1. INTRODUCTION

CPTree is a web-based Phylogenetic Tree Generator using Chloroplast Genomes. It defined two methodologies to generate the phylogenetic trees, one uses single ribosomal RNA in cpDNA to provide a fast preview of the evolutionary relationship; the other uses full cpDNA to calculate a relatively accurate tree. Meanwhile, the key model of CPTree, Chloroplast Database (CPDB), contains a local database of all available cpDNA from Genome Information by Organism in NCBI https://www.ncbi.nlm.nih.gov.

DATA SOURCE

The Fast Mode uses ribosomal RNA data as the material for phylogenetic analysis. Meanwhile, an input of the whole cpDNA is supported in the Normal Mode.

CPDB synchronized with NCBI https://www.ncbi.nlm.nih.gov maintains over 1000 cpDNAs and their key features extracted from its original NCBI database, including: full Sequence file(.fa), GenBank file(.gb), Nucleotide and Protein in CDS, Feature table, stand-alone 16S and 23S ribosomal RNA Sequence, and also Summary pages for each cpDNA.

DATA FORMAT

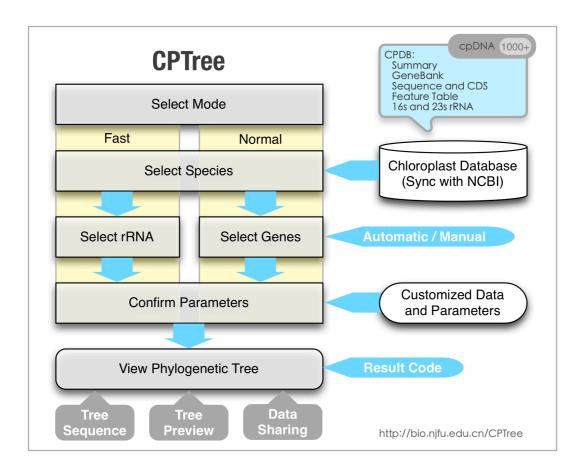
GeneBank (.gb): GenBank format is a flat file format for sequence data related to genomes. The file format we used here is **NMPDR** (http://www.nmpdr.org/FIG/wiki/view.cgi/FIG/GBK).

FASTA (.fa): FASTA format is a text-based format for representing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are represented using single-letter codes. The file format we used here is https://en.wikipedia.org/wiki/FASTA_format.

Txt feature table: The table, which is synchronized with NCBI https://www.ncbi.nlm.nih.gov, organizes information of Chloroplast genomes including Organism/Name, RefSeq, INSDC, number of genes, RNAs, GC percentage and so on.

WORKFLOW

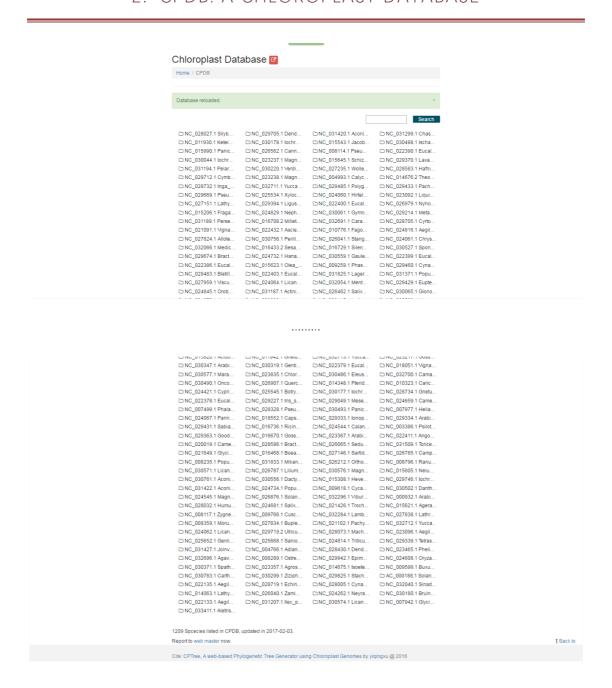
The flow chart is as follows:



When you choose the fast mode to build a tree, the first step is to select the species you need. What follows is a ribosomal RNA selection. After confirming parameter, the calculation starts. About one to five minutes later, the result of data is presented in newick tree. You can also view the result in tree view and circular view. Moreover, you can send the data to iTOL to open an editable tree view.

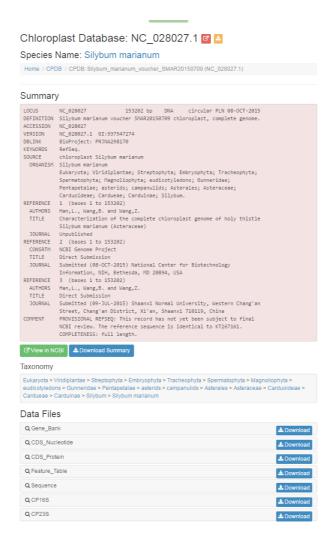
When the normal mode is selected, the first step is to select species. What follows is genes selection. You can use the automated selected genes. After confirming parameter, the calculation starts. About five to thirty minutes, the result of data is presented in newick tree. You can also view the result in tree view and circular view. Moreover, you can send the data to iTOL to open an editable tree view.

2. CPDB: A CHLOROPLAST DATABASE



CPDB contains all available cpDNA from genome information by organisms in NCBI. It maintains over 1000 cpDNAs in the current version. To one cpDNA correspond to a link and once click it, the page for the cpDNA will be opened.

Besides, the amount of the species listed in CPDB and the latest date updated are displayed at the bottom of the page.



The key features extracted from NCBI database include: full sequence file(.fa), GenBank file(.gb), nucleotide and protein in CDS, feature table, stand-alone 16S and 23S ribosomal RNA sequence, and also the summary information for each cpDNA. The information in red implies the summary of the species. Below, there are two buttons. Click



(https://www.ncbi.nlm.nih.gov). The button is used to download the summary information for the cpDNA.

Taxonomy

Eukaryota > Viridiplantae > Streptophyta > Embryophyta > Tracheophyta > Spermatophyta > Magnoliophyta > Liliopsida > Poales > Poaceae > PACMADclade > Panicoideae > Panico

Below the "Taxonomy" title, we provide the taxonomy links towards NCBI (https://www.ncbi.nlm.nih.gov.), so the users can access the taxonomy information of the species.

Below the "Data Files" title, seven accordions all have two buttons: and and Download. The details in accordions will be shown after clicking the left-most button while the right-most button can download file of these details. The file format downloaded by the first five buttons is txt and the others' file format is FASTA.

If you require downloading all these files, you should use the button on the right at the top of the page. It will compress all these file into zip.

The button has the same feature as the "View in NCBI" button to open a new page from NCBI which introduces the detail of the species.

3. STANDALONE PERL SCRIPT

DATA EXTRACTOR FROM NCBI

Download the species data from NCBI by typing the command: perl batchDownload.pl -i list.txt -o cpdb

- -i: The -i command allows you to input the name of the text file with information about species' refseqs.
- -o: The -o command allows you to input the name of the output directory.
 For example: -o cpdb outputs species' data to the folder named cpdb.

FAST MODE

If you choose the fast mode to build a tree, just type the command: perl rRNA2tree.pl -i list.txt -g cp_16s -c default_config.txt -o result_16s

- -i: The -i command allows you to input the name of the text file with information about species' refseqs.
- -g: The -g command allows you to select gene type between cp_16s and cp_23s.
- -c: The command allows you to input the name of the file with information about parameter value of phylogenetic tree drawn by ClustalW.
- -o: The -o command allows you to input the name of the output directory. For example: -o result_16s outputs the result data to the folder named result 16s.

NORMAL MODE

When you select the normal mode to generate a **phylogenetic tree**, the first step is to type the command: perl pickGene.pl -i list.txt > gene.txt

• -i: The **-i** command allows you to input the name of the text file with information about species' refseqs.

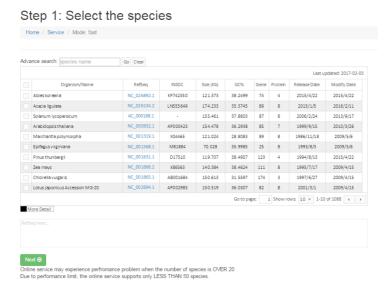
The next step is to install clustalw. Refer to http://www.clustal.org/clustal2 for more information about installing and using clustalw.

Then type the command: perl Protein2Tree.pl -i list.txt -g gene.txt -c default_config.txt -o result_protein.

- -i: The -i command allows you to input the name of the text file with information about species' refseqs.
- -g: The -g command allows you input the name of the text file with information about genes used to build the tree.
- -c: The command allows you to input the name of the file with information about parameter value of phylogenetic tree drawn by ClustalW.
- -o: The -o command allows you to input the name of the output directory.
 For example: -o result_protein outputs the result data to the folder named result_protein.

4. ONLINE SERVICES

FAST MODE	
STEP 1: SELECT SPECIES	



After choosing the fast mode to build a tree, the first step is to select the species you need. The table in the page of "Select the species" organizes information of Chloroplast genomes including Organism/Name, RefSeq, INSDC, number of genes, RNAs, GC percentage and so on, and the top right of the table shows this update time. The table provides paging, sorting and filtering facilities to ease the selection of the species.

How to page

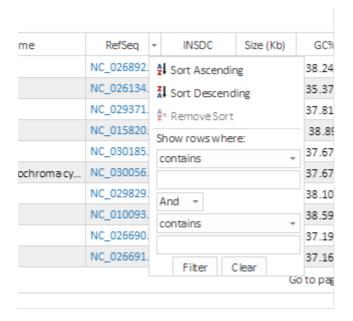
On the bottom right corner, besides the" go to page" label, enter the page number in the text field, the table will update the information. To scroll back and forth through the pages of data, click arrow symbols.

How to filter

Search the species by inputting terms into the Search box located at the top left of the table and clicking the "Go" button. The left-most column lists the filters, which are selected by checking the boxes at the left of the species name. Once the filters are selected, its RefSeq information appears in the text field below the table. To clear all the filters, click the "clear" button. After selecting the species, proceed to the next step via clicking the "next" button. There is one important caution that needs to be noted: Online service may experience performance problem when the number of species is **OVER 20**

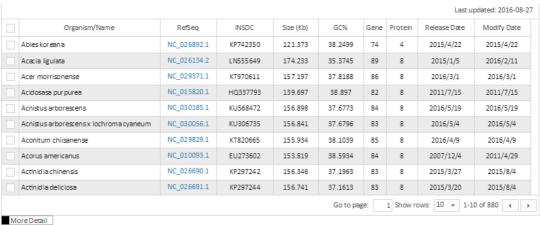
Due to performance limit, the online service supports only LESS THAN 50 species.

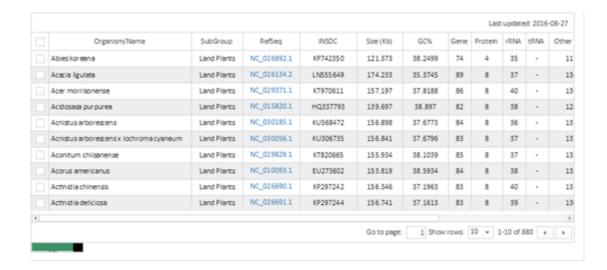
How to sort



Hover your mouse over the any column of the first row and click the arrow symbols, it produces a new dialog allowing you to sort the column cells.

Detailed information





To see all of the columns, you need to click on More Detail at the lower left

table. Clicking on the arrow symbol besides the "show rows" label, you can choose the number of species on this page from the drop down menu. Click on the "**↑**Back to Top" at the lower-right side of the page to go back to the top of the page.

STEP 2: SELECT RIBOSOMAL RNA Step 2: Parameter Confirm Mode Fast No. 029371.1.NC_015820.1.NC_030185.1.NC_030056.1 Gene Selected 165 ribosomal RNA Custom Sequence 165 ribosomal RNA Custom Tree-building 156 Use Default Tree Parameters 1 Clustalw Para -ALIGN_TREE_OUTPUT=NEXUS_TYPE=ENA_BOOTSTRAP=1000 Confirm Cancel Cite CFTree, A web-based Phylogenetic Tree Generator using Chloroplast Genomes by Ethan @ 2016

There are four text areas and one drop down menu vertically arranged in the "Parameter Confirmation" page. You can select the ribosomal RNA in the drop down menu .The first text area shows user-selected mode. The gene sequences are displayed in the second text

area. The users have to upload custom sequence in FASTA format to the third one, if they need additional sequence. The tree parameters are presented in the last text area.

How to select Ribosomal RNA

Clicking the arrow symbol in the lower-right hand corner of the second box will appears a drop-down menu including 16s RNA and 23s RNA, which the user can choose from.

How to use additional Sequence

Custom Sequence	
Sequence in Fasta	

Click the small icon besides "Use Additional Sequence", and then upload custom sequence in FASTA format.

Tree Parameter

Custom Tree-building ✓ Use Default Tree Parameters ?		
Clustalw Para	-ALIGN -TREE -OUTPUT=NEXUS -TYPE=DNA -BOOTSTRAP=1000	/

If the users don't want to use the default parameters to build the tree, click the small icon with checkmark besides "Use Default Tree Parameters" and the checkmark will disappear. Then input the new parameter value in the text area below.

After data determination, click the "confirm" button located below the boxes to submit the data. The user needs reselect data by checking the "cancel" button.

STEP 3: CALCULATION



Cite: CPTree, A web-based Phylogenetic Tree Generator using Chloroplast Genomes by Ethan @ 2016

The page of "data Submitted" where you can view the progress of computing and download file, will be displayed after parameter confirmation. If "Data accepted" are seen in green, it shows that the data has been submitted successfully.

View the result



You can view the result of data by clicking on the link named 'Result code'. If the phylogenetic tree hasn't been constructed, a progress bar will be shown to indicate the progress of construction. The user can share the results by simply sharing the link whenever the final result is still in process or finalized. If the process takes too much calculation time, the users can use the code to retrieve the final result without waiting online. The running log is also represented in the text area below the progress bar.

Download

The gene sequences and ribosomal RNA are available for download in textual format by clicking button.

155.934 153.819

38.5934

2011/4/29

After choosing the normal mode, the first step is to select the species you need. The table in the page of "Select the species" organizes information of Chloroplast genomes including Organism/Name, RefSeq, INSDC, number of genes, RNAs, GC percentage and so on, and the top right of the table shows this update time. The table provides paging, sorting and filtering facilities to ease the selection of the species.

may experience perfromance problem when the number of species is OVER 20 ance limit, the online service supports only LESS THAN 50 species.

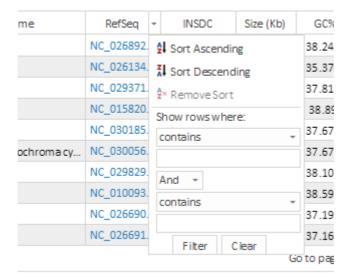
How to page

On the bottom right corner, besides the "go to page" label, enter the page number in the text field, the table will update the information. To scroll back and forth through the pages of data, click arrow symbols.

How to filter

Search the species by inputting terms into the Search box located at the top left of the table and clicking the "Go" button. The left-most column lists the filters, which are selected by checking the boxes at the left of the species name. Once the filters are selected, its RefSeq information appears in the text field below the table. To clear all the filters, click the "clear" button. After selecting the species, proceed to the next step to choose an appropriate mode via clicking the "next" button.

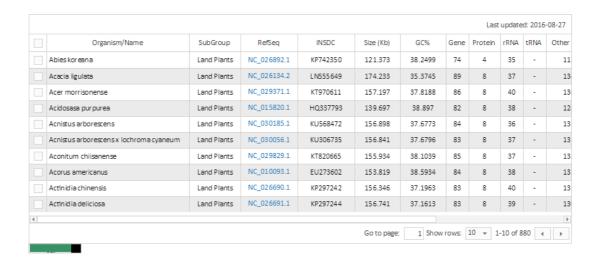
How to sort



Hover your mouse over the any column of the first row and click the arrow symbols, it will produce a new dialog allowing you to sort the column cells.

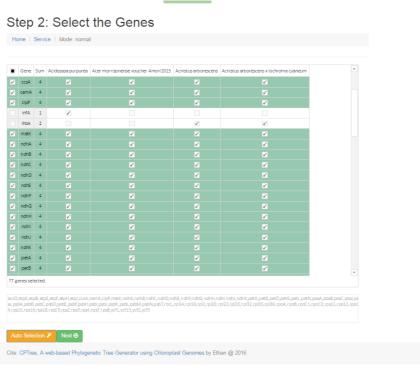
Detailed information





To see all of the columns, you need to click on More Detail at the lower left table. Clicking on the arrow symbol besides the "show rows" label, you can choose the number of species on this page from the drop down menu. Click on the "↑Back to Top" at the lower-right side of the page to go back to the top of the page.

STEP 2: SELECT GENE



The selected species are listed in the first row of the table. The first column of lists all the cpDNA genes contained in the selected species. All genes are filtered and matched for

each species. The text field under the table shows all the genes used to **build phylogenetic** trees.

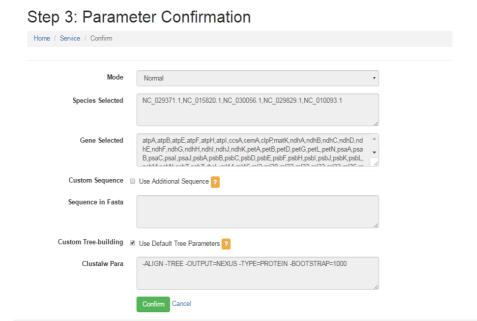
How to select genes

To search the cpDNA genes, move the mouse to the left-most column and select the check box on the left of the name of the gene molecules needed. Once the filters are selected, these gene molecules will be listed in the text area at the bottom of the page and the number of the gene molecules selected will appear below the table. If you want to select all the genes, you can choose the check box at the top of the left-most column.

Moreover, if you click

Auto Selection at the bottom of the page, all genes will be filtered and matched for each species in order to build phylogenetic trees.

Parameter confirmation



There are five text areas vertically arranged in the middle of the page. The first area shows user-selected mode. The RefSeq and genes you have chosen are displayed in the second and third text areas respectively. To see all genes, you need click on the arrow symbol in the right of the third area. The users have to upload custom sequence in FASTA format to the fourth one, if they need additional sequence. The tree parameters are presented in the last area.

How to use additional Sequence

Custom Sequence	✓ Use Additional Sequence
Sequence in Fasta	
	a a second and a second a seco

Click the small icon besides "Use Additional Sequence", and then upload custom sequence in FASTA format to the text area below.

Tree Parameter



If the users don't want to use the default parameters to build the tree, click the small icon with checkmark besides "Use Default Tree Parameters" and the checkmark will disappear. Then input the new parameter value in the text area below.

After data determination, click the "confirm" button to submit the data. The user needs reselect data by checking the "cancel" button.

STEP 3: CALCULATION

Data Submitted

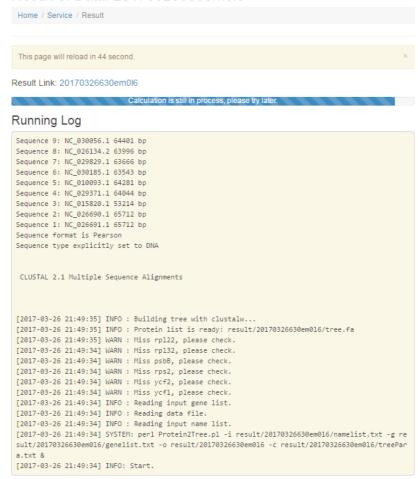


Cite: CPTree, A web-based Phylogenetic Tree Generator using Chloroplast Genomes by yiqingxu @ 2016

The page of "data Submitted" where you can view the progress of computing and download files, will be displayed after parameter confirmation. If "Data accepted" are seen in green, it shows that the data has been submitted successfully.

View the result

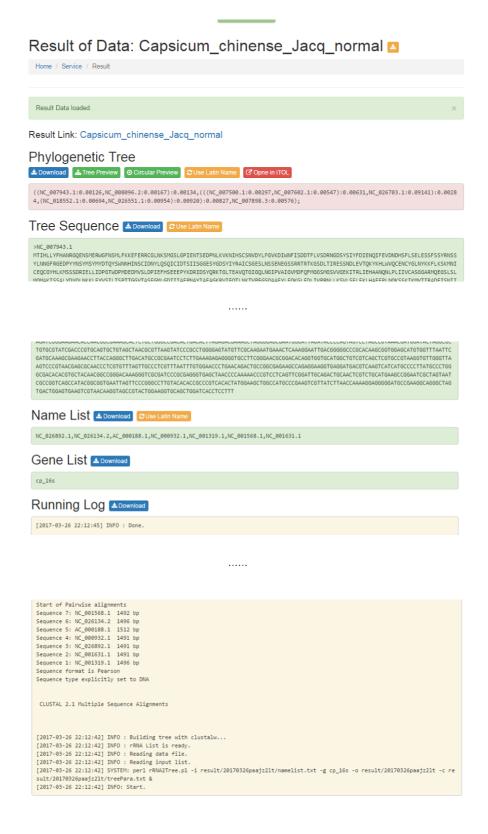
Result of Data: 20170326630em0l6



There is a link named 'Result code' that you can view the result of data by clicking on. If the phylogenetic tree hasn' t been constructed, a progress bar will be shown to indicate the progress of construction. The running log is represented in the text area below the progress bar.

Download

The name list of selected species and gene list are available for download in textual format by clicking button.



After calculation, click on the result link and the "result of data" page will appear.

Phylogenetic Tree

 $(NC_016730.1:0.00000, (((NC_016728.1:0.00717, (NC_023358.1:0.00000, NC_016729.1:0.00044):0.00279):0.00098, NC_023359.1:0.000345):0.00035, ((((NC_007578.1:0.00137, NC_007977.1:0.00077):0.00261, NC_008096.2:0.00629):0.00300, NC_010776.1:0.00795):0.00826, NC_023357.1:0.00107):0.00001):0.00000, NC_016727.1:0.00000);$

Below "Phylogenetic Tree" title, there are five buttons and a text area. The phylogenetic tree in red area is **in Newick format**, which is available for download by clicking

Language State S

are used to watching the result in the tree view and in the circular view. The users can also download the generated plots as either PNG raster image or SVG vector graphics. To send

the tree view to iTOL http://itol.embl.de/help.cgi#manage, click the

button. Click Cuse Latin Name button, the RefSeq in the red area will be replaced by Latin name of species and vice versa.

Tree Sequence Download Use Latin Name

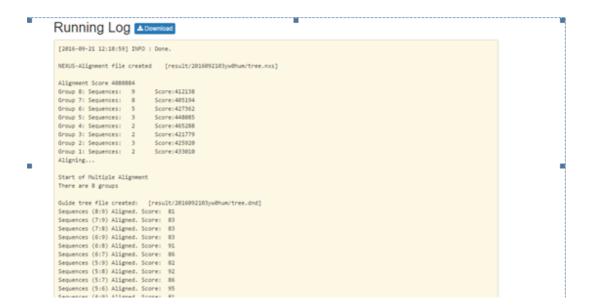
The tree sequence shown in the green area below the title of "Tree Sequence" is the re-organized sequence (in fasta format) for phylogenetic calculation, which is

downloadable by clicking button. Click Suse Latin Name button, the RefSeq will be replaced by Latin name of species and vice versa.

Name List ≜Download Suse Latin Name

The RefSeqs in the green area below the title of "Gene list" lists the selection of species, which can be downloaded in the textual format by clicking button. Click button, the RefSeq will be replaced by Latin name of species.

The genes you have selected are listed in the green area below the title of "Gene List", which is available for download by clicking button.



The content in the red area below the title of "Running Log" records the running log, which can be downloaded in the log format by clicking button besides the "Running Log" title.

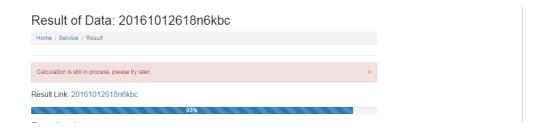
If all the files are needed to be downloaded, the download button at the top of the page can compress all files into zip format. Click on the "↑Back to Top" at the lower-right side of the page to go back to the top of the page.

5. DATA VISUALIZATION AND SHARING

The Phylogenetic Tree in the result page installed a data sharing interface, so it can transmit the tree data to iTOL (http://itol.embl.de/help.cgi#manage) and will generate an online tree for further manipulation.

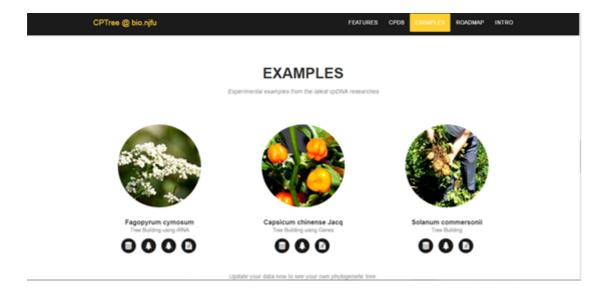


Each result page is numbered by an experiment code, and user can share the result by simple share the link, whenever the final result is still in process or finalized.



In this way, if the process cost too much time calculating, user can use the code to retrieve the final result without waiting online.

6. SAMPLES



The service homepage of CPTree http://bio.njfu.edu.cn/CPTree/service lists three experimental examples including the cpDNA research of Fagopyrum cymosum, Capsicum chinense Jacq and Solanum commersonii in 2015-2016.

There are four buttons below the research of Fagopyrum cymosum, using ribosomal RNA

to build trees. The left-most button allows you to open the "Step 1: Select the species" page of fast mode. Click on the green

button Use Fagopyrum_cymosum Sample

to select ribosomal RNA in the "Step 2:

Parameter Confirmation" page. Next, click the button and skip to the page of "Data Submitted". You can click the link beside "Result code" to view result of the research. You can also download the name lists and gene lists by checking the

button .The second button on the left opens the "Result of Data:

Fagopyrum_cymosum_cp_16s" page, which displays the result of tree building using 16s

ribosomal RNA. Meanwhile, the third button on the left allows you to view the tree,

which is built from 23s ribosomal RNA. After clicking the right-most button, you can read the article which provides theoriginal tree in the cpDNA research of Fagopyrum cymosum.

There are three buttons respectively below the research of Capsicum chinense Jacq and

Solanum commersonii using genes to build trees. The left-most button
to open the "Step 1: Select the species" page of fast mode. Click on the green
button
Use Fagopyrum_cymosum Sample to go to the "Step 2: Select the Genes"
page, where you need to check the button
Use Capsicum_chinense_Jacq Sample Setting and Next
to open the page of "Step 3:

Parameter Confirmation" . Next, click the button

Confirm and skip to the page of "Data Submitted" . You can click the link beside "Result code" to view result of the research. You can also download the name list and gene list by checking button. The

building using genes. After clicking the right-most button , you can read the article which provides the original tree in the cpDNA research of Capsicum chinense Jacq and Solanum commersonii.