



TissueScan qPCR Array

Survey gene expression in cancer tissues using archived tumor samples

Abstract

TissueScan is a unique qPCR array of first-strand cDNA from thousands of clinical tumor samples. It provides fast and accurate gene expression profiling across a large number of cancer tissues. High-quality cDNAs were prepared from well-documented cancer biopsy samples, normalized and assembled into ready-to-use gene expression panels. This alleviates for researchers the tedious work of sample collection and the meticulous work of RNA/cDNA preparation, while facilitating quick and reliable profiling of gene expression levels across cancer progression stages. TissueScan combines the high sensitivity and specificity of the RT-PCR methodology with a well-designed multi-sample format. It is an excellent tool for validation of potential cancer markers such as those obtained by microarray or differential display.

Introduction

Despite the incredible potential of cancer biomarkers, very few have been sufficiently validated to justify their use in developing drugs or making patient care decisions. The number of cancer biomarkers in current use for clinical therapy is quite small. One of the major obstacles in biomarker validation is the difficulty in accessing a sufficient number of clinical samples with adequate documentation. Much promising, preliminary research ends after obtaining data from cancer cell lines or after microarray screening using a small number of paired cancer/normal tissues. With the availability of OriGene's TissueScan oncology panel containing many primary samples per plate, that preliminary research can now be extended with relevant human material.

TissueScan qPCR arrays were invented to provide researchers with easy access to a large number of well-documented primary human biopsy specimens.. Each TissueScan panel is contains 48, 96 or 384 first-strand cDNAs prepared from individual tumor tissue blocks. The researcher can simply aliquot a real-time PCR master mix with gene-specific primers into the plate, load onto a PCR machine and walk away. Two hours later, the survey of gene expression levels across numerous samples are available. The whole process is quick, sensitive, accurate and painless.

Protocol at a glance

Before using TissueScan, design a pair of real-time PCR primers specific for each gene of interest.

Step 1:

Prepare a qPCR master mix with gene-specific primers, and aliquot into the PCR panel.

Step 2:

Perform thermal cycling (see appendix for list of compatible thermal cyclers).

Step 3:

Plot the expression profile of your gene of interest using the Ct (threshold cycle) method.

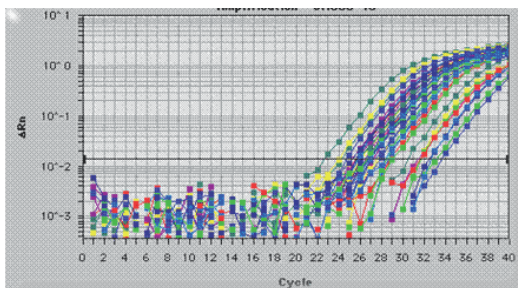
The TissueScan Advantage

Unlike microarrays that explore expression changes of multiple genes in a single tissue, TissueScan reveals the expression changes of a single gene in multiple tissues by a simple qPCR experiment. Therefore TissueScan can be considered a “reverse-microarray”. It is especially useful for validation of a large number of leads from microarray experiments.

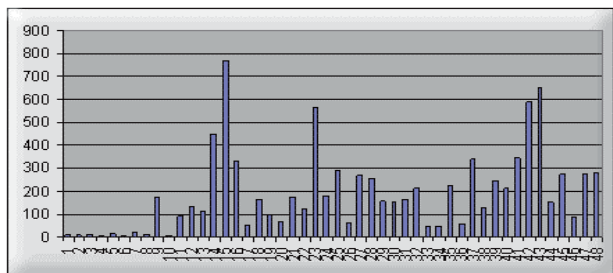
- **Convenient** - Remove the hurdle of tissue procurement so you can focus on validation and discovery
- **Comprehensive** – 48-384 unique samples per panel covering normal and all progression stages
- **Economical** – As low as \$2.00 per sample
- **Reliable** - Pathologist verified tissues obtained with informed consent, provided with detailed pathology reports and histology images
- **Fast** - Results in less than 2 hours

Application example

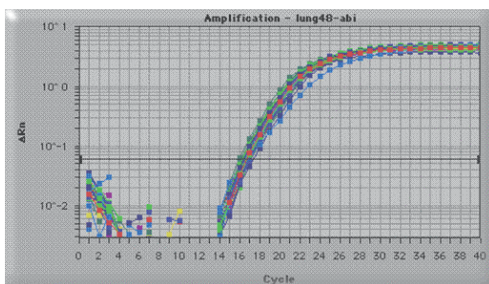
To illustrate the validity of TissueScan, we used Lung Cancer Panel (HLRT-101) to evaluate the expression level of Topoisomerase II alpha, which has been known to be upregulated in a large percentage of lung cancers (Syahrudin et al, Liu et al). A pair of primers was designed to detect the transcript of this gene (NCBI accession #NM_001067). SYBR Green I based real-time PCR was performed in a Perkin Elmer thermal cycler (model #PE7700). The raw data (Figure 1A) and the expression levels calculated by the Ct method (Figure 1B) are shown below. A second panel in the kit was used with beta-actin control primers provided in the kit (Figure 1C) to demonstrate the normalization of the cDNAs.



1A. Real-time PCR results with TOP2A .



1B. Normalized relative expression of TOP2A among 48 lung samples



1C. Real-time PCR results with beta-actin primers

The TissueScan experiment demonstrated that the topoisomerase II alpha gene is expressed at very low levels in normal samples of lung tissue (Samples 1-8). In all but one cancerous tissue (well 10, tumor stage IA), topoisomerase II alpha expression levels are markedly higher than in the normal tissues, supporting the notion that this gene can serve as a valid biomarker for lung cancer. After primer design, the entire experiment took about 2 hours, with 20 minutes of hands-on time.

Quality is the key for TissueScan

The quality of the source tissue ultimately determines the value of a product such as this. OriGene is proud to declare that all tissues are provided by Cytomyx (www.cytomyx.com), a reputable biorepository with one of the largest collections of highly characterized human tissue samples in the world. Each sample was obtained with informed consent and is accompanied by a detailed pathology report. Histology images corresponding to the tissue from which the cDNA was derived are available in two levels of detail. An electropherogram measuring the RNA integrity is included as well; the sharp 18S and 28S peaks represent high quality RNA. Finally, quality control data indicating a lack of genomic DNA contamination are also provided, assuring our customers that only the highest quality RNA preparations were used to produce the cDNA in every TissueScan Oncology Panel.

The quality of the panel is demonstrated in the following experiment. A breast cancer panel (BCRT101) was used to evaluate Her-2 (ERBB2, NCBI accession number NM_00100005862) expression levels in 48 breast tissues. Her-2 is known to be overexpressed in 25-30% of breast cancer patients. Due to its value in predicting prognosis and responsiveness to the drug Herceptin, Her-2 expression levels are routinely checked in breast cancer patients. Out of the 48 tissues in BCRT101 panel, 39 have accompanying Her-2 immunohistochemistry (IHC) data. Taking advantage of this valuable clinical information, we compared our real-time PCR results with the provided IHC data. Not only did the analysis indicate upregulation of Her-2 in cancer tissues as compared to normal, the data provided with the samples gave us further useful information. It was exciting to find that all TissueScan samples indicated to be upregulated for Her-2 by our real-time PCR experiment were also identified as positive for Her-2 by IHC (Fig 2).

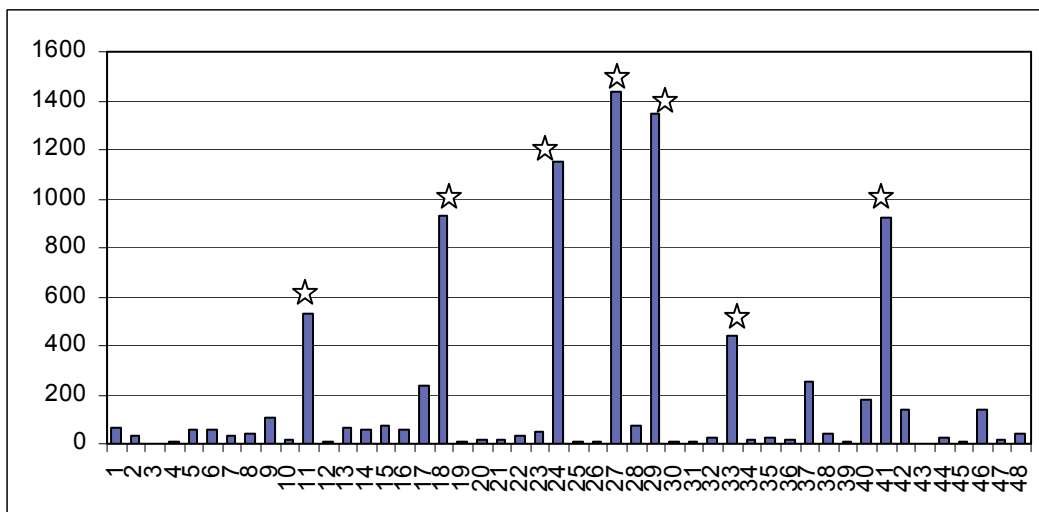


Fig 2. Quantitative measurements of Her-2 expression levels correlate with IHC data for Her-2. TissueScan breast cancer panel (BCRT101) was screened by real-time PCR using primers for Her-2. Clinical data provided with the panel was used to identify Her-2 positive



samples as determined by IHC; stars indicate positive samples by IHC as indicated in accompanying pathology reports. (No IHC data was available for tissue samples #17 and #37.)

Who needs TissueScan?

Scientists in any of the following research areas would benefit from TissueScan.

1. Scientists who have obtained the leads for potential biomarkers derived from studies with a relatively small sample size. To validate such candidate genes, it is crucial to investigate with a larger sample pool to gain statistical relevance.
2. Scientists with biomarker candidates derived from a cancer cell line study or from animal studies. It is pivotal to profile the genes in human cancer tissues to understand their significance in clinical diagnosis and therapy.
3. Scientists validating cancer staging markers. One key feature of TissueScan is that most panels contains one cancer type across all stages, providing a comprehensive landscape for a gene expression survey.
4. Scientists studying SNP/chromosomal aberrations and cancer risk. As the 48 samples are derived from 48 individuals, they can be used studied for the frequency of a particular SNP or chromosomal rearrangement. Comparing the diseased with the normal tissues may reveal a potential association of a genotype with cancer.
5. Scientists who wish to determine the relevance of a cancer biomarker to other cancer types. Many genes that have aberrant expression in one cancer type are also present in other types of cancer. OriGene's growing TissueScan repertoire currently includes multiple cancer types, facilitating the work of those who wish to expand the validation process into other cancer types.

Product offerings

Thanks to Cytomyx's large human tissue bank, OriGene has been expanding the TissueScan product line with a new panel released monthly. In addition to the growing repertoire of panels that are focused on a cancer type of single tissue origin, OriGene has launched a brand new TissueScan panel: the Cancer Survey Panel. This Cancer Survey Panel is a 96-well qPCR plate, containing 8 cancer types with 12 samples for each type. Three of the 12 samples are normal controls that are surgical samples from the cancer patients but defined by the pathologist as normal tissue.

Cancer Survey 96 - I (96 Tissues)	2 Panel Pack	10 Panel Pack	QC
Cancer Survey 384 - I (381 Tissues)	2 Panel Pack	10 Panel Pack	QC
Prostate Cancer I (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Prostate Cancer II (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Breast Cancer I (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Breast Cancer II (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Breast Cancer III (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Breast Cancer IV (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Colon Cancer (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Colon Cancer II (48 Tissues)	2 Panel Pack	10 Panel Pack	QC



Colon Cancer III - Matched Pairs (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Colon Cancer IV (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Bladder Cancer I (24 Tissues)	2 Panel Pack	10 Panel Pack	QC
Liver Cancer (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Sarcoma (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lung Cancer I (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lung Cancer II (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lung Cancer III (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lung Cancer IV - Matched Pairs (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lung Cancer V - (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Ovarian Cancer I (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Ovarian Cancer II (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Ovarian Cancer III (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Ovarian Cancer IV (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Melanoma (43 Tissues)	2 Panel Pack	10 Panel Pack	QC
Thyroid Cancer (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Endometrium (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Lymphoma Cancer (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Kidney Cancer (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Gastroesophageal (48 Tissues)	2 Panel Pack	10 Panel Pack	QC
Crohn's and Colitis (48 Tissues)	2 Panel Pack	10 Panel Pack	QC

Beyond gene-expression patterns

TissueScan is an excellent tool to study the expression pattern of specific genes in cancer versus normal tissues. However, if complementary analysis is needed (such as immunohistochemistry, genotyping, analysis of the patient treatment history and outcome, etc.), additional information or other source material would be required. Thanks to OriGene's excellent relationship with the biorepository Cytomyx, some related material (tissue, RNA, DNA, or protein) for many TissueScan samples can be acquired. Using the identification number for each sample (identified in the pathology report, e.g. CI0000015482), customers can request additional information and various related materials for many TissueScan samples.

Additional clinical data: As all TissueScan samples originate from the Cytomyx tissue collection, they are all accompanied by extensive clinical and pathology data available at the time the sample was originally collected. For about 50% of the samples ("linked samples"), Cytomyx can obtain new data relevant to the donor such as treatment outcomes. The clinical data team, operating strictly within the guidelines of HIPAA, is able to undertake new data retrieval projects for clients in order to identify samples that may be suitable for a project that has highly specific requirements unmet by the data obtained at the time of sample collection.

Related source products: RNA, DNA, protein and tissue sections are generated from a meticulously maintained, highly annotated tissue collection. Prior to RNA/DNA/protein extraction, the histology laboratory prepares an H&E stained slide from each individual tissue sample. The H&E slide is then reviewed by a pathologist to determine an accurate, sample-level diagnosis and a description of sample cellularity. Each RNA, DNA, or protein sample is supplied with an abstracted pathology report, digital H&E images captured at 4x and 20x magnification and a tabular presentation of additional patient data.

With the high quality human tissue biorepository from Cytomyx and scientific expertise from OriGene, we are creating new cost-effective tools that accelerate the process for discovering and validation of more biomarkers. Please contact us at 1-888-267-4436 (outside the US, call 301-340-3188) or write to us at techsupport@origene.com for more information on the TissueScan product line.

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Recent TissueScan Citations:

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Estrogen receptor {beta}1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells., O Treeck, G Pfeiler,



D Mitter, C Latrich, G Piendl, and O Ortmann, [J Endocrinol. 2007 Jun;193\(3\):421-33](#)

Appendix

TissueScan compatible thermocyclers :

TissueScan panels are made with PCR plates from Abgene (AB-0600). According to the plate manufacturer, the plates will fit in the thermocyclers listed below. If your thermocycler is not listed, please contact Technical Support for more information. Custom panels using other multiwell plates can be generated upon request.

Standard thermocyclers

Biometro Uno, Uno II, T1, Tgradient, TRobot

BioRad iCycler and MyCycler

Eppendorf Mastercycler Gradient and Mastercycler EP Gradient

Ericomp SingleBlock system, TwinBlock system, and Deltacycler I

ThermoHybaid PCR Express, Px2, PxE, MultiBlock System and MBS, Touchdown, Omnigene, and Omn-E

MJ Research PTC-200 DNA Engine, PTC-225 DNA Tetrad/PTC-220/221 DNA Dyad, and PTC-100 with 96 well block

MWG Primus 96 and TheQ Lifecycler

ABI GeneAmp 2700/2720, GeneAmp 9600, and GeneAmp 9700

Stratagene Robocycler

TaKaRa TP3000

Techne TC-412/512, Touchdown Gradient, Flexigene, and Genius

qPCR thermocyclers

ABI Prism 7000, 7700, 7300, 7500, and 9500 (except fast block formats)

BioRad iCycler and MyiQ

Stratagene MX4000, MX3000p, and MX3005p

You can find this information at the following links

http://www.abgene.com/Static_Pages.asp?page=25

http://www.abgene.com/Static_Pages.asp?page=26