



Use cases and improvements of REPET package : pipeline and tools to identify and annotate TEs in genomic sequences

Véronique Jamilloux, Olivier Inizan, Sandie Arnoux, Timothée Flutre, Claire Hoede, Nathalie Choisne, Hadi Quesneville

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Use cases and improvements of REPET package : pipelines and tools to identify and annotate TEs in genomic sequences

CNET Lyon 4th – 6th July 2011

V. Jamilloux



Introduction

- **TEs play a major role in structure, functions and evolution of genomes.**
- **This role is relative to their particular dynamics: burst and degeneration.**
- **Their nature and their mobile capability make them very particular biological elements**

Goals

- **To correctly annotate TEs, the tools have to take into account these specificities and the TE diversity**
- **With the acceleration of genome sequencing, the tools have to treat lot of data and to annotate automatically to help the biologist**

REPET package

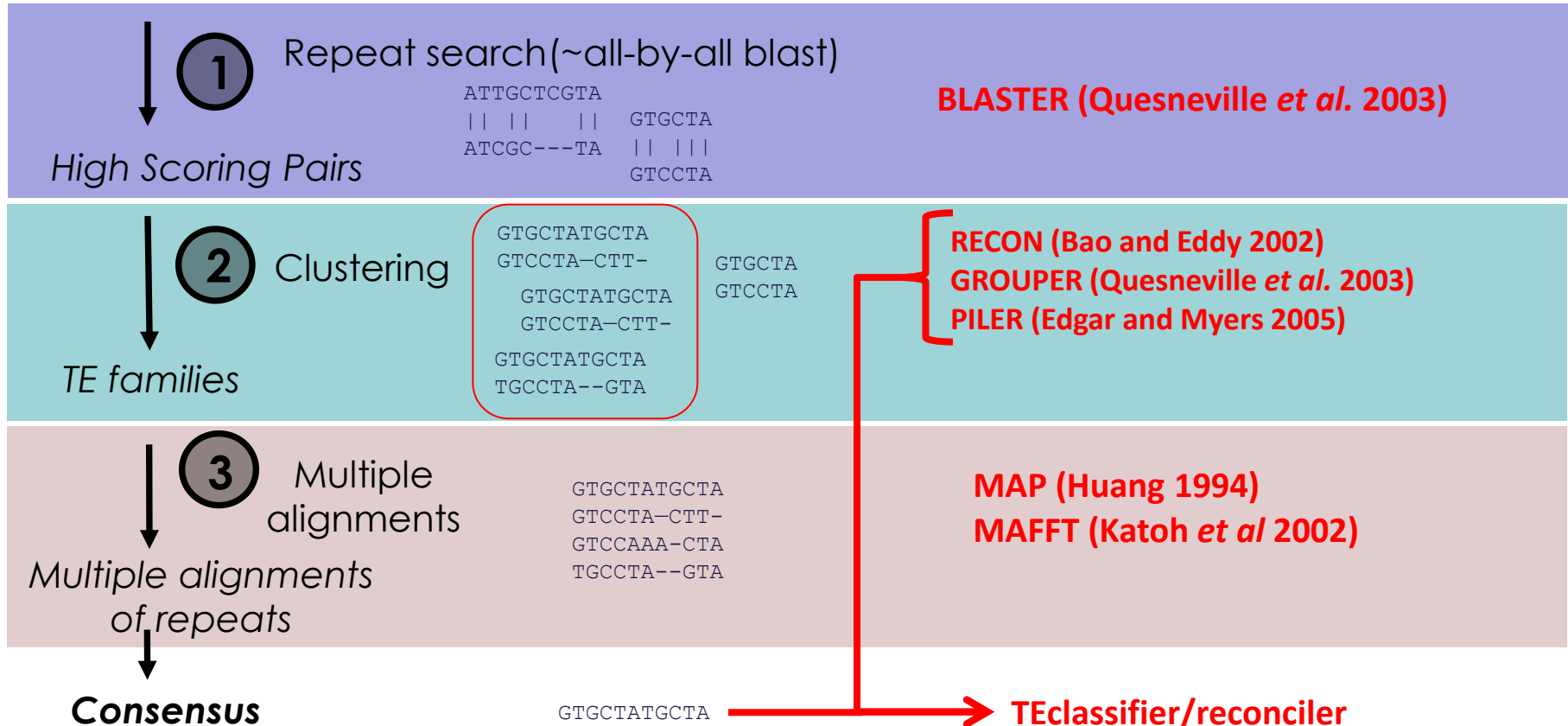
- Automatic detection and annotation of repeats in genomic sequences
- Version 1.4 available
<http://urgi.versailles.inra.fr/tools/REPET>
- 2 pipelines TEdenovo and TEannot
- Several tools to analyze the different results of the 2 pipelines
- Run on a compute cluster, SGE
- Results files and database (MySQL)
- Development and validation phases on benchmark with *D. melanogaster* (release 4) and *A. thaliana* (release 9)

TEdenovo

Pipeline TEdenovo
(Flutre *et al.* 2011)

Genomic sequence

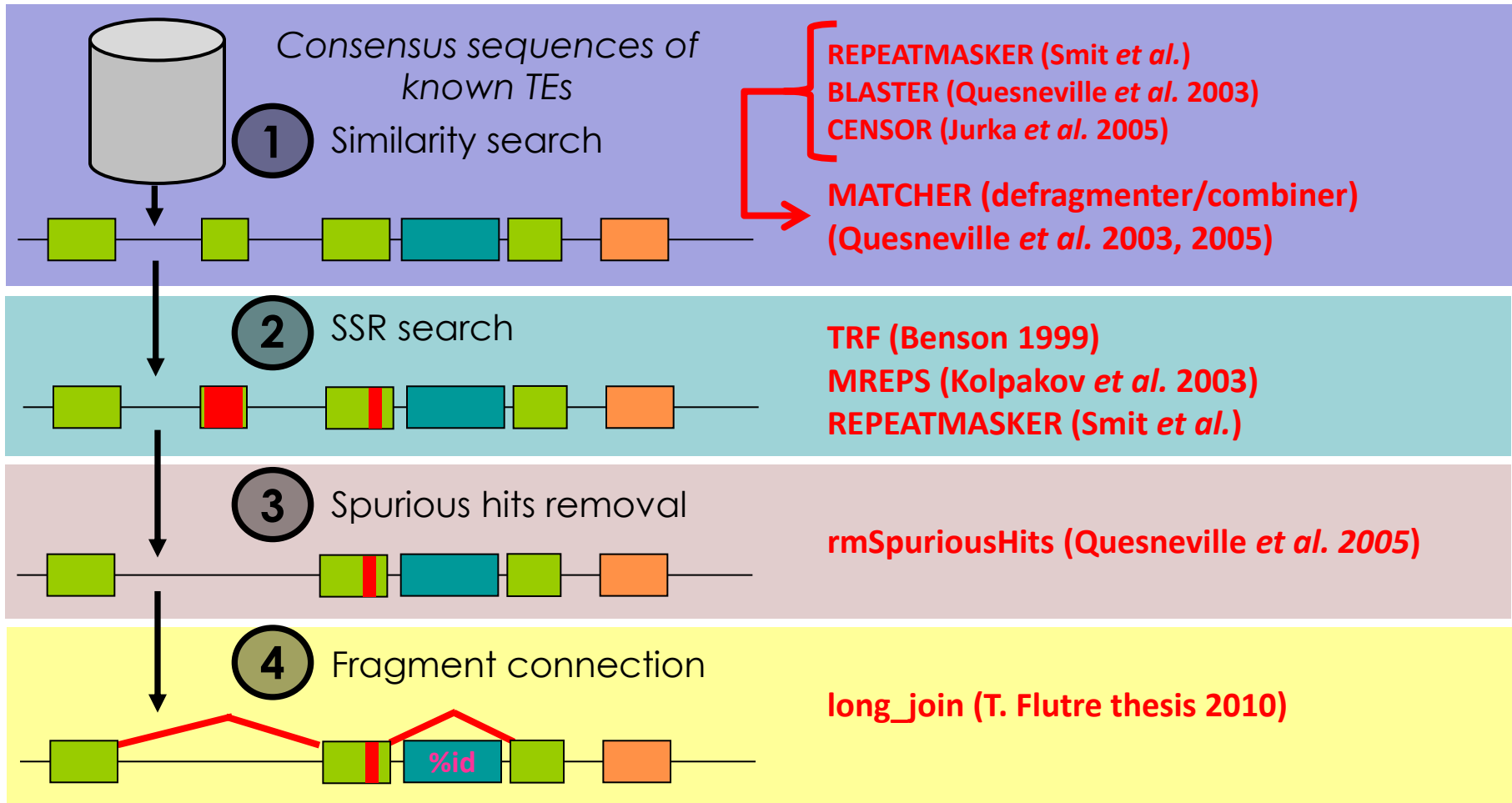
... .TATGTGCTATTACTATTACATTACCATGCGT.....



2 new steps included

- **Filter out SSR and not classified consensus (built from less than 10 sequences).**
- **Consensus clustering (with Blastclust) to investigate the similarity relationships among the consensus that have been built.**

TEannot



Tools

- **ClusterConsensus, 3 steps :**
 - ◆ Consensus clustering with Blastclust
 - ◆ Data organization : 1 directory by cluster (considered like a TE family), containing fasta files (consensus, “all-by-all” sequences)
 - ◆ For each cluster, building a multiple alignment and phylogeny (between consensus and “all-by-all” sequences or annotated copies)
- **GiveInfoTEannot**
 - ◆ After the TE annotation, this tool gives some statistics about genome annotation (coverage, copies, ...)

More than 35 annotated genomes

Espèces	Publications
D. melanogaster	<i>Quesneville et al. PLoS Comp. Biol</i> 2005 <i>Bergman et al. Genome Biology</i> 2006 Browsable at: http://flybase.org
12 Drosophila genomes	<i>Clark AG et al. Nature.</i> 2007
A. thaliana	<i>Buisine et al. Genomics.</i> 2008 Browsable at: http://www.arabidopsis.org
Fusarium graminearum	<i>Cuomo et al. Science.</i> 2007
Rice	
Laccaria bicolor	<i>Martin et al. Nature.</i> 2008
Meloidogyne incognita	<i>Abad et al. Nature Biotech</i> 2008
Acyrtosiphon pisum	<i>IAGC PLoS Biology</i> 2010
Aedes aegypti	<i>Nene et al. Science.</i> 2007
Spodoptera frugiperda, Helicoverpa armigera	<i>Alençon et al. PNAS</i> 2010 Browsable at: http://genouest.org/
Tuber melanosporum	<i>Martin et al. Science</i> 2010
Ectocarpus siliculosus	<i>Cock et al. Nature</i> 2010.
Leptosphaeria maculans	<i>Rouxel et al Nature Comm</i> 2011
Blumeria graminis	<i>Spanu et al Science</i> 2010
Melampsora larici-populina and Puccinia graminis	<i>Duplessis et al. (submitted)</i>

Several use cases



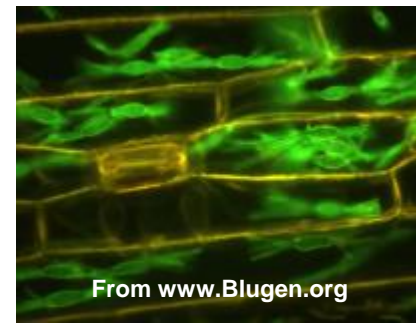
- *Vitis vinifera* genome 12X
- *Blumeria graminis* v3
- *Aphid Acyrthosiphon*
- *Ectocarpus*
- *Nicotiana tabacum*
- *Ixodes scapularis*
- *Brassica rapa*

Vitis vinifera



- **Analysis aim** : Study the TE distribution and their evolution.
For REPET, improvement for larger genomes
- **The genome** : 486 Mbp, whole genome 33 built chromosomes, 12X
- **REPET package v1.3.12.1** , adapt settings for sensitivity and parallel computing.
- **Results** :
 - ♦ TEdenovo : a library of 6315 TE consensus (11621 consensus before SSR and noCat filtering)
 - ♦ Classification of the 6315 TE consensus :
Class I => 69,90% ; Class II => 15,15% ; Confused/Unknown => 14,95%
 - ♦ TEannot : 375737 copies (8425 full length copies) using 6315 TE consensus
=> 65,07% of the genome

Blumeria graminis



- **Analysis aim** : to annotate the genome and to learn about the TE impact on genome dynamics and evolution.
- **The genome** : 130 Mbp, whole genome sequences 8X
- **REPET package v1.4** with a new setting on Grouper (TEdenovo step 2). This specific setting improves clustering in order to avoid false positive chimeric consensus.
- **Results** :
 - ♦ TEdenovo : a library of 2226 TE consensus
 - ♦ Classification of the 2215 TE consensus :
Class I => 69,03% ; Class II => 1,40% ; Confused/Unknown => 29,57%
 - ♦ TEannot : 165505 copies using 2215 TE consensus => 75,87% of the genome
 - ♦ Genome coverage according to classification :
Class I => 60,33% ; Class II => 1,18% ; Confused/Unknown => 14,36%

See J. Amselem's talk : "Fungal genome analysis of TEs".

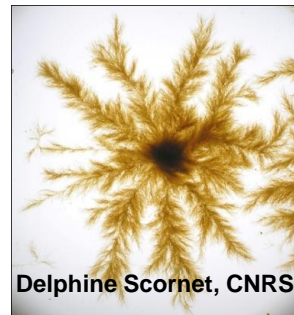
Aphid acyrtosiphon



- **Analysis aim** : This genome is holocentric, make the TEs annotation and see their distribution.
- **The genome** : 464 Mbp, (1130 scaffolds)
- **REPET package V1 with default settings**
- **Results** :
 - ♦ TEdenovo : a library of 1883 TE consensus (with manual curation)
 - ♦ Classification of the 1883 TE consensus :
Class I => 19,60% ; Class II => 16,46% ; unclassified => 64,58%
 - ♦ TEannot : 506309 copies using these 1883 consensus => 37,86% of the genome.
 - ♦ Genome coverage according to classification :
Class I => 5,43% ; Class II => 5,31% ; unclassified => 27,12%;

**See : “Genome sequence of the Pea Aphid *Acyrthosiphon pisum*”.
Plos biology, Feb 2010**

Ectocarpus siliculosus



- **Analysis aim** : identify the TE complement to evaluate their contribution to the genome size and to analyze the phylogenetic relationship with LTR retrotransposons from other micro algae.
- **The genome** : 195 Mbp, 1561 scaffolds (N50 = 504,468)
- **REPET package V1**
- **Results** :
 - ♦ TEdenovo : a library of 770 TE consensus (with manual curation)
 - ♦ Classification of the 770 TE consensus :
 Class I => 9,35% ; Class II => 8,18% ; Tandem repeat => 2,99% ; unclassified => 79,48%
 - ♦ TEannot : 95004 copies using these 770 consensus => 22.4% of the genome.
 - ♦ Genome coverage according to classification :
 Class I => 9,4% ; Class II => 2,7% ; Tandem repeat => 0,4% ; unclassified => 9,9%

See F. Maumus's talk : "Transposable elements in marine stramenopiles".

Nicotiana tabacum



- **Analysis aim** : Identification of new retrotransposons families and estimation of their copy numbers
- **Initial genome data** : 382 Mbp (300158 seq from SGN assembly)
- **Part of genome analyzed** : 72,2Mbp (~1,6% of estimated genome size); (16892 contigs > 3 kpb) , N50 => 4116;
- **REPET package** : **TEdenovo v1.3.12.1** and **TEannot v1.4** with default settings.
- **Results** :
 - ◆ TEdenovo : a library of 1022 TE consensus
 - ◆ Classification of the 1022 TE consensus :
Class I => 3,52% ; Class II => 1,17% ; SSR => 0,19%; unclassified => 95,1%
 - ◆ TEannot : 60504 copies using 1022 TE consensus => 36,30% of the genome.

Ixodes scapularis



- **Analysis aim** : TEs like characterization tool for polymorphism, MITEs because they are near the genes
- **Initial genome data** : 1,39 Gbp (570 637 contigs); genome estimated size 2,1 Gbp
- **Part of genome analyzed** : 694,2 Mbp; (88 851 contigs)
- **REPET package V 1.3.9** with setting MITE_max_length: 800 for TEclassifier.
- **Results** :
 - ♦ TEdenovo : a library of 3802 TE consensus
 - ♦ Classification of the 3802 TE consensus :
Class I => 28,09% ; Class II => 18,62% (MITEs => 5,6%) ; unclassified => 53,29%
 - ♦ TEannot : Only on contigs > 10 Kbp, 24035 copies using 1789 TE consensus (LTR, LINE, TIR, MITE => 9%) => 10,26% of the analyzed genome (2,05% of estimated genome).

Master 2 report “Recherche *in silico* des éléments transposables dans le génome de la tique *ixodes scapularis*” S. Aubernon

Brassica rapa



- **Analysis aim** : To predict Brassica TEs and then study their activation during the polyploidization in *brassica napus*.
- **Initial genome data** : 529 Mpb
- **Part of genome** : 184,4Mbp; (1377 BACs) , N50 => 130740; (34,82% of the genome)
- **REPET package** : **V1.3.12.1** with default settings.
- **Results** :
 - ◆ TEdenovo : a library of 4123 TE consensus after redundancy elimination
 - ◆ Classification of the 4123 TE consensus :
 Class I => 15,45% ; Class II => 9,99% ; SSR => 0,41%; unclassified => 74,14%

Note : V. Sarilar's thesis "Activation des éléments transposables suite à la polyploïdisation du colza *Brassica napus*"

Few useful tips

- **Analyze your data**
 - ◆ Sort your sequences according to their size and filter out short ones
- **Specify the biological question**
 - ◆ Help for parameters settings
- **Contact: urg-repet@versailles.inra.fr**
 - ◆ Support
 - ◆ Collaborations within projects

Perspectives

- **TEdenovo**

- ◆ Add a new dedicated structural detection tool : LTRharvest
- ◆ Improve combined approach. See poster P8 «Strategies for TEs detection among genomes » O. Inizan
- ◆ Replace the classifier with PASTEC. See Poster P9 “PASTEC” S. Arnoux poster

- **ClusterConsensus:**

- ◆ Improve the analysis of structural variants.

- **GiveInfoTEannot**

- ◆ Improve statistics (e.g. by cluster of consensus)

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Thank you for your attention