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NEBNext dA-Tailing Module (NEB #E6053) V.1

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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.

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

MATERIALS

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

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

STEP MATERIALS

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

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Protocol materials

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Troubleshooting

Before start

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**

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

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

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

Note

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**).