

# High-throughput microRNA Target Screening: miR-122 Case Study

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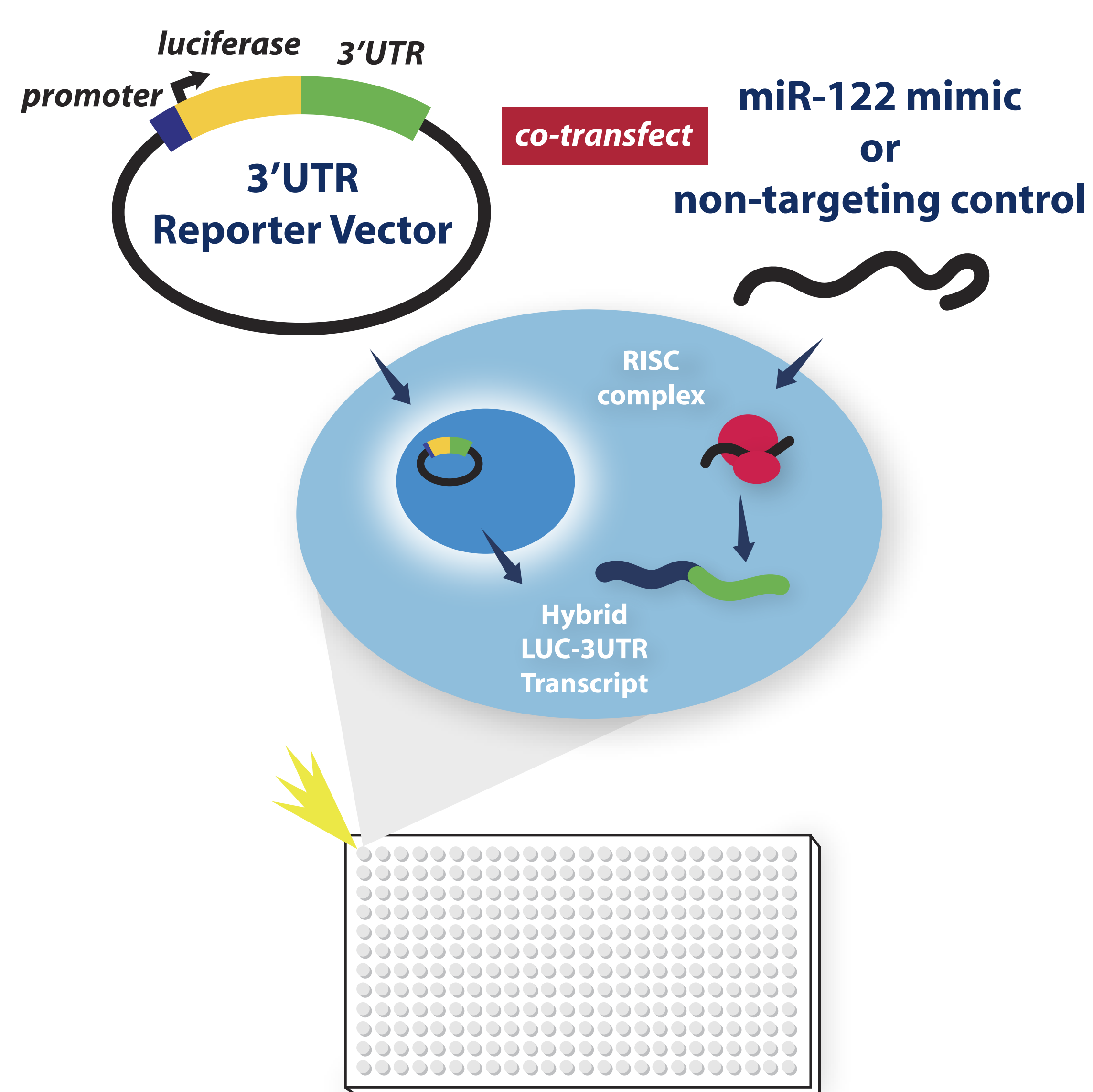
## INTRODUCTION

MicroRNAs are important regulators of gene expression. Computational predictions of miRNA-UTR interactions represent an important starting point for experimental analysis, however there is little functional data on which to train prediction algorithms. Similarly, genome-wide expression analysis identifies candidate target transcripts but cannot identify genes that translationally repressed. We have created a genome-wide library of human 3'UTR-luciferase reporter constructs to enable researchers to screen thousands of potential miRNA targets in a single experiment. Using this high throughput strategy, we sought to identify new targets of miR-122, an important regulator in liver cells and the cholesterol biosynthesis pathway.

### GOALS

- ▶ Measure the functional effects of miR-122 on putative target UTRs
- ▶ Characterize dose response patterns of miR-122 targets
- ▶ Identify necessary seed sites by site-directed mutagenesis
- ▶ Compare luciferase knockdown to endogenous transcript levels by qRT-PCR

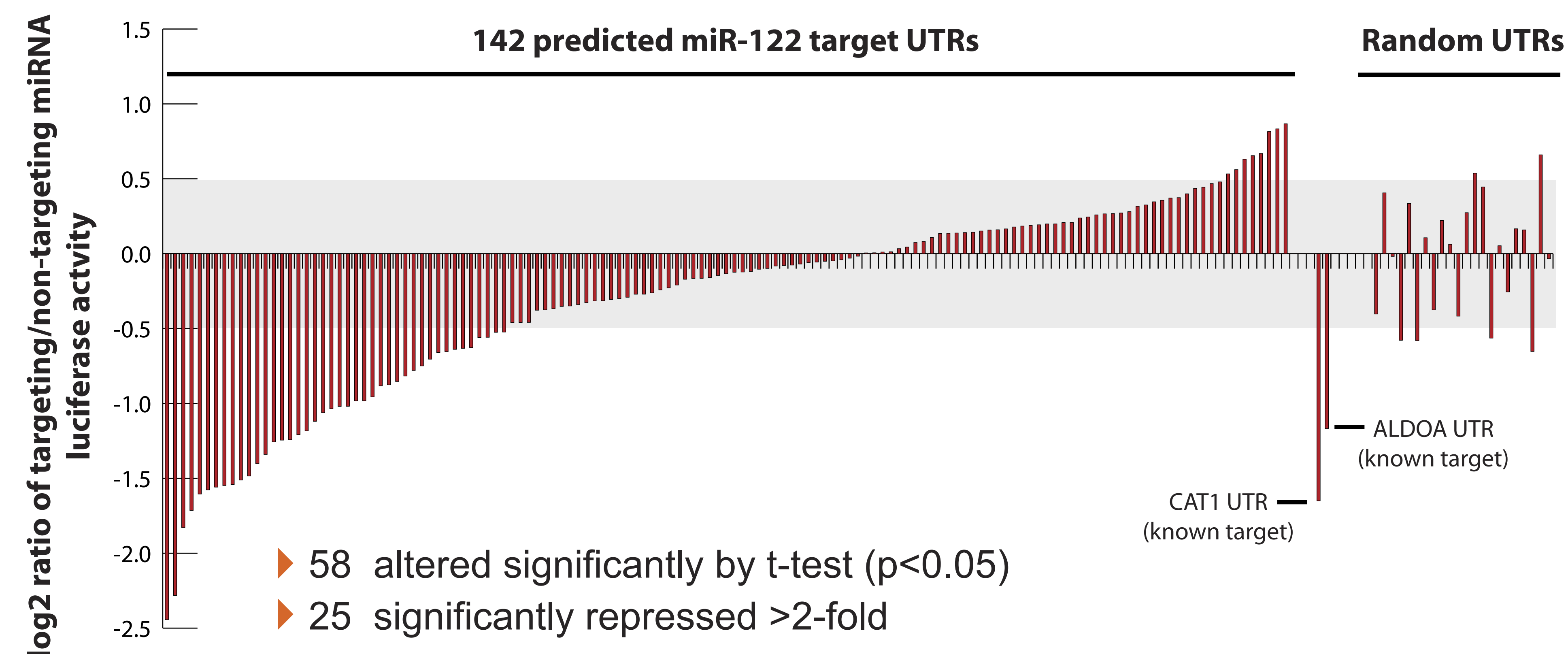
## EXPERIMENTAL DESIGN



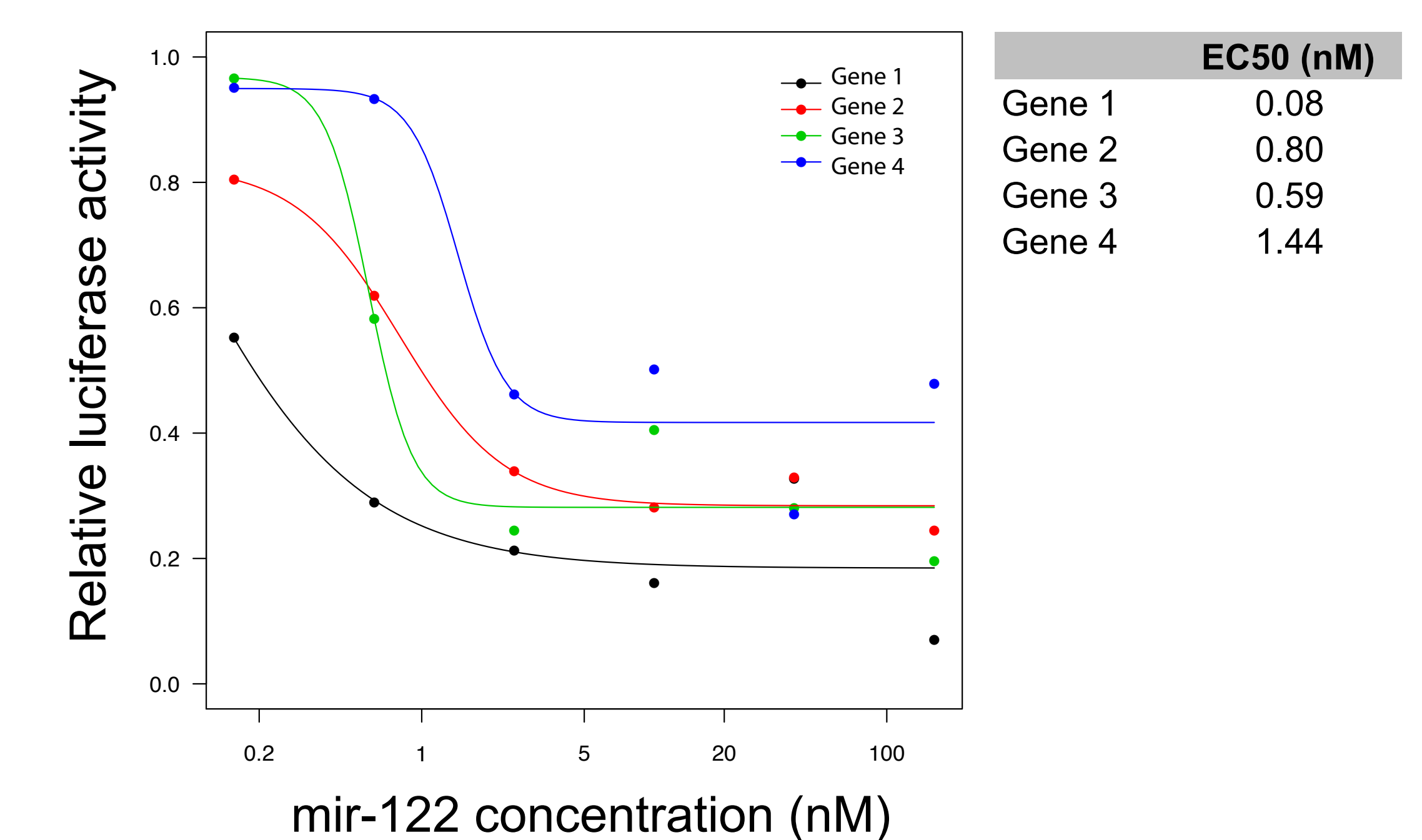
**miR-122 3'UTR panel**  
188 human 3'UTRs with predicted miR-122 sites and controls

## RESULTS

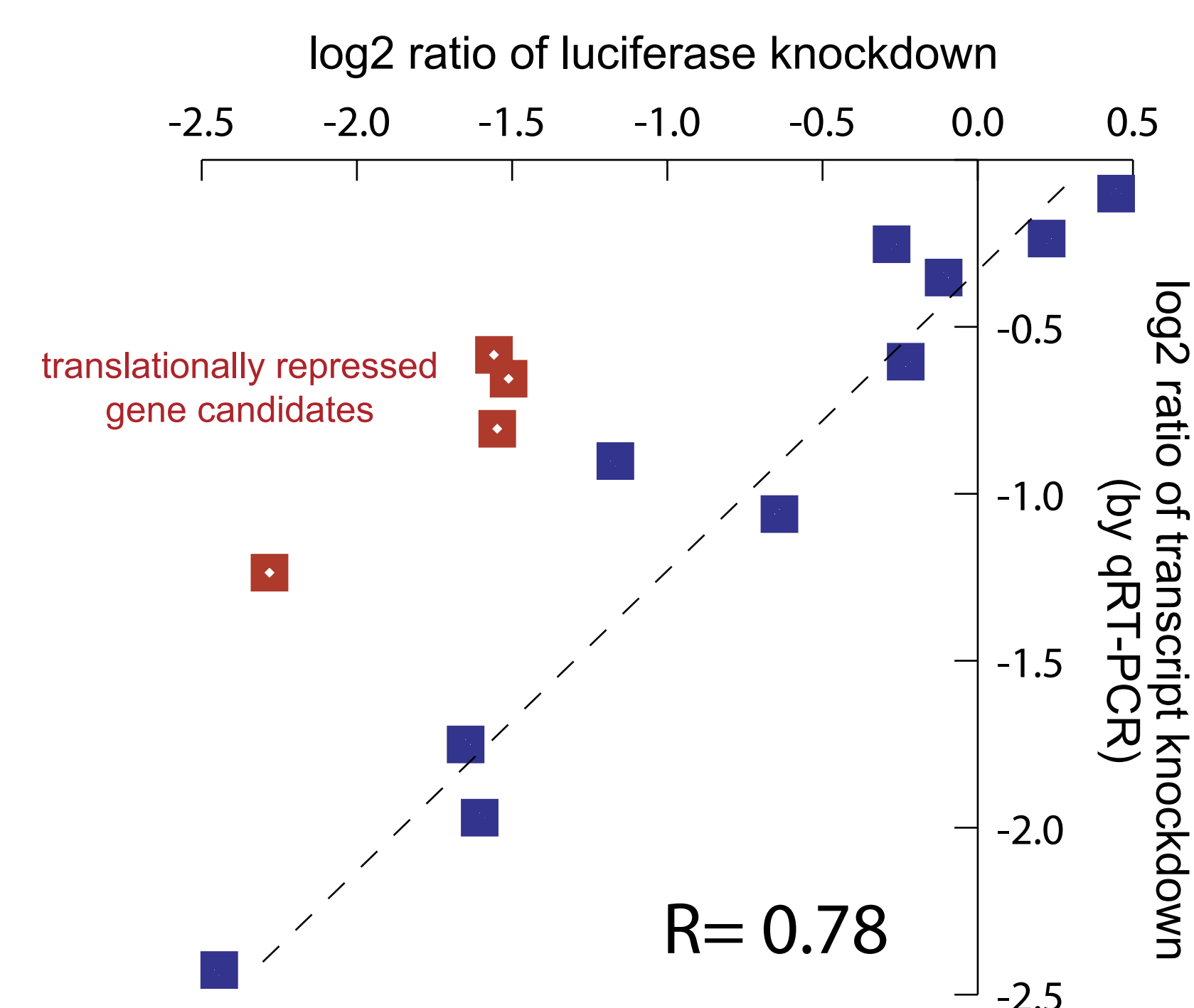
$$\frac{\text{Light output with miR-122 mimic}}{\text{Light output with control}} = \text{Activity of miR-122 on target 3'UTR}$$



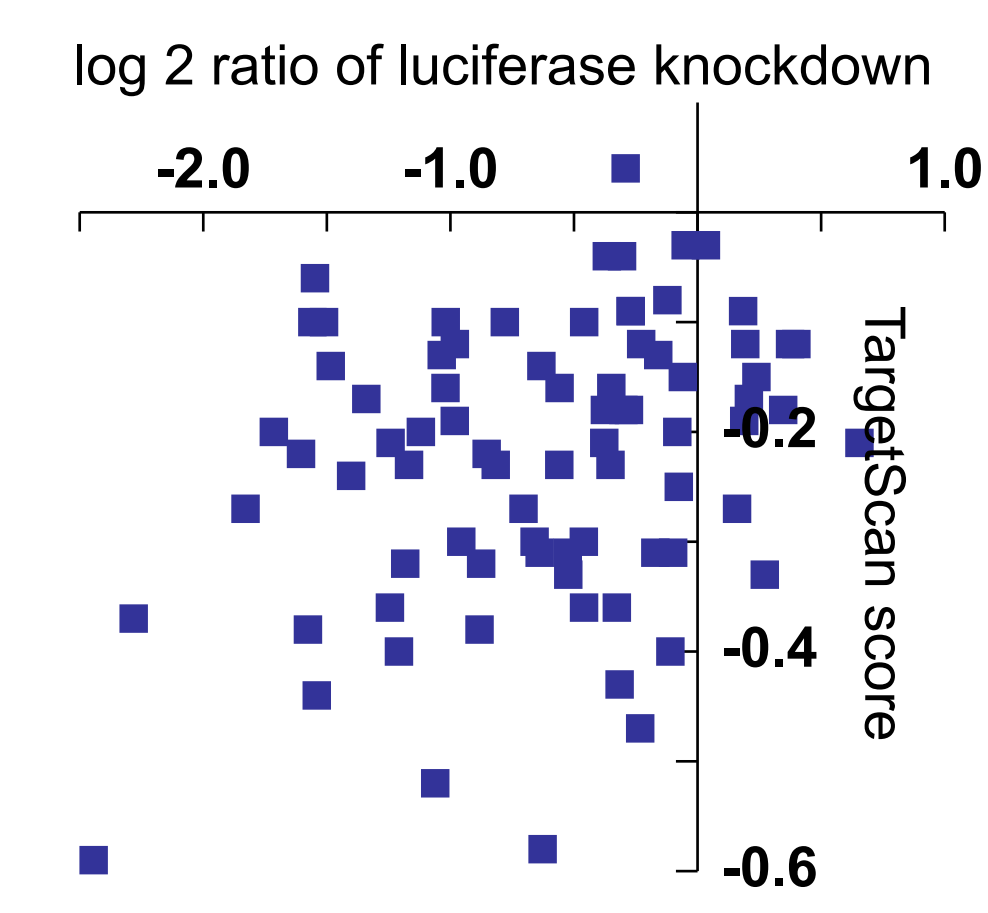
### miR-122 dose response curve



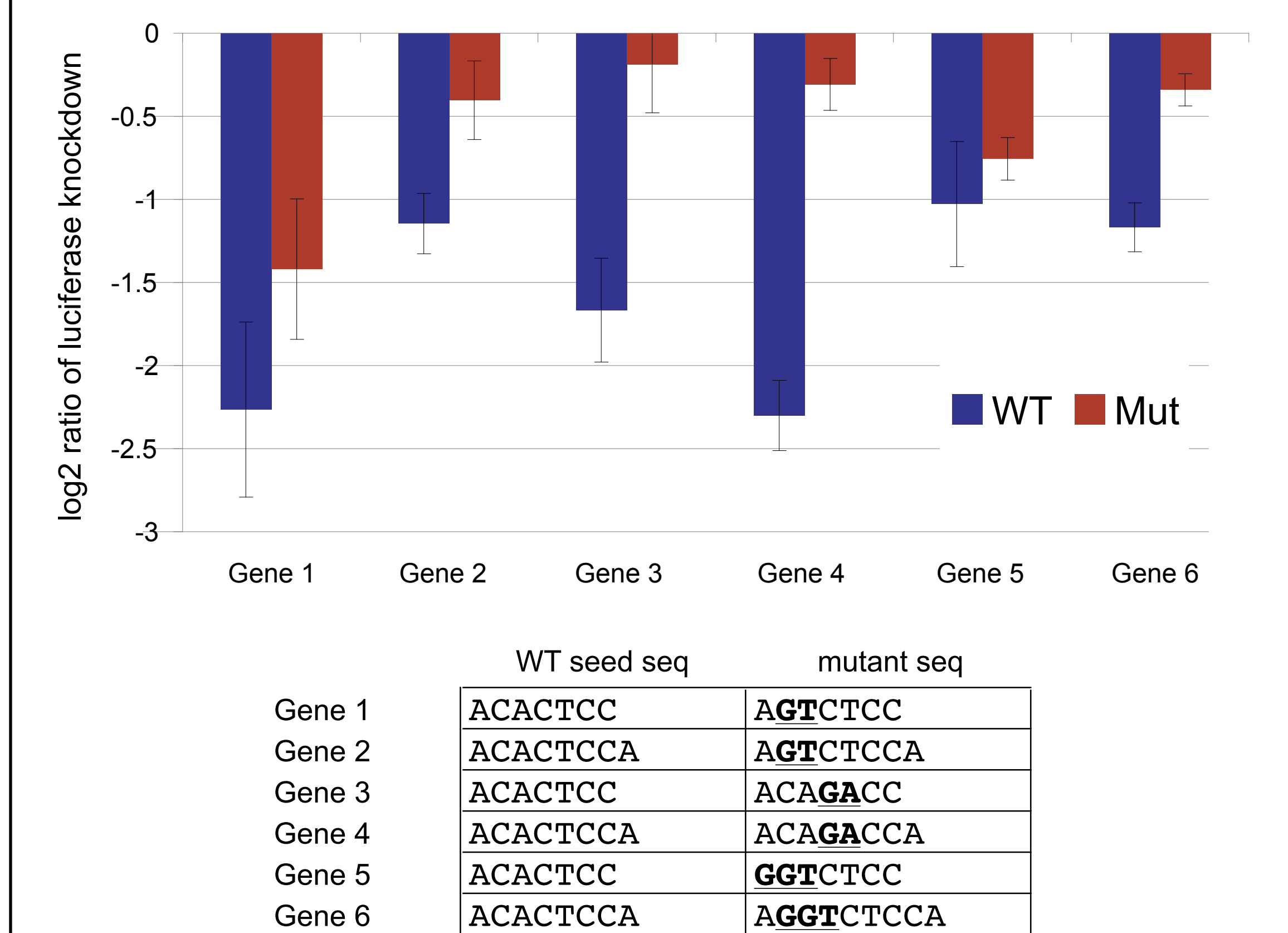
### 3'UTR-luciferase activity is highly correlated with endogenous transcript levels



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



### Mutagenesis of seed sequence disrupts miR-122 function



## CONCLUSIONS

- ▶ Identified 58 UTRs that respond specifically to miR-122
- ▶ Identified 4 genes that may be translationally repressed by miR-122
- ▶ Mutagenesis experiments identified seed sites that are necessary for miR-122 function
- ▶ UTR targets show a variety of dose response patterns to miR-122 concentration

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