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Feb 14, 2022

Blunting ends by 3’ overhang removal and 3’ recessed (5’ overhang) end fill-in using T4 DNA Polymerase (M0203) V.3

New England Biolabs[1]

[1]New England Biolabs

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dx.doi.org/10.17504/protocols.io.be6ejhbe

New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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

DOI

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https://www.neb.com/protocols/2014/01/13/protocol-for-blunting-ends-by-3-overhang-removal-and-fill-in-of-3-recessed-5-overhang-ends-using1

New England Biolabs 2022. Blunting ends by 3’ overhang removal and 3’ recessed (5’ overhang) end fill-in using T4 DNA Polymerase (M0203). **protocols.io** https://dx.doi.org/10.17504/protocols.io.be6ejhbe New England Biolabs

Blunting, M0203, T4, T4 DNA pol, DNA polymerase

protocol ,

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35750

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.

MATERIALS

T4 DNA Polymerase - 150 units **New England Biolabs Catalog #M0203S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 Dissolve DNA in any [M]**1 X reaction buffer** supplemented with [M]**100 Micromolar (µM) dNTPs** .

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.

2 

Add 1 unit of T4 DNA Polymerase per microgram DNA.

3 

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Incubate **00:15:00** at **12 °C** .

4

Stop reaction by adding EDTA to a final concentration of **10 Milimolar (mM)** .

See references 1, 2 for information on stopping reaction using EDTA and 75°C.

5

Heat for **00:20:00** at **75 °C** .

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.

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