Database, 2019, 1–7
doi: 10.1093/database/baz127
Original article

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PCIR: a database of Plant Chloroplast Inverted Repeats

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Citation details: Zhang,R., Ge,F., Li,H. *et al.* PCIR: a database of Plant Chloroplast Inverted Repeats. *Database* (2019) Vol. 2019: article ID baz127; doi:10.1093/database/baz127

Received 16 July 2019; Revised 26 September 2019; Accepted 7 October 2019

Abstract

Inverted repeats (IRs) serve as potential biomarkers for genomic instability, DNA replication and other genetic processes. However, little information can be found in databases to help researchers recognize potential IR nucleotides, explore junction sites and annotate related functional genes. Plant Chloroplast Inverted Repeats (PCIR) is an interactive, web-based platform containing various sequenced chloroplast genomes that enables detection, searching and visualization of large-scale detailed information on IRs. PCIR contains many datasets, including 21 433 IRs, 113 plants chloroplast genomes, 16 948 functional genes and 21 659 visual maps. This database offers an online prediction tool for detecting IRs based on DNA sequences. PCIR can also analyze phylogenetic relationships using IR information among different species and provide users with high-quality marker maps. This database will be a valuable resource for IR distribution patterns, related genes and architectural features.

Database URL: http://biodb.sdau.edu.cn/pcir/index.php

Introduction

Inverted repeats (IRs) are DNA secondary structures that consist of six or more nucleotides in reverse complementary sequence orientation (1,2). Shortly after the β-helix structure of DNA was proposed by Watson and Crick (3), the existence of IRs was recognized to account for some biological processes of cells (4,5). IRs are distributed abundantly in a variety of eukaryote and prokaryote plastids (2). When the transcription or replication processes occurring, two strands separating and IRs might produce with nucleotides transiting from inter-strand to intra-strand base pairing. When the two DNA strands are separated during transcription or replication processes, IRs can result in nucleotides transitioning from inter-strand to intra-strand base pairing. If the symmetric region has a complementary interaction in only one strand, a hairpin structure is formed. However, the extrusion of two opposite hairpins in both strands can lead to the formation of a cruciform structure (4,6). This biological mechanism has been proposed to account for the role of long IRs (>500 bp) in stimulating recurrent chromosomal translocations and generating chromosomally unbalanced offspring (7–9). In contrast, short IRs (≤30 bp), which are enriched in the human genome (7), can prevent DNA replication forks to stimulate DNA double-strand breakage (10).

IRs play significant roles in regulating genetic instability (11), gene splicing (12, 13) and replication stalling (14). *In vivo*, the most distinct property of IRs is their tendency to decrease genomic stability in various kinds of organisms. The repeat length, motif composition, sequence position and genetic background of IRs all have crucial roles in influencing genetic stability (4). In eukaryotes, endogenous transcripts, especially transcribed IRs, produce small RNAs to trigger gene splicing (15–18). In bacteria, replication forks stop at IR positions depending on the length of the central spacer (19).

Many tools are available to detect IRs, such as Mfold (20), findIR (21), detectIR (22), IRF (23), Emboss (24) and Palindrome analyser (25). However, most of these tools require high-level computing skills and lack visualization functions. Mfold is an available online tool (http://unafold.rna.albany.edu), but it can only detect IRs with the input sequences no more than 9000 bases. FindIR and DetectIR are two comprehensive tools for detecting IRs, but they are used based on the commercial software MATLAB. Another tool allowing IR detection is IRF, which only contains a command line version of the IR algorithm. Besides these, Emboss as a standalone application for UNIX can detect IR structures without any presentation and visualization abilities. Palindrome analyser is also a web tool that can be easily performed to detect IRs, but they limit the amount of IRs that can be shown to 5000, and only the registered users can upload/import sequences and store results. In addition, as increasing numbers of chloroplast genomes have been sequenced, IRs have been explored in the chloroplast genomes of many species, such as *Quercus acutissima* (26), *Aster tataricus* (27), *Carnegiea gigantea* (28), *Strobilanthes cusia* (29), *Colobanthus apetalus* (30) and *Sanguisorba* (31). Therefore, a comprehensive platform for IR analysis in plant chloroplast genomes is urgently needed.

In this study, the Plant Chloroplast Inverted Repeats (PCIR) database was established to provide a reliable platform to identify, annotate and analyze IRs in the cpDNAs of sequenced plants and to provide a series of webpages to display IR junction features. PCIR should greatly contribute to research on the distribution, characteristics and functions of IRs.

Materials and Methods

Chloroplast genome sequencing data

The chloroplast genome datasets of 113 plant species were screened from the GenBank repository of NCBI and downloaded without annotations (Table S1). The minimum criteria for these genomes in the PCIR relational database were as follows:

(i) all plant genomes from public published studies;
(ii) the genome sequence is complete, not partial; and
(iii) the chloroplast genome reads length is at least 50 kb.

These standards ensured that reads from plant samples were sequenced with similar experimental techniques, and search queries could be established using consistent criteria to minimize variations in reads between samples.

Identification of IRs

An IR usually comprises two DNA sequences of equal length and includes bases in a reverse complementary direction that form a palindrome. Based on IR structures, a pipeline composed of Perl scripts was used to scan each chloroplast genome. The parameters of IR motif length were set to 6–30 and no mismatches or gaps were allowed among the sequences. The IR positions were also identified by this pipeline.

Phylogenetic analysis and annotation

The phylogenetic relationships of the 113 species were determined from their complete chloroplast genome sequences. HomBlocks (32) was used to align nucleic acid sequences, and MEGA X (33) was used to generate a phylogenetic neighbor-joining (NJ) tree with 1000 bootstrap replicates. Furthermore, each cpDNA genome was annotated using the online web tool Dual Organellar GenoMe Annotator (34) with default parameters. The circular organelle genome maps of these species were drawn with OGDRAW (35), and the linear IR junction sites were predicted using IRScope (36).

Database implementation

The database was constructed based on a LAMP framework (Centos 6.5, Apache 2.4.27, MySQL 14.4, Xampp 3.2.2 and ThinkPHP 3.2.3/PHP7.1.9). All IR data were imported into MySQL for efficient management, searching and display. The web-friendly interface was based on HTML5 and PHP. Perl and Java scripts were used to enhance the Common Gateway Interface programs. A complete workflow portraying the content exhibition procedures used in setting up PCIR is shown in Figure 1.

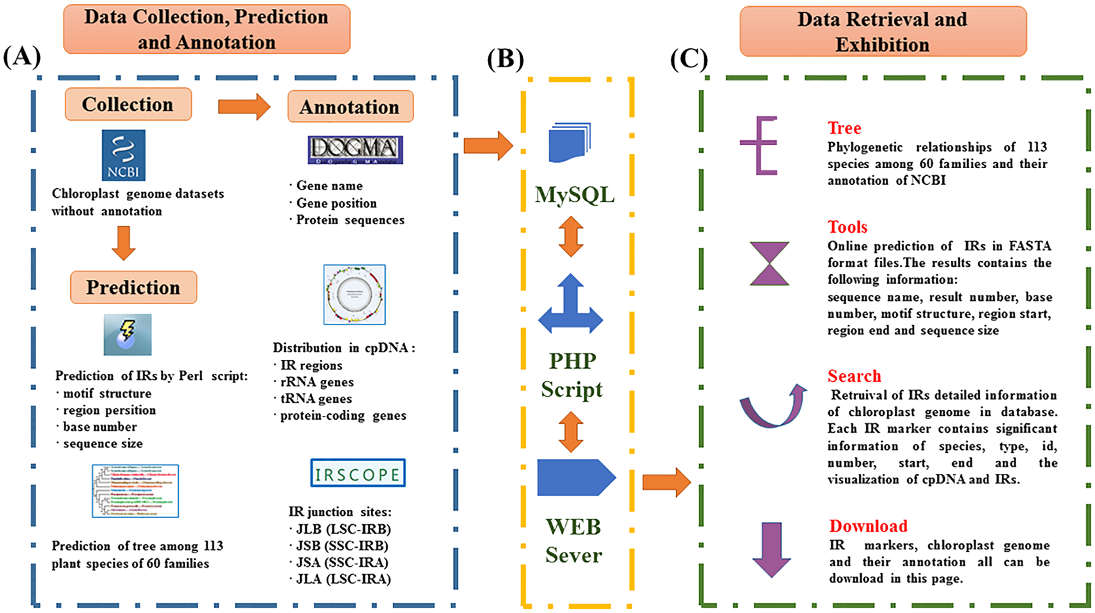

Data Collection, Prediction and Annotation
(A)
Collection
Annotation
NCBI
Chloroplast genome datasets without annotation
DOGMA
· Gene name
· Gene position
· Protein sequences
Prediction
Prediction of IRs by Perl script:
· motif structure
· region persition
· base number
· sequence size
Distribution in cpDNA :
· IR regions
· rRNA genes
· tRNA genes
· protein-coding genes
IRSCOPE
IR junction sites:
· JLB (LSC-IRB)
· JSB (SSC-IRB)
· JSA (SSC-IRA)
· JLA (LSC-IRA)
Prediction of tree among 113 plant species of 60 families
(B)
MySQL
PHP Script
WEB Sever
Data Retrieval and Exhibition
(C)
Tree
Phylogenetic relationships of 113 species among 60 families and their annotation of NCBI
Tools
Online prediction of IRs in FASTA format files.The results contains the following information:
sequence name, result number, base number, motif structure, region start, region end and sequence size
Search
Retruival of IRs detailed information of chloroplast genome in database. Each IR marker contains significant information of species, type, id, number, start, end and the visualization of cpDNA and IRs.
Download
IR markers, chloroplast genome and their annotation all can be download in this page.

Figure 1. Schematic overview of IR database. (**A**) Data sources of PCIR. (**B**) Workflow of PCIR. (**C**) Data acquisition in the platform.

Table 1. Numbers, frequencies and proportion of IRs according to size

IR size	Amount in dataset	IR/KB	Proportion of all	IR size	Amount in dataset	IR/KB	Proportion of all
6	7260	0.4214	0.3387	19	283	0.0164	0.0132
7	3468	0.2013	0.1618	20	250	0.0145	0.0117
8	2069	0.1201	0.0965	21	231	0.0134	0.0108
9	1398	0.0811	0.0652	22	216	0.0125	0.0101
10	1107	0.0643	0.0516	23	191	0.0111	0.0089
11	917	0.0532	0.0428	24	174	0.0101	0.0081
12	685	0.0398	0.0320	25	148	0.0086	0.0069
13	567	0.0329	0.0265	26	130	0.0075	0.0060
14	497	0.0288	0.0232	27	108	0.0063	0.0050
15	437	0.0254	0.0204	28	95	0.0055	0.0044
16	382	0.0222	0.0178	29	84	0.0049	0.0039
17	349	0.0203	0.0163	30	74	0.0043	0.0035
18	313	0.0182	0.0146				

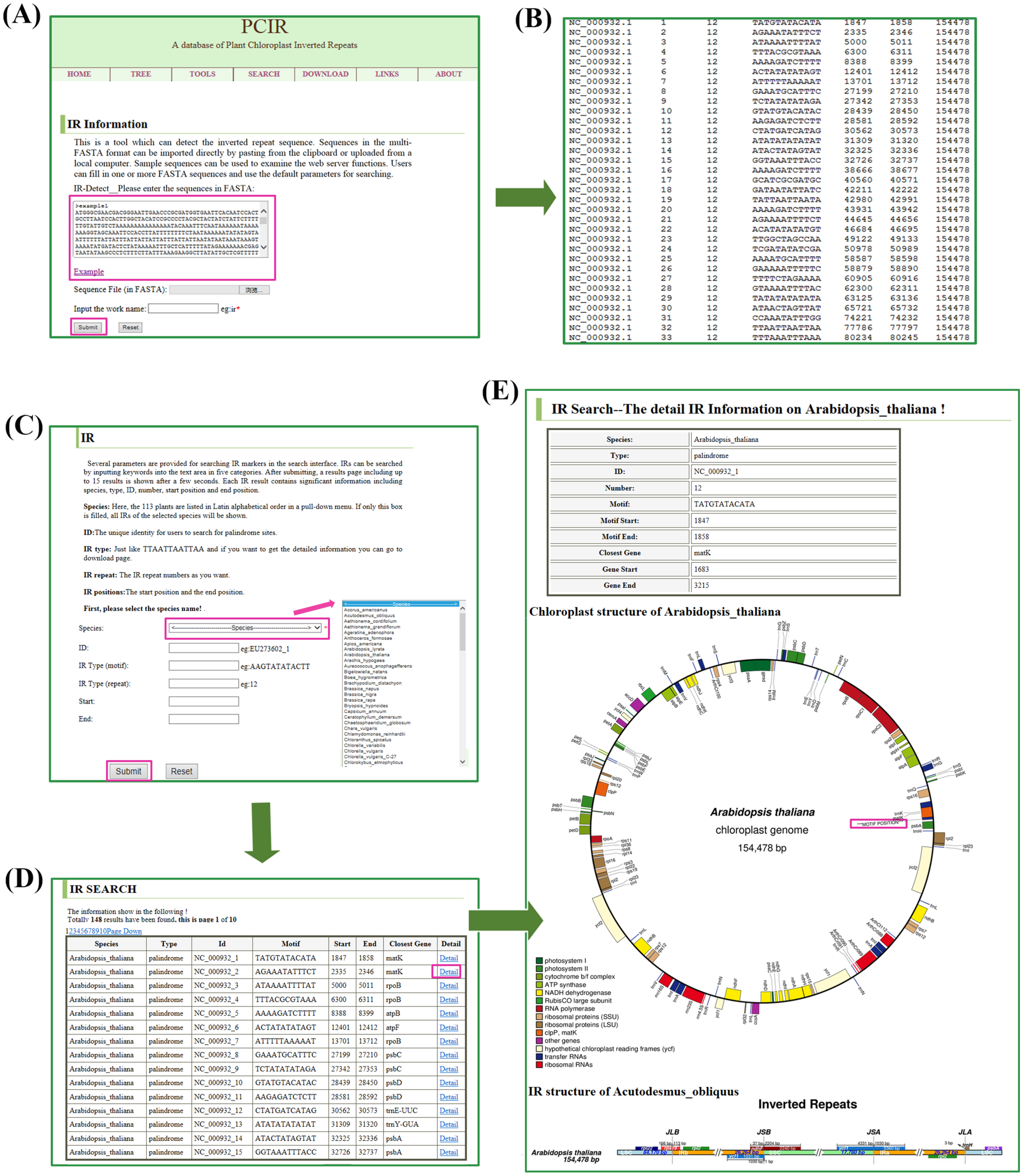
Database content

Distribution characteristics of IRs

A total of 21 433 IRs were characterized in the 113 species. The lengths of the chloroplast genome sequences varied from 64 335 bp in Prasinophyceae, which had only 72 chloroplast genes, to 269 044 bp in *Dunaliella salina*, which had 107 genes (Supplementary Table S1). According to motif length, the IR sequences were categorized into 25 groups. Hexamers were the most abundant type with a percentage of 33.87%. With increasing IR length, the IR distribution frequency ranged from 0.4214 to 0.0043 (/KB). This result showed that the numbers of IR sites were negatively correlated with repeat length (Table 1).

Developing IRs online

Sequences in the multi-FASTA format can be imported directly by pasting from the clipboard or uploading from a local computer (Figure 2A). Sample sequences can be used to examine the web server functions. Users can fill in one or more FASTA sequences and use the default parameters for searching. The web application provides the results in text format. The results contain the following information: sequence name, result number, base number, motif



PCIR

A database of Plant Chloroplast Inverted Repeats

IR Information

This is a tool which can detect the inverted repeat sequence. Sequences in the multi-FASTA format can be imported directly by pasting from the clipboard or uploaded from a local computer. Sample sequences can be used to examine the web server functions. Users can fill in one or more FASTA sequences and use the default parameters for searching.

IR-Detect__Please enter the sequences in FASTA:

```
>example1
ATGGGCGAACGACGGGAATTGAACCCGCGATGGTGAATTCACAATCCACT
GCCTTAATCCACTTGGCTACATCCGCCCCTACGCTACTATCTATTCTTTT
TTGTATTGTCTAAAAAAAAAAAAAAAATACAAATTTCAATAAAAAATAAAA
AAAGGTAGCAAATTCCACCTTATTTTTTTTCTAATAAAAAATATATAGTA
ATTTTTTATTATTTATTATTATTATTTATTATTAATATAATAAATAAAGT
AAAATATGATACTCTATAAAAATTTGCTCATTTTTATAGAAAAAAACGAG
TAATATAAGCCCTCTTTCTTATTTAAAGAAGGCTTATATTGCTCGTTTTT
```

Sequence File (in FASTA):

Input the work name: eg:ir*

```
NC_000932.1    1     12    TATGTATACATA    1847    1858    154478
NC_000932.1    2     12    AGAAATATTTCT    2335    2346    154478
NC_000932.1    3     12    ATAAAATTTTAT    5000    5011    154478
NC_000932.1    4     12    TTTACGCGTAAA    6300    6311    154478
NC_000932.1    5     12    AAAAGATCTTTT    8388    8399    154478
NC_000932.1    6     12    ACTATATATAGT    12401   12412   154478
NC_000932.1    7     12    ATTTTTAAAAAT    13701   13712   154478
NC_000932.1    8     12    GAAATGCATTTC    27199   27210   154478
NC_000932.1    9     12    TCTATATATAGA    27342   27353   154478
NC_000932.1    10    12    GTATGTACATAC    28439   28450   154478
NC_000932.1    11    12    AAGAGATCTCTT    28581   28592   154478
NC_000932.1    12    12    CTATGATCATAG    30562   30573   154478
NC_000932.1    13    12    ATATATATATAT    31309   31320   154478
NC_000932.1    14    12    ATACTATAGTAT    32325   32336   154478
NC_000932.1    15    12    GGTAAATTTACC    32726   32737   154478
NC_000932.1    16    12    AAAAGATCTTTT    38666   38677   154478
NC_000932.1    17    12    GCATCGCGATGC    40560   40571   154478
NC_000932.1    18    12    GATAATATTATC    42211   42222   154478
NC_000932.1    19    12    TATTAATTAATA    42980   42991   154478
NC_000932.1    20    12    AAAAGATCTTTT    43931   43942   154478
NC_000932.1    21    12    AGAAAATTTTCT    44645   44656   154478
NC_000932.1    22    12    ACATATATATGT    46684   46695   154478
NC_000932.1    23    12    TTGGCTAGCCAA    49122   49133   154478
NC_000932.1    24    12    TCGATATATCGA    50978   50989   154478
NC_000932.1    25    12    AAAATGCATTTT    58587   58598   154478
NC_000932.1    26    12    GAAAAATTTTTC    58879   58890   154478
NC_000932.1    27    12    TTTTCTAGAAAA    60905   60916   154478
NC_000932.1    28    12    GTAAAATTTTAC    62300   62311   154478
NC_000932.1    29    12    TATATATATATA    63125   63136   154478
NC_000932.1    30    12    ATAACTAGTTAT    65721   65732   154478
NC_000932.1    31    12    CCAAATATTTGG    74221   74232   154478
NC_000932.1    32    12    TTAATTAATTAA    77786   77797   154478
NC_000932.1    33    12    TTTAAATTTAAA    80234   80245   154478
```

IR

Several parameters are provided for searching IR markers in the search interface. IRs can be searched by inputting keywords into the text area in five categories. After submitting, a results page including up to 15 results is shown after a few seconds. Each IR result contains significant information including species, type, ID, number, start position and end position.

Species: Here, the 113 plants are listed in Latin alphabetical order in a pull-down menu. If only this box is filled, all IRs of the selected species will be shown.

ID: The unique identity for users to search for palindrome sites.

IR type: Just like TTAATTAATTAA and if you want to get the detailed information you can go to download page.

IR repeat: The IR repeat numbers as you want.

IR positions: The start position and the end position.

First, please select the species name! .

Species: *

ID: eg:EU273602_1

IR Type (motif): eg:AAGTATATACTT

IR Type (repeat): eg:12

Start:

End:

Acorus_americanus
Acutodesmus_obliquus
Aethionema_cordifolium
Aethionema_grandiflorum
Ageratina_adenophora
Anthoceros_formosae
Apios_americana
Arabidopsis_lyrata
Arabidopsis_thaliana
Arachis_hypogaea
Aureococcus_anophagefferens
Bigelowiella_natans
Boea_hygrometrica
Brachypodium_distachyon
Brassica_napus
Brassica_nigra
Brassica_rapa
Bryopsis_hypnoides
Capsicum_annuum
Ceratophyllum_demersum
Chaetosphaeridium_globosum
Chara_vulgaris
Chlamydomonas_reinhardtii
Chloranthus_spicatus
Chlorella_variabilis
Chlorella_vulgaris
Chlorella_vulgaris_C-27
Chlorokybus_atmophyticus

IR SEARCH

The information show in the following !
Totally **148** results have been found, **this is page 1 of 10**

Species	Type	Id	Motif	Start	End	Closest Gene	Detail
Arabidopsis_thaliana	palindrome	NC_000932_1	TATGTATACATA	1847	1858	matK	Detail
Arabidopsis_thaliana	palindrome	NC_000932_2	AGAAATATTTCT	2335	2346	matK	Detail
Arabidopsis_thaliana	palindrome	NC_000932_3	ATAAAATTTTAT	5000	5011	rpoB	Detail
Arabidopsis_thaliana	palindrome	NC_000932_4	TTTACGCGTAAA	6300	6311	rpoB	Detail
Arabidopsis_thaliana	palindrome	NC_000932_5	AAAAGATCTTTT	8388	8399	atpB	Detail
Arabidopsis_thaliana	palindrome	NC_000932_6	ACTATATATAGT	12401	12412	atpF	Detail
Arabidopsis_thaliana	palindrome	NC_000932_7	ATTTTTAAAAAT	13701	13712	rpoB	Detail
Arabidopsis_thaliana	palindrome	NC_000932_8	GAAATGCATTTC	27199	27210	psbC	Detail
Arabidopsis_thaliana	palindrome	NC_000932_9	TCTATATATAGA	27342	27353	psbC	Detail
Arabidopsis_thaliana	palindrome	NC_000932_10	GTATGTACATAC	28439	28450	psbD	Detail
Arabidopsis_thaliana	palindrome	NC_000932_11	AAGAGATCTCTT	28581	28592	psbD	Detail
Arabidopsis_thaliana	palindrome	NC_000932_12	CTATGATCATAG	30562	30573	trnE-UUC	Detail
Arabidopsis_thaliana	palindrome	NC_000932_13	ATATATATATAT	31309	31320	trnY-GUA	Detail
Arabidopsis_thaliana	palindrome	NC_000932_14	ATACTATAGTAT	32325	32336	psbA	Detail
Arabidopsis_thaliana	palindrome	NC_000932_15	GGTAAATTTACC	32726	32737	psbA	Detail

IR Search--The detail IR Information on Arabidopsis_thaliana !

Species:	Arabidopsis_thaliana
Type:	palindrome
ID:	NC_000932_1
Number:	12
Motif:	TATGTATACATA
Motif Start:	1847
Motif End:	1858
Closest Gene	matK
Gene Start	1683
Gene End	3215

Chloroplast structure of Arabidopsis_thaliana

Arabidopsis thaliana
chloroplast genome
154,478 bp

IR structure of Acutodesmus_obliquus

Figure 2. Retrieval of IR sites in the database. (**A**) The tool interfaces. The sequences in FASTA format can be filled into the test area to find IR sites. (**B**) The display of a sample tool's results. The results contain sequence name, result number, base number, motif structure, region start, region end and sequence size. (**C**) The search interfaces. IR sites can be searched by species name, markers ID, motif structure and repeat positions. (**D**) The display of a sample search's results. The results include species, type, ID, motif, closest genes, start position and end position. (**E**) A sample (e.g. *A. thaliana*) detail information of IRs. The results contain species, type, id, number, position, circular complete chloroplast genome map and IR regions map.

structure, region start, region end and sequence size. All files can be downloaded (Figure 2B).

Query interface

Several parameters are provided for searching IR markers in the search interface. IRs can be searched by inputting keywords into the text area in the following five categories (Figure 2C): (i) species names, where in the 113 plants are listed in Latin alphabetical order in a pull-down menu. If only this box is filled, all IRs of the selected species will be shown; (ii) marker ID, the unique identity for users to search for palindrome sites; (iii) IR motif (such as TATGTATACATA); (iv) IR repeat number; and (v) IR positions, including start position and end position. After submitting, a results page including up to 15 results is shown after a few seconds (Figure 2D). Each IR result contains significant information including species, type, ID, motif, closest genes, start position and end position. If more than 15 results appear in the search interface, the navigation bar at the top of the results table can be used to switch pages. The number of queried results is recorded at the top of the Search page. All query information can be downloaded in batches of 'Markers' from the Download webpage. More detailed information can be obtained by clicking on the details, including a visualization of the chloroplast genome and IRs.

Visualization of IRs

PCIR provides two ways to visualize IRs; one is by showing the circular complete genome and the other is by showing the linear regional genome. *Arabidopsis thaliana* (GeneBank: NC_000932.1) was selected as an example (Figure 2E). In the high-quality circle map, different-colored diamonds represent various structures, and the complete cpDNA genome size is marked. The inverted repeats and functional genes including rRNA, tRNA and protein-coding genes are displayed in the outer ring. In the regional map, an elaborate comparison of four junctions, JLB (LSC-IRB), JSB (SSC-IRB), JSA (SSC-IRA) and JLA (LSC-IRA), between the two single-copy regions (LSC/SSC) and two IRs (IRA/IRB) is shown and the length of each region can be observed. In *A. thaliana*, the JLB junction is covered by *rps19* and the gene *ndhF* can be seen to be 37 bp away from the IRB-SSC border. Correspondingly, JSA is located in *ycf1*, which is a pseudogene for plant viability, and the *trnH* gene is located in the LSC region, 3 bp away from the IRA-LSC border. These maps can be found in the Search page of detailed species information and can be downloaded from the Download page.

Phylogenetic analysis and annotations

Based on the cpDNA genome multi-sequence alignments, a phylogenetic tree of individual groups is shown on the Tree webpage. The dendrogram contains 81 species in 38 families of angiosperms or gymnosperms and 32 species in 22 families of green algae (Figure 3). Selecting a species will open a new page of detailed species information from NCBI, including accession number, version, organism and features. To further explore the related genes in these chloroplast genomes, all genomes were subjected to annotation analysis. The gene information includes start position, end position, plus or minus strand and protein sequence and can be downloaded from the Download page. Related sister species had similar IR border positions, distribution

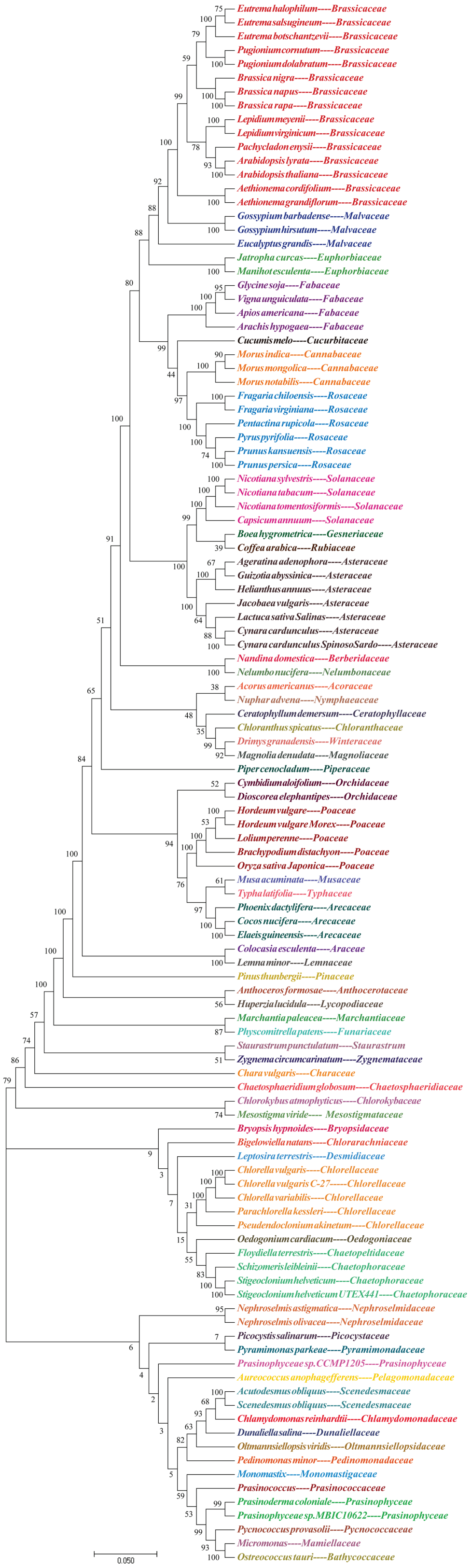

Eutremahalophilum----Brassicaceae
Eutremasalsugineum----Brassicaceae
Eutremabotschantzevii----Brassicaceae
Pugioniumcornutum----Brassicaceae
Pugioniumdolabratum----Brassicaceae
Brassica nigra----Brassicaceae
Brassica napus----Brassicaceae
Brassica rapa----Brassicaceae
Lepidiummeyenii----Brassicaceae
Lepidiumvirginicum----Brassicaceae
Pachycladon enysii----Brassicaceae
Arabidopsis lyrata----Brassicaceae
Arabidopsis thaliana----Brassicaceae
Aethionemacordifolium----Brassicaceae
Aethionemagrandiflorum----Brassicaceae
Gossypium barbadense----Malvaceae
Gossypium hirsutum----Malvaceae
Eucalyptus grandis----Malvaceae
Jatropha curcas----Euphorbiaceae
Manihot esculenta----Euphorbiaceae
Glycine soja----Fabaceae
Vigna unguiculata----Fabaceae
Apios americana----Fabaceae
Arachis hypogaea----Fabaceae
Cucumis melo----Cucurbitaceae
Morus indica----Cannabaceae
Morus mongolica----Cannabaceae
Morus notabilis----Cannabaceae
Fragaria chiloensis----Rosaceae
Fragaria virginiana----Rosaceae
Pentactina rupicola----Rosaceae
Pyrus pyrifolia----Rosaceae
Prunus kansuensis----Rosaceae
Prunus persica----Rosaceae
Nicotiana sylvestris----Solanaceae
Nicotiana tabacum----Solanaceae
Nicotiana tomentosiformis----Solanaceae
Capsicum annuum----Solanaceae
Boea hygrometrica----Gesneriaceae
Coffea arabica----Rubiaceae
Ageratina adenophora----Asteraceae
Guizotia abyssinica----Asteraceae
Helianthus annuus----Asteraceae
Jacobaea vulgaris----Asteraceae
Lactuca sativa Salinas----Asteraceae
Cynara cardunculus----Asteraceae
Cynara cardunculus SpinosoSardo----Asteraceae
Nandina domestica----Berberidaceae
Nelumbo nucifera----Nelumbonaceae
Acorus americanus----Acoraceae
Nuphar advena----Nymphaeaceae
Ceratophyllum demersum----Ceratophyllaceae
Chloranthus spicatus----Chloranthaceae
Drimys granadensis----Winteraceae
Magnolia denudata----Magnoliaceae
Piper cenocladum----Piperaceae
Cymbidium aloifolium----Orchidaceae
Dioscorea elephantipes----Orchidaceae
Hordeum vulgare----Poaceae
Hordeum vulgare Morex----Poaceae
Lolium perenne----Poaceae
Brachypodium distachyon----Poaceae
Oryza sativa Japonica----Poaceae
Musa acuminata----Musaceae
Typha latifolia----Typhaceae
Phoenix dactylifera----Arecaceae
Cocos nucifera----Arecaceae
Elaeis guineensis----Arecaceae
Colocasia esculenta----Araceae
Lemna minor----Lemnaceae
Pinus thunbergii----Pinaceae
Anthoceros formosae----Anthocerotaceae
Huperzia lucidula----Lycopodiaceae
Marchantia paleacea----Marchantiaceae
Physcomitrella patens----Funariaceae
Staurastrum punctulatum----Staurastrum
Zygnema circumcarinatum----Zygnemataceae
Chara vulgaris----Characeae
Chaetosphaeridium globosum----Chaetosphaeridiaceae
Chlorokybus atmophyticus----Chlorokybaceae
Mesostigma viride---- Mesostigmataceae
Bryopsis hypnoides----Bryopsidaceae
Bigelowiella natans----Chlorarachniaceae
Leptosira terrestris----Desmidiaceae
Chlorella vulgaris----Chlorellaceae
Chlorella vulgaris C-27-----Chlorellaceae
Chlorella variabilis----Chlorellaceae
Parachlorella kessleri----Chlorellaceae
Pseudendoclonium akinetum----Chlorellaceae
Oedogonium cardiacum----Oedogoniaceae
Floydiella terrestris----Chaetopeltidaceae
Schizomeris leibleinii----Chaetophoraceae
Stigeoclonium helveticum----Chaetophoraceae
Stigeoclonium helveticum UTEX441----Chaetophoraceae
Nephroselmis astigmatica----Nephroselmidaceae
Nephroselmis olivacea----Nephroselmidaceae
Picocystis salinarum----Picocystaceae
Pyramimonas parkeae----Pyramimonadaceae
Prasinophyceae sp.CCMP1205----Prasinophyceae
Aureococcus anophagefferens----Pelagomonadaceae
Acutodesmus obliquus----Scenedesmaceae
Scenedesmus obliquus----Scenedesmaceae
Chlamydomonas reinhardtii----Chlamydomonadaceae
Dunaliella salina----Dunaliellaceae
Oltmannsiellopsis viridis----Oltmannsiellopsidaceae
Pedinomonas minor----Pedinomonadaceae
Monomastix----Monomastigaceae
Prasinococcus----Prasinococcaceae
Prasinoderma coloniale----Prasinophyceae
Prasinophyceae sp.MBIC10622----Prasinophyceae
Pycnococcus provasolii----Pycnococcaceae
Micromonas----Mamiellaceae
Ostreococcus tauri----Bathycoccaceae
0.050

Figure 3. Evolutionary tree of plants. NJ phylogenetic tree reconstruction contains 113 species among 60 families based on concatenated sequences using a maximum likelihood method of all chloroplast genomes.

architectures and gene annotations. This finding strongly supports the idea that IRs play significant roles in species evolution and can be used to examine genetic diversity.

Conclusion and future works

PCIR is an interactive, web-based platform that enables browsing, searching and downloading of IR site information. It contains 21 433 IRs in 113 plant plastid genomes with gene annotations, protein resources and region visualization. This database of IRs will accelerate effective analyses of nucleic acid sequences and help researchers to discover plant regulatory mechanisms.

New IRs will be added from all existing species, and cpDNA sequences from new species with identified IR markers will be appended in an upgraded version. Users can offer useful notes on IR sequences such as polymorphisms, occurrence conditions and experiment results to make the information more useable.

Supplementary data

Supplementary files are available at *Database* Online.

Funding

Foundation of Shandong Province Modern Agricultural Technology System Innovation Team (SDAIT-25-02 to L.Y.).

Conflict of interest. None declared.

References

1. Cechova,J., Lysek,J., Bartas,M. *et al.* (2018) Complex analyses of inverted repeats in mitochondrial genomes revealed their importance and variability. *Bioinformatics*, **34**, 1081–1085.
2. Lavi,B., Levy Karin,E., Pupko,T. *et al.* (2018) The prevalence and evolutionary conservation of inverted repeats in Proteobacteria. *Genome Biol. Evol.*, **10**, 918–927.
3. Platt,J.R. (1955) Possible separation of intertwined nucleic acid chains by transfer-twist. *Proc. Natl. Acad. Sci. USA*, **41**, 181–183.
4. Lobachev,K.S., Rattray,A. and Narayanan,V. (2007) Hairpin- and cruciform-mediated chromosome breakage: causes and consequences in eukaryotic cells. *Front. Biosci.*, **12**, 4208–4220.
5. Zhao,J., Bacolla,A., Wang,G. *et al.* (2010) Non-B DNA structure-induced genetic instability and evolution. *Cell. Mol. Life Sci.*, **67**, 43–62.
6. Allers,T. and Leach,D.R.F. (1995) DNA palindromes adopt a methylation-resistant conformation that is consistent with DNA cruciform or hairpin formation in-vivo. *J. Mol. Biol.*, **252**, 70–85.
7. Wang,Y. and Leung,F.C. (2006) Long inverted repeats in eukaryotic genomes: recombinogenic motifs determine genomic plasticity. *FEBS Lett.*, **580**, 1277–1284.
8. Ho,B., Baker,P.M., Singh,S. *et al.* (2012) Localized DNA cleavage secondary to genotoxic exposure adjacent to an Alu inverted repeat. *Genes Chromosomes Cancer*, **51**, 501–509.
9. Krasilnikova,M.M. and Mirkin,S.M. (2004) Replication stalling at Friedreich's ataxia (GAA)n repeats *in vivo*. *Mol. Cell. Biol.*, **24**, 2286–2295.
10. Lu,S., Wang,G., Bacolla,A. *et al.* (2015) Short inverted repeats are hotspots for genetic instability: relevance to cancer genomes. *Cell Rep.*, **10**, 1674–1680.
11. Leach,D.R. (1994) Long DNA palindromes, cruciform structures, genetic instability and secondary structure repair. *BioEssays*, **16**, 893–900.
12. Smith,N.A., Singh,S.P., Wang,M.B. *et al.* (2000) Total silencing by intron-spliced hairpin RNAs. *Nature*, **407**, 319–320.
13. Lacomme,C., Hrubikova,K. and Hein,I. (2003) Enhancement of virus-induced gene silencing through viral-based production of inverted-repeats. *Plant J.*, **34**, 543–553.
14. Mizuno,K., Miyabe,I., Schalbetter,S.A. *et al.* (2013) Recombination-restarted replication makes inverted chromosome fusions at inverted repeats. *Nature*, **493**, 246–249.
15. Okamura,K., Chung,W.J. and Lai,E.C. (2008) The long and short of inverted repeat genes in animals: microRNAs, mirtrons and hairpin RNAs. *Cell Cycle*, **7**, 2840–2845.
16. Tijsterman,M., Ketting,R.F. and Plasterk,R.H. (2002) The genetics of RNA silencing. *Annu. Rev. Genet.*, **36**, 489–519.
17. Wroblewski,T., Matvienko,M., Piskurewicz,U. *et al.* (2014) Distinctive profiles of small RNA couple inverted repeat-induced post-transcriptional gene silencing with endogenous RNA silencing pathways in *Arabidopsis*. *RNA*, **20**, 1987–1999.
18. Zhu,A., Guo,W., Gupta,S. *et al.* (2016) Evolutionary dynamics of the plastid inverted repeat: the effects of expansion, contraction, and loss on substitution rates. *New Phytol.*, **209**, 1747–1756.
19. Voineagu,I., Narayanan,V., Lobachev,K.S. *et al.* (2008) Replication stalling at unstable inverted repeats: interplay between DNA hairpins and fork stabilizing proteins. *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 9936–9941.
20. Zuker,M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*, **31**, 3406–3415.
21. Sreeskandarajan,S., Flowers,M.M., Karro,J.E. *et al.* (2014) A MATLAB-based tool for accurate detection of perfect overlapping and nested inverted repeats in DNA sequences. *Bioinformatics*, **30**, 887–888.
22. Ye,C., Ji,G., Li,L. *et al.* (2014) detectIR: a novel program for detecting perfect and imperfect inverted repeats using complex numbers and vector calculation. *PLoS One*, **9**, e113349.
23. Warburton,P.E., Giordano,J., Cheung,F. *et al.* (2004) Inverted repeat structure of the human genome: the X-chromosome contains a preponderance of large, highly homologous inverted repeats that contain testes genes. *Genome Res.*, **14**, 1861–1869.
24. Rice,P., Longden,I. and Bleasby,A. (2000) EMBOSS: the European molecular biology open software suite. *Trends Genet.*, **16**, 276–277.
25. Brazda,V., Kolomaznik,J., Lysek,J. *et al.* (2016) Palindrome analyser—a new web-based server for predicting and evaluating

inverted repeats in nucleotide sequences. *Biochem. Biophys. Res. Commun.*, **478**, 1739–1745.

26. Li,X., Li,Y., Zang,M. *et al.* (2018) Complete chloroplast genome sequence and phylogenetic analysis of *Quercus acutissima*. *Int. J. Mol. Sci.*, **19**, 2443.
27. Shen,X., Guo,S., Yin,Y. *et al.* (2018) Complete chloroplast genome sequence and phylogenetic analysis of *Aster tataricus*. *Molecules*, **23**, 2426.
28. Sanderson,M.J., Copetti,D., Burquez,A. *et al.* (2015) Exceptional reduction of the plastid genome of saguaro cactus (*Carnegiea gigantea*): loss of the ndh gene suite and inverted repeat. *Am. J. Bot.*, **102**, 1115–1127.
29. Chen,H., Shao,J., Zhang,H. *et al.* (2018) Sequencing and analysis of *Strobilanthes cusia* (Nees) Kuntze chloroplast genome revealed the rare simultaneous contraction and expansion of the inverted repeat region in angiosperm. *Front. Plant Sci.*, **9**, 324.
30. Androsiuk,P., Jastrzębski,J.P., Paukszto,Ł. *et al.* (2018) The complete chloroplast genome of *Colobanthus apetalus* (Labill.) Druce: genome organization and comparison with related species. *PeerJ.*, **6**, e4723.
31. Meng,X.X., Xian,Y.F., Xiang,L. *et al.* (2018) Complete chloroplast genomes from *Sanguisorba*: identity and variation among four species. *Molecules*, **23**, 2137.
32. Bi,G., Mao,Y., Xing,Q. *et al.* (2018) HomBlocks: a multiple-alignment construction pipeline for organelle phylogenomics based on locally collinear block searching. *Genomics*, **110**, 18–22.
33. Kumar,S., Stecher,G., Li,M. *et al.* (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, **35**, 1547–1549.
34. Wyman,S.K., Jansen,R.K. and Boore,J.L. (2004) Automatic annotation of organellar genomes with DOGMA. *Bioinformatics*, **20**, 3252–3255.
35. Lohse,M., Drechsel,O. and Bock,R. (2007) OrganellarGenomeDRAW (OGDRAW): a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Curr. Genet.*, **52**, 267–274.
36. Amiryousefi,A., Hyvonen,J. and Poczai,P. (2018) IRscope: an online program to visualize the junction sites of chloroplast genomes. *Bioinformatics*, **34**, 3030–3031.