ligation criteria were met with cohesive ends of 2–4 bp in length.

Biology: Some DNA sequences are not easy to clone. Sequences that form structures, including inverted and tandem repeats, are selected against by *E. coli*. Some recombinant proteins are not well tolerated by *E. coli* and can result in poor transformation or small colonies.

Usage Notes:
Cells: Competent cells can vary by several logs in their competence. Perceived ligation efficiency directly correlates with the competence of the cells used for transformation. Always transform uncut vector as a control for comparison purposes.

Electroporation: While electroporation can dramatically increase transformation efficiency, Instant Sticky-end Ligase Master Mix is not directly compatible with transformation by electroporation. It is necessary to reduce the PEG concentration. We recommend purification of the ligated DNA by spin column.

DNA: Purified DNA for ligations can be dissolved in dH₂O (Milli-Q® water or equivalent is preferable); TE or other dilute buffers also work well. For optimum ligation, the amount of vector DNA should be 20–100 ng and the insert should be added at a 3-fold molar excess. For ligation volumes greater than 10 μl, increase the volume of Instant Sticky-end Ligase Master Mix such that it remains 50% of the reaction. Insert:vector ratios between 2 and 6 are optimal for single insertions. Ratios below 2:1 result in lower ligation efficiency. Ratios above 6:1 promote multiple inserts. If you are unsure of your DNA concentrations, perform multiple ligations with varying ratios.