Abstract

The progress on the field of RNA interference makes the sequence-specific knockdown of genes at larger scale feasible. Three pivotal methods are needed to conduct RNAi experiments: Knockdown of a specific gene (via siRNA or shRNA transfections), verification of knockdown (via reporter system or qPCR) and finally expression profiling for a selected knockdown (via microarrays).

The objective of this work was to establish the basic techniques for a silencing system and therefore focuses mainly on two points: firstly the transfection of 3T3L1 mouse fibroblasts with different methods, including on-slide reverse transfections and secondly readout with LUX™Primer-qPCR.

This work shows that 3T3L1 cells are hard to transfect with plasmids using standard transfection methods (cationic lipid, activated dendrimere, calcium phosphate coprecipitation) applied to adherent undifferentiated and differentiated cells.

Reverse transfections using gelatin-embedded plasmid DNA activated with Effectene® or TransMessenger™ on GAPS- or poly-L-lysine coated glass slides were successful. During the experiments the combination of TransMessenger™ Transfection Reagent and poly-L-lysine slides showed most stable results.

The qPCR was able to verify data obtained from a microarray experiment using RNA of 8 timepoints during a 3T3L1 fibroblast to adipocyte differentiation study. Although the use of LUX™Primers on the AbiPrism7000 realtime PCR machine was possible, the primers couldn't show their full potential on this specific machine.

Keywords: fibroblast, transfection, qPCR, reverse transfection, microarray