Application Note

ProtAnnot: visualizing effects of alternative splicing and transcription on protein sequence and function in a genome browser

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ABSTRACT

Summary: One gene can produce multiple transcript variants encoding proteins with different functions. To facilitate visual analysis of transcript variants, we developed ProtAnnot, which shows protein annotations in the context of genomic sequence. ProtAnnot searches InterPro and displays profile matches (protein annotations) alongside gene models, exposing how alternative promoters, splicing, and 3' end processing add, remove, or remodel functional motifs. To draw attention to these effects, ProtAnnot color-codes exons by frame and displays a cityscape graphic summarizing exonic sequence at each position. These techniques make visual analysis of alternative transcripts faster and more convenient for biologists.

Availability and Implementation: ProtAnnot is a plug-in for Integrated Genome Browser, an open source desktop genome browser available from http://www.bioviz.org. Videos showing ProtAnnot in action are available at http://bit.ly/igbchannel.

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1 INTRODUCTION

Many genes produce multiple transcript variants due to alternative splicing, alternative promoters, and alternative 3' end processing. Often these transcript variants encode proteins with different amino acid sequences and thus different functions. We and other groups have often used protein annotation methods to detect when this occurs. For example, we used BLOCKS, InterPro, and TMHMM to show that alternative transcription frequently remodels or deletes conserved regions and trans-membrane spans in human and mouse proteins (Cline, et al., 2004; Loraine, et al., 2002).

However, even now it is not easy for biologists to perform similar analysis on individual genes. Using Web tools, biologists can upload and annotate single protein sequences, but mapping those annotations back onto gene structures is time-consuming and error-prone.

To solve these problems, we developed ProtAnnot, a new plug-in extension for the Integrated Genome Browser (IGB). IGB (pronounced "Ig-Bee") is a highly interactive, desktop genome browser that helps biologists explore and analyze experimental data from genomics, especially RNA-Seq data (Nicol, et al., 2009). Using ProtAnnot together with IGB, users can achieve deeper insight into how alternative transcription affects protein sequence and function.

2 RESULTS

ProtAnnot enables fast, efficient visual analysis of the impact of alternative transcription on proteins by extending standard genome browser iconography, in which linked blocks represent transcript structures and block thickness indicate translated regions.

ProtAnnot improves on this in three ways. First, it uses exon fill colors to show the frame of translation, revealing frame shifts across transcript variants. By comparing exon colors between transcripts, a user can quickly determine if they encode the same protein without having to zoom in to see the amino acid sequence, as is required in most genome browser tools.

Second, ProtAnnot introduces an exon summary graphic, a series of blocks at the bottom of the display whose heights indicate the number of exons overlapping each position. Height differences between adjacent blocks signal that models differ at that position. By scanning the exon summary, users can easily identify so-called "difference regions" (English, et al., 2010), sequences that are differentially included in transcripts due to alternative splicing, promoters, or 3'-end processing. The exon summary graphic draws attention to these regions by exploiting our native ability to notice discontinuities in a horizon (Fig. 1).

Third, ProtAnnot exposes how different regions of a gene may encode different functions by displaying protein annotations next to their respective transcripts. In ProtAnnot, these protein annotations appear as single- or multi-span linked blocks beneath the transcripts that encode them. A thin line links spans from the same motif; note that these matches often span introns. Discontinuous spans from the same match are shown in alternating shades.

To use ProtAnnot in conjunction with IGB, users open the Plug-Ins tab and select ProtAnnot, which triggers download of the ProtAnnot plug-in from a repository located on BioViz.org to a local plug-in cache. A new menu item labeled "Start ProtAnnot" then appears in the IGB Tools menu. Next, the user selects one or more gene models on the same strand within the IGB main display window and selects "Tools > Start ProtAnnot". This opens ProtAnnot in a new window, which shows the selected gene models with color-coded exons above the exon summary graphic.

As with IGB, we developed ProtAnnot using the GenoViz SDK, a Java toolkit for building genome browsers (Helt, et al., 2009). By using GenoViz, we were able to implement advanced visualization techniques familiar to IGB users with minimal effort. These include: user-settable zoom focus indicated by a zoom stripe graphic, fast animated zooming, edge matching of selected items, and selectable Glyphs.

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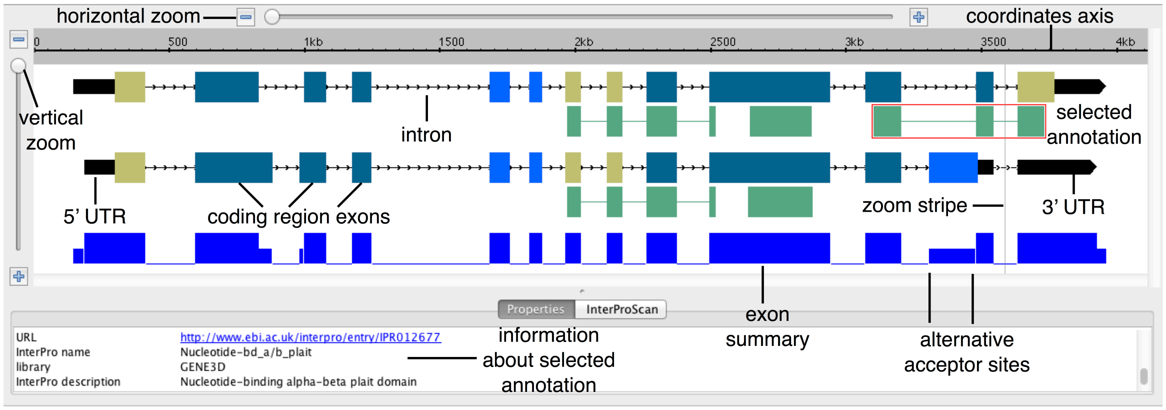

URL	http://www.ebi.ac.uk/interpro/entry/IPR012677
InterPro name	Nucleotide-bd_a/b_plait
library	GENE3D
InterPro description	Nucleotide-binding alpha-beta plait domain

Fig. 1. ProtAnnot visualization of *Arabidopsis thaliana* gene AT4G36690 encoding splicing regulator U2AF65 shows how alternative splicing deletes a nucleotide-binding domain. The coordinates axis indicates base positions relative to start of the gene. Exon fill colors indicate frame of translation. Untranslated regions (UTR) are shown in black. Gene model AT4G36690.4 (upper) and AT4G36690.3 (lower) are shown. AT4G36690.3 is the dominant isoform in pollen, but in leaves, AT4G36690.4 predominates (Loraine, et al., 2013).

To search InterPro using ProtAnnot, users select the InterProScan tabbed panel and click a button labeled "Run InterProScan," which opens a new window listing databases and search methods available from the InterProScan Web service hosted at the European Bioinformatics Institute. Users then select one or many databases to search, enter an email address, and run the search. Note that the InterProScan Web service maintainers require an email address so that they can contact users in case of problems.

The search happens in a separate thread, enabling users to continue using ProtAnnot and IGB. Status updates appear in the InterProScan tab. When the search finishes, ProtAnnot adds newly found protein annotations to the display, below their respective transcripts. ProtAnnot also updates the status message with a link to an XML file hosted on the EBI Web site containing the "raw" results; this is mainly a convenience for developers.

Clicking on a protein annotation opens the Properties tab, listing all information about that particular profile or motif. Depending on the database, this can include the name of the domain or motif identified, a brief description, as well as a link to additional information. Users can also shift-click to select multiple annotations, putting all of the available information side-by-side, allowing for direct comparison.

These results can be saved and reopened later, allowing the user to avoid having to re-run InterProScan each session, as well as reducing server load on the InterProScan Web service. ProtAnnot saves results to an XML format file (extension ".paxml") that specifies the genomic sequence surrounding the analyzed gene models, contains a genomic sequence surrounding the gene models, an offset indicating the relationship between this sequence and the reference genome, transcript structures using coordinates relative to the sequence, and protein annotations in protein sequence coordinates. Users can open the saved files and share them with colleagues.

It is also possible to modify the files and change how the data appear within ProtAnnot. The ProtAnnot XML format includes a general-purpose "descriptor" tag that can be used to add arbitrary properties to a gene model or protein sequence hit. The contents of these tags appear in the Properties table when users select the corresponding items in the main display.

3 CONCLUSION

ProtAnnot benefits users by exposing how gene structures affect protein sequence and function. As such, ProtAnnot complements the MI Bundle, another IGB extension that links genomic features to protein interaction and structure viewers (Céol and Müller, 2015). Like MI Bundle, ProtAnnot highlights relationships between the language of DNA (exons, introns, codons) and the more structure-oriented language of protein sequence, thus helping biologists achieve deeper understanding of gene function.

ACKNOWLEDGEMENTS

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