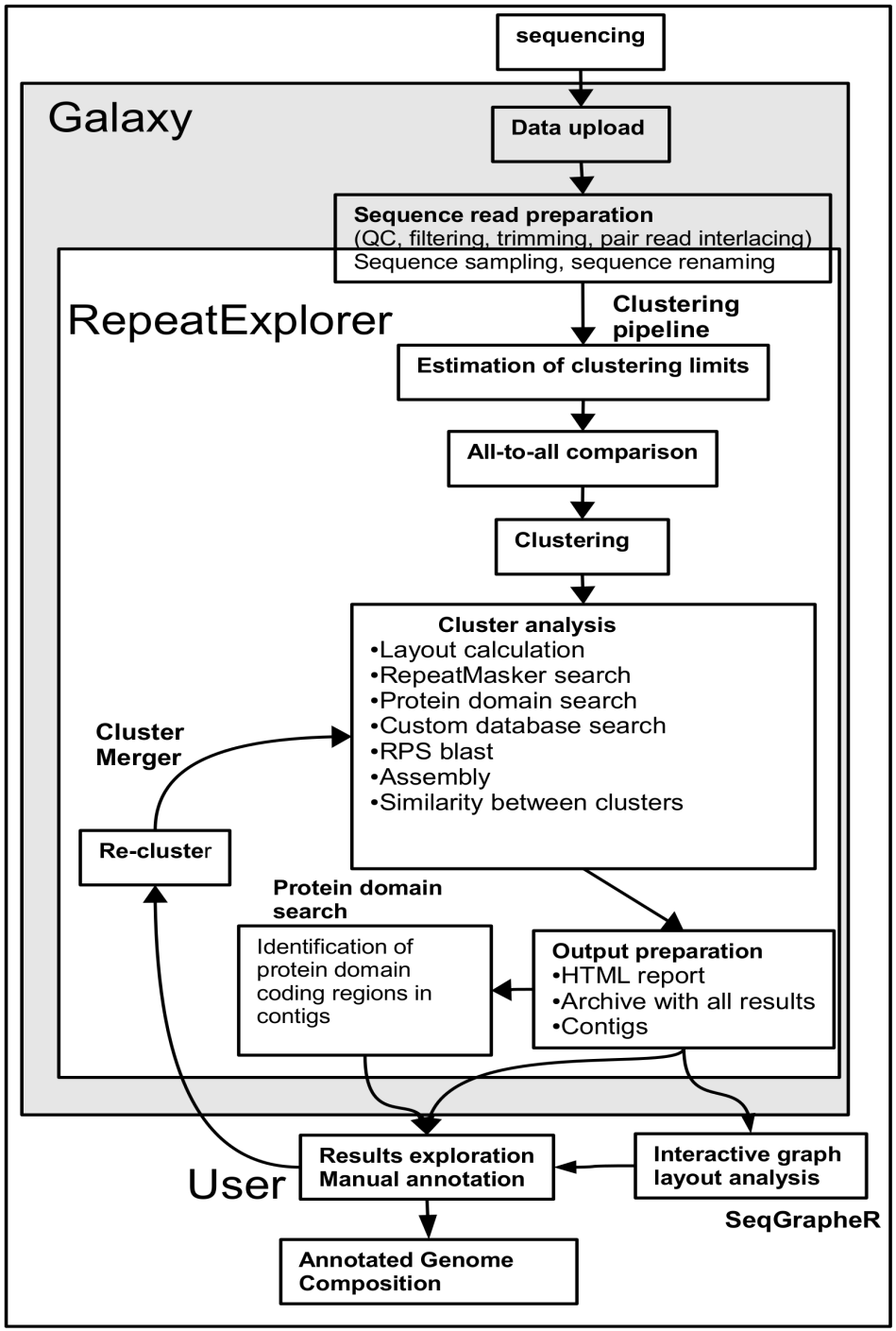
Using *RepeatExplorer* output for repeat annotation and quantification

RepeatExplorer pipeline



The pipeline combines all principles of repeat identification:

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

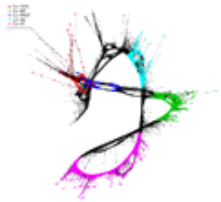
Structure-based approaches
- shape of cluster graphs
- investigation of assembled contigs

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

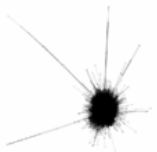
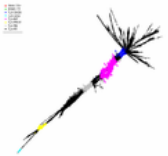
Summary table – new features

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

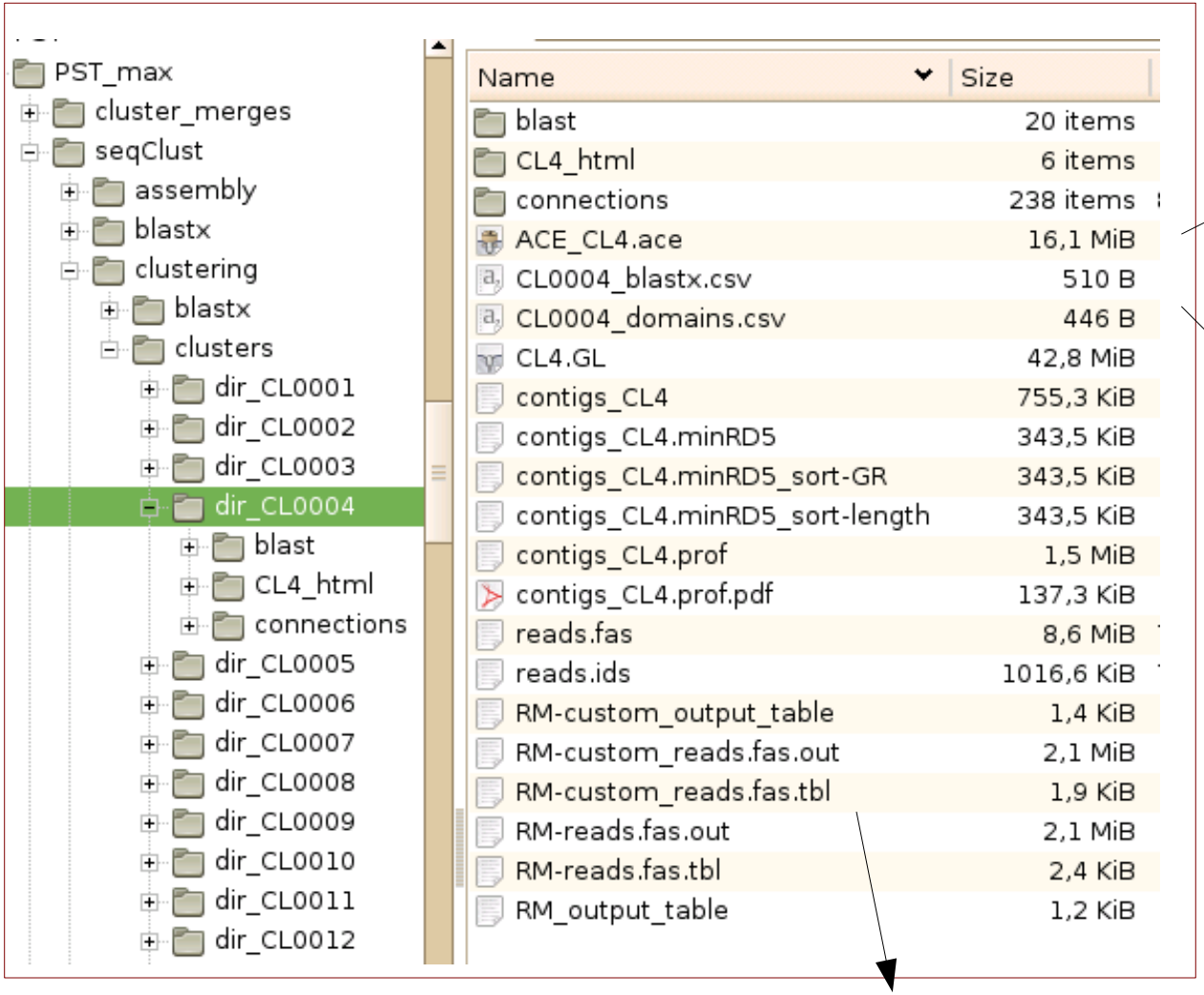
Automatic classification / super-clusters are not yet available in the public RE

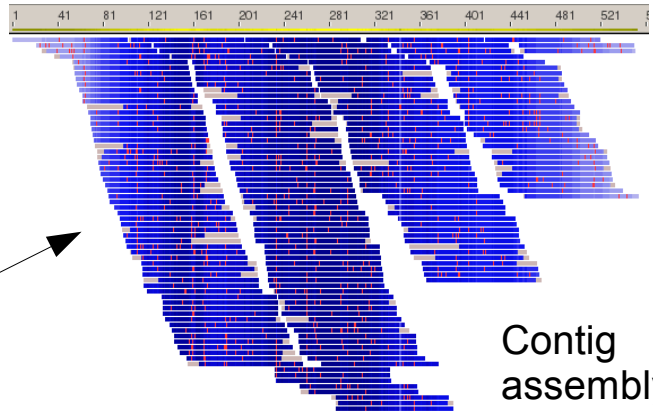
Top clusters

	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 [%]	Missin mates with n simila 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%)	group content VSA_ 0 (0%) VSP2 0 (0%) VPN_ 15853 (2.77%) VML_ 20856 (2.59%)	3.10	0.62
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 (.....	group content VSA_ 2832 (1.6%) VSP2 8141 (2.17%) VPN_ 7966 (1.39%) VML_ 13390 (1.66%)	15.20	5.40
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,	group content VSA_ 1809 (1.02%) VSP2 1822 (0.487%) VPN_ 3838 (0.67%)	17.87	6.06

Detailed information for the largest clusters

RepeatExplorer output files





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	IpoB1_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 in query begin	position in query end	position in query (left)	matching repeat	repeat class/family	position in repeat begin	position in repeat end	position in 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	

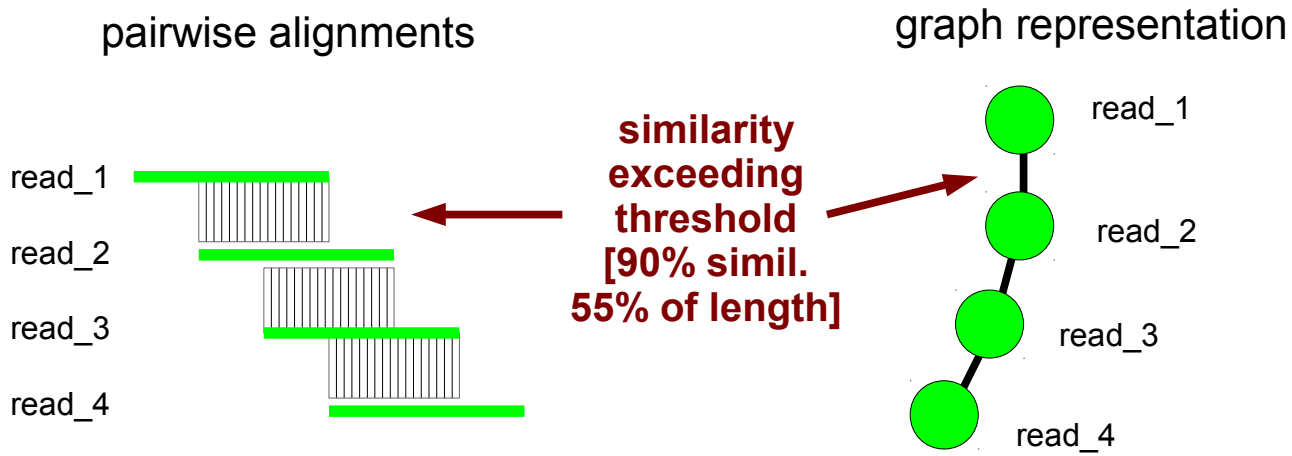
Repeat annotation

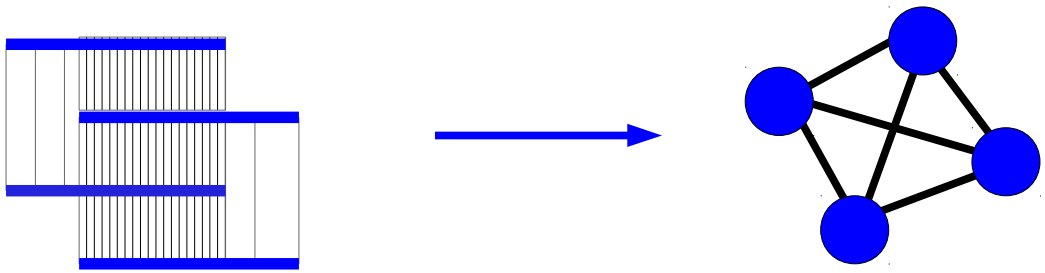
For repeat annotation in individual clusters, consider:

- 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
- *Automated classification*

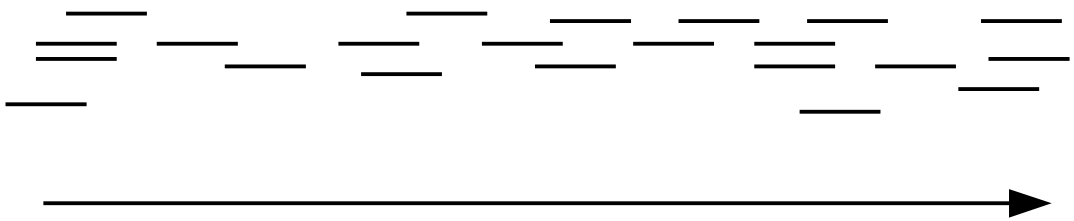
Be prepared to leave some clusters without annotation !

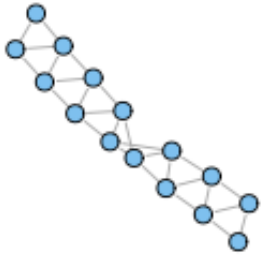
Graph shapes





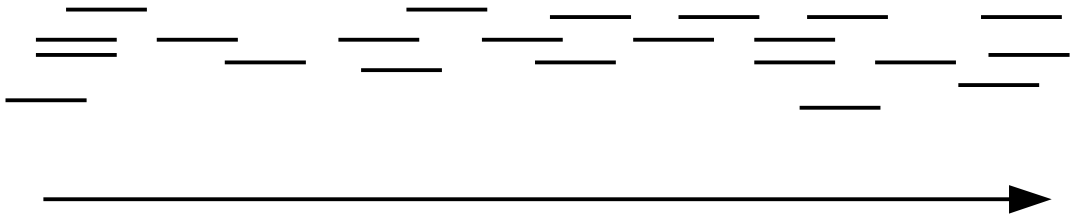
Linear graphs

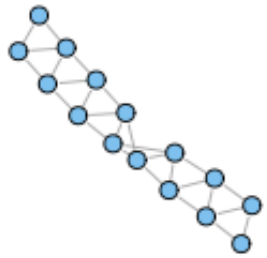




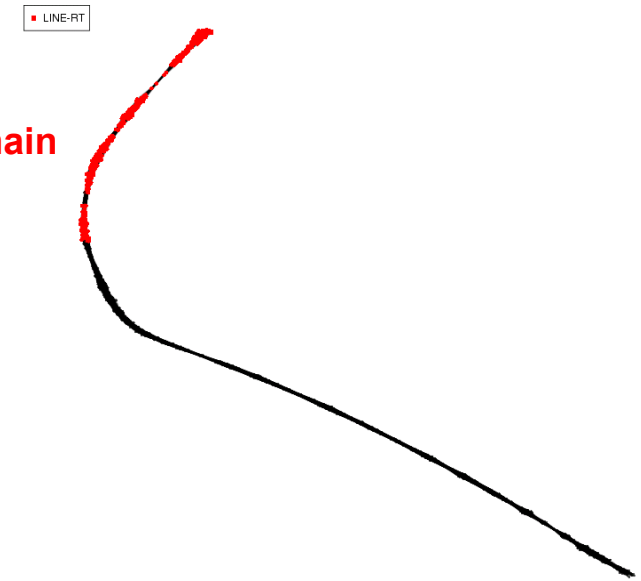
DNA transposons, LINEs, ...

Linear graphs

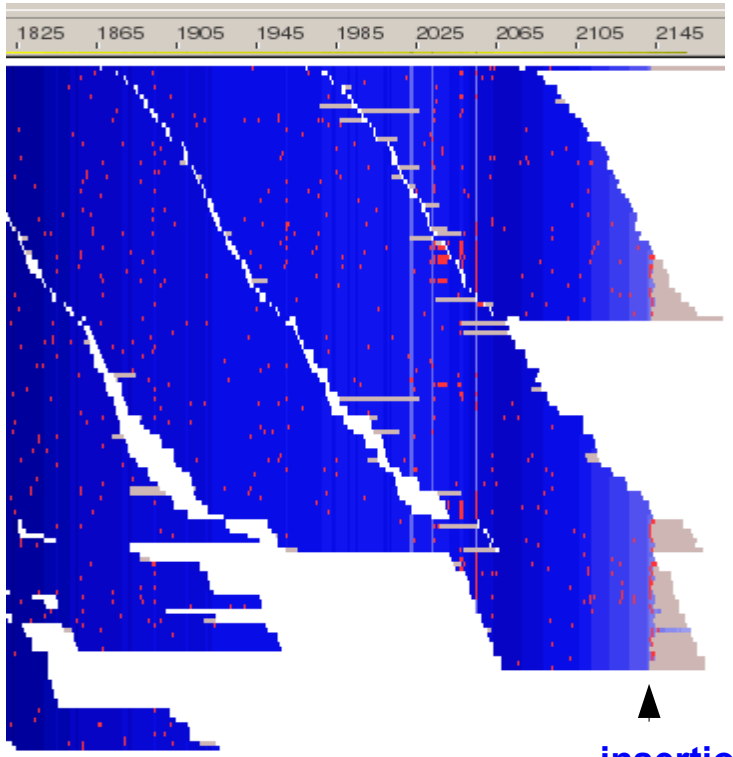




DNA transposons, LINEs, ...



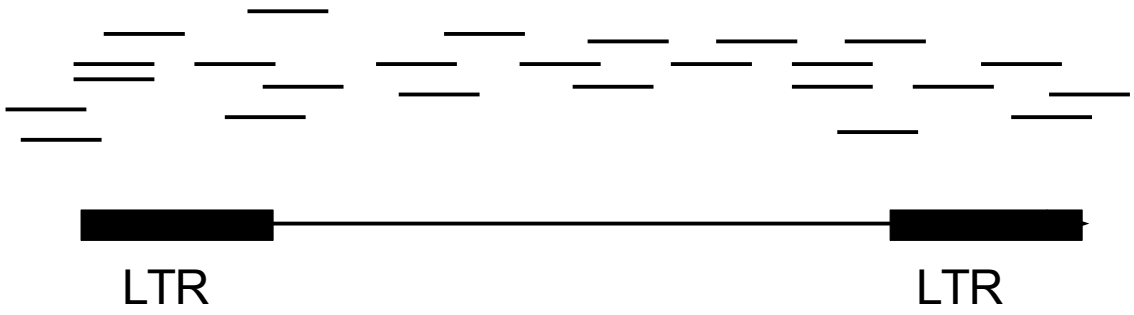
RT domain (LINE)



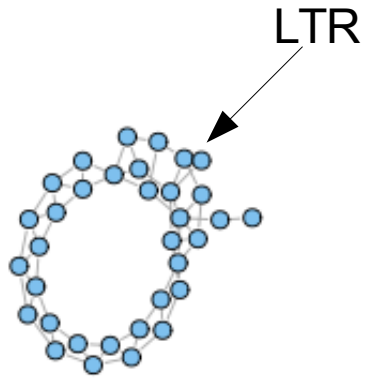
assembled contig

insertion sites

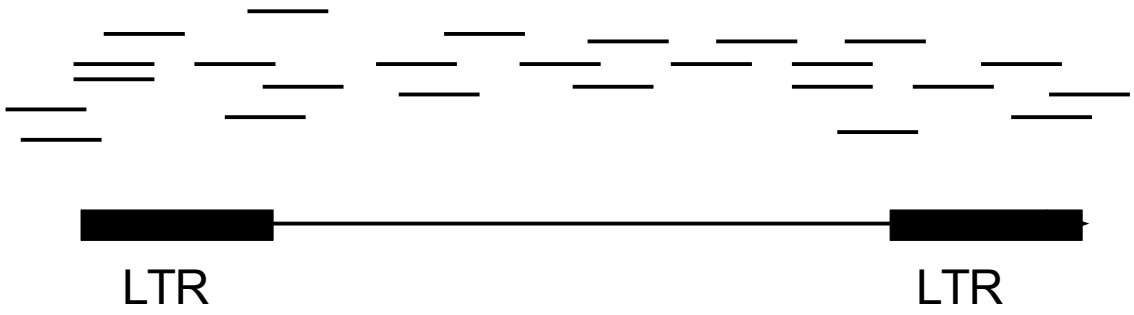
Linear / large circular graphs

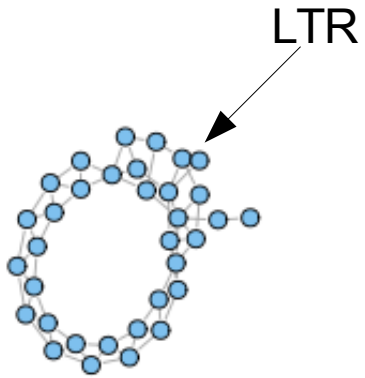


LTR-retrotransposons

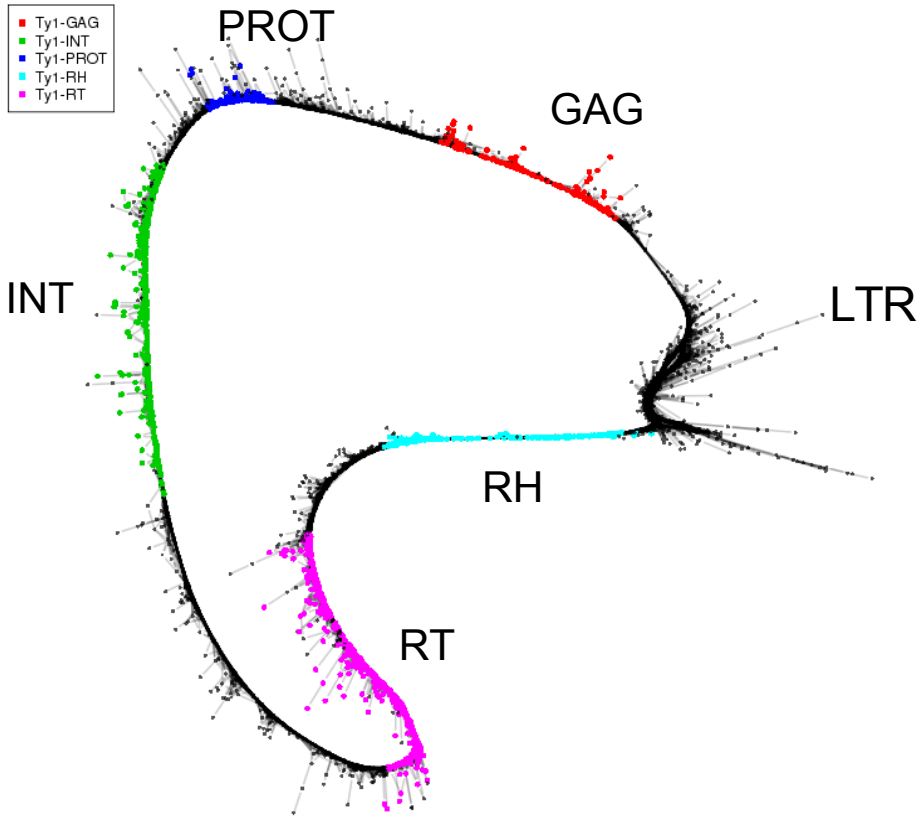


Linear / large circular graphs

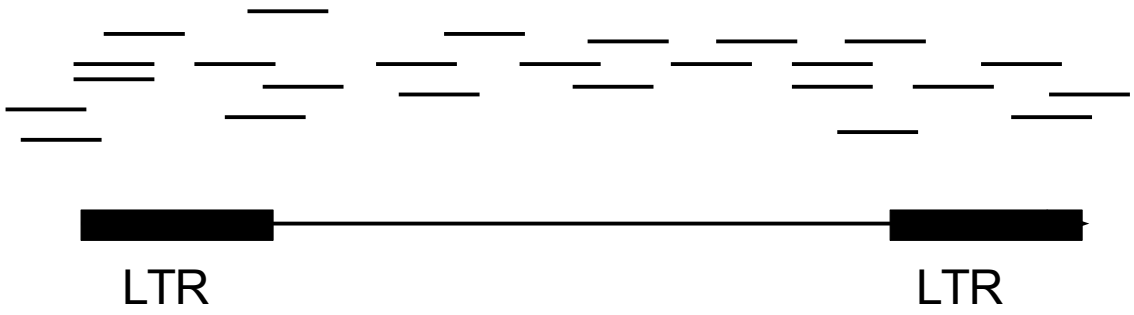


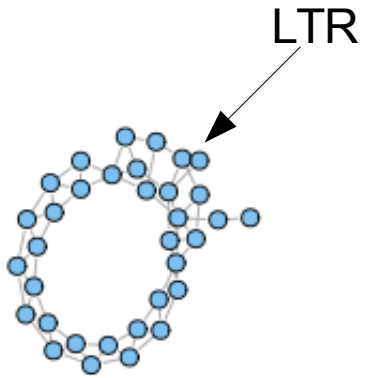


LTR-retrotransposons

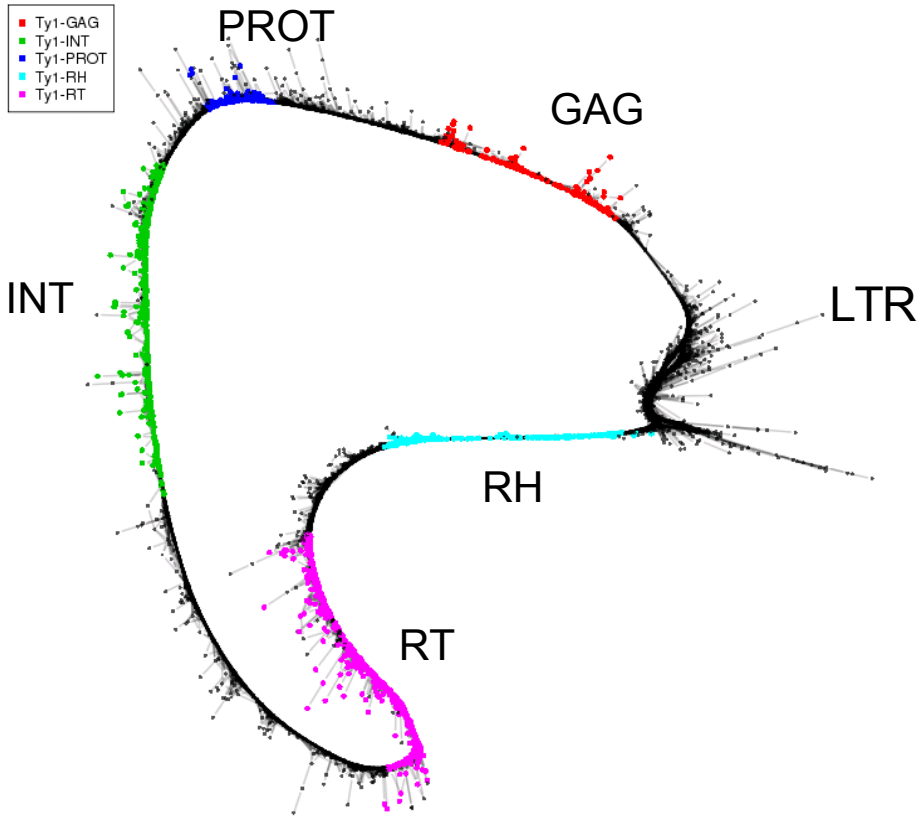


Linear / large circular graphs





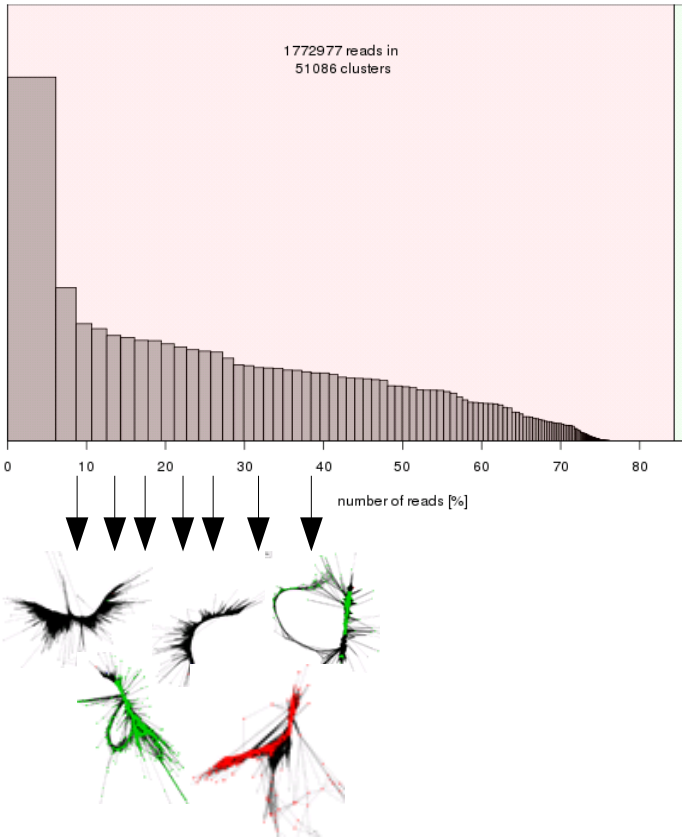
LTR-retrotransposons



Cluster fragmentation leads to linear graphs !

Linear / large circular graphs

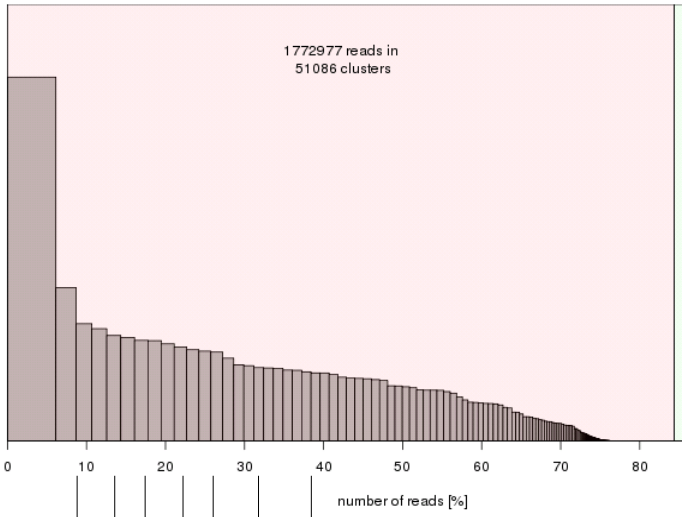
Cluster fragmentation leads to linear graphs !

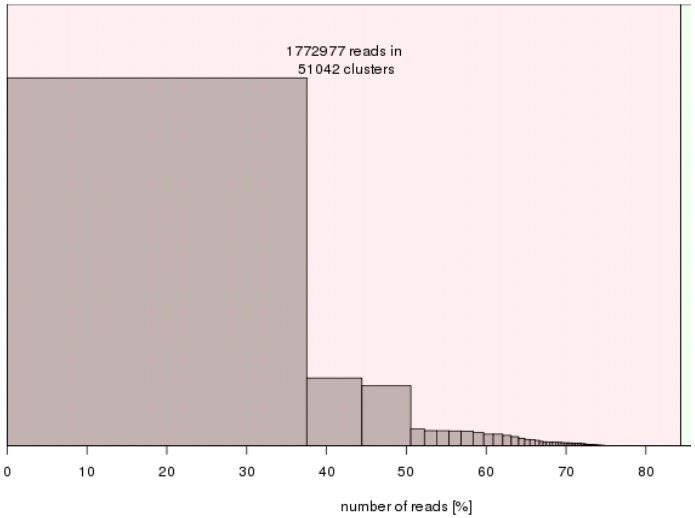


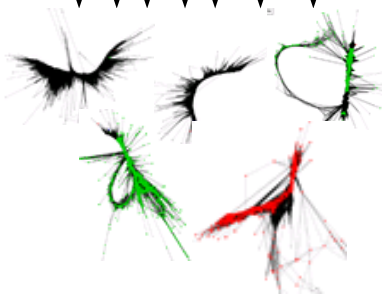
LTR-retrotransposon Ogre in *Vicia pannonica* (~ 40% of the genome)

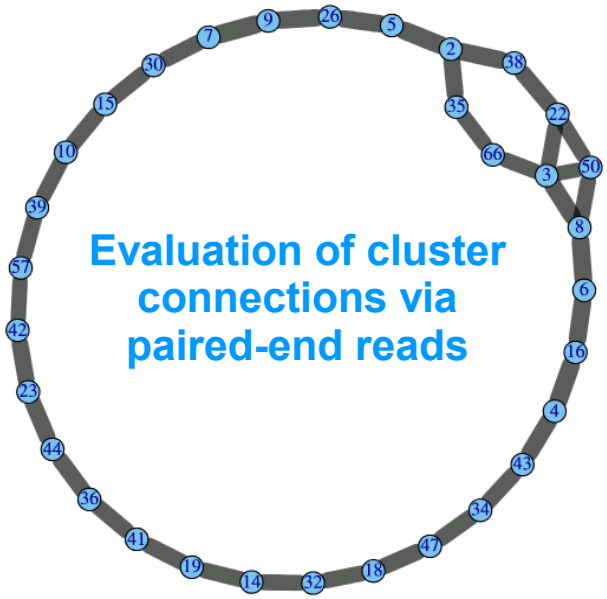
Linear / large circular graphs

Cluster fragmentation leads to linear graphs !

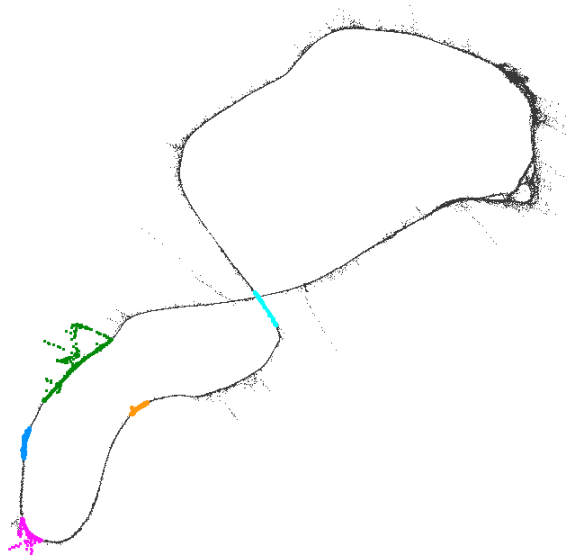








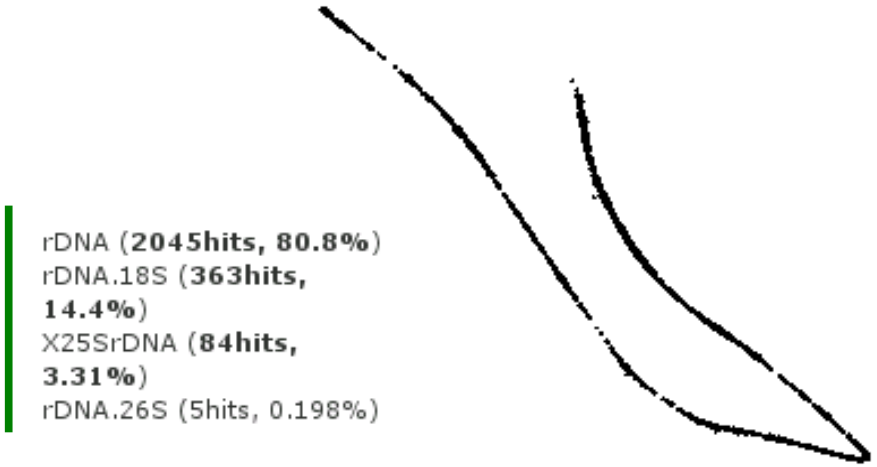
Evaluation of cluster connections via paired-end reads



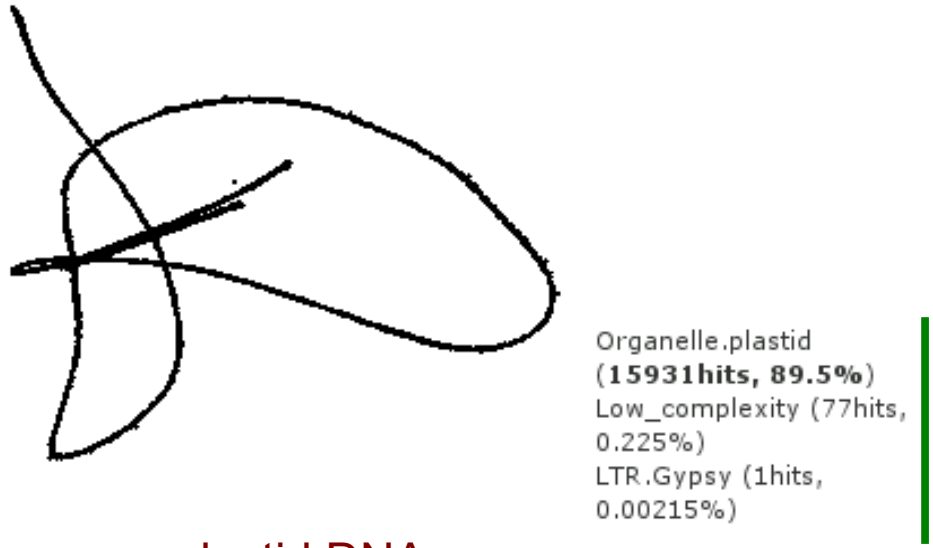
LTR-retrotransposon Ogre in *Vicia pannonica* (~ 40% of the genome)

Linear / large circular graphs

Cluster fragmentation leads to linear graphs !



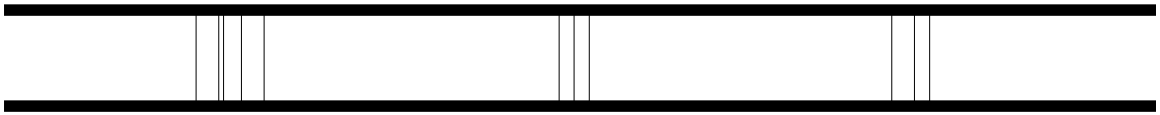
45S rDNA



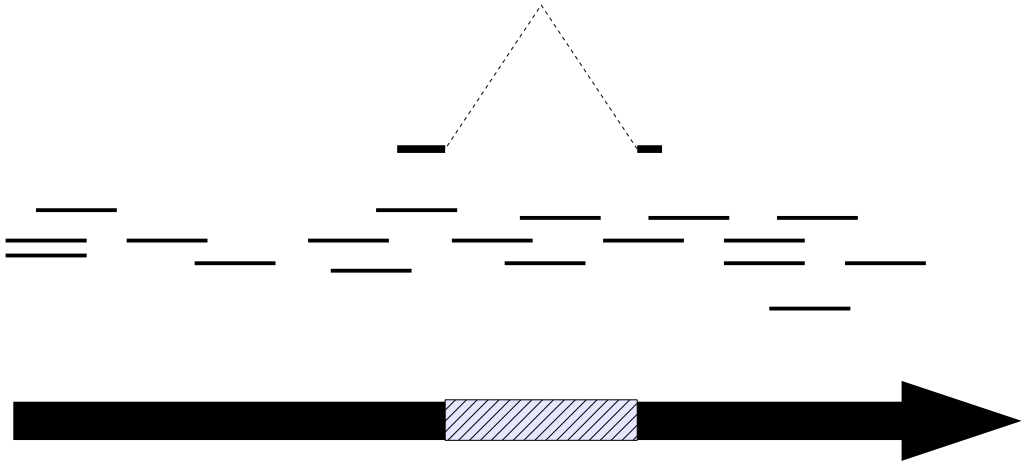
plastid DNA
(contamination)

Both can be easily recognized by similarity searches

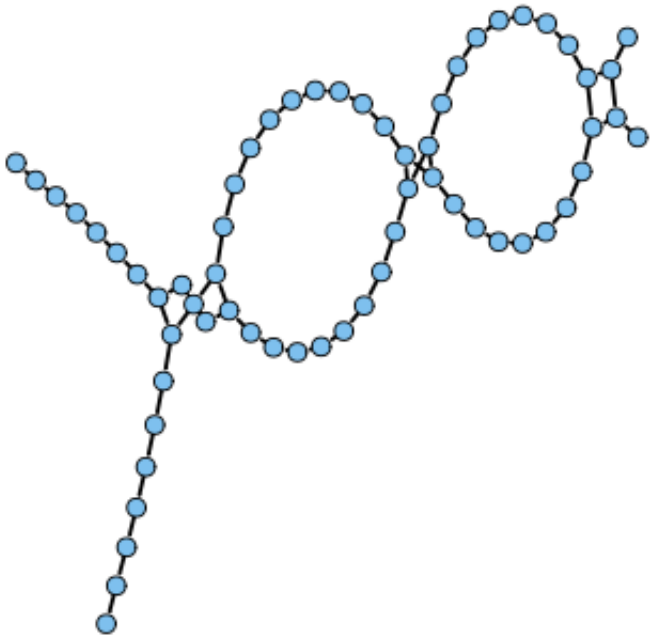
It's getting complicated...

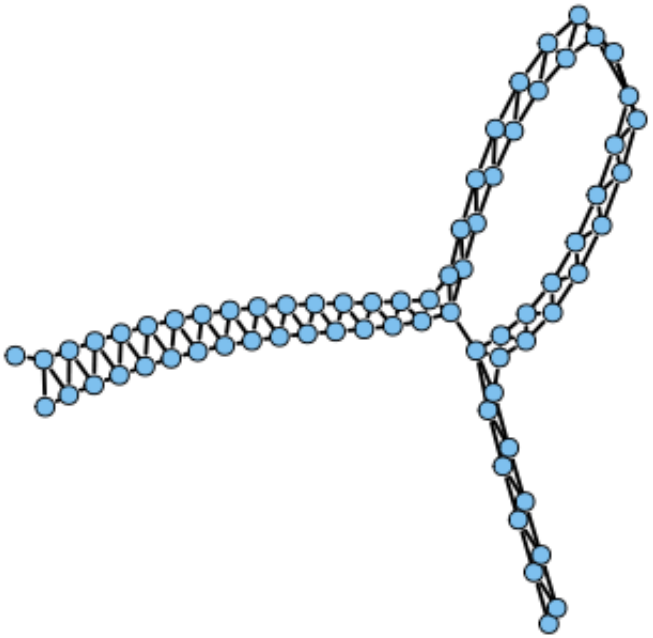


Repeat variants with partial similarity
(depends on similarity threshold)

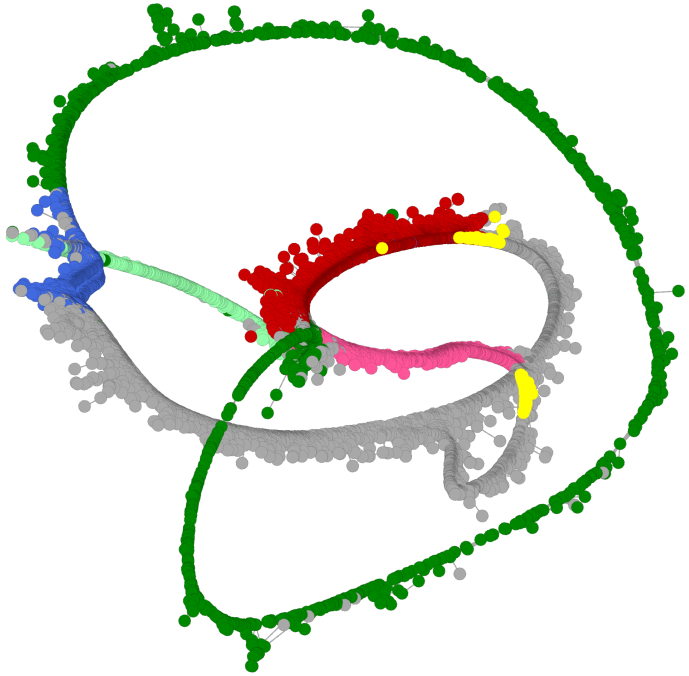


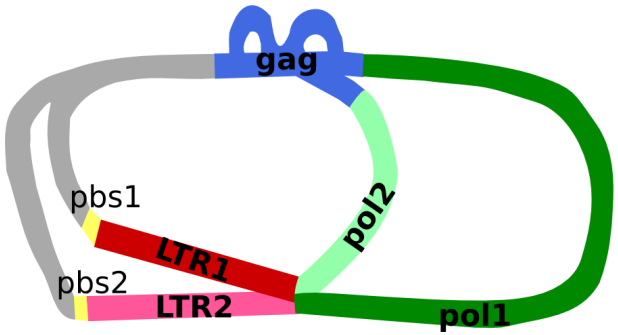
Variants differing in indel





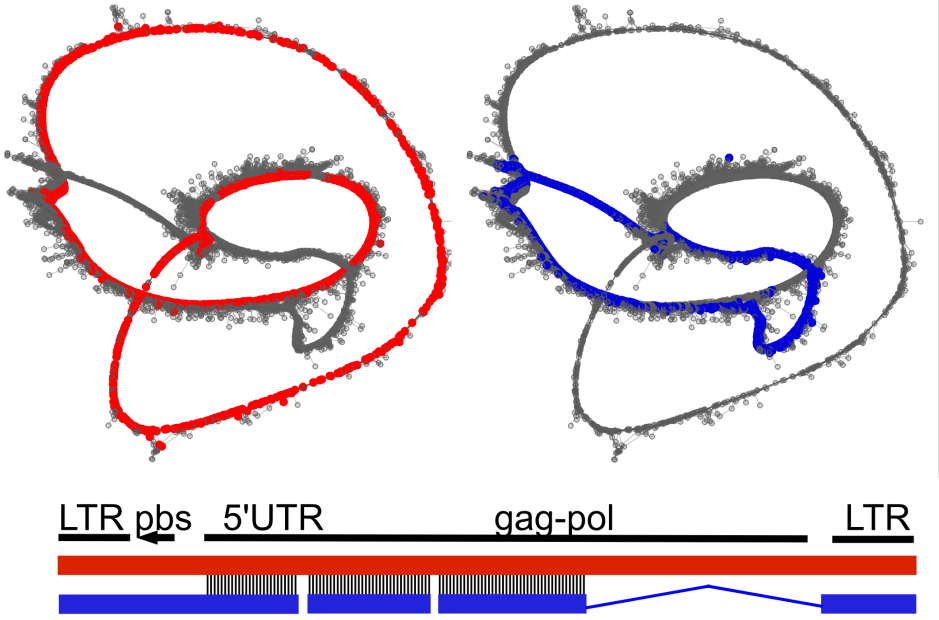
It's getting complicated...





...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

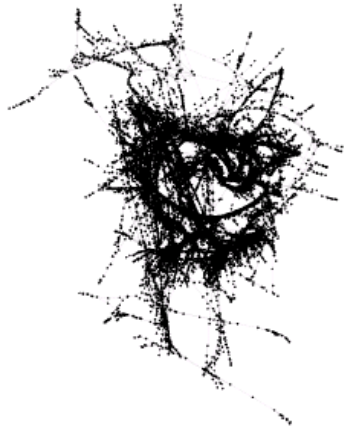
Number of reads: 42851
Number of pairs: 717667
Density: 0.0007817
Diameter: NA
Mean edge weigth: 130.31
Max. degree: 257

CL2

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

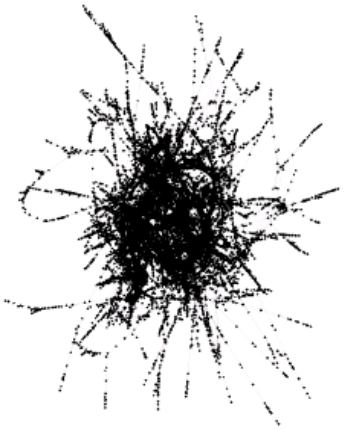
CL3

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

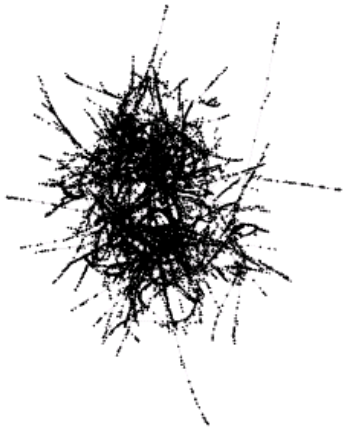


CL4

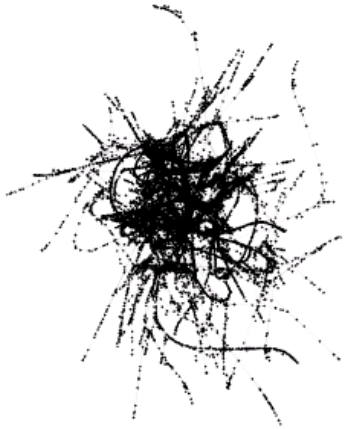
Number of reads: 46876
Number of pairs: 1022899
Density: 0.000931
Diameter: NA
Mean edge weigth: 132.18
Max. degree: 290



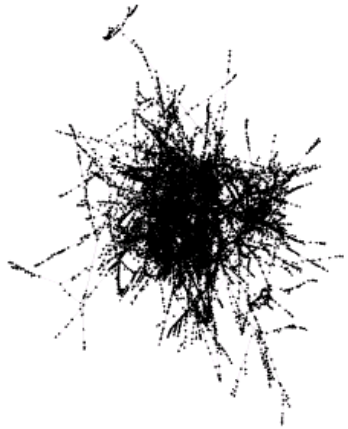
CL5



CL6

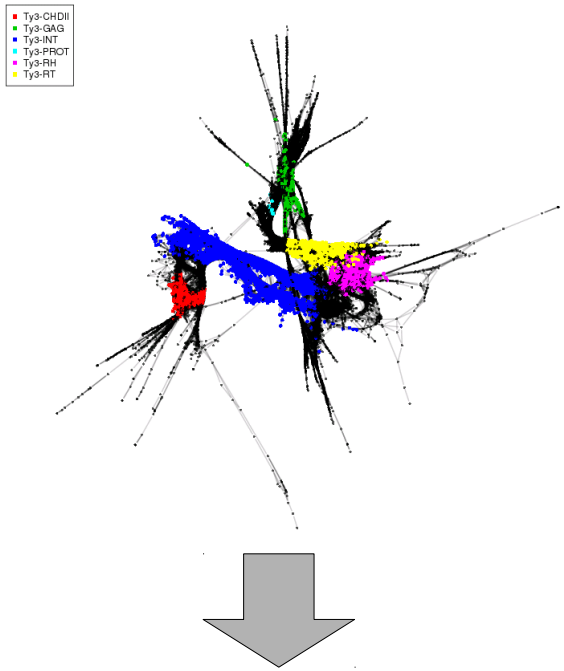


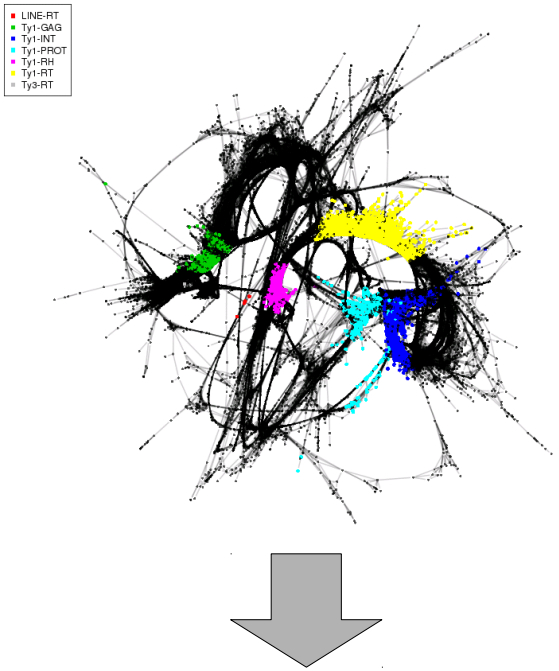
CL7

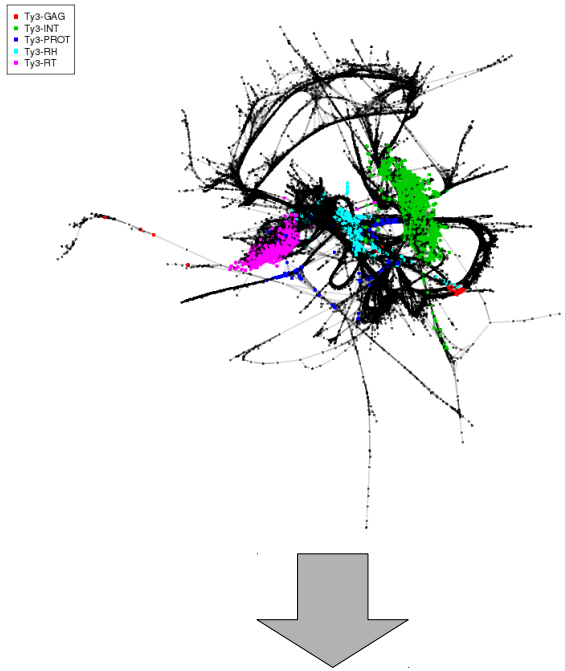


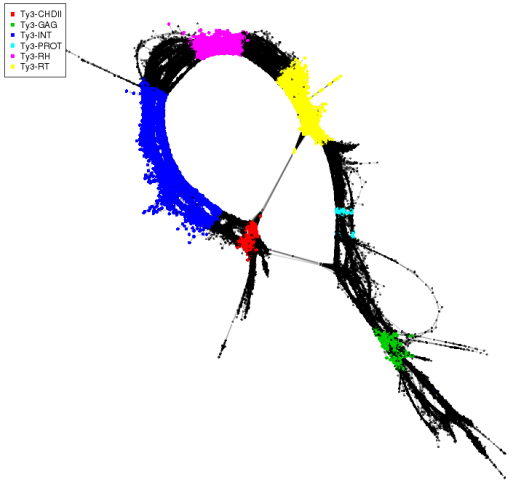
CL8

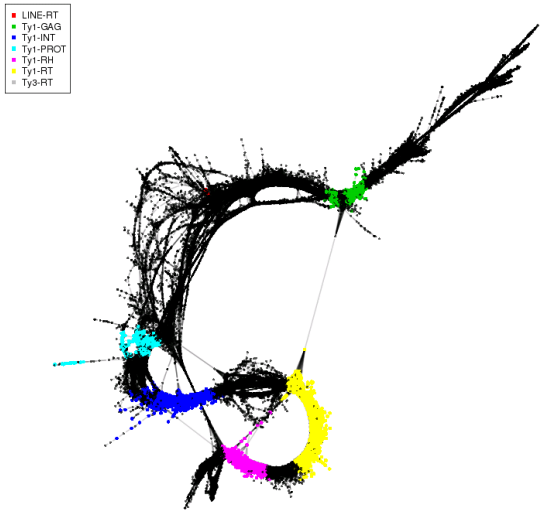
Using different algorithms for calculating graph topology

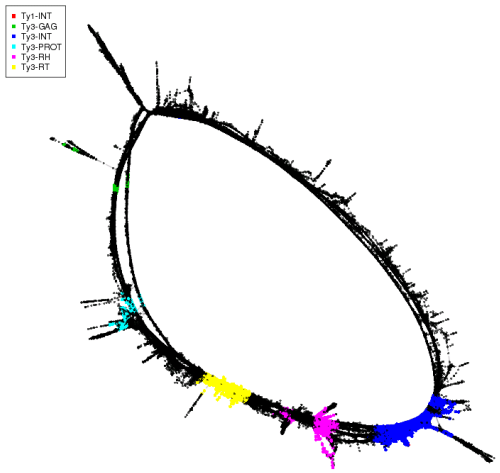








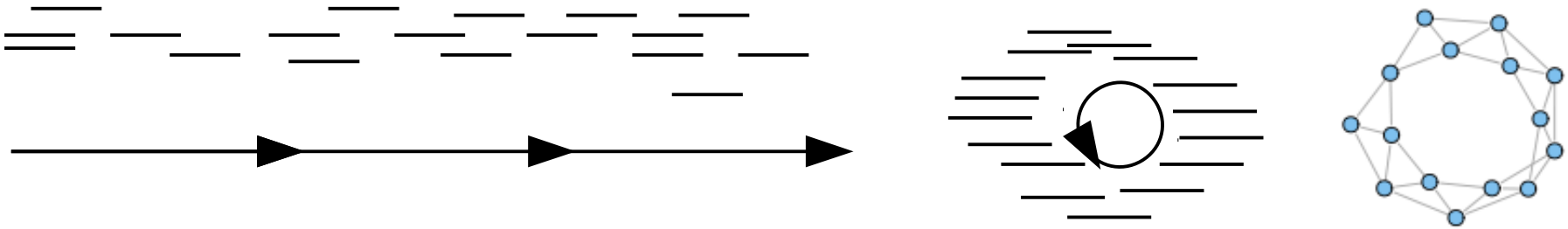




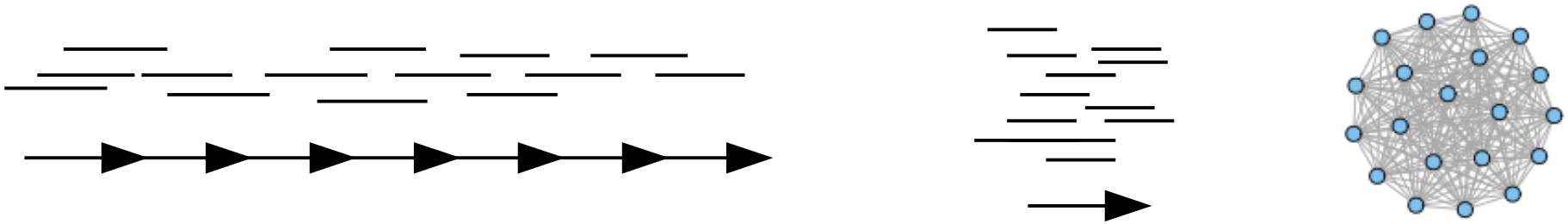
Circular graphs

TANDEM REPEATS

Read length << monomer

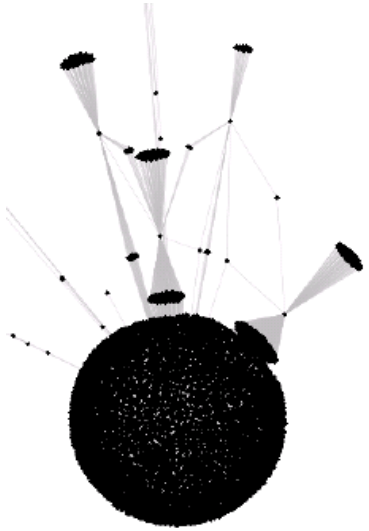


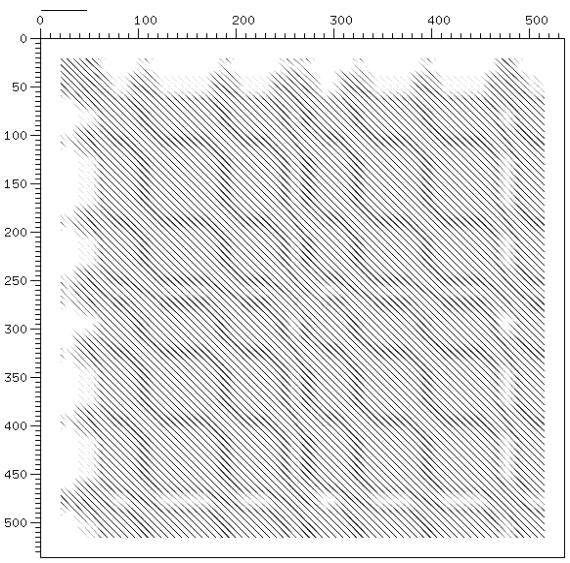
Read length >= monomer



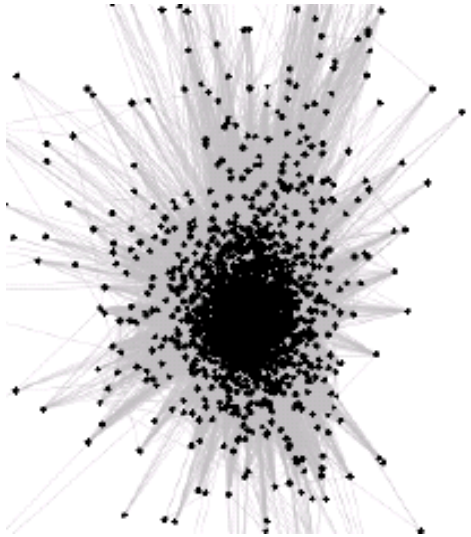
Circular graphs

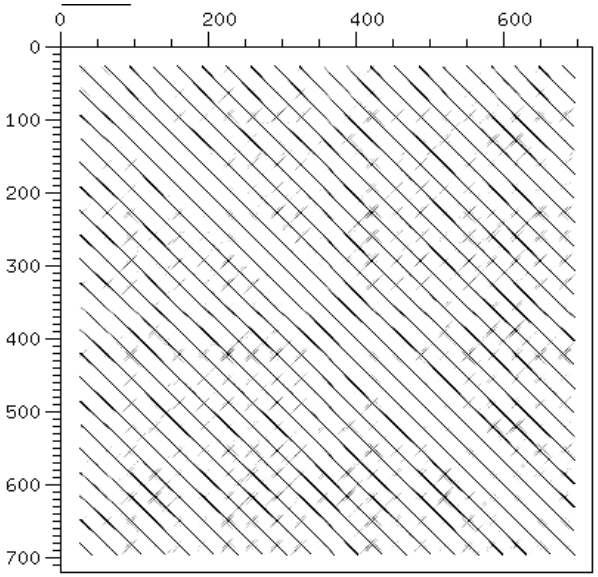
microsatellite (6bp)
(GAACCT)n



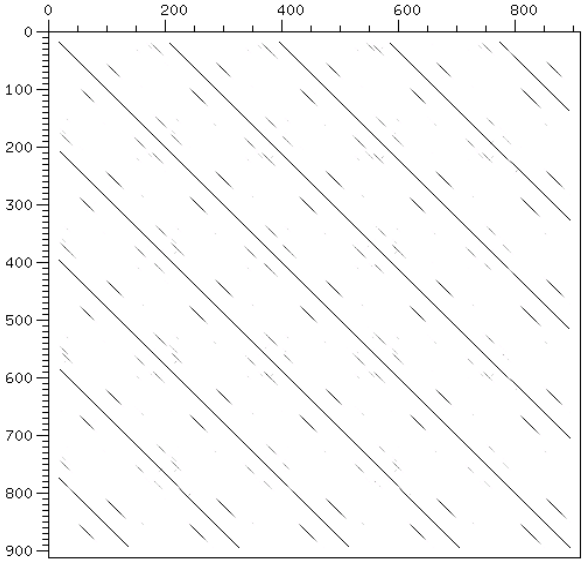


satellite 35 bp



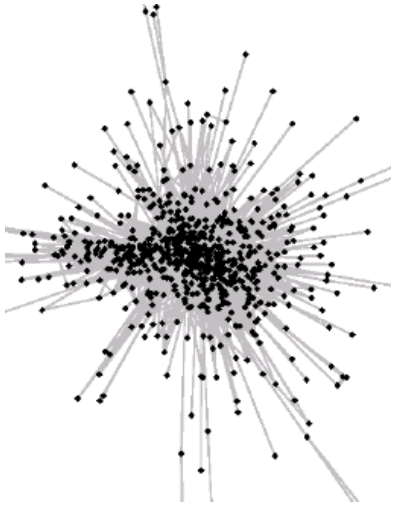


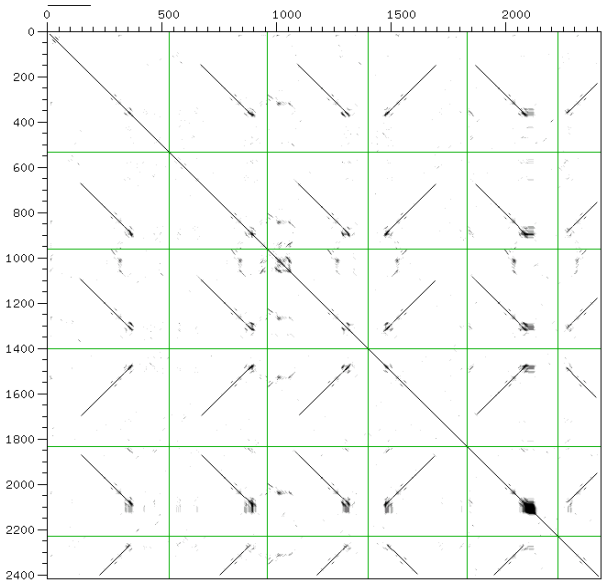
satellite 190 bp



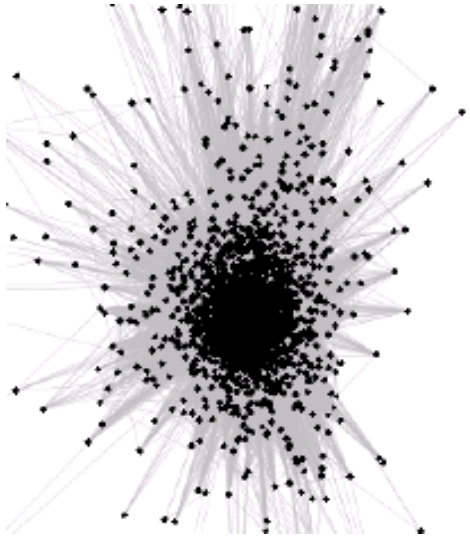
Circular graphs

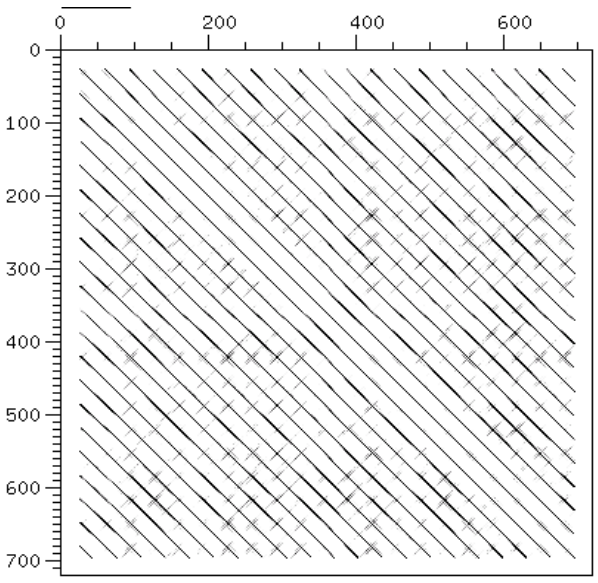
SINE



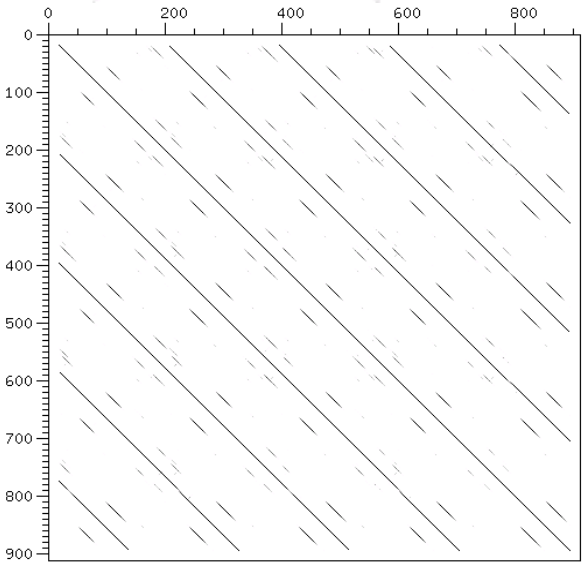


satellite 35 bp



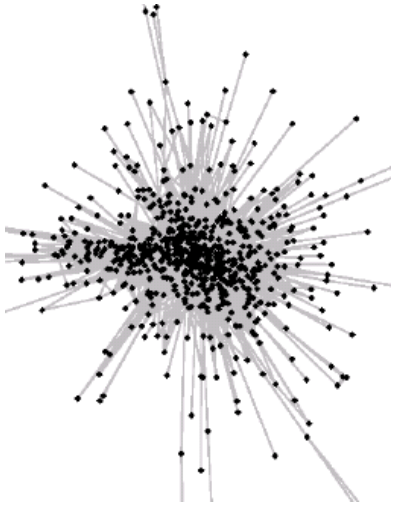


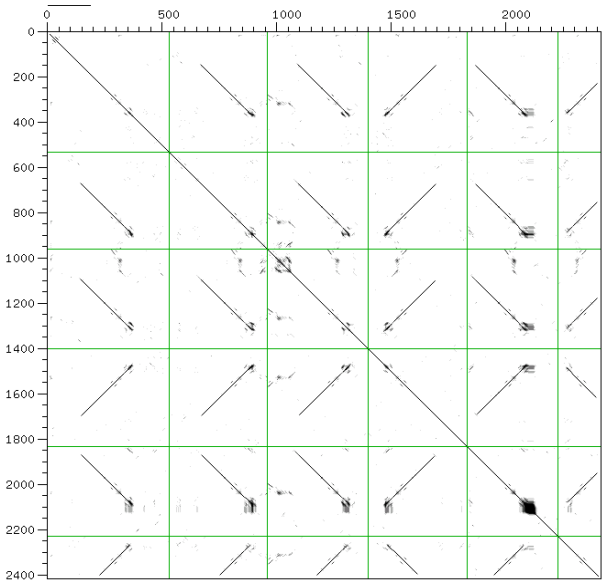
satellite 190 bp



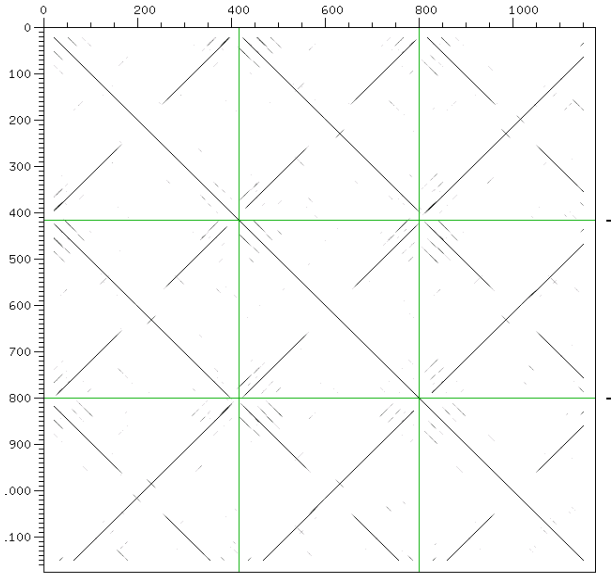
Circular graphs

SINE

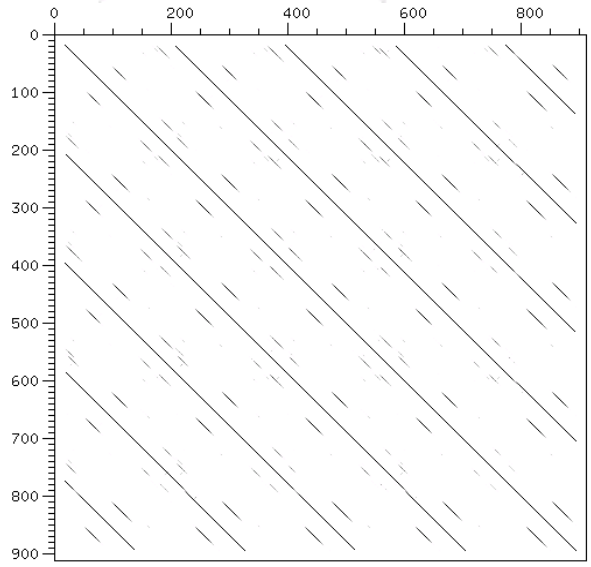




MITE
(foldback)

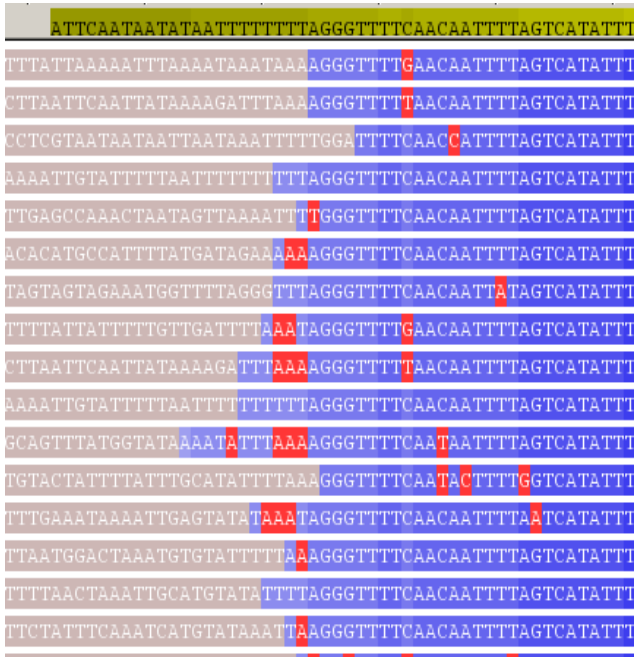


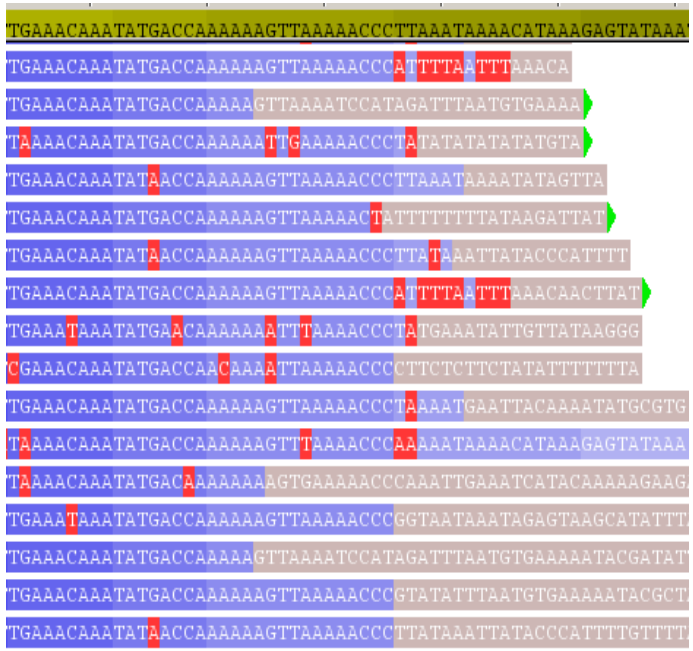
satellite 190 bp

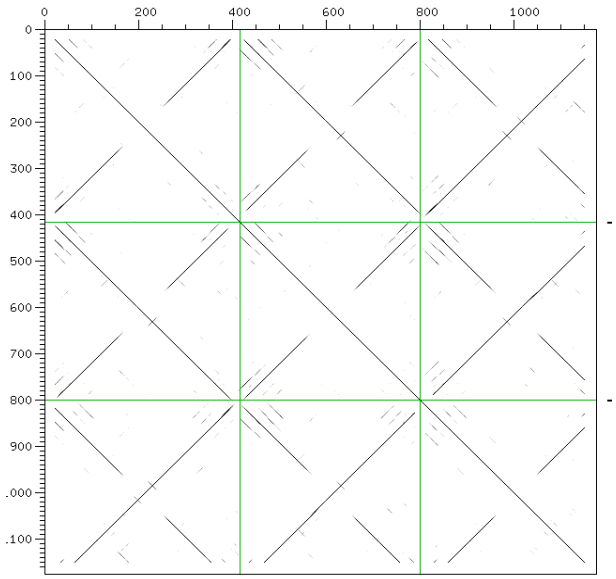


Insertion sites of mobile elements

MITE
(foldback)

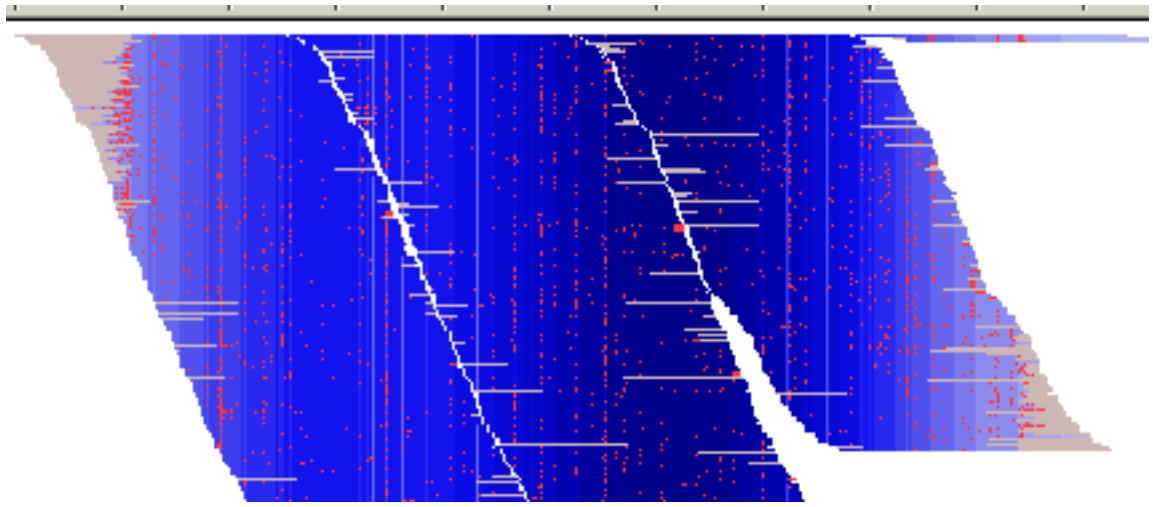






TIR

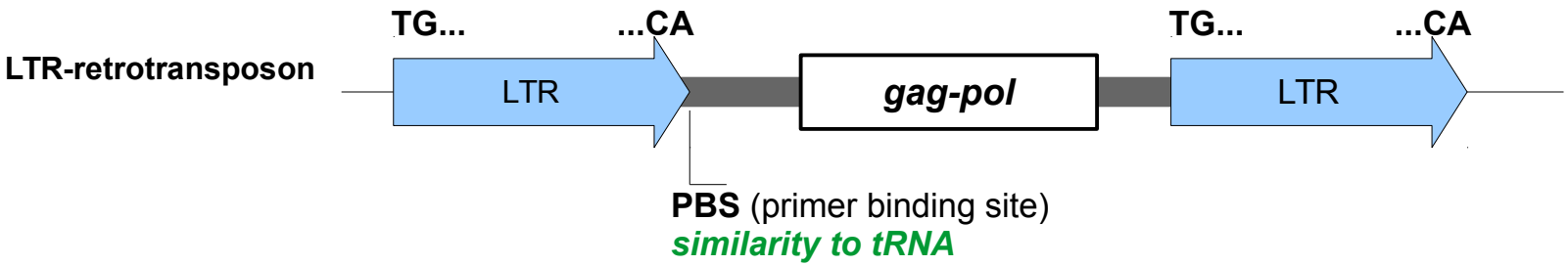
TIR



contig

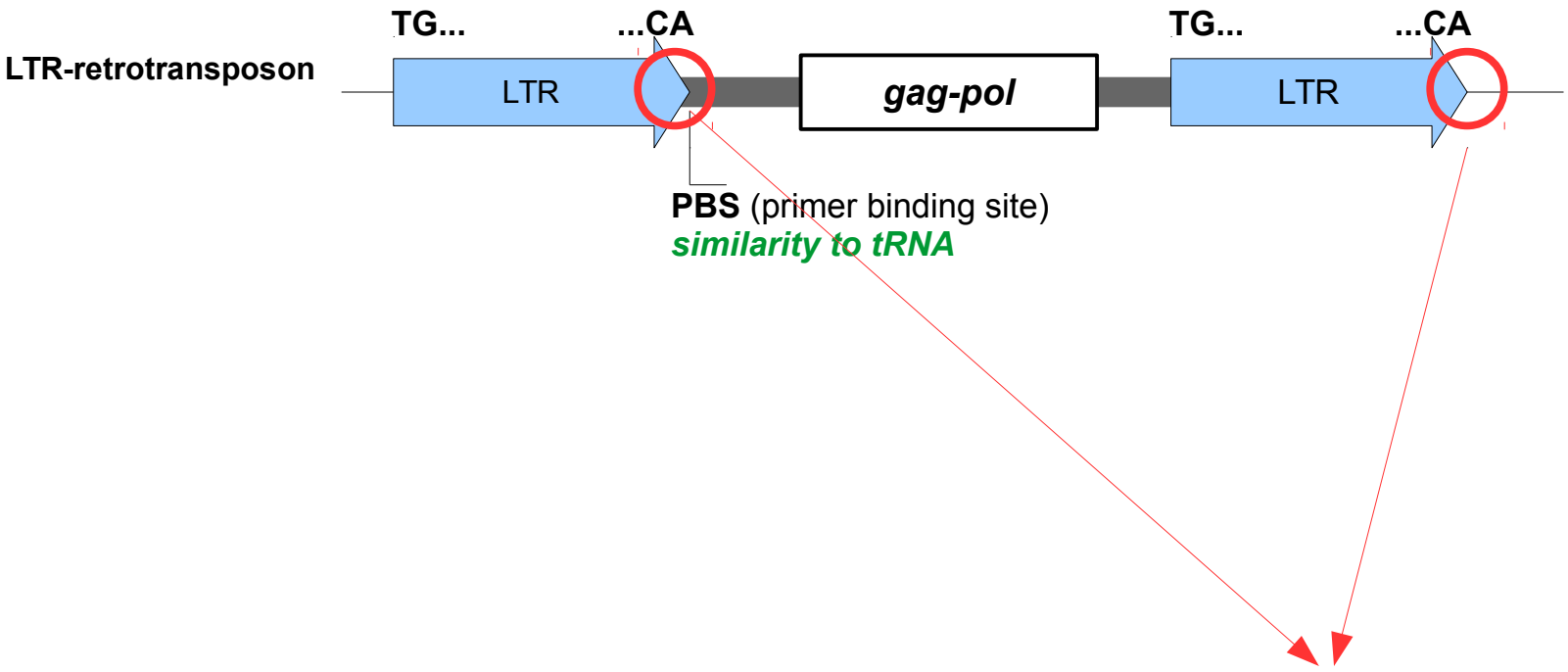
Insertion sites of mobile elements

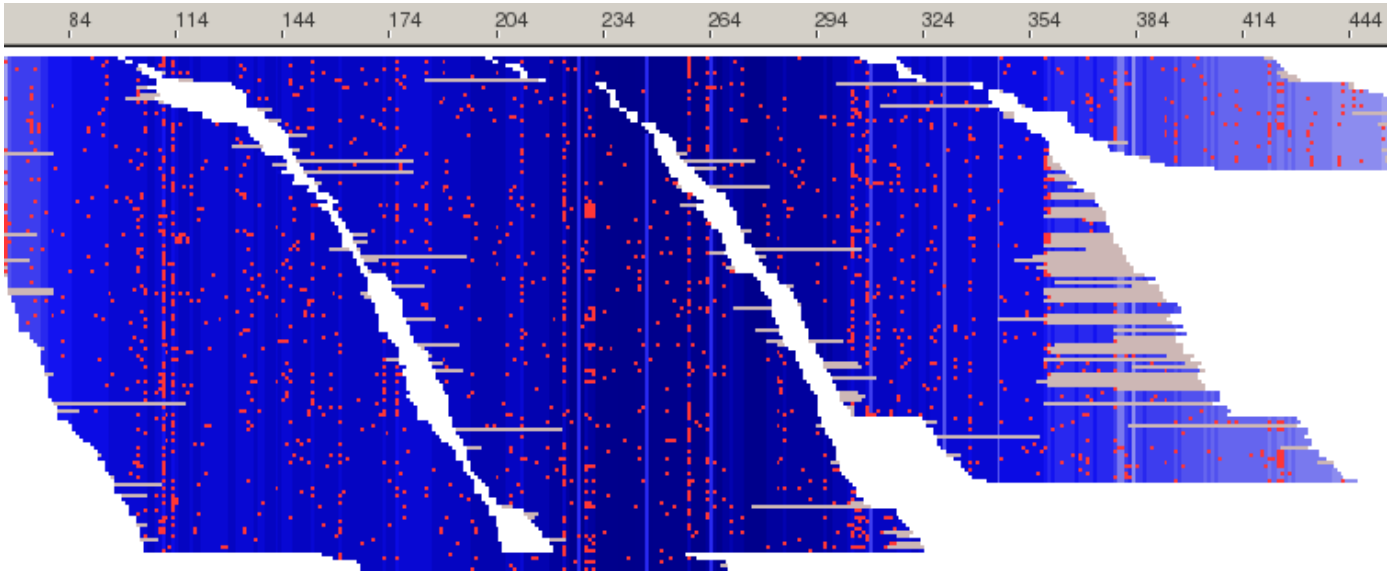
A new tool for detection of LTR / PBS sites



Insertion sites of mobile elements

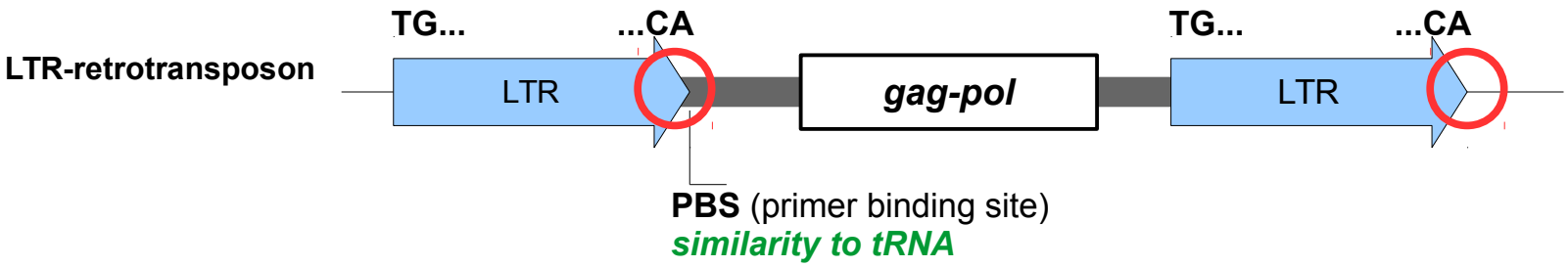
A new tool for detection of LTR / PBS sites





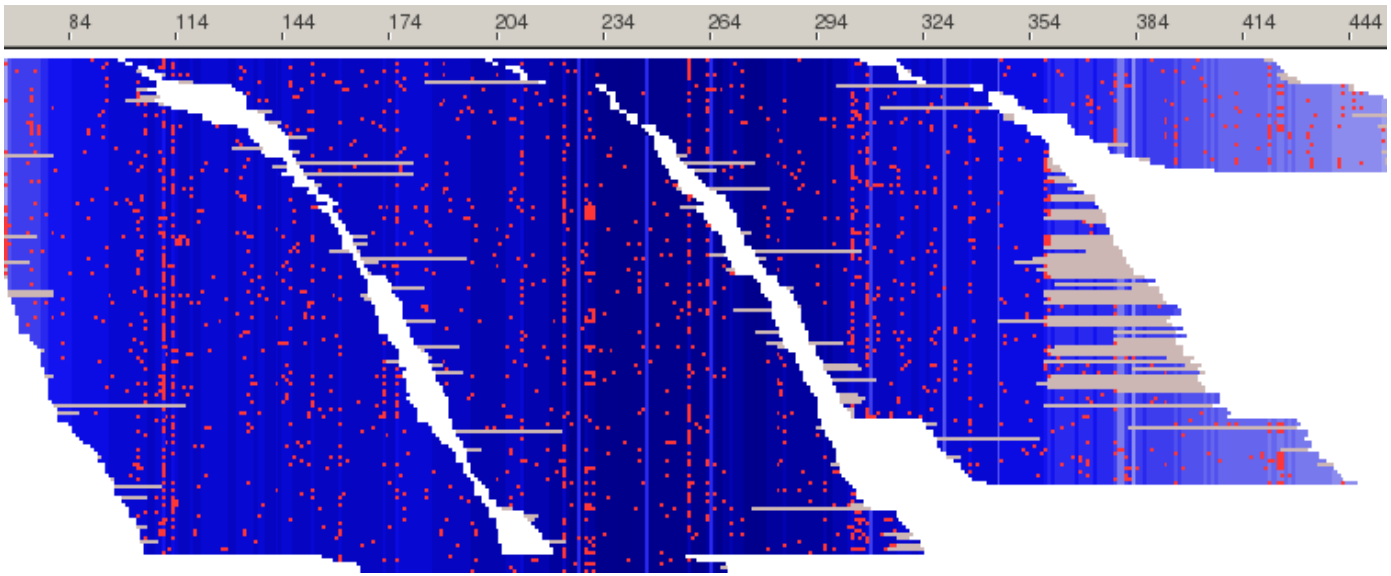
Insertion sites of mobile elements

A new tool for detection of LTR / PBS sites



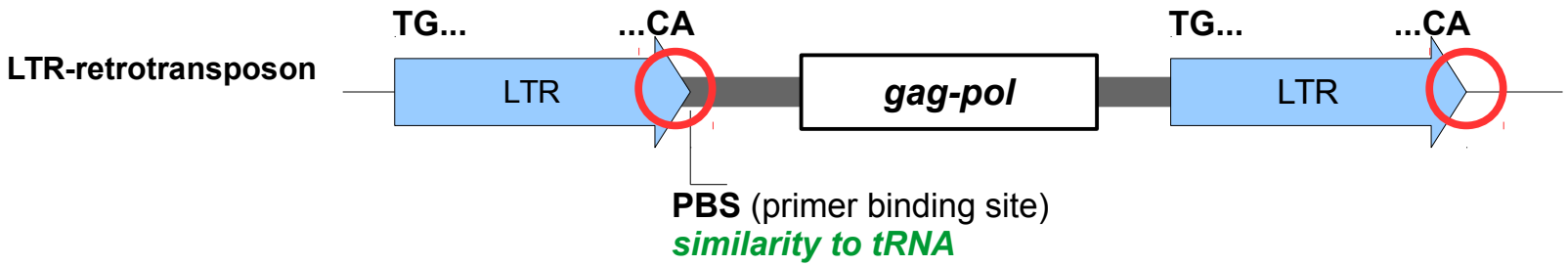
The tool searches assembled contigs (ACE files) for **differences in proportions of masked reads** accompanied by **occurrence of TG/CA**

...and **similarity to tRNA**



Insertion sites of mobile elements

A new 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

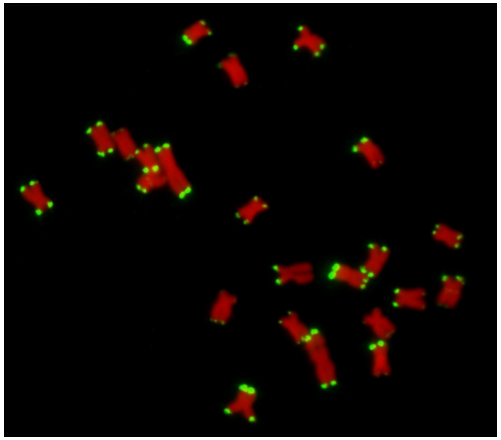
(window size 7)

“Chimeric” clusters ?

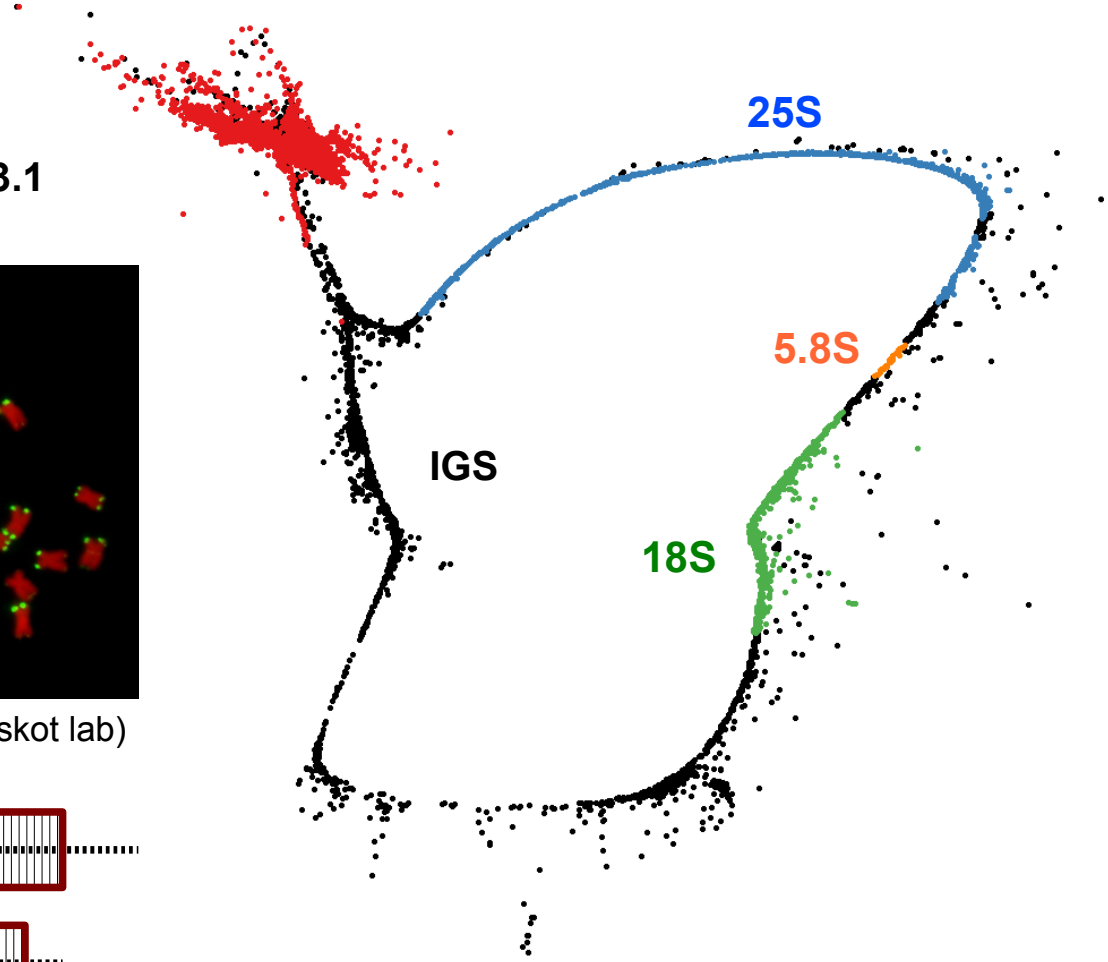
Reconstruction of rDNA gene cluster in *Silene latifolia*

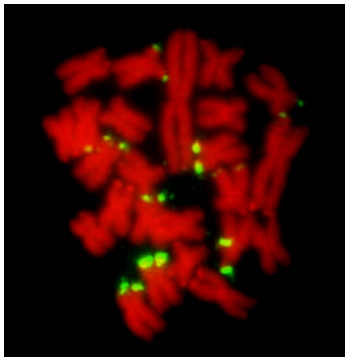
TANDEM REPEAT

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

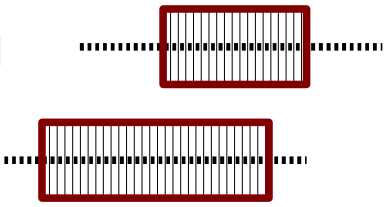


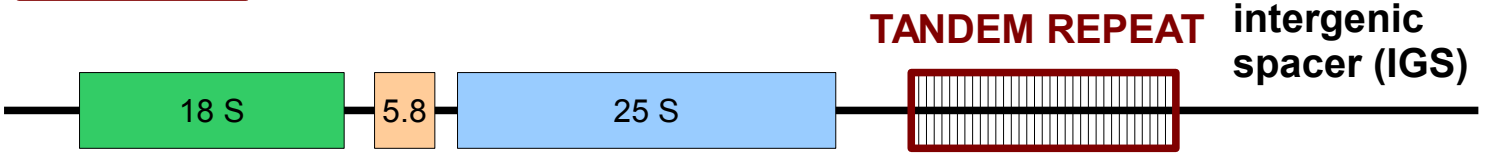
(Vyskot lab)





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	copia	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	copia	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	copia	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	copia	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			?

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