Labelling DNA ends with the Klenow fragment of the *E.coli* DNA polymerase I: a cautionary note

Gloria del Solar and Manuel Espinosa*

Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006 Madrid, Spain

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One of the most employed methods to label the 3' ends of double stranded DNA fragments generated by restriction cutting is the use of the Klenow fragment of the *E. coli* DNA Polymerase I (PolIK). It is accepted (1–3) that the method has two main advantages: i) it yields a high efficiency of labelling, and ii) labelling can be performed immediately after digestion with the desired restriction enzyme, which does not need to be removed or inactivated (3). We show here that the second point may not be necessarily correct, as observed while purifying DNA fragments labelled with PolIK. In the Figure, lanes 1 and 2 depict the label incorporated into two fragments resulting from digestion of a plasmid DNA with *Apa*LI followed by labelling with PolIK and digestion with *Pst*II. In lane 1, the *Apa*LI enzyme was inactivated by heating (65°C, 15 min), before treatment with PolIK and *Pst*II. The fragment of 602 bp shows an intensity about 3 times higher than the band of 297 bp. In lane 2, the DNA was phenol-extracted and ethanol-precipitated after digestion with *Apa*LI, followed by the labelling and second digestion: both bands show similar label. In another experiment, the same DNA was digested with *Hinf*I, heated (65°C, 15 min) and labelled with PolIK, ³²P-dATP and ³²P-dTTP: the radioactivity in bands of 855 and 213 bp is lower than in the rest (lane 3). To know if one of the strands was being preferentially labelled (or discriminated), the 213-bp band (having the sequence 5'-G/AAT-C- 3' in both ends) was cut from the gel and purified. After denaturation (92°C, 5 min, in 30% dimethyl sulfoxide), the strands were separated on a 2 mm-thick 8% polyacrylamide

sequencing gel (4), and the strands identified as described (5). One strand (in this case, the plasmid coding strand) was preferentially labelled (lane 4). The radioactivity in the coding (C) strand was about five times higher than that of the noncoding strand (NC). When the same samples were treated with phenol and ethanol-precipitated (after digestion with *Hinf*I), labelling of all the restriction fragments was uniform (lane 5), and both strands of the 213 bp fragment were equally labelled (lane 6). It seems that the restriction enzymes used were able to remain bound to the DNA (even after heating), hindering the correct activity of the PolIK. Phenol treatment of the samples avoided the problem, which can be of importance if the strand to work with were the noncoding strand.

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* To whom correspondence should be addressed