Abstract

Genomic *E.coli* DNA was prepared from a saturated culture by lysing the cells with SDS and proteinase K, precipitating protein fragments, polysaccharides and other cellular debris with CTAB, removing the CTAB complexes through extraction with mixed organic solvent, and precipitating the DNA with isopropanol. The genomic DNA was used as a template for PCR amplification of the *trp* B gene. The PCR product was purified using the QIAquick® protocol and ligated into a pGEM-T easy® vector containing an amp^R gene. The vector was pre-opened at the insertion site such that the *lac* Z' coding sequence was interrupted by incorporated insert. The vector/insert combination was used to transfect JM 109 competent *E. coli* cells. A colony of transfected cells containing the vector/insert combination (identified by its lack of blue color when grown on an agar plate containing ampicillin/IPTG/X-gal) was transferred to 5 mL of growth medium and grown to saturation. The vector/insert combination was purified from the saturated culture by the Promega Wizard® Plus SV Minipreps DNA Purification System protocol. The insert was excised using Eco R1 restriction endonuclease, analyzed by gel electrophoresis, and a sample was sent to Alphabiolabs for sequencing. The length of the insert (in base pairs) matched the length of the *trp* B gene. The insert sequence corresponded almost exactly to the *trp* B sequence from a reference *E. coli* strain, thus confirming the identity of the insert as *trp* B.