Identification and characterization of human non-coding RNAs with tissue specific expression

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Abstract

We have examined the expression profile of selected non-coding RNAs (ncRNAs) in 11 human tissues. Among 5489 full-length cDNA clones annotated as non-protein-coding transcripts in the H-Invitational database, we chose 150 clones for further analysis based on their gene structure and EST information. Expression profiling using quantitative RT-PCR and Northern blot hybridization revealed that the majority of the selected ncRNAs exhibited tissue specificity: 67% are predominantly expressed in a restricted subset of tissues. The absolute quantification of representative ncRNAs revealed that the majority of ncRNAs are expressed as low abundance transcripts. A comparative genomic analysis revealed that only 27% of the selected ncRNAs have mouse counterparts. Since the expression patterns of the human ncRNAs having no mouse counterparts remain to be similar to those of the mouse ncRNAs, the expression patterns of the selected ncRNAs may be conserved between human and mouse.

Keywords: non-coding RNA; H-Invitational; expression profile; quantitative RT-PCR; northern blot hybridization

Post-genomic transcriptome analysis has revealed the existence of large numbers of transcripts that lack protein-coding potential and are therefore expected to exist as non-protein-coding RNAs [1, 2, and refs therein]. Since this transcriptome analysis is based on sequencing of a full-length cDNA library constructed from RNA molecules possessing both a 5' cap structure and 3' poly(A) tail, these ncRNAs have mRNA-like features at their termini. Whether all these transcripts are functional, independent transcription units or just experimental artifacts derived from truncated mRNAs is still in dispute. However, a number of these mRNA-like ncRNAs appear to play pivotal roles in mammalian cells, including transcriptional regulation, genomic imprinting, and protein transport [3, 4, 5]. Such evidence raises the intriguing possibility that ncRNAs play an important role in controlling various aspects of gene expression [1, 2, and refs therein].

The primary dataset of the human transcriptome is available in the H-Invitational database (H-InvDB, <http://www.h-invitational.jp>). In the primary dataset from 2002, 1377 transcripts in which the longest ORF is less than 80 amino acids were isolated from 21,037 human full-length cDNAs. The transcripts in the H-InvDB have been classified based on their overlap with other transcripts, and the features of their 3' end, as well as the

existence of any corresponding EST clones. The putative ncRNAs include transcripts that are at a distance of >5kb from neighboring genes and are found in at least one EST database, and are thus likely to be genuine ncRNAs.

Here, we investigated the expression profiles of 150 selected human ncRNAs. Potential ncRNAs were selected from 5489 transcripts annotated in the H-InvDB2.2, updated in 2005, by several criteria. The resultant 150 ncRNAs were employed for expression profiling in 11 human tissues. Our RT-qPCR and Northern blot hybridizations revealed that the majority of human ncRNA were expressed in one or a few tissues at relatively low levels, suggesting that ncRNAs play specific biological roles in the tissues in which they are expressed.

Materials and Methods

The dataset of full-length cDNAs from which the ncRNAs are derived

The original dataset of human ncRNAs was selected from the H-InvDB2.2. We extracted 5489 non-protein-coding transcripts from the annotated database. Currently, the updated dataset has been released as H-Inv3. Non-protein-coding transcripts in the previous release are now annotated as hypothetical short proteins, but their IDs and sequences are unaltered. The dataset of the originally selected ncRNAs from H-InvDB2.2 is available in our original ncRNA database (<http://www.ncrna.org/> [6]).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNAs from adult human tissues were purchased from Clontech. Total RNA (1 µg) was reverse transcribed using the QuantiTect reverse transcription kit (Qiagen). The primers, designed by Primer3 software (<http://www-genome.wi.mit.edu/ftp/distribution/software/>), were purchased from Invitrogen. Aliquots of cDNA were subjected to real time PCR, performed using a LightCycler 480 SYBR Green I Master (Roche Diagnostics) following the manufacturer's protocol. For each reaction, no amplification was observed when reverse transcription was omitted. Information for primers used in this study is available upon request. To estimate relative abundance among ncRNAs, we used the "cross point" (CP) value as a reference. CP is the point at which the fluorescence of a sample rises above the background fluorescence and is given in number of amplification cycles. The CP of a sample depends on the initial concentration of target DNA in the sample. For some ncRNA targets, we performed absolute quantification analyses in which serial dilutions of an external standard of known concentration were used to create a standard curve. cDNA probes for Northern blot analyses were used as the external standards.

Northern blot hybridization

A 10 µg aliquot of total RNA per lane was separated on a 0.8% agarose gel containing 2% formaldehyde and transferred to a positively charged nylon membrane (HybondN+; Amersham Biosciences). After UV crosslinking, blots were hybridized to ³²P-labeled riboprobes at 60 °C, overnight, in hybridization buffer (5X SSC, 5X Denhardt solution, 50% formamide, 1% SDS and 0.1 mg/ml denatured salmon sperm DNA). An autoradiographic image was captured and quantified using a Fuji BAS2500 Bio-imaging analyzer. For low abundance ncRNAs, we optimized the hybridization efficiency by using a high specific activity (~2X10⁹ cpm/µg) cDNA probe and by increasing the probe concentration to 10 ng/ml. Hybridization was performed at 68 °C in ExpressHyb Hybridization Solution (Clontech) overnight. Blots containing polyA⁺ RNA from different human tissues (Human 12-lane MTN Blot or Human MTN Blot II) were purchased from Clontech.

Comparative genomics

Mouse counterparts of human ncRNAs were identified by sequence comparison and mapped onto the mouse genome. The mapping was performed using the liftOver program in the UCSC genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway> [7]) with the following changes in parameters. We lowered the minMatch and minBlocks parameters to 0.01. Flanking known genes of both query human ncRNA and putative counterpart sequence were withdrawn to validate whether the two genes were aligned in the same order in syntenic regions of each genome. In parallel, human ncRNAs were subjected to BLASTN searches against the FANTOM3 mouse cDNAs database (<ftp://fantom.gsc.riken.jp/fantomdb/3.0/>). The resultant hits were examined by manual inspection to determine if they were present in a mouse syntenic region.

Results and Discussion

Selection of ncRNAs from the database

The selection procedure is summarized in Fig. 1. We used several sets of selection criteria to identify putative ncRNAs, as follows. For the initial selection, the transcript must: 1) have no overlap with other gene transcripts, 2) not be truncated due to errors during cDNA synthesis, and 3) not be a transcript derived from retrotransposons or transposons. To select such ncRNAs, 5489 transcripts annotated in the H-InvDB2.2 were employed as the starting material. Among these 5489 transcripts, 712 "putative ncRNA" (as defined in H-InvDB) were subjected to the following additional selection criteria.

First, transcripts composed of >30% repetitive elements were excluded. Second, only transcripts possessing a poly (A) signal (AAUAAA or AUUAAA) within approximately 50 bp from the 3' end of the cDNA were included. Because relatively large numbers of cDNAs in the H-InvDB have poly (A) tail-like sequences at their 3' termini but lack the poly (A) signal, this selection step is important to exclude truncated cDNA clones. In such cases, the cDNA synthesis may have initiated from an adenine (A)-rich region in the middle of transcripts, resulting in 3'-truncated cDNAs. Third, transcripts that overlap with any other full-length cDNA clones in the H-InvDB were excluded. In case multiple ncRNAs are aligned to the same cluster, we adopt the longest one as a representative clone. This step was done in order to exclude the cDNAs that correspond to a portion of protein-coding genes. Finally, transcripts whose expression was validated by the existence of any EST clones were adopted for "ncRNA candidates". In a second selection protocol, transcripts which had >70% sequence homology to mouse transcripts were selected from 2564 "uncharacterized transcripts" (as defined in H-InvDB), and were then subjected to the additional selection criteria described above.

It has been reported that considerable fraction of cDNAs is a product of internal priming from the A-rich region [8,9]. We examined whether A-rich sequence is present at the 3' end of all the ncRNAs in the list. We also examined its presence in the genomic region immediately downstream of all the ncRNAs. The survey resulted in six clones with adjacent A-rich regions. However, four out of these six clones possess typical polyadenylation signals near their 3' termini, suggesting that polyA tails were added posttranscriptionally at those positions rather than produced by the internal priming. The remained two clones (HIT000002513 and HIT000018844) are likely to be internally primed, since they lack the typical polyadenylation signals and are followed by consecutive polyA stretches. The resultant 150 ncRNAs are listed in Supplemental Table 1. These 150 ncRNAs are expected to be intact and independent transcription units, and, thus, provide an appropriate ncRNA pool, free of the controversies described above, for an initial analysis of ncRNAs.

We noticed that a number of cDNAs are located near the 3' end of other protein-coding genes. Although it is possible that they are the 5' truncated cDNAs derived from the read-through transcripts of the upstream genes, we temporarily adopt these clones as the candidates of "the independent ncRNA" unless they overlap with the upstream cDNA clones. Our preliminary results of RNase protection assay indicated that at least two cDNAs in our list, which aligned close to the upstream protein-coding genes, possess their own 5' ends, therefore are the independent ncRNAs (data not shown). We cannot exclude the possibility that the selected ncRNAs code for small polypeptides; this

possibility could be addressed by determining the presence of each transcript in polysomal fractions.

The levels of ncRNAs expressed in HeLa cells were observed to decrease upon treatment with α -amanitin for 48 h, indicating that the majority of human ncRNAs are transcribed with RNA polymerase II, as are mRNAs (data not shown). Examination of these 150 ncRNAs revealed that only 60 ncRNAs (40%) are produced from intron-containing genes (Supplemental Table 1). This characteristic of ncRNA genes is distinct from protein-coding genes in which >90% are intron-containing genes. In mRNA biogenesis, pre-mRNA splicing is critical to determine the fate of the resultant mRNA as well as to remove introns [10]. These data raise an interesting possibility that the characteristic gene structure influences the intracellular behavior of ncRNAs, which may be distinct from that of mRNAs.

The majority of ncRNAs are expressed in a tissue-specific manner

To examine the distribution and relative abundance of the selected ncRNAs in human tissues, we used RT-qPCR with 11 adult human tissues. Experiments were repeated at least twice for each primer-pair using template tissue RNAs from different individuals with some exceptions. Representative profiling results are shown in Fig. 2A. We divided tissue-specific expression patterns into three categories: 1) ncRNAs exclusively or predominantly expressed in a single tissue; 2) ncRNAs expressed in a limited number of tissues; and 3) ncRNAs ubiquitously expressed. To quantify expression of ncRNAs, the ncRNAs exclusively or predominantly expressed in a single tissue were scored for the corresponding tissue. Clones expressed in a few tissues were scored only once as being expressed in the tissue with the highest expression, and were not counted for the rest of tissues expressing the clone. Clones ubiquitously expressed in all tissues were scored separately as “ubiquitous”.

Of the 150 candidate ncRNAs, we detected expression of 121 ncRNAs in at least one of the tissues tested (Fig. 2B). Forty-four ncRNAs (29%) were expressed exclusively or predominantly in one tissue, and 56 ncRNAs (38%) were expressed in a small subset of tissues. The rest of the ncRNAs were ubiquitously expressed (21 ncRNAs; 14%). These data show that 81% of ncRNAs were expressed in at least one of the 11 tissues examined, and 67% of ncRNAs exhibited a tissue-specific expression pattern. In contrast, no expression was detected for 29 ncRNAs (19%), at least in the 11 tissues we examined and with the PCR primer pairs we used. These data reveal that the majority of human ncRNAs are expressed in a tissue-specific manner, and suggest that the selected ncRNAs play specific physiological roles in distinct tissues rather than housekeeper roles in every tissue.

The result also excludes the possibility that ncRNA-like transcripts merely originate from genomic DNA contamination and, therefore, are non-functional.

We tabulated the tissue-distribution patterns by classifying each ncRNA with respect to tissue specificity, according to the three categories defined above (Supplemental Table 2). These data are summarized in Fig. 2C. The number of ncRNAs predominantly expressed in testis was conspicuous; testis specific ncRNAs formed the largest subset, containing 29% of all the expressed ncRNAs. The second largest group was those ncRNAs predominantly expressed in brain, which contained 17% of all the expressed ncRNAs. The ncRNAs specifically expressed in thymus followed with 7%. More than 50% of ncRNAs belong to these three groups. Similar tendencies were observed in mouse ncRNAs [9], implying that ncRNAs may be involved in complex regulatory mechanism(s) in testis and brain in mammals. Recently, Pollard et al. reported that HAR1, an RNA that has evolved more rapidly in humans than in other mammals, was expressed in a specific region of the neocortex in the developing human brain [11]. It will be of interest to compare the brain-specific ncRNAs from this study with respect to the sequence conservation across various mammalian species. Some ncRNAs specifically expressed in testis have also been reported in mouse [12]. Furthermore, novel classes of small RNAs (piRNA or rasiRNA) are expressed only in germ line organs in mammals and in *Drosophila* [13]. Together with small RNA species, testis-specific ncRNAs may impart yet another dimension of gene regulatory mechanisms to the testis.

The majority of ncRNAs are low abundance transcripts

To confirm the RT-qPCR results, we performed Northern blot analyses. We used riboprobes to verify the transcript orientation because some ncRNA-like transcripts were registered with the opposite orientation in the H-InvDB and, in some cases; the antisense strand was also transcribed. For ncRNAs that exhibited relatively high abundance in RT-qPCR experiments, we detected bands closely corresponding to the registered length in the H-InvDB (Fig. 3: brain, thymus, heart, lung, testis, placenta, skeletal muscle and ubiquitous). We observed additional bands in the blots of the representative ncRNAs for brain, testis, and placenta. Interestingly, the transcript distribution pattern of the representative placenta ncRNA obtained by RT-qPCR analysis (Fig. 2A) was most similar to the distribution of the additional band (the open arrowhead in the panel of Placenta of Fig. 3) rather than the distribution of the expected size band.

ncRNAs whose "cross point" (CP) values in RT-qPCR (see Materials and Methods) were over 30 were undetectable in conventional Northern blot hybridization conditions (e.g. Supplemental Figure upper panel: liver, spleen, kidney, prostate). Therefore, we switched

to a cDNA probe and employed poly (A)⁺ RNA blots in order to maximize hybridization sensitivity. This change was successful only for the liver specific ncRNA. For the rest of the ncRNAs, changing the probe and/or RNA blot did not enable us to detect hybridization (Supplemental Figure lower panel: spleen, kidney, prostate). This prompted us to perform an absolute quantification of ncRNA transcripts in tissue samples. We estimated the amount of target RNA in 10 µg of total tissue RNA, since this amount corresponded to the amount of RNA per lane in the Northern blot gels. The amount of target ncRNA was estimated to be 0.46 fg in prostate (CP=33), 0.96 fg in kidney (CP=32), 9.83 fg in liver (CP=30) and 1.17 pg in skeletal muscle (CP=22). Thus, the CP values roughly reflected the actual ncRNA levels.

Our hybridization conditions were able to detect as little as 0.1 pg RNA per lane; therefore, this quantification data was consistent with the Northern hybridization results in which a clear detection threshold existed at approximately CP30. Even for clones where bands were successfully detected, CP values in the RT-qPCR experiments were much higher, and band intensity in Northern blot hybridizations were much lower, than values for abundant mRNAs such as β-actin (CP=20), or glycerol-3-phosphate dehydrogenase (CP=17) (data not shown). The CP values for the majority of ncRNAs were over 30. Thus, we concluded that most of the ncRNA candidates are low abundant transcripts. This low abundance may indicate that tissue-specific ncRNA expression is restricted to certain cell types in the tissues. In situ hybridization against human tissue samples would confirm this interesting possibility.

Expression-patterns exhibited by the human ncRNAs are shared between the human and mouse ncRNAs

Our analyses above revealed characteristic expression profiles of human ncRNAs that mirrored the expression profiles of mouse ncRNAs. Using comparative genomics, we assessed the degree of overlap between human ncRNAs and mouse ncRNAs. First, the presence of the syntenic region in the mouse genome was searched by identifying the neighboring genes of each of the 150 ncRNAs in the human genome. The syntenic regions in the mouse genome were mapped for 93 ncRNAs. Second, the presence of any mouse transcripts with significant sequence similarity to the human ncRNAs was searched using the BLASTN program, and 74 ncRNAs were found to correspond to mouse cDNA clones in the FANTOM3 datasets (P-value=<0.1). Finally, we examined whether the 74 mouse cDNA clones mapped to syntenic regions, and found that 40 mouse cDNAs mapped to syntenic regions of the genome. A data collection of this search is available at our website (<http://www.ncrna.org/papers/sasaki/>). We concluded that 40 ncRNAs (27% of 150

ncRNAs) are conserved between human and mouse. We merged the result of this counterpart search with the tissue-specificity profiling data to assess the degree of overlap in expression between human and mouse ncRNA. Fig. 2D shows that human ncRNAs having mouse counterparts exist in most of the tissues examined, with the exception of spleen, liver and placenta. It should be noted that three human ncRNAs for which we did not detect expression did indeed have mouse counterparts, suggesting that they may be transcribed in tissues other than the 11 tissues we examined. We found that a number of brain- or testis-specific human ncRNAs lacked mouse counterparts. This evidence raises the intriguing possibility that these ncRNAs are involved in phenomena specific to humans. Recently, a pattern of tissue specificity similar to human has been reported using microarray analysis of a much larger population of mouse ncRNAs [9]. Our comparative genomic analysis combined with the expression profiling data support the hypothesis that the characteristic expression profile exhibited by human ncRNAs is conserved in the mouse transcriptome despite of low degree of overlap between human and mouse ncRNAs.

In summary, we have identified large numbers of tissue-specific ncRNAs in human, most of which are present as low abundance transcripts. Some ncRNAs are conserved between human and mouse, but the majority of ncRNAs found in human were not present in mouse. Further analysis of individual ncRNAs will reveal important functions in the respective human tissues. Our selected ncRNA collection will provide an important resource for that line of analysis.

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Figure Legends

Figure 1

Screening procedure to identify ncRNAs from H-InvDB2.2. Putative ncRNAs and uncharacterized cDNA entries were subjected to manual inspection by several criteria. See text for details.

Figure 2

Expression profile of ncRNA candidates. (A) Tissue-distribution pattern of the representative ncRNAs. Relative abundance among 11 different tissues was examined by RT-qPCR for each ncRNA. Representative ncRNA clones for each of 11 tissues and a clone representing the “ubiquitous” pattern are shown. The abundance in each tissue was normalized and aligned in following order: (from left to right) brain, thymus, heart, lung, liver, spleen, kidney, testis, placenta, prostate, and skeletal muscle. Bars in the histogram were color-coded as follows: black, exclusive or predominant in a single tissue; dark gray, expressed in several tissues; light gray, ubiquitous. See Supplemental Table 3 for ID of representative clones. (B) Summary of RT-qPCR for 150 ncRNAs. The same color-code is used as in the histograms in (A) and white indicates the ncRNAs whose expression was not detected in any of the 11 tissues. The results are tabulated in Supplemental Table 2. (C) The tissue-specific expression pattern of all ncRNAs is summarized. (D) Comparison of tissue specificity of the selected ncRNAs in human and mouse. The results of the human-mouse counterpart search were merged onto the human ncRNA tissue specific distribution histogram shown in (C). The ncRNAs having no mouse counterparts are shown in black in each bar.

Figure 3

Northern blot analysis of representative ncRNAs. Eight representative clones probed with riboprobes. Total RNAs from 11 tissues and HeLa cells were analyzed. Positions of RNA size markers are shown to the left of each blot. CP value is shown in parentheses after the name of each tissue. Filled arrowheads indicate the bands corresponding to the expected size while open arrowheads indicate additional hybridization bands. Tissue abbreviations: Br; brain, Th; thymus, He; heart, Lu; lung, Li; liver, Sp; spleen, Ki; kidney, Te; testis, Pl; placenta, Pr; prostate, Mu; skeletal muscle. IDs and sizes of ncRNAs are given in Supplemental Table 3.

Fig. 1 Sasaki et al.

Fig. 2 Sasaki et al.

Fig. 3 Sasaki et al.

Supplemental Figure Sasaki et al.

Supplemental Figure Legend

Supplemental Figure

Northern blot analysis of representative ncRNAs. Extremely rare ncRNAs were hybridized with high specific activity cDNA probes. Positions of RNA size markers are shown to the left of each blot. CP value is shown in parentheses after the name of each tissue. Upper panels. Blots are the same as in Fig. 3. Lower panels. Commercially obtained poly(A)+RNA blots from 12 human tissues were probed with ncRNAs representing liver and kidney. Commercially obtained poly(A)+ blots from 8 tissues were probed with ncRNAs representing spleen and prostate. Tissue abbreviations: Br; brain, Co; colon, He; heart, In; small intestine, Ki; kidney, Leu; peripheral blood leukocyte, Li; liver, Lu; lung, Mu; skeletal muscle, Ov; ovary, Pl; placenta, Pr; prostate, Sp; spleen, Te; testis, Th; thymus. IDs and sizes of ncRNAs are given in Supplemental Table 3.

Supplemental Table 1. A list of 150 selected human ncRNA candidates

ncRNA candidate	Accession No.	Category* ¹	Conservation	intron	EST* ²	cDNA library	H-Angel	Tissue-specificity	ncRNA candidates
HIT000002513	AK000038	-		-	A	adipose tissue	eye, placenta, lung	* ³ lung, liver	HIT000002513
HIT000003532	AK001058	+		-	A	whole embryo, mainly head	placenta	lung, placenta, heart	HIT000003532
HIT000004025	AK001551	+		+	B	NA	liver		HIT000004025
HIT000004056	AK001582	+		+	B	NA	cerebrum		HIT000004056
HIT000004377	AK001903	unch	+	-	A	placenta	ubiquitous		HIT000004377
HIT000004808	AK021534	unch	+	-	A	placenta	lung or ubiquitous	testis, lung, brain, thymus, prostate	HIT000004808
HIT000006301	AK023027	unch	+	+	A	NA	cerebrum	brain, testis	HIT000006301
HIT000007366->	AK024092	unch	+	-	A	whole embryo, mainly head	lung or ubiquitous	* ³ lung, brain, thymus, testis, heart	HIT000007366->92364
HIT000007377	AK024103	unch	+	-	A	whole embryo, mainly head	ubiquitous	* ³ muscle	HIT000007377
HIT000007630	AK024356	unch	+	-	A	placenta	spine, breast	heart, muscle, brain	HIT000007630
HIT000008220	AK024946	+		-	A	colon	ubiquitous	heart, brain	HIT000008220
HIT000009660	AK026386	+		+	C	human small intestine	cerebrum	testis	HIT000009660
HIT000009690	AK026416	+		+	C	ileal mucosa	ubiquitous	kidney, liver	HIT000009690
HIT000010157	AK026883	+		+	A	NA	ubiquitous	* ³ lung, testis, spleen	HIT000010157
HIT000010473	AK027199	unch	+	-	A	lung	ubiquitous	brain, testis, lung, placenta, thymus	HIT000010473
HIT000010507	AK027233	+		+	C	lung	?	lung, testis	HIT000010507
HIT000011439	AK054825	+		+	C	cerebellum	cerebrum, colon, kidney	heart, brain	HIT000011439
HIT000011547	AK054933	+		-	B	cerebellum	adipose, breast	brain, thymus	HIT000011547
HIT000011622	AK055008	+		-	C	cerebellum	cerebrum	muscle, brain, heart	HIT000011622
HIT000011636	AK055022	+		-	A	cerebellum	pancreas	thymus, lung, spleen	HIT000011636
HIT000011828	AK055214	unch	+	-	A	NA	ubiquitous	prostate, testis, skeletal	HIT000011828
HIT000011885	AK055271	+		-	C	brain	?	testis, heart, brain	HIT000011885
HIT000011965	AK055351	+		+	B	brain	colon		HIT000011965
HIT000012073	AK055459	+		+	A	brain	breast	brain, prostate, lung	HIT000012073
HIT000012128	AK055514	+		+	B	NA	testis	testis	HIT000012128
HIT000012261	AK055647	+		-	A	NA	cerebrum	brain, testis	HIT000012261
HIT000012319	AK055705	+		-	B	NA	cerebrum		HIT000012319
HIT000012733	AK056119	+		+	B	NA	colon, kidney	testis, (lung, prostate)	HIT000012733
HIT000012825	AK056211	+		+	C	NA	ovary	* ³ testis	HIT000012825
HIT000012901	AK056287	+		+	C	NA	?		HIT000012901
HIT000013099	AK056485	+		+	C	NA	?		HIT000013099
HIT000013398	AK056784	+		+	A	placenta	heart	placenta, brain, lung	HIT000013398
HIT000013588	AK056974	unch	+	-	A	skeletal muscle	ubiquitous	lung, brain, testis, heart, placenta	HIT000013588
HIT000013730->	AK057116	+		+	A	spleen	?	muscle, testis, heart, thymus, brain	HIT000013730->251316
HIT000013840	AK057226	+		-	C	testis	?		HIT000013840
HIT000013867	AK057253	+		+	C	testis	?	testis	HIT000013867
HIT000013911	AK057297	+		+	C	testis	?		HIT000013911
HIT000013965	AK057351	+		+	C	testis	?	testis	HIT000013965
HIT000014168	AK057554	+		+	B	thymus	?	thymus	HIT000014168
HIT000014470	AK057863	+		+	A	brain	cerebrum	brain, (testis, heart)	HIT000014470

HIT000014545	AK057938	+		-	C	NA	testis		HIT000014545
HIT000015843	AK090976	+		-	C	amygdala	liver	testis	HIT000015843
HIT000016553	AK091686	+		-	B	brain	cerebrum	spleen, brain	HIT000016553
HIT000016555	AK091688	+		+	A	brain	placenta	brain, testis, prostate	HIT000016555
HIT000016590	AK091723	unch	+	-	A	NA	retina	*3brain	HIT000016590
HIT000016701	AK091834	+		+	A	lung	?	Lung, (prostate)	HIT000016701
HIT000017066	AK092199	+		+	B	NA	brain stem	testis	HIT000017066
HIT000017408	AK092541	+		+	B	prostate	?	*3testis, (kidney, lung)	HIT000017408
HIT000017445	AK092578	+		+	A	prostate	?	*3kidney	HIT000017445
HIT000017869	AK093002	+		+	A	spleen	?	*3prostate	HIT000017869
HIT000018283	AK093416	+		+	A	testis	testis, placenta	testis, lung, placenta	HIT000018283
HIT000018359	AK093492	+		+	C	testis	ubiquitous	testis, kidney	HIT000018359
HIT000018388	AK093521	+		-	A	testis	cerebrum, placenta, muscle	thymus	HIT000018388
HIT000018698	AK093832	unch	+	-	A	trachea	ubiquitous	heart, lung, brain	HIT000018698
HIT000018839	AK093982	unch	+	-	A	uterus	kidney, artery/aorta, prostate, muscle		HIT000018839
HIT000018844	AK093987	+		?	B	uterus	prostate, colon	prostate, placenta	HIT000018844
HIT000019009	AK094154	+		+	C	NA	muscle	placenta, testis, brain	HIT000019009
HIT000019317	AK094462	+		+	C	cerebellum	cerebellum	brain, kidney	HIT000019317
HIT000019431	AK094576	+		+	B	amygdala	?	testis, (lung, brain)	HIT000019431
HIT000019460	AK094605	-	-	-	C	amygdala	?		HIT000019460
HIT000019599	AK094744	+		-	A	brain	cerebrum, kidney	brain, kidney, spleen	HIT000019599
HIT000019668	AK094813	+		-	B	brain	muscle	prostate, testis, brain	HIT000019668
HIT000019901	AK095046	-	+	-	A	hippocampus	cerebrum, stomach	brain	HIT000019901
HIT000020054	AK095199	+	+	+	B	subthalamic nucleus	cerebrum, breast		HIT000020054
HIT000020137	AK095282	+		+	B	tongue, tumor tissue	artery/aorta		HIT000020137
HIT000020163	AK095308	+		-	B	tongue, tumor tissue	testis, muscle		HIT000020163
HIT000020554	AK095699	+		+	B	brain	?	testis	HIT000020554
HIT000020699	AK095844	+		-	B	NA	?	brain	HIT000020699
HIT000020920	AK096065	+		-	A	kidney	pineal gland	testis	HIT000020920
HIT000020989	AK096134	+		+	B	liver	liver	liver, kidney	HIT000020989
HIT000020998	AK096143	+		+	A	liver	breast, liver	liver	HIT000020998
HIT000021556	AK096701	-	-	-	A	pericardium	ubiquitous	heart, muscle	HIT000021556
HIT000021635	AK096780	+		-	C	prostate	lung, kidney, pancreas	*3testis, kidney	HIT000021635
HIT000021759->	AK096904	unch	+			skeletal muscle	ubiquitous	muscle	HIT000021759->45761
HIT000022361	AK097507	+		-	C	testis	?	testis	HIT000022361
HIT000022501	AK097647	+		-	B	testis	?	*3testis, (thymus)	HIT000022501
HIT000022977	AK098142	+		-	A	trachea	adipose, lung, cerebrum	lung, brain, heart, spleen, brain, placenta, heart	HIT000022977
HIT000023168	AK098338	unch	+			uterus		thymus	HIT000023168
HIT000023427	AK098597	+		+	B	testis	cerebrum, breast	*3thymus, spleen, kidney, brain, lung,	HIT000023427
HIT000023486	AK098656	+		+	B	testis	?		HIT000023486
HIT000023747	AL049321	unch	+			brain	ubiquitous	testis, heart, lung	HIT000023747
HIT000024044	AL050166	-	-	-	A	uterus	ubiquitous	lung, brain	HIT000024044
HIT000024203	AL080082	+	+	-	B	brain	placenta	thymus, testis, prostate	HIT000024203
HIT000024337	AL080216	unch	+			uterus	ubiquitous	testis, lung, heart, brain, thymus	HIT000024337

HIT000024429	AL110163	unch	+	-	A	uterus	cerebrum	testis, heart, brain, lung, placenta, heart, lung	HIT000024429
HIT000024446	AL110180	unch	+	-	A	brain	colon, liver	testis, lung, brain, prostate, spleen	HIT000024446
HIT000024455	AL110189	-	+	-	C	testis	?		HIT000024455
HIT000024721	AL117559	+		-	B	kidney	cerebrum	*3thymus, (brain)	HIT000024721
HIT000024815	AL117653	unch	+			uterus		heart, muscle	HIT000024815
HIT000025044	AL133577	unch	+	-	A	testis	ubiquitous	testis	HIT000025044
HIT000025071	AL133604	+		-	C	testis	muscle	testis	HIT000025071
HIT000025686	AL137398	+		-	B	testis	testis	testis	HIT000025686
HIT000025786	AL137498	unch	+			amygdala	ubiquitous	*3brain, kidney	HIT000025786
HIT000026049	AL137761	+		-	A	uterus	ubiquitous	testis, kidney, prostate, lung, brain	HIT000026049
HIT000026243	AL162073	+		-	A	melanoma	ubiquitous	lung, prostate, placenta, muscle, testis	HIT000026243
HIT000027034	AL832122	+	+	-	A	cervix	prostate, cerebrum, muscle	testis, (thymus)	HIT000027034
HIT000027048	AL832136	+	+	-	B	cervix	endocrine, exocrine	*3thymus, spleen	HIT000027048
HIT000027165	AL832253	+		-	B	lymph node	muscle		HIT000027165
HIT000027207	AL832295	+		+	C?	lymph node	ubiquitous		HIT000027207
HIT000027585	AL832673	unch	+			adipose	ubiquitous	brain, testis, spleen, lung, prostate	HIT000027585
HIT000027821	AL832909	unch	+	-	A	melanoma	ubiquitous	testis, heart, brain	HIT000027821
HIT000028053	AL833141	unch	+			heart	peripheral nerve, pancreas	muscle, heart, brain	HIT000028053
HIT000028152	AL833240	+		-	A	amygdala	lung, cerebrum	brain, (lung)	HIT000028152
HIT000028245	AL833333	+		+	C	testis	testis, cerebrum	testis	HIT000028245
HIT000042993	AK123120	+		+	B	cerebellum	?	testis, (prostate, brain)	HIT000042993
HIT000043067	AK123194	+		+	B	cerebellum	breast	testis, brain, prostate	HIT000043067
HIT000043322	AK123449	+		-	A	subthalamic nucleus	cerebrum, bone		HIT000043322
HIT000043631	AK123758	+		+	B	NA	?		HIT000043631
HIT000043639	AK123766	+		+	B	NA	?	prostate, heart, lung	HIT000043639
HIT000043674	AK123801	+		+	B	ovary	?	spleen, testis	HIT000043674
HIT000044126	AK124253	unch	+			kidney, tumor tissue	cerebrum, ovary	thymus, (brain)	HIT000044126
HIT000044150	AK124277	+		+	C	liver, tumor tissue	liver	liver	HIT000044150
HIT000044154	AK124281	+		-	A	liver, tumor tissue	breast		HIT000044154
HIT000044215	AK124342	+		+	C	uterus	?	brain, testis, muscle	HIT000044215
HIT000044222	AK124349	+		+	C	uterus	colon, liver, lung		HIT000044222
HIT000044272	AK124399	+		+	A	bladder	?	*3thymus, testis, kidney, spleen, heart	HIT000044272
HIT000044649	AK124776	+		-	A	brain	cerebrum, skin	brain, kidney	HIT000044649
HIT000044812	AK124939	+		-	A	subthalamic nucleus	?	brain, heart, liver	HIT000044812
HIT000044831	AK124958	+		-	A	subthalamic nucleus	ubiquitous	brain, prostate, lung	HIT000044831
HIT000044861	AK124988	+		-	C	thalamus	eye, muscle		HIT000044861
HIT000044963	AK125090	+		-	A	tongue, tumor tissue	artery/aorta, placenta, colon	placenta, testis, lung	HIT000044963
HIT000045023	AK125150	unch	+	-	A	brain	ubiquitous	brain, thymus	HIT000045023
HIT000045537	AK125664	+		-	A	synovial membrane tissue from	ubiquitous	brain, testis, kidney	HIT000045537
HIT000046198	AK126325	+		+	A	trachea	cerebrum, lung		HIT000046198
HIT000046322	AK126449	+		?	A	uterus	?	lung, testis, kidney, muscle, placenta	HIT000046322
HIT000047747	AK127874	+		+	C	prostate	cerebrum, breast	testis, (brain)	HIT000047747
HIT000048359	AK128486	+		+	A	trachea	?	brain, (lung)	HIT000048359
HIT000048747	AK128874	+		+	A	hippocampus	?	brain, spleen, liver, placenta, testis	HIT000048747

HIT000048811	AK129540	+		-	C	pancreas	breast	* ³ testis	HIT000048811
HIT000049265	AK129994	+		+	C	kidney	colon		HIT000049265
HIT000049320	AK130049	-	+	-	A	kidney	artery/aorta, vein, placenta, muscle, breast	kidney, testis, heart, lung	HIT000049320
HIT000049338	AK130067	+		-	A	lung	brain stem	heart, lung, kidney, placenta	HIT000049338
HIT000049924	AK130653	unch	+			spleen	ubiquitous	testis, thymus, lung, heart, brain	HIT000049924
HIT000050135	AK130864	+		+	A	testis	artery/aorta, muscle	testis	HIT000050135
HIT000050297	AK131026	+		+	B	testis	ubiquitous		HIT000050297
HIT000050965	BC029043	+		-	B	brain	?		HIT000050965
HIT000051024	BC031235	+		+	B	testis	ubiquitous	* ³ testis	HIT000051024
HIT000051342	BC035189	+		-	B	testis	ubiquitous	* ³ testis	HIT000051342
HIT000054382	BX537597	unch	+			human fetal kidney	?	testis, thymus, lung, heart, brain	HIT000054382
HIT000054391	BX537606	+		-	A	human rectal tumor	bone		HIT000054391
HIT000054535	BX537750	unch	+			retina	ubiquitous	thymus, testis, lung, heart, brain	HIT000054535
HIT000054547	BX537762	unch	+	-	A	liver	?	testis, heart, lung	HIT000054547
HIT000054926	BX538144	-	-	-	A	fetal kidney	cerebrum, adipose, colon	testis, heart, brain, kidney, thymus	HIT000054926
HIT000055032	BX538250	+		+	A	testis	heart	testis, brain, thymus	HIT000055032
HIT000055119	BX538337	unch	+			rectum tumor	?	testis, heart	HIT000055119
HIT000055794	BX647147	-	+	-	A	human uterine endothelium prim	cerebrum, skin, testis, muscle, kidney	heart, testis, brain	HIT000055794
HIT000056511	BX647864	+		+	B	adipose	bladder, cerebrum	kidney, testis	HIT000056511
HIT000056687	BX648040	+		-	C	liver	muscle	heart, muscle	HIT000056687
HIT000057296	BX648649	+		-	C	fetal kidney	lung, cerebrum	kidney, heart, lung	HIT000057296
HIT000057775	BX649128	unch	+	-	A	amygdala	ubiquitous	* ³ brain, thymus, spleen, lung, prostate	HIT000057775

*1Category: '+'; putative ncRNA, unch; uncharacterized ncRNA, '-'; all others

*2 No. of supported ESTs: A; >10, B; 5-9, C; 1-4

*3Result of single experiment

*4A-rich region: to be met following two criteria: 1) a polyA tract consecutive of at least seven nucleotides, 2) eight As out of ten nucleotides immediately downstream of 3'-end

key:

testis, (brain)
heart, testis, brain
testis, prostate, liver, lung, heart

predominantly expressed in one tissue (i.e., testis), with minor expression elsewhere (i.e., brain)

expressed in limited number of tissues, i.e., heart, testis, and brain

ubiquitously expressed, only top 5 tissues are listed

no expression was observed in 11 tissues examined

Supplemental Table 2. ncRNAs that exhibited tissue-specific expression patterns.

	Brain	Thymus	Heart	Lung	Liver	Spleen	Kidney	Testis	Placenta	Prostate	Muscle	Ubiquitous no expression	
1	6301	11636	7630	2513	20989	16553	9690	9660	13398	11828	7377	4808	4025
2	11547	14168	8220	3532	20998	43674	17445	11885	19009	17869	11622	7366	4056
3	12073	18388	11439	10157	44150		49320	12128	44963	18844	21759	10473	4377
4	12261	23168	18698	10507			56511	12733		19668	28053	13588	11965
5	14470	24203	21556	16701			57296	12825		43639		13730	12319
6	16555	24721	24815	24044				13867				22977	12901
7	16590	27048	49338					13965				23427	13099
8	19317	44126	55794					15843				24337	13840
9	19599		56687					17066				24429	13911
10	19901							17408				24446	14545
11	20699							18283				26049	18839
12	25786							18359				26243	19460
13	28152							19431				27585	20054
14	44215							20554				44272	20137
15	44649							20920				46322	20163
16	44812							21635				48747	23486
17	44831							22361				49924	24455
18	45023							22501				54382	27165
19	45537							23747				54535	27207
20	48359							25044				54926	43322
21								25071				57775	43631
22								25686					44154
23								27034					44222
24								27821					44861
25								28245					46198
26								42993					49265
27								43067					50297
28								47747					50965
29								48811					54391
30								50135					
31								51024					
32								51342					
33								54547					
34								55032					
35								55119					
36													
37													
38													
39													
40													

The last 5-digits of the H-InvDB ID (HIT number) are shown.

Clones exclusively or predominantly expressed in a given tissue are typed in blue.

Clones expressed in a limited number of tissues are typed in black.

Clones used in Northern blot hybridization experiments are highlighted.

Clones having a putative mouse counterpart are typed in bold.

Supplemental Table 3. The representative ncRNA clones shown in Fig. 2A and used in the Northern blot hybridization analyses.

Tissue	H-Inv ID	Accession No.	Length (bp)	CP**
Brain	HIT000014470	<u>AK057863</u>	816	25
Thymus	HIT000014168	<u>AK057554</u>	2596*	24
Heart	HIT000056687	<u>BX648040</u>	3995	27
Lung	HIT000016701	<u>AK091834</u>	3093	25
Liver	HIT000020998	<u>AK096143</u>	1826	31
Spleen	HIT000016553	<u>AK091686</u>	2256	31
Kidney	HIT000009690	<u>AK026416</u>	1948	32
Testis	HIT000025686	<u>AL137398</u>	1921	25
Placenta	HIT000044963	<u>AK125090</u>	2408	29
Prostate	HIT000018844	<u>AK093987</u>	2283	33
Skeletal Muscle	HIT000045761	<u>AK125888</u>	4939	22
Ubiquitous	HIT000027585	<u>AL832673</u>	5270	29

*: A probe was designed to also detect the abundant 0.5 kb splice variant.

** CP = "crossing point"