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We attempted to increase the thermostability of Moloney murine leukemia virus (MMLV) reverse transcriptase (RT). The eight-site saturation mutagenesis libraries corresponding to Ala70−Arg469 in the whole MMLV RT (Thr24−Leu671), in each of which 1 out of 50 amino acid residues was replaced with other amino acid residue, were constructed. Seven-hundred and sixty eight MMLV RT clones were expressed using a cell-free protein expression system, and their thermostabilities were assessed by the temperature of thermal treatment at which they retained cDNA synthesis activity. One clone D200C was selected as the most thermostable variant. The highest temperature of thermal treatment at which D200C exhibited cDNA synthesis activity was 57ºC, which was higher than for WT (53ºC). Our results suggest that a combination of site saturation mutagenesis library and cell-free protein expression system might be useful for generation of thermostable MMLV RT in a short period of time for expression and selection.

Site saturation mutagenesis library of MMLV RT was constructed and expressed using cell-free protein expression system.

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