ISSN: 2659-2029
Proceedings of 2nd Research Symposium of the Faculty of Allied Health Sciences
University of Ruhuna, Galle, Sri Lanka
December 05, 2019

OP 16

Optimization of a Protocol for the Extraction of DNA from Human Blood and Isolation of the Human Gene *CtsK* Using Polymerase Chain Reaction (PCR) Amplification Techniques

Cooray R.[1#], Madubashetha H.[1,5], Wicramasinghe S.[2,5], De Silva N.[3], Warnakula L.[1,4]
[1]*Section of Genetics, Institute for Research and Development in Health and Social Care*
[2]*Department of Chemistry, Faculty of Science, University of Colombo*
[3]*Department of Nanotechnology, Faculty of Technology, Wayamba University of Sri Lanka*
[4]*National Science Foundation, Colombo, Sri Lanka*
[5]*Department of Biotechnology, Faculty of Science, Horizon Campus*

[#]***Corresponding author:*** *krncooray@live.co.uk*

Background: Cathepsin K, encoded by *CtsK* gene involves in bone remodeling through ossification. Besides this orthopaedic importance, it demonstrates other physiological importances including metastasis of prostate, ovarian, breast cancers and human growth regulation. Therefore, it is a timely concern that further molecular characterization be done on *CtsK* gene to facilitate further biomedical research and other aspects such as recombinant production of Cathespin K.

Objectives: To develop an optimized protocol for isolation of DNA from human blood and PCR amplification of a catalytic domain of *CtsK* gene.

Methodology: Genomic DNA was extracted from four human blood samples using FlexiGene®-QIAGen®, by subjecting blood to action of cell lysis, denaturation and resuspension buffers. Incubation time and number of 70% ethanol washings, volumes of isopropanol added were increased and absorbance of eluted DNA was measured spectrophotometricaly. PCR amplification of a catalytic domain of *CtsK* gene using forward primer 5'ACGCGTCGACGTGTACCATCAGTACCTCGCAC3' and reverse primer 5'ACGCAAGCTTCTTCCAAAGTGCATCGTTACAC3' was done. PCR conditions were optimized as; 94 ºC initial denaturation, 94 ºC denaturation, 55 ºC annealing, 72 ºC elongation, 72 ºC final elongation and 4 ºC final hold for 3 minutes, 30 seconds, 30 seconds, 40 seconds, 5 minutes and infinite, respectively.

Results: Extracted DNA showed a concentration of nearly 500 ng/µL which increased drastically upon addition of more isopropanol to better pellet out DNA. İn terms of purity, A_{260}/A_{280} ratio for DNA revealed to be between 1.7 and 1.8 while A_{260}/A_{230} revealed to be between 1.7 and 2.2, reflecting optimum purity. As a result of optimization of PCR conditions, expected band size, as a clearband of size 265bp (between 200bp-300bp) was generated in 1.5% TAE-agarose gel, which was verified to be a catalytic domain of Cathepsin K by previous literature.

Conclusions: An optimized protocol for extraction of quality DNA with high concentration was developed successfully and a catalytic domain of *CtsK* gene was successfully amplified.

Keywords: *Cathepsin K, CtsK, DNA Extraction, Human, PCR*

Acknowledgement: Funding from the Section of Genetics at the Institute for Research and Development in Health and Social Care, Sri Lanka, is acknowledged.