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INHIBITION OF REPORTER GENES BY SMALL INTERFERING RNAS IN CELL CULTURE AND LIVING FISH

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RNA interference is a mechanism for silencing specific genes. It has been applied in cell culture to inhibit expression of genes involved in disease including viral genes as recently shown for the fish pathogenic rhabdovirus viral haemorrhagic septicaemia virus or VHSV (Bohle et al., 2011). But evidence of specific siRNA inhibition in living fish is still needed. Using the small interfering RNAs (siRNAs), messenger RNA (mRNA) can be targeted resulting in degradation of targeted transcript or translational repression. Reporter genes such as luciferase and green fluorescence protein (GFP) can be used to observe the knock down effect by siRNAs designed to target these reporters. One aim of this project is to verify the specific knock down effect of siRNAs in cell culture and in living fish and to establish easy-read out models for testing the effect especially *in vivo*. Cell culture from human embryonic kidney HEK293t cells was used because they are easy to transfect and generally show high expression of transfected genes. Two types of fish including albino trouts were used as animal models to get better visualization of reporter gene expression. The luciferase gene was used as reporter gene as it provides low background compared to other reporter genes such as green fluorescence protein (GFP). In cell culture, the luciferase can be used as reporter gene to see the effect of gene silencing. In the living fish, the bioluminescence signal detected is influenced by the melanin pigment. Timing between coinjection and the assay is important in order to detect knock down by siRNA. Our experiment reveal *in vivo* knock down at 72 hours post injection of reporter gene and siRNA, but further dose-response experiments are required to confirm specificity.

Bohle, H., Lorenzen, N. & Schyth, B. D. 2011. Species specific inhibition of viral replication using dicer substrate siRNAs (DsiRNAs) targeting the viral nucleoprotein of the fish pathogenic rhabdovirus viral haemorrhagic septicaemia virus (VHSV). *Antiviral Research*, 90(3): 187-194.