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

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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

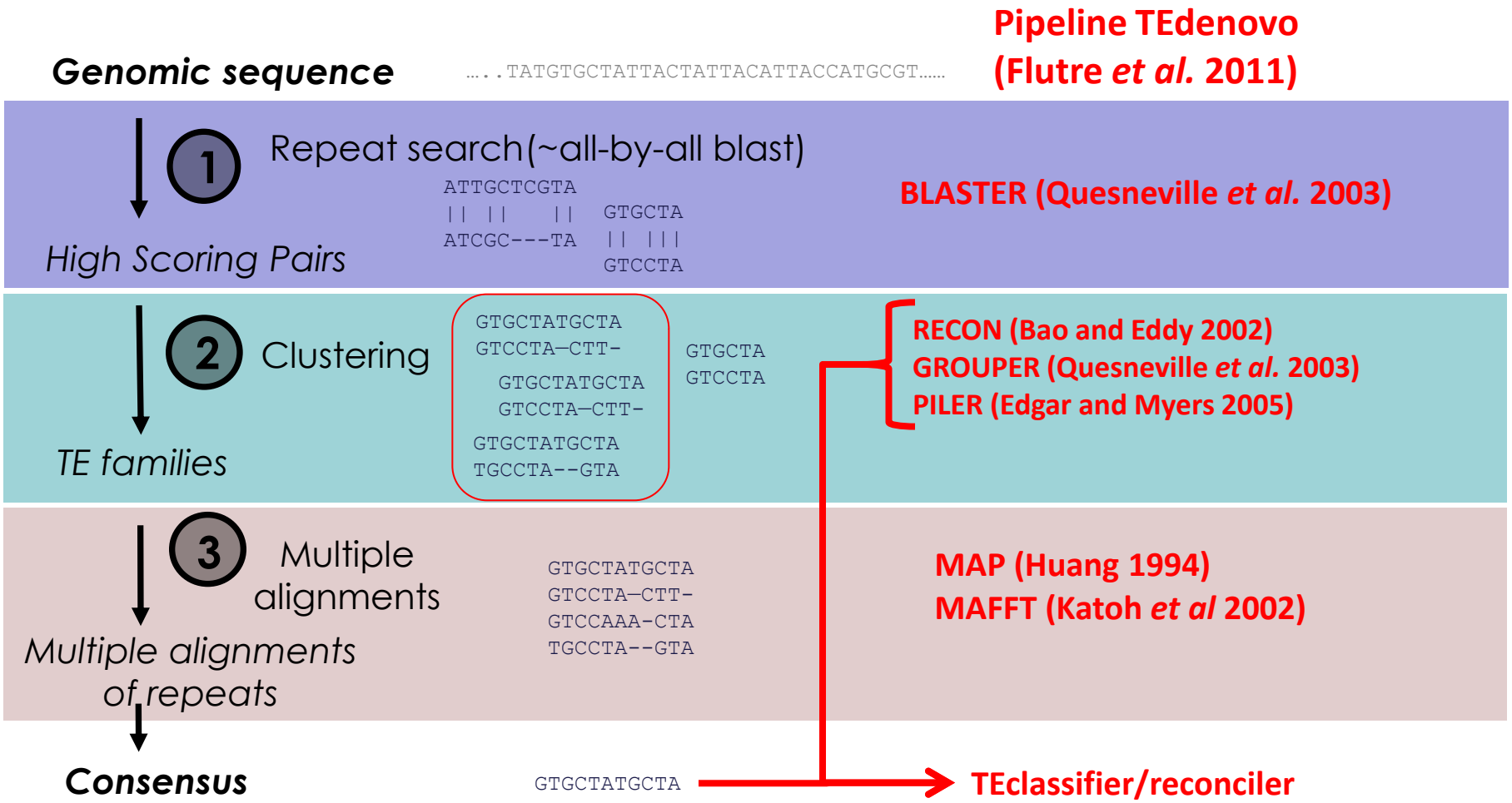
- **To correctly annotate TEs, the tools have to take into account these specificities and the TE diversity**

- **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)**

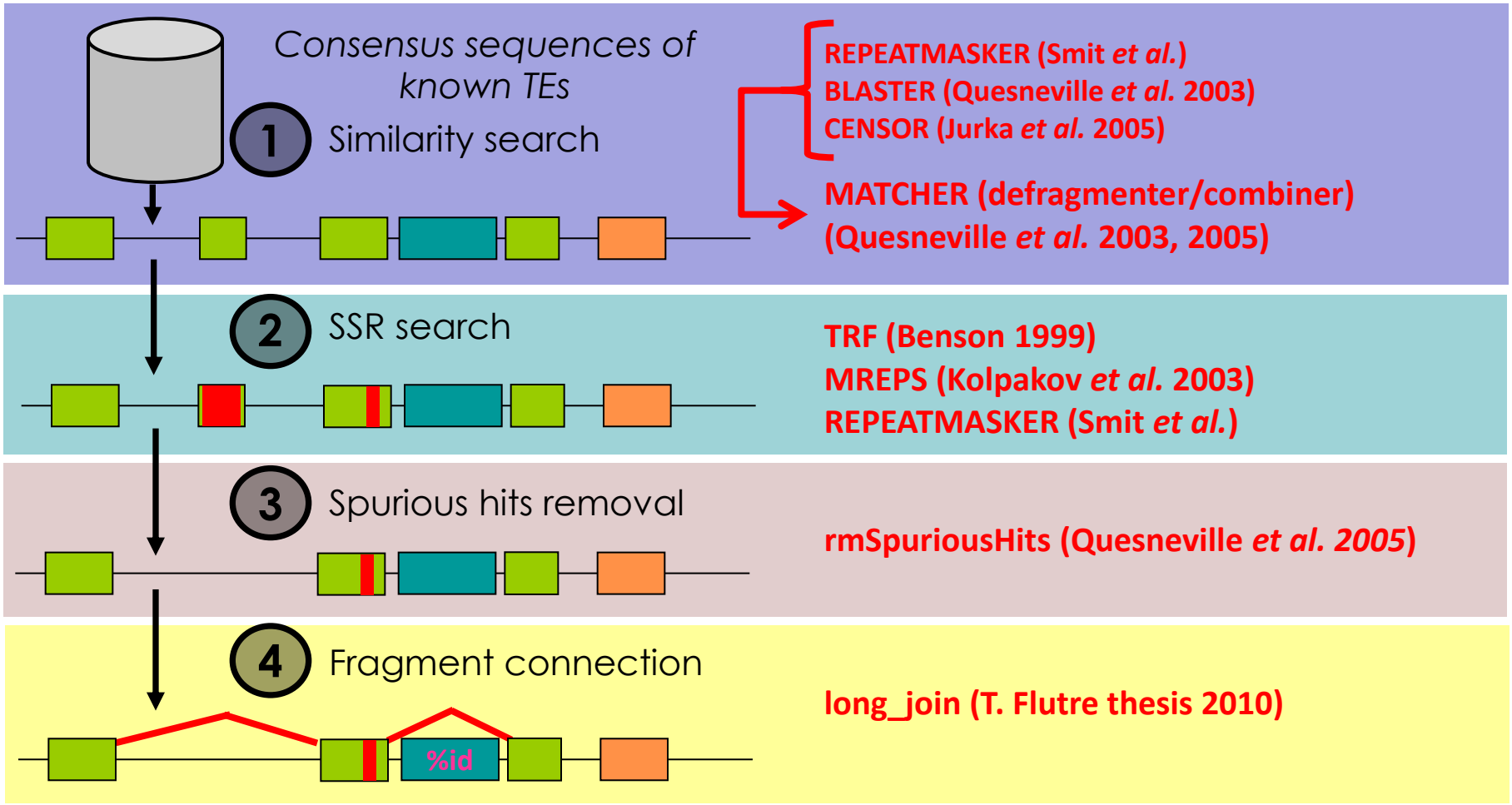
TEdenovo


Pipeline TEdenovo
(Flutre et al. 2011)
Genomic sequence
…..TATGTGCTATTACTATTACATTACCATGCGT……
1
Repeat search(~all-by-all blast)
ATTGCTCGTA
|| || || GTGCTA
ATCGC---TA || |||
GTCCTA
BLASTER (Quesneville et al. 2003)
High Scoring Pairs
2
Clustering
GTGCTATGCTA
GTCCTA—CTT-
GTGCTATGCTA
GTCCTA—CTT-
GTGCTATGCTA
TGCCTA--GTA
GTGCTA
GTCCTA
RECON (Bao and Eddy 2002)
GROUPER (Quesneville et al. 2003)
PILER (Edgar and Myers 2005)
TE families
3
Multiple alignments
GTGCTATGCTA
GTCCTA—CTT-
GTCCAAA-CTA
TGCCTA--GTA
MAP (Huang 1994)
MAFFT (Katoh et al 2002)
Multiple alignments of repeats
Consensus
GTGCTATGCTA
TEclassifier/reconciler

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


Consensus sequences of known TEs
1
Similarity search
REPEATMASKER (Smit et al.)
BLASTER (Quesneville et al. 2003)
CENSOR (Jurka et al. 2005)
MATCHER (defragmenter/combiner) (Quesneville et al. 2003, 2005)
2
SSR search
TRF (Benson 1999)
MREPS (Kolpakov et al. 2003)
REPEATMASKER (Smit et al.)
3
Spurious hits removal
rmSpuriousHits (Quesneville et al. 2005)
4
Fragment connection
%id
long_join (T. Flutre thesis 2010)

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

Several use cases

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

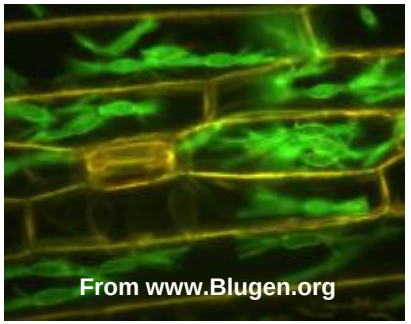
Vitis vinifera

- **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

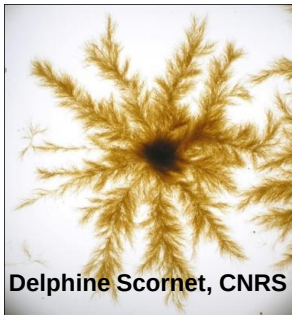
- **Analysis aim :** This genome is holocentric, make the TEs annotation and see their distribution.
- **The genome :** 464 Mbp, (1130 scaffolds)
- **REPET package V1 with default settings**
- **Results** :
  - TEdenovo : a library of 1883 TE consensus (with manual curation)
  - Classification of the 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


Delphine Scornet, CNRS

- **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 851contigs)
- **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: urgi-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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  - M. Deloger *(Nicotiana)*, V. Sarilar *(Brassica)*, S. Aubernon *(I. scapularis)*

- **All URGI team members**

Thank you for your attention