RapidScan™
Gene Expression cDNA Panels

APPLICATION GUIDE

Table of Contents

PACKAGE CONTENTS AND STORAGE CONDITIONS

The following components are included:

- Two identical, sealed Rapid-Scan™ multi-well PCR plates containing a group of dried, serially diluted, PCR†-ready, first-strand cDNAs
- Two adhesive cover sheets for sealing the multi-well PCR plates
- Control primers

Rapid-ScanTM Gene Expression Panel	Cat. #	Format (Wells)	# of plates provided	Control primers	See Page
Human-24	HSCA-101	96 (8x12)	2	Human β-actin	10-11
Human Brain	BSCD-101	48 (4x12)	2	Human β-actin	12-13
Human Breast	TSCE-101	96 (8x12)	2	Human β-actin	14-15
Mouse-24	MSCB-101	96 (8x12)	2	Mouse β-actin	16-17
Mouse Brain	NSCF-101	96 (8x12)	2	Mouse β-actin	18-19
Drosophila	DSCC-101	48 (4x12)	2	Drosophila RP49	20-21

- Free sample of DNA Quanti-Ladder™, a ready-to-use molecular marker for easy quantitation and fragment-size determination
- Free sample of Rapid-Load™ direct-loading dye for electrophoresis of PCR products

The above components are shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, they have a 12-month shelf life.

The following components are required but are not provided:

- Reagents for PCR amplification (buffer, Taq DNA polymerase, dNTPs)
- Gene-specific primers
- A 96-well thermocycler with heated lid

†The polymerase chain reaction (PCR) is protected by patents held by Hoffmann-La Roche. Purchase of any of OriGene's PCR-related products does not convey a license to use the PCR process, covered by these patents. Purchasers of these products must obtain a license before performing PCR.

NOTE: FOR RESEARCH PURPOSES ONLY! NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

We will replace, at no cost, any product of ours that does not meet our standard product specifications. No other warranties, expressed or implied, are given with our products. OriGene Technologies, Inc. is not liable for any damages due to the use of this product nor are we liable for the inability to use this product.

Related Products

TissueScan Real-Time Human Tissue Panels (HMRT101; HBRT101)
TissueScan Real-Time Mouse Developmental Tissue Panels (MDRT101)
TissueScan Real-Time Disease Expression Panels (HCRT101; HORT101; HLRT101)

INTRODUCTION

With the availability of technologies to support high-throughput gene discovery, comes the need for complementary technologies for large-scale functional discovery. An essential first step in this process is to determine the comprehensive expression profiles of the newly identified genes, information that may provide key clues to our understanding of biological functions. OriGene Technologies, Inc. is a biotechnology company focused on developing products to support high-throughput gene cloning and analysis. Rapid-Scan™ Gene Expression Panels were developed to serve this market.

Rapid-Scan is a PCR-based system, using high-quality first-strand cDNAs derived from different tissues and/or developmental stages, to generate a "comprehensive" expression profile of any cloned gene or identified expressed sequence tag (EST). Individual first-strand cDNAs have been tested to assure that low abundance and long transcripts are represented, and have been normalized using a generally accepted house-keeping cDNA as an internal standard, β-actin in the human and mouse panels; and RP49 cDNA in the Drosophila panel.

The cDNAs were then serially diluted over a 4-log range and arrayed into a multi-well PCR plate, in order to ensure that the amplification reaction will be within the linear range and, hence, to facilitate semi-quantitative determination of relative mRNA accumulation in the various tissues and/or developmental stages. With this non-radioactive technique, one can generate a comprehensive expression profile for any given human or mouse or Drosophila gene in just three hours. All that is required is a multi-well PCR using gene-specific primers, followed by electrophoretic analysis of the products in an agarose gel.

Benefits of the Rapid-Scan panel:

- Fast and non-radioactive (no radio labeling of probe/no blot hybridization/no filter washing)
- Simultaneous examination of a wide range of different tissues and/or developmental stages
- High-sensitivity and semi-quantitative
- Simultaneous analysis of alternatively spliced or familial transcripts

PRODUCTION AND QUALITY ASSURANCE:

Rapid-Scan panels were assembled by selecting either frequently studied tissues and/or developmental stages. To avoid detection of individual differences in gene expression, tissues were pooled, whenever possible, from multiple individuals. For the human panel, tissues were from individuals of different ethnicity. For the mouse panel, adult tissues were from out bred Swiss Webster mice, the embryos from Inbred of FVB, and breast tissues from out bred CD1 mice. For the Drosophila panel, the tissues were from Drosophila melanogaster Canton S strain.

Total RNA was isolated and subjected to oligo(dT) selection. The recovered poly A^+ RNA was then examined by Northern blot hybridization, using a β-actin cDNA probe as control, to confirm RNA integrity. The poly A^+ RNA was then used to synthesize first-strand cDNA, using oligo(dT) primers and MMLV reverse transcriptase. Individual cDNA pools were confirmed to be free of genomic DNA contamination and to contain complete reverse transcripts of selected rare and long mRNAs, such as those for the transferrin receptor (5 kb) and the ataxia telangiectasia gene (9.4 kb). The first-strand cDNAs from each human and mouse tissue were then subjected to normalization, such that they all contain an equivalent concentration of β-actin cDNA, while Drosophila cDNAs all contain an equivalent concentration of RP49 cDNA . Each cDNA was diluted in water to a series of four concentrations (labeled 1000x, 100x, 10x and 1x), with the lowest concentration (1x) being approximately 1 pg. The diluted cDNAs were subsequently arrayed into a multi-well PCR plate in the order indicated in Figs. 1, 3, 5, 7, 9 and 11.

PROTOCOL FOR RAPID-SCAN PANELS

Primer design

Primer design is a crucial factor in obtaining success in PCR. There are no simple rules, but there is a general set of guidelines for primer design that are reported to aid in the amplification of specific product. Primers should be between 17 and 25 nucleotides in length, have about a 50% G/C content and do not form strong secondary structures. Avoid sequences that are susceptible to primer-dimer formation. There are several commercially available computer programs for designing PCR primers.

In addition:

- The primers should ideally correspond to the coding region of the gene of interest. The positions of the forward and the reverse primers should be between 300 and 1000 bp apart, so as to facilitate efficient template amplification and easy detection of the PCR product in an agarose gel.

- If possible, a pilot PCR amplification should be performed using the genespecific primers and a test template, such as a plasmid cDNA clone or a first-strand cDNA preparation that is known to contain the target sequence of interest.

Experimental procedure

- Remove the Rapid-Scan plate from -20ºC storage and allow it to warm to room temperature.
- Rapid-Scan™ has two different formats, 48-well and 96-well. Prepare a PCR pre-mix* according to the Rapid-Scan™ you are using.

  * Note: Please add an appropriate amount of magnesium to your premix if your 10X PCR buffer does not contain magnesium.

	for 96-well	*for 48-well*	
Stock Solution	**Volume**	**Volume**	**Final**
10x PCR Buffer	250 uL	125 uL	1x
dNTP (2 mM each)	250 uL	125 uL	0.2 mM
Forward Primer (10 pmol/uL)	100 uL	50 uL	10 pmol/well
Reverse Primer (10 pmol/uL)	100 uL	50 uL	10 pmol/well
ddH_2O	1790 uL	895 uL	–
Taq DNA Polymerase (5 U/uL)	10 uL	5 uL	0.5 U/well
Total	*2500 uL*	*1250 uL*	

- Remove the sealing film from the Rapid-Scan plate. Aliquot 25 uL of the PCR pre-mix to each well, avoiding cross-contamination during pipetting. This can best be achieved using a multichannel pipettor.
- Cover the top of the plate with a new adhesive cover sheet (provided). Seal each well tightly by pressing the cover around each well. Remove any air bubbles that may have been trapped at the bottom of the tubes by gently tapping the plate. Let the plate sit on ice for 15 min to allow the dried cDNA to dissolve. Gentle vortexing facilitates the re-suspension of the DNA.
- NOTE: ***Tight seals are critical for the successful use of Rapid-Scan products.***
- Mount the plate snugly into the block of a 96-well thermal cycler, so that each well makes tight contact with the heating unit. If a 48-well plate is used here, place it into the four rows at the center of the heating unit. Tighten the lid to ensure a direct contact between the lid and the top of the 48 or 96-well plate to prevent any evaporation from the wells. These steps are very critical for the even amplification of the template.
- Use a thermal cycling parameter optimized for your gene-specific primers.

The following conditions are recommended:

Pre-soak	94°C for 3 min
Denaturation	94°C for 30 sec
Annealing	55°C for 30 sec†
Extension	72°C for 2 min
Cycles	35††
Soak	72°C for 5 min

† The annealing condition for control primers (β-actin and RP49). The annealing temperature should be 5°C below that of the Tm of the gene-specific primers.

†† Use 31 cycles for the control primers (β-actin and RP49) to obtain the results shown in Fig. 2, 4, 6 and 8.

- When the reaction is completed, remove the cover sheet and add to each well 12.5 uL of 2.5x DNA loading buffer which can be prepared by diluting the 5x Rapid-Load Buffer (provided) with an equal volume of water.
- Load 15 uL of each sample into the gel, starting from the lowest to the highest concentration and changing pipette tips after each tissue group and arranging the samples in such way that the row with the lowest concentration (1x) is nearest the positive electrode. Include molecular weight markers as desired (e.g., 5 uL DNA Quanti-Ladder). Store the remaining samples at –20°C.
- Separate the samples by electrophoresis and document the results.

Positive control PCR

Two individual multi-well Rapid-Scan plates are provided for your convenience. Ideally, the two plates may be used for the analysis of two different genes or ESTs. Alternatively, after obtaining an adequate expression profile for a test gene, the second plate may be used for determining the β-actin or RP49 profile using the control primers provided.

To perform the control PCR substitute the gene-specific primers with 200 uL of the β-actin Control Primers or 100 uL of RP49. (The single tube contains both the forward and reverse primers). The expected size of the b-actin PCR product is 640 bp for human, 570 bp for mouse and 433 bp for Drosophila RP49.

In the event that the gene-specific primers give only a marginal signal with the first plate, indicative of the presence of a very low-abundance transcript, the second plate may be used in a repetition of the reactions but choosing more cycles and/or altered annealing temperature.

SAMPLE GENE PROFILING BY RAPID-SCAN

To facilitate comparison of transcript accumulation in different tissues, the first-strand cDNAs have been normalized using β-actin as an internal control. Fig. 2 shows equivalent β-actin distribution in all 24 human tissues, when products of a 31-cycle PCR amplification (using primers provided with the Rapid-Scan panels) were analyzed in a 1% agarose gel. Of the four cDNA concentrations used (labeled from 1000x to 1x), only the lowest concentration (1x) gave an amplification signal that was within the linear range. The 10x concentration signal was just sub-plateau and the signals for both 100x and 1000x were already at plateau. It should be noted that the slight variation in β-actin transcript accumulation was detected only at cDNA concentrations that were in the linear range but became obscured when the cDNA concentrations were at saturation. This finding illustrates the need to perform the PCR analysis over a range of cDNA concentrations. The concentration range selected assures a better chance of detecting any unknown gene transcript, which almost invariably will accumulate at a lower level than the β-actin transcript.

As an example to illustrate the utility of the Rapid-Scan panel, it was used to determine the relative accumulation of the muscle-specific β-actinin 2 transcript (Fig. 2). As expected, β-actinin 2 mRNA (1, 2) was detected predominantly in heart and muscle (lanes 2 and 9). There was less than a 10-fold difference in mRNA accumulation between the two tissues: at 10x concentration cDNA levels in muscle were about 2.5-fold higher than that in heart. The β-actinin 2 transcript was not detected in any other tissues other than brain (lane 1), where the β-actinin 2 protein has been shown to anchor the N-methyl-D-asparate (NMDA) receptor on neurons (2). From a comparison of PCR signals at different cDNA concentrations, it was observed that the β-actinin 2 mRNA level in brain was one-hundredth of that found in muscle.

The Rapid-Scan panel is also an effective tool to obtain an expression profile of an uncharacterized EST. The result shown in Fig. 8 demonstrates such an example. Using a mouse Rapid-Scan panel, a nominal skin-specific EST sequence (6) was found to be expressed in stomach, skin and 19 day-old embryo (Fig. 8, lanes 7, 12 and 20).

The expressions of the actin genes in Drosophila are developmentally regulated, therefore, we used RP49 , the gene encoding a ribosomal protein, and a commonly used standard, as internal control to normalize each Drosophila cDNAs (Fig. 12). The specificity of the tissues used in the panel was confirmed by examining the profiles of several known transcripts. Some of the data are shown in Fig 12. The male-specific Fruitless transcript (7) is only detected in the adult male head. The alternatively spliced male form of Doublesex (DSX) (8) was detected mainly in mixed flies started from 3rd instar and male tissues(Fig 12, lane 7, 8, 9 and 11) and is not presented in female tissues (Fig. 12, lane 10 and 11). The Eyeless(EY) transcripts were detected in whole embryos collected starting from 4 hour and adult heads (lane 2, 3, 4, 5, 6, 7, 8, 9, 10), but not in adult body (lane 11 and 12).

When using the Rapid-Scan panels to determine the relative levels of mRNA accumulation, one has to be reminded that the PCR amplification has to be carried out with excess primers and that the PCR readout has to be within the linear range. This frequently implies that one should be cautious to not over amplify the PCR product by incorporating too many cycles of amplification. However, too few rounds of amplification may not suffice to detect transcripts with a low abundance of accumulation. Since it is difficult to predict a balance between sensitivity and nonlinearity, we suggest that investigators start by performing a 35-cycle amplification with the first 96-well plate to ensure detection of a PCR signal and, if necessary, use the second 96-well plate for optimization and quantitation.

TROUBLE-SHOOTING GUIDE

No PCR product detected

May have omitted a PCR component. Be sure to use a written checklist when assembling the reaction mix.

May have used an inappropriate annealing temperature. If at all possible, test primers against a known template, which can be diluted cloned DNA, to determine the optimal condition for amplification.

May be due to poor primer design. Follow the general guidelines when designing PCR primers.

More than one PCR product detected

It is possible that all of the products are specific, resulting from the amplification of either alternatively spliced mRNAs or transcripts derived from distinct members of a gene family. This may be resolved by either DNA sequencing of the PCR products or use of "nested" primers for re-amplification.

Detection of both specific band(s) and a background smear

This may be the result of the annealing temperature used, giving rise to both specific component(s) and non-specific background noise. The non-specific components may be reduced by an increase in the stringency of the PCR amplification.

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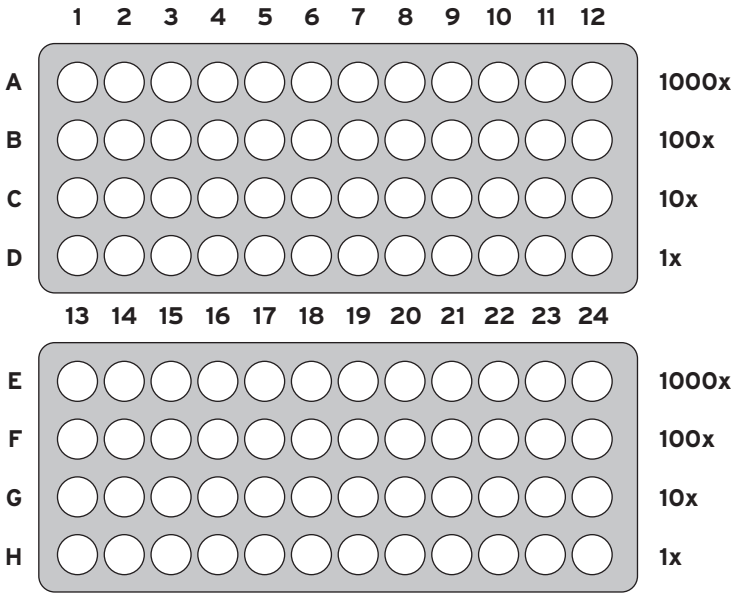
FIGURES

Figure 1: HSCA101 – Human 24 Tissue Rapid-Scan Layout



1. Brain
2. Heart
3. Kidney
4. Spleen
5. Liver
6. Colon
7. Lung
8. Small Intestine
9. Muscle
10. Stomach
11. Testis
12. Placenta
13. Salivary
14. Thyroid
15. Adrenal Gland
16. Pancreas
17. Ovary
18. Uterus
19. Prostate
20. Skin
21. PBL
22. Bone Marrow
23. Fetal Brain
24. Fetal Liver

Figure 2: Sample Profiling of Genes using HSCA101.

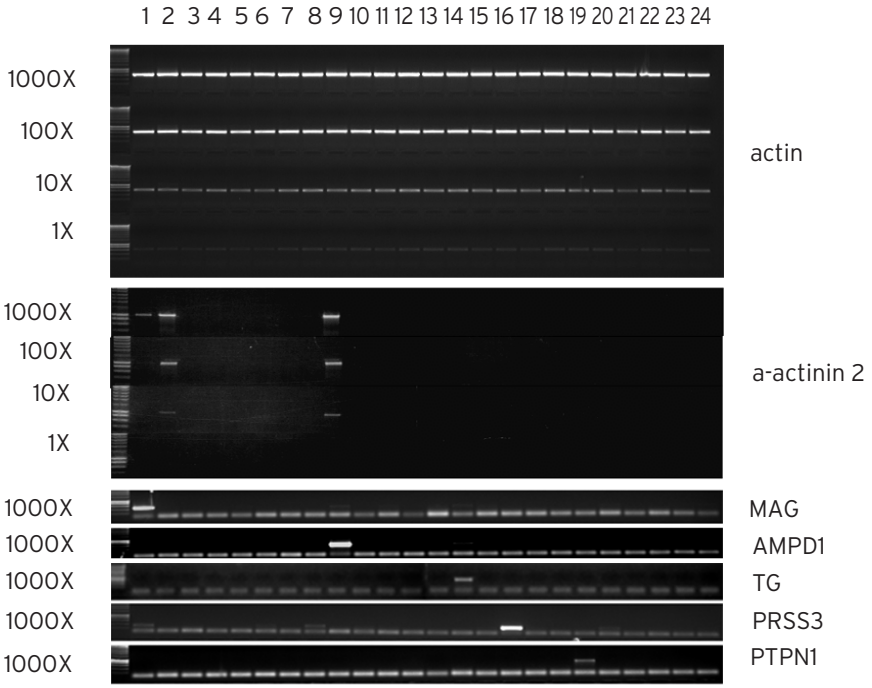
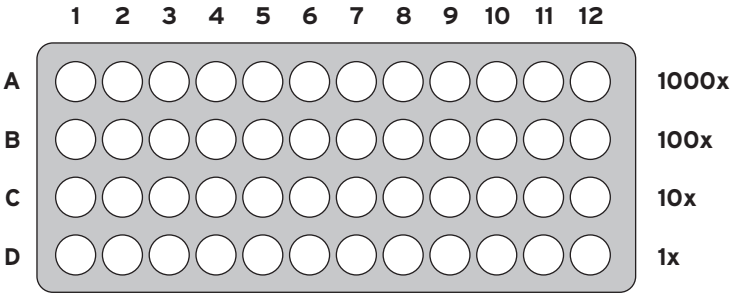


Figure 3: BSCD101 – Human Brain Rapid-Scan Layout



1. Frontal Lobe
2. Temporal Lobe
3. Cerebellum
4. Hippocampus
5. Substantia Nigra
6. Caudate Nucleus
7. Amygdal
8. Thalamus
9. Hypothalamus
10. Pons
11. Medulla
12. Spinal Cord

Figure 4: Sample Profiling of Genes using BSCD101

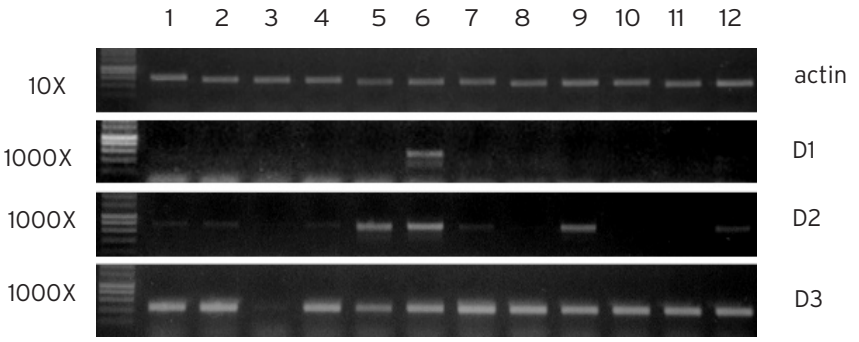
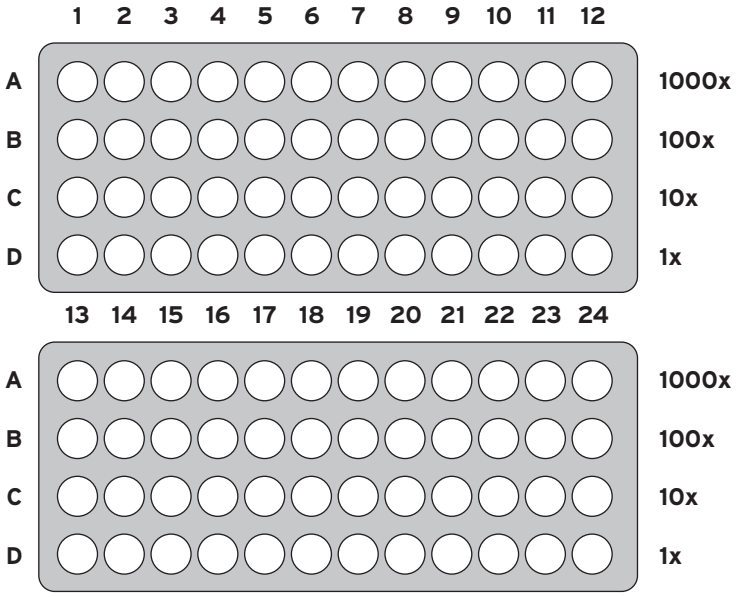
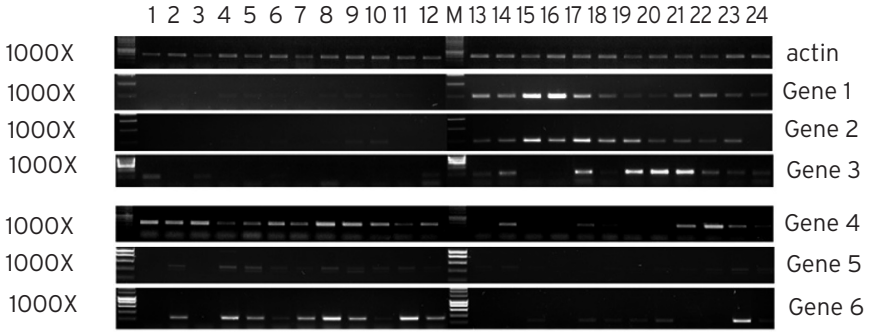


Figure 5: TSCE101- Human Breast Cancer Rapid-Scan Layout



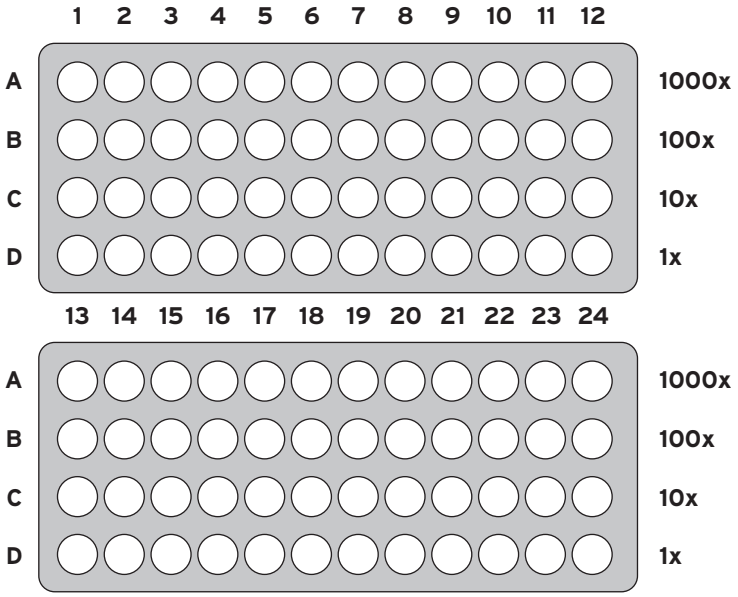
1. Normal Breast 1
2. Normal Breast 2
3. Normal Breast 3
4. Normal Breast 4
5. Normal Breast 5
6. Normal Breast 6
7. Normal Breast 7
8. Normal Breast 8
9. Normal Breast 9
10. Normal Breast 10
11. Normal Breast 11
12. Normal Breast 12
13. Breast Cancer 1
14. Breast Cancer 2
15. Breast Cancer 3
16. Breast Cancer 4
17. Breast Cancer 5
18. Breast Cancer 6
19. Breast Cancer 7
20. Breast Cancer 8
21. Breast Cancer 9
22. Breast Cancer 10
23. Breast Cancer 11
24. Breast Cancer 12

Figure 6: Sample Profiling of Genes using TSCE101



Sample	Tumor Type	Grade	Characteristics	ER Value fmol/mg	PR Value fmol/mg
13.	Invasive mixed tubular carcinoma	5	ER+/PR+++	7	233
14.	Invasive ductal carcinoma	9	ER+/PR+++	14	99
15.	Invasive lobular carinoma	6	ER+++++/PR+++++	142	528
16.	Invasive ductal carcinoma	7	ER++/PR-	20	9
17.	Invasive ductal carcinoma	-	ER++/PR-	18	7
18.	Invasive ductal carcinoma	6	ER+++/PR+	65	30
19.	Invasive ductal carcinoma	5	ER++/PR+	30	32
20.	Invasive ductal carcinoma	6	ER+PR-	9	0.5
21.	Adenoid cystic carcinoma	-	ER++/PR+	22	14
22.	Invasive ductal carcinoma	5	ER-/PR-	3	0
23.	Ductal carcinoma in-situ	-	ER+/PR+/-	19	13
24.	Invasive ductal carcinoma	8	ER+/PR+	6	26

Figure 7: MSCB101 - Mouse Rapid-Scan Layout



1. Brain
2. Heart
3. Kidney
4. Spleen
5. Thymus
6. Liver
7. Stomach
8. Small Intestine
9. Muscle
10. Lung
11. Testis
12. Skin
13. Adrenal Gland
14. Pancreas
15. Uterus
16. Prostate Gland
17. Embryo / 8.5 day
18. Embryo / 9.5 day
19. Embryo / 12.5 day
20. Embryo / 19 day
21. Breast / Virgin
22. Breast / Pregnant
23. Breast / Lactating
24. Breast / Involuting

Figure 8: Sample Profiling of Genes using BSCD101

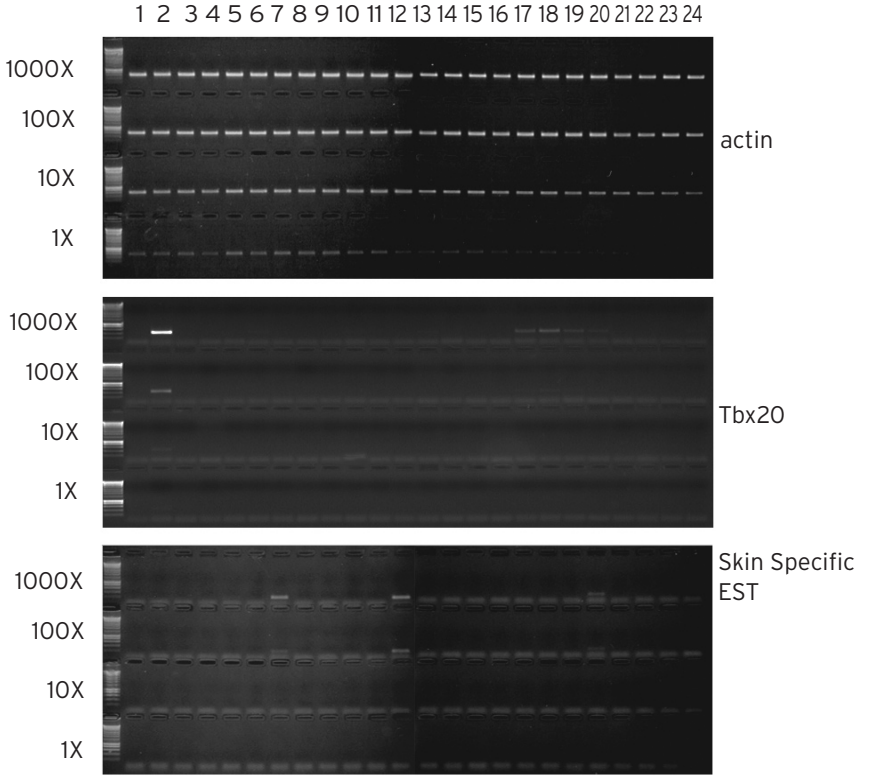
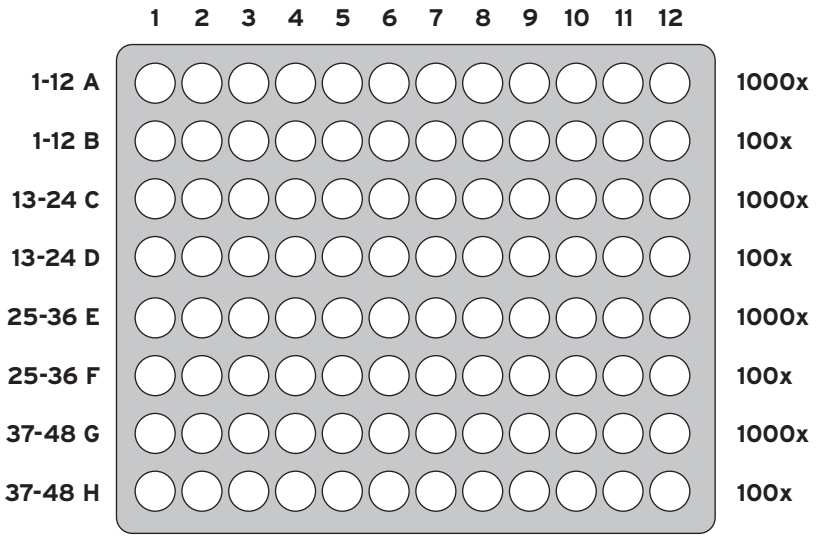


Figure 9: NSCF101- Mouse Brain Rapid-Scan Layout



Location	Tissue	Location	Tissue
	Embryo Day 13		Postnatal Day 7
1	Telencepahlon/ Diencephalon	23	Frontal Cortex
2	Mesencephelon (Midbrain)	24	Posterior Cortex
3	Rhombencephalon (Hindbrain)	25	Entorhinal Cortex
4	Spinal Cord	26	Olfactory Bulb
	Embryo Day 15	27	Hippocampus
5	Telencephalon	28	Striatum
6	Diencephalon	29	Thalamus
7	Midbrain	30	Hypothalamus
8	Pons	31	Cerebellum
9	Medulla	32	Midbrain
10	Spinal Cord	33	Pons
	Embryo Day 18	34	Medulla
11	Frontal Cortex	35	Spinal Cord
12	Posterior Cortex		Adult 5 Week
13	Entorhinal Cortex	36	Frontal Cortex
14	Olfactory Bulb	37	Posterior Cortex
15	Hippocampus	38	Entorhinal Cortex
16	Striatum	39	Olfactory Bulb
17	Thalamus	40	Hippocampus
18	Hypothalamus	41	Striatum
19	Midbrain	42	Thalamus
20	Pons	43	Hypothalamus
21	Medulla	44	Cerebellum
22	Spinal Cord	45	Midbrain
		46	Pons
		47	Medulla
		48	Spinal Cord

Figure 10: Sample Profiling of Genes using NSCF101.

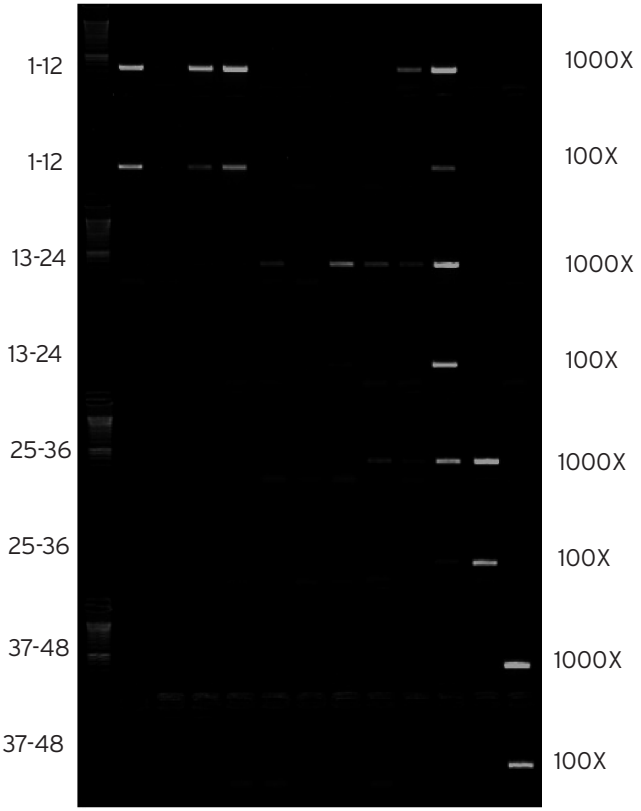
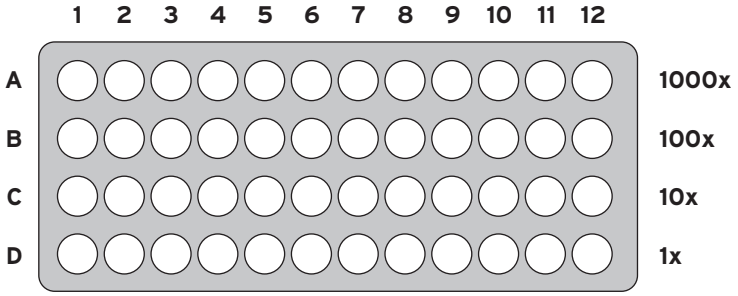


Figure 11: DSCC101 – Drosophila Rapid-Scan Layout



1. Embryo 0-4 Hour
2. Embryo 4-8 Hour
3. Embryo 8-12 Hour
4. Embryo 12-14 Hour
5. 1st Instar
6. 2nd Instar
7. 3rd Instar
8. Pupae
9. Male Head
10. Female Head
11. Male Body
12. Female Body

Figure 12: Sample Profiling of Genes using DSCC101