

Arraystar provides easy-to-use qPCR data analysis tool for nrStar™ Human tRNA Repertoire PCR Array. You can download it free after registration on our website.

## Instructions for use

1. Export the raw Ct values using the software supplied with the real-time PCR instrument.
2. Paste your test sample Ct values to the sheet "Test Sample Data" in the same well positions.

Transcript name	Well	exp1	exp2	exp3	exp4	exp5	exp6	exp7	exp8	exp9	exp10	exp11	exp12	exp13	exp14	exp15	exp16	exp17	exp18	exp19	exp20	AVG	SD	
AB073614	A01																					35.00	N	
AF339813	A02																						35.00	N
CLinc01	A03																						35.00	N
CLinc02	A04																						35.00	N
ENST00000456816	A05																						35.00	N
ENST00000434223	A06																						35.00	N
CTB-89H12.4	A07																						35.00	N
CHL1-AS2	A08																						35.00	N
KRT7-AS	A09																						35.00	N
LINC00237	A10																						35.00	N
KRASP1	A11																						35.00	N
anti-NOS2A	A12																						35.00	N

3. Paste your control sample Ct values to the sheet "Control Sample Data" in the same well positions.

Transcript name	Well	exp1	exp2	exp3	exp4	exp5	exp6	exp7	exp8	exp9	exp10	exp11	exp12	exp13	exp14	exp15	exp16	exp17	exp18	exp19	exp20	AVG	SD	
AB073614	A01																						35.00	N/A
AF339813	A02																						35.00	N/A
CLinc01	A03																						35.00	N/A
CLinc02	A04																						35.00	N/A
ENST00000456816	A05																						35.00	N/A
ENST00000434223	A06																						35.00	N/A
CTB-89H12.4	A07																						35.00	N/A
CHL1-AS2	A08																						35.00	N/A
KRT7-AS	A09																						35.00	N/A
LINC00237	A10																						35.00	N/A
KRASP1	A11																						35.00	N/A
anti-NOS2A	A12																						35.00	N/A

4. The software will perform all the calculations and mapping automatically.
5. Check the results in Excel sheets "All expressed genes", "Scatter Plot", "Volcano Plot", etc.

## Description of automatic procedures

1. The software may not work if the sample numbers participating in the comparison are less than 4.
2. The automatic procedures include:

### 2.1 Data pre-processing

2.1.1. Set all Ct values  $\geq 35$  or N/A (not detected) to 35. From this point on, any Ct value equal to 35 is considered a negative result.

2.1.2. Examine the Ct values of the Genomic DNA Control (GDC) wells. If the value is 35, no genomic DNA contamination is detected and no action is needed. If the value is less than 35, genomic DNA contamination is evident and the result may be compromised.

2.1.3. Before initiating the data analysis, the RNA spike-in wells are compared. Outlier

samples may be identified and considered for exclusion in the further data analysis.

2.1.4. Inter-plate calibration (IPC) can be performed using the PPC assay replicates either with the data analysis software or manually. For each plate, verify that the replicates have Ct standard deviation  $\leq 0.5$ . If this is not the case, exclude the outlier if it can be identified. Calculate the average of the replicates for each plate, the overall average (average of IPC values from all plates). The calibration factor for each plate is calculated as the difference between the plate average and the overall average:

$$\text{calibration factor} = \text{IPC}(\text{plate } n) - \text{IPC}(\text{overall})$$

The Ct value is corrected with the calibration factor as

$$Ct_{RNA} = Ct_{RNA}(\text{Raw value, plate } n) - \text{IPC}(\text{plate } n) + \text{IPC}(\text{overall})$$

or

$$Ct_{RNA} = Ct_{RNA}(\text{Raw value, plate } n) - \text{calibration factor}$$

	Plate 1	Plate 2	Plate 3
snoRD71	20.26	20.83	20.44
IPC Plate average	16.70	16.70	16.40
IPC overall average	16.60	16.60	16.60
Calibration factor	0.10	0.10	-0.20
snoRD71 (Calibrated)	20.16	20.73	20.64

2.1.5. Calculate the  $\Delta Ct$  for each RNA in the plate.

$$\Delta Ct_{RNA} = Ct_{RNA} - \text{average}(Ct_{HKs})$$

Where average ( $Ct_{HKs}$ ) is the average of the Ct values derived from the multiple housekeeping genes (HKs). These most stably expressed housekeeping reference genes were selected from a broad range of samples by our stringent algorithm that evaluates the optimal properties and the number for endogenous controls.

## 2.2 Data analysis

2.2.1. Calculate the  $\Delta\Delta Ct$  for each RNA

$$\Delta\Delta Ct = \Delta Ct(\text{sample 1}) - \Delta Ct(\text{sample 2}), \text{ between samples}$$

or

$$\Delta\Delta Ct = \Delta Ct(\text{group 1}) - \Delta Ct(\text{group 2}), \text{ between groups}$$

2.2.2. Calculate the fold change for each gene from sample 1 to sample 2 or group 1 to group 2 as following:

$$\text{Fold Change} = 2^{-\Delta\Delta Ct}$$

**NOTE:** By convention, if the fold change is greater than 1, the result is reported as a fold up-regulation. If the fold change is less than 1, its negative inverse is reported as a fold down-regulation.

2.2.3. When comparing profiling differences between two groups (such as disease versus control) with biological replicates, the statistical significance of the difference can be estimated as p-value by *t*-test. RNAs having fold changes  $\geq 2$  and *p*-values  $\leq 0.05$  are selected as the significantly differentially expressed RNAs.

**NOTE:** Fold change is related to biological effect size. Ranking by fold change is preferred over *p*-value. qPCR as commonly used in confirmation has a limit of quantification of 0.5  $\Delta C_t$ , which is equivalent to approximately 1.5 fold change.

2.2.4. Other analyses such as scatter plots, volcano plots, and bar graph of expression differences for the tRNAs are performed and included in the standard analysis package.

### Sample data analysis results

The sample data were generated from RNAs extracted from two glioblastoma cell lines LN-229 and LN-18. The normalization was carried out using the average of the housekeeping genes.

#### 1. Fold Change and P-value

Transcript name	Well	AVG $\Delta C_t$ (Ct(GOI) - Ave Ct (HKG))		$2^{-\Delta C_t}$		Fold Difference	T-TEST	Fold Up- or Down- Regulation	Comments
		Test	Control	Test	Control	Test /Control	p value	Test /Control	
Arg-CCG	A06	5.18	4.09	2.8E-02	5.9E-02	0.47	0.0000	-2.14	OKAY
Asp-ATC	A12	5.23	4.25	2.7E-02	5.3E-02	0.51	0.0240	-1.98	OKAY
Asp-GTC	B01	-3.48	-2.79	1.1E+01	6.9E+00	1.62	0.0016	1.62	OKAY
Gln-CTG-2	B04	5.19	3.70	2.7E-02	7.7E-02	0.36	0.0125	-2.80	OKAY
His-GTG	C01	-0.13	0.64	1.1E+00	6.4E-01	1.70	0.0000	1.70	OKAY
Leu-CAG	C07	2.56	3.97	1.7E-01	6.4E-02	2.66	0.0246	2.66	OKAY
Ser-ACT	D08	5.21	4.29	2.7E-02	5.1E-02	0.53	0.0028	-1.89	OKAY
Sup-CTA	E02	5.87	4.73	1.7E-02	3.8E-02	0.45	0.0122	-2.22	OKAY
Thr-AGT-2	E05	3.31	5.96	1.0E-01	1.6E-02	6.26	0.0000	6.26	OKAY
Val-CAC-1	F02	7.22	6.02	6.7E-03	1.5E-02	0.43	0.0233	-2.30	OKAY
Val-CAC-2	F03	5.68	4.52	1.9E-02	4.4E-02	0.45	0.0001	-2.25	OKAY
mt-Arg-TCG	F08	-13.27	-14.33	9.9E+03	2.1E+04	0.48	0.0002	-2.08	OKAY

Figure 1. A snapshot of the data file for the fold change and p values.

#### 2. Scatter Plot

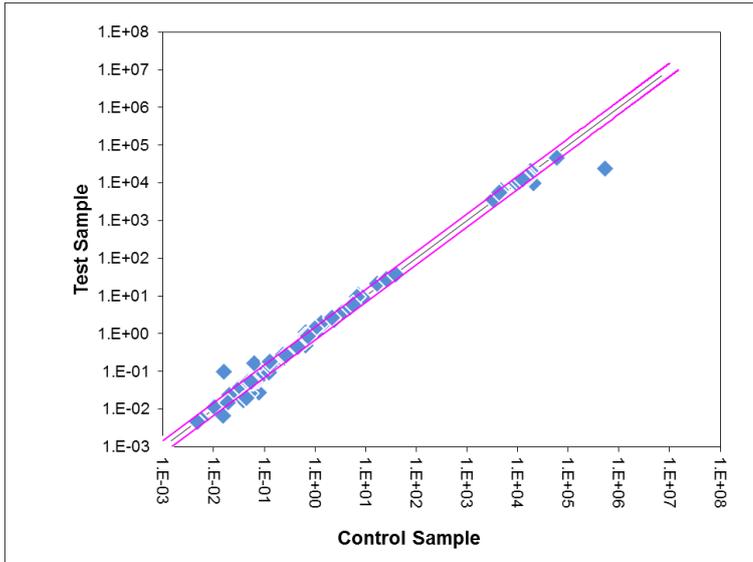


Figure 2. The black diagonal line indicates fold change being 1 (no change). The pink diagonal lines indicate the up- or down- fold changes set at 1.5 (default).

### 3. Volcano Plot

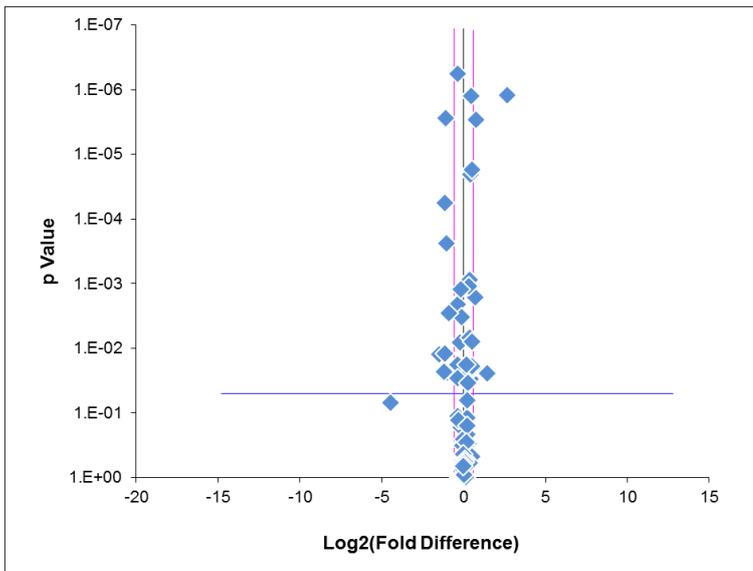


Figure 3. The middle vertical black line indicates the fold change at 1, or  $\log_2(\text{FC})$  at 0. The vertical pink lines indicate the thresholds of up- or down- fold changes at 1.5. The horizontal blue line indicates P-value threshold of 0.05.

### 4. TOP20 Up-Fold change RNAs

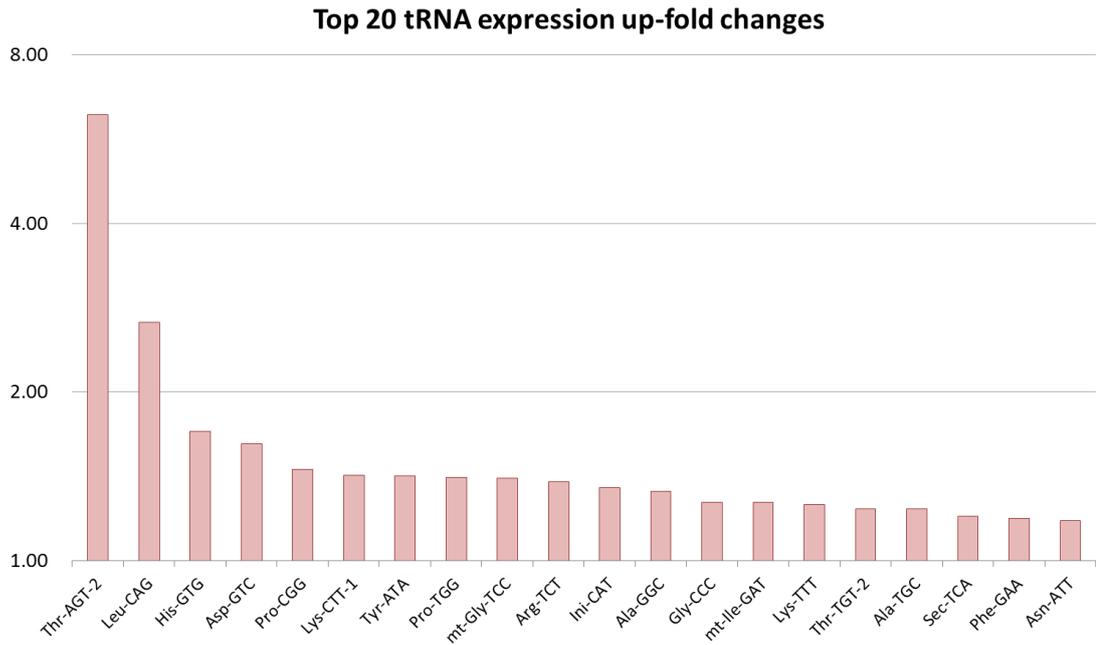


Figure 4. Bar graph of top 20 up-fold change tRNAs.

5. TOP20 Down-Fold change RNAs

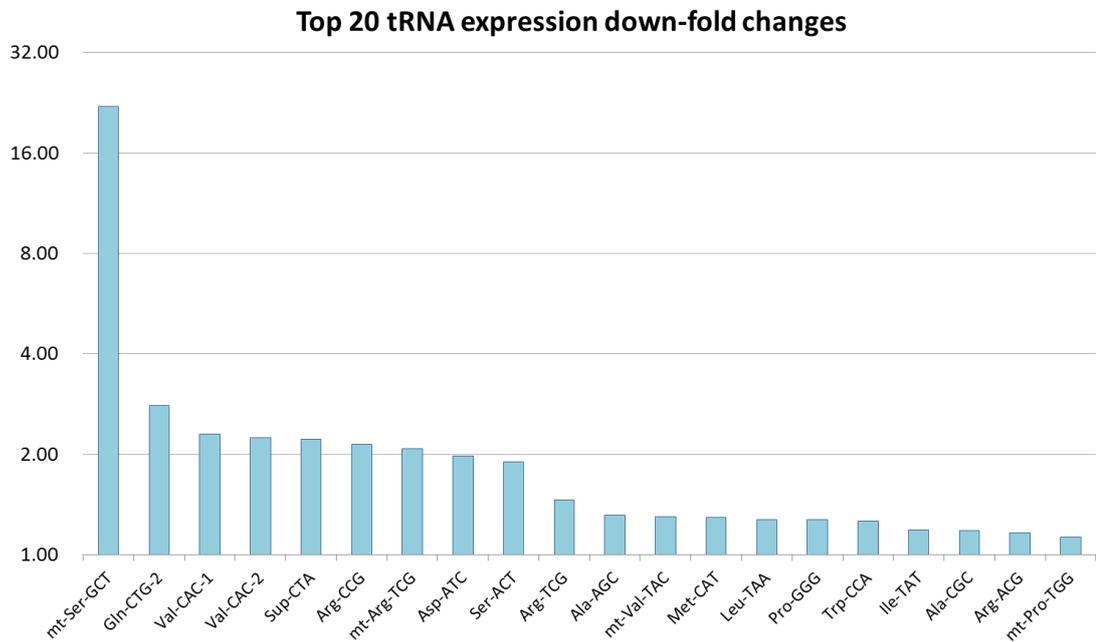


Figure 5. Bar graph of top 20 down-fold change tRNAs