FILLING RECESSED 3' ENDS OF DOUBLE-STRANDED DNA

The reaction conditions are identical to those used for nick translation of DNA except that:

- The Klenow fragment of E. coli DNA polymerase is used instead of the holoenzyme.
- No DNase is used.
- Generally, only one of the four dNTPs is labeled.
- Which dNTPs are added to the reaction depends on the sequence of the protruding 5' termini at the ends of the DNA; e.g., to fill in recessed 3' ends created by cleavage of DNA by EcoRI, only dATP and TTP need be present in the reaction:

\[
\begin{align*}
p\text{-Cp-Tp-Tp-Ap}^5' & \quad \text{dATP} & \quad \text{Cp-Tp-Tp-Ap}^5' \\
pGOH^3' & \quad \text{TTP} & \quad \text{G-pA-pA-pT-pTOH}^3'
\end{align*}
\]

On the other hand, all four dNTPs are required to fill recessed ends created by HindIII:

\[
\begin{align*}
p\text{-Tp-Tp-Cp-Gp-Ap}^5' & \quad \text{dATP} & \quad \text{dGTP} & \quad \text{Tp-Tp-Cp-Gp-Ap}^5' \\
p\text{A}_{OH}^3' & \quad \text{dCTP} & \quad \text{A-pA-pG-pC-pTOH}^3'
\end{align*}
\]

Finally, to repair the ends left after treatment of DNA with nuclease S1 or BAL31, all four dNTPs should be present during the reaction. A typical reaction contains 1 µg of DNA in 20 µl. However, the reaction works well over a wide range of DNA concentrations (1-500 µg/ml).

1. Mix:
   - DNA 1 µg
   - 10X nick-translation buffer 2 µl
   - unlabeled dNTPs (as needed) 2 nmoles of each (1 µl of a 2 mM solution)
   - \([a^{32}\text{P}]\text{dTNTP}\) 2 pmoles (sp.act.>400 Ci/m mole)
   - Klenow fragment of DNA polymerase 1 unit
   - \(dH_2O\) to 25 µl

2. Incubate at room temperature for 30 minutes.

3. Stop the reactions by adding 1 µl of 0.5 M EDTA. Extract once with
phenol/chloroform.

4. Separate the DNA from unincorporated dNTPs by chromatography on or centrifugation through small columns of Sephadex G-50.

Procedure from Maniatis cloning manual.