Thymine-DNA Glycosylase (TDG) Protocol for Removal of dU:dG or dT:dG Mispairs (NEB #M0766)

Materials

- Thymine-DNA Glycosylase (NEB #M0766)
- TDG Reaction Buffer (included)
- Nuclease-free water (NEB #B1500)

Overview

Thymine-DNA glycosylase (TDG) is a DNA repair enzyme that specifically removes thymine or uracil bases mispaired with guanine in double-stranded DNA, such as those arising from 5-methylcytosine (5mC) or cytosine deamination. This protocol describes the steps for TDG treatment to generate abasic sites, which can be further processed for downstream applications. TDG treatment is particularly useful in reducing sequencing artifacts in FFPE DNA samples and is active only on dsDNA substrates.

Protocol

1. Reaction Setup

COMPONENTS	20 µl REACTION
T:G or U:G-containing dsDNA substrate	Up to 2 pmol
TDG Reaction Buffer (10X)	2 µl
Thymine-DNA Glycosylase (TDG)	1 µl
Nuclease-free Water	to 20 µl

Gently mix the reaction by pipetting up and down.

2. Incubation

Incubate at 37°C for 1 hour.

3. Optional Enzyme Inactivation

Heat inactivate TDG at 65°C for 10 minutes, if required.

Post-reaction Treatment

After enzymatic generation of the abasic (AP) site:

- Chemical Cleavage Option: Treat the AP-containing DNA with 200 mM NaOH for 10 minutes at 65°C.
- Enzymatic Cleavage Option: Treat the AP-containing DNA with an AP endonuclease for strand cleavage at the AP site.