nrStar™ Human snoRNA PCR Array

Cat#: AS-NR-003

Instruction Manual Version 1.0

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I. Introduction

A. Overview

small nucleolar RNAs (snoRNAs) are intermediate-length small noncoding RNAs (sncRNAs), ranging from 60–300 nt, and associated with small nucleolar ribonucleoproteins (snoRNPs) [1]. In Vertebrates, the snoRNA genes are embedded within the introns of protein coding or noncoding genes and are post-transcriptionally processed [2]. snoRNAs are involved in rRNA processing and regulation of splicing, translation, and oxidative stress [3]. Recent studies, however, suggest that snoRNAs may have broader roles, including in genetic disorders[4], human variation[5], hematopoiesis[6], metabolism[7, 8] and neoplasia[3, 9]. snoRNA expression is also dysregulated in human neurodegenerative disorders. snoRNAs are relatively stable and have been detected in blood plasma, sputum, and urine samples, presenting a promising target for diagnostics and treatment of human pathologies. Now studying snoRNAs has become a hot field in research.

Arraystar nrStar™ PCR Arrays have the most comprehensive, up to date, and the best contents on the market. nrStar™ Human snoRNA PCR Array profiles 359 snoRNAs, which are derived from two authoritative snoRNA databases (SnoPY and snoRNA-LBME-db). This panel covers all the prevalently detected snoRNAs and makes it a powerful tool for snoRNA profiling and functional research.

B. snoRNA in biological functions and human diseases

■ Biogenesis of snoRNAs

There are two types of snoRNAs: Box C/D or Box H/ACA snoRNAs (**Fig. 1A** and **1B**)[9]. Most of snoRNAs are located within introns of genes transcribed via RNA polymerase II (**Fig. 1C**)[9]. However, snoRNAs can also be processed from introns of long non-coding RNAs (lncRNAs). For example, GAS5, an lncRNA, encodes 9 C/D box snoRNAs (snoRNDs 74-81) [10]. After liberation from introns, snoRNAs are processed to remove excess nucleotides from either end via exonuclease activity. Signal sequences within the snoRNAs centered at boxes C and D or H and ACA direct binding of protein interacting partners that represent the functional snoRNP complex.

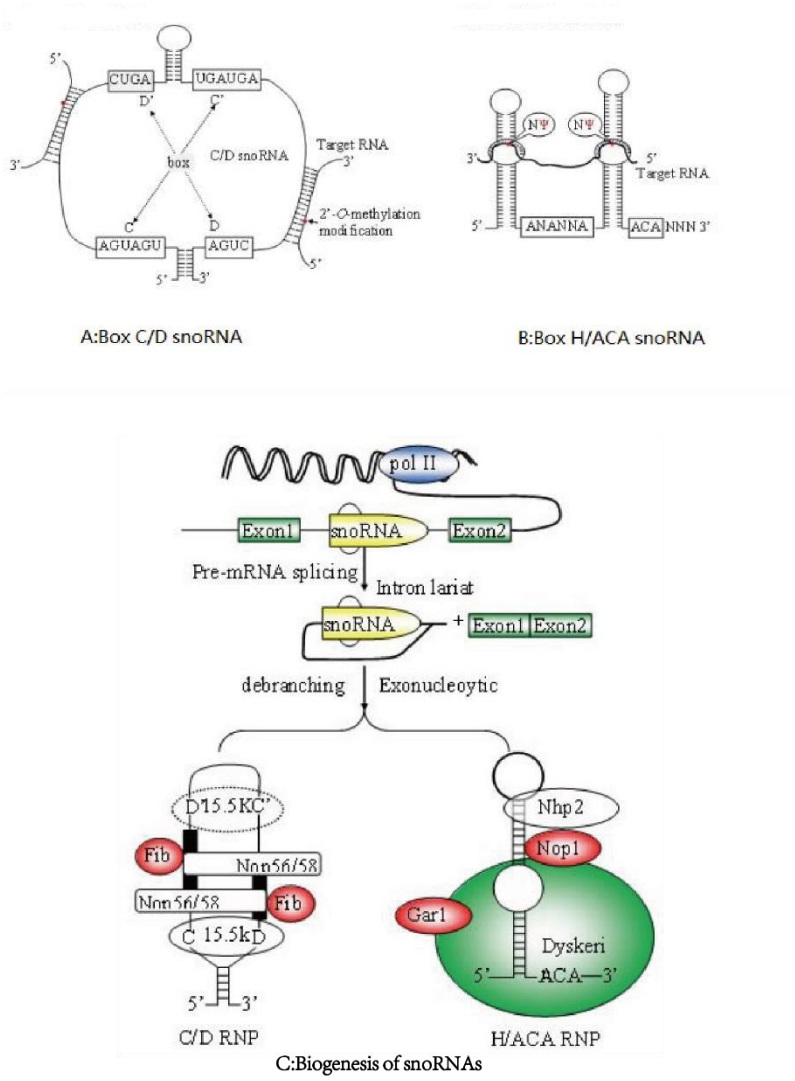


Fig 1. The structure and biogenesis of snoRNAs. A. The 2'-O-methylated nucleotides located five nucleotides upstream of the D or D' box sequences are indicated as red. B. Positions and consensus sequences of the conserved C, C' box (UGAUGA), and D, D' boxes (CUGA). The uridine residues selected for pseudouridylation are shown as Ψ. C. Biogenesis of snoRNA: The great majority of mammalian box C/D and H/ACA snoRNAs are processed from pre-mRNA introns. snoRNA families are not independently transcribed but processed from the pre-mRNA introns, in most cases by exonucleolytic digestion of the debranched lariat. Box C/D snoRNAs contain four evolutionarily conserved, essential proteins, fibrillarin (methyltransferase), Nop56, Nop58, and 15.5kDa. Proteins common to H/ACA snoRNAs include dyskerin (pseudouridine synthase), Gar1, Nhp2, and Nop10p.[9]

■ snoRNA Functions

snoRNAs in processing of rRNAs

The snoRNAs have two basic mechanisms: 2'-O-methylation and pseudouridylation of rRNAs [11]. 2'-O-methylation of rRNAs is carried out by the C/D box snoRNA family. The box C sequence (RUGAUGA, where R stands for A or G)

is typically located close to the 5’ terminus, whereas box D sequence (CUGA) is situated near the 3’ end (**Fig. 1A**). The two motifs are generally brought together in a typical 5’-3’ terminal stem-box structure involving 4-5 nucleotides at both termini, which is critical for snoRNA biogenesis and nuclear localization [12].Many C/D box snoRNAs contain a less conserved copy of the box C motif C’ in the central position and an additional box D motif, termed box D’ in their 5’ half, where RNA protein interactions occur to direct the proper assembly of the functional snoRNP complexes [13]. The pseudouridylation of rRNAs is accomplished by the H/ACA box of snoRNAs family composed of two large hairpin domains linked by a hinge and followed by short tail [13]. The conserved motif called box H (ANANNA, N stands for any nucleotide) and ACA (a trinucleotide always found three nucleotides away from the 3’ end) are located in the hinge and tail (**Fig. 1B**). Guide sequences that direct the snoRNAs to the appropriate rRNA sequence are in one or both of the hairpin loop domains. A combination type of snoRNAs that comprises features of both SNORAs and SNORDs localizes specifically to Cajal bodies, Cajal body-specific RNAs (SCARNAs). SCARNAs can guide both methylation and pseudouridylation of the RNA Pol II transcribed spliceosomal RNAs[14].

snoRNAs as precursor of miRNAs

Processing of snoRNAs into smaller fragments has been reported with convincing evidence suggesting that some snoRNAs act as precursors for functional miRNA[15].The first report that identified fragments of the H/ACA box snoRNA ACA45 in association with Ago proteins from the miRNA RISC complex showed that processing of ACA45 is dependent on Dicer. Furthermore, resulting 20-22 nucleotide fragments were validated to function as a miRNA that represses expression of an identified target (CDC2L6) [15]. Recently, 11 C/D box snoRNAs that are processed to miRNA-sized species were identified and verified to indicate gene silencing activity[16].

snoRNAs for alternative splicing

The snoRNA transcripts also serve as regulators of alternative splicing. The 18-nucleotide-conserved target recognition element of a HBII-52 C/D box snoRNA is complementary to the serotonin receptor 5-HT (2C) mRNA [17]. The snoRNA HBII-52 can control processing of mRNA expression of the serotonin receptor 2C by regulating its alternative splicing and hence contributes to the Prader-Willi syndrome.

snoRNAs in stress response

The nucleolus is one of the key participants of the cell response to stress. Various stress conditions can cause changes in the nucleolus and even its destruction. It was observed that snoRNAs could contribute to stress response. The expression of SNORD14A and SNORD83B is significantly increased under hypoxia conditions [18]. Moreover, SNORDs 32A, 33, and 35A are significantly increased under oxidative stress upon treatment of cells with palmitate. Cells become resistant against palmitate treatment when the expression of SNORD32A, SNORD33, and SNORD35A is inhibited [7]. Under metabolic stresses, the full length forms of snoRNAs are accumulated in the cytoplasm but not in the nucleus. For instance, independently transcribed snoRNAs (SNORD3, SNORD13, and SNORD118) complementarily interact and regulate their target mRNAs translation in the cytoplasm. The pathways of snoRNA involvement in the regulation of cellular processes can be even more diverse.

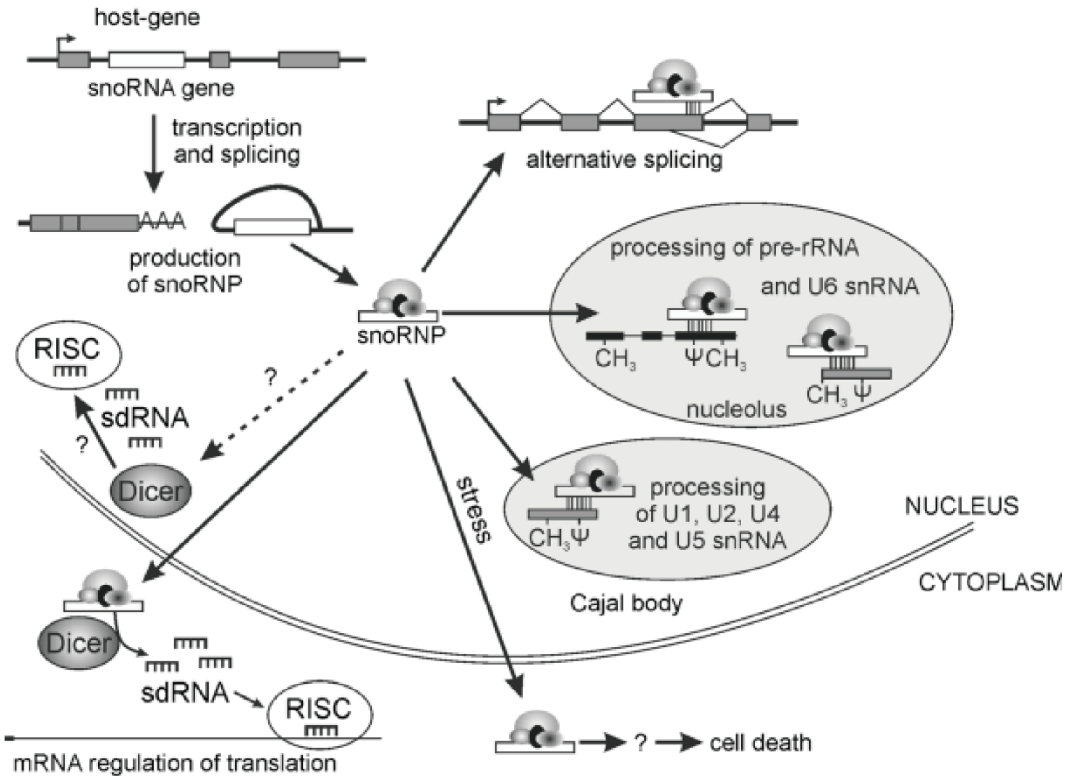


Fig 2. Involvement of snoRNAs in various cellular processes.[19]

■ snoRNAs in cancer

snoRNA involvement in molecular pathology of cancer

Insights into the potential roles of snoRNAs in cancer began with a study that reported substantial downregulation of snoRNAs in meningiomas compared with normal brains[20]. More recently, it was also shown that various snoRNAs are differentially expressed in non-small cell lung cancer in comparison with the corresponding matched tissue[21]. Other studies have shown that a germline homozygous 2 bp (TT) deletion of the snoRNA U50 is associated with prostate cancer development [22] and that U50 undergoes frequent somatic heterozygous deletion and transcriptional downregulation in breast cancer [23]. Other studies showed that growth arrest specific 5 (GAS5) — a gene that hosts ten intronic snoRNAs but that also encodes an snoRNA — controls cell survival by inducing or sensitizing cells to apoptosis. A substantial decrease of GAS5 mRNA level in breast cancer samples compared with adjacent unaffected normal breast epithelial tissues also suggests that it functions as a tumor-suppressor gene[24].

The association between snoRNAs and tumorigenesis also extends to their associated proteins. snoRNPs are divided into two main classes — C/D box and H/ACA box — according to their conserved secondary structural characteristics and associated modification reactions. The C/D box snoRNPs consist of a core of four proteins — fibrillarin (the methyltransferase), nucleolar protein 56 (NOP56), NOP58 and NHP2-like 1 (NHP2L1)—whereas the H/ACA box snoRNPs contain dyskerin (the pseudouridine synthase), GAR1, NHP2 and NOP10. Fibrillarin is essential for development, and its depletion is lethal in embryos. Mutations in the human dyskerin (DKC1) gene,

NOP10 and NHP2 are associated with the X-linked genetic disorder dyskeratosis congenita, one of the characteristics of which are susceptibility to epithelial cancers [25].

snoRNAs and snoRNPs are likely to contribute to tumorigenesis through an effect on ribosomes and protein translation, given that translation is often perturbed in cancer cells. However, snoRNAs might also be involved in the regulation of gene expression by giving rise to other regulatory RNA species, such as miRNAs. Of particular interest for future research are the orphan snoRNAs, the functions and targets of which remain unknown.

snoRNAs	class	changes	Proposed role	Cancer type
U50	C/D	Decreased	TS	Breast & Prostate cancer
H5sn2	H/ACA	Decreased	TS	Meningioma
RNU43	C/D	Decreased	TS	Breast cancer & HNSCC
RNU44	C/D	Decreased	TS	Breast cancer & HNSCC
snoRD43	C/D	Increased	OG	NSCLC
snoRD66	C/D	Increased	OG	NSCLC
snoRD76	C/D	Increased	OG	NSCLC
snoRA42	H/ACA	Increased	OG	NSCLC
snoRD44	C/D	Increased	OG	Breast cancer

Table 1. Representative snoRNAs involved in cancer and their proposed roles in tumorigenesis. TS: tumor suppressor; OG: oncogene.

snoRNAs as potential diagnostic and prognostic biomarkers in cancer

It has been shown that snoRNAs are stable and measurable in peripheral blood plasma and serum samples [9]. Cancer specific snoRNAs have the potential to become circulating biomarkers of cancer (**Table 1)** [9]. Increased levels of C/D box snoRNAs, SNORD33, SNORD66, and SNORD76, are detected not only in tumors but also in plasma, with observed sensitivity and specificity in distinguishing NSCLC patients. Measuring circulating plasma snoRNAs early will serve as potential noninvasive approach for the diagnosis of NSCLC [21]. Increased expression of H/ACA snoRNA SNORA42 in tissues correlates the unfavorable outcome of the disease and can be used for predicting the disease course [26]. Recent observation further supported the development of snoRNAs as potential biomarkers. The three C/D box snoRNAs, SNORD43, SNORD44, and SNORD48, seem to be suppressors of breast cancer and head and neck squamous cell carcinomas[27]. Likewise, snoRNA U50 encoded by introns of the non-coding host gene (U50HG) seems to be suppressor of breast and prostate cancer (**Table 1**) [22, 23]. SNORD113-1 is shown to be downregulated in hepatocellular carcinoma (HCC) and functions as a tumor suppressor in HCC [28]. Taken together, many snoRNAs have shown their potentials as biomarkers for both diagnosis and prognosis of malignancies.

snoRNAs as potential therapeutic targets in cancer

The molecular mechanism of snoRNAs functioning in cancer is still not fully understood. However, some snoRNAs are ideal candidates for therapeutic intervention. Silencing of transcriptional gene pathway mediated by snoRNA may be of therapeutic benefit; also, the use of RNAi·mediated gene silencing could be used for selective silencing of oncogenic snoRNAs. For example, snoRA42 is expressed highly in lung tumor tissues; silencing the expression of snoRA42 was tested in a lung cancer cell line [26]. Knockdown of snoRA42 significantly decreased proliferation and viability in NSCLC cells. These findings provide an evidence to indicate the potential in developing snoRNA·mediated therapies.

snoRNAs and other diseases

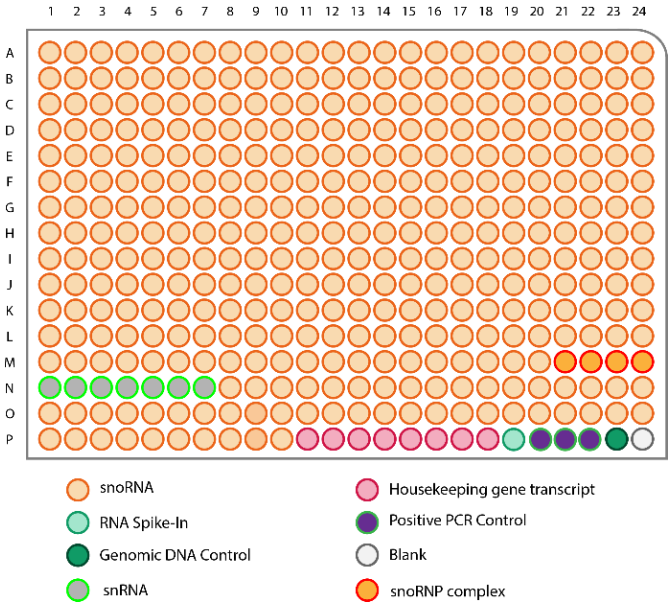
The relation between box C/D RNAs and neurodegenerative disease progression has also been described [29, 30]. In a series of independent studies of the genetic disorder, Prader Willi syndrome (PWS), was shown to be caused by the loss of paternal gene expression from a maternally imprinted region 15q11–q13 on chromosome 15. Locus 15q11–q13 contains numerous copies of two box C/D RNAs—SNORD115 (HBII·52) and SNORD116 (HBII·85) [31]. Box C/D RNA SNORD115 may have an impact on 5·HT2CR serotonin receptor mRNA level in brain [17].The loss of the SNORD116 snoRNAs can be a significant contribution to the etiology of PWS. A recent study revealed that the change in box C/D RNA level also takes place in brain cells during abnormal fetal development caused by maternal alcohol consumption during pregnancy .Particularly, the increase in SNORD115 and the decrease in SNORD116 snoRNA levels were demonstrated[32]. The observed change in SNORD115 level led to various psychic and behavioral aberrations typical of autism [33]. Recently, the first data on upregulation of the series of snoRNA genes in virus·infected human cells have been obtained[34]. On the one hand, snoRNA can act as mediators of host antiviral response; on the other hand, the activity of regulatory RNAs can be utilized by viruses to evade innate immunity and complete their life cycle[35].

C. Product summary

Kit Contents

Catalog Number	Contents
AS-NR-003-1	nrStar™ Human snoRNA PCR Array, dried down assays in 384-well plate, Package size of 1 plate

Figure 4 The array layout for nrStar™ Human snoRNA PCR Array



All 359 snoRNAs chosen for the array are from the most updated authoritative databases. The panel also includes four snoRNP complex members, seven target snRNAs, eight housekeeping genes for qPCR normalization, one RNA Spike-In for overall reactions efficiency, three positive PCR control (PPC) for PCR efficiency and inter-plate calibration, and Genomic DNA Control (GDC) for genomic DNA contamination detection.

Description of the control assays

nrStar™ Human snoRNA PCR Array includes a series of external and internal controls for effective correction and normalization of sample and qPCR variabilities. In addition, Positive PCR Control and Genomic DNA Control are included to monitor the experiment process and the quality of RNA sample. These controls are described below.

- **HK** (Housekeeping genes; Internal Controls): 8 human housekeeping genes ACTB, B2M, Gusb, Hsp90ab1, GAPDH, 5S rRNA, 28S rRNA and 18S rRNA are included as the internal qPCR normalization references. nrStar™ PCR system provides multiple reference genes selected among commonly used reference genes by

using a stringent bioinformatic algorithm, which offers the flexibility of choosing the most valid reference gene(s) for qPCR normalization for your sample types.

- **RNA Spike-In** (External Control): One External RNA Spike-In Mix is added in the RNA sample prior to the first strand cDNA synthesis. The RNA Spike-In control assay indicates the overall success and the efficiency of the reaction beginning from the cDNA synthesis to the final qPCR. Any problem(s) in these steps will result in a failed or compromised RNA Spike-In outcome. RNA spike-in assay results for samples are compared and outliers or failed reactions may be identified and excluded from further data analysis.

- **PPC** (Positive PCR control)**:** 3 replicates of one artificial DNAs and the PCR primer pairs to indicate the qPCR amplification efficiency. A Ct value greater than 25 is an indication of low qPCR amplification efficiency. More importantly, the PPC are used as inter-plate calibrator (IPC) between PCR plate runs to give the same Ct value for the calibrator, thereby reducing run-to-run variance. Inter-plate calibration (IPC) can easily be performed with the data analysis software avaliable on our website (www.arraystar.com).

- **GDC** (Genomic DNA Control): The control assay consists of PCR primers for an untranscribed genomic region. Non-RT sample or RNA sample are added during the qPCR Process. The Ct values should be greater than 35. A positive GDC signal indicates the array result may have been compromised with genomic DNA contamination.

Shipping and Storage

nrStar™ PCR Arrays are shipped at ambient temperature, on ice, or on dry ice depending on the destination and accompanying products. Store at –20°C upon receipt. The contents are stable for at least 6 months.

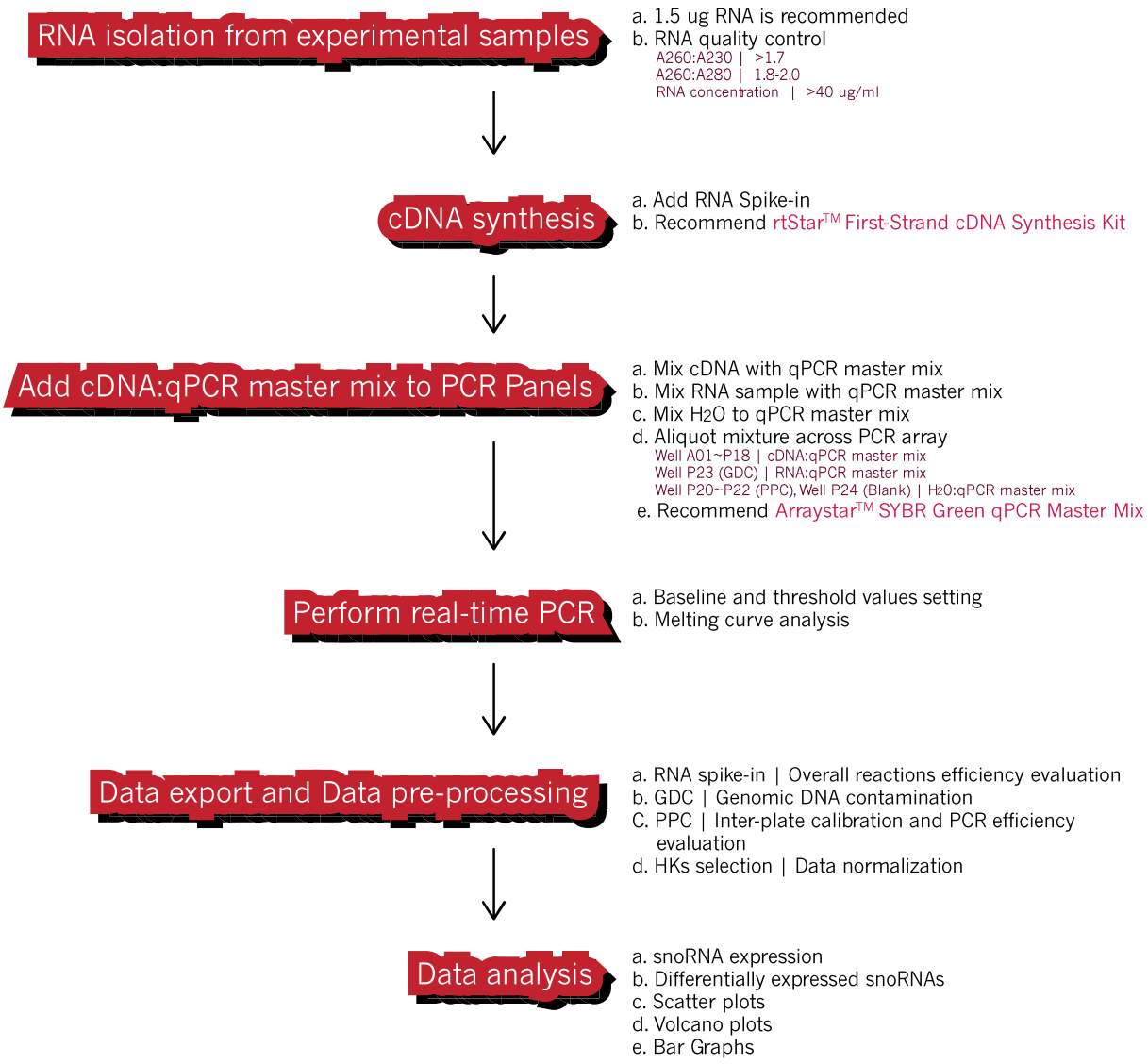
Additional Required Equipment

- Thermal cycler
- Real time qPCR instrument, compatible with 384-well format

Additional Required Reagents

- rtStar™ First-Strand cDNA Synthesis Kit (Cat# AS-FS-001)
- Arraystar™ SYBR Green qPCR Master Mix (Cat# AS-MR-006-5)
- Nuclease free PCR-grade water

D. Protocol overview



II. Protocol

IMPORTANT: Please read through this entire protocol before your first experiment. Prepare a workspace and use labwares free of DNA, RNA, and nuclease contamination. Wear gloves while performing this protocol.

A. RNA sample preparation and quality control

For best results from the PCR array, RNA samples must meet the QC standards of integrity and purity from protein, organics, genomic DNA contamination and excessive RNA degradation. You can measure the RNA concentration and purity by UV spectrophotometry. You can also check 18S and 28S ribosomal RNA as an indicator of RNA integrity by denaturing gel electrophoresis or by using an Agilent BioAnalyzer.

- A260:A230 ratio greater than 1.7.
- A260:A280 ratio between 1.8 and 2.0.
- Total RNA concentration greater than 40 ng/μl

Eliminating genomic DNA contamination is essential for accurate gene expression profiling by qPCR, which is particularly important for genes at low expression levels. Due to the presence of pseudogenes, even cross-intron primers are not a guarantee of avoiding amplification from contaminating genomic DNA. The Genomic DNA Control (GDC) in the PCR Array specifically detects potential genomic DNA contamination. A Ct for GDC less than 35 indicates the presence of genomic DNA contamination that may compromise the qPCR results

The recommended input total RNA amount is 1.5 μg. Lower amounts may reduce the assay sensitivity particularly for genes at lower expression levels.

B. First-strand cDNA synthesis

Use the same amount of total RNA in this reaction for every sample. High quality cDNA synthesis is vital for the following qPCR performance. We highly recommend using rtStar™ First-Strand cDNA Synthesis Kit (Cat# AS-FS-001), which is specifically optimized for and fully compatible with the nrStar™ PCR Arrays.

> **NOTE:** The first time to use this kit, please reconstitute the RNA spike-in by adding 20 μl Nuclease-free water to the tube. Mix by vortexing and spin down. Leave on ice for 20 ~ 30 min to fully dissolve the RNA spike-in. Vortex again, then spin down.

1. Mix the following components in a 200 μL PCR tube for each sample.

If performing first-strand cDNA synthesis on multiple RNA samples, it is recommended to prepare an Annealing Mix of the Primer, dNTP Mix and RNA Spike-in (in the proportion indicated in Table). 10% excess volume for pipetting losses is recommended.

Random Primers	1.0 µl
dNTP Mix	1.5 µl
RNA Spike-in	1.0 µl
Template Total RNA	10.5 µl
Total volume	14.0 µl

2. Incubate in a thermal cycler at 65°C for 5 min, and then immediately place on ice for at least 1 min. Collect the contents of the tube by brief centrifugation.

3. Add the following components directly to the product from STEP 2. The final volume will be 20 µL.

5 × RT Reaction buffer	4.0 µl
0.1 M DTT	1.0 µl
RNase Inhibitor	0.6 µl
Reverse Transcriptase	0.4 µl
Total volume	20 µl

4. Incubate at 25°C for 5–10 min, followed by 60 min at 50°C

5. Terminate the reactions at 85°C for 5 min. Hold the finished First Strand cDNA Synthesis Reaction on ice until the next step. OK to store overnight at -20°C.

C. Perform qPCR for the PCR array

1. Dilute the cDNA in nuclease free water. If 1.5 µg input RNA is used with rtStar™ First-Strand cDNA Synthesis Kit (Cat# AS-FS-001), the dilution factor is 1:80. Mix well and spin down. The diluted cDNA is used as the qPCR template in the wells for SnoRNA Transcript assays, Housekeeping gene Internal Controls, and Spike-in External Controls.

2. For GDC Controls, combine 1 µL NRT (no RT) sample or 1 µL RNA sample, 5 µL SYBR Green Master Mix, and 4 µL Nuclease-free water. Mix well and spin down

3. For Blank Controls, combine 25 µL SYBR Green Master Mix and 25 µL Nuclease-free water. Mix well and spin down.

4. Use Arraystar SYBR Green Real-Time Quantitative PCR Master Mix to prepare the qPCR Master Mix. There are total of 384 wells of PCR reaction. Some extra amount is included for consumption by the liquid dispensing operation. Prepare the cocktail according to the following table.

2010 μL	SYBR Green Master Mix
1600 μL	diluted cDNA template
390 μL	ddH_2O
4000 μL	total volume

5. Loading the 384-Well PCR Array

   a. CAREFULLY remove the plate seal from the PCR Array;

   b. Add 10 μL of the cocktail from STEP 4 to each PCR Array plate well (except well P20-P24);

   c. Add 10 μL GDC Mixture aliquot from STEP 2 into the wells P23 to detect genomic DNA contamination.

   d. Add 10 μL Blank Mixture aliquot from STEP 3 into the wells P20~P22 and wells P24.

   e. CAREFULLY but tightly seal the PCR Array plate with the optical adhesive cover. Be sure that no bubbles appear in any of the wells. To remove bubbles, tap the plate gently on the bench top or centrifuge the plate briefly.

   f. Keep the plate on ice while setting up the PCR program described in “Running Real-Time PCR Detection” below.

6. Running Real-Time PCR Detection

Cycles	Temperature	Time
1	95 ℃	10 minutes
40	95 ℃	10 seconds
	60 ℃	1 minute
Melting curve analysis		

D. Data pre-processing and data analysis

Pre-process the qPCR run data according to the qPCR instrument manufacturer's instructions. If using baseline and threshold method for Ct calculations, such as with ABI 7900HT, the optimal baseline and threshold manual settings applied consistently across all assays on the plate are preferred over the software automatic settings for better reliability and accuracy.

Inspect the melting curve analysis of the post-PCR products to verify the amplification specificity. If the melting curve has multiple peaks or poor peak morphology deviated from that of the known single amplicon product, it may indicate non-specific off-target amplification or primer dimer formation, which will compromise the quantification. In such cases, gel electrophoresis or DNA fragment analysis can be performed to verify whether the product is from a single correct amplicon.

Export the raw Ct values to Excel. Free, easy-to-use data analysis software is available online, please refer to www.arraystar.com for detailed instruction. The data analysis procedures include:

■ Data pre-processing

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

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.

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.

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 ≤ 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:

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

The Ct value is corrected with the calibration factor as

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

or

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

	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

5. Calculate the ΔCt for each RNA in the plate.

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

Where average (Ct_{HKs}) is the average of the Ct values derived from the multiple HK genes. 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.

Data analysis

1. Calculate the ΔΔCt for each RNA

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

or

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

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

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

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.

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 ≥ 2 and *p*-values ≤ 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 ΔCt, which is equivalent to approximately 1.5 fold change.

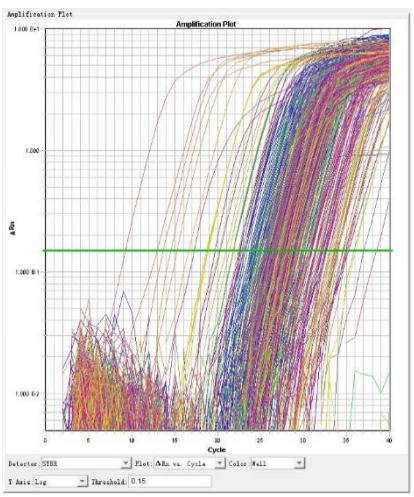
4. Other analyses such as scatter plots, volcano plots, and bar graph of expression differences for the snoRNAs are performed and included in the standard analysis package.

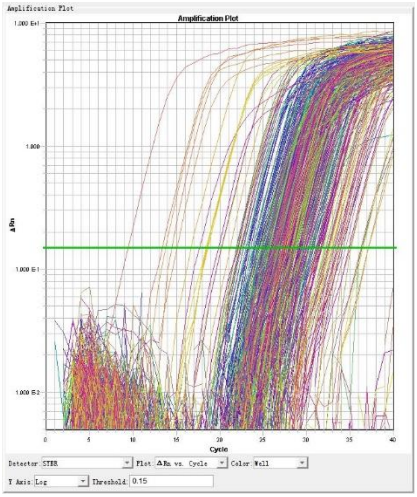
III. Quality Control and Sample Data

A. Human snoRNA PCR Array validation

Real-time qPCR Validation

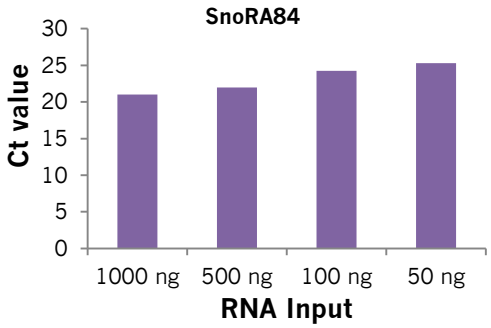
The performance of Human snoRNA PCR Panel was tested using a cohort of 10 cell line samples. The extracted RNA samples were converted to cDNA using rtStar™ First-Strand cDNA Synthesis Kit. The cDNAs were profiled using the PCR array according to aforementioned protocol without modification. The results are charted on the real-time amplification plots for the entire plate for the cell lines.

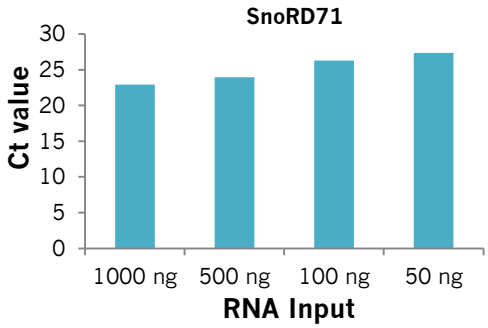




Sensitivity Test

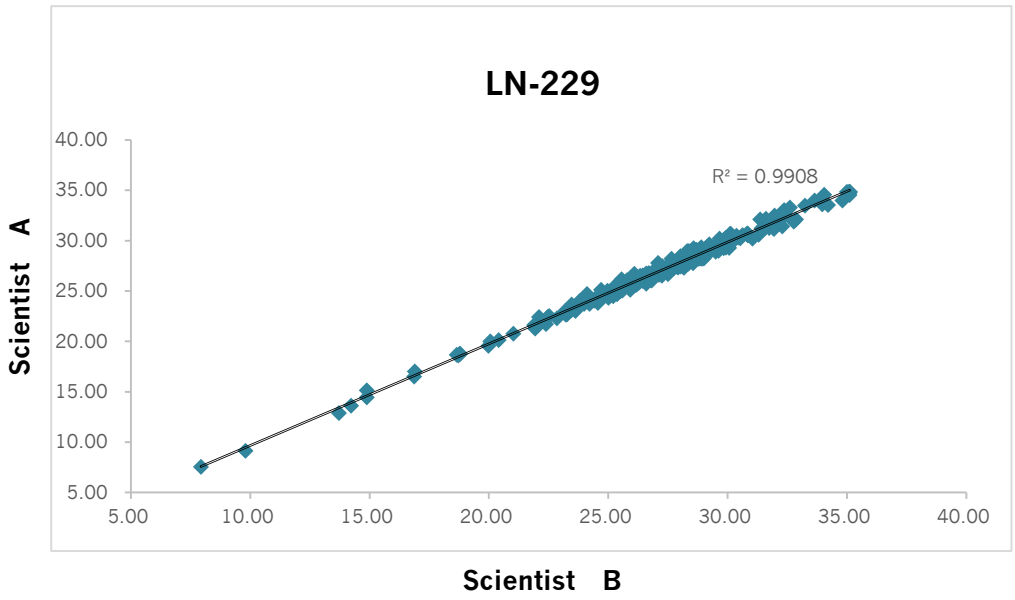
The decreasing input amounts of total RNAs from human glioblastoma cell lines were converted to cDNAs and profiled by the PCR arrays. The Ct values were determined using the software default automatic baseline and Ct settings. snoRA84 and snoRD71 transcripts were detected at Ct values of 25.2 and 27.0 respectively, with the input RNA amount as low as 50 ng.

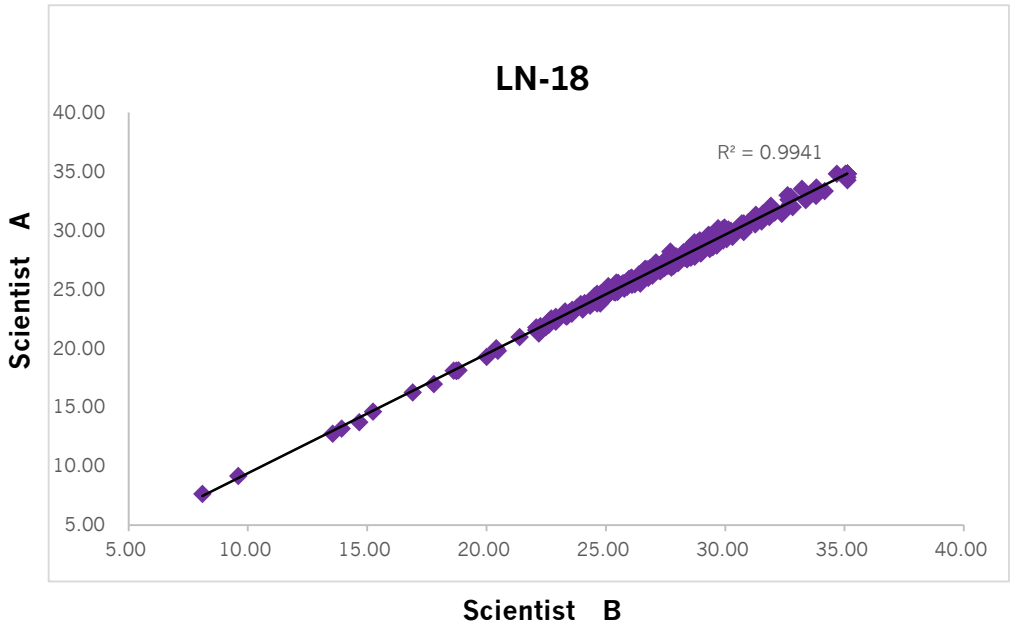




Reproducibility Test

Two independent runs of Human snoRNA PCR Array were conducted by two different scientists A and B at two different times using two different cell lines. The results demonstrate a high degree of reproducibility with correlation R^2>0.98.

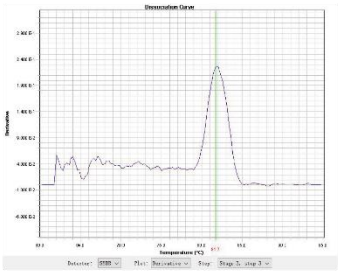




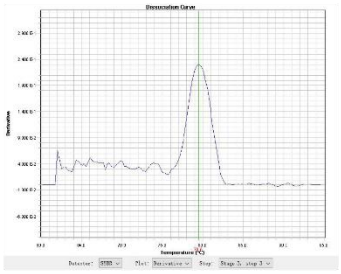
Specificity Test

The amplification products of transcripts and the isoforms of 9 snoRNA genes were analyzed by melting curves, all of which had single sharp melting peaks. The results demonstrate the high amplification specificities for the transcript isoforms with the assays on the array.

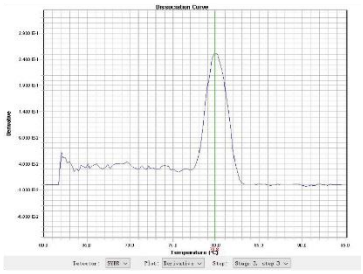
SNORA3



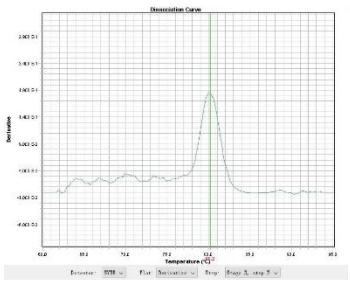
SNORA31



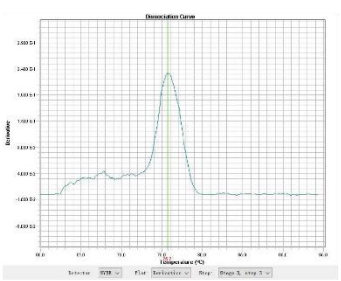
SNORA33



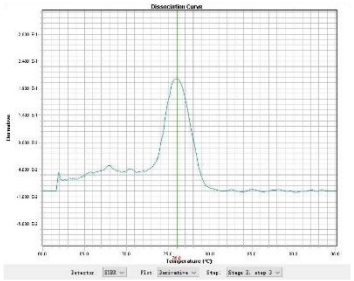
SNORA46



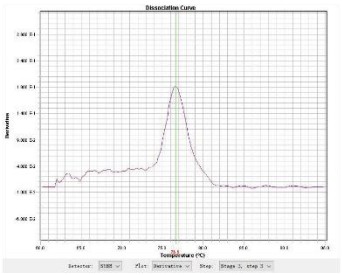
SNORD1B



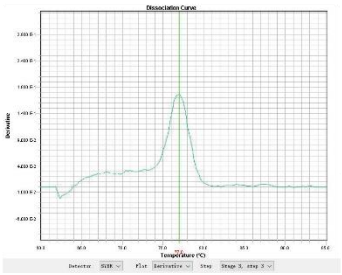
SNORD2



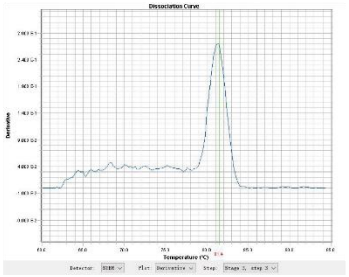
SNORD91A



SNORD105

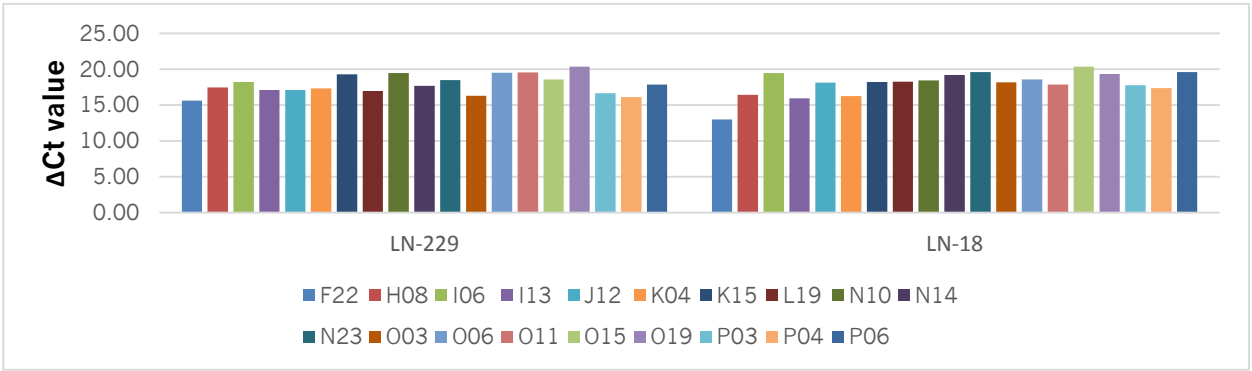


SNORD124



B. Sample data: Analysis of human snoRNA levels in cell lines

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. ΔCt for the snoRNA gene transcripts (well positions) in LN-229 vs. LN-18 cells are graphed in the bar chart below.



IV. Troubleshooting

Problem	Possible solution
qPCR background too high	• Reduce the amount of cDNA used in the SYBR Green Master Mix.
No qPCR signals	• Inspect if the Internal Controls have valid qPCR signal • Set SYBR Green as the Detector's Reporter Dye • Use more cDNA in the Master Mix • Lower the annealing temperature in Protocol STEP C.6 from 60°C to 50°C.
Baseline and threshold settings	• Follow the instructions of the qPCR system manufacturer. • Contact their technical support as necessary.

V. References

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VI. Technical Support

For additional information, manual download or technical assistance, please visit our website at www.arraystar.com, or contact us at:

Arraystar Inc.
9430 Key West Ave #128
Rockville, MD 20850, USA

Tel: 888-416-6343
Fax: 240-238-9860
Email: support@arraystar.com

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Arraystar warrants that the Product will meet the specifications stated on the technical data sheet for that product, and agrees to replace the product free of charge if the product does not conform to the specifications. Notice for non-conformity and request for replacement must be given within 30 days of receipt of Products. In consideration of the above warranty by Arraystar, the buyer agrees to and accepts the following conditions:

That the buyer's sole remedy shall be to obtain replacement of the product from Arraystar; and

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