

Genome-wide collection of 3' UTR reporters for functional screening of miRNA targets

Patrick Collins, Michael Rose, Shelley Force Aldred, Nathan Trinklein
SwitchGear Genomics, Menlo Park, CA

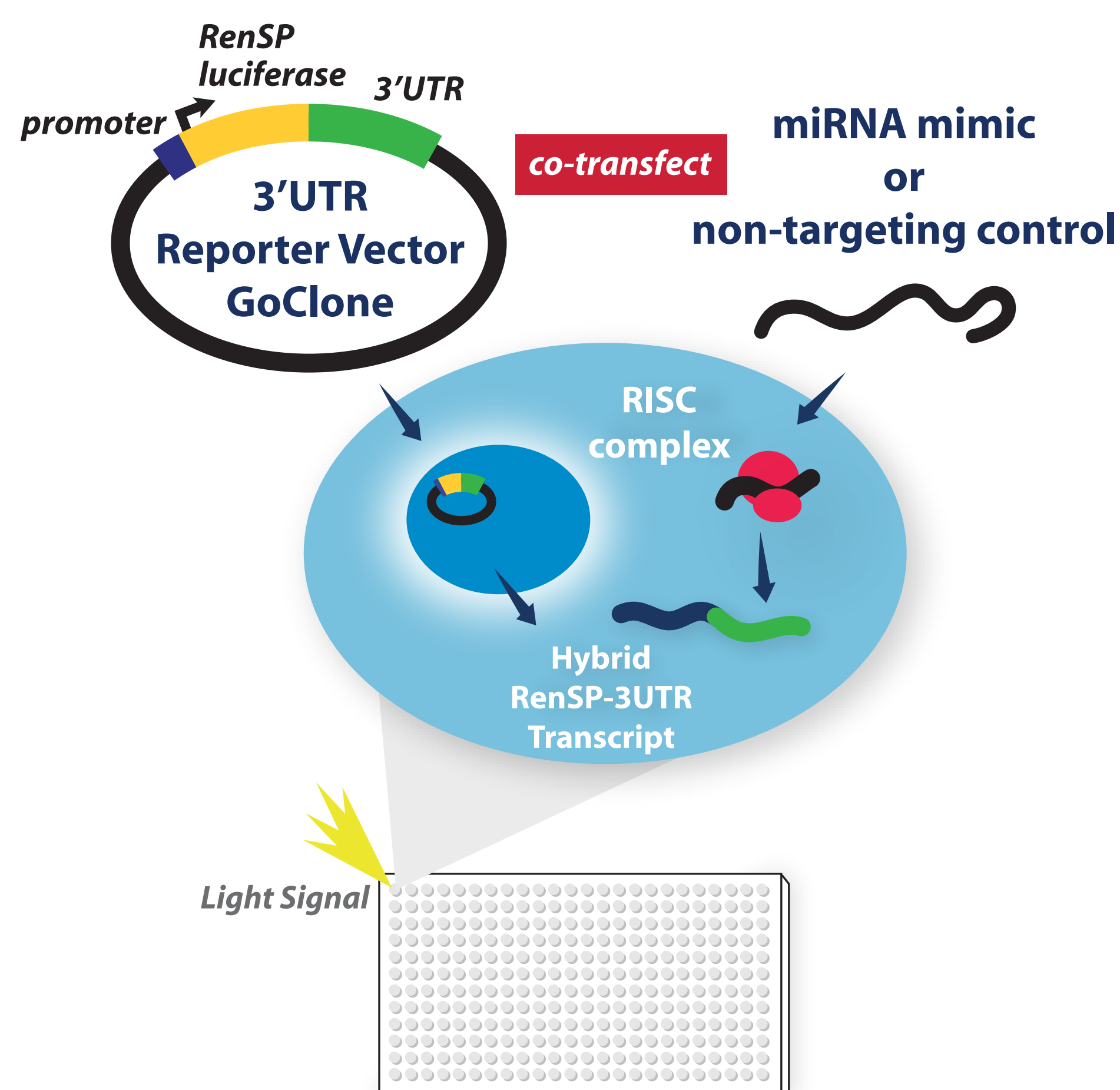
INTRODUCTION

MicroRNAs are important regulators of gene expression involved in both normal and pathological processes. To better understand miRNA-UTR interactions, we have created a genome-wide collection of 12,000 3' UTR luciferase reporters. This collection can be used to validate miRNA targets identified by prediction algorithms or other experimental methodologies. We performed a screen of a large set of predicted miR-122 targets using 3' UTR luciferase GoClone reporters and identified several novel targets. Several of these targets were tested for reproducibility and specificity using RT-PCR, dose responsiveness and site-directed mutagenesis. The results demonstrate that the 3' UTR luciferase reporter collection represents a sensitive, specific and economical means to probe miRNA-UTR interactions.

GOALS

- ▶ Identify functional targets of miR-122
- ▶ Compare luciferase data with endogenous transcript and protein levels
- ▶ Characterize dose responsiveness of luciferase reporters
- ▶ Verify specificity of miRNA/reporter interaction

EXPERIMENTAL DESIGN



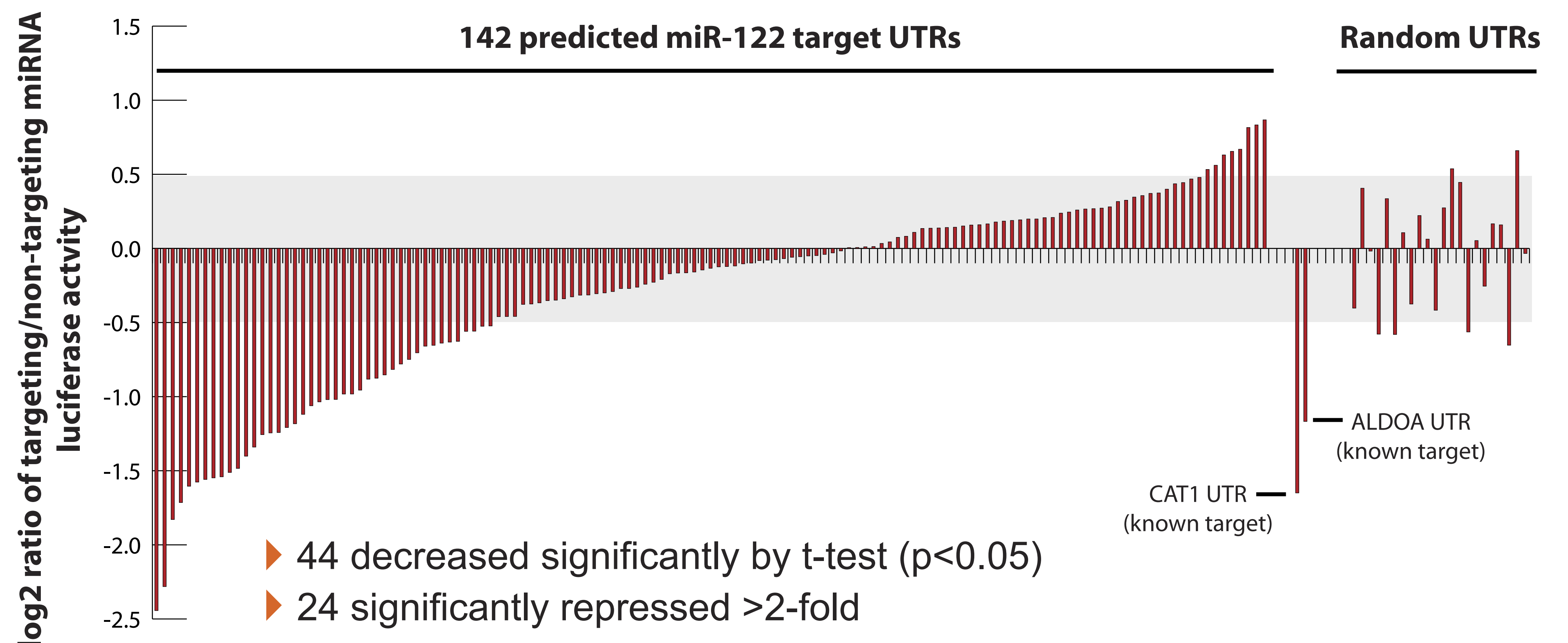
<http://www.switchdb.com>



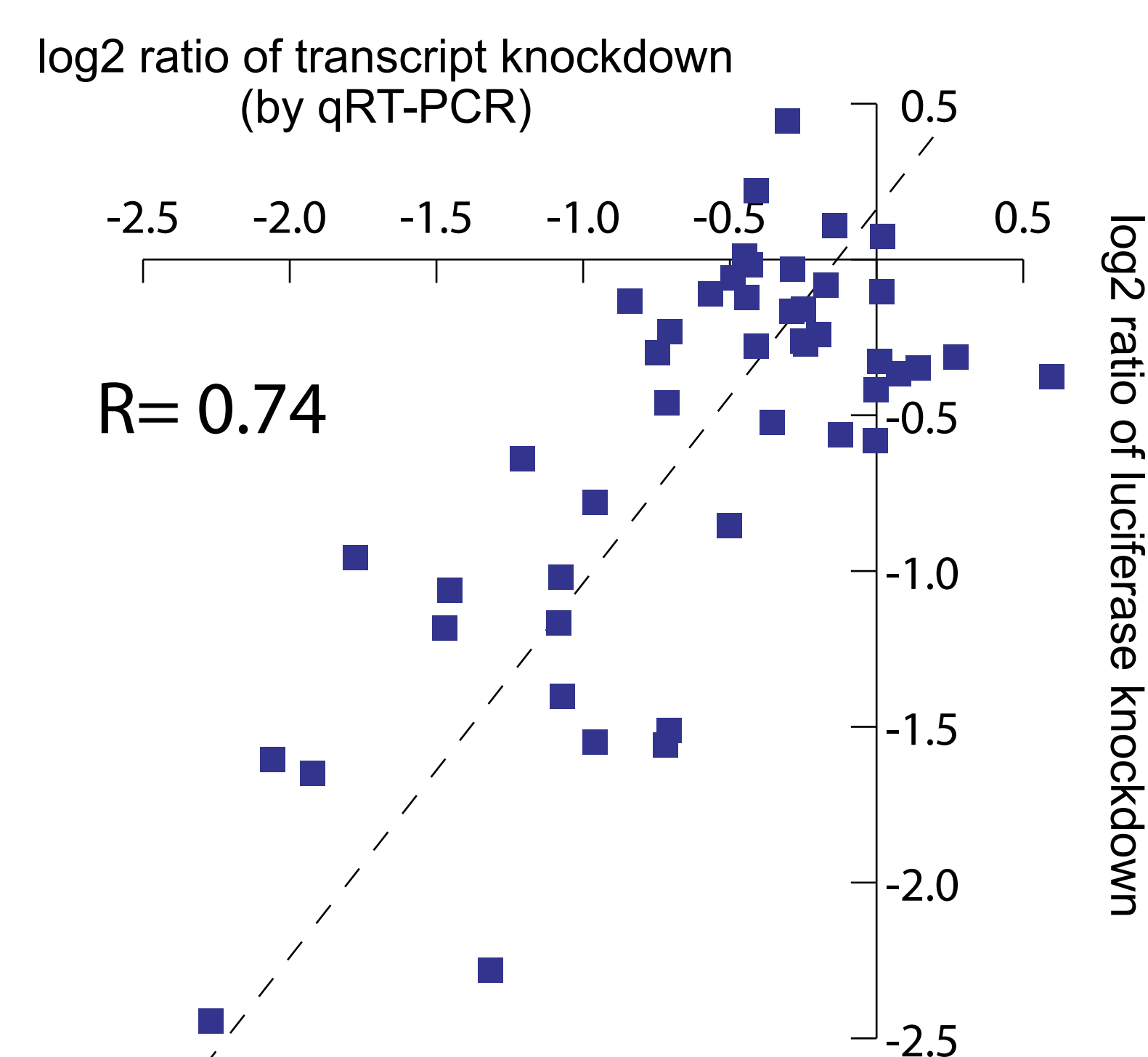
search human UTR database by miRNA

RESULTS

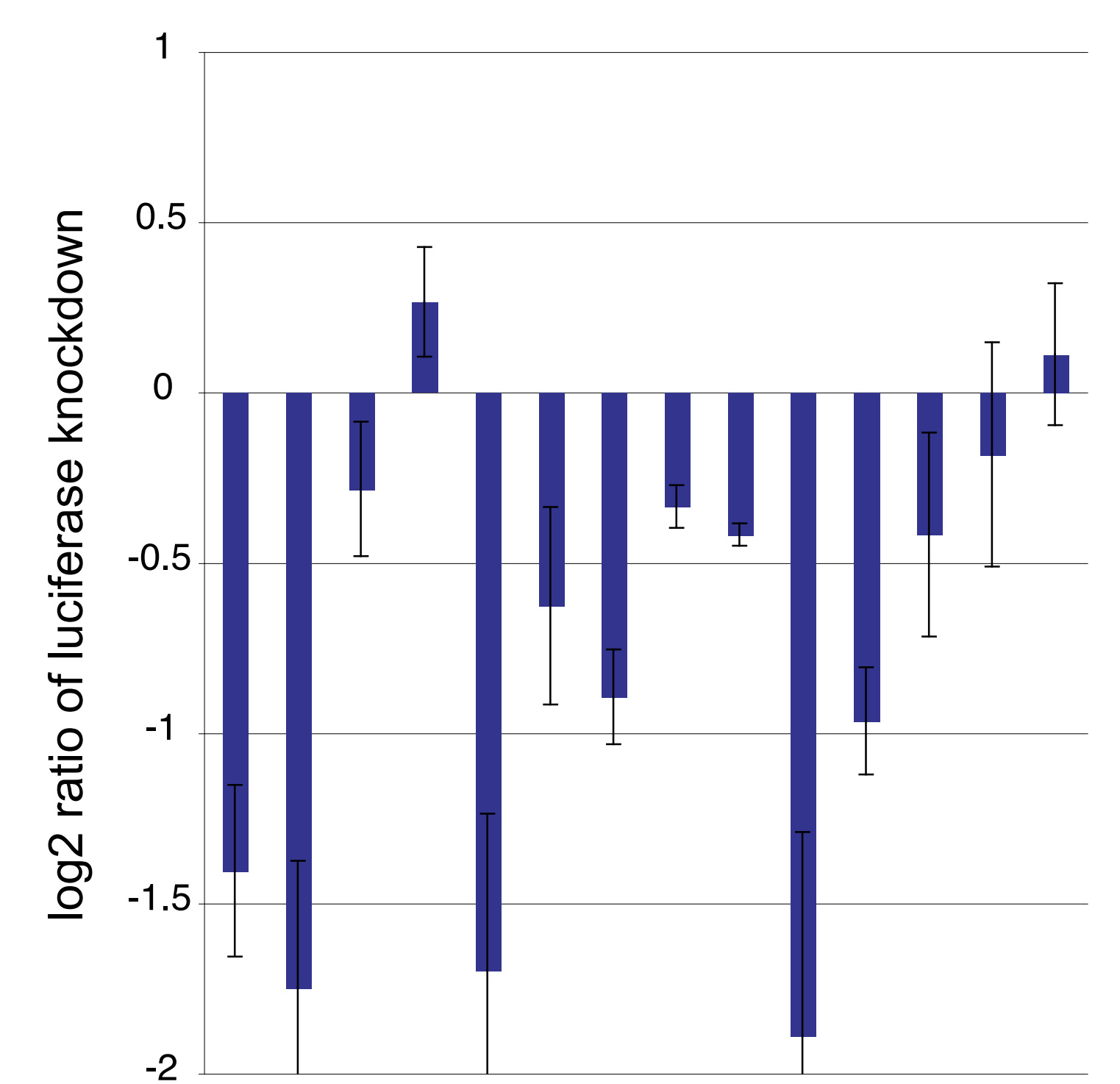
$$\frac{\text{Light output with miR-122 mimic}}{\text{Light output with control}} = \text{Activity of miR-122 on target 3'UTR}$$



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



11/14 miR-122 targets identified by proteomics are validated by luciferase



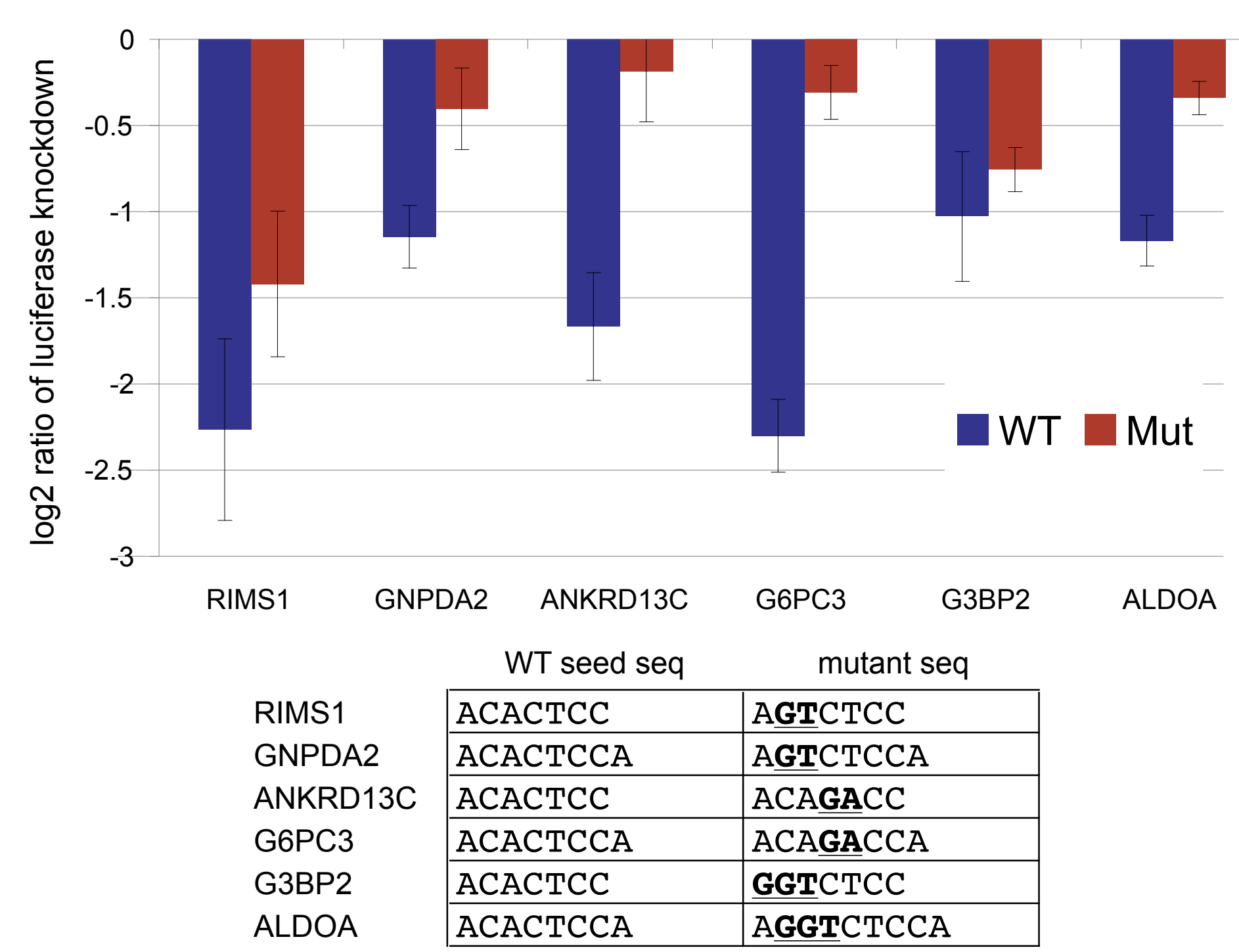
miR-122 Dose Response Data

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

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

Mutagenesis of seed sequence disrupts miR-122 function



CONCLUSIONS

- ▶ Identified 44 UTRs that respond specifically to miR-122
- ▶ Luciferase activity correlates with endogenous transcript levels
- ▶ 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