Application Note

Detection of siRNA-mediated gene modulation using SmartFlare™ Detection Probes

Introduction

Modulation of gene expression using techniques such as RNAi has become a fundamental tool in the study of gene function and biological pathways.[1] To determine the efficiency of gene knockdown using most widely used detection methods, cell samples must be sacrificed by lysis or permeabilization and fixation. In addition to sample destruction, another disadvantage of these techniques is that they yield results that only reflect the average expression of the gene in the collected cell population. One nondestructive option is the use of transfected reporter constructs. However, although the cells remain alive, the technique cannot reveal endogenous gene expression and can have negative effects on cell health. An ideal RNA detection agent provides a noninvasive approach to interrogating gene expression while enabling sorting of live cells that can be separated and directly used for downstream studies.

SmartFlare™ RNA detection probes can detect target mRNA and microRNA levels in live, intact cells. SmartFlare™ probes require no carrier agent to enter cells and have no toxic effect on cell health.[2] SmartFlare™ probes enable users to quickly verify gene expression, and, in conjunction with cell sorting, isolate desired cell populations. Furthermore, these reagents require no sample preparation and leave cells intact after the detection event, allowing for downstream studies.

Using SmartFlare™ RNA detection probes, we have demonstrated accurate, efficient detection of siRNA-mediated gene knockdown using a SmartFlare™ probe specific for a target of interest. SmartFlare™ probes enable fast and accurate detection of target gene expression at single cell resolution. The ability to specifically detect RNA levels on a cell–by-cell basis provides new opportunities to link biological pathways and physiological processes to gene functions.

Materials and Methods

mRNA knockdown

SCC12 (human squamous cell carcinoma) and LNCaP (human prostate adenocarcinoma) cells were seeded in 96-well plates at 10,000 cells/well. 24 hours after seeding, cells were transiently transfected with control or survivin-targeted siRNA and incubated for 48 hours. Survivin is an antiapoptotic gene that is highly upregulated in many cancer cell lines.[3] After the incubation period, the medium was changed to remove the siRNA and transfection agent.

Modulation detection

After the siRNA treatment, the SCC12 and LNCaP cells were incubated with 1000x dilution of stock survivin-specific SmartFlare™ probe or control SmartFlare™ probe overnight in cell growth medium. The next morning, cells were trypsinized and analyzed using the guava easyCyte™ 8HT flow cytometer. Concurrently, siRNA-treated cells not interrogated with SmartFlare™ probes were also harvested for comparative analysis by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

qRT-PCR

Total RNA was extracted using the RNeasy™ kit (Qiagen) and added to the TaqMan® RNA-to-Ct™ 1-step kit (Life Technologies). qRT-PCR was carried out using a LightCycler® 480 system (Roche).

Human Survivin[4]

TaqMan® probe:	5'-TGGTGCCACCAGCCTTCCTGTG-3'
Sense primer:	5'-GCACCACTTCCAGGGTTTATTC -3'
Antisense primer:	5'-TCTCCTTTCCTAAGACATTGCTAAGG-3'

Human GAPDH[5]

TaqMan® probe:	5'-ACCACAGTCCATGCCATCACTGCCA-3'
Sense primer:	5'-CAAGGTCATCCATGACAACTTTG-3'
Antisense primer:	5'-GGCCATCCACAGTCTTCTGG-3'

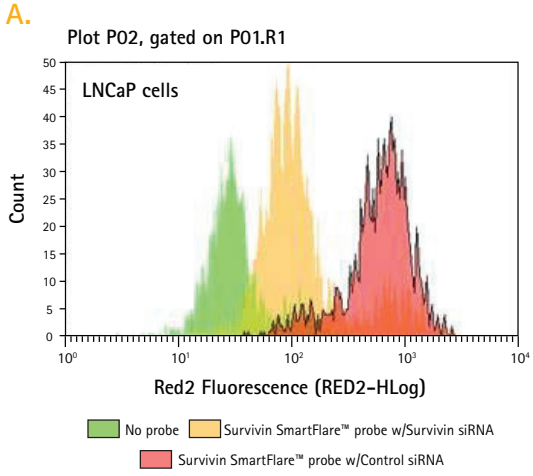
Results

mRNA detection by flow cytometry

To detect gene knockdown, survivin-targeted SmartFlare™ probes were incubated with SCC12 and LNCaP cells that had been treated with survivin and control siRNAs. Differences in expression levels of the target gene were detectable using flow cytometry analysis of cells treated with target-specific SmartFlare™ probes (Figure 1).

This experiment highlights the performance of Gamma Stable Opticap® XL 50 with Millipore Express® SPG membrane under typical cell culture conditions. Even when exposed to condensate during the cell culture process, Gamma Stable Opticap® XL 50 with Millipore Express® SPG membrane retained high flow rates, ensuring minimal loss of exhaust flow rate and minimal process disruption due to filter blinding.

A.



B.

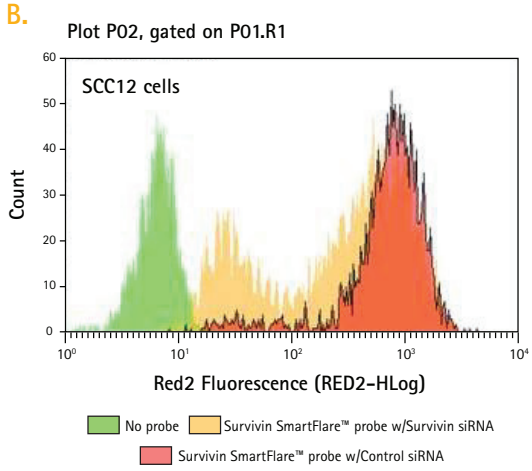


Figure 1.
Survivin gene knockdown in LNCaP and SCC12 cells were distinguishable by measuring relative SmartFlare™ signals. Histograms of SmartFlare™ signals corresponding to survivin expression in A. LNCaP cells and B. SCC12 cells, with or without siRNA knockdown of survivin.

mRNA detection by qRT-PCR

To detect gene knockdown using qRT-PCR, survivin-targeted SmartFlare™ probes and control SmartFlare™ probes were incubated with SCC12 and LNCaP cells that had been treated with survivin and control siRNAs. Relative gene expression levels determined using SmartFlare™ technology were plotted as bar graphs and compared with qRT-PCR data. This comparison showed that the relative gene expression levels were qualitatively similar regardless of whether SmartFlare™ technology or qRT-PCR was used to measure knockdown (Figure 2).

Because SmartFlare™ technology enabled us to analyze expression in individual, intact cells, we obtained additional information about the population distribution of the various treated cells. Specifically, we observed that LNCaP cells treated with a survivin-specific SmartFlare™ probe exhibited a unimodal distribution of survivin signal, while SCC12 cells displayed a bimodal distribution.

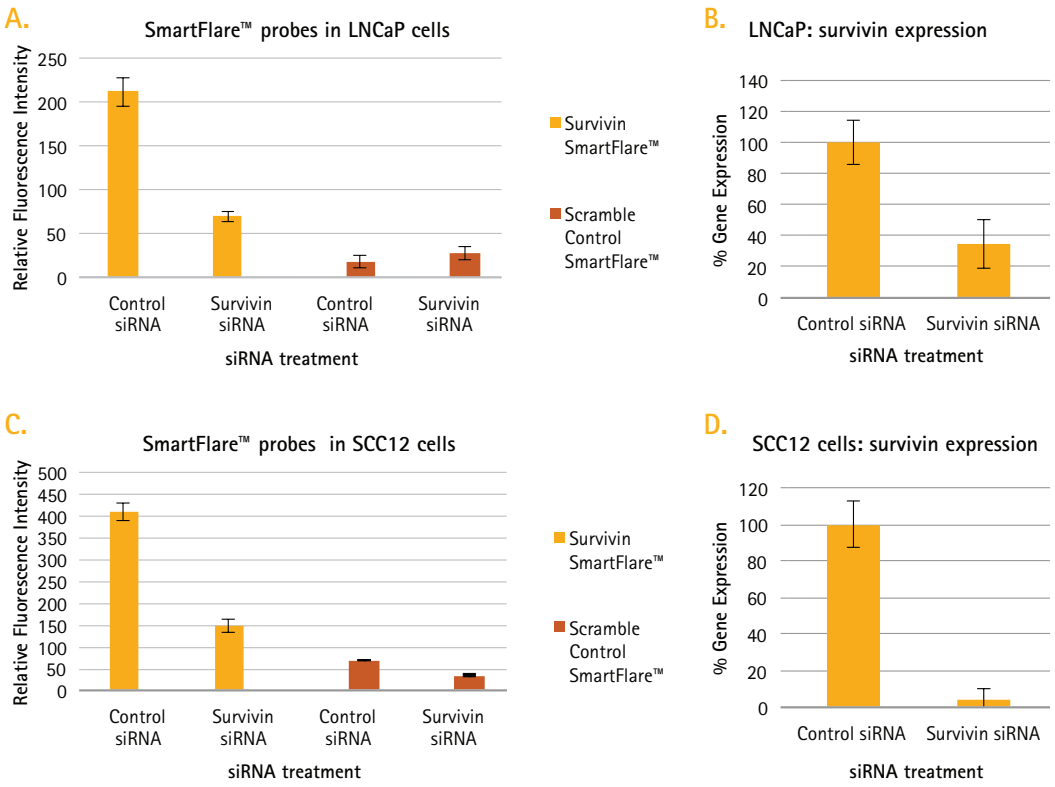


Figure 2.
Survivin gene knockdown in LNCaP and SCC12 cells by RNAi are distinguishable by SmartFlare™ detection as well as by qRT-PCR. A. Survivin expression in LNCaP cells with or without survivin knockdown as determined using SmartFlare™ technology and analyzed by flow cytometry. B. Confirmation of relative survivin expression levels with or without survivin knockdown in LNCaP cells by qRT-PCR. C. Survivin expression in SCC12 cells with or without survivin knockdown as determined using SmartFlare™ technology and analyzed by flow cytometry. D. Confirmation of relative survivin expression levels with or without survivin knockdown in SCC12 cells by qRT-PCR.

Conclusion

We have demonstrated the ability to detect intracellular gene expression in individual, live cells using SmartFlare™ technology. This ability is crucial when performing RNAi-mediated gene knockdown studies, in which it has been traditionally difficult to determine the cause of incomplete knockdown. Specifically, traditional methods of RNA measurement (which measure average RNA levels) cannot distinguish between inefficient knockdown due to a poorly designed siRNA sequence, inefficient entry of the siRNA into target cells, or vast differences in endogenous gene expression within the target cells. We have shown that using SmartFlare™ technology reveals the degree of gene expression knockdown in individual cells, thereby providing information on cell-to-cell variation in expression, knockdown and efficiency of siRNA entry. Such information may greatly facilitate the interpretation of analyses performed subsequent to RNAi treatment.

Furthermore, using SmartFlare™ technology makes it possible to sort cells and return them to culture, enabling downstream analyses, such as antibody staining, flow cytometry and qRT-PCR. As a result, it is now possible to measure physiological changes in the exact same cell samples assessed for gene knockdown, increasing the strength of observed correlations between gene expression and cell phenotype.

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