TranSignal™ Function-Specific Protein/DNA Arrays

(Spin Column Separation Version)

Cat. # MA1500, MA1505 & MA1510
Product User Manual
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1. INTRODUCTION

Eukaryotic gene expression is regulated by a group of proteins called transcription factors (TFs). By interacting with specific DNA-binding elements present in the promoters of certain genes, TFs modulate the frequency of transcriptional initiation. The expression or activity of TFs may be regulated in a cell-type, tissue-specific, or cell cycle-dependent manner. Regulation can also be mediated by interactions with other proteins. Through different combinations of these regulatory mechanisms, eukaryotes are able to elicit myriad gene expression patterns (1). The key to a full understanding of how gene expression is regulated is the analysis of the biochemical activity of TFs.

Panomics' TranSignal Protein/DNA Arrays simplify the functional analysis of eukaryotic TFs. Our array-based technology is a significant improvement over cumbersome gel mobility-shift assays, which permit the characterization of only a single TF at a time. With TranSignal™ Arrays, you can profile the activities of multiple TFs simultaneously.

TranSignal Function-Specific Protein/DNA Arrays are designed for researchers who need to focus on transcription factors involved in specific biological processes. We currently offer three of these function-specific arrays:

TranSignal cAMP/Calcium Protein/DNA Array

Second messengers like cAMP and calcium regulate a number of physiologic processes—including metabolism, cellular proliferation, and neuronal signaling—by altering patterns of gene expression through changes in the DNA-binding properties of transcription factors. A good first step toward teasing apart these layers of regulation is profiling the activities of cAMP/calcium-regulated transcription factors. With the TranSignal™ cAMP/Calcium Protein/DNA Array, you can easily determine how the activities of cAMP- and calcium-regulated transcription factors change under different conditions. The array profiles the activity of 20 TFs that are known to be regulated by cAMP and calcium.

Transignal Nuclear Receptor Protein/DNA Array

The nuclear hormone receptor superfamily encompasses receptors for small lipotranscription factors, binding to the *cis*-elements that mediate expression of genes that control the growth, differentiation, and

reproduction of multicellular eukaryotes. The TranSignal Nuclear Receptor Protein/DNA Array is designed for researchers who are interested in how the activities of these nuclear receptors change under different conditions. With the TranSignal Nuclear Receptor Protein/DNA Array, you can profile the activities of 15 nuclear receptors.

TranSignal Cell Growth Protein/DNA Array

Cell growth and differentiation demand a delicate balance between signaling and regulation of transcription. This balance depends on a series of events initiated when an extracellular stimulus binds to a cell surface receptor. Binding triggers a signaling cascade, which migrates through the cytoplasm and into the nucleus. Once transmitted to the nucleus, the signal regulates transcription factors, which turn on and off genes that mediate growth and differentiation. The TranSignal Cell Growth Protein/DNA Array provides a good starting point for researchers who are looking to dissect this series of events. The array profiles the activity 20 TFs that are key players in cell growth and differentiation.

Each consensus sequence spotted on each array corresponds to a specific transcription factor. These transcription factors were selected because they are well charaterized and widely available in published literature.

Principle of TranSignal™ Technology

TranSignal Arrays use a proprietary, patent-pending technology developed by Panomics for the high-throughput analysis of TF activation. The TranSignal procedure is simple and straightforward (Figure 1). Three basic steps are involved: (1) a set of biotin-labeled DNA binding oligonucleotides (TranSignal Probe Mix) are preincubated with a nuclear extract of interest to allow the formation of DNA/protein complexes; (2) the protein/DNA complexes are separated from the free probes; and (3) the probes in the complexes are then extracted and hybridized to the TranSignal Array. Each kit includes the reagents for HRP-based chemiluminescence detection.

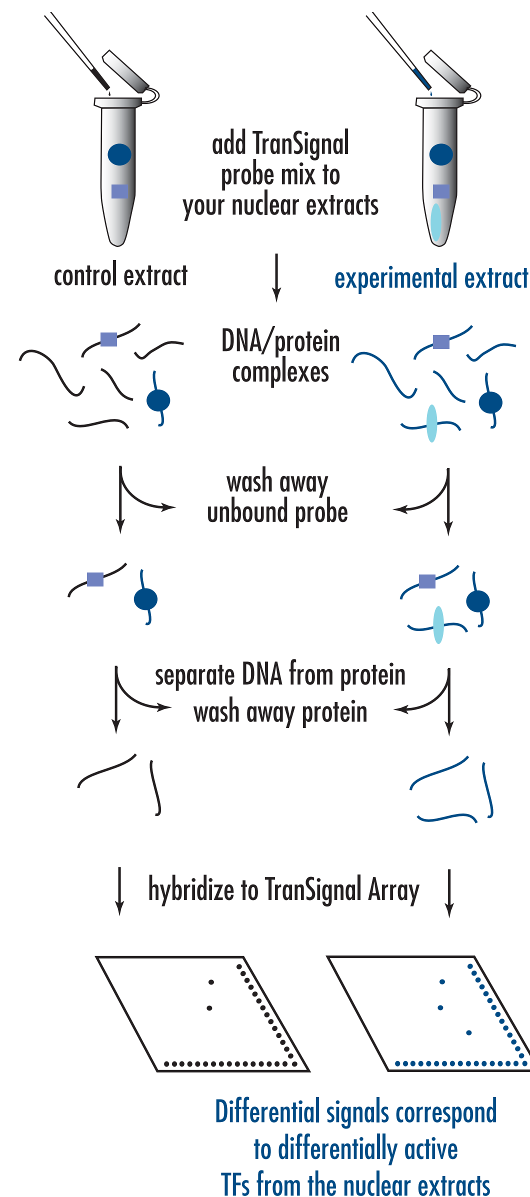

Figure 1: Schematic flow chart of the TranSignal Function-Specific Protein/DNA Array procedure.

2. MATERIALS PROVIDED

Box 1: Array Membranes & Hybridization Reagents

- TranSignal™ Protein/DNA Array (3 each)
- Spin Column (3 each)
- Spin Column Collection Tube (6 each)
- 1X Column Incubation Buffer (2 ml)
- 1X Column Wash Buffer (10 ml)
- Hybridization Buffer (15 ml)
- 20X SSC (32 ml)
- 20% SDS (15 ml)
- Streptavidin-HRP conjugate (60 µl)
- 4X Wash Buffer (45 ml)
- Solution I (750 µl)
- Solution II (750 µl)
- Solution III (8 ml)

Box 2: Reaction Kit

- TranSignal™ Probe Mix (30 µl)
- 1X Column Elution Buffer (200 µl) **Bring to room temperature before use.**
- Distilled H_2O (RNase, DNase free; 500 µl)
- Control Nuclear Extract (from HeLa cells; 5 µl)
- 2X Blocking Buffer (30 ml)
- 1X Detection Buffer (60 ml)

** These reagents are provided as stock solutions. See Appendix A for instructions on how to dilute these solutions/buffers.*

*** See Product Info sheet for specific information about the Function-Specific Protein/DNA Array and probe mix.*

3. ADDITIONAL MATERIALS REQUIRED

3.1 Reagents and Solutions

- Nuclear Extraction kit (e.g., Panomics Nuclear Extraction Kit, Cat. # AY2002)
- Hybridization Wash I (2X SSC/0.5% SDS)*
- Hybridization Wash II (0.1X SSC/0.5% SDS)*
- Deionized H_2O

* See Appendix A for recipes.

3.2 Materials and Equipment

- 0.5-ml and 1.5-ml microfuge tubes
- Pipetman and tips
- Microcentrifuge
- Hybridization oven and bottles (Stratagene, Cat.# 401030)
  Note: skirted centrifuge tubes with screw caps may be used in place of the hybridization bottles (VWR, Cat. # 21008-480). Hybridization bottle dimensions: 150 mm x 35-mm diameter tubes.
- Plastic containers (~4.5" x 3.5"; equivalent to the size of a container for 200-µl pipet tips)
- Shaker
- Plastic sheet protectors, or overhead transparency (used for detection, see Section 7.6)
- Hyperfilm ECL (Amersham, Cat.# RPN3114K)

OR

- Chemiluminescence imaging system (e.g., FluorChem™ from Alpha Innotech Corp.)

4. PREPARING NUCLEAR EXTRACT FROM CELLS OR TISSUES

Nuclear extracts can be prepared using the method described by Dignam *et al.* (6). Alternatively, you can use a commercially available kit, such as Panomics' Nuclear Extraction Kit (Cat. # AY2002). For best results, your nuclear extraction sample should have at least 3-5 µg of protein per µl.

5. PREPARING TF-BOUND DNA

In this Section, you will allow the DNA probes to bind transcription factors from your nuclear extract. Be sure to dilute solutions/buffers as described in Appendix A.

5.1 For each nuclear extract sample, combine the following components into a sterile 0.5-ml microcentrifuge tube (* = provided):

Nuclear extract (3–5 µg/µl)	5	µl
TranSignal Probe Mix*	10	µl
dH2O (RNase, DNase free)	5	µl
Total Volume	20	µl

NOTE: If the protein concentration of your nuclear extract does not fall within the recommended range, you can replace the dH_2O with additional nuclear extract. If sample is still too dilute (less than 15-25 ug total protein), you can concentrate with a Microcon Centrifuge Tube (10,000 NWML).

5.2 Mix well by pipeting. Incubate samples at 15°C for 30 min.

6. ISOLATING TF-BOUND PROBES

In this section, you will isolate the protein-bound probes from the non-bound probes. All centrifuge steps should be carried out on a regular benchtop centrifuge, at 7,000 rpm at 4°C, unless otherwise stated.

6.1 Wash Spin Column by adding 500µl chilled 1X Column Incubation Buffer and centrifuging at 7,000 rpm for 30 sec at 4°C.

6.2 Add 20µl 1X Column Incubation Buffer to the TF-Probe mix, from step 5.2. Transfer all of this mix into the center of the Spin Column.

6.3 Incubate the Spin Column **on ice** for 30 min.

6.4 Centrifuge column at 7,000rpm for 30 sec at 4°C and discard the flow through.

6.5 Place the Spin Column in a new Spin Column Collection Tube (provided).

6.6 Add 600µl 1X Column Wash Buffer to the Spin Column and incubate for 10 min, **on ice**.

6.7 Centrifuge column at 7,000rpm for 30 sec at 4°C and discard the flow through.

6.8 Wash the column by adding 600µl 1X Column Wash Buffer to the Spin Column and centrifuging at 7,000rpm for 30 sec at 4°C.

6.9 Repeat step 6.8 a further 3 times.

6.10 Remove residual Wash Buffer by an additional centrifugation at 10,000rpm for 30 sec at 4°C

6.11 Add 60µl 1X Column Elution Buffer to the center of the Spin Column and incubate at room temperature for 5 min.

6.12 Place the Spin Column in a clean 1.5ml microcentrifuge tube and centrifuge for 1 minute at 10,000rpm at room temperature.

6.13 Place the microfuge tube, containing the collected flow through on ice and use for further steps.

7. HYBRIDIZATION

In this Section, you will hybridize the labeled probe (prepared in Section 6) to the array membrane. Before you begin, warm the hybridization buffer to 42°C in a water bath. If you notice a cloudy or soapy appearance, make sure the particulates are completely dissolved before proceeding with hybridization. It may require overnight heating in a water bath.

7.1 Place each array membrane into a hybridization bottle. Wet the membrane by filling the bottle with deionized H_2O. Then, carefully decant the water. Be sure to place the membrane in the hybridization bottle such that the spotted oligos face the center of the tube (away from the walls). *NOTE: When the membrane is properly oriented, the notched corner will be in the top right*.

7.2 Add 3–5 ml of prewarmed Hybridization Buffer (provided) to each hybridization bottle that contains an array membrane. Place each bottle in the hybridization oven at 42°C for 2 hr.

7.3 Denature the eluted probe (prepared in Section 6) by heating it at 95°C for 3 min and quickly chill in ice for 2 min. Add the eluted probe to each hybridization bottle and hybridize at 42°C overnight.

7.4 Decant the hybridization mixture from each hybridization bottle, and wash each membrane as follows (*See Appendix A for recipes):

7.4.1 Add 50 ml of prewarmed Hybridization Wash I*, incubate at 42°C

for 20 min in a rotating hybridization oven. Decant liquid and repeat wash.

7.4.2 Add 50 ml of prewarmed Hybridization Wash II*, incubate at 42°C for 20 min in a rotating hybridization oven. Decant liquid and repeat wash.

8. DETECTION

IMPORTANT: Do not allow the membrane to dry during the detection.

8.1 Using forceps, carefully remove each membrane from its hybridization bottle and transfer to a new container containing 20 ml of 1X Blocking Buffer; each membrane needs its own container. (We use a container that is equivalent to the size of a 200-µl pipet-tip container, ~4.5" x 3.5".)

8.2 Block the membrane by incubating at room temperature with the 1X Blocking Buffer for 15 min with gentle shaking.

8.3 Dilute 20 µl of Streptavidin-HRP conjugate 1:1000 with the 1X Blocking Buffer from the blot container. (Prepare the dilution by removing 1 ml of the 1X blocking buffer to a clean microcentrifuge tube and adding 20 µl of the stock Streptavidin-HRP conjugate.) Vortex the diluted streptavidin and transfer back into the 1X Blocking Buffer from Step 8.2 (containing the membrane), making sure not to pour directly on the membrane. Continue shaking the membrane for 15 min at room temperature.

8.4 Decant the diluted Streptavidin-HRP solution. Wash each membrane three times with 20 ml of 1X Wash Buffer for 8 min at room temperature.

8.5 Add 20 ml of 1X Detection Buffer to each membrane and incubate for 5 min at room temperature.

8.6 Overlay each blot with 2.5 ml of working substrate solution, prepared by mixing thefollowing, in order: 250 µl Solution I with 250 µl Solution II. Briefly vortex and add 2.0 ml Solution III. Throughly mix. Using a plastic sheet protector or overhead transparency film (whichever is readily available), place each membrane on a plastic sheet. Then, pipet 2.5 ml of the mixed substrate solution onto each membrane and overlay each with a second plastic sheet. **Ensure that substrate is evenly distributed over the membrane with no air bubbles.** Incubate at room temperature for 5 min.

8.7 Remove excess substrate by gently applying pressure over the top sheet. Using a paper towel, remove excess substrate that might be remaining on the surface of the sheets. Expose the membranes using either Hyperfilm™ ECL (2-10 min) or a chemiluminescence imaging system (5-15 min), such as the FluorChem™ imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposure times.

8.8 Obtain quantitative analysis, if desired. If you are using a chemiluminescence imaging system, follow the instructions that are provided with that system's software. If you are using Hyperfilm ECL, you will need to scan the film to obtain numerical data for comparison.

9. RESULTS & ANALYSIS

The main advantage of the TranSignal Protein/DNA Arrays is that you can simultaneously analyze multiple transcription factors. TranSignal Arrays give you quick answers when you want to identify those activated transcription factors through comparison of two (or more) samples. Follow these guidelines to analyze your results:

9.1. Acquire the images using either x-ray film or a chemiluminescence imaging system.

9.2. Adjust the exposure time such that the majority of the spots have equal signal intensity.

9.2.1 If you are acquiring your image with an imaging system, such as

FlourChem, measure the density of the spots and convert denisty to numbers using applicable software. (Pruchase of a FlourChem Imaging System includes software that allows the density of each spot to be measured. At Panomics, we use the local area surrounding the individual spots for background subtraction).

9.2.2 If you are suing x-ray film, obtain an electronic image of your blot. Then analyze the density of each spot using software with this ability.

9.3 Save data in an Excel spreadsheet and calculate the ratio of the data collected from the images. (For example, if your experiment contaings one control and two experimentals, you may want to collect the ratios of sample 1 vs. sample 2; and sample 1 vs. sample 3; and perhaps, sample 2 vs. sample 3).

9.4 Any spots with two-fold increase or decrease are considered significant, and should be confirmed by EMSA and/or luciferase reporter assay. *NOTE: During the quantification process, some spots may show a two-fold increase or decrease without a visible spot present on your membrane.These data points are insignificant and should be considered background on the arrays.*

10. TROUBLESHOOTING GUIDE

Problem	Cause	Recommendation
Uneven background	Substrate is not evenly distributed on the membrane.	Shake 1:10 diluted substrate for 30 min.
		Expose longer—10 to 15 min
High Background	Incubation with substrate is too long.	Incubation should not exceed 5 min.
	Samples ran slowly in the gel— insufficient separation of protein/DNA complex occurred.	Repeat Steps 5.1 to 5.6.
Signal is too weak	The density of the detected spots are weak, but alignment spots are OK	The yield of recovered DNA probe is low. Confirm using control nuclear extract (provided). Also, check the concentration of your nuclear extract.
	Gel was not completely dissolved in Extraction Buffer A.	Use 300 µl of Extraction Buffer A for each 100 mg of gel.

11. REFERENCES

1. Chodosh, L.A., Olesen, J., Hahn, S., Baldwin, A.S., Guarente, L., and Sharp, P.A. (1988) A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. *Cell* **53**:25–35.

2. Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Research* **11**:1475–1489.

TRANSIGNAL FUCNTION-SPECIFIC PROTEIN/DNA ARRAYS

Please visit our website at www.panomics.com for the most up-to-date information about our products.

Products	Size	Catalog#
TranSignal cAMP/Calcium Protein/DNA Array	1 kit	MA1500
TranSignal Cell Growth Protein/DNA Array	1 kit	MA1505
TranSignal Nuclear Receptor Protein/DNA Array	1 kit	MA1510
TranSignal cAMP/Calcium Protein/DNA Refill Kit	1 kit	MA1600
TranSignal Cell Growth Protein/DNA Refill Kit	1 kit	MA1605
TranSignal Nuclear Receptor Protein/DNA Refill kit	1 kit	MA1610

RELATED PRODUCTS

Nuclear Extraction Kit	10 rxn	AY2002
EMSA Kit	25 rxn	many

APPENDIX A: Recipes & instructions for diluting stock solutions

SECTION 6

- **300 ml of 2X SSC/0.5% SDS (Hybridization Wash I)**

  To 262.5 ml of deionized H_2O, add 30 ml of 20X SSC (provided) and 7.5 ml of 20% SDS (provided). Mix well.

- **300 ml of 0.1X SSC/0.5% SDS (Hybridization Wash II)**

  To 291ml of deionized H_2O, add 1.5 ml of 20X SSC (provided) and 7.5 ml of 20% SDS (provided). Mix well.

SECTION 7

- **60 ml of 1X Blocking Buffer**

  To 30 ml of deionized H_2O, add 30 ml of 2X Blocking Buffer (provided). Mix well and store at 4°C.

- **180 ml of 1X Wash Buffer**

  To 135 ml of deionized H_2O, add 45 ml of 4X Wash Buffer (provided). Mix well and store at room temperature.

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APPENDIX B: Schematic diagram of the TranSignal cAMP/Calcium Protein/DNA Array

LEGEND:

	1	2	
A	AP1	AP1	normal
B	AP1	AP1	diluted 1:10

	1	2	3	4	5	6	7	8
A	AP1	AP1	c/EBP	c/EBP	CBF	CBF	CREB	CREB
B	AP1	AP1	c/EBP	c/EBP	CBF	CBF	CREB	CREB
C	E4F, ATF	E4F, ATF	EGR	EGR	Ets	Ets	GATA-3	GATA-3
D	E4F, ATF	E4F, ATF	EGR	EGR	Ets	Ets	GATA-3	GATA-3
E	GATA-4	GATA-4	HNF-4	HNF-4	HSE	HSE	MEF-2	MEF-2
F	GATA-4	GATA-4	HNF-4	HNF-4	HSE	HSE	MEF-2	MEF-2
G	NFAT	NFAT	NF-E1	NF-E1	NFkB	NFkB	OCT-1	OCT-1
H	NFAT	NFAT	NF-E1	NF-E1	NFkB	NFkB	OCT-1	OCT-1
I	PPAR-γ	PPAR-γ	Rel	Rel	Smad SBE	Smad SBE	Sp1	Sp1
J	PPAR-γ	PPAR-γ	Rel	Rel	Smad SBE	Smad SBE	Sp1	Sp1
K								
	1	2	3	4	5	6	7	8

Figure 3. Schematic diagram of the TranSignal cAMP/Calcium Protein/DNA Array. *The genes on the array are spotted in duplicate: the first row is DNA spotted normally, the second row is DNA diluted 1:10. The dark grey columns along the right and bottom sides of the array indicate where biotinylated DNA has been spotted. These spots are intended for alignment. (Note that the notch is at the top, right-hand corner.)*

APPENDIX C: Schematic diagram of the TranSignal Cell Growth Protein/DNA Array

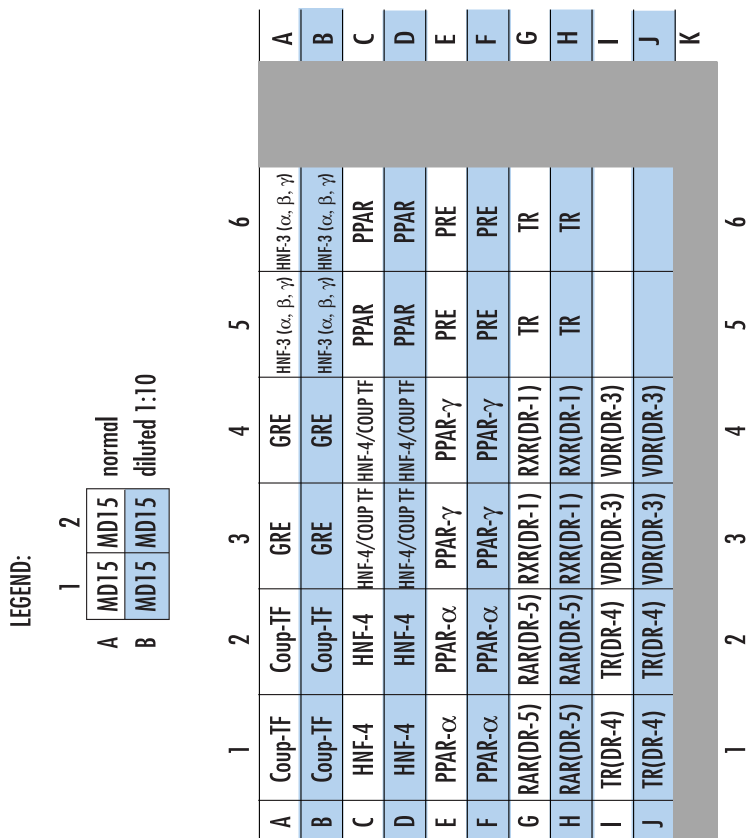
LEGEND:

	1	2	
A	AP1	AP1	normal
B	AP1	AP1	diluted 1:10

	1	2	3	4	5	6	7	8
A	AP1	AP1	c-Myb	c-Myb	CREB	CREB	E2F-1	E2F-1
B	AP1	AP1	c-Myb	c-Myb	CREB	CREB	E2F-1	E2F-1
C	EGR	EGR	Ets	Ets	FKHR	FKHR	Myc-Max	Myc-Max
D	EGR	EGR	Ets	Ets	FKHR	FKHR	Myc-Max	Myc-Max
E	NF-E1 (YY1)	NF-E1 (YY1)	NFkB	NFkB	OCT-1	OCT-1	p53	p53
F	NF-E1 (YY1)	NF-E1 (YY1)	NFkB	NFkB	OCT-1	OCT-1	p53	p53
G	pax-2	pax-2	Pax-3	Pax-3	pax-6	pax-6	PPAR-γ	PPAR-γ
H	pax-2	pax-2	Pax-3	Pax-3	pax-6	pax-6	PPAR-γ	PPAR-γ
I	Smad SBE	Smad SBE	Sp1	Sp1	SRE	SRE	Stat3	Stat3
J	Smad SBE	Smad SBE	Sp1	Sp1	SRE	SRE	Stat3	Stat3
K								
	1	2	3	4	5	6	7	8

Figure 4. Schematic diagram of the TranSignal Cell Growth Protein/DNA Array. *The genes on the array are spotted in duplicate: the first row is DNA spotted normally, the second row is DNA diluted 1:10. The dark grey columns along the right and bottom sides of the array indicate where biotinylated DNA has been spotted. These spots are intended for alignment. (Note that the notch is at the top, right-hand corner.)*

APPENDIX D: Schematic diagram of the TranSignal Nuclear Receptor Protein/DNA Array



LEGEND:

	1	2	
A	MD15	MD15	normal
B	MD15	MD15	diluted 1:10

	1	2	3	4	5	6
A	Coup-TF	Coup-TF	GRE	GRE	HNF-3 (α, β, γ)	HNF-3 (α, β, γ)
B	Coup-TF	Coup-TF	GRE	GRE	HNF-3 (α, β, γ)	HNF-3 (α, β, γ)
C	HNF-4	HNF-4	HNF-4/COUP TF	HNF-4/COUP TF	PPAR	PPAR
D	HNF-4	HNF-4	HNF-4/COUP TF	HNF-4/COUP TF	PPAR	PPAR
E	PPAR-α	PPAR-α	PPAR-γ	PPAR-γ	PRE	PRE
F	PPAR-α	PPAR-α	PPAR-γ	PPAR-γ	PRE	PRE
G	RAR(DR-5)	RAR(DR-5)	RXR(DR-1)	RXR(DR-1)	TR	TR
H	RAR(DR-5)	RAR(DR-5)	RXR(DR-1)	RXR(DR-1)	TR	TR
I	TR(DR-4)	TR(DR-4)	VDR(DR-3)	VDR(DR-3)		
J	TR(DR-4)	TR(DR-4)	VDR(DR-3)	VDR(DR-3)		
K						

Figure 5. Schematic diagram of the TranSignal™ Nuclear Receptor Protein/DNA Array. *The genes on the array are spotted in duplicate: the first row is DNA spotted normally, the second row is DNA diluted 1:10. The dark grey columns along the right and bottom sides of the array indicate where biotinylated DNA has been spotted. (Note that the notch is at the top, right-hand corner.) These spots are intended for alignment.*

APPENDIX E: Stripping Procedure for TranSignal Arrays

Note: We do not encourage stripping the TranSignal array membranes more than two times.

Procedure

1. Wash membranes in 0.4M NaOH at 45°C for 30 min .
2. Wash membranes in 0.2M Tris-HCL, pH 7.6; 0.1X SSC, and 0.1% SDS at 45°C for 15 min .
3. To ensure that stripping was successful, run it through the standard chemiluminescence detection procedure as described in this user manual.
4. After detection, wash the membrane in 1X Washing Buffer at 42ºC for 30 min.
5. Membranes are ready for hybridization or dry the membrane in an 80ºC incubator for later use.