As genome sequencing projects for different organisms are completed, the identification and characterization of the gene function becomes fundamental to understanding the complexity of these systems. This creates a need for comprehensive studies to fill the gap between sequence and function. Many investigative approaches for identifying gene function have been introduced but RNA interference (RNAi) studies have gained the most prominence due to their potential to enable rapid genome-wide loss-of-function screens in mammalian systems. Quantification of the mRNA target is typically used to validate successful gene knockdown, and therefore, genome-wide screens require a high-throughput approach for isolating and quantifying mRNA level. Standard methods for isolating mRNA can be laborious, time-consuming, and not amenable to automation. Therefore, an automated system has been developed for the isolation and subsequent analysis of mRNA that uses Sigma’s SpyLine™ Poly A+ Capture kit, a novel system for the rapid isolation of poly A+ mRNA from cultured mammalian cells without centrifugation or vacuum filtration. With SpyLine, mRNA from the cell lysate is selectively bound in a 96-well PCR plate for direct use in quantitative reverse transcriptional PCR (qRT-PCR) analysis. The automated method was used to identify effective RNAi gene knockdown. Results indicate that this approach has both the sensitivity and reproducibility necessary for measuring transcript levels following gene knockdown.

**Materials**

Unless otherwise indicated, all reagents and materials used in this study were obtained from Sigma-Aldrich (St. Louis, MO). SpyLine Poly A+ Capture Kit (Cat. No. SP16K) was used to isolate mRNA from cultured HeLa cells after the treatments with targeting siRNA, or non-targeting siRNA or without any treatment. SYBR® Green-QuantiTect™ RT-PCR Kit (Cat. No. QR0200) was used to detect qRT-PCR products of SURF-4 mRNA after capture by SpyLine. Quantitative RT-PCR ReadyMix (Cat. No. QR0200) was used to quantify mRNA levels of eight genes before and after RNAi treatments. The M1215 Reverse Transcription Kit (Cat. No. M1210) was used for reverse transcription. The primers for amplifying SURF-4 gene were obtained from Sigma-Genosys (Woodlands, TX). The fluorogenic probes were obtained from Applied Biosystems (Foster City, CA). mRNA isolated from human kidney tissue (BioChain Institute Inc., Hayward, CA) was used as the standards for quantitative RT-PCR.

**Methods**

**Cell Culture**

HeLa cells were cultured in DMEM medium with 10% fetal bovine serum (FBS) and plated into a 96-well culture plate at 1×10^4 cells/well 24 hours before mRNA preparation using SpyLine Poly A+ Capture kit or transfection with siRNA.

**Transfection**

The targeting or non-targeting siRNAs were transfected into HeLa cells using commercially available transfection reagent. After 24 hours, the cells were re-plated with 100 μL of 10% FBS medium. After 48 hours, mRNA was isolated and quantified.

**mRNA Isolation and Quantitation by RT-PCR**

The SURF-4 and CHUK genes were utilized. Quantitative RT-PCR was performed in an ABI PRISM® 7700 Sequence Detection System. The SYBR Green dye was used to detect amplified RT-PCR product of SURF-4 gene. All 88 samples showed consistent amplification with a standard deviation of 0.3 Ct. The arrows indicate the amplification plot of the standards with and without transfection by siRNA treatment.

**Application of SpyLine Poly A+ Capture Kit in the Detection of Gene Knockdown by RNA Interference**

The automated method was applied to identify effective RNAi gene knockdown with both the sensitivity and reproducibility necessary for measuring transcript levels following gene knockdown.

**Conclusions**

- SpyLine Poly A+ Capture Kit offers a simple, rapid, and cost-effective method for isolating mRNA from cultured mammalian cells for direct qRT-PCR analysis.
- An automated protocol for SpyLine Poly A+ Capture Kit developed on Sciclone ALH 3000 workstation enables high-throughput mRNA preparations and subsequent analysis. 96 samples can be processed in 50 minutes from mRNA isolation to RT-PCR reaction setup.
- The automated method was applied to identify effective RNAi gene knockdown with both the sensitivity and reproducibility necessary for measuring transcript levels following gene knockdown.