Abstract

Non-radioactive DNA detection methods have been used to overcome the disadvantages associated with radioactivity. Plasmid and chromosomal DNA have been labeled in vivo with thymidine analogs, using 5-fluorodeoxyuridine (FdUrd) as inhibitor of thymidylate synthase (FdUrd method). In order to avoid the use of FdUrd we have obtained a thymidineless (thy-) Escherichia coli strain for in vivo labeling of plasmid DNA with BrdUrd (thy- method). E. coli DH5a strain was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine. A single colony was selected by screening colonies grown on properly supplemented minimal medium and supplemented minimal medium plus thymidine (0.6 g/L). The strain exhibited a mutation reversion index less than 3.93 · 10⁻⁸ on supplemented minimal medium. The comparative study of in vivo labeling systems was carried out. The detection of single and double BrdUrd labeled DNA stranded was performed immunoenzymatically. The detection limit values of single stranded DNA by thy- method was 1 ng whereas by FdUrd method was 5 ng. The detection limit value is related to the amount of single or double stranded DNA available for detection by the anti-5-BrdUrd antibody. The sensitivity of the system is comparable to those of other non-radioactive nucleic acids labeling systems. These results demonstrate the capacity of the thy- strain for efficient in vivo DNA labeling as a system capable to produce large quantities of labeled probes.

Keywords

Bromodeoxyuridine, in vivo DNA-labeling, thymidineless strain.