# Improvement of Xylanase Activity by Error-prone PCR

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Error-Prone PCR conducted on a weakly active chimeric xylanase gene APNc produced a mutation library from which mutant forms with enhanced xylanase activity were effectively identified. From 1766 mutant clones, a clone showing strong xylanase activity was selected. DNA sequencing of the clone showed that it had one amino acid substitution: $E^{127}\rightarrow V$. The E127V mutant enzyme and APNc chimeric enzyme were purified by affinity chromatography using Ni-NTA agarose. The E127V displayed thermal stability similar to APNc, up to 35℃ stable. The respective optimum pH and optimum temperature of the two enzymes were observed at pH 7.2, 45℃ for E127V, and at pH 7.2, 40℃ for APNc. The pH stability profiles showed that the two enzymes retained above 80% of their original activity between pH 3.7-8.7 for APNc, and between 5.1-10.0 for E127V. The kinetic parameters of the two enzymes were measured employing the *p*-nitrophenyl-β-D-xylobioside as the substrate. The $K_m$ values for E127V and APNc were 0.035 mM and 0.065 mM, respectively. The $K_{cat}$ value was increased by 10-fold in the case of the mutant E127V enzyme (0.270 $s^{-1}$) compared with that of the chmeric APNc enzyme (0.027 $s^{-1}$).