

Protocol

Product	Catalog #
PRMCK002 (G2041)	2 Panorama Mouse Cytokine Gene Arrays
PRLMC001 (CDLBL-MC)	Panorama Mouse Cytokine cDNA Labeling and Hybridization Kit
GPMCK001 (C5978)	Mouse Cytokine cDNA Labeling Primers
PRHY0001 (P5485)	Panorama Hybridization Solution
PRRT0001 (A8970)	AMV Reverse Transcriptase

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Panorama

Mouse Cytokine Gene Arrays

Protocol Booklet

For use with Catalog Number PRMCK002 (Sigma-G2041)

The Mouse Cytokine Gene Arrays are manufactured by Sigma-Genosys, Inc. and are developed in collaboration with R&D Systems, Inc.

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Notice to the Customer

Panorama Gene Arrays are to be used for research purposes only. The responsibility of all patent considerations in the use of this kit rests solely with the user. Suggestions and recommendations for the use of this kit are not to be taken as license to operate under or infringe upon any patent.

The Panorama Gene Arrays have been manufactured by Sigma-Genosys, Inc. using PCR technology owned by Hoffmann-La Roche, Inc. under a license granted by Hoffmann-La Roche, Inc. to Sigma-Genosys, Inc. The sale and purchase of this product does not convey any license to the purchaser to practice PCR or any other technology owned by Hoffmann-La Roche, Inc.

Trademarks & Patents

Panorama is a trademark of Sigma-Genosys, Inc.

Genosys® is a registered trademark of Sigma-Genosys, Inc.

Sephadex® is a registered trademark of Pharmacia Biotech AB.

Microsoft® is a registered trademark of the Microsoft Corporation.

ImageQuant® is a registered trademark of Molecular Dynamics, Inc.

ArrayVision is a trademark of Imaging Research, Inc.

Genbank® is a registered trademark of The United States Department of Health and Human Services.

TRI Reagent® is a registered trademark of Molecular Research Center, Inc.

The PCR process is covered by patents owned by Hoffmann-La Roche, Inc.

Limitations of the Procedure

- FOR RESEARCH PURPOSES ONLY.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can alter the performance of the kit.

Components

- **Panorama Gene Arrays.** Two charged, nylon membranes containing PCR products spotted in duplicate. Each array contains 514 different mouse cytokine-related genes, 8 housekeeping genes, 3 *E. coli* genes, pUC19, 1x TE Buffer and mouse genomic DNA.
- **Protocol booklet.**
- **Transparency.** An array template to facilitate spot location within a field on autoradiographs.
- **Floppy diskette.** Containing a spreadsheet of gene-specific information and showing the layout of genes on the arrays. The spreadsheet is in Microsoft® Excel (for Office 2000) format.

Storage of Components

Storage at room temperature: Panorama Gene Arrays, keep dry and protected from light.

Optional Components

Panorama Mouse Cytokine cDNA Labeling and Hybridization Kit* (Catalog No. PRLMC001, Sigma: CDLBL-MC):

- ◆ Hybridization Solution, 100 mL, store at 4°C.
- ◆ cDNA Spin columns, 10 columns, store at 4°C.
- ◆ 10 mM dGTP, 15 µL, store at -20°C.
- ◆ 10 mM dATP, 15 µL, store at -20°C.
- ◆ 10 mM dTTP, 15 µL, store at -20°C.
- ◆ 10 mM dCTP, 15 µL, store at -20°C.
- ◆ 500 units AMV Reverse Transcriptase, 20 µL, store at -20°C.
- ◆ 5x Reverse Transcriptase Buffer, 100 µL, store at -20°C.
- ◆ Mouse Cytokine cDNA Labeling Primers, lyophilized. Upon receipt, store desiccated at 4°C. After resuspension with 40 µL sterile distilled water, store in the short-term (several days) at 4°C. For longer-term storage of several weeks, it is recommended that the primers are stored at -20°C.

* The above reagents are sufficient to perform 10 labeling reactions and hybridizations.

Additional reorder items:

- Panorama Hybridization Solution (Catalog No. PRHY0001, Sigma P5485)
- AMV Reverse Transcriptase (Catalog No. PRRT0001, Sigma A8970)
- Mouse Cytokine cDNA Labeling Primers (Catalog No. GPMCK001, Sigma C5978)

Protocol version 1.2

Materials and Equipment Required but Not Provided

Materials

- Total RNA Isolation Reagent, TRI Reagent (Sigma catalog number T-9424) and Poly(A)+ RNA Isolation Kit (optional). Phenol, pH 4.3, optional (Sigma P4682).
- cDNA Labeling and Hybridization Kit (Catalog number PRLMC001).
- [α -³³P]-deoxycytidine 5-triphosphate (dCTP) (i.e. NEN Life Science Products, Inc., NEG 613H, 2,000-3,000 Ci/mmol). [α -³²P]-deoxycytidine 5-triphosphate (dCTP) may also be used (i.e. NEN Life Science Products, Inc., NEG 513H, 2000-3000 Ci/mmol).
- Mouse Cytokine Gene cDNA Labeling Primers (Catalog number GPMCK0001, Sigma C5978) or oligo(dT)₂₀₋₂₅ reverse transcriptase primer.
- Ribonuclease inhibitor (from mouse placenta) (Sigma R2520).
- RNase-free DNase I (optional; Sigma AMP-D1).
- Microcentrifuge tubes (0.5 mL, 1.5 mL), sterile, nuclease-free.
- Conical tubes (50 mL screwcap).
- Scintillation vials and scintillation fluid (optional).
- 20x SSPE (Sigma S2015).
- Panorama Hybridization Solution (Catalog number PRLMC001, Sigma-CDLBL-MC (kit) & PRHY0001, Sigma-P5485 (component)).
- Panorama Mammalian Array Wash Pack (Sigma M 0935).
- X-ray film (Kodak BioMax MR, 8 in x 10 in; Catalog number 870-1302). Use with Kodak BioMax intensifying screens; standard autoradiography cassette.
- Kodak Low Energy Storage Phosphor Screens HD measuring 20.3 cm x 25.4 cm (Molecular Dynamics LE177-956) or Fujifilm screens (Fuji Medical Systems YBIP2025MS, 20 cm x 25 cm); standard autoradiography cassette.
- Micropipettors and nuclease-free disposable tips: 1.0 μ L to 1000 μ L capacity.

Equipment

- Spectrophotometer.
- Geiger-Mueller Counter.
- Heating block (90-95°C, 70°C and 42°C) or thermal cycler.
- Centrifuge (capable of 1100 x g) for spin column purification of cDNA labeling reaction.
- Microcentrifuge.
- Scintillation counter.
- Tweezers for handling the gene array membrane.
- Hybridization oven and roller bottles.
- An alternative system to roller bottles for the hybridization/wash steps is to use the following: sealable storage bags; water bath; container with cover (large enough to accommodate an 8 x 12 cm membrane and 200 mL minimum capacity); and shaking platform to accommodate the gene array hybridization and wash steps.
- Film developer and/or phosphorimager for obtaining the gene array image.
- Computer (PC capable of running Microsoft Excel, Office 2000) for viewing the cDNA array spreadsheet and analyzing phosphorimages.
- Radioisotope solid and liquid waste containers.

Overview

The Panorama Mouse Cytokine Gene Array provides researchers with a rapid, semi-quantitative tool to identify differentially-expressed cytokine and cytokine-related genes. The development of gene array technology allows researchers to study the relative mRNA levels of hundreds to thousands of genes simultaneously, in a single experiment. This method can be used to determine which genes are “turned on” or “turned off” in response to developmental cues, a particular physiological or pathological condition, external stimuli, or a variety of stresses and cell treatments. In the past, an equivalent amount of information could only be derived using differential screening methods, such as, differential display RT-PCR, differential cDNA library screening or by performing gene-specific methods such as, hundreds of Northern blot experiments or RT-PCR reactions. Figure 1 shows the steps that are involved in an expression profiling experiment using gene arrays.

The Panorama Mouse Cytokine Gene Array represents a comprehensive collection of cytokines, chemokines and other immunomodulatory factors, and their receptors. The array consists of 514 different cytokine-related cDNAs printed as PCR products onto charged nylon membranes. A pair of macroarrays are provided to allow differential gene expression analysis of two different samples. Each gene is printed in duplicate at 10 ng per spot. The array contains coding sequence-specific PCR products derived from fully sequence-characterized cDNA clones. The amplified gene sequences are derived from coding sequences devoid of repetitive sequence elements (i.e. *Alu* repeats) that are often associated with untranslated regions of cDNAs. Also included on the gene arrays are eight positive control "housekeeping" genes, mouse genomic DNA and five negative controls. The signal from one or more of the positive control genes can be used to normalize signals of all genes between two comparative samples. Mouse genomic DNA is spotted at the four corners of the array. During any expression profiling experiment, the genomic DNA spots will act as a positive control, showing some degree of hybridization signal. The genomic DNA spots can also be used to orient the corners of the array. In addition, the top right-hand corner of each array is clipped for orientation purposes. Detailed gene information and layout of genes on the array are described in Appendix C and are included in the accompanying Microsoft Excel spreadsheet. High-quality expression data is obtained provided that the protocols detailed in the methods section are closely followed.

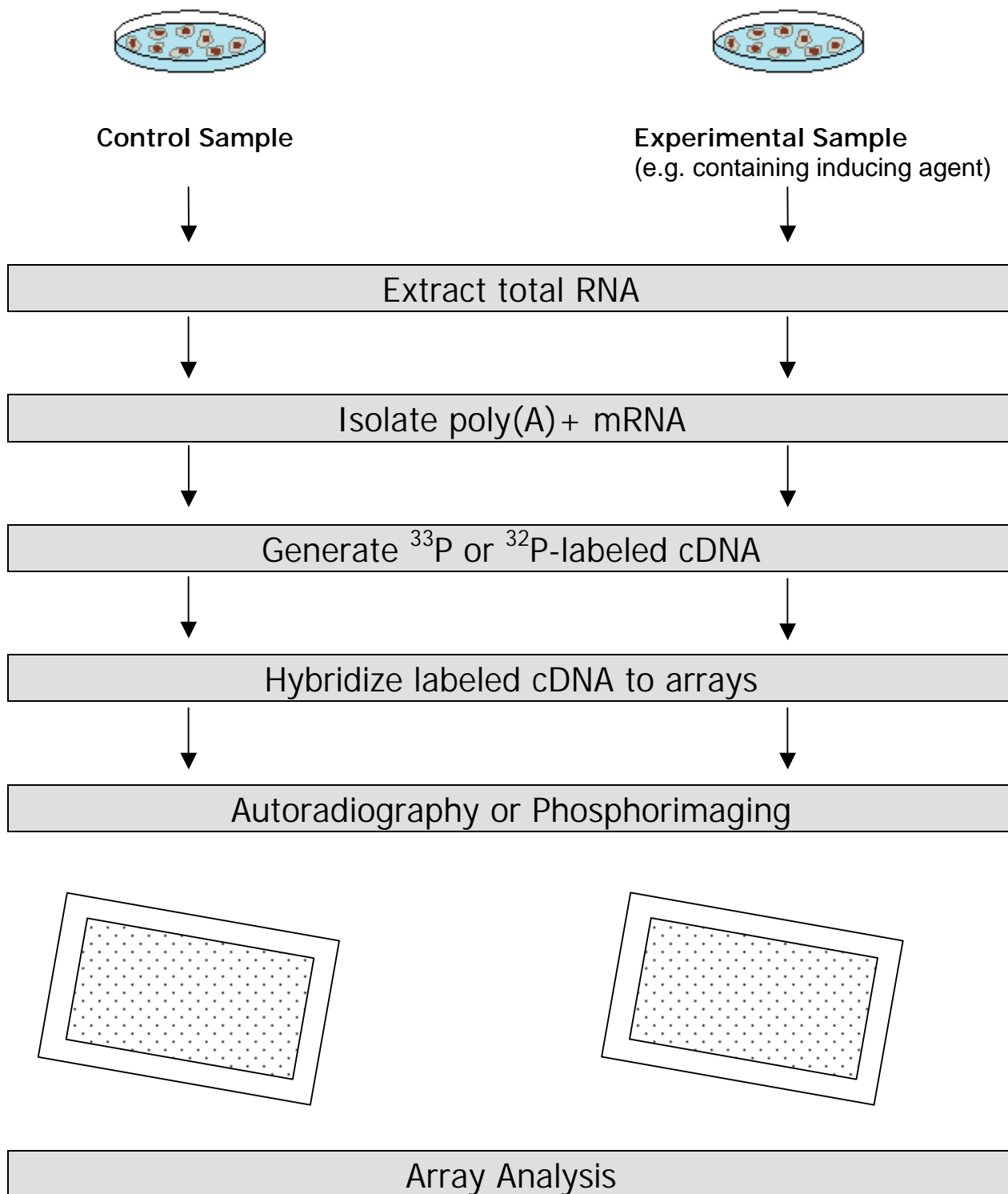


Figure 1. *Diagrammatic representation of the expression profiling process using Panorama gene macroarrays.*

An example of differential gene expression in unstimulated versus stimulated murine macrophage cell line RAW 264.7 cells is shown in Figure 2. RNA was prepared from unstimulated RAW 264.7 cells and RAW 264.7 cells that were stimulated for 24 hours with interferon gamma (IFN γ) and bacterial lipopolysaccharide (LPS). Equal amounts of total RNA from each sample were then reverse transcribed using cDNA labeling primers and the resulting radiolabeled cDNA samples were hybridized to the Panorama Mouse Cytokine Gene Arrays. The membranes were then exposed to phosphorimaging plates and the signals quantified using the ArrayVisionTM software.

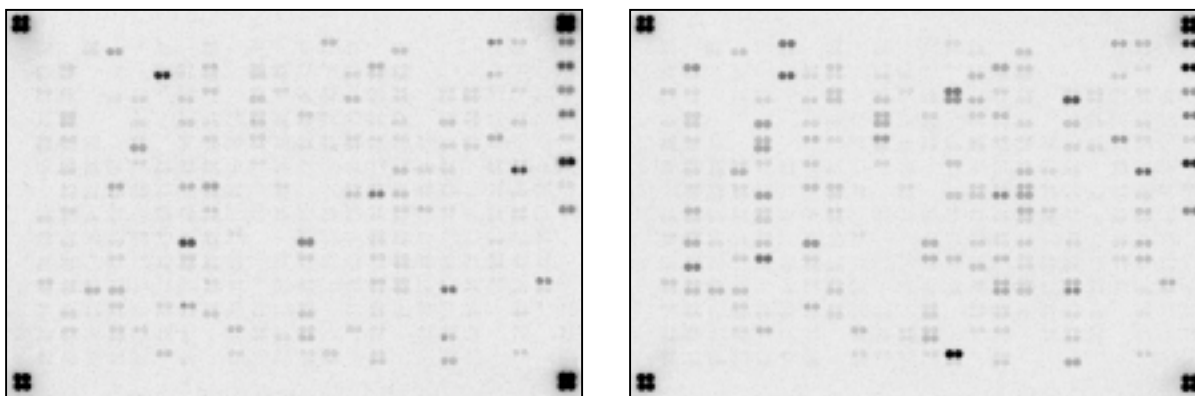


Figure. 2 Differential gene expression in a stimulated murine macrophage cell line, RAW 264.7 cells. Two Panorama Mouse Cytokine Gene Arrays were probed with labeled cDNA from unstimulated RAW 264.7 cells (left panel) or RAW 264.7 cells stimulated with IFN γ (1 unit/mL) and LPS (1 μ g/mL) for 24 hr (right panel). Equal amounts of total RNA were reverse transcribed using the mouse cytokine cDNA labeling primers and ³³P-dCTP. The labeled cDNAs hybridized to the arrays overnight at 65°C. The images were obtained following an overnight exposure to low-energy phosphorimaging screens.

Methods

Part One – RNA Extraction

A. Total RNA Isolation

The methodology for RNA extraction is critical for the success of any expression profiling experiment. It is essential to purify total RNA without any contaminating genomic DNA. Traces of genomic DNA in the RNA preparation may contribute to background hybridization.

For high quality total RNA isolation with minimal genomic DNA contamination, we recommend the use of the *TRI Reagent* (Sigma, Catalog Number T-9424). An abbreviated protocol for using the *TRI Reagent* is shown below. An additional step is suggested to reduce genomic DNA contamination (see *):

Abbreviated Protocol:

1. HOMOGENIZATION Use 1 mL *TRI Reagent* per:
50-100 mg tissue,
5-10 x 10⁶ cells in suspension or
10 cm² of cell culture plate.

* Prior to the phase separation step, pellet insoluble material (extracellular membranes, genomic DNA, polysaccharides) by centrifugation at 12,000 x g for 10 minutes at 4°C. Transfer the clear supernatant to a fresh tube and proceed to phase separation step.

2. PHASE SEPARATION Homogenate + 0.2 mL chloroform.
 3. RNA PRECIPITATION Aqueous phase + 0.5 mL isopropanol. Pellet by centrifugation.
 4. RNA WASH Wash pellet with 1 mL 75% ethanol. Air dry.
 5. RNA SOLUBILIZATION Sterile distilled water.
-

After washing the precipitate with 75% ethanol (step 4), discard the supernatant, taking care NOT to discard the RNA pellet. Drain well. The pellet may easily dislodge from the bottom of the tube. Air-dry the pellet for 10-15 minutes. When the RNA pellet appears clear or translucent, dissolve the RNA in 100-200µL of sterile, RNase-free water or 1xTE Buffer. Place the tube at 37°C for 15-30 minutes and then pipet repeatedly to thoroughly dissolve the RNA. Keep on ice for immediate use or store at -20°C until ready to proceed with the poly(A)+ isolation step.

B. Quantitation of RNA

1. Quantitate the RNA sample by measuring the absorbance at 260nm of an appropriate dilution (5-10 µL of RNA in 1 mL water) in a spectrophotometer. For example, dilute 10

μL sample to 1 mL with water, which is equivalent to a 1:100 dilution (dilution factor = 100). Read the absorption in optical density units (OD) of this dilution at 260nm.

2. Calculate the concentration of your RNA sample:

Concentration of RNA sample ($\mu\text{g}/\text{mL}$) = OD units \times Dilution factor \times 40 $\mu\text{g}/\text{mL}$

C. Check the Quality of the Total RNA Sample

1. A total RNA sample that is essentially free of proteins should have an $A_{260\text{nm}}/A_{280\text{nm}}$ absorbance ratio of 1.6 - 1.9. If the RNA is resuspended in water with a pH <7.0, then the $A_{260\text{nm}}/A_{280\text{nm}}$ ratio may be falsely decreased, giving the impression of protein contamination in the sample. It is important to check both the integrity of the RNA and the amount of genomic DNA contamination by agarose gel electrophoresis. A non-denaturing agarose gel is used so that any genomic DNA contamination can be easily observed. Mix 5-10 μg RNA sample with non-denaturing loading dyes. Load the sample on a 1.2% agarose gel and separate the nucleic acids by electrophoresis. The 28S (~4.8 kb) and 16S (~1.9 kb) ribosomal RNA bands should be clearly visible at a staining intensity of about 2:1 ratio (28S:16S). A significantly lower ratio of staining may be indicative of RNA degradation and hence, the RNA sample may not be suitable for use. If genomic DNA is present in the RNA sample, it will be seen as high molecular weight-staining material. Note that some RNA species may appear as discrete bands of sizes up to ~15 kb. Typically, if the *TRI Reagent* total RNA extraction procedure is strictly followed, then genomic DNA contamination will be minimal. If excessive amounts of genomic DNA are present, it may be necessary to consider treating the RNA sample with RNase-free DNase I (Appendix-E). Note: For details on running agarose gel electrophoresis, refer to Sambrook, J. *et al.*, (1989) *Gel Electrophoresis of DNA*. In *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, N.Y., p. 6.1.

D. Poly(A)+ mRNA Isolation

The *Mouse Cytokine cDNA Labeling Primers* may be used to synthesize cDNA with total RNA as template. However, using poly(A)+ mRNA (rather than total RNA) with the cDNA labeling primers may help to achieve a slightly greater sensitivity of detection. The majority of total RNA is comprised of ribosomal RNA (rRNA) and transfer RNA (tRNA). Messenger RNAs comprise a small percentage of the total RNA (<5%). Hence, any mRNA enrichment step will

help to increase the specific activity of the cDNA probe needed for hybridization to the arrays (in *Parts Two* and *Three* of this protocol). Poly(A)+ mRNA isolation kits are available from a number of suppliers. An oligo-(dT)-cellulose or oligo-(dT)-latex bead method should work well for these purposes. We recommend the use of GenElute™ mRNA Miniprep Kit (MRN-10) from Sigma for purification of mRNA from total RNA or isolation of mRNA directly (GenElute™ mRNA Miniprep Kit, DNM-10 from Sigma). Ensure that all RNA samples to be used with the Panorama gene arrays are processed in the same manner. Do not compare two samples where the poly(A)+ mRNAs have been isolated by two different approaches.

Part Two – Generating Labeled cDNA

Panorama gene arrays can be used with either ^{33}P or ^{32}P for expression profiling. The signals from cDNAs probes synthesized using ^{33}P generate well-defined spots on the arrays that allow easier quantitation. Spot signals from images generated with ^{32}P may tend to bleed into one another, necessitating the analysis of several exposures from the same array. Whenever possible, Sigma-Genosys recommends the use of ^{33}P .

A. Preparation of Labeled cDNA Using *Mouse Cytokine cDNA Labeling Primers*

The cDNA labeling reactions are performed in two steps. In the first step, the Mouse Cytokine Labeling Primers are annealed to the RNA template. During the second step, radiolabeled nucleotide and reverse transcriptase are added to initiate the cDNA synthesis reaction. Components for the cDNA labeling reaction can be obtained from Sigma-Genosys (PRLMC001 or Sigma CDLBL-MC, Panorama cDNA Labeling and Hybridization Kit).

Table 1.

Final Concentration	Stock Reagent	Volume for one reaction
2 µg total RNA or ~0.5 µg poly(A)+ RNA		X µL
Mouse Cytokine cDNA Labeling Primers, resuspended (Sigma-Genosys, GPMCK001, Sigma C5978)		4 µL
Sterile distilled water to		15 µL

Table 2.

Final Concentration	Stock Reagent	Volume for one reaction
Components from Table 1		15 µL
1x Reverse Transcriptase Buffer	5x	6 µL
333 µM dATP	10 mM	1 µL
333 µM dGTP	10 mM	1 µL
333 µM dTTP	10 mM	1 µL
1.67 µM dCTP (if using labeled dCTP)	100 µM	0.5 µL
20 µCi [α - ³³ P] dCTP or 20 µCi [α - ³² P] dCTP (2,000-3,000 Ci/mmol)	10 µCi/µL	2 µL
20 U Ribonuclease Inhibitor (from mouse placenta)	40 U/µL	0.5 µL
50 U AMV Reverse Transcriptase	25 U/µL	2 µL
Sterile, distilled water to a final volume of		30 µL

1. For the annealing step, assemble the reaction in a 0.5 mL tube using the components listed in Table 1.
2. Anneal the *Mouse Cytokine cDNA Labeling Primers* to the RNA template by placing the tube in a thermal cycler. Program the cycler to heat to 90°C for 2 minutes and then ramp to 42°C over a period of 20 minutes. Alternatively, place the tube in a heat block at 90°C. After 2 minutes, remove the block from the heating device and place on the work bench. Allow to cool to 42°C.
3. Once the thermal cycler (or heat block) has reached 42°C, add the components for the cDNA labeling step as listed in Table 2. NOTE: If using the Sigma-Genosys *cDNA Labeling and Hybridization Kit* (PRLMC001 or CDLBL-MC), remember to dilute the

10mM dCTP stock 1:100 (100 μ M final concentration) prior to setting up the reaction shown in Table 2. **The final reaction volume = 30 μ L.**

4. Mix carefully by pipetting and return to the thermal cycler or heat block. Incubate at 42°C for 2-3 hours.

NOTE: If the desired radioactive nucleotide is other than dCTP, then include unlabeled dCTP at 333 μ M in the reaction and reduce the unlabeled nucleotide corresponding to the labeled nucleotide being used to 1.67 μ M.

** Use appropriate personal protective equipment and adopt your institutions handling and waste disposal procedures for use of radioactive materials.*

5. The unincorporated radiolabeled nucleotide must be removed from labeled cDNA by purification over a Sephadex G-25 gel-filtration spin column. Use the spin column protocol detailed below.
6. After removal of unincorporated radiolabeled nucleotides, the labeled cDNA probe is ready to be used in a hybridization with the Panorama gene array (*Part Three* of this protocol).

B. Preparation of Labeled cDNA Using Oligo(dT) Primers

The protocol for generating cDNA with oligo(dT) primers is similar to that described above with the following exceptions:

1. Use 40-50 pmol (~300 ng) of 20-25mer oligo(dT) primers instead of the "Mouse Cytokine cDNA Labeling Primers". Assemble the reaction as in step 1 of section A, above. Heat the RNA to 70°C for 3 minutes, then immediately chill on ice. Assemble the remainder of the reaction on ice, adding the components listed in Table 1 (step 3, section A).
2. After assembling the reaction, mix carefully by pipetting and incubate at 42°C for 2-3 hours. After the incubation period, proceed to section C to remove unincorporated radiolabeled nucleotides.

C. Purification of Labeled cDNA Using Spin Columns

It is important to remove the excess, unincorporated-radioactive nucleotides from the labeling reaction. Removing the unincorporated nucleotides will help prevent background during the hybridization to the gene arrays. In addition, removing the excess nucleotides allows a general determination of the efficiency of incorporation into the cDNA. Use a Sephadex G-25 spin column (Sigma-Genosys, component of the kit PRLMC001 or CDLBL-MC).

1. Gently invert the gel-filtration spin column several times to resuspend the column matrix.
2. Carefully remove the top cap from the column, then remove the bottom cap and allow the buffer to drain by gravity into a 1.5 mL microcentrifuge tube. Discard the tube with buffer.
3. Place the column in a collection tube (1.5 mL microcentrifuge tube) and then place the whole device in a 50 mL screw-top conical tube.
4. Centrifuge at 1100 x g for 2 minutes to remove all of the buffer from the column.
5. Add the sample to the center of the column bed (make sure that the column is in an upright position) and place the column in a fresh 1.5 mL collection tube within the 50 mL screw-top conical tube.
6. Spin the column at 1100 x g for 4 minutes and save the eluate. This is the purified cDNA sample.
7. A hand-held Geiger-Mueller counter can be used to determine a rough estimate of the percentage incorporation of radioactive nucleotide into the cDNA. Measure the radioactivity left in the column (unincorporated radioactive nucleotide, "U") and the amount of radioactivity in the collection tube (labeled nucleotide incorporated into the cDNA, "I"). To determine the approximate percentage incorporation, calculate $(I/(I+U)) \times 100$. Typically, an incorporation of 20%-50% will yield acceptable results. Alternatively, an aliquot of the sample before (T) and after the column purification (I) can be counted in a scintillation counter to determine percent incorporation $(I/T \times 100)$.

Part Three – Hybridization and Analysis of Arrays

A. Hybridization and Washing of the Gene Array

After preparing the radioactively-labeled cDNA in *Part Two*, the next step is to perform a hybridization to the Panorama gene array. The hybridizations are best performed in roller bottles in a hybridization oven, where minimal volumes of hybridization solutions are employed. Hybridizations can also be performed in sealed plastic bags in a heated air incubator or immersed in a water bath, with agitation. The following protocol presumes the use of roller bottles in a hybridization oven (volumes may have to be increased for use with sealed bag hybridizations). See Appendix A for buffer compositions.

1. Rinse the arrays in 50 mL 2x SSPE at room temperature for 5 minutes. Drain and discard the solution.
2. Pre-warm the hybridization oven to 65°C. Warm the *Hybridization Solution*, Cat. No. PRHY0001 (Sigma P5485) or Kit Cat. No. PRLMC001 (Sigma CDLBL-MC), to 65°C prior to use. Pre-hybridize the Panorama gene array in 5 mL *Hybridization Solution* for at least 1 hour at 65°C, using roller bottles at 6 r.p.m. (or continuously agitate if using sealed bags).
3. Add the entire labeled cDNA generated from *Part Two* of this protocol to 2-3 mL *Hybridization Solution*, in a 15 mL conical screw-top tube. Denature the cDNA at 90-95°C for 10 minutes in a water bath or heat block.
4. Decant and discard the *Hybridization Solution* from the pre-hybridized array. Add the denatured labeled cDNA in *Hybridization Solution* to the array in the roller bottle.
5. Hybridize overnight (12-18 hours) at 65°C.
6. Decant the *Hybridization Solution* and save for future use or discard appropriately.
7. Add 40-50 mL of *Wash Solution I* to the roller bottle. Wash the array by inverting the roller bottle by-hand, at room temperature for 2-3 minutes. Decant and discard the *Wash Solution I* in an appropriate manner for radioactive waste solutions.
Note: An alternative wash method may be adopted for the arrays. Arrays may be washed in a suitably-sized plastic food container (dedicated for use with radioactive materials). Agitate the container on a rocking table or use a shaking water bath.
8. Repeat step 7 two more times.

9. Pre-warm the *Wash Solution I* to 65°C. Add 80-100 mL *Wash Solution I* to the roller bottle. Wash the arrays in the hybridization oven at 65°C for 20 minutes (6 r.p.m.). Decant and discard the *Wash Solution I* to an appropriate radioactive waste container.
10. Repeat step 9 one more time.
11. Pre-warm the *Wash Solution II* to 65°C. Add 80-100 mL *Wash Solution II* to the roller bottle. Wash the arrays in the hybridization oven at 65°C for 20 minutes (6 r.p.m.).
12. Remove the array from the roller bottle (or the alternative washing container). Lay the array on a sheet of blotting paper.
13. Air-dry the array for 2-5 minutes.

Warning: do NOT let the array dry completely. If allowed to dry completely, then stripping of the array for re-probing will be significantly less efficient. A protocol for stripping the gene arrays can be found in Appendix B.

14. Wrap the array in clear plastic food wrap and subject it to autoradiography using Kodak BioMax MR (for ³³P-labeled cDNA), BioMax MS (for ³²P-labeled cDNA) X-ray film or expose phosphor screens for analysis by phosphorimager. When performing phosphorimager with ³³P, we strongly recommend the use of Kodak Low Energy Storage Phosphor Screens HD (Molecular Dynamics LE177-956, 20.3 cm x 25.4 cm) or Fujifilm screens (YBIP2025MS, 20 cm x 25 cm). Images with these screens have a significantly higher resolution than with other general purpose phosphor screens. Imaging screens should be scanned at a 50µm rather than a 100 µm or 200 µm pixel size, for greater resolution of spots and more accurate quantitation of pixel values. The file sizes of 50 µm scans may be extremely large. It is advisable to crop the images after scanning to one field per image file. If using phosphorimager screens, care must be taken to ensure that there are no wrinkles in the clear plastic wrap separating the screen from the array. Also, make sure that the entire array is pressed firmly against the imaging screen. Typically, a 2-4 day exposure to X-ray film or an overnight exposure to a phosphorimager screen will yield quantifiable results. It may be necessary to perform several exposures for different time periods to distinguish between extremes of expression levels.

NOTE: For all experiments it is recommended that an autoradiograph be generated for each array. Depending upon the phosphor screen being used,

spots from phosphorimaging may appear diffuse. Autoradiographs typically show spots with sharper edge boundaries and provide a qualitative "hard copy" of the array image.

15. If the arrays are to be reused, it is essential that they be stripped before the membrane dries out. It is advisable to strip the arrays as soon as possible following imaging (see Appendix B).

B. Analysis of the Gene Arrays

Quantitation of gene expression signals is best determined from phosphorimager-generated image files. The image files may be analyzed using the phosphorimager manufacturer's software, e.g. ImageQuant from Molecular Dynamics, Quantity One from Bio-Rad Laboratories, OptiQuant from Packard Instrument Company or MacBas from Fuji. Sigma-Genosys has created an image analysis "template" for use with the ImageQuant software (version 5.0) from Molecular Dynamics as well as ArrayVision™ from Imaging Research. Pixel data that has been obtained using these templates can be exported to a Microsoft Excel spreadsheet. This data can then be copied and pasted into another spreadsheet that contains the gene names in relation to their position in the template. The ImageQuant template, the ArrayVision™ template and the appropriate Microsoft Excel spreadsheets (containing the appropriate gene names corresponding to each spot) may be downloaded from the Sigma-Genosys website (<http://www.genosys.com/expression/frameset.html>).

Refer to Appendices C and D for detailed information of the genes and to determine the layout of genes on the Panorama Mouse Cytokine Gene Arrays.

The process of analysis involves the following steps:

1. Set up a quantitation template to analyze pixel intensity in each spot of the array.
2. Subtract background signal from each spot. Use signal from a clear area of the array or from the 1x TE Buffer negative control spots as a background value.
3. Export signal values to a spreadsheet file for manipulation in a program such as Microsoft Excel.
4. Determine the average signal (pixel intensity) of the pair of duplicate spots representing each gene.

5. Normalize relative signals from different arrays by representing the "averaged" spot signal of sample gene as a percentage of the "averaged" signal from a housekeeping gene known not to change between the samples. The signals from certain housekeeping genes may change following different cell treatments. In these cases, it may be necessary to normalize all gene signals to the averaged signal from several or all housekeeping genes.
6. Compare normalized signals of corresponding spots from different samples on different arrays. Divide the normalized values from the "experimental" array by the normalized values from the "control" array. This will determine fold-induction or fold-reduction in expression of gene-specific mRNAs between samples.

Appendices

Appendix A - Solution Compositions

1x TE BUFFER

10 mM Tris-HCl, pH 8.0
1 mM EDTA

5x REVERSE TRANSCRIPTASE BUFFER

250 mM Tris-HCl, pH 8.5
40 mM MgCl₂
150 mM KCl
5 mM dithiothreitol (DTT)

HYBRIDIZATION SOLUTION

5x SSPE
2% SDS
5x Denhardt's Reagent
100 µg/mL sonicated, denatured salmon testes DNA

WASH SOLUTION I (*Panorama Mammalian Array Wash Pack (Sigma M 0935)*)

0.5x SSPE
1% SDS

WASH SOLUTION II (*Panorama Mammalian Array Wash Pack (Sigma M 0935)*)

0.1x SSPE
1% SDS

1x SSPE

0.18 M NaCl
10 mM sodium phosphate, pH 7.7
1 mM EDTA

NOTE: Na₂HPO₄ is added to NaH₂PO₄ to bring sodium phosphate to pH 7.7.

Typically, different concentrations of SSPE are prepared by dilution of a 20x stock solution.

1x DENHARDT'S REAGENT

0.02% Ficoll (MW 400,000)
0.02% polyvinylpyrrolidone (PVP; MW 40,000)
0.02% bovine serum albumin (BSA)

STRIPPING SOLUTION (*Sigma S3312*)

10 mM Tris-HCl, pH 8.0
1 mM EDTA
1% (w/v) SDS

Appendix B - Stripping the Arrays

It is important to make sure that the arrays do not dry out before performing the stripping procedure. If arrays are allowed to dry, prior to stripping, then the labeled probe may be irreversibly bound to the membrane.

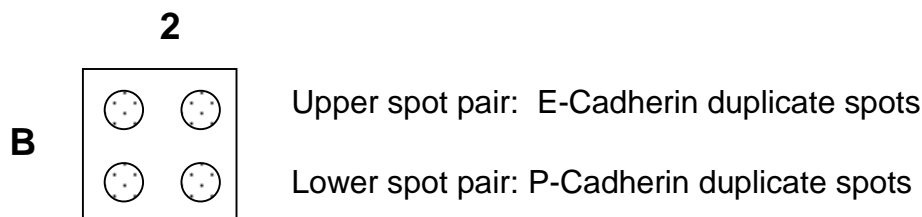
- 1) Prepare 500 mL *Stripping Solution* (Sigma S3312, Appendix A). In a "Tupperware" container or in a Pyrex dish covered with clear plastic food wrap, bring the *Stripping Solution* to a boil using a microwave oven (about 5 minutes at full power). NOTE: If a microwave oven is not available, then add the *Stripping Solution* to a Pyrex dish and place the dish on a variable temperature hotplate. Bring the solution to a boil on the hotplate.
- 2) Add the array to the heated solution and continue a low boil using the "defrost" setting (about 33% power) for 20 minutes. If using a hotplate, lower the temperature to a simmer rather than a "hard" boil.
- 3) Drain the excess *Stripping Solution* and re-wrap the array in clear plastic wrap. Expose the array to phosphorimaging plates or subject it to autoradiography. The duration of the exposure should be same as for a typical experiment, i.e. overnight exposure.
- 4) Analyze the image by phosphorimagery (or develop the autoradiograph). Compare the signals from the stripped array with the experimental signals obtained prior to stripping. Typically, >95% of the signal should have been removed from the array. If significant signals persist, then repeat the stripping procedure one more time, using fresh *Stripping Solution*.
- 5) If not proceeding directly to a new hybridization experiment, then store the array in plastic wrap at -20°C until ready to use. If additional strippings are to be performed, ensure that the gene arrays do not dry completely.

Each time an array is stripped, there will be a slight loss of bound DNA from the filter. Also, depending on the signal strength of the probe used, some labeled spots are likely to remain after stripping. For new experiments, it is advisable to compare signals only from similarly stripped arrays. For example, do not use a fresh array for the control sample and a stripped array for the test sample.

Appendix C - Layout of Gene Groups on the Array

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A-u	GEN																							GEN	
A-l	GEN																							GEN	
B-u		AM		AR		CSP	C		CYT-R		DF	EGF	FGF	INT	IL-R							TGF	TNF		HR
B-l		AM		AR	BP		C		CYT-R		DF		FGF	INT	IL-R		IS					TGF	TNF		
C-u		AM	AR		BP		C	CYT-R	CYT-R		DF	EPH	FGF	INT	IL-R	IS	IS					TGF	TNF		HR
C-l		AM	AR		BP		C	CYT-R	CYT-R		DF	EPH	FGF		IL-R	IS	IS					TGF	TNF		
D-u		AM	AR		BP	C	C	CYT-R	CYT-R		DF	EPH	FGF	IL		IS	IS		NO	TR	TGF	TNF	WR	HR	
D-l		AM	AR		BP	C		CYT-R	CYT-R		DF	EPH	FGF	IL	IL-R	IS	IS		NO	TR	TGF	TNF	WR		
E-u		AM	AR			C		CYT-R	CYT-R	DF	DF	EPH	FGF	IL	IL-R	IS	IS		NO	TR	TGF	TNF	WR	HR	
E-l		AM	AR			C		CYT-R	CYT-R	DF	DF	EPH	FGF	IL	IL-R	IS	IS		NO	TR	TGF	TNF	WR		
F-u		AM	AR	BP		C		CYT-R	CYT-R	DF	DF	EPH	FGF	IL	IL-R	IS	IS	NF	PRF	TR	TGF	TNF	WR	HR	
F-l		AM	AR	BP		C		CYT-R	CYT-R	DF	DF	EPH	FGF	IL	IL-R	IS	IS	NF	PRF	TR	TGF	TNF	WR		
G-u		AM	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	DF	EPH	FGF	IL	IL-R	IS	IS	NF	PRF		TGF	TNF	WR	HR	
G-l		AM	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	DF	EPH		IL	IL-R	IS	IS	NF	PRF		TGF	TNF	WR		
H-u			AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	DF	EPH		IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR	HR	
H-l			AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF		EPH		IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
I-u		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	DF	EPH	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR	HR	
I-l		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	DF	EPH	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
J-u		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF			INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
J-l		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF			INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
K-u		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF		FGF	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
K-l		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF		FGF	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR		
L-u		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF		FGF	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR	TE	
L-l		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF		FGF	INT	IL	IL-R	IS	IS	NF	PRF	TGF	TGF	TNF	WR	TE	
M-u		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	EGF	FGF	INT		IL-R	IS	IS	NF	PRF			TNF	WR	pUC19	
M-l		AF	AR	BP	CSP	C	C-R	CYT-R	CYT-R	DF	EGF	FGF	INT		IL-R	IS	IS	NF	PRF	TGF		TNF		<i>E. coli</i>	
N-u		AF	AR	BP	CSP	C	C-R	CYT-R		DF	EGF	FGF	INT		IL-R	IS		NF	PRF	TGF		TNF		<i>E. coli</i>	
N-l		AF	AR	BP	CSP	C	C-R	CYT-R		DF	EGF	FGF	INT			IS		NF	PRF	TGF		TNF		<i>E. coli</i>	
O-u		AF	AR	BP	CSP	C	C-R	CYT-R		DF	EGF	FGF	INT	IL-R		IS			PRF	TGF		TNF			
O-l		AF	AR	BP	CSP	C		CYT-R		DF	EGF	FGF	INT	IL-R		IS			PRF	TGF					
P-u	GEN																							GEN	
P-l	GEN																								GEN

Note: Some array coordinates are intentionally left blank. Within each coordinate, two genes (or control) are arrayed in duplicate. For example, B2 contains the genes, E-cadherin and P-Cadherin:



KEY TO GENE GROUPS

AM	Adhesion Molecule
AF	Angiogenic Factor
AR	Apoptosis-Related
BP	Binding Protein
CSP	Cell Surface Protein
C	Chemokine
C-R	Chemokine Receptor
CYT-R	Cytokine and Receptors

DF	Developmental Factors
EGF	EGF Family
EPH	Eph Family
FGF	FGF Family
INT	Integrin
IL	Interleukin
IL-R	Interleukin Receptor
IS	Intracellular Signaling

NF	Neurotrophic Factor
NO	NO Metabolism
PRF	Protease or Related Factor
TR	Telomerase Related
TGF	TGF-beta Superfamily
TNF	TNF Superfamily
WR	Weight Regulation
HR	Housekeeping Gene
GEN	Genomic DNA

Appendix D - Gene List and Locating spots of Interest

All PCR products used on the arrays are derived from cloned cDNA sequences. For all genes listed below, a representative Genbank DNA sequence accession number has been assigned. Note that the PCR products used on the gene arrays contain complete or partial coding sequences only. Use the hyperlinks in the spreadsheet file on the accompanying disk to access the Genbank sequence files with an internet browser.

The following list shows genes organized by *Gene Group*. To identify gene information for spots of interest on an array, it may be more convenient to use the gene listings within the spreadsheet file on the accompanying disk. The spreadsheet file contains two gene lists: 1) gene information organized by array rows and 2) gene information organized by array columns.

If analyzing an autoradiogram, use the accompanying transparency orientation template to determine array coordinates of spots of interest on the gene arrays. Simply align the corners of the orientation grid with the genomic DNA positive control spots located at the corners of the array.

U = upper gene pair and L = lower gene pair within an array coordinate.

Array Coordinate	Gene Group	Gene Name	Accession Number
A1U	Genomic DNA	Genomic DNA	N/A
P1U	Genomic DNA	Genomic DNA	N/A
B2U	Adhesion Molecule	E-Cadherin	NM_009864
B2L	Adhesion Molecule	P-Cadherin	X06340
C2U	Adhesion Molecule	VE-Cadherin	NM_009868
C2L	Adhesion Molecule	VE-Cadherin 2	Y08715
D2U	Adhesion Molecule	ICAM-1	X52264
D2L	Adhesion Molecule	ICAM-2	NM_010494
E2U	Adhesion Molecule	OCAM	NM_010954

Array Coordinate	Gene Group	Gene Name	Accession Number
E2L	Adhesion Molecule	VCAM-1	NM_011693
F2U	Adhesion Molecule	E-Selectin	NM_011345
F2L	Adhesion Molecule	L-Selectin	NM_011346
G2U	Adhesion Molecule	P-Selectin	NM_011347
G2L	Adhesion Molecule	VAP-1	NM_009675
I2U	Angiogenic Factor	Angiopoietin-1	NM_009640
I2L	Angiogenic Factor	Angiopoietin-2	NM_007426
J2U	Angiogenic Factor	Angiopoietin-3/4	NM_009641
J2L	Angiogenic Factor	ARP2	NM_011923
K2U	Angiogenic Factor	TIE-1	NM_011587
K2L	Angiogenic Factor	TIE-2	X71426
L2U	Angiogenic Factor	VEGF-A	NM_009505
L2L	Angiogenic Factor	VEGF-B	NM_011697
M2U	Angiogenic Factor	VEGF-C	NM_009506
M2L	Angiogenic Factor	VEGF-D	NM_010216
N2U	Angiogenic Factor	PLGF	NM_008827
N2L	Angiogenic Factor	VEGF R1	NM_010228
O2U	Angiogenic Factor	VEGF R2	NM_010612
O2L	Angiogenic Factor	VEGF R3	NM_008029
C3U	Apoptosis-Related	A1	L16462
C3L	Apoptosis-Related	AIF	NM_012019
D3U	Apoptosis-Related	Apaf1	NM_009684
D3L	Apoptosis-Related	Bad	NM_007522
E3U	Apoptosis-Related	Bag-1	NM_009736
E3L	Apoptosis-Related	BAK	NM_007523
F3U	Apoptosis-Related	Bax-a	NM_007527
F3L	Apoptosis-Related	Bcl-2	NM_009741
G3U	Apoptosis-Related	Bcl-w	NM_007537
G3L	Apoptosis-Related	Bcl-x	NM_009743
H3U	Apoptosis-Related	BID	NM_007544
H3L	Apoptosis-Related	BLK	NM_007546
I3U	Apoptosis-Related	Caspase-1	NM_009807
I3L	Apoptosis-Related	Caspase-3	NM_009810
J3U	Apoptosis-Related	Caspase-7	NM_007611
J3L	Apoptosis-Related	Caspase-11	NM_007609
K3U	Apoptosis-Related	Cox-1	NM_008969
K3L	Apoptosis-Related	Cox-2	NM_011198
L3U	Apoptosis-Related	Cytochrome p450 Oxidoreductase	NM_008898
L3L	Apoptosis-Related	FLIPL	NM_009805
M3U	Apoptosis-Related	NF-k B inducing kinase	NM_016896
M3L	Apoptosis-Related	TRP53/p53	NM_011640
N3U	Apoptosis-Related	PARP	NM_007415
N3L	Apoptosis-Related	pRB	NM_009029

Array Coordinate	Gene Group	Gene Name	Accession Number
O3U	Apoptosis-Related	SARP-1	NM_009144
O3L	Apoptosis-Related	sFRP-3	NM_011356
B4U	Apoptosis-Related	Survivin	NM_009689
B4L	Apoptosis-Related	XIAP	NM_009688
F4U	Binding Protein	Cerberus	NM_009887
F4L	Binding Protein	Chordin	NM_009893
G4U	Binding Protein	DAN/NBL1	NM_008675
G4L	Binding Protein	Endoglin	NM_007932
H4U	Binding Protein	Follistatin	NM_008046
H4L	Binding Protein	Gremlin	NM_011824
I4U	Binding Protein	Noggin	U79163
I4L	Binding Protein	Dkk-1	NM_010051
J4U	Binding Protein	Dkk-2	AJ243963
J4L	Binding Protein	Dkk-3	NM_015814
K4U	Binding Protein	Soggy	NM_015789
K4L	Binding Protein	Hip	AF116865
L4U	Binding Protein	IGF Binding Protein 1	NM_008341
L4L	Binding Protein	IGF Binding Protein 2	NM_008342
M4U	Binding Protein	IGF Binding Protein 3	NM_008343
M4L	Binding Protein	IGF Binding Protein 4	NM_010517
N4U	Binding Protein	IGF Binding Protein 5	NM_010518
N4L	Binding Protein	IGF Binding Protein 6	NM_008344
O4U	Binding Protein	IGF Binding Protein 7	AB012886
O4L	Binding Protein	IL-18 Binding Protein	NM_010531
B5L	Binding Protein	LPS Binding Protein	NM_011029
C5U	Binding Protein	LTB-1	AF022889
C5L	Binding Protein	LTBP-2	AF004874
D5U	Binding Protein	LTBP-3	NM_008520
D5L	Binding Protein	Biglycan	NM_007542
G5U	Cell Surface Protein	B7-1	X60958
G5L	Cell Surface Protein	B7-2	L25606
H5U	Cell Surface Protein	C5A R	NM_007577
H5L	Cell Surface Protein	CD3z	M33158
I5U	Cell Surface Protein	CD4	X04836
I5L	Cell Surface Protein	CD6	NM_009852
J5U	Cell Surface Protein	CD8	M12052
J5L	Cell Surface Protein	CD28	NM_007642
K5U	Cell Surface Protein	CD45/PTPRC	NM_011210
K5L	Cell Surface Protein	CTLA-4	NM_009843
L5U	Cell Surface Protein	Decorin	NM_007833
L5L	Cell Surface Protein	MD-1	NM_010745
M5U	Cell Surface Protein	RP105	NM_008533
M5L	Cell Surface Protein	ST2	D13695

Array Coordinate	Gene Group	Gene Name	Accession Number
N5U	Cell Surface Protein	Toll-like R2	AF185284
N5L	Cell Surface Protein	Toll-like R4	AF110133
O5U	Cell Surface Protein	Toll-like R6	NM_011604
O5L	Cell Surface Protein	Tolloid-like protein	NM_009390
B6U	Cell Surface Protein	Vanin-1	NM_011704
D6U	Chemokine	6Ckine/CCL21	AF006637
D6L	Chemokine	C10/CCL6	NM_009139
E6U	Chemokine	Eotaxin/CCL11	NM_011330
E6L	Chemokine	MIP-1a/CCL3	NM_011337
F6U	Chemokine	MIP-1b/CCL4	M35590
F6L	Chemokine	MIP-1g/CCL9	NM_011338
G6U	Chemokine	MIP-2a/GROb/CXCL2	NM_009140
G6L	Chemokine	MIP-3a/CCL20	NM_016960
H6U	Chemokine	MIP-3b/CCL19	NM_011888
H6L	Chemokine	JE/MCP-1/CCL2	NM_011333
I6U	Chemokine	MARC/MCP-3/CCL7	Z12297
I6L	Chemokine	MCP-5/CCL12	NM_011331
J6U	Chemokine	TCA-3/CCL1	NM_011329
J6L	Chemokine	MDC/CCL22	NM_009137
K6U	Chemokine	RANTES/CCL5	M77747
K6L	Chemokine	c-Tack/CCL27	NM_011336
L6U	Chemokine	TARC/CCL17	AJ242587
L6L	Chemokine	TECK/CCL25	NM_009138
M6U	Chemokine	BLC/BCA-1/CXCL13	AF044196
M6L	Chemokine	BRAK	AF152377
N6U	Chemokine	CRG-2/CXCL10	M86829
N6L	Chemokine	GCP-2/CXCL6	NM_009141
O6U	Chemokine	GROa/CXCL1	NM_008176
O6L	Chemokine	Lungkine/CXCL15	NM_011339
B7U	Chemokine	MIG/CXCL9	NM_008599
B7L	Chemokine	SDF-1/CXCL12	NM_013655
C7U	Chemokine	Neurotactin/CX3CL1	NM_009142
C7L	Chemokine	Lyphotactin	NM_008510
D7U	Chemokine	CCL28	no seq 11/99
G7U	Chemokine Receptor	CCR-1	NM_009912
G7L	Chemokine Receptor	CCR-2	NM_009915
H7U	Chemokine Receptor	CCR-3	NM_009914
H7L	Chemokine Receptor	CCR-4	NM_009916
I7U	Chemokine Receptor	CCR-5	NM_009917
I7L	Chemokine Receptor	CCR-6	NM_009835
J7U	Chemokine Receptor	CCR-7	NM_007719
J7L	Chemokine Receptor	CCR-8	NM_007720
K7U	Chemokine Receptor	CCR-9	NM_009913

Array Coordinate	Gene Group	Gene Name	Accession Number
K7L	Chemokine Receptor	D6	NM_007721
L7U	Chemokine Receptor	Duffy	NM_010045
L7L	Chemokine Receptor	CXCR-3	NM_009910
M7U	Chemokine Receptor	CXCR-4	NM_009911
M7L	Chemokine Receptor	CXCR-5	NM_007551
N7U	Chemokine Receptor	APJ/AGTRL1	NM_011784
N7L	Chemokine Receptor	RDC-1	NM_007722
O7U	Chemokine Receptor	CX3CR-1	AF074912
C8U	Cytokines and Receptors	CT-1	NM_007795
C8L	Cytokines and Receptors	CTGF	NM_010217
D8U	Cytokines and Receptors	Epo	M12930
D8L	Cytokines and Receptors	Flt-3/Flk-2 Ligand	U04807
E8U	Cytokines and Receptors	Insulin	NM_008386
E8L	Cytokines and Receptors	GAS6	X59846
F8U	Cytokines and Receptors	G-CSF	NM_009971
F8L	Cytokines and Receptors	GM-CSF	X02333
G8U	Cytokines and Receptors	M-CSF	NM_007778
G8L	Cytokines and Receptors	HGF	X84046
H8U	Cytokines and Receptors	IGF-I	NM_010512
H8L	Cytokines and Receptors	IGF-II	NM_010514
I8U	Cytokines and Receptors	LIF	NM_008501
I8L	Cytokines and Receptors	MSP	NM_008243
J8U	Cytokines and Receptors	OSM	D31942
J8L	Cytokines and Receptors	OPN	NM_009263
K8U	Cytokines and Receptors	PDGF-a	NM_008808
K8L	Cytokines and Receptors	PDGF-b	NM_011057
L8U	Cytokines and Receptors	SCF	NM_013598
L8L	Cytokines and Receptors	Tpo	NM_009379
M8U	Cytokines and Receptors	Axl	NM_009465
M8L	Cytokines and Receptors	Dtk	U18933
N8U	Cytokines and Receptors	c-kit	Y00864
N8L	Cytokines and Receptors	c-met	NM_008591
O8U	Cytokines and Receptors	Endothelin-b R	NM_007904
O8L	Cytokines and Receptors	G-CSF R	NM_007782
B9U	Cytokines and Receptors	Epo R	NM_010149
B9L	Cytokines and Receptors	Flt-3 (Flk-2/Stk-1)	NM_010229
C9U	Cytokines and Receptors	GM-CSF Ra	NM_009970
C9L	Cytokines and Receptors	IFNA R1	NM_010508
D9U	Cytokines and Receptors	IFNA R2	NM_010509
D9L	Cytokines and Receptors	IFN-b	NM_010510
E9U	Cytokines and Receptors	IFN-g	K00083
E9L	Cytokines and Receptors	IFN-g R1	NM_010511
F9U	Cytokines and Receptors	IFN-g R2	NM_008338

Array Coordinate	Gene Group	Gene Name	Accession Number
F9L	Cytokines and Receptors	IGF-I R	AF056187
G9U	Cytokines and Receptors	Insulin R	NM_010568
G9L	Cytokines and Receptors	LIF R	D26177
H9U	Cytokines and Receptors	M-CSF R	NM_007779
H9L	Cytokines and Receptors	Mer	U21301
I9U	Cytokines and Receptors	MSP R	NM_009074
I9L	Cytokines and Receptors	OSM Rb	NM_011019
J9U	Cytokines and Receptors	PDGF Ra	NM_011058
J9L	Cytokines and Receptors	PDGF Rb	NM_008809
K9U	Cytokines and Receptors	PREF-1	NM_010052
K9L	Cytokines and Receptors	RYK	M98547
L9U	Cytokines and Receptors	Tpo R	NM_010823
L9L	Cytokines and Receptors	Galectin-9	NM_010708
M9U	Cytokines and Receptors	Mannose R	NM_008625
M9L	Cytokines and Receptors	Mannose 6-phosphate R	U04710
E10U	Developmental Factors	DANCE	NM_011812
E10L	Developmental Factors	Dhh	NM_007857
F10U	Developmental Factors	Ihh	U85610
F10L	Developmental Factors	Shh	X76290
G10U	Developmental Factors	Sema3A	NM_009152
G10L	Developmental Factors	Sema3B	NM_009153
H10U	Developmental Factors	Sema3C	X85994
H10L	Developmental Factors	SLIT-1	AF144627
I10U	Developmental Factors	SLIT-2	AF144628
I10L	Developmental Factors	SLIT-3	AF088902
J10U	Developmental Factors	Lefty-1	NM_010094
J10L	Developmental Factors	WNT-1	K02593
K10U	Developmental Factors	WNT-3	NM_009521
K10L	Developmental Factors	WNT-4	NM_009523
L10U	Developmental Factors	WNT-5a	NM_009524
L10L	Developmental Factors	WNT-5b	NM_009525
M10U	Developmental Factors	WNT-8b	NM_011720
M10L	Developmental Factors	WNT-8d	NM_009290
N10U	Developmental Factors	WNT-10a	NM_009518
N10L	Developmental Factors	WNT-10b	NM_011718
O10U	Developmental Factors	WNT-11	NM_009519
O10L	Developmental Factors	WNT-13	NM_009520
B11U	Developmental Factors	WNT-16	AF172064
B11L	Developmental Factors	DCC	NM_007831
C11U	Developmental Factors	Mahogany	AF116897
C11L	Developmental Factors	Netrin-1	NM_008744
D11U	Developmental Factors	Neuropilin-1	NM_008737
D11L	Developmental Factors	Neuropilin-2	AF022854

Array Coordinate	Gene Group	Gene Name	Accession Number
E11U	Developmental Factors	Notch-1	NM_008714
E11L	Developmental Factors	Notch-2	D32210
F11U	Developmental Factors	Notch-3	NM_008716
F11L	Developmental Factors	Notch-4	M80456
G11U	Developmental Factors	Plexin1	NM_008881
G11L	Developmental Factors	Plexin2	D86949
H11U	Developmental Factors	Plexin3	NM_008883
I11U	Developmental Factors	Smo	AF089721
I11L	Binding Protein	WIF-1	NM_011915
M11U	EGF Family	Cripto	NM_011562
M11L	EGF Family	EGF	J00380
N11U	EGF Family	EGF R	NM_007912
N11L	EGF Family	erbB2	U71126
O11U	EGF Family	erbB3	L47240
O11L	EGF Family	erbB4	AF059176
B12U	EGF Family	NRG-3	NM_008734
C12U	Eph Family	Ephrin-A1	NM_010107
C12L	Eph Family	Ephrin-A2	NM_007909
D12U	Eph Family	Ephrin-A4	NM_007910
D12L	Eph Family	Ephrin-B1	NM_010110
E12U	Eph Family	Ephrin-B2	NM_010111
E12L	Eph Family	EphA2	NM_010139
F12U	Eph Family	EphA3	M68513
F12L	Eph Family	EphA4	NM_007936
G12U	Eph Family	EphA6	NM_007938
G12L	Eph Family	EphA7-d1	X79082
H12U	Eph Family	EphA8	NM_007939
H12L	Eph Family	EphB2	L25890
I12U	Eph Family	EphB3	Z49086
I12L	Eph Family	EphB4	NM_010144
K12U	FGF Family	FGF-1	NM_010197
K12L	FGF Family	FGF-2	M30644
L12U	FGF Family	FGF-3	NM_008007
L12L	FGF Family	FGF-4	NM_010202
M12U	FGF Family	FGF-5	NM_010203
M12L	FGF Family	FGF-6	M92416
N12U	FGF Family	FGF-7	NM_008008
N12L	FGF Family	FGF-8	Z48746
O12U	FGF Family	FGF-9	NM_013518
O12L	FGF Family	FGF-10	NM_008002
B13U	FGF Family	FGF-11	NM_010198
B13L	FGF Family	FGF-12	U66201
C13U	FGF Family	FGF-13	NM_010200

Array Coordinate	Gene Group	Gene Name	Accession Number
C13L	FGF Family	FGF-14	NM_010201
D13U	FGF Family	FGF-15	NM_008003
D13L	FGF Family	FGF-17	NM_008004
E13U	FGF Family	FGF-18	NM_008005
E13L	FGF Family	FGF R1	NM_010206
F13U	FGF Family	FGF R2	NM_010207
F13L	FGF Family	FGF R3	NM_008010
G13U	FGF Family	FGF R4	NM_008011
I13U	Integrin	Integrin-a1	AA008624
I13L	Integrin	Integrin-a2	NM_008396
J13U	Integrin	Integrin-a3	D13867
J13L	Integrin	Integrin-a4	NM_010576
K13U	Integrin	Integrin-a5	NM_010577
K13L	Integrin	Integrin-a6	X69902
L13U	Integrin	Integrin-a7	NM_008398
L13L	Integrin	Integrin-a8	AF041409
M13U	Integrin	Integrin-aL	M60778
M13L	Integrin	Integrin-aV	NM_008402
N13U	Integrin	Integrin-b1	Y00769
N13L	Integrin	Integrin-b2	NM_008404
O13U	Integrin	Integrin-b3	AF026509
O13L	Integrin	Integrin-b4	L04678
B14U	Integrin	Integrin-b5A	NM_010580
B14L	Integrin	Integrin-b6	AF115376
C14U	Integrin	Integrin-b7	M95632
D14U	Interleukin	IL-1a	NM_010554
D14L	Interleukin	IL-1b	NM_008361
E14U	Interleukin	IL-2	X01772
E14L	Interleukin	IL-3	NM_010556
F14U	Interleukin	IL-4	M25892
F14L	Interleukin	IL-5	NM_010558
G14U	Interleukin	IL-6	X54542
G14L	Interleukin	IL-7	NM_008371
H14U	Interleukin	IL-9	NM_008373
H14L	Interleukin	IL-10	NM_010548
I14U	Interleukin	IL-11	NM_008350
I14L	Interleukin	IL-12 p35	M86672
J14U	Interleukin	IL-12 p40	M86671
J14L	Interleukin	IL-13	NM_008355
K14U	Interleukin	IL-15	NM_008357
K14L	Interleukin	IL-16	NM_010551
L14U	Interleukin	IL-17	NM_010552
L14L	Interleukin	IL-18	NM_008360

Array Coordinate	Gene Group	Gene Name	Accession Number
O14U	Interleukin Receptor	IL-1ra	M57525
O14L	Interleukin Receptor	IL-1 RI	NM_008362
B15U	Interleukin Receptor	IL-1 RII	NM_010555
B15L	Interleukin Receptor	IL-2 Ra	NM_008367
C15U	Interleukin Receptor	IL-2 Rb	NM_008368
C15L	Interleukin Receptor	IL-2 Rg	L20048
D15L	Interleukin Receptor	IL-3 Rb	NM_007781
E15U	Interleukin Receptor	IL-4 Ra	NM_010557
E15L	Interleukin Receptor	IL-5 Ra	NM_008370
F15U	Interleukin Receptor	IL-6 Ra	X51975
F15L	Interleukin Receptor	IL-7 Ra	NM_008372
G15U	Interleukin Receptor	IL-8 R	NM_009909
G15L	Interleukin Receptor	IL-9 Ra	NM_008374
H15U	Interleukin Receptor	IL-10 Ra	NM_008348
H15L	Interleukin Receptor	IL-10 Rb	NM_008349
I15U	Interleukin Receptor	IL-11 Ra	U14412
I15L	Interleukin Receptor	IL-12 Rb1	NM_008353
J15U	Interleukin Receptor	IL-12 Rb2	NM_008354
J15L	Interleukin Receptor	IL-13 Ra1	S80963
K15U	Interleukin Receptor	IL-13 Ra2	NM_008356
K15L	Interleukin Receptor	IL-15 Ra	NM_008358
L15U	Interleukin Receptor	IL-17 R	NM_008359
L15L	Interleukin Receptor	IL-18 R	NM_008365
M15U	Interleukin Receptor	IL-1 R AcP	NM_008364
M15L	Interleukin Receptor	IL-18 R AcP	NM_010553
N15U	Interleukin Receptor	gp130	NM_010560
C16U	Signal Transduction	14-3-3 eta	NM_011738
C16L	Signal Transduction	c-fos	NM_010234
D16U	Signal Transduction	CIS1	NM_009895
D16L	Signal Transduction	CIS2	NM_007706
E16U	Signal Transduction	CIS3	NM_007707
E16L	Signal Transduction	SOCS-5	AF033187
F16U	Signal Transduction	c-jun	NM_010591
F16L	Signal Transduction	c-myb	M16449
G16U	Signal Transduction	c-myc	NM_010849
G16L	Signal Transduction	EBF	NM_007897
H16U	Signal Transduction	En-1	L12703
H16L	Signal Transduction	En-2	NM_010134
I16U	Signal Transduction	FAST-2/FOXH1	AF079514
I16L	Signal Transduction	FTa	NM_008033
J16U	Signal Transduction	FTb	AI151779
J16L	Signal Transduction	GATA-3	NM_008091
K16U	Signal Transduction	I-kB	NM_010908

Array Coordinate	Gene Group	Gene Name	Accession Number
K16L	Signal Transduction	IRS-1	NM_010570
L16U	Signal Transduction	Jak-1	S63728
L16L	Signal Transduction	Jak-2	NM_008413
M16U	Signal Transduction	Jak-3	NM_010589
M16L	Signal Transduction	NF-k Bp65	NM_009045
N16U	Signal Transduction	NIK(Ste20-related kinase)	NM_008696
N16L	Signal Transduction	NSG1/p21	NM_010942
O16U	Signal Transduction	P38	U10871
O16L	Signal Transduction	AKT/PKB	NM_009652
B17L	Signal Transduction	RhoA	NM_016802
C17U	Signal Transduction	SAPK	U18310
C17L	Signal Transduction	SKI	U14173
D17U	Signal Transduction	SMAD1	NM_008539
D17L	Signal Transduction	SMAD2	NM_010754
E17U	Signal Transduction	SMAD3	AF016189
E17L	Signal Transduction	SMAD4	NM_008540
F17U	Signal Transduction	SMAD5	NM_008541
F17L	Signal Transduction	SMAD6	NM_008542
G17U	Signal Transduction	SMAD7	NM_008543
G17L	Signal Transduction	SnoN	NM_011386
H17U	Signal Transduction	SOCS-1	NM_009896
H17L	Signal Transduction	Stat 1	NM_009283
I17U	Signal Transduction	Stat 2	AF088862
I17L	Signal Transduction	Stat 3	U06922
J17U	Signal Transduction	Stat 4	NM_011487
J17L	Signal Transduction	Stat 5a	NM_011488
K17U	Signal Transduction	Stat 6	NM_009284
K17L	Signal Transduction	STRAP	NM_011499
L17U	Signal Transduction	TAK1	D76446
L17L	Signal Transduction	TANK	NM_011529
M17U	Signal Transduction	TIAF1	AF104984
M17L	Signal Transduction	Tyk2	AF173032
F18U	Neurotrophic Group	Artemin	NM_009711
F18L	Neurotrophic Group	b-NGF	M35075
G18U	Neurotrophic Group	BDNF	NM_007540
G18L	Neurotrophic Group	CNTF	U05342
H18U	Neurotrophic Group	GDNF	D49921
H18L	Neurotrophic Group	Midkine	NM_010784
I18U	Neurotrophic Group	Neurturin	NM_008738
I18L	Neurotrophic Group	Persephin	NM_008954
J18U	Neurotrophic Group	NT-3	NM_008742
J18L	Neurotrophic Group	NT-4	AI322264
K18U	Neurotrophic Group	CNTF Ra	AF068615

Array Coordinate	Gene Group	Gene Name	Accession Number
K18L	Neurotrophic Group	GFR a1	NM_010279
L18U	Neurotrophic Group	GFR a2	NM_008115
L18L	Neurotrophic Group	GFR a3	NM_010280
M18U	Neurotrophic Group	NGF R	AF105292
M18L	Neurotrophic Group	Ret	NM_009050
N18U	Neurotrophic Group	TrkB	NM_008745
N18L	Neurotrophic Group	TrkC	AF035400
D19U	NO Metabolism	eNOS	NM_008713
D19L	NO Metabolism	iNOS	NM_010927
E19U	NO Metabolism	nNOS	NM_008712
E19L	NO Metabolism	PIN	AA041883
F19U	Protease or Related Factor	BMP-1	L35281
F19L	Protease or Related Factor	EMMPRIN	Y16256
G19U	Protease or Related Factor	Granzyme F	NM_010374
G19L	Protease or Related Factor	ADAM-10	NM_007399
H19U	Protease or Related Factor	MMP-2	NM_008610
H19L	Protease or Related Factor	MMP-3	NM_010809
I19U	Protease or Related Factor	MMP-24	NM_010808
I19L	Protease or Related Factor	MMP-7	NM_010810
J19U	Protease or Related Factor	MMP-8	NM_008611
J19L	Protease or Related Factor	MMP-10	Y13185
K19U	Protease or Related Factor	MMP-11	NM_008606
K19L	Protease or Related Factor	MMP-12	NM_008605
L19U	Protease or Related Factor	MMP-13	NM_008607
L19L	Protease or Related Factor	MMP-14	NM_008608
M19U	Protease or Related Factor	MMP-15	NM_008609
M19L	Protease or Related Factor	TIMP-1	NM_011593
N19U	Protease or Related Factor	TIMP-2	NM_011594
N19L	Protease or Related Factor	TIMP-3	NM_011595
O19U	Protease or Related Factor	Plasminogen	NM_008877
O19L	Protease or Related Factor	uPAR1	NM_011113
D20U	Telomerase Related	TP1	NM_009351
D20L	Telomerase Related	TERT/TP2	NM_009354
E20U	Telomerase Related	TRF1	NM_009352
E20L	Telomerase Related	TRF2	NM_009353
F20U	Telomerase Related	Tin2	AF214013
F20L	Telomerase Related	TR/TeRc	U33831
H20U	TGF-beta Superfamily	Activin B (bB subunit)	X83376
H20L	TGF-beta Superfamily	Activin C (bC subunit)	NM_010565
I20U	TGF-beta Superfamily	Activin E (bE subunit)	NM_008382
I20L	TGF-beta Superfamily	Inhibin A (asubunit)	NM_010564
J20U	TGF-beta Superfamily	BMP-2	NM_007553
J20L	TGF-beta Superfamily	BMP-3	AI325452

Array Coordinate	Gene Group	Gene Name	Accession Number
K20U	TGF-beta Superfamily	BMP-4	NM_007554
K20L	TGF-beta Superfamily	BMP-5	NM_007555
L20U	TGF-beta Superfamily	BMP-6	NM_007556
L20L	TGF-beta Superfamily	BMP-7	NM_007557
M20L	TGF-beta Superfamily	BMP-8	NM_007559
N20U	TGF-beta Superfamily	BMP-9	AF188286
N20L	TGF-beta Superfamily	BMP-10	NM_009756
O20U	TGF-beta Superfamily	BMP-11	AF092734
O20L	TGF-beta Superfamily	BMP-15	AF082348
B21U	TGF-beta Superfamily	GDF-1	NM_008107
B21L	TGF-beta Superfamily	GDF-3	NM_008108
C21U	TGF-beta Superfamily	GDF-5	NM_008109
C21L	TGF-beta Superfamily	GDF-6	U08338
D21U	TGF-beta Superfamily	GDF-7	U08339
D21L	TGF-beta Superfamily	GDF-8	NM_010834
E21U	TGF-beta Superfamily	GDF-9	NM_008110
E21L	TGF-beta Superfamily	GDF-10	S82648
F21U	TGF-beta Superfamily	TGF-b	NM_011577
F21L	TGF-beta Superfamily	TGF-b2	NM_009367
G21U	TGF-beta Superfamily	TGF-b3	NM_009368
G21L	TGF-beta Superfamily	Activin RIB	NM_007395
H21U	TGF-beta Superfamily	Activin RIIA	NM_007396
H21L	TGF-beta Superfamily	Activin RIIB	NM_007397
I21U	TGF-beta Superfamily	ALK-1	NM_009612
I21L	TGF-beta Superfamily	BMP RIA	NM_009758
J21U	TGF-beta Superfamily	BMP RIB	NM_007560
J21L	TGF-beta Superfamily	BMP R2	NM_007561
K21U	TGF-beta Superfamily	TGF-b RI	NM_007394
K21L	TGF-beta Superfamily	TGF-b RII	NM_009371
L21U	TGF-beta Superfamily	TGF-b RIII	AF039601
L21L	TGF-beta Superfamily	GDF-15	NM_011819
B22U	TNF Superfamily	TNF-a/TNFSF2	M13049
B22L	TNF Superfamily	TNF-b/TNFSF1	NM_010735
C22U	TNF Superfamily	OX40L/TNFSF4	NM_009452
C22L	TNF Superfamily	CD40L/TNFSF5	NM_011616
D22U	TNF Superfamily	FasL/TNFSF6	NM_010177
D22L	TNF Superfamily	CD27L/TNFSF7	NM_011617
E22U	TNF Superfamily	CD30L/TNFSF8	NM_009403
E22L	TNF Superfamily	4-1BBL/TNFSF9	NM_009404
F22U	TNF Superfamily	TRAIL/TNFSF10	NM_009425
F22L	TNF Superfamily	RANK L/TRANCE/TNFSF11	NM_011613
G22U	TNF Superfamily	TWEAK/TNFSF12	AF030100
G22L	TNF Superfamily	THANK/BAFF/TNFSF13B	AF119383

Array Coordinate	Gene Group	Gene Name	Accession Number
H22U	TNF Superfamily	LIGHT/TNFSF14	AB029155
H22L	TNF Superfamily	TNF RI/TNFRSF1A	NM_011609
I22U	TNF Superfamily	TNF RII/TNFRSF1B	NM_011610
I22L	TNF Superfamily	LT-b R/TNFRSF3	U29173
J22U	TNF Superfamily	OX40/TNFRSF4	NM_011659
J22L	TNF Superfamily	CD40/TNFRSF5	NM_011611
K22U	TNF Superfamily	Fas/TNFRSF6	NM_007987
K22L	TNF Superfamily	CD27/TNFRSF7	L24495
L22U	TNF Superfamily	CD30/TNFRSF8	NM_009401
L22L	TNF Superfamily	4-1BB/TNFRSF9	NM_011612
M22U	TNF Superfamily	TRAIL R2/DR5/TNFRSF10B	AF176833
M22L	TNF Superfamily	RANK/TNFRSF11A	NM_009399
N22U	TNF Superfamily	OPG/TNFRSF11B	NM_008764
N22L	TNF Superfamily	GITR/TNFRSF18	NM_009400
O22U	TNF Superfamily	TNFRSF19	AF173166
D23U	Weight Regulation	Agouti	L06451
D23L	Weight Regulation	ART	NM_007427
E23U	Weight Regulation	CRFR1	NM_007762
E23L	Weight Regulation	Leptin	NM_008493
F23U	Weight Regulation	MCH	BB175332
F23L	Weight Regulation	NPY	AI385504
G23U	Weight Regulation	Orexin	NM_010410
G23L	Weight Regulation	POMC1	NM_008895
H23U	Weight Regulation	Leptin R	U49109
H23L	Weight Regulation	MC1 R	NM_008559
I23U	Weight Regulation	MC2 R	NM_008560
I23L	Weight Regulation	MC3 R	NM_008561
J23U	Weight Regulation	MC4 R	AB009664
J23L	Weight Regulation	MC5 R	NM_013596
K23U	Weight Regulation	NPY Y5R	AF022948
K23L	Weight Regulation	UCP1	NM_009463
L23U	Weight Regulation	UCP2	NM_011671
L23L	Weight Regulation	UCP3	NM_009464
M23U	Weight Regulation	CART	NM_013732
A24U	Genomic DNA	Genomic DNA	N/A
B24U	Housekeeping Genes	b2M	NM_009735
C24U	Housekeeping Genes	b-Actin	X03672
D24U	Housekeeping Genes	Cyclophilin	NM_008907
E24U	Housekeeping Genes	GAPDH	NM_008084
F24U	Housekeeping Genes	HPRT	J00423
G24U	Housekeeping Genes	L19	NM_009078
H24U	Housekeeping Genes	Transferrin R	X57349
I24U	Housekeeping Genes	a-Tubulin	M13446

Array Coordinate	Gene Group	Gene Name	Accession Number
L24U	Negative Controls	1xTE Buffer	N/A
L24L	Negative Controls	1xTE Buffer	N/A
M24U	Negative Controls	pUC19	M77789
M24L	Negative Controls	E. coli b0658 gene	AE000170
N24U	Negative Controls	E. coli b1444 gene	AE000241
N24L	Negative Controls	E. coli b3535 gene	AE000430
P24U	Genomic DNA	Genomic DNA	N/A

Appendix E - DNase I treatment of total RNA

1. Pipette 50-100 μg of RNA sample into a fresh 1.5 mL microfuge tube add 5 μL of DNase I buffer (10X) and 5 units of DNase I (1 unit/ μL) (*Sigma-AMP-D1*). Make up to a total volume of 50 μL with sterile, distilled water.
2. Incubate at room temperature for 15 minutes.
3. Add 150 μL of water to bring up the volume to 200 μL .
4. Add 200 μL of hot acidic phenol (pH 4.3, Sigma P4682), mix thoroughly with a vortex and incubate for 3 minutes at 65°C.
5. Cool tubes on ice for 3 minutes.
6. Separate the phases by centrifuging the sample in a microcentrifuge at maximum speed ($\sim 12,000 \times g$) for 2-3 minutes. Transfer the upper aqueous phase to a fresh tube.
7. Repeat the phenol extraction two more times.
8. Add 200 μL of acidic phenol:chloroform:isoamyl alcohol (25:24:1), vortex, and separate the phases by centrifuging the sample in a microcentrifuge at $\sim 12,000 \times g$ for 2-3 minutes. Transfer the upper aqueous phase to a fresh tube.
9. Add 200 μL of chloroform:isoamyl alcohol (24:1), vortex, and separate the phases by centrifuging the sample in a microcentrifuge at $\sim 12,000 \times g$ for 2-3 minutes. Transfer the upper aqueous phase to a fresh tube.
10. Precipitate the RNA by adding 0.1x volumes of 3M sodium acetate (pH 5.2) and vortex to mix. Add 3 volumes of absolute ethanol and mix by inverting the tube several times. Samples may be stored at -20°C indefinitely or proceed directly to the next step.
11. Pellet the RNA by centrifugation at 12,000 $\times g$ for 30 minutes. Carefully discard the supernatant either by decanting or by pipetting.
12. Wash the RNA pellet by adding 1 mL 70% ethanol. Gently invert the tube several times and centrifuge at maximum speed for 15 – 20 minutes.
13. Air dry the pellet for 10-15 minutes.
14. Dissolve the RNA in 50 μL of sterile water. Place tube at 37°C for 15 – 30 minutes to thoroughly dissolve the RNA. Keep on ice for immediate use or store at -20°C for long term storage.

It is advisable to check equivalent amounts of RNA before and after the DNase I digestion by non-denaturing agarose gel electrophoresis to ensure the quality of the RNA and to observe the effect of the DNase I treatment.

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