NICK TRANSLATION OF DNA

Stock Solutions:

10X Nick-translation buffer

0.5 M Tris-Cl (pH 7.2)
0.1 M $MgSO_4$
1 mM dithiothreitol
500 µg/ml bovine serum albumin (BSA Pentax Fraction V)
-Divide into small aliquots and store at -20°C.

DNase I Prepare a stock solution containing 1 mg/ml of DNase I in 0.15 M NaCl and 50% glycerol. (Electrophoretically pure DNase I can be obtained from Worthington Biochemicals). Divide into small aliquots and store at -20°C.

Deoxynucleotide triphosphates

dATP (1 mM)
dGTP (1 mM)
dCTP (1 mM)
TTP (1 mM)

E. Coli DNA Polymerase I Most commercial suppliers sell the enzyme in buffer containing 50% glycerol. Usually 1 µl of the preparation contains 5 units of enzyme. (For a definition of a unit, see Richardson et al. 1964).

Escherichia coli DNA polymerase I adds nucleotide residues to the 3'-hydroxyl terminus that is created when one strand of a double-stranded DNA molecule is nicked. In addition, the enzyme, by virtue of its 5' to 3' exonucleolytic activity, can remove nucleotides from the 5' side of the nick. The elimination of nucleotides from the 5' side and the sequential addition of nucleotides to the 3' side results in movement of the nick (nick translation) along the DNA (Kelly et al. 1970). By replacing the pre-existing nucleotides with highly radioactive nucleotides, it is possible to prepare ^{32}P-labeled DNA with a specific activity well in excess of 10^8 cpm/µg (Maniatis et al. 1975; Rigby et al. 1977).

Procedure:

1. A typical reaction contains 1 µg of DNA in a volume of 50 µl. However, the reaction can be scaled down to volumes as small as 5 µl.

2. Although E. coli DNA polymerase I will work with concentrations of dNTPs as low as 2 µM, the enzyme synthesizes DNA much more efficiently when supplied with higher concentrations of substrates. For reasons of cost, nick-translation reactions usually contains minimal concentrations (2 µM) of labeled dNTPs and much greater

concentrations (20 µM) of unlabeled dNTPs. A 50-µl reaction therefore contains 1 nmole of each unlabeled dNTP and 100 pmoles of each labeled dNTP. The specific activity of the final, nick-translated DNA depends in part on the ratio of labeled to unlabeled dNTPs in the reaction. When high specific activities (>10^8 cpm/µg DNA) are required (e.g., for screening of recombinant DNA libraries or for detecting single-copy sequences in Southern hybridizations of eukaryotic DNA), the nick translation should contain 200 pmoles of each of the four dNTPs labeled in the a position with ^{32}P (sp. act. >800 Ci/mmole).

For most other purpose, it is adequate to use one dNTP labeled with a-^{32}P and three unlabeled dNTPs, or to dilute each [a-^{32}P]dNTP with an appropriate amount of the unlabeled dNTP.

3. Most commercial suppliers sell [a-^{32}P]dNTPs in a concentrated aqueous solution which can be added directly to the nick-translation reaction The specific activity ranges from 400 to 2000 Ci/mmole. If [a-^{32}P]dNTPs in an ethanol/water mixture are used, remove the ethanol and water.
4. Set up the nick-translation reaction as follows:

10X nick-translation buffer	5 µl
DNA	1 µg
unlabeled dNTPs (if needed)	1 nmole of each (1 µl of a 1 mM solution)
[a-^{32}P]dNTPs	100 pmoles
dH_2O	to 44 µl

Chill the mixture to 0°C. Make a 10^4 fold dilution of a small quantity of a stock solution of DNase (1 mg/ml) in ice-cold, nick-translation buffer containing 50% glycerol. The diluted enzyme is stable when stored at -20°C in this buffer.

5. Add 0.5 µl of diluted Dnase I (0.1 µg/ml) to the reaction mixture. Mix by vortexing.

6. Add 5 units (as defined by Richardson et al. 1964) of E. coli DNA polymerase I. Mix.

7. Incubate at 16 C for 60 minutes.

Note. If the reaction is carried out at higher temperature, a considerable amount of "snapback" DNA may be generated by DNA polymerase copying the newly synthesized strand.

8. Stop the reaction by adding 2 µl of 0.5 M EDTA.

9. Using the DE-81 binding or TCA precipitation assays determine the proportion [a-^{32}P]dNTPs that have been incorporated into DNA.

10. Separate the nick-translated DNA from unincorporated dNTPs either by chromatography on or centrifugation through a small column of Sephadex G-50.

Notes:

i. The specific activity of the nick-translated DNA depends not only on the specific activity of the dNTPs, but also on the extent of nucleotide replacement of the template. This can be controlled by varying the amount of DNase I in the reaction. The aim is to establish conditions that will result in incorporation of about 30% of the [a-^{32}]dNTPs into DNA.

The size of DNA after nick translation also depends on the amount of DNase I added to the reaction and the amount of DNase contaminating the preparation of DNA polymerase I. Because the amount of contaminating nuclease differs widely between different batches of commercial DNA polymerase I and DNase I, each new preparation should be tested as described in Maniatis cloning manual.

ii. Nick translation usually yields uniformly labeled DNA (Rigby et al.1977), indicating that any bias in nicking by DNase I (Ehrlich et al. 1973) or in synthesis by DNA polymerase is too small to produce significant distortion in the pattern of labeling.

iii. The enzymes used in nick translation are sensitive to contaminants in agarose. Thus, DNA samples eluted from agarose gels should be carefully purified before they are used in nick-translation reactions.

iv. For some purposes (e.g., analysis of DNA-RNA hybrids by nuclease S1), it is desirable to obtain nick-translated DNA whose chain length is significantly longer than 400-800 nucleotides. In this case:

a. Carry out the nick-translation reaction using the amount of DNase I determined to be optimal.
b. Add 2 µl of 0.5 M EDTA. Heat at 70°C for 5 minutes to inactivate DNA polymerase and DNase.
c. Add 150 µl of 10 mM $MgCl_2$ and 20 µl of 10x ligation buffer.
d. Add 2 units of T4 ligase.
e. Incubate at room temperature for 2 hours.
f. Separate the nick-translated and ligated DNA from unincorporated dNTPs either by chromatography through a column of Sephadex G-50 or by spun-column chromatography.
g. Analyze the size of the DNA by electrophoresis through an alkaline agarose gel.
h. If necessary, isolate DNA of the required size form a preparative alkaline agarose gel.

References:

Procedure from Maniatis cloning manual

Ehrlich, S.D. et al. (1973) E.J.B. 40, 143.
Kelley, R.G. et al.(1970) J.B. C. 245, 39.
Maniatis, T.A. et al. (1975) PNAS (USA) 72, 1184.
Richardson, C.C. et al.(1964) J.B.C. 239, 22.
Rigby, P.W. J. et al. (1977) J.M.B. 113 237.