A-Tailing with Taq Polymerase

New England Biolabs

Abstract

This protocol can be used to add As to the blunt-ends of DNA fragments that have been amplified using a high-fidelity polymerase (such as Q5® High Fidelity DNA Polymerase).

Citation: New England Biolabs A-Tailing with Taq Polymerase. **protocols.io**
dx.doi.org/10.17504/protocols.io.crvv65
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Materials

- ThermoPol Reaction Buffer Pack - 6.0 ml B9004S by New England Biolabs
- Taq DNA Polymerase with ThermoPol Buffer - 400 units M0267S by New England Biolabs

Protocol

Step 1.
Clean-up the amplified DNA from the PCR components

NOTES
New England Biolabs 03 Feb 2015
This can be done by using a PCR-column purification protocol.
New England Biolabs 03 Feb 2015
This step is essential because the robust exonuclease activity associated with the high-fidelity enzyme will remove any untemplated nucleotides that are added by Taq DNA Polymerase.

Step 2.
Set-up the reaction by adding the following components:

PROTOCOL

. A-Tailing with Taq Mixture

CONTACT: New England Biolabs
Step 2.1.
PCR-amplified DNA – X

Step 2.2.
10X ThermoPol® Buffer – **5ul**

AMOUNT
5 µl Additional info:

REAGENTS
- ThermoPol Reaction Buffer Pack - 6.0 ml B9004S by New England Biolabs

Step 2.3.
1 mM dATP – 10 µl

Step 2.4.
Taq DNA Polymerase - **0.2 ul**

AMOUNT
2 µl Additional info:

REAGENTS
Taq DNA Polymerase with ThermoPol Buffer - 400 units M0267S by New England Biolabs

Step 2.5.
H2O to **50 µl**

NOTES
New England Biolabs 03 Feb 2015
This volume can be adjusted based on the volume of PCR-amplified DNA that needs to be added

Step 3.
Incubate the reaction at 72 °C for 20 minutes

DURATION
00:20:00

ANNOTATIONS
Chi-Yu Lee 29 Jan 2018

Dear Protocol Author,
I am a phd student at UCL. I want to use Taq polymerase to add A-tail on DNA fragments and then put these fragments into pCRII vector by topo cloning. Do I need to purify the DNA fragments after adding A-tailing to get rid of taq polymerase and other molecules?

Best Regards

Chi-Yu

New England Biolabs 29 Jan 2018

Dear Chi-Yu,

Thank you for your question. Yes, the A-tailing reaction should be cleaned up to remove the DNA Polymerase and dNTPs to avoid any unwanted polymerization in the ligation step.

Should you need additional support, please contact NEB tech support directly at 1-800-NEB-LABS or info@neb.com

Best,

NEB

Warnings

The DNA cleanup step is essential because the robust exonuclease activity associated with the high-fidelity enzyme will remove any untemplated nucleotides that are added by Taq DNA Polymerase.