Jul 23, 2019

NEBNext dA-Tailing Module (NEB #E6053)

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[1]New England Biolabs

1 Works for me dx.doi.org/10.17504/protocols.io.4t3gwqn

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ABSTRACT

The NEBNext dA-Tailing Module has been optimized to efficiently incorporate a non-templated dAMP on the 3′ end of a blunt DNA fragment (1). 3′-dA DNA tailing prevents concadamer formation during subsequent ligation steps.
DNA tailed with the NEBNext dA-Tailing module may be ligated to adaptors or cloning vectors with complementary dT overhangs. The NEBNext dA-Tailing Module is provided as a master mix to maximize efficiency and convenience in DNA sample preparation workflows.

The NEBNext dA-Tailing Module has been validated by sequencing with the Il- lumina Genome Analyzer II (Illumina, Inc.) in conjunction with the NEBNext End Repair Module, NEBNext Quick Ligation Module and Phusion® High-Fidelity PCR Master Mix.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

EXTERNAL LINK

https://www.neb.com/products/e6053-nebnext-da-tailing-module#Product%20Information

GUIDELINES

Safe Stop Point: This is a point where you can safely stop the protocol and store the samples prior to proceeding to the next step in the protocol.

Caution: Signifies a step in the protocol that has two paths leading to the same point.

Color: A color listed before or after a reagent name indicates the cap color of the reagent to be added.

MATERIALS

NAME	CATALOG #	VENDOR
NEBNext dA-Tailing Reaction Buffer	E6055	New England Biolabs
Klenow Fragment (3→5 exo–)	E6054 in Kit E6053	New England Biolabs

STEPS MATERIALS

NAME	CATALOG #	VENDOR
NEBNext dA-Tailing Reaction Buffer	E6055	New England Biolabs
Klenow Fragment (3→5 exo–)	E6054 in Kit E6053	New England Biolabs

BEFORE STARTING

Starting Material: 1–5 µg of of end repaired, blunt DNA (100–1000 bp).

dA Tailing

1 Mix the following components in a sterile microfuge tube:

Component	Volume

End Repaired, Blunt DNA	variable
NEBNext dA-Tailing Reaction Buffer	5 µl
Klenow Fragment (3'→5' exo-)	3 µl
Sterile water	variable
Total Volume	**50 µl**

NEBNext dA-Tailing Reaction Buffer
by New England Biolabs
Catalog #: E6055

Klenow Fragment (3→5 exo–)
by New England Biolabs
Catalog #: E6054 in Kit E6053

30m

2 Incubate in a thermal cycler for **00:30:00** at **37 °C**.

3 Purify DNA sample on one spin column or using AMPure XP beads.

Note: for details on how this module is used in the NEBNext Library Prep for Illumina workflow, please see the manual for NEBNext DNA library Prep Master Mix Set for Illumina (NEB #E6040).