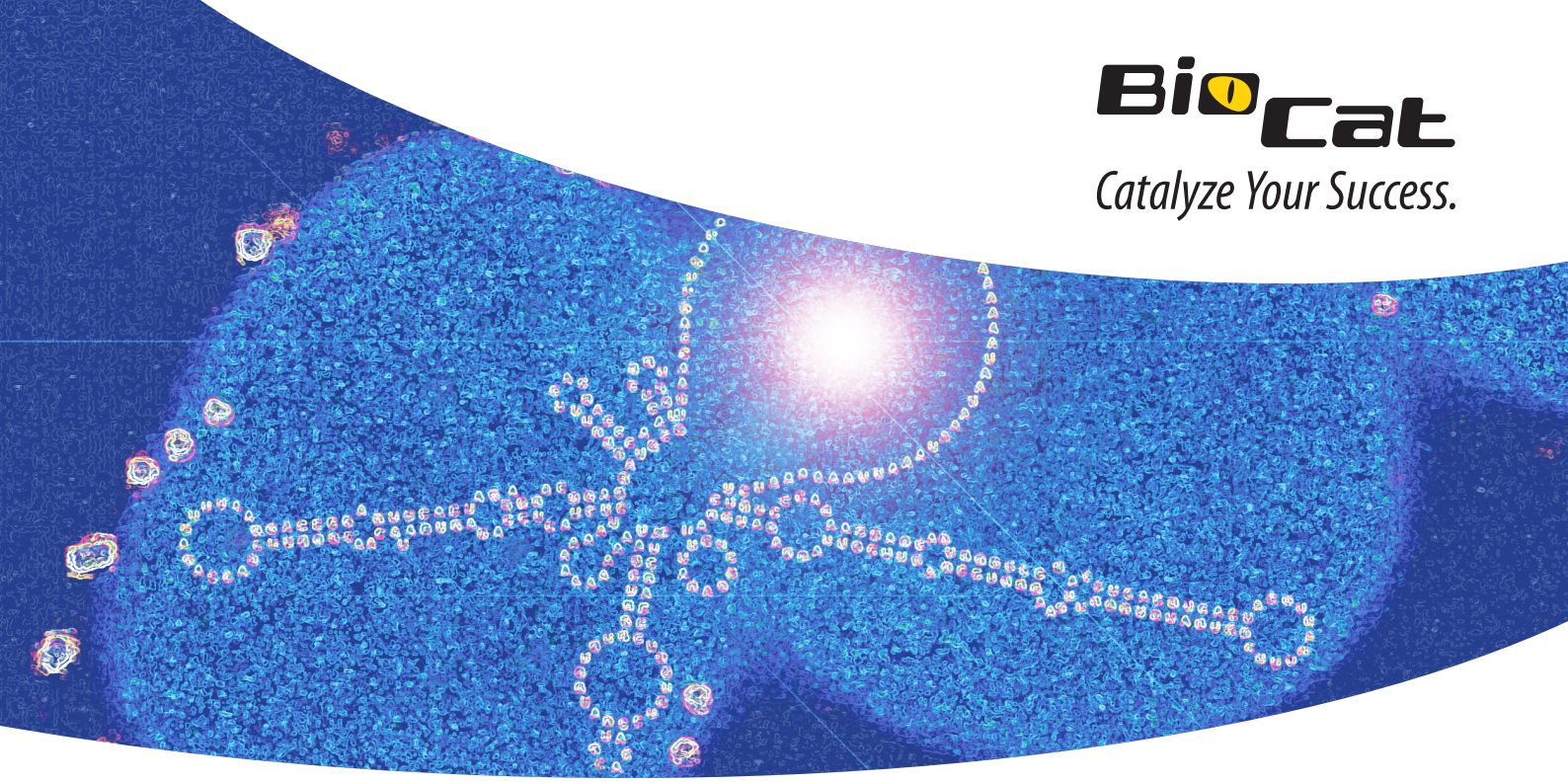
BioCat

Catalyze Your Success.



microRNA

microRNAs are a class of naturally occurring, small non-coding RNAs that control gene expression by translational repression or mRNA degradation. They modulate a variety of cellular pathways, including development, differentiation, cell proliferation, apoptosis, and multiple forms of cancer.

microRNAs are widely expressed throughout the plant and animal kingdoms and may comprise 1-5% of animal genes. Since the discovery of lin-4 and let-7 in *Caenorhabditis elegans*, over six thousand microRNAs have been identified through genomic and bioinformatic approaches. Innovative technologies for the purification, expression profiling, overexpression and knockdown of microRNAs are offered to enhance your research discoveries.

- microRNA Purification
- microRNA Expression Profiling
- microRNA Overexpression
- microRNA Knockdown
- Long Non-Coding RNA Profiling

Long Non-Coding RNA

Long non-coding RNAs (lncRNAs) are emerging as master regulators of embryonic pluripotency, differentiation, patterning of the body axis and promoting developmental transitions. LncRNAs are larger than 200 nucleotides in length and are pervasively expressed across the genome.

LncRNAs function as molecular scaffolds regulating histone modifications and influence the epigenetic programs of the transcriptome. Dysregulated expression of lncRNAs has been shown to be associated with a broad range of defects in development and diseases. Studying the expression patterns of lncRNAs will be a crucial method to understanding the roles they play in many model systems.

www.biocat.com/microrna

www.biocat.com/lncrna

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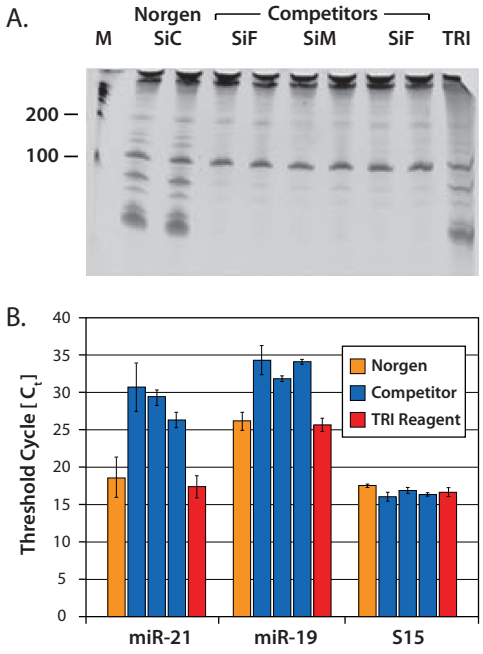
microRNA Purification

Total RNA Kit

- High-purity total RNA
- Complete RNA profile including microRNA
- Rapid spin column format, protocol completed in < 20min

The Norgen Total RNA Isolation Kit provides a rapid method for isolating total RNA of the highest purity from a wide range of tissues and cells as well as serum and plasma. The kit is based on a silicon carbide (SiC) resin and purifies all sizes of RNA, from large mRNA and rRNA down to microRNA, without the use of phenol/chloroform.
Both microRNA and mRNA can be studied from the same sample without further purifications, thus offering considerable advantages when comparing and relating expression of microRNA to other RNA.



Silicon carbide-based RNA purification isolates a complete profile of RNA species enabling microRNA detection by qPCR at low C_t values.
Total RNA was isolated from HeLa cells using RNA isolation kits with spin column formats based on silicon carbide (SiC), silica fiber (SiF) or silica matrix (SiM). Guanidinium thiocyanate/phenol-based TRI Reagent isolation was used as positive control for isolation of the complete size range of RNA. (A) Equal portions of the isolated RNA were resolved on an 8% Urea-PAGE gel. (B) The relative expression of miR-21, miR-19 and S15 rRNA was determined by qRT-PCR, the resulting threshold cycle (C_t) values are shown.

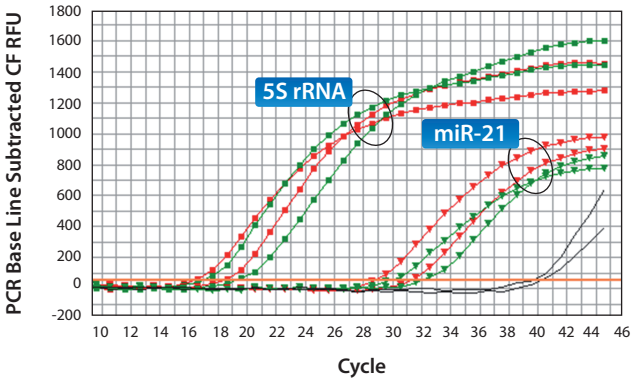
The Total RNA Purification Kit works very well with small sample inputs and does not require carrier RNA for the extraction. Total RNA can be isolated from as little as a single cell. The purified RNA is of the highest integrity and can be used in a number of downstream applications including real-time RT-PCR, RT-PCR, Northern Blot analysis, RNase protection and primer extension, expression profiling, microRNA cloning as well as amplification and Next Generation Sequencing.

www.biocat.com/rna_purification

Plasma/Serum Circulating RNA Purification Kit

- Purification of all sizes of circulating RNA incl. microRNA
- Versatile serum and plasma input volumes
- Convenient spin column format, processing time < 30 min

Free-circulating RNA in plasma and serum has the potential to provide biomarkers for certain cancers and disease states and includes tumor-specific extracellular RNA in the blood. It is usually present as short fragments of less than 1,000 nt, and includes free-circulating microRNA.
The Norgen Plasma/Serum Circulating RNA Purification Kit provides an efficient method for the purification of all sizes of these fragmented free-circulating RNAs from human plasma or serum (1-5 ml). The kit does not require extension tubes for the purification of free-circulating RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples, and the kit allows for the concentration of RNA that is present in low concentrations (1-100 ng/ml circulating RNA in human plasma).
The purified plasma/serum free-circulating RNA is eluted in a solution that is compatible with reverse transcription qPCR, reverse transcription PCR, Northern Blot analysis, RNase protection and primer extension as well as expression array assays.



Isolation and detection of circulating RNA from serum and plasma.
The Plasma/Serum Circulating RNA Purification Kit was used to isolate circulating RNA from 5 ml samples of serum and plasma that had been spiked with 1 x 10^6 HeLa cells. Five microlitres of the purified RNA were then used as template in qRT-PCR reactions to detect expression of the human 5S gene and the miR-21 gene. Both 5S rRNA and miR-21 could be detected from plasma (red and green triangles) and serum (red and green circles), indicating the high quality of the purified RNA. The black line corresponds to the non-template control.

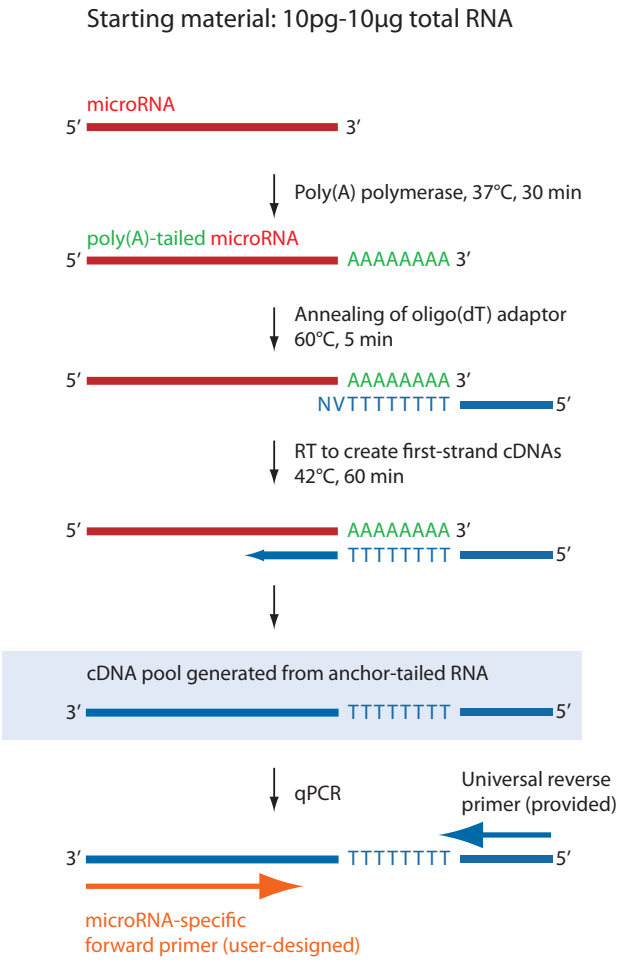
www.biocat.com/circulating_rna

microRNA Expression Profiling

QuantiMir Small RNA Quantitation System

- Rapid conversion of small RNAs into cDNA
- High cDNA yields even from picograms of input total RNA
- cDNA yield sufficient for 5,000 qPCR measurements

The QuantiMir Small RNA Quantitation System developed by SBI is designed to generate PCR-ready cDNA from total RNA in a robust manner. Highly efficient poly(A) tailing and reverse transcription in a single reaction tube provide uniform cDNA synthesis of microRNAs.
The optimized reaction conditions and buffer components maximize cDNA yield using anywhere from several micrograms to picograms of input total RNA. The universal 3′ tag sequence incorporated during reverse transcription enables the ease of scalable and accurate microRNA expression analysis by qPCR. The cDNA yield from one reaction is sufficient for 5,000 qPCR measurements.



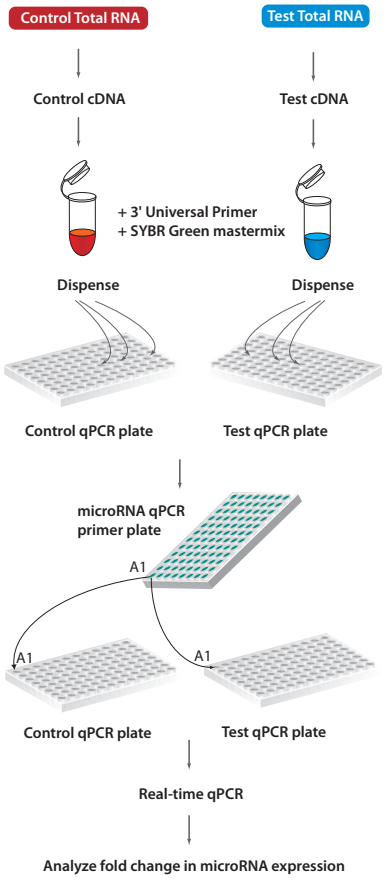
Conversion of microRNA into qPCR-ready cDNA.
First a poly(A) tail is added to the 3′ end of small non-coding RNAs in the presence of poly(A) polymerase. An oligo(dT) adaptor primer is used to initiate the subsequent reverse transcription reaction. The resulting cDNAs are ready-to-use for either end-point PCR experiments or to perform real-time qPCR analysis.

www.biocat.com/quantimir

miRNome microRNA qRT-PCR Arrays

- Genome-wide microRNA expression profiling
- High sensitivity and accuracy
- Dynamic detection range > 6 logs

The SBI microRNA qRT-PCR Arrays (human, mouse, rat) are based on the latest Sanger miRBase microRNA database entries. All microRNAs, including all miR star forms, are represented. The microRNA qRT-PCR Arrays are designed for the accurate microRNA expression analysis by real-time qRT-PCR. First, the sensitive QuantiMir cDNA synthesis technology is used to tag and convert small RNAs into quantifiable cDNA. Then microRNA expression is quantified applying the qPCR assays provided in pre-formatted 384-well plates for either the full compliment of human, mouse or rat individual microRNAs with three endogenous reference RNA controls on each plate. Pre-formatted microRNA qRT-PCR Arrays for profiling the expression of cancer-, stem cell-related or exosomal microRNAs are also available.



Expression profiling using microRNA qRT-PCR Arrays.

www.biocat.com/microRNA_detection

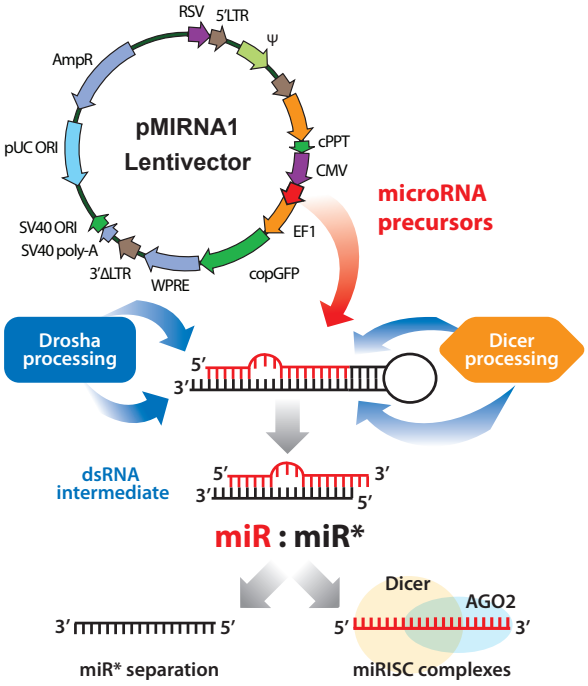
microRNA Overexpression

Lentiviral microRNA Precursor Vectors

- Overexpression of microRNA precursors
- Correct expression ensured by native context
- Individual or clustered Lenti-miR constructs available

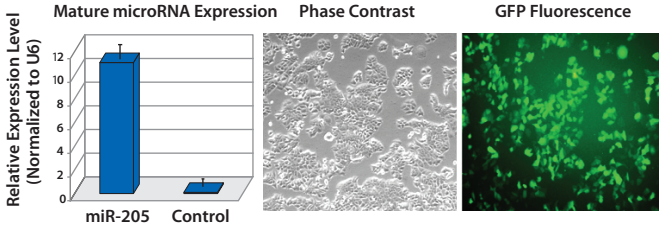
An extensive collection of microRNA precursors in lentiviral vectors* that can be used to modulate the expression of their targeted mRNAs *in vivo*, and thereby study microRNA function, is offered. The sequence-verified constructs are built in a lentiviral vector backbone, so they can be packaged into lentiviral particles and delivered to primary cells, stem cells or other hard-to-transfect cell lines. A pooled library of pre-packaged viral particles containing the entire microRNA precursor collection for high-throughput phenotypic screens is also available.

*in addition, microRNA precursor constructs are provided in retroviral, adenoviral or standard expression vectors.



Overexpression of mature microRNAs.

The SBI microRNA precursors consist of the native stem-loop structure and 200-400 bp upstream and downstream flanking genomic sequence ensuring stable expression and correct processing into mature microRNAs.



MCF-7 breast cancer cells transduced with Lenti-miR-205.

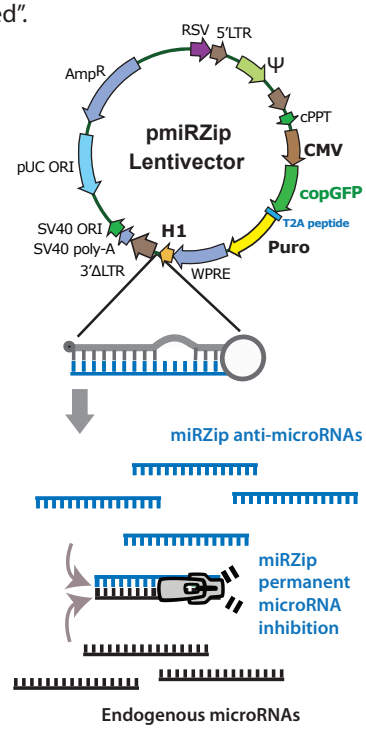
www.biocat.com/microRNA_precursors

microRNA Knockdown

Lentiviral miRZip anti-sense microRNA Constructs

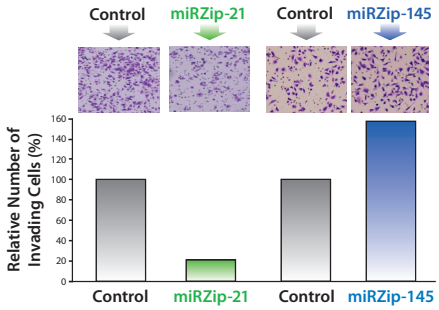
- Expression of RNAi hairpins with anti-microRNA activity
- Efficient and specific microRNA knockdown
- Reliable delivery to dividing and non-dividing cells

miRZip anti-sense microRNAs are stably expressed short hairpins that have anti-microRNA activity. These miRZip shRNAs produce short, single-stranded anti-microRNAs that competitively bind to their endogenous microRNA target and inhibit its function. The result is the derepression and elevation of the protein levels of the transcripts targeted by the microRNA being "zipped".



microRNA knockdown.

The SBI miRZip short hairpin RNAs are rationally designed for asymmetry such that the upper strand of the hairpin (in gray) does not contain the endogenous microRNA sequence and the lower strand produces anti-sense microRNAs (in blue) that are fully complementary to a specific microRNA target.



Tumor invasion assays using miRZips.

Inhibition of miR-21 decreases tumor cell invasion, while inhibition of miR-145 increases tumor cell invasion.

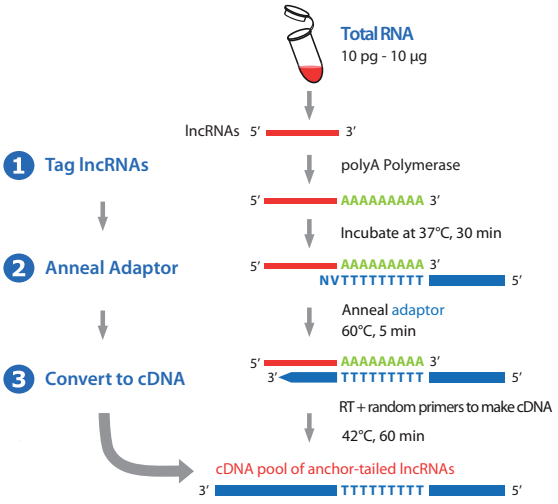
www.biocat.com/mirzip

Long Non-Coding RNA Profiling

Human and Mouse LncRNA Profiler qPCR Array Kits

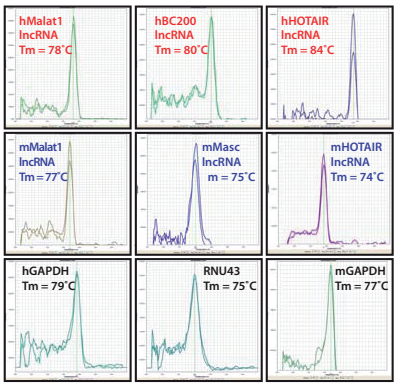
- Quantitate 90 cancer and stem cell lncRNAs
- Highly sensitive and specific real-time qPCR assay
- Provided as complete kit

Long non-coding RNAs (lncRNAs) function as molecular scaffolds regulating histone modifications and influence the epigenetic programs of the transcriptome. Dysregulated expression of lncRNAs has been shown to be associated with a broad range of defects in development and diseases. Studying the expression patterns of lncRNAs will be a crucial method to understanding the roles they play in many model systems. The Human and Mouse LncRNA Profiler qPCR Array Kits developed by SBI are based on a sensitive, accurate and robust qPCR array enabling researchers to closely profile the expression changes of the top lncRNAs known to date. They are provided as complete cDNA synthesis kits combined with a 96-well qPCR assay to quantitate 90 different lncRNAs, 5 positive housekeeping reference controls and one negative control.
All of the lncRNAs represented in the LncRNA Profiler qPCR Array Kits have validated primer sets for well-annotated lncRNAs that are registered in the lncRNA database created by Dr. John Mattick, see *www.lncrnadb.org*



cDNA preparation for lncProfiler qPCR Array analyis.

The qPCR assays have been validated across numerous cell types for robust and specific performance. Some lncRNAs have endogenouse polyA tails, while other lncRNAs do not. To enhance qPCR assay performance, the cDNA synthesis kit includes reagents to polyadenylate all lncRNAs before cDNA conversion using the tagged oligo(dT) adaptor and random primers. This combined RNA tailing and oligo dT plus random primers boosts cDNA yield significantly and enables strand-specific lncRNA qPCR profiling.

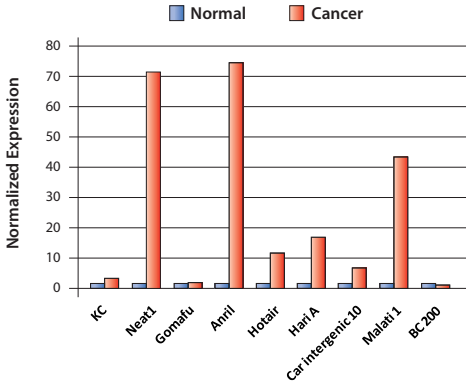


Various human and mouse lncRNA qPCR assay products were analyzed using standard dissociation analysis.

Disease-related Human LncRNA Profiler qPCR Array

- Quantitate 83 disease-related lncRNAs
- Highly sensitive and specific real-time qPCR assay
- Provided as pre-printed 96-well primer sets

Multiple lines of evidence increasingly link lncRNAs to diverse human diseases. The SBI Disease-related Human LncRNA Profiler qPCR Array allows for the quantification of differential expression of 83 individual lncRNAs among various experimental RNA samples. All 83 lncRNAs chosen for the array are based on publications and they are implicated in diseases ranging from neurodegeneration to cancer.



Profiling of lncRNAs in skin cancer.

The array plate also includes house keeping genes and small RNA transcripts for normalization purposes. Using real-time RT-PCR, you can easily and reliably analyze expression of a panel of lncRNAs that have potential roles in a variety of cellular processes such as cell cycle progression, differentiation, self-renewal and apoptosis. The Disease-related Human LncRNA Profiler qPCR Array is easy, convenient, sensitive and specific. It is suitable for cDNA synthesized by either random primer, oligo dT primer, or strand specific primer. It can be used for research in cancer, stem cells, immunology, biomarker discovery & validation, as well as phenotypic analysis of cells. The Disease-related Human LncRNA Profiler qPCR Array is available in 3 different plate formats, each tailored to a specific subset of real-time PCR instruments.

www.biocat.com/lncrna

BioCat

- Genome Engineering
- cDNA, ORF, shRNA and sgRNA Clones
- Antibodies and ELISA Kits
- Recombinant Proteins
- Cell-based Assays
- Stem Cell Research
- Metabolism Assays
- microRNA Analysis
- Epigenetics
- Exosome Research
- Sample Preparation
- PCR, Sequencing and Cloning
- Lenti-, Retro- and Adenoviral Systems
- Tissue-specific Analysis

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