

## **LPL Sequencing and Validation Protocol**

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## **Abstract**

The methods used for re-sequencing the full *LPL* gene in Kuwaiti Arabs and to analyse the sequence variation and identify variants that could attribute to variation in plasma lipid levels for further genetic association is described in details. Samples (n=100) of an Arab ethnic group from Kuwait were intially selected for the full sequencing of the 30 kb *LPL* gene locus and flanking regions by Sanger sequencing across the 30 Kb gene and its flanking sequences. A set of 76 primers were designed to cover the entire target sequence yielding on average between 500-700 bp PCR products inlcuding overlapping regions. The PCR products were then subjected to purification using nucleospin columns. Each sequence reaction was run in duplicates with one reaction including the forward primer and the other with the reverse primer for quality assurance. Sequence alignment was perfomed to compare the obtained sequences with the genbank reference sequence to screen and identify all varaints. A selected number of novel variants were selected for validation using realtime PCR.

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## **Protocol**