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DNA Ligation

Addgene The Nonprofit Plasmid Repository[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.4g7gtzn

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ABSTRACT

This protocol is for how to perform DNA ligation. To see the full abstract and other resources, visit https://www.addgene.org/protocols/dna-ligation/.

EXTERNAL LINK

https://www.addgene.org/protocols/dna-ligation/

GUIDELINES

Tips and General Guidelines

Do controls: When doing ligations you should ALWAYS do a **vector alone + ligase** control. This will allow you to verify that the vector was completely digested and if phosphatase treated, that the phosphatase treatment worked. This control should, in principle, be free of colonies, but the reality is that it will have some amount of background. What you want to see is that your vector + insert ligation has many more colonies than your vector alone ligation.

Additional controls are encouraged, but may only be required for troubleshooting failed ligations. The following table indicates the various controls:

Control	Ligase	Interpretation
Uncut vector	-	Checks viability of competent cells and verifies the antibiotic resistance of the plasmid
Cut vector	-	Background due to uncut vector
Cut vector	+	Background due to vector re-circularization - most useful for phosphatase treated vector
Insert or water	+	Any colonies indicate contamination of intact plasmid in ligation or transformation reagents

Optimizing the Vector:Insert Ratio: Although a 3:1 insert to vector ratio is usually sufficient, you can optimize the amount of insert and vector to improve ligation efficiency in situations where the 3:1 ratio is not working or when doing more complicated cloning. While 3:1 will get you in the ballpark for average size genes and vectors, this ratio is really meant to refer to the molarity of DNA ends available for ligation. Simply put, there are only two ends on any given piece of DNA no matter how long it is, and therefore we need to adjust the amount of DNA used in a ligation based on the length of the DNA to get a proper ratio of 3 available insert ends for every available vector end. Ligation calculators are easily found on the web. Just enter the concentration, lengths of your insert and vector, and what ratio you want, and it will tell you exactly how much of each to use.

1 Before setting up the ligation reaction itself, it is important to determine the amount of cut insert and vector to use for the ligation reaction. The volume of vector DNA and insert DNA used in the ligation will vary depending on the size of each and their concentration. However, for most standard cloning (where the insert is smaller than the vector) a 3 insert : 1 vector molar ratio will work just fine. We recommend around **100 ng** of total DNA in a standard ligation reaction. Use a ligation calculator to easily quantify how much vector and insert DNA to use.

Combine the following in a PCR or Eppendorf tube:

- Vector DNA
- Insert DNA
- Ligase Buffer (**1 µl** / **10 µl** reaction for 10X buffer, and **2 µl** / **10 µl** reaction for 5X buffer)
- **0.5 µl** - **1 µl** T4 DNA Ligase
- H_2O to a total of **10 µl**

Notes:

- If the DNA concentrations are low such that you cannot get all **100 ng** of DNA, buffer and ligase into a **10 µl** reaction, scale the reaction size as necessary - being sure to increase the amount of buffer proportionally. **1 µl** of ligase should be sufficient for larger ligation reactions.
- Because ligase buffer contains ATP, which degrades upon freeze/thaw cycles, it is a good idea to take a fresh tube, thaw it one time and aliquot individual tubes of **5 µl** , **10 µl** or **20 µl** for storage at **-20 °C** . Whenever you need to set up ligations in the future you can thaw a new tube that you know has only been thawed once before.
- Always do controls. See the tips and general guidelines section for details
- Try different vector to insert ratios to optimize the ligation reaction. See the tips and general guidelines section for details.

2 Incubate at **Room temperature** for **02:00:00** , or at **16 °C** overnight (following the manufacturer's instructions).

Note, for many ligation reactions, especially if using "high concentration" ligase, **00:05:00** at **Room temperature** is enough. For trickier ligations (such as ligation of annealed oligos) the efficiency of ligation can be improved by incubation at **37 °C** .

3 Proceed with bacterial transformation.