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

J. Jagath-Reddy, N. Appaji Rao, H. S. Savithri

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

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.

SITE-directed 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[1–8]. 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′

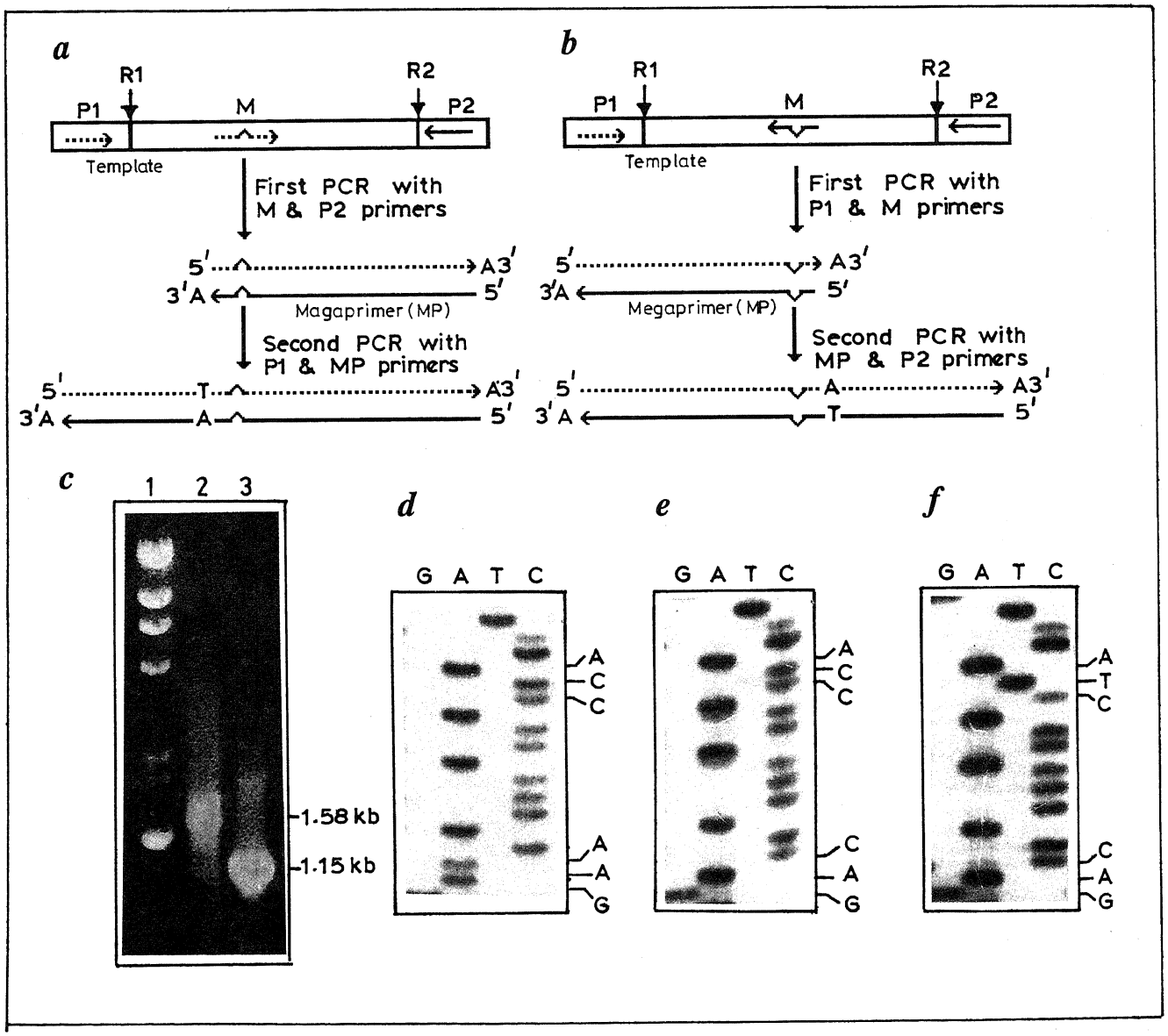

Figure 1 *a–c*. A megaprimer method for the construction of single and double mutants. *a*, *b*, Schematic diagram for the construction of single and double mutants. The coding strand of PCR product is shown as dotted lines and non-coding strand in solid lines. Sense and antisense primers are shown as (....) and (←). *c*, Agarose gel with lane 1. Lambda *Hind* III/pUC 19 *Hinf* 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).