Ori-Finder: A web-based system for finding oriCs in unannotated bacterial genomes
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Abstract
Background: Chromosomal replication is the central event in the bacterial cell cycle. Identification of replication origins (oriCs) is necessary for almost all newly sequenced bacterial genomes. Given the increasing pace of genome sequencing, the current available software for predicting oriCs, however, still leaves much to be desired. Therefore, the increasing availability of genome sequences calls for improved software to identify oriCs in newly sequenced and unannotated bacterial genomes.

Results: We have developed Ori-Finder, an online system for finding oriCs in bacterial genomes based on an integrated method comprising the analysis of base composition asymmetry using the Z-curve method, distribution of DnaA boxes, and the occurrence of genes frequently close to oriCs. The program can also deal with unannotated genome sequences by integrating the gene-finding program ZCURVE 1.02. Output of the predicted results is exported to an HTML report, which offers convenient views on the results in both graphical and tabular formats.

Conclusion: A web-based system to predict replication origins of bacterial genomes has been presented here. Based on this system, oriC regions have been predicted for the bacterial genomes available in GenBank currently. It is hoped that Ori-Finder will become a useful tool for the identification and analysis of oriCs in both bacterial and archaeal genomes.

Background
With the increasing availability of complete genome sequences, it has become clear that an essential factor influencing bacterial proliferation is the organization of the oriC region. In bacteria, chromosome replication initiates at a single chromosome locus, called the replication origin (oriC), from which replication proceeds bidirectionally to the terminus. At the beginning of replication, ATP binds DnaA, resulting in a large oligomeric complex consisting of DnaA monomers bound to a series of 9-mer consensus elements termed DnaA boxes [1]. As a general rule, bacterial chromosomes are characterized by one bidirectional origin of replication and one terminus that define the boundaries of replichores [2]. The replication process is asymmetric with respect to the two DNA strands from the fact that the leading strand is synthesized continuously, whereas the lagging strand is synthesized as Okazaki fragments. DNA strand asymmetry can also be described in terms of gene content and nucleotide composition. Moreover, all rRNA genes, the majority of ribosomal protein coding genes and essential genes are mainly transcribed in the same direction as DNA replication (leading strand) [2]. Therefore, identifying the oriCs of bacteria is one of the key steps not only in understanding...
the mechanisms of bacterial replication but also in gaining insight into gene distribution and function of these bacterial genomes.

The pioneer work to identify oriCs in silico is the GC-skew analysis, which is deemed as a routine method [3,4]. Based on the GC-skew analysis, Frank and Lobry also developed Oriloc, a program for predicting bacterial oriCs [5]. Meanwhile, an oligomer-skew method was also proposed to predict oriC regions in bacterial genomes [6], and later used to identify oriCs in 200 bacterial and archaeal genomes [7]. Use of GC-skew analysis together with location of the dnaA gene and distribution of DnaA boxes led to more accurate prediction of oriC regions [8].

Identification of oriCs is necessary for almost all newly sequenced bacterial genomes. Given the increasing pace of genome sequencing, the currently available software for predicting oriCs, however, still leaves much room to be improved. For instance, 1) Most, if not all, available software only predicts one approximate position instead of the boundaries of oriCs. Since the oriC is an intergenic region rather than one site, it is necessary to present oriC regions with definite boundaries. 2) Most software does not provide the distribution of species-specific DnaA boxes, which are strong indicators of oriCs. 3) No automated web server is available so far, e.g., Oriloc, in which the program can be downloaded and run locally by users. Web-based tools are more convenient, since they are platform independent and there is no need to download and install the software. 4) Only the GC-skew and/or AT-skew are provided. In addition to G/C and A/T bases, R/Y and M/K bases can also show asymmetrical distributions around oriCs. It is more informative to show all the base composition asymmetries than using GC and/or AT-skews alone.

To identify oriC regions of unannotated bacterial genomes, we have developed an online tool, Ori-Finder, based on an integrated method comprising gene identification, analysis of base composition asymmetry using the Z-curve method, distribution of DnaA boxes, occurrence of genes frequently close to oriCs and phylogenetic relationships. Output results that contain detailed information of predicted oriCs, DnaA box distributions, base composition disparity curves, and coordinates of identified genes by ZCURVE 1.02, are exported to an HTML report, which offers convenient views in both graphical and tabular formats.

Implementation
The web server Ori-Finder is implemented on Apache server and the web interface is designed using CGI (Common Gateway Interface) Perl scripts. The algorithms to predict oriC regions of bacterial genomes in silico are complemented with the language of C++. The output graphs are generated by gnuplot graphic routine [9].

The software kit consists of the following programs.

1. The program to calculate the coordinates of the RY, MK, GC and AT disparity curves [10].

2. The program to calculate the distribution of DnaA boxes for the input genome sequence.

3. The program to assign and rank the priority of every intergenic sequence.

4. The gene-finding program ZCURVE 1.02 [11].

5. The Perl script integrating the above programs into one system.

Input to server
Ori-Finder has a user-friendly and intuitive input interface. Users can choose to paste the sequence into the input box or upload the sequence (FASTA format) in a file. Additionally, the server requires the specification of some optional parameters listed as follows.

1. Select ‘species-specific’ DnaA boxes according to the species. It defaults to the Escherichia coli perfect DnaA box (TTATCCACA). In addition, ‘species-specific’ DnaA boxes have also been provided, and some of them are proposed based on the phylogenetic relationships, which have not been reported previously, such as ‘TTTTCGACA’ for Chlamydiae, ‘TTATCGAAA’ for Dehalococcoides and ‘TTTTTCGAC’ for Bradyrhizobiaceae etc. For example, the motif ‘TGTTTCGAC’ is proposed to server as DnaA boxes for the genomes of the family Bradyrhizobiaceae (including Rhodopseudomonas, Nitrobacter and Bradyrhizobium). Currently, ten genomes of Bradyrhizobiaceae have been fully sequenced. Note that the predicted oriC regions of them are all closely next to gidA gene as well as the switch of GC disparity curve, and all contain the palindromic sequence ‘TGTTTCAGTAAA’, which is composed of two proposed DnaA boxes ‘TGTTTCGAC’. Consequently, the ‘species-specific’ DnaA boxes proposed by us are consistent with the phylogenetic relationships. Users can also choose to search for the DnaA boxes defined themselves. By default, only nonamers differing in no more than one position from the selected DnaA boxes are scanned, but this can be changed according to the requirements of users.

2. Whether to upload a protein table file (”.ptt” file) containing the coordinates of genes in the input genome sequence. The ”.ptt” file is a table of protein features, which is usually provided by GenBank in NCBI’s RefSeq
ftp site [12]. Users can choose to download the ".ptt" file provided by GenBank or produce their own genome annotation that has the same format as the ".ptt" file according to their requirements. For Ori-Finder, if no ".ptt" file is uploaded, the gene-finding program ZCURVE 1.02 will be called to generate the gene list. In general, ZCURVE 1.02 or other gene-finding software alone couldn’t annotate gene function. To obtain the ".ptt" file with annotation of genes related to oriCs, a database of indicator genes (such as dnaA, dnaN, hemE, gidA etc) has been collected by us, and BLAST program has also been installed locally for users’ convenience (click 'Database' on the home page for more detail). Therefore, users can Blast the query sequences and find the homologues of these genes in the sequences.

(3) Whether to display RY, MK, GC or AT disparity curves and the DnaA box distribution in the output graphs. It defaults to displaying all of them in the output graphs.

Output from the sever
The output web page shows the process of Ori-Finder, and provides links to the output results by Ori-Finder: (i) the genome size, GC content, location, length, DnaA box number, the motif of DnaA boxes, the conserved genes adjacent to the predicted oriCs, the precise coordinates of extremes of the four disparity curves and the sequence of identified oriC regions as an HTML table; (ii) the DnaA box distribution and the coordinates of the four disparity curves as text files; (iii) the integrated plot for the original sequence and (iv) that for the rotated sequence to display the obtained results, such as general genome information, the four disparity curves, distribution of DnaA boxes, locations of indicator genes and oriC regions in PNG format. A typical example of the output results obtained by Ori-Finder is shown in Figure 1. If no ".ptt" file is uploaded, the gene list file generated by ZCURVE1.02 will also be outputted. Users can do a Blast search using each output result by Ori-Finder against Doric, a database of oriC regions [13], to confirm the reliability of the prediction. Refer to Figure 2 for the complete procedure of Ori-Finder to identify oriC regions. The output results by Ori-Finder for the newly sequenced genomes in GenBank are also available at our website.

Results
Based on this online system, we have predicted oriC regions for the bacterial genomes available in GenBank currently. To estimate the accuracy of the predicted oriCs by Ori-Finder, a simple and straight way is to compare the predicted oriCs with those confirmed by experimental data. Consequently, the experimental confirmed oriCs so far are in accordance with those predicted by Ori-Finder [13]. In addition, other lines of evidence from oriC types, the coherence of all the three methods (i.e., typical base composition asymmetry, DnaA box distribution and indicator genes positions), and comparative genomic analysis, have shown the high reliability of the predicted oriCs by Ori-Finder.

In what follows we will discuss the unique merits of the present method, showing the evidence why this method is superior to other methods currently available. First of all, the in silico methods currently available to identify oriCs include the GC-skew analysis, and the oligomer-skew method etc. Jointly using the three methods (GC-skew, location of the dnaA gene and distribution of DnaA boxes) resulted in better prediction of oriC regions by Mackiewicz et al [8]. Although DnaA and its binding sites are well conserved throughout the bacterial kingdom, replication origins from different species show considerable diversity in terms of the number, arrangement and even the consensus sequence of DnaA boxes. In addition, genes adjacent to oriCs include not only dnaA gene itself but also dnaN, hemE, gidA or hemB etc. The information mentioned above has all been taken into consideration by the present approach, such as ‘species-specific’ DnaA boxes, possible genes adjacent to oriCs and phylogenetic relationships. However, by analyzing the GC-skew, Blasting for the dnaA gene and searching for DnaA boxes, the oriCs for only ~60% of genomes can be predicted. For example, the oriCs were identified for only 76 chromosomes out of the 120 chromosomes (76/120 = 63.3%) [8], which has also been confirmed again by our result for 568 chromosomes available currently in GenBank. Out of the 568 chromosomes, there are only 342 chromosomes (342/568 = 60.2%) whose oriCs are adjacent to dnaA gene and near the switch of GC-skew. Second, the present work can find oriC regions with definite boundaries with the accuracy of single base pair. Consequently, the integrated method presented in this paper outperforms those jointly applying the three methods mentioned above, as reflected by the fact that using the integrated method, the oriC regions can be identified for over 98% chromosomes out of the 568 bacterial chromosomes analyzed. For example, the genome of Synechococcus sp. JA-3-3Ab (NC_007775) does not exhibit any typical GC skew pattern. Therefore, based on the location of the dnaA gene, the origin is tentatively assigned to the rpmH-dnaA intergenic region [14]. However, in the family of Synechococcus genomes the oriC regions are closely adjacent to dnaN gene rather than dnaA gene and the DnaA box motif is ‘TITTCCACA’ rather than ‘TTATCCACA’. Here, by taking the above information into account, the oriC of Synechococcus sp. JA-3-3Ab can be easily predicted by Ori-Finder. For another example, applying the methods based on GC-skew, dnaA gene location and E. coli perfect DnaA box distribution to the genome of Synechococcus sp. WH 8102 (NC_005070), each of the three methods identifies a different region, respectively [8]. Consequently, none oriC can be identified for this
A typical example of the output results obtained by Ori-Finder. (A) The information of genome and oriC region for *Escherichia coli* K12 as an HTML table. (B) The integrated plot as a PNG figure for the original sequence and (C) the integrated plot as a PNG figure for the rotated sequence, displaying the obtained results, such as general genome information, four disparity curves, distribution of DnaA boxes, locations of putative indicator genes and the predicted oriC region. Note that the coordinate origin of the rotated sequence begins and ends in the maximum of the GC disparity curve.
Bacterial genome

Annotation file uploaded?  Yes

Specification of input parameters

Run Ori-Finder

Is the result satisfying?  No

Blast DoriC to confirm the result, if necessary

The output result in both graphical and tabular formats

Figure 2
The procedure of Ori-Finder to identify oriC regions. Flow chart schematically showing the procedure to identify oriC regions by Ori-Finder.

For the convenience of users’ query, the predicted oriC regions have been organized into a MySQL database, called DoriC, which is freely available online [15].

Conclusion
With the availability of an increasing number of bacterial genomes, the prediction will be more accurate and reliable since the DnaA boxes or genes frequently close to oriCs can be analyzed by comparative genomics. Currently, the web server Ori-Finder is designed only for the identification of oriCs in bacterial genomes. However, the architecture of the system is also suitable for archaeal genomes by changing DnaA box into ORB (origin recognition boxes) elements. The current version contains an input option by which users can define their own DnaA boxes or ORB elements. It is hoped that Ori-Finder will become a useful tool for the identification and analysis of oriCs in both bacterial and archaeal genomes.

Availability and requirements
Project name: Ori-Finder

Project homepage: http://tubic.tju.edu.cn/Ori-Finder

Operating systems: platform independent

Programming language: C++

License: Freely available

Any restrictions on use by non-academics: None

Authors’ contributions
FG designed the computer program and drafted the manuscript. CTZ supervised the study and revised the manuscript. Both authors read and approved the final manuscript.

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genome [8], whereas a reliable oriC for this genome has been identified by the integrated method Ori-Finder.

Finally, we should point out the limitation of the present method. Note that the replication origins from different species show considerable diversity and sometimes annotation mistakes affect the performance. Therefore, the present method couldn’t solve the problem of identifying the oriC for every bacterial genome currently available, of course, nor could other methods. Accordingly, oriCs for only ~2% of 568 chromosomes remain unresolved currently using the integrated method presented here, whereas ~40% of 568 genomes cannot be solved using only the information of GC-skew, location of the dnaA gene and distribution of DnaA boxes. However, for the chromosomes whose oriCs remain unresolved by Ori-Finder, the distribution of DnaA boxes and the extremes of GC (AT, RY and MK) disparity curves are still provided by Ori-Finder, which are helpful to obtain the approximate position of oriC region.
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