

siRNA

Turbo si-Designer

- Bioneer's proprietary siRNA design algorithm
- Identify highly effective siRNA target sites with high success rates
- Highly effective in selecting functional siRNAs: 80% of the tested siRNA showed >75% knockdown and 38% elicited >90% knockdown

Successful siRNA experiments in mammalian cultured cells depend upon several factors. Specifically it is important to design and identify effective and specific siRNA sites and to perform efficient and specific delivery of siRNA to the desired target cell types. To facilitate design process, Bioneer, in collaboration with the National Genome Information Center (NGIC), has developed Turbo siRNA designer, (a proprietary siRNA selection algorithm). Turbo si-Designer can identify highly effective siRNA target sites with superior success rates. The performance of the Turbo si-designer was evaluated by designing hundreds of siRNAs and testing their knockdown efficacy by Real-time PCR analysis. When compared with other web-based design tools, Turbo si-Designer algorithm successfully predicted functional siRNAs at high probability of efficient knockdown. Notably, siRNAs with the low score were mostly nonfunctional, indicating that ineffective siRNAs are efficiently removed by Turbo si-Designer (Fig. 1).



Figure 1. Knockdown efficiency of siRNAs designed by Turbo si-Designer was analyzed by Northern blot and real-time PCR analysis. A) Knockdown efficiency of high score siRNAs. B) Knockdown efficiency of low score siRNAs. (a: siRNA 15% Acrylamide gel electrophoresis, b: Northern blot analysis, c: Real time PCR analysis)

AccuTarget™ Genome-wide Predesigned siRNAs

- AccuTarget™ Genome-wide Predesigned siRNAs are available for about 44,000 genes of the human, mouse, and rat genomes.
- Three top-scoring siRNAs per target gene are available.
- At least two of the three siRNAs candidates will reduce target mRNA level by >70% when transfected at 100 nM concentration.

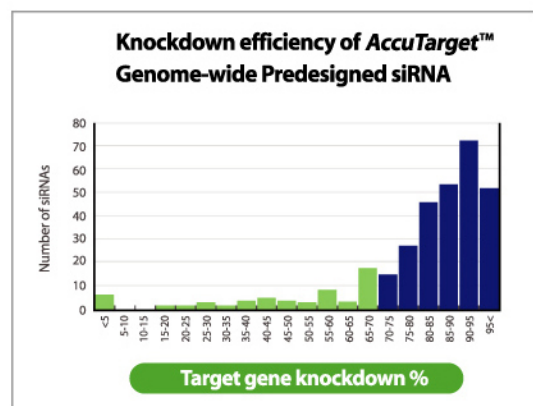


Figure 2. AccuTarget™ Pre-designed siRNAs are highly effective

To determine knockdown efficiency of predesigned siRNAs, HeLa cells were transfected with siRNAs at 100 nM concentrations. Twenty-four hour after transfection, total RNA was isolated and the level of target mRNA was measured by quantitative Real-time PCR analysis. This data demonstrates the effectiveness of the Turbo si-Designer algorithm: 83.8% of tested siRNAs induced >70% knockdown and 38.1% of tested siRNAs elicited >90% knockdown.

AccuTarget™ *siRNA* library Human

| AccuTarget™ <i>siRNA</i> library | | | |
|----------------------------------|----------------|----------------------------------|----------------|
| Gene Family/ Functional Class | Human Genes | Gene Family/ Functional Class | Human Genes |
| Caspase pathway | 37 | Isomerase | 104 |
| Kinase | 701 | Ligase | 272 |
| Phosphatase | 188 | Ligase | 123 |
| NF-κB pathway | 22 | Oxidoreductase | 551 |
| Apoptosis | 290 | Transferase | 1432 |
| Cell cycle | 113 | Motor | 122 |
| Antioxidant | 38 | Receptor | 1526 |
| Nucleic acid binding | 2589 | Ubiquitin | 77 |
| Cyclase | 22 | Tubulin | 20 |
| Deaminase | 22 | Transporter | 1023 |
| Helicase | 115 | Cytochrome P450 | 52 |
| Peptidase | 495 | GPCR signaling | 732 |

- Choose to purchase either 1, 2, or 3 *siRNA*(s) per target gene
- New library sets are continuously being updated
- siRNA* library is grouped by function of gene and pathway
- Twenty four *siRNA* library sets are currently available
- Validated *siRNA* library sets are available
- Flexible validated *siRNA* library sets for customer specified genes are also available; minimum order 10 *siRNAs*

AccuTarget™ validated *siRNA* library Human

| AccuTarget™ Validated <i>siRNA</i> library Human | |
|--|----------------|
| Gene Family/ Functional Class | Human Genes |
| Apoptosis | 53 |
| Caspase | 18 |
| Cell cycle | 107 |
| GPCR signaling pathway | 1 |
| Kinase | 43 |
| Ligase | 7 |
| Motor | 1 |
| Nucleic acid binding | 28 |
| Peptidase | 9 |
| Phosphatase | 1 |
| Receptor | 2 |
| Transferase | 38 |
| Transporter | 1 |
| Ubiquitin | 1 |

AccuTarget™ validated *siRNA* *siRNA* with proven knockdown efficiency

- When transfected at 100 nM concentration, greater than 70% of knockdown is proven.
- Validated *siRNA* are provided as Tube types or Plate types at various scales (2, 10, 20, 50, and 100 nmole).
- Validated real time PCR primer sets are also available for measuring knockdown efficiencies by SYBR® green based real-time PCR analysis.

Functionally validated *siRNAs* with guaranteed knockdown

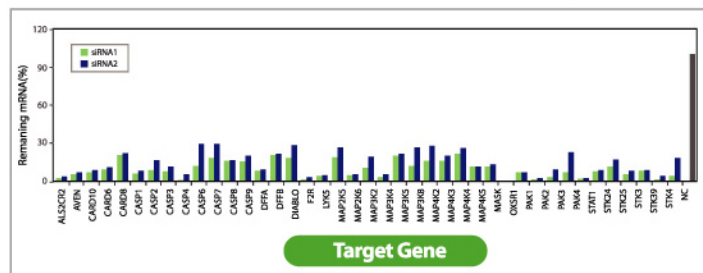


Figure 3. Knockdown efficiency of AccuTarget™ Validated *siRNAs* HeLa cells were transfected with validated *siRNAs* and the knockdown efficiency was measured by real-time PCR. The level of target mRNA was expressed as a percent of mRNA remaining in cells treated with the target gene-specific *siRNA* compared to cells treated with a negative control *siRNA* (AccuTarget™ Negative control *siRNA*).

AccuTarget™ Control *siRNA*

- AccuTarget™ Positive Control *siRNAs* (Human GAPDH, GFP, Luciferase)
- AccuTarget™ Negative Control *siRNAs* (commonly used for Human, Mouse, and Rat)
 - Fluorescently labeled *siRNAs* can be used for monitoring transfection efficiency AccuTarget™ Control *siRNA* Sets (Positive & Negative)
 - Convenient and cost-effective
- Various scales (5, 10, 20 nmole) and the option of purification methods (BioRP or HPLC)

• Positive control siRNA

● GAPDH-siRNA

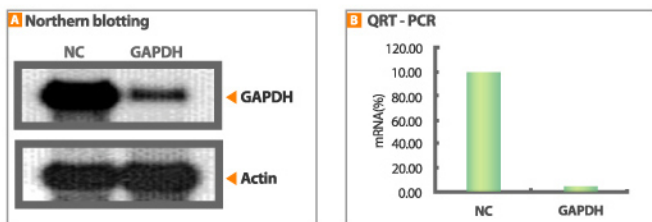


Figure 4. HeLa cells were transfected with GAPDH and NC(negative control)-siRNA at 100 nM final concentration using transfection reagent. Twenty four hour after transfection, total cellular RNA was isolated from transfected cells and subjected to Northern blot and real-time PCR analysis. As can be seen in Fig. 4B, only about 3% GAPDH mRNA remained after 24 hr treatment with GAPDH siRNA, indicating that highly efficient knockdown of GAPDH mRNA can be easily achieved using our positive control GAPDH siRNA.

● Luciferase-siRNA

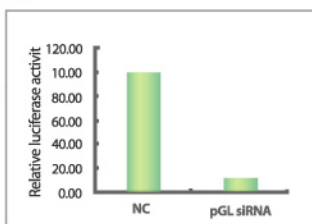


Figure 6. HeLa cells in a 6-well plate were cotransfected with 400 ng of CMV-luc plasmid and 10 nM of luciferase siRNA using lipofectamine 2000 transfection reagent. Next day, cells were harvested and assayed for luciferase activity. As shown in Fig. 6, cotransfection with our positive control luciferase siRNA led to efficient knockdown of luciferase activity (85% - 95% knockdown compared to luciferase activity of NC-siRNA-transfected cells).

● GFP-siRNA

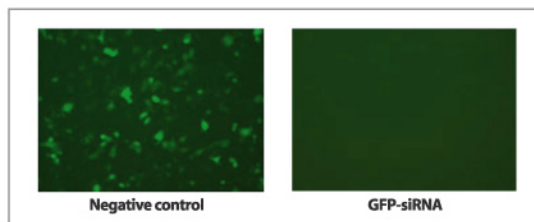


Figure 5. HeLa cells in a 24-well plate were cotransfected with 200 ng of CMV-GFP plasmid and 10 nM of GFP siRNA using transfection reagent. Next day, the expression of GFP was observed by using a Nikon Eclipse TS100 epifluorescence microscope. In contrast to bright green fluorescence of GFP protein in NC-siRNA-transfected cells, no fluorescence was detected from GFP-siRNA-transfected cells, indicating efficient knockdown of GFP by using our positive control GFP-siRNA.

• Negative control siRNA (NC-FITC)

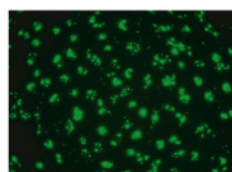


Figure 7. HeLa cells transfected with FITC-labeled siRNA (Cat No.: SN-1021) was observed by confocal microscopy. The fluorescent cells indicate that the target cells were successfully transfected with the siRNA.

Custom siRNA

- High-throughput synthesis system (384 parallel Synthesizer)
- High quality (100% MALDI-TOF QC) - see Fig. 8A
- Automatic Purification System (BioRP & HPLC)
- Clean Process (Manufactured in clean room) - see Fig. 8B
- 100% satisfaction guarantee

Bioneer's High-throughput RNA synthesis platforms produce high quality siRNA at an affordable price. Synthesized RNA strands are purified by either Bioneer's proprietary BioRP technology (OPC, free of charge) or by HPLC (additional charge). The synthesized siRNA is quality controlled (QC) utilizing two different methods: Single-strand RNAs are checked via MALDI-TOF analysis and Double-stranded siRNA duplexes are confirmed by PAGE analysis. Custom synthesized siRNA are provided in various formats and amounts, and many different types of modifications including fluorescent labels.

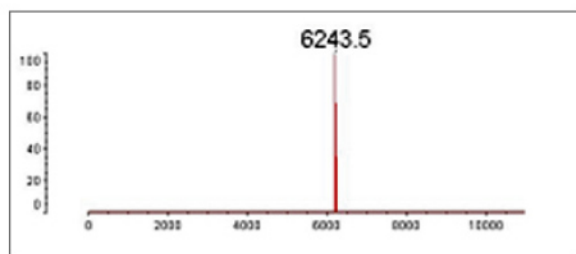


Figure 8A. MALDI-TOF mass spectrometry analysis of the synthesized siRNA. All shipped siRNAs are processed through quality control (QC) procedures, including MALDI-TOF and gel analysis



Figure 8B. All siRNAs are manufactured in clean room.

Ordering Information

| Cat. No | Product Name | Purification | Guaranteed nmole |
|--------------|---|--------------|--------------------------------------|
| SDO-1001 | AccuTarget™ Genome-wide Predesigned siRNA | BioRP | 10 nmole |
| SDO-1002 | | | 20 nmole |
| SDO-1003 | | | 50 nmole |
| SDO-1004 | | | 100 nmole |
| SL-1001/2/3 | AccuTarget™ siRNA library | BioRP | 1 siRNA (0.1, 0.5, 2nmole) |
| SL-2001/2/3 | | | 2 siRNAs (0.1, 0.5, 2nmole) |
| SL-3001/2/3 | | | 3 siRNAs (0.1, 0.5, 2nmole) |
| SVL-1001/2/3 | AccuTarget™ Validated siRNA library | BioRP | 1 siRNA (0.1, 0.5, 2nmole) |
| SVL-2001/2/3 | | | 2 siRNAs (0.1, 0.5, 2nmole) |
| SVL-3001/2/3 | | | 3 siRNAs (0.1, 0.5, 2nmole) |
| SFVL-1001 | AccuTarget™ Flexible Validated siRNA library | BioRP | 2 nmole (minimum order 10 siRNAs) |
| SV-1005 | AccuTarget™ Validated siRNA | BioRP | 2 nmole |
| SV-1001/11 | | BioRP/HPLC | 10 nmole |
| SV-1002/12 | | | 20 nmole |
| SV-1003/13 | | | 50 nmole |
| SV-1004/14 | | | 100 nmole |
| SVP-1001 | AccuTarget™ Validated Real Time PCR primer | Tube | 200 rxns |
| SVP-1002 | | | 100 rxns |
| SVP-1003 | | | 50 rxns |
| SP-1001/11 | AccuTarget™ GAPDH siRNA | BioRP/HPLC | 5 nmole |
| SP-1002/12 | | | 10 nmole |
| SP-1003/13 | | | 20 nmole |
| SP-2001/11 | AccuTarget™ GFP siRNA | BioRP/HPLC | 5 nmole |
| SP-2002/12 | | | 10 nmole |
| SP-2003/13 | | | 20 nmole |
| SP-3001/11 | AccuTarget™ Luciferase siRNA | BioRP/HPLC | 5 nmole |
| SP-3002/12 | | | 10 nmole |
| SP-3003/13 | | | 20 nmole |
| SN-1001/11 | AccuTarget™ Negative control siRNA | BioRP/HPLC | 5 nmole |
| SN-1002/12 | | | 10 nmole |
| SN-1003/13 | | | 20 nmole |
| SN-1021 | AccuTarget™ Fluorescein labeled negative control | BioRP | 5 nmole |
| SN-1022 | | | 10 nmole |
| SN-1023 | | | 20 nmole |
| SS-1001 | AccuTarget™ GAPDH Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | BioRP | (5P + 2N) nmole |
| SS-1002 | AccuTarget™ GFP Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | BioRP | (5P + 2N) nmole |
| SS-1003 | AccuTarget™ Luciferase Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | BioRP | (5P + 2N) nmole |
| SS-1011 | AccuTarget™ GAPDH Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | HPLC | (5P + 2N) nmole |
| SS-1012 | AccuTarget™ GFP Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | HPLC | (5P + 2N) nmole |
| SS-1013 | AccuTarget™ Luciferase Control siRNA Set, 5 nmole(Positive) + 2 nmole(Negative) | HPLC | (5P + 2N) nmole |

Contact

Bioneer Corporation

49-3 Munpyeong - dong Daedeok - gu,
Daejeon 306-220 South Korea
Domestic Phone: 82-42-930-8521 Fax: 82-42-930-8622
International Phone: 82-42-930-8523 Fax: 82-42-930-8688
Email: sales@bioneer.com

Bioneer, Inc.

1000 Atlantic Avenue,
Alameda, CA 94501-1147 USA
Toll Free: (877)264-4300 Fax: (510) 865-0350
Email: order.usa@bioneer.com

VWR (For North America & Mexico customers)

1310 Goshen Parkway
West Chester, PA 19380
Orders: 1-800-932-5000
Web Orders: www.vwr.com
Phone: (610) 431-1700 Fax: (610) 431-9174