

MIAME Checklist

EXPERIMENT DESIGN

Goal:

Analysis of transcripts regulated by Dicer and Argonaute proteins in human HEK-293 cells

Description of the experiment:

HEK-293-derived cell lines depleted of Dicer or one of four Argonaute proteins were generated. Microarray analysis of these knock down cell lines and various control cell lines was performed to address how large the proportion of genes is that are regulated by RNA silencing in an individual cell type. Comparison of different arrays revealed commonly (up) regulated genes. The highest overlap was observed between Ago2 and Dicer knockdown cells.

Experimental factors:

Different time points (non-induced, 2 days or 6 days) of induction (10µg/ml Tetracycline or 1µg/ml Doxycycline) of stable mammalian 293 knock down cell lines compared to various control cell lines.

Experimental design:

Stable cell lines that knock down components of the RNA silencing pathway upon induction were compared to control cell lines and/or the non-induced state.

Quality control:

Three control cell lines (T-Rex, shRNA, empty vector), two individual Argonaute 1 and 4 (Ago1kd, Ago4kd) knock down cell lines, one Argonaute 2 and 3 (Ago2kd, Ago3kd) and two individual Dicer knock down cell lines were analyzed as biological replicates.

Data originating from three different array hybridization experiments were uploaded and simultaneously normalized into Analyst from Genedata AG (Basel, Switzerland). Quality control and background normalization was performed using Refiner from Genedata AG and expression values were estimated using the GC-RMA algorithm provided by Genedata. Statistical analysis was performed using Analyst. Genes were required to

pass an N-way ANOVA with a $P < 0.05$ and/or have a median fold change of 1.5 or greater between one or more pairs of conditions.

Links:

Raw data from 38 Microarray experiments were submitted to the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo>). The platform ID is GPL570. The accession ID for the set of experiments described is GSE4246.

SAMPLES USED

HEK-293 human embryonic kidney cells (female), stably expressing the tet-repressor (T-REx). After induction for 48 hrs with 10 μ g/ml of Tetracycline these cells express a hairpin against Ago1-4. T-REx-Dicer knockdown cells were induced with 1 μ g/ml Doxycycline for 2- or 6 days. T-REx cells, cells harboring an empty vector (EV) or cells expressing a scramble hairpin (shRNA) served as controls.

Cells were grown, induced and processed separately as biological replicates.

!Series_sample_id = T-REx rep1 exp1
!Series_sample_id = T-REx rep2 exp1
!Series_sample_id = shRNA rep1 exp1
!Series_sample_id = shRNA rep2 exp1
!Series_sample_id = Ago2kd rep1
!Series_sample_id = Ago2kd rep2
!Series_sample_id = Ago3kd rep1
!Series_sample_id = Ago3kd rep2
!Series_sample_id = Ago1kd #1 rep1
!Series_sample_id = Ago1kd #1 rep2
!Series_sample_id = Ago1kd #2 rep1
!Series_sample_id = Ago1kd #2 rep2
!Series_sample_id = Ago4kd #1 rep1
!Series_sample_id = Ago4kd #1 rep2
!Series_sample_id = Ago4kd #2 rep1
!Series_sample_id = Ago4kd #2 rep2

!Series_sample_id = T-REx rep1 exp2
!Series_sample_id = T-REx rep2 exp2
!Series_sample_id = shRNA rep1 exp2
!Series_sample_id = shRNA rep2 exp2
!Series_sample_id = EV rep 1 not induced
!Series_sample_id = EV rep 2 not induced
!Series_sample_id = Dcr2-2kd rep 1 not induced
!Series_sample_id = Dcr2-2kd rep 2 not induced
!Series_sample_id = Dcr2b2kd rep 1 not induced
!Series_sample_id = Dcr2b2kd rep 2 not induced
!Series_sample_id = EV rep 1 2 days induced
!Series_sample_id = EV rep 2 2 days induced
!Series_sample_id = Dcr2-2kd rep 1 2 days induced
!Series_sample_id = Dcr2-2kd rep 2 2 days induced
!Series_sample_id = Dcr2b2kd rep 1 2 days induced
!Series_sample_id = Dcr2b2kd rep 2 2 days induced
!Series_sample_id = EV rep 1 6 days induced
!Series_sample_id = EV rep 2 6 days induced
!Series_sample_id = Dcr2-2kd rep 1 6 days induced
!Series_sample_id = Dcr2-2kd rep 2 6 days induced
!Series_sample_id = Dcr2b2kd rep 1 6 days induced
!Series_sample_id = Dcr2b2kd rep 2 6 days induced

HYBRIDIZATION PROCEDURES AND PARAMETERS

5 µg of total RNA (extracted with Absolutely RNA Miniprep kit from Stratagene) was reverse transcribed using the Affymetrix cDNA synthesis kit and cRNA was produced by *in vitro* transcription (IVT) by T7 RNA polymerase using the Affymetrix IVT kit as per manufacturer's instructions. 20 µg of biotinylated cRNA was fragmented by heating with magnesium (as per Affymetrix's instructions) and 15 µg of fragmented cRNA was hybridized to Human U133 plus 2.0 GeneChips™.

MEASUREMENT DATA AND SPECIFICATIONS

Raw data put reference to CEL files submitted to GEO database

Data extraction and processing protocols

Affymetrix U133 Plus 2.0 microarrays were scanned with the GeneChipScanner 3000 and analyzed with GeneChip Operating Software Version 1.4 from Affymetrix.

Array results represent median-normalized expression values from three independent microarray experiments, which produced a good-quality data.

In order to compare data from three independent experiments within one environment, all of the arrays were condensed together using the GC-RMA algorithm with quantile normalization composed of 100 bins using Genedata's Refiner application. Per chip normalization was performed by scaling the median of the genes called present (detection P-value < 0.04) to a value of 500. The per chip normalized data are referred to as "raw" expression values in this paper. In addition, a per gene normalization was performed using a point-wise division of gene (probe set) in each experimental sample with the median expression profile of the same gene from the matched control samples processed on the same day. The results of this point wise division are referred to here as "normalized" data.

ARRAY DESIGN

Commercially available Affymetrix U133 Plus 2.0 microarrays