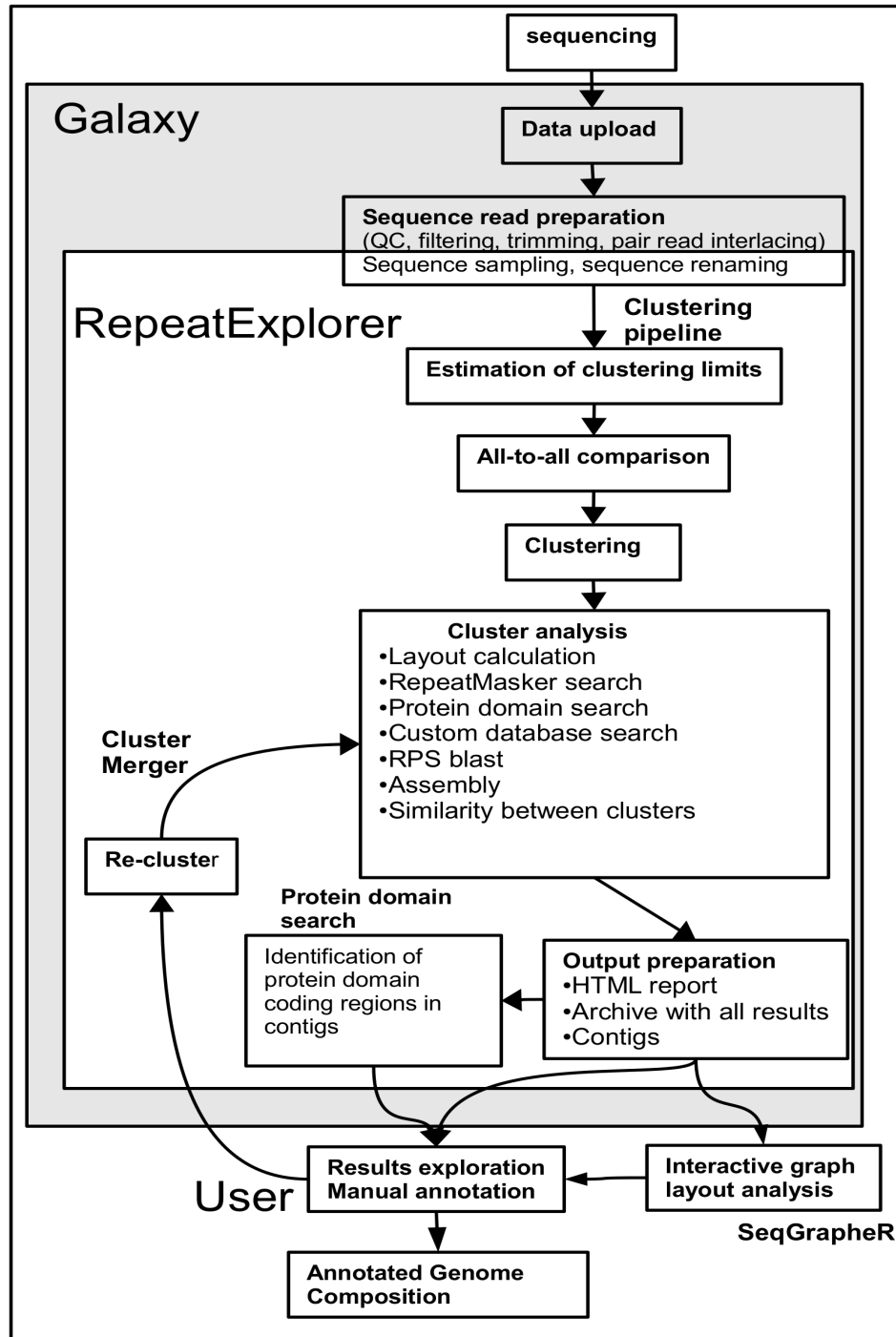


Using *RepeatExplorer* output for repeat
annotation and quantification

RepeatExplorer pipeline



The pipeline combines all principles of repeat identification:

De novo (“repetitiveness”) (graph-based clustering can handle millions of reads)

Structure-based approaches

- shape of cluster graphs
- investigation of assembled contigs

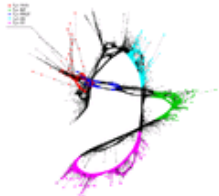
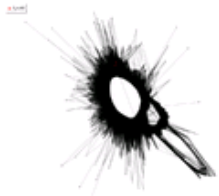
Similarity searches

- protein domains (blastx)
- CDD (RPS-BLAST)
- RepeatMasker

Repeat annotation is achieved by combining all three approaches

Summary table

Top clusters

cluster	total length [bp]	number of reads	Genome proportion[%]	cumulative GP [%]	Repeat Masker	Domain hits	Layout	All missing mates [%]	Missing mates with no similarity hit [%]	Portion of similarity hits to other clusters[%]	Outside reads with similarity [%]
1 CL1	1611900	16119	18.00	18	rRNA (8531hits, 50.7%) DNA.MULE.MuDR. (368hits, 2.08%) LTR.Gypsy (7hits, 0.0161%) Satellite (1hits, 0.0023%)			4.746	4.746	0.00000	0.000
2 CL2	988500	9885	11.10	29	LTR.Copia (5818hits, 51.2%) Low_complexity (50hits, 0.202%) Simple_repeat (51hits, 0.168%) LTR.Gypsy (6hits, 0.0245%)	Ty1-RT Ty1/copia Ivana/Oryco (1346 hits 13.6%) Ty1-PROT Ty1.....		9.944	9.924	0.00000	0.000
3 CL3	922400	9224	10.30	39	LTR (2524hits, 19.1%) Satellite (312hits, 2.28%) LTR.Gypsy (54hits, 0.378%) DNA.CMC.EnSpm (63hits, 0.287%) DNA.hAT.Tip100 (3hits, 0.0194%)	Ty3-RT Ty3/gypsy chromovirus (2 hits 0.0217%)		25.520	25.520	0.00012	0.043
4 CL4	900800	9008	10.10	50	Low_complexity (340hits, 1.23%) LTR.Copia (100hits, 0.502%) Simple_repeat (1hits, 0.00422%) LTR.Gypsy (1hits, 0.00389%)	Ty3-RT Ty3/gypsy Ogre/Tat (1 hits 0.0111%)		6.083	6.083	0.00000	0.000

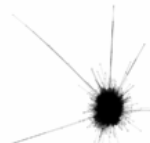
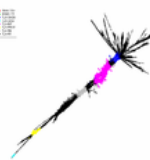
Summary table – new features

Automatic Classification Summary

	repeat_name	Content [%]	number_of_clusters
1	ltr/copia/unspecified	0.515	3
2	ltr/copia/maximus	2.280	8
3	ltr/copia/tork	0.260	1
4	ltr/copia/angela	0.155	1
5	ltr/copia/ivana	0.268	1
6	ltr/gypsy/unspecified	3.200	7
7	ltr/gypsy/chromo	5.080	8
8	ltr/gypsy/ogre_tat	40.500	66
9	ltr/gypsy/athila	1.010	3

Automatic classification / super-clusters are not yet available in the public RE

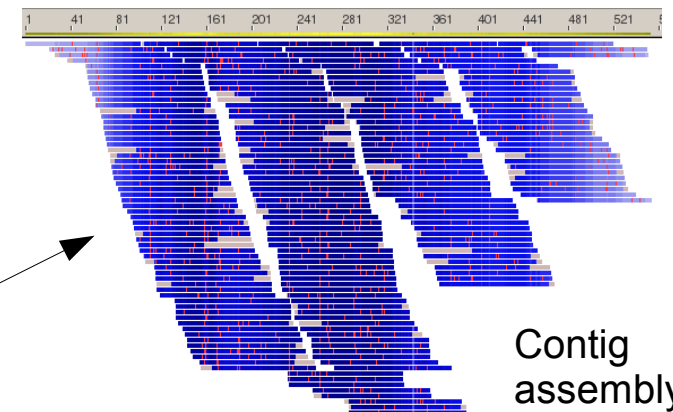
Top clusters

cluster	total length [bp]	number of reads	Genome proportion[%]	cumulative GP [%]	Automatic classification	Super cluster	Repeat Masker	Protein domain hits	blastn hits	Layout	Repeat Masker custom library	group - number of reads	All missing mates [%]	Missing mates with n similar hit [%]											
1	CL1	3670900	36709	1.900	1.9	nd	1	Satellite (34392hits, 86.3%) Low_complexity (45hits, 0.0331%) LINE.Penelope (17hits, 0.0283%) DNA (5hits, 0.00627%) Simple_repeat (3hits, 0.00204%) LTR.Gypsy (1hits, 0.00185%) RC.Helitron (1hits,.....		organelle/plastid (36 hits 0.0981%)		Satellite.VicTR.A (31921hits, 82.3%) Telomeric.Arabidopsis (19hits, 0.031%)	<table><tr><th>group</th><th>content</th></tr><tr><td>VSA_</td><td>0 (0%)</td></tr><tr><td>VSP2</td><td>0 (0%)</td></tr><tr><td>VPN_</td><td>15853 (2.77%)</td></tr><tr><td>VML_</td><td>20856 (2.59%)</td></tr></table>	group	content	VSA_	0 (0%)	VSP2	0 (0%)	VPN_	15853 (2.77%)	VML_	20856 (2.59%)	3.10	0.62
group	content																								
VSA_	0 (0%)																								
VSP2	0 (0%)																								
VPN_	15853 (2.77%)																								
VML_	20856 (2.59%)																								
2	CL2	3232900	32329	1.670	3.6	ltr/gypsy /chromo	2	LTR.Gypsy (13535hits, 38.9%) LTR.Gypsy.peabody (9877hits, 28.3%) Low_complexity (1453hits, 1.7%) LTR (88hits, 0.224%) Simple_repeat (144hits, 0.184%) LTR.gypsy (29hits, 0.0663%) Organelle.....	Ty3-INT Ty3/gypsy chromovirus (7349 hits 22.7%) Ty3-RT Ty3/gypsy chromovirus (4605 hits 14.2%) Ty3-RH Ty3/gypsy chromovirus (2807 hits 8.68%) Ty3-CHDII Ty3/gypsy chromovirus (725 hits 2.24%) Ty3-PROT Ty3/gyps.....	organelle/mitochondria (13 hits 0.0402%) organelle/plastid (1 hits 0.00309%)		LTR.Gypsy.peabody (15124hits, 43.8%) Organelle.Mitochondrion (194hits, 0.484%) LTR.Gypsy.centromeric (65hits, 0.152%) LTR.Gypsy.Ogre (2hits, 0.00424%) LTR.Gypsy (1hits, 0.00294%) Organelle.Plastid (.....	<table><tr><th>group</th><th>content</th></tr><tr><td>VSA_</td><td>2832 (1.6%)</td></tr><tr><td>VSP2</td><td>8141 (2.17%)</td></tr><tr><td>VPN_</td><td>7966 (1.39%)</td></tr><tr><td>VML_</td><td>13390 (1.66%)</td></tr></table>	group	content	VSA_	2832 (1.6%)	VSP2	8141 (2.17%)	VPN_	7966 (1.39%)	VML_	13390 (1.66%)	15.20	5.40
group	content																								
VSA_	2832 (1.6%)																								
VSP2	8141 (2.17%)																								
VPN_	7966 (1.39%)																								
VML_	13390 (1.66%)																								
3	CL3	3192600	31926	1.650	5.2	ltr/gypsy /ogre_tat	3	LTR.Gypsy (1551hits, 3.57%) LTR.Gypsy.Ogre (1614hits, 3.37%) Low_complexity (695hits, 0.803%) Simple_repeat (67hits, 0.113%)	DTA-CD1 NA NA (1 hits 0.00313%) Ty3-INT Ty3/gypsy chromovirus (1 hits 0.00313%)		LTR.Gypsy.Ogre (2281hits, 4.76%) LTR.Gypsy.Ogre_PA (47hits, 0.0929%) LTR.Gypsy.peabody (3hits, 0.00417%) LTR.Gypsy (2hits, 0.00244%) Satellite.VicTR.A (2hits, 0.00417%)	<table><tr><th>group</th><th>content</th></tr><tr><td>VSA_</td><td>1809 (1.02%)</td></tr><tr><td>VSP2</td><td>1822 (0.487%)</td></tr><tr><td>VPN_</td><td>3838 (0.67%)</td></tr></table>	group	content	VSA_	1809 (1.02%)	VSP2	1822 (0.487%)	VPN_	3838 (0.67%)	17.87	6.06			
group	content																								
VSA_	1809 (1.02%)																								
VSP2	1822 (0.487%)																								
VPN_	3838 (0.67%)																								

Detailed information for the largest clusters

RepeatExplorer output files

The screenshot shows the RepeatExplorer file explorer. The left pane displays a directory tree with 'PST_max' at the root, containing 'cluster_merges', 'seqClust', 'assembly', 'blastx', 'clustering', and 'clusters'. The 'clusters' folder is expanded, showing subfolders 'dir_CL0001' through 'dir_CL0012'. 'dir_CL0004' is selected and highlighted in green. The right pane shows the contents of 'dir_CL0004', listing files such as 'blast', 'CL4_html', 'connections', 'ACE_CL4.ace', 'CL0004_blastx.csv', 'CL0004_domains.csv', 'CL4.GL', 'contigs_CL4', 'contigs_CL4.minRD5', 'contigs_CL4.minRD5_sort-GR', 'contigs_CL4.minRD5_sort-length', 'contigs_CL4.prof', 'contigs_CL4.prof.pdf', 'reads.fas', 'reads.ids', 'RM-custom_output_table', 'RM-custom_reads.fas.out', 'RM-custom_reads.fas.tbl', 'RM-reads.fas.out', 'RM-reads.fas.tbl', and 'RM_output_table'.



Contig assembly

Protein domains

Domain	ID	Type	Lineage	Hits	MeanScore
Ty3-INT	MedT2_ID89	Ty3/gypsy	chromovirus	96	49.7
Ty3-INT	PopT1_ID21	Ty3/gypsy	chromovirus	74	46.7
Ty3-RT	PetI1_ID72	Ty3/gypsy	chromovirus	71	60.4
Ty3-RT	CRR3_ID17	Ty3/gypsy	chromovirus	56	48.4
Ty3-GAG	PetI1_ID72	Ty3/gypsy	chromovirus	49	35.5
Ty3-INT	MedT1_ID93	Ty3/gypsy	chromovirus	42	58.8
Ty3-INT	PopT2_ID20	Ty3/gypsy	chromovirus	41	45.3
Ty3-PROT	lpoB1_ID87	Ty3/gypsy	chromovirus	37	45.8
Ty3-PROT	PetI1_ID72	Ty3/gypsy	chromovirus	36	45.9
Ty3-RH	BraR5_ID117	Ty3/gypsy	chromovirus	31	57.7
Ty3-INT	CRA2a_ID5	Ty3/gypsy	chromovirus	31	53.4
Ty3-GAG	PetI2_ID76	Ty3/gypsy	chromovirus	30	35.0

Similarity searches

SW score	perc div.	perc del.	perc ins.	query sequence	position begin	position end	query (left)	matching repeat	repeat class/family	position begin	position end	repeat (left)	ID
602	17.0	0.0	0.0	SJM_1000031f	1	100	(0)	C Gypsy-33_ST-I-int	LTR/Gypsy	(4879)	1441	1342	1
256	14.3	0.0	0.0	SJM_1000031r	1	42	(58)	+ Gypsy-33_ST-I-int	LTR/Gypsy	1224	1265	(5055)	2 *
581	6.8	0.0	0.0	SJM_1000031r	28	100	(0)	+ Gypsy-33_ST-I-int	LTR/Gypsy	1197	1269	(5051)	2
885	3.0	0.0	0.0	SJM_1000270f	1	100	(0)	+ Gypsy-33_ST-I-int	LTR/Gypsy	3002	3101	(3219)	3
802	5.0	5.0	0.0	SJM_1000270r	1	100	(0)	C Gypsy-33_ST-I-int	LTR/Gypsy	(2983)	3337	3233	4
680	11.0	0.0	0.0	SJM_1002242f	1	100	(0)	+ Gypsy-33_ST-I-int	LTR/Gypsy	2328	2427	(3893)	5

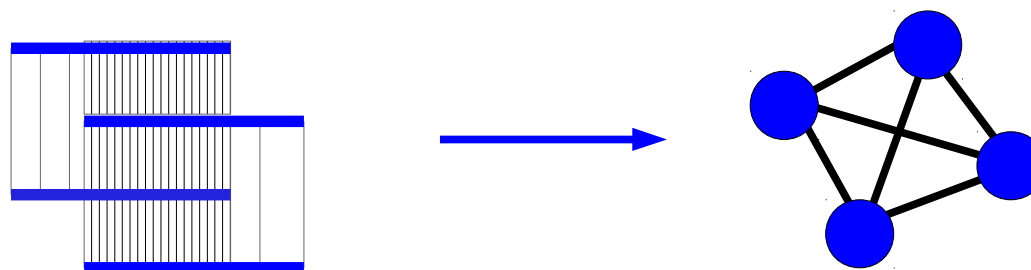
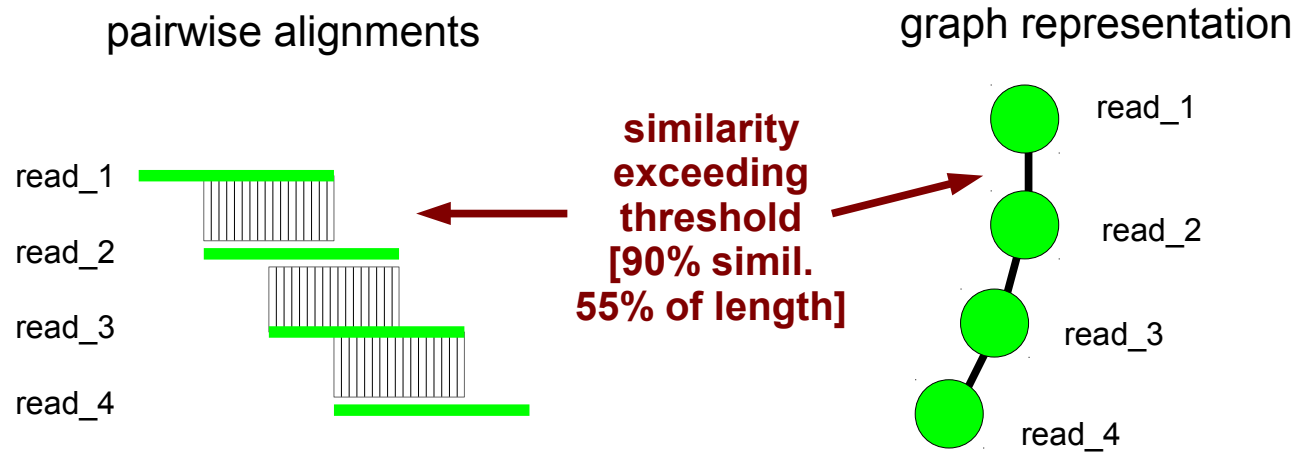
Repeat annotation

For repeat annotation in individual clusters, consider:

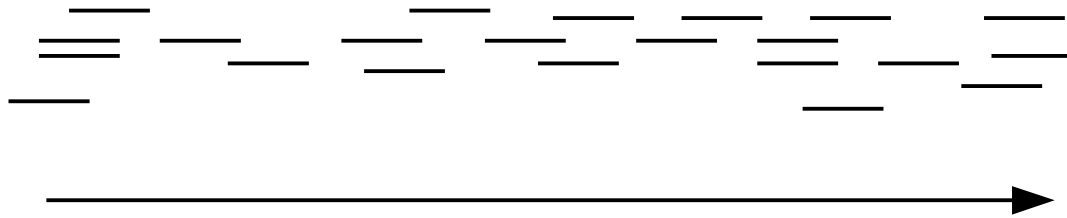
- Shape of cluster graph
- Significant similarity to conserved types of repeats (45S and 5S rDNA, *cpDNA*)
- Similarity to conserved protein domains
- Structural features of assembled contigs (dot-plot)
- Variability and masked regions of reads in contig assembly
- (blastn and blastx to GenBank)
- Cluster connections via paired-end reads
- LTR + primer binding site (PBS) detection
- **New !** Automated classification

Be prepared to leave some clusters without annotation !

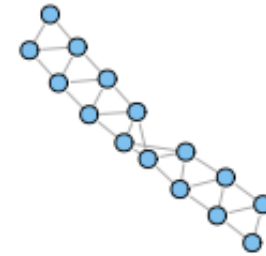
Graph shapes



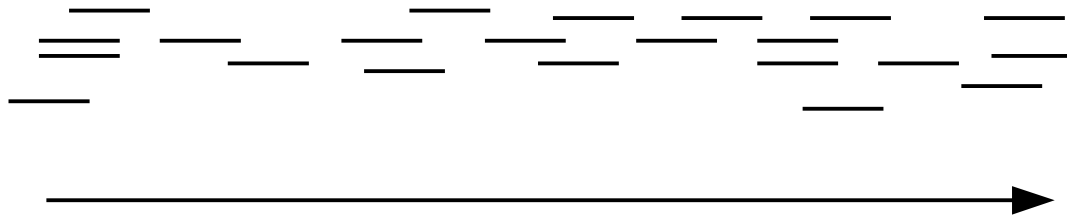
Linear graphs



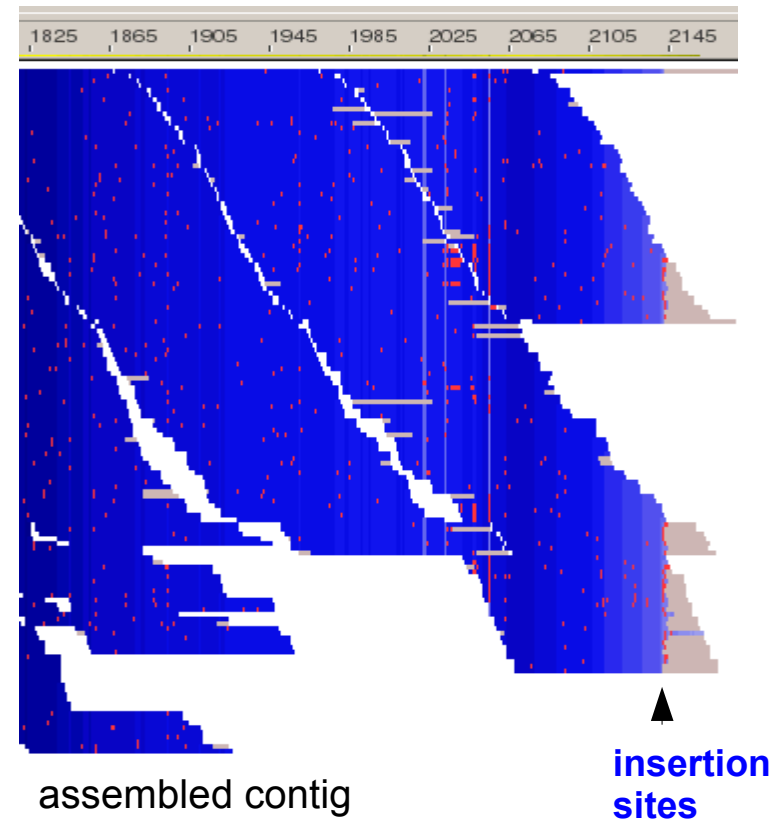
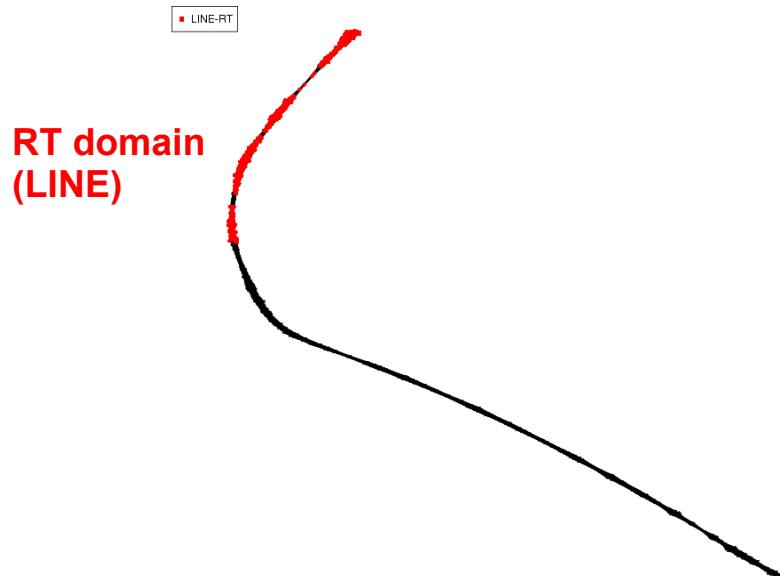
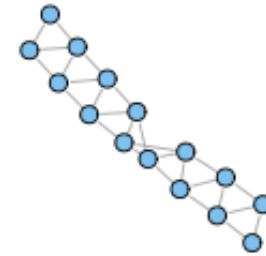
DNA transposons, LINEs, ...



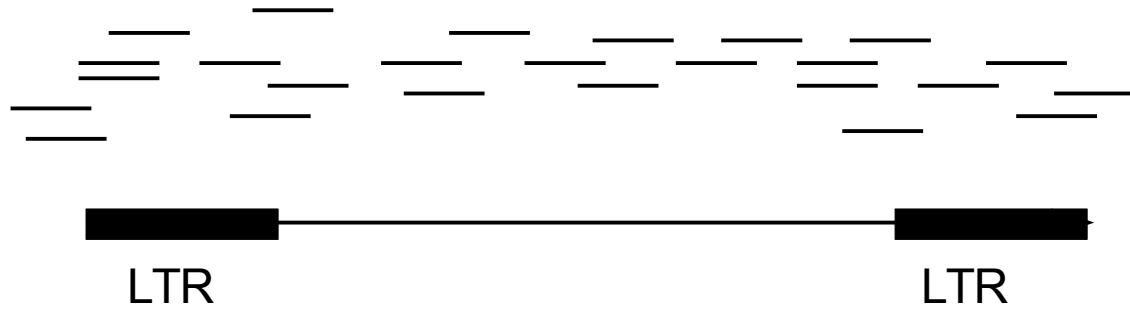
Linear graphs



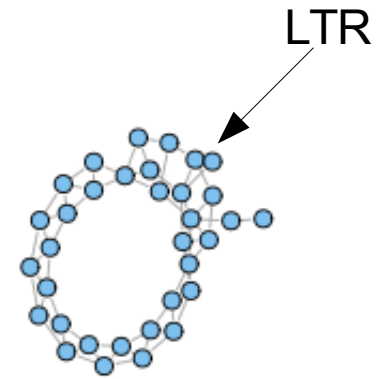
DNA transposons, LINEs, ...



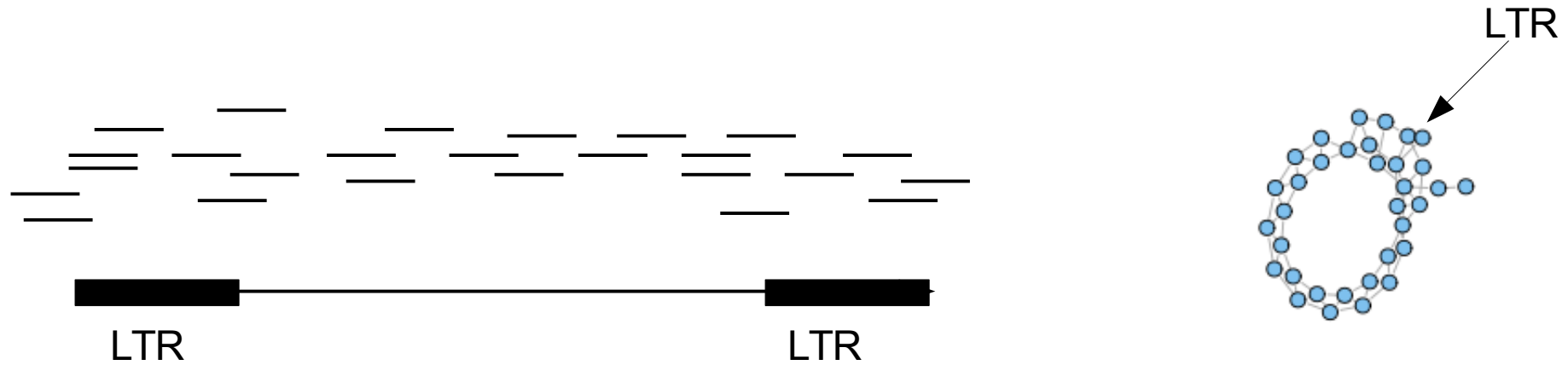
Linear / large circular graphs



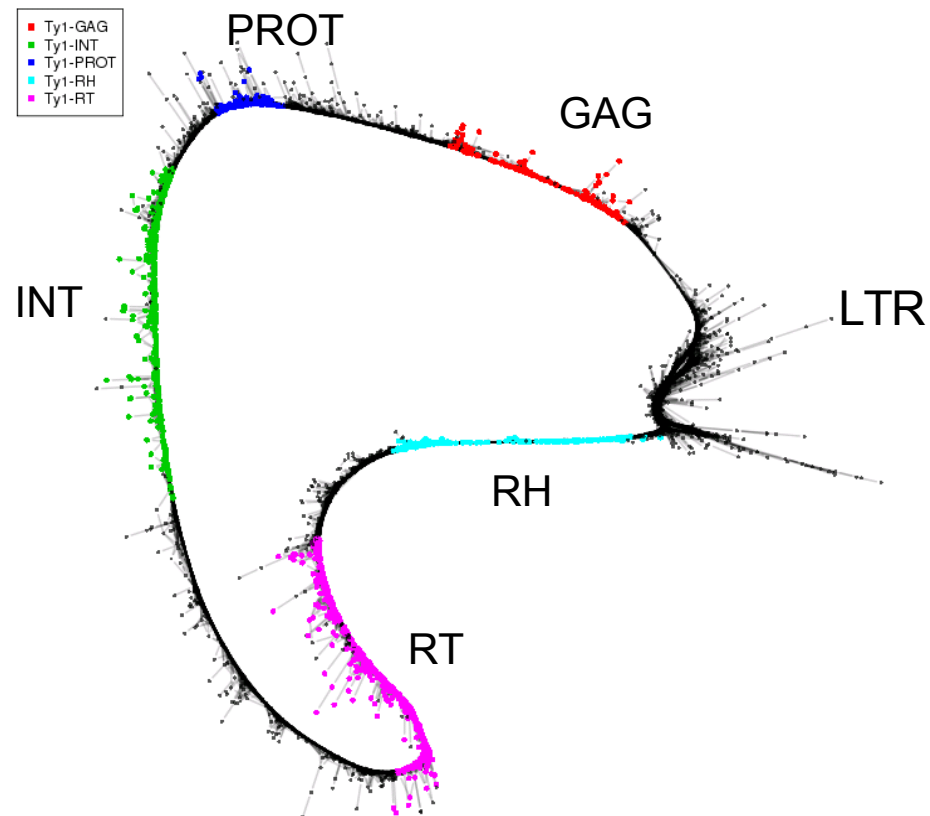
LTR-retrotransposons



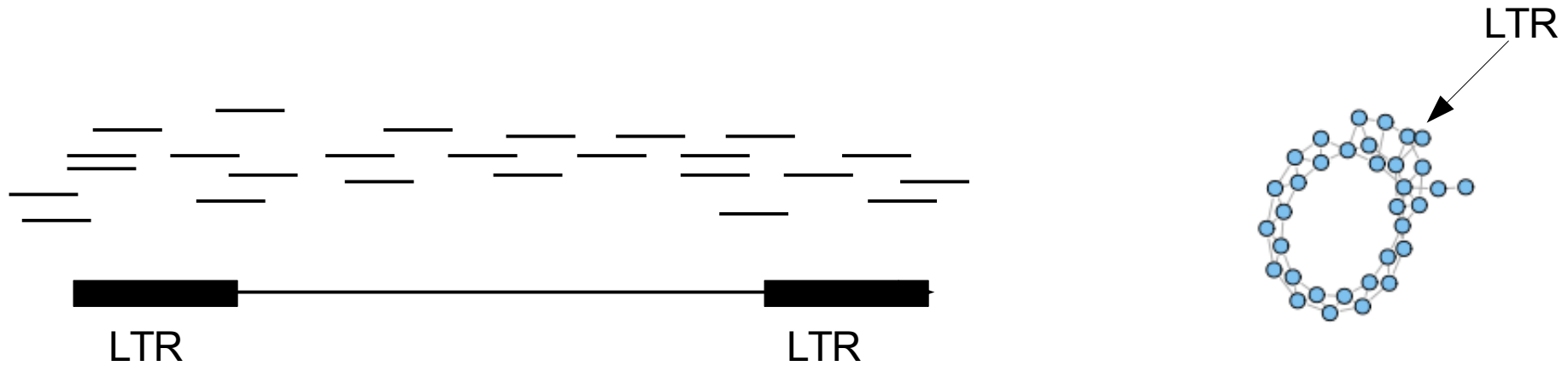
Linear / large circular graphs



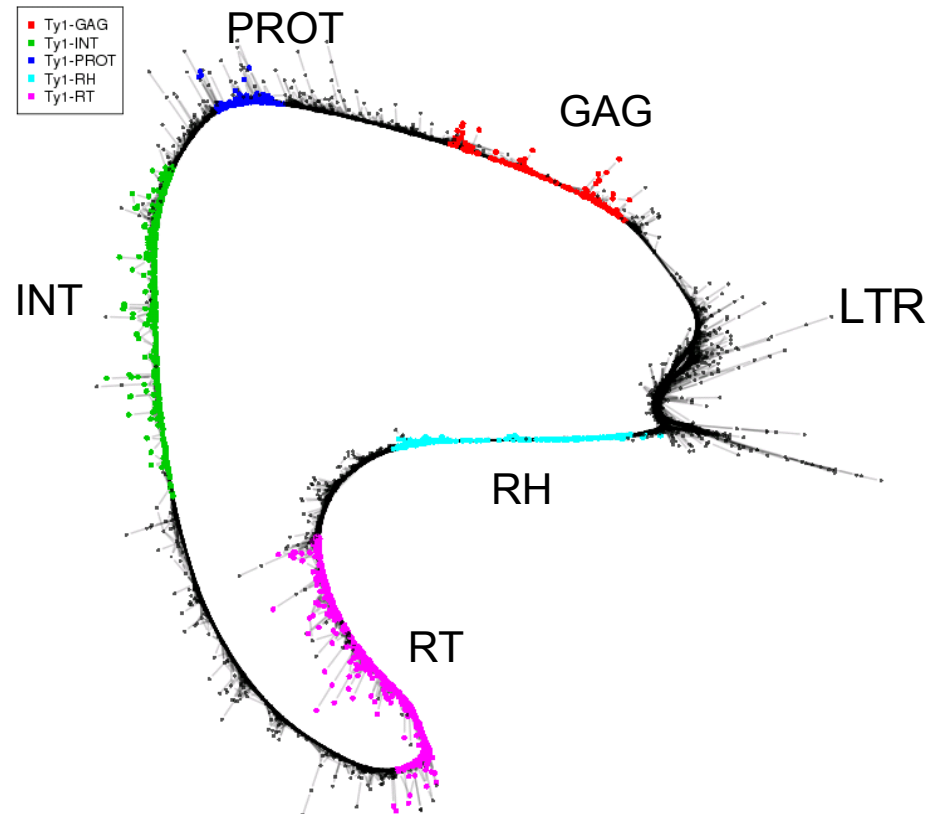
LTR-retrotransposons



Linear / large circular graphs



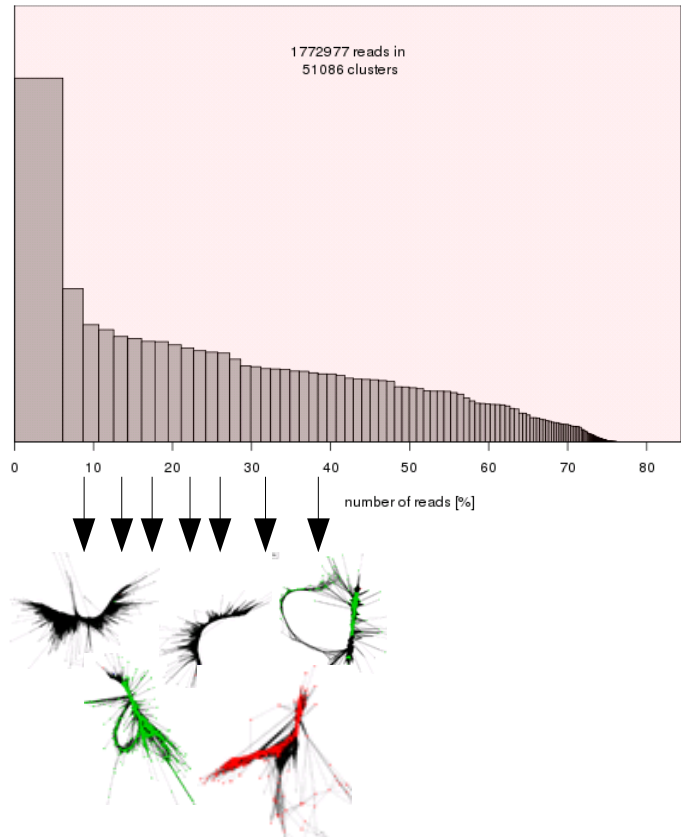
LTR-retrotransposons



Cluster fragmentation
leads to linear graphs !

Linear / large circular graphs

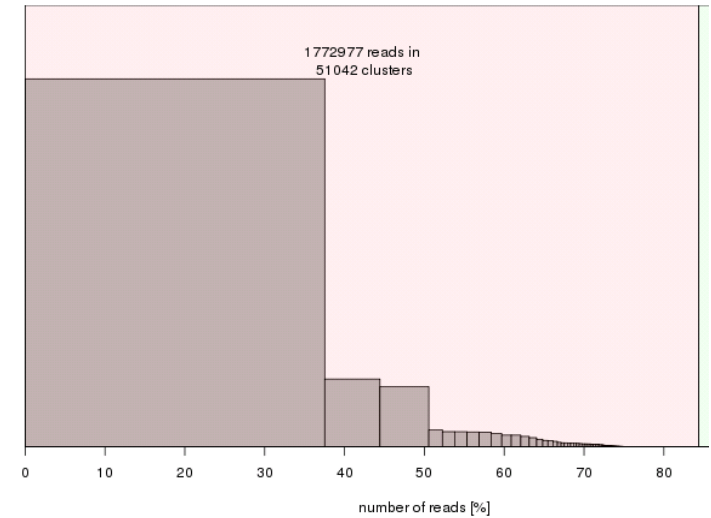
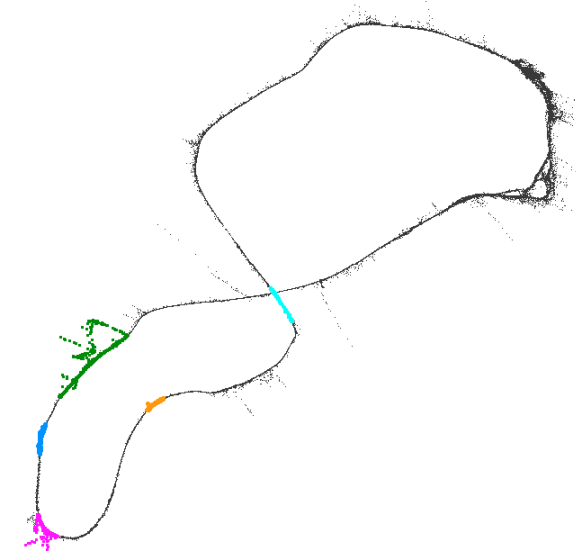
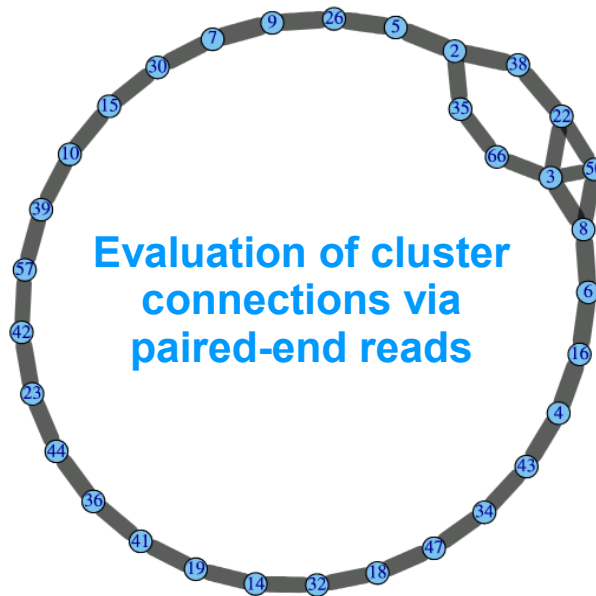
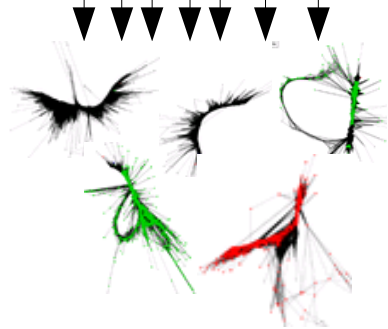
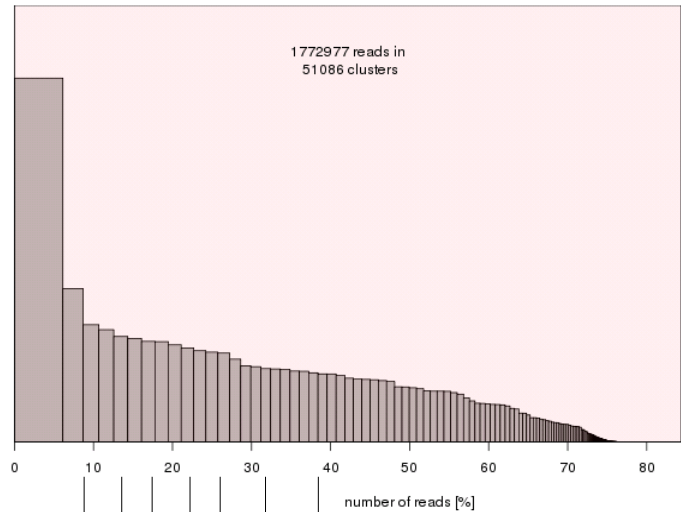
Cluster fragmentation leads to linear graphs !



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

Linear / large circular graphs

Cluster fragmentation leads to linear graphs !

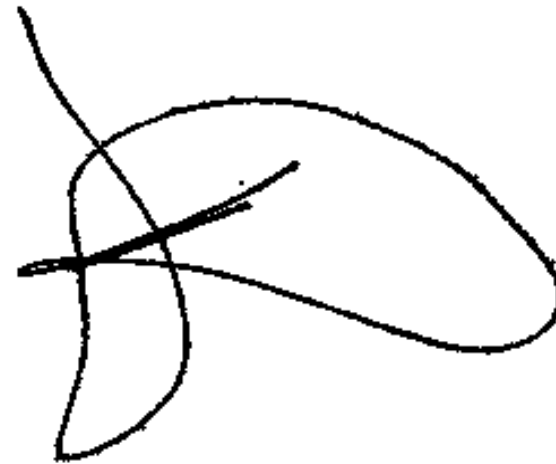


Linear / large circular graphs

Cluster fragmentation leads to linear graphs !

rDNA (2045hits, 80.8%)
rDNA.18S (363hits, 14.4%)
X25SrDNA (84hits, 3.31%)
rDNA.26S (5hits, 0.198%)

45S rDNA

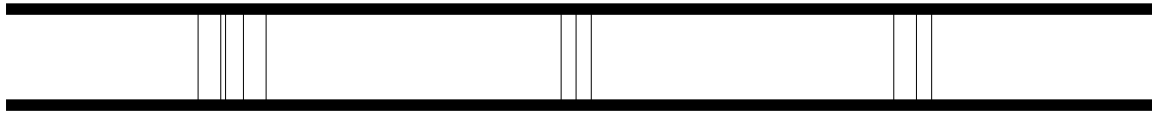


plastid DNA
(contamination)

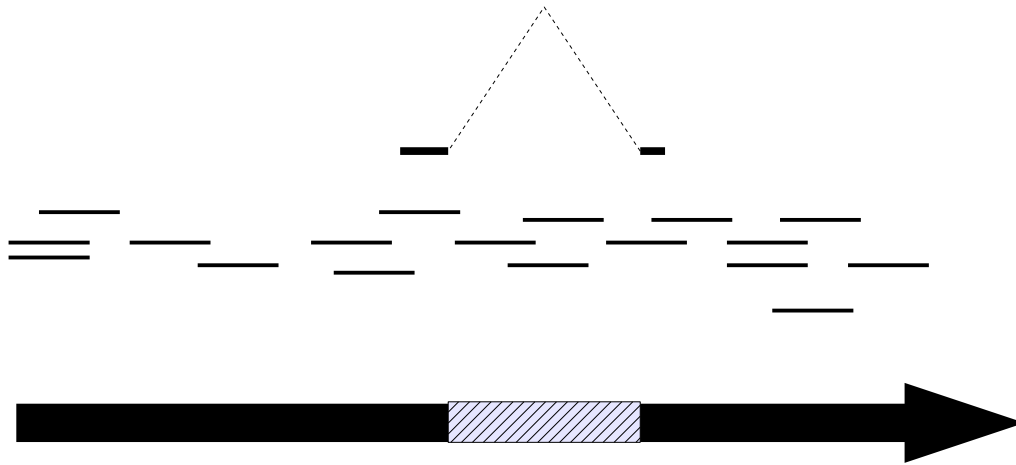
Organelle.plastid (15931hits, 89.5%)
Low_complexity (77hits, 0.225%)
LTR.Gypsy (1hits, 0.00215%)

Both can be easily recognized by similarity searches

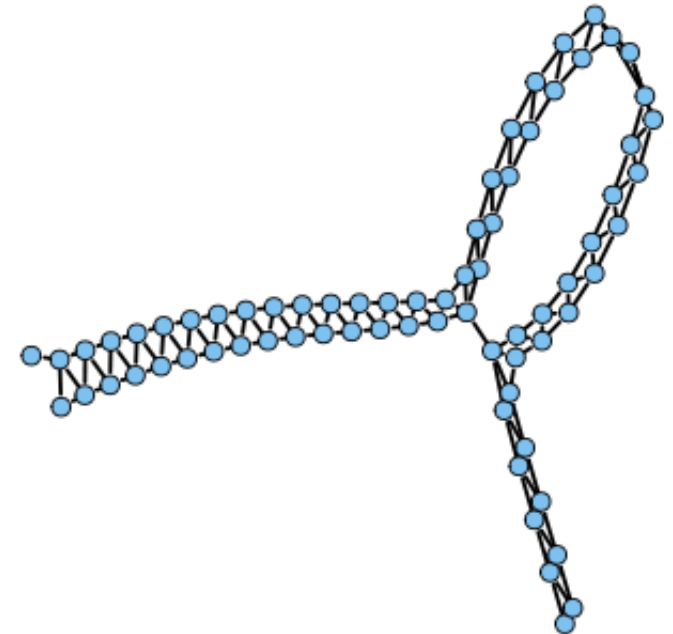
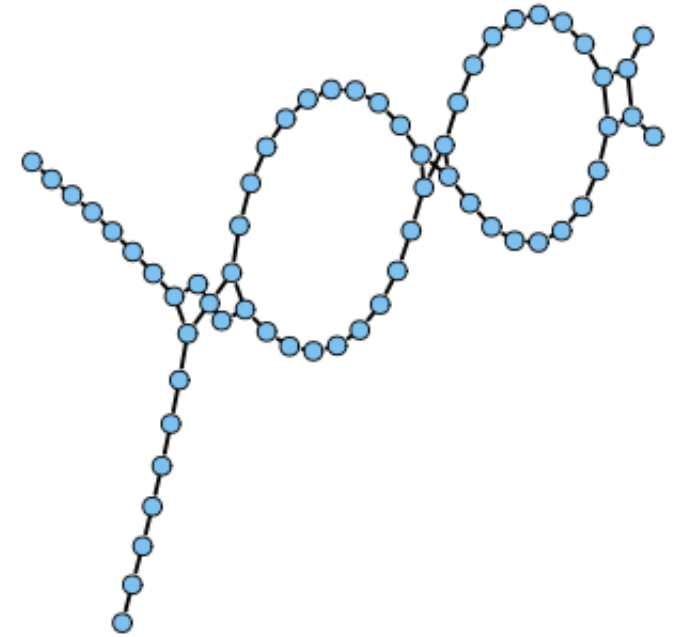
It's getting complicated...



Repeat variants with partial similarity
(depends on similarity threshold)



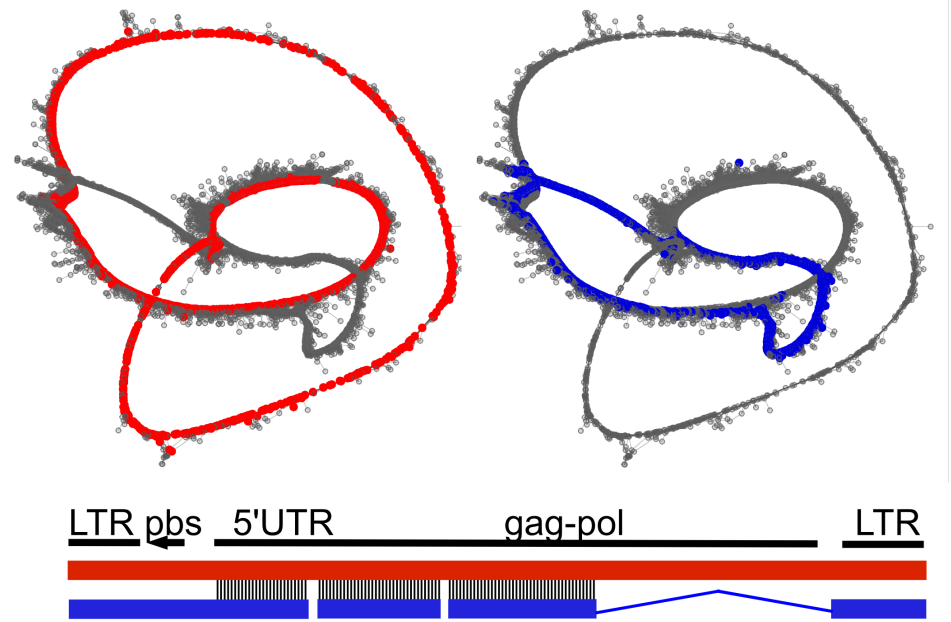
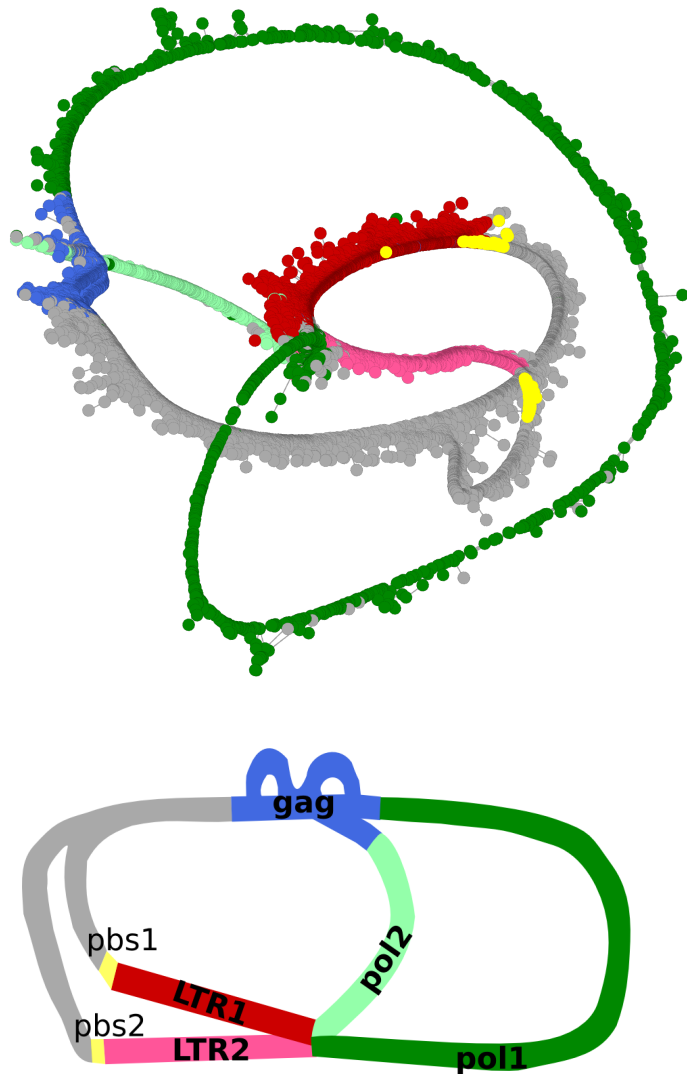
Variants differing in indel



It's getting complicated...

...but it has some meaning

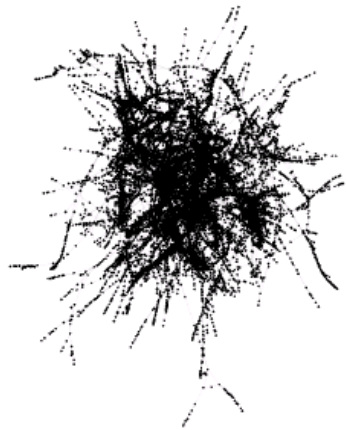
(in this case, presence of element variants differing in LTR sequence and in deletion within gag-pol region)



It's getting complicated...

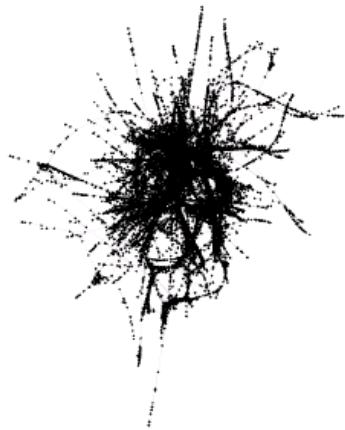
...but it has some meaning

OR NOT ? ;-)



CL1

Number of reads: 42851
Number of pairs: 717667
Density: 0.0007817
Diameter: NA
Mean edge weight: 130.31
Max. degree: 257



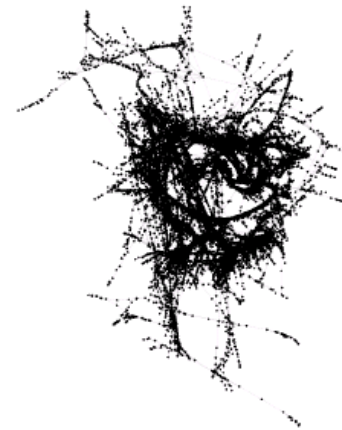
CL2

Number of reads: 47505
Number of pairs: 1460012
Density: 0.001294
Diameter: NA
Mean edge weight: 129.94
Max. degree: 647



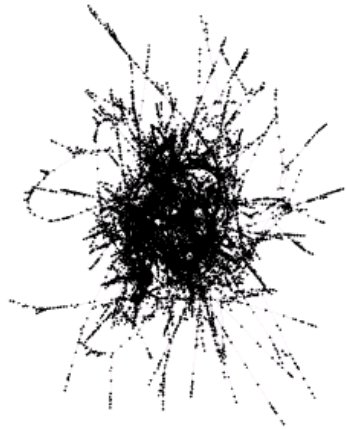
CL3

Number of reads: 44258
Number of pairs: 473407
Density: 0.0004834
Diameter: NA
Mean edge weight: 129.42
Max. degree: 186

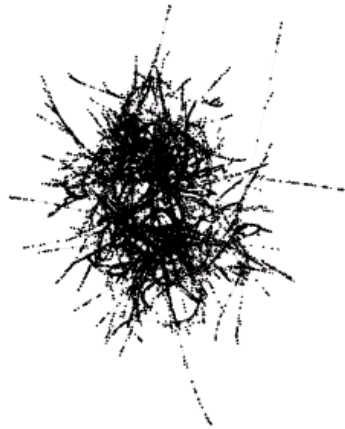


CL4

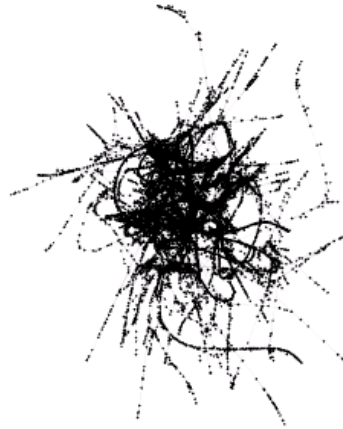
Number of reads: 46876
Number of pairs: 1022899
Density: 0.000931
Diameter: NA
Mean edge weight: 132.18
Max. degree: 290



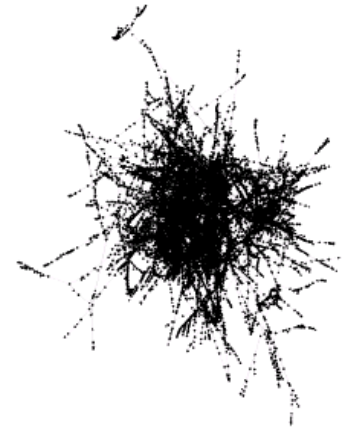
CL5



CL6



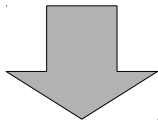
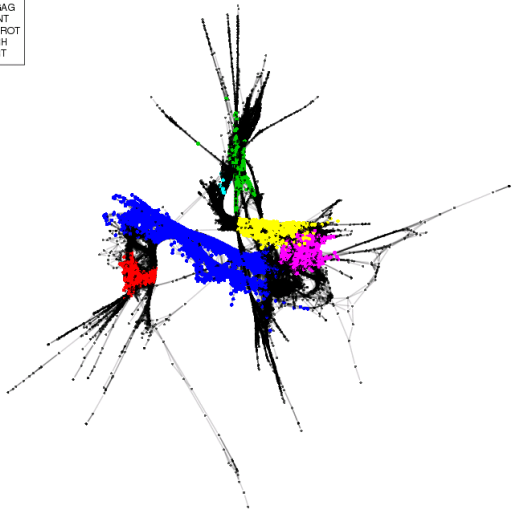
CL7



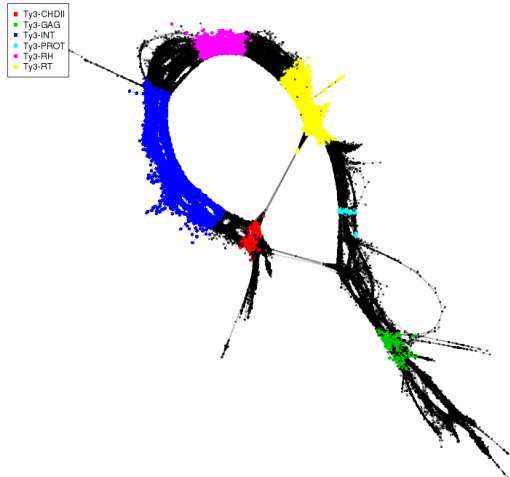
CL8

Using different algorithms for calculating graph topology

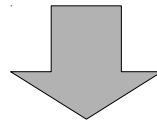
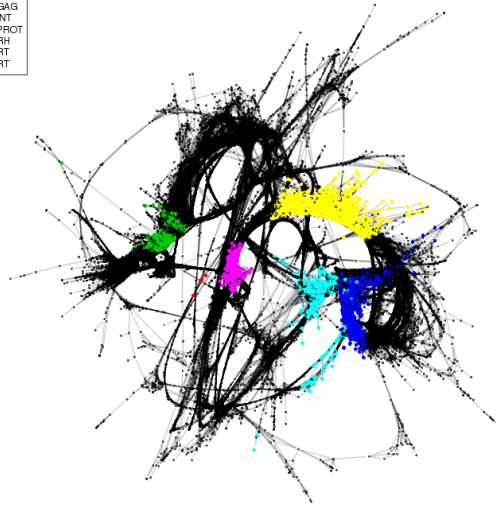
Ty3-CHDII
Ty3-GAG
Ty3-INT
Ty3-PROT
Ty3-RH
Ty3-RT



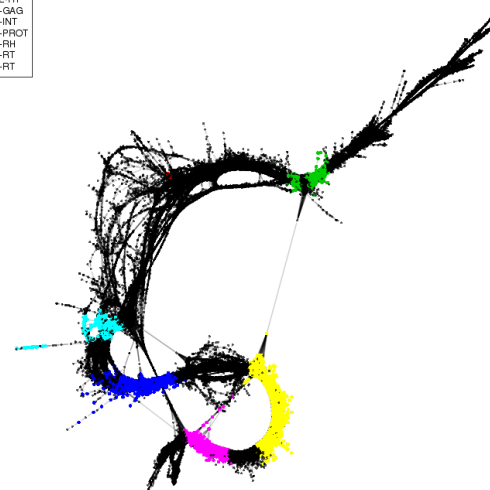
Ty3-CHDII
Ty3-GAG
Ty3-INT
Ty3-PROT
Ty3-RH
Ty3-RT



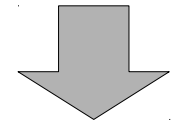
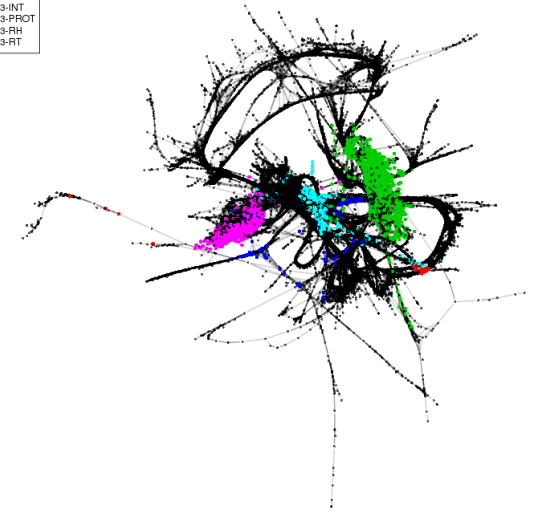
LINE-RT
Ty1-GAG
Ty1-INT
Ty1-PROT
Ty1-RH
Ty1-RT
Ty3-RT



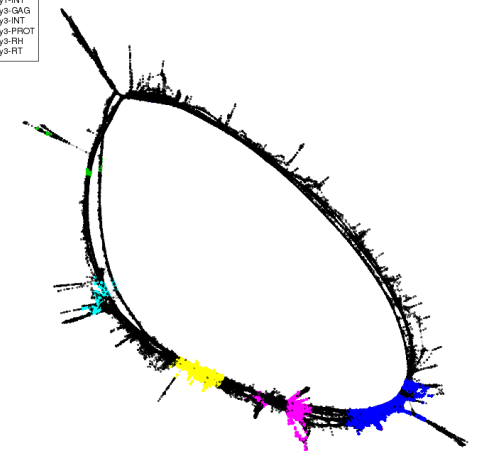
LINE-RT
Ty1-GAG
Ty1-INT
Ty1-PROT
Ty1-RH
Ty1-RT
Ty3-RT



Ty3-GAG
Ty3-INT
Ty3-PROT
Ty3-RH
Ty3-RT



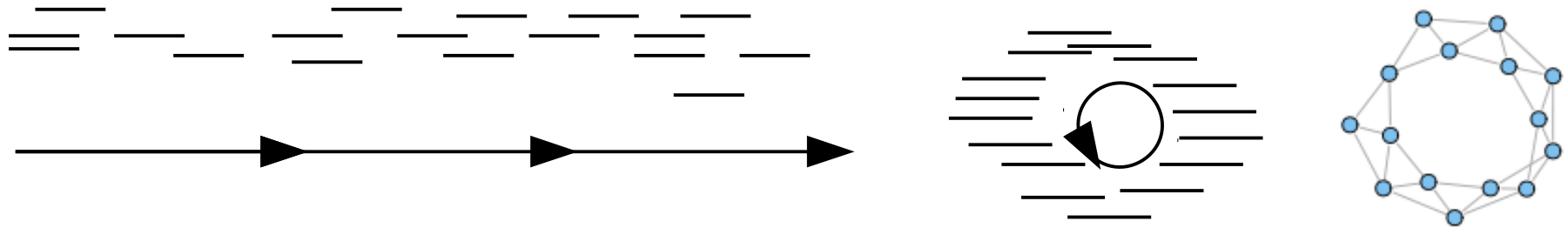
Ty1-INT
Ty3-GAG
Ty3-INT
Ty3-PROT
Ty3-RH
Ty3-RT



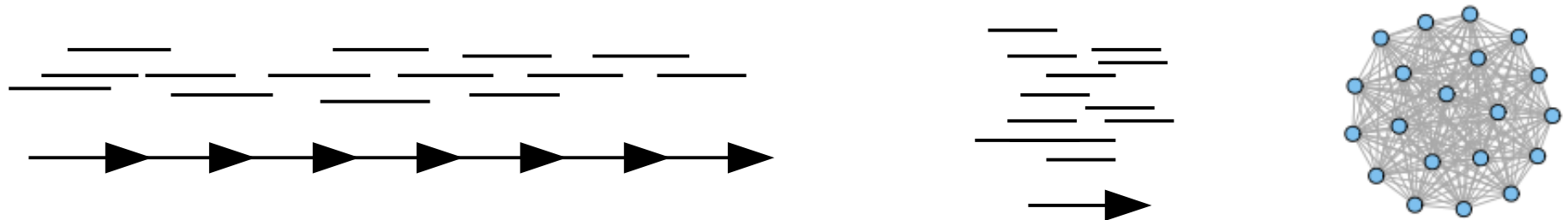
Circular graphs

TANDEM REPEATS

Read length $\ll$ monomer

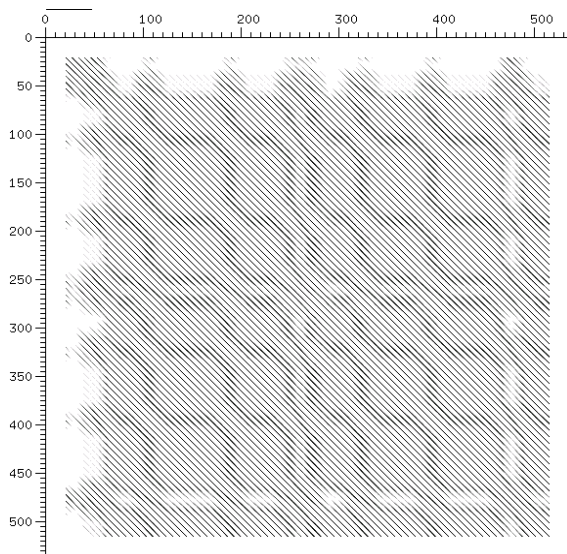
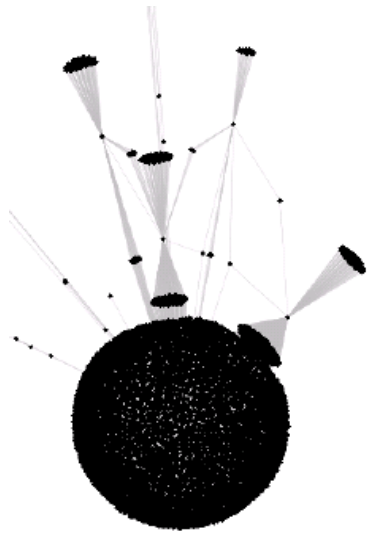


Read length $\geq$ monomer

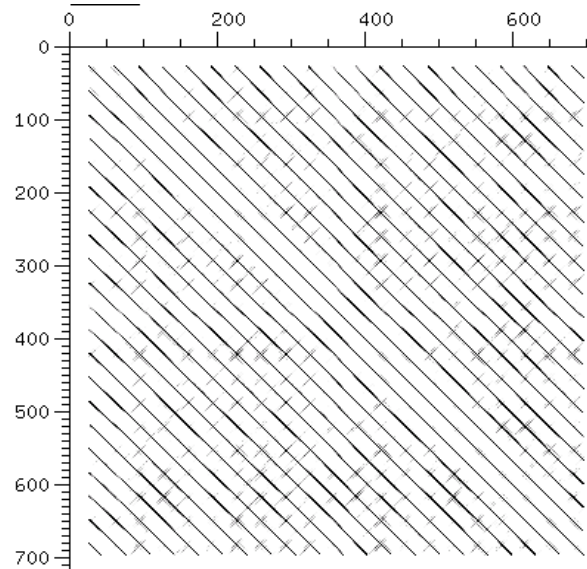
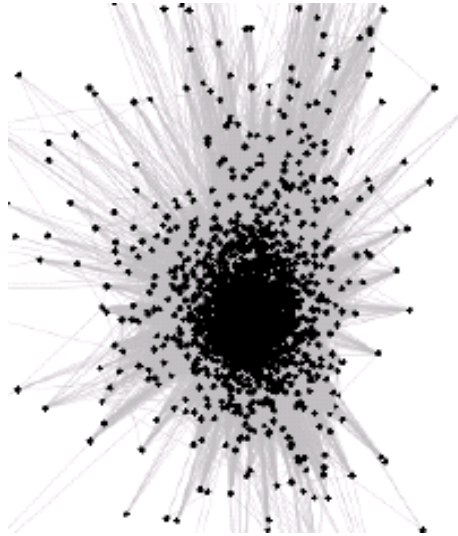


Circular graphs

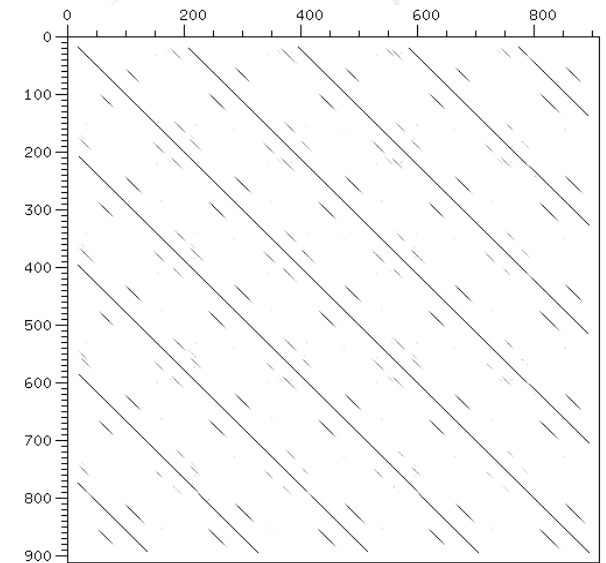
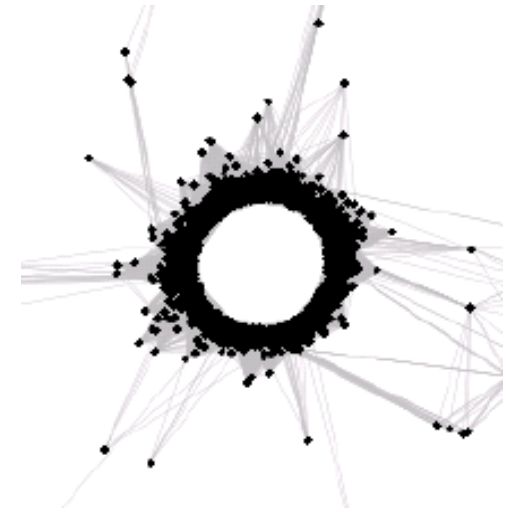
microsatellite (6bp)
(GAACCT)_n



satellite 35 bp

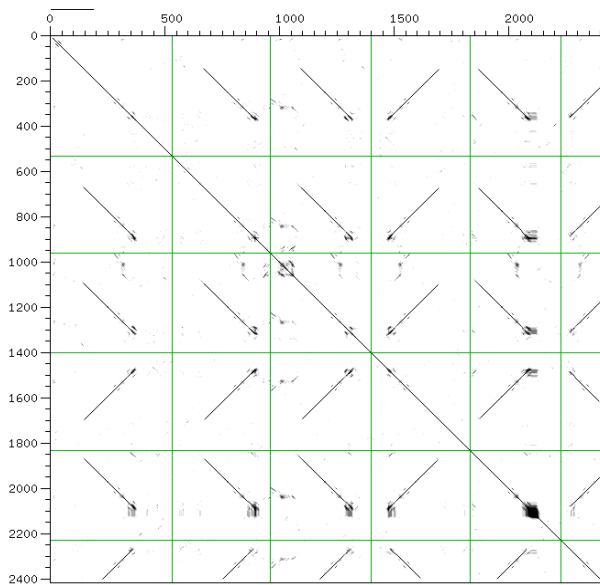
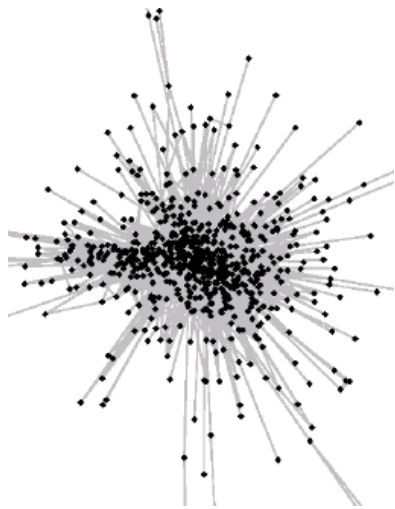


satellite 190 bp

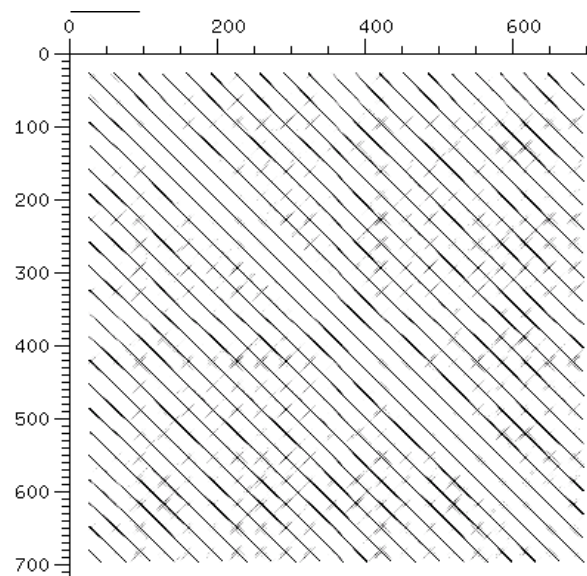
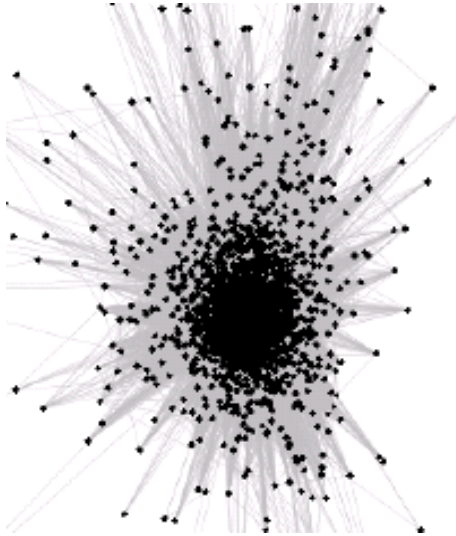


Circular graphs

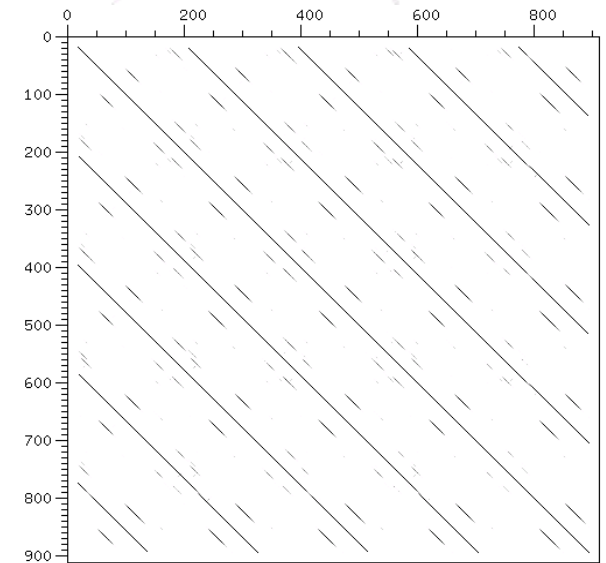
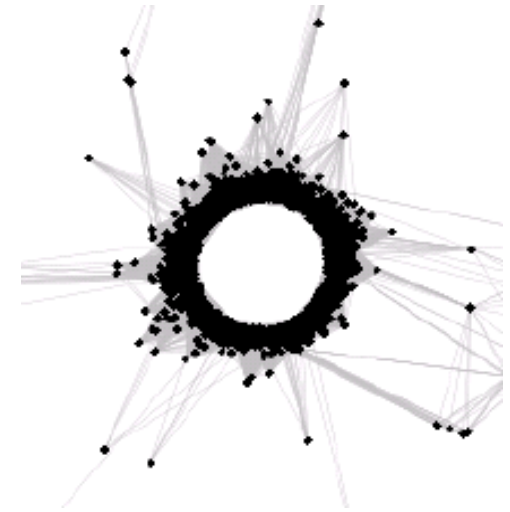
SINE



satellite 35 bp

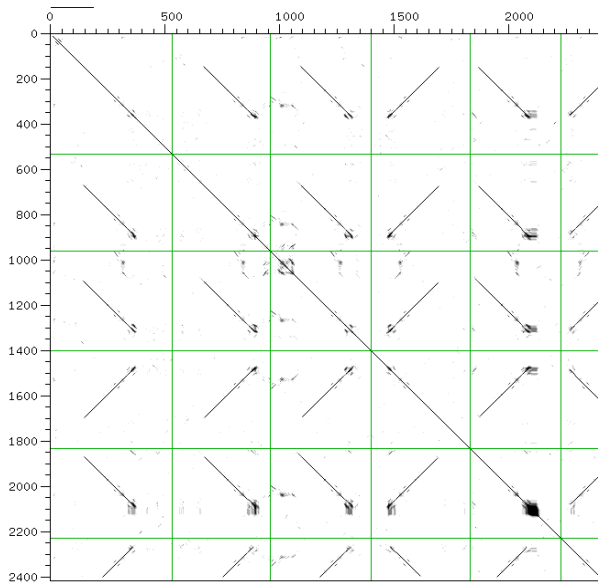
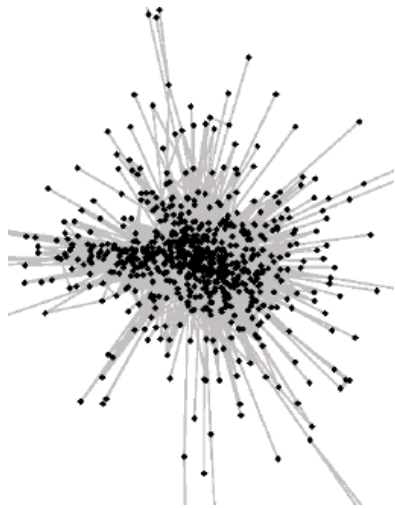


satellite 190 bp

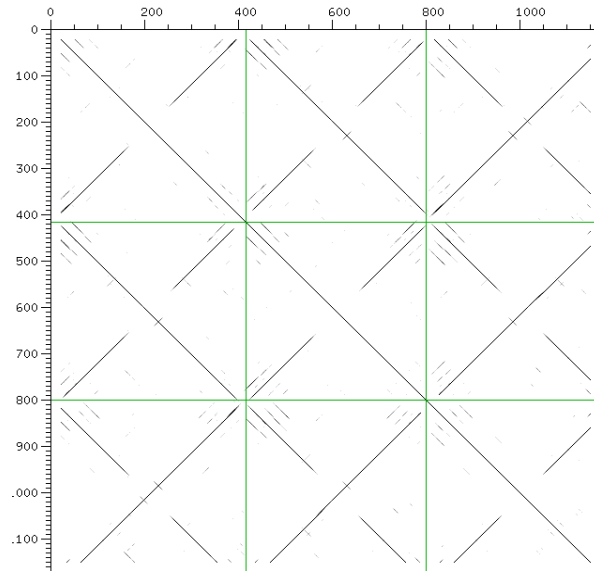


Circular graphs

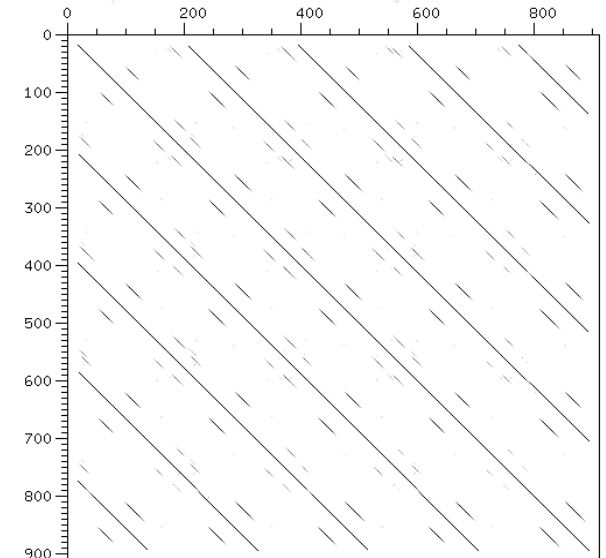
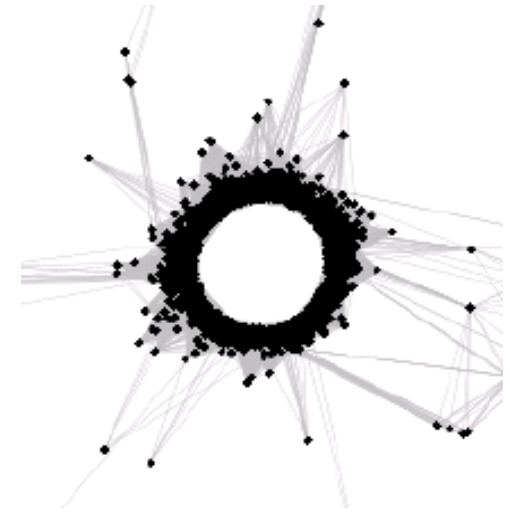
SINE



MITE
(foldback)

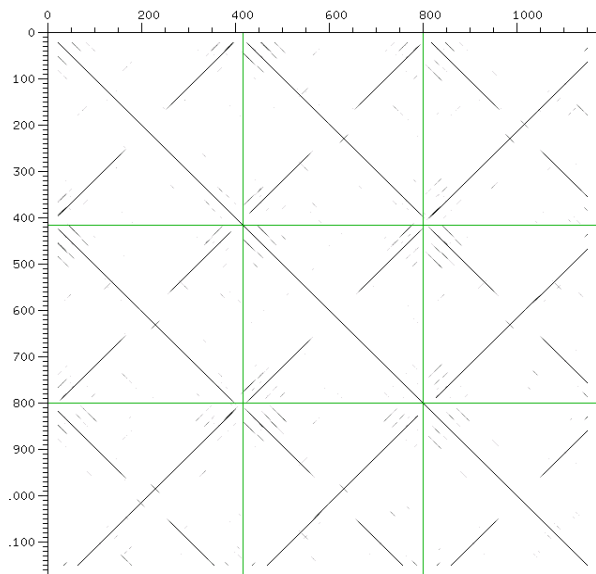


satellite 190 bp



Insertion sites of mobile elements

MITE (foldback)

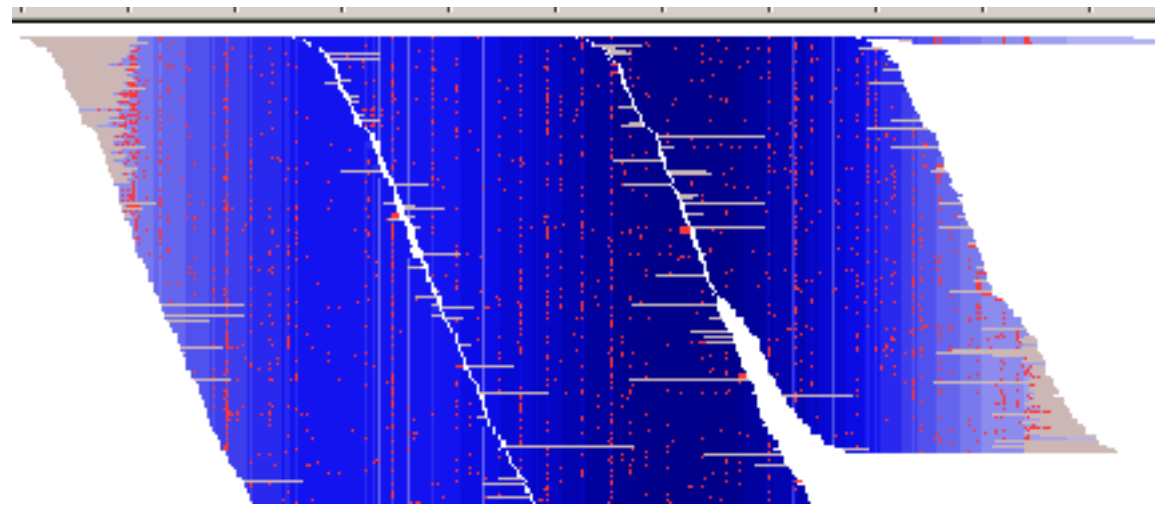


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CCTCGTAATAATAATTAATAAATTTTTGGATTTTCAACCATTTTAGTCATATT  
AAAATTGTATTTTAAATTTTTTTTAGGGTTTTCAACAATTTTAGTCATATT  
TTGAGCCAAACTAATAGTTAAATTTTAGGGTTTTCAACAATTTTAGTCATATT  
ACACATGCCATTTATGATAGAAAAAGGGTTTTCAACAATTTTAGTCATATT  
TAGTAGTAGAAATGGTTTTAGGGTTTAGGGTTTTCAACAATTTTAGTCATATT  
TTTTATTATTTTGTGATTTTAAATAGGGTTTTGAACAATTTTAGTCATATT  
CTTAATTCAATTATAAAAGATTTAAAGGGTTTTTAACAATTTTAGTCATATT  
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GCAGTTTATGGTATAAAATATTAAAGGGTTTTCAATAATTTTAGTCATATT  
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TTCTATTTCAAATCATGTATAAATTAAGGGTTTTCAACAATTTTAGTCATATT
```

← TIR

```
TGAAACAAATATGACCAAAAAAGTTAAAAACCCCTTAAATAAAACATAAAGAGTATAAA  
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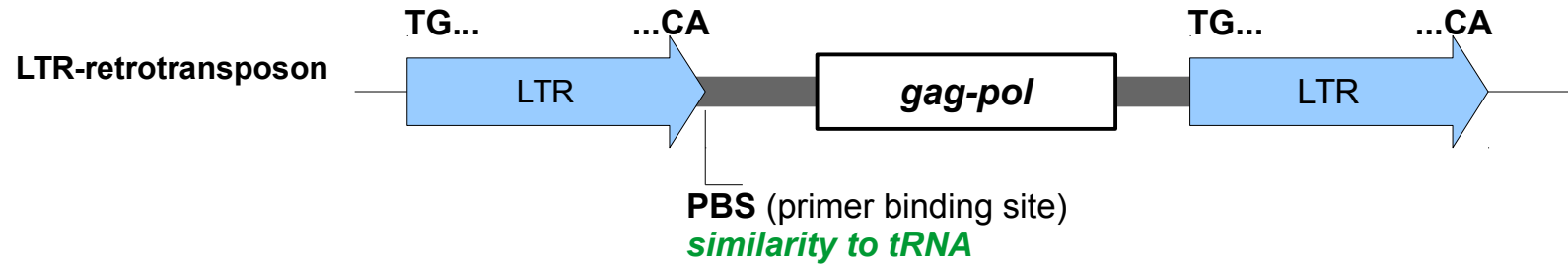
TIR →



contig

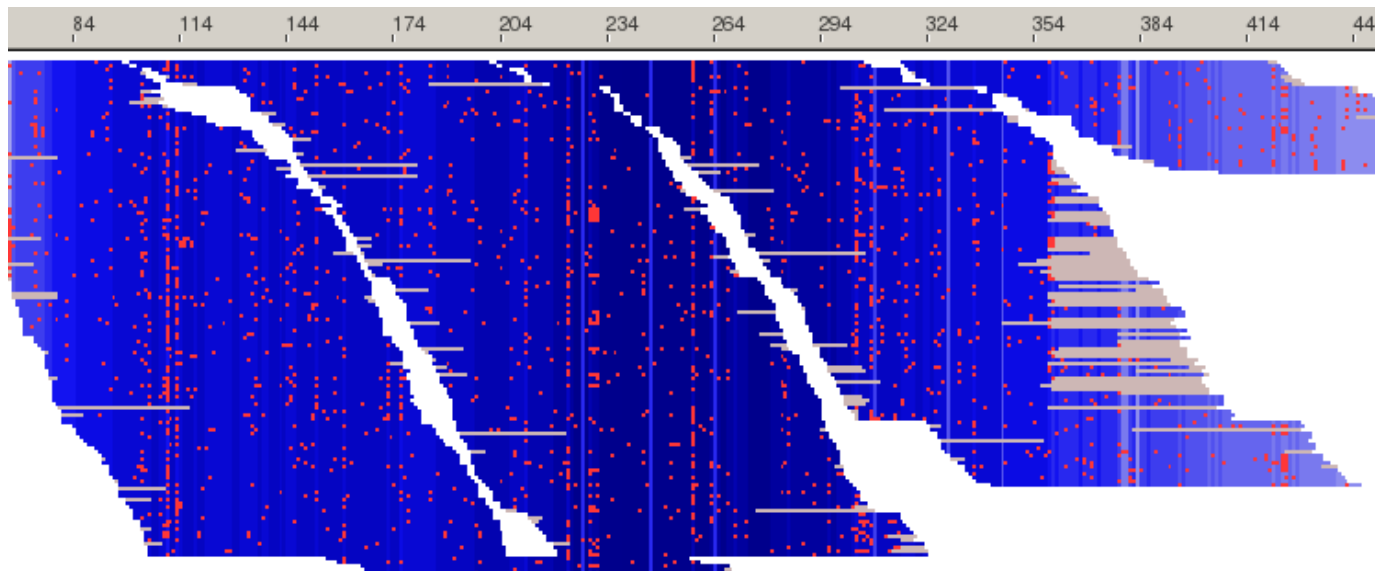
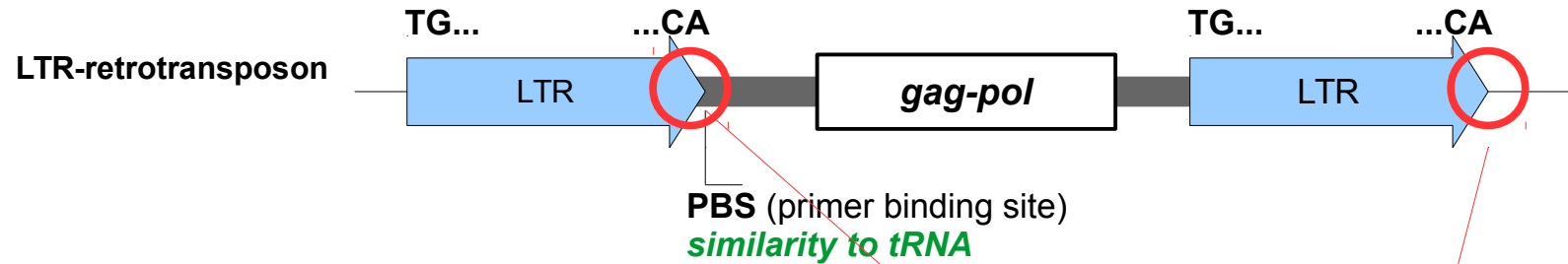
Insertion sites of mobile elements

A new tool for detection of LTR / PBS sites



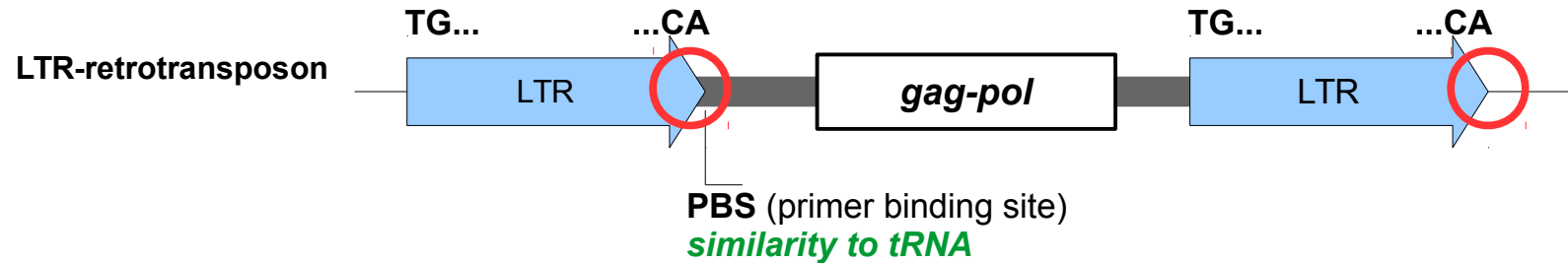
Insertion sites of mobile elements

A new tool for detection of LTR / PBS sites



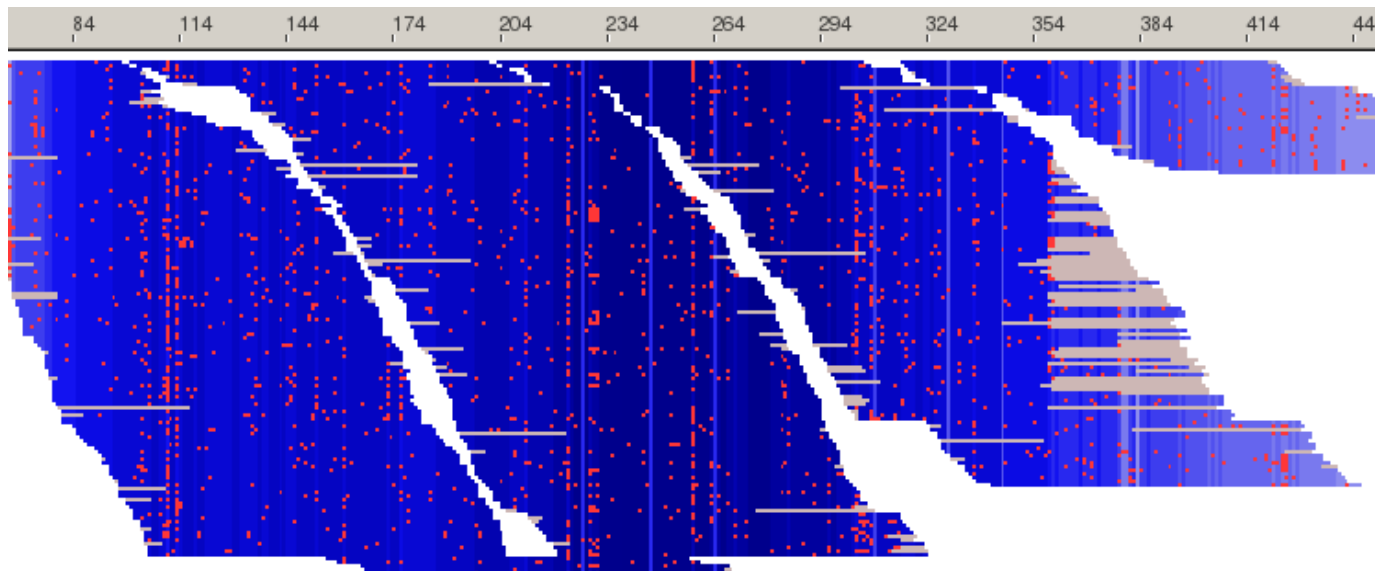
Insertion sites of mobile elements

A new tool for detection of LTR / PBS sites

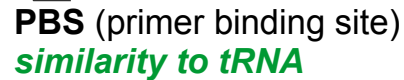


The tool searches assembled contigs (ACE files) for **differences in proportions of masked reads** accompanied by **occurrence of TG/CA**

↓
...and **similarity to tRNA**

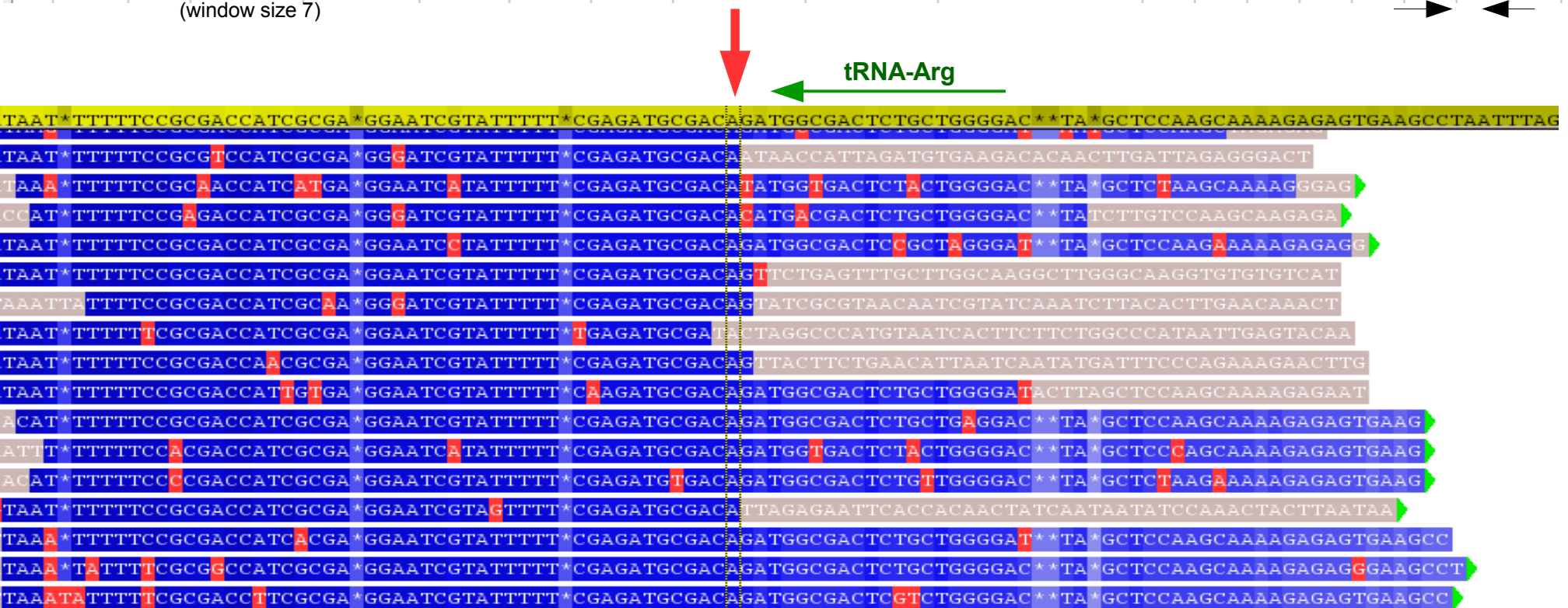


A new tool for detection of LTR / PBS sites



CL	contig	pos.	site	site_depth	out_ma	maske	maske	region_in	region_out	blast to tRNA	%	length		site from	to	tRNA from	to	E-val	
19	400	364	TGCGACA	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)

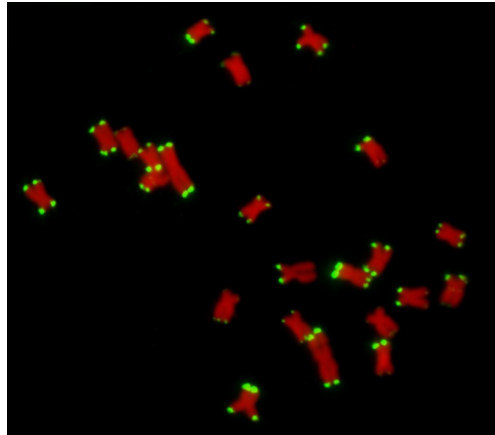


“Chimeric” clusters ?

Reconstruction of rDNA gene cluster in *Silene latifolia*

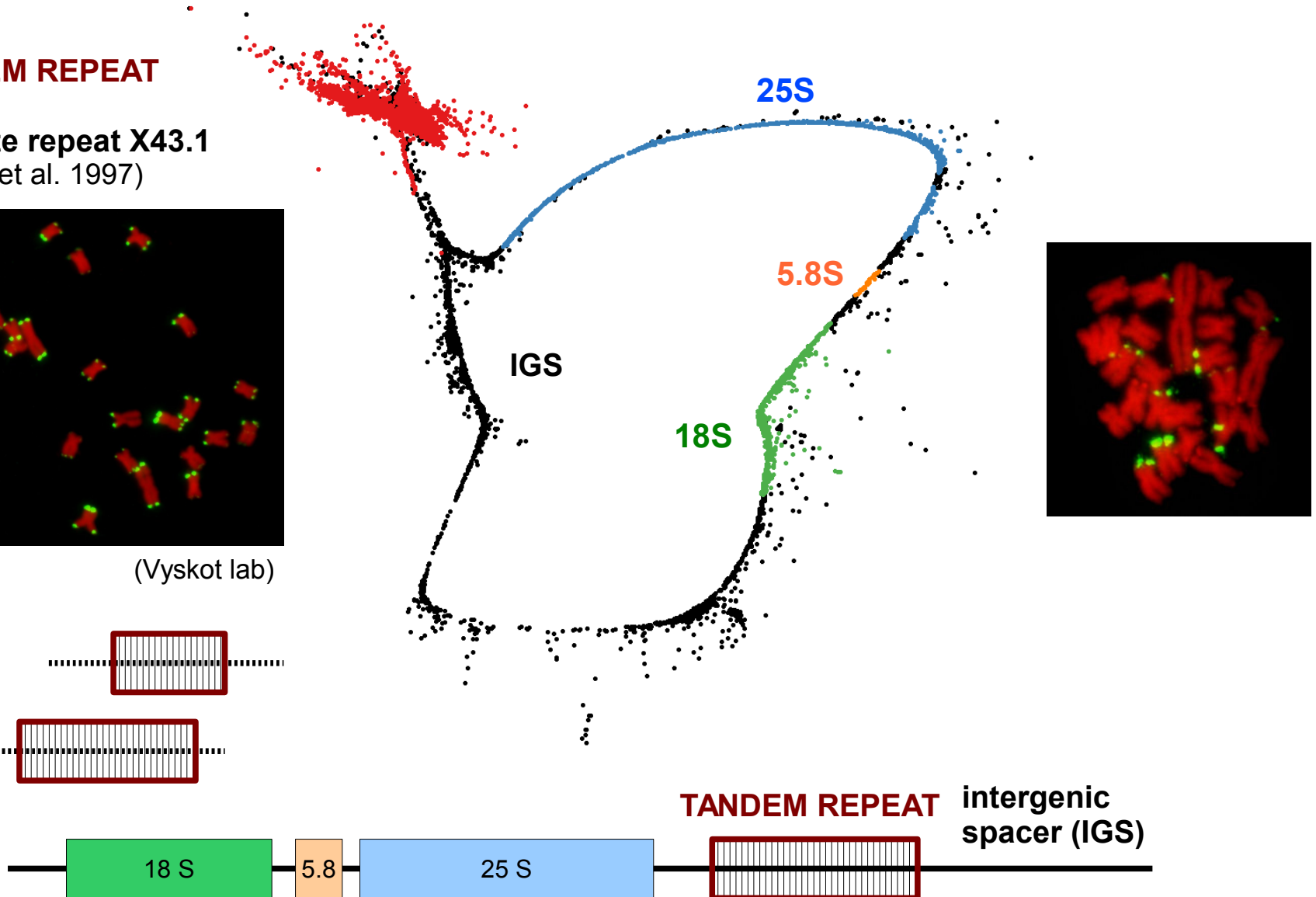
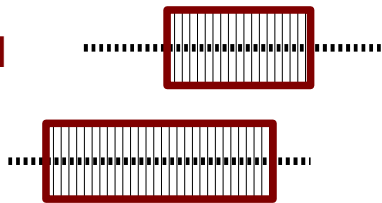
TANDEM REPEAT

Satellite repeat X43.1
(Buzek et al. 1997)



(Vyskot lab)

Additional
loci of X43.1
occur
outside
rDNA IGS



Repeat quantification

	7192836 (= 100%)	64.1			
CL	reads	genome %	class	type	note
1	304159	4.229	gypsy	Tat	PROT-RT/RH-INT
2	234749	3.264			?, PE->24,18
3	216307	3.007	gypsy	chromo	ALL domains
4	202822	2.820	copla	Maximus	ALL domains
5	149693	2.081	gypsy	Athila	ALL domains
6	145911	2.029	gypsy	Tat	ALL domains
7	143766	1.999	gypsy	chromo	ALL domains
8	142608	1.983	copla	Maximus	ALL domains
9	141836	1.972	LINE		RT
10	123886	1.722	gypsy	chromo	GAG
11	79345	1.103			?,PE->21,95
12	72781	1.012	copla	Angela	ALL domains
13	67096	0.933	gypsy	Tat	ALL domains
14	65455	0.910	gypsy	Athila	GAG, PE->1(!!!),36
15	62334	0.867	gypsy	Tat	ALL domains
16	53845	0.749	copla	Ivana/Oryco	ALL domains
17	49341	0.686			? DNA transp ?? + TR
18	45062	0.626			?,PE->2,63
19	44762	0.622			?,PE->28
20	43332	0.602	tandem		monom ?? ~400 (~1200)
21	42344	0.589	gypsy	chromo	ALL domains
22	40125	0.558	gypsy	Tat	PE->15
23	39923	0.555			?,PE->7,73
24	36353	0.505	gypsy	chromo	(GAG), PE->2,3,28
25	35977	0.500			?,PE->2,81
26	35674	0.496			?
27	34829	0.484	rDNA	5S	
28	34534	0.480	gypsy	chromo	PE->29,19,24
29	34302	0.477	gypsy	chromo	PE->28
30	33114	0.460			?
31	32930	0.458			?
32	32122	0.451	LINE		RT

Based on numbers of reads
in individual clusters

(more info during practical training)

Using paired-end read
information for improving
cluster annotation

Repeat quantification

Cluster annotation and quantification

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

Proportions of various repeat types in a genome

