

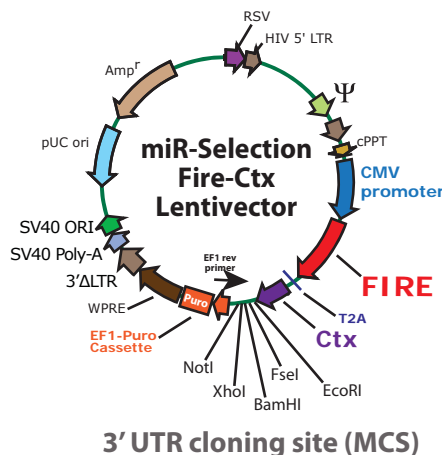
MicroRNA Target Selection System

Dual sensor system to identify 3' UTR targets featuring luciferase and a cytotoxic sensor

Current experimental approaches to verifying predicted interactions are laborious and time-consuming. We have developed a unique technology for identifying the microRNAs that bind to 3'UTRs using a cellular selection system. The miR-Selection lentivector features a dual reporter system with firefly luciferase (Fire) and a cytotoxic sensor (Ctx). The miR-Selection platform captures the 3'UTR to microRNA binding event using a survival screen by modulating the reduction of the cytotoxic sensor. Quantitative validation is made simple using the built-in luciferase reporter. This powerful and elegant technology enables the accurate identification of microRNA targets.

How to use the miR-Selection system

First, clone the 3'UTR of interest into the multiple cloning site (MCS); this will place the expression levels of the dual reporter Fire-Ctx sensors under the control of microRNA binding sites present in this 3'UTR. Next, create a stable screening cell line using the constitutive EF1-Puro selection cassette built in to the system. Once the cell line is established, the selection can begin. Add the Ctx drug to the cell culture medium. The cells will die rapidly and be completely wiped out after 4 days unless a valid microRNA to 3'UTR binding event occurs. Cells surviving the Ctx drug treatment is due by detecting direct microRNA-3'UTR interactions when overexpressing a microRNA or pools of microRNAs during the selective screen.

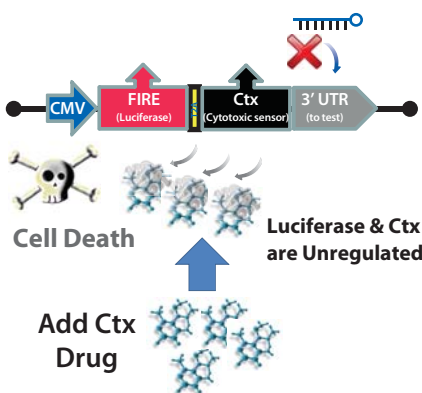


Highlights

- Dual sensor 3'UTR lentivector system
- Use Ctx drug in survival screens
- Quantify microRNA binding using luciferase assays
- Easily make miR-Selection stable cell lines using the EF1-Puro cassette
- Validate microRNA to 3'UTR predictions
- Discover new microRNA binding sites in 3'UTRs in genome-wide miR screens

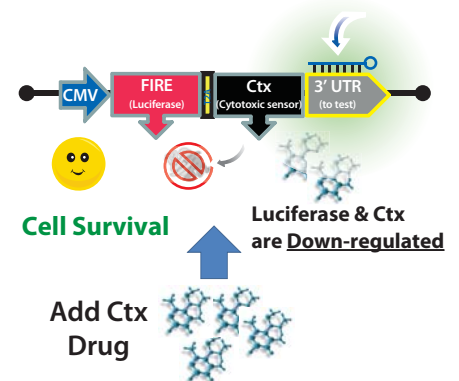
Novel cellular screen to identify microRNA binding sites in 3'UTRs

No miR Binding



Survival screen based upon successful microRNAs binding to the 3' UTR cloned in the miR-Selection lentivector construct.

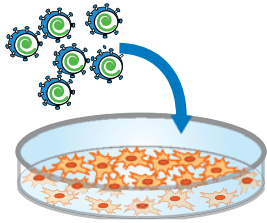
Successful miR Binding



MicroRNA Target Selection System

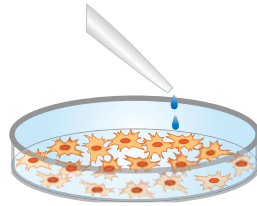
Validation data of the miR-Selection system using miR-145 and c-Myc 3'UTR

SBI's Lenti-miR-145 Virus (GFP)



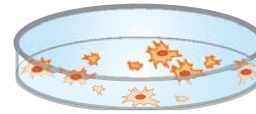
Transduction with MicroRNA

Add Ctx drug to begin selection



Ctx Toxin Treatment

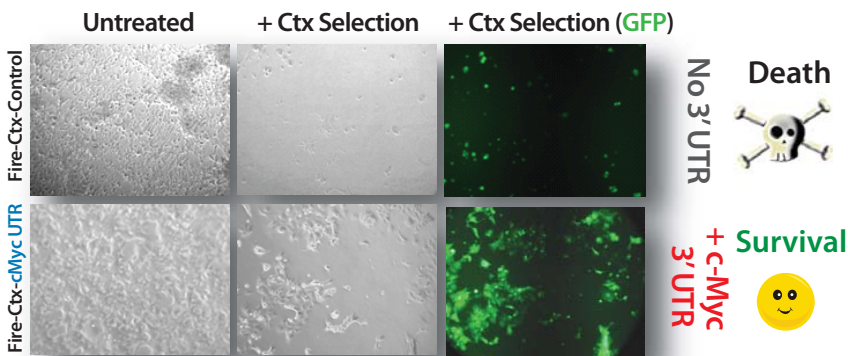
4 day selection with Ctx in media



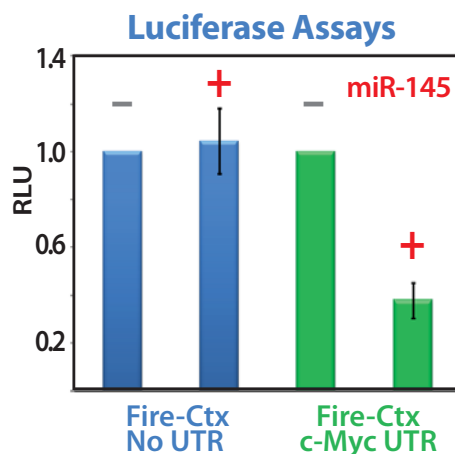
Selection for Survivors

The cytotoxic screen was performed using control (no 3'UTR) and c-Myc 3'UTR miR-Selection lentivectors. Both stable cell lines were transduced with equal amounts or Lenti-miR-145 virus (GFP marker). The cells were treated for 4 days with 1x Ctx cytotoxin drug.

Ctx Selection Cell Images



The Ctx sensor is down regulated by miR-145 binding to c-Myc 3' UTR which enables survival in Ctx screens and also reduces Luciferase activity by 63%.



The 3'UTR of the human c-Myc gene was cloned into the miR-Selection vector and transduced into 293 cells. If microRNAs bind to the 3' UTR being tested, then the expression levels of both luciferase and the Ctx sensors will be greatly reduced. Lowering the amount of the Ctx sensor is what will enable the cells to survive in the presence of the Ctx drug. This interaction between microRNAs and the 3' UTR is key to the selection system and is what is being measured during the screen. Measurements of the luciferase (Fire) activities of the +/- c-Myc 3' UTR in the miR-Selection vector infected with or without Lenti-miR-145 virus without Ctx selection show that the levels of luciferase were unchanged in the No UTR controls as expected. A pronounced 63% reduction in luciferase activity in the c-Myc 3'UTR plus Lenti-miR-145 cells was observed in experimental cells when compared to the controls.

The real power of the technology will be to use it as a discovery tool to identify and validate, without prejudice, microRNAs that can bind and regulate a 3' UTR of interest during a cellular screen.

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