Phylo-Node: a molecular phylogenetic toolkit using Node.js

Damien M. O'Halloran[1,2*]

1. Department of Biological Sciences, The George Washington University, Science and Engineering Hall, Rm 6000, 800 22nd Street N.W., Washington DC 20052, USA.
2. Institute for Neuroscience, The George Washington University, 636 Ross Hall, 2300 I St. N.W. Washington DC 20052, USA.

***Corresponding author address:**
Damien O'Halloran, The George Washington University, 636 Ross Hall, 2300 I St. N.W. Washington DC 20052, USA.
Tel: 202-994-8955
Fax: 202-994-6100
EMAIL: damienoh@gwu.edu

ABSTRACT

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

Results: To address this problem, I have developed, *Phylo-Node*, which was developed using Node.js and provides a fast, stable, and scalable toolkit that allows the user to go from sequence retrieval to phylogeny reconstruction. Phylo-Node can execute the analysis and process the resulting outputs from a suite of software options that provides tools for sequence retrieval, alignment, primer design, evolutionary modeling, and phylogeny reconstruction. Furthermore, Phylo-Node provides simple integration and interoperation with other Node modules to develop workflows across multiple components and languages using Node inheritance patterns and a customized piping module to support the production of diverse pipelines.

Conclusions: Phylo-Node is open-source and freely available to all users without sign-up or login requirements. All source code and user guidelines are openly available at the GitHub repository: https://github.com/dohalloran/Phylo-Node

Keywords: Node.js, JavaScript, phylogenetics

BACKGROUND

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

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

Node.js provides server-side back-end JavaScript. Node.js is written in C, C++, and JavaScript and uses the Google Chrome V8 engine to offer a very fast cross-platform environment for developing server side Web applications. Node is a single-threaded environment, which means that only one line of code will be executed at any given time; however, Node employs non-blocking techniques for I/O tasks to provide an asynchronous ability, by using *callback* functions to permit the parallel running of code. Node holds much potential for the bioinformatic analysis of molecular data. A community of Node developers provides modules for bioinformatic sequence workflows (http://www.bionode.io/) which in time will likely parallel the BioJS community (http://www.biojs.io/) for the number of modules versus components. However, as of now there are no robust tools for phylogenetic analysis pipelines currently available using the Node.js codebase. To fill this void I have developed, *Phylo-Node*, which provides a Node.js toolkit that goes from sequence retrieval, to primer design, to alignment, to phylogeny reconstruction, all from a single toolkit. MolPhylo is fast, easy to use, and offers simple customization and portability options through various inheritance patterns. The Node package manager, *npm* (https://www.npmjs.com/), provides a very easy and efficient way to manage dependencies for any Node application. Phylo-Node is available at both GitHub (https://github.com/dohalloran/Phylo-Node) and npm (https://www.npmjs.com/package/phylo-node).

IMPLEMENTATION

Phylo-Node was developed using the Node.js codebase. The Phylo-Node core contains methods for remote sequence retrieval, and phylogenetic analysis using a suite of popular software tools. A base wrapper object is used to prepare the arguments and directory prior to program execution. The base wrapper module is contained within the 'Wrapper_core' directory. An individual software tool can be easily accessed and executed by importing the module for that tool so as to get access to the method properties on that object (Figure 1). These method properties are available to the user by using the 'module.exports' reference object. Inside a driver script file, the user can import the main module object properties and variables by using the 'require' keyword which is used to import a module in Node.js. The 'require' keyword is actually a global variable, and a script has access to its context because it is wrapped prior to execution inside the 'runInThisContext' function (for more details, refer to the Node.js source code: https://github.com/nodejs). Once imported, the return value is assigned to a variable which is used to access the various method properties on that object. For example: a method property on the 'phyml' object is 'phyml.getphyml()', which invokes the 'getphyml' method on the 'phyml' object to download and decompress the PhyML executable. For a complete list of all methods, refer to the 'README' file at the GitHub repository (https://github.com/dohalloran/Phylo-Node/blob/master/README.md). In order to correctly wrap and run each executable, new shells must be spawned so as to execute specific command formats for each executable. This was achieved by using 'child.process.exec', which will launch an external shell and execute the command inside that shell while buffering any output by the process. Binary files and executables were downloaded and executed in this manner and the appropriate file and syntax selected by determining the user's operating system. Phylo-Node was validated on Microsoft Windows 7 Enterprise ver.6.1, MacOSX El Capitan ver.10.11.5, and Linux

Ubuntu 64-bit ver.14.04 LTS.

RESULTS AND DISCUSSION

Phylo-Node is a toolkit to interface with key applications necessary in building a phylogenetic pipeline (Figure 2). Firstly, Phylo-Node allows the user to remotely download sequences by building a unique URL and passing this string to the NCBI e-utilities API (http://www.ncbi.nlm.nih.gov/books/NBK25501/). Any number of genes can be supplied as command-line arguments to Phylo-Node by accessing the *fetch_seqs.fasta* method on the *fetch_seqs* object in order to retrieve sequence information in FASTA format. The module for remote sequence retrieval is contained within the ‘Sequence’ directory. Phylo-Node also provides methods on specific objects to download various executable files using the ‘download’ module. Any binary can be downloaded using the base module ‘get_executable’ contained within the ‘Download’ directory, however objects pertaining to specific tools such as PhyML also contain methods for downloading and unpacking binaries (see README.md file for details). Phylo-Node then provides modules to execute the following programs from within the ‘./Tool/Run’ directory: Primer3 [20-22] to facilitate primer design; Clustal Omega [23], K-align [24], and MUSCLE [25, 26] for multiple sequence alignments; jModelTest2 [27] and ProtTest3 [28] to determine the best-fit model of evolution, and PhyML [29, 30] for phylogeny reconstruction. The PhyML executable is also employed by jModelTest2 and ProtTest3. Primer3 is the most popular software for primer design, and takes a very lengthy list of input variables to optimize primer selection. Clustal Omega, K-align, and MUSCLE are very fast and accurate multiple sequence alignment tools that are commonly used to build robust DNA, RNA, or protein alignments. PhyML is a popular program for building phylogenies using maximum likelihood, and Prottest3 determines the best-fit model of evolution for protein sequences across 120 different potential models, while jModelTest2 determines best-fit models of nucleotide substitution from DNA sequence alignments. Together, Phylo-Node provides a novel toolkit that allows the user to go from raw sequence to phylogeny using Node.

Phylo-Node is highly scalable and customizable, and was inspired by projects such as BioPerl [31] which provides Perl modules for many bioinformatic tasks and also provides parsers and wrappers for diverse sequence formats and applications. BioPerl's open source structure and architecture allows users to plug new modules into BioPerl pipelines to design new applications. Node.js implements prototypal inheritance as per JavaScript but also provides access to the 'module.exports' object which permits easy portability between the Phylo-Node toolkit and any other modules, and also interoperation between different languages by using the 'child.process.exec' process. Therefore, Phylo-Node can be integrated with existing Node.js bioinformatics tools [32, 33] or software written in other languages. For example, both jModelTest2 and Prottest3 require a Java runtime environment (http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html), and by using 'require' to import each module, the user can execute the analysis of jModelTest2 and Prottest3. The 'prottest' and 'jmodeltest2' modules and driver scripts (index.js) are contained within the respective 'Prottest3' and 'jModelTest2' directories and sample input is provided in the 'COX2_PF0016' sub-directory of the 'Input_examples' folder for ProtTest3 and the sample FASTA file aP6.fas (also contained within the ‘Input_examples’ folder) can be used for testing jModelTest2.

To further facilitate the ease of interoperation between various applications and components, the Phylo-Node package also contains a module called ‘phylo-node_pipes’ inside the ‘Pipes’ directory. The ‘phylo-node_pipes’ module allows the user to easily pipe data between different applications by requiring the ‘child_process’ module which provides the ability to spawn child processes. Through ‘phylo-node_pipes’, the user can chain commands together that will be executed in sequence to build consistent, and extensive pipelines. The ‘Pipes’ directory contains sample driver scripts for using the ‘phylo-node_pipes’ module.

CONCLUSIONS

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

AVAILABILITY AND REQUIREMENTS

- Project name: Phylo-Node
- Project home page: https://github.com/dohalloran/phylo-node
- Operating system(s): Platform independent
- Programming language: Node.js
- Other requirements: none
- License: MIT
- Any restrictions to use by non-academics: no restrictions or login requirements

ACKNOWLEDGMENTS

I thank members of the O'Halloran lab for critical reading of the manuscript, and would like to thank The George Washington University (GWU) Columbian College of Arts and Sciences, GWU Office of the Vice-President for Research, and the GWU Department of Biological Sciences for Funding.

AUTHOR CONTRIBUTIONS

D.O'H. conceived the idea for *Phylo-Node*, wrote and tested the code, and wrote the manuscript.

COMPETING INTERESTS

The author declares no competing interests.

References

1. Shaffer C: **Next-generation sequencing outpaces expectations.** Nat Biotechnol 2007, **25**(2):149.

2. Wade N: **The quest for the $1,000 human genome: DNA sequencing in the doctor's office? At birth? It may be coming closer.** N Y Times Web 2006, :F1, F3.

3. Mardis ER: **Anticipating the 1,000 dollar genome.** Genome Biol 2006, **7**(7):112.

4. Service RF: **Gene sequencing. The race for the $1000 genome.** Science 2006, **311**(5767):1544-1546.

5. Hayden EC: **The $1,000 genome.** Nature 2014, **507**(7492):294-295.

6. Yachdav G, Goldberg T, Wilzbach S, Dao D, Shih I, Choudhary S, Crouch S, Franz M, Garcia A, Garcia LJ, Gruning BA, Inupakutika D, Sillitoe I, Thanki AS, Vieira B, Villaveces JM, Schneider MV, Lewis S, Pettifer S, Rost B, Corpas M: **Anatomy of BioJS, an open source community for the life sciences.** Elife 2015, **4**:10.7554/eLife.07009.

7. Gomez J, Garcia LJ, Salazar GA, Villaveces J, Gore S, Garcia A, Martin MJ, Launay G, Alcantara R, Del-Toro N, Dumousseau M, Orchard S, Velankar S, Hermjakob H, Zong C, Ping P, Corpas M, Jimenez RC: **BioJS: an open source JavaScript framework for biological data visualization.** Bioinformatics 2013, **29**(8):1103-1104.

8. Salazar GA, Meintjes A, Mulder N: **PPI layouts: BioJS components for the display of Protein-Protein Interactions.** F1000Res 2014, **3**:50-50.v1. eCollection 2014.

9. Gomez J, Jimenez R: **Sequence, a BioJS component for visualising sequences.** F1000Res 2014, **3**:52-52.v1. eCollection 2014.

10. Cui Y, Chen X, Luo H, Fan Z, Luo J, He S, Yue H, Zhang P, Chen R: **BioCircos.js: an interactive Circos JavaScript library for biological data visualization on web applications.** Bioinformatics 2016, **32**(11):1740-1742.

11. Buels R, Yao E, Diesh CM, Hayes RD, Munoz-Torres M, Helt G, Goodstein DM, Elsik CG, Lewis SE, Stein L, Holmes IH: **JBrowse: a dynamic web platform for genome visualization and analysis.** Genome Biol 2016, **17**(1):66-016-0924-1.

12. Salavert F, Garcia-Alonso L, Sanchez R, Alonso R, Bleda M, Medina I, Dopazo J: **Web-based network analysis and visualization using CellMaps.** Bioinformatics 2016, .

13. Franz M, Lopes CT, Huck G, Dong Y, Sumer O, Bader GD: **Cytoscape.js: a graph theory library for visualisation and analysis.** Bioinformatics 2016, **32**(2):309-311.

14. Vanderkam D, Aksoy BA, Hodes I, Perrone J, Hammerbacher J: **pileup.js: a JavaScript library for interactive and in-browser visualization of genomic data.** Bioinformatics 2016, .

15. Garcia L, Yachdav G, Martin MJ: **FeatureViewer, a BioJS component for visualization of position-based annotations in protein sequences.** F1000Res 2014, **3**:47-47.v2. eCollection 2014.

16. Kalderimis A, Stepan R, Sullivan J, Lyne R, Lyne M, Micklem G: **BioJS DAGViewer: A reusable JavaScript component for displaying directed graphs.** F1000Res 2014, **3**:51-51.v1. eCollection 2014.

17. Villaveces JM, Jimenez RC, Habermann BH: **KEGGViewer, a BioJS component to visualize KEGG Pathways.** F1000Res 2014, **3**:43-43.v1. eCollection 2014.

18. Villaveces JM, Jimenez RC, Habermann BH: **PsicquicGraph, a BioJS component to visualize molecular interactions from PSICQUIC servers.** F1000Res 2014, **3**:44-44.v1. eCollection 2014.

19. Yachdav G, Hecht M, Pasmanik-Chor M, Yeheskel A, Rost B: **HeatMapViewer: interactive display of 2D data in biology.** F1000Res 2014, **3**:48-48.v1. eCollection 2014.

20. You FM, Huo N, Gu YQ, Luo MC, Ma Y, Hane D, Lazo GR, Dvorak J, Anderson OD: **BatchPrimer3: a high throughput web application for PCR and sequencing primer design.** BMC Bioinformatics 2008, **9**:253-2105-9-253.

21. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG: **Primer3--new capabilities and interfaces.** Nucleic Acids Res 2012, **40**(15):e115.

22. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JA: **Primer3Plus, an enhanced web interface to Primer3.** Nucleic Acids Res 2007, **35**(Web Server issue):W71-4.

23. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG: **Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega.** Mol Syst Biol 2011, **7**:539.

24. Lassmann T, Sonnhammer EL: **Kalign--an accurate and fast multiple sequence alignment algorithm.** BMC Bioinformatics 2005, **6**:298.

25. Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput.** Nucleic Acids Res 2004, **32**(5):1792-1797.

26. Edgar RC: **MUSCLE: a multiple sequence alignment method with reduced time and space complexity.** BMC Bioinformatics 2004, **5**:113.

27. Darriba D, Taboada GL, Doallo R, Posada D: **jModelTest 2: more models, new heuristics and parallel computing.** Nat Methods 2012, **9**(8):772.

28. Darriba D, Taboada GL, Doallo R, Posada D: **ProtTest 3: fast selection of best-fit models of protein evolution.** Bioinformatics 2011, **27**(8):1164-1165.

29. Guindon S, Gascuel O: **A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood.** Syst Biol 2003, **52**(5):696-704.

30. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O: **New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0.** Syst Biol 2010, **59**(3):307-321.

31. Stajich JE, Block D, Boulez K, Brenner SE, Chervitz SA, Dagdigian C, Fuellen G, Gilbert JG, Korf I, Lapp H, Lehvaslaiho H, Matsalla C, Mungall CJ, Osborne BI, Pocock MR, Schattner P, Senger M, Stein LD, Stupka E, Wilkinson MD, Birney E: **The Bioperl toolkit: Perl modules for the life sciences.** Genome Res 2002, **12**(10):1611-1618.

32. Kim J, Levy E, Ferbrache A, Stepanowsky P, Farcas C, Wang S, Brunner S, Bath T, Wu Y, Ohno-Machado L: **MAGI: a Node.js web service for fast microRNA-Seq analysis in a GPU infrastructure.** Bioinformatics 2014, **30**(19):2826-2827.

33. Page M, MacLean D, Schudoma C: **blastjs: a BLAST+ wrapper for Node.js.** BMC Res Notes 2016, **9**:130-016-1938-1.

FIGURE LEGENDS

Figure 1. Workflow for Phylo-Node.

Phylo-Node is organized into a workflow of connected modules and driver scripts. In order to interface with a phylogenetic tool, the base wrapper module is invoked to process command-line requests that are then passed into the software specific module. The input for the specific software can be passed into the base wrapper from: a) sample input directory; b) from a folder specified by the user; or c) by using the sequence retrieval module which is contained within the 'Sequence' directory. The 'Pipes' folder contains a module for easy piping of data between applications in Phylo-Node. Binaries can be downloaded using the 'get_executable' module from within the 'Download' folder.

Figure 2. Graphical overview of Phylo-Node applications within the *./Tools/Run* directory.

Phylo-Node provides a toolkit for interacting with various applications including: the sequence alignment software Clustal Omega [23], K-align [24], and MUSCLE [25, 26]; the primer design software, Primer3 [20-22]; software for determining the best-fit models of evolution: jModelTest2 [27] and Prottest3 [28]; and also the phylogeny reconstruction software, PhyML [29, 30]. Phylo-Node also enables the user to retrieve sequences remotely from the NCBI database using Entrez Programming Utilities. A key feature of Phylo-Node is interoperability between languages and other Node modules, which can be easily leveraged to form stable and scalable pipelines. This concept of interoperation and inheritance is highlighted by the brown cog at the bottom of Figure 2 that represents the potential to integrate any other module(s) [*require('./module');*] with Phylo-Node.

Figure 1

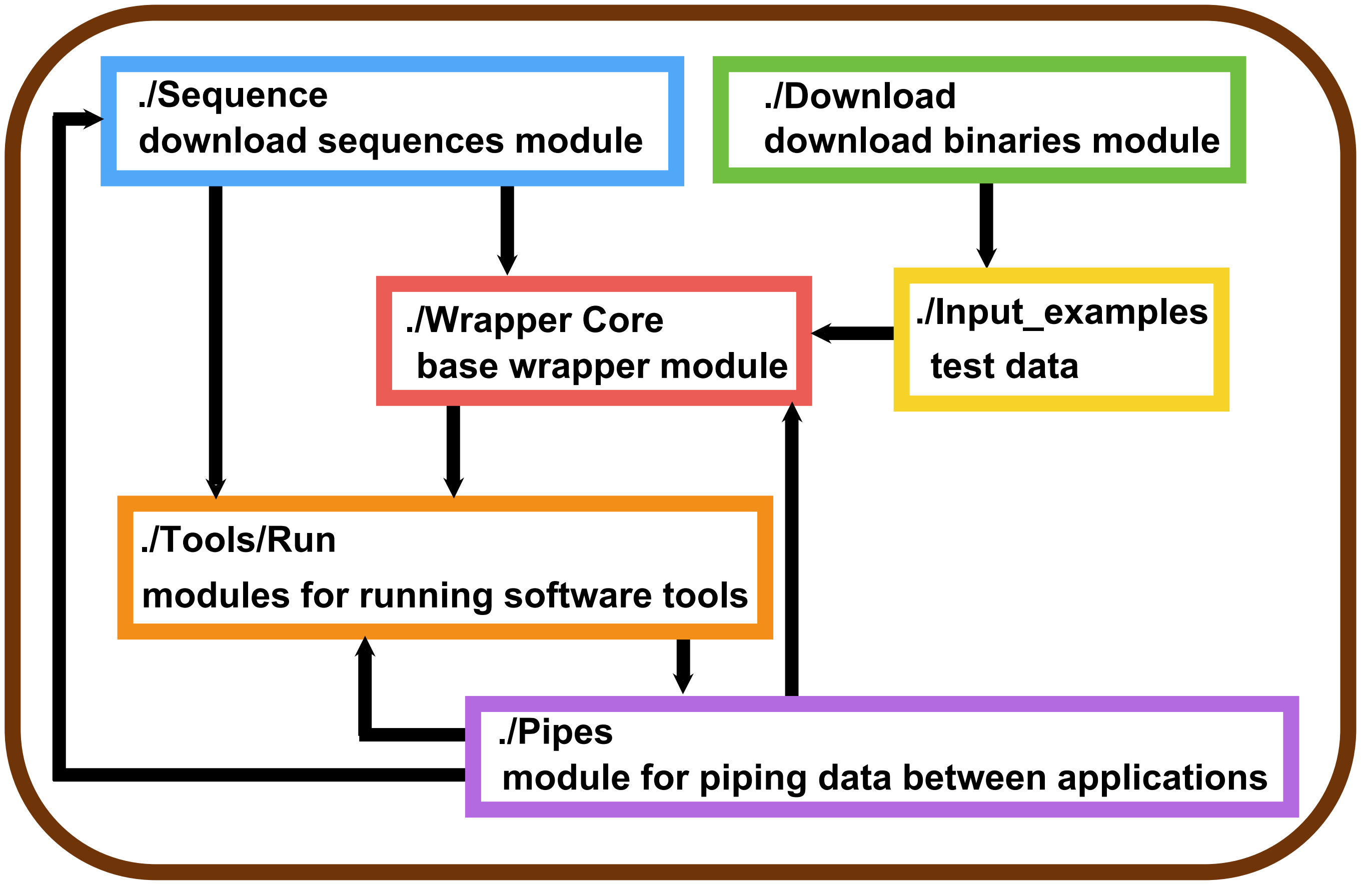
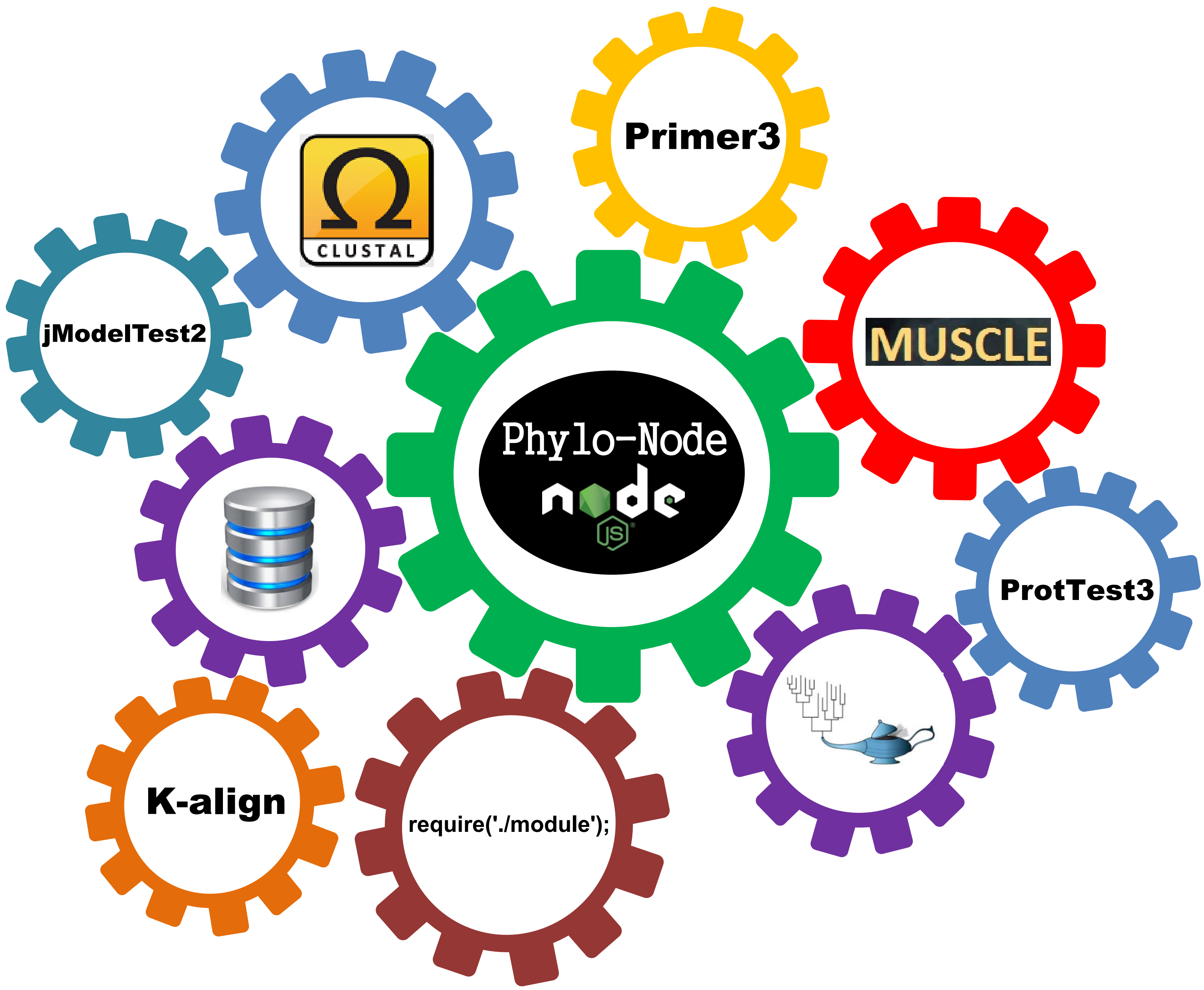

./Sequence
download sequences module
./Download
download binaries module
./Wrapper Core
base wrapper module
./Input_examples
test data
./Tools/Run
modules for running software tools
./Pipes
module for piping data between applications

Figure 2


CLUSTAL
Primer3
jModelTest2
MUSCLE
Phylo-Node
node
JS
ProtTest3
K-align
require('./module');