

1 ***phylo-node: A Molecular Phylogenetic Toolkit using Node.js***

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26 **ABSTRACT**

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28 **Background:** Node.js is an open-source and cross-platform environment that
29 provides a JavaScript codebase for back-end server-side applications. JavaScript
30 has been used to develop very fast and user-friendly front-end tools for bioinformatic
31 and phylogenetic analyses. However, no such toolkits are available using Node.js to
32 conduct comprehensive molecular phylogenetic analysis.

33 **Results:** To address this problem, I have developed, *phylo-node*, which was
34 developed using Node.js and provides a stable and scalable toolkit that allows the
35 user to perform diverse molecular and phylogenetic tasks. *phylo-node* can execute
36 the analysis and process the resulting outputs from a suite of software options that
37 provides tools for read processing and genome alignment, sequence retrieval,
38 multiple sequence alignment, primer design, evolutionary modeling, and phylogeny
39 reconstruction. Furthermore, *phylo-node* enables the user to deploy server
40 dependent applications, and also provides simple integration and interoperation with
41 other Node modules and languages using Node inheritance patterns, and a
42 customized piping module to support the production of diverse pipelines.

43 **Conclusions:** *phylo-node* is open-source and freely available to all users without
44 sign-up or login requirements. All source code and user guidelines are openly
45 available at the GitHub repository: <https://github.com/dohalloran/phylo-node>

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47 **Keywords:** Node.js, JavaScript, phylogenetics

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51 **BACKGROUND**

52 The cost of whole genome sequencing has plummeted over the last decade and as
53 a consequence, the demand for genome sequencing technology has risen
54 significantly [1]. This demand has meant that producing large complex datasets of
55 DNA and RNA sequence information is common in small research labs, and in terms
56 of human health this boom in sequence information and precipitous drop in
57 sequencing costs has had a direct impact in the area of personalized medicine [2-5].
58 However, once the sequence information becomes available, perhaps the greater
59 challenge is then processing, analyzing, and interpreting the data. To keep pace with
60 this challenge, the development of new, fast, and scalable software solutions is
61 required to visualize and interpret this information.

62 JavaScript is a lightweight programming language that uses a web browser as
63 its host environment. JavaScript is cross-platform and supported by all modern
64 browsers. Because JavaScript is client-side, it is very fast as it doesn't have to
65 communicate with a server and wait for a response in order to run some code. Web
66 browsers are ubiquitous and require no dependencies to deploy and operate, and so
67 JavaScript represents an obvious solution for visualizing sequence information.
68 Front-end developments using JavaScript have proven to be extremely efficient in
69 providing fast, easy-to-use, and embeddable solutions for data analysis [6-14]. A
70 very active community of developers at BioJS (<http://www.biojs.io/>) provides diverse
71 components for parsing sequence data types, data visualization, and bioinformatics
72 analysis in JavaScript [6,7,15-19].

73 Node.js provides server-side back-end JavaScript. Node.js is written in C,
74 C++, and JavaScript and uses the Google Chrome V8 engine to offer a very fast
75 cross-platform environment for developing server side Web applications. Node is a

76 single-threaded environment, which means that only one line of code will be
77 executed at any given time; however, Node employs non-blocking techniques for I/O
78 tasks to provide an asynchronous ability, by using *callback* functions to permit the
79 parallel running of code. Node holds much potential for the bioinformatic analysis of
80 molecular data. A community of Node developers provides modules for bioinformatic
81 sequence workflows (<http://www.bionode.io/>) which in time will likely parallel the
82 BioJS community for the number of modules versus components. However, as of
83 now there are no robust tools for phylogenetic analysis pipelines currently available
84 using the Node.js codebase. To fill this void I have developed, *phylo-node*, which
85 provides a Node.js toolkit that provides sequence retrieval, primer design, alignment,
86 phylogeny reconstruction and as well as much more, all from a single toolkit. *phylo-*
87 *node* is fast, easy to use, and offers simple customization and portability options
88 through various inheritance patterns. The Node package manager, *npm*
89 (<https://www.npmjs.com/>), provides a very easy and efficient way to manage
90 dependencies for any Node application. *phylo-node* is available at GitHub
91 (<https://github.com/dohalloran/phylo-node>), npm
92 (<https://www.npmjs.com/package/phylo-node>), and also BioJS
93 (<http://www.biojs.io/d/phylo-node>).

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95 **IMPLEMENTATION**

96 *phylo-node* was developed using the Node.js codebase. The *phylo-node* core
97 contains a base wrapper object that is used to prepare the arguments and directory
98 prior to program execution. The base wrapper module is contained within the
99 *Wrapper_core* directory (Figure 1). An individual software tool can be easily
100 accessed and executed by importing the module for that tool so as to get access to

101 the method properties on that object. These method properties are available to the
102 user by using the *module.exports* reference object. Inside a driver script file, the user
103 can import the main module object properties and variables by using the *require*
104 keyword which is used to import a module in Node.js. The *require* keyword is
105 actually a global variable, and a script has access to its context because it is
106 wrapped prior to execution inside the *runInThisContext* function (for more details,
107 refer to the Node.js source code: <https://github.com/nodejs>). Once imported, the
108 return value is assigned to a variable which is used to access the various method
109 properties on that object. For example: a method property on the *phyml* object is
110 *phyml.getphyml()*, which invokes the *getphyml* method on the *phyml* object to
111 download and decompress the PhyML executable. For a complete list of all
112 methods, refer to the *README.md* file at the GitHub repository
113 (<https://github.com/dohalloran/phylo-node/blob/master/README.md>). In order to
114 correctly wrap and run each executable, new shells must be spawned so as to
115 execute specific command formats for each executable. This was achieved by using
116 *child.process.exec*, which will launch an external shell and execute the command
117 inside that shell while buffering any output by the process. Binary files and
118 executables were downloaded and executed in this manner and the appropriate file
119 and syntax selected by determining the user's operating system. phylo-node was
120 validated on Microsoft Windows 7 Enterprise ver.6.1, MacOSX El Capitan
121 ver.10.11.5, and Linux Ubuntu 64-bit ver.14.04 LTS.

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123 **RESULTS AND DISCUSSION**

124 phylo-node is a toolkit to interface with key applications necessary in building a
125 phylogenetic pipeline (Table 1). Firstly, phylo-node allows the user to remotely

126 download sequences by building a unique URL and passing this string to the NCBI
127 e-utilities API (<http://www.ncbi.nlm.nih.gov/books/NBK25501/>). Any number of genes
128 can be supplied as command-line arguments to phylo-node by accessing the
129 *fetch_seqs.fasta* method on the *fetch_seqs* object in order to retrieve sequence
130 information in FASTA format. The module for remote sequence retrieval is contained
131 within the *Sequence* directory. phylo-node also provides methods on specific objects
132 to download various executable files using the *download* module (Figure 1). Any
133 binary can be downloaded using the base module *get_executable* contained within
134 the *Download* directory, however objects pertaining to specific tools such as PhyML
135 also contain methods for downloading and unpacking binaries (see README.md file
136 for details). phylo-node also enables the user to create a web server to deploy
137 embeddable applications such as JBrowse [11] which provides genome visualization
138 from within a web browser. To facilitate interoperability between various applications
139 and components, the phylo-node package also contains a module called *phylo-*
140 *node_pipes* inside the *Pipes* directory. The *phylo-node_pipes* module allows the user
141 to easily pipe data between different applications by requiring the *child_process*
142 module which provides the ability to spawn child processes. Through *phylo-*
143 *node_pipes*, the user can chain commands together that will be executed in
144 sequence to build consistent, and extensive pipelines. The *Pipes* directory contains
145 sample driver scripts for using the *phylo-node_pipes* module.

146 phylo-node is highly scalable and new modules for diverse applications can
147 easily be plugged in. The modules required to wrap and execute applications are all
148 contained within the *Run* directory. The following tools can be implemented using
149 phylo-node from within the *./Tool/Run* directory: Trimmomatic [20] to process reads
150 prior to read alignment; Bowtie2 [21] for read alignment to a reference sequence;

151 Primer3 [22-24] to facilitate primer design; Clustal Omega [25], K-align [26], and
152 MUSCLE [27,28] for multiple sequence alignments; Codeml [29], PAL2NAL [30], and
153 Slr [31] for selection analysis; jModelTest2 [32] and ProtTest3 [33] to determine the
154 best-fit model of evolution; and PhyML [34,35] for phylogeny reconstruction. The
155 PhyML executable is also employed by jModelTest2 and ProtTest3. These specific
156 tools were selected because they are some of the most popular choices and
157 applications in many bioinformatics pipelines: for example, Primer3 is the most
158 popular software (over 15,000 citations) for primer design [36]; Clustal Omega, K-
159 align, and MUSCLE are very fast and accurate multiple sequence alignment tools
160 that are commonly used to build robust DNA, RNA, or protein alignments [37];
161 Codeml is part of the PAML suite [29], and alongside PAL2NAL [30] and Slr [31] are
162 commonly used to determine rates of selection [38,39]. ProtTest3 and jModelTest2
163 are widely used to determine best-fit models of amino-acid replacements and
164 nucleotide substitution [40-42]; PhyML is also a popular program (over 12,000
165 citations) for building phylogenies using maximum likelihood [34]; for next generation
166 sequencing data, Trimmomatic and Bowtie2 are commonly implemented in read
167 processing and mapping pipelines [43]. Sample input files for all applications
168 deployed by phylo-node can be found in the *Input_examples* directory and sub-
169 directories. Taken together, phylo-node provides a diverse toolkit that allows the user
170 to develop robust pipelines and instances using Node.

171 phylo-node is highly scalable and customizable, and was inspired by projects
172 such as BioPerl [44] which provides very diverse tools that include Perl modules for
173 many bioinformatic tasks and also parsers and wrappers for diverse sequence
174 formats and applications. BioPerl's open source structure and architecture allows
175 users to plug new modules into BioPerl pipelines to design new applications. Node.js

176 implements prototypal inheritance as per JavaScript but also provides access to the
177 *module.exports* object which permits easy portability between the phylo-node toolkit
178 and any other modules, and also interoperation between different languages by
179 using the *child.process.exec* process. Therefore, *phylo-node* can be integrated with
180 existing Node.js bioinformatics tools [45,46] or software written in other languages.
181 For example, jModelTest2, ProtTest3 and Trimmomatic require a Java runtime
182 environment ([http://www.oracle.com/technetwork/java/javase/downloads/jre8-](http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html)
183 [downloads-2133155.html](http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html)), and by using *require* to import each module, the user can
184 execute the analysis of these tools.

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186 **CONCLUSIONS**

187 In conclusion, phylo-node is a novel package that leverages the speed of Node.js to
188 provide a robust and efficient toolkit for researchers conducting molecular
189 phylogenetics. phylo-node can be easily employed to develop complex but
190 consistent workflows, and integrated with existing bioinformatics tools using the
191 Node.js codebase.

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202 **AVAILABILITY AND REQUIREMENTS**

- 203 • Project name: phylo-node
- 204 • Project home page: <https://github.com/dohalloran/phylo-node>
- 205 • Operating system(s): Platform independent
- 206 • Programming language: Node.js
- 207 • Other requirements: none
- 208 • License: MIT
- 209 • Any restrictions to use by non-academics: no restrictions or login requirements

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217 **AUTHOR CONTRIBUTIONS**

218 D.O'H. conceived the idea for *phylo-node*, wrote and tested the code, and wrote the
219 manuscript.

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221 **COMPETING INTERESTS**

222 The author declares no competing interests.

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367 **FIGURE LEGENDS**

368

369 **Figure 1. Workflow for *phylo-node*.**

370 *phylo-node* is organized into a workflow of connected modules and application
371 scripts. In order to interface with a software tool, the base wrapper module is invoked
372 to process command-line requests that are then passed into the software specific
373 module. The input for the specific software can be passed into the base wrapper
374 from a folder specified by the user or by using the sequence retrieval module which
375 is contained within the *Sequence* directory. The *Pipes* directory contains a module
376 for easy piping of data between applications while binaries and executables can be
377 downloaded using the *get_executable* module from within the *Download* folder to
378 deploy software specific modules within the *Run* directory or to provide applications
379 to a web server from within the *Server* directory.

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392 **Table 1.** Summary of *phylo-node* applications.

Module	Description	Source	Application	Citation
<i>fetch_seqs</i>	Remotely retrieve sequence data	https://www.ncbi.nlm.nih.gov/books/NBK25501/	ASN.1 and FASTA sequences	-
<i>get_executable</i>	Download binaries and executables	https://www.npmjs.com/package/download	batch, exe, jar etc..	-
<i>http_server</i>	Creates a web server	https://nodejs.org/api/http.html and http://ibrowse.org/ibrowse-1-12-1/	local version of JBrowse	Skinner et al. 2009 [47]
<i>bowtie2</i>	Sequence aligner	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml	align fastq reads onto reference genome	Langmead and Salzberg (2012) [21]
<i>trimmomatic</i>	Read Trimming	http://www.usadellab.org/cms/?page=trimmomatic	preprocessing for reference alignment	Bolger et al. (2014) [20]
<i>phyml</i>	Maximum-Likelihood Phylogenies	http://www.atgc-montpellier.fr/phyml/binaries.php	model testing (ProtTest3 and jModelTest2) and tree building	Guindon et al. (2010) [35]
<i>primer3</i>	Primer design	https://sourceforge.net/projects/primer3/	PCR and sequencing	Untergasser et al. (2012) [23]
<i>muscle</i>	MSA	http://www.drive5.com/muscle/downloads.htm	sequence alignment	Edgar (2004a); Edgar (2004b) [27,28]
<i>clustal_Omega</i>	MSA	http://www.clustal.org/omega/#Download	sequence alignment	Sievers et al. (2011) [25]
<i>kalign</i>	MSA	http://msa.sbc.su.se/cgi-bin/msa.cgi	sequence alignment	Lassmann and Sonnhammer (2005) [26]
<i>pal2nal</i>	Generate codon alignments	http://www.bork.embl.de/pal2nal/	processing of alignments for selection analysis	Suyama et al. (2006) [30]
<i>Slr</i>	Selection analysis	http://www.ebi.ac.uk/goldman-srv/SLR/#download	detect rates of selection in coding DNA	Massingham and Goldman (2005) [31]
<i>codeml</i>	Selection analysis	http://abacus.gene.ucl.ac.uk/software/paml.html	ML analysis of coding DNA using codon substitution models	Yang (1997); Yang (2007) [29,48]
<i>prottest</i>	Model selection	https://github.com/ddarriba/prottest3	best-fit model determination of amino-acid replacement	Darriba et al. (2011) [33]
<i>jmodeltest2</i>	Model selection	https://github.com/ddarriba/jmodeltest2	best-fit model determination of nucleotide substitutions	Darriba et al. (2012) [32]
<i>base_wrap</i>	Base module for program execution	https://nodejs.org/api/child_process.html	handles arguments and spawns child processes	-
<i>phylo-node_pipes</i>	Module for chaining commands	-	used to pipe data between applications	-

