TIRfinder: A Web Tool for Mining Class II Transposons Carrying Terminal Inverted Repeats

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Abstract: Transposable elements (TEs) can be found in virtually all known genomes; plant genomes are exceptionally rich in this kind of dispersed repetitive sequences. Current knowledge on TE proliferation dynamics places them among the main forces of molecular evolution. Therefore efficient tools to analyze TE distribution in genomes are needed that would allow for comparative genomics studies and for studying TE dynamics in a genome. This was our main motivation underpinning TIRfinder construction—an efficient tool for mining class II TEs carrying terminal inverted repeats. TIRfinder takes as an input a genomic sequence and information on structural properties of a TE family, and identifies all TEs in the genome showing the desired structural characteristics. The efficiency and small memory requirements of our approach stem from the use of suffix trees to identify all DNA segments surrounded by user-specified terminal inverse repeats (TIR) and target site duplications (TSD) which together constitute a mask. On the other hand, the flexibility of the notion of the TIR/TSD mask makes it possible to use the tool for de novo detection. The main advantages of TIRfinder are its speed, accuracy and convenience of use for biologists. A web-based interface is freely available at http://bioputer.mimuw.edu.pl/tirfindertool/.

Keywords: transposon, terminal inverted repeat, suffix tree, comparative genomics

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Introduction
Transposable elements (TEs) are DNA segments capable of changing their position in the genome. They are a major component of genomes of many organisms, comprising 66%–69% of the human genome\(^1\) and even up to 85% of genetic material of certain plants (eg, maize).\(^2\)

Recently, genomes of many species have been fully or partially sequenced and several bioinformatic tools have been created facilitating a more systematic identification and annotation of numerous TE families. On the basis of the mechanism of transposition, TEs were divided into two groups, ie, class I (retrotransposons) and class II (DNA transposons).\(^3\)\(^,\)\(^4\) Retrotransposons transpose via an RNA intermediate (copy-and-paste mechanism), each transposition event leading to an increase of their copy number. DNA transposons change their chromosomal localization by physical excision of the element from the donor site and reintegration in the acceptor site. This group contains two subclasses, depending on the number of DNA strands cut during transposition. Subclass 1 is mobilized in accordance with the cut-and-paste mechanism, where both strands are cut at each end resulting in the complete excision of the TE. Most Subclass 1 DNA transposons carry Terminal Inverted Repeats (TIR) and create Target Site Duplication (TSD) upon insertion. Subclass 2 elements transpose on the basis of the rolling circle mechanism, where only one strand is cut. Classes and subclasses are further divided into orders, superfamilies, and families.

The effect of TE proliferation on genome evolution is noticeable.\(^5\) Moreover, the importance of accurate TE annotation and masking for the structural characterization of newly sequenced genomes drives the interest in developing new methods for TE detection and analysis.\(^6\) The exhaustive list of tools and resources for TE analysis compiled by Bergman Lab (http://bergmanlab.smith.man.ac.uk/) contains about 120 items. A large number of them are designed for particular TE families (eg, Helitrons,\(^7\) LTR retrotransposons\(^8\)) or for analysis of particular species (like Drosophila melanogaster\(^9\)), several of these are for general use, ie, for all kinds of repeats (RepeatMasker,\(^10\) REPuter,\(^11\) PILER,\(^12\) RepeatScout,\(^13\) RECON\(^14\)), and finally six of them are suitable for the structural analysis of class II elements (Inverted Repeat Finder,\(^15\) MAK,\(^16\) MITE-hunter,\(^17\) MUST,\(^18\) STAN,\(^19\) TRANSPO\(^20\)), with the latter three providing a web interface.

MUST\(^18\) allows the user to search for all Miniature Inverted-repeat TEs (MITEs) that satisfy given criteria corresponding to minimum and maximum length of TIR, TSD and size of MITEs (up to 1000 bp). TRANSPO\(^20\) in addition to the functionality of MUST, enables the user to specify the sequence of TIRs and maximum number of errors allowed in TIRs. STAN\(^19\) is the most flexible tool. It finds all sequences containing a given pattern specified in the SVG grammar. However, STAN uses fixed (non-parameterized) definition of inverted repeats which makes it less suitable for searching class II transposons.

All of the tools mentioned above have been developed to facilitate identification of MITEs, ie, elements that have TIRs but lack any coding capacity. Our TE discovery tool, TIRfinder, (which also captures specific structural features) offers functionality that goes beyond the proposed methods. It combines an efficient approach based on suffix trees that allows de novo TE detection, with the possibility of a deep analysis of a specific TE family, based on its structural characteristics. In particular, while searching for all putative TEs, TIRfinder allows the user to specify TIR and TSD patterns as a sequence of A, C, T, G or symbols from extended IUPAC nomenclature.\(^21\) This provides the ability to define TIR/TSD patterns as consensus sequences which combine conserved and non-conserved positions. A detailed description of the tool has not been provided previously, however the prototype, stand-alone version of TIRfinder was used for mining TEs in Medicago truncatula.\(^22\)\(^,\)\(^23\)

Recently, deep sequencing projects have increased dramatically, raising the possibility of the repetitive DNA characterization of not completely sequenced organisms. Notice that our approach based on a suffix tree requires a large contiguous genomic sequence. Therefore for analysis of non-assembled raw reads produced by next generation sequencing, recently developed tools which utilize a clustering approach\(^24\) should be applied.

Methods of TE Detection and Analysis
TIRfinder analysis workflow
TIRfinder allows efficient searches of the DNA for structured set of motifs that define TEs to be conducted. Then it performs a BLAST search to find transposase
TIRfinder: a web tool for mining class II transposons

or any other TE-related open reading frames (ORFs), aiming at the detection of autonomous copies, as well as elements directly derived from them. The method works in three stages: structural analysis, functional analysis and MITE analysis (see Fig. 1).

In the first step, TIRfinder scans the input sequence for all putative TEs, based on the given structural characteristic of a particular TE family, ie, patterns describing TIR, TSD, the number of allowed mismatches and size limits of desired TEs. The algorithmic details of the structural analysis are described further in this section.

Next, all elements identified in the structural analysis stage are analyzed to check whether they contain the ORF coding for a protein specified by the user, most often it would be a transposase. For this purpose, they are aligned with the protein sequence (using an appropriate version of the BLAST algorithm)\(^\text{25}\) and elements with statistically significant similarity are marked as putative autonomous. Each autonomous element is then used to search for so called Derivatives. These are non-autonomous elements that have emerged from an autonomous elements during the course of evolution, often by internal deletions disrupting the ORFs. To classify the TE as a derivative of a given autonomous element, we require that both pairs of subterminal regions share a significant level of similarity which is defined by the user (eg, BLAST E-value < \(e^{-10}\)).

Finally, the remaining set of putative TEs (ie, those not classified as autonomous TEs and their derivatives) is analyzed to find short elements which cluster together based on their sequence similarity. The level of similarity is defined by the user. Clustering is performed using BLAST-clust algorithm.\(^\text{25}\) The TEs assigned to clusters are labeled as MITEs.

**Structural analysis**

Our application follows a structure-based approach, ie, it relies on the detection of specific models of TE architecture consisting of a pair of TIRs (inverted repeats) that are flanked by TSDs (direct repeats).

For detecting TEs we use suffix trees which are very efficient data structures, commonly used in computer science over last four decades.\(^\text{26}\) They consist of a root, nodes and labeled edges representing one or more characters from input sequence. All suffixes of the input sequence can be obtained by traversing the suffix tree from the root to leaves. The core idea is that all suffixes that share the common prefix are hanged off on the common node, which reduces the total number of nodes and memory usage.

The algorithm implemented in TIRfinder takes as an input a DNA sequence, a mask corresponding to combined TSD and TIR patterns, and other parameters (ie, the number of mismatches), see Figure 2 for the pseudocode of the algorithm and Figure 3 for graphical explanation of the mask notion.

First, the DNA sequence is divided into a set of smaller fragments of the same length, corresponding to the maximal TE size. A suffix tree is built for every two consecutive fragments, with overlaps of one fragment length (see Fig. 3B). This is to ensure that we do not miss any match. The algorithm for each fragment independently searches all matches
Input: DNA sequence G, TIR pattern, TSD pattern, max_size, mismatch_thresholds
Output: all regions flanked by TIR's and TSD up to predefined mismatch threshold

(1) split the sequence G into fragments \( g_i \), \( i = 1 \ldots n \) of size = max_size
(2) MASK = TSD \( \cdot \) TIR
(3) foreach sequence \( g_i \cdot g_{i+1} \)
    (4) build the suffix tree \( ST_i \);
    (5) find all matches to MASK in \( g_{i+1} \),
    (6) say \( m_1, m_2, \ldots, m_j, \ldots, m_k \);
    (3) foreach \( m_j \)
(8) find in the suffix tree \( ST_i \) all positions that match to \( \text{revcomp}(m) \);
    (9) // revcomp = reverse complement
(10) check the number of mismatches between MASK and \( \text{revcomp}(m) \);

Figure 2. TIRfinder algorithm.

for the mask. In this step, all potential 3'-ends of a TIR should be found. Then we determine if the complementary (5’-end) part of the repeat exists, which is the most time-consuming part of the algorithm. In order to do this efficiently the suffix tree is used (it is built in linear time with respect to a length of the fragment). For each match (TIR + TSD) found in the previous step, the reverse complement of TIR followed by TSD is searched in the suffix tree (see Fig. 2).

The principal advantage of our approach is memory efficiency: the genomic DNA sequence is split into smaller fragments and the suffix tree data structure needs only a linear amount of space. Thus, we do not impose any limits to the total length of the sequence and the stand-alone version of TIRfinder can be run even on standard PC computers.

Usage
The user must first select the genomic sequence to be searched for TEs. Several plant genomes are currently available in the TIRfinder website (Arabidopsis thaliana, Arabidopsis lyrata, Oryza sativa japonica, Oryza sativa indica and Medicago truncatula); other genomes can be provided upon request. Alternatively, the user can provide any sequence by uploading a FASTA file not exceeding 100 MB.

Next, the user has to define several parameters such as a minimal and maximal distance between TIRs and patterns of inverted and direct repeats. Patterns
of the particular TIR or TSD can be determined by means of a finite string composed of extended IUPAC nucleotide alphabet.\textsuperscript{21}

Note that the user has to define only one side of the repeat. The second part will be computed as a reverse complement in the case of TIR or simply duplicated in the case of TSD. Furthermore, there is a possibility to specify a maximal number of mismatches between TSDs and TIRs flanking each copy of identified TEs. TIRfinder reads a DNA sequence given in a FASTA file to search for results and saves them in a simple text format (see Appendix for the detailed description). Then the file can be further processed in order to obtain more detailed and accurate data.

Carefully prepared input data, such as TIR and TSD masks, are extremely important in order to get satisfactory results. One possible way is to take TIR sequences from individual copies of TEs representing the desired family and their corresponding flanking TSDs, and create consensus for TSD and TIR sequences. Afterwards, the user can set up other parameters of TIRfinder, which allow for the control of similarity between mined elements and the mask (MaskMismatches) or between corresponding 5’- and 3’- TIRs and TSDs of found elements (SeqMismatches) see Figure 3A. The ability to manipulate these parameters makes it easier to search for known, well conserved elements as well as to mine new (sub)families of TEs de novo.

Subsequently, the user may specify parameters for the identification of TE copies carrying ORFs, which for simplicity was dubbed functional analysis. First, the protein sequence (in FASTA format) is provided to detect ORFs (a significance threshold is required). Then, long non-autonomous copies—direct derivatives are identified as elements sharing similarity of subterminal regions (length parameter in bp and

Figure 3. TIRfinder—structural analysis. (A) Explanation of TIR and TSD mask concept. (B) Overview of TEs detection phase.
alignment significance threshold are provided by the user) with one of the previously found autonomous TEs. If the ORF sequence is unknown to the user, the functional analysis step can be omitted in the course of analysis.

Finally, the user defines constraints for MITE analysis, ie., minimal and maximal length of MITEs, maximal number of MITE clusters and the level of in-cluster similarity.

TIRfinder implementation

Current release of TIRfinder is an open source web application built with Java, Perl, Apache and other tools (ie, BLAST, BLAST-clust). Previous, stand-alone release of TIRfinder, used for case studies reported in, we fixed TIR and TSD strings as consensus subsequences from given sequences of ATHPOGO, ATHPOGON1, ATHPOGON2 and ATHPOGON3 from Repbase. Finally, we obtained the following parameters: TIR pattern = CAGTARAAMCTC-TATAAATTAATA, TSD pattern = TA. We decided to set min distance = 300, max distance = 5000, max TSD mask and TSD seq mismatches = 0, max TIR mask and TIR seq mismatches = i ∈ (0, 1, 2, 3, 4, 5). The experiment allowed for efficient mining of pogo-like transposons in A. thaliana and M. truncatula. The number of TEs found by TIRfinder and annotated in Repbase for each chromosome of A. thaliana is shown in the Table 1 (see also Appendix). The breakdown analysis of the found TE sizes (shown in Fig. 4) reveals that lengths of the majority of putative TEs correspond to the sizes of known pogo-like elements. Most of these elements were MITEs, while in M. truncatula we found only 28 pogo-like elements and no MITEs. It fully confirmed previous reports on these elements, reflecting species-specific behavior of related TEs in the two species and confirming TIRfinder efficiency.

Moreover, we performed similar search of M. truncatula genome for PIF/Harbinger TEs family (using TIR pattern = GNNNNGTTNNNN and TSD pattern = TWA). The exemplary results of this analysis, presented by TIRfinder (see Fig. 5),

| chr1 | chr2 | chr3 | chr4 | chr5 | Sum | Positive predictive value |
|------|------|------|------|------|-----|---------------------------|
| 0    | 1    | 2    | 0    | 4    | 2   | 9                         | 100 |
| 1    | 11   | 6    | 7    | 10   | 9   | 43                        | 100 |
| 2    | 20   | 10   | 16   | 17   | 13  | 76                        | 95  |
| 3    | 25   | 20   | 18   | 20   | 14  | 97                        | 94  |
| 4    | 29   | 22   | 22   | 22   | 17  | 112                       | 88  |
| 5    | 33   | 22   | 25   | 24   | 20  | 124                       | 84  |
| Rep. | 25   | 22   | 20   | 22   | 8   | 97                        |      |

Notes: aNumber of allowed Tir mask and Tir seq mismatches; †% of TIRfinder output masked by Repbase data. ‡numbers of ATHPOGO elements (~300 bp) annotated in Repbase.

Case Studies

As an example of TIRfinder application, we searched the genome of Arabidopsis thaliana and Medicago truncatula for the ATHPOGON3 class II TE family. We fixed TIR and TSD strings as consensus subsequences from given sequences of ATHPOGO, ATHPOGON1, ATHPOGON2 and ATHPOGON3 from Repbase. Finally, we obtained the following parameters: TIR pattern = CAGTARAAMCTC-TATAAATTAATA, TSD pattern = TA. We decided to set min distance = 300, max distance = 5000, max TSD mask and TSD seq mismatches = 0, max TIR mask and TIR seq mismatches = i ∈ (0, 1, 2, 3, 4, 5). The experiment allowed for efficient mining of pogo-like transposons in A. thaliana and M. truncatula. The number of TEs found by TIRfinder and annotated in Repbase for each chromosome of A. thaliana is shown in the Table 1 (see also Appendix). The breakdown analysis of the found TE sizes (shown in Fig. 4) reveals that lengths of the majority of putative TEs correspond to the sizes of known pogo-like elements. Most of these elements were MITEs, while in M. truncatula we found only 28 pogo-like elements and no MITEs. It fully confirmed previous reports on these elements, reflecting species-specific behavior of related TEs in the two species and confirming TIRfinder efficiency.

Moreover, we performed similar search of M. truncatula genome for PIF/Harbinger TEs family (using TIR pattern = GNNNNGTTNNNN and TSD pattern = TWA). The exemplary results of this analysis, presented by TIRfinder (see Fig. 5),
TIRfinder: a web tool for mining class II transposons

Evolutionary Bioinformatics 2013:9

Figure 5. The example of TIRfinder outcomes: search for PIF/Harbinger TEs family in chromosome 5 of M. truncatula genome.

reveals the occurrence of all functional classes of PIF/Harbinger TEs, ie, putative autonomous, derivatives and MITEs.

Discussion

Efficient methods for computational analysis of TEs are of great interest in the light of the TE contribution to genome structure and evolution. We developed TIRfinder to provide users with a fully functional web server that allows structure-based detection and clustering of class II TEs carrying TIRs.

There are several unique features of TIRfinder, in comparison with other tools designed for mining TEs carrying TIRs. It provides flexibility in defining TIR/TSD masks, allowing for a range of actions, from de novo identification of TEs belonging to a given superfamily in newly annotated genomes to extensive mining of all copies belonging to a particular family (eg, ATHPOGO), depending on the mask specificity.

Although our tool is highly efficient for well-determined TSD/TIR mask, the complexity of the method may significantly increase with relaxing the mask constraints (eg, allowing large number of mismatches between TIR/TSD). Moreover, TIRfinder helps identifying elements with coding capacity, however some additional downstream processing, eg, ORF prediction, should be performed for all copies classified as autonomous, if a more detailed functional characteristics is required.

Author Contributions

TG, MS, JP developed the software and performed tests. AG, KW and DG supervised all the software developments, and carried case studies. TG, MS, DG, and AG wrote the manuscript. All authors reviewed and approved of the final manuscript.

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Competing Interests
Author(s) disclose no potential conflicts of interest.

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Appendix

Output file formats

TE identifiers

All groups of detected TE elements, i.e., all putative TEs, putative autonomous TEs, derivatives and MITEs can be downloaded as separate multi fasta files. Names of each TE sequence in the file is composed as in the following example:

For the name “1-102-101894195-101894898-ATHPOGO”

1 = chromosome number
102 = ID of putative TE
101894195 = TE start position
101894898 = TE end position
ATHPOGO = TE family name

Hits file

The output of structural analysis (containing all putative TEs) can be downloaded as txt file. First 5 lines consists of TIR/TSD mask parameters used to search for TEs. Next the total number of found TEs is presented. Subsequent lines describes each found TE including:

- element ID
- start and stop genomic position
- TSD/TIR sequence and mask mismatches
- TSD and TIR sequence alignment
- Extracted sequence

The beginning of the hits file corresponding to the results shown in Figure A2 is presented below:

```
MASK ID = 0
MASK(TSD:TIR) = TA:CAGTARAAMCTCTATAAATTAATA
max mask mismatches(tsd:tir) = 0:5
max seq mismatches(tsd:tir) = 0:5
distance(min:max) = 200:5000
------------------------------------------------------------------------
Number of found elements = 126
------------------------------------------------------------------------
ID: 0 begin: 784927 end: 785436 length: 509
mismatches seq1 to mask(tsd:tir) = 0 : 0
mismatches seq2 to mask(tsd:tir) = 0 : 2
mismatches between seq1 and seq2(tsd:tir:extra) = 0 : 2 : 62
TA: CAGTAAAAACCTCTATATAATTAATA:GTATTGAGACCACAAAAATTGATTAATTGAGAG ...
||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:||:|
ATAATATATATTAGTAAATTTATAAAATTATTAATTTATACATATTGATGGGAGCATATAT
TTATATAAGATTTTCAATAAAAAATATTATCCTTATAGTTTATCGATTTATACATTTTT
ATAATTGGTCCTACTCTGCAACCAAAAAAATTATTAATTTATAAAAAATTATATTATC
GAATATTAATTTATAAAGATTTTACTGTAA

*************SEQ END*************

ID: 1 begin: 6759166 end: 6759752 length: 586
mismatches seq1 to mask(tsd:tir) = 0 : 0
mismatches seq2 to mask(tsd:tir) = 0 : 2
mismatches between seq1 and seq2(tsd:tir:extra) = 0 : 2 : 59
TA:CATGACACCTCTCATATAAATAAATAATAAAATTGATCGAAATATATAGATTTCAAA
AAAAAAATTTATTAATTTATAAAAATTATTAATTTAGAAG ... ||:|| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |... TA:CATGAAAACCTCTCATATAAATAAATAATAAAATTGATCGAAATATATAGATTTCAAA
AAAAAAATTTATTAATTTATAAAAATTATTAATTTAGAAG ...

*************SEQ START*************
TACAGTAAACCTCTATATAAATAAATAAATTTTGGACCAAAAAATTTATTAATTTTAGAGA
GGTATATTTTATCGATAAAATATAAAAARTTGTACAAAAATTCTCTAACAAAAAT
TCGATATAAAATATATGTTTTTTTCCTTTATAATACATTTGATTTCTAACAAAAAT
TGAAACGATATATTAAAGTGGAAAAACAAATACATTTTTTCGATATTTGAAATATTAG
AATTGGAACACTAACAAATGAAATACACAAAAAGATTGAAAITAATGACATACATC
ACATGAATATTTTAATATATATTGAAAATATATTATTTATAATTTAAATATTTATCTTATTTGA
TGACCAATATATATTATAAGATTTCATAAAATATTATATCTTTATAATTATATTTTCGATATT
TTATCCAAATATTGACTACAAATCTGTCAGAATACATGTAAAATATTAGTTTTGCTGTA

*************SEQ END*************

ATHPOGO TEs in A. thaliana genome

Figure A1. TiRfinder results of search for pogo-like family in chromosome 2 of A. thaliana genome.
Figure A2. TIRfinder results of search for pogo-like family in the whole A. thaliana genome.

Notes: The single known autonomous TE was detected (see the green row in the table) as well as 125 other putative TEs. The 63 of them were classified as MITEs.