Optimization of reverse transcriptase PCR for selected hepatic cytokines in Wistar Rats

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Expression patterns of hepatic cytokines elucidates the immune and pathological pathways involved in inflammatory responses. Cytokine mRNA quantification is widely used approach in this regard that involves RNA extraction, cDNA synthesis and real-time polymerase chain reaction of selected targets. In the present study, we optimized the reverse transcriptase PCR conditions for selected hepatic cytokines; TNF alpha and IL 6 in Wistar Rats.

Liver tissues obtained from Wistar rats were washed with diethylpyrocarbonate (DEPC) treated water and frozen immediately in liquid nitrogen. Samples were stored at -80^{0}C. Total RNA was extracted from 0.1 g of liver tissue using Trizol$^@$ according to the manufacturer‘s instructions. Subsequently, cDNA was synthesized from 2000ng of RNA using random primers and M-MLV reverse transcriptase enzyme. PCR for target cytokines was carried out using newly synthesized cDNA based on following PCR conditions.

For TNF alpha, 5'-TTC TGT CTA CTG AAC TTG GGG GTG ATC GGT CC-3' and 5'-GTA TGA GAT AGC AAA TCG GCT GAC GGT GTG GG -3' were used as primers. PCR was optimized with initial denaturation at 94^{0}C for 1 and 30 sec followed by 35 cycles of 30 sec denaturation at 94^{0}C, 1 min annealing and 1 min extension at 72^{0}C. A temperature gradient of 53^{0}C, 55^{0}C and 57^{0}C was used for annealing step. Final extension was done at 72^{0}C for 3 min.

For IL 6, 5‘-TCC TAC CCC AAC TTC CAA TGC TC-3‘ and 5‘-TTG GAT GGT CTT GGT CCT TAG CC-3‘were used as primers. PCR was optimized with initial denaturation at 94^{0}C for 1 and 30 sec followed by 35 cycles of 30 sec denaturation at 94^{0}C, 1 min annealing and 1 min extension at 72^{0}C. A temperature gradient of 57^{0}C, 59^{0}C, and 61^{0}C was used for annealing step. Final extension was done at 72^{0}C 3 min.

Based on PCR products of TNF alpha and IL-6 separated by agarose gel electrophoresis, annealing temperatures for both genes were decided as 55^{0}C and 59^{0}C respectively.

Key Words: Rat hepatic cytokines, TNF alpha, IL 6, reverse transcription, Cytokine primers

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