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

New England Biolabs[1]

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

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https://www.neb.com/protocols/0001/01/01/blunting-protocol-e1202

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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.

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.

Reaction volume may be scaled up or down as necessary.

1 

Mix the following components in a sterile microfuge tube:

A	B
COMPONENT	**AMOUNT**
Purified DNA (up to 5 µg)	1-19 µl
10X Blunting Buffer	2.5 µl
1 mM dNTP Mix	2.5 µl
Blunt Enzyme Mix	1.0 µl
Sterile dH20	variable
Total volume	**25 µl**

2 Determine whether your reactions are using DNA digested by restriction enzymes or are sheared/nebulized or PCR products and move forward with the following steps:

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

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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.

Step 2 includes a Step case.

RE DNA

Shear/nebulized

step case

RE DNA

3 

Incubate the reactions containing restriction enzyme digested DNA at **Room temperature** for **00:15:00** .

4 

Immediately inactivate enzyme in the blunting reaction by heating at **70 °C** for **00:10:00** .

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

Blunt ligation reactions using standard T4 DNA Ligase should be incubated **Overnight** at **Room temperature** .

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