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Genomics 2019: Molecular Cloning and In Vitro Expression of a Selected Catalytic Domain of the Human Cathepsin K Gene (CtsK) in Top 10 Escherichia coli Expression System

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Cathepsin K, encoded by CtsK gene in human, is involved in bone remodeling through ossification. Objective of the work conducted here was to express catalytic domains of CtsK gene in bacterial expression system as an initial step, facilitating recombinant production of human Cathepsin K for downstream applications in pharmacology in Sri Lanka. Genomic DNA was extracted using FlexiGene® whole blood DNA extraction kit from four healthy blood samples. Upon quantification of DNA through NanodropTM spectrophotometer, sufficient quantity and quality was observed. CtsK gene was amplified by PCR using two pairs of primers tagged with restriction endonuclease sites of Sal1 and HindIII and visualized by Agarose Gel Electrophoresis (AGE). Two different bands of size 545bp and 265bp were observed. Bands were dissected and gel purified using GenaxxonTM gel purification kit and sequentially double digested by the above restriction enzymes. Vector PBS was also subjected to sequential double digestion using same enzymes and visualized via AGE. Double digested insert of size 265bp and vector were ligated using T4 DNA Ligase (all enzymes from PromegaTM). In addition, ligation of PCR product with band size 265bp to pGEM-TTM easy vector system (from PromegaTM) was also done. Recombinant PBS was transformed to Top10 Escherichia coli competent cells for expression separately. Cells were grown in LB media in presence of XGAL, IPTG and Ampicillin and transformed cells were screened. In restriction enzyme digestion and ligation, since insert and vector were both double digested, it is confirmed that white colonies obtained were transformed cells with desired recombinant vector and is confirmatory. In pGEM-TTM ligation, a colony PCR was done using white colonies obtained and product size was confirmed via AGE. In conclusion, objective was successfully achieved, by expressing selected catalytic domains of CtsK. Developments and improvements could be made for expression of entire gene in Sri Lanka.

The precious stone design of wild-type CTSK (PDB ID: 1BY8) and I249T freak (PDB ID: 5Z5O) without water and ligand was utilized for sub-atomic reproductions utilizing CHARMm power field with CFF incomplete charges in Discovery Studio variant 4.1 (Biovia, San Diego, CA, USA). The missing buildups in the area of ace/develop connection of I249T freak were produced by 'Construct and Edit Protein' module of Discovery Studio. Nuclear places of the particles were at first improved by 200 patterns of steepest plummet (SD) minimizer keeping the consonant limitation of the spine. The advanced protein structures were completely solvated with the TIP3P unequivocal water model and charges were killed by adding counter particles to the framework. The solvated structures were again limited with 200 cycles at first with symphonious limitations applied to spine particles and afterward with 1000 cycles permitting the spine loosened up utilizing form slope (CG) minimizer. The resultant designs of wild-type and I249T freak pro-CTSK were recreated for 12 ns of which creation run was directed for 10 ns in 2 fs time venture with an underlying warming from 50 to 300 K in 100 ps. Reenactment was acted in a NPT group at steady temperature (300 K) and consistent pressing factor (1 atm) utilizing Leapfrog Verlet strategy. Long-range electrostatics was treated with the particle-mesh Ewald technique and a 12-å cut-off was utilized for nonbonded short-range associations. Co-ordinates were saved in each 10 ps during creation run. Root mean square changes (RMSF) of the spine iotas (C, Cα and N particles) were processed utilizing the Analysis module of Discovery Studio form 4.1.

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