Ligation Protocol for NEB PCR Cloning Kit (E1202)

New England Biolabs

Abstract

Ligation protocol for the PCR Cloning Kit (E1202)

Citation: New England Biolabs Ligation Protocol for NEB PCR Cloning Kit (E1202). **protocols.io** dx.doi.org/10.17504/protocols.io.cp5vq5
Published: 02 Feb 2015

Guidelines

	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 unpurified PCR amplicons from reactions yielding a specific product, use 1 µl or less of the reaction as insert. Do not use larger amounts, as carryover material from the PCR reactions can inhibit ligation or transformation. To estimate the concentration of PCR product for the above calculations, compare the reaction yield to known amounts of DNA fragments in a marker lane, such as our Quick-Load® Purple 2-Log DNA Ladder (NEB #N0550S).

Before start

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.

Materials

NEB PCR Cloning Kit - 20 rxns E1202S by New England Biolabs

Protocol

Step 1.
Mix the first 3 components of the reaction.

PROTOCOL

. E1202 Ligation Mixture

CONTACT: New England Biolabs
Step 1.1.
Linearized pMiniT Vector (25 ng/µl), **1 µl**

AMOUNT
1 µl Additional info:
Step 1.2.
Insert, **1-4 µl**

Step 1.3.
H2O to **5 µl**

Step 2.
Add Cloning Master Mix (2X), **5 µl,** to a total of 10 µl per ligation reaction.

AMOUNT
5 µl Additional info:
Step 3.
Incubate at room temperature (25°C) for 5–15 minutes.

NOTES
New England Biolabs 25 Jan 2015
While 5 minutes is sufficient, 15 minutes will increase transformation levels for amplicons with single base overhangs
Step 4.
Incubate on ice for 2 minutes.

DURATION
00:02:00
Step 5.
Transform immediately into NEB 10-beta Competent E. coli or store at -20°C.