Blunting Protocol for NEB PCR Cloning Kit (E1202)

New England Biolabs

Abstract

This is the blunting protocol for NEB PCR Cloning Kit (E1202)

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Guidelines

Reaction volume may be scaled up or down as necessary.

PCR generated DNA must be purified before blunting by using a commercial purification kit, phenol extraction/ethanol precipitation, or gel electrophoresis.

Restriction enzyme digested DNA can be blunted directly without purification. The Blunt Enzyme Mix has been optimized in Blunting Buffer, but is also active in NEBuffers 1.1, 2.1, 3.1, and CutSmart™ Buffer in addition to NEBuffers 1-4, BamHI, EcoRI and DpnII unique buffers when supplemented with dNTPs and dithiothreitol. There is a small reduction in ligation fidelity in these buffers. Transformation efficiency is lowest in NEBuffer 1 and 1.1 where the total yield is about 50% of optimum.

Before start

Reaction volume may be scaled up or down as necessary.

Materials

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

Protocol

Step 1.
Mix the following components in a sterile microfuge tube:

PROTOCOL

. E1202 Blunting Mixture

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Step 1.1.

Purified DNA (up to 5 µg): **1-19 µl**

Step 1.2.
10X Blunting Buffer: **2.5 µl**

AMOUNT
3 µl Additional info:

Step 1.3.
1 mM dNTP Mix: **2.5 µl**

AMOUNT
3 µl Additional info:

Step 1.4.
Blunt Enzyme Mix: **1.0 µl**

AMOUNT
1 µl Additional info:

Step 1.5.
Sterile dH20 up to **25 µl**

Step 2.
Reactions containing restriction enzyme digested DNA are incubated at room temperature for 15 minutes. Reactions with sheared/nebulized DNA or PCR products are incubated at room temperature for 30 minutes.

NOTES
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Step 3.
Immediately inactivate enzyme in the blunting reaction by heating at 70°C for 10 minutes.

DURATION
00:10:00

Step 4.
Proceed directly to the ligation step using the Quick Ligation Kit (NEB #M2200) or standard T4 DNA Ligase (NEB #M0202).

NOTES
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Blunt ligation reactions using standard T4 DNA Ligase should be incubated overnight at room temperature.