3' Non-templated ‘A’ addition by Taq DNA polymerase: An advantage in the construction of single and double mutants

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The 3'-end non-templated ‘A’ addition by Taq DNA polymerase has been described as a disadvantage in the generation of site-specific mutants as this ‘A’ leads to a unplanned second mutation. Here we demonstrate the utility of this 3’ non-templated ‘A’ addition in the simultaneous construction of single and double mutants of serine hydroxymethyltransferase.

Sitedirected mutagenesis (SDM) has been widely used for research in molecular biology and protein engineering. Several methods for SDM using polymerase chain reaction (PCR) have been described. Megaprimer method is one of the most rapid and universal, in which one mutagenic primer and two universal flanking primers are required. A possible problem associated with this method is the addition of an adenosine residue at 3’

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**Figure 1** a-c. A megaprimer method for the construction of single and double mutants. a, b, Schematic diagram for the construction of single and double mutants. The coding strand of PCR product is shown as dotted lines and non-coding strand in solid lines. Sense and antisense primers are shown as (--) and (—), c, Agarose gel with lane 1. Lambda Hind III/pUC 19 Hind I marker, lane 2. Final PCR product with expected size of 1.58 kb and lane 3. First PCR product (megaprimer) with an expected size of 1.15 kb. Part of sequencing gel confirming d, wild type SHMT sequence; e, single mutant (AAG → CAG); f, double mutant (AAG → CAG and ACC → ATC).

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