NEBNext® dA-Tailing Module

NEB #E6053S/L

20/100 reactions

Version 5.0_6/22

Table of Contents

The dA-Tailing Module Includes

The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6053S) and 100 reactions (NEB #E6053L). All reagents should be stored at –20°C.

Klenow Fragment (3´ → 5´ exo⁻)

NEBNext dA-Tailing Reaction Buffer

The NEBNext dA-Tailing Module is Designed for use with the Following:

NEBNext Quick Ligation Module (NEB #E6056)

NEBNext End Repair Module (NEB #E6050)

NEBNext Q5® Hot Start HiFi PCR Master Mix (NEB #M0543)

NEBNext Oligo kit options can be found at neb.com/oligos

Alternatively, customer supplied adaptor and primers can be used, please see information in link below:
https://www.neb.com/faqs/2019/03/08/can-i-use-this-nebnext-kit-with-adaptors-and-primers-from-other-vendors-than-neb

Please note: This manual is not for use with UNIQUE DUAL INDEX UMI ADAPTORS.

Required Materials Not Included

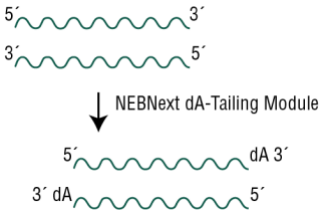
- Thermal cycler
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect® Reagent Kit (Beckman Coulter, Inc. #B23317)
- Vortex Mixer
- Microcentrifuge
- DNase RNase free PCR strip tubes (USA Scientific® 1402-1708)
- Magnetic rack/stand (NEB #S1515, Alpaqua®, cat. #A001322 or equivalent)
- 10 mM Tris-HCl or 0.1X TE

Description

The NEBNext dA-Tailing Module enables incorporation of a non-templated dAMP on the 3´ end of a blunt-ended DNA fragment. The module is optimized for use with the NEBNext dA-Tailing Module (NEB #E6053), and is part of the original standard DNA library prep workflow for Illumina sequencing, which is suitable for 1–5 µg of input DNA.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of an indexed library on the Illumina sequencing platform.

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.



Applications

DNA sample preparation

dA-Tailing of 1-5 µg fragmented and end repaired DNA

Advantages

- Efficient – Converts 1–5 µg blunt DNA to DNA with 3´-dAMP overhangs
- Convenient – Reactions are provided in master mix format to reduce steps during DNA sample prep workflows
- Automation Friendly

Protocol for use with NEBNext dA-Tailing Module

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

1. NEBNext dA-tailing

1.1. Mix the following components in a sterile microfuge tube:

COMPONENT	VOLUME (µl) PER REACTION
• End Repaired, Blunt DNA	variable
• NEBNext dA-Tailing Reaction Buffer	5 µl
• Klenow Fragment (3´ → 5´ exo⁻)	3 µl
Sterile H_2O	variable
Total Volume	50 µl

1.2. Incubate in a thermal cycler for 30 minutes at 37°C with the heated lid set to ≥ 45°C.

2. Cleanup of dA-tailed DNA

2.1. Vortex AMPure XP or SPRIselect Beads to resuspend.

2.2. Add 90 µl (1.8X) of resuspended AMPure XP or SPRIselect Beads to the ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

2.3. Incubate for up to ~5 minutes at room temperature.

2.4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (~5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.

2.5 Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.6 Repeat Step 2.5 once for a total of two washes.

2.7. Air dry beads for up to 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

2.8 Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 30 µl of 10 mM Tris-HCl or 0.1 X TE.

2.9 Mix well on a vortex mixer or by pipetting up and down 10 times and incubate for 2 minutes at room temperature.

2.10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 25 µl of the supernatant to a fresh, sterile microfuge tube.

Kit Components

NEB #E6053S Table of Components

NEB #	PRODUCT	VOLUME
E6054A	Klenow Fragment (3´ → 5´ exo⁻)	0.06 ml
E6055A	NEBNext dA-Tailing Reaction Buffer	0.1 ml

NEB #E6053L Table of Components

NEB #	PRODUCT	VOLUME
E6054AA	Klenow Fragment (3´ → 5´ exo⁻)	0.30 ml
E6055AA	NEBNext dA-Tailing Reaction Buffer	0.5 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	3/12
2.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages	4/18
3.0	Add "Designed for Use", "Materials not Included". Update the introduction text and the protocol.	5/19
4.0	New format applied.	9/19
5.0	Update protocol and required materials not included	6/22

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