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Ligation Protocol for NEB PCR Cloning Kit (E1202) V.2

New England Biolabs[1]

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Ligation protocol for the PCR Cloning Kit (E1202).

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https://www.neb.com/protocols/2013/12/27/ligation-protocol-e1202

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Assemble ligation reactions using the chart below as a guide. Mix the first 4 components before adding 5 µl of the cloning mix consisting of 4 µl Cloning Mix 1 and 1 µl Cloning Mix 2, for a total of 10 µl per ligation reaction. This ensures the ligase is not allowed to recircularize the vector backbone before this insert is present. It is recommended that first-time users of this kit perform the positive control ligation

reaction.

A	B	C
	LIGATION REACTION	**POSITIVE CONTROL**
Linearized pMiniT Vector (25 ng/µl)	1 µl (25 ng)	1 µl (25 ng)
Insert*	1–4 µl*	–
Amplicon Cloning Control (1 kb) (15 ng/µl)	–	2 µl (30 ng)
H2O	to 5 µl	2 µl
Cloning Master Mix (2X)	5 µl	5 µl
Total Volume	10 µl	10 µl

*For purified PCR amplicon products, the amount of insert to be added can be calculated by relative length or molar calculations. Formulas below use the recommended values of 25 ng of linearized vector (2525 bp) per reaction and an insert-to-vector ratio of 3:1.

a. Relative length calculations:
ng insert to be added = (3)(25 ng vector) (bp of insert/2525 bp of vector)

b. Molar calculations:

i. Convert the 25 ng vector present in the ligation reaction to pmoles:
(25 ng vector)(1000)/(650 daltons per base pair)(number of base pairs in vector or
2525) = (25)(1000)/(650)(2525) = 25000/1641250 = 0.015 pmoles vector

ii. Calculate a 3-fold molar amount of insert to add to each ligation:
(3)(0.015 pmoles vector) = 0.045 pmoles insert

iii. Convert the pmoles insert amount to ng insert to be added:
ng insert to be added = (0.045 pmoles insert)(base pairs in insert)(650 daltons per
base pair)/1000

As examples, these calculations will yield insert levels of 15 ng (500 bp insert), 30 ng (1 kb insert) or 60 ng (2 kb insert).

For unpurifiedPCR amplicons, analyze 5% of your reaction by agarose gel electrophoresis both to confirm the specificityof the product and to estimate the DNA concentration of the product by comparing amplicon yield to known amounts of DNA fragments in a marker lane, such as our Quick-Load® Purple 1 kb Plus DNA Ladder (NEB #N0550). This quantitation allows estimating the appropriate amount of PCR volume to achieve a 3:1 molar ratio of insert:vector backbone. Both too low a level of inset, or such high level of insert that insert ligates to both ends of the linearized vector, will decrease cloning efficiency. Do not use more than 1 µl of a PCR for cloning reactions to avoid carrying over PCR components that will interfere with cloning.

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MATERIALS

NEB PCR Cloning Kit - 20 rxns **New England Biolabs Catalog #E1202S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

For purified PCR amplicon products, the amount of insert to be added can be calculated by relative length or molar calculations. See the Guidelines for the formulas.

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Assemble ligation reactions using the chart below as a guide. Mix the first 4 components **before** adding **5 µL cloning mix** consisting of **4 µL Cloning Mix 1** and **1 µL Cloning Mix 2** , for a total of **10 µL** per ligation reaction.

This ensures the ligase is not allowed to recircularize the vector backbone before this insert is present. It is recommended that first-time users of this kit perform the positive control ligation reaction.

A	B	C
	LIGATION REACTION	**POSITIVE CONTROL**
Linearized pMiniT Vector (25 ng/µl)	1 µl (25 ng)	1 µl (25 ng)
Insert*	1–4 µl*	–
Amplicon Cloning Control (1 kb) (15 ng/µl)	–	2 µl (30 ng)
H2O	to 5 µl	2 µl
Cloning Master Mix (2X)	5 µl	5 µl
Total Volume	10 µl	10 µl

*For purified PCR amplicon products, the amount of insert to be added can be calculated by relative length or molar calculations. For illustrative purposes calculations are shown below; however, the NEBiocalculator web tool is a quick and convenient way to determine the insert amounts for all cloning reactions. Formulas below use the recommended values of 25 ng of linearized vector (2588 bp) per reaction and an insert-to-vector ratio of 3:1.

1. Relative length calculations:
   ng insert to be added = (3)(25 ng vector) (bp of insert/2588 bp of vector)

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2. Molar calculations:
   Convert the 25 ng vector present in the ligation reaction to pmoles:
   (25 ng vector)(1000)/(650 daltons per base pair)(number of base pairs in vector or 2588) = (25)(1000)/(650)(2588) = 0.015 pmoles vector

   Calculate a 3-fold molar amount of insert to add to each ligation:
   (3)(0.015 pmoles vector) = 0.045 pmoles insert

   Convert the pmoles insert amount to ng insert to be added:
   ng insert to be added = (0.045 pmoles insert)(base pairs in insert)(650 daltons per base pair)/1000

As examples, these calculations will yield insert levels of 15 ng (500 bp insert), 30 ng (1 kb insert) or 60 ng (2 kb insert).

For unpurified PCR amplicons, analyze 5% of your reaction by agarose gel electrophoresis both to confirm the specificity of the product and to estimate the DNA concentration of the product by comparing amplicon yield to known amounts of DNA fragments in a marker lane, such as our Quick-Load® Purple 1 kb Plus DNA Ladder (NEB #N0550). This quantitation allows estimating the appropriate amount of PCR volume to achieve a 3:1 molar ratio of insert:vector backbone. Both too low a level of inset, or such high level of insert that insert ligates to both ends of the linearized vector, will decrease cloning efficiency. Do not use more than 1 µl of a PCR for cloning reactions to avoid carrying over PCR components that will interfere with cloning.

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Incubate at **Room temperature** (**25 °C**) for 5-15 minutes.

While 5 minutes is recomended, 15 minutes will increase transformation levels for inserts suspected as being difficult to clone.

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Incubate **On ice** for **00:02:00** .

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Transform immediately or store at **-20 °C** . For best results, transform into NEB 10-beta Competent *E. coli* (NEB#C3019), which are supplied with NEB#E1202.

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