PASTEC: An Automatic Transposable Element Classification Tool

Claire Hoede, Sandie Arnoux, Mark Moisset, Timothée Chaumier, Olivier Inizan, Véronique Jamilloux, Hadi Quesneville

Abstract

Summary: The classification of transposable elements (TEs) is key step towards deciphering their potential impact on the genome. However, this process is often based on manual sequence inspection by TE experts. With the wealth of genomic sequences now available, this task requires automation, making it accessible to most scientists. We propose a new tool, PASTEC, which classifies TEs by searching for structural features and similarities. This tool outperforms currently available software for TE classification. The main innovation of PASTEC is the search for HMM profiles, which is useful for inferring the classification of unknown TE on the basis of conserved functional domains of the proteins. In addition, PASTEC is the only tool providing an exhaustive spectrum of possible classifications to the order level of the Wicker hierarchical TE classification system. It can also automatically classify other repeated elements, such as SSR (Simple Sequence Repeats), rDNA or potential repeated host genes. Finally, the output of this new tool is designed to facilitate manual curation by providing to biologists with all the evidence accumulated for each TE consensus.

Availability: PASTEC is available as a REPET module or standalone software (http://urgi.versailles.inra.fr/download/repet/REPET_linux-x64-2.2.tar.gz). It requires a Unix-like system. There are two standalone versions: one of which is parallelized (requiring Sun grid Engine or Torque), and the other of which is not.

Introduction

Transposable elements account for a high proportion of eukaryotic genomes. They are involved in a number of important processes, including genome rearrangement, heterochromatin formation and the regulation of gene expression. Wicker et al. [1] published a detailed classification for eukaryotic TEs. Their goals were to harmonize and clarify TE classifications and names. This classification includes both well-known classes of TEs: class I (retrotransposons) and II (DNA transposons). Five orders (LTR, DIRS, PLE, LINE, SINE) are defined for class I, and four (TIR, Crypton, Helitron, Maverick) for class II. Each order is also divided into one or several superfamilies, resulting in 29 superfamilies total. Other categories comprising non-autonomous TEs (LARD, TRIM and MITE) are considered in this classification, together with various forms of non-autonomous entities related to TEs to various degrees. This classification is based on the transposition mechanism, sequence similarities and structural relationships. Wicker et al. described the protein-coding domains present in each TE superfamily in particular detail.

Several computational tools have been proposed for the ab initio identification of repeat families (see reviews [2]; [3]). These tools generally reconstruct the consensus sequences of repeated regions. It is then useful to characterize and classify these consensus sequences, to study the TE repertoire of a genome. Two tools for the automatic classification of a large range of TEs have been proposed: TEclass [4] and REPCLASS [5]. TEclass classifies TE sequences only into two classes: Retro (class I in Wicker’s classification) and DNA (class II in Wicker’s classification). This tool can classify class I elements into LTRs and non-LTRs and, where possible, into SINE or LINE elements. However, TEclass cannot distinguish between the various orders of class II elements. The SVM (Support Vector Machine: supervised learning model) classifiers in this tool were trained with RepBase Update, a database of eukaryotic repetitive sequences [6], using different class lengths and vectors of tetramer and pentamer frequencies. This approach is useful when TEs show no clear similarity to known repeats.

REPCCLASS can classify TEs into classes I and II, and into the orders LTR retrotransposon (grouping LTR, LARD, TRIM, DIRS and PLE from Wicker’s classification), DNA transposon (grouping TIR, crypton and Polinton from Wicker’s classification), LINE/SINE and helitron. In some cases, this tool can also identify the superfamilies of the TE. REPCCLASS consists of three modules. The first is based on homology and has the greatest impact on final decision. At this step, the software carries out a tblastx analysis against the RepBase databank. The second module (in terms of order of priority for classification) searches for structural charac-
Materials and Methods

PASTEC was developed in the REPET package [7]. In this context, we used PASTEC to classify the consensus TE sequences found de novo in a genome. PASTEC uses several features of TE to classify TE consensus sequences. It searches for structural evidence and sequence similarities stored in a MySQL database obtained during a preprocessing step. The structural features considered are TE length, presence of a LTR (long terminal repeat) or TIR (terminal inverted repeat) detected with a custom-built tool (with a minimum length of 10 bp, a minimum identity of 80%, the taking into account of reciprocal orientations of terminal repeats and a maximal length of 7000 bp), the presence of SSRs (simple sequence repeats detected with the tandem repeat finder (TRF) tool [8]), the polyA tail and an ORF (open reading frame). The blastx and tblastx routines are used to search for similarities to signals and SSR. Finally, the third module searches for target site duplication (TSD) around TE copies within the genome.

Neither TEclass nor RepClass can classify TE at the level of detail given by Wicker’s classification (see Table 1). We therefore developed PASTEC (Pseudo Agent System for Transposable Element Classification), a modular tool able to classify TE automatically to order level, for all nine orders of autonomous TE defined in Wicker’s classification plus three orders of non-autonomous TE (LARD, TRIM and MITE). If PASTEC cannot classify a TE to order level, it classifies it to class level. PASTEC is also designed to filter out false-positive repeated sequences identified by de novo approaches. It can therefore distinguish SSRs, rDNA sequences and potential host genes from real TE. Moreover, PASTEC can classify and recognize incomplete TE and potentially chimeric TE (detection of an anomaly in the evidence used to select the classification or several classifications possible). In this last case, PASTEC chooses the best classification if it has sufficient evidence to make a decision. All evidence is written to the output file, to facilitate the manual curation of potential chimeric TE. We tested this tool by comparing the results of these three tools on three different datasets.

Table 1. Comparison of the classifications obtained with the PASTEC, TEClass and RepClass tools.

| PASTEC (Wicker’s) Class | PASTEC (Wicker’s) Order | TEClass Class | TEClass Order | RepClass Class | RepClass Order |
|-------------------------|-------------------------|---------------|---------------|----------------|----------------|
| **Class I (Retrotransposons)** | | | | | |
| LTR | Retro | LTR | Class I | LTR/DIRS/PLE |
| DIRS | Retro | LTR | Class I | LTR/DIRS/PLE |
| PLE | Retro | LINE | Class I | LTR/DIRS/PLE |
| LARD* | Retro | na | Class I | na |
| TRIM* | Retro | na | Class I | na |
| LINE | Retro | LINE | Class I | LINE/SINE |
| SINE | Retro | SINE | Class I | LINE/SINE |
| **Class II (DNA transposons)** | | | | | |
| TIR | DNA | DNA | Class II | TIR/Crypton/Polinton |
| MITE* | DNA | DNA | Class II | TIR/Crypton/Polinton |
| Crypton | DNA | na | Class II | TIR/Crypton/Polinton |
| Hellitron | DNA | na | Class II | Hellitron |
| Maverick | DNA | na | Class II | TIR/Crypton/Polinton |

Notes: (*) Non-autonomous element. na: not considered by the tool.

doi:10.1371/journal.pone.0091929.t001
and formats the output file. It resolves conflicts by using a confidence index normalized to 100. For example, the LTR agent calculates a confidence index with the following rules: presence of ENV profiles (+2 because this condition is sufficient for classification), presence of a long terminal repeat and an INT, GAG, RT, RH or AP profile (+1 for each profile combined with the long terminal repeat), +1 for each profile (ENV, AP, RT, RH and GAG) found in the same frame in the same ORF. If the consensus matches at least one known LTR retrotransposon, the LTR agent adds +2 for each type of blast (blastx or tblastx) at the confidence index. Finally, the length of the TE is taken into account because we add +1 if the TE without the long terminal repeat is between 4000 and 15000 bp in length, and we decrease the confidence index by 1 if the TE without the long terminal repeat is less than 1000 bp or more than 15000 bp long. The super-agent uses the maximum confidence index defined for each classifier agent to normalize the confidence index for each classification to 100 and then compare the different classifications. Advanced users can edit all decision rules and maximum confidence indices in the Decision_rules.yaml file.

The output can be read by humans and is biologist-friendly. A single line specifies the name of the TE, its length, orientation of the sequence, chimeric/non-chimeric status (OK indicating that the element is not potentially chimeric), class (class I in this case), order. In the first line of the example provided, the TE is a LTR. We presume that the element is complete because we have no evidence to suggest that it is incomplete, and the confidence index is 71/100. The last column summarizes all the evidence found: coding sequence evidence, such as the results of blastX queries against the Repbase database (TE_BLRx evidence), blastX queries against the Repbase database (TE_BLRx evidence) and profiles. A blast match is taken account if coverage exceeds 5%, and a profile is taken into account if its coverage exceeds 20% (these parameters can be edited in the configuration file). For each item of coding sequence evidence, the coverage of the subject is specified. The structural evidence is also detailed: >4000 bp indicates that TE length without terminal repeats is between 4000 and 15000 bp, the next item of information presented in the comments column is the presence of terminal repeats: we have a LTR in this case, with an LTR length of 433 bp; two long ORFs have been identified, the last of which contains four profiles in the same frame and is up to 3000 bp long. Other evidence provided for this example includes the partial match with a *Drosophila melanogaster* gene (coverage 16.55% and the TE contains 18% SSRs). The super-agent determines whether a TE is complete based on whether it is sufficiently long, whether the expected terminal repeats or polyA tail are present, whether blast match coverage exceeds 30% and profile coverage exceeds 75%. The second line of the example corresponds to a potentially chimeric TE, for which human expertise is required.

**Results and Discussion**

We compared the results of PASTEC with those of the two other classification tools, REPCLASS and TEclass, for three datasets: (i) the *A. thaliana* consensuses found in Repbase update.
version 15.09 (referred to hereafter as Repbase-atha), (ii) the TEs present in Repbase update 15.09 but not in Repbase 13.07 (referred to hereafter as Repbase-diff), and (iii) the whole Repbase update 15.09 from which we removed redundant TEs, i.e., those with strictly identical sequences (referred to hereafter as Repbase-all). For RepClass and PASTEC, which require a database for blast analyses, we used RepBase update 15.09 from which we removed the A. thaliana TEs in the first case, RepBase 13.07 in the second case and no blast database in the last case. The second case (Repbase-diff) mimics standard use: we wanted to classify TEs obtained from a new genome and, therefore, not yet known in the Repbase database. Indeed, Repbase-diff contains the TEs present in Repbase Update 15.09, but not in Repbase Update 13.07, and we provided Repbase Update 13.07 for sequence similarity searches by REPCLASS and PASTEC. So, as is usually the case in such analyses, we have unknown TEs (Repbase-diff) and a database of known TEs (Repbase Update 13.07). This made it possible to check whether the classification results for Repbase-diff were consistent with the classification given in Repbase Update 15.09. The last case is the most difficult, with attempts to classify TEs as if no known TEs are present in the database.

We compared the classification performance, in terms of the percentages of TEs well classified, misclassified relative to their annotation in the Repbase database or not classified at the level of class (Table 2) or order (Table 3), for each of the three tools. It was not possible to compare sensitivity and specificity for each order, because the three tools do not use the same classification. Nevertheless, sensitivity and specificity data are presented in the supplementary data for the orders for which this was possible. We mapped the PASTEC classification onto those used by TEclass or REPCLASS, to improve comparisons. Table 3 shows the performances obtained with PASTEC after mapping.

PASTEC misclassified very few TEs, regardless of the dataset tested (Table 2 and 3). These rare ambiguities mostly concerned closely related TEs: essentially MITE/TIR (427/605 = 70.6% - TEs misclassified at order level in the Repbase subtraction dataset). For Repbase-diff, PASTEC was frequently more specific than the other two tools (see suppl. Data: Venn diagram and table of sensitivity and specificity of the three tools for this second dataset Tables S2, S3, S4, S5 S6, Figures S1, S2, S3, S4, S5 S6). However, it failed to classify many TEs due to a lack of useful evidence. PASTEC HMM profile detection was found to be particularly useful if the database contained no similar TE to that for which the search was carried out. For Repbase-all without blast searches, we had profile information for 3408 TEs, making it possible to classify these elements at least partially. For classification to order level, PASTEC gave better results than REPCLASS. PASTEC correctly classified more TEs than REPCLASS for the Repbase-all library (31.9% of the TEs were well classified by PASTEC on the basis of REPCLASS order, whereas REPCLASS classified only 12.4% of TEs well, and PASTEC misclassified fewer TEs for all datasets (see Table 2 and 3).

REPCLASS performed well on the Repbase-atha dataset, particularly for helitrons, thanks to its helitron_scan tool, if no TSDs were found. It was efficient, but required the genome sequence. This tool searches for 5’ and 3’ termini, including the flanking sequences of all copies found in the genome, and a subterminal hairpin-like GC-rich motif. For the other datasets, we provided no genome sequence because the TEs came from many different genomes. In such conditions, the TSD module of REPCLASS cannot be used, but good results were nevertheless obtained with this tool for the Repbase-diff dataset (67% of TEs well classified to order level, see Table 3). For this dataset, REPCLASS was more sensitive (83.6% vs 52.7%) but less specific (89.7% vs 99.9%) than PASTEC for classification to order level. By contrast, for the Repbase-all dataset (without blast homology searches), this tool gave the worst results (12.4% of TEs well classified to order level).

TEclass classified the A. thaliana dataset well, but despite the number of TEs correctly classified with each of the three test datasets, the percentage of TEs misclassified remained high. TEC class between 40% and 60% of TEs from the Repbase-diff and Repbase-all datasets, misclassifying more than half of the TEs at order level for these two datasets. This suggests that this tool does not recognize effectively the situations in which it cannot give a correct classification. For interpretation purposes, a lower level of misclassification with more TEs unclassified and requiring manual checking would be preferable. It should also be noted that, in this study, TEclass was at an advantage over the other tools, as we could not train TEclass with a database containing none of the TEs in the test set.

### Table 2. Comparison of the performances of PASTEC, REPCLASS, and TECLASS for classification into TE classes.

| Dataset (sequence) | Performance | PASTEC | REPCLASS | TECLASS |
|--------------------|-------------|--------|----------|---------|
| Repbase-atha (318) | Well classified | 80.7 | 83.6 | 98.4 |
|                    | Misclassified | **0.95** | 3.5 | 1.3 |
|                    | Not classified | 18.4 | 12.9 | 0.3 |
| Repbase-diff (5546) | Well classified | 63.7 | 26.1 | 59.2 |
|                    | Misclassified | **2.9** | 31.8 | **40.2** |
|                    | Not classified | 33.4 | 42.1 | 0.5 |
| Repbase-all (9665) | Well classified | 52.7 | 20.35 | 53.8 |
|                    | Misclassified | **5.9** | 27.09 | **45.8** |
|                    | Not classified | 41.3 | 52.6 | 0.3 |

[do10.1371/journal.pone.0091929.t002](http://do10.1371/journal.pone.0091929.t002)
Conclusions

Furthermore, PASTEC highlights the TEs for which manual information required for manual curation by a biologist. Generation of a file readable by humans, containing all the particular attention in the development of this tool to the of TEs (almost four times more than PASTEC).

REPCLASS classified 67% of TEs correctly and misclassified 11% TEs well and misclassified only 3% of TEs. In the same conditions, REPCLASS levels of classification, PASTEC classified 59% of classified and 11% were misclassified for classification to order level (for the nine available orders). If we consider only the four dataset, which simulates a standard dataset: 51% of TEs were well classified, with only 11% misclassified). This is particularly useful when looking at a HMM profile searches (22% of TEs are well classified and only 16% are misclassified). This is particularly useful when looking at a species phylogenetically distant from the species from which the known TEs were obtained. It performed well for the Repbase-atha TEs well classified, with only 3% misclassified, 67% of Repbase-diff TEs well classified, with only 11% misclassified). However for the Repbase-all dataset, for which no homology search was possible, it performed poorly (12% of TEs well classified for 33% misclassified).

PASTEC was found to have many advantages over the other tools. First, it can classify TEs into no less than nine orders, according to Wicker’s classification (versus only four for TEclass and REPCLASS). It performs well for classification into these nine orders, particularly for the dataset for which blast homology searches were not possible, due to the possibility of carrying out HMM profile searches (22% of TEs are well classified and only 16% are misclassified). This is particularly useful when looking at a species phylogenetically distant from the species from which the known TEs were obtained. It performed well for the Repbase-diff dataset, which simulates a standard dataset: 51% of TEs were well classified and 11% were misclassified for classification to order level (for the nine available orders). If we consider only the four REPCLASS levels of classification, PASTEC classified 59% of TEs well and misclassified only 3% of TEs. In the same conditions, REPCLASS classified 67% of TEs correctly and misclassified 11% of TEs (almost four times more than PASTEC).

A second advantage of PASTEC is its output format. We paid particular attention in the development of this tool to the generation of a file readable by humans, containing all the information required for manual curation by a biologist. Furthermore, PASTEC highlights the TEs for which manual curation is particularly necessary, by specifying whether the classification was ambiguous.

Finally, PASTEC’s modular architecture makes it possible to add new analyses or decision rules without difficulty. Expert users can access a configuration file, to change diverse parameters.

The main perspective for improving PASTEC concerns classification to the superfamily level, not only through blast searches, but also on the basis of the HMM profiles detected in the TEs. http://urgi.versailles.inra.fr/Tools/REPET

Supporting Information

Figure S1 Venn diagram (Repbase-diff dataset) for class I TEs. The number of well classified class I TEs is shown in brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools. (TIF)

Figure S2 Venn diagram (Repbase-diff dataset) for class II TEs. The number of well classified class II TEs is shown in brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools. (TIF)

Figure S3 Venn diagram (Repbase-diff dataset) for LINE/SINE TEs. The number of well classified LINE/SINE TEs is shown in brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools. (TIF)

Figure S4 Venn diagram (Repbase-diff dataset) for helitron TEs. The number of well classified helitron TEs is shown in brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools. Note: TECLASS does not classify helitron TEs to order level. (TIF)

Figure S5 Venn diagram (Repbase-diff dataset) for LTR TEs. The number of well classified LTR TEs is shown in
brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools.

Figure S6 Venn diagram (Repbase-diff dataset) for TIR TEs. The number of well classified TIR TEs is shown in brackets. The numbers within the Venn diagram are the numbers of TEs well classified by each tool, with the overlaps indicating those well classified by several tools. Note: TECLASS does not classify TIR TEs to order level.

Table S1 An example of PASTEC output. We present two TEs: the first is classified as a LTR and the second is a potentially chimeric TE (chimera between a helitron and LARD). The output is a tabular file providing the maximum amount of information to facilitate interpretation by biologists.

Table S2 Sensitivity/specificity for ClassI and ClassII TEs.

Table S3 Sensitivity/specificity for LINE/SINE TEs.

Table S4 Sensitivity/specificity for helitrons TEs.

Table S5 Sensitivity/specificity for LTR TEs.

Table S6 Sensitivity/specificity for TIR TEs.

Acknowledgments

We thank the REPET team for their support in software development, and Joelle Amselem, Nathalie Choisne and Florian Maumus, for user feedback. We also thank Aurélie Kapusta for her help with REPCLASS and the reviewers for their helpful comments.

Author Contributions

Conceived and designed the experiments: CH, HQ. Performed the experiments: CH. Analyzed the data: CH, HQ. Wrote the paper: CH, HQ. Designed the software used in analysis: CH, SA, TC, MM, OI, VJ.

References

1. Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, et al. (2007) A unified classification system for eukaryotic transposable elements. Nature Rev. Genet. 8: 973–982.
2. Permal E, Flutre T, Quesneville H (2012) Roadmap for annotating transposable elements in eukaryote genomes. Methods Mol. Biol. 859: 53–68.
3. Bergman CM, Quesneville H (2007) Discovering and detecting transposable elements in genome sequences. Brief. Bioinform. 8: 382–392.
4. Abrusaén G, Grundmann N, DeMeester L, Makalowski W (2009) TEclass: a tool for automated classification of unknown eukaryotic transposable elements. Bioinformatics 25: 1329–1330.
5. Feschotte C, Kowani C, Kranzathanan N, Guibotsy ML, Levine D (2009) Exploring Repetitive DNA Landscapes Using REPCLASS, a Tool That Automates the Classification of Transposable Elements in Eukaryotic Genomes. Genome Biol. Evol. 1: 205–220.
6. Jurka J, Kapitonov VV, Pavlicek A, Klokovskii P, Kohany O, et al. (2005) Repbase update, a database of eukaryotic repetitive elements. Cytogenet. Genome Res. 110: 462–467.
7. Flutre T, Duprat E, Feuillet C, Quesneville H (2011) Considering Transposable Element Diversification in De Novo Annotation Approaches. PLoS ONE 6(1).
8. Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27(2): 573–580.
9. Eddy SR (2011) Accelerated Profile HMM Searches. PLoS Comput. Biol. 7(10).