Supplemental methods

1. Optimized oligo-capping method

One hundred μg of total RNA was treated for 60 min at 37 ℃ with 5 U of bacterial alkaline phosphatase (TaKaRa) in 200 μl of 0.1 M Tris-HCl pH7.0 containing 10 mM 2-mercaptoethanol (2-ME) and 216 U of RNase inhibitor (Promega). After extraction with phenol-chloroform (1:1) twice and ethanol precipitation, the RNAs were treated for 60 min at 37 ℃ with 1 U of tobacco acid pyrophosphatase (TAP) in 100 μl of 50 mM sodium acetate pH 6.5 containing 1 mM EDTA, 10 mM 2-ME and 108U of RNase inhibitor. After extraction with phenol-chloroform (1:1) and ethanol precipitation, the RNAs were ligated with 1.2 μg of 5'-oligo RNA (5'-AGC AUC GAG UCG GCC UUG UUG GCC UAC UGG-3') using 525 U of RNA ligase (TaKaRa) in 300 μl of 50 mM Tris-HCl pH 7.0 containing 10 mM 2-ME, 5 mM $MgCl_2$, 0.5 mM ATP, 300 U of RNase inhibitor and 25% PEG8000, followed by incubation of the mixture at 20 ℃ for 3 hr. The ligation mixture was diluted with 600 μl of TE buffer, extracted with phenol-chloroform (1:1) and then the RNAs were precipitated using ethanol. From this mixture, the oligo-capped poly A(+) RNAs were isolated using oligo-dT cellulose (STRATAGENE) according to the standard poly A(+) purification method. The first strand cDNA was synthesized from the oligo-capped poly A (+) RNAs using 200 U Superscript II reverse transcriptase (Invitrogen) in 325 μl of 50 mM Tris-HCl pH 8.3 containing 75 mM KCl, 3 mM $MgCl_2$, 0.01 M DTT, 0.8 mM each dNTP mixture and 5 pmol of oligo dT-adaptor (5'- GCG GCT GAA GAC GGC CTA TGT GGC CTT TTT TTT TTT TTT TTT-3') by incubating the reaction mixture at 16 ℃ for 60 min and then at 42 ℃ for another 60 min. The mixture was diluted with an equal volume of H_2O and then extracted with phenol-chloroform (1:1). Next, 7.5 μl of 0.1 N NaOH and 1 μl of 0.5 M EDTA were added to the mixture and incubated 65 ℃ for 60 min to degrade RNAs. After adding 10 μl of 1 M Tris-HCl pH7.8 and ethanol precipitation, the first strand cDNAs were dissolved in 50 μl of TE buffer. One fifth of this cDNA solution was utilized for PCR amplification using the Gene Amp XL PCR kit (ABI) with oligo cap primer (5'-AGC ATC GAG TCG GCC TTG-3') and dT adaptor primer (5'-GCG GCT GAA GAC GGC CTA TGT-3'). The following cycling steps were used for amplification: 10 - 15 cycles at 95 ℃ 1 min, 58 ℃ 1 min, 72 ℃ 10 min. The PCR amplified products were extracted with phenol-chloroform (1:1), precipitated with ethanol and then digested with the restriction enzyme SfiI (NEB). Next, SfiI-digested cDNA fragments longer than about 2 kb were isolated by agarose gel electrophoresis and subsequently cloned into the DraIII-digested pME18SFL3 vector following routinely used methods. More detailed methods were described in "Ota, T., Wakamatsu, A., Isogai, T., Saito, K., Suzuki, Y., Sugano, S. 2001, METHOD FOR CONSTRUCTING FULL-LENGTH cDNA LIBRARY, WO 01/04286 A1".

2. The 5'-end fullness rate of the optimized oligo-capped cDNA libraries

The method which evaluated the 5'-end fullness rate of the constructed oligo-capped cDNA libraries was described previously in references 22 and 23.

The 5'-end fullness rate of most of the oligo-capped cDNA libraries was described previously in the Supplementary Table 1 of reference 14. ESTs listed in this Supplementary Table 1 were obtained from the cDNA libraries, all of which were constructed by the optimized oligo-capping method: ADRGL, BEAST, NOVAR, BRALZ, BRAMY, ASTRO, BLADE, BRCAN, CD34C, D3OST, D6OST, D9OST, BRACE, HCHON, COLON, HCASM, BRCOC, DFNES, HHDPC, NESOP, FEBRA, FEHRT, FEKID, FELNG, HEART, BRHIP, KIDNE, LIVER, HLUNG, MESAN, NB9N(NB9N4), NHNPC, 3NB(3NB69), SKNSH, PERIC, PEBLM, PROST, PUAEN, RECTM, SKMUS, SMINT, SPLEN, STOMA, BRSSN, BRSTN, SYNOV, HSYRA, NT2RI, NT2NE, TESTI, BRTHA, THYMU, NTONG, CTONG, TRACH, TBAES, TCERX, TCOLN, TESOP, TKIDN, TLIVE, TLUNG, TOVAR, TSTOM, TUTER, UTERU and BRAWH. The average 5'-end fullness rate of these cDNA libraries was 90.3%.

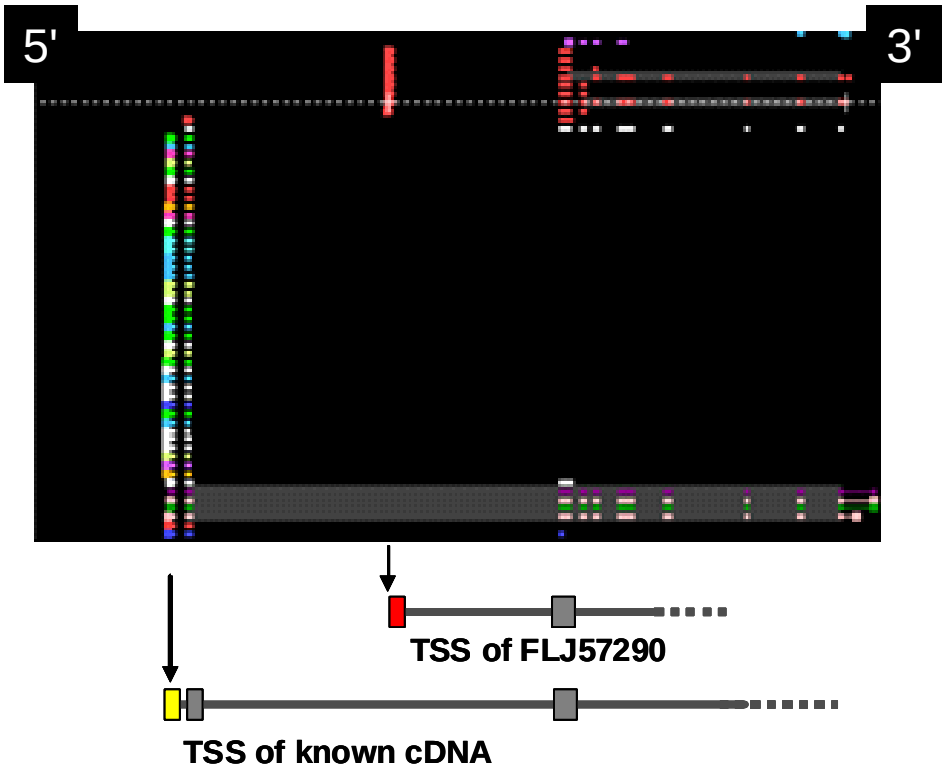
For the present work, we used about 1.45 million human 5'-ESTs for analysis, among which about 1.4 million 5’-ESTs were constructed by the optimized oligo-capping method. These ESTs were obtained from 99 human cDNA libraries, which were constructed using RNAs purified from different tissues and cells.

3. Method for the analysis of the tissue specific expression

Methods used for the genome mapping and clustering analyses are described in section 2.2**.** Mapping results of all cDNA splicing variants are shown in detail in each cluster. Since a large number of different tissues and cell lines were used for constructing nearly one-hundred cDNA libraries from which these ESTs were obtained, each tissue or cell was indicated using a color code. Thus, one could easily understand the expression pattern of the TSS from the indicated color.

Example: FLJ57290 (Locus position chr8+2717)

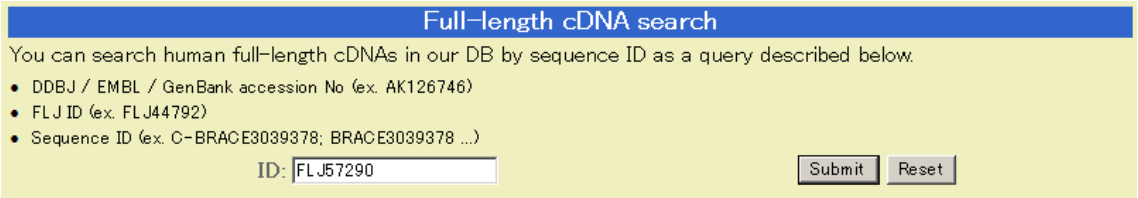
Mapping results of FLJ57290 is shown using the dotted line in the following figure. Each color indicates a specific tissue or cell, such as: Red: trachea, Green: tongue tumor, Blue: NT2 cells induced with RA and then treated with mitotic inhibitor, Yellow: NT2 cell induced by RA, Purple: fetal brain, Orange: thymus; etc.



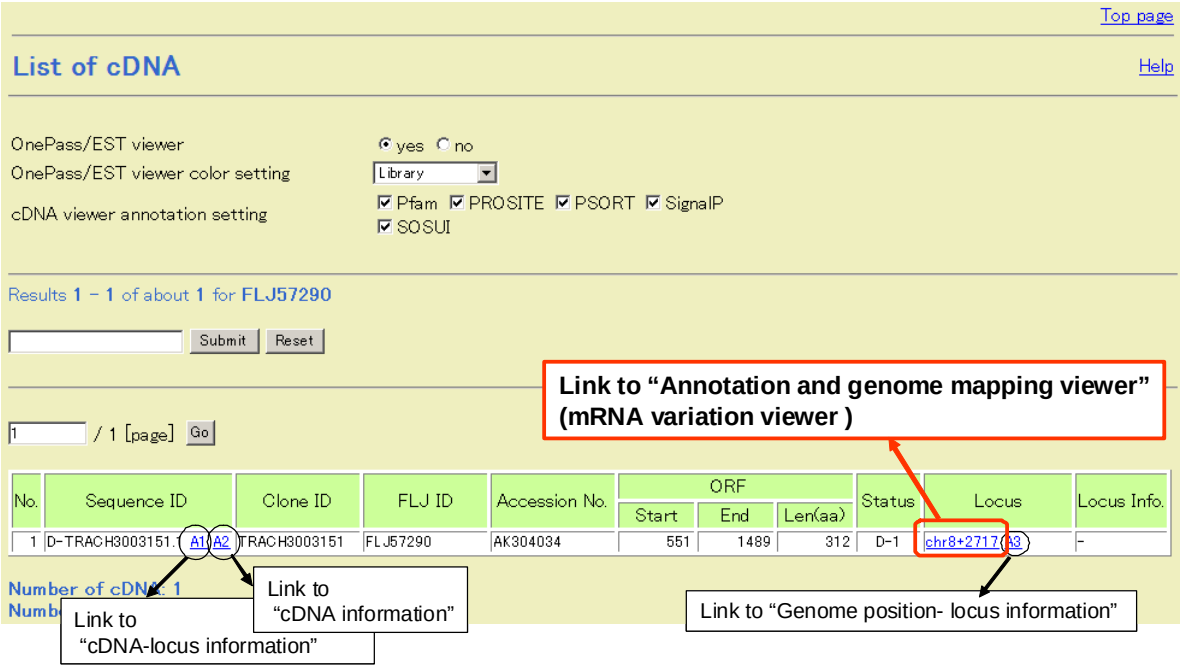
The TSS we identified in the FLJ57290 cDNA was highly expressed in the trachea as it is shown only in red. However, the TSS of the known cDNA was expressed at equal levels in various tissues because it is shown in different colors.

4. Analysis of AS by using the information available in "the FLJ Human cDNA Database ver. 3.0"

A. To begin a search, the Sequence ID (for example, FLJ57290) is entered into the text box of the page “Full-length cDNA search”.



B. When a query is entered in our FLJ Human cDNA Database, a "List of cDNA" page appears. The "Locus" column (second from the lower right – circled in red in the Figure shown below) represents the "Locus position" obtained by genome mapping analysis.



C. Button "Locus position" (for example, chr8+2717) is linked to "Annotation and genome mapping viewer". A lot of information regarding AS can be found from this page of the site.

In the central gray area, the genome mapping results of the query cDNAs are shown. We can also find information on the splicing patterns as well as the predicted functions of the genes. In addition, we can get genome mapping results of the ESTs. By using these information, we are able to analyze the expression profile and predict reliability of splicing pattern.


Expression profile by ESTs
Mapping results of ESTs
Mapping results of cDNAs
chr8+2717 - Windows Internet Explorer
5'(F-):52
Improved
TRACH 10/52865
CTONG 8/32043
NT2RI 7/32042
NT2RP 5/24763
OCBBF 3/48042
ASTRO 2/17226
BNGH4 2/7739
BRTHA 2/53818
CHONS 2/2694
FCBBF 2/32305
THYMU 2/71574
BRAMY 1/59232
BRHIP 1/58498
NT2NE 1/16468
SKNMC 1/7656
SKNSH 1/8692
SYNOV 1/27671
Original
HEMBB 1/2699
3'(R-):6
Improved
TRACH 3/3011
BRHIP 1/2577
NT2RI 1/1102
Original
HEMBB 1/2620
76059263
76062000
76068000
76074000
76080000
76086000
76092000
76098000
76104000
76109348
D-TRACH3003151.1 D-1 A1A2
D-TRACH3029284.1 D-1 A1A2
G-NT2RI2008013_F-01 A1A2
NM_031461.3 A1A2
ENST00000262207 A1A2
AL834301.1 A1A2
BC020514.1 A1A2
same ORF region, same reading frame as selected reference cDNA
same ORF region, 2nd frame
same ORF region, 3rd frame
different ORF region
5' ---> 3'
chr8+2717
Help
Onepass annotation setting window
Intron setting
Uncompressed Compressed
Settings
Sorted
Genome upstream
Color setting
Library
Seq ID
Shown Not shown
Abscissa scale
Apply
cDNA annotation setting window
Annotation
Pfam PROSITE PSORT SignalP SOSUI
ORF setting
Reference cDNA
D-TRACH3003151.1

Supplemental Table 1. List of total RNAs used in this study

List of cDNA libraries used full-length sequencing in this study

1) Cell lines

Library Name	Cell type
3NB69	NB69, neuroblastoma
AHMSC	mesenchymal cells (HMSC)
BGGI1	GI1, glioma separated from gliosarcoma
BNGH4	H4, neuroglioma
CHONS	chondrocytes
ERLTF	TF-1, erythroleukemia
HELAC	HeLa, HeLa cells
IMR32	IMR32, neuroblastoma
JCMLC	leukemia cell line (myelogenous)
MESTC	mesenchymal stem cells
N1ESE	mesenchymal stem cells
NB9N3	NB9, neuroblastoma
NB9N4	NB9, neuroblastoma
NCRRM	embryonal carcinoma
NCRRP	embryonal carcinoma, after retinoic acid (RA) induction.
NT2NE	NT2, teratocarcinoma, NT2 neuron after the differentiation of NT2 neuronal precursor cells.
NT2RI	NT2, teratocarcinoma, NT2 neuronal precursor cells treated 2-weeks mitotic inhibitor after 5-weeks retinoic acid (RA) induction., majorly NT2 neuron
NT2RM	NT2, teratocarcinoma, NT2 neuronal precursor cells.
NT2RP	NT2, teratocarcinoma, NT2 neuronal precursor cells after 2days or 2-weeks or 5-weeks retinoic acid (RA) induction.
SKNMC	SK-N-MC, neuroepithelioma
SKNSH	SK-N-SH, neuroblastoma
T1ESE	mesenchymal stem cells, mesenchymal stem cells treated with trichostatin and 5-azacytidine.
Y79AA	Y79, retinoblastoma
ACTVT	primary culture, activated T-cells
ASTRO	primary culture, normal astrocytes (NHA5732)
DFNES	primary culture, normal dermal fibroblasts (Neonatal Skin) (NHDF2564)
HCASM	primary culture, coronary artery smooth muscle cells (HCASMC)
HCHON	primary culture, chondrocytes (HC)
HHDPC	primary culture, dermal papilla cells (HDPC)
HSYRA	primary culture, synoviocytes from rheumatoid arthritis (HS-RA)
LYMPB	primary culture, lymphoblasts (EB virus transferred B cell)
MESAN	primary culture, normal mesangial cells (NHMC56046-2)
NETRP	primary culture, neutrophils
NHNPC	primary culture, normal neural progenitor cells (NHNP5958)
PEBLM	primary culture, peripheral blood mononuclear cells (HPBMC5939)
PUAEN	primary culture, pulmonary artery endothelial cells (HPAEC)
VESEN	primary culture, endothelial cells, umbilical vein endothelial cells (HUVEC)
CD34C	cord blood, primary culture, CD34+ cells
D3OST	cord blood, primary culture, CD34+ cells after 3-days ODF induction.
D6OST	cord blood, primary culture, CD34+ cells after 6-days ODF induction.
D9OST	cord blood, primary culture, CD34+ cells after 9-days ODF induction.

2) Tissues

Library Name	Tissue type
ADRGL	adrenal gland
BEAST	breast
BLADE	bladder
BRACE	cerebellum
BRALZ	alzheimer cortex
BRAMY	amygdala
BRASW	alzheimer cortex, subtracted library (BRALZ - BRAWH)
BRAWH	brain
BRCAN	caudate nucleus
BRCOC	corpus callosum
BRHIP	hippocampus
BRSSN	substantia nigra
BRSTN	subthalamic nucleus
BRTHA	thalamus
CERVX	cervix
COLON	colon
CORDB	cord blood
CTONG	tongue, tumor tissue
FCBBF	brain, fetal
FEBRA	brain, fetal
FEHRT	heart, fetal
FEKID	kidney, fetal
FELNG	lung, fetal
HEART	heart
HEMBA	whole embryo, mainly head
HEMBB	whole embryo, mainly body
HLUNG	lung
KIDNE	kidney
LIVER	liver
MAMGL	mammary gland
MAMMA	mammary gland
NESOP	esophagus
NOVAR	ovary
NTONG	tongue
OCBBF	brain, fetal
OVARC	ovary, tumor tissue
PANCR	pancreas
PERIC	pericardium
PLACE	placenta
PROST	prostate
RECTM	rectum
SALGL	salivary gland
SKMUS	skeletal muscle
SMINT	small intestine
SPLEN	spleen
STOMA	stomach
SYNOV	synovial membrane tissue from rheumatoid arthritis
TBAES	breast, tumor tissue
TCERX	cervix, tumor tissue
TCOLN	colon, tumor tissue
TESOP	esophagus, tumor tissue
TESTI	testis
THYMU	thymus
THYRO	thyroid gland
TKIDN	kidney, tumor tissue
TLIVE	liver, tumor tissue
TLUNG	lung, tumor tissue
TOVAR	ovary, tumor tissue
TRACH	trachea
TSTOM	stomach, tumor tissue
TUTER	uterus, tumor tissue
UTERU	uterus

Supplemental Table 2. Human cDNA sequences used in this study

1) Human cDNA sequences of cDNAs by oligo-capping method

- **Full-length cDNA sequences**
  - **a) FLJ full-length cDNA sequences by human cDNA sequencing project I : 30,326**
  - **b) FLJ full-length cDNA sequences by human cDNA sequencing project II focused on splicing variants of mRNAs : 25,076**
- **FLJ ESTs : 5'-EST 1,456,213 & 3'-EST 109,283**

2) Other human cDNA sequences from public databeses

- **Full-length sequenced cDNAs**
  - **a) KIAA (Kazusa DNA Research Institute) : 2,459 (KIAA : 2039, FLJ-PJ : 420)**
  - **b) MGC (Mammalian Gene Collection) : 25,778**
  - **c) DKFZ (Deutsches Krebsforschungszentrum) : 8,212**
  - **d) Other institutions : 15,677**
- **RefSeq, human, ver. 2005.10.17**
  - **a) NM : 24,210**
  - **b) NR : 251**
  - **c) XM : 5,239**
  - **d) XR : 61**
- **Ensembl, human gene transcripts, human-38.36g/UCSC hg18 : 47,585**
- **UniGene, human ESTs* : 5'-EST 2,699,311 & 3'-EST 1,638,884**

*** not including deposited about 1.6 million of FLJ EST sequences**

Detail informations of those sequences were shown in FLJ Human cDNA Database ver. 3.0 (http://flj.lifesciencedb.jp)

Supplemental Table 4. List of total RNAs used for the real-time PCR in this study

No	RNA Name	total RNA type
1	Brain, whole	Clontech (Cat.636530)
2	Brain, cerebellum	STRATAGENE (Cat.540007), Ambion (Cat.6820)
3	Fetal Brain	Clontech (Cat.636526)
4	Spinal Cord	Clontech (Cat.636554)
5	Colon	Clontech (Cat.636521)
6	Heart	Clontech (Cat.636532)
7	Kidney	Clontech (Cat.636529)
8	Liver	Clontech (Cat.636531)
9	Lung (whole)	Clontech (Cat.636524)
10	Stomach	Clontech (Cat.636522)
11	Trachea	Clontech (Cat.636541)
12	Prostate	Clontech (Cat.636550)
13	Testis	Clontech (Cat.636533)
14	Ovary	Clontech (Cat.636555)
15	Placenta	Clontech (Cat.636527)
16	Uterus	Clontech (Cat.636551)
17	Spleen	Clontech (Cat.636525)
18	Bone Marrow	Clontech (Cat.636548)
19	Thymus	Clontech (Cat.636549)
20	Tumor Mix	Mixed equal volume No.22, 23, 24, 25, 26, 27, 28
21	Normal Mix	Mixed equal volume No.1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19
22	Kidney Tumor	Clontech (Cat.636632)
23	Lung Tumor	Clontech (Cat.636633)
24	Stomach Tumor	Clontech (Cat.636629)
25	Uterus Tumor	Clontech (Cat.636628)
26	Colon Tumor	Clontech (Cat.636634)
27	Liver Tumor	Clontech (Cat.636531)
28	Ovary Tumor	Clontech (Cat.636631)
29	NT2 RA(-)	NT2 Cell, teratocarcinoma, Total RNA from uninduced NT2 neuronal precursor cells.
30	NT2 RA(+) 24hr A	NT2 Cell, teratocarcinoma, Total RNA ffrom NT2 neuronal precursor cells after 24hrs retinoic acid (RA) induction.
31	NT2 RA(+) 1week A	NT2 Cell, teratocarcinoma, Total RNA ffrom NT2 neuronal precursor cells after 1-week retinoic acid (RA) induction.
32	NT2 RA(+)	NT2 Cell, teratocarcinoma, Total RNA ffrom NT2 neuronal precursor cells after 5-weeks retinoic acid (RA) induction.
33	NT2 RA(+)Inh(+)	NT2 Cell, teratocarcinoma, Total RNA from NT2 neuronal precursor cells treated 2-weeks mitotic inhibitor after 5-weeks retinoic acid (RA) induction.
34	NT2 Neuron	NT2 Cell, teratocarcinoma, Total RNA from NT2 neuron after the differentiation of NT2 neuronal precursor cells.

Supplemental Table 5. Sequences of primers and probes used in the real-time PCR

1) List of primers detected specific FEV region

Gene	Target cDNA	Primer set Name	Forward primer Name	Forward primer sequence	Reverse primer Name	Reverse primer sequence	TaqMan probe Name	TaqMan probe sequence
AKT1	FLJ53606	019_01	019_01F	TCAGGGTGTACGTGCTGTAGGT	019_01R	ACATGGAAGGTGCGTTCGA		
SPRED2	FLJ52731	031_01	031_01F	GGCTCCACAATGTAGAGGAAAT	031_01R	GGTCATAACCACAGCCTTGACA		
SEMA5B	FLJ55460	039_01	039_01F	TGGCTGCGTGAAGGTCAGA	039_01R	ACTCACTCGATCCCTTCGCTTCT		
FGF13	FLJ57884	003_01	003_01F	GCTGCCCGCTGAAGCA	003_01R	TTGTCGGCTGTATAGCTTGGTAAC	003_01TP	AACACAGAGCCGGAAGAGCCTCAGC
FGF13	FLJ57068	003_02	003_02F	GTGACCAAGCCCAAAGAGGA	003_02R	TCAATGGTTCCATCCGCCT	003_02TP	GATGCTTCTAAGGAGCCTCAGCTTAAGGGT
CACNB3	FLJ58949, FLJ58411	041_01	041_01F	GCTGCCCGGCTTAGCT	041_01R	GGGCGGCTGGTGTAGGA		
C6orf142	FLJ58494	075_01	075_01F	TGCATCTTATCAGGGAGCATT	075_01R	TGAAGATCAGAGGTTTGGCTTCA		
OXR1	FLJ56044	090_02	090_02F	CACAGAAATGACGAAGGACAAA	090_02R	ACCAGCCAAAGGGTTGAAC		
PLD5	FLJ57051	035_01	035_01F	CAGAGGCTGTGGGAAGCA	035_01R	CTGGGACATTGCGACCAATC		
BACE1	FLJ54690	054_01	054_01F	CAATGGTTCCCTTCATCTATCTG	054_01R	CCACTTGCCCTGGGTGTAG		
FGD4	FLJ55905	040_02	040_02F	GAATGCCTATAGTTTCTACCAACAAATT	040_02R	CTGCTGCAGCCCCCCATA		
PTPN4	FLJ53929	009_01	009_01F	CCATTAACAGCAATAAAGTGTTCTGA	009_01R	TCTGCTTACTACTTCCCAATCCAT		
BTNL8	FLJ51528	074_01	074_01F	ACACCTTGAGAGGCTGAGACA	074_01R	CTGAGCCCAGTGCATATGC	074_01TP	AGGATTGCTTGAGTCCAGGAGCTCGA
C6orf32	FLJ56137	026_02	026_02F	TGTCCTCTCCAAGATGCAGTT	026_02R	TTCACCAAAAACATCATCCTCTTC		
C6orf32	FLJ56038	026_01	026_01F	CGCGATTACACTGGATCAGA	026_01R	TGGTCGGTAGTCGGTTGAG		

2) List of primers detected control TSS region

Gene	Target cDNA	Primer set Name	Forward primer Name	Forward primer sequence	Reverse primer Name	Reverse primer sequence	TaqMan probe Name	TaqMan probe sequence
AKT1	NM_005163.2	019_02	019_02F	GGTTGGCTGCACAAACGA	019_02R	GAGCCTCACGTTGGTCCACAT		
SPRED2	NM_181784.1	031_02	031_02F	ACAGGCGTCTAGGTAACAAGAAA	031_02R	CAGCCTTGACACGCACAATA		
SEMA5B	NM_001031702.1	039_03	039_03F	CCGCCTCTGGGAGTGAGT	039_03R	CCACAGGGCATCCAAGAGA		
FGF13	NM_004114.2	003_04	003_04F	TCAAACTCTTCGGCTCCAAGA	003_04R	TGGTAGCCTTGTCGGCTGTAT	003_04TP	CGCAGAAGAAGACCAGAGCCTCAGCTT
CACNB3	NM_000725.2	041_03	041_03F	CGTGCCCGGGTTTGAG	041_03R	GGGCGGCTGGTGTAGGA		
C6orf142	NM_138569.1	075_02	075_02F	TGGAACTTGAAAAGCGTGAAA	075_02R	GGACAAATGTGAAGATCAGAGGTT		
OXR1	NM_181354.3	090_04	090_04F	GGTGCGCTTCGAAGATTACC	090_04R	TTCTTGTCTAGGGTCTTCTTTTGG		
PLD5	NM_152666.1	035_02	035_02F	CGTCTGGCTTCGGAGGAA	035_02R	GGGCAAAGATCACGATGCA		
BACE1	NM_012104.3	054_02	054_02F	CCGTGCTCTGCGGATCT	054_02R	GCTTCTCAGGAGAGGGAGCTT		
FGD4	NM_139241.1	040_03	040_03F	GCTCGCTGGAAGAACAAATG	040_03R	AGGTACTGCTGCCTTATAAAAGCT		
PTPN4	NM_002830.2	009_02	009_02F	TTGTGGAAAGCATGTGTAGAACATC	009_02R	TTCTCCCACAGTACCGGAATTT		
BTNL8	NM_024850.1	074_03	074_03F	GAAGGCCATCTGGGAGCTA	074_03R	CATATCCCGTGATGGAAATGA	074_03TP	AGGTGTCAGCACTGGGCTCAGTTCC
C6orf32	AB002384.1	026_03	026_03F	GGAGGCTTTTATATGCCAGAGA	026_03R	CCTAACGACGACGGCTCCTT		
C6orf32	NM_015864.2	026_04	026_04F	TCTTCAATCTGGGCAGTAACTATTT	026_04R	TCGGTAGTCCATTGTCTTAGCA		

3) List of primers detected common region

Gene	Target cDNA	Primer set Name	Forward primer Name	Forward primer sequence	Reverse primer Name	Reverse primer sequence	TaqMan probe Name	TaqMan probe sequence
AKT1	AKT1	019_03	019_03F	CCGCGTCCTGCAGAACTC	019_03R	CCCGTTGGCGTACTCCAT		
SPRED2	SPRED2	031_03	031_03F	GACCCCGAGGGAGACTATACAGA	031_03R	ATCCACCGGAGGCAAAACT		
SEMA5B	SEMA5B	039_05	039_05F	TGAGGAGGAGTGTCAGAACTACGT	039_05R	AAGGCATTGGTTCCACACATG		
FGF13	FGF13	003_05	003_05F	CAGCTCATTTTCTGCCTAAACCA	003_05R	CAGCACGCCAGAGACACTTC	003_05TP	AGCCATCACTGCACGATCTCACGGA
CACNB3	CACNB3	041_04	041_04F	TCCAGCCTGAGTGACATTGG	041_04R	TCCGCCTGCTTTTGCTTCT		
C6orf142	C6orf142	075_05	075_05F	TCTGATGTAGTGCGTCCCAAAA	075_05R	TTGTATTGCTGCCCATGCTTT		
OXR1	OXR1	090_06	090_06F	TGGCACAAGCTTGAAAACTCT	090_06R	CCATCACTGTCTTTAATCACCATCA		
PLD5	PLD5	035_03	035_03F	AGCAGAAGTGCATCGTGATCT	035_03R	CATGATGTCCACGGCTGAAA		
BACE1	BACE1	054_03	054_03F	CTCTGCCTCATGGTGTGTCAGT	054_03R	CATGGGCCTCCTCACTTCAG		
FGD4	FGD4	040_04	040_04F	CAGGAAGGCATGGATTGACA	040_04R	CACCCTGAGTCTTATCTGCATCAT		
PTPN4	PTPN4	009_03	009_03F	ATGCCTGTGATTGTGTCTCGAGTA	009_03R	TGTCCCGACCATTGATCAGTAC		
BTNL8	BTNL8	074_04	074_04F	CCACAGCGAAGTGGAAAGGT	074_04R	GCCGCATGGAACAGGATATG	074_04TP	CTCTGACCGTCCAAGAGAACGCCG
C6orf32	C6orf32	026_05	026_05F	CAGGCTCCCCTTCCAACTC	026_05R	TGGTGTCATCCATACCCTCATTC		
GAPDH	GAPDH	GAPDH	GAPD_01F	CCAGGTGGTCTCCTCTGACTTC	GAPD_01R	GTGGTCGTTGAGGGCAATG	GAP_01TP	ACAGCGACACCCACTCCTCCACCTT

Supplemental Table 6. Quantitative evaluation of expression of 13 selected genes (TypeA1) by real-time PCR

Relative expression to Normal Mix (Log10RQ)

Target gene	SPRED2	SPRED2	SPRED2	SEMA5B	SEMA5B	SEMA5B	CACNB3	CACNB3	CACNB3	C6orf142	C6orf142	C6orf142	PLD5	PLD5	PLD5	BACE1	BACE1	BACE1
Primer set name	031_01	031_02	031_03	039_01	039_03	039_05	041_01	041_03	041_04	075_01	075_02	075_05	035_01	035_02	035_03	054_01	054_02	054_03
Brain,whole	1.00	0.29	0.37	0.46	0.56	0.70	-0.80	0.69	0.59	1.29	-2.40	0.15	0.71	0.63	0.76	0.41	0.91	0.59
Brain,cerebellum	-1.31	0.02	0.17	-0.57	-0.35	-0.35	-0.52	0.65	0.65	-1.18	-4.06	-0.90	1.32	0.79	1.26	0.44	0.71	0.49
Fetal Brain	-0.56	0.40	0.40	1.60	1.17	1.40	-0.58	0.97	1.10	0.86	-3.37	-0.14	0.02	0.17	0.17	0.36	0.28	0.58
Spinal Cord	-0.43	-0.04	-0.09	0.24	-0.14	0.14	-0.80	-0.44	-0.54	-1.78	-3.20	0.05	-1.12	0.48	0.16	0.89	0.60	0.78
Colon	0.16	0.03	0.00	-0.46	-0.18	-0.34	-0.13	-0.24	-0.01	Undet.	-2.51	-2.04	-3.19	-1.02	-1.49	-0.17	-0.95	-0.40
Heart	Undet.	-0.64	-0.69	Undet.	0.04	-0.07	-1.38	-1.06	-1.16	-1.88	0.62	0.29	Undet.	-1.44	-1.88	-2.07	-0.19	-0.97
Kidney	-1.28	0.12	0.03	0.50	0.47	0.46	-0.20	-0.56	-0.42	Undet.	-3.04	-1.43	-2.51	-0.39	-0.72	-0.38	-0.02	-0.02
Liver	-1.30	0.11	0.06	-0.54	-0.42	-0.46	-1.23	-1.37	-1.27	-0.96	-2.69	0.46	-2.38	-1.65	-1.84	-0.48	-0.21	-0.13
Lung (whole)	0.51	0.91	0.91	0.08	-0.42	0.77	0.53	0.47	0.55	Undet.	-0.74	-0.91	-2.21	0.20	-0.20	-0.35	0.52	0.48
Stomach	0.03	0.20	0.15	-0.10	-0.42	-0.14	-0.12	-0.49	-0.13	Undet.	-2.60	-1.72	-2.08	-0.18	-0.56	-0.29	-0.46	-0.12
Trachea	0.19	0.31	0.26	-0.49	-0.42	-0.10	-0.09	0.07	0.09	Undet.	-2.81	-0.93	-1.52	0.14	-0.20	-0.20	0.05	-0.11
Prostate	0.23	0.28	0.26	-0.07	-0.42	-0.17	-0.16	-0.02	-0.02	-2.61	-0.81	-0.79	-1.47	0.18	-0.16	-0.09	0.04	0.12
Testis	-1.49	-0.14	-0.06	-0.33	-0.42	-0.21	0.20	0.19	0.22	-1.73	-2.67	-1.14	-1.17	0.03	0.00	0.57	-0.08	0.17
Ovary	-0.97	0.15	0.18	0.17	-0.42	0.23	1.21	0.39	0.72	Undet.	-3.08	-2.38	-1.12	0.43	0.14	0.40	0.33	0.44
Placenta	-0.82	0.38	0.32	-0.10	-0.42	0.45	0.11	0.08	0.03	-2.00	Undet.	-1.60	-1.15	0.55	0.19	-0.13	0.07	0.10
Uterus	-0.37	0.46	0.40	0.48	-0.42	0.34	0.98	0.57	0.64	-2.45	-2.37	-1.77	-1.28	0.56	0.30	0.21	0.24	0.33
Spleen	-0.10	0.22	0.15	Undet.	-0.42	-0.58	-0.45	-0.41	-0.37	Undet.	Undet.	-2.04	-0.96	0.18	-0.08	-0.03	0.25	0.23
Bone Marrow	-1.47	-0.68	-0.69	-1.14	-0.42	-1.42	-1.42	-1.21	-0.98	-2.31	-2.42	-2.09	-2.87	-1.20	-1.76	-0.44	-1.23	-0.65
Thymus	-0.18	0.23	0.29	-0.52	-0.42	-0.69	-0.89	-0.11	0.06	Undet.	-2.52	-1.88	-1.27	-0.28	-0.58	-0.24	-0.77	-0.46
Tumor Mix	-0.57	-0.22	-0.27	-0.28	-0.42	-0.14	-0.35	-0.37	-0.28	-2.24	-1.86	-0.37	-2.51	-1.57	-1.81	-0.49	-0.09	-0.28
Normal Mix	0.00	0.00	0.00	0.00	-0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Target gene	FGD4	FGD4	FGD4	PTPN4	PTPN4	PTPN4	BTNL8	BTNL8	BTNL8	C6orf32	C6orf32	C6orf32	C6orf32	C6orf32
Primer set name	040_02	040_03	040_04	009_01	009_02	009_03	074_01	074_03	074_04	026_01	026_02	026_03	026_04	026_05
Brain,whole	-0.09	0.33	-0.01	-1.09	0.42	0.47	-0.57	-0.34	-0.95	-1.38	-1.41	0.28	-2.10	-0.06
Brain,cerebellum	-0.42	0.37	-0.04	-1.22	1.01	1.08	-0.95	-0.63	-1.39	-2.17	-2.08	-0.07	-2.94	Undet.
Fetal Brain	-0.49	0.76	0.24	-2.27	0.09	-0.01	-0.48	-0.22	-1.06	-2.23	-1.52	0.77	-1.68	0.33
Spinal Cord	0.47	0.37	0.23	-0.55	-0.10	-0.13	-0.42	-0.15	-0.85	-1.12	-1.03	-0.18	-2.54	-0.47
Colon	-0.94	-1.17	-0.26	-0.24	-0.73	-0.44	-1.08	1.08	1.22	-0.25	-0.73	-0.61	-2.93	-0.70
Heart	-1.11	-1.40	-1.39	-0.49	-1.10	-1.30	0.26	-0.88	-0.76	-1.09	-0.35	-1.39	-3.89	-1.01
Kidney	-0.66	-0.37	0.07	-0.61	-0.04	-0.13	0.02	-0.06	-0.57	-0.23	-0.91	-0.60	-2.97	-0.76
Liver	-0.29	0.07	0.00	-0.17	-0.64	-0.58	-0.27	-0.08	-0.58	-0.21	-0.48	-0.82	-2.76	-0.59
Lung (whole)	1.27	-0.33	0.71	1.18	0.41	0.66	1.70	-0.39	1.15	0.54	0.75	0.29	-1.47	0.44
Stomach	-0.06	0.65	0.62	0.20	-0.03	0.06	0.50	1.21	0.80	0.25	0.38	-0.07	-1.73	0.12
Trachea	-0.23	-0.43	-0.04	0.05	-0.33	-0.29	-0.30	0.53	-0.58	0.12	-0.07	-0.10	-2.09	-0.23
Prostate	-0.08	-0.58	0.39	0.16	0.21	0.22	Undet.	-0.06	-0.76	-0.06	-0.23	0.43	-1.52	0.11
Testis	-0.60	0.76	0.40	-0.92	0.14	0.08	-0.94	0.13	0.80	-1.02	-0.78	-0.39	-2.05	-0.11
Ovary	-0.26	0.20	0.70	-0.35	0.10	0.11	Undet.	0.55	-0.82	-0.62	-1.10	0.00	-2.37	-0.43
Placenta	0.15	-0.66	0.47	-0.31	-0.31	-0.35	0.27	0.12	-0.21	-0.71	0.11	0.12	1.69	0.86
Uterus	-0.52	-0.85	-0.42	-0.13	-0.37	-0.41	-1.06	-1.09	-0.96	-0.25	-0.51	0.97	-0.98	0.53
Spleen	0.76	-1.39	0.19	1.47	0.06	0.43	1.15	0.32	0.59	1.31	1.52	0.60	-0.74	1.22
Bone Marrow	1.21	-0.92	0.32	0.53	-0.36	-0.11	1.57	-0.24	0.81	0.38	0.76	-0.05	-1.37	0.46
Thymus	-0.85	-1.62	-0.98	-0.04	-0.43	-0.34	-0.55	-0.49	-0.76	1.16	0.56	0.40	-1.34	0.41
Tumor Mix	-0.48	-0.14	0.08	-0.38	-0.18	-0.17	-0.21	0.12	-0.14	-0.79	-0.66	-0.89	-2.28	-0.83
Normal Mix	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Target gene	AKT1	AKT1	AKT1
Primer set name	019_01	019_02	019_03
Brain,whole	-0.46	-0.06	-0.05
Brain,cerebellum	0.42	-0.11	-0.06
Fetal Brain	0.14	0.40	0.37
Spinal Cord	-0.28	-0.04	-0.25
Colon	0.12	0.19	0.21
Heart	-0.35	0.12	0.07
Kidney	-0.22	0.05	0.04
Liver	-0.12	0.23	0.19
Lung (whole)	0.24	0.75	0.68
Stomach	0.07	0.37	0.26
Trachea	0.36	0.34	0.27
Prostate	0.27	0.50	0.46
Testis	0.32	0.24	0.16
Ovary	0.85	0.29	0.30
Placenta	0.70	0.49	0.47
Uterus	0.00	0.31	0.34
Spleen	0.68	0.57	0.51
Bone Marrow	0.19	-0.09	-0.13
Thymus	0.60	0.11	0.04
Tumor Mix	-0.44	-0.04	-0.01
Normal Mix	0.00	0.00	0.00
NT2 RA(-)	-1.19	-0.78	-0.76
NT2 RA(+) 24hr A	-0.77	-0.95	-0.94
NT2 RA(+) 1week	-0.23	-0.63	-0.52
NT2 RA(+)	1.46	-0.08	-0.04
NT2 RA(+)Inh(+)	0.13	-0.09	-0.05
NT2 Neuron	-1.18	-1.15	-1.74

Target gene	FGF13	FGF13	FGF13	FGF13	OXR1	OXR1	OXR1
Primer set name	003_01	003_02	003_04	003_05	090_02	090_04	090_06
Brain,whole	0.45	0.49	1.27	1.11	1.04	-1.00	0.57
Brain,cerebellum	0.05	-0.06	-1.14	-0.42	-0.34	-0.63	0.95
Fetal Brain	1.50	1.94	1.42	1.53	-0.39	-1.16	-0.22
Spinal Cord	0.14	0.12	0.14	0.16	-0.79	-0.29	0.07
Colon	-0.09	0.01	-0.15	-0.10	-1.87	-0.37	-0.67
Heart	-1.45	-1.46	-1.21	-1.21	Undet.	-1.54	-1.60
Kidney	-0.05	0.33	-0.12	0.01	0.11	-0.92	-0.39
Liver	-0.33	-0.24	-0.79	-0.61	Undet.	-0.55	-0.67
Lung (whole)	-0.28	-0.07	0.43	0.31	-1.57	0.19	0.28
Stomach	0.45	0.46	0.19	0.26	0.15	0.20	0.17
Trachea	0.14	0.45	-0.20	0.10	-0.87	0.19	-0.21
Prostate	1.00	1.21	0.29	0.67	-0.39	0.28	0.24
Testis	0.40	0.30	0.25	0.27	0.62	0.59	0.31
Ovary	0.08	0.27	0.37	0.20	-1.75	0.27	0.00
Placenta	0.61	0.63	0.43	0.47	-0.94	-0.14	-0.33
Uterus	0.10	0.16	0.50	0.38	-0.80	0.04	-0.22
Spleen	-0.74	-0.43	0.66	0.56	-1.73	0.47	0.09
Bone Marrow	-0.32	0.04	-0.02	-0.02	-1.35	-0.01	-0.15
Thymus	-0.91	-0.60	-0.55	-0.70	-0.71	0.01	-0.61
Tumor Mix	-0.26	0.10	-1.02	-0.43	0.87	-0.35	0.04
Normal Mix	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kidney Tumor	-0.05	0.50	-0.80	-0.02	1.47	-0.68	0.13
Lung Tumor	-0.68	-0.45	-1.67	-0.47	0.24	-1.40	-1.28
Stomach Tumor	-0.15	0.07	-1.27	-0.12	-1.11	-0.37	-0.03
Uterus Tumor	-1.70	-0.98	-0.66	-0.93	-1.67	-0.05	-0.05
Colon Tumor	-0.64	-0.79	-0.66	-0.45	Undet.	-0.68	-0.87
Liver Tumor	-0.39	-0.26	-0.44	-0.35	-1.48	0.43	0.28
Ovary Tumor	-0.37	-0.22	-1.01	-0.61	Undet.	-0.13	-0.54

Undet.: undetective data