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AnnotationBustR: An R package to extract subsequences from GenBank annotations

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Background. DNA sequences are pivotal for a wide array of research in biology. Large sequence databases, like GenBank, provide an amazing resource to utilize DNA sequences for large scale analyses. However, many sequences on GenBank contain more than one gene or are portions of genomes, and inconsistencies in the way genes are annotated and the numerous synonyms a single gene may be listed under provide major challenges for extracting large numbers of subsequences for comparative analysis across taxa. At present, there is no easy way to extract portions from multiple GenBank accessions based on annotations where gene names may vary extensively. **Results.** The R package *AnnotationBustR* allows users to extract sequences based on GenBank annotations through the ACNUC retrieval system given search terms of gene synonyms and accession numbers. *AnnotationBustR* extracts portions of interest and then writes them to a FASTA file for users to employ in their research endeavors. **Conclusion.** FASTA files of extracted subsequences and accession tables generated by *AnnotationBustR* allow users to quickly find and extract subsequences from GenBank accessions. These sequences can then be incorporated in various analyses, like the construction of phylogenies to test a wide range of ecological and evolutionary hypotheses.

1 ***AnnotationBustR: An R package to extract subsequences from GenBank annotations***

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Abstract

Background. DNA sequences are pivotal for a wide array of research in biology. Large sequence databases, like GenBank, provide an amazing resource to utilize DNA sequences for large scale analyses. However, many sequences on GenBank contain more than one gene or are portions of genomes, and inconsistencies in the way genes are annotated and the numerous synonyms a single gene may be listed under provide major challenges for extracting large numbers of subsequences for comparative analysis across taxa. At present, there is no easy way to extract portions from multiple GenBank accessions based on annotations where gene names may vary extensively.

Results. The R package *AnnotationBustR* allows users to extract sequences based on GenBank annotations through the ACNUC retrieval system given search terms of gene synonyms and accession numbers. *AnnotationBustR* extracts portions of interest and then writes them to a FASTA file for users to employ in their research endeavors.

Conclusion. FASTA files of extracted subsequences and accession tables generated by *AnnotationBustR* allow users to quickly find and extract subsequences from GenBank accessions. These sequences can then be incorporated in various analyses, like the construction of phylogenies to test a wide range of ecological and evolutionary hypotheses.

Introduction

The use of DNA sequence data is vital for a wide variety of research in evolutionary biology and ecology. Molecular phylogenies, which rely on DNA sequences for their construction, are extremely prevalent in biological research. Whether being used to correct for shared ancestry among organisms (Felsenstein, 1985), or to test hypotheses related phylogeography (Avice et al., 1987), diversification (Hey, 1992; Maddison, 2006), and trait evolution (Bollback, 2006; O'Meara et al., 2006), phylogenies are required. Additionally, the use of phylogenies is important in community ecology to place systems into an evolutionary framework (Webb et al., 2002; Cavender-Bares et al., 2009). The construction of molecular phylogenies for systematic purposes is also a popular tool for taxonomists to identify new taxa and classify organisms (De Queiroz & Gauthier, 1994; Tautz et al., 2003). Some DNA sequences, like the mitochondrial gene cytochrome oxidase subunit I (COI), are also gaining utility as a method to identify and catalog species using DNA barcoding (Hebert et al., 2003; Ratnasingham & Hebert, 2007; Ratnasingham & Hebert, 2013).

Sequence databases like GenBank provide an extremely valuable resource for using DNA sequence data to test evolutionary and ecological hypotheses. With the reduction in cost of DNA sequencing and the advancement of methods to analyze sequence data, the amount of sequence data available for use is growing at a rapid pace. Given that GenBank has over one-trillion sequences from over 370,000 species (Benson et al., 2017) and recent advances in methods to create massive phylogenies using either super-matrix (Driskell et al., 2004; Ciccarelli et al., 2006) or mega-phylogeny approaches (Smith et al., 2009; Izquierdo-Carrasco et al., 2014), many generate large DNA sequence data sets for comparative analyses (Leslie et al., 2012; Rabosky et al., 2013; Spriggs et al., 2014; Zanne et al., 2014; Shi & Rabosky, 2015). Additionally, sequence retrieval within common scripting environments for biological analyses, like R (R Development Core Team, 2017), are made possible with packages like *ape* (Paradis et al., 2004), *rentrez* (Winter, 2016), *reutils* (Schofl, 2015), and *seqinr* (Charif & Lobry, 2007).

While GenBank provides a wealth of sequence data for researchers to use, some of it is rather difficult to manipulate into a useful form. For example, some sequences may be concatenated together, or the only gene sequence available for a species for the locus of interest may be within a mitochondrial or chloroplast genome. Although GenBank's annotation system provides a means to see where a locus of interest is in a genome or concatenated sequence and provides the ability to download it manually, this is extremely time consuming when many accessions are involved and not a feasible way to extract mass amounts of sequence data for use in research.

Alternative methods to increase the speed of which one could extract out loci in a concatenated sequence could involve aligning it to a known sequence of the locus of interest using an alignment program like BLAST (Altschul et al., 1990). However, BLAST and similar programs only align sequences that are similar, and the gene region aligned may not be entirely homologous to the gene of interest. Given that alignment programs use homologous sequences for their input, this can cause alignments that are not useful and provide the wrong phylogenetic inference, affecting downstream analyses (Lassmann & Sonnhammer, 2005).

Another major challenge to obtaining large amounts of sequence data is the highly variable nomenclature of gene names. Most genes have several alternative names and symbols that are present in sequence databases. Among distant taxa, it is common for homologous genes to vary considerably in nomenclature (Tuason et al., 2003). Even within a group of closely related taxa or within a single taxa itself, how genes are annotated may differ substantially from record to record and a wide variety of alternative gene names may be found for a single gene (Morgan et al., 2004; Fundel & Zimmer, 2006). This poses serious problems when searching through databases for data extraction like molecular sequence data (Mitchell et al., 2003; Tamames & Valencia, 2006).

Here we present the R package *AnnotationBustR* to solve the issues discussed above. *AnnotationBustR* reads GenBank annotations in R and pulls out the gene(s) of interest given a set of search terms and a vector of taxon accession numbers supplied by the user. It then writes the sequence for the gene(s) of interest to FASTA formatted files for each locus that users can then use in further analyses. For a more in depth introduction to using *AnnotationBustR* users should consult the vignette in R through `vignette("AnnotationBustR-vignette")`, which provides instructions on how to use the different functions and their respective options. Other details about the package can be accessed through the documentation via `help("AnnotationBustR")`.

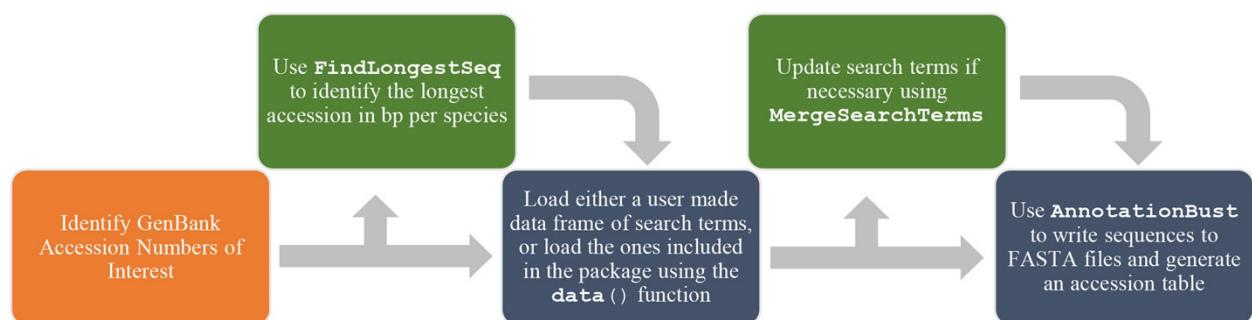
Description

AnnotationBustR is written in R (R Development Core Team, 2017), a popular language for analyzing biological data. It uses the existing R packages *ape* (Paradis et al., 2004) and *seqinr* (Charif & Lobry, 2007). *AnnotationBustR* uses *seqinr*'s interface to the online ACNUC database to extract gene regions of interest from concatenated gene sequences or genomes (Gouy et al., 1985; Gouy & Delmotte, 2008). ACNUC's storage of subsequence strings allows easy access and manipulation of complex sequences, such as trans-spliced genes that may be on opposite strands of DNA. A list of the currently implemented commands is given in Table 1 and a flow chart of function usage is shown in Figure 1.

96 **Table 1: Functions and data included in the package *AnnotationBustR*.**

Function/Data Name	Description
<code>AnnotationBust</code>	Writes found subsequences for loci of interest to a FASTA file for a vector of GenBank accessions and writes a corresponding accession table.
<code>data(cpDNATerms)</code>	Loads a data frame of search terms for chloroplast genes.
<code>data(mtDNATerms)</code>	Loads a data frame of search terms for mitochondrial genes.
<code>data(rDNATerms)</code>	Loads a data frame of search terms for ribosomal DNA genes and spacers.
<code>FindLongestSeq</code>	Finds the longest sequence for each species in a set of GenBank accession numbers.
<code>MergeSearchTerms</code>	Merges two or more data frames containing search terms of features to extract into a single data frame.

97 The main function of *AnnotationBustR*, `AnnotationBust`, takes a vector of accession
98 numbers and a data frame of synonym search terms to extract loci of interest and write them to a
99 FASTA formatted file. This function also returns a pre-made accession table of all the loci of
100 interest and the corresponding accession numbers the loci were extracted from for each species
101 that can then be written to a csv file by the user. Users can specify duplicate genes be extracted
102 as well, although we caution the use of doing this as they can be misleading to use in
103 comparative analyses due to issues of paralogy (Goodman et al., 1979; Maddison, 1997). If
104 extracting coding sequences, users can also specify if they would like to translate the sequence
105 into the corresponding peptides by specifying the GenBank numerical translation code for the
106 taxa of interest.



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108 **Figure 1: Flow chart of functions for a complete usage of *AnnotationBustR*.** Blue boxes
109 indicate a step using the package *AnnotationBustR* while orange boxes represent steps that need
110 to be completed outside of *AnnotationBustR*. Boxes in green represent optional steps in the
111 *AnnotationBustR* pipeline.

112 We have included pre-made data frames with search terms in *AnnotationBustR* for
113 mitochondrial genomes, chloroplast genomes, and rDNA. These can be used to easily extract
114 DNA barcodes, like cytochrome oxidase subunit I (COI) for animals in mitochondrial genomes

(Hebert et al., 2003), the internal transcribed spacers (ITS) in rDNA for fungi and plants (Kress et al., 2005; Schoch et al., 2012), and maturase K (matK) and ribulose-bisphosphate carboxylase (rbcL) genes in the chloroplast genome of plants (Hollingsworth et al., 2009). These pre-made data frames consist of three columns with the column `Locus` containing the output file name, `Type` containing the type of sequence it is (i.e. CDS, tRNA, rRNA, misc_RNA, D-loop), and the third column, `Name`, containing a possible synonym of the loci to search for. For example, for cytochrome oxidase subunit I, GenBank includes gene names of COI, CO1, COX1, cox1, COXI, cytochrome c oxidase subunit I, and COX-I. These search terms can be loaded into the workspace using the `data()` function. Annotations files for each accession are read in through `seqinr` and regular expressions matching of the synonyms provided by the user to the feature annotations are performed to identify the subsequence to extract. As certain loci may have numerous synonymous listings in GenBank feature tables that may not be included in the pre-made data frames of search terms, *AnnotationBustR* has the function `MergeSearchTerms` which allows users to easily add additional search terms to a pre-existing data frame of search terms if users follow the basic three column formatting stated above. An additional feature of *AnnotationBustR* is the function `FindLongestSeq` which finds the longest sequence for each species in a set of GenBank accessions.

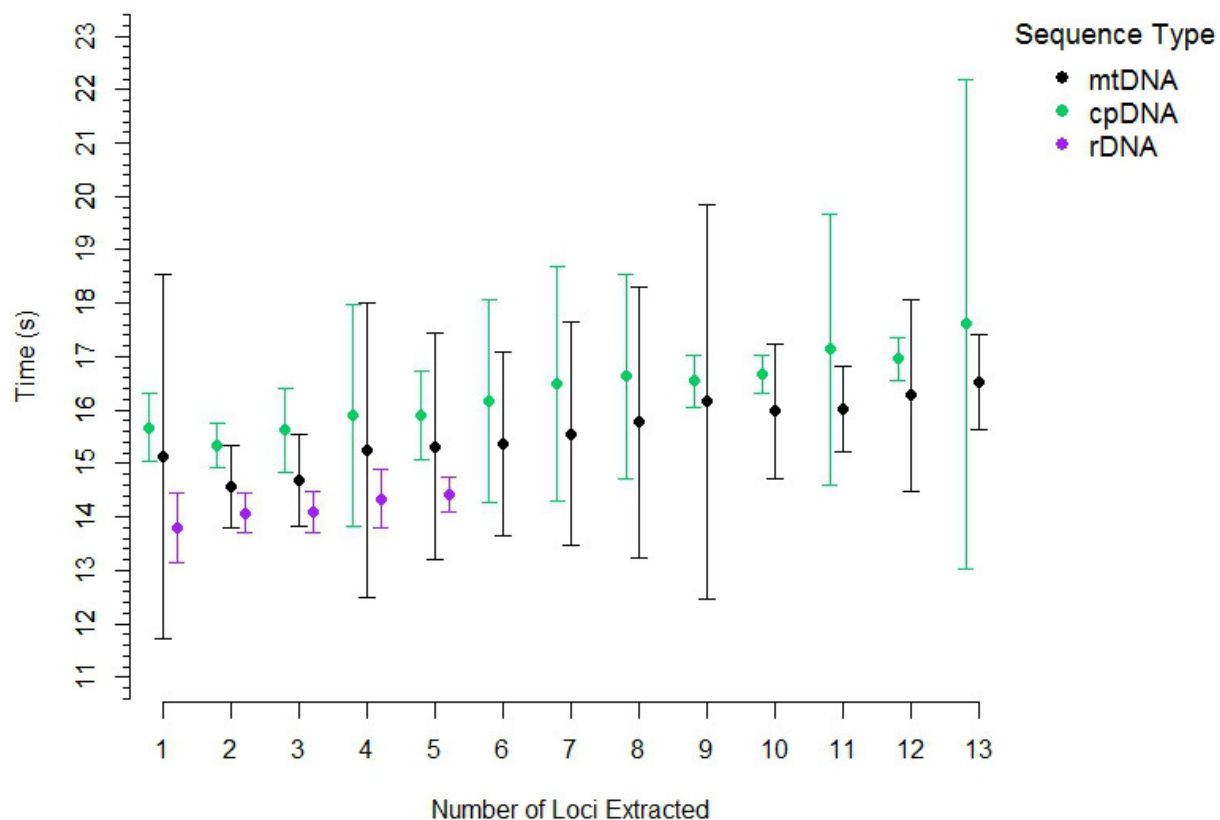


Figure 2: Timings of subsequence extraction using AnnotationBust for thirteen mitochondrial coding sequences (black), thirteen chloroplast subsequences (green), and five rDNA subsequence (purple). Points represent the mean time in seconds with bars representing +/- one standard deviation.

To demonstrate the performance of *AnnotationBustR*, we timed how long it took to extract thirteen popular coding sequences from 100 chloroplast genomes, the thirteen coding sequences from 100 metazoan mitochondrial genomes, and the three ribosomal RNA genes and internal transcribed spacers 1 and 2 from 100 metazoan rDNA sequences (Figure 2, see code in Supplemental Data S1). Timing trials were performed on a Windows desktop with a 3.8 GHz Intel Core i7 processor and 64 GB of RAM. For each accession, we timed the how long it took to extract one through the full number of subsequences sought. Our timings indicate that *AnnotationBustR* can efficiently extract these loci into FASTA files and that performance scales well as the number of loci to extract increases.

AnnotationBustR is available through CRAN (<https://cran.r-project.org/package=AnnotationBustR>) and is developed on GitHub (<https://github.com/sborstein/AnnotationBustR>). New extensions in development and fixes can be seen under the issues section on the packages GitHub page.

Conclusions

AnnotationBustR provides a quick and effortless way for users to extract subsequences from concatenated sequences or plastid and mitochondrial genomes where gene names for subsequences may vary substantially. The major limitation to the functionality of *AnnotationBustR* is that it is only as good as the annotations in the features table it is using for extraction. For instance, some concatenated sequences do not have the individual gene positions annotated for the record and just state that it contains the genes, therefore making it impossible to extract a gene from it (ex. [GenBank KM260685.1](#), [GenBank KT216295.1](#)). Additionally, some loci may be present in the sequence yet missing from the features table completely (ex. mitochondrial D-loop missing in [GenBank KU308536.1](#)). Another limitation is that some popular loci are intergenic spacers and are not annotated in the features table, making them impossible to extract. A good example of this is the trnH-psbA intergenic spacer, a proposed locus for plant DNA barcodes (Kress et al., 2005).

Citation

Researchers publishing a paper that has used *AnnotationBustR* should cite this article and indicate the version of the package they are using. Package citation information can be obtained using citation("AnnotationBustR").

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