

SBIINSIGHTS

**Against the Central
Dogma: The
Involvement of Non-
Coding RNAs**

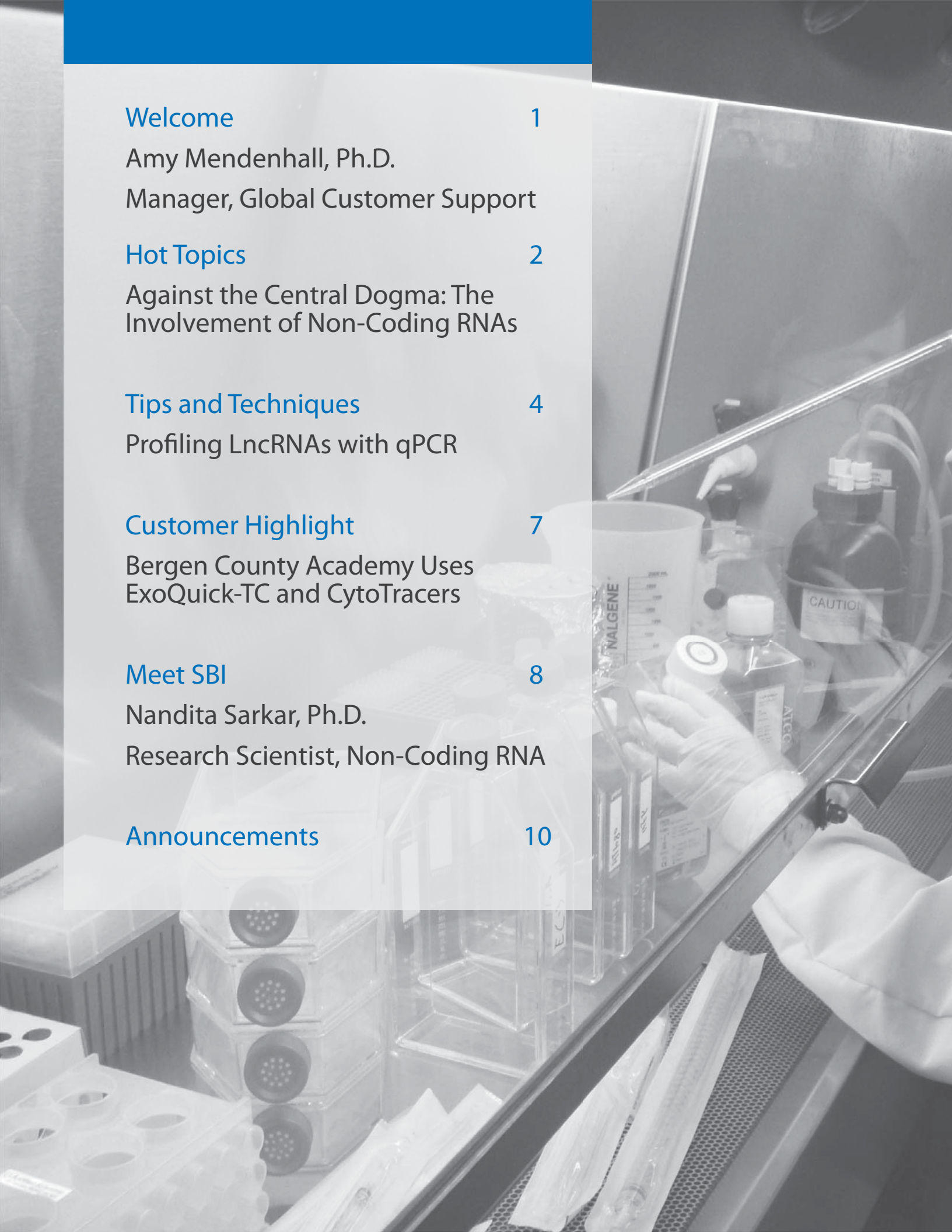
**Profiling LncRNA with
qPCR**

**Bergen County
Academy in New Jersey
Uses ExoQuick and
CytoTracers**

Issue.

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ACCELERATING DISCOVERIES THROUGH INNOVATIONS



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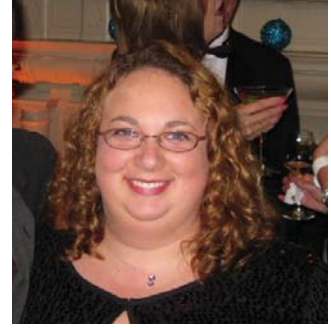
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Dear SBI Customer,

Welcome to the 4th volume of SBI's quarterly e-newsletter, **SBINSIGHTS**. The focus of this issue is non-coding RNAs. We have a series of articles highlighting new methods for studying non-coding RNAs that are available from SBI.

SBI has also recently launched a new **SBIinsiders** VIP customer program. Look inside to find out how you and your lab can become an **SBIinsiders**.

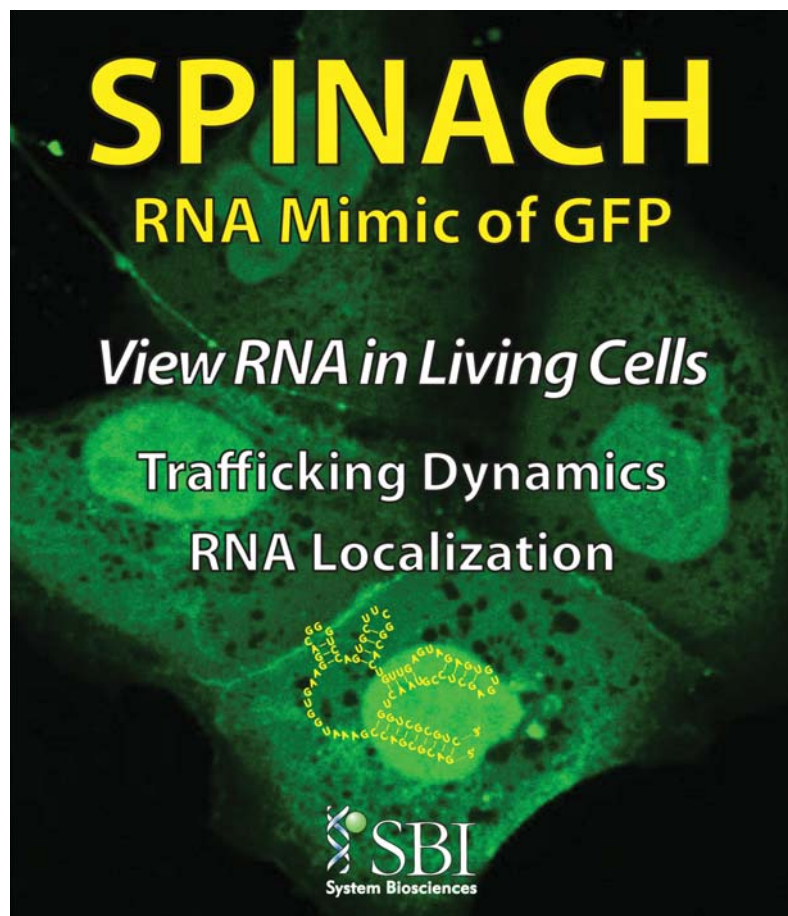
If you have anything that you would like to contribute to **SBINSIGHTS**, please contact tech@systembio.com. We would enjoy hearing your comments and questions. If you have published a paper or presented your work at a meeting or conference using products or services from SBI, let us know! We would love to hear your feedback and may even feature your work in a future volume.



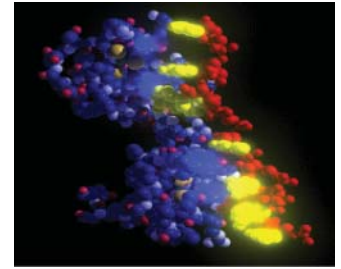
We hope you enjoy reading **SBINSIGHTS**.

A handwritten signature in black ink that reads "Amy Mendenhall". The signature is written in a cursive, flowing style.

Amy Mendenhall, Ph.D.
Manager, Global Customer Support



Against the Central Dogma: The Involvement of Non-Coding RNAs



For the last few decades of the 20th century, the underlying dogma of molecular biology has been that the purpose of RNA is to direct the assembly of proteins from amino acids. A few exceptions to this paradigm were known (for example, ribosomal RNA and transfer RNA, which are functional RNA macromolecules that do not code for protein, or viral genomes that exist as or pass through an RNA phase as part of total genome replication). However, more recent studies have uncovered a new role for RNA that is involved more indirectly in protein synthesis. Thousands of RNA species are transcribed in the genome that serve as regulatory elements in the genome without coding for proteins to exert their effects. These RNAs are collectively known as non-coding RNAs (ncRNAs). They appear to be numerous in the genome, and while remarkable functions have been described for some, most are still of unknown importance to gene regulation.

ncRNAs include the familiar “housekeeping” RNAs (ribosomal, transfer, small nuclear, and small nucleolar RNAs) and the thousands of regulatory RNAs that are the subject of recent intense exploration. Regulatory ncRNAs are arbitrarily classified by size: small ncRNAs (sncRNA) being less than 200 bp, and long ncRNAs (lncRNA) greater than 200 bp. The sncRNAs include other sub-classifications: microRNA (miRNA), endogenous small inhibitor RNA (endo-siRNA), and PIWI-associated RNA (piRNA). miRNAs have a further characteristic that define them as a group, a universal requirement for posttranscriptional processing by a complex of enzymes and cofactors, including the protein Dicer. Subgroups have not been defined for lncRNAs, and unlike miRNAs there does not appear to be a modification process common to all lncRNAs.

The roles of lncRNAs in the regulation of gene expression and organismal development are diverse and just beginning to be discovered. Biological processes dependent upon lncRNAs include imprinting and gene dosage regulation, stem cell pluripotency, embryonic development and segmentation, hematopoiesis, and neural cell fate determination. lncRNAs may employ a number of mechanisms to impact gene expression via cis and trans processes.

Gene imprinting

While the function of parental gene imprinting is still unclear, lncRNAs have been found to participate in imprinting processes. Imprinting Control Regions (ICRs) are DNA regions that are differentially methylated depending on their parental origins. Unmethylated ICRs cause specific expression of nearby lncRNAs, which then suppress neighboring genes in cis. *Air* and *Kcnq1ot1* are examples of lncRNAs that cause suppression of paternally inherited genes.

Gene dosage and X chromosome inactivation

The discovery of *Xist* was one of the defining moments in the realization that ncRNAs can have profound roles in the control of gene expression. *Xist* is an lncRNA that suppresses the inactive, non-coding X chromosome (Xi) in female cells. In all, 7 ncRNAs are found as part of the X inactivation center on the X chromosome, including *Xist*. Initially, *Xist* and its antisense transcript *Tsix* are expressed on both X chromosomes. However, *Tsix* expression continues on the X that will remain active (Xa) and this activity recruits DNMT3A to suppress *Xist* from being transcribed on Xa. On Xi, it is *Tsix* that is suppressed, potentially via another lncRNA that is part of the X inactivation center, *Jpx*. With *Tsix* suppressed, the protein PRC2 is recruited to induce histone modification marks at the 5' end of *Xist*. This upregulates *Xist* expression on Xi and causes further propagation of these silencing marks throughout Xi, which are maintained across the lifetime of the organism.

Flies have developed a different, essentially reverse approach to balancing gene dosage in males that still depends upon lncRNAs. roX1 and roX2 are lncRNAs that upregulate gene expression on the male X chromosome via activation of histone H4 acetylation. Unlike Xist and Tsix, these lncRNAs appear to have functionality in trans, as transgenic absence of roX1 and roX2 can be functionally rescued by their exogenous replacement. Additionally, flies have hundreds of binding sites for these lncRNAs throughout the X chromosome with differing affinities, suggesting they can exert their upregulatory effects to differing degrees.

Embryonic development and segmentation

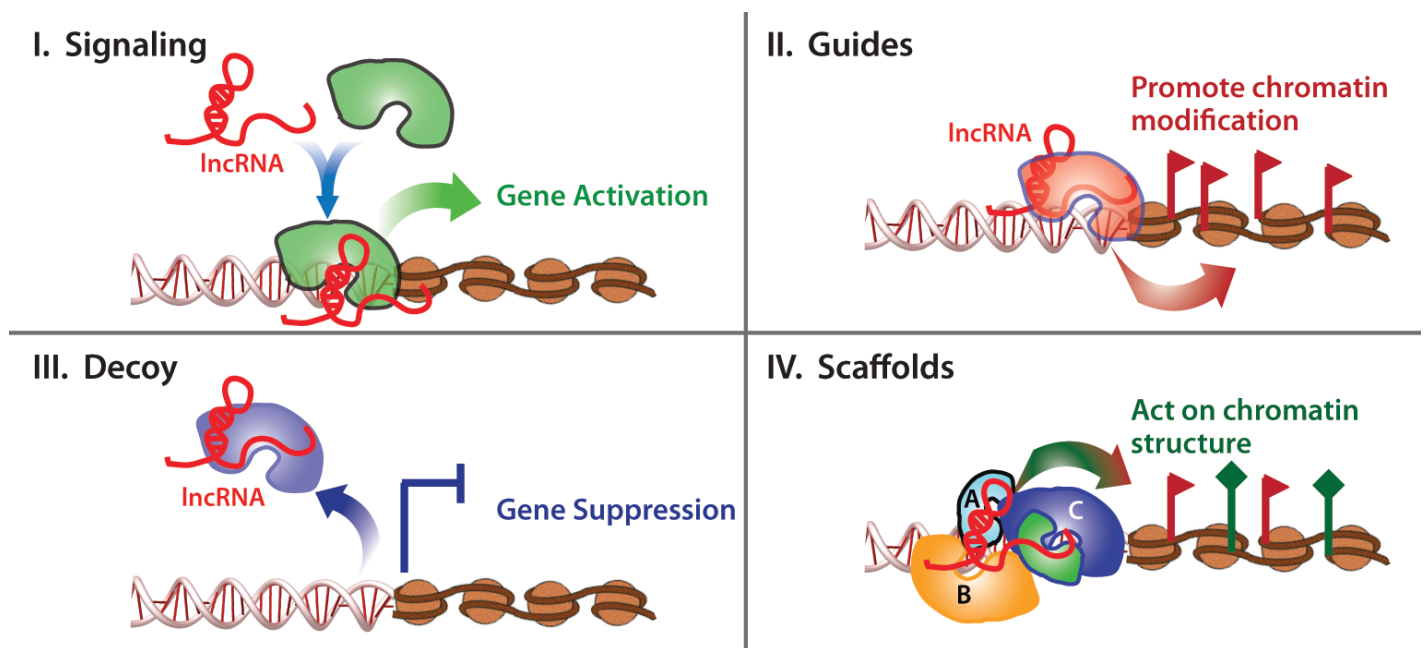
The expression of HOX genes is also regulated by lncRNAs. Some HOX-related lncRNAs operate in cis, having either enhancing or repressive effects. However, some like the human HOTAIR work in trans, and may function as scaffolds for histone-modifying complexes. It is not clear if trans-acting lncRNAs like HOTAIR are involved in the process of identifying the DNA sites to which the complexes will be recruited, or if that function is retained by the protein elements of the complex.

Stem cell pluripotency

The promoters of more than 100 lncRNAs are bound by stem cell factors. Disruption of these lncRNAs can alter cell differentiation. lincRNA-RoR is involved in the reprogramming of fibroblasts back to a pluripotent state. Thus, lncRNAs are likely to play important roles in both normal development and processes that require maintenance of adult stem cell pools.

Cell fate determination

lncRNAs are implicated in cell fate determination events in multiple cell lineages, including the nervous system. TUG1 is an lncRNA that may enhance rod gene expression and suppress cone gene expression in the developing eye. Evf2 is a mouse lncRNA that appears to have both cis and trans effects to repress Dlx5, Dlx6, and Gad1 during forebrain development.



Graphic adapted from: Wang, KC and Chang HY, Molecular Mechanisms of Long Noncoding RNAs. Mol Cell. 2011 Sep 16;43(6):904-14.

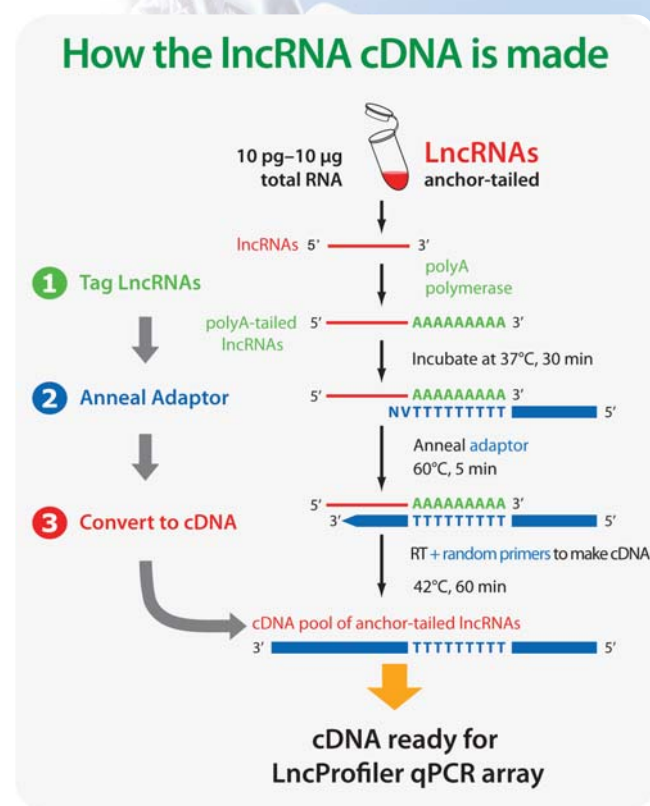
Profiling Long Non-coding RNAs using qPCR-based Methods

In order to help researchers study expression of long non-coding RNAs (lnc RNAs), SBI has developed two different lncRNA profiling kits. These kits can be used to study expression lncRNAs in different tissues, disease states, or treatment models. You can even study lncRNAs in exosomes isolated from biological fluids by combining the use of ExoQuick or ExoQuick-TC, followed by lncRNA profiling.

Cancer and Stem Cell Lnc Profilers

The Cancer and Stem Cell Related Lnc Profiling kit combines a cDNA synthesis kit with a 96-well assay that allows you to profile 90 lncRNAs and 5 internal controls. After you extract total RNA from your cells,

a polyA tail is added to the RNA. (lncRNAs, microRNAs, and mRNAs are all tailed in this step, enabling you to profile several types of RNA from one sample.) Next, a polyT adaptor is added that also contains a flanking sequence that allows for strand-specific lncRNA profiling. Last, the RNA is converted to cDNA by a combination of reverse transcriptase and random priming, which boosts cDNA yield and enables strand-specific lncRNA profiling.



The lncRNA primers are provided in a 96-well plate as stock primers. All of the lncRNAs on the LncProfiler™ qPCR array have validated primer sets for well-annotated human lncRNAs that are registered in the lncRNA database created by Dr. John Mattick. The lncRNA profiling begins by adding the produced lncRNA cDNA, 2xSYBR Green, and the lncRNA primers provided to your favorite qPCR plate. The delta-Ct values obtained from the qPCR run can be normalized against the 5 internal positive controls found on each plate, and compared across samples. Up to 20 samples can be profiled from one kit, and you can purchase the kit either with or without the SYBR green, depending on your needs. The figure on page 5 shows a diagram of the workflow used in the Cancer and Stem Cell LncProfilers. The total workflow takes about 1 day.

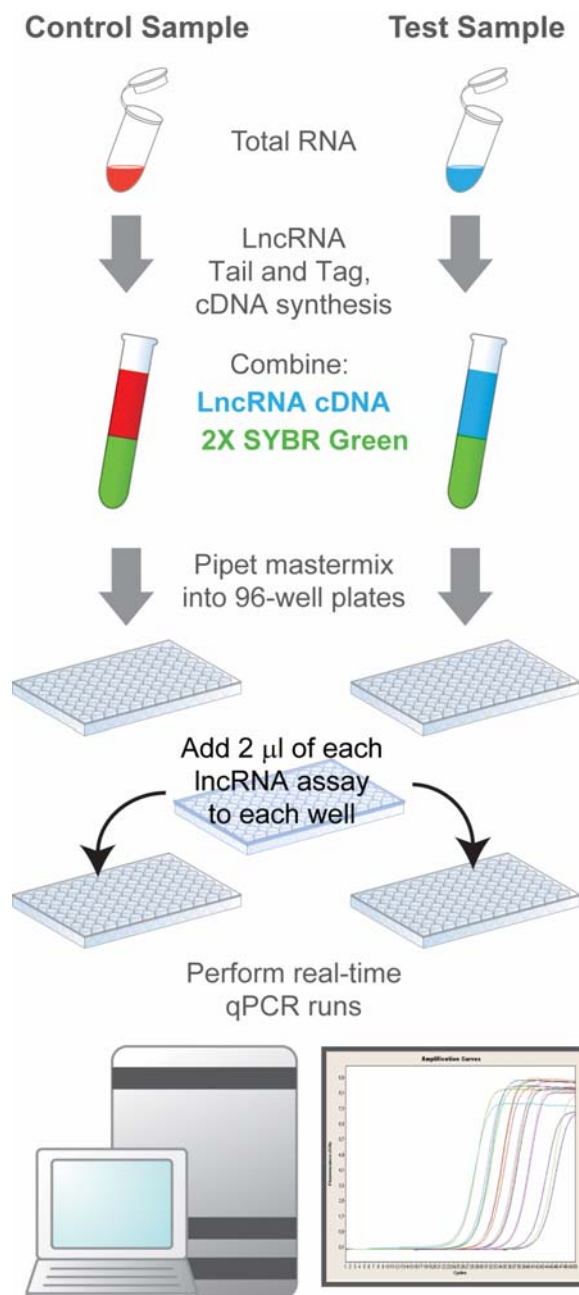
One trick to studying lncRNAs is that some lncRNAs are antisense, meaning that they lie on the "negative" or bottom strand of DNA. In several cases, when the lncRNAs are produced within the cell, the antisense region is not produced as abundantly as the sense region. Moreover, the sense and antisense regions of the lncRNA are overlapping each other. This can cause problems in detecting the antisense lncRNAs in your samples, as random primers cannot discriminate top, sense strands with bottom, antisense strands. Luckily, the lncRNA primers on the LncProfiler™ qPCR array are strand-specific. The forward primer has been designed to a specific strand of the lncRNA cDNA produced. The reverse primer has been generated to the adaptor sequence. This enables you to detect differences in expression of lncRNAs that are only present on one strand of the cDNA.

For a full list of the primers available for the Cancer and Stem Cell Lnc Profilers, please see the SBI website: <http://www.systembio.com/lncrna-research/long-non-coding-rna/array-details>



Disease-Related Human LncProfiler

The Disease-Related Human LncProfiler kit contains 83 primer sets for studying human LncRNAs, along with 12 primer sets for internal controls and housekeeping genes. Each primer set has been designed according to several individual peer-reviewed literature references that demonstrate the importance of lncRNAs in diseases such as neurodegeneration and cancer.



Cross-compare $\Delta\Delta C_t$ measurements between Control and Tumor Samples

The workflow for the Disease-Related Human LncProfiler kit starts with extraction of RNA from your cells. Using your own cDNA synthesis reagents and techniques, you then create cDNA from the extracted RNA. (The Disease-Related Human LncProfiler does not come with the cDNA synthesis reagents). There is no tailing and tagging step involved in the Disease-Related Human LncProfiler kit, which decreases the time involved in creating the cDNA used in the qPCR reactions by about 30 minutes.

The lncRNA primers are provided as a 96-well plate as stock primers. You add the produced cDNA to a qPCR reaction, containing the Disease-Related Human LncRNA primers. Up to 20 samples can be profiled from one stock primer plate. The total workflow for the protocol takes about 1 day to complete.

Both the forward and reverse primers contained within the Disease-Related Human LncProfiler kit are designed for a specific lncRNA. Since there is no tagging and tailing reaction, the kit cannot detect strand-specific lncRNAs, but can detect overall changes within a lncRNA region.

The Disease-Related Human LncProfilers are also available in small sizes (2 profiling reactions), pre-aliquoted onto qPCR plates for specific qPCR machines.

For a full list of the primers available for the Disease-Related Human LncProfilers, please see the SBI website:

<http://www.systembio.com/lncrna-research/disease-long-non-coding-rna/array-details>

Easy Data Analysis

Both the Cancer and Stem Cell LncProfilers and the Disease-Related Human LncProfiler come with a free data analysis spreadsheet that allows you to plug the deltaCt values in from your qPCR run into the spreadsheet. The corresponding geometric mean normalization values and fold change levels are computed and graphed automatically for your convenience.

Comparison of LncProfilers from SBI

SBI has chosen to offer these 2 different formats of LncRNA profilers so that our customers can choose the kit that works the best for their specific research. We recommend the Disease-Related Human LncProfilers for researchers who prefer to use their own reagents for cDNA synthesis, or for researchers who are looking for a quick way to profile LncRNAs at the start of a project. We recommend the Cancer and Stem Cell LncProfilers to researchers who need a more robust assay that is capable of differentiating between strand-specific LncRNAs, or who want a more standardized complete kit. SBI recommends that LncRNAs that show changes in expression in your biological system be further characterized.

| | Cancer and Stem Cell LncProfilers | Disease-Related Human Lnc Profilers |
|-----------------------------------|--|--|
| Species Specificity | Human, Mouse (coming soon) | Human |
| cDNA Synthesis Reagents | Provided | Not Provided |
| Strand-Specificity | Yes. Able to detect both sense and anti-sense LncRNAs if present. | No. |
| Internal Controls Provided | 18S rRNA, RNU43, GAPDH, LAMIN A/C, U6, negative control | 7SL scRNA, 5.8S rRNA, U87 scaRNA, ACTB, B2M, PGK1, GAPDH, HPRT1, RPL1A, RPL13A, negative control |
| Length of Workflow | 1 day | 1 day |
| Validated Primer Sets | Yes. Primer sets based on LncRNA database created by John Mattick | Internal validation of some primers performed at SBI. |
| Other Benefits | <p>Can be used in conjunction with SBI's microRNA profiling assays to simultaneously profile microRNAs and LncRNAs from the same sample.</p> <p>Can be used in conjunction with ExoQuick or ExoQuick-TC to monitor LncRNA expression within exosomes.</p> <p>Individual primers for validating single LncRNAs can also be made available upon request.</p> | |
| Catalog Numbers | RA900A-1/ RA910A-1/ RA910B-1 | RA920A, B, C, and D-1 |

Bergen County Academy in New Jersey Uses ExoQuick and CytoTracers to Study Mitochondrial DNA Transfer



Recently SBI was contacted by a new customer, Donna Leonardi, science teacher from the Bergen County Academy in Hackensack, New Jersey. The Bergen County Academy is a public, magnet high school servicing the county of Bergen in New Jersey. Ms. Leonardi leads a group of high school students competing for a place in Intel's International Science and Engineering fair, which is the world's largest pre-college science and engineering fair. One of her students, Ysa Esquilin, was doing a project on exosomal transfer and they were looking for products and advice on how to study exosome-mediated gene transfer between cells.

SBI's technical support staff recommended two products to her: 1) ExoQuick-TC, which is SBI's proprietary exosome precipitation solution for use in tissue culture media and 2) the CD63- CytoTracer, which has a GFP tag linked to CD63, which is an exosomal marker. By combining the use of these two products, Ms. Esquilin was able to show successful transfer of exosomes from HT1080 cells to a fresh plate of HT1080 cells. Additionally, she was able to show successful exosome transfer from her cell-line of interest and that the exosomes contained mitochondrial DNA. The mitochondrial DNA was also transferred into the target cells by the exosomes. SBI's technical support staff also assisted by providing protocols for using the CytoTracer, ExoQuick-TC, and electron microscopy of the precipitated exosomes.

" Thanks so much for all of your help. I will be working feverishly with the students over the next few weeks to ensure they finish their college applications and their INTEL Science Talent Search research submissions. Your graciousness is noteworthy and you should be applauded for helping promote science research in our youth. I will continue to use your company for our research program as you seem to have the same mission and vision as our school." --Donna Leonardi (pictured above)

SBI wishes Ms. Esquilin a successful start to her career in research and commends the Bergen County Academy for providing an excellent education in science and technology to our future scientists!

Introducing SBInsiders Club!

The SBInsiders Club is SBI's new frequent customer incentive program. Members enjoy benefits such as free shipping on handling, 10:00 am delivery, priority order processing, advance notification of upcoming products, individualized webinars, and private technical support. Contact your sales representative to find out how your lab can enroll in the program.

<http://www.systembio.com/company/salesreps>

(Membership is open to US customers only at this time.)



Meet Nandita Sarkar, Ph.D.

Dr. Sarkar is a Research Scientist in our Non-coding RNA Department and has been working here since April 2009.

Where did you do your training?

I completed my graduate studies at University of Nebraska-Lincoln. My research was focused on understanding the molecular mechanisms of DNA repair and gene silencing by small RNA in unicellular algae *Chlamydomonas reinhardtii*. After my graduation, I joined the Gladstone Institute at the University of California San Francisco for my post-doctoral training where I worked on understanding the role of post-transcriptional modification of HDAC7 and MF2D during T cell activation.

How did you get interested in science and research?

As a little girl, I got excited about science because I grew up in an industrial township where many of my father's colleagues were scientists. I used to visit their labs and used to find colorful liquids in beakers and test tubes very fascinating. My undergraduate professor also had a very strong influence on me to decide on my career path. I had the opportunity to work with him in a plant tissue culture lab and he taught me the nuances of research and instilled in me a strong sense of scientific curiosity.

What do you like about working at SBI and what do you do there?

I like the work culture at SBI as it encourages research and fosters innovation and creative thinking. Here, I find a collaborative spirit and yet have freedom to work on projects from conception to completion very exciting. Most importantly, I value the strong relationships and support of my colleagues.

We want to hear from you!

Would you like to be featured in SBI's next newsletter? Send us a description of your work, what products or services are your favorite from SBI, and some photos. We will review your submission and contact you when we are ready to feature you and your lab!

Would you like to join the SBI team?

SBI is currently hiring a Technical Support representative.

SBI is located in Mountain View, California in the heart of Silicon Valley and is a growing company.

Qualified candidates must have a Ph.D. in molecular biology or a similar field, be fluent in English, and have a willingness to help customers. Excellent verbal and written communication skills are required for the position. Applicants must be reliable, organized, punctual, self-motivated, friendly, courteous and willing to work with customers and colleagues. Job duties include technical assistance for customers and potential customers by phone and email, communication with Research and Development concerning customer feedback, and interaction with the Sales and Marketing teams. Previous teaching experience or technical support experience are desirable, although not required.

Applicants must be able to work on-site at SBI during regular business hours. If interested, please submit your resume or CV to amendenhall@systembio.com.



SBI's 2011 Holiday Party



Top Left: General Manager, Joseph Huang and his wife. Above: (left to right), Sayoni (summer intern), Chandreyee (Production Team), Rohini (fall intern), Peggy (Customer Service), Ranjita (Production and Custom Services Manager), and Nandita (MicroRNA Department). Left: Jane (Shipping), and Fangting, (Cloning Scientist).



LIVE WEBINARS

All webinars are free and open to the public. Space is limited, so register on-line at:
<http://www.systembio.com/webinars>

Advances in Induced Pluripotent Stem Cell Research Tools

Thursday, February 2, 2012, 10:00 AM Pacific Time

This webinar on iPS cell technology is for people who are new to iPS cell culture techniques and who would like to learn more about the basics of iPS techniques. Attendees will learn the various ways of reprogramming source cells to iPS cells. They will be able to understand the pros and cons of different iPS reprogramming methods and become familiar with human iPS cell culture techniques. We will also learn how to successfully characterize iPS cells.

Monitoring Differentiation and Transcriptional Activity with SBI's Lentivector Reporter Constructs

Thursday, Feb 9, 2012, 10:00 AM Pacific Time

Thursday, Feb 16, 2012, 8:00 AM Pacific Time

This webinar will talk about the different reporter systems available from SBI. We will talk about the benefits of lentiviral constructs, construct design and validation, transduction into target cells, experimental best-practices, and creation of reporter cell lines.

Long Non-coding RNA Profiling and Spinach, RNA mimic of GFP

Thursday, March 1, 2012, 10:00 AM Pacific Time

Long noncoding 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. Join us for a fascinating discussion on LncRNAs and learn more about SBI's new sensitive, accurate and robust qPCR arrays – The LncRNA Profilers.