# Using *RepeatExplorer* output for repeat annotation and quantification

# RepeatExplorer ver. 2

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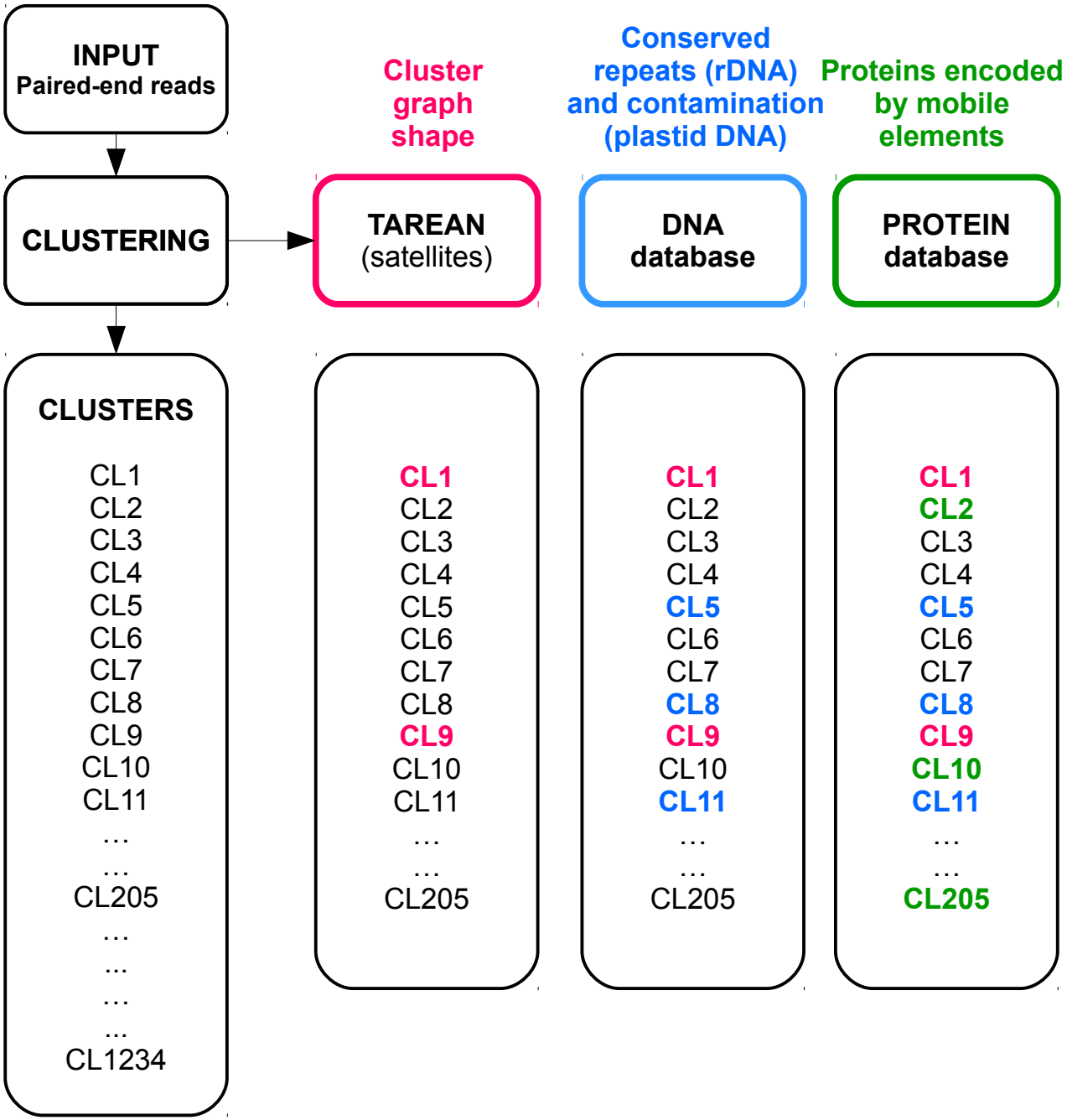
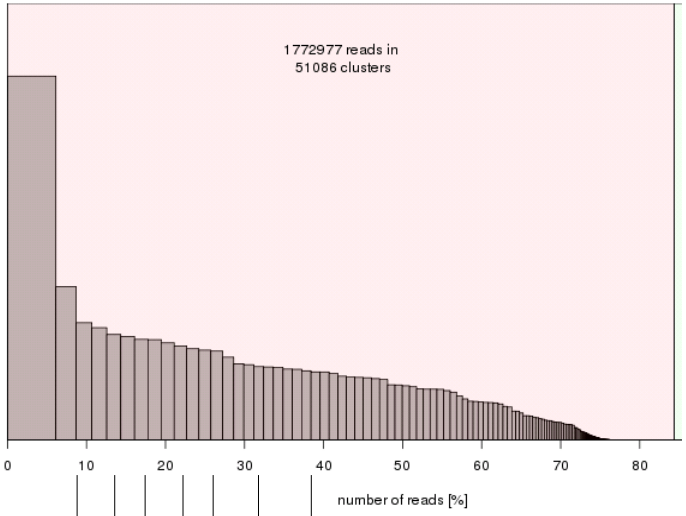
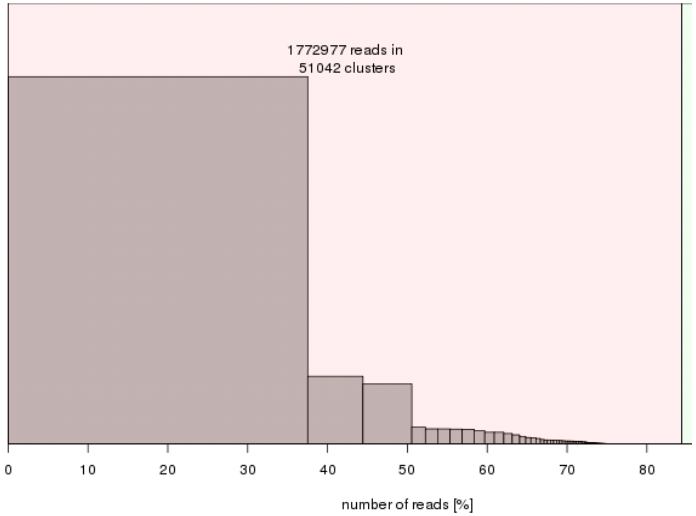


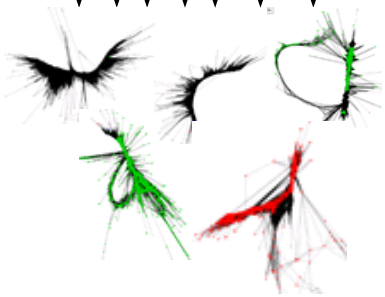

Many repeats are split to multiple clusters

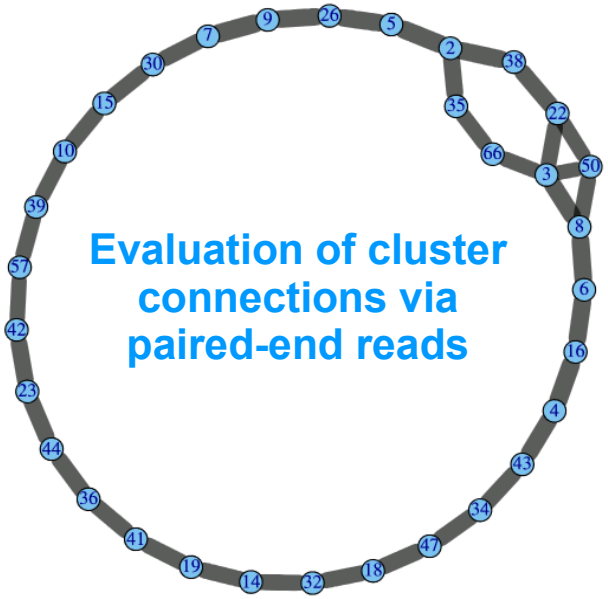
(feature of the clustering algorithm; depends on many factors)

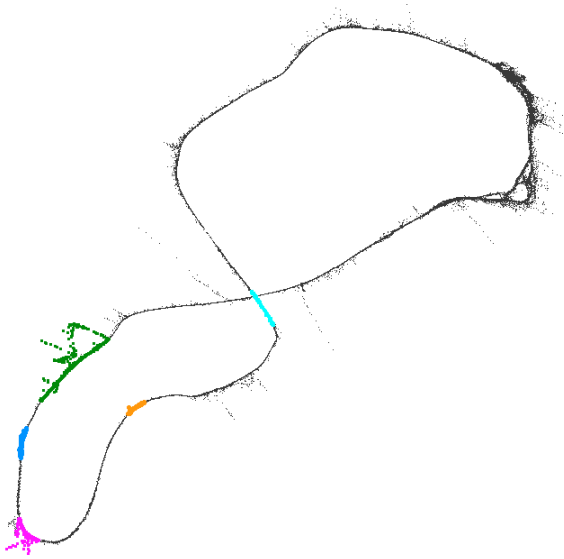
# Cluster fragmentation



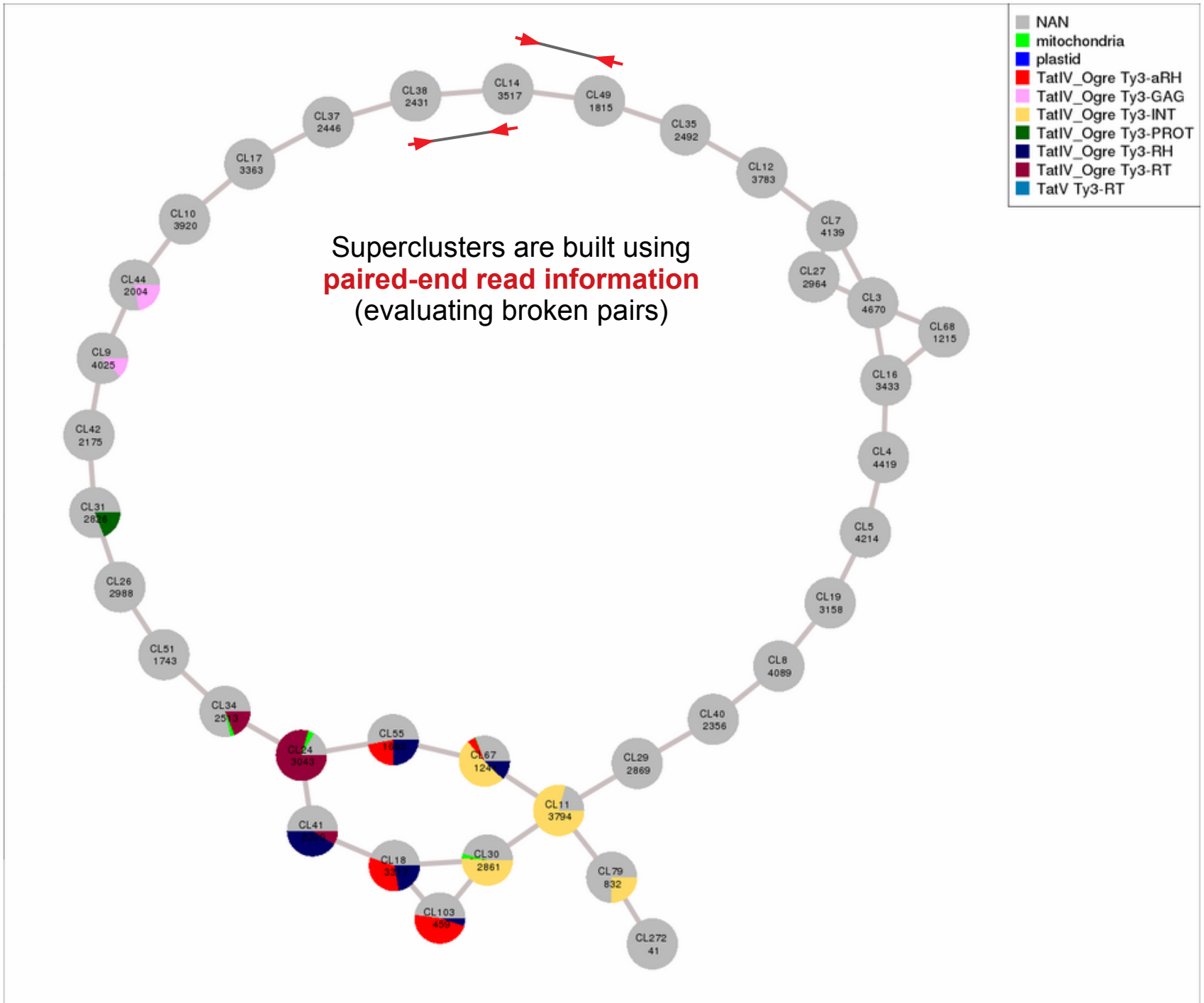





LTR-retrotransposon Ogre in *Vicia pannonica* (~ 40% of the genome)

# RepeatExplorer 2 – automatic detection of superclusters



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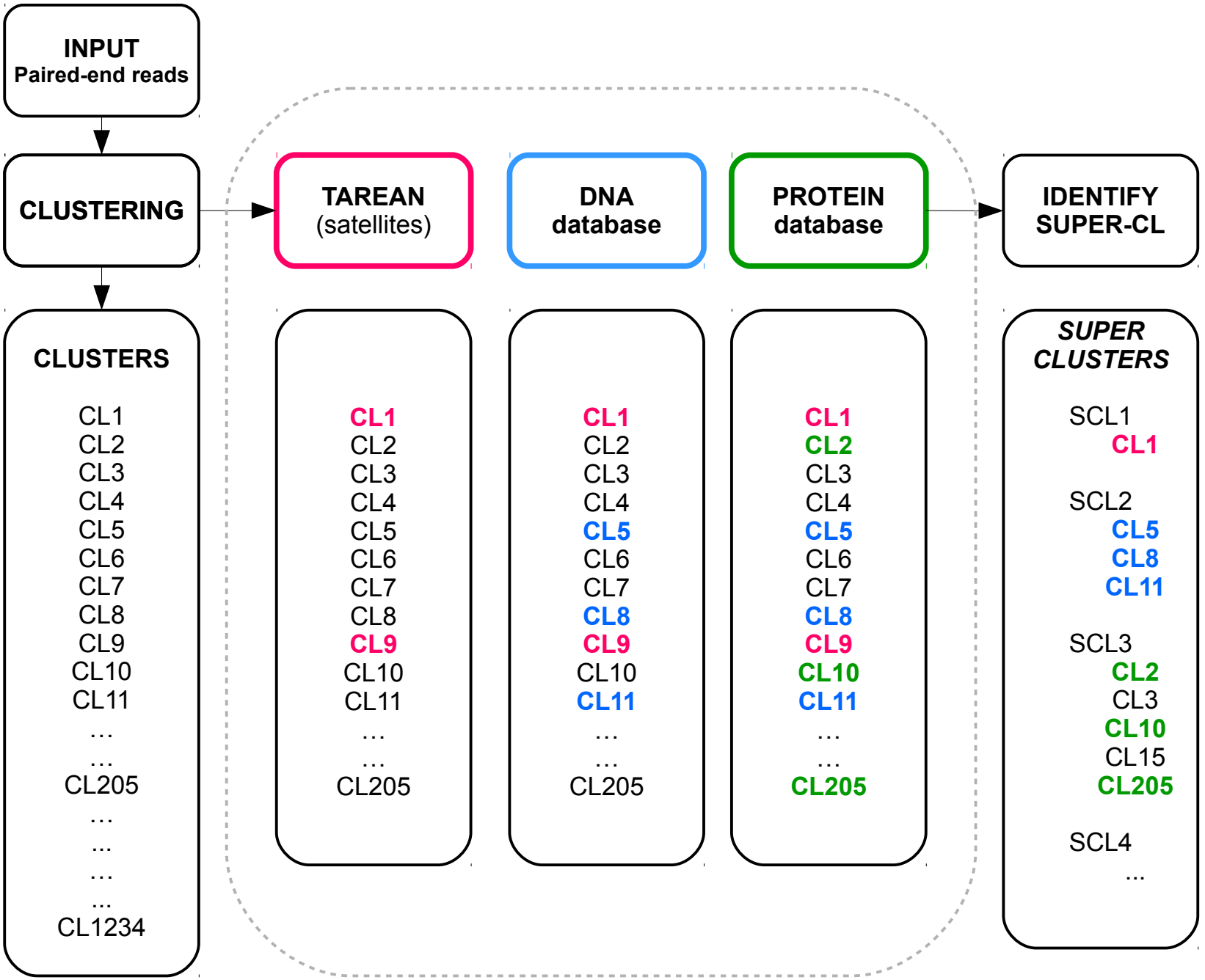
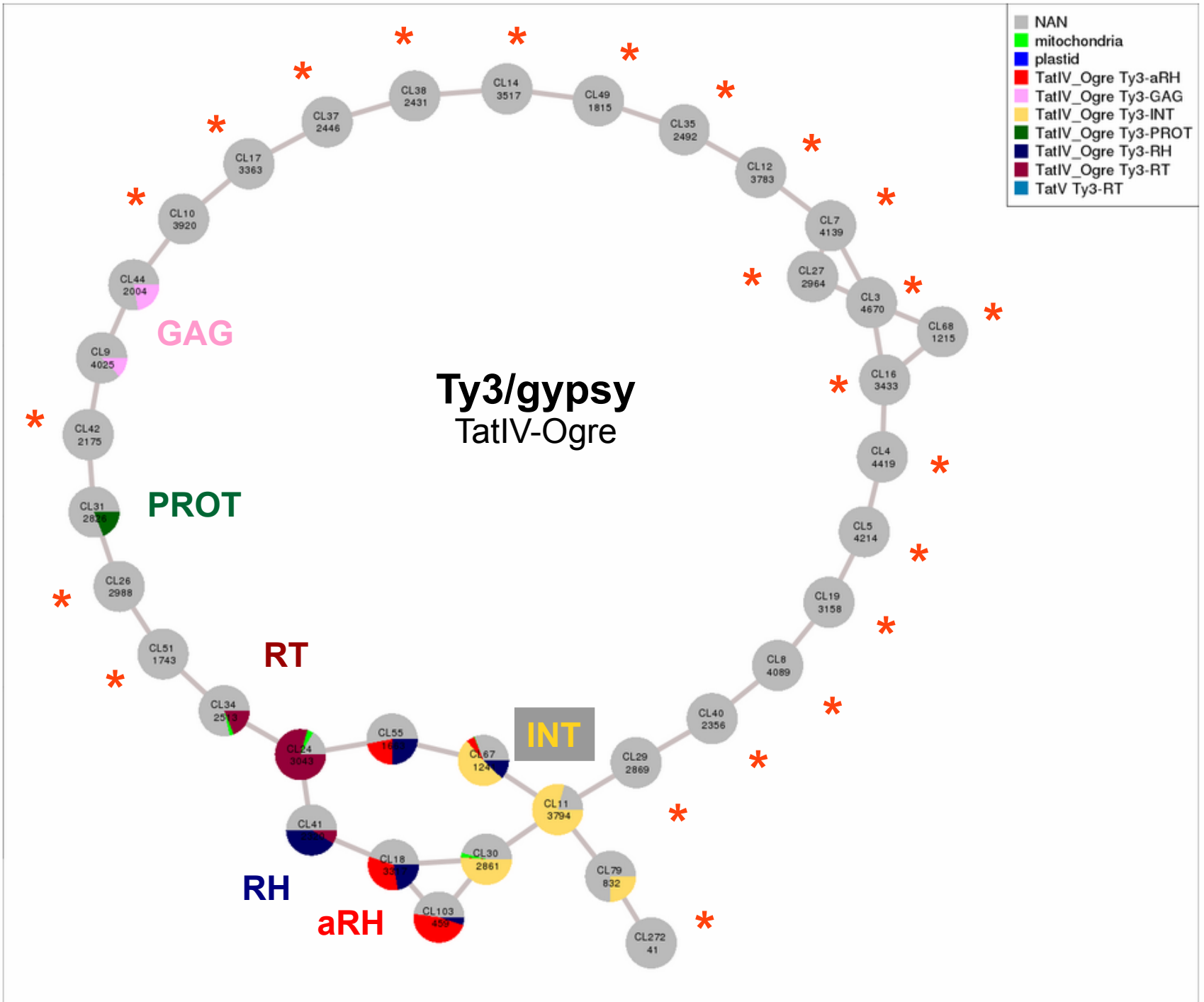


# Superclusters provide more complete annotation



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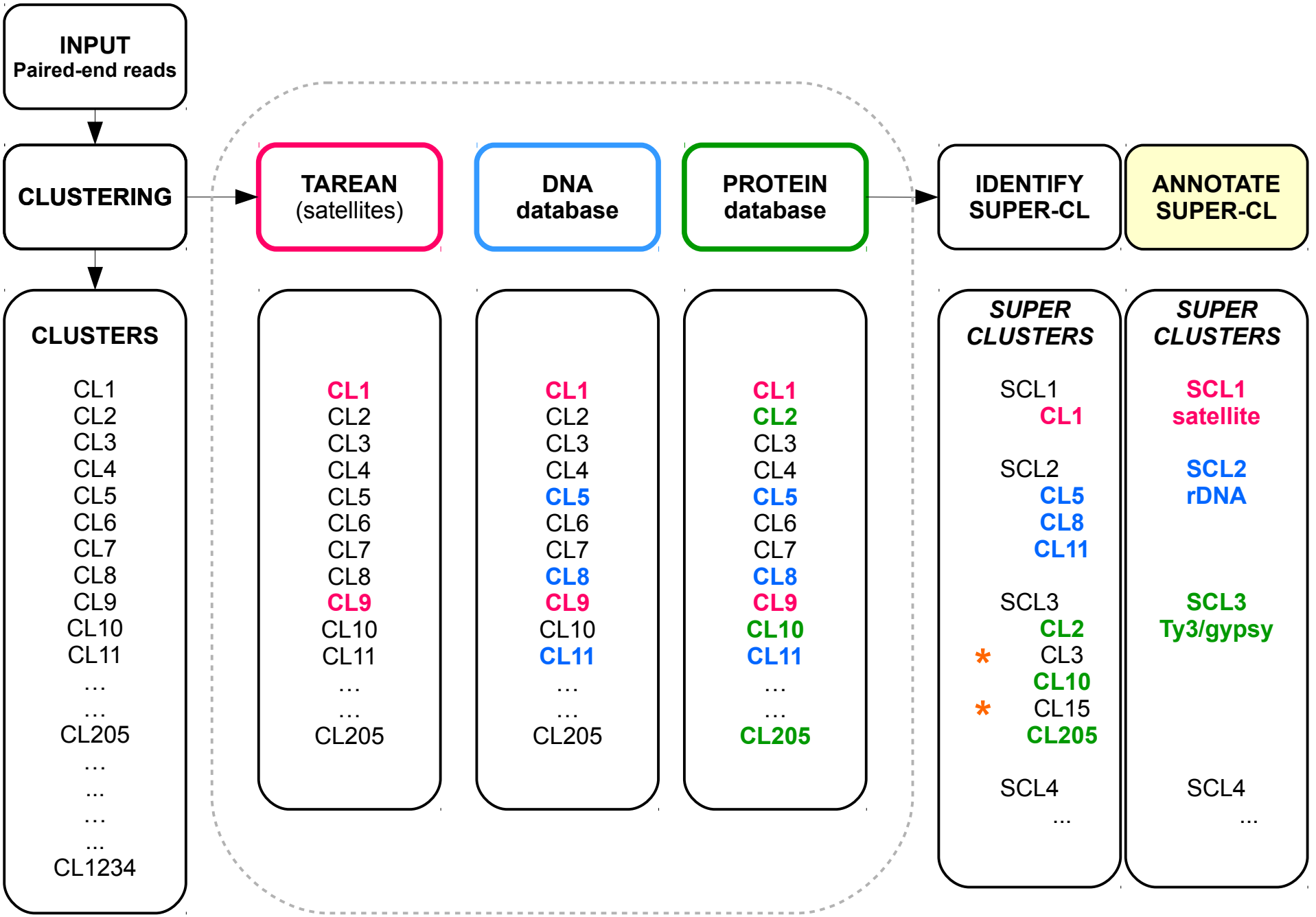


# Repeat quantification

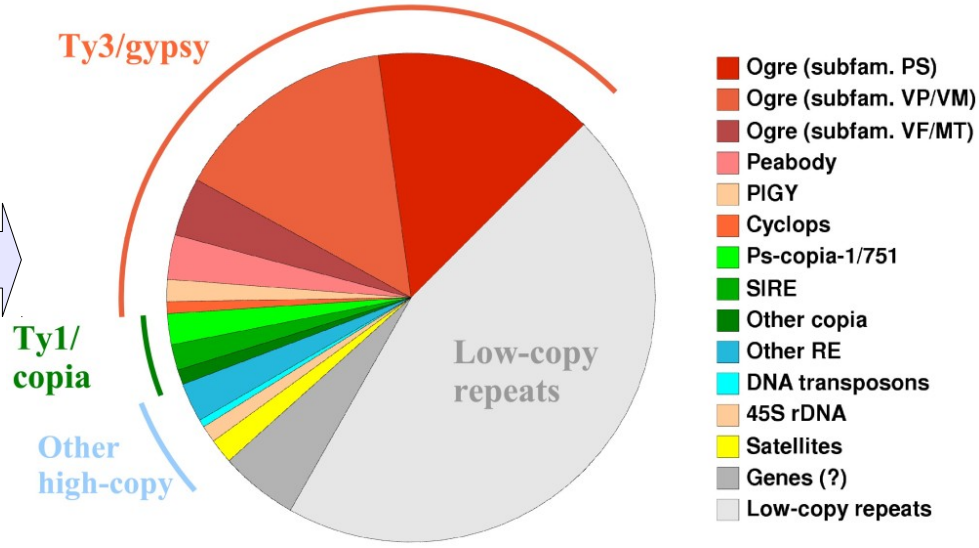
**Cluster sizes (numbers of reads) are proportional to genomic abundance of corresponding repeats**

| | 7192836 (= 100%) | 64.1 |
|---|---|---|
| CL | reads | genome % |
| 1 | 304159 | 4.229 |
| 2 | 234749 | 3.264 |
| 3 | 216307 | 3.007 |
| 4 | 202822 | 2.820 |
| 5 | 149693 | 2.081 |
| 6 | 145911 | 2.029 |
| 7 | 143766 | 1.999 |
| 8 | 142608 | 1.983 |
| 9 | 141836 | 1.972 |
| 10 | 123886 | 1.722 |
| 11 | 79345 | 1.103 |
| 12 | 72781 | 1.012 |
| 13 | 67096 | 0.933 |
| 14 | 65455 | 0.910 |
| 15 | 62334 | 0.867 |
| 16 | 53845 | 0.749 |
| 17 | 49341 | 0.686 |
| 18 | 45062 | 0.626 |
| 19 | 44762 | 0.622 |
| 20 | 43332 | 0.602 |
| 21 | 42344 | 0.589 |
| 22 | 40125 | 0.558 |
| 23 | 39923 | 0.555 |
| 24 | 36353 | 0.505 |
| 25 | 35977 | 0.500 |
| 26 | 35674 | 0.496 |
| 27 | 34829 | 0.484 |
| 28 | 34534 | 0.480 |
| 29 | 34302 | 0.477 |
| 30 | 33114 | 0.460 |
| 31 | 32930 | 0.458 |

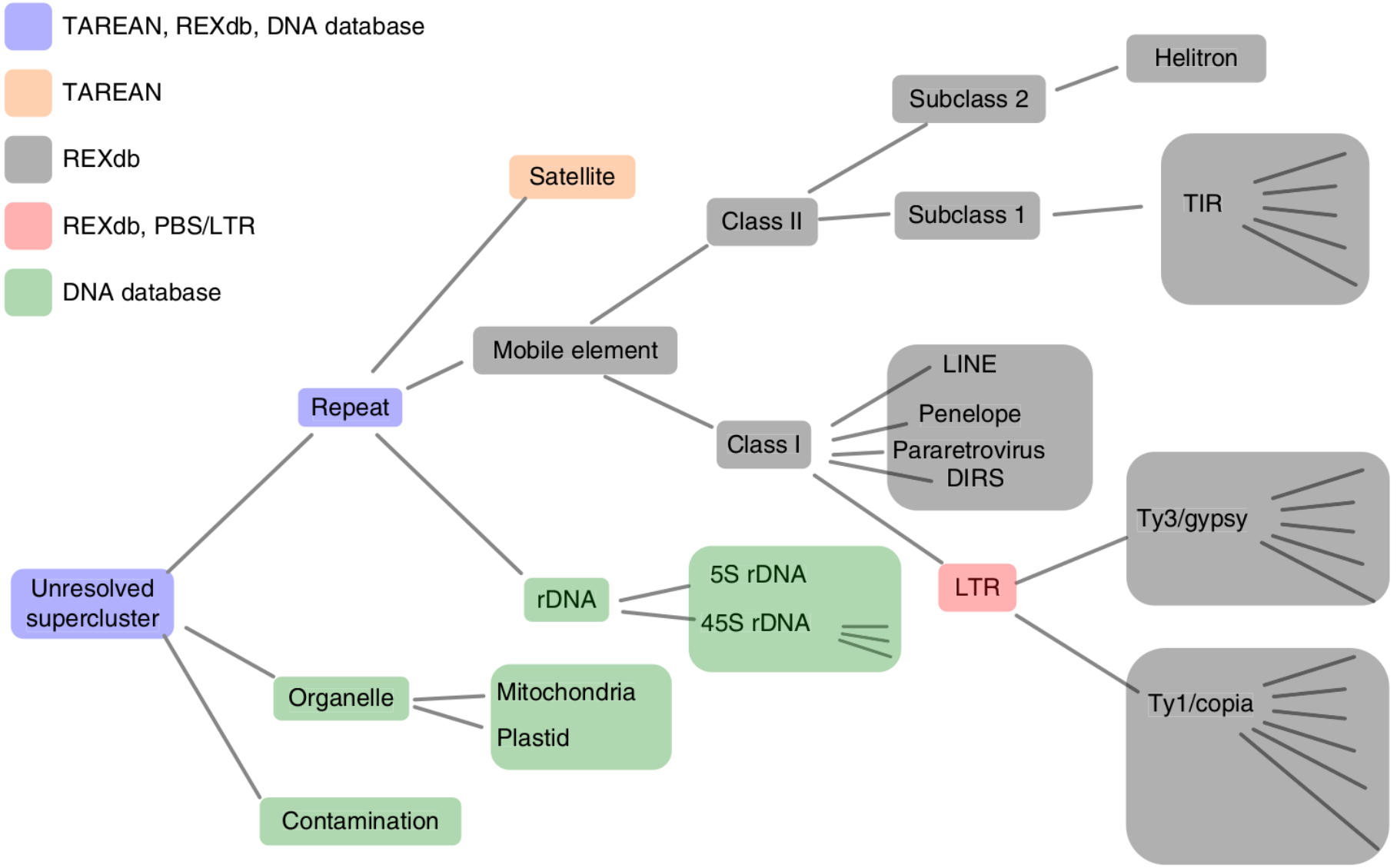
**IDENTIFY SUPER-CL** → **ANNOTATE SUPER-CL**

| *SUPER CLUSTERS* | *SUPER CLUSTERS* |
|---|---|
| SCL1 **CL1** | **SCL1 satellite** |
| SCL2 **CL5** **CL8** **CL11** | **SCL2 rDNA** |
| SCL3 **CL2** CL3 **CL10** CL15 **CL205** | **SCL3 Ty3/gypsy** |
| SCL4 ... | SCL4 ... |

**Proportions of various repeat types in a genome**



# Decision tree for automatic annotation



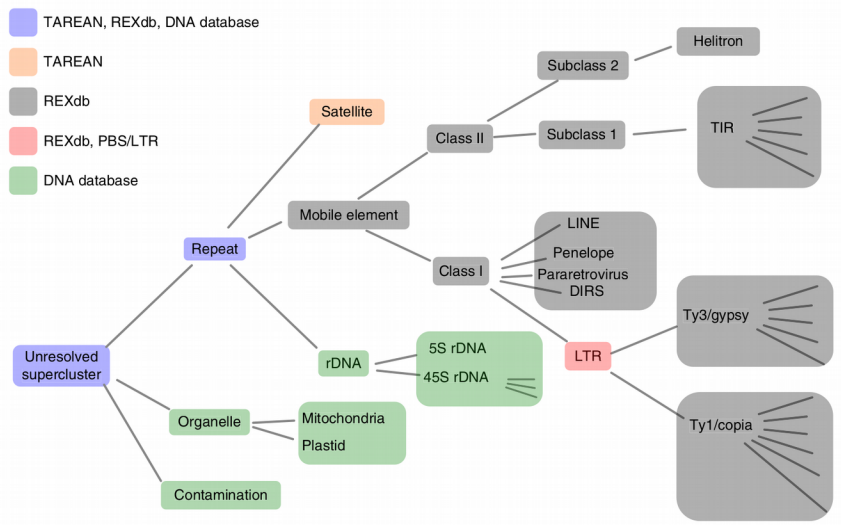
# How reliable is automatic annotation ?

- conserved repeats (rDNA)
- organelles, contamination

- satellite DNA (structure-based annotation)

- mobile elements (autonomous)
  - plants
  - other organisms

- non-autonomous mobile elements
  - LTR detection
  - insertion site detection (coming soon)



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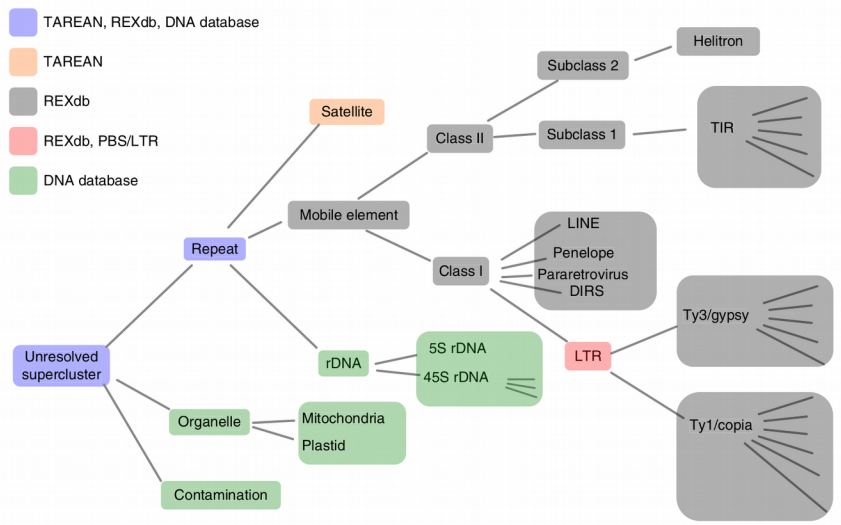


**Working with organisms not covered in REXdb ?**

- use custom repeat database (and share it !)
- help us to improve REXdb ;-)

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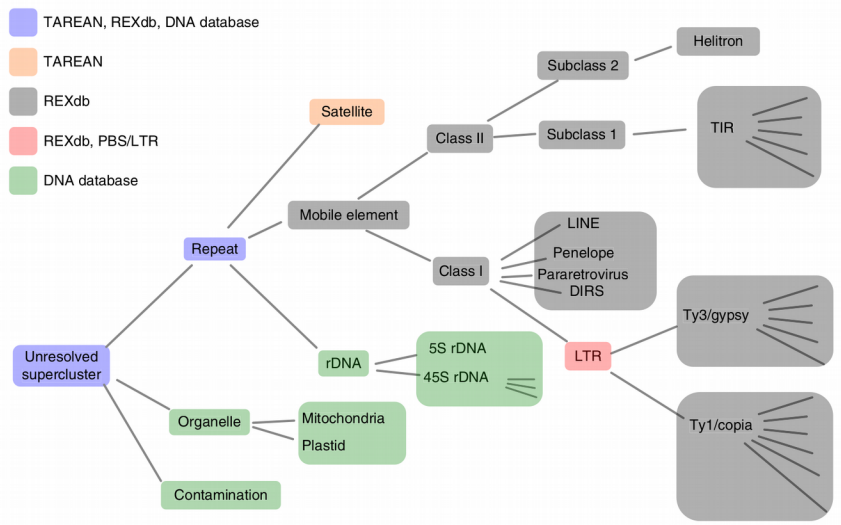


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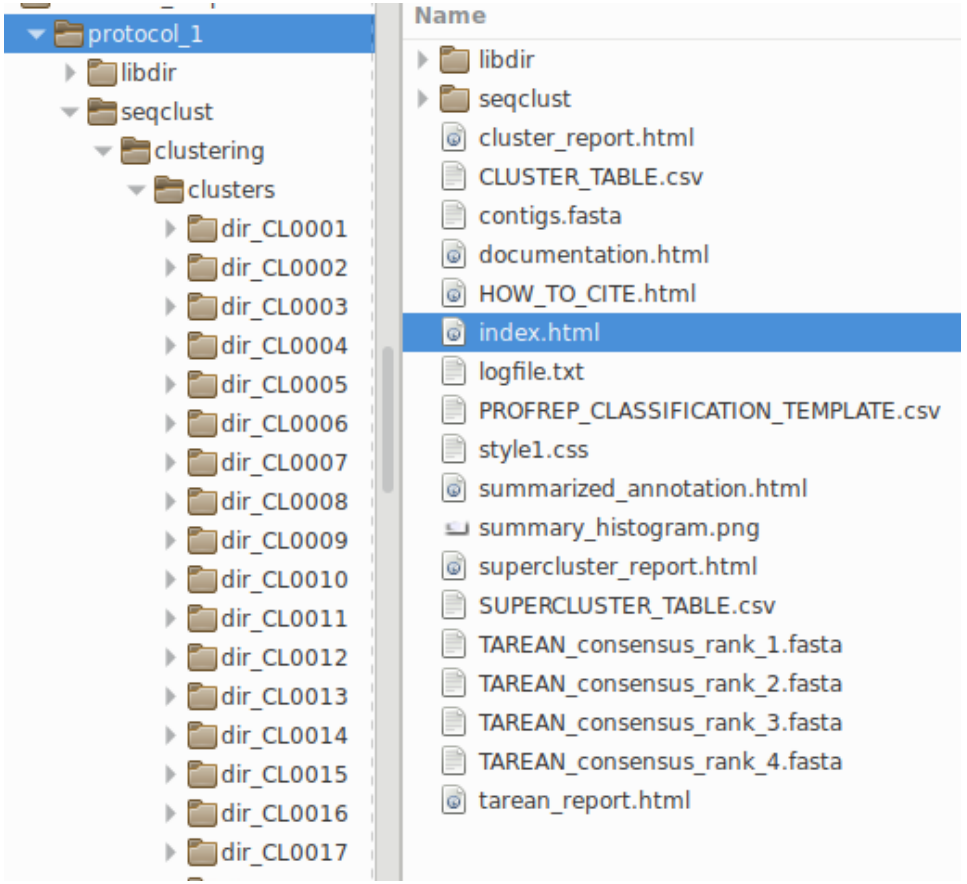
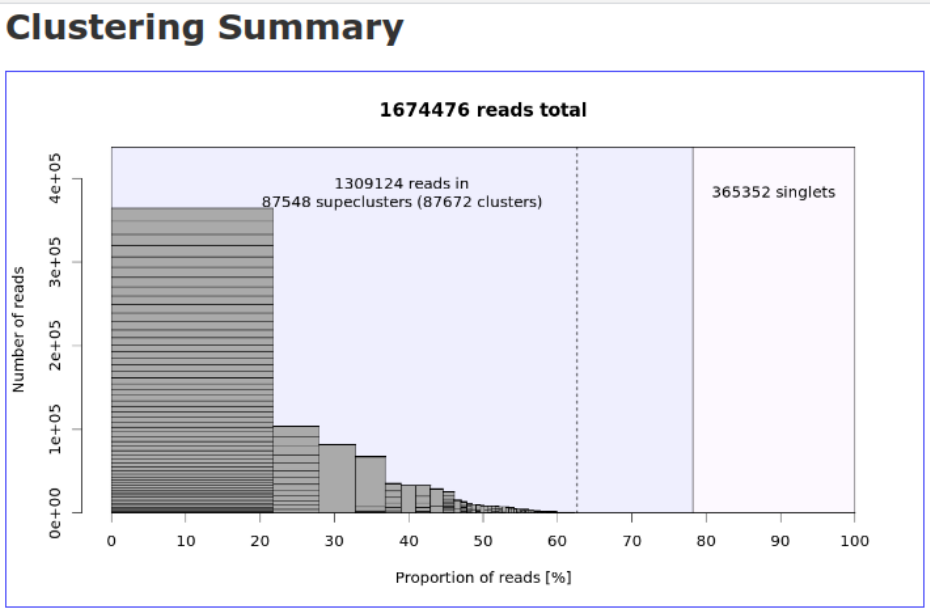
**Clusters without annotation**

- The lack of annotation does not mean these are not repeats (all repeats are detected)
- More info available in cluster directories

# RepeatExplorer output files




**Graphical summary of the clustering results.** Bars represent superclusters, with their heights and widths corresponding to the numbers of reads in the superclusters (y-axis) and to their proportions in all analyzed reads (x-axis), respectively. Rectangles inside the supercluster bars represent individual clusters. If the filtering of abundant satellites was performed, the affected clusters are shown in green, and their sizes correspond to the adjusted values. Blue and pink background panels show proportions of reads that were clustered and remained single, respectively. Top clusters are on the left of the dotted line.

## Run information:

Number of input reads: 2935772

Number of analyzed reads: 1674476

Proportion of reads in top clusters : 63 %

Cluster merging: No

Paired-end reads: Yes

## Available analyses:

Tandem repeat analysis

Cluster annotation

Supercluster annotation

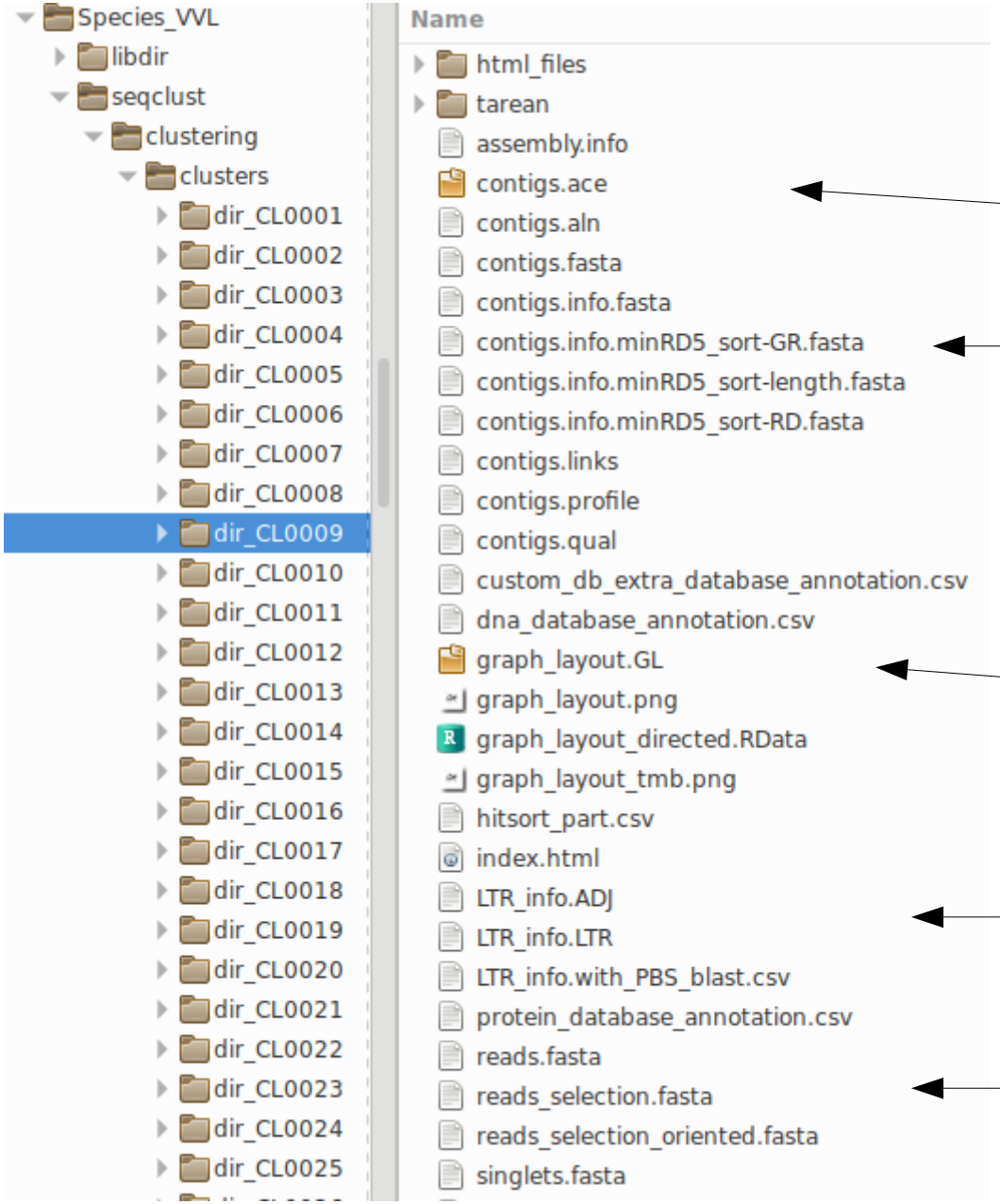
Repeat annotation summary

Always download and use archive with complete output of the analysis

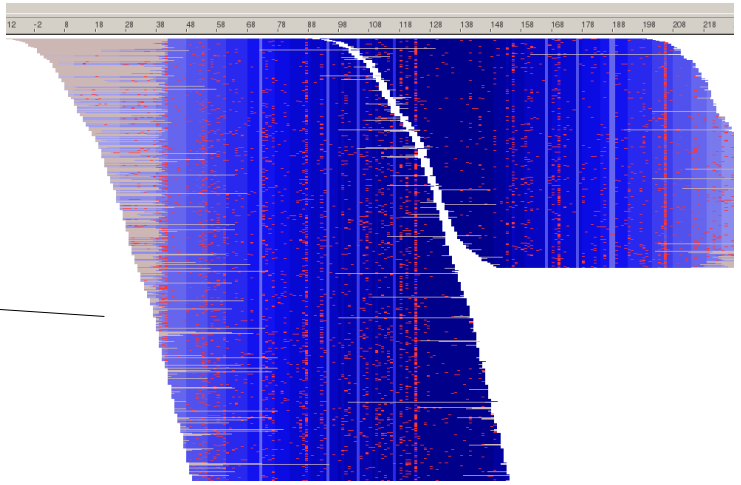
- do not work with HTML only

# RepeatExplorer output files

## Cluster directories

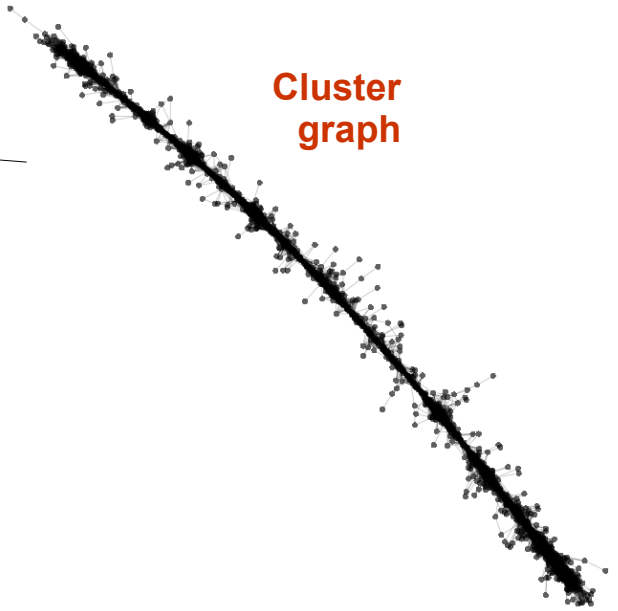



**Contigs**



**assembly**

**Cluster graph**

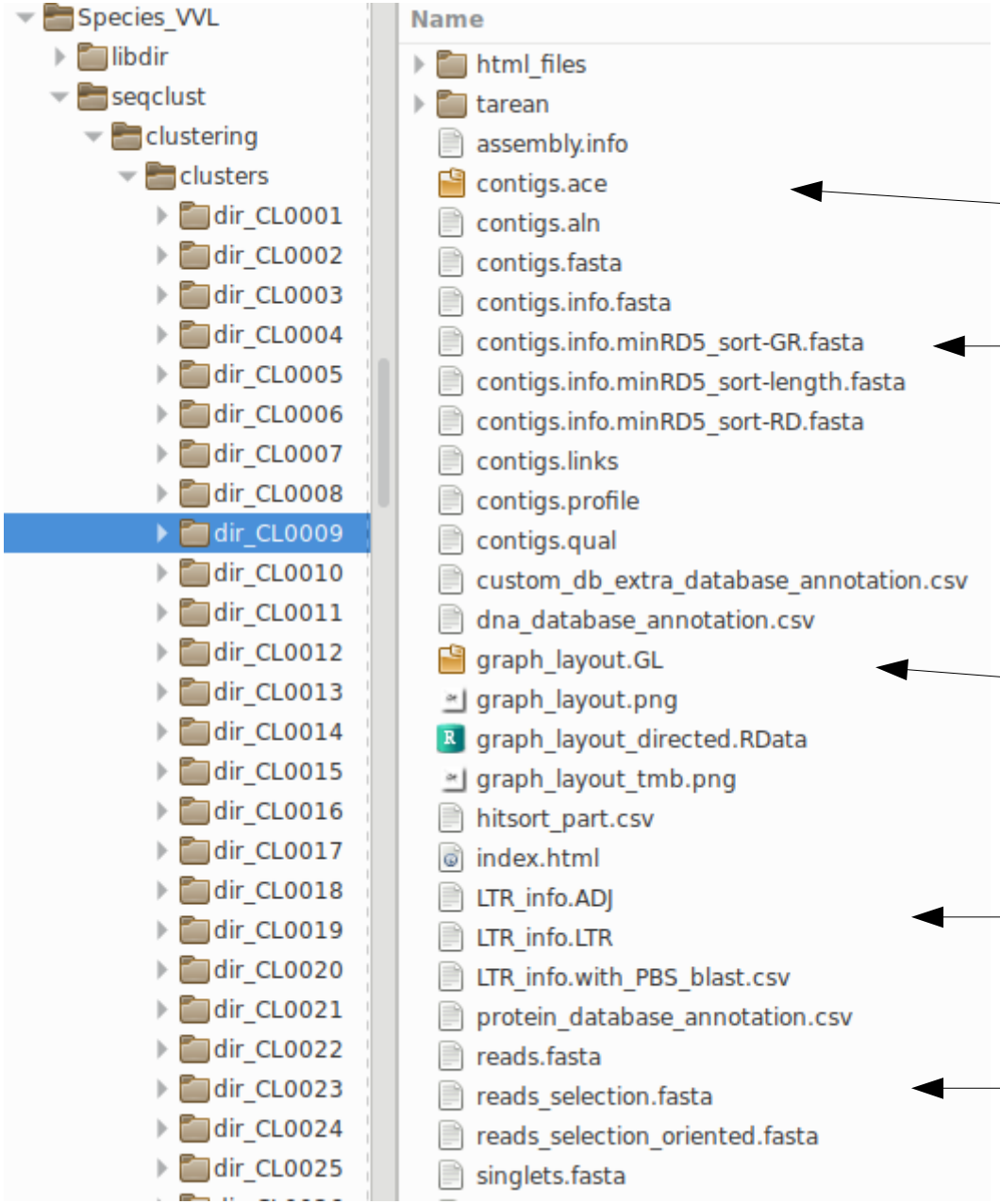


**LTR-detection**

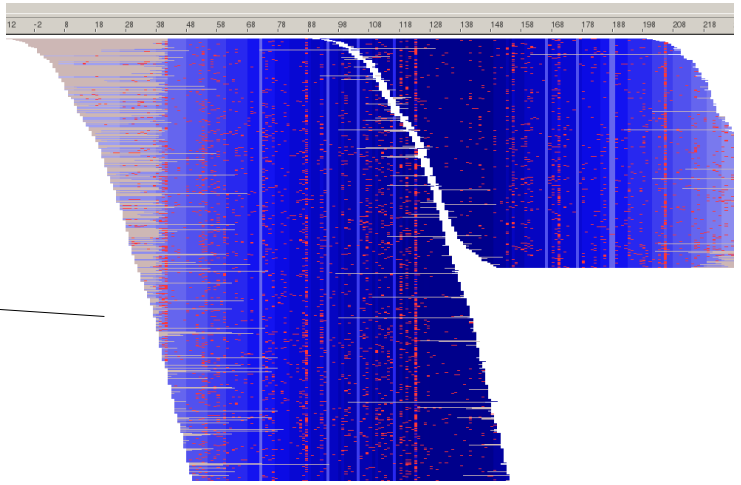
**Read sequences**

# RepeatExplorer output files

## Cluster directories

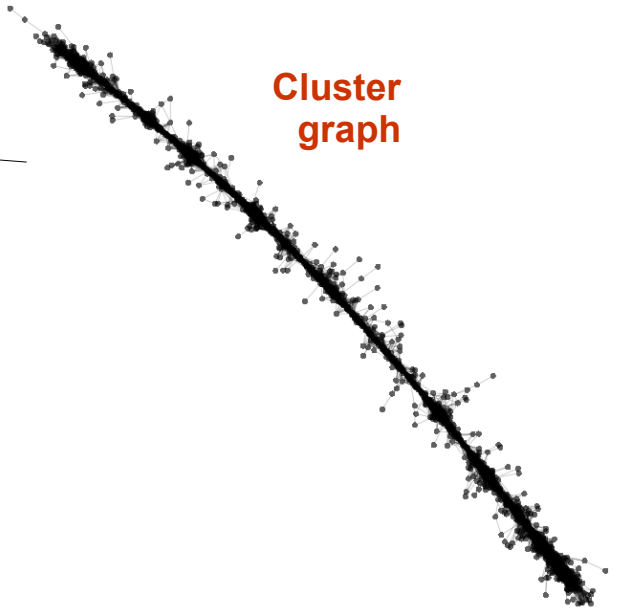



Contigs



assembly

Cluster graph

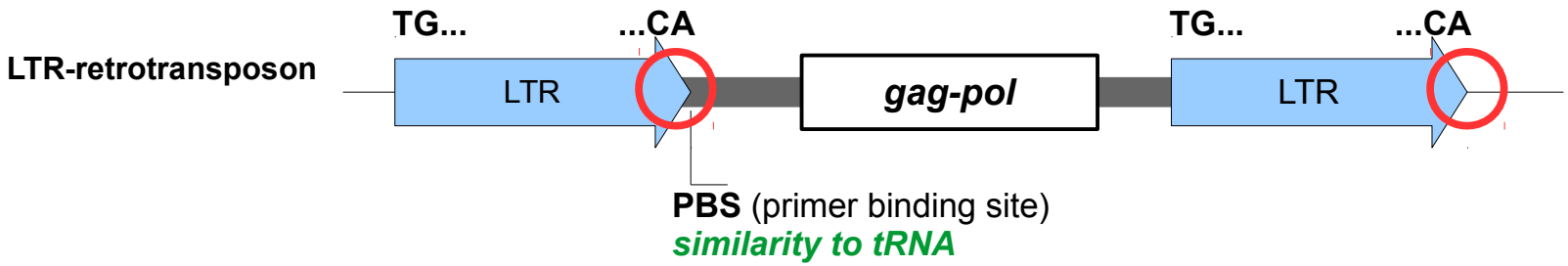


LTR-detection

Read sequences

# Insertion sites of mobile elements

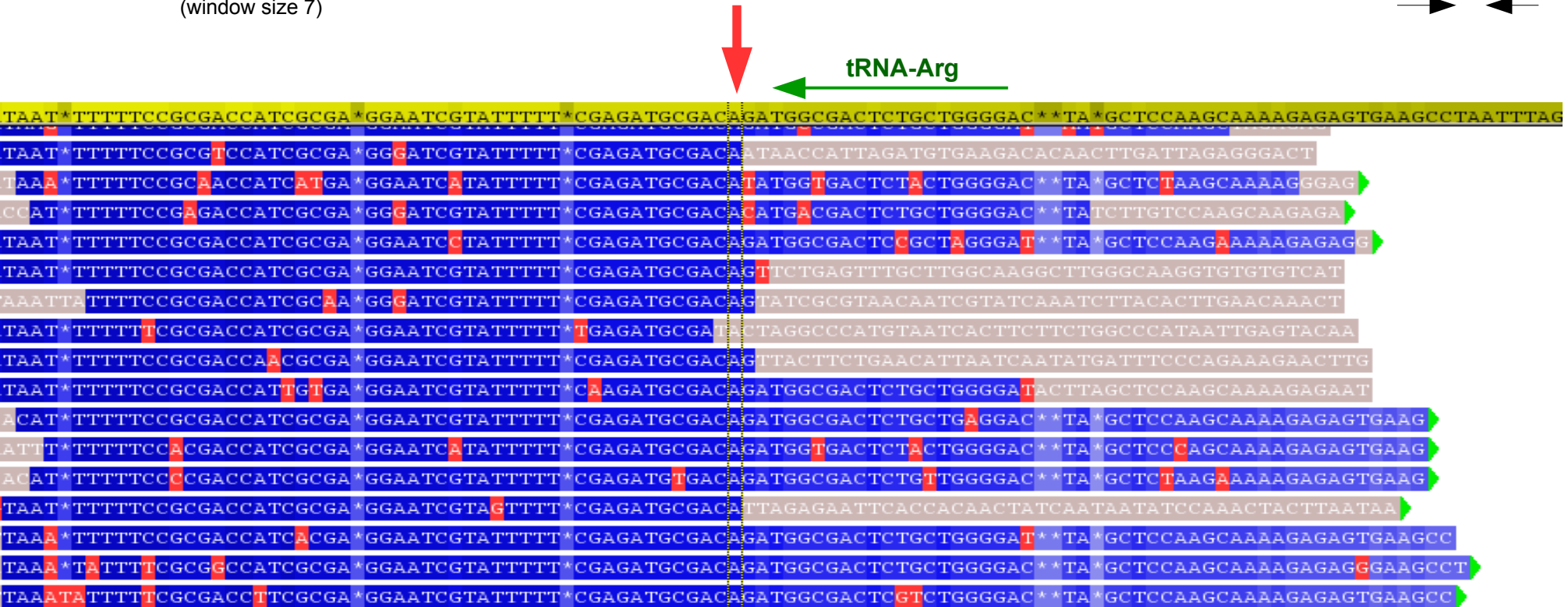
## A tool for detection of LTR / PBS sites



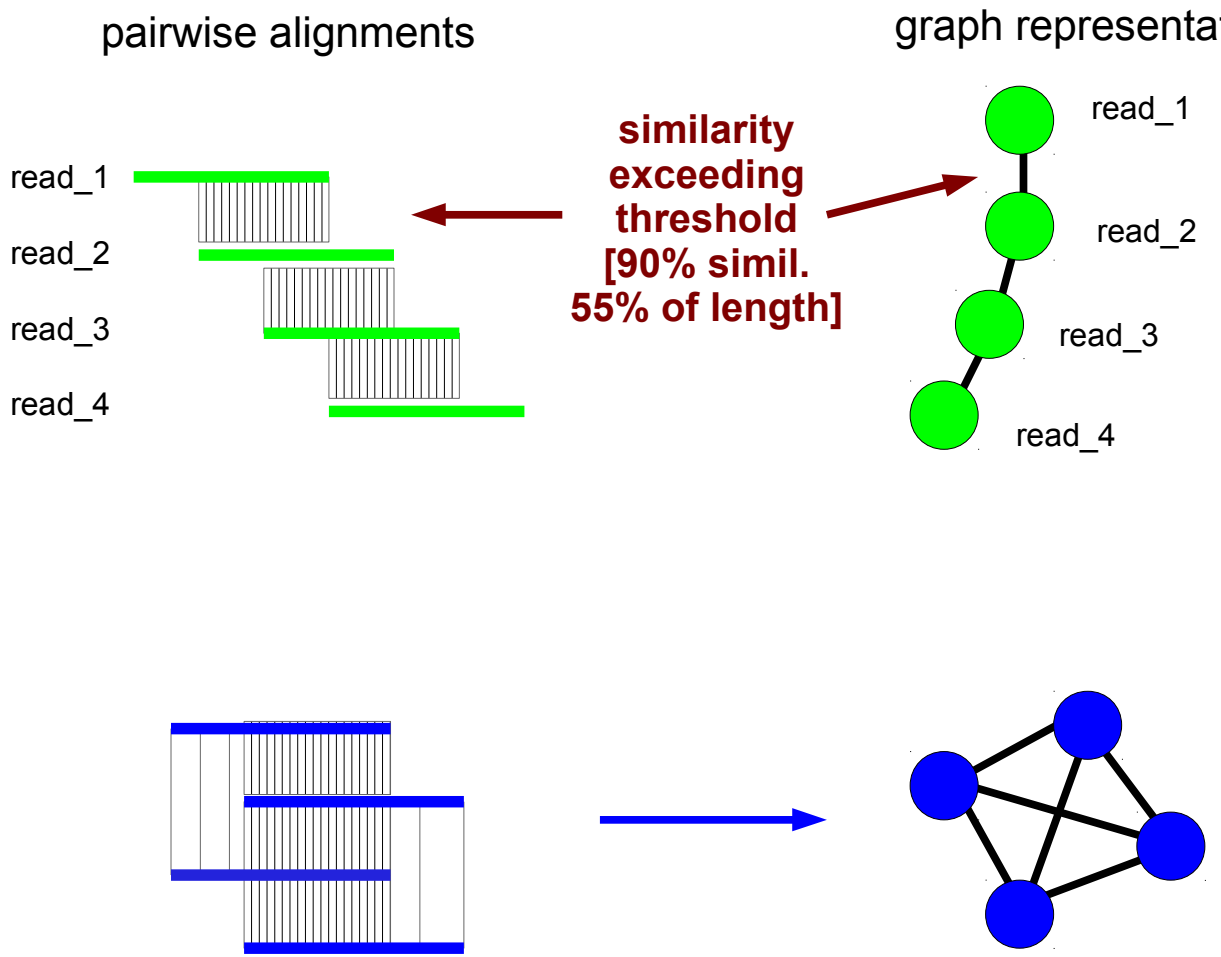

## Program output:

| CL | contig | pos. | site | site_depth | out_ma | maske | maske | region_in | region_out | blast to tRNA | % | length | | | site from | site to | tRNA from | tRNA to | E-val |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 400 | 364 | TGCGA**CA** | 106.6 | 30.4 | 0.0362 | 0.2975 | GCGAGGAA | GATGGCGA | At-chr2.tRNA28-Arg | 100 | 18 | 0 | 0 | 3 | 20 | 23 | 6 | 7E-007 |

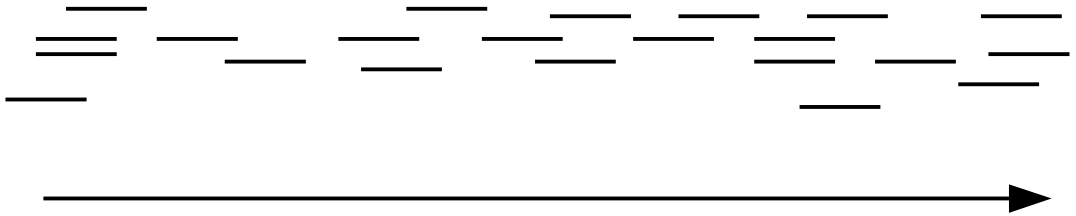
(window size 7)

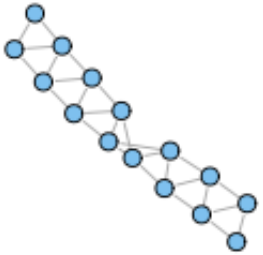


# Graph shapes



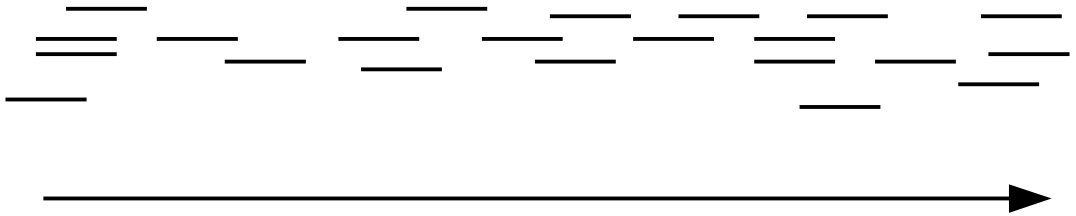
# Linear graphs

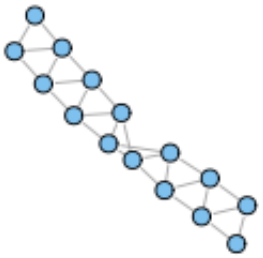




DNA transposons, LINEs, ...

# Linear graphs





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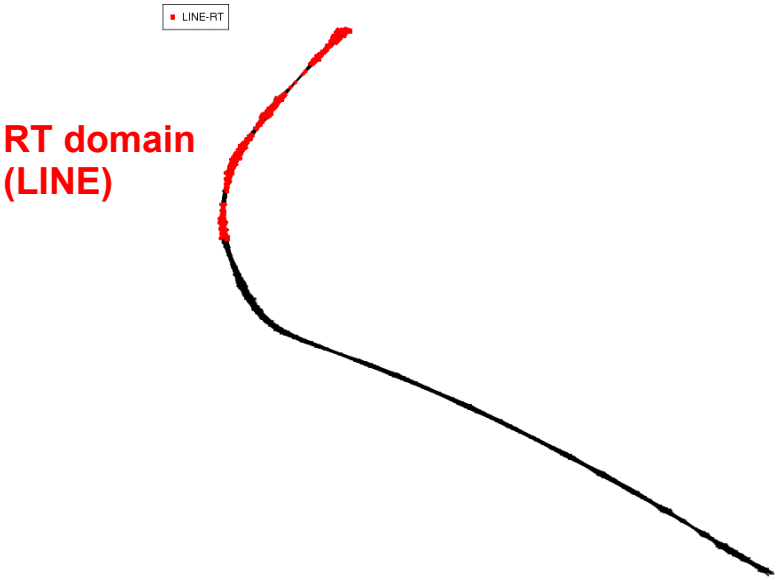
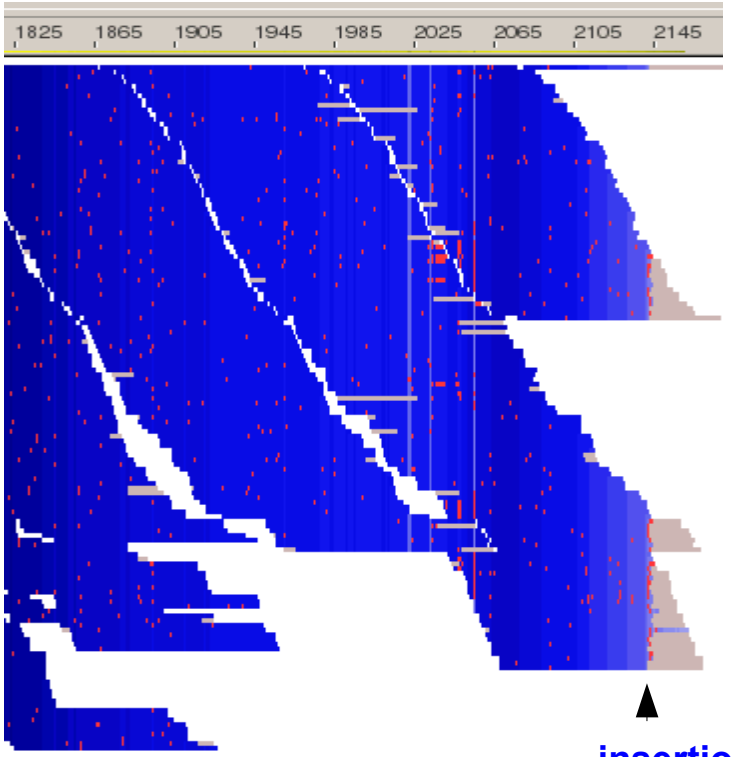


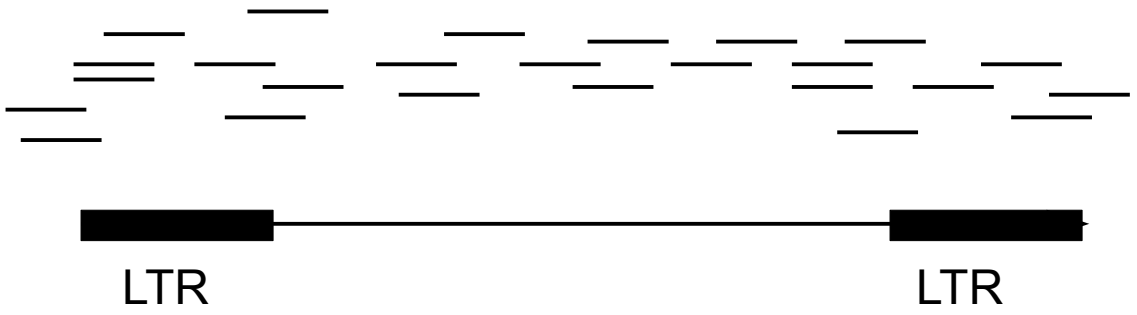

**RT domain (LINE)**

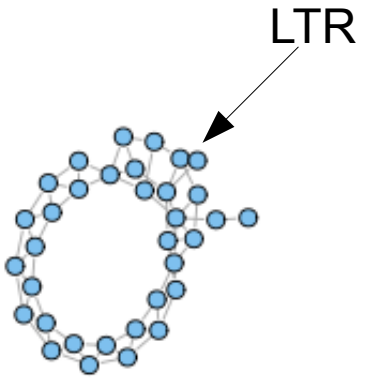



assembled contig

**insertion sites**

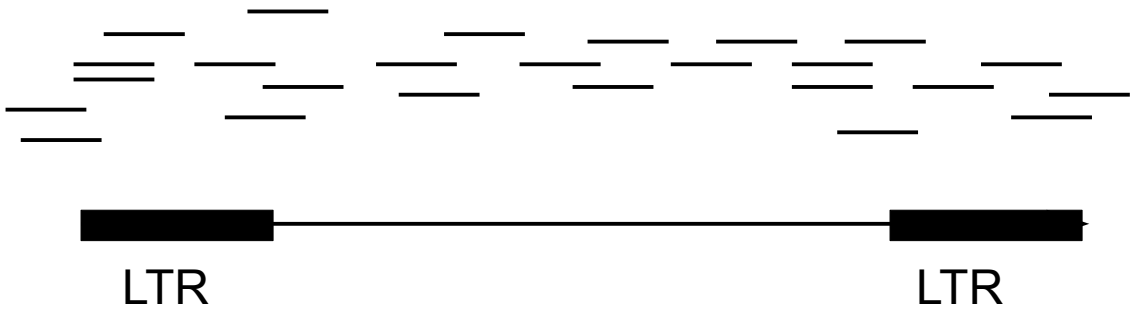
# Linear / large circular graphs

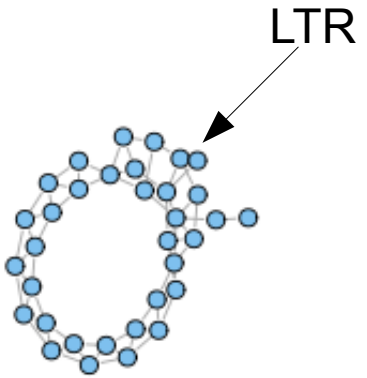


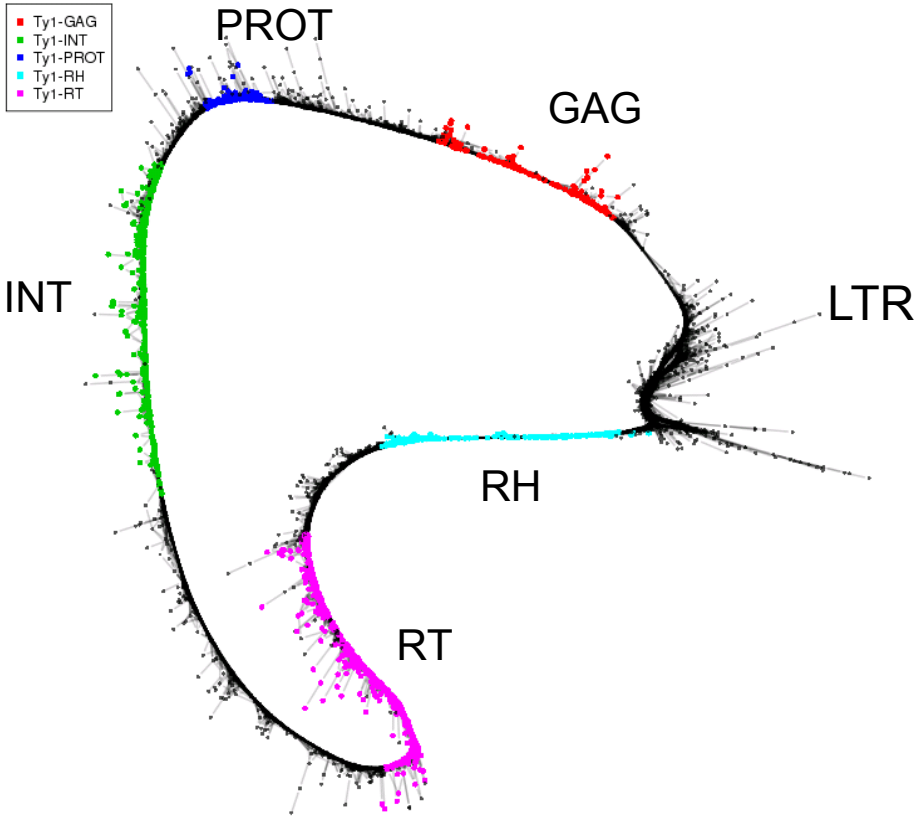

LTR-retrotransposons

# Linear / large circular graphs

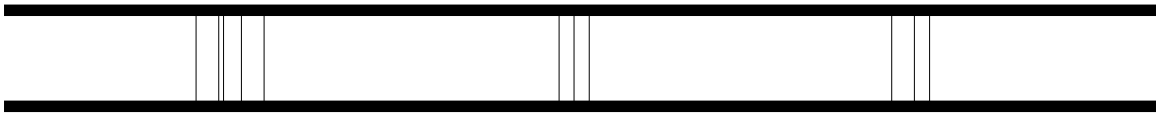


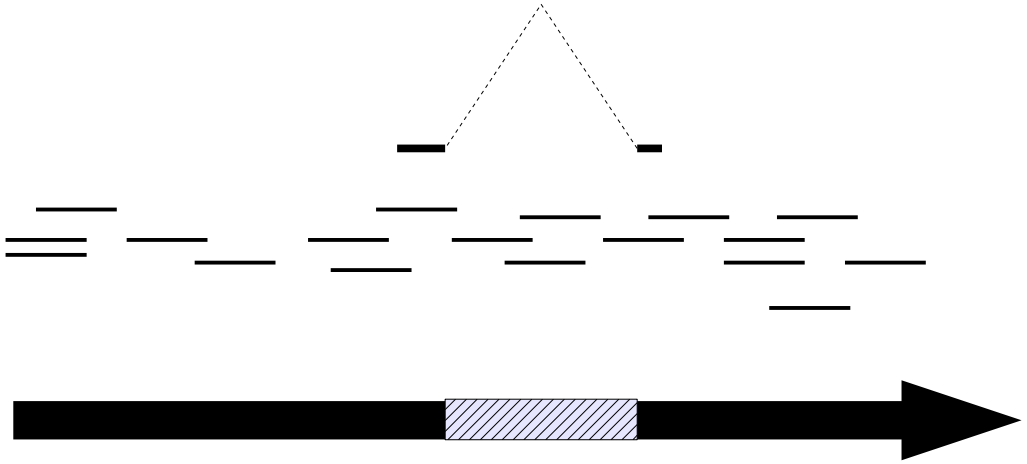

LTR-retrotransposons



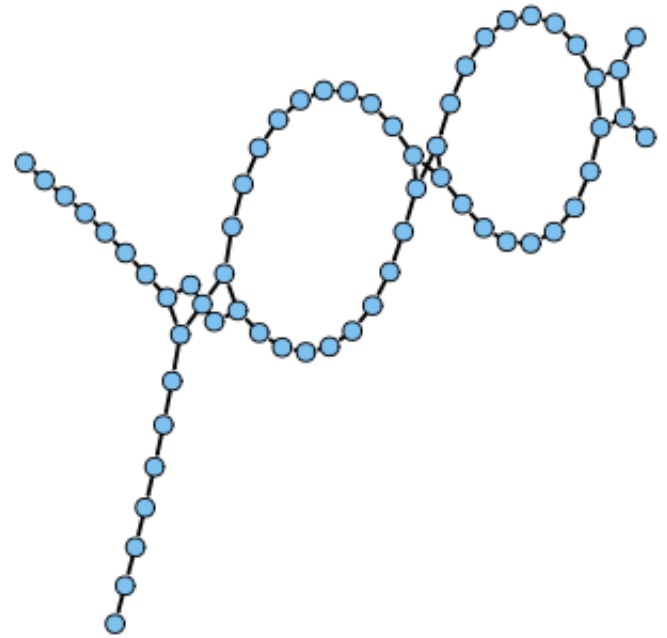
# It's getting complicated...

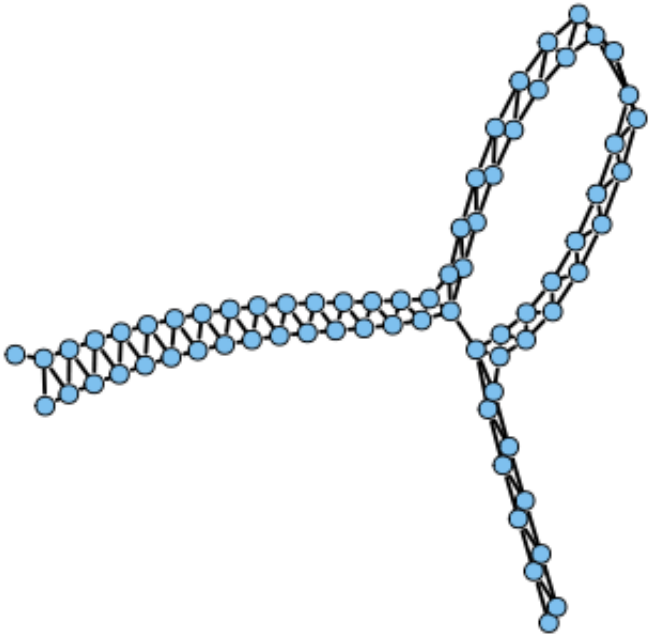


Repeat variants with partial similarity
(depends on similarity threshold)



Variants differing in indel





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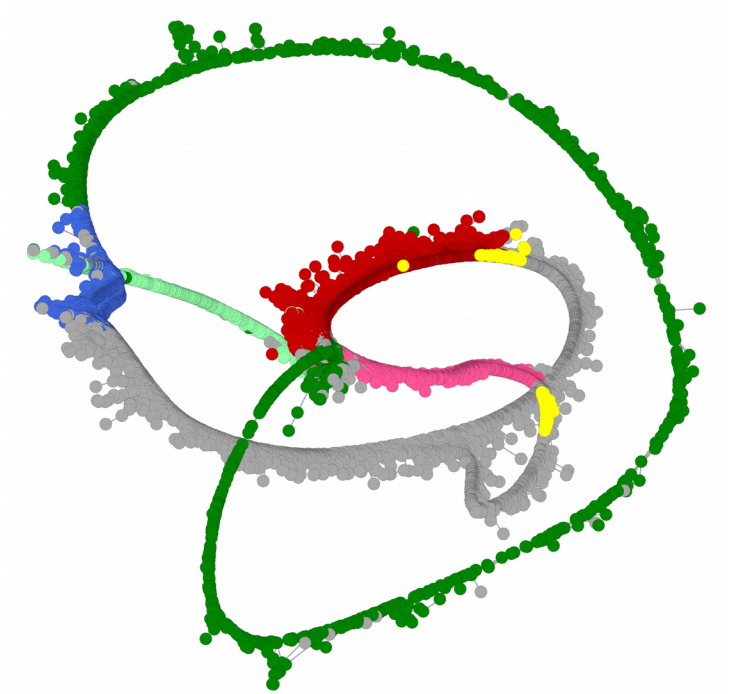
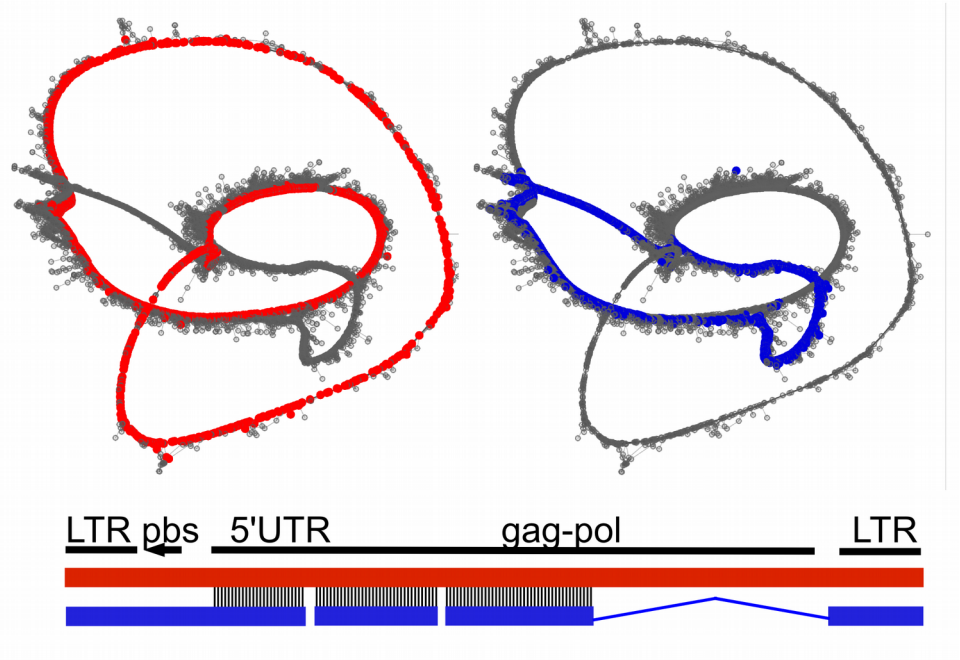



## ...but it has some meaning

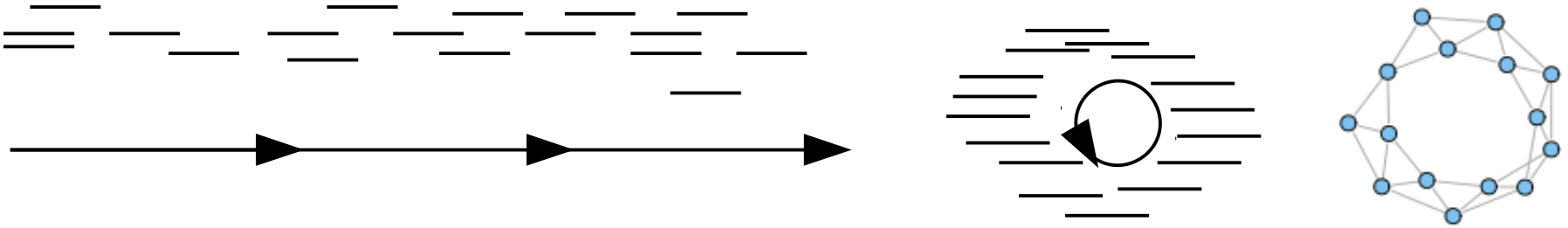
(in this case, presence of element variants differing in LTR sequence and in deletion within gag-pol region)



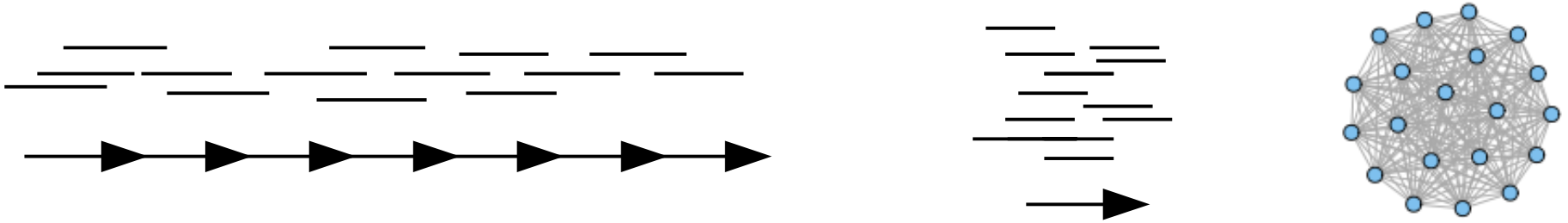
# Circular graphs

## TANDEM REPEATS

Read length << monomer

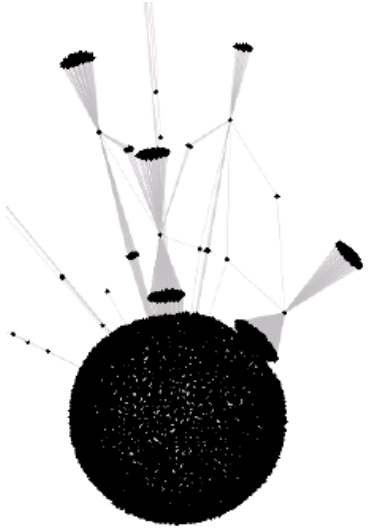


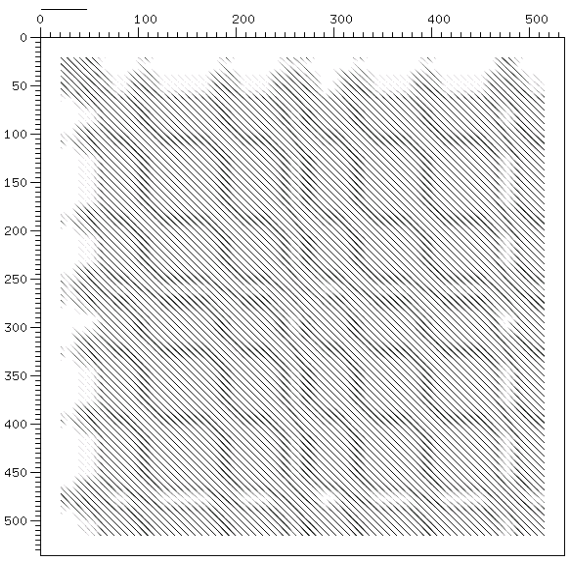
Read length >= monomer



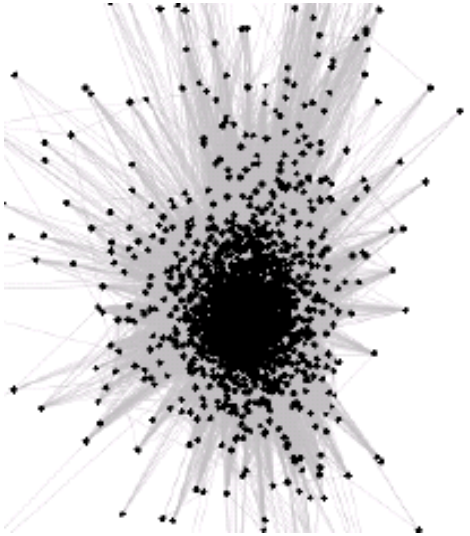
# Circular graphs

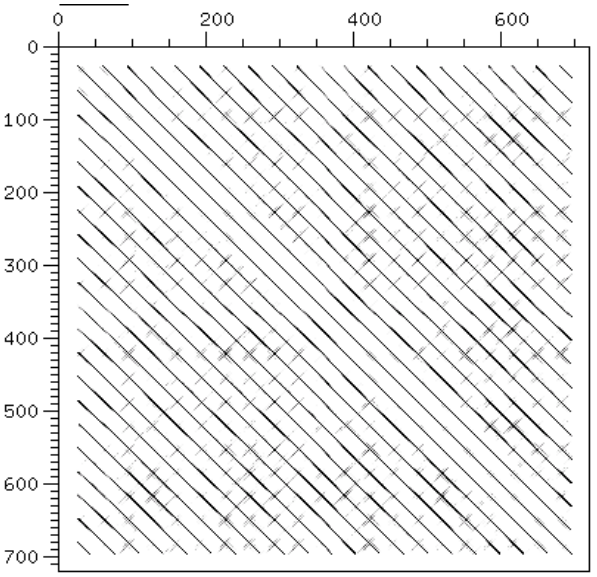
**microsatellite (6bp)**
**(GAACCT)n**



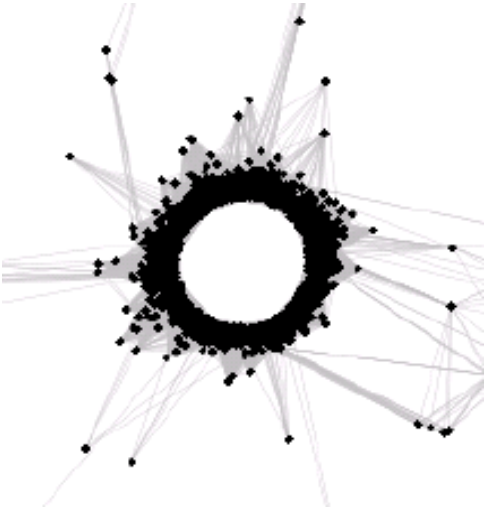


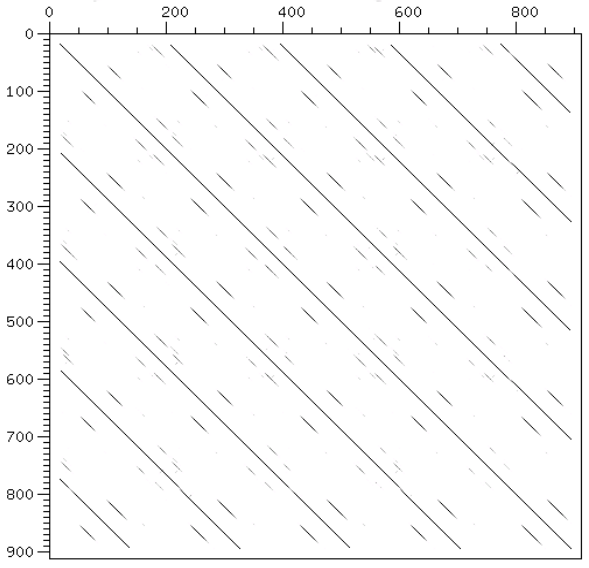

**satellite 35 bp**





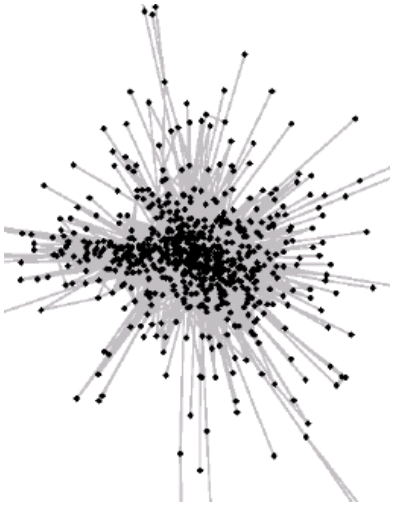

**satellite 190 bp**

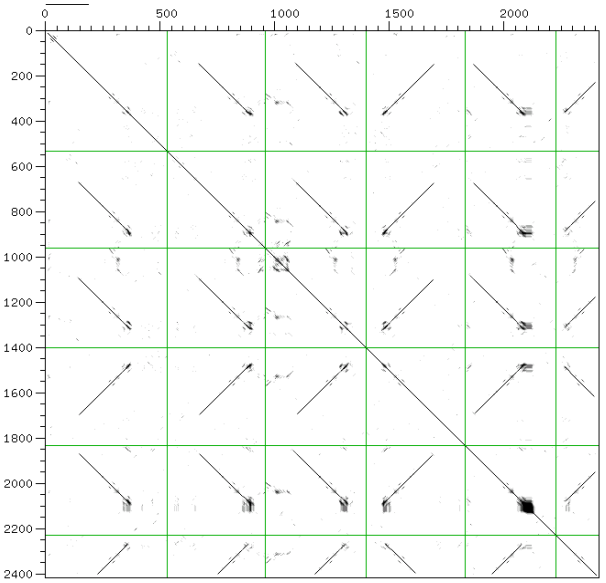




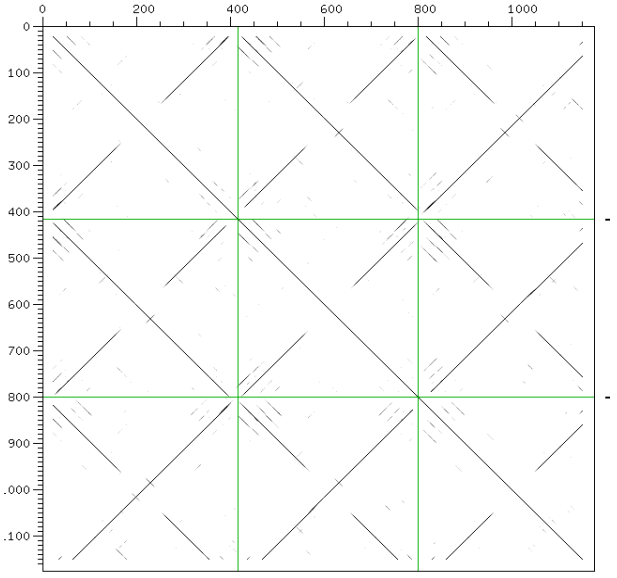
# Circular graphs

**SINE**

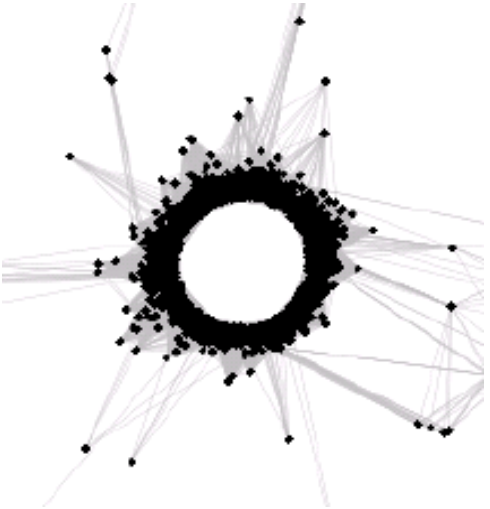


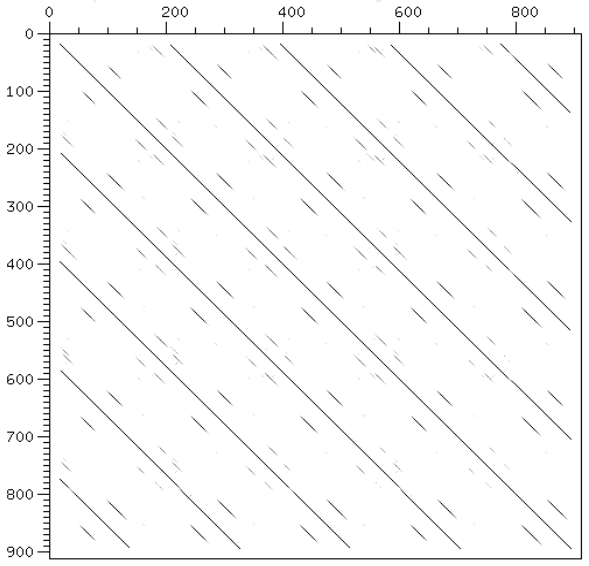



**MITE**
**(foldback)**



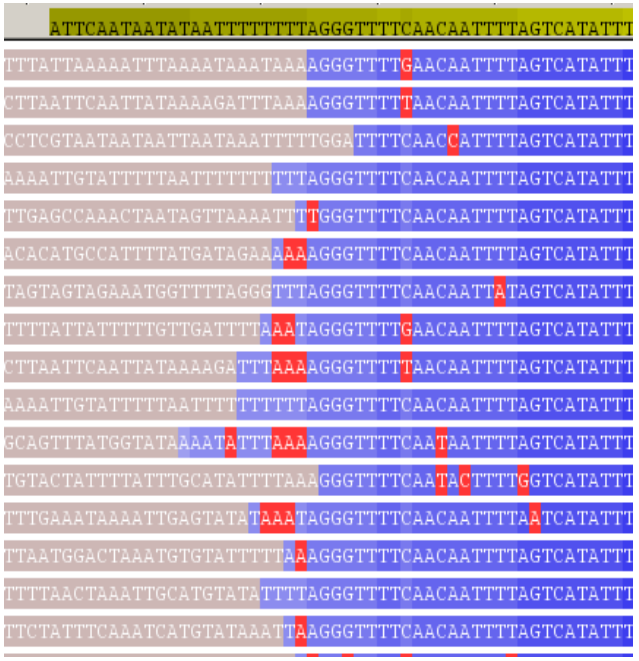

**satellite 190 bp**

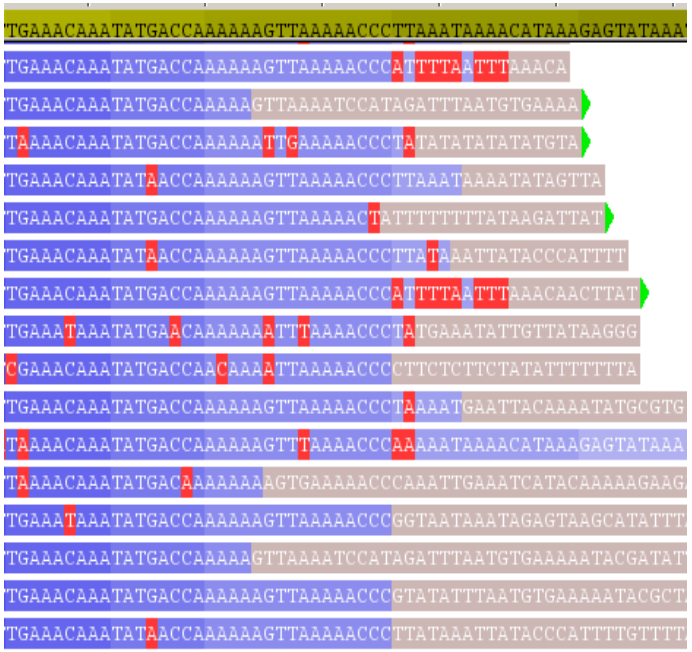


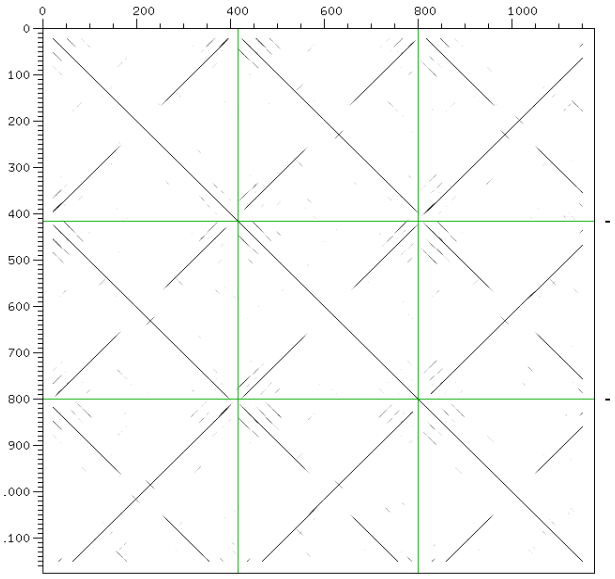


# Insertion sites of mobile elements

**MITE**
**(foldback)**

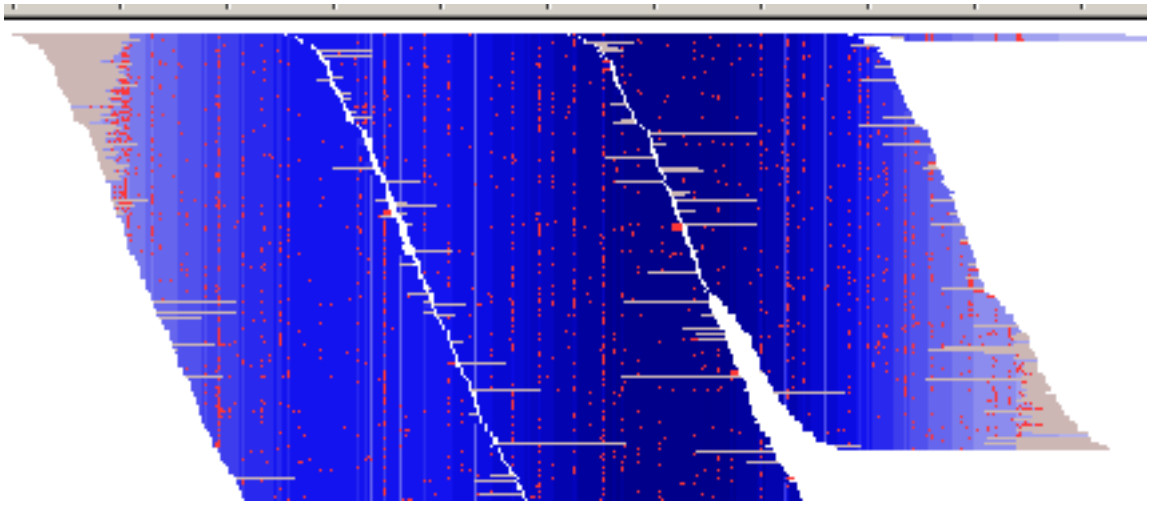







TIR

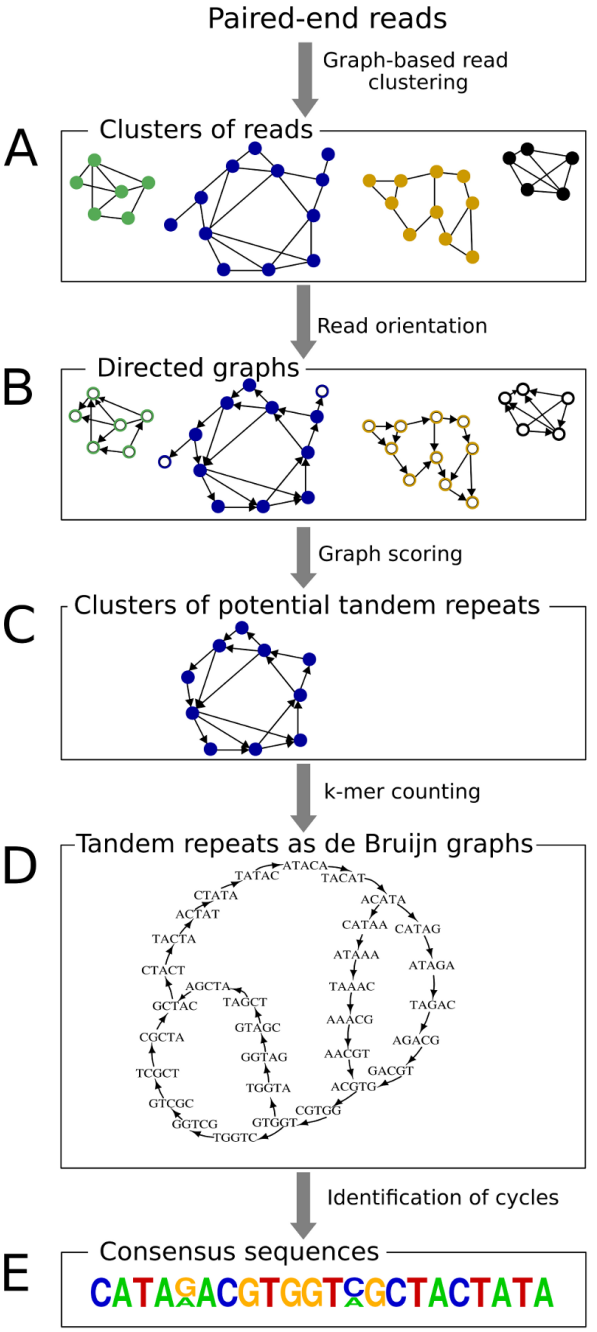
TIR



contig

# TAREAN

- Detects clusters with circular graphs automatically
- Calculates consensus sequences (alignment-free)
- Uses various parameters to distinguish tandem repeats from mobile elements
- *It is recommended to run TAREAN with cluster merging option as a complementary analysis to RepeatExplorer*



# Using *RE* output for repeat annotation and quantification

- Check / correct automatic annotation
- Use all data available from the analysis archive (not just HTML output)
- Interpret the results considering your experimental setup
  - e.g., proportion of reads in clusters *vs.* singlet reads depends on coverage
  - efficiency of automatic annotation depends on organism (taxon, genome composition)

- *More info in our practical tutorials*