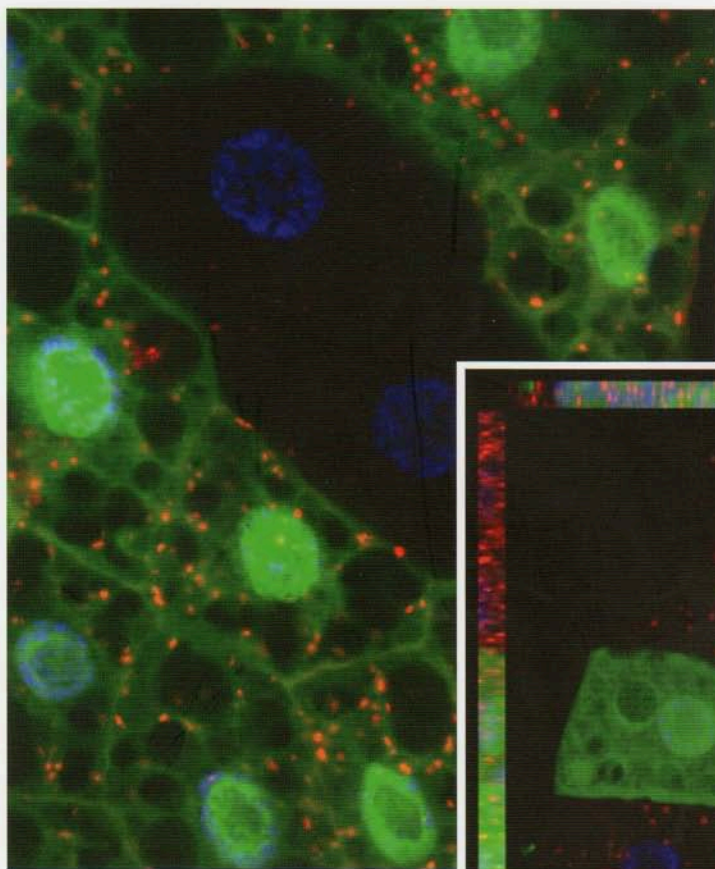
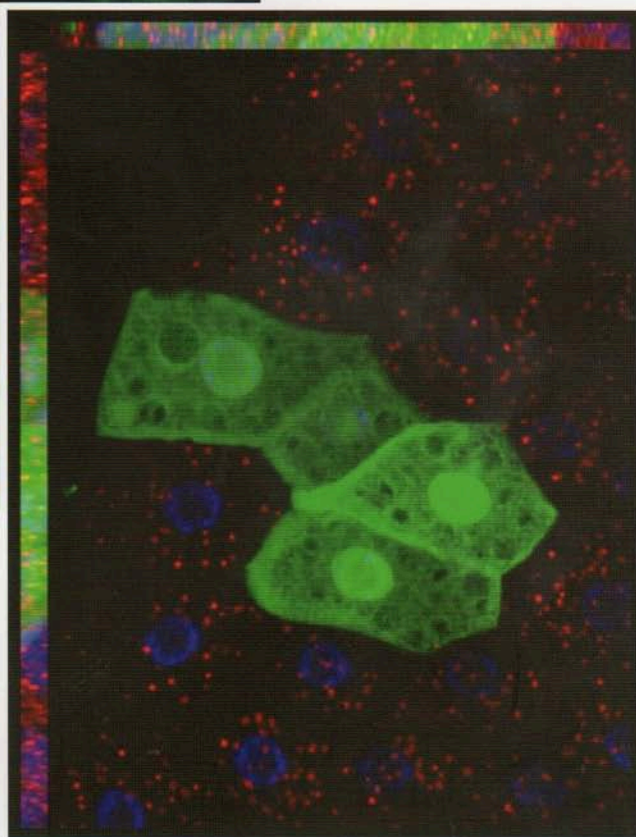


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VALIDATION OF RABBIT MICRORNA SEQUENCES IDENTIFIED BY SOLID SEQUENCING

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Over the past few decades microRNAs have emerged as important regulators of gene expression networks. Since then, numerous studies tried to identify microRNAs and analyse their function. It is proven, that they have important regulatory roles in proliferation, apoptosis, cell differentiation, and link to numerous types of cancer specific microRNA expression patterns are known.

MicroRNAs are 21-26 nucleotide long, non-coding, single stranded RNAs, which have an important role in the sensitive adjustment of basic functions by posttranscriptional gene regulation. They target sites are in the three prime untranslated regions (3' UTR) of messengerRNA (mRNA), where they repress the translation or cleavage the mRNA.



According to data of miRbase, more than 1000 human (*Homo sapiens*) and more than 800 mouse (*Mus musculus*) microRNAs are known, but not any yet in rabbit (*Oryctolagus cuniculus*). Numerous studies try to identify specific gene expression patterns of specific cell types, or cancers.

In this study we would like to test the hypothesis according to which the number of reads, what we get by Solid sequencing, could be used to estimate the gene expression level. Moreover, we would like to devise a method to compare the data of Solid sequencing with the results of real-time PCR analysis. With this object we searched known human and mouse microRNA sequences in sequenced mouse fibroblast, rabbit fibroblast and 13.5-day-old rabbit embryo. After that, we isolated RNA from mouse and rabbit fibroblasts, and rabbit 13.5-day-old embryo, than analysed it by real-time PCR to identify some of the searched microRNAs.

As a result of comparison, it could be established that the read numbers of Solid sequencing cannot be directly compared to the relative expression levels of real-time PCR analysis. Although in some cases we could estimate the order of magnitude, these are not really reliable, and we could not identify exact scales or expression patterns.

We suppose it could be possible to perform more exact comparison, if we used RNeasy Reagent for RNA isolation. It let us to isolate separately the microRNA fraction, and we could use cDNA for the real-time PCR from microRNA fraction. In this case it could be possible to compare each microRNA's expression to the all microRNA expression level. It could be done the same analysis with the read numbers of Solid sequencing, because we know the number of the total microRNA reads. So, we could make more exact comparison between the two methods.

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