

Ligation Protocol for NEB PCR Cloning Kit

Overview

Protocols.io also provides an [interactive version of this protocol](#) where you can discover and share optimizations with the research community.

1. 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.

	Ligation Reaction	Positive Control
Linearized pMiniT 2.0 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)
H ₂ O	to 5 µl	2 µl
Cloning Mix 1	4 µl	4 µl
Cloning Mix 2	1 µl	1 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 [NEBcalculator 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.

a. Relative length calculations:

$$\text{ng insert to be added} = (3)(25 \text{ ng vector}) (\text{bp of insert}/2588 \text{ bp of vector})$$

b. Molar calculations:

i. Convert the 25 ng vector present in the ligation reaction to pmoles:

$$(25 \text{ ng vector})(1000)/(650 \text{ daltons per base pair})(\text{number of base pairs in vector or } 2588) = (25)(1000)/(650)(2588) = 0.015 \text{ pmoles vector}$$

ii. Calculate a 3-fold molar amount of insert to add to each ligation:

$$(3)(0.015 \text{ pmoles vector}) = 0.045 \text{ pmoles insert}$$

iii. Convert the pmoles insert amount to ng insert to be added:

$$\text{ng insert to be added} = (0.045 \text{ pmoles insert})(\text{base pairs in insert})(650 \text{ daltons per base pair})/1000$$

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 insert, 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.

2. Incubate at room temperature (25°C) for 5–15 minutes. While 5 minutes is recommended, 15 minutes will increase transformation levels for inserts suspected as being difficult to clone.
3. Incubate on ice for 2 minutes.
4. 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.

NEB® PCR Cloning Kit

NEB® PCR Cloning Kit (Without Competent Cells)