

Request for siRNA duplex and miRNA reagent re-confirmation information

We are collecting information about the activity of individual siRNA duplexes and of miRNA mimics and inhibitors in the ICCB-L RNAi libraries and we need your help.

We believe that information about the reconfirmation activity of individual duplexes and miRNA reagents will be very valuable to all screeners when available from all of the screening projects that are completed at ICCB-L. With siRNA assays, most screeners simultaneously screen all 4 duplexes from a pool that scored as positive in their genome screen. This information may allow us to identify the "better" siRNA pools (those in which multiple individual duplexes produce the same phenotype in a given assay) and also those that might be prone to off-target effects (those for which most individual duplex components generally don't re-test as positive). With miRNA assays, screeners retest the same reagents that scored as positives in the primary screen.

We have worked out a way to post the information in Screensaver that is "screen anonymous"-- assay title and protocol will not be linked to the duplex results in the database user interface. For each siRNA pool or miRNA mimic/inhibitor in our collection, we will provide the information: "This siRNA pool (or miRNA mimic/inhibitor) has scored as a positive in x out of y assays in which it was tested." For each individual siRNA duplex or miRNA mimic/inhibitor in our collection we will provide similar retest information: "This individual siRNA duplex induces the same phenotype as the corresponding pool in x out of y assays" or "This miRNA mimic/inhibitor induces the same phenotype as in the primary screen in x out of y re-confirmation assays".

The siRNA reagents for some genes are expected to score positive in an assay, but under screening conditions do not score (false negatives). We believe that this information, when properly presented, is also valuable to share among screeners. Again, it will be posted in Screensaver in a screen anonymous manner. We will provide the information: "This pool has been identified as a putative false negative in x of y screens." Defining which genes are expected to score in any assay can be quite subjective. We would like to include only those genes that are expected based on validated experimental evidence. For example, different siRNA reagents do score as positive in the same or equivalent assay, or other molecular biological/genetic data support that knock down of the gene product should produce a positive in the assay. We ask screeners to use their best judgment in identifying these potential false negatives and expect that, for most screens, there will only be one or two pools that fall into this category.

Annotating activity of individual siRNA duplexes or miRNA mimics/inhibitors (see attached files): Please complete the last two columns of the first worksheet (Cherry Pick Platemap).

1. The next to last column is entitled "Re-test Result." Please categorize each reagent as one of the following (enter only Y, N, NT, INC, or NA):

Y --Yes this reagent induces the same phenotype as the corresponding primary screen well (siRNA pool or miRNA reagent).

N --No, this reagent does NOT induce the same phenotype as the corresponding primary screen well.

NT --This reagent was not re-tested.

INC --The data from the re-test of this reagent was inconclusive.

NA --Not applicable, this reagent did not score as a positive (or was never assayed) in the primary screen.

2. In the last column, "Comments" please write any comments about an individual duplex or miRNA mimic/inhibitor that you think might provide helpful information to others.

Annotating putative false negative siRNA pools: In the second worksheet "Potential false negatives," please list the genes, one gene per row, that you identify as putative false negatives in your whole genome screen. The more information that you can provide about the gene, the better. At the minimum, we ask you to provide the Entrez Gene ID for each putative false negative gene. If you could also provide the pool catalog number and the ICCB-L plate/well location of the pool that would be great.

We appreciate the time that you will take to provide this information. Thanks for your help.