MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.1

Product name: DNA Homopolymeric Tailing Kit

Cat #: NGMA-100, NGMA-200, NGMT-100, NGMT-200, NGMC-100, NGMC-200, NGMG-100, NGMG-200

Description:

DNA Homopolymeric Tailing Kit provides qualified reagents for the addition of homopolymer tails to the 3′ ends of DNA with terminal deoxynucleotidyl transferase (TdT). Under the optimized assay condition, approximately average of 30~70nt oligo(dA) and oligo(dT) or 15~45nt oligo(dC) and oligo(dG) could be added to the target substrate.

TdT is a template-independent DNA polymerase that catalyzes the repetitive addition of deoxynucleotides to the 3′ hydroxyl terminus of DNA molecules. The enzyme was generated from an *E. coli* strain that carries the cloned TdT gene from calf thymus with selected mutations. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT. The addition of dNTPs to 3'-overhanging ends is more efficient than with 3′-recessed or blunt ends. TdT incorporates dATP and dTTP with higher efficiency than dCTP and dGTP. The optimized master mixture stimulates the tailing of the 3′-ends of DNA fragments, even applicable for incorporating ribonucleotides and modified nucleotides (e.g., fluorescein-, biotin-, aminoallyl-labeled nucleotides and dideoxynucleotides).

Storage:

DNA Homopolymeric Tailing Kit should be stored at -20°C. The MasterMix should be prevented from light.

Protocol:

We recommend assembling reactions on ice from pre-chilled components. This protocol is for a reaction size of 10 µL. The reaction size may be adjusted as desired.

1. Set up reaction as below.

Amount	Description	Final Concentration
5 µL	2x Master Mix	1X
0.5 µL	Tailing Enzyme	N/A
X µL	DNA termini	Around 1µM
X µL	Nuclease free water	N/A
10 µL	Total volume	

2. Incubate at 37°C for 15 to 45 minutes, depends on the tail length expected.
3. Inactivate the TdT and stop the reaction by heating to 70°C for 10 minutes or directly add 2 µl of 5 M EDTA.

Note:

Input quantity of DNA substrate is critical to the tail length of final product. Reaction time could be adjusted according to expected tail length.
Repeated freeze-thaw cycles may reduce kit performance.
Due to the presence of $CoCl_2$, the tailing reaction mixture is incompatible with downstream applications. It is necessary to remove $CoCl_2$ by spin column or phenol/chloroform extraction and subsequent ethanol precipitation.

Reference:

1. F. J. Bollum, Terminal deoxynucleotidyl transferase, The Enzymes, the third edition (Boyer, P.D., ed.3, Academic Press, New York, vol.10, 1974) pp. 145-171.
2. G. R. Deng, R. Wu, Terminal transferase: Use in the tailing of DNA and for in vitro mutagenesis. Meth. Enzymol. 100, 96-116 (1983).

Figures:

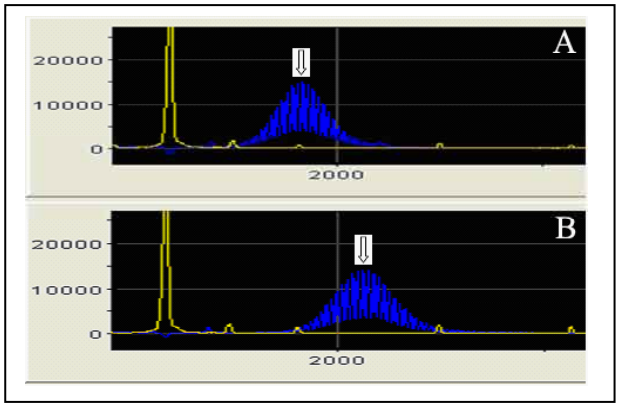

Figure 1. Fragment analysis through 3730 xl DNA Analyzer shows an oligo(dA) tail was added to 5′ fluorescent labeled single strand DNA oligo using DNA Homopolymeric Tailing Master Mix (dA) Kit. Arrow: Tailing reaction final product. A: Fifteen minutes at 37°C reaction product with a 50 nt average length of homopolymer dA tail. B: Thirty minutes at 37°C reaction product with a 65 nt average length of homopolymer dA tail.

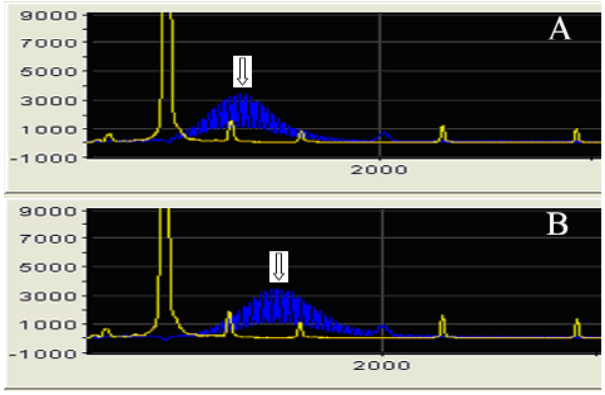

Figure 2. Fragment analysis through 3730 xl DNA Analyzer shows an oligo(dC) tail was added to 5′ fluorescent labeled single strand DNA oligo using DNA Homopolymeric Tailing Master Mix (dC) Kit. Arrow: Tailing reaction final product. A: Twenty minutes at 37°C reaction product with a 38 nt average length of homopolymer dC tail. B: Forty minutes at 37°C reaction product with a 45 nt average length of homopolymer dC tail.