Biology-on-Array siRNA Handbook

For regulation studies using gene silencing



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Product Use Limitations

The Biology-on-Array siRNA Plate is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN[®] products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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I. Introduction

Biology-on-Array identifies proteins that regulate the expression of any gene. Each array includes synthetic siRNAs targeting 84 biologically relevant transcription factors. Following siRNA transfection, users measure changes in cellular phenotype (such as measurement of changes in gene expression by real-time PCR). The Biology-on-Array analysis software facilitates the identification of the transcription factor(s), epigenetic modification enzyme(s) or signaling pathway(s) that regulate the expression of specific genes following real-time PCR analysis.

Using Biology-on-Array siRNA Plates allows users to easily and rapidly determine

- Molecular basis for gene expression regulation
- Mode of action of potential drug candidates
- Transcription regulators involved in phenotypic changes

Benefits of the Biology-on-Array siRNA System:

- **Complete Solution:** Screen 672 transcription factors and epigenetic modification enzymes involved in transcription regulation.
- Simple Protocol: Simple siRNA experimental procedure routinely used.
- **Discovery Tool:** Uncover the transcriptional regulation mechanism of any gene, up to 100 genes, with one experiment.

	1	2	3	4	5	6	7	8	9	10	11	12
А	Target 1	Target 2	Target 3	Target 4	Target 5	Target 6	Target 7	Target 8	Target 9	Target 10	Target 11	Target 12
В	Target 13	Target 14	Target 15	Target 16	Target 17	Target 18	Target 19	Target 20	Target 21	Target 22	Target 23	Target 24
С	Target 25	Target 26	Target 27	Target 28	Target 29	Target 30	Target 31	Target 32	Target 33	Target 34	Target 35	Target 36
D	Target 37	Target 38	Target 39	Target 40	Target 41	Target 42	Target 43	Target 44	Target 45	Target 46	Target 47	Target 48
Е	Target 49	Target 50	Target 51	Target 52	Target 53	Target 54	Target 55	Target 56	Target 57	Target 58	Target 59	Target 60
F	Target 61	Target 62	Target 63	Target 64	Target 65	Target 66	Target 67	Target 68	Target 69	Target 70	Target 71	Target 72
G	Target 73	Target 74	Target 75	Target 76	Target 77	Target 78	Target 79	Target 80	Target 81	Target 82	Target 83	Target 84
Н	NEC	NEC	NEC	NEC	NEC	NEC	POS	POS	TEC1	TEC2	NTC	NTC

Layout of a Biology-on-Array siRNA Plate:

Figure 1: Layout of a Biology-on-Array siRNA Plate.

Gene-Specific siRNA

Wells A1 through G12 each contain siRNA pairs targeting a panel of 84 relevant, biology-focused transcription regulator genes.

Negative Control for RNA Interference

Wells H1 through H4 contain a negative control siRNA (NEC). Compare or normalize the results of your phenotype assay to this negative control to ascertain the gene-specific siRNA effects on the assay.

H5-H6 negative control siRNA samples, which are without treatment, can also serve as the baseline to check the influence of desired treatment on gene expression regulation when compared with treated negative control siRNA samples (wells H1-H4).

Knockdown Efficiency control

Wells H7 through H8 contain validated siRNA (POS) that target the GAPDH gene. These wells combined with wells H5-H6, which have negative control siRNA pairs, serve as the knock down efficiency control for the array. There should be no biological treatment (control or experimental) on these wells. The total RNA from these wells can be used to check GAPDH knockdown level to confirm the siRNA delivery and knockdown efficiency. We strongly recommend checking the GAPDH knockdown efficiency before analyzing the cellular phenotype.

Transfection Efficiency Control

Wells H9 and 10 contain the siRNA for indicating the transfection efficiency. H9 contains fluorescently labeled negative control siRNA (TEC1) and H10 contains cell death control siRNA (TEC2) targeting ubiquitously expressed human genes that are essential for cell survival (knockdown induces cell death which is visible by light microscopy). Wells H11 and 12 do not contain siRNA and can be plated with normal cells and serve as no transfection control (NTC) or MOCK control (with transfection reagent).

II. Materials Provided:

Biology-on-Array siRNA Plate is shipped as an individual siRNA plate.

Biology-on-Array siRNA Plate

Components	Specifications	3				
96-well cell culture plate pre-	Individually	wrapped	under	sterile,	RNase-free	
dispensed with siRNA	conditions in a	a foil pouch				

OPEN THE FOIL POUCH ONLY UNDER STERILE CONDITIONS INSIDE YOUR CELL CULTURE HOOD.

Storage Conditions:

Biology-on-Array siRNA Plates are shipped on dry ice.

Keep siRNA plates at -20°C for long-term storage (4°C storage can be used, lower than -20°C is not recommended). The arrays are stable for six (6) months at the recommended temperature. Do not use the kit beyond the expiration date printed on the label.

III. Additional Required Reagents:

A. For Transfection

- 1. HiPerFect Transfection Reagent, QIAGEN, Cat # 301704
- 2. Opti-MEM[™] I Reduced-Serum Medium, Invitrogen, Cat #51985-034
- 3. Mammalian cell culture equipment and media
- 4. Optional: Fluorescence Microscope to monitor transfection efficiency control (TEC)

B. Optional protocol reagents Real-Time RT-PCR Verification of Gene Expression and Positive Target Gene Compression

- 1. 96-well total RNA isolation kit, such as RNeasy[®] 96 Kit (QIAGEN, Cat # 74181)
- 2. RT² HT First Strand Kit (QIAGEN, Cat # 330411)
- 3. QuantiTect[®] SYBR[®] Green PCR Kit/QuantiFast[®] SYBR Green PCR Kit

- 4. QuantiTect Primer Assays for your genes of interest
- 5. QuantiTect Primer Assay for human GAPDH
- 6. QuantiTect Primer Assays for a housekeeping gene, such as 18S rRNA, ACTB and RPL13A, to normalize the real-time RT-PCR results

IV. Protocol:

Read the entire Protocol carefully before starting your experiment.

A. Before starting the experiment:

1. <u>Transfection Protocol Optimization</u>: The success of the Biology-on-Array experiment depends on the efficiency of the reverse transfection. The transfection efficiency, in turn, depends upon the cell line, transfection reagent and the protocol. Therefore, we strongly recommend optimizing the transfection conditions before the Biology-on-Array is used. We recommend optimizing the protocol by using the Biology-on-Array Optimize siRNA Plate (Cat# 336581) with HiPerFect Transfection Reagent (Cat# 301704).

<u>NOTE:</u> To reliably observe the effects of key transcription regulators on a gene's expression, a minimum transfection efficiency of 80 percent must be reproducibly achieved.

2. <u>Condition of Cells</u>: Cells should be healthy in order to obtain an optimal siRNA transfection. The cells should be at low passage number (cells that have been passed fewer than 10 times are recommended). Importantly, passage the cells one day before the reverse transfection so that the cells are between 70% to 80% confluent at the time of transfection.

3. <u>Calibrate all pipettors</u>: All pipettors should be calibrated routinely. Use sterile, low-retention filter (barrier) tips. For each experiment, $200-\mu$ l tips and $10-\mu$ l tips are needed.

4. Important recommendations for best results:

- a. Inspect the siRNA plate foil pouches. Damage to the seal or pouch may result in contamination of the plates, thus compromising the experiment. Do not open the pouch until immediately before the experiment. Open the plate in a cell culture hood.
- b. Allow the siRNA plates to warm to room temperature.
- c. Prepare reagents, such as transfection reagent and medium, and materials, such as tips and reservoirs.
- d. Ensure that a sufficient number of cells is available at the beginning of the protocol.

B. Experimental Protocol:

Day 1: Reverse Transfection

The following protocol uses HiPerFect Transfection Reagent (Cat. No. 301704). This is a suggested protocol; the conditions and amounts should be adjusted according to the cell type, transfection reagent used and the experimental requirements.

- 1. For one siRNA array plate, dilute 115 μl of HiPerFect Transfection Reagent with 4485 μl of Opti-MEM I (1 μl HiPerFect + 39 μl Opti-MEM I per well). Mix well.
- Add 40 μl of diluted HiPerFect Transfection Reagent to each well <u>and mix by</u> <u>pipetting up and down once.</u> Resuspend siRNAs by gently tapping the side of the plate, while slightly rocking the plate back and forth, then left to right, five-seven times each.
- 3. Incubate the plate at room temperature for 20 minutes.
- 4. Prepare cells:
 - a. For adherent cells, trypsinize cells and collect by centrifugation. If using nonadherent cells, simply collect cells by centrifugation.
 - b. Wash cells once with cell culture media by resuspension and recentrifugation.
 - c. Suspend cells in fresh cell culture media.
 - d. Count the cells.

Dilute to a concentration of 40 to 80 cells per μI with normal cell culture medium.

The final concentration may depend on the cell line and experimental conditions.

- 6. When the siRNA resuspension is complete, mix the cells very well. Add 160 μ l of the cell suspension (6,000 to 12,000 cells) to each well. Mix by carefully pipetting up-and-down once.
- 7. Shake each plate back and forth then left and right gently three times to thoroughly mix the cells and the siRNA-transfection reagent complexes.
- 8. Incubate at 37°C, 5% CO₂ (or the cell's normal growth conditions) for 72 hours. The duration of the experiment should be adjusted to reflect the maximum knockdown <u>at the protein level.</u>

Day 2: Change Media and Assess Transfection

Transfection Efficiency Measurement:

1. After 24 hours of transfection, gently wash wells H9 and H11 (H9 is a transfection efficiency control well (TEC1) and H11 is a no transfection control well (NTC)) with

200 μ l of PBS, re-fill with 200 μ l of fresh PBS, and then examine the cells under a fluorescence microscope using a GFP or 488-nm filter (we recommend using 20× or greater magnification for observation).

- Count the total number of cells and the number of fluorescent cells from several randomly-chosen microscope fields in wells H9. The fields should be from the interior, as opposed to the edges. Observe H11 wells to make sure that there is no autofluorescence and compare them with TEC1 wells. Calculate the percentage of transfected cells.
- 3. Transfection efficiencies greater than 80 percent should provide sufficient knockdown for phenotype characterization.

<u>NOTE</u>: The transfection media may be replaced with fresh media after 24 hours of transfection, if your cell line requires normal serum concentrations and/or removal of the chosen transfection reagent for viability.

Day 3-4: Transfection Efficiency Measurement

Transfection Efficiency Measurement:

- After 72 hours (48-96 hours) of transfection, observe the cells in H10 and H12 (H10 is transfection efficiency control wells TEC2 and H12 is no transfection control wells –NTC) under a microscope. Significant cell death should be observed for H10 compared with H12 if the transfection efficiency is good.
- 2. Users can do cell viability assay for the H10 and H12 (assay reagent not included) for quantitative measurement.
- 3. A reduction in cell number by greater than 70 percent indicates the sufficient siRNA delivery for phenotypic characterization.

Optional Assay: Measure Gene Expression by qRT-PCR

Day 3 to 4: Experimental Treatment and qRT-PCR assays

Experimental treatment

Most proteins are significantly "knocked-down" 72 hours after siRNA transfection. Therefore, if an experimental treatment such as the addition of a small molecule or exogenous protein is planned, be sure to have the treatment and knockdown endpoints coincide. While performing a treatment, make sure that all wells from A1 to G12, as well as the NEC control wells (H1 through H4) receive the same treatment.

Total RNA extraction (96-well total RNA isolation)

Total RNA extraction can be accomplished with a 96-well RNA isolation system (such as the QIAGEN RNeasy 96 Kit). We recommend referring to the manufacturer's detailed protocol for the best results.

<u>NOTE:</u> In order to obtain reliable qRT-PCR results, it is important to begin with high quality total RNA. Therefore, we recommend that all of the RNA samples have comparable concentrations <u>(concentrations greater than 30 ng/µl are</u> <u>recommended)</u> with no more than a 6-fold difference among each sample. The concentration of RNA samples should be greater than 10 ng/µl and 260/280 OD ratio should be higher than 1.8. Please be aware that elution volumes from some RNA extraction protocols should be reduced in order to ensure higher RNA concentrations.

GAPDH Knockdown assay (Knockdown efficiency control assay)

We recommend testing knockdown efficiency of the positive control (GAPDH siRNA) before proceeding to 96 well cDNA preparation. We recommend using human GAPDH primers for GAPDH mRNA level detection and ACTB primers for the housekeeping gene control. Greater than 70% knockdown of GAPDH mRNA levels confirms an efficient siRNA delivery and siRNA potency. If the GAPDH knockdown is less than 70%, please do not proceed with the experiment and call technical support.

Reverse transcription (96-well cDNA preparation)

Considerations of RNA amount to be used:

The RT² HT First Strand Kit (QIAGEN, Catalog Number 330411) yields results with as little as 25 ng or as much as 5 µg total RNA per well reaction. However, the optimal amount of starting material depends on the relative abundance of the transcripts of interest. Lower abundance transcripts require more RNA; higher abundance transcripts require less RNA. Greater amounts of input total RNA yield a greater number of positive calls; that is, genes expressed in the linear dynamic range of the method. It is also important to use a consistent amount of total RNA for all samples to be characterized and compared in a single experiment.

- Remove the Buffer GE2 Plate from -20°C storage and leave at room temperature for 3 minutes.
- **2.** Centrifuge the Buffer GE2 Plate at 1000 rpm for 1 minute.
- **3.** Put the Buffer GE2 plate with provided rack on lab bench.
 - a. Remove the strip-caps carefully
 - b. Add 8 µl RNA sample to each well with a multi-channel pipette and mix by pipetting up and down
 - c. Completely seal the plate with the aluminum foil sealing film.

Note: The amount of total RNA should be in the range of 25 ng to 5 μ g.

- **4.** Centrifuge the plate at 1000 rpm for 1 minute.
- 5. Incubate the plate at 37°C for 5 minutes (or room temperature for 10 minutes).
- 6. Remove the aluminum seal carefully
- 7. Aliquot BC4 Reverse Transcriptase Mix into provided strip tubes
 - a. If using a 12-channel micropipettor, aliquot 53 µl per tube
 - b. If using a 8-channel micropipettor, aliquot 80 µl per tube
- **8.** Pipette 6 μl BC4 Reverse Transcriptase Mix from the provided 8-well Strip Tube with a multi-channel pipette to each well of the plate and mix by pipetting up and down
 - a. Completely seal the plate with a new piece of aluminum foil sealing film.
- **9.** Centrifuge the plate at 1000 rpm for 1 minute.
- **10.** Perform reverse transcription (this can be performed in a regular PCR cycler)
 - a. Set up a program for 42°C 15 minutes, 95°C 5 minutes, 4°C forever (without multiple cycling)
 - b. Put the plate in the PCR cycler with the reusable compression mat on top of the plate
 - c. Close lid of the PCR instrument, and run the program.
- 11. Hold the finished reaction on ice until ready to use for real-time PCR, or place at -

20°C for long-term storage.

Note: For real-time PCR, the cDNA should not be more than 5% of the total PCR volume.

Gene-of-Interest specific qPCR assay

Terminology used below

- <u>PCR plate:</u> For selecting PCR plates please refer to the qPCR machine manual or manufacturer's customer service to match a plate to a specific instrument.
- <u>PCR primer plate:</u> Customers can prepare the plate by dispensing 1× qPCR primers pair to each well of the PCR plate.
- <u>PCR reaction mix.</u> A PCR reaction mix includes dNTPs, necessary enzymes, buffers, cDNA and water.
- Prepare two PCR primer plates: one for a gene-of-interest and another for a housekeeping gene. For the gene-of-interest qRT-PCR plate, add 5 μl primer mix (1 μl of a gene-specific PCR primer pair and 4 μl nuclease-free H₂O) to each well

of a 96-well PCR plate (choose the PCR plates based on the qPCR machine). Likewise for a housekeeping gene (control) specific qRT-PCR plate, add 5 μ l primer mix (1 μ l of housekeeping gene-specific PCR primers pair and 4 μ l nuclease-free H₂O) to each well of a 96-well PCR plate.

2. In a third 96-well PCR plate, prepare the PCR reaction mix for two plates by combining PCR Master Mix, a specific cDNA sample and water into each well (as detailed below) and mix.

SYBR Green qPCR Master Mix	= 12.5 µl × 3 = 37.5 µl
Sample cDNA	= 1.0 µl × 3 = 3.0 µl
Nuclease-free H ₂ O	= 6.5 µl × 3 = 19.5 µl

General guidance for preparation of PCR reaction mix for Z number of 96-well PCR primer plates:

SYBR Green qPCR Master Mix	= 12.5 µl × (Z + 1)
Sample cDNA	$= 1 \mu I \times (Z + 1)$
Nuclease-free H ₂ O	= 6.5 µl × (Z + 1)
Nuclease-free H ₂ O	= 6.5 µl × (Z + 1)

- 3. Add 20 µl PCR reaction mix to each well of the PCR primer plates.
- 4. Run real-time PCR.
- 5. Analyze expression data with Excel[®] Data Analysis Template.

Note: QIAGEN recommends determining the expression of any gene under the mock experimental condition before the final assay. Use 100 ng total RNA for one standard RT reaction. The Ct value should be less than 30, thus ensuring a range that facilitates the identification of either positive or negative regulators. Ct values greater than 30 will make the final qPCR analysis unreliable.

C. Data Analysis Following Optional qRT-PCR Assay

Access the complimentary Biology-on-Array siRNA Data Analysis Software from the following address:

http://www.sabiosciences.com/BiologyonArrayData.php

The Excel-based data analysis software for Biology-on-Array siRNA Plates automatically performs all $\Delta\Delta$ Ct based fold-change calculations from uploaded raw threshold cycle data for the gene-specific and housekeeping gene real time-PCR assays. The spreadsheet delivers results in a tabular format and helps in automatic outlier identification and "hit" (transcription factor) identification.

Detailed instructions for using the data analysis software is given on the webpage above as well as within the spreadsheet. Provided below is a brief overview of the analysis.

- 1. Read and follow detailed instruction given on "Instruction" sheet.
- 2. Copy siRNA-plate gene information from the website and paste on "Gene Table" worksheet.
- 3. Copy the gene-specific Ct values from the PCR machine and paste them into the "Test Data" worksheet.
- 4. Copy the housekeeping gene Ct values from the PCR machine and paste them into the "Control Data" sheet.

<u>NOTE:</u> Any Ct values reported as greater than or equal to 35 or as N/A (not detected) is considered a negative call, and recognized as outlier and marked as Check, and Low input.

5. Check "QC report" sheet to identify outliers and any other possible problem.

The "QC Report" sheet is designed to provide a quality assessment of the data generated.

- <u>Gene-of-interest expression level in negative control siRNA samples</u>: If the gene-ofinterest expression Ct value for the negative control siRNA sample is greater than 35, the calculated fold changes may not reflect the true expression changes between the experimental samples and controls and hence will not be analyzed and will be marked as "Your target gene expression level is low, the result may not be reliable".
- <u>Column and row data reliability analysis:</u> will help the user identify possible positionrelated data errors, for example, a treatment was skipped for one column that results in significantly higher or lower data compared with all of the other columns or rows. Researchers should be aware of these events and carefully judge the results. If a significant number of hits come from a single row or column, it might reflect false positives.
- If an experimental treatment is used: We recommend users treat wells A1 to G12 and wells H1 to H4. Wells H5-H6 should not be treated. Significant differences between the treated and control wells will provide a wide range for identification of "hits".
 - <u>Z' score</u> is used to check the quality of the differences between the positive control (treatment negative control) and negative control (no treatment negative control). >0 indicates that they are well separated. If no experimental treatment is used, this score is not necessary.

<u>SSMD</u> (Strictly Standardized Mean Difference). SSMD, which was developed for use with RNAi screening, is the ratio between the difference of the means and the standard deviation of the difference between two populations (here the software uses the treated samples as positive and the non-treated samples as negative controls). A higher positive value (such as >2.5) indicates good separation between controls. If no treatment was used, it is not necessary to check this score.

- 6. Users should enter in experimental information and conditions in the "Summary" worksheet and automatically identify list of "hits" (proteins involved in regulating the expression of the gene-of-interest).
- 7. Select the "Result 2D" worksheet to view the potential hits. The threshold for a "hit" can also be adjusted within this worksheet.
- 8. Select "3D" worksheet to view the 3D figure for any position related bias.

Calculation:

The "hits" selection uses the Median Absolute Deviation (MAD) method to identify the positive and negative transcriptional regulators. This method can assess the strength of a "hit" and is a superior hit selection method compared to traditional methods, especially if the user chooses to perform fewer replicate experiments. The threshold is calculated for each individual experiment. If the user wants to use a fixed threshold, such as a 2-fold change, users can set it manually in "Result 2D" worksheet.

If a note reads "**Please note you are manually setting the threshold!**" shows up, it means the user has selected the manual threshold setting. Users can switch back to default setting just by deleting the number on the "Result 2D" worksheet.

NOTE: Change data only in yellow highlighted cells of data analysis software. Please do not change Gray and White cells because they contain formulas for calculation or results.

D. Suggestions for target validation

The Biology-on-Array siRNA Plates identify transcription factors associated with a userselected gene's expression. Users should validate the "hits" identified with the Biologyon-Array siRNA Plates. Typical validation experiments include the following controls/conditions:

- Choose two different RNAi designs for each identified transcription factor to eliminate possible off-target effects.
- Carry out siRNA experiments with and without treatments during the validation process to ensure the specificity of results.

• Use three different biological and technical replicates to obtain statistically significant data.

VI. Troubleshooting and FAQs

A. Why do I not observe any positive hits?

Low Transfection Efficiency

Check the transfection efficiency using transfection efficiency control (TEC) wells to insure that the transfection efficiency was high enough (> 80%) to provide knockdown results. If the transfection efficiency is < 80%, re-optimize your transfection conditions and protocol.

B. How can I optimize or improve low transfection efficiencies?

We highly recommend you to use our Biology-on-Array Optimize siRNA Plate to optimize your transfection conditions. For better estimation of transfection efficiency, we also recommend staining cells with a nuclear DNA stain to count both transfected (green-positive) cells and the total number of cells (nuclear DNA stain) in the same fluorescent view of the same microscopic field. Be sure to obtain numbers from several different randomly-chosen microscopic fields in the interior (not toward the edges) of the cell culture well.

C. What transfection method should I use with the Biology-on-Array siRNA Plates?

- 1. If you have already optimized a reverse transfection reagent and protocol for your cell line of interest, you may use that reagent and protocol for reverse transfection of the same cell line in the Biology-on-Array siRNA Plate.
- 2. If you have never optimized reverse transfection of your cell line of interest before, we first recommend trying high-efficiency, low-toxicity reverse transfection reagent, such as HiPerFect Transfection Reagent from QIAGEN. Follow the manufacturer's instructions and recommendations for optimizing your reverse transfection protocol.
- 3. When optimizing any reverse transfection reagent or method, use a marker and/or method that count cells, such as fluorescently-labeled siRNA.
- 4. If your cells do not tolerate standard or traditional transfection methods, electroporation may be a useful alternative.

D. Can I use electroporation to transfect in the Biology-on-Array siRNA Plates?

Yes. However, test and optimize the chosen electroporation device and method with your cell line of interest first, using the following protocol:

- 1. Suspend the siRNA in the Biology-on-Array siRNA Plate wells into an appropriate volume of the electroporation buffer.
- 2. Transfer the contents of each Biology-on-Array siRNA Plate well into the corresponding wells of the 96-well electroporation plate.
- 3. Cells may be loaded before or after the addition of the siRNA. See the recommendations given by the electroporation plate manufacturer.
- 4. Follow the device manufacturer's instructions for electroporation conditions.
- 5. Transfer the transfected cells back into the correct wells of the original siRNA plate or a fresh 96-well cell culture plate suitable for your phenotype assay.

E. Can I use the Biology-on-Array siRNA Plates with primary cells or macrophages?

Yes, with a validated method. With the improvement of transfection technology, many new methods have been used in primary cells. Please validate them before use with Biology-on-Array siRNA Plates for those cell lines that tend to be difficult to transfect by standard or traditional methods. The Biology-on-Array siRNA Plates are meant for use with *in vitro* cultured human cell lines only.

F. What shall we do after some "hits" are identified?

Further validation is needed to confirm the results. Several ways can be used to confirm the transcription regulator's effect on the gene of interest.

- a. Use two or more validated individually designed siRNA to prove the same gene expression change under the same experimental condition. We recommend high-purity QIAGEN siRNA for this purpose (www.giagen.com/GeneGlobe).
- b. Use ChIP qPCR to confirm the binding enrichment with the antibody of the "hit". Please refer to our ChIP product information at: <u>http://www.sabiosciences.com/chipqpcr.php</u>
- c. Use a rescue method by expressing active 'hit' protein with an ORF construct while knocking down the "hit' gene with an siRNA targeting the 3' UTR.

G. How many replicates do I need for Biology-on-Array siRNA Plates?

For screening purposes, usually one test is fine if cost is a barrier. Duplicates will help identify occasional false negative hits that may occur due to individual transfection failure and missing samples. More than three replicates are not necessary.

For any other troubleshooting or technical questions about the Biology-on-Array siRNA Plates, please call one of our Technical Support representatives at 1-888-503-3187 or 301-682-9200 or email at <u>support@SABiosciences.com</u>

Ordering Information

Product	Contents	Cat. no.
Biology-on-Array siRNA Plate	siRNA plate targeting a focused panel of 84 transcription factors	Varies
Biology-on-Array Optimize siRNA Plate	siRNA plate for transfection optimization	336581

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