

NEBNext[®] dA-Tailing Module

NEB #E6053S/L

20/100 reactions

Version 4.0_9/19

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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 (10X)

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 Singleplex or Multiplex Oligos for Illumina[®]
 (NEB #E7350, #E7335, #E7500, #E6609, #E7710, #E7730 or #E7600)

Required Materials Not Included

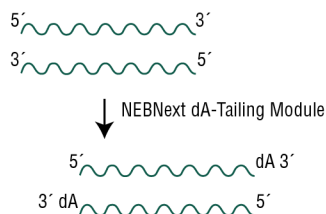
- Thermocycler
- AMPure[®] XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect[®] Reagent Kit (Beckman Coulter, Inc. #B23317)
- 10 mM Tris-HCl, pH 7.5–8.0 or 0.1 μM Tris-HCl, pH 8.0

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

NEBNext dA-Tailing Module Protocol

Starting Material

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

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 (10X) | 5 µl |
| Klenow Fragment (3' → 5' exo ⁻) | 3 µl |
| Sterile H ₂ O | variable |
| Total Volume | 50 µl |

2. Incubate in a thermocycler for 30 minutes at 37°C with the heated lid set to ≥ 45°C.
3. Purify DNA sample on one purification 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 manual for NEBNext DNA Library Prep Master Mix Set for Illumina (NEB #E6040).

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 |

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