

## *Frequently asked questions*

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### USB<sup>®</sup> Ligate-IT<sup>™</sup> Rapid Ligation Kit [PN 78400/10]

**Can the Ligate-IT Kit be used to ligate PCR products into TA cloning vectors?**

Yes, the Ligate-IT Kit can be used to ligate PCR products into TA cloning vectors, although no vector is provided in the kit.

**Is it necessary to purify PCR products before ligation reactions?**

Usually, unincorporated nucleotides and primers do not inhibit ligation reactions. Gel purification is recommended in situations where primer-dimers and non-specific products appear upon gel electrophoresis, especially if cloning into TA or TOPO<sup>®</sup> vectors.

**Why do I observe few positive constructs and high background?**

Vector self-ligation is probably dominating the ligation reaction. In order to reduce or eliminate the self-ligation of linearized plasmid DNA, a 5'-end dephosphorylation reaction should be performed with recombinant Shrimp Alkaline Phosphatase [PN 78390]. If possible, use a directional cloning strategy with two different restriction enzymes.

**Why does the ligation reaction appear successful by gel analysis, but I observe few transformants?**

There is a possibility that host cells may have lost their competency. Check cell competency by transforming 1 ng of supercoiled pUC18/19 vector into the host cells. If few transformants arise following this condition, purchase or make new competent cells.

**What is the transformation efficiency when using the Ligate-IT Rapid Ligation Kit?**

Each kit lot exceeds  $1 \times 10^6$  white cfu/ $\mu$ g from both cohesive- and blunt-end ligations of a 1200 bp insert encoding the lac repressor (lacI) into pUC19. Competent cells used for transformation routinely yield  $10^8$ - $10^9$  white cfu/ $\mu$ g from uncut, supercoiled pUC19-lacI. Use 25-100 ng vector per reaction for optimum transformation efficiency.

**Why does my electroporation arc?**

The 5X Reaction Buffer has reagents which interfere with electroporation. Prior to electroporation, purify ligation reactions with a commercial spin column. Alternatively, perform chloroform extraction (3 volumes) and alcohol precipitate.

**What is the optimum insert to vector molar ratio for ligation?**

The recommended insert to vector molar ratio for standard reactions is 3:1. If performing linker or adaptor ligation, up to 10:1 insert to vector molar ratio is acceptable.

**Why do I observe constructs with multiple inserts?**

In order to avoid insert concatemers, do not exceed an insert to vector molar ratio of 3:1.

### How do I calculate the proper molar ratio of insert to vector?

Here is an example equation for molar ratio determination.

Equation to calculate moles of double-stranded DNA ends:

$$\frac{2 \times (\text{gm of DNA})}{(\text{number of base pairs}) \times (650 \text{ daltons/bp})} = \text{moles of dsDNA ends}$$

Example:

Vector calculation: 50 ng of cut pUC19 (2686 bp) vector has how many moles dsDNA ends?

$$\frac{2 \times (50 \times 10^{-9} \text{ gm})}{(2686 \text{ bp}) \times (650 \text{ daltons/bp})} = 5.72 \times 10^{-14} \text{ mol or } 0.057 \text{ pmol ends}$$

Insert calculation: How many ng of a 1000 bp insert must be used for a 3:1 insert:vector molar ratio?

Step 1: 3:1 molar ratio insert:vector is  $3 \times 0.057 \text{ pmol pUC19} = 0.171 \text{ pmol insert ends required}$ .

Step 2: Rearrange former equation to solve for mass given molar amount:

$$\frac{(\text{moles of ends}) \times (\text{number of base pairs}) \times (650 \text{ daltons/bp})}{2} = \text{gm of insert DNA needed}$$

Thus:

$$\frac{(0.171 \times 10^{-12} \text{ mol insert ends}) \times (1000 \text{ bp}) \times (650 \text{ daltons/bp})}{2} = 5.5 \times 10^{-8} \text{ gm or } 55 \text{ ng insert DNA}$$

Therefore, mixing 55 ng of a 1000 bp insert with 50 ng of pUC19 will satisfy a 3:1 molar ratio in this example.

### Are there any steps that can be taken to enhance the transformation efficiency?

Yes, use of SOC medium instead of LB medium following heat shock treatment may enhance transformation efficiency. Also, do not heat inactivate the ligation reaction prior to transformation.  $\text{Mg}^{2+}$  at higher temperatures might nick DNA.

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