

Application of 3'-UTR Reporter Clones for miRNA Target Validation

A case study with mir205

Mir205 expression in HEK293T cell and down-regulation of GFP-mir205 target

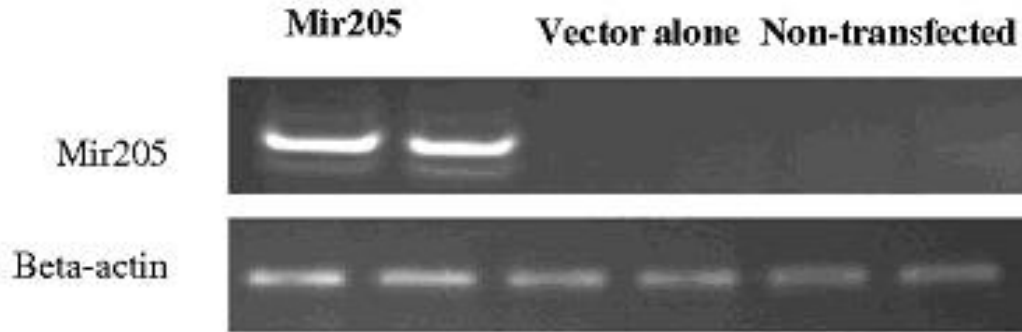


Figure 1. Overexpression of mir205 in HEK293 cells (RT-PCR amplification assessed by agarose gel electrophoresis).

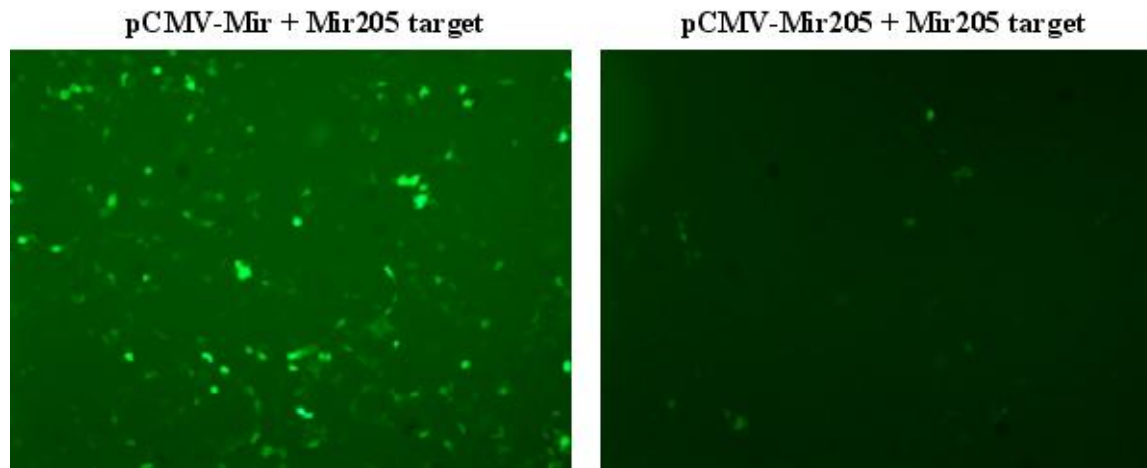


Figure 2. Mir205 down-regulated GFP expression when the GFP transcript was fused to the mir205 target sequence

Interaction of Luciferase-3'UTR reporters with over-expressed Mir205 in HEK293T cells.

Table of experiment set-ups

Transfection	Experiment	Control
pMirTarget-3UTR	+	+
Mir205 in expression vector	+	-
pCMVMIR Empty Vector)	-	+

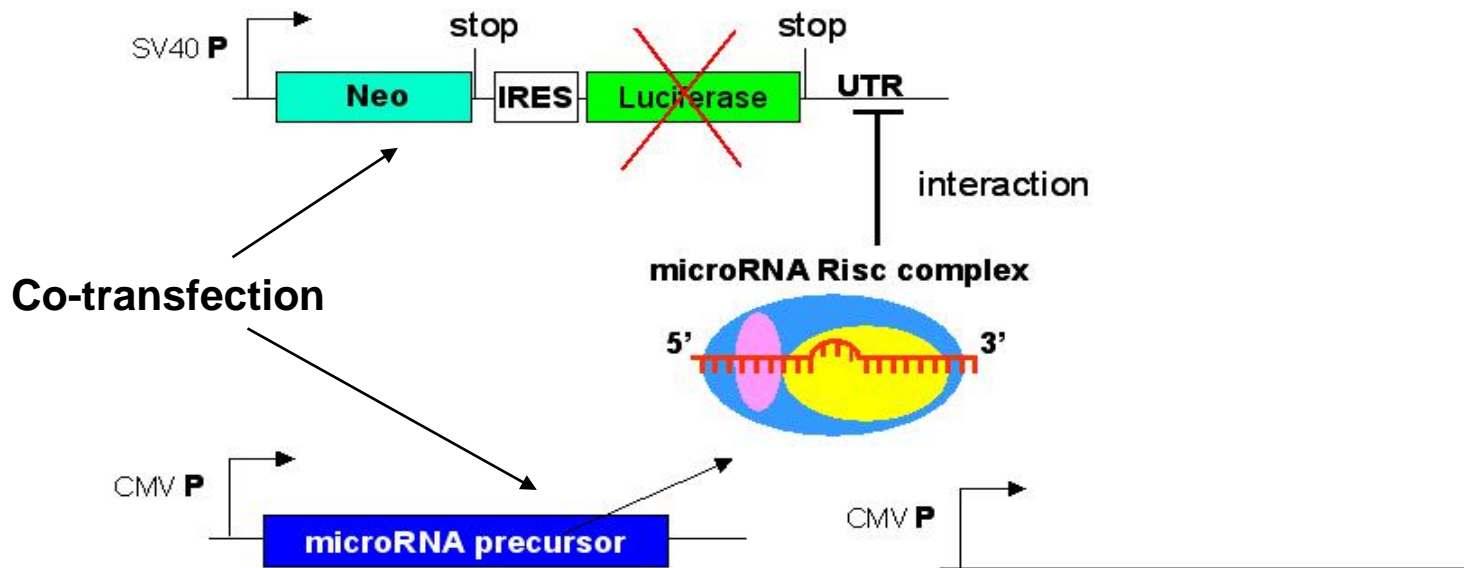


Diagram of pMir-Target 3'UTR clone system

Validation with seeding sequence mutants of 3'UTR reporter clones

Table of experiment set-ups

Transfections	Control	Exp1	Exp2
pMir-Target-3UTR	+	+	
pMir-Target-3UTR mutant			+
Mir205 in pCMVMir		+	+
PCMVMir(Empty Vector)	+		

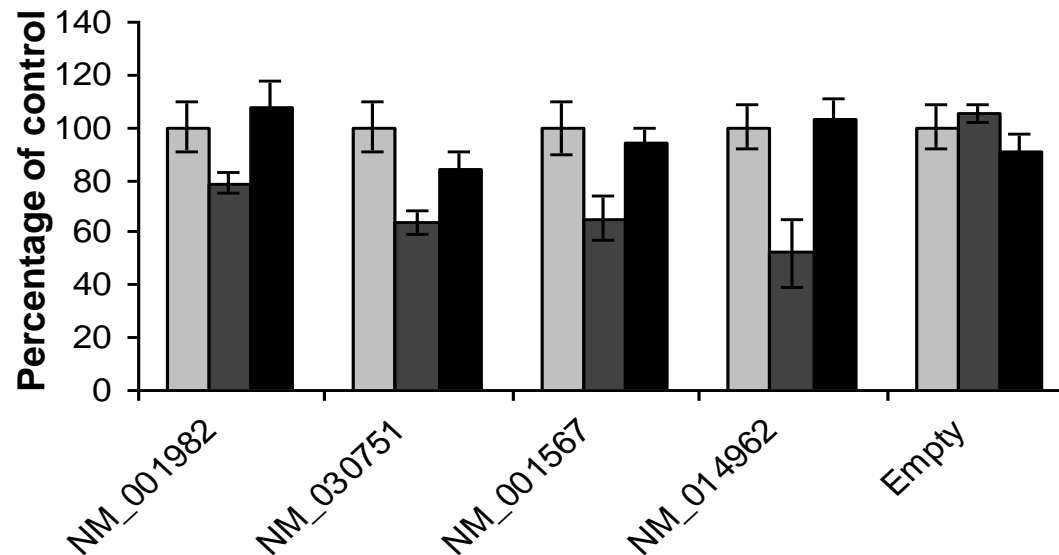


Figure 4. Abolishment of Mir205 effects by mutated seeding sequences in the 3'UTR-luciferase reporter clones (grey bar, control with 3' UTR reporter clones; darker grey bar, mir205 with 3' UTR reporter clones; black bar, mir205 with mutated 3' UTR reporter clones.)

Dose dependence of the interaction between target sequence and Mir205

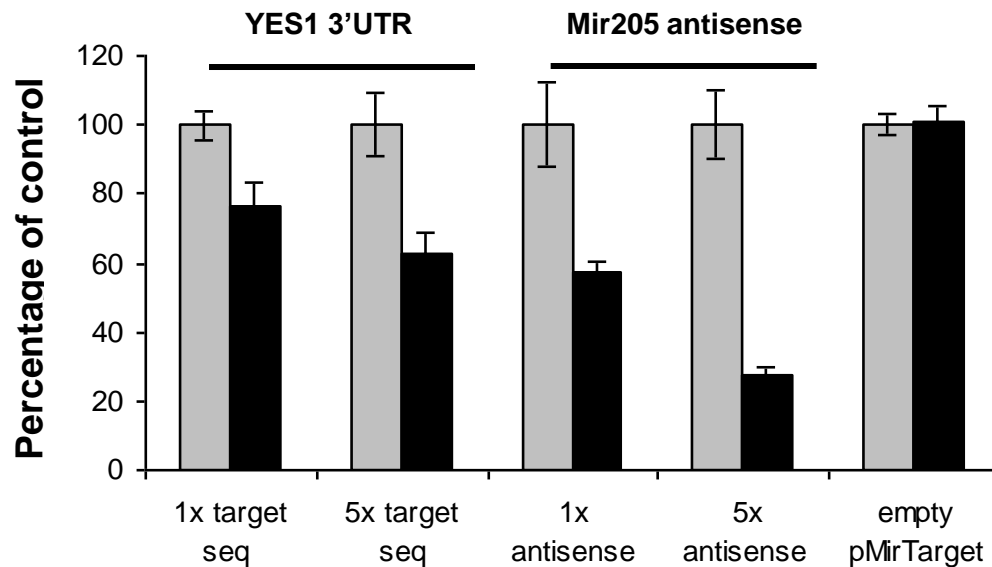


Figure 5. Enhanced interaction between mir205 and its targets by tandem repeats of targeting sequences (grey bar, empty pCMVMir with reporter clones; black bar, mir205 with reporter clones.)