

# Application Forum

## High-throughput microRNA target screening: miR-122 case study

Patrick Collins, Michael Rose, Shelley Force Aldred, and Nathan Trinklein  
SwitchGear Genomics, Inc. Contact [info@switchgeargenomics.com](mailto:info@switchgeargenomics.com).

### Introduction

Although current computational predictions of miRNA-UTR interactions provide important guidance for experimental analysis of miRNAs, little functional data exists to verify prediction algorithms. Genome-wide transcript analysis can identify candidate target transcripts but cannot measure the translational efficiency of messages attributable to miRNAs. We have created a genome-wide library of human 3' UTR-luciferase reporter constructs to enable researchers to efficiently screen thousands of potential miRNA targets. Using this strategy, we sought to identify new targets of miR-122, an important regulator of cholesterol and fatty-acid metabolism in liver, and a therapeutic target for metabolic disease.

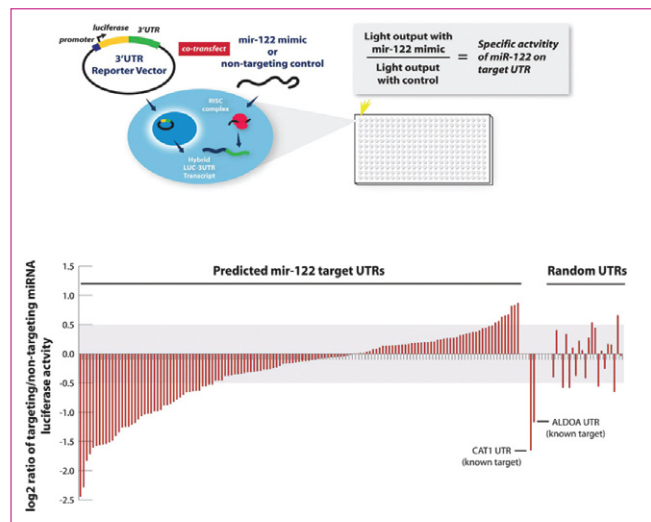
### Materials and methods

SwitchGear Genomics has created genome-wide collections of promoters and 3' UTR regions cloned into an optimized luciferase reporter vector system containing Promega's Luc2P reporter cassette. This destabilized form of luciferase greatly facilitates detailed kinetic studies, especially those focusing on repression, which might otherwise be obscured by reporter protein accumulation.

Using the SwitchGear protocol (Figure 1), we co-transfected HT-1080 cells with individual 3' UTR-luciferase reporter plasmids and either the miRNA mimic or a non-targeting control mimic. Luciferase reagent was added post-transfection and signal was read on a luminometer. Knockdown of activity for each construct was quantified as the ratio of luciferase signal with the miR-122 mimic over the non-targeting control. SwitchGear control vectors were used in this assay to account for non-specific effects.

### Results

Luminescence for 58/142 (40.8%) of the predicted targets was significantly different in the mimic co-transfection compared to the non-targeting control ( $P < 0.05$ , t-test) (Figure 1). Of those 3' UTRs with significantly altered luminescence, 25/58 (43.1%) were repressed 2-fold or more by the miR-122 mimic. In addition to performing a screen of putative targets using the 3' UTR reporter collection,



**Figure 1.** Experimental design and results summary for a large-scale functional screen of potential miR-122 target UTRs in HT1080 cells

we validated the resulting data with specificity and dose-dependence studies. The complete results can be viewed at <http://switchgeargenomics.com/technote>.

### Summary

Computational predictions and transcript-based expression analysis alone cannot measure functional roles of miRNAs, and this 3' UTR reporter screen demonstrates the ability to quantify miRNA function. Using several complementary approaches, we show that the results from the reporter screen were dose-dependent, specific, and reproducible. Luciferase assays provide another advantage by measuring both translational efficiency and message stability. Our data (see complete study), revealed several UTRs exhibiting more repression by luminescence than by qRT-PCR, highlighting translationally repressed targets. Our genome-wide library of human 3' UTR-luciferase reporter constructs enables researchers to understand the roles of miRNAs by screening thousands of potential miRNA targets in a single experiment.

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