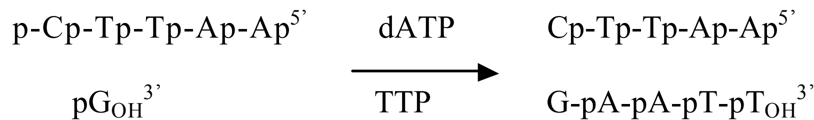
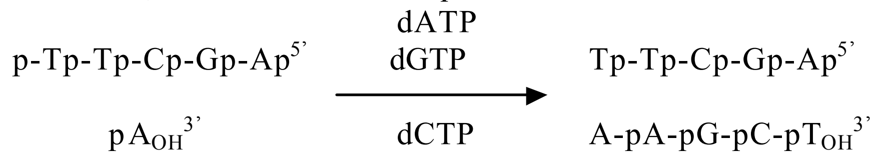
FILLING RECESSED 3' ENDS OF DOUBLE-STRANDED DNA

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:



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



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}P]dNTP	2pmoles (sp.act.>400 Ci/mmole)
Klenow fragment of DNA polymerase	1 unit
dH_20	to 25µl

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.