

P027 pSJ1: novel gapped DNA substrate for forward mutation assay

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The majority of DNA polymerases are extremely accurate and catalyse DNA synthesis with error rates ranging from 10^{-4} to 10^{-7} per incorporated base. Recently the most widely used method measuring fidelity and “the molecular signature” of DNA polymerases is M13mp2 forward mutation assay developed by Kunkel. The method is an excellent molecular tool useful to characterize DNA polymerases; however, preparation of phage M13mp2 gapped DNA is technically tedious. We have developed novel gapped DNA substrate for the forward mutation assay which is easier to prepare and give us possibility for studying mutations introduced during synthesis of coding and non coding strand of the α -LacZ reporter. To generate our novel gapped DNA pUC18 was chosen and after two rounds of site-directed mutagenesis we have developed pSJ1 vector which contains the α -LacZ gene flanked with nicking enzymes sites NtBpu10I and NbBpu10I on coding and non-coding strand respectively. Specific nicking of the pSJ1 plasmid facilitate removal of one of the strands coding for the reporter, resulting in a gapped DNA substrate. The pSJ1 gapped DNA was successfully validated as a useful for measuring fidelity of DNA polymerases. We have chosen *Taq Pfu* and *T4* to test functionality of our method. The gathered data during the validation of pSJ1 forward mutation assays indicate the highest accuracy of *Pfu* polymerase. In summary we are convinced that using our pSJ1 gapped substrate improves forward mutation assay methodology.