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H-DBAS: Alternative splicing database of completely sequenced and manually annotated full-length cDNAs based on H-Invitational

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ABSTRACT

The Human-transcriptome DataBase for Alternative Splicing (H-DBAS) is a specialized database of alternatively spliced human transcripts. In this database, each of the alternative splicing (AS) variants corresponds to a completely sequenced and carefully annotated human full-length cDNA, one of those collected for the H-Invitational human-transcriptome annotation meeting. H-DBAS contains 38 664 representative alternative splicing variants (RASVs) in 11 744 loci, in total. The data is retrievable by various features of AS, which were annotated according to manual annotations, such as by patterns of ASs, consequently invoked alternations in the encoded amino acids and affected protein motifs, GO terms, predicted subcellular localization signals and transmembrane domains. The database also records recently identified very complex patterns of AS, in which two distinct genes seemed to be bridged, nested or degenerated (multiple CDS): in all three cases, completely unrelated proteins are encoded by a single locus. By using AS Viewer, each AS event can be analyzed in the context of full-length cDNAs, enabling the user's empirical understanding of the relation between AS event and the consequent alternations in the encoded amino acid sequences together with various kinds of affected protein motifs. H-DBAS is accessible at http://jbirc.jbic.or.jp/h-dbas/.

INTRODUCTION

Alternative splicing (AS) is a phenomenon in which various combinations of exons are integrated into different types of transcripts. By utilizing AS, diverse transcripts can be produced. Although it might not be always true that all the variants are translated, this mechanism at least enables a single locus to encode functionally divergent proteins. Actual abundant cases have been reported for such diversification of the gene functions mediated by AS, in which the binding site of a growth factor receptor or an activation site of transcription factor are modified. Especially in mammals, use of AS is widespread [it is reported that 40–60% of entire human genes have AS variants (1)] and is supposed to provide a molecular basis for highly fabricated systems, such as immune systems and neural networks.

Because of the growing interests in AS, a number of databases were launched, such as ASD [http://www.ebi.ac.uk/asd/; (2)] and ASAP [http://www.bioinformatics.ucla.edu/ASAP/; (3)]. However, most of these preexisting AS databases are still incomplete in a sense that they are mainly based on the fragmented information of partially and imprecisely sequenced cDNAs (ESTs) or computationally divided information of the exons. In order to elucidate the functional

*To whom correspondence should be addressed. Tel: +81 3 3599 8800; Fax: +81 3 3599 8801; Email: imanishi@jbirc.aist.go.jp relevance of the alternative variants to the protein functions, comprehensive information about the cDNA sequences is indispensable because sometimes protein motifs are embedded over a wide region of the protein sequences, and all of the combinations of the AS exons may not be allowed. Besides, for certain types of subcellular targeting signals, such as signal peptides, the position within the protein sequence is critical. Also, very recent reports, including ours (4), have demonstrated that many loci are subjected to complex patterns of AS in which two distinct genes seemed to be bridged (in which a variant uses exons from two adjacent loci), nested (in which a variant is located inside long intron of another locus) or degenerated (in which two variants use different reading frames in the shared exons. Its another name is multiple CDS): in all three cases, completely unrelated proteins are encoded by a single locus. These cases might not be regarded as alterative splicing in a strict sense. However, when those cases are also considered as extreme cases of functional diversification of a single locus and are subjected to be functional annotations, it is impossible to precisely characterize the combination of the exon usages.

Here, we introduce our new database of AS database, H-DBAS. We constructed this database exclusively using our unique dataset of completely sequenced and carefully annotated full-length cDNAs, which was produced by a human annotation meeting, H-Invitational (5,6). In H-Invitational, 56 419 cDNA sequences of human genes, which were fully sequenced with a sequence reliability higher than 99% [Phred values greater than 30; (7)] and whose potentially problematic sequences such as vectors and polyA tails were precisely trimmed, were subjected to manual annotation of AS variants. These cDNAs were clustered into 24 425 loci and of these, 6877 AS-containing loci, represented by 18 297 AS variants, were identified (4). As a specialized AS database, H-DBAS enables multifaceted analyses from various viewpoints, comprehensively aiming at elucidating functional consequences of widespread AS in human genes. [Note: We will use the word, 'locus', for the transcript cluster for the purpose of simplicity. However, the wording might be reconsidered, having observed highly diverse nature of the human transcriptome. Also see the reference (8)].

DATABASE CONTENTS

Data resources

In H-DBAS, the set of 167 992 so-called H-Invitational cDNAs was used (available from the URL). In addition, an option in which ASs represented by 23 210 RefSeq and 33 411 Ensembl transcripts were also considered is also implemented. In total, 167 564 transcripts were presented in the context of corresponding human genomic information as of UCSC hg17 (http://hgdownload.cse.ucsc.edu/downloads.html#human), cDNA information as of H-Invitational cDNA dataset (Table 1). The mapping and clustering procedures for the cDNAs were followed the annotation pipelines of the H-Invitational cDNAs. For details, see the help page of H-InvDB [http://jbirc.jbic.or.jp/hinv/; (9)].

Data processing

Patterning alternative splicings. Using the positional information for each of the transcripts on the human genome, representative AS patterns were defined for each locus as follows. First, in order to remove possible 5′/3′-end-truncated cDNAs, we excluded cDNAs whose 5′/3′-ends were located inside the second or later exons of any other cDNAs with compatible exon structure in the same locus. We accepted the cDNAs whose 5′/3′-ends were located inside of the first/last exons and considered as variations in the exact transcriptional starting/terminating sites. We also assumed that those cDNAs whose 5′-ends were located outside of the exonic regions of any other clones could not be truncated forms of any known types of transcripts, at least [for further detailed discussion of this subject, see reference (10)]. Second, using the resulting filtered set of putative full-length cDNAs, the genomic position of each exon–intron boundary

Table 1. Statistics of the data processing and of the AS variants and exons identified by genomic structure

	#Locus	#cDNA	#Total exon	#Alternative exon[a]	#Constitutive exon
H-Invitational cDNAs	35 005	167 992	1 164 482[b]	184 649	979 833
Successfully mapped	34 678	167 564	1 164 482	184 649	979 833
⩾2 cDNAs per locus	15 445	89 687	795 175	184 649	610 526
Identified AS variants	11 744	74 378	687 841	184 649	503 192
Identified RASVs[c]	11 744	38 664	378 024	98 156	279 868
5′-end	7488	15 920	38 664	15 920	22 744
Internal	10 030	26 443	300 696	69 359	231 337
3′-end	5978	12 877	38 664	12 877	25 787
Retrotransposons[d]	7435	14 534	22 583	12 735	9848
LINEs	3548	5360	6620	3863	2757
SINEs	5849	10 188	14 114	8724	5390
Alu elements	4487	7323	10 240	6379	3861
Identified RASVs[c] including full-length ORF	11 382	30 389	311 409	78 078	233 331
5′-UTR	6660	14 230	26 310	10 238	16 072
CDS	11 382	30 389	272 780	64 270	208 510
3′-UTR	3519	5259	12 319	3570	8749

[a] The number of exons was simply counted in which indicated AS relation was not associated.

[b] Unmapped transcripts' exons could not be counted.

[c] Representative AS Variants.

[d] They were detected by RepeatMasker (A.F.A. Smit, R. Hubley & P. Green RepeatMasker at http://repeatmasker.org).

was compared with those of the other transcripts belonging to the same locus. For the comparison, a 10 bp allowance was made. If a cDNA had a part of the exonic sequence in the first/last exon inside confirmed intronic regions of the other isoforms, it was regarded as being a '5′/3′-end' AS variant. If a cDNA had a part of an internal exonic sequence inside a confirmed intronic region of other isoforms, it was recognized as being an 'internal' AS variant (4). At this point, we removed annotated genomic rearrangement genes such as Immunoglobulin (Ig) and T-cell receptor (TCR) and anomalistic high polymorphic genes such as Major histocompatibility complex (MHC).

Merging alternative splicing patterns with functional annotations of the encoded proteins. Obtained information of patterns of AS was merged with that of detailed ORF prediction and functional annotation of H-Invitational cDNAs regarding protein motifs, GO terms, predicted subcellular localization signals and transmembrane domains. The protein motif and GO term were identified by InterProScan (11), the subcellular localization was predicted by WoLF PSORT (12) and TargetP (13) and the transmembrane domain was predicted by TMHMM (14) and SOSUI (15). For further details in functional annotation pipeline, see H-InvDB help page (http://jbirc.jbic.or.jp/hinv/). The results of the computational identification and annotation of the AS were visually inspected by the members of the AS annotation team and whenever annotations were considered to be controversial, the caveats were inserted to flag possible annotation errors.

Complex patterns of alternative splicing. Several 'complex' patterns of AS were defined as follows and registered in the database: (i) 'bridged': a locus in which two AS variants were arrayed tandemly without sharing any exons and another transcript 'bridged' these two isoforms, sharing at least some of its exons with both of them; (ii) 'nested': a locus in which CDS region of one AS variant was not shared with another variant and (iii) 'multiple CDS': a locus in which different ORFs >200 bp in length were annotated independently for different AS isoforms sharing at least some of the exons but not sharing any reading frame.

Current statistics

Current statistics of the database are as summarized in Tables 1 and 2 (updated from those presented in the reference (4). In total, 38 664 AS patterns were identified from 11 744 loci. When focused on the consequence of the AS to the encoded amino acid sequences, 30 389 AS variants in 11 382 loci caused changes of 97 amino acids in length on average. Further detailed statistics about how the ASs changed amino acid sequences are presented in 'Statistics' page in the database. Especially, 14 550 AS variants changed the protein motifs. In 14 248 cases, different GO terms were assigned to different AS variants, thus, they could be considered as good targets for further analyzing functional diversification of the genes. Similarly, AS changing subcellular localization signals and transmembrane domains were identified in 17 718 and 3995 AS variants in 5323 and 1248 loci, respectively. As for 'complex' AS, 2336, 3629 and 258 AS variants in 472, 1223 and 101 loci were identified and registered in the database as bridged, nested and multiple CDS, respectively.

Table 2. Numbers of the loci in which AS variants should influence the possible protein functions

	#Locus	#cDNA
AS affecting function total	7630	24 092
Motif-changed	4624	14 550
GO-changed	4150	14 248
Subcellular localization-changed	5323	17 718
Transmembrane domain-changed	1248	3995
Complex AS pattern total	1512	5394
Bridged	472	2336
Nested	1223	3629
Multiple CDS	101	258

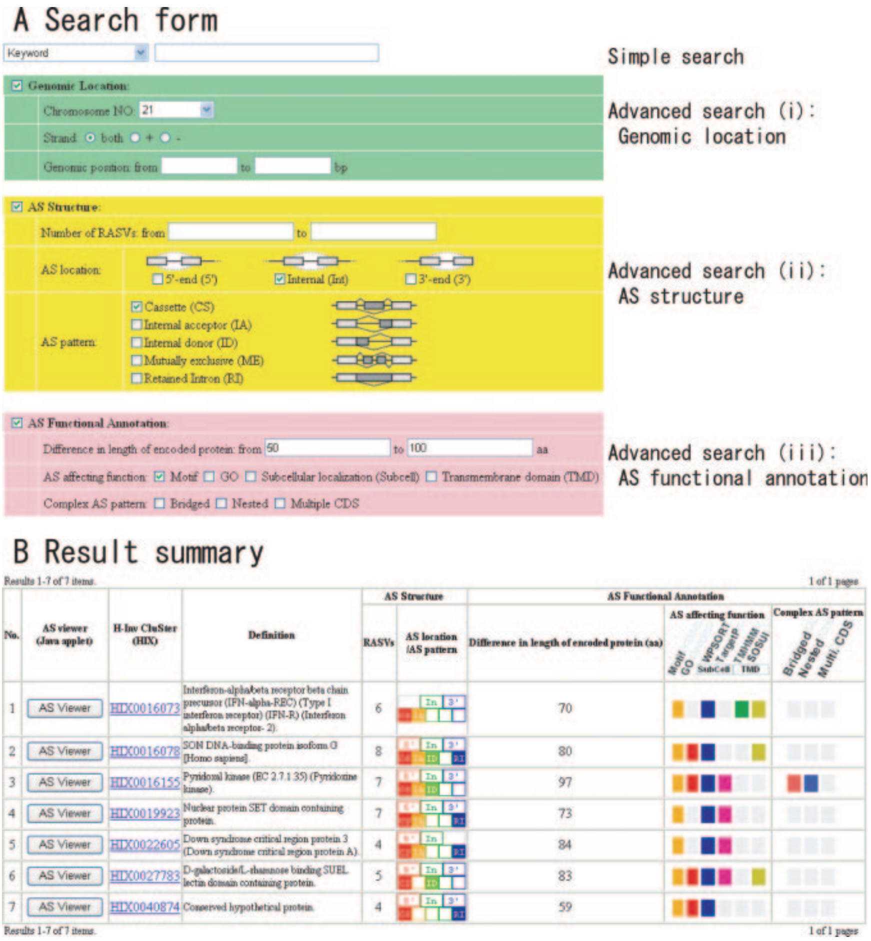
ACCESS TO DATABASE

Search system

A simple search form in the top page allows the user to retrieve from within H-DBAS by inputting word(s) of selected categories such as Keyword, HIX (H-Invitational cluster ID), HIT (H-Invitational transcript ID), corresponding Accession/Refseq/Emsembl ID, HUGO gene symbol and definition. In the advanced search form, the user can search the database by more detailed features of AS. The advanced search form consists of three categories: (i) 'Genomic Location' in which the user can specify in which chromosome and where in the chromosome the AS should be searched; (ii) 'AS Structure' in which the user can look for the number of representative AS variants in the locus, particular patterns of AS (such as cassette, internal acceptor, internal donor, mutually exclusive and retained intron) and their locations (5′/3′-end and internal); (iii) 'AS Functional Annotation' in which the user can specify the length difference of encoded protein, protein motifs, GO terms, predicted subcellular localization signals and transmembrane domains invoked by the AS: 'Complex' AS patterns can be also specified here. It is possible to use any combinations of the above search conditions which are within the same or different categories (Figure 1A). For example, the users can perform the search by querying the AS, which should be located on 'chromosome 21', having 'internal' 'cassette' exons, affecting '50–100 amino acids' and 'protein motif'. When multiple entries are hit, the user can see the Result summary and select which should be further examined (Figure 1B). Text-based summarized information can be also selected instead of showing a Java-based dynamic user interface.

AS Viewer

A main part of H-DBAS is a user-friendly Java-based interface, which is subjected to dynamic operations of the user (Figure 2). The browser can be zoomed from the genomic level to the sequence level (genomic/cDNA and amino acid sequences can be viewed). RefSeq and Ensembl transcripts can be viewed together with H-Invitational cDNAs as references. By using the clone view controller, the users can select which items should be viewed. Functional annotation view controllers can be used for selecting which protein motifs identified in the locus should be highlighted/erased. This page is designed so that the user can empirically recognize the positions and patterns of AS in the context of the



A Search form

Keyword ▾ [ ]

Simple search

☑ Genomic Location:

Chromosome NO: 21 ▾

Strand: ● both ○ + ○ -

Genomic position: from [ ] to [ ] bp

Advanced search (i): Genomic location

☑ AS Structure:

Number of RASVs: from [ ] to [ ]

AS location: □ 5'-end (5') ☑ Internal (Int) □ 3'-end (3')

AS pattern: ☑ Cassette (CS) □ Internal acceptor (IA) □ Internal donor (ID) □ Mutually exclusive (ME) □ Retained Intron (RI)

Advanced search (ii): AS structure

☑ AS Functional Annotation:

Difference in length of encoded protein: from 50 to 100 aa

AS affecting function: ☑ Motif □ GO □ Subcellular localization (Subcell) □ Transmembrane domain (TMD)

Complex AS pattern: □ Bridged □ Nested □ Multiple CDS

Advanced search (iii): AS functional annotation

B Result summary

Results 1-7 of 7 items. 1 of 1 pages

No.	AS viewer (Java applet)	H-Inv CluSter (HIX)	Definition	AS Structure		AS Functional Annotation									
				RASVs	AS location /AS pattern	Difference in length of encoded protein (aa)	AS affecting function						Complex AS pattern		
							Motif	GO	SubCell WPSORT	SubCell TargetP	TMD TMHMM	TMD SOSUI	Bridged	Nested	Multi. CDS
1	AS Viewer	HIX0016073	Interferon-alpha/beta receptor beta chain precursor (IFN-alpha-REC) (Type I interferon receptor) (IFN-R) (Interferon alpha/beta receptor- 2).	6	In 3'	70	■		■		■	■			
2	AS Viewer	HIX0016078	SON DNA-binding protein isoform G [Homo sapiens].	8	5' In 3' ID RI	80	■	■	■			■			
3	AS Viewer	HIX0016155	Pyridoxal kinase (EC 2.7.1.35) (Pyridoxine kinase).	7	5' In 3' ID	97	■	■	■	■			■	■	
4	AS Viewer	HIX0019923	Nuclear protein SET domain containing protein.	7	5' In 3' RI	73	■		■	■					
5	AS Viewer	HIX0022605	Down syndrome critical region protein 3 (Down syndrome critical region protein A).	4	5' In RI	84	■		■	■					
6	AS Viewer	HIX0027783	D-galactoside/L-rhamnose binding SUEL lectin domain containing protein.	5	5' In 3' ID	83	■	■	■	■		■			
7	AS Viewer	HIX0040874	Conserved hypothetical protein.	4	5' In 3' RI	59	■	■	■						

Results 1-7 of 7 items. 1 of 1 pages

Figure 1. Search system of H-DBAS is shown. (**A**) Search form. H-DBAS has two sorts of search form named simple search and advanced search. The simple search allows the user to find AS locus by using keyword, H-Inv cluster ID (HIX), H-Inv transcript ID (HIT), accession number, RefSeq ID, Ensembl ID, HUGO gene symbol and definition. The advanced search allows the user to find AS locus by using various combinations of three categories such as Genomic location (i), AS structure (ii) and AS functional annotation (iii). In this search system, any combinations including simple search are available. (**B**) Result summary. The result of the query written in text and Figure 1A is shown.

full-length form of each transcript. It should be especially advantageous that the user can view possible influence of the AS on various kinds of protein motifs. When an AS exon in 'Exonic Segment' window is clicked, the positions which are regarded as mutually AS are highlighted in 'AS Event View' window. At the same time, if the protein motifs and transmembrane domains are identified, the corresponding exonic region of the cDNA(s) in 'Entry cDNA' window is colored aqua on the ORF region colored pink.

Example of the search

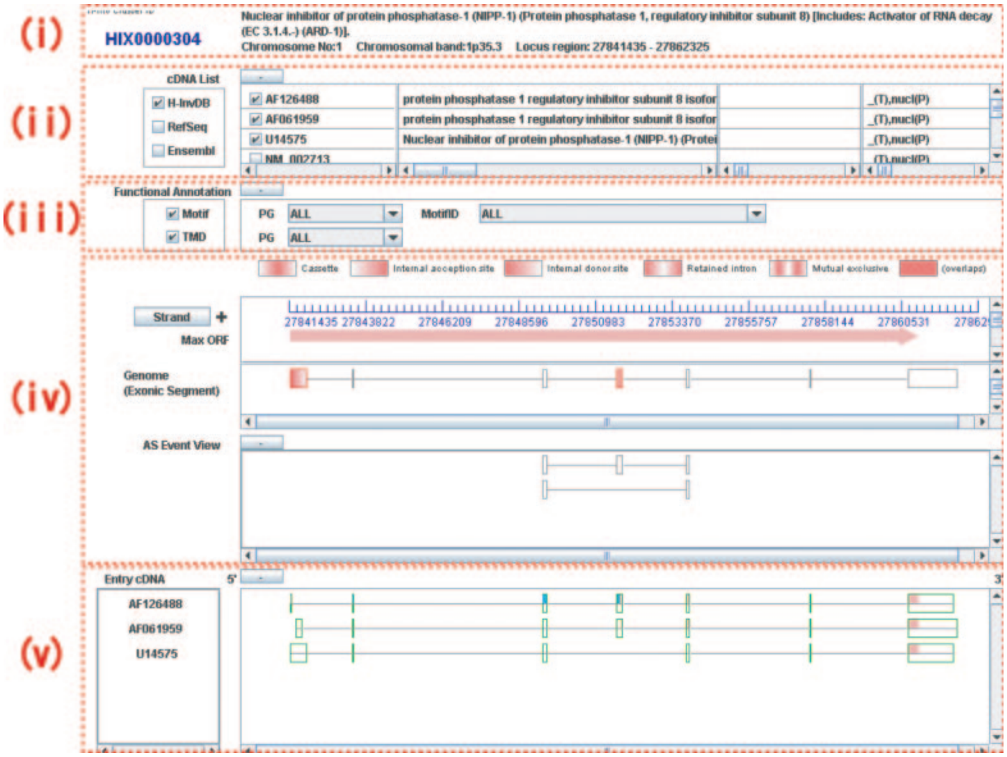
In Figure 3A, we show an example of AS affecting a motif by using AS Viewer. This is the IKK-related kinase epsilon gene. In this gene, while a cDNA (D63485) contains a prtein motif, 'protein kinase (InterPro ID; IPR000719)', another cDNA (AK093798) does not contain it. The lack of exons 2–7 in the latter cDNA because of cassette type AS is responsible for this putative functional difference. Figure 3B shows an example from complex AS pattern. AJ276409, which is Ssu72-like protein family protein looks as if 'bridging' AK127149 and AK023110 (Figure 3B), both of the latter two transcripts are of known genes and are reported to be protein-coding.

Glossary and download

Use of the database as well as the archives of the raw data is freely available to anonymous public users without any restrictions. A detailed user manual and technical terms used, definitions and parameters for the annotations are precisely described in the 'Glossary' page in H-DBAS. The users can follow the links to further detailed information from each items displayed here. In the 'Download' page, archives of raw data, containing all kinds of AS information and sequence data about all AS variants in our database, are made publicly and anonymously downloadable.

FUTURE DEVELOPMENTS

We are currently interconnecting H-DBAS with H-ANGEL [http://jbirc.jbic.or.jp/hinv/h-angel/; (16)], in which gene expression patterns of the H-Invitational cDNAs are registered. We are also adding precisely annotated mouse



HIX0000304

Nuclear inhibitor of protein phosphatase-1 (NIPP-1) (Protein phosphatase 1, regulatory inhibitor subunit 8) [Includes: Activator of RNA decay (EC 3.1.4.-) (ARD-1)].

Chromosome No:1 Chromosomal band:1p35.3 Locus region: 27841435 - 27862325

cDNA List

☑ H-InvDB
□ RefSeq
□ Ensembl

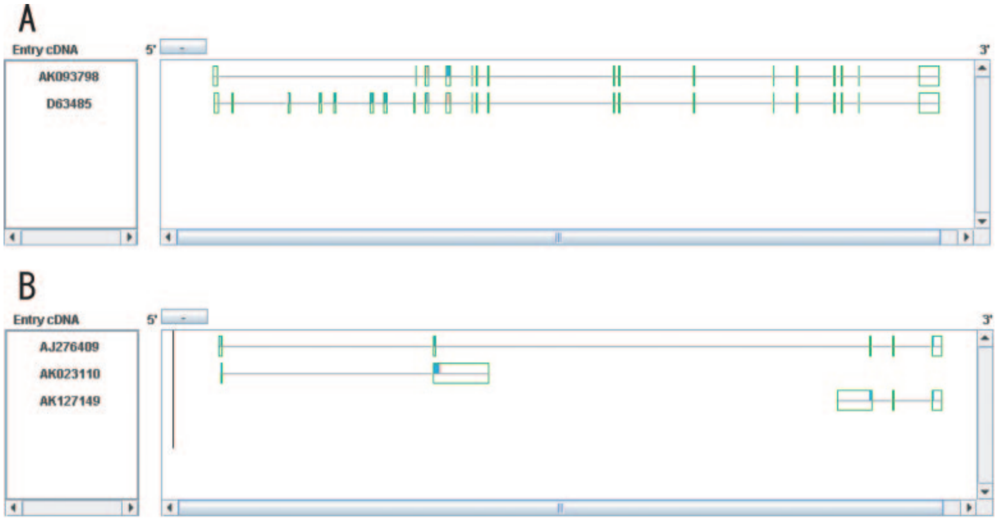
☑ AF126488	protein phosphatase 1 regulatory inhibitor subunit 8 isofor		_(T),nucl(P)
☑ AF061959	protein phosphatase 1 regulatory inhibitor subunit 8 isofor		_(T),nucl(P)
☑ U14575	Nuclear inhibitor of protein phosphatase-1 (NIPP-1) (Protei		_(T),nucl(P)
□ NM_002713			(T),nucl(P)

Functional Annotation

☑ Motif PG ALL MotifID ALL
☑ TMD PG ALL

AF126488
AF061959
U14575

Figure 2. AS Viewer of H-DBAS is shown. Java applet for operating AS event and checking AS exon and protein functions such as protein motif and transmembrane domain. The user can also compare with representative AS variants (RASVs) by nucleic and amino acid sequence level. AS Viewer is separated following parts: (i) Definition and genomic information of the locus; (ii) Selection function and definition of RASVs including RefSeq and Ensembl transcripts as references; (iii) Selection function of protein motif and transmembrane domain; (iv) All exons of selected RASVs are located on genome and AS exons are colored red. By clicking an AS exon in Exonic Segment field, the AS events on the location are shown in AS Event Viewer. Max ORF means total ORF range on genome of selected RASVs; (v) Selection function of AS structure on genome and on cDNA. Selected RASVs' structures are shown and these ORFs are colored pink and protein motifs and transmembrane domains are colored aqua. They are also shown nucleic and amino acid sequences by using zoom function.



AK093798
D63485

AJ276409
AK023110
AK127149

Figure 3. Examples of the alternative splicing affecting motif (**A**) and bridged complex AS pattern (**B**) from AS Viewer in H-DBAS. Exons and introns are represented by boxes and lines. ORF region is colored pink and protein motif region is colored aqua.

full-length cDNA information as well and developing comparative genomics interfaces. The upcoming two major categories of extensive data will allow us to start determining how the ASs were acquired during evolution and how they fulfill the functional diversification of a single locus in various cellular circumstances. Furthermore, in the phase of further detailed experimental validation of the AS, H-DBAS should serve as an important interface for looking for cDNA clone resources, as the H-DBAS represents physical full-length cDNAs, which should serve as indispensable reagents for many kinds of experimental purposes.

Finally, we realize that we have a long way ahead for improving the web-page and database contents. We sincerely welcome any feedbacks from the users.

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Conflict of interest statement. None declared.

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