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Optimization of a Standard Molecular Protocol for Amplification of *CtsK* Gene Responsible for the Disease Pycnodysostosis: A Preliminary Study

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Incidence of bone fragility and related fractures due to genetic disorders indicate a high prevalence in Asia. Pycnodysostosis (PKND) is an autosomal recessive disorder caused by mutations in *CtsK* gene encoding for Cathepsin K. PKND causes bone fragility due to retarded ossification; hence precise screening or early detection is important. Overall aim of the proposed study is to develop a complete and precise gene based biomarker, to identify *CtsK* mutations contributing to PKND. This plot reports the startup work on one objective achieved, based on control optimization of a molecular protocol to amplify and isolate a selected region in exon 2 of *CtsK* from healthy blood samples. Human DNA was extracted using FlexiGene™ QIAGEN DNA extraction kit with additional cell lysis, washing and purification steps using cell lysis buffer, ethanol and isopropanol respectively. Nanodrop™ quantification indicated DNA of sufficient quantity (average 500ng/µl) and quality; A_{260}/A_{280}; 1.6-1.8 and A_{260}/A_{230}; 1.4-2.2. Polymerase Chain Reaction (PCR) was performed using known primers 5′CTCTGTTTCCCTGCCAAATG′3 and 5′CTCAGGTCTCAGCCTTCCTG′3 of concentration 10pmol/µL each with dNTP concentration each of 200µM using 1X FIREPol® Master Mix at conditions; initial denaturation; 95℃, 3 minutes, denaturation; 95℃, 30 seconds, annealing; 55℃, 30 seconds, elongation; 72℃, 40 seconds and final elongation; 72℃, 5 minutes. PCR amplicons were subjected to Agarose Gel Electrophoresis (1.7%, 40V; 3 hours). Results showed a clear single band of 220bp; validated by previously published work. In conclusion, the objective was achieved. Results imply that local infrastructure and expertise could be exploited to complete the proposed aim of the study.

Key words: *Bone health, Cathepsin K, CtsK, Fragility Bone disease, Pycnodysostosis*

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