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Simple and low cost method for amplification of GC rich segments by touchdown PCR

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Amplification of human genomic segments by PCR is a common technique widely used for research and or diagnostic purposes. Tissue specific genes that contain high GC segments are difficult to amplify by normal PCR. Generation of non-specific products and false negatives in PCR also hinders the specificity and intensity of the desired band. In the present study we report an improved method for successful amplification of a >70% GC rich 291 bp region consisting G1057D of Insulin receptor substrate 2 (IRS2), an intermediate in the downstream of insulin signaling pathway. Touchdown PCR was performed setting the annealing temperature below the melting temperature (Tm) of primers. Secondary structure formation during amplification was removed by introducing betaine as co-solvent in PCR mixture which increased band intensity and specificity. In conclusion, TD-PCR is a better, well known method applied to amplify GC rich segements. The method proposed by us is different in 1) Applying an initial annealing temperature below the Tm as performed in our study may also provide good results in contrast to the conventional method of using high annealing temperature 5^0c above to 5^0c below Tm in TD-PCR. 2) The amplicon generated by our method remains good enough which would also be useful for further applications. 3) less time taking 4) Economic, requiring lower volumes of reaction mixture and 5) Absence of spurious products that interfere with further steps of RFLP, DNA sequencing. The proposed method also has implication in amplifying other GC rich regions of human genome.

Biography

D D Pratyush is a senior research fellow and pursuing his PhD from Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University under the supervision of Prof.S K Singh.