# Welcome !

# Program at a glance

***Tuesday (May 24)***

**Introduction and update on RepeatExplorer**

- Principles and history of RepeatExplorer *(J. Macas)*
- Diagnostic features of repetitive elements – Part I *(P. Neumann)*

*Coffee break*

- Diagnostic features of repetitive elements – Part II *(P. Neumann)*
- RepeatExplorer tools for genome annotation *(P. Novák)*

*Lunch*

**Practical training I**

Basic Protocols 1-4: troubleshooting & advanced data analysis

**Dinner at CITYgastro restaurant**

***Wednesday (May 25)***

**Short talks**

- Nicola Schmidt
- Ludwig Mann
- Zirlane Portugal da Costa

*Coffee break*

- Pol Fernández Mató
- Yennifer Mata-Sucre
- Nusrat Sultana

*Lunch*

**Practical training II**

- using RE output for annotating genome assemblies
- REXdb and DANTE
- structure-based annotation of complete LTR-retrotransposons

***Thursday (May 26)***

**Short talks**

- Matej Lexa
- Monika Čechová
- Sophie Maiwald
- Vratislav Peška

*Coffee break*

- Camila do Nascimento Moreira
- Veit Herklotz
- Alice Krumpolcová

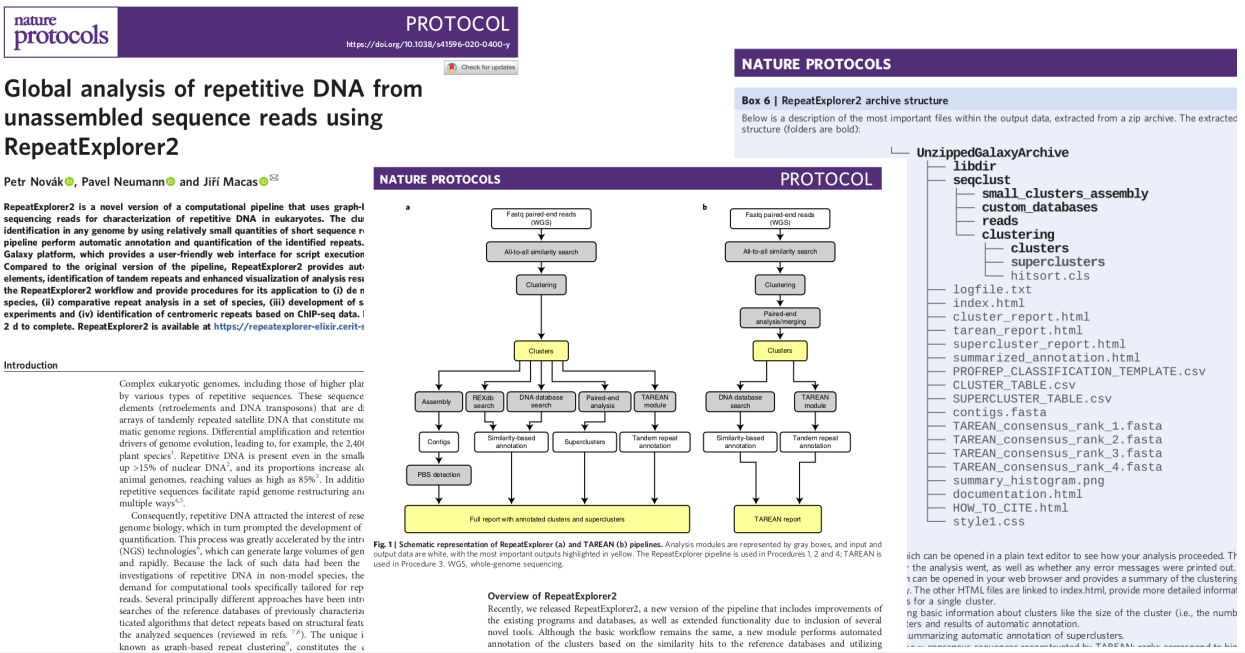
*Lunch*

**Practical training III**

- reconstructing phylogenetic relationships
- local installation and running RE tools from a command line
- topics proposed by the participants & individual consultations

# Protocols and tutorials

- RepeatExplorer principles and example protocols published
- Corresponding video tutorials available from YouTube



nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-020-0400-y

Check for updates

## Global analysis of repetitive DNA from unassembled sequence reads using RepeatExplorer2

Petr Novák, Pavel Neumann and Jiří Macas

**RepeatExplorer2 is a novel version of a computational pipeline that uses graph-l sequencing reads for characterization of repetitive DNA in eukaryotes. The clu identification in any genome by using relatively small quantities of short sequence r pipeline perform automatic annotation and quantification of the identified repeats. Galaxy platform, which provides a user-friendly web interface for script execution Compared to the original version of the pipeline, RepeatExplorer2 provides aut elements, identification of tandem repeats and enhanced visualization of analysis resu the RepeatExplorer2 workflow and provide procedures for its application to (i) de n species, (ii) comparative repeat analysis in a set of species, (iii) development of s experiments and (iv) identification of centromeric repeats based on ChIP-seq data. 2 d to complete. RepeatExplorer2 is available at https://repeatexplorer-elixir.cerit-s**

Introduction

Complex eukaryotic genomes, including those of higher plar by various types of repetitive sequences. These sequence elements (retroelements and DNA transposons) that are d arrays of tandemly repeated satellite DNA that constitute ma matic genome regions. Differential amplification and retentio drivers of genome evolution, leading to, for example, the 2,40 plant species[1]. Repetitive DNA is present even in the small up >15% of nuclear DNA[3], and its proportions increase ale animal genomes, reaching values as high as 85%[2]. In additio repetitive sequences facilitate rapid genome restructuring an multiple ways[4,5].

Consequently, repetitive DNA attracted the interest of rese genome biology, which in turn prompted the development of quantification. This process was greatly accelerated by the intro (NGS) technologies[6], which can generate large volumes of gen and rapidly. Because the lack of such data had been the investigations of repetitive DNA in non-model species, the demand for computational tools specifically tailored for rep reads. Several principally different approaches have been intr searches of the reference databases of previously characteriz ticated algorithms that detect repeats based on structural featu the analyzed sequences (reviewed in refs. [7,8]). The unique known as graph-based repeat clustering[9], constitutes the

NATURE PROTOCOLS PROTOCOL



**Fig. 1 | Schematic representation of RepeatExplorer (a) and TAREAN (b) pipelines.** Analysis modules are represented by gray boxes, and input and output data are white, with the most important outputs highlighted in yellow. The RepeatExplorer pipeline is used in Procedures 1, 2 and 4; TAREAN is used in Procedure 3. WGS, whole-genome sequencing.

Overview of RepeatExplorer2

Recently, we released RepeatExplorer2, a new version of the pipeline that includes improvements of the existing programs and databases, as well as extended functionality due to inclusion of several novel tools. Although the basic workflow remains the same, a new module performs automated annotation of the clusters based on the similarity hits to the reference databases and utilizing

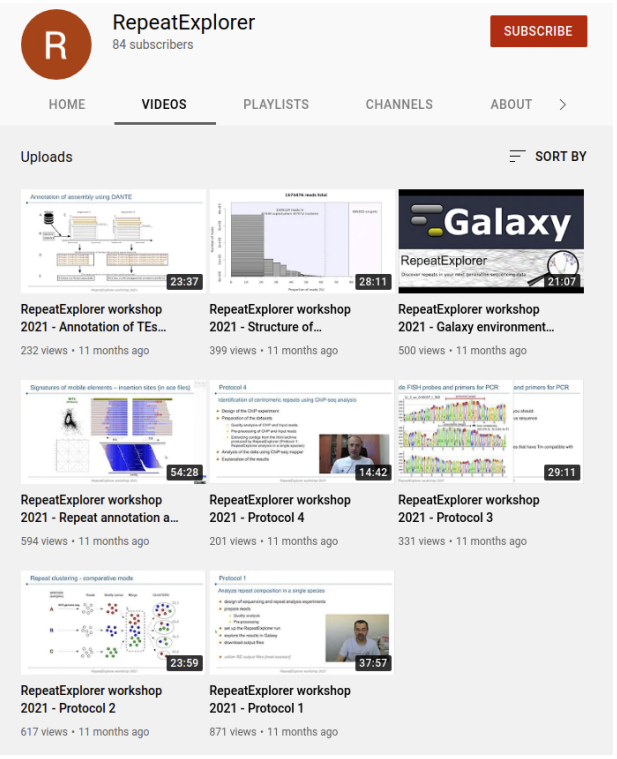
NATURE PROTOCOLS

**Box 6 | RepeatExplorer2 archive structure**

Below is a description of the most important files within the output data, extracted from a zip archive. The extracted structure (folders are bold):

```
└── UnzippedGalaxyArchive
    ├── libdir
    ├── seqclust
    │   ├── small_clusters_assembly
    │   ├── custom_databases
    │   ├── reads
    │   └── clustering
    │       ├── clusters
    │       ├── superclusters
    │       └── hitsort.cls
    ├── logfile.txt
    ├── index.html
    ├── cluster_report.html
    ├── tarean_report.html
    ├── supercluster_report.html
    ├── summarized_annotation.html
    ├── PROFREP_CLASSIFICATION_TEMPLATE.csv
    ├── CLUSTER_TABLE.csv
    ├── SUPERCLUSTER_TABLE.csv
    ├── contigs.fasta
    ├── TAREAN_consensus_rank_1.fasta
    ├── TAREAN_consensus_rank_2.fasta
    ├── TAREAN_consensus_rank_3.fasta
    ├── TAREAN_consensus_rank_4.fasta
    ├── summary_histogram.png
    ├── documentation.html
    ├── HOW_TO_CITE.html
    └── style1.css
```

ich can be opened in a plain text editor to see how your analysis proceeded. Th the analysis went, as well as whether any error messages were printed out. can be opened in your web browser and provides a summary of the clustering The other HTML files are linked to index.html, provide more detailed informa s for a single cluster.
ng basic information about clusters like the size of the cluster (i.e., the numb ters and results of automatic annotation.
ummarizing automatic annotation of superclusters.



RepeatExplorer
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# Principles and history of RepeatExplorer

# Principles and history of RepeatExplorer

**2007**

First paper on repeat clustering from NGS data

**BMC Genomics**

BioMed Central

Research article

Open Access

**Repetitive DNA in the pea (*Pisum sativum* L.) genome: comprehensive characterization using 454 sequencing and comparison to soybean and *Medicago truncatula***

Jiří Macas*, Pavel Neumann and Alice Navrátilová

Address: Biology Centre ASCR, Institute of Plant Molecular Biology, Branišovská 31, České BudEjovice, CZ-37005, Czech Republic

Email: Jiří Macas* - macas@umbr.cas.cz; Pavel Neumann - neumann@umbr.cas.cz; Alice Navrátilová - navratil@umbr.cas.cz

* Corresponding author

Published: 21 November 2007

*BMC Genomics* 2007, **8**:427 doi:10.1186/1471-2164-8-427

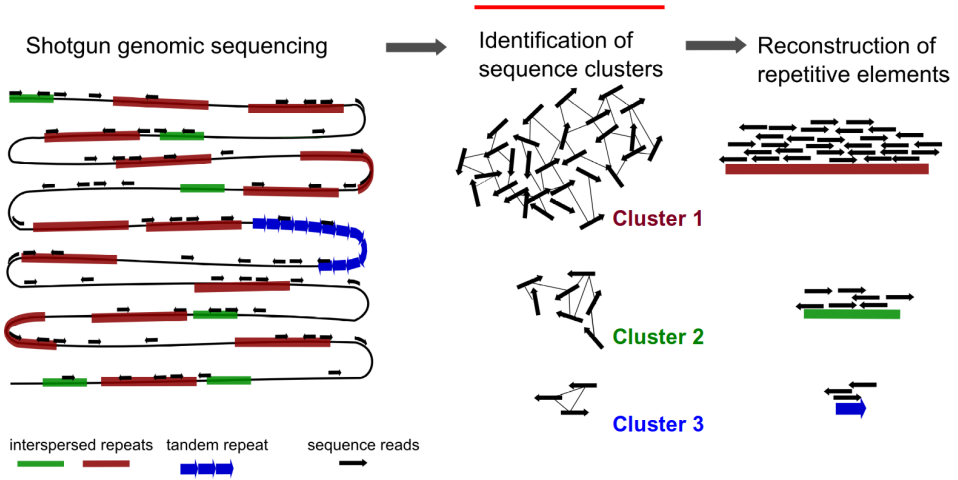
Received: 13 August 2007

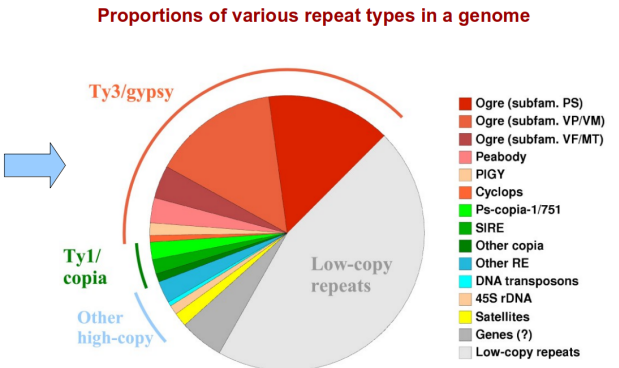
Accepted: 21 November 2007

# Principles and history of RepeatExplorer

**2007**

First paper on repeat clustering from NGS data




CLUSTER = a set of frequently overlapping reads = REPEAT FAMILY

# Principles and history of RepeatExplorer

**2007**

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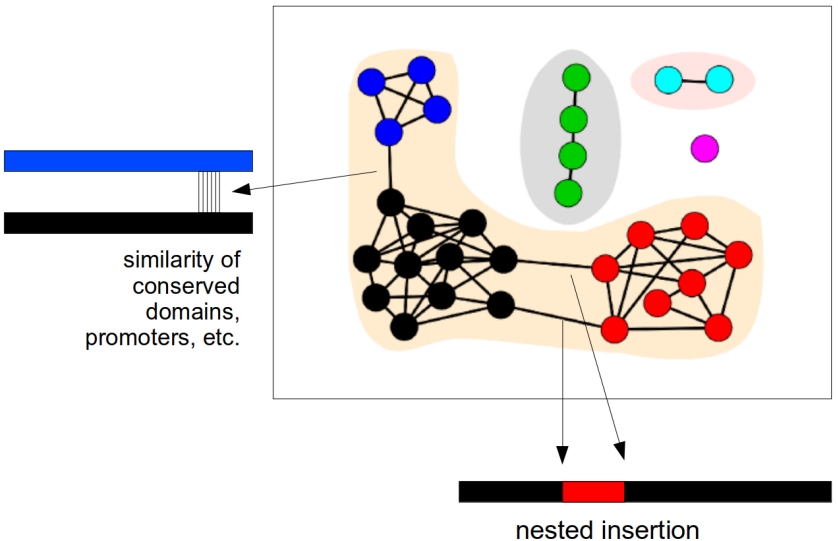



**Chimeric clusters !**

Single linkage clustering => *connected components*

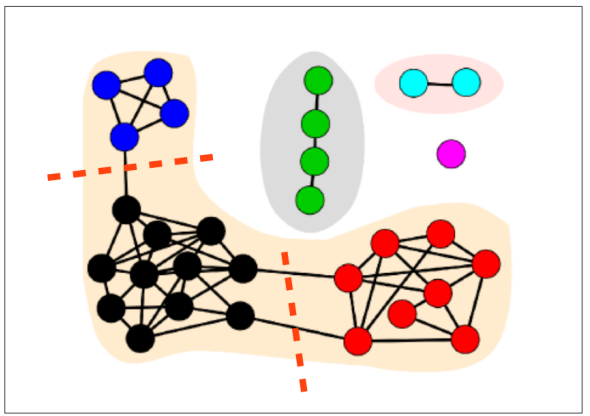
**TGICL**
**(TIGR Gene Indices clustering tool)**
*Pertea et al., 2003*

# Principles and history of RepeatExplorer

**2007** … **2010**

First paper on repeat clustering from NGS data

Introduction of **graph-based clustering** (Novak et al. 2010)



**Graph-based clustering**

- Sequence overlaps between the reads are transformed to a graph where the reads are represented as nodes and their similarities as edges connecting the nodes
- Graph structure is examined to detect ***communities of frequently connected nodes*** which **are split to separate clusters**

# Principles and history of RepeatExplorer

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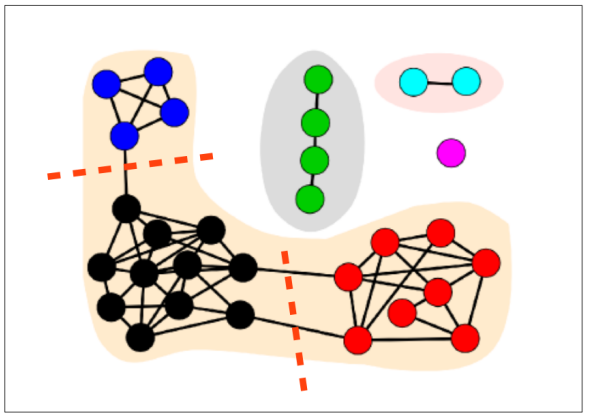

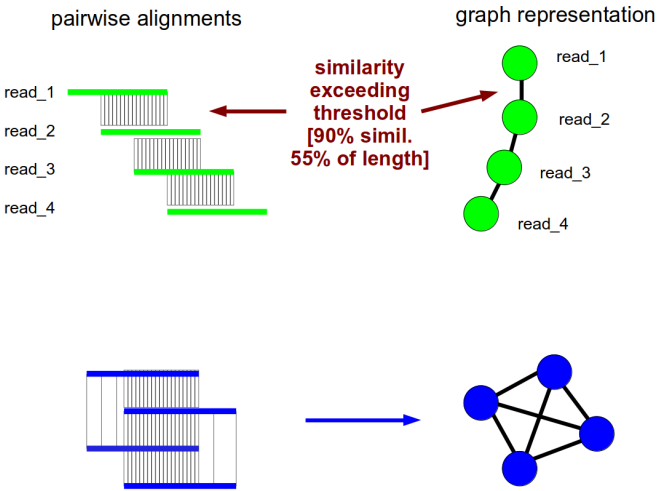


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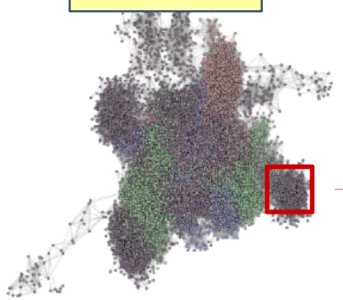
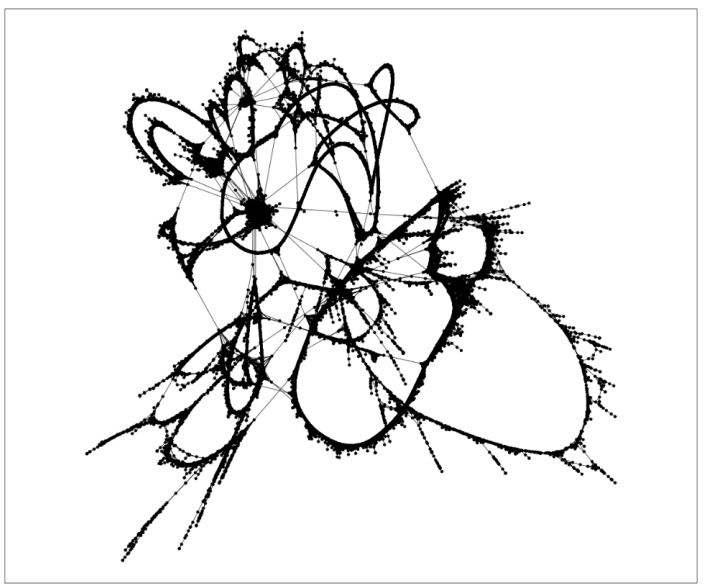
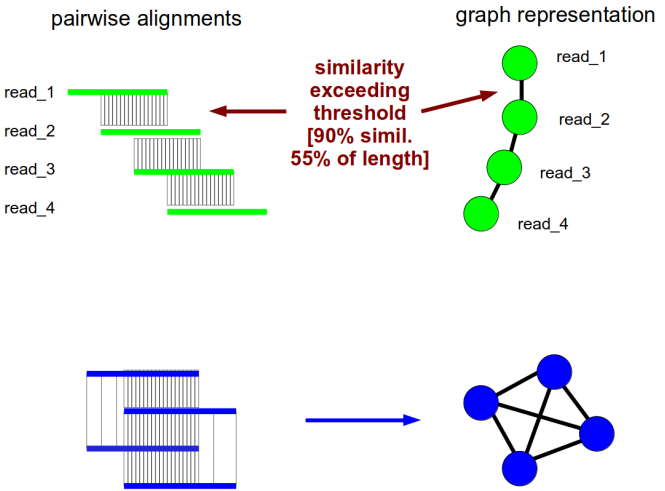


**Virtual graphs** used to analyze real data contain **up to millions of nodes** (reads)





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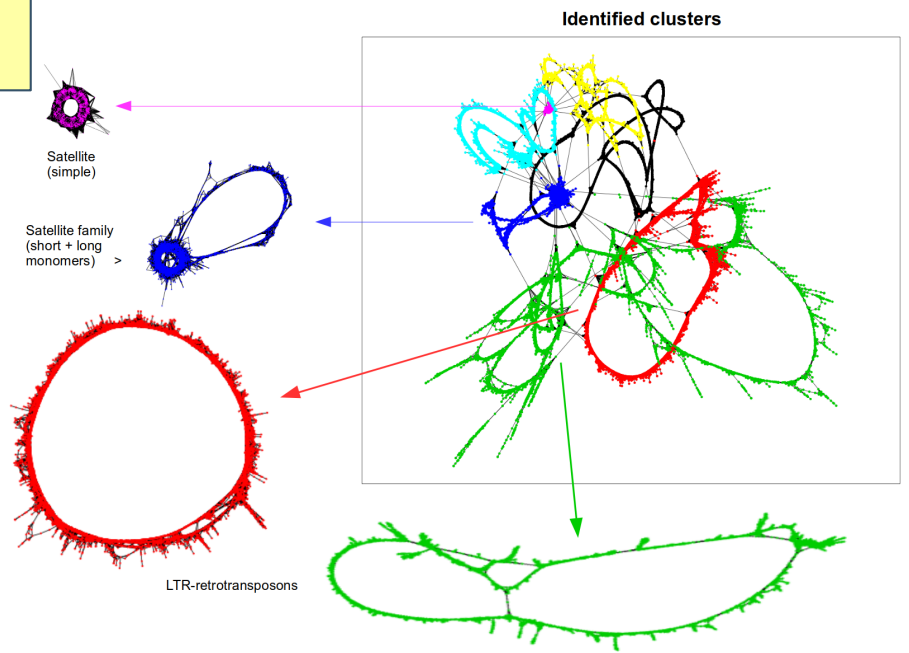


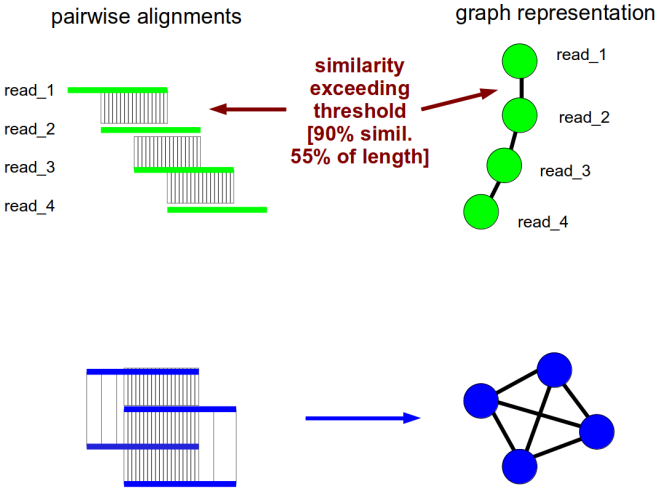

# Principles and history of RepeatExplorer

**2007 … 2010**

First paper on repeat clustering from NGS data

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***command-line version***

**FIRST WORKSHOP !**

# Principles and history of RepeatExplorer

**2007** … **2010** … **2013**

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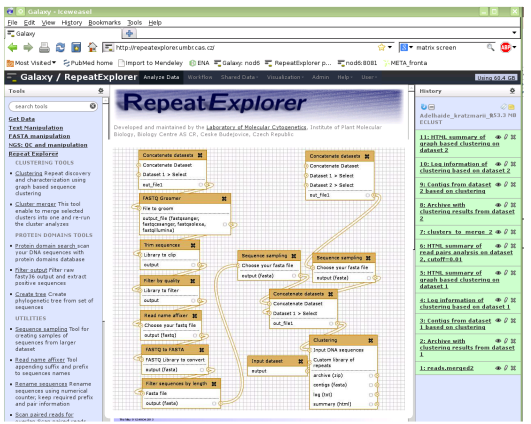
***command-line version***

**Repeat Explorer in Galaxy** (Novak et al. 2013)

***Public web-based server***

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**ELIXIR $$$**

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**2007** … **2010** … **2013** **2014** … **2016** **2017**

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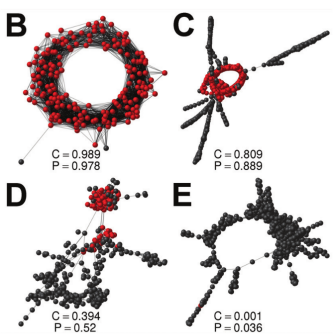
**TAREAN**

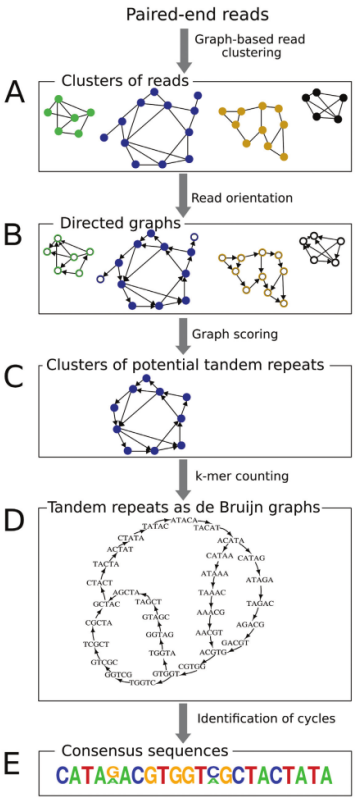



*Nucleic Acids Research, 2017* **1**
*doi: 10.1093/nar/gkx257*

**TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads**

**Petr Novák, Laura Ávila Robledillo, Andrea Koblížková, Iva Vrbová, Pavel Neumann and Jiří Macas***

Institute of Plant Molecular Biology, Biology Centre CAS, České Budějovice CZ-37005, Czech Republic



# Principles and history of RepeatExplorer

**2007** … **2010** … **2013** **2014** … **2016** **2017** **2018**

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**Repeat Explorer in Galaxy** (Novak et al. 2013)

***Public web-based server***

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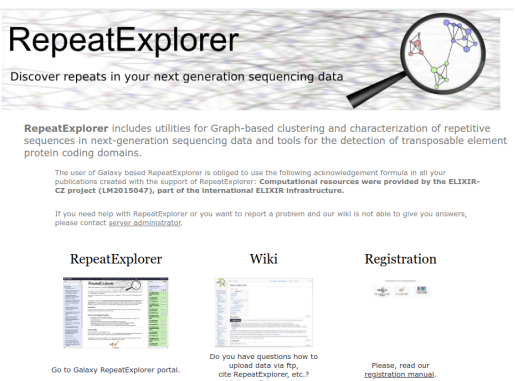
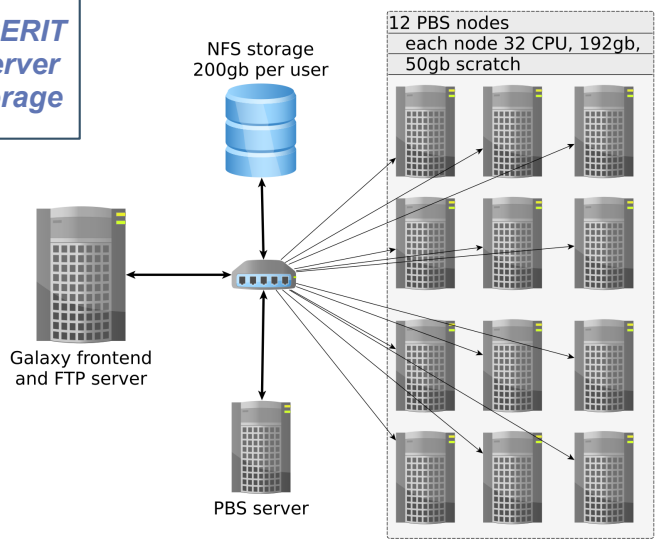
**ELIXIR $$$**

**TAREAN**

***ELIXIR / CERIT Galaxy server + data storage***



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***Public web-based server***

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**ELIXIR $$$**

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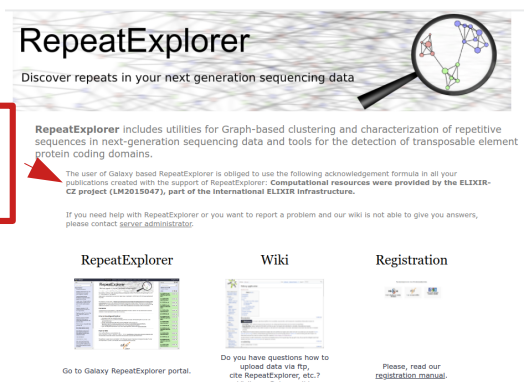


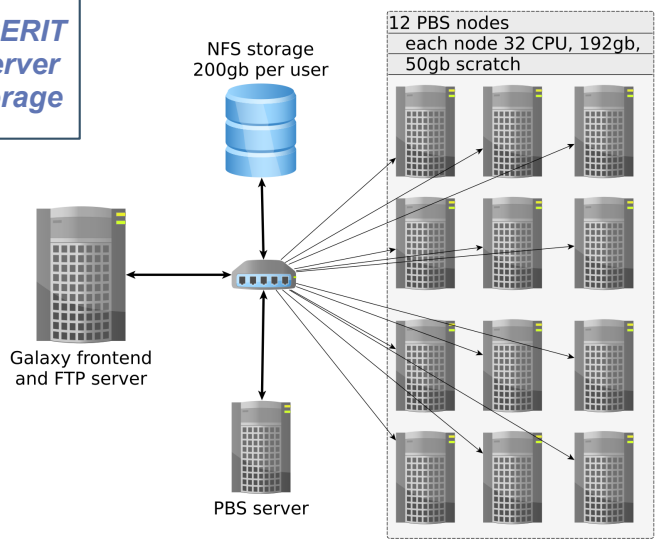


The user of Galaxy based RepeatExplorer is obliged to use the following acknowledgement formula in all your publications created with the support of RepeatExplorer: **Computational resources were provided by the ELIXIR-CZ project (LM2015047), part of the international ELIXIR infrastructure.**

Please, acknowledge ELIXIR in your publications !

# Principles and history of RepeatExplorer

**2007** … **2010** … **2013** **2014** … **2016** **2017** **2018** **2019**

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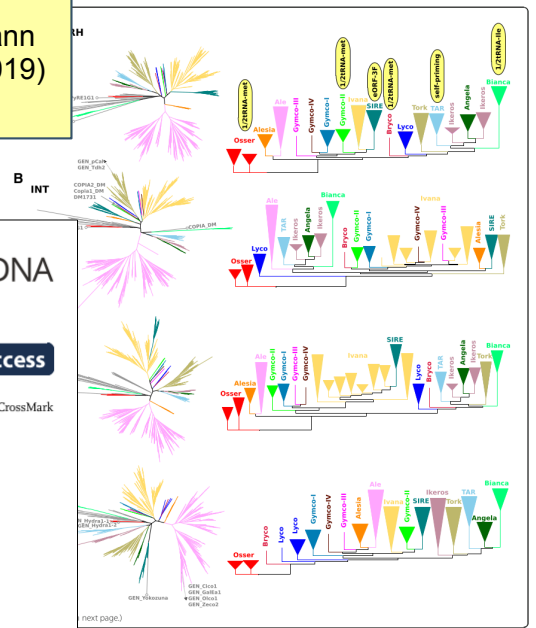
**ELIXIR $$$**

**TAREAN**

*ELIXIR / CERIT Galaxy server + data storage*

**REXdb** database

(Neumann et al. 2019)



Neumann *et al. Mobile DNA* (2019) 10:1
https://doi.org/10.1186/s13100-018-0144-1

Mobile DNA

RESEARCH Open Access

CrossMark

Systematic survey of plant LTR-retrotransposons elucidates phylogenetic relationships of their polyprotein domains and provides a reference for element classification

Pavel Neumann*, Petr Novák, Nina Hoštáková and Jiří Macas

# Principles and history of RepeatExplorer

**2007** … **2010** … **2013** **2014** … **2016** **2017** **2018** **2019** **2020**

First paper on repeat clustering from NGS data

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**Repeat Explorer in Galaxy** (Novak et al. 2013)

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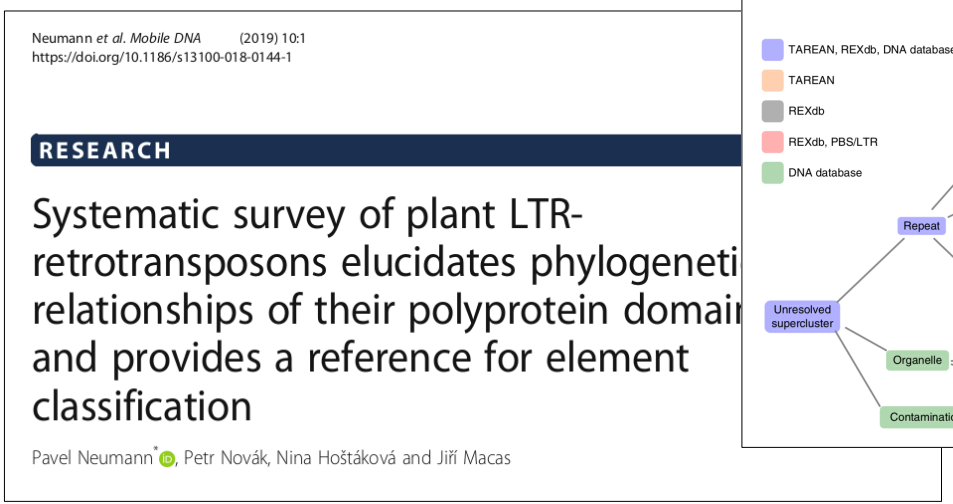
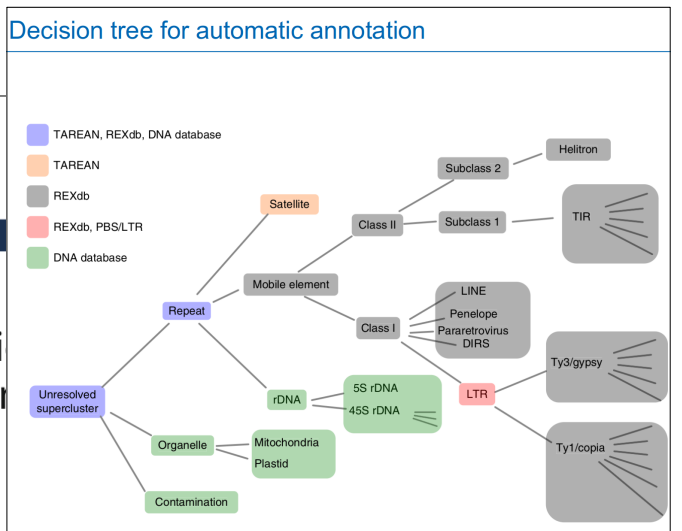
**TAREAN**

**REXdb** (Neumann et al. 2019)

**Repeat Explorer ver. 2**



# Principles and history of RepeatExplorer

2007 … 2010 … 2013 2014 … 2016 **2017** **2018** **2019** **2020**

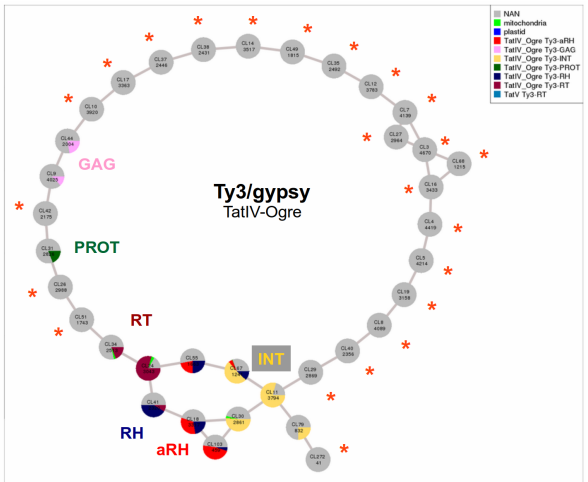
**TAREAN**

**REXdb**

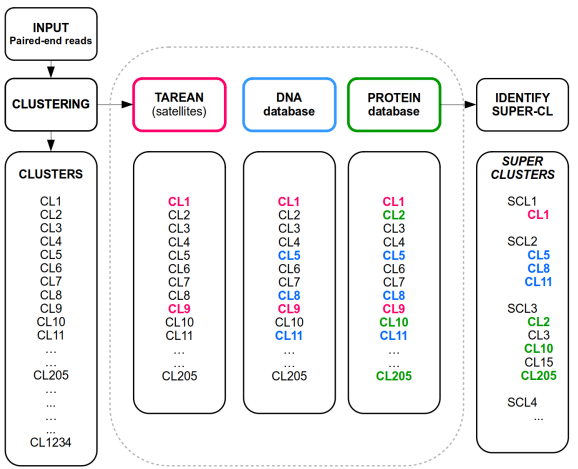
(Neumann et al. 2019)

**Repeat Explorer ver. 2**

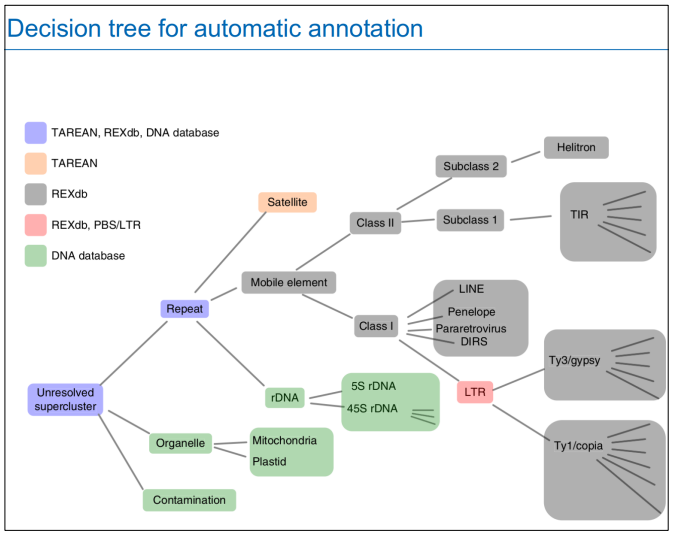
## Superclusters provide more complete annotation




## RepeatExplorer 2 – automatic detection of superclusters




## Decision tree for automatic annotation



# Principles and history of RepeatExplorer

2007 … 2010 … 2013 2014 … 2016 **2017** **2018** **2019** **2020**

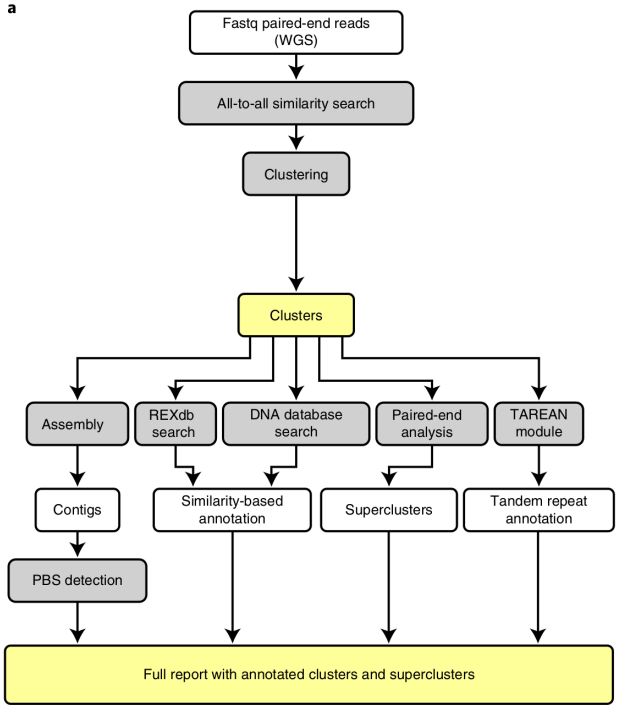
**TAREAN**

**REXdb**

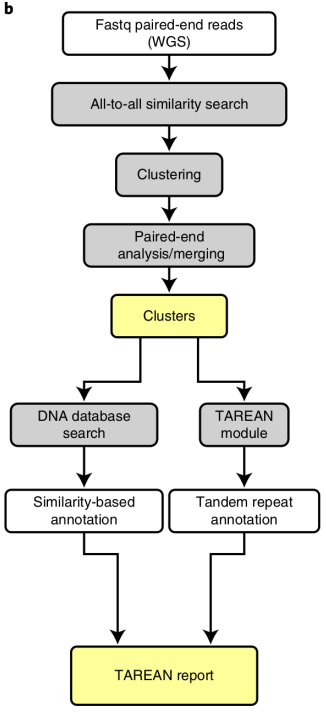
(Neumann et al. 2019)

**Repeat Explorer ver. 2**

**RepeatExplorer pipeline**




**TAREAN pipeline**



# Principles and history of RepeatExplorer

2007 … 2010 … 2013 2014 … 2016 **2017** **2018** **2019** **2020** **2021** **2022**

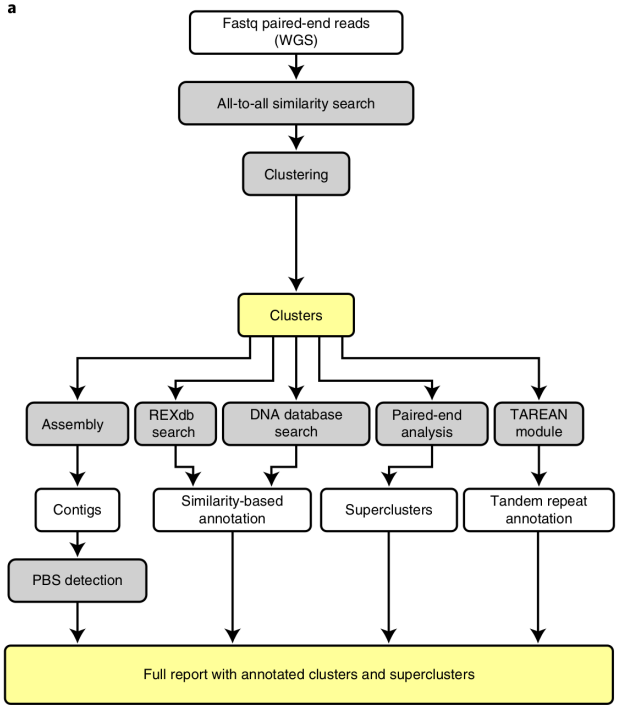
**TAREAN**

**REXdb**

(Neumann et al. 2019)

**Repeat Explorer ver. 2**

**RepeatExplorer pipeline**

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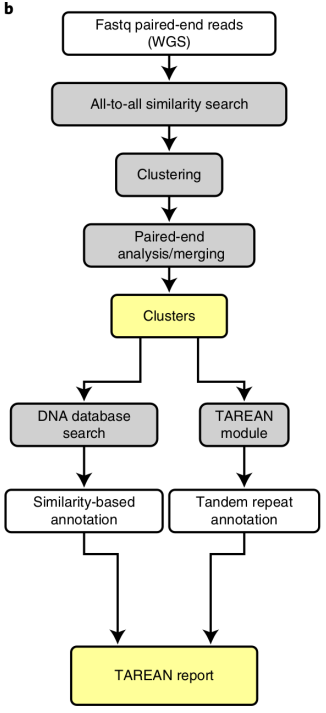


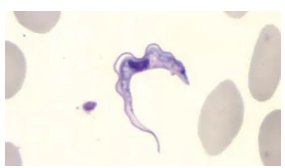

***Additional tools*:**

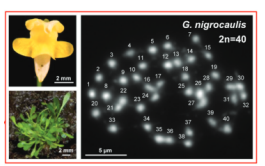
- **ChIP-seq Mapper**
- **DANTE**
- **Long reads**
- **Assembly annotation**

# Applications

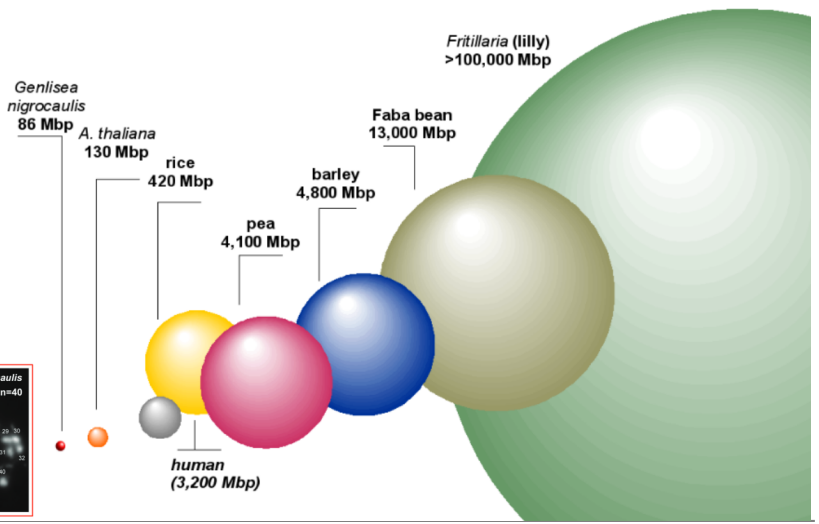
# Applications

- Repeat composition
  - single species



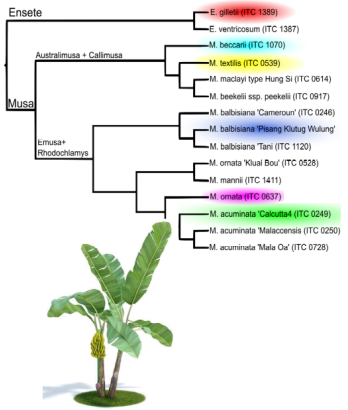


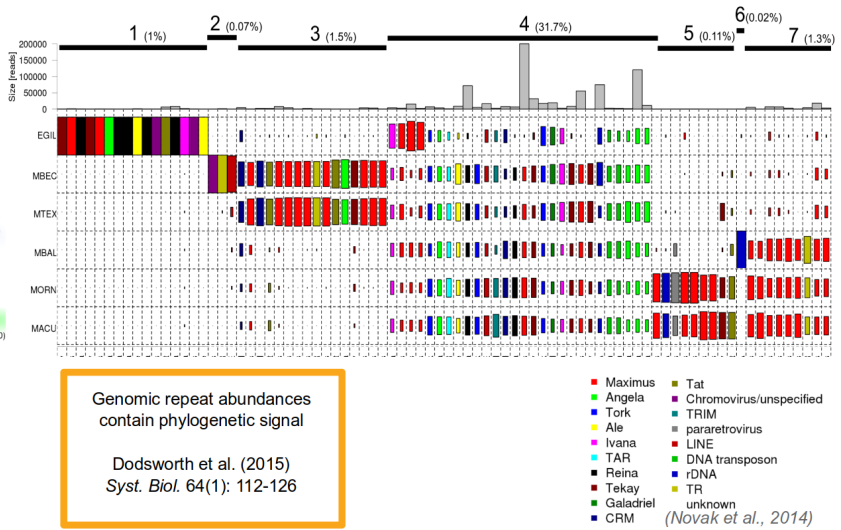

Plant species varying ~2,000-fold
in their genome sizes



# Applications

- Repeat composition
  - single species
  - comparative analysis



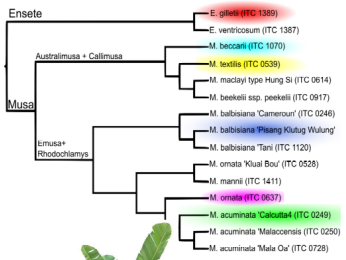

Genomic repeat abundances contain phylogenetic signal

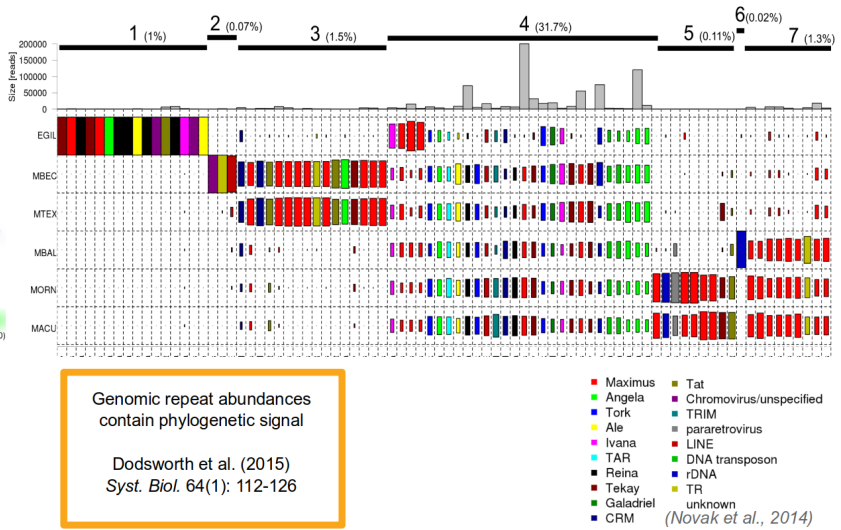
Dodsworth et al. (2015)
*Syst. Biol.* 64(1): 112-126

*(Novak et al., 2014)*

# Applications

- Repeat composition
  - single species
  - comparative analysis




Genomic repeat abundances contain phylogenetic signal

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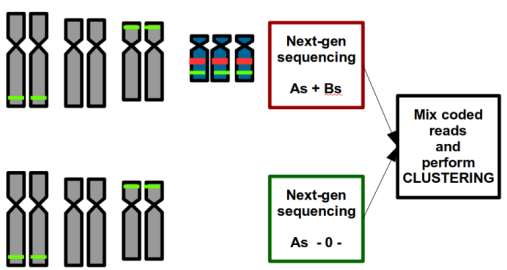
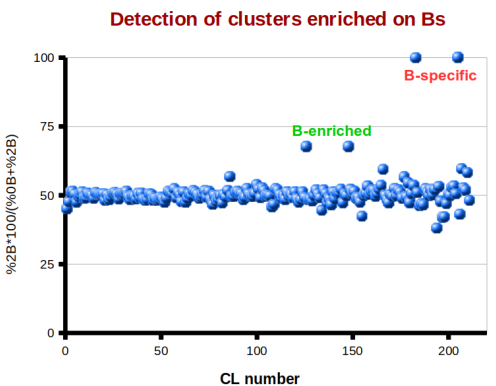
## Identification of chromosome B-specific repeats

### Comparative analysis of B+/- plants



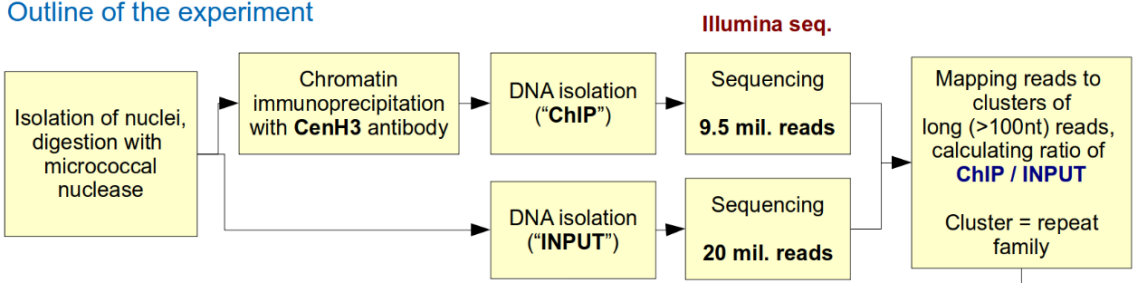



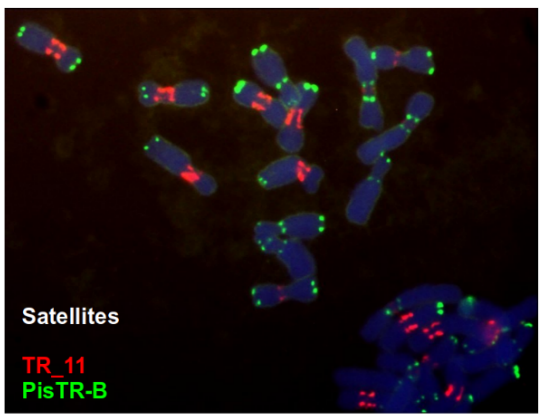
# Applications

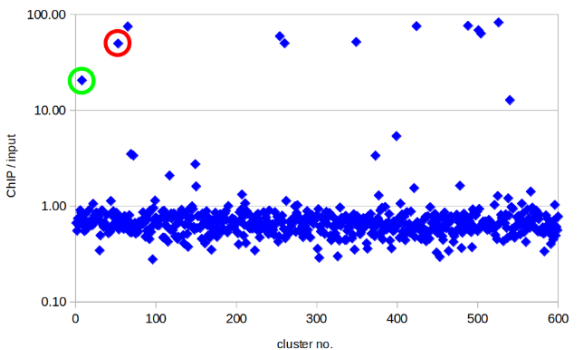
- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq

## Identification of centromeric repeats by ChIP-seq

Outline of the experiment




Neumann et al. (2012) *PLoS Genetics* **8**:e1002777

# Applications

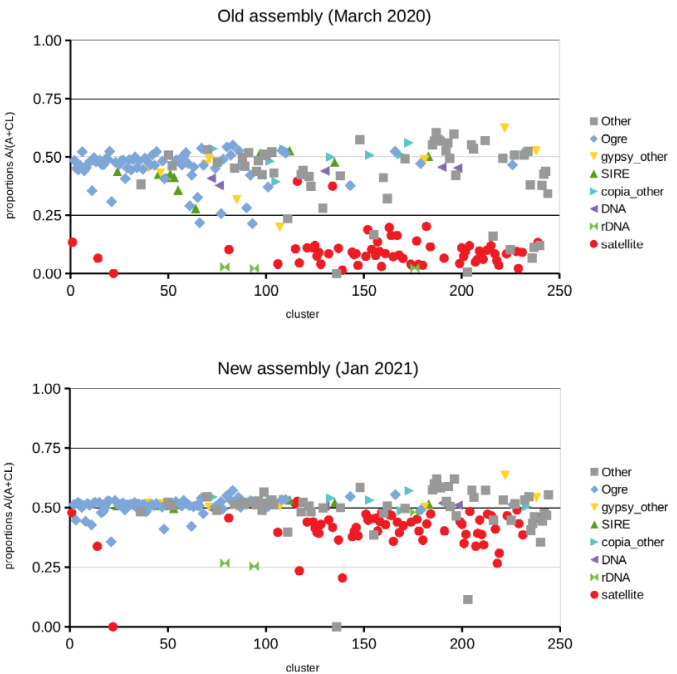
- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq
  - assembly

## Assessing completeness of genome assemblies

Illumina WGS reads (cluster size)

Mapped fragments from assembly

| | | | | sum of hits 133232913 | blasted fragments 207900483 | CL/(CL+BLAST) |
|---|---|---|---|---|---|---|
| Size_adjusted | Proportion [%] | Final_annotation | CL | hits | | |
| | | | 0 | 6171084 | 2.968 | |
| 85804 | 4.187 | All/repeat/satellite/FabTR_FokI | 1 | 1343009 | 0.646 | 0.13 |
| 38954 | 1.901 | All/repeat/mobile_element/Class_I | 2 | 3723933 | 1.791 | 0.49 |
| 37146 | 1.813 | All/repeat/mobile_element/Class_I | 3 | 3091188 | 1.487 | 0.45 |
| 37051 | 1.808 | All/repeat/mobile_element/Class_I | 4 | 3014725 | 1.450 | 0.45 |
| 36830 | 1.797 | All/repeat/mobile_element/Class_I | 5 | 3221042 | 1.549 | 0.46 |
| 33547 | 1.637 | All/repeat/mobile_element/Class_I | 6 | 3719091 | 1.789 | 0.52 |
| 32220 | 1.572 | All/repeat/mobile_element/Class_I | 7 | 2574963 | 1.239 | 0.44 |
| 31149 | 1.520 | All/repeat/mobile_element/Class_I | 8 | 2619726 | 1.260 | 0.45 |
| 30972 | 1.511 | All/repeat/mobile_element/Class_I | 9 | 2791506 | 1.343 | 0.47 |
| 30361 | 1.482 | All/repeat/mobile_element/Class_I | 10 | 2857979 | 1.375 | 0.48 |
| 29548 | 1.442 | All/repeat/mobile_element/Class_I | 11 | 1649483 | 0.793 | 0.35 |
| 29227 | 1.426 | All/repeat/mobile_element/Class_I | 12 | 2924386 | 1.407 | 0.50 |
| 27341 | 1.334 | All/repeat/mobile_element/Class_I | 13 | 2544864 | 1.224 | 0.48 |
| 26964 | 1.316 | All/repeat/satellite/FabTR_53 | 14 | 189991 | 0.091 | 0.06 |
| 26082 | 1.273 | All/repeat/mobile_element/Class_I | 15 | 2496449 | 1.201 | 0.49 |
| 25651 | 1.252 | All/repeat/mobile_element/Class_I | 16 | 2280990 | 1.097 | 0.47 |
| 25147 | 1.227 | All/repeat/mobile_element/Class_I | 17 | 2239097 | 1.077 | 0.47 |
| 23553 | 1.149 | All/repeat/mobile_element/Class_I | 18 | 2304645 | 1.109 | 0.49 |
| 22914 | 1.118 | All/repeat/mobile_element/Class_I | 19 | 2227830 | 1.072 | 0.49 |
| 22829 | 1.114 | All/repeat/mobile_element/Class_I | 20 | 2547541 | 1.225 | 0.52 |
| 22696 | 1.108 | All/repeat/mobile_element/Class_I | 21 | 1024482 | 0.493 | 0.31 |
| 22481 | 1.097 | All/repeat/satellite/FabTR_83 | 22 | 6 | 0.000 | 0.00 |
| 21364 | 1.043 | All/repeat/mobile_element/Class_I | 23 | 1971842 | 0.948 | 0.48 |
| 20442 | 0.998 | All/repeat/mobile_element/Class_I | 24 | 1610084 | 0.774 | 0.44 |
| 19605 | 0.957 | All/repeat/mobile_element/Class_I | 25 | 1753755 | 0.844 | 0.47 |
| 19289 | 0.941 | All/repeat/mobile_element/Class_I | 26 | 1835955 | 0.883 | 0.48 |
| 19234 | 0.939 | All/repeat/mobile_element/Class_I | 27 | 1842540 | 0.886 | 0.49 |
| 18932 | 0.924 | All/repeat/mobile_element/Class_I | 28 | 1318285 | 0.634 | 0.41 |
| 18345 | 0.895 | All/repeat/mobile_element/Class_I | 29 | 1533938 | 0.738 | 0.45 |
| 18112 | 0.884 | All/repeat/mobile_element/Class_I | 30 | 1753161 | 0.843 | 0.49 |
| 17991 | 0.878 | All/repeat/mobile_element/Class_I | 31 | 1457392 | 0.701 | 0.44 |
| 17167 | 0.838 | All/repeat/mobile_element/Class_I | 32 | 1663803 | 0.800 | 0.49 |
| 16726 | 0.816 | All/repeat/mobile_element/Class_I | 33 | 1693448 | 0.815 | 0.50 |



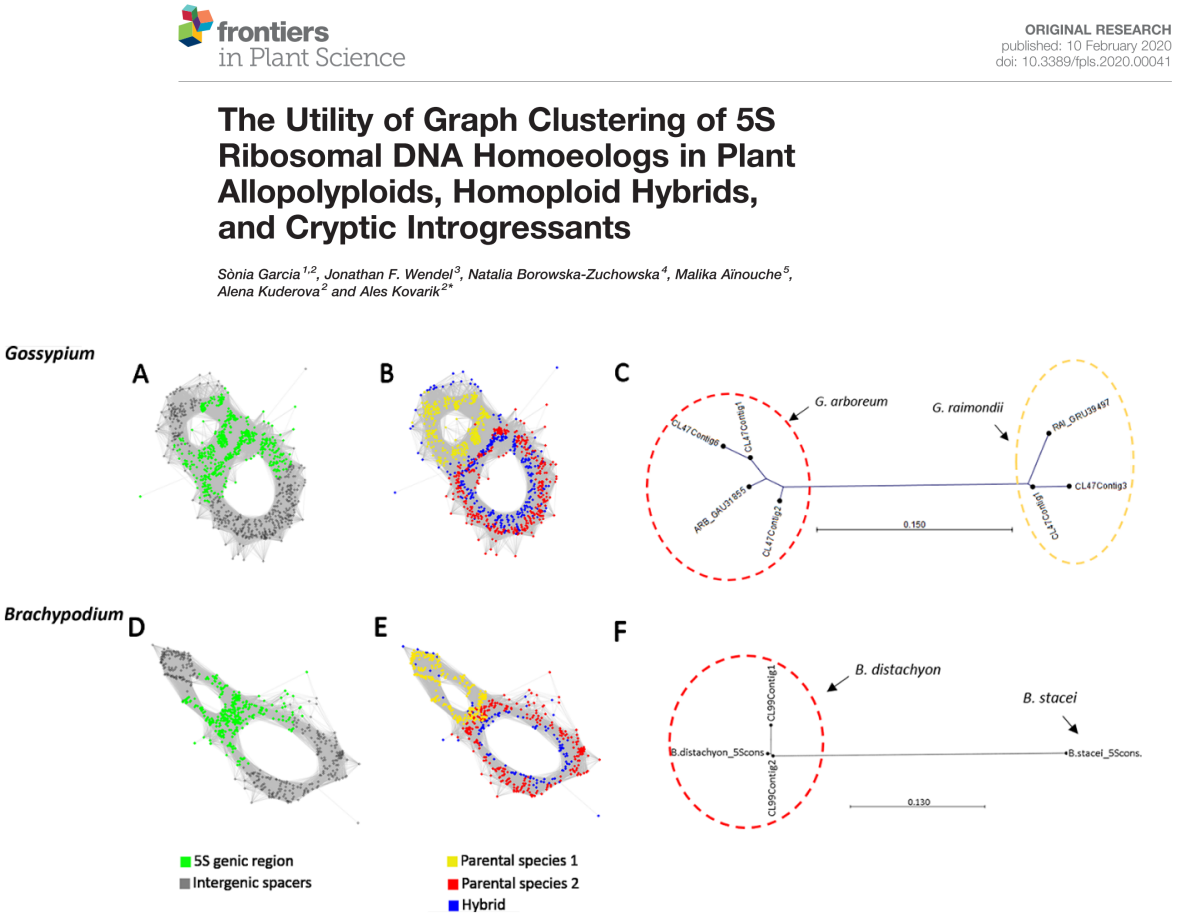
# Applications

- Repeat composition
  - single species
  - comparative analysis

- Repeat clusters as a reference
  - ChIP-seq
  - assembly
    - reference databases for repeat annotation

# Applications

- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq
  - assembly
  - *graph shapes*



# Applications

- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq
  - assembly
  - *graph shapes*
- Satellite DNA
  - cytogenetic studies

## Cytogenetic markers (FISH probes)

NGS data (Illumina)

↓

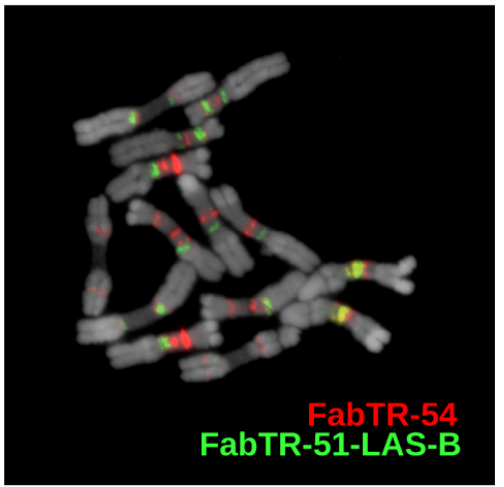
RepeatExplorer / **TAREAN**

↓

Satellite DNA

↓

FISH probe




*Lathyrus sativus*
(FISH by L. Avila Robledillo)

# Applications

- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq
  - assembly
  - *graph shapes*
- Satellite DNA
  - cytogenetic studies
  - diagnostic markers

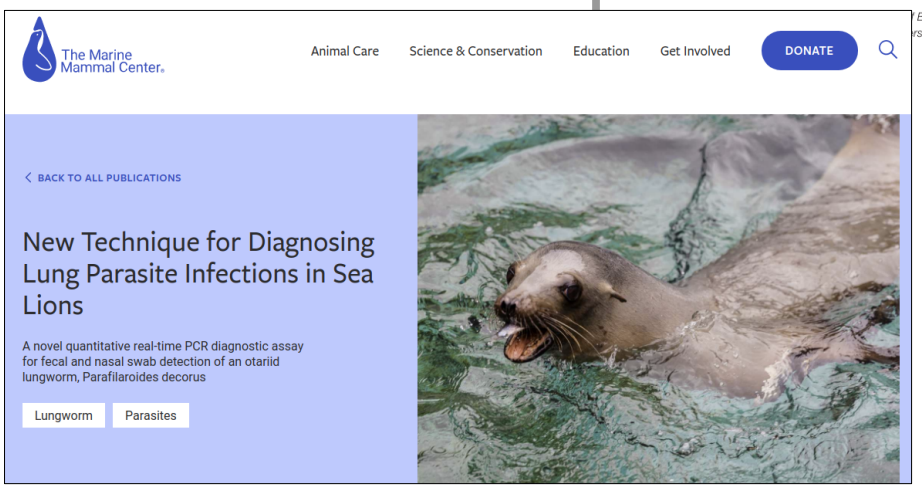

frontiers in Genetics

REVIEW
published: 23 September 2019
doi: 10.3389/fgene.2019.00883

A Case for Using Genomics and a Bioinformatics Pipeline to Develop Sensitive and Species-Specific PCR-Based Diagnostics for Soil-Transmitted Helminths

Jessica R. Grant[1]*, Nils Pilotte[1,2] and Steven A. Williams[1,2]

[1] Biological Sciences, Smith College, Northampton, MA, United States, [2] Molecular and Cellular Biology ersity of Massachusetts, Amherst, MA, United States




# Applications

- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
  - ChIP-seq
  - assembly
  - *graph shapes*
- Satellite DNA
  - cytogenetic studies
  - diagnostic markers
- RE utilization in other pipelines

SCIENTIFIC REPORTS

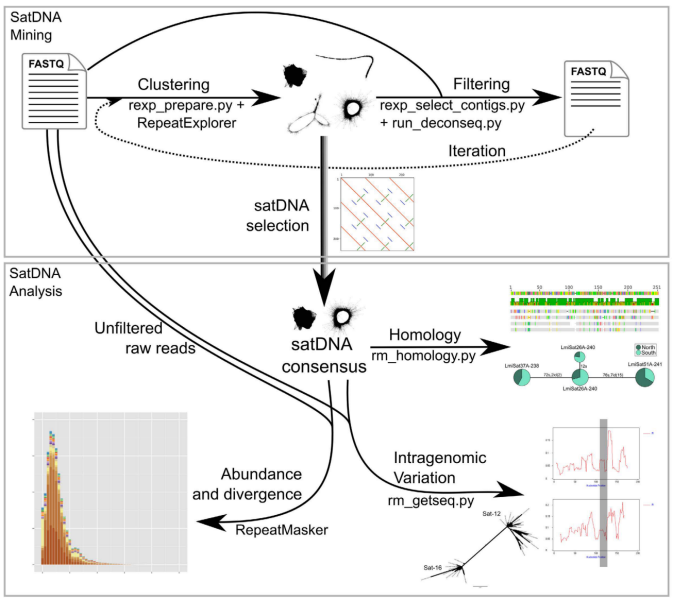
OPEN

**High-throughput analysis of the satellitome illuminates satellite DNA evolution**

Received: 14 January 2016
Accepted: 02 June 2016

Francisco J. Ruiz-Ruano, María Dolores López-León, Josefa Cabrero & Juan Pedro M. Camacho

**satMiner**



# Applications

- Repeat composition
  - single species
  - comparative analysis
- Repeat clusters as a reference
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  - assembly
  - *graph shapes*
- Satellite DNA
  - cytogenetic studies
  - diagnostic markers
- RE utilization in other pipelines

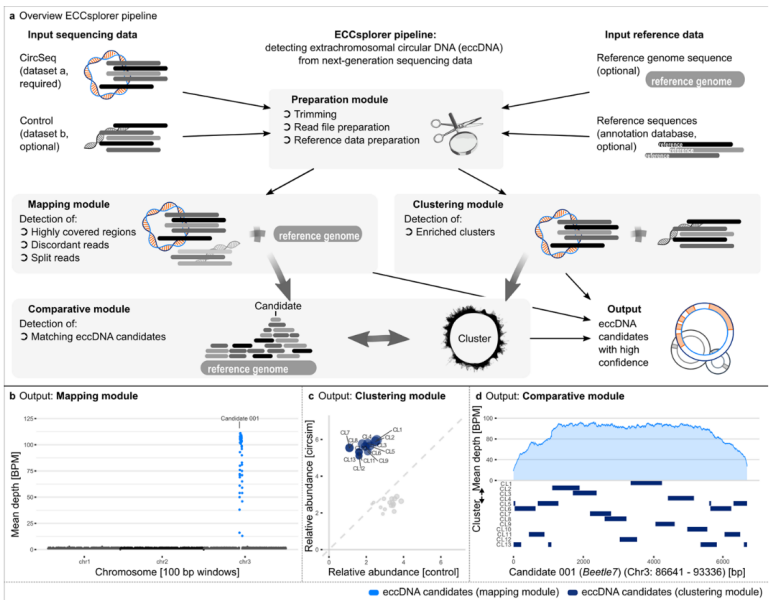
Mann *et al. BMC Bioinformatics* (2022) 23:40
https://doi.org/10.1186/s12859-021-04545-2

BMC Bioinformatics

SOFTWARE Open Access

## ECCsplorer: a pipeline to detect extrachromosomal circular DNA (eccDNA) from next-generation sequencing data

Ludwig Mann, Kathrin M. Seibt, Beatrice Weber and Tony Heitkam*



# Applications

- Repeat composition
  - single species ***
  - comparative analysis ***
- Repeat clusters as a reference
  - ChIP-seq ***
  - assembly ***
  - *graph shapes*
- Satellite DNA
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- RE utilization in other pipelines

**Enjoy the workshop !**