DNA 3‘-end Labeling by Fill-in of 5‘-overhangs with Klenow Fragment* or Bsm DNA Polymerase, Large Fragment

Protocol

1. Prepare the following reaction mixture:

Linear DNA (aqueous solution)	0.1-4 µg
10X reaction buffer for Klenow Fragment or **10X Bsm buffer**	2 µl
[alpha-32P]-dNTP, ~15-30 TBq/mmol (400-800 Ci/mmol) or **[alpha-32P]-dNTP, ~110 TBq/mmol (3000 Ci/mmol)**	0.74 MBq (20 µCi) 2.96 MBq (80 µCi)
3 dNTP Mix, 2 mM each (without a labeled dNTP)	2.5 µl (0.25 mM final concentration)
Klenow Fragment or **Klenow Fragment, exo- or** **Bsm DNA Polymerase, Large Fragment**	0.1 µl (1 u) 0.2 µl (1 u) 0.125 µl (1 u)
Water, nuclease-free	to 20 µl
Total volume	**20 µl**

2. Incubate at 37°C for 15 min.
3. Stop the reaction by heating at 75°C for 10 min.

Note

* This protocol is suitable labeling of the following Fermentas DNA markers, composed of DNA fragments with 5’-overhangs:

Lambda DNA EcoRI Marker, #SM028

Lambda DNA HindIII Marker, #SM0101

Lambda DNA EcoRI/HindIII Marker, #SM0191

Lambda DNA Eco91I Marker, #SM0111

phiX174 DNA HinfI Marker, #SM0261

- The modified version of this protocol can be used for non-radioactive labeling of DNA markers. Substitute a part of dTTP nucleotide with a modified nucleotide (e.g. Biotin-11-dUTP or Fluorescein-12-dUTP) at a molar ratio of 1:2.
- For estimation of pmol of DNA ends, see REviewer™.

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