Blunting Protocol (M0203) Version 2

New England Biolabs

Abstract

Protocol for blunting ends by 3' overhang removal and fill-in of 3' recessed (5' overhang) ends using T4 DNA Polymerase.

Citation: New England Biolabs Blunting Protocol (M0203). **protocols.io**
dx.doi.org/10.17504/protocols.io.iswcefe
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Guidelines

CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´ → 5´ exonuclease activity of the enzyme.

* T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, and CutSmart Buffer as well as NEBuffers 1, 2, and 4 and T4 DNA Ligase Reaction Buffer. Optimal activity is observed in NEBuffer 2.1. BSA supplementation is recommended when using a buffer that does not already contain BSA.

References:

1. Tabor, S. and Struhl, K. (1989). DNA-Dependent DNA Polymerases. In F. M. Ausebel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl(Ed.), Current Protocols in Molecular Biology. 3.5.10-3.5.12. New York: John Wiley & Sons, Inc.

2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. (2nd ed.), 5.44-5.47. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Protocol

Step 1.

Dissolve DNA in any 1X reaction buffer supplemented with 100 µM dNTPs.

NOTES
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Step 2.

Add 1 unit of T4 DNA Polymerase per microgram DNA.

Step 3.
Incubate 15 minutes at 12°C.

DURATION
00:15:00

Stop reaction

Step 4.
Stop reaction by adding EDTA to a final concentration of 10 mM.

NOTES
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Add 0.5 microL of 0.5M EDTA

Stop reaction

Step 5.
Heat for 20 minutes at 75°C (see references 1,2).

DURATION
00:20:00

NOTES
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CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

Warnings

Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.