Investigating the heterogeneity of repeat regions in the human genome

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Overview

Classification of repeated sequences:

- Microsatellites (1-7bp)
- Minisatellites (<100bp)
- Macrosatellites (>100bp)

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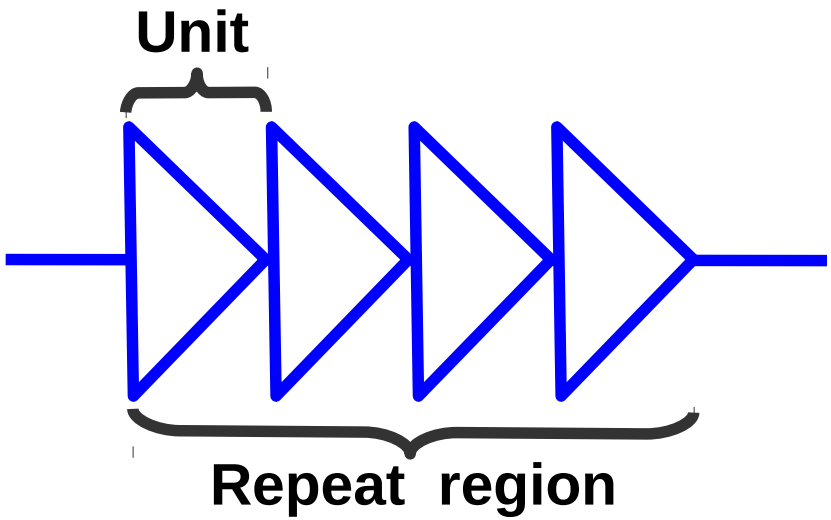
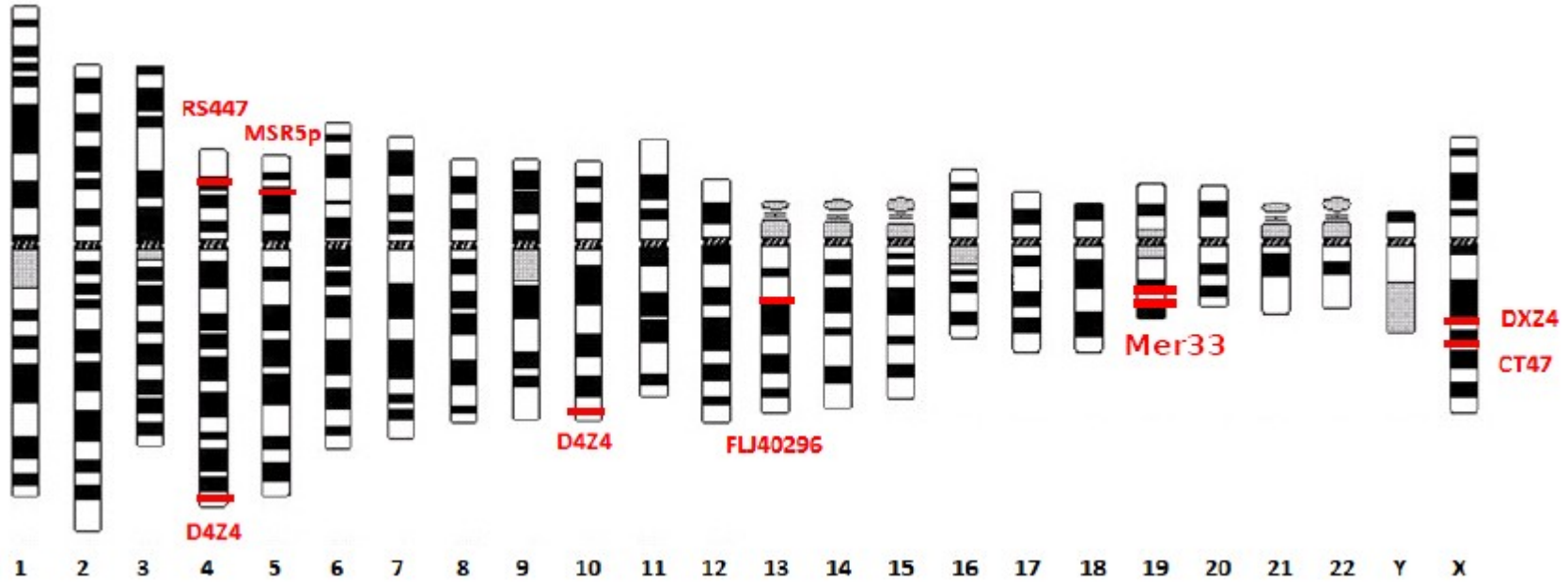


Overview

- 9 MSRs on different chromosome
- 3.0-6.6 kb
- Units within an array: 95-99% homologous



Relevance of project

- ***Medical relevance***

The development of the FSHD-disease depend on amount of units in repeat region D4Z4 chr4 (<11 units FSHD)

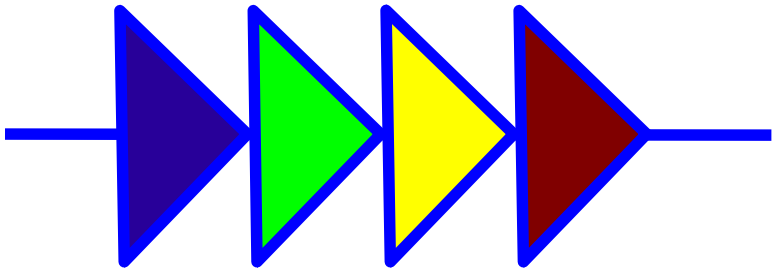
- ***Bioinformatic relevance***

The complexity of genome assembling in the repeat regions leads to necessity of development new approaches to their study

Aims

Investigating the heterogeneity of repeat regions:

- Heterogeneity among units of one repeat region



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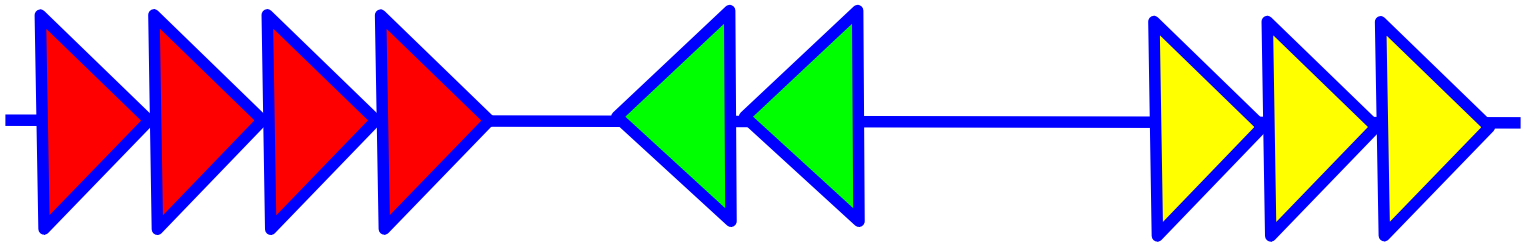
- Heterogeneity among repeat regions of one genome



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- Heterogeneity among repeat regions of one genome
- Heterogeneity of repeat regions among genomes

Next Generation Sequencing data

- Millions of short fragments (reads)
- Length of reads 36-100 nt

Typical fastq-file (file containing reads):

```
……
@ERR000624.129 EAS254_13:8:1:7:874/2
AGGCAAAAAGAAACAGGCAAAACTAATATTTTAAATATCCCAACGTAACCA
+
,I3$5=243&);3.8=(41315+91."84B)7"""/"+-3)+3/+0+*,'*
……
```

Two different approaches

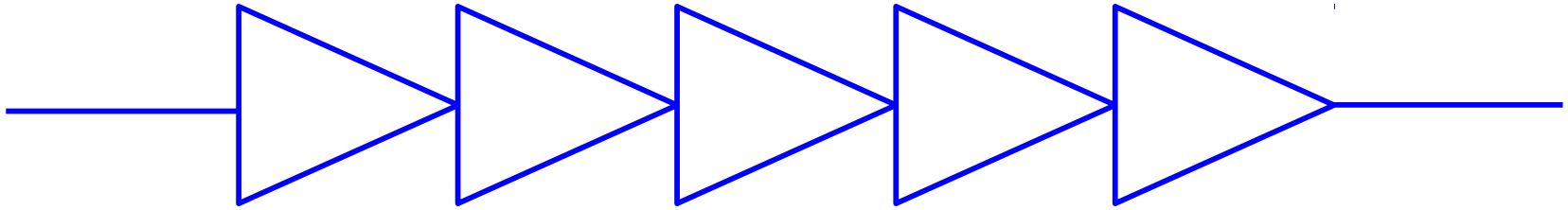
- ***Work with consensuses of repeat units***

(N. Gulyaeva)

- ***K-mer profiling***

(P. Sinitsyn)

Find boundaries of units of MSRs in the reference genome



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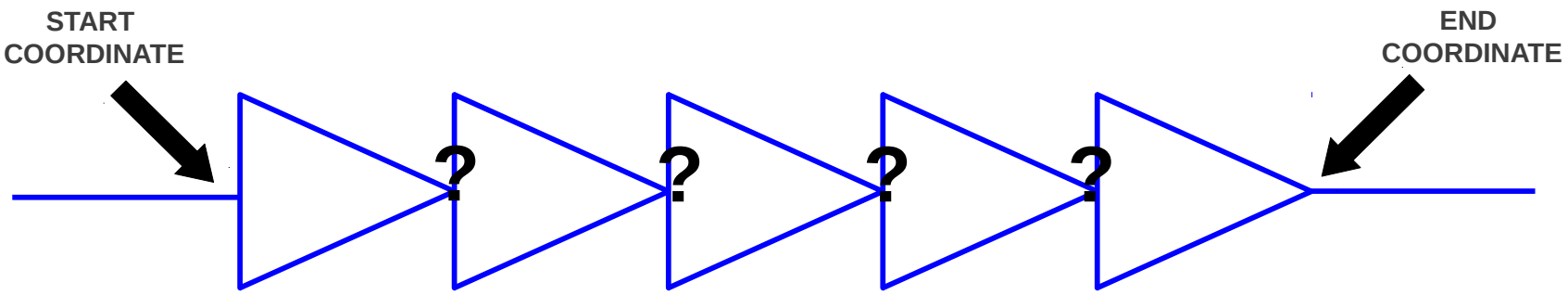

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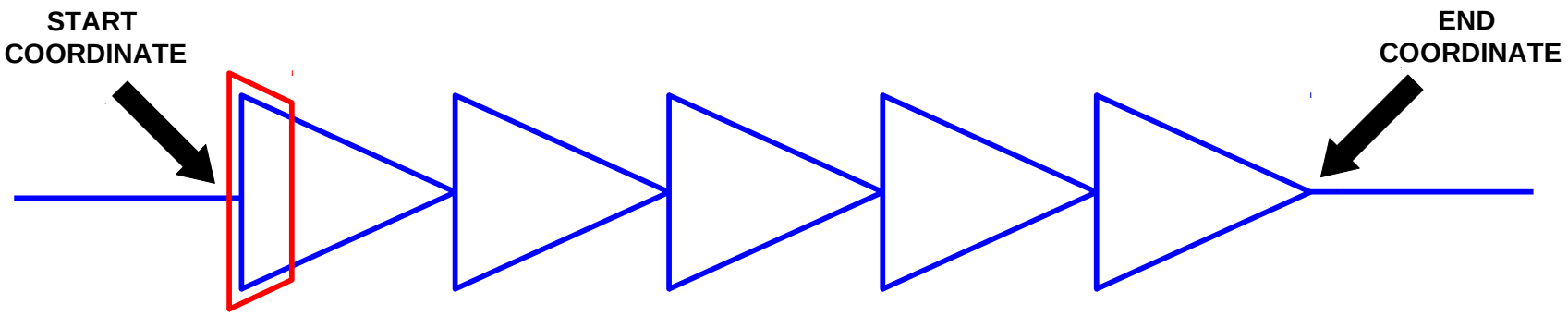

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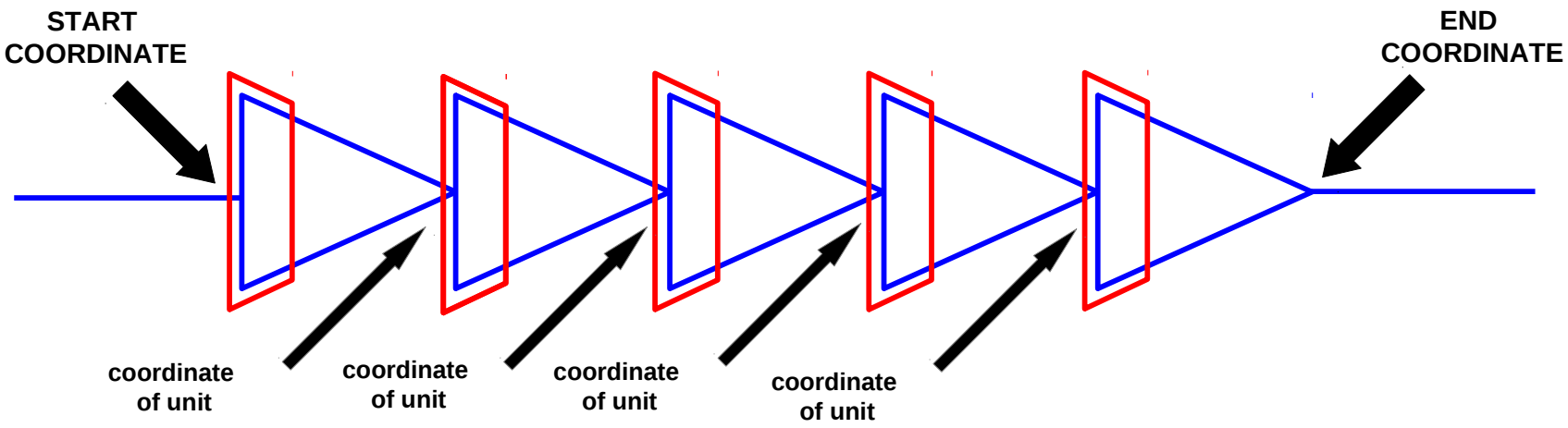
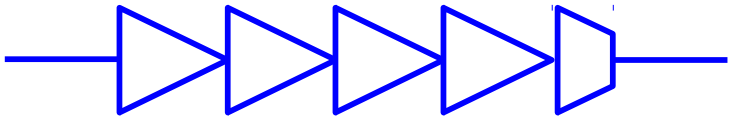
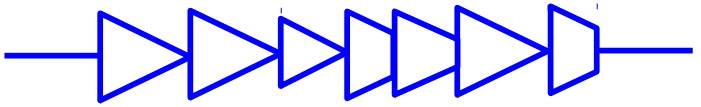
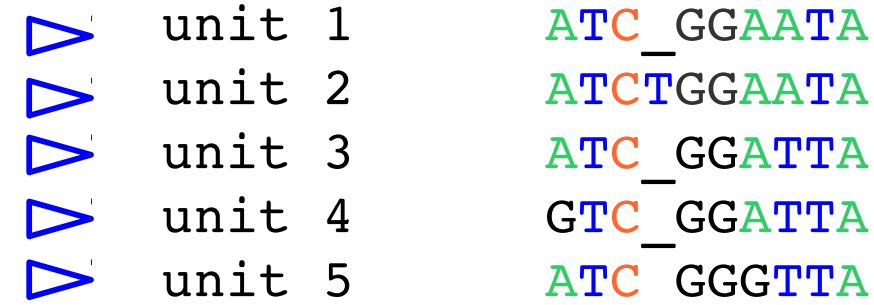
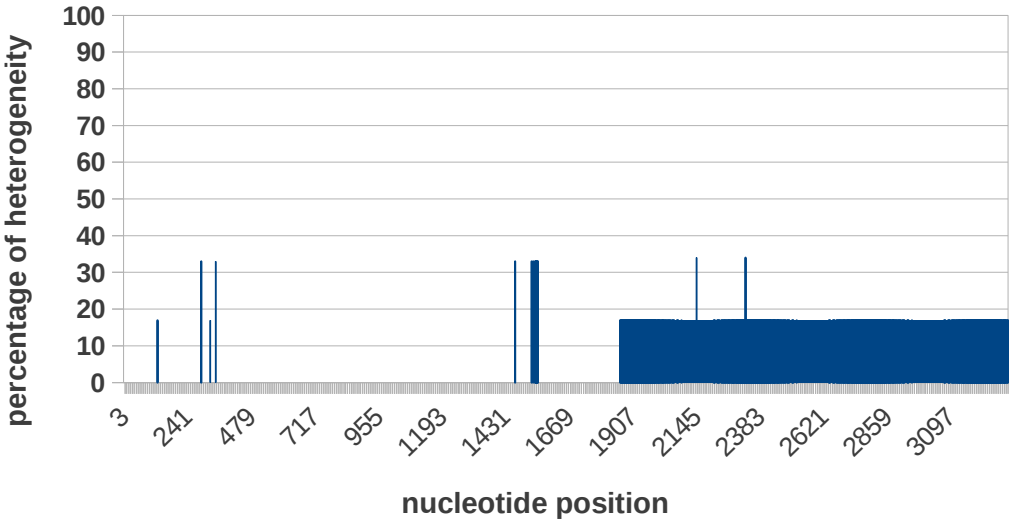


MSR	Number of units	Sequence structure
D4Z4_4q	8	
D4Z4_10q	6	
FLJ40296	5	
RS447	10	
Mer33_1	11	
CT47	14	
MSR5p	11	
DXZ4	14	
Mer33_2	12	

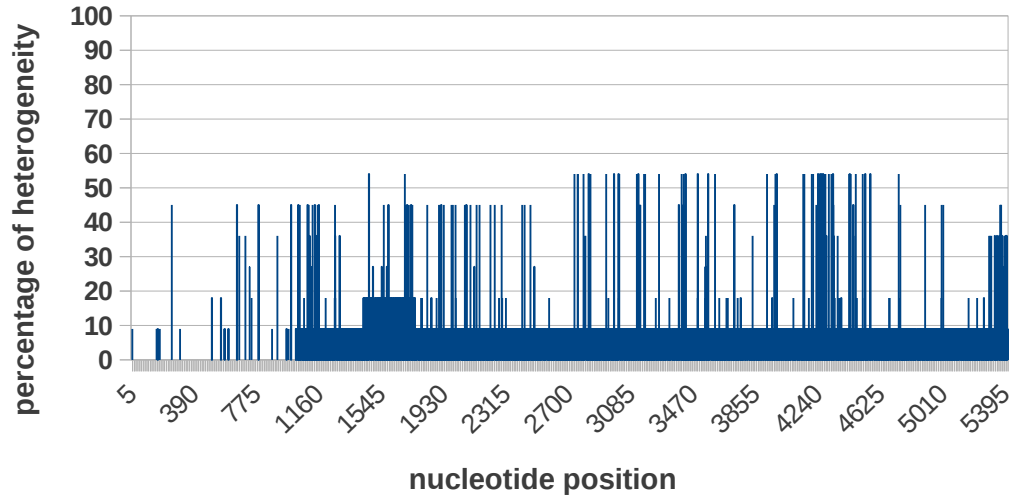
Multiple alignment of units for each MSR (clustalw)



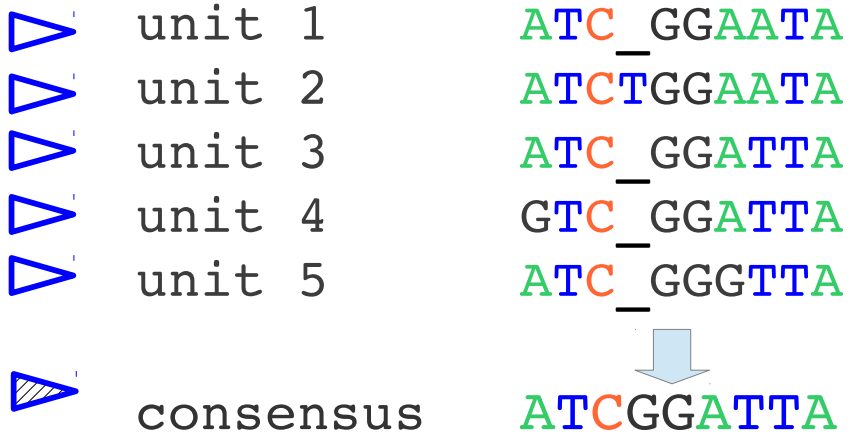
D4Z4_10q



Mer33_1



Build consensus unit for each MSR

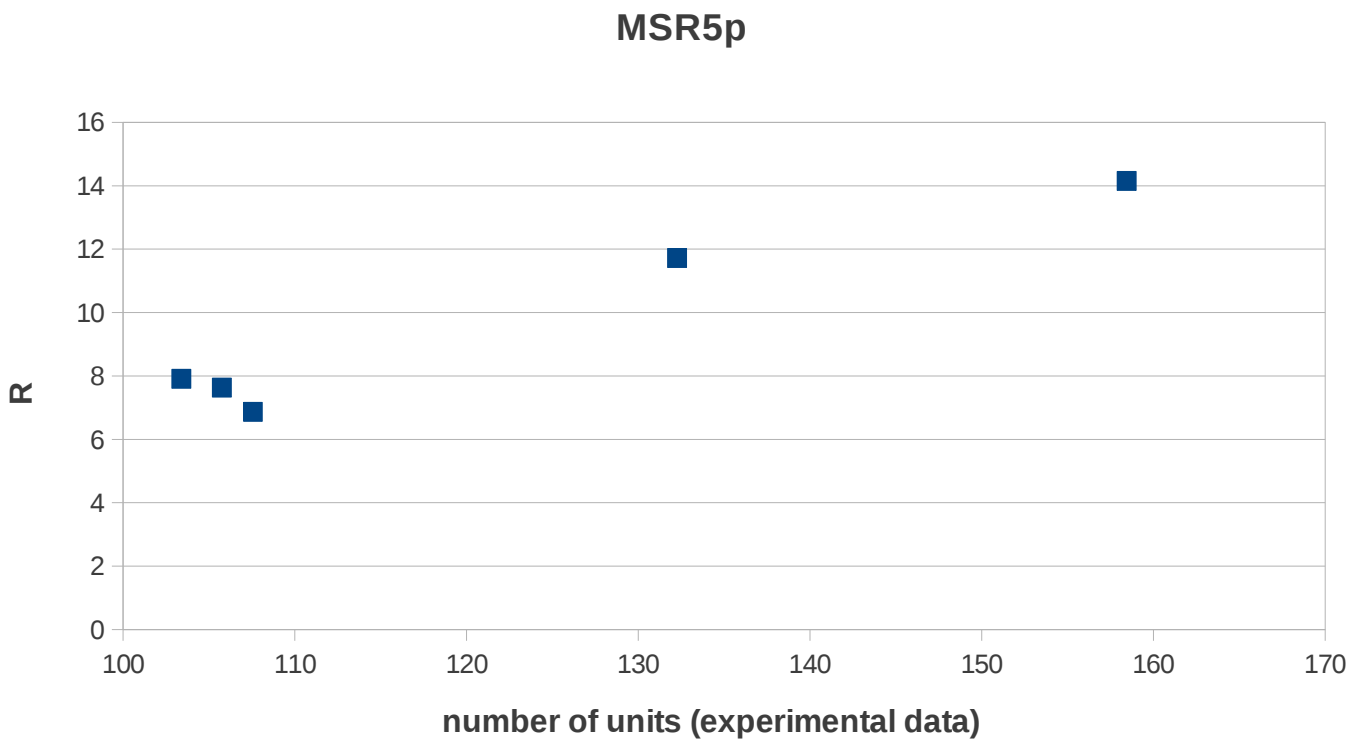


Calculations based on alignment results

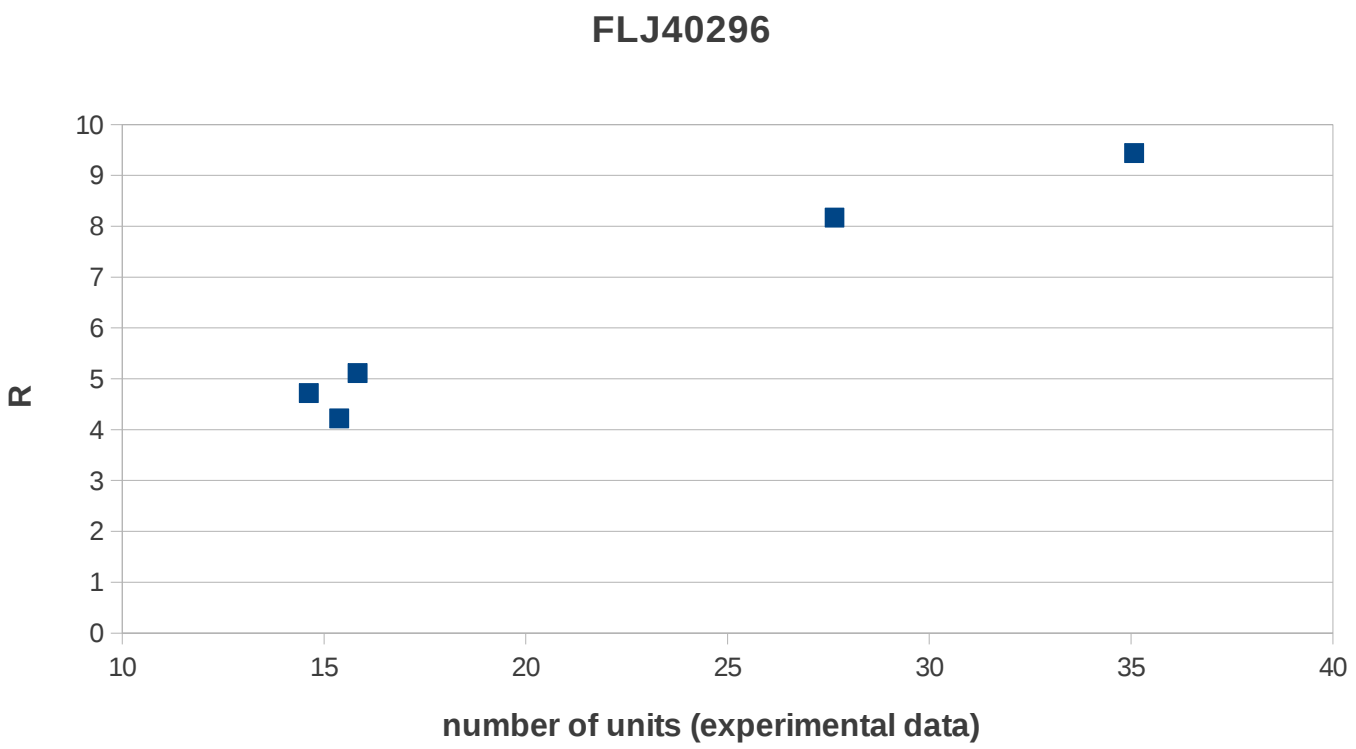
$$R = \frac{n}{T \times l} \times 10^9$$

- R – proportion of reads per nucleotide of MSR
- n – number of reads that map to consensus unit of MSR
- T – total number of reads
- l – length of consensus unit

Calculations based on alignment results



Calculations based on alignment results



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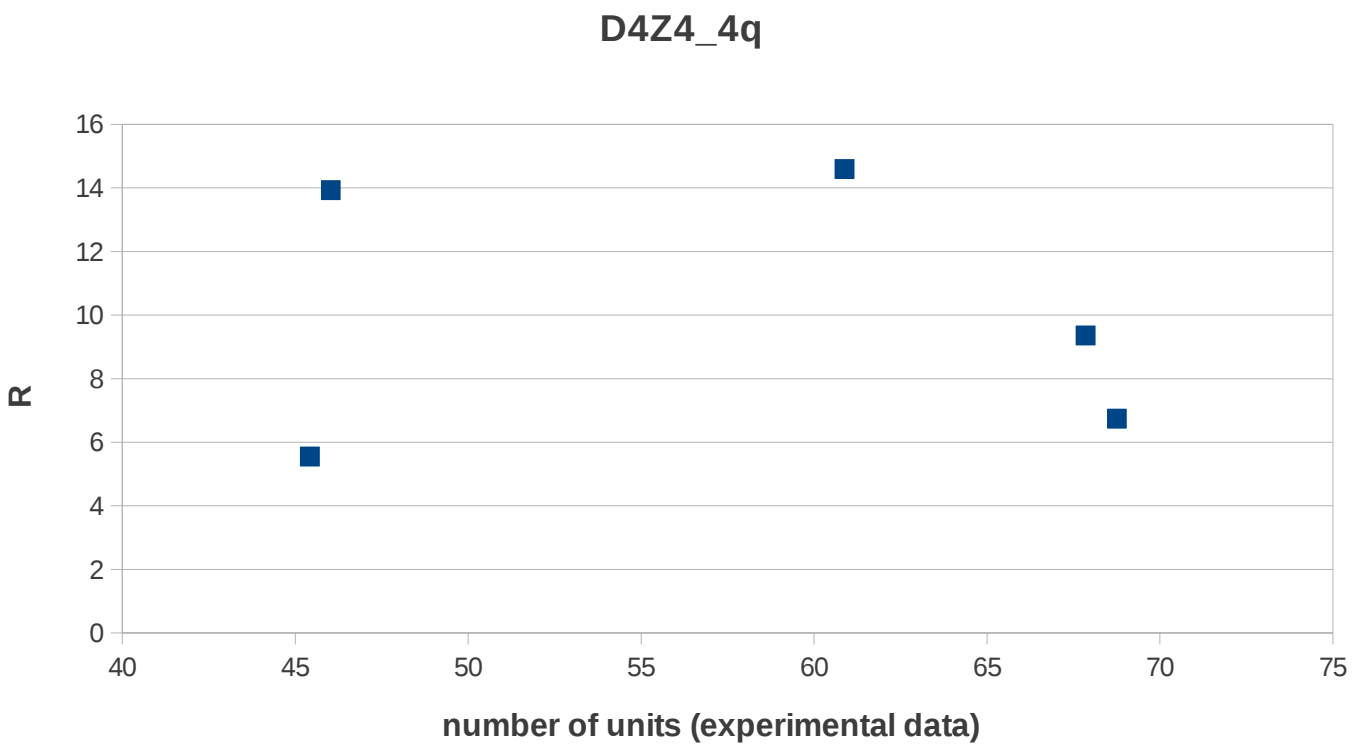
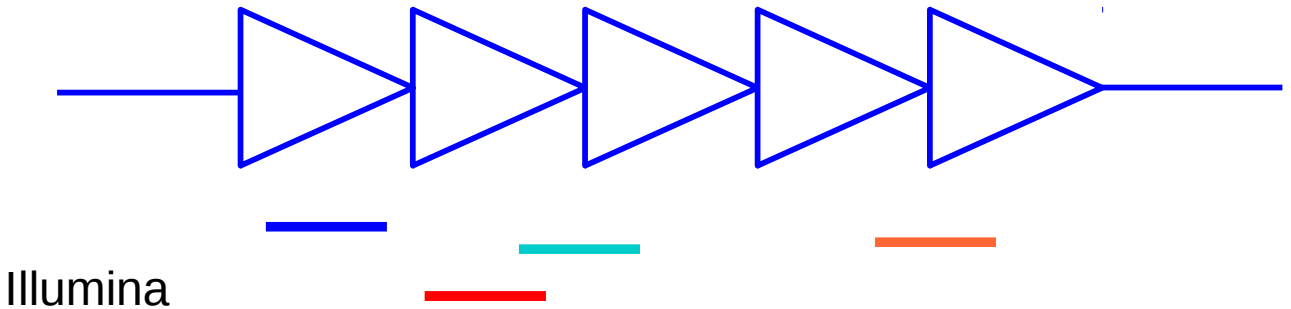


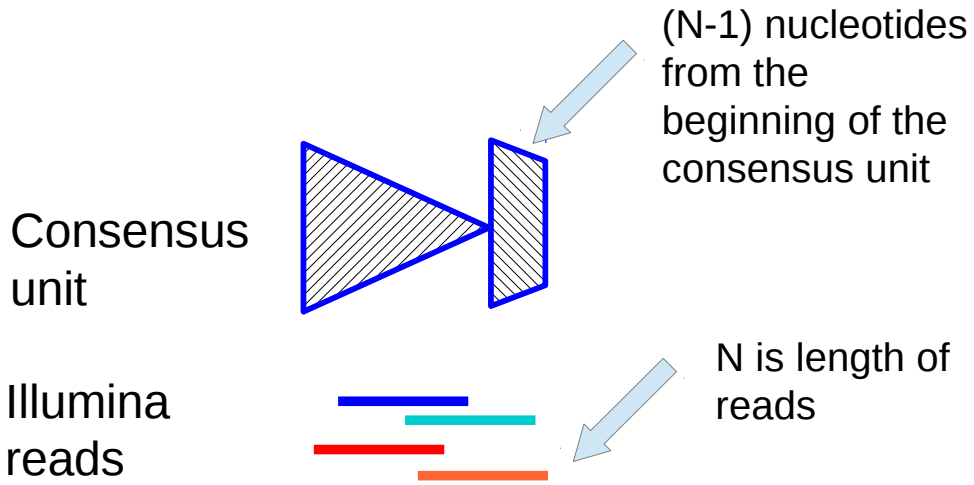
Calculations based on alignment results

Sample GM12878

	CT47	D4Z4_4q	D4Z4_10q	DXZ4	FLJ40296	Mer33_1	Mer33_2	MSR5p	RS447
reads – 36 nucleotides	87.72	6.74	6.63	20.9	4.72	151.76	176.27	7.63	9.41
reads – 37 nucleotides	86.83	7.59	7.55	16.81	4.08	146.14	175.73	6.54	8.97
reads – 67 nucleotides	5.99	1.63	1.82	8.1	1.68	10.33	17.44	5.73	14.83

Alignment of reads on consensus units (bwa)





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How to do: k-mer profiling

Sequence of repeat unit X:

AATGC

Sequence of genome with 2 repeat region X: AAA AATGC AATGC AA

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Look at the frequency all possible

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Look at the frequency all possible sub-sequences length 2nt for whole genome and reapeat region

***Scientific Slang**: create k-mer profiling (k=2) for whole genome and repeat region*

K-mer profiling (k=2)

Forward strand

AAA AATGCAATGC AA

Reverse strand

TT GCATTGCATT TTT

Repeat region

	forward	reverse	Sum
AA	2		
AT			
TG			
GC			
CA			
TT			

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Repeat region

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AA	2		
AT	2		
TG			
GC			
CA			
TT			

K-mer profiling (k=2)

Forward strand

AAA AATGCAATGC AA

Reverse strand

TT GCATTGCATT TTT

	Repeat region		
	forward	reverse	Sum
AA	2	0	2
AT	2	2	4
TG	2	2	4
GC	2	2	4
CA	2	2	4
TT	0	2	2

K-mer profiling (k=2)

Forward strand

AAA AATGCAATGC AA

Reverse strand

TT GCATTGCATT TTT

	Repeat region/Genome		
	forward	reverse	Sum
AA	2/6	0/0	2/6
AT	2/2	2/2	4/4
TG	2/2	2/3	4/5
GC	2/2	2/2	4/4
CA	2/3	2/2	4/5
TT	0/0	2/6	2/6

Extraction of unique k-mers (k=2)

Unique k-mer is a sequence whose frequency at genome is equal to frequency at repeat region

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AA	2/6	0/0	2/6
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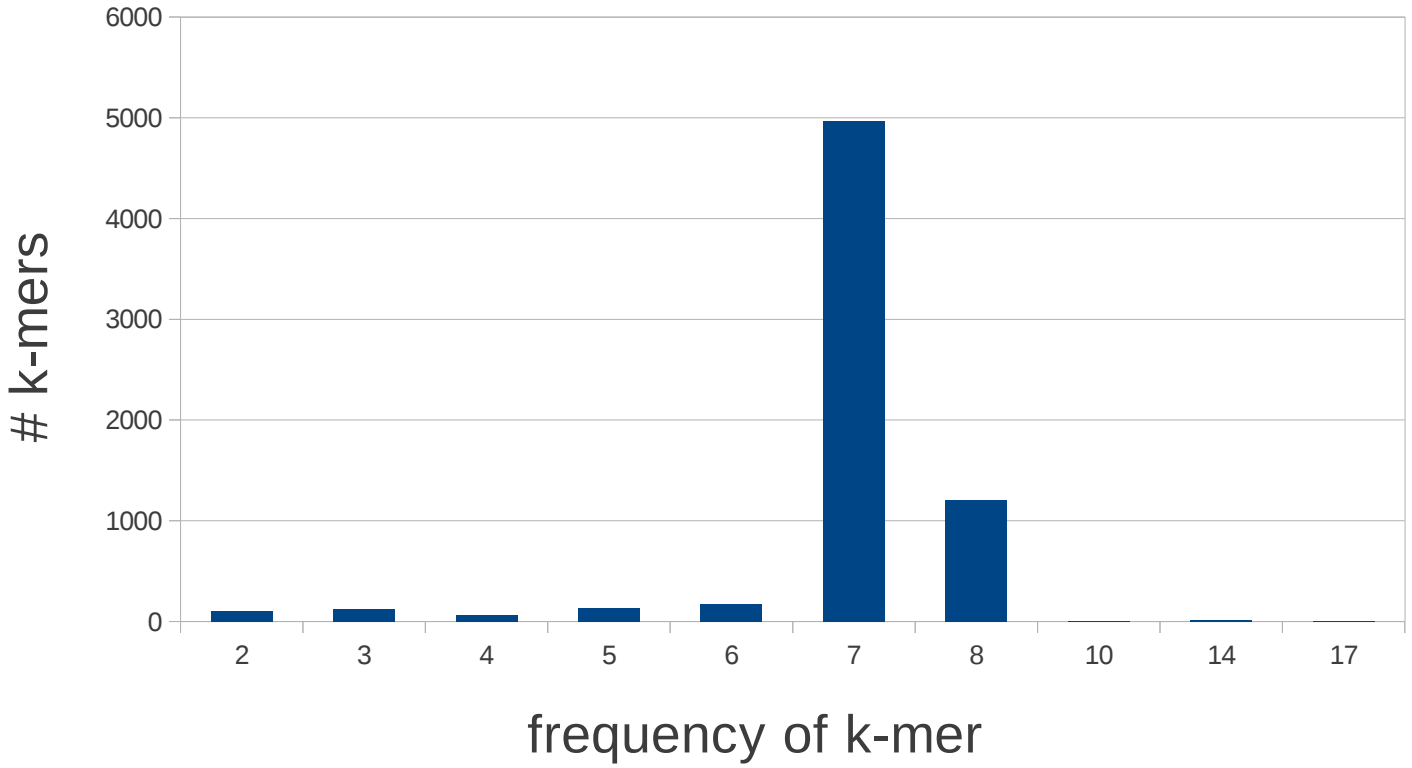
GC	2/2	2/2	4/4
CA	2/3	2/2	4/5
TT	0/0	2/6	2/6

Unique 2-mers for repeat region X is:
AT and GC

Result: k-mer profiling (k=17)

17-mer profiling for D4Z4_4q repeat region



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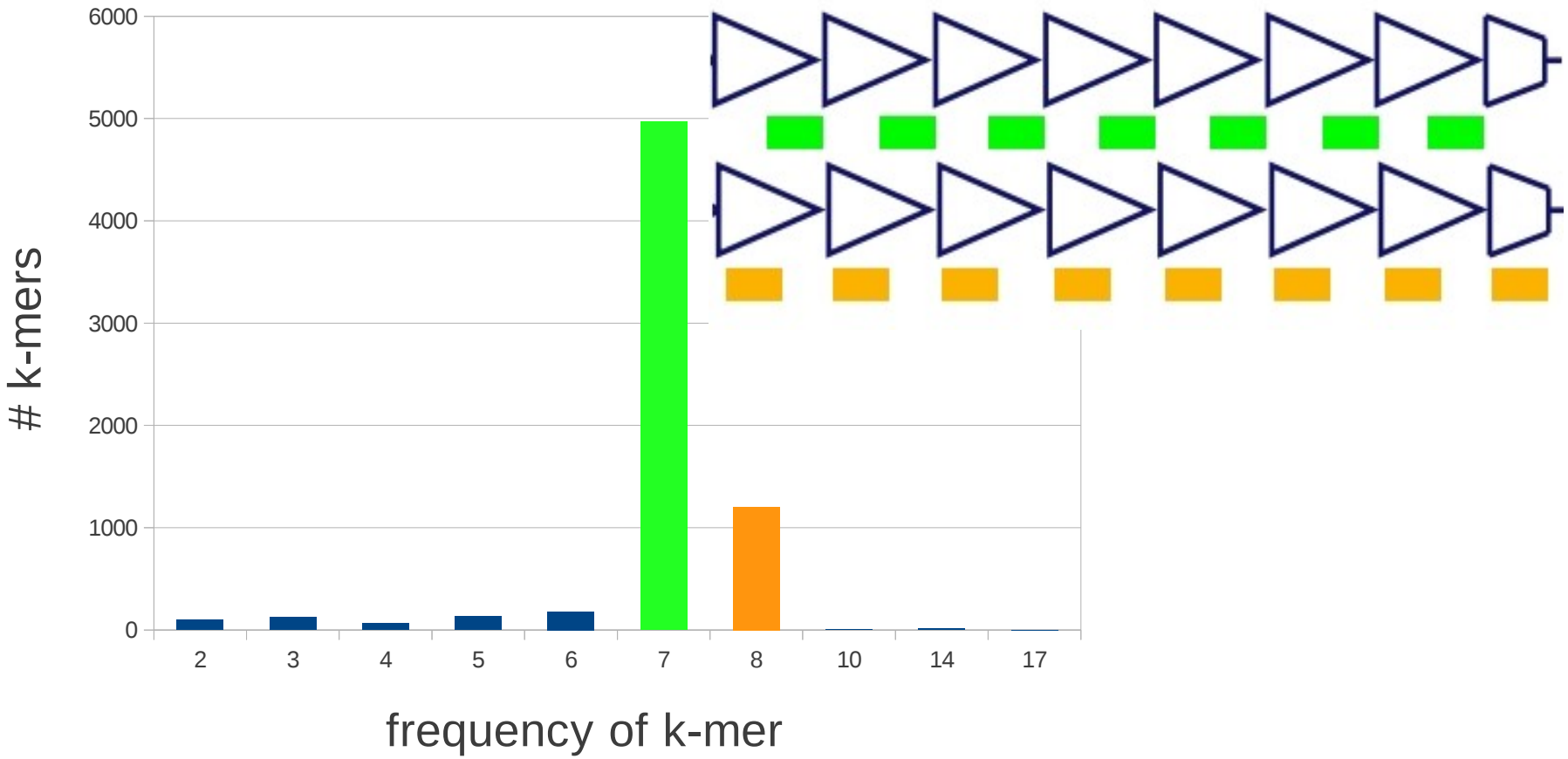
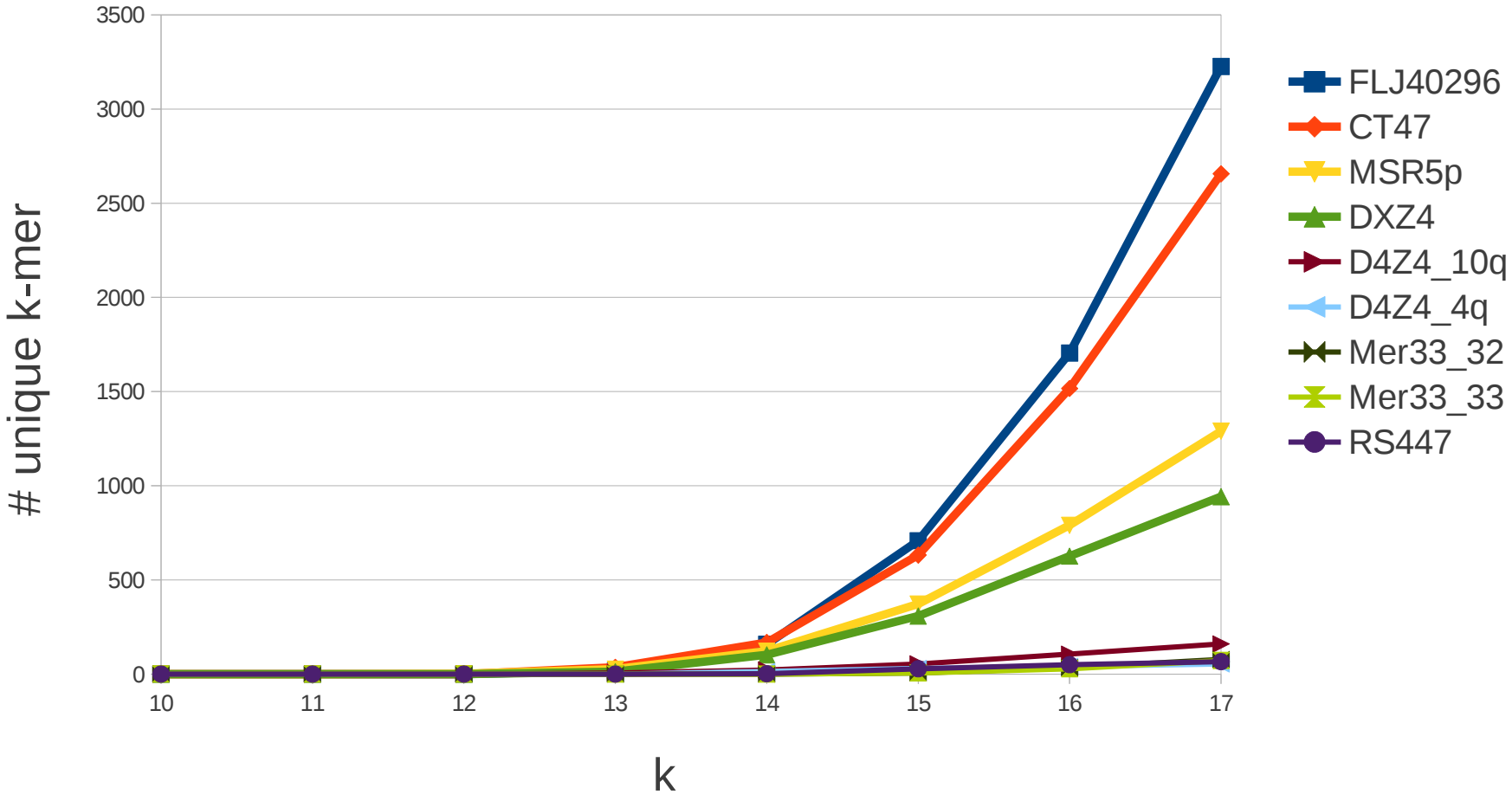
17-mer profiling for D4Z4_4q repeat region



Result: k-mer profiling (k=17)

rep.region	*# units*	*Nastya's #units*
CT47	**11-13**	**14**
D4Z4_4q	**6-8**	**8**
D4Z4_10q	**5-6**	**6**
DXZ4	**14-15**	**14**
FLJ40296	**4-5**	**5**
RS447	**9**	**10**
MSR5p	**7-11**	**11**
Mer33_32	**9-11**	**11**
Mer33_33	**7-11**	**12**

Result: unique k-mer (k=10..17)



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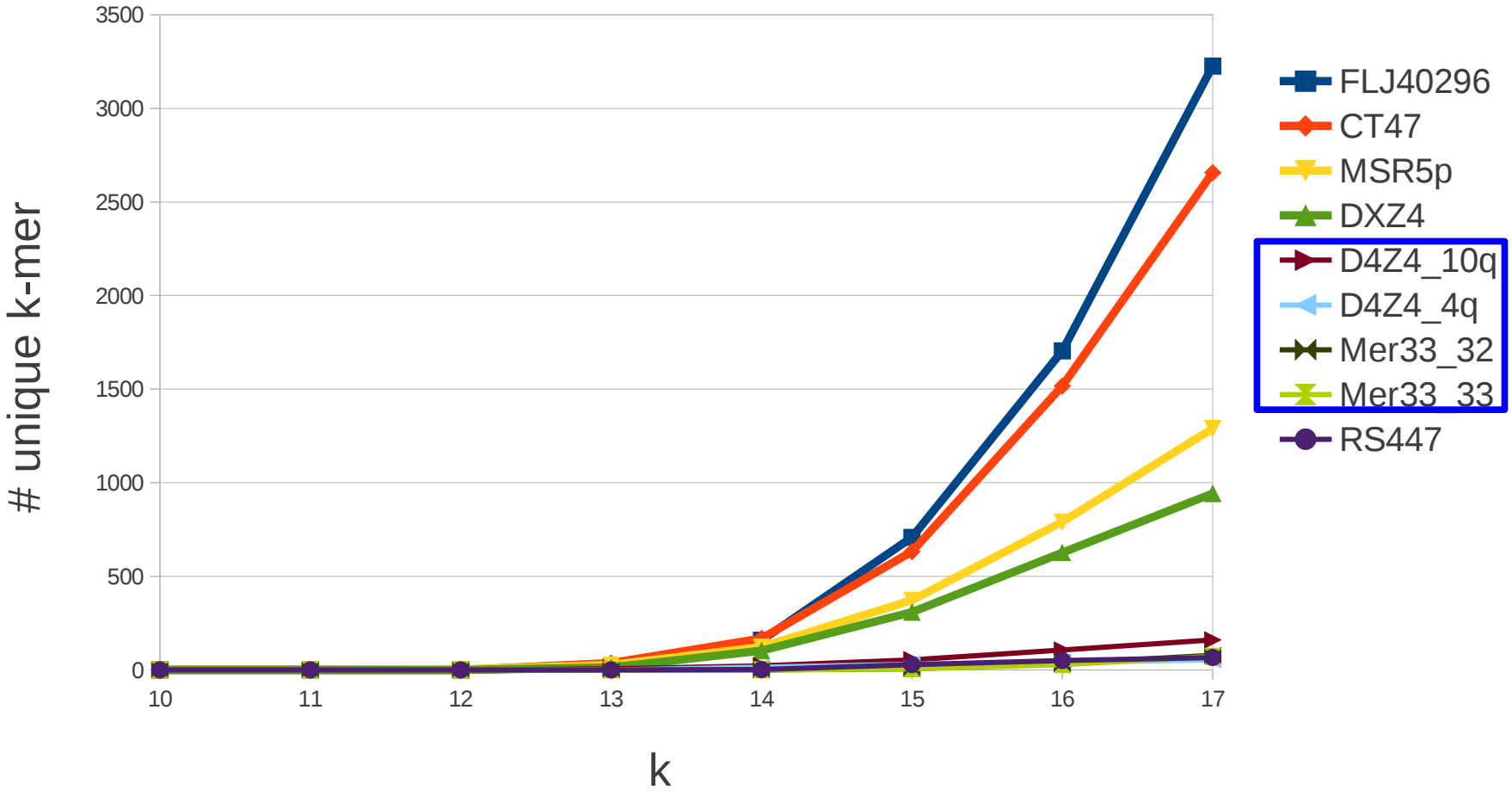

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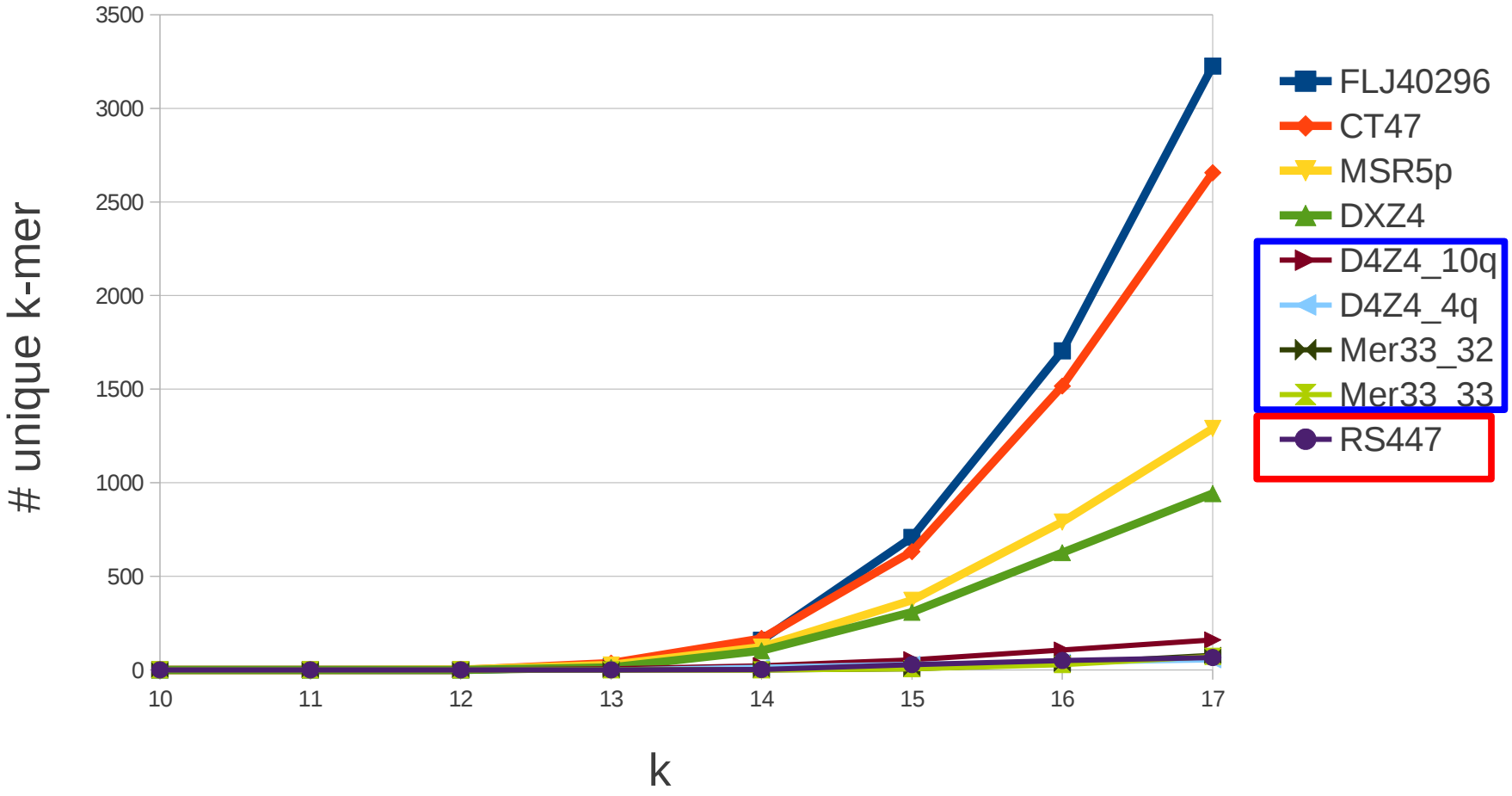

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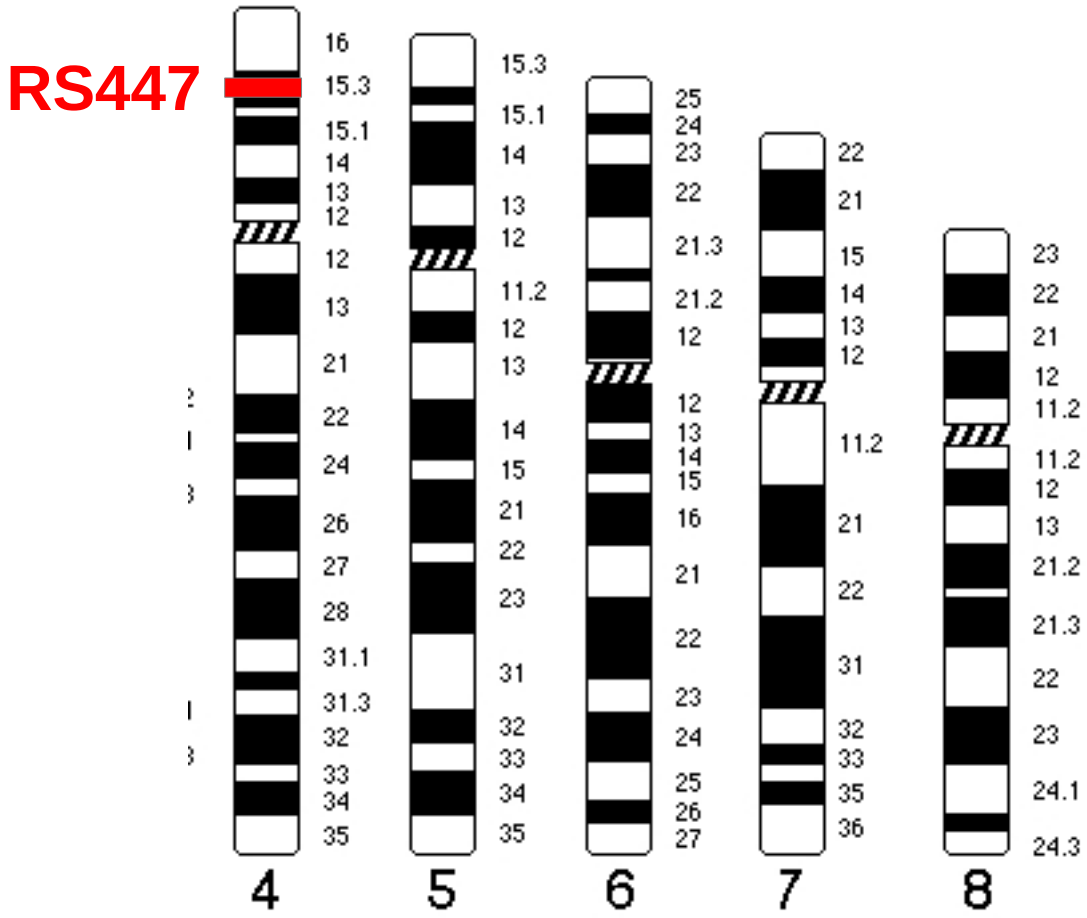

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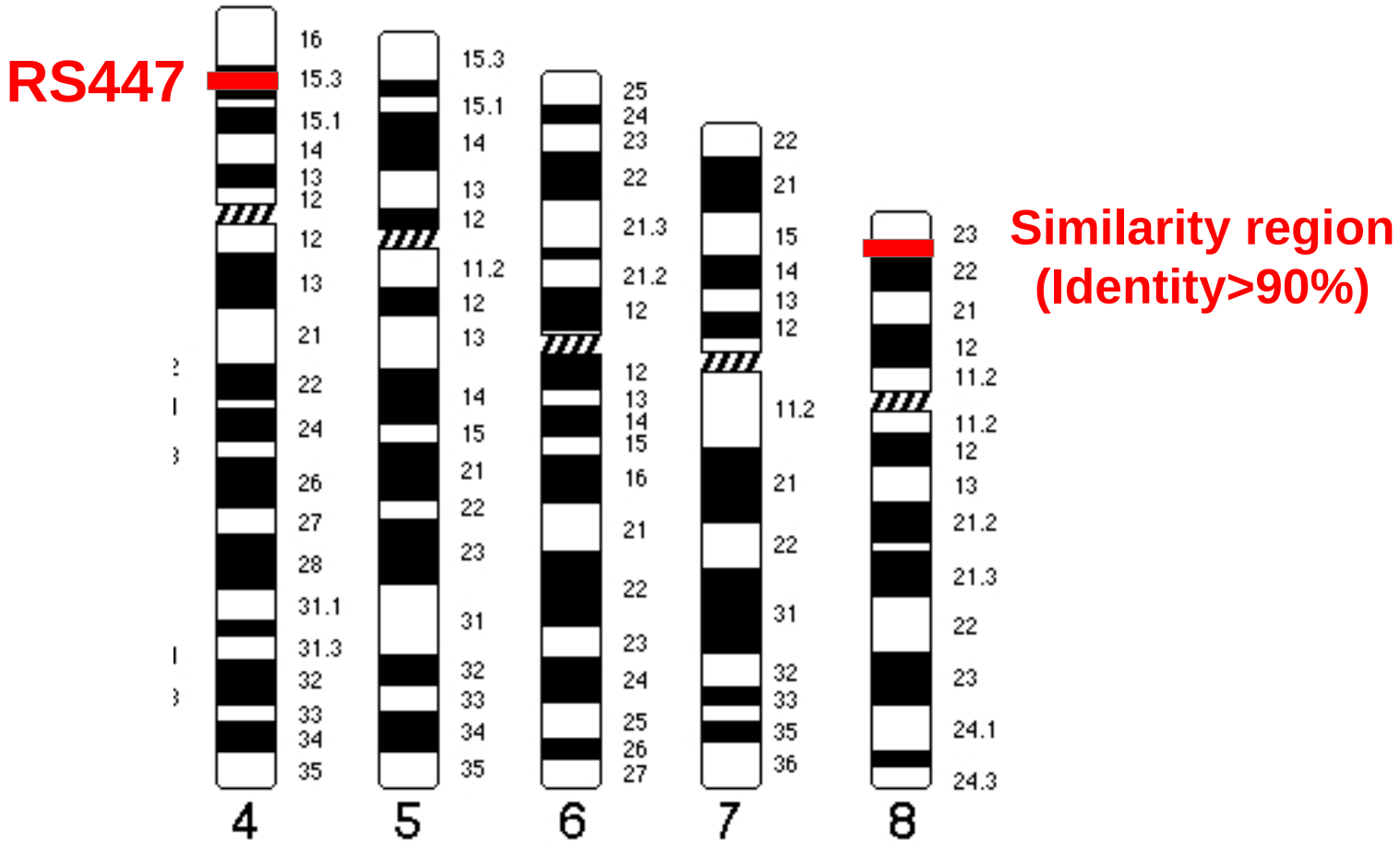

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- Heterogeneity of repeat regions among genomes

Future

- There are NextGenerationSequencing sample from 80 different individuals
- Number of units in repeat-region (experimental results for all 80 ind.) and unique k-mers for repeat regions (previous result) are known

Future

- we'll catch reads containing those unique k-mers
- we correlate this result with experimental data

Result: 'predictive power' to infer how many units are in repeate region

Acknowledgment

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