A genome-wide 3´UTR collection for high-throughput functional screening of miRNA targets

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INTRODUCTION

MicroRNAs (miRNAs) are important regulators of gene expression and have been shown to play a role in numerous biological processes such as cellular signaling, cell differentiation, growth, development, and apoptosis. To enable researchers to gain a better understanding of miRNA and 3´UTR interactions, we have created a genome-wide collection of 12,000 3´UTR luciferase reporters along with synthetic miRNA biosensor reporter vectors. The 3´UTR reporter vectors use a novel and highly optimized luciferase gene and assay reagent specifically for gene regulation studies, the LightSwitch™ Assay System. This collection can be used to validate miRNA targets identified by prediction algorithms or other experimental methodologies. In this study, we performed a screen of a large set of predicted miR-122 targets and identified several novel targets. A selection of these targets was further tested for reproducibility and specificity using RT-PCR, dose responsiveness and site-directed mutagenesis of the seed sequence. This miR-122 case study demonstrates that the 3´UTR luciferase reporter collection represents a sensitive, specific and economical means to probe miRNA-UTR interactions and functionally validate miRNA targets.

The LightSwitch™ System for miRNA target screens

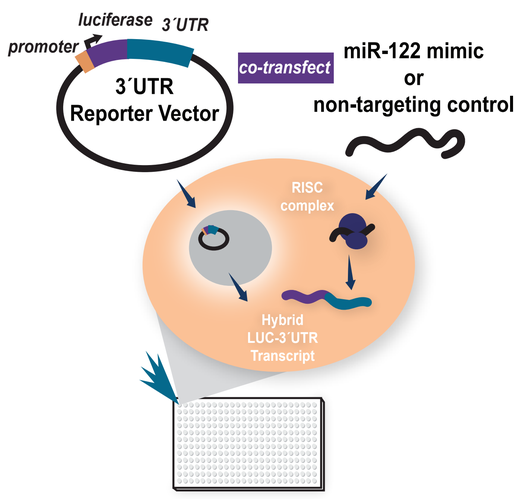


Figure 1. Experimental design for miR-122 functional screen using the LightSwitch™ System. Cells are co-transcfected with an individual 3´UTR reporter vector and either a miR-122 mimic or a non-targeting control using DharmaFECT Duo. Luciferase reagent is added to cells 24-48 hours after transfection and the plate incubated for 30 minutes in the dark before being read on a plate luminometer. Knockdown of activity is calulated as the ratio of the luciferase signal of the mimic over non-targeting control.

$$\frac{\text{Light output with miR-122 mimic}}{\text{Light output with control}} = \text{Effect of miR-122 on target 3´UTR}$$

RenSP - a superior *Renilla* luciferase

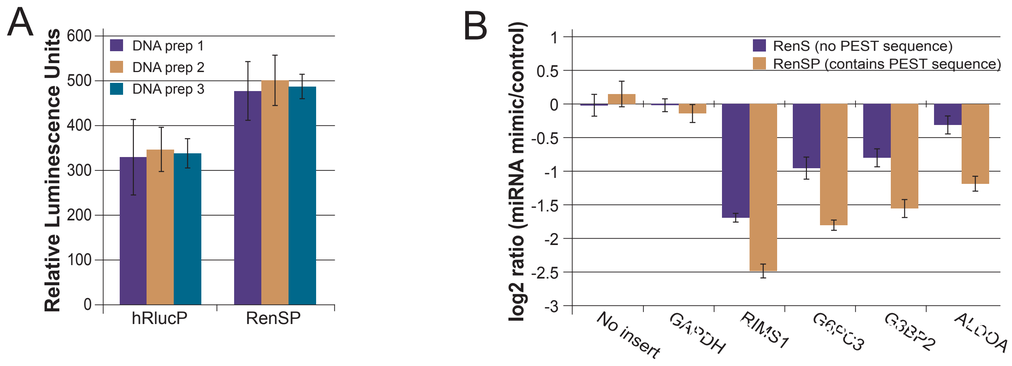
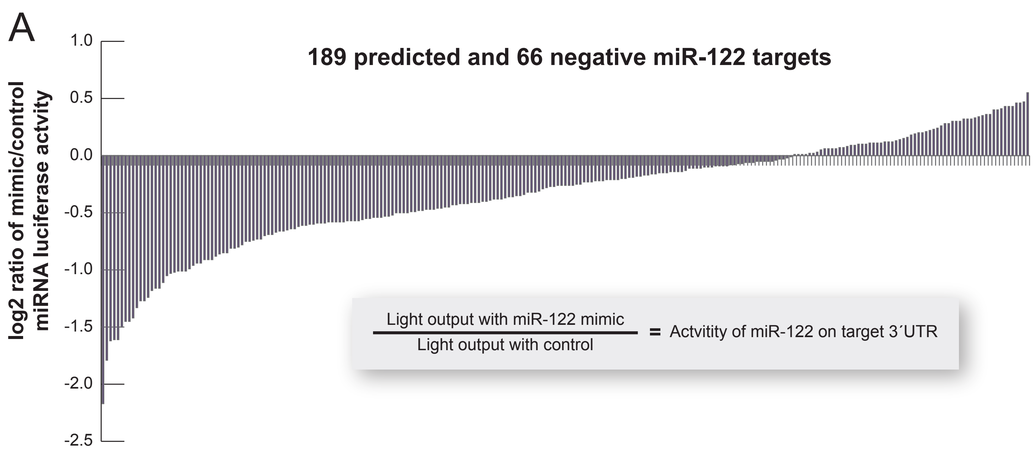


Figure 2. RenSP exhibits increased enzymatic activity and brightness. We have created an optimized *Renilla* luminescent reporter gene by increasing its enzymatic activity and adding a protein destabilization domain. The increased signal and short half-life provides a sensitive measure of the induction or repression of gene activity. **A.** The absolute signal of RenSP is significantly brighter than hRlucP. **B.** RenSP with PEST increases the knock-down of 3´UTR targets in the presence of miR-122.

miRNA Mimics & Inhibitors to study miRNA function



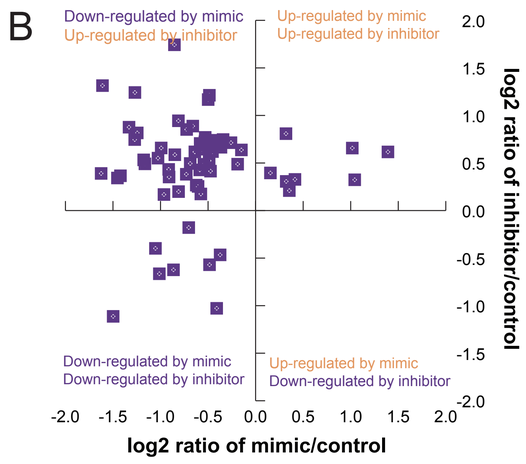
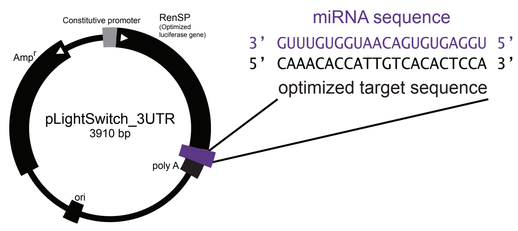


Figure 3. A miR-122 target screen confirms the utility of mimics and inhibitors in studying miRNA function. LightSwitch miRNA mimics and inhibitors can be used to study the over-expression and loss of function of a particular miRNA, respectively. **A.** To determine the utility of miRNA mimics and inhibitors in miRNA target screens we performed a miR-122 screen of 255 3´UTR reporters in Huh-7 cells. Interestingly, putative targets identified by microarray expression validated at a higher rate than computationally predicted targets. **B.** 3´UTR-luciferase activity agrees strongly between miRNA mimics and inhibitors, supporting the use of these chemically optimized RNAs to alter miRNA abundance and study their function.

Synthetic miRNA reporters serve as Biosensors



Reporter vectors with synthetic target sequences for miRNAs based on miRBase 16

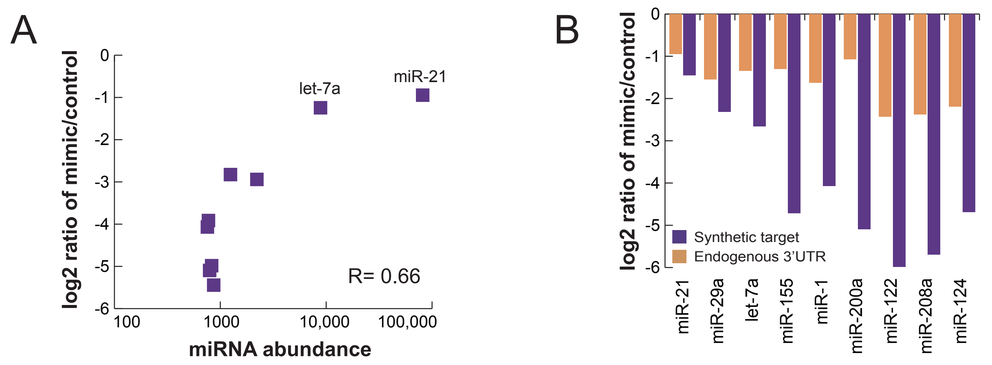
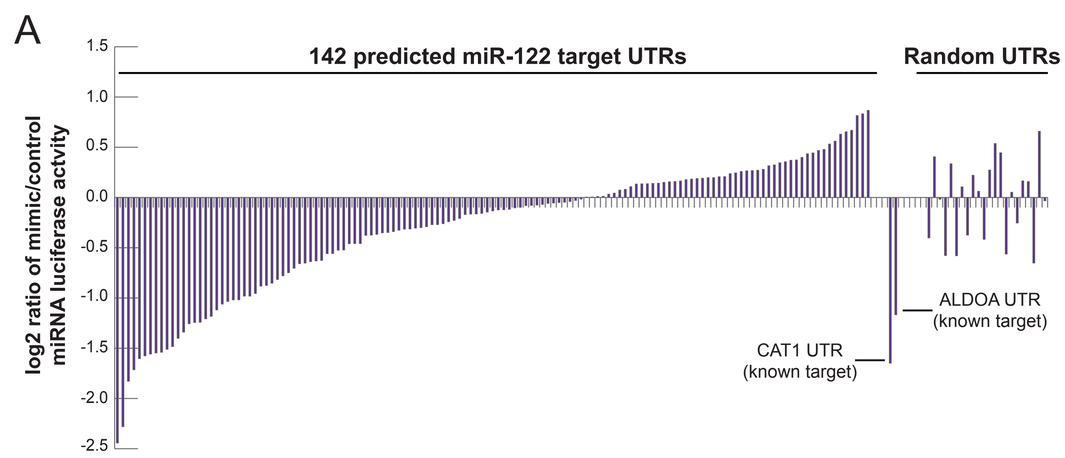
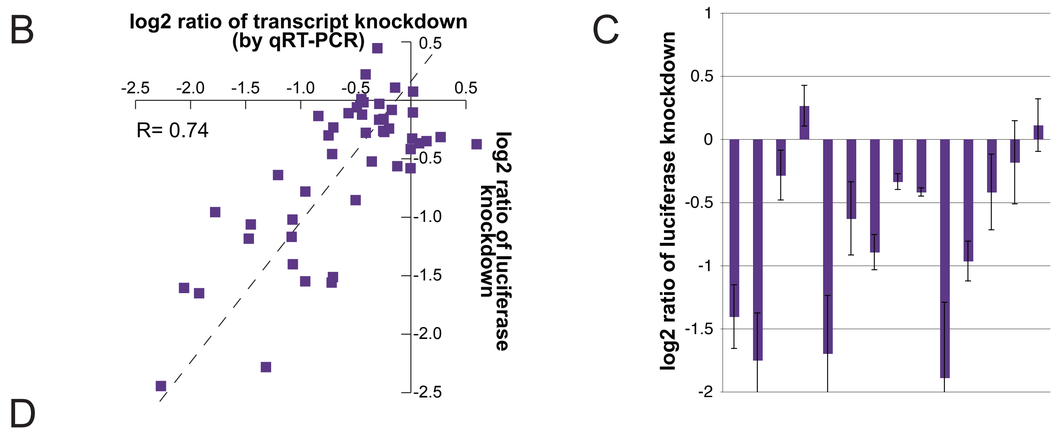
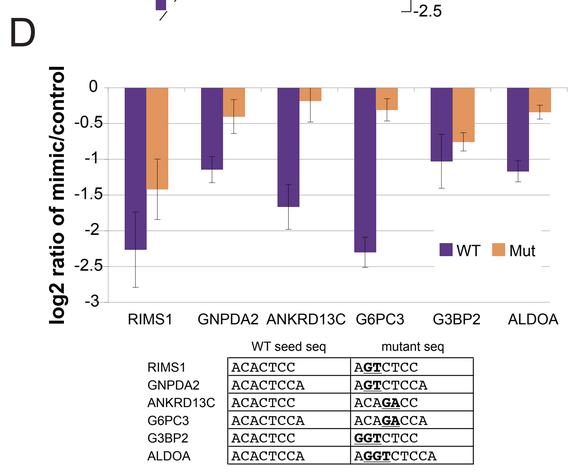


Figure 4. Synthetic miRNA reporter vectors as biosensors for miRNA studies. We have created a selection of reporter vectors with synthetic target sequences for 918 miRNAs based on miRNA sequence information from miRBase 16. **A.** Knockdown of synthetic miRNA targets correlates with endogenous miRNA levels in HeLa cells. **B.** Synthetic miRNA targets respond more strongly than endogenous 3´UTRs.

A genome-wide screen for novel miR-122 targets







	WT seed seq	mutant seq
RIMS1	ACACTCC	AGTCTCC
GNPDA2	ACACTCCA	AGTCTCCA
ANKRD13C	ACACTCC	ACAGACC
G6PC3	ACACTCCA	ACAGACCA
G3BP2	ACACTCC	GGTCTCC
ALDOA	ACACTCCA	AGGTCTCCA

Correlation with Prediction Algorithms

miRNA Target Prediction Algorithm	Correlation with knockdown (R)
TargetScan	0.29
Miranda	0.24
TargetScan conserved	0.14
Pictar	0.11

miR-122 Dose Response Data

Gene	EC50 (nM)
RIMS1	0.08
CHST12	0.80
G6PC3	0.50
CAT1	1.44

Figure 5. A high-throughput miR-122 target screen identitifed and validated novel miR-122 specific targets. A. We performed a functional screen of 142 human 3´UTRs with predicted miR-122 sites in HT-1080 cells. 44 of the targets decreased significantly by t-test ($p<0.05$) and 24 were significantly repressed (>2-fold). **B.** 3´UTR luciferase activity is highly correlated with endogenous transcript levels as measured by RT-PCR. **C.**11/14 miR-122 targets identified by proteomics were validated by luciferase. Further, out of the tested miRNA target prediction algorithms TargeScan exhibited the highest correlation with R=0.29. **D.** Mutagenesis of 2-3 bases of the miR-122 seed sequence disrupted miR-122 function in all six selected targets (shown to be highly repressed). Furthermore, when subjecting these targets to miR-122 mimic concentrations ranging from 0.0625 to 100nM they responded in a dose-dependent fashion, with EC50 at or below 1.5 nM.

CONCLUSIONS

- The LightSwitch System demonstrates optimal performance when measuring changes in activity attributable to miRNA mimics or inhibitors
- A genome-wide miRNA target screen identified 44 novel 3´UTRs responding specifically to miR-122
- The observed luciferase levels was shown to correlate with both endogenous transcript and protein levels
- Mutagenesis experiments verified the miRNA/reporter interactions and identified seed sites that are necessary for miR-122 function

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