Jun 02, 2018

3' Azide labelling with TdT

DOI
https://dx.doi.org/10.17504/protocols.io.qnmdvc6

Benjamin Emert[1]

[1]University of Pennsylvania

Human Cell Atlas Metho... RajLab

Benjamin Emert
University of Pennsylvania

DOI: https://dx.doi.org/10.17504/protocols.io.qnmdvc6

Protocol Citation: Benjamin Emert 2018. 3' Azide labelling with TdT. **protocols.io** https://dx.doi.org/10.17504/protocols.io.qnmdvc6

Protocol status: In development
We are still developing and optimizing this protocol

Created: June 02, 2018

Last Modified: April 05, 2024

Protocol Integer ID: 12717

Keywords: end of unmodified dna olgionucleotide, unmodified dna olgionucleotide, modified nucleotide, tdt protocol, using terminal transferase, terminal transferase, dna, azide

Abstract

Protocol for add azide modified nucleotide to 3' end of unmodified DNA olgionucleotide using terminal transferase. Protocol adapted from **Winz et al. 2015**.

Materials

MATERIALS

- Terminal Transferase - 2,500 units **New England Biolabs Catalog #**M0315L
- ethanol
- N6-(6-Azido)hexyn-3dATP **Jena Bioscience Catalog #**NU-1707
- 3M Sodium Acetate solution

TdT reaction

1 Set up TdT reaction

- 2.5 µL 10x Terminal transferase buffer
- 10 µL 2.5mM CoCl2
- 2 µL 400µM template oligo
- 1 µL 10mM N6(6-azido)hexyl-3'-dATP
- 2.5 µL recombinant TdT (20U/µL)
- 7 µL nuclease free water

Note

Probably useful to scale up reaction.

2 Incubate at 37ºC overnight.

- 37 °C Incubate on thermal cycler
- 50 °C thermal cycler lid temperature
- 12:00:00 incubate overnight

Ethanol precipitate to cleanup

3 Add 1/10th volume sodium acetate and 3x volume ethanol to precipitate azide labelled oligo.

- 2.5 µL 3M sodium acetate
- 82.5 µL 200 proof ethanol

Note

If you want to get rid of excess N6-(6-azido)hexyl-3'-dATP, you may want to use 1/2 volume 7.5M ammonium acetate.

4 Place solution at -80ºC for 1 hour to precipitate.

Note

Fine to incubate at -20ºC or -80ºC for longer.

- -80 °C

01:00:00

5 Centrifuge for 10 minutes to precpitate

00:10:00 Centrifuge at 16,000 x g

4 °C

6 Remove supernatant. Careful not to disturb pellet.

7 Wash sample with 500 µL 70% ethanol

500 µL 70% ethanol

8 Centrifuge for 2-5 minutes.

00:02:00 Centrifuge at 16,000 x g

9 Remove supernatant. Careful not to disturb pellet.

10 Centrifuge for 1 minute to collect supernatant from the lid and walls.

00:01:00 Centrifuge at 16,000 x g

11 Remove supernatant then dry pellet under vacuum or ambient air.

12 Add 1.8 µL nuclease free water to dissolve dry pellet. Let sit at room temp for ≥20 minutes.

1.8 µL nuclease free water

25 °C incubate at room temperature

00:20:00 incubate at room temperature.

Note

Adding 1.8 µL and assuming ~10% loss. Can be more precise and measure final concentration but beware of residual dATP.

13 Store azide modified oligo at -20ºC indefinitely.

-20 °C