Applied Biosystems

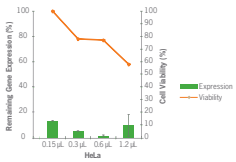
Using siRNAs to Delineate Gene Function: A Step by Step Guide

With RNA interference, you can elucidate gene function in a matter of days—but utilizing the right protocol with optimized reagents is essential. Applied Biosystems and Ambion, now an Applied Biosystems Business, provide the most complete suite of validated, quality reagents for RNA interference experiments available anywhere. Our potent, validated siRNAs, optimized TaqMan® qRT-PCR Gene Expression Assays, reagents, instruments, and the protocols provided with them, streamline each step of an RNAi experiment and remove the technical variability from your experiments, letting you focus on making critical biological discoveries.

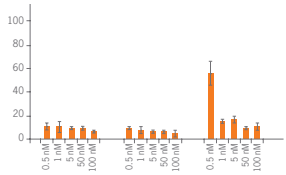
In this article, we describe step-by-step how easy it is to use these reagents together to accelerate gene function analysis. We then apply these steps to a real research example, the role of survivin (BIRC5) in promoting oncogenesis.

1 Obtain Effective siRNAs: www.ambion.com/siRNA

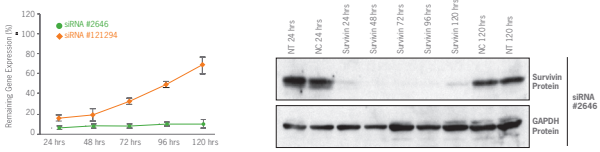
2 Optimize siRNA Delivery



3 Test siRNA Silencing Efficiency



4 Examine Biological Impact of Silencing Target Gene(s)



5 Correlate Silencing Efficiency with Biological Impact

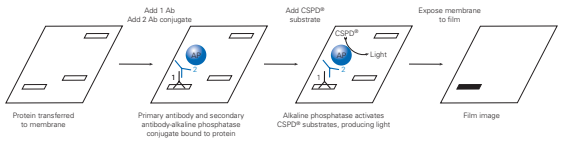


Figure 1.
The Five Key Steps of Delineating Gene Function Using siRNAs.

Ambion®

Step 1. Obtain Effective siRNAs

To obtain gene silencing, potent and specific siRNAs must be designed. Effective siRNA design and rigorous quality control of synthesis and purification is key to avoid wasting both time and money. In addition, good experimental design dictates that at least two effective siRNAs be used in the experiment in order to confirm that the biological results obtained are the result of knocking down the gene of interest.

Researchers can choose to custom design their siRNA, select pre-designed siRNAs, or obtain validated siRNAs.

Case Study: Selecting Effective siRNAs to Survivin

For this study, three *Silencer*® Pre-designed siRNAs targeting human survivin were selected. To accomplish this, we simply searched the Ambion siRNA Database (**www.ambion.com/siRNA**; Figure 2) and ordered siRNAs ID# 2646, 2734, and 121294 in standard purity (Cat# AM16708A).

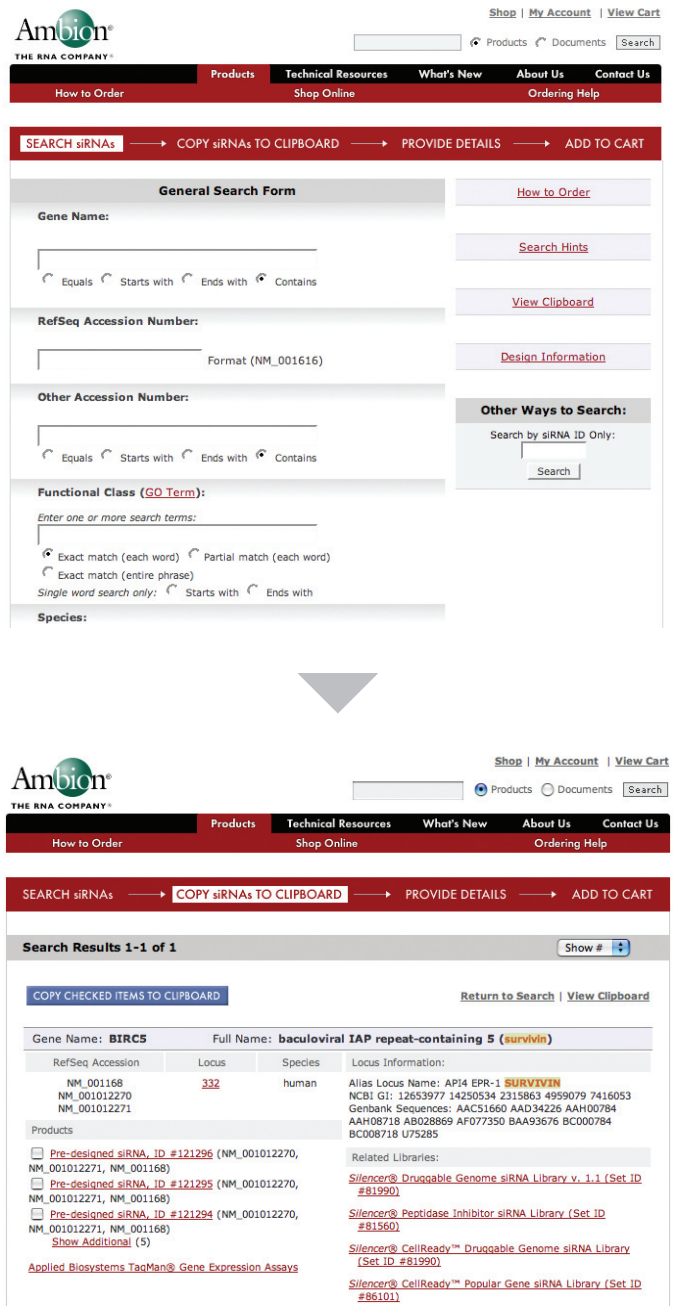


Figure 2. Selecting siRNAs Using the Ambion siRNA Database.

Case Study Background

Linking Survivin Expression to Oncogenesis

Survivin (BIRC5) expression is linked to a broad range of cancers [1]; all major cancer types express survivin while survivin is not expressed in noncancerous, differentiated cells [2] (see Survivin Biology, this page). The survivin complex and its constituents appear to be very important for cancer cell survival and for apoptotic control [3]. In spite of these observations, the exact mechanism by which survivin promotes oncogenesis is unclear.

By altering survivin expression through gene silencing we can assess the effect survivin has on known indicators of apoptosis with the aim of better understanding the role of survivin in cancer.

Survivin Biology

Survivin (BIRC5) is a 17 kDa bifunctional protein that plays critical roles in the regulation of both cell division and survival [4]. In many cell types, survivin blocks apoptosis by inhibiting members of the caspase cysteine protease family, such as caspase-9 [5], caspase-3 and caspase-7 [6]. The phosphorylation of survivin at Thr 34 by the cyclin-dependent kinase cdc2 is believed to promote physical interactions with caspase-9 enzyme which result in caspase-9 inhibition [7]. Survivin and cdc2 proteins comigrate at the mitotic spindles and appear to be important for proper progression through the cell cycle [8]. Survivin contains a single BIR (baculovirus IAP repeat) domain, a caspase inhibitory domain, like other IAP (Inhibitor of Apoptosis) proteins that inhibit caspases [3]. The BIR domain facilitates binding to caspases as well as to the HBXIP (hepatitis B X-interacting protein), which is proposed to be a cofactor for survivin [9].

Silencer® Pre-designed and Validated siRNAs, and Custom siRNA Synthesis

Silencer® Pre-designed siRNAs are designed with one of the most rigorously tested siRNA design algorithms in the industry and then manufactured to exacting quality standards.

The strength of siRNA performance (Figures 3 and 4) means that Ambion can guarantee that when three *Silencer* Pre-designed siRNAs are obtained to the same target, at least two will reduce target mRNA levels by 70% or more.

Furthermore, *Silencer* Validated siRNAs are able to provide the ultimate guarantee such that each *Silencer* Validated siRNAs will reduce target mRNA levels by 70% or more.

If you are unable to find what you are looking for with either validated or pre-designed siRNAs, Ambion offers premium quality siRNA custom synthesized to your specifications.

Learn more at **www.ambion.com/siRNA**.

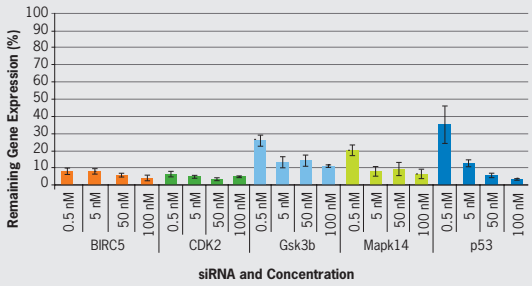


Figure 3. Potency of *Silencer*® siRNAs at Low Concentrations. siRNAs were transfected into HeLa cells at the indicated concentrations, and target mRNA levels were monitored by real-time RT-PCR 48 hr after transfection using the appropriate TaqMan® Gene Expression Assay. Transfections were performed in triplicate, and the data are shown relative to nontargeting siRNA (*Silencer* Negative Control #1 siRNA; Ambion, Cat.#AM4611) transfected cells.

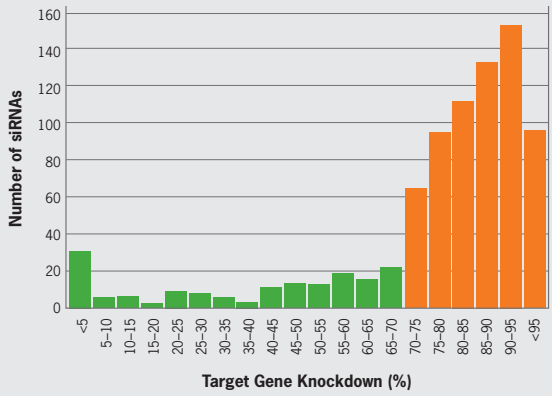


Figure 4. Effectiveness of *Silencer*® Pre-designed siRNAs. This graph shows the distribution of gene silencing measured for 808 *Silencer*® Pre-designed siRNAs targeting >300 endogenously expressed human genes. Target mRNA levels were measured by qRT-PCR 48 hr after transfection into HeLa cells. More than 82% of the siRNAs successfully silenced their targets, which means that Ambion can guarantee that *Silencer* siRNAs will be effective. Data courtesy of Cenix BioScience.

Step 2. Optimize siRNA Delivery

Maximize Gene Knockdown, Minimize Toxicity

Efficient, reproducible siRNA delivery is essential for successful RNAi experiments. Because siRNA delivery is so critical, it is highly worthwhile to invest the time and effort to determine the best siRNA delivery method and conditions for each cell line studied. Careful optimization of procedures limits siRNA delivery variability and enhances the quality of the results.

The best siRNA delivery protocol provides good gene knockdown (i.e., effective siRNA delivery), while maintaining an acceptable level of cell viability (i.e., low cytotoxicity).

Some of the conditions that improve gene knockdown, such as higher amounts of transfection agent, also result in decreased cell viability. Therefore, it is important to assay for both gene knockdown and cell viability when developing siRNA delivery conditions.

Controls Are Key

Negative controls that do not target any endogenous transcript are needed to control for non-specific effects on gene expression caused by introducing any siRNA. Easy-to-assay positive controls are needed to optimize transfection conditions, ensure that siRNAs are delivered, and ascertain that a particular downstream assay is working. Because positive controls are required for many different aspects of an RNAi experiment, often more than one control is required.

Ambion scientists use and recommend a number of different controls for siRNA experiments. Most of these coincide with the suggested controls detailed in an editorial published in *Nature Cell Biology* [Whither RNAi? (2003) *Nature Cell Biology* 5: 489–490].

Case Study: Optimizing siRNA Delivery in HeLa Cells

Transfection Agent

In this experiment, we chose siPORT™ *NeoFX*™ Transfection Agent as our siRNA delivery vehicle. This transfection agent efficiently delivers siRNA into a variety of cell types with minimal cytotoxicity compared to other commercially available transfection reagents. It is serum tolerant, compatible with reverse transfection, and yields highly reproducible results.

siRNA Controls

For optimizing transfection conditions, we typically use *Silencer*® GAPDH siRNA and *Silencer* Negative Control #1 siRNA. Both of these siRNAs have been extensively validated for use in human, mouse, and rat cells. GAPDH mRNA is a particularly useful target for a positive control siRNA for use in transfection optimization experiments because it is ubiquitously expressed across cell types and is easy to assay. *Silencer* Negative Control #1 siRNA has been tested in multiple cell lines and found to have minimal effects on cell survival or cell proliferation. This control is useful in essentially all siRNA experiments to control for the effects of delivering any siRNA.

Optimization Process and Results

To determine the best transfection conditions to use in our HeLa cell system, we transfected *Silencer* GAPDH siRNA or *Silencer* Negative Control #1 siRNA using increasing amounts of siPORT *NeoFX* Transfection Agent (Figure 5). We then analyzed GAPDH mRNA levels by qRT-PCR to monitor siRNA delivery efficiency, and examined relative cell numbers using the KDalert™ GAPDH Assay Kit to monitor transfection induced cytotoxicity. Based on these data, as well as on additional experiments not shown, we decided to use 0.3 µL siPORT *NeoFX* reagent for subsequent experiments.

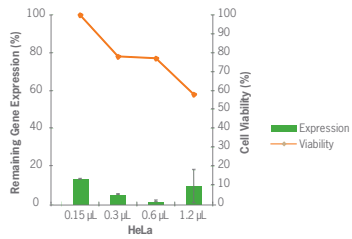


Figure 5. Optimizing Transfection Conditions with siPORT™ *NeoFX*™ Transfection Agent. HeLa cells were transfected with either GAPDH or Negative Control #1 siRNA using the indicated amount of siPORT™ *NeoFX*™ Transfection Agent (Cat. #AM4510). GAPDH mRNA levels were measured 48 hrs after transfection using a corresponding TaqMan Gene Expression Assay. Percent remaining gene expression is depicted versus negative control siRNA-treated cells. Percent cell viability was measured with the KDalert™ GAPDH Assay Kit and is shown relative to non-transfected cells.

Step 3. Test siRNA Silencing Efficiency

Once siRNAs are obtained, the next step in a typical gene silencing experiment is to assess the silencing efficiency of the selected siRNAs. siRNAs exert their effects at the mRNA level. Therefore, the preferred assay for siRNA validation is one that monitors target mRNA levels. The simplest and most sensitive assay for siRNA validation relies on qRT-PCR to measure target transcript levels in gene specific siRNA-treated cells versus negative control siRNA-treated cells.

Case Study: Verifying Survivin siRNA Silencing Efficiency

Methods

To test the efficacy and potency of the three different *Silencer*® siRNAs targeting survivin, selected in Step 1, we individually reverse transfected HeLa cells with various concentrations of the survivin siRNAs or *Silencer* Negative Control #1 siRNA. 48 hours after transfection, RNA was isolated using the MagMAX™ Total RNA Isolation Kit, which simplified sample prep in our chosen 96-well format. The RNA was then converted to cDNA and the appropriate TaqMan® Gene Expression Assay with Master Mix (NEW! TaqMan® Gene Expression Master Mix) was used to monitor down regulation of survivin mRNA using an Applied Biosystems 7900HT Fast Real-time PCR System.

Results

Figure 6 demonstrates that all three of the siRNAs provided excellent silencing of survivin. Indeed survivin mRNA levels were reduced >85% at 1 nM siRNA. We chose two *Silencer* survivin siRNAs that provided different silencing efficiency (#2646 and #12194) for further study to help determine if the differences in knockdown level impacted the biological response observed.

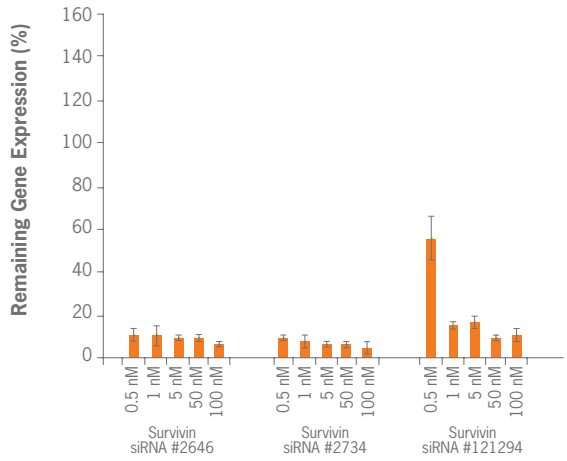


Figure 6. Validation of siRNAs Targeting Survivin. Three *Silencer*® Pre-designed siRNAs targeting human survivin and nontargeting *Silencer* Negative Control #1 siRNA (Ambion, Cat #AM4611) were reverse transfected into HeLa cells in triplicate at 6 different concentrations using 0.3 µL siPORT™ *NeoFX*™ Transfection Agent (Ambion, Cat. #AM4510). 48 hr post transfection, RNA was isolated using the MagMAX™-96 Total RNA Isolation Kit (Ambion, Cat #AM1830). cDNA was synthesized and 2 µL cDNA was used to amplify survivin mRNA in real time RT-PCR reactions with Survivin Hs00977611_g1 TaqMan® Gene Expression Assay (Applied Biosystems). Percent gene expression remaining is expressed as the relative amount of survivin mRNA in cultures transfected with survivin siRNAs vs cells transfected with the nontargeting control siRNA. TaqMan Gene Expression Assays against 18S rRNA were used to normalize for differences in total RNA concentration.

TaqMan® Gene Expression Assays

TaqMan® Gene Expression Assays—which are available for human, mouse, rat, *Rhesus*, *C. elegans*, *Drosophila*, and *Arabidopsis* genes—provide exquisite sensitivity, linearity and dynamic range in real-time PCR applications and are the most widely trusted and referenced real-time PCR detection method. These advantages, as well as their convenient availability and alignment for the same transcripts to which Ambion *Silencer*® siRNAs are targeted and their ability to be used under universal cycling conditions, make TaqMan Gene Expression Assays ideal for analyzing siRNA induced knockdown by qRT-PCR.

Step 4. Examine Biological Impact of Silencing Target Gene(s)

Assays that measure the effects of gene silencing are varied and diverse. Morphological, enzymatic, biochemical, and immunological assays can all be useful depending on the goals of the experiment. In general, it is best to choose the simplest, most reproducible assay that is relevant to the biological process you are studying. However, first it is important to define the time course over which silencing takes place so that this window can be targeted in the biological assays. Because siRNAs exert their effects at the mRNA level, but phenotypes are usually induced by protein reduction, it is highly recommended that siRNA-induced silencing be measured at both the mRNA and protein levels. This is typically accomplished by qRT-PCR and Western blotting, respectively.

Case Study: Examining Impact of Survivin Silencing on Several Apoptosis Parameters

Silencing Time Course Methods

The window of mRNA and protein silencing was defined by delivering survivin and nontargeting siRNAs to cells and measuring mRNA (Figure 7) and protein levels (Figure 8) at 24, 48, 72, 96, and 120 hours post transfection. mRNA levels were monitored using qRT-PCR and the same TaqMan® Gene Expression Assay as used for verifying siRNA induced silencing in Step 3. To analyze survivin protein levels, Western blot analysis was performed on cell lysates using the Western-Light™ Immunodetection System. The Western-Light System provides a sensitive, chemiluminescent based protein detection method.

Methods for Measuring Apoptosis

A series of biochemical and morphological assays were performed to measure the effect of siRNA-mediated silencing of survivin on several apoptotic indicators over the time course described above.

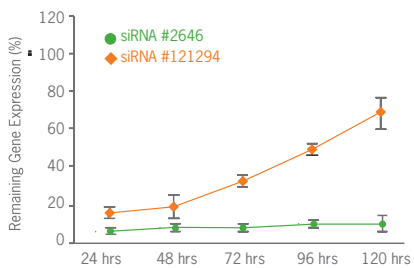


Figure 7. Time Course of Survivin mRNA Reduction in HeLa cells Transfected with Survivin siRNAs. *Silencer*® siRNAs (#2646 and #121294) were transfected at 30 nM in triplicate into HeLa cells using 0.3 µL siPORT™ *NeoFX*™ Transfection Agent in 96 well plates (4000 cells/well). Total RNA was isolated from each sample at the indicated time point using the MagMAX™-96 Total RNA Isolation Kit. Survivin mRNA levels were measured using Survivin Hs00977611-g1TaqMan® Gene Expression Assay. Percent gene expression remaining was expressed as the relative amount of survivin mRNA in cultures transfected with survivin siRNA versus cells transfected with *Silencer* Negative Control #1 siRNA.

Cell Survival

Relative cell survival was measured using fluorescein diacetate (FDA), a fluorogenic nonspecific esterase substrate.

Nuclear Condensation

Chromatin condensation is a late apoptosis indicator and was monitored by fluorescence microscopy after DAPI staining of the cells.

Increased phosphatidyl serine externalization

Induction of membrane asymmetry, as evidenced by phosphatidyl serine externalization, is an early apoptosis indicator and was measured by labeling with fluorescent annexin V and then assaying with an 8200 Cellular Detection System (Applied Biosystems).

Pro-caspase-3 activation

Caspase-3 activation is an early to mid-stage apoptosis indicator and was assayed using a fluorogenic caspase-3 substrate.

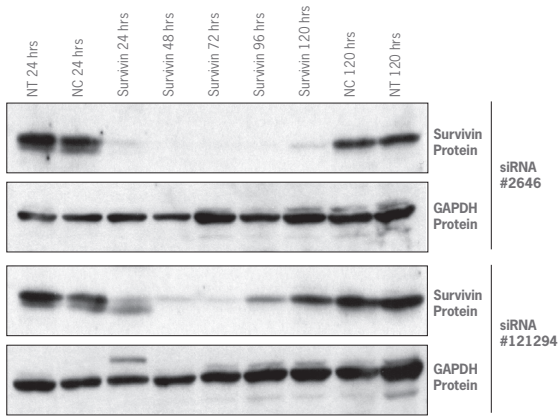


Figure 8. Time Course of Survivin Protein Reduction in HeLa Cells Transfected with Survivin siRNAs. *Silencer*® siRNAs (#2646 and #121294) were transfected in triplicate (30 nM siRNA) into HeLa cells using 5 µL siPORT™ *NeoFX*™ Transfection Agent in 6 well plates (2.5×10^5 cells/well). Survivin protein was detected in cell lysates harvested at the indicated time points by immunoblot using a rabbit polyclonal antibody to survivin (1:2000 dilution; Abcam Cat #AB469) and the Western-*Light*™ Immunodetection System (Applied Biosystems Cat #T1047). GAPDH was detected for use as a loading control (Ambion Cat. #AM4300). NT=Nontransfected; NC=*Silencer* Negative Control #1 siRNA.

Silencing Time Course

Transfection of survivin siRNA into HeLa cells reduced survivin mRNA levels >80% compared to negative control transfected cells. However, the patterns of silencing were not identical. siRNA #2646 knocked down survivin mRNA over 80% consistently over the time course while survivin siRNA #121294 knocked down survivin mRNA 80% at 48 hours, but less efficiently at the later time points (Figure 7). The survivin protein expression pattern paralleled that of survivin mRNA expression (Figure 8).

Cell Survival

Survivin supports cell proliferation and thus, silencing of survivin might be expected to result in a decrease in cell number. This was tested and results are shown in Figure 9. As compared to negative control siRNA transfected cells, the two survivin siRNAs did not lead to significantly decreased cell numbers at any of the time points monitored.

Nuclear Condensation

Specific silencing of survivin has been shown to cause apoptotic events, one of which is characteristic changes in nuclear morphology due to nuclear condensation. Nuclear morphology was therefore observed 48–72 hours post transfection by staining cells with DAPI. At 48 hours, many survivin siRNA transfected cells exhibited nuclear condensation, while *Silencer*® Negative Control #1 siRNA transfected cells did not (Figure 10).

Phosphatidyl Serine Externalization

One of the earliest hallmarks of apoptosis is phosphatidyl serine externalization. To see whether survivin silencing would cause an increase in phosphatidyl serine externalization, this parameter was measured by labeling with fluorescent Annexin V. HeLa cells were transfected with either the survivin siRNAs or negative control siRNA. 72 hours after transfection, fluorescent annexin V labeling was assayed using the 8200 Cellular Detection System (Figure 11). The 8200 instrument enables mix-and-read assays with live cells and beads. As with cell number, the observed magnitude of the effect varied somewhat between the two siRNAs.

Caspase-3 Activation

A series of caspases are typically activated in the early stages of apoptosis. These proteases cleave key structural and nuclear proteins, which leads to chomosomal cleavage and nuclear condensation. Caspase-3 is generally the last caspase activated in the caspase cascade. Therefore we monitored the impact of survivin knockdown on activated caspase-3 levels. siRNA-mediated survivin silencing caused little or no effect on caspase-3 activity (data not shown). Our observation agrees with published reports stating that inhibition or silencing of survivin results in the activation of apoptosis events but does not activate caspases [6,7].

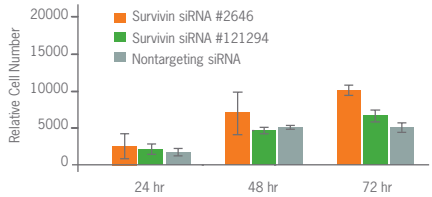
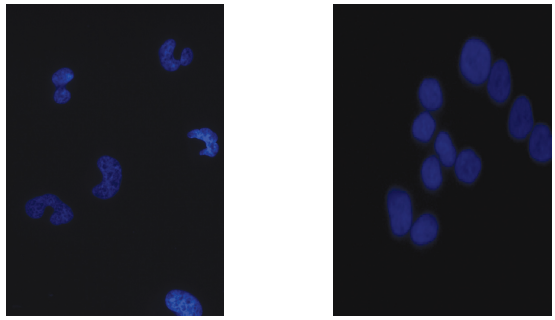
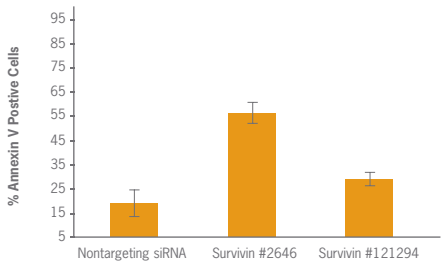


Figure 9. Effect of Survivin Silencing by on Cell Number. *Silencer*® siRNAs #2646 and #121294 were transfected in triplicate (30 nM siRNA) into HeLa cells (4000 cells/well in 96 well plates) using 0.3 µL siPORT™ *NeoFX*™ Transfection Agent. At various time points post transfection, cells were harvested and lysed. Cell extract (8 µL) was added to 384 well plate wells containing 32 µL (0.01 mg/mL) fluorescein diacetate solution. Relative cell number/well was determined by measuring the increase in fluorescence (ex=488 nm, em=529 nm) over 4 min at room temperature.

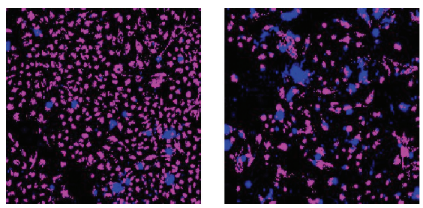


Panel A. Survivin siRNA **Panel B. Nontargeting Negative Control siRNA**

Figure 10. Survivin Silencing Causes Changes in Nuclear Morphology. HeLa cells transfected with (A) survivin siRNA #2646 (30 nM) and (B) *Silencer*® Negative Control #1 siRNA (Ambion, Cat. #AM4611), were fixed 48 hr post transfection, and stained with DAPI. Nuclear morphology was assessed using an Olympus BX60 fluorescent microscope.



Panel A. Induced Phophatidyl Serine Externalization.



Panel B. Cells Showing Phosphatidyl Serine Externalization (Blue).

Figure 11. Phosphatidyl Serine Externalization Caused by Silencing of Survivin. (A) HeLa cells were transfected at 4000 cells/well with 30 nM *Silencer*® survivin siRNA #2646, #121294, or *Silencer* Negative Control #1 siRNA (Ambion, Cat #AM4611) in 6 replicates using siPORT™ *NeoFX*™ Transfection Agent (0.3 µL). Cells were harvested at five time points (24, 48, 72, 96, and 120 hr; 72 hr time point shown) post transfection and assayed for phosphatidyl serine externalization using fluorescent labeled Annexin V (blue) and CentriRed™ DNA binding dye (pink) to enumerate cells. Cells were analyzed on the Applied Biosystems 8200 Cellular Detection System. (B) Cells transfected with negative control siRNA and *Silencer* siRNA #2646.

Step 5. Correlating Silencing Efficiency with Biological Effect

In order to fully understand the biological impact of reducing expression of a target gene, it is necessary to correlate the observed phenotype with the level of knockdown induced. Time course and siRNA titration experiments are particularly useful for this endeavor.

Case Study: Correlating Survivin RNA and Protein Levels with Apoptosis Parameters

The time course of siRNA silencing of survivin mRNA (Figure 7) and protein (Figure 8) correlated well with the time frames where decreased cell survival/proliferation; changes in nuclear and plasma membrane shape, chromosomal condensation and fragmentation; and phosphatidyl serine externalization were observed. These observations are summarized in the chart in Figure 12 and strongly suggest that specific silencing of survivin by siRNA in HeLa cells is sufficient to cause an increase in apoptosis in the absence of additional apoptotic insults. Our observations are consistent with other investigations on the role of survivin in cell biology and regulation of apoptosis [10].

Conclusions

Overexpression of survivin is rapidly becoming a diagnostic and prognostic marker for malignant cancers. Its restricted expression in malignant cells makes it a promising target for novel therapies for cancer treatment. To fully exploit survivin as a target for cancer therapy, we need to more completely understand how survivin inhibits apoptosis, and to identify the biological mechanisms that are critical for its inhibition. We, along with others, have demonstrated that siRNA mediated silencing of survivin in cell culture models is a convenient and efficacious model to understand survivin function. Cell culture is exquisitely sensitive to knockdown by siRNA which will facilitate more in-depth research into this important biological pathway.

This case study provides an example of how gene function can be elucidated in a matter of days by:

- effective siRNAs
- an efficient siRNA delivery method
- sensitive assays for monitoring siRNA induced knockdown
- specific assays for the biological parameters of interest

Success is maximized when optimized reagents are used. Applied Biosystems, now including Ambion, provide the most complete suite of validated, quality reagents for RNA interference experiments available for this purpose.

Hours Post Transfection		24	48	72	96	120
Assay	siRNA					
mRNA Expression (% decrease in normal expression)	2624	●	●	●	●	●
	121294	●	●	●	●	●
Protein Expression (% decrease in normal expression)	2624	●	●	●	●	●
	121294	●	●	●	●	●
Cell Number (% reduction)	2624	●	●	●	●	ND
	121294	●	●	●	●	ND
Nuclear Morphology (% cells affected)	2624	●	●	●	●	ND
	121294	●	●	●	●	ND
Externalization (% Increase in Annexin V staining)	2624	●	●	●	●	ND
	121294	●	●	●	●	ND

Scale: From most affected (%) to most similar (%) to the nontransfected or nontargeting control:

● ● ● ● ● ND

Most affected — 50% altered — Most similar — Not Done

Figure 12. Correlating Survivin Silencing with Apoptosis Parameters. Data from the experiments in Figure 7-11 is summarized here showing the relationship between silencing survivin over time and the phenotypic effects assayed over this same time frame.

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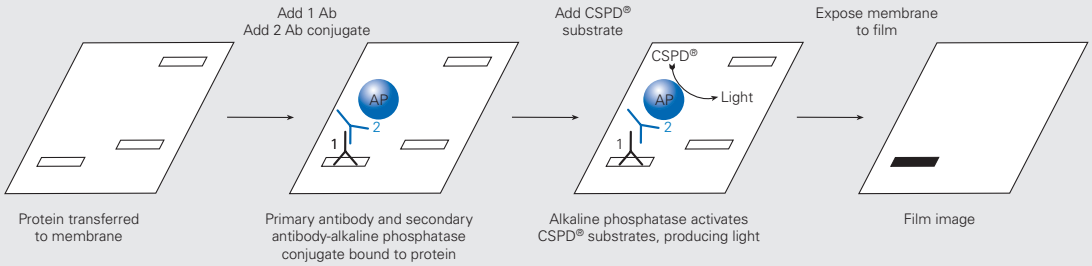
mRNA Expression Analysis Products

Applied Biosystems offer a complete portfolio of technologies dedicated for the investigation of mRNA expression analysis: from off-the-shelf gene-specific probe and primer sets to Custom TaqMan® probes and primers manufactured to your desired sequences, and everything in between. All products use TaqMan probe-based chemistry and are designed for use on the suite of Applied Biosystems Real-Time PCR Systems—together the gold standard in quantitative gene expression offering the greatest sensitivity, specificity, reproducibility, and the broadest dynamic range. Some of the products include: TaqMan® Gene Expression Assays, TaqMan® Gene Expression Master Mix, High Capacity cDNA Reverse Transcription Kit, and Applied Biosystems 7900HT Fast Real-Time PCR System.

Applied Biosystems 7900HT Fast Real-Time PCR System.

Protein Analysis Products

Applied Biosystems provides two highly sensitive chemiluminescent immunodetection systems for Western blotting: the Western-*Star*™ and Western-Light™ Immunodetection Systems. Both systems detect proteins in protein extracts from a variety of sources, including cell cultures and tissues, and both make use of a high intensity chemiluminescent signal that persists from hours to days.



Immunodetection with Western-Light™ System.

Ensure RNAi Success

Applied Biosystems, now including its Ambion business, provides a convenient, complete, optimized, and validated solution for gene silencing experiments. *Silencer*® siRNAs from Ambion eliminate the guesswork—and the tedious labwork—associated with siRNA design and testing. And gene-specific, ready-to-run TaqMan® Gene Expression Assays from Applied Biosystems give you a fast, simple way to measure knock-down effectiveness. Below is just a partial list of these and the many other kits and reagents from Ambion and Applied Biosystems that together serve to accelerate RNAi based gene function analyses and assist you in answering important biological questions.

Applied Biosystems and Ambion Reagents and Instruments for Each Step of an RNAi Experiment

1 Obtain Effective siRNAs

For successful siRNA experiments, you need two or more effective siRNAs per target, a positive control siRNA, and a negative control siRNA. Independent use of multiple individual siRNAs per target improves confidence in data and is generally required for publication of RNAi results.

PRODUCTS

Already designed, guaranteed-to-silence siRNAs to human, mouse, and rat genes

- *Silencer*® Pre-designed siRNAs
- *Silencer*® Validated siRNAs
- *Silencer*® Control siRNAs
- *Silencer*® siRNA Libraries
- Custom siRNA Design
- Custom siRNA Synthesis

2 Optimize Delivery

Transfection and electroporation are commonly used to deliver siRNAs into cultured mammalian cells.

PRODUCTS

Transfection

- siPORT™ *NeoFX*™ Transfection Agent
- siPORT™ *Amine* Transfection Agent
- *Silencer*® siRNA Transfection Kit II
- KDalert™ Kit
- *Silencer*® siRNA Starter Kit

Electroporation

- siPORT™ Electroporation Buffer
- siPORT™ Electroporation Kit

3 Test Silencing Efficiency

PRODUCTS

RNA Isolation

- PARIS™ Kit (Protein and RNA Isolation System)
- RNAqueous®-4PCR Kit
- MagMAX™-96 Total RNA Isolation Kit

qRT-PCR

- TaqMan® Gene Expression Assays
- TaqMan® Gene Expression Master Mix
- High Capacity cDNA Reverse Transcription Kit
- 7900HT Fast Real-time PCR System
- 7500 Real-time PCR System

4 Examine Biological Impact

PRODUCTS

Cell-Based Assays

- 8200 Cellular Detection System
- Tropix® Cell Signaling and Reporter Gene Assays

Protein Detection

- Western-Light™ Immunodetection System
- Antibodies Matched to *Silencer*® Control siRNAs

qRT-PCR

- TaqMan® Gene Expression Assays
- TaqMan® Gene Expression Master Mix
- High Capacity cDNA Reverse Transcription Kit
- 7900HT Fast Real-time PCR System
- 7500 Real-time PCR System

5 Correlate Silencing with Biological Impact

PRODUCTS

RNA Isolation

- PARIS™ Kit (Protein and RNA Isolation System)
- RNAqueous®-4PCR Kit
- MagMAX™-96 Total RNA Isolation Kit

qRT-PCR

- TaqMan® Gene Expression Assays
- TaqMan® Gene Expression Master Mix
- High Capacity cDNA Reverse Transcription Kit
- 7900HT Fast Real-Time PCR System
- 7500 Real-time PCR System

Protein Detection

- Western-Light™ Immunodetection System
- Antibodies Matched to *Silencer*® Control siRNAs

Green: Ambion kits and reagents used in the presented case study.

Blue: Applied Biosystems reagents and instruments used in the presented case study.

For more information on these products, contact us or visit www.ambion.com (green listings) and www.appliedbiosystems.com (blue listings).

Simplify Gene Silencing Experiments

Silencer® Pre-designed siRNAs

- Ready to use siRNAs for human, mouse, and rat genes
- Searchable database makes it simple to find siRNAs to your genes of interest
- Efficient gene knockdown guaranteed

Tired of wasting time with poorly designed siRNAs? *Silencer*® Pre-designed siRNAs—highly effective, ready-to-use siRNAs available for all human, mouse, and rat genes—provide guaranteed gene silencing. Each one has been designed using a carefully optimized algorithm and then manufactured to exacting quality standards to provide potent and specific gene silencing.

Journal article reviewers generally require that you verify gene silencing results with a second siRNA. [Whither RNAi? (2003) *Nat Cell Bio* **5(6):**489-490]. *Silencer* Pre-designed siRNAs make it easy to comply. Ambion guarantees that at least two *Silencer* Pre-designed siRNAs will knockdown target mRNA levels by ≥70% when three are purchased for the same gene.

Find *Silencer* Pre-designed siRNAs and corresponding TaqMan® Gene Expression Assays on the web at **www.ambion.com/info/siRNA.**

Verify Gene Silencing

TaqMan® Gene Expression Assays

- Gene-specific TaqMan® probe and primer sets for quantitative gene expression studies in human, mouse, rat, *Rhesus*, *C. elegans*, *Drosophila*, and *Arabidopsis*
- Convenient single tube format and 20X formulation
- Universal thermal cycling conditions

TaqMan® Gene Expression Assays are a comprehensive collection of more than 600,000 predesigned primer and probe sets that let researchers quickly and easily perform quantitative gene expression studies on human, mouse, rat, *Rhesus*, *C. elegans*, *Drosophila*, and *Arabidopsis* genes. These assays provide the exquisite sensitivity, linearity and dynamic range that you've come to expect from the TaqMan chemistry, and all perform under universal thermal cycling conditions. These properties, as well as their availability for the same transcripts to which Ambion *Silencer* siRNAs are targeted, make TaqMan Gene Expression Assays ideal for analyzing siRNA induced knockdown by qRT-PCR.

Find TaqMan Gene Expression Assays and corresponding *Silencer* siRNAs for your genes of interest at **www.allgenes.com**.

For research use only. Not for use in diagnostic procedures.

ORDERING INFORMATION

Description	Quantity	Part Number
siRNAs		
Silencer® Pre-designed siRNA, standard purity (when 3 or more are purchased to the same target)	5 nmol	AM16708A
Silencer® Validated siRNAs, standard purity	5 nmol	AM51331
Silencer® Control siRNAs For additional Controls, see www.ambion.com/prod/controls	5 nmol	Various
Silencer® siRNA Libraries	Variable	Various
Transfection		
siPORT™ *NeoFX*™ Transfection Agent	0.4 mL	AM4510
siPORT™ *Amine* Transfection Agent	0.4 mL	AM4502
Silencer® siRNA Transfection Kit II	2 x 0.4 mL + Controls	AM1631
Electroporation		
siPORT™ siRNA Electroporation Buffer	12 x 1.5 mL	AM8990
siPORT™ siRNA Electroporation Kit	60 rxns	AM1629
RNA Isolation		
PARIS™ Kit (Protein and RNA Isolation System)	50 purifications	AM1921
RNAqueous®-4PCR Kit	30 rxns	AM1914
MagMAX™-96 Total RNA Isolation Kit	96 rxns	AM1830
qRT-PCR		
TaqMan® Gene Expression Assays, Small Scale	360 µL	4351372
TaqMan® Gene Expression Assays, Inventoried	250 µL	4331182
For more information, visit **www.appliedbiosystems.com**		
NEW! TaqMan® Gene Expression Master Mix	200 rxns	4369016
High Capacity cDNA Reverse Transcription Kit	200 rxns	4368814
Applied Biosystems 7900HT Fast Real-time PCR System with Fast 96-Well Block Module	1 instrument	4351405
Applied Biosystems 7500 Real-time PCR System with Notebook Computer	1 instrument	4351104
Protein Detection		
Western-Light™ Immunodetection System with Goat Anti-Mouse IgG+IgM AP Conjugate, Size B For more information, visit **www.appliedbiosystems.com**	30 blots	T1045
anti-GAPDH, mouse monoclonal 6C5 For more primary Antibodies for siRNA Research, see **www.ambion.com/siRNA**	100 µg	AM4300

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