# 行政院國家科學委員會專題研究計畫　期中進度報告

## 血管新生微列陣的構建及在人類胃癌的研究(1/2)

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# 行政院國家科學委員會補助專題研究計畫 □ 成 果 報 告 ■期中進度報告

（計畫名稱）

# 血管新生微陣列的建構及在人類胃癌的研究

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**中文摘要**

關鍵字: 去氧核糖核酸微列陣 彩色杜卜勒血管密度 胃癌

腫瘤的生長是須要血管新生才能繼續成長和造成腫瘤細胞的轉移，人類癌症的血管新生的評估最常用的方法是微細血管密度，最近我們用彩色杜卜勒超音波來偵測腸胃道癌症的血管新生，而這個方法—彩色杜卜勒血管密度，可用於活體病人使用，可重覆偵測，且不具侵犯性，而且超音波是容易獲得的檢查儀器，這是微細血管密度無法做到的。我們利用這個方法評估大腸癌病人發現彩色杜卜勒血管密度可預測大腸癌病人的遠處轉移和存活。

在胃癌病人方面，彩色杜卜勒血管密度是一個獨立的預後因子，更有趣的是當彩色杜卜勒血管密度大於 11%，可預測第三期胃癌病人有很差的預後，且術後皆於兩年內死亡，但是小於 11%的病人，其三年存活率卻高於 60%。所以彩色杜卜勒血管密度可挑選出第三期胃癌病人中，真正的高危險病人，這對臨床上胃癌病人的治療是非常重要，可以挑險選出應該接受進一步治療的病人 但是彩色杜卜勒超音波對胃癌的可偵測率不高，僅約 39%，且無法偵測早期胃癌，因此無法適用所有胃癌病人。

目前為止有關血管新生的研究中發現腫瘤的血管新生，其實是由很多不同種類的細胞互相作用造成的，其中包括腫瘤細胞本身，間質細胞、免疫細胞、血管內皮細胞等，而這些細胞的互動則須靠很多調節因子的相互作用，與血管新生相關的至少包括(1)血管促進因子(proangiogenic factors)，如血管內皮生長因子(VEGF)、胎盤生長因子(PlGF)等。(2)血管抑制因子(antiangiogenic factors)，如 angiostatin，endostatin 和 thrombospondin 等。(3)腫瘤基因(oncogenes)，如 ras，myc，src，eIF4 等。(4)腫瘤抑制基因(tumor suppressor genes)，如 $P^{53}$(5)發炎相關基因(inflammation-related genes)，如環氧化 (COX)和氧化氮生成 (NOS)等。(6)蛋白質分解 (protease)基因，如 MMP。(7)黏附基因(adhesive molecules)，如 selectin 家族，integrin 家族等。(8)Interleukin 家族，如 IL-1，IL-6 和 IL-8 等。(9)CD 家族，如 CD31，34 等。(10) 腫瘤內皮細胞特有基因。由於單一分子(molecule)僅是這個複雜網路的一員，很少單一分子可被利用當作選擇高危險病人的指標，尤其是針對單一病人少有選擇性。

我們在臨床上胃癌的 COX-1，COX-2，iNOS，VEGF，PLGF 和 eIF4E 等相關血管促進因子的研究中發現 COX-2，iNOS，PLGF 和微細血管密度有顯著相關，但僅 iNOS 是一預後因子，但它卻無法像彩色杜卜勒血管密度(CDVI)，對單一病人有很好的選擇性，即 iNOS 可預測某一人群人的整體存活，但 CDVI 卻有更強的能力去預測單一病人的存活，為何 CDVI 有此能力，我們並不清礎，我們發現 CDVI 與上述 6 種血管新生相關因子並無顯著相關，這可能意謂著 CDVI 是由眾多的血管新生相關基因一起作用而產生的結果，並不是少數的基因就可決定，因此，若分析大量血管新生相關基因的表現特徵或尋找真正重點基因 (hot spot genes) 或許可提供這個問題的答案。因此我們期望可以建構一血管新生相關基因微陣列，以提供一準確且可廣泛利用的方法去偵測胃癌的基因表現。

在本年度的計畫中我們已完成血管新生微列陣(angiochip)的構建。我們收集了前述的十大類與血管新生相關的基因，和一部份未知的內皮細胞的特殊基因，再加上之前我們利用 9600 個基因的微列陣方法，比較 CDVI 高低不同的胃癌的基因表現而篩選出的重要基因，總共約 350 個與血管新生有重要關係的基因。在本院的第三共同研究室（包括微列陣專門人員，相關技術和儀器設備）進行這個以血管新生相關基因所構建的血管新生微列陣(angiochip)。同時並以兩組胃癌病人的組織抽取 RNA 製備 cDNA probe 進行此血管新生微列陣的測試，並找出此血管新生微列陣最適之偵測條件，確保下一年度計劃能順利進行。

**ABSTRACT**

Angiogenesis is a very complex phenomenon and essential for the growth of solid tumors measuring more than a few millimeters.[1] It permits rapid tumor growth and potential presence of tumor metastasis. At present the most widely used method to assess angiogenesis in human malignancies is the quantification of microvessel density (MVD) of primary tumors using specific markers for endothelial cells including factor VIII-related antigen, CD31 and CD34. Recently we have developed a new method, color Doppler vascularity index (CDVI), to evaluate tumor angiogenesis. This is a non-invasive, in vivo, easily accessible method that can be performed repeatedly in the patients preoperatively. Using CDVI, we can predict distant metastasis and survival in colon cancer patients. In gastric cancer patients, CDVI were independent prognostic factors. There was an intriguing finding that the stage III gastric cancer patients with the CDVI > 11% could not survive beyond 2 years after curative resection, however, 3-yaer survival rate of those with CDVI > 11% was greater 60%. Therefore, CDVI can select high risky patients with gastric cancer, and this is very important for gastric cancer diagnosis and treatment. The major technical limitation of CDVI is low detectability rate. Only 39% of gastric cancer can be visualized in abdominal ultrasound examination. We can't apply this method to evaluate all gastric cancer patients.

Tumor angiogenesis is controlled by different cell types, including cancer cell itself, stromal cells, immune cells, and endothelial cells in the tumor tissue. The interaction of these cells is mediated by many regulatory factors such as (1) angiogenic factors: vascular endothelial growth factor(VEGF), placenta growth factor(PlGF), and et al. (2) antiangiogenic factors: angiostatin, endostatin, thrombospondin, and et al. (3) oncogenes: ras, myc, src, eIF4E, and et al. (4) tumor suppressor genes: p53. (5) inflammation related genes: cyclooxygenase-1(COX-1, -2(COX-2), inducible nitric oxide synthase(iNOS), and et al. (6) protease genes: matrix metalloprotease family and et al. (7) adhesive molecules: selectin family, integrin family, and et al. (8) interleukin: IL-1, IL-6, IL-8, and et al. (9) CD family: CD31, CD34, CD44, et al. (10)

tumor specific endothelial antigens: known and unknown genes.

In our previous preliminary study on clinicopathologic correlation of COX-1, COX-2, iNOS, VEGF, PlGF, and eIF4E with angiogenesis and clinical outcome in human gastric cancer, iNOS expression was significantly correlated with microvessel density and patients' survival. INOS is an important gene in angiogenesis and its expression is significantly correlated with gastric cancer patients' survival, but it is not as specific as CDVI to single patient. So we can predict a flock of patients' survival by INOS. However it is better to predict a patient's survival by CDVI than INOS. We don't know why CDVI has such ability. But we found CDVI wasn't significantly correlated with above six angiogenesis factors. This means that CDVI is determined by a lot of angiogenesis-related genes not just a few genes. Therefore, from analysis the feature of the expression of large angiogenesis-related genes and finding out hot spot genes for angiogenesis may provide the answer of this question. So we would like to develop an angiogenesis related microarray to provide an unbiased and broad approach to detecting gene expression profile of gastric cancer.

In 1st year study, we have constructed an angiogenesis microarray (Angiochip) including 350 genes of above 10 categories, and genes that were obtained from 9600-gene microarray differential expression of high and low CDVI gastric cancer to evaluate angiogenesis of gastric cancer. We optimized conditions of RNA extraction from human gastric cancer tissues and prepared cDNA probes from two pairs of gastric cancer tissue and corresponding adjacent noncancerous gastric tissue RNA to test of this newly developed angiochip and optimized angiomicroarray testing conditions. This will make sure experimental progress in the next year study.

**INTRODUCTION**

Angiogenesis is a very complex phenomenon and essential for the growth of solid tumors measuring more than a few millimeters.[1] It permits rapid tumor growth and potential presence of tumor metastasis.[2,3] At present the most widely used method to assess angiogenesis in human malignancies is the quantification of microvessel density (MVD) of primary tumors using specific markers for endothelial cells including factor VIII-related antigen, CD31 and CD34.[4] Pathologic angiogenesis is a hallmark of malignant diseases. Concentrated efforts in this area of research are resulting in the discovery of a growing number of pro- and anti-angiogenic molecules and other angiogenesis related molecules, some of which are already in clinical trials. The complex interactions among these molecules and how they affect the vascular structure and function in different environments are now beginning to be elucidated. This integrated understanding is leading to the development of a number of approachs to treat cancer. Therefore, it is important appropriate to study angiogenesis by using

DNA microarray, which can measure hundreds to thousands of genes simultaneously on nylon membrane.[5] This technology is expected to enable development of a more precise cancer classification system, using comprehensive profiles of the molecular alterations in tumors to identify distinct subset of patients.[6-8] It is believed recognizing exquisitely detailed "tumor signatures" and learning how they correlate with key clinical criteria will revolutionize cancer diagnosis and drug development.[9] Therefore, we would like to develop an angiogenesis related microarray to provide an unbiased and broad approach to detecting gene expression profile of gastric cancer.

Recently we have developed a new method, color Doppler vascularity index (CDVI), to evaluate tumor angiogenesis. This is a non-invasive, in vivo, easily accessible method that can be performed repeatedly in the patients preoperatively. Using CDVI, we can predict distant metastasis and survival in colon cancer patients.[10] There was an intriguing finding that the stage III gastric cancer patients with CDVI > 11% couldn't survive beyond 2 years after curative resection, however, 3-yaer survival rate of those with CDVI > 11% was greater 60%.[11] Therefore, CDVI can select high risky patients with gastric cancer, and this is very important for gastric cancer diagnosis and treatment. This is why we try to construct this angiomicroarray that includes known angiogenesis-related genes and genes selected from differential expression of high and low CDVI gastric cancer using 9600-gene microarray. Because the major technical limitation of CDVI is low detectability rate, CDVI can not be applied to all gastric cancer patients. If microarray pattern can be identified, it is very useful in diagnosis and treatment of gastric cancer. Microarray technique combined with CDVI may provide the unbiased, large-scale information in the reseach of angiogenesis.

In our previous preliminary study on clinicopathologic correlation of COX-1, COX-2, iNOS, VEGF, PlGF, and eIF4E with angiogenesis and clinical outcome in human gastric cancer, iNOS expression was significantly correlated with microvessel density and patients' survival. INOS is the enzyme that catalyzes the high output pathway of production of NO from L-arginine. the role of NO and iNOS in human gastric cancer is more uncertain. It is important to go back to laboratory and study whether iNOS is involved in angiogenesis and whether iNOS inhibitor or constitutively expressed iNOS in gastric cancer cells will change the expression pattern of angiomicroarray.

iNOS is an important gene in angiogenesis and its expression is significantly correlated with gastric cancer patients' survival. Therefore, it is interesting to find out hot spot genes for angiogenesis. We would like to use angiomicroarray constructed in this protocol to test 60 gastric cancer tissue specimens and try to find out common, significant expression of certain genes in our microarray, and prove it.

## MATERIALS AND METHODS

**Construction of angiogenesis microarray (angiochip).** About 350 known and unknown genes selected from differential expression of high and low CDVI gastric cancer using 9600-gene microarray are amplified by PCR and spotted onto a membrane by an arraying robust system. Total RNA will be extracted from five pairs of high and low CDVI gastric cancer tissues, and be converted to probe cDNA. After hybridization with cDNA probes and multi-color detection procedure, the color spots on membrane are digitized and quantified by an inhouse developed image analysis software. The location and expression level of genes of interest are identified and presented by the computer program. Details are described below:

**Colony amplification.** Above gene clones were obtained from Unigene and Incyte through its distributor. The colonies we selected were inoculated in 1 ml 96 microliter plates. One microliter of bacteria culture from each well of 96-well plates was deposited in one well of V-bottomed 96-well polycarbonate microtiter plate and amplified with polymerase chain reaction using primer specific to each individual library construct. DNA sequencing was used to verify these PCR-amplified cDNA inserts. Three plant genes, lhcI, rbcL, rca, and one human GAPDH gene were served as internal controls for gene expression quantification.

The PCR amplification products were concentrated by evaporation at 95°C to obtain a concentration of 2-3μg/μl before they were spotted onto nylon membranes.

An arraying machine fitted with steel pins spotted the double-stranded cDNA fragments onto a positively charged nylon membrane (Boehringer Mannheim, Mannheim, Germany). The arraying machine was a personal computer-controlled XYZ translation system (PM500, Newport, Inc., Fountain Valley, CA) outfitted with Teflon-coated (Teflon-AF, DuPont, Wilmington, DE) tool steel pins for sample delivery. Samples were held at the tip of the pins for delivery by the action of surface tension, and the delivery volume was governed by the diameter of the tips. Depending on the array density needed and the number of elements in an array, arraying tools consisting of 1 to 24 pins were used. The time required for printing arrays also varied by the number of pins used and the number of arrays to print in a batch. For instance, it took 5 h to print an array consisting of 9600 elements by using a 6-pin tool, but the time required per array is much shorter when multiple arrays are printed in a batch. The arraying system is presently capable of placing 100-μm-diameter spots on nylon membranes with spots spaced 150 μm apart. Each 100-μm-diameter spot is estimated to have around 10 ng of DNA. With this capacity, approximately 85,000 gene transcripts can be placed on a piece of nylon membrane measuring 35 by 55 mm by using a 24-pin (4 × 6 pins) arraying tool.

**cDNA probe preparation and membrane hybridization.** To com-ply with the

terms commonly used in hybridization technologies, "probes" refers to the DNA molecules in the hybridization solution while "targets" refers to the DNA molecules immobilized on the membrane surface. To prepare hybridization probes, RNAs were extracted from gastric cancer tissue by using Trizol reagent (Gibco Life technologies). Thirty microgram of each total RNA sample was labeled with biotin, in single-color detection. The labeling reactions were performed during reverse transcription in the presence of 50 μM random primers and denatured at 70℃ for 10 min and then at 0℃ for 5min. Subsequently the labeling reactions were added in RT mixture (25 mM each dATP, dCTP and dGTP; 2 mM dTTP; 50 nmol-16-biotin-dUTP or 40μM digoxigenin-11-dUTP (Boehringer Mannheim);0.1 M DTT; 0.5 μl RNasin (GIBCO BRL, Gaithersburg, MD)) and incubated at 42℃ for 3 min and then 200 units of Super Script reverse transcriptase (invitrogene) are added in a 50-μl solution.

The reaction mixture was incubated at 25℃ for 10 min, 42℃ for 90 min and was stopped by heating of the reaction mixture to 95℃ for 5 min . The RNA was degraded by addition of 5.5μl of 3 M NaOH followed by a 30-min incubation at 50℃. The labeled samples were neutralized by addition of 5.5μl of 3 M acetic acid and then precipitated by addition of 5μl of 7.5 M ammonium acetate, 20μg of linear polyacrylamide as carrier, 380μl of absolute alcohol, and water to make a total of 550μl.

The membrane carrying the double-stranded cDNA targets was pre-hybridized in 1ml hybridization buffer (5× SSC, 0.1% N-lauroylsar-cosine, 0.1% SDS, 1% BM blocking reagent made by Boehringer Mann-heim, and 50μg/ml salmon sperm DNA) at 63℃ for 1h before hybridization was carried out. In single-color detection, the cDNA probe was in 13μl hybridization buffer containing 200μg/ml d(A)10 and 300-400μg/ml human COT-1 DNA to prevent nonspecific binding and were hybridized to the cDNA fragments on the membrane by Southern hybridization procedure. The 13-μl reaction mixture was sealed and then incubated at 63℃ for 17 h. The membrane was then washed with 2× SCC containing 0.1% SDS for 5 min at room temperature followed by three washes with 0.1× SCC containing 0.1% SDS at 63℃ for 15 min each.

**Colorimetry detection and image analysis.** After hybridization, the membrane was blocked by 1ml of 1% blocking regent (Boehringer Mannheim) containing 2%dextran sulfate at room temperature for 1 h. The sensitive single-color detection studies employed the streptavidin and alkaline phosphatase-conjugated antibody. The membrane was incubated with a 1-ml mixture containing 2500 * diluted streptavidin and alkaline phosphatase-conjugated antibody, 4% polyethylene glycol 8000 (Sigma), and 0.3% BSA in 1 *PBST buffer for 1 h. The membrane was stained with 2 ml NBT/BCIP substrate for alkaline phosphatase rection. The color development

reactions were then stopped by 1 * PBS containing 20 mM EDTA.

After color development the cDNA molecules labeled with biotin yielded a blue chromogen. To determine the results from arrays of different densities, we performed image digitization using a drum scanner (ScanView, Foster City, CA) which provides 3000 dpi optical resolution andis suitable for larger arrays such as arrays of 9600 elements.

For quantitative measurements using single-color detection, the color yielded by the enzymatic reatction was converted to gray levels. In dual-color mode, the colors of the spots were separated into the artist's subtractive primaries (cyan, magenta, and yellow) to semiquantitatively identify differentially expressed genes by a computer program written in-house. The computer program was written in C+ + on the Windows 95 operating system. The program includes image processing algorithms such as image deskewing, object recognition, color separation, and color filtering. The program isolates differentially expressed genes by measuring the integrated density of the three primary color components(cyan, magenta, and yellow) of each spot, performing regression analysis of the integrated density data, and locating the statsti-cal outliers as differentially expressed genes. The computer program can be made available upon request.

## RESULTS

**Construction of an angiogenesis-related mini-microarray (angiochip).** A high performance cDNA microarray with colorimetry detection system will be developed to simultaneously monitor the expression of many genes in a microarray format on nylon membrane. Angiogenesis-related genes are expressed by this system. We select about 350 genes to construct theangiogenesis-related mini-microarray (angiochip). The 350 clones, including (1) proangiogenic factors: placenta growth factor(PlGF), and et al. (2) antiangiogenic factors: angiostatin, endostatin, thrombospondin, and et al. (3) oncogenes: ras, myc, src, eIF4E, and et al. (4) tumor suppressor genes: p53. (5) inflammation-related genes: cyclooxygenase-1(COX-1, -2(COX-2), inducible nitric oxide synthase(iNOS), and et al. (6)protease genes: matrix metalloprotease family and et al. (7) adhesive molecules: selectin family, integrin family, and et al. (8) interleukin: IL-1, IL-6, IL-8, and et al. (9) CD family: CD31, CD34, CD44, et al. (10) tumor specific endothelial antigens.(11) genes selected from differential expression of high and low CDVI gastric cancer using 9600gene microarray (Table 1). The image of original (unhybridized) angioachip is shown as Figure 1.

**Preliminary test of this angiochip with gastric cancer tissue.** Two pairs of the advanced gastric cancer (T1-T2) and corresponding adjacent noncancerous gastric tissue (N1-N2) were obtained from patients who underwent gastrectomy at NTUH.

Tissues were homogenized and lysed directly in Trizol reagent (Gaibco Life technologies). Total RNA was extracted according to the manufacturer's instruction (Figure 2). To prepare hybridization probes, 30 microgram of each RNA sample was labled with biotin in single color detection. The labeling reaction was performed by RT-PCR. The cDNA probe was prepared and hybridized to the cDNA fragments on the membrane by Southern hybridization procedure. After hybridization, the membrane was washed and blocked. To detect the spots on the membrane, Streptavidin- alkaline phosphatase conjugated antibody was employed. The membrane subsequently stained with NBT/BCIP substrate for alkaline phosphatase rection. The color development reactions were then stopped After color development the cDNA molecules labeled with biotin yielded a-purple dark chromogen. To determine the results from arrays of different densities, we performed image digitization using imaging devices: a flatbed scanner (Scanjet 4c, Hewlett-Packard, Palo Alto, CA) which provides 3000 dpi optical resolution and is-suitable for angiochip (Figure 3).

**DISCUSSION**

RNA quality determines the results of microarrays. In the beginning, we got a lot of trouble in extracting total RNA from gastric cancer tissue. RNA quality is checked by agarose gel electrophoresis. From the gel, we often saw the bands are smearing that means RNA is degraded (Figure 2A). After modifying the protocol of RNA extraction, we finally saw two single, discrete bands (28S and 18S RNA) in the gel (Figure 2B). That means such RNA quality is good enough to carry out following procedure. The key point of getting good quality total RNA is always maintaining tissues in liquid nitrogen when grinding solid tissues into powder and keeping RNA in ice. After solving the trouble of extraction total RNA from tissue, we optimized angiochip hybridization conditions. Following are several points should be paid attention to obtain good results of arrays.

1. Good RNA quality.
2. Accuracy RT-PCR procedure.
3. RT-PCR product should be dissolved carefully.
4. Prevent to leak when seal the cDNA probe to hybridize with array.
5. Buffers should be prepared freshly.
6. Because NBT/BCID substrate is easy to degrade, be sure to keep it well.

Therefore, we have constructed a new angiochip as we promised in the first year study and we have optimized RNA extraction from gastric cancer tissue and hybridization condition of angiochip. This will keep next year study going smoothly.

Table 1. Gene list of angiochip.

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| HAT4 | — | — | — | 1 | A1 | 1 |
| CD3G antigen, gamma polypeptide (TiT3 complex) | pT7T3-Pac | 1038 | V | 1 | B1 | 25 |
| endothelin 3 | pT7T3-Pac | 1651 | V | 1 | C1 | 49 |
| CD38 antigen (p45) | pT7T3-Pac | 853 | V | 1 | D1 | 73 |
| protein disulfide isomerase-related protein | pT7T3-Pac | 1038 | V | 1 | E1 | 97 |
| CD48 antigen (B-cell membrane protein) | pT7T3-Pac | 1446 | V | 1 | F1 | 121 |
| cyclin-dependent kinase 7 | pT7T3-Pac | 1202 | V | 1 | G1 | 145 |
| RCA | — | — | — | 1 | H1 | 169 |
| platelet/endothelial cell adhesion molecule (CD31 antigen) | pT7T3-Pac | — | NV | 1 | A2 | 2 |
| Prolactin | pT7T3-Pac | 0.9 kb | NV | 1 | B2 | 26 |
| MMP11,stromelysin 3 | pT7T3-Pac | 2.3 kb | NV | 1 | C2 | 50 |
| mouse double minute 2, human homolog of; p53-binding protein | pT7T3-Pac | 837 | V | 1 | D2 | 74 |
| lymphatic vessel endothelial hyaluronan receptor 1 | pT7T3-Pac | 2.1 kb | NV | 1 | E2 | 98 |
| CCAAT/enhancer binding protein (C/EBP), beta | pT7T3-Pac | 679 | V | 1 | F2 | 122 |
| retinoid X receptor, beta | pT7T3-Pac | 2.4 kb | NV | 1 | G2 | 146 |
| cyclin-dependent kinase 9 (CDC2-related kinase) | pT7T3-Pac | 2000+ | V | 1 | H2 | 170 |
| selectin E (endothelial adhesion molecule 1) | pT7T3-Pac | 1.8 kb | NV | 1 | A3 | 3 |
| 2252 | pT7T3-Pac | 1500 | V | 1 | B3 | 27 |
| small inducible cytokine subfamily A (Cys-Cys), member 14 | pT7T3-Pac | 1.7 kb | NV | 1 | C3 | 51 |
| neuropilin 2 | pT7T3-Pac | 1.9 kb | NV | 1 | D3 | 75 |
| chemokine (C-C motif) receptor 2 | pT7T3-Pac | 1202 | V | 1 | E3 | 99 |
| transforming growth factor, beta receptor III (betaglycan, 300kD) | pT7T3-Pac | 2000+ | V | 1 | F3 | 123 |
| nidogen 2 | pT7T3-Pac | 2.0 kb | NV | 1 | G3 | 147 |
| nidogen 2 | pT7T3-Pac | 2.0 kb | NV | 1 | H3 | 171 |
| von Hippel-Lindau syndrome | pT7T3-Pac | 1084 | V | 1 | A4 | 4 |
| EGFR | pT7T3-Pac | 1.4 kb | NV | 1 | B4 | 28 |
| breast cancer 1, early onset | pT7T3-Pac | 1000 | V | 1 | C4 | 52 |
| CD36 antigen (collagen type I receptor, thrombospondin receptor) | pT7T3-Pac | 1393 | V | 1 | D4 | 76 |
| plasminogen activator inhibitor, type I | pT7T3-Pac | — | NV | 1 | E4 | 100 |
| neuropilin 2 | pT7T3-Pac | 1.8 kb | NV | 1 | F4 | 124 |
| chemokine (C-C motif) receptor 6 | pT7T3-Pac | 1328 | V | 1 | G4 | 148 |
| eukaryotic translation initiation factor 2B, subunit 1 (alpha, 26kD) | pT7T3-Pac | 1654 | V | 1 | H4 | 172 |
| eukaryotic translation initiation factor 2B, subunit 1 (alpha, 26kD) | pT7T3-Pac | 1654 | V | 1 | A5 | 5 |

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| eukaryotic translation initiation factor 2B, subunit 1 (alpha, 26kD) | pT7T3-Pac | 1654 | V | 1 | B5 | 29 |
| MCF.2 cell line derived transforming sequence | pT7T3-Pac | 1629 | V | 1 | C5 | 53 |
| v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog | pT7T3-Pac | 1184 | V | 1 | D5 | 77 |
| spleen focus forming virus (SFFV) proviral integration oncogene spi1 | pT7T3-Pac | 1817 | V | 1 | E5 | 101 |
| chemokine (C-X3-C) receptor 1 | pT7T3-Pac | 961 | V | 1 | F5 | 125 |
| CD22 antigen | pT7T3-Pac | 1651 | V | 1 | G5 | 149 |
| interleukin 15 | pT7T3-Pac | 1169 | V | 1 | H5 | 173 |
| MAX protein | pT7T3-Pac | 458 | V | 1 | A6 | 6 |
| pim-1 oncogene | pT7T3-Pac | 862 | V | 1 | B6 | 30 |
| eukaryotic translation initiation factor 4 gamma, 3 | pT7T3-Pac | 1225 | V | 1 | C6 | 54 |
| basic transcription element binding protein 1 | pT7T3-Pac | 2000+ | V | 1 | D6 | 78 |
| eukaryotic translation initiation factor 4A, isoform 2 | pT7T3-Pac | 725 | V | 1 | E6 | 102 |
| glycogenin 2 | pT7T3-Pac | 2.1 kb | NV | 1 | F6 | 126 |
| melanoma adhesion molecule,CD146 | pT7T3-Pac | 2.2 kb | NV | 1 | G6 | 150 |
| melanoma adhesion molecule,CD146 | pT7T3-Pac | 2.2 kb | NV | 1 | H6 | 174 |
| Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains | pT7T3-Pac | 1.7 kb | NV | 1 | A7 | 7 |
| Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | pT7T3-Pac | 1.4 kb | NV | 1 | B7 | 31 |
| collagen, type I, alpha 1 | pT7T3-Pac | 3.0 kb | NV | 1 | C7 | 55 |
| interleukin 6 (interferon, beta 2) | pT7T3-Pac | 1500 | V | 1 | D7 | 79 |
| phosphatase and tensin homolog (mutated in multiple advanced cancers 1) | pT7T3-Pac | 956 | V | 1 | E7 | 103 |
| fibroblast growth factor 2 (basic) | pT7T3-Pac | 1253 | V | 1 | F7 | 127 |
| hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) | pT7T3-Pac | — | V | 1 | G7 | 151 |
| Fas-activated serine/threonine kinase | pT7T3-Pac | 924 | V | 1 | H7 | 175 |
| angiogenin | pT7T3-Pac | 1.2 kb | NV | 1 | A8 | 8 |
| fibroblast growth factor 1 (acidic) | pT7T3-Pac | 2000+ | V | 1 | B8 | 32 |
| TAT-INTERACTIVE PROTEIN, 72-KD | pT7T3-Pac | — | NV | 1 | C8 | 56 |
| CD3D antigen, delta polypeptide (TiT3 complex) | pT7T3-Pac | 875 | V | 1 | D8 | 80 |
| CD1C antigen, c polypeptide | pT7T3-Pac | 1500 | V | 1 | E8 | 104 |
| IGF-II mRNA-binding protein 3 | pT7T3-Pac | 1248 | V | 1 | F8 | 128 |
| CD5 antigen-like (scavenger receptor cysteine rich family) | pT7T3-Pac | 961 | V | 1 | G8 | 152 |

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| angiogenin, ribonuclease, RNase A family, 5 | pT7T3-Pac | 956 | V | 1 | H8 | 176 |
| prostaglandin I2 (prostacyclin) synthase | pT7T3-Pac | 1.0 kb | NV | 1 | A9 | 9 |
| caveolin-2 | pT7T3-Pac | 1.4 kb | NV | 1 | B9 | 33 |
| chemokine (C-C motif) receptor 1 | pT7T3-Pac | 858 | V | 1 | C9 | 57 |
| IL14 | pT7T3-Pac | 1.7 kb | NV | 1 | D9 | 81 |
| interleukin 1, beta | pT7T3-Pac | 1549 | V | 1 | E9 | 105 |
| IL19 | pT7T3-Pac | 0.7 kb | NV | 1 | F9 | 129 |
| — | — | — | — | 1 | G9 | 153 |
| — | — | — | — | 1 | H9 | 177 |
| eukaryotic translation initiation factor 4E | pT7T3-Pac | 2000+ | V | 1 | A10 | 10 |
| v-rel avian reticuloendotheliosis viral oncogene homolog B | pT7T3-Pac | 1500 | V | 1 | B10 | 34 |
| CD44 antigen (homing function and Indian blood group system) | pT7T3-Pac | 1563 | V | 1 | C10 | 58 |
| CD8 antigen, beta polypeptide 1 (p37) | pT7T3-Pac | 1697 | V | 1 | D10 | 82 |
| CD9 antigen (p24) | pT7T3-Pac | 965 | V | 1 | E10 | 106 |
| Lymphocyte-specific protein tyrosine kinase | pT7T3-Pac | 0.8 kb | NV | 1 | F10 | 130 |
| cyclin-dependent kinase 5 | pT7T3-Pac | 1225 | V | 1 | G10 | 154 |
| IGF-II mRNA-binding protein 2 | pT7T3-Pac | 797 | V | 1 | H10 | 178 |
| eukaryotic translation initiation factor 2 alpha kinase 3 | pT7T3-Pac | 1328 | V | 1 | A11 | 11 |
| retinoic acid receptor, beta | pT7T3-Pac | 2000+ | V | 1 | B11 | 35 |
| breakpoint cluster region | pT7T3-Pac | 636 | V | 1 | C11 | 59 |
| ephrin-B1 | pT7T3-Pac | 1184 | V | 1 | D11 | 83 |
| cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 1 | pT7T3-Pac | 961 | V | 1 | E11 | 107 |
| neurofibromin 2 (bilateral acoustic neuroma) | pT7T3-Pac | 457 | V | 1 | F11 | 131 |
| cyclin E2 | pT7T3-Pac | 1.0 kb | NV | 1 | G11 | 155 |
| CD4 antigen (p55) | pT7T3-Pac | 603 | V | 1 | H11 | 179 |
| RBCL | — | — | — | 1 | A12 | 12 |
| CD34 antigen | pT7T3-Pac | 1038 | V | 1 | B12 | 36 |
| CD8 antigen, alpha polypeptide (p32) | pT7T3-Pac | 1248 | V | 1 | C12 | 60 |
| catechol-O-methyltransferase | pT7T3-Pac | 1612 | V | 1 | D12 | 84 |
| v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 | pT7T3-Pac | 2000+ | V | 1 | E12 | 108 |
| proliferating cell nuclear antigen | pT7T3-Pac | 1500 | V | 1 | F12 | 132 |
| CD1D antigen, d polypeptide | pT7T3-Pac | 915 | V | 1 | G12 | 156 |
| ASAR | — | — | — | 1 | H12 | 180 |
| ATPS | — | — | — | 2 | A1 | 193 |

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| ornithine decarboxylase 1 | pT7T3-Pac | 1202 | V | 2 | B1 | 217 |
| BCL2-like 2 | pT7T3-Pac | — | NV | 2 | C1 | 241 |
| eukaryotic translation initiation factor 4 gamma, 1 | pT7T3-Pac | 617 | V | 2 | D1 | 265 |
| eukaryotic translation initiation factor 3, subunit 5 (epsilon,47kD) | pT7T3-Pac | 674 | V | 2 | E1 | 289 |
| adenomatosis polyposis coli | pT7T3-Pac | — | NV | 2 | F1 | 313 |
| HAT22 | pT7T3-Pac | 1768 | V | 2 | G1 | 337 |
| plasminogen activator, tissue | pT7T3-Pac | — | NV | 2 | B2 | 218 |
| leukotriene A4 hydrolase | pT7T3-Pac | — | NV | 2 | C2 | 242 |
| PDGFA | pT7T3-Pac | 2000 | V | 2 | D2 | 266 |
| small inducible cytokine subfamily B (Cys-X-Cys), member 11 | pT7T3-Pac | 1.3 kb | NV | 2 | E2 | 290 |
| Retinoblastoma 1 (including osteosarcoma) | pT7T3-Pac | 2.3 kb | NV | 2 | F2 | 314 |
| p27 | pT7T3-Pac | 2.2 kb | NV | 2 | G2 | 338 |
| IL2 | pT7T3-Pac | 2.0 kb | NV | 2 | H2 | 362 |
| Non-metastatic cells 1, protein (NM23A) expressed in | pT7T3-Pac | 1.0 kb | NV | 2 | A3 | 195 |
| nitric oxide synthase 2A (inducible, hepatocytes) | pT7T3-Pac | 1.7 kb | NV | 2 | B3 | 219 |
| bone morphogenetic protein 1 | pT7T3-Pac | 1000 | V | 2 | C3 | 243 |
| interleukin 6 (interferon, beta 2) | pT7T3-Pac | 1.9 kb | NV | 2 | D3 | 267 |
| tumor necrosis factor (TNF superfamily, member 2) | pT7T3-Pac | 1.2 kb | NV | 2 | E3 | 291 |
| signaling lymphocytic activation molecule | pT7T3-Pac | 1.6 kb | NV | 2 | F3 | 315 |
| cyclin-dependent kinase (CDC2-like) 10 | pT7T3-Pac | 2.0 kb | NV | 2 | G3 | 339 |
| nidogen (enactin) | pT7T3-Pac | 1094 | V | 2 | H3 | 363 |
| nitric oxide synthase 3 (endothelial cell) | pT7T3-Pac | 1.9 kb | NV | 2 | A4 | 196 |
| phospholipase C, beta 4 | pT7T3-Pac | 1343 | V | 2 | B4 | 220 |
| eukaryotic translation initiation factor 4B | pT7T3-Pac | 1.0 kb | NV | 2 | C4 | 244 |
| eukaryotic translation initiation factor 3, subunit 10 | pT7T3-Pac | 1084 | V | 2 | D4 | 268 |
| lyphotoxin alpha (TNF superfamily, member 1) | pT7T3-Pac | 1817 | V | 2 | E4 | 292 |
| Colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog | pT7T3-Pac | 1.1 kb | NV | 2 | F4 | 316 |
| acidic FGF | pT7T3-Pac | 2.0 kb | NV | 2 | G4 | 340 |
| CD3E antigen, epsilon polypeptide (TiT3 complex) | pT7T3-Pac | 2.9 kb | NV | 2 | H4 | 364 |
| deleted in colorectal carcinoma | pT7T3-Pac | 654 | V | 2 | A5 | 197 |
| CD2 antigen (p50), sheep red blood cell receptor | pT7T3-Pac | 899 | V | 2 | B5 | 221 |
| selectin E (endothelial adhesion molecule 1) | pT7T3-Pac | 617 | V | 2 | C5 | 245 |
| Membrane metallo-endopeptidase | pT7T3-Pac | 0.8 kb | NV | 2 | D5 | 269 |
| mucosal vascular addressin cell adhesion molecule 1 | pT7T3-Pac | 1.9 kb | NV | 2 | E5 | 293 |

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| CD3Z antigen, zeta polypeptide (TiT3 complex) | pT7T3-Pac | 0.7 kb | NV | 2 | F5 | 317 |
| epidermal growth factor (beta-urogastrone) | pT7T3-Pac | 1000 | V | 2 | G5 | 341 |
| cytochrome P450, subfamily IID | pT7T3-Pac | 1000 | V | 2 | H5 | 365 |
| LIM domain binding 2 | pT7T3-Pac | 1446 | V | 2 | A6 | 198 |
| eukaryotic translation initiation factor 2B, subunit 5 | pT7T3-Pac | 2.5 kb | NV | 2 | B6 | 222 |
| Tissue inhibitor of metalloproteinase 1 | pT7T3-Pac | 965 | V | 2 | C6 | 246 |
| Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) | pT7T3-Pac | 1.4 kb | NV | 2 | D6 | 270 |
| TGF-B2 | pT7T3-Pac | 1.9 kb | NV | 2 | E6 | 294 |
| TIMP4 | pT7T3-Pac | 1.2 kb | NV | 2 | F6 | 318 |
| interleukin 11 | pT7T3-Pac | 1.3 kb | NV | 2 | G6 | 342 |
| endothelin 2 | pT7T3-Pac | 968 | V | 2 | H6 | 366 |
| caveolin-1 | pT7T3-Pac | — | V | 2 | A7 | 199 |
| interleukin 18 (interferon-gamma-inducing factor) | pT7T3-Pac | 1.9 kb | NV | 2 | B7 | 223 |
| pro-platelet basic protein (includes platelet basic protein, beta-thromboglobulin, connective tissue-activating peptide III, neutrophil-activating peptide-2) | pT7T3-Pac | 931 | V | 2 | C7 | 247 |
| Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | pT7T3-Pac | 1.0 kb | NV | 2 | D7 | 271 |
| Platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit (29kD) | pT7T3-Pac | 1.5 kb | NV | 2 | E7 | 295 |
| interleukin 10 | pT7T3-Pac | 1.1 kb | NV | 2 | F7 | 319 |
| cyclin-dependent kinase 5, regulatory subunit 2 (p39) | pT7T3-Pac | — | V | 2 | G7 | 343 |
| prostaglandin D2 synthase, hematopoietic | pT7T3-Pac | 544 | V | 2 | H7 | 367 |
| interleukin 13 | pT7T3-Pac | 0.9 kb | NV | 2 | A8 | 200 |
| insulin-like growth factor binding protein 6 | pT7T3-Pac | 1383 | V | 2 | B8 | 224 |
| CD1A antigen, a polypeptide | pT7T3-Pac | 2.0 kb | NV | 2 | C8 | 248 |
| B-cell CLL/lymphoma 2 | pT7T3-Pac | 1041 | V | 2 | D8 | 272 |
| Cyclin-dependent kinase inhibitor 1B (p27, Kip1) | pT7T3-Pac | 1.2 kb | NV | 2 | E8 | 296 |
| Werner syndrome | pT7T3-Pac | 1.6 kb | NV | 2 | F8 | 320 |
| Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog) | pT7T3-Pac | 1795 | V | 2 | G8 | 344 |
| cyclin E1 | pT7T3-Pac | 1.7 kb | NV | 2 | H8 | 368 |
| cyclin E1 | pT7T3-Pac | 0.9 kb | NV | 2 | A9 | 201 |
| serum deprivation response (phosphatidylserine-binding protein) | pT7T3-Pac | 0.9 kb | NV | 2 | B9 | 225 |

| gene name | vector | inserted length | sequence verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| chemokine (C-C motif) receptor-like 2 | pT7T3-Pac | 2.5 kb | NV | 2 | C9 | 249 |
| leukotriene C4 synthase | pT7T3-Pac | 723 | V | 2 | D9 | 273 |
| interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) | pT7T3-Pac | 961 | V | 2 | E9 | 297 |
| CD1B antigen, b polypeptide | pT7T3-Pac | — | V | 2 | F9 | 321 |
| CD6 antigen | pT7T3-Pac | 1000 | V | 2 | G9 | 345 |
| alpha V (vitronectin receptor, alpha polypeptide, antigen CD51) | pT7T3-Pac | 899 | V | 2 | H9 | 369 |
| interleukin 6 signal transducer (gp130, oncostatin M receptor) | pT7T3-Pac | 1.0 kb | NV | 2 | A10 | 202 |
| MMP14, membrane-inserted | pT7T3-Pac | 853 | V | 2 | B10 | 226 |
| MMP14, membrane-inserted | pT7T3-Pac | 1.6 kb | NV | 2 | C10 | 250 |
| IL17B | pT7T3-Pac | 0.8 kb | NV | 2 | D10 | 274 |
| matrix metalloproteinase 12 | pT7T3-Pac | 2000+ | V | 2 | E10 | 298 |
| intercellular adhesion molecule 4, Landsteiner-Wiener blood group | pT7T3-Pac | 1.1 kb | NV | 2 | F10 | 322 |
| Thrombospondin-2 | pT7T3-Pac | 1.0 kb | NV | 2 | G10 | 346 |
| interleukin 7 | pT7T3-Pac | 2.1 kb | NV | 2 | H10 | 370 |
| v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog | pT7T3-Pac | — | V | 2 | A11 | 203 |
| chemokine (C-C motif) receptor 5 | pT7T3-Pac | — | V | 2 | B11 | 227 |
| BCL2-associated X protein | pT7T3-Pac | — | V | 2 | C11 | 251 |
| neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease) | pT7T3-Pac | — | V | 2 | D11 | 275 |
| androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease) | pT7T3-Pac | — | V | 2 | E11 | 299 |
| caveolin-3 | pT7T3-Pac | — | V | 2 | F11 | 323 |
| cyclinD1 | pT7T3-Pac | 1.1 kb | NV | 2 | G11 | 347 |
| GA4 | pT7T3-Pac | 2.5 kb | NV | 2 | H11 | 371 |
| CD7 antigen (p41) | — | — | — | 2 | A12 | 204 |
| cyclin-dependent kinase 2 | pT7T3-Pac | — | V | 2 | B12 | 228 |
| small inducible cytokine subfamily A (Cys-Cys), member 14 | pT7T3-Pac | — | V | 2 | C12 | 252 |
| colony stimulating factor 2 (granulocyte-macrophage) | pT7T3-Pac | 0.6 kb | NV | 2 | D12 | 276 |
| E-selectin | pT7T3-Pac | 0.8 kb | NV | 2 | E12 | 300 |
| chemokine (C-C motif) receptor 7 | pT7T3-Pac | 0.8 kb | NV | 2 | F12 | 324 |
| LHC | pT7T3-Pac | — | V | 2 | G12 | 348 |

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|---|---|---|---|---|---|---|
| HAT4 | — | — | — | 2 | H12 | 372 |
| retinoid X receptor, alpha | — | — | — | 3 | A1 | 13 |
| Bcl-2 | pT7T3-Pac | 1.0 kb | NV | 3 | B1 | 37 |
| MMP26 | pT7T3-Pac | 1.2 kb | NV | 3 | C1 | 61 |
| tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K,neuropeptide gamma) | pT7T3-Pac | 1.1 kb | NV | 3 | D1 | 85 |
| platelet factor 4 | pT7T3-Pac | 1.1 kb | NV | 3 | E1 | 109 |
| thromboxane A synthase 1 | pT7T3-Pac | 0.7 kb | NV | 3 | F1 | 133 |
| thromboxane A synthase 1 (platelet, cytochrome P450, subfamily V) | pT7T3-Pac | 1.5 kb | NV | 3 | G1 | 157 |
| RCA | — | — | — | 3 | H1 | 181 |
| alpha V | pT7T3-Pac | 0.7 kb | NV | 3 | A2 | 14 |
| EGF | pT7T3-Pac | 1.7 kb | NV | 3 | B2 | 38 |
| Transcription factor AP-2 alpha | pT7T3-Pac | 1.9 kb | NV | 3 | C2 | 62 |
| MMP10 (stromelysin 2) | pT7T3-Pac | 1.6 kb | NV | 3 | D2 | 86 |
| Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor | pT7T3-Pac | 1.5 kb | NV | 3 | E2 | 110 |
| PIGF | pT7T3-Pac | 1.3 kb | NV | 3 | F2 | 134 |
| (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2) | pT7T3-Pac | 1.8 kb | NV | 3 | G2 | 158 |
| Dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) | pT7T3-Pac | 1.5 kb | NV | 3 | H2 | 182 |
| phosphodiesterase I - nucleotide pyrophosphatase 2 (autotaxin | pT7T3-Pac | 1.9 kb | NV | 3 | A3 | 15 |
| MutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1) | pT7T3-Pac | 2.2 kb | NV | 3 | B3 | 39 |
| hyaluronan synthase 3 | pT7T3-Pac | 1.8 kb | NV | 3 | C3 | 63 |
| KIAA1071 protein(angiostatin?) | pT7T3-Pac | 1.5 kb | NV | 3 | D3 | 87 |
| angiopoietin 1 | pT7T3-Pac | 2.3 kb | NV | 3 | E3 | 111 |
| MMP23A | pT7T3-Pac | — | V | 3 | F3 | 135 |
| tenascin R (restrictin, janusin | pT7T3-Pac | 1.4 kb | NV | 3 | G3 | 159 |
| MMP20, enamelysin | pT7T3-Pac | — | NV | 3 | H3 | 183 |
| IL17C | pT7T3-Pac | — | NV | 3 | A4 | 16 |
| N-acetyltransferase 2 (arylamine N-acetyltransferase) | pT7T3-Pac | — | NV | 3 | B4 | 40 |
| Calreticulin | — | 1.2 kb | NV | 3 | C4 | 64 |
| collagen, type XII, alpha 1 | pCMV-SPORT6 | 2.4 kb | NV | 3 | D4 | 88 |
| prostaglandin E synthase | pCMV-SPORT6 | 1.2 kb | NV | 3 | E4 | 112 |
| prostaglandin E synthase | pCMV-SPORT6 | 1.9 kb | NV | 3 | F4 | 136 |

| gene name | vector | inserted sequence length | verifided | plate | position | localization code |
|---|---|---|---|---|---|---|
| neuropilin 1 | pCMV-SPORT6 | 1.9 kb | NV | 3 | G4 | 160 |
| MMP9,gelatinase B, 92kD gelatinase, 92kD type IV collagenase) | pCMV-SPORT6 | 2.0 kb | NV | 3 | H4 | 184 |
| PECAM1 | pCMV-SPORT6 | 2.3 kb | NV | 3 | A5 | 17 |
| Flap structure-specific endonuclease 1 | pCMV-SPORT6 | 2.3 kb | NV | 3 | B5 | 41 |
| EphB3 | pCMV-SPORT6 | 2.0 kb | NV | 3 | C5 | 65 |
| Thy-1 cell surface antigen | pCMV-SPORT6 | 1.7 kb | NV | 3 | D5 | 89 |
| insulin-like growth factor binding protein 7,IGFBP7 | pCMV-SPORT6 | 1.9 kb | NV | 3 | E5 | 113 |
| TGF-alpha | pCMV-SPORT6 | 2.9 kb | NV | 3 | F5 | 137 |
| TIMP3,(Sorsby fundus dystrophy, pseudoinflammatory) | pCMV-SPORT6 | 1.9 kb | NV | 3 | G5 | 161 |
| MMP19, | pCMV-SPORT6 | 2.6 kb | NV | 3 | H5 | 185 |
| PDGFC | pCMV-SPORT6 | 2.0 kb | NV | 3 | A6 | 18 |
| monokine induced by gamma interferon | pCMV-SPORT6 | 2.1 kb | NV | 3 | B6 | 42 |
| splicing factor, arginine/serine-rich 5,TEM3 | pCMV-SPORT6 | 2.0 kb | NV | 3 | C6 | 66 |
| splicing factor, arginine/serine-rich 5,TEM3 | pCMV-SPORT6 | 2.5 kb | NV | 3 | D6 | 90 |
| cystatin S | pCMV-SPORT6 | 0.9 kb | NV | 3 | E6 | 114 |
| dickkopf (Xenopus laevis) homolog 3, DKK3 | pCMV-SPORT6 | 2.9 kb | NV | 3 | F6 | 138 |
| MMP_2 | pCMV-SPORT6 | 2.4 kb | NV | 3 | G6 | 162 |
| integrin, alpha 1 | pCMV-SPORT6 | 2.2 kb | NV | 3 | H6 | 186 |
| SPARC-like 1 (mast9, hevin) | pCMV-SPORT6 | 3.5 kb | NV | 3 | A7 | 19 |
| met proto-oncogene (hepatocyte growth factor receptor) | pCMV-SPORT6 | 2.6 kb | NV | 3 | B7 | 43 |
| thrombospondin 4 | pCMV-SPORT6 | 2.0 kb | NV | 3 | C7 | 67 |
| MCF.2 cell line derived transforming sequence-like | pCMV-SPORT6 | 2.2 kb | NV | 3 | D7 | 91 |
| secreted protein, acidic, cysteine-rich (osteonectin),SPARC | pCMV-SPORT6 | 1.5 kb | NV | 3 | E7 | 115 |
| hepatocyte growth factor (hepapoietin A; scatter factor) | pCMV-SPORT6 | 2.6 kb | NV | 3 | F7 | 139 |
| interferon induced transmembrane protein 1 (9-27) | pCMV-SPORT6 | 1.2 kb | NV | 3 | G7 | 163 |
| cysteine-rich protein 2 | pCMV-SPORT6 | 2.0 kb | NV | 3 | H7 | 187 |
| lectomedin-2,KIAA0821 | pCMV-SPORT6 | 1.8 kb | NV | 3 | A8 | 20 |
| Human variant urokinase plasminogen activator receptor (uPAR2) mRNA, partial cds | pCMV-SPORT6 | 1.6 kb | NV | 3 | B8 | 44 |
| prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) | pCMV-SPORT6 | 1.4 kb | NV | 3 | C8 | 68 |
| Laminin, gamma 2 (nicein (100kD), kalinin (105kD), BM600 (100kD), Herlitz junctional epidermolysis bullosa)) | pCMV-SPORT6 | 2.0 kb | NV | 3 | D8 | 92 |
| tensin, DKFZp434G162 | pCMV-SPORT6 | 1.5 kb | NV | 3 | E8 | 116 |
| MMP17,membrane-inserted | pCMV-SPORT6 | 2.3 kb | NV | 3 | F8 | 140 |

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|---|---|---|---|---|---|---|
| platelet/endothelial cell adhesion molecule (CD31 antigen) | pCMV-SPORT6 | 1.5 kb | NV | 3 | G8 | 164 |
| integrin, beta 5 | pCMV-SPORT6 | 2.3 kb | NV | 3 | H8 | 188 |
| collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant) | pCMV-SPORT6 | 3.1 kb | NV | 3 | A9 | 21 |
| MMP15,membrane-inserted | pCMV-SPORT6 | 3.1 kb | NV | 3 | B9 | 45 |
| Ephrin-B2 | pCMV-SPORT6 | 2.3 kb | NV | 3 | C9 | 69 |
| IGF1 | pCMV-SPORT6 | 1.8 kb | NV | 3 | D9 | 93 |
| tenascin XB | pCMV-SPORT6 | 1.9 kb | NV | 3 | E9 | 117 |
| p16 | pCMV-SPORT6 | 2.3 kb | NV | 3 | F9 | 141 |
| collagen, type VI, alpha 3 | pCMV-SPORT6 | 1.3 kb | NV | 3 | G9 | 165 |
| Cadherin 1, type 1, E-cadherin (epithelial | pCMV-SPORT6 | 3.5 kb | NV | 3 | H9 | 189 |
| endocytic receptor (macrophage mannose receptor family) | pCMV-SPORT6 | 1.8 kb | NV | 3 | A10 | 22 |
| matrix Gla protein | pCMV-SPORT6 | 2.4 kb | NV | 3 | B10 | 46 |
| thrombospondin 3 | pCMV-SPORT6 | 2.2 kb | NV | 3 | C10 | 70 |
| transforming growth factor, beta 3 | pCMV-SPORT6 | 2.4 kb | NV | 3 | D10 | 94 |
| transforming growth factor, beta 3 | pCMV-SPORT6 | 2.7 kb | NV | 3 | E10 | 118 |
| Laminin, alpha 4 | pCMV-SPORT6 | 1.9 kb | NV | 3 | F10 | 142 |
| von Willebrand factor | pCMV-SPORT6 | 1.8 kb | NV | 3 | G10 | 166 |
| insulin-like growth factor-binding protein 4 | pCMV-SPORT6 | 2.4 kb | NV | 3 | H10 | 190 |
| transforming growth factor, beta receptor II (70-80kD) | pCMV-SPORT6 | 2.3 kb | NV | 3 | A11 | 23 |
| tumor endothelial marker 1 precursor,TEM1 | pCMV-SPORT6 | 2.6 kb | NV | 3 | B11 | 47 |
| colony stimulating factor 1 (macrophage) | pCMV-SPORT6 | 1.8 kb | NV | 3 | C11 | 71 |
| Restin (Reed-Steinberg cell-expressed intermediate filament-associated protein) | pCMV-SPORT6 | 2.4 kb | NV | 3 | D11 | 95 |
| CD14 antigen | pCMV-SPORT6 | 1.7 kb | NV | 3 | E11 | 119 |
| tumor endothelial marker 8 | pCMV-SPORT6 | 1.5 kb | NV | 3 | F11 | 143 |
| hyaluronan synthase 1 | pCMV-SPORT6 | 2.2 kb | NV | 3 | G11 | 167 |
| RBCL | pCMV-SPORT6 | — | NV | 3 | H11 | 191 |
| small inducible cytokine subfamily B (Cys-X-Cys), member 5 (epithelial-derived neutrophil-activating peptide 78) | — | — | — | 3 | A12 | 24 |
| protein kinase C, eta | pCMV-SPORT6 | — | NV | 3 | B12 | 48 |
| C-reactive protein, pentraxin-related | Bluescript SK- | 1.5 kb | NV | 3 | C12 | 72 |
| chemokine (C-X-C motif), receptor 4 (fusin) | Bluescript SK- | 2.0 kb | NV | 3 | D12 | 96 |
| matrix metalloproteinase 12 (macrophage elastase) | Bluescript SK- | 937 | V | 3 | E12 | 120 |
| v-myb avian myeloblastosis viral oncogene homolog | Bluescript SK- | — | NV | 3 | F12 | 144 |

| gene name | vector | inserted length | sequence verified | plate | position | localization code |
|---|---|---|---|---|---|---|
| ASAR | Bluescript SK- | 1919 | V | 3 | G12 | 168 |
| ATPS | — | — | — | 3 | H12 | 192 |
| MMP-1 | — | — | — | 4 | A1 | 205 |
| interleukin 8 | Bluescript SK- | 2.1 kb | NV | 4 | B1 | 229 |
| placental growth factor, vascular endothelial growth factor-related protein | Bluescript SK- | 1937 | V | 4 | C1 | 253 |
| KIAA0337 gene product, TEM4 | Bluescript SK- | 732 | V | 4 | D1 | 277 |
| IGF-II mRNA-binding protein 1 | Bluescript SK- | 1.3 kb | NV | 4 | E1 | 301 |
| basic FGF | Bluescript SK- | 1732 | V | 4 | F1 | 325 |
| HAT22 | Bluescript SK- | 2.6 kb | NV | 4 | G1 | 349 |
| interleukin 16 (lymphocyte chemoattractant factor) | — | — | — | 4 | H1 | 373 |
| transforming growth factor, beta receptor II (70-80kD) | Bluescript SK- | 1294 | V | 4 | A2 | 206 |
| cyclin-dependent kinase 4 | Bluescript SK- | 1629 | V | 4 | B2 | 230 |
| eukaryotic translation initiation factor 3, subunit 4 (delta, 44kD) | Bluescript SK- | 1225 | V | 4 | C2 | 254 |
| collagen, type IV, alpha 1 | Bluescript SK- | 728 | V | 4 | D2 | 278 |
| hyaluronan synthase 2 | Bluescript SK- | 2.8 kb | NV | 4 | E2 | 302 |
| KIAA1075 protein | Bluescript SK- | 1.6 kb | NV | 4 | F2 | 326 |
| KIAA1075 protein | Bluescript SK- | 2.2 kb | NV | 4 | G2 | 350 |
| slit (Drosophila) homolog 3 | Bluescript SK- | 1.9 kb | NV | 4 | H2 | 374 |
| collagen, type VI, alpha 2 | Bluescript SK- | 2.8 kb | NV | 4 | A3 | 207 |
| Glutathione S-transferase pi | Bluescript SK- | 2.4 kb | NV | 4 | B3 | 231 |
| ephrin-A1 | Bluescript SK- | 1.6 kb | NV | 4 | C3 | 255 |
| interleukin 1, alpha | Bluescript SK- | 1768 | V | 4 | D3 | 279 |
| MMP13,(collagenase 3) | Bluescript SK- | 1253 | V | 4 | E3 | 303 |
| GTP-binding protein | Bluescript SK- | 1.1 kb | NV | 4 | F3 | 327 |
| hepatocyte growth factor (hepapoietin A; scatter factor) | Lafmid BA | 1.9 kb | NV | 4 | G3 | 351 |
| t-PA | Lafmid BA | 1697 | V | 4 | H3 | 375 |
| endothelin 1 | Lafmid BA | 1.7 kb | NV | 4 | A4 | 208 |
| CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1 | Lafmid BA | 1107 | V | 4 | B4 | 232 |
| angiopoietin 2 | Lafmid BA | 2.4 kb | NV | 4 | C4 | 256 |
| Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog | pT7T3-Pac | 837 | V | 4 | D4 | 280 |
| Wilms tumor 1 | pT7T3-Pac | 2000+ | V | 4 | E4 | 304 |
| guanine nucleotide binding protein 11 | pT7T3-Pac | 671 | V | 4 | F4 | 328 |
| GAPDH | pT7T3-Pac | 0.8 kb | NV | 4 | G4 | 352 |

| gene name | vector | inserted sequence | | plate | position | localization code |
|---|---|---|---|---|---|---|
| | | Length | verifided | | | |
| NS12 | — | — | — | 4 | H4 | 376 |
| SN26 | — | — | — | 4 | A5 | 209 |
| D310 | — | — | — | 4 | B5 | 233 |
| D45 | — | — | — | 4 | C5 | 257 |
| NS54 | — | — | — | 4 | D5 | 281 |
| A1 | — | — | — | 4 | E5 | 305 |
| A2 | — | — | — | 4 | F5 | 329 |
| Sex | — | — | — | 4 | G5 | 353 |
| SYN2 | — | — | — | 4 | H5 | 377 |
| MNRP | — | — | — | 4 | A6 | 210 |
| NP-1 | — | — | — | 4 | B6 | 234 |
| NP-2 | — | — | — | 4 | C6 | 258 |
| S-3B | — | — | — | 4 | D6 | 282 |
| S-3C | — | — | — | 4 | E6 | 306 |
| S-3E | — | — | — | 4 | F6 | 330 |
| S-3F | — | — | — | 4 | G6 | 354 |
| Mucin 1 | — | — | — | 4 | H6 | 378 |
| Mucin 2 | — | — | — | 4 | A7 | 211 |
| Mucin 3 | — | — | — | 4 | B7 | 235 |
| Mucin 3 | — | — | — | 4 | C7 | 259 |
| Mucin 4 | — | — | — | 4 | D7 | 283 |
| Mucin 5B | — | — | — | 4 | E7 | 307 |
| Mucin 6 | — | — | — | 4 | F7 | 331 |
| Mucin 7 | — | — | — | 4 | G7 | 355 |
| Mucin 8 | — | — | — | 4 | H7 | 379 |
| — | — | — | — | 4 | A8 | 212 |
| — | — | — | — | 4 | B8 | 236 |
| — | — | — | — | 4 | C8 | 260 |
| — | — | — | — | 4 | D8 | 284 |
| — | — | — | — | 4 | E8 | 308 |
| — | — | — | — | 4 | F8 | 332 |
| — | — | — | — | 4 | G8 | 356 |
| — | — | — | — | 4 | H8 | 380 |
| — | — | — | — | 4 | A9 | 213 |
| — | — | — | — | 4 | B9 | 237 |

| gene name | vector | inserted length | sequence verified | plate | position | localization code |
|---|---|---|---|---|---|---|
| — | — | — | — | 4 | C9 | 261 |
| — | — | — | — | 4 | D9 | 285 |
| — | — | — | — | 4 | E9 | 309 |
| — | — | — | — | 4 | F9 | 333 |
| — | — | — | — | 4 | G9 | 357 |
| — | — | — | — | 4 | H9 | 381 |
| — | — | — | — | 4 | A10 | 214 |
| — | — | — | — | 4 | B10 | 238 |
| — | — | — | — | 4 | C10 | 262 |
| — | — | — | — | 4 | D10 | 286 |
| — | — | — | — | 4 | E10 | 310 |
| — | — | — | — | 4 | F10 | 334 |
| — | — | — | — | 4 | G10 | 358 |
| — | — | — | — | 4 | H10 | 382 |
| — | — | — | — | 4 | A11 | 215 |
| — | — | — | — | 4 | B11 | 239 |
| — | — | — | — | 4 | C11 | 263 |
| — | — | — | — | 4 | D11 | 287 |
| — | — | — | — | 4 | E11 | 311 |
| — | — | — | — | 4 | F11 | 335 |
| — | — | — | — | 4 | G11 | 359 |
| — | — | — | — | 4 | H11 | 383 |
| G A 4 | — | — | — | 4 | A12 | 216 |
| — | — | — | — | 4 | B12 | 240 |
| — | — | — | — | 4 | C12 | 264 |
| — | — | — | — | 4 | D12 | 288 |
| — | — | — | — | 4 | E12 | 312 |
| — | — | — | — | 4 | F12 | 336 |
| LHC | — | — | — | 4 | G12 | 360 |
| — | — | — | — | 4 | H12 | 384 |

Figure 1.Image of original angiochip.

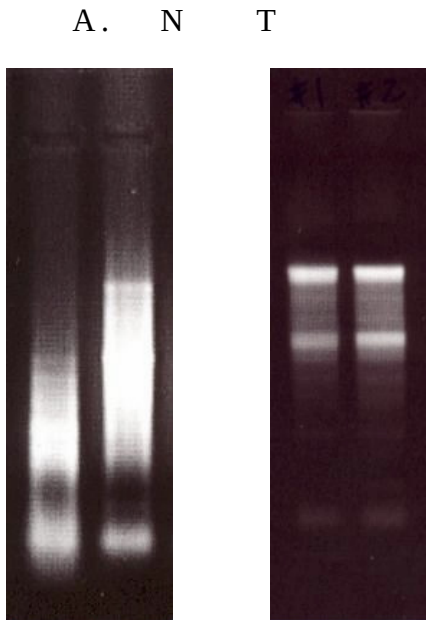


Figure 2. Check the quality of total RNA extracted from gastric cancer (T) and noncancerous (N) tissue byagarose gel electrophoresis A. RNA is smearing before extraction protocol was modified. B. RNAquality is much improved after extraction protocol was modified.

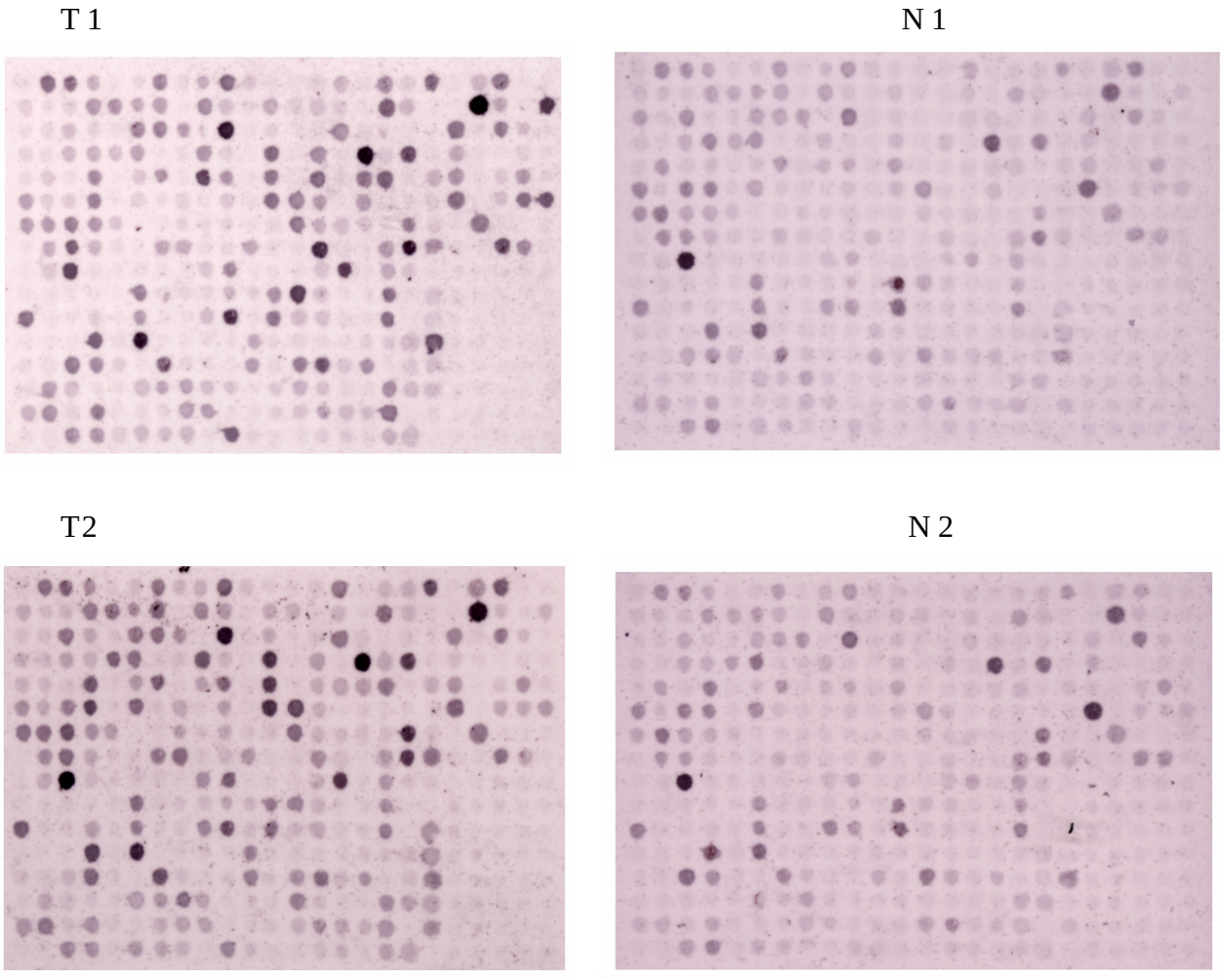


Figure 3. Hybridization of angiochip with two pairs of gastric cancer tissuescDNA probe prepared from two pairs of gastric cancer (T1-T2) and corresponding adjacent noncancerous gastric tissue (N1-N2) RNA.