

## 6. SUMMARY

Side-effects of siRNA were tested on cell lines after silencing the cyclooxygenase gene. The theoretical basis for this experiment was the observation that siRNAs may induce immune response. For this reason, IFN-induced genes expression was tested in non-influenced Hep2 cell line and three other Hep2 derivative cell lines in which the siRNA was inserted by different methods.

Cell lines were derived from Hep2 by using various methods for transfection of siRNA (i.e. nucleofection, Effectene technology and further cloning). RNA was isolated from these cell lines and cDNA was obtained by reverse transcription.

Also, dilution series ( $10^7$  to 1 copy number/reaction) were prepared from the plasmid which contained the tested sequence.

Gene expression was measured by QRT PCR and quantified as copy number/  $\mu\text{g}$  RNA. The degree of expression was compared to non-influenced Hep2 cell line. The housekeeping gene used was NUP. The results were evaluated using REST version 2 and Rotor Gene version 6 softwares.

Initially, nine IFN-induced genes, RNL, PKR, IRF1, IRF3, IFITM, IFIT1, OAS1, OAS2 and OAS3 were to be tested. Out of these, OAS1 and OAS2 were impossible to isolate and thus, impossible to measure. PKR expression was up-regulated, and all the other tested genes were down-regulated. There are many possible explanations for such result, but whatever the reason, much more extensive measurements are necessary.