



SOFTWARE TOOL ARTICLE

periodicDNA: an R/Bioconductor package to investigate k-mer periodicity in DNA [version 1; peer review: 1 approved, 2 approved with reservations]

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Abstract

Periodic occurrences of oligonucleotide sequences can impact the physical properties of DNA. For example, DNA bendability is modulated by 10-bp periodic occurrences of WW (W = A/T) dinucleotides. We present periodicDNA, an R package to identify k-mer periodicity and generate continuous tracks of k-mer periodicity over genomic loci of interest, such as regulatory elements. periodicDNA will facilitate investigation and improve understanding of how periodic DNA sequence features impact function.

Keywords

DNA periodicity, gene regulation, promoter



This article is included in the **Bioconductor** gateway.




This article is included in the **RPackage** gateway.

Open Peer Review

Reviewer Status

Invited Reviewers

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Introduction

Short DNA sequence motifs provide key information for interpreting the instructions in DNA, for example by providing binding sites for proteins or altering the structure of the double-helix. A less studied but important feature of DNA sequence motifs is their periodicity¹⁻⁴. Two famous examples are the universal 3bp periodic (RNY)_n pattern (R = A or G, Y = C or U, N = any base) in exons⁵ and 10-bp periodic k-mers in nucleosome positioning (reviewed in 6 and 7). However, despite the wealth of software focusing on motif discovery and analysis, no tool provides an easy way to quantify the periodicity of a given motif, i.e. the extent to which a given motif is repeated at a regular interval in specific sequences. For instance, HeliCis and SpaMo identify conserved distances between two motifs in sequences of interest, but they do not assess larger scale periodic occurrences of motifs^{8,9}.

Here we present periodicDNA, an R package to investigate k-mer periodicity. periodicDNA provides a framework to quantify the periodicity of any k-mer of interest in DNA sequences. It can identify which periods are statistically enriched in a set of sequences by using a randomized shuffling approach to compute an empirical p-value and can also generate continuous linear tracks of k-mer periodicity strength over genomic loci.

Methods

Operation

The two main functions of periodicDNA are `getPeriodicity()` and `getPeriodicityTrack()`. The `getPeriodicity()` function interrogates the extent to which a given k-mer periodically occurs, and at which periods, in one sequence or a set of sequences. It uses a randomized shuffling approach to compute an empirical p-value and identify k-mer periods that are statistically enriched in the sequences of interest. `getPeriodicityTrack()` generates a linear .bigWig track over genomic loci of interest, representing the periodicity strength of a chosen k-mer at a given period.

periodicDNA is available as an R package on Github. Package dependencies and system requirements are documented here: <https://js2264.github.io/periodicDNA/>. periodicDNA has been tested using R (version 4.0.2) on Mac OS (versions 10.11 and later) and Ubuntu 18.04.2 machines.

Implementation

Internally, `getPeriodicity()` and `getPeriodicityTrack()` functions both compute the power spectral density (PSD) of an input k-mer in input sequence(s) to estimate its average periodicity. The magnitude of the PSD reflects the strength of the k-mer signal at periodic positions¹⁰. To compute the power spectral density (PSD) of a k-mer of interest in one or several sequences, the following steps are executed (Figure 1):

1. The k-mer occurrences are mapped and their pairwise distances are calculated (Figure 1A).
2. The distribution of all the resulting pairwise distances (also called “distogram”) is generated (Figure 1B).
3. The distogram is transformed into a frequency histogram (Figure 1C) and smoothed using a moving window of 3 to mask the universal three-base genomic periodicity¹¹ (Figure 1D). To normalize the frequency for distance decay, the local average (obtained by averaging the frequency with a moving window of 10) is subtracted from the smoothed frequency (Figure 1E).
4. Finally, the power spectral density (PSD) is estimated using a Fast Fourier Transform (Figure 1F). The magnitude of the PSD values indicates the contribution of a given period to the overall periodicity of the k-mer of interest. In Figure 1, TT dinucleotides are generally 10bp periodic.

The package relies on BSGenome packages to handle genome assemblies. Genomic loci can be imported from BED files with rtracklayer and are handled in R by the GenomicRanges classes. Biostrings functions are used to map k-mer occurrences in sequences of interest.

Workflow

Installation

To install the current release of periodicDNA from Bioconductor, use:

```
> if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
> BiocManager::install("periodicDNA")
> library("periodicDNA")
```

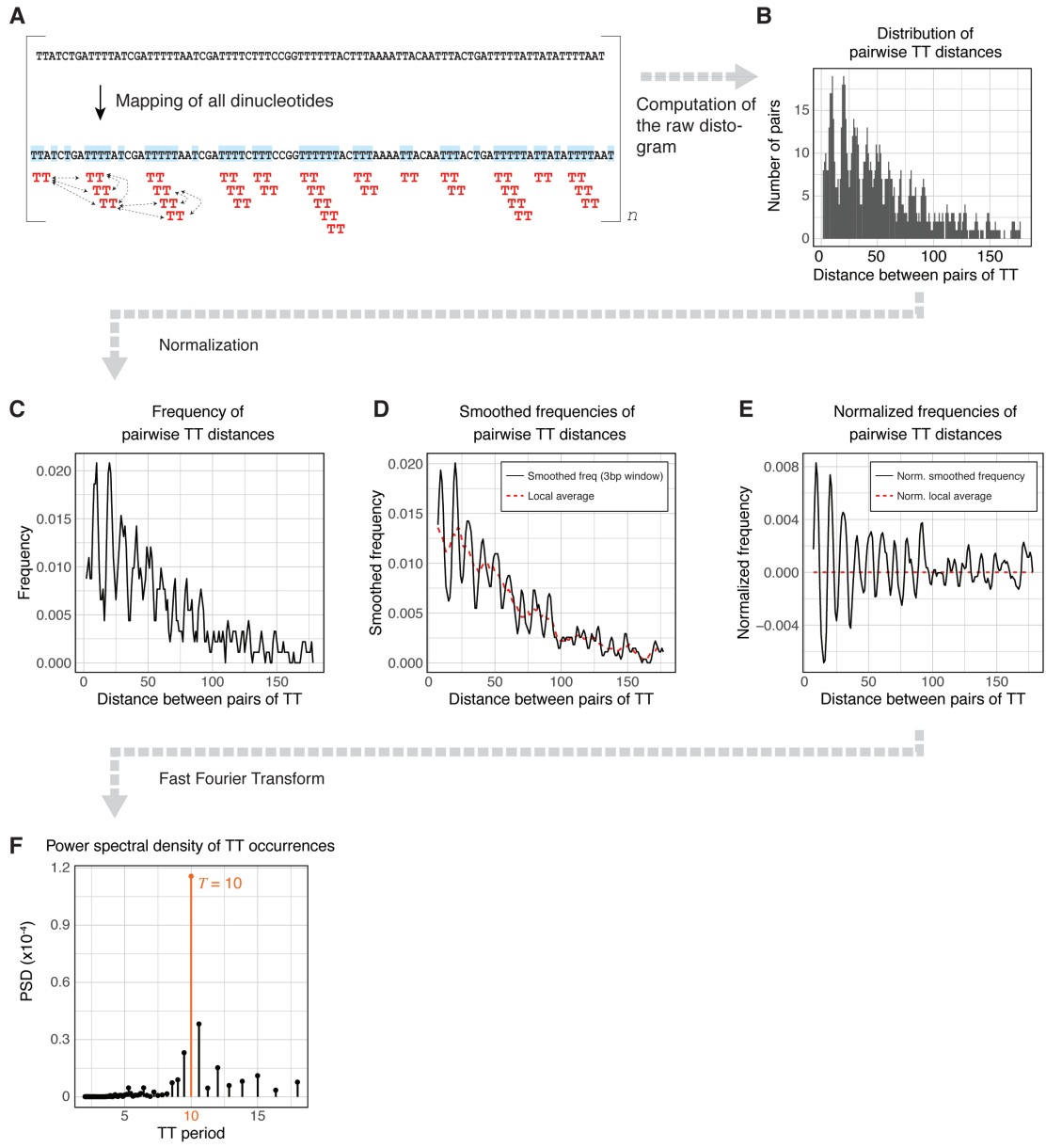


Figure 1. Estimation of k-mer power spectral density with periodicDNA. (A) The k-mer of interest is mapped in the input sequence and all pairwise distances are calculated. (B) The distribution of all resulting pairwise distances (also called the “distogram”) is generated. (C) The distogram is transformed into a frequency histogram and (D) smoothed using a moving window of 3 to mask the universal three-base genomic periodicity¹⁷. (E) To normalize the frequency for distance decay, the local average (obtained by averaging the frequency with a moving window of 10) is subtracted from the smoothed frequency. (F) Finally, the power spectral density (PSD) is estimated using a Fast Fourier Transform (F). In this example, TT shows strong 10bp periodicity.

Alternatively, the developing branch of periodicDNA can be installed from Github as follows:

```
> remotes::install_github("js2264/periodicDNA")
> library("periodicDNA")
```

Quantifying k-mer periodicity over a set of sequences

Using a provided k-mer (e.g. WW, motif argument) and a set of sequences of interest (seqs argument), getPeriodicity() computes several different metrics:

1. The k-mer power spectral density (PSD) values at different periods obtained by Fourier Transform (following the approach described in the Implementation section). For each individual period T , the corresponding PSD_T indicates the relative contribution of the period to the overall periodicity of the k-mer of interest¹⁰.
2. The log₂ fold-change, for each individual period T , between the observed PSD_T and the median of the PSD_T values measured after shuffling n times the input sequences ($l2FC = \log_2 \left(\frac{PSD_{T,observed}}{\text{median}(PSD_{T,shuffled})} \right)$).
3. Associated empirical p-values and false discovery rates (FDR) indicating, for each individual period T , whether the observed $PSD_{T,observed}$ is significantly greater than $PSD_{T,shuffled}$ values measured after shuffling n times the input sequences ($p = \frac{\sum_{i=1}^n (PSD_{T,shuffled} \geq PSD_{T,observed}) + 1}{n + 1}$, 12) Note that empirical p-values are only an estimation of the real p-value. Notably, small p-values are systematically over-estimated as their lower bound is $1/(n + 1)$.

These metrics are accessible in the `periodicityMetrics` table obtained when running `getPeriodicity()`. For each Frequency (or Period) analysed by Fourier Transform, the resulting PSD value, a log₂ fold-change, its associated p-value as well as its false-discovery rate (FDR) are returned (see tables in the examples below).

We ran `getPeriodicity()` on a set of 6,533 300-bp long sequences centered at all *S. cerevisiae* TSSs, to investigate WW periodicity, comparing to 500 shufflings as default. Using 12 cores in parallel, this function took approximately 15 minutes to run. The results were then plotted using `plotPeriodicityResults()` (Figure 2A), demonstrating the known underlying 10-bp WW periodicity present at promoter sequences in the yeast genome¹³.

```
> # ----- Get the sequences of S. cerevisiae TSSs
> library(TxDb.Scerevisiae.UCSC.sacCer3.sgdGene)
> library(GenomicFeatures)
> library(magrittr)
> genes <- genes(TxDb.Scerevisiae.UCSC.sacCer3.sgdGene)
> sacCer3_TSSs <- genes %>%
  resize(fix = 'center', 300) %>%
  '['(. %within% GRanges(seqinfo(genes)))
> # ----- Run getPeriodicity()
> sacCer3_results <- getPeriodicity(
  sacCer3_TSSs,
  genome = 'sacCer3',
  motif = 'WW',
  n_shuffling = 500,
  cores_shuffling = 12
)
> # ----- Plot results with plotPeriodicityResults()
> plotPeriodicityResults(sacCer3_results, xlim = 150)
> # ----- Print the computed periodicity metrics
> sacCer3_results$periodicityMetrics

## | Freq| Period|PSD_observed | l2FC| pval | fdr|
## |-----|-----|:-----|-----|:-----|-----|
## | 0.005| 200.000|3.32e-08 | -0.5313|1.00e+00 | 1.0000|
## | 0.010| 100.000|5.83e-09 | -0.6259|1.00e+00 | 1.0000|
## | 0.015| 66.667|1.45e-09 | -0.7594|1.00e+00 | 1.0000|
## | 0.020| 50.000|9.38e-10 | 0.3728|2.55e-01 | 0.9125|
## | 0.025| 40.000|1.78e-10 | -0.7144|7.49e-01 | 1.0000|
## | 0.030| 33.333|3.18e-10 | 0.4657|3.25e-01 | 1.0000|
## | 0.035| 28.571|1.18e-10 | -0.9550|7.31e-01 | 1.0000|
```

```

## | 0.040| 25.000|3.84e-11 | -2.5141|9.28e-01 | 1.0000|
## | 0.045| 22.222|6.63e-12 | -4.9433|9.88e-01 | 1.0000|
## | 0.050| 20.000|3.66e-11 | -1.9689|8.70e-01 | 1.0000|
## | 0.055| 18.182|2.49e-11 | -2.6349|8.94e-01 | 1.0000|
## | 0.060| 16.667|3.40e-10 | 1.4118|1.50e-01 | 0.7879|
## | 0.065| 15.385|9.89e-11 | -0.3535|6.01e-01 | 1.0000|
## | 0.070| 14.286|1.27e-11 | -3.2144|9.42e-01 | 1.0000|
## | 0.075| 13.333|1.29e-11 | -3.2219|9.52e-01 | 1.0000|
## | 0.080| 12.500|4.05e-10 | 1.8788|8.38e-02 | 0.5240|
## | 0.085| 11.765|5.45e-10 | 2.4637|2.59e-02 | 0.2162|
## | 0.090| 11.111|4.66e-11 | -0.9672|7.21e-01 | 1.0000|
## | 0.095| 10.526|9.78e-10 | 3.5220|2.00e-03 | 0.0499|
## | 0.100| 10.000|3.48e-09 | 5.7209|2.00e-03 | 0.0499|
## | 0.105| 9.524|1.34e-09 | 4.3497|2.00e-03 | 0.0499|
## | 0.110| 9.091|1.28e-10 | 1.1208|2.22e-01 | 0.8862|
## | 0.115| 8.696|2.37e-10 | 2.1408|4.19e-02 | 0.3224|
## | 0.120| 8.333|4.38e-10 | 3.2951|3.99e-03 | 0.0544|
## | 0.125| 8.000|3.65e-10 | 3.0512|3.99e-03 | 0.0544|
## | 0.130| 7.692|3.37e-10 | 3.0458|5.99e-03 | 0.0544|
## | 0.135| 7.407|7.23e-12 | -2.5883|8.96e-01 | 1.0000|
## | 0.140| 7.143|1.84e-11 | -1.2455|7.11e-01 | 1.0000|
## | 0.145| 6.897|1.35e-10 | 1.7676|9.38e-02 | 0.5518|
## | 0.150| 6.667|3.43e-11 | -0.0344|5.05e-01 | 1.0000|
## ...

```

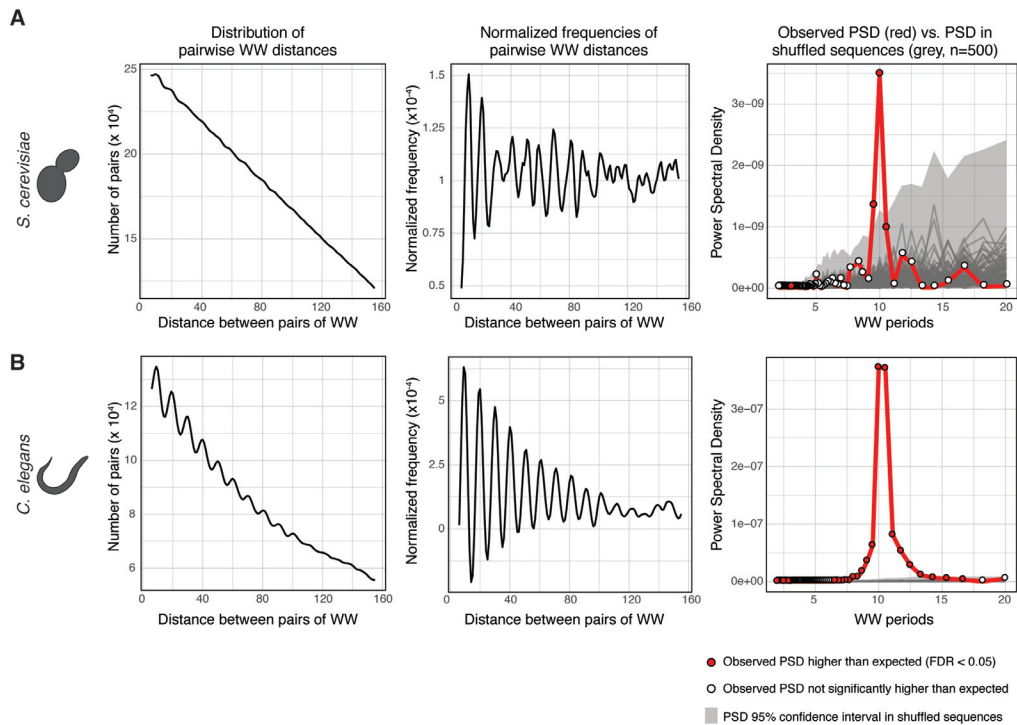


Figure 2. Output of the plotPeriodicityResults() function run on getPeriodicity() results. To identify periodicity of WW dinucleotides, getPeriodicity() was run on (A) a set of 300-bp long sequences centered at 6,533 *S. cerevisiae* TSSs and (B) a set of 300-bp long sequences centered at 2,295 ubiquitous *C. elegans* TSSs¹⁴. The plotPeriodicityResults() function was run on the getPeriodicity() results to generate three plots as shown. Left, frequency histogram of distribution of pairwise WW distances; middle, normalised frequency histogram of distribution of pairwise WW distances; right, power spectral densities (PSDs) of a set of experimental sequences (red) and 500 iterations of shuffled sequences (grey). Grey ribbon represents the 95% confidence interval of the PSD values obtained after sequence shuffling. Red-filled dots represent PSD values in experimental sequences statistically higher than those from shuffled sequences (FDR < 0.05).

Using the same approach, we measured the WW periodicity around ubiquitous TSSs in *C. elegans*, which have been characterized as largely enriched for WW 10-bp periodic sequences¹⁴ (Figure 2B). The 10-bp WW periodicity at ubiquitous *C. elegans* TSSs is stronger than at all *S. cerevisiae* TSSs.

```
> # ----- Run getPeriodicity()
> data(cell_TSSs)
> cell_results <- getPeriodicity(
  cell_TSSs[['Ubiq.']],
  genome = 'cell',
  motif = 'WW',
  n_shuffling = 500,
  cores_shuffling = 12
)
> # ----- Plot results with plotPeriodicityResults()
> plotPeriodicityResults(cell_results, xlim = 150)
> # ----- Print the computed periodicity metrics
> cell_results$periodicityMetrics

## | Freq|   Period|PSD_observed |   l2FC|  pval  |   fdr|
## |-----:|-----:|:-----|-----:|:-----|-----:|
## | 0.005| 200.000|4.59e-08   |  0.0716|1.04e-01 | 0.2414|
## | 0.010| 100.000|8.96e-09   |  0.1241|2.02e-01 | 0.3733|
## | 0.015| 66.667|8.15e-10   | -1.4410|1.00e+00 | 1.0000|
## | 0.020| 50.000|3.07e-09   |  2.2148|2.00e-03 | 0.0080|
## | 0.025| 40.000|4.48e-09   |  3.7005|2.00e-03 | 0.0080|
## | 0.030| 33.333|1.59e-09   |  2.5195|5.99e-03 | 0.0222|
## | 0.035| 28.571|1.05e-09   |  1.8486|7.78e-02 | 0.2162|
## | 0.040| 25.000|2.88e-10   |  0.0116|4.95e-01 | 0.6770|
## | 0.045| 22.222|1.55e-10   | -1.0142|7.29e-01 | 0.8471|
## | 0.050| 20.000|5.22e-09   |  4.2517|2.00e-03 | 0.0080|
## | 0.055| 18.182|2.18e-10   | -0.2869|5.51e-01 | 0.7155|
## | 0.060| 16.667|3.16e-09   |  3.6181|2.00e-03 | 0.0080|
## | 0.065| 15.385|5.03e-09   |  4.4401|2.00e-03 | 0.0080|
## | 0.070| 14.286|6.47e-09   |  4.6155|2.00e-03 | 0.0080|
## | 0.075| 13.333|1.12e-08   |  5.6508|2.00e-03 | 0.0080|
## | 0.080| 12.500|2.79e-08   |  6.9992|2.00e-03 | 0.0080|
## | 0.085| 11.765|5.27e-08   |  8.0068|2.00e-03 | 0.0080|
## | 0.090| 11.111|8.08e-08   |  8.7938|2.00e-03 | 0.0080|
## | 0.095| 10.526|3.71e-07   | 10.9426|2.00e-03 | 0.0080|
## | 0.100| 10.000|3.72e-07   | 11.3303|2.00e-03 | 0.0080|
## | 0.105|  9.524|6.26e-08   |  8.8359|2.00e-03 | 0.0080|
## | 0.110|  9.091|3.56e-08   |  8.3330|2.00e-03 | 0.0080|
## | 0.115|  8.696|1.75e-08   |  7.4185|2.00e-03 | 0.0080|
## | 0.120|  8.333|7.48e-09   |  6.6215|2.00e-03 | 0.0080|
## | 0.125|  8.000|7.21e-09   |  6.4817|2.00e-03 | 0.0080|
## | 0.130|  7.692|1.43e-09   |  4.1824|2.00e-03 | 0.0080|
## | 0.135|  7.407|1.75e-09   |  4.5476|2.00e-03 | 0.0080|
## | 0.140|  7.143|8.75e-10   |  3.5609|2.00e-03 | 0.0080|
## | 0.145|  6.897|1.04e-09   |  3.7575|2.00e-03 | 0.0080|
## | 0.150|  6.667|4.38e-10   |  2.6277|1.40e-02 | 0.0466|
## |  ....
```

Generating tracks of k-mer periodicity

The `generatePeriodicityTrack()` function calculates the strength of a given k-mer at a particular periodicity across genomic regions of interest, generating a linear genomic track in .bigWig format (Figure 3). The user specifies a genome and a set of genomic loci, a motif and a period of interest, and a sliding window size (`window_size`, 100 bp by default) and step value (`step_size`, 2 bp by default). The

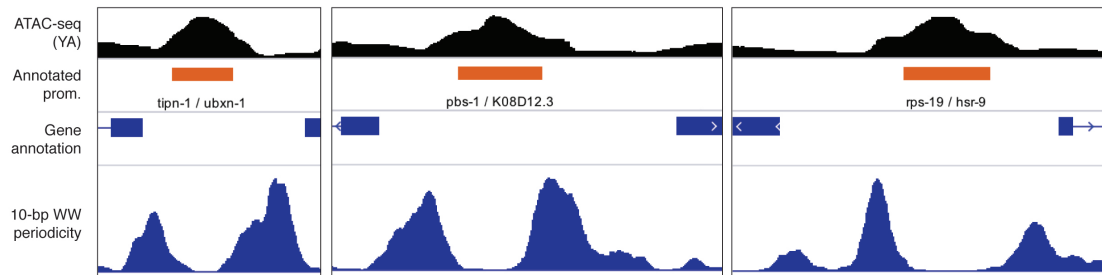


Figure 3. Example of WW dinucleotide 10-bp periodicity track at promoters in *C. elegans*. ATAC-seq signal and 10-bp WW dinucleotide periodicity at three *C. elegans* promoters. 10bp WW dinucleotide periodicity signal was generated at 1 kb centered at annotated *C. elegans* promoters¹⁴ using the `generatePeriodicityTrack()` function. The data are visualised using IGV.

input genomic loci are split into small sliding windows and for each window, the k-mer periodicity is quantified as described in the Implementation section. The PSD value at the period of interest (e.g. `period = 10`) is then retrieved and assigned to the center of the corresponding window. Finally, the resulting .bigwig track is smoothed using a rolling window (`smooth_track = 20`). `generatePeriodicityTrack()` should be run in parallel across many cores using the `BPPARAM` argument from BiocParallel. Using 12 cores, this command takes approximately half a day to produce a periodicity track over ~ 15,000 1-kb-long GRanges with default parameters.

As an example, we generated a WW 10-bp periodicity linear track over annotated promoters in *C. elegans* genome. In the previous section, we have shown that sequences in the vicinity of ubiquitous TSSs were statistically enriched for WW 10-bp periodicity. Here, the .bigwig track highlights the increased WW 10-bp periodicity in the sequences immediately flanking ubiquitous promoters, where the -1 and +1 nucleosomes are positioned¹⁴.

```
> data(cell_proms)
> track <- getPeriodicityTrack(
  genome = 'cell',
  granges = cell_proms,
  motif = 'WW',
  period = 10,
  window_size = 100,
  step_size = 2,
  smooth_track = 20,
  BPPARAM = setUpBPPARAM(12),
  bw_file = 'WW-10-bp-periodicity_over-proms_cell.bw'
)
```

Conclusion

periodicDNA is an R package that provides functions to investigate the periodicity of k-mers of interest in DNA sequences. It is primarily designed to analyse individual or sets of sequences (typically few hundred bases long and up to a kilobase) to identify overall periodicity of a chosen k-mer. It can also generate linear .bigwig tracks of k-mer periodicity at a chosen period (e.g. 10-bp WW periodicity), over genomic loci of interest. periodicDNA is well integrated within the Bioconductor environment and can easily fit in standard genomic analysis workflows.

Data availability

Underlying data

This project contains underlying data published in Serizay *et al.*, 2020¹⁴. All the data are also available from the original reference.

Software availability

periodicDNA is released as an R package on Bioconductor: <http://www.bioconductor.org/packages/release/bioc/html/periodicDNA.html>

Source code available from: <https://github.com/js2264/periodicDNA>.

Archived source code as at time of publication: <https://doi.org/10.5281/zenodo.4533704¹⁵>.

License: GPL-3

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Open Peer Review

Current Peer Review Status: ? ✓ ?

Version 1

Reviewer Report 13 April 2021

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Eugene Korotkov

Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russian Federation

The manuscript is aimed at solving an important problem - the study of the structure of promoter sequences. The calculations are performed at a good scientific level, the results are beyond doubt. I have only three, as it seems to me, small comments.

1. It seems to me that it is not entirely correct to represent the data as a file printout. Probably it would be better to present this data in the form of tables.
2. Figure 3 evokes some skepticism. It seems to me that it is very difficult to draw a conclusion on 3 promoters. Maybe this is a purely coincidence. I believe that it is necessary to study much more promoters in this way in order to draw any conclusions. Probably more promoter sequences need to be studied.
3. There are a lot of publications on the search for periodicity in promoter sequences. For example, Kravatskaya GI *et al.* (2011)¹ and Nov Klaiman T *et al.* (2009)²

It seems to me that a little comparative analysis could be done in the discussion.

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Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 12 April 2021

<https://doi.org/10.5256/f1000research.54274.r81589>

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Sabarinathan Radhakrishnan 

National Centre for Biological Sciences, Bangalore, India

The manuscript by Serizay and Ahringer presents an R/Bioconductor package to investigate the k-mer periodicity in DNA. Both the manuscript and the package details are informative and will be useful to the researchers. The following are some minor comments and suggestions:

a) Regarding the shuffling of input sequences, it is not clear if there is an option to preserve the doublet or triplet nucleotide counts. Is the parameter "order" in getPeriodicity() function meant for that (through ushuffle)? If so, please do mention this in the manuscript and its default value.

b) In addition to the shuffling of input sequences, it would also be useful if the function allows for random selection of N number of sequences (with similar GC content) from the genome or an option for the users to define their own set of input random sequences (through DNAStringSet or bed file).

c) In Figure 1A, under the “Mapping of all dinucleotides” it is not clear why the single occurrence of ‘T’ is also shaded.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

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Yes

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Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Cancer genomics, mutational processes and gene regulation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 26 March 2021

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Ilya Ioshikhes

University of Ottawa, Ottawa, Canada

The software offered is potentially helpful to the researchers in the area. However the paper (and probably the software itself) should be modified to enable its complete evaluation for indexing.

1. Comparison to other existing state of the art approaches should be added, e.g. to PerPlot and PerScan tools by Mrazek and colleagues¹, ID/MPA by Shelenkov *et al.*, 2008². The progress obtained in the present submission should be clarified.
2. All the examples shown in the paper and related links are focused on TT dinucleotides. It is

not clear whether oligo nucleotide combinations involving W, S, R, Y, N, and others from the IUPAC nucleotide codes are supported by the present software version.

3. It is not clear whether there any other restrictions on the software parameters, e.g. oligonucleotide and sequences lengths.
4. The software is composed at R language with key functions provided. To make it really practically helpful, the graphic user interface should be provided, to enable entering oligonucleotides of interest and other parameters from a window or dialogue line, without necessitating the changes in the functions text. As a very minimum, the clear instructions for such changes should be provided.

References

1. Mrázek J, Chaudhari T, Basu A: PerPlot & PerScan: tools for analysis of DNA curvature-related periodicity in genomic nucleotide sequences. *Microb Inform Exp*. 2011; **1** (1): 13 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Shelenkov A, Skryabin K, Korotkov E: Classification analysis of a latent dinucleotide periodicity of plant genomes. *Russian Journal of Genetics*. 2008; **44** (1): 101-114 [Publisher Full Text](#)

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Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, gene regulation, nucleosomes, algorithms

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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