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Feb 07, 2022

A-Tailing with Taq Polymerase V.2

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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).

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https://www.neb.com/protocols/2013/11/01/a-tailing-with-taq-polymerase

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A-tail, Taq, Taq pol, Taq polymerase

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MATERIALS

ThermoPol Reaction Buffer Pack - 6.0 ml **New England Biolabs Catalog #B9004S**

Taq DNA Polymerase with ThermoPol Buffer - 400 units **New England Biolabs Catalog #M0267S**

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

1 Clean-up the amplified DNA from the PCR components

This can be done by using a PCR-column purification protocol. 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.

2 

Set-up the reaction by adding the following components:

A	B
REAGENT	AMOUNT
PCR-amplified DNA	X µl
10X ThermoPol® Buffer (NEB#B9004)	5 µl
1mM dATP	10 µl
Taq DNA Polymerase (NEB#M0267)	0.2 µl
H2O	X µl
Total Reaction Volume	*50 µl**

*This volume can be adjusted based on the volume of PCR-amplified DNA that needs to be added).

3 

Incubate the reaction at **72 °C** for **00:20:00** .

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