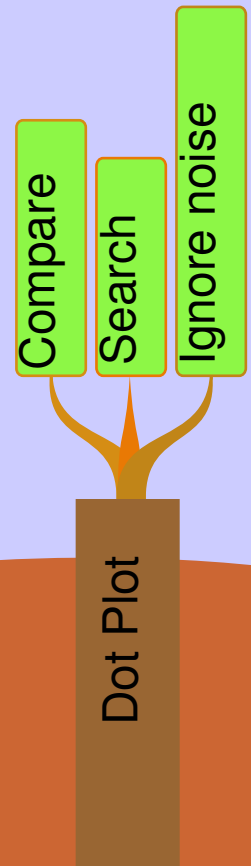
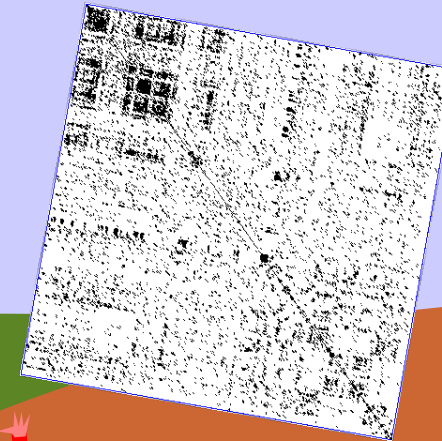
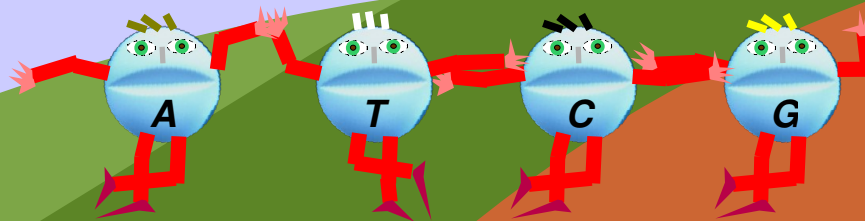


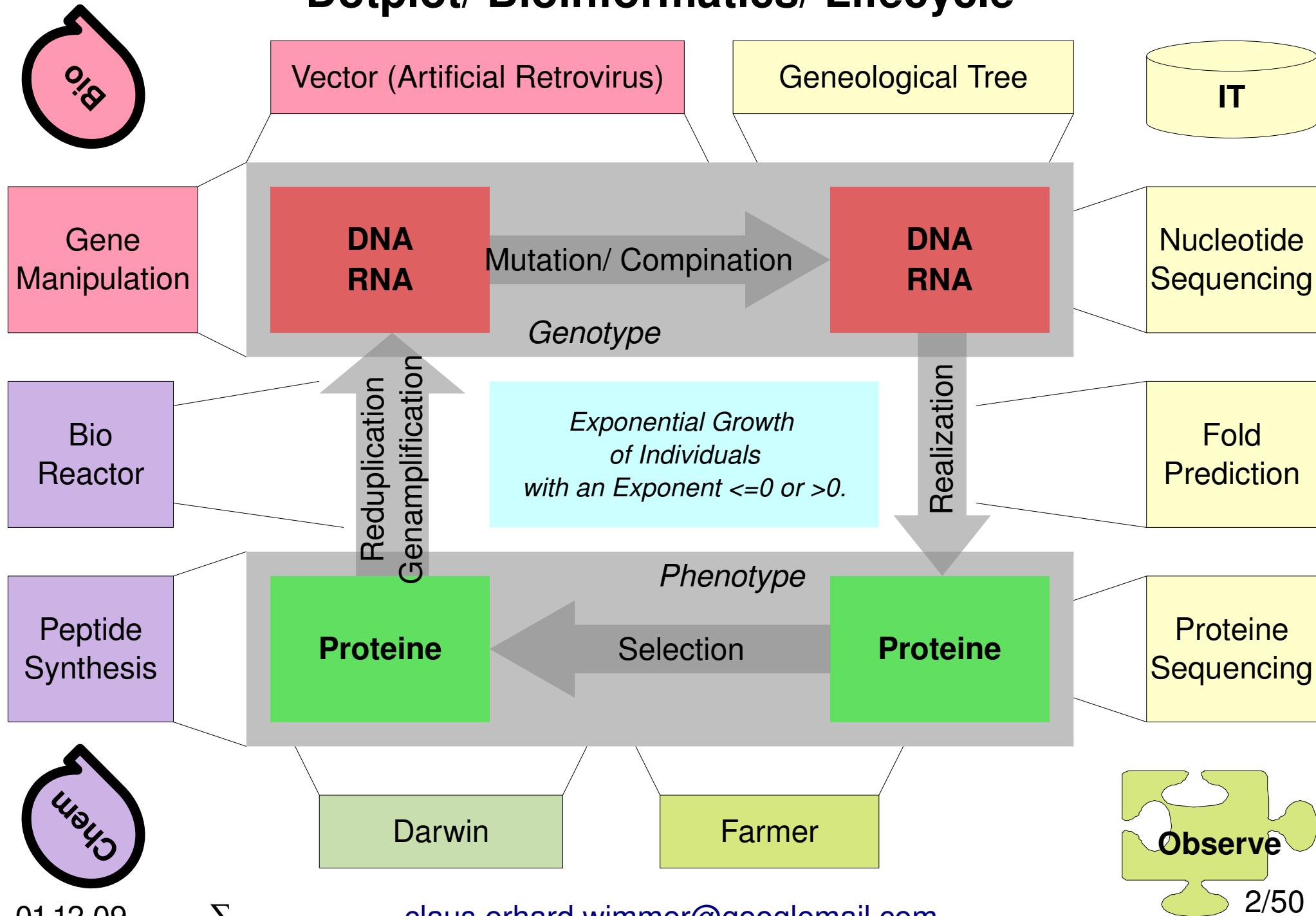
Dotplot Presentation



- A) **Bioinformatics**
- B) **Principle**
- C) **In Use**
- D) **Implementation**
- E) **Software**
- F) **Proteine Synthesis**
- G) **Misc**



Dotplot/ Bioinformatics/ Lifecycle



Dotplot/ Bioinformatics/ NCBI GenBank

→ <http://www.ncbi.nlm.nih.gov/Genbank/>

- Collection of biological and medical data without fee.
- Find nucleotide (RNA/ DNA) and protein (amino acids) sequences.
- Have a look at the metadata such as *organism publisher* and *part-position*!
- Copy & Paste the sequences from the webbrowser into your program!
- Enter a piece of 20 bp into the BLAST search engine!

→ <http://www.uniprot.org/>

- Collection only of knowledge about proteins without fee.
- Find proteine sequences and fold prediction tools!
- Try the **SWISSPROT/TOOLS/SEQUENCE_ALIGNMENT/DOTLET**!
- Combine the GenBank data with the Uniprot data!

Dotplot/ Bioinformatics/ Bioclipse

➔ <http://www.bioclipse.net/>

- IBM eclipse rich client.
- Java based, several OS.
- User can write extensions.
- Graphical view-plugins.
- Meta data storage.
- Open source license.
- ~40 downloads/ day.

The screenshot displays the Bioclipse application window. On the left is a 'Bioclipse Navigator' pane showing a file tree with folders like 'demo', 'Sample Data 1', and 'Virtual'. The main area contains a table with columns: '2D-structure', 'cdkTitle', 'Consensus', 'Signature Signifi...', 'Bursi Toxicopho...', 'Bursi Nearest Ne...', and 'Bursi Exact Match'. The table lists five molecules: 1. trazodone (Generated), 2. trimethoprim (Generated), 3. valproic acid (Generated), 4. warfarin (Generated), and 5. zalcitabine (Generated). The 'Consensus' column shows 'NEGATIVE' for all. The 'Signature Signifi...' column shows 'NEGATIVE [1 hits]' for all. The 'Bursi Toxicopho...' column shows 'NEGATIVE' for all. The 'Bursi Nearest Ne...' column shows 'NEGATIVE' for all. The 'Bursi Exact Match' column shows 'INCONCLUSIVE' for all. On the right, there is a 'Decision Support' pane with a 'Tests not started' button and a '2D-Structure' pane showing a chemical structure of a molecule with a pyridine ring and a methoxy group.

	2D-structure	cdkTitle	Consensus	Signature Signifi...	Bursi Toxicopho...	Bursi Nearest Ne...	Bursi Exact Match
1		trazodone	NEGATIVE	NEGATIVE [1 hits]	NEGATIVE	NEGATIVE	INCONCLUSIVE
2		trimethoprim	POSITIVE	POSITIVE [1 hits]	NEGATIVE	POSITIVE [1 hits]	INCONCLUSIVE
3		valproic acid	NEGATIVE	NEGATIVE [1 hits]	NEGATIVE	NEGATIVE [13 hits]	INCONCLUSIVE
4		warfarin	NEGATIVE	NEGATIVE [1 hits]	NEGATIVE	NEGATIVE	INCONCLUSIVE
5		zalcitabine	NEGATIVE	NEGATIVE [1 hits]	NEGATIVE	NEGATIVE	INCONCLUSIVE

Comment:

A number of developers has tried to use the offered eclipse client - intended for writing Java-programs - to create a tool processing chemical and biological data. The user may earn benefits from the lateral connections between the particular plugins. But the price is the existence of another instance of information outside from the filesystem and therefore not reachable by native applications. However
→ A dotplot plugin has not been found within the bioclipse.

Dotplot/ Bioinformatics/ Search & Comparison

- ◆ Input as nucleotide sequence : ... **301 xxxxxxxxxxxx** ... **[ATCG]**
- ◆ Input as proteine sequence : ... **61 xxxxxxxxxxxx** ... **nearly [A-Z]**
- ◆ Noise is present in the genome, nucleotide is more noise sensitive than proteine.
- ◆ Data collections stored, for example, in GenBank and Uniprot.
- ◆ Quick-Fuzzy-Search and Fuzzy-Comparison required.
- ◆ Comparison must not have too much parameters (equal for all samples).

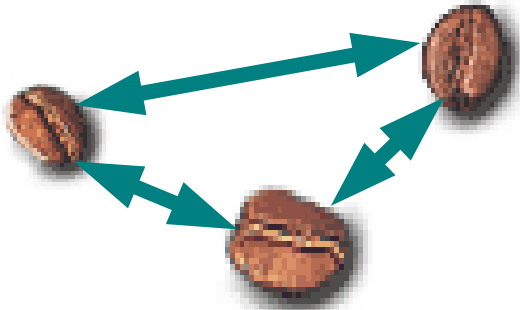
BLAST Algo:

Search method, provided as an interface of the large genome data collections. As the result the user obtains an alignment from short pieces of the both comparison operands. Multiple hits are possible. Outside of these pieces the both genomes may be completely different.

Dotplot Algo (subject of this presentation):

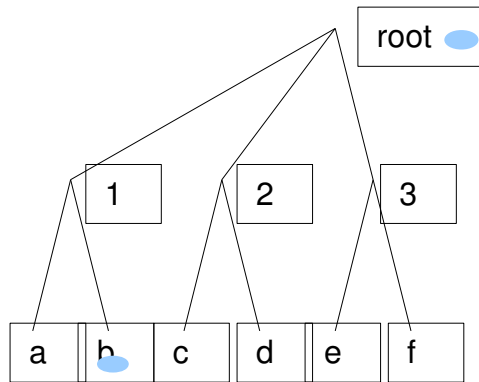
Graphical comparison of two genomes as an area. Both whole genomes are visible. This algo has the same age as the UNIX operating system and can also be used for the evaluation of electrical signals and for detection of plagiarism.

Dotplot/ Bioinformatics/ Fold-Prediction, Docking



- RNA and proteins are able to fold relating to their temperature.
- Folding is necessary to calculate the realization of the genome.
- RNA and proteins consist of atoms (shown as coffee beans).
- Forces and temperature lead to optimum distances between the atoms.
- Atom's electrons lead to optimum angles between more than two atoms.
- A fold prediction program has to calculate a minimum penalty.
- Many connected variables plus graphical view => expensive software.
- A PC bit consists of a number of atoms => PC slower than reality.
- Details omitted in fold programs => result not exact but available.

Dotplot/ Bioinformatics/ Geneological Tree

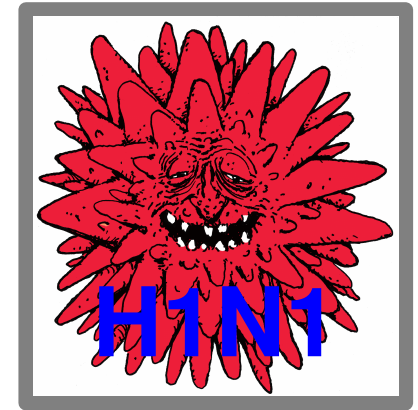


(1)
Is **root** the ancestor
of **a** and **e**?

(1) – Construct virtual parents of **a/b** **c/d** and **e/f** from their genomes (these are **1/2/3**). Merge the genomes with minimal modifications (*maximum mean*). Construct **root** from **1/2/3** in the same manner! Compare it with the candidates. Method is unsafe because of the assumptions during merging.

(2) – Like (1). Virtual parents **1/2/3** don't need have to exist. Root doesn't need to be calculated. $N*(N-1)/2$ virtual parents found while combining **a-f**. $N=6 \rightarrow 15$ virtual parents to compare. Parent 1 will have a good *maximum mean*.

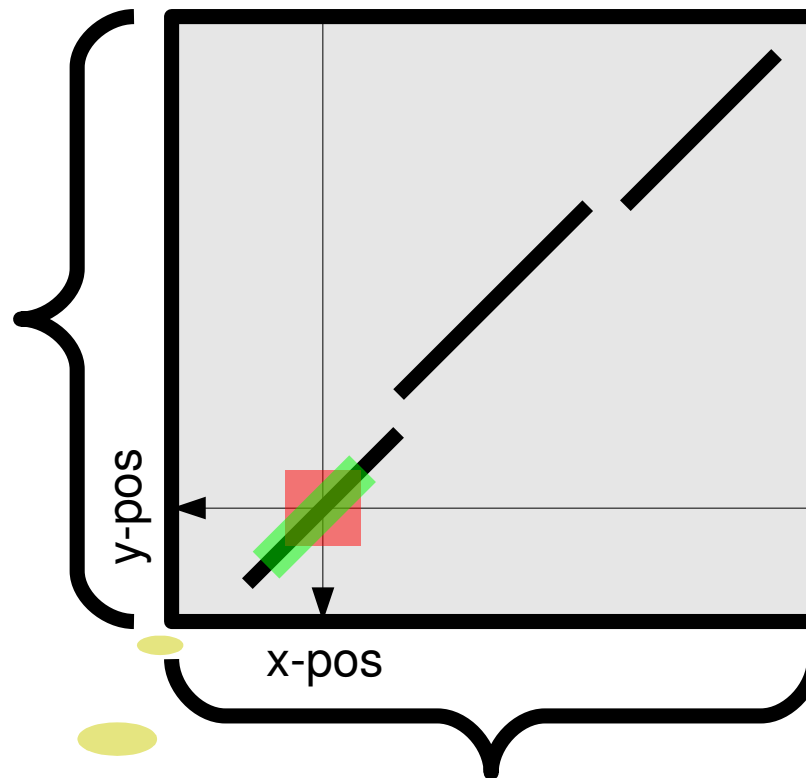
Dotplot/ Bioinformatics/ Employment



- Field for researchers => Should be controlled by the government.
- Field for industry => Pollution of the environment and of the genetic code-space may be the result if there are only poor restrictions.
- Career-chances and profit-constraints have often generated inventions with benefits only for a minority of persons.
- Mix of IT and chemical data processing involved into created biological organisms/ knowledge about genomes/ decisions based on genomes.
- Universities but also Universities of applied sciences offer studies.
- Only 7 jobs found at <http://jobsuche.monster.de> on 2009-11-21 under keyword „Bioinformatik“ in GERMANY.
- 2 XING-Bioinformatics-Groups: Only members can read = Exclusive club.

Dotplot/ Principle/ Drawing

Second genome with another but similar length.



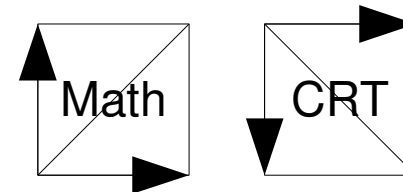
When ≥ 7 of 20 points in **env** are equal then set black dot on **pos**.

Diagram-env

Diagram-pos

First genome as char string, length ~ 1000.

Hacker & Sniffer tool for home & commercial use



Dotplot/ Principle/ Probability

Variable	Meaning
p	Probability of a random hit (= noise).
k	Number of items which must be equal at least.
m	Number of characters in the alphabet (= nucleotide bases, amino acids).
n	Number of compared items at one time (= sliding window size).

$$p \simeq \binom{n}{k} * \frac{(m-1)^{n-k}}{(m)^n}$$

Variable	Nucleotide	Proteine
k	15	7
m	4	20
n	20	20
p	~3.4/ (1Mio.)	~31/ (1Mio.).

/*/ Wolff, Hauck, Küchlin: „Mathematik für Informatik und Bioinformatik“, Isbn: 3-540-20521-7, Springer 2004, Page 459.

Dotplot/ Principle/ Genome Sizes

Organism	~Nucleotide Size	~Prot. Size	~Prot. Dotplot Area
Virus - H1N1	13 000	3 000	9 000 000
Bacterium <i>sabaldella termididis</i>	4 400 000	800 000	640 000 000 000
Human	3 000 000 000	90 000 000	8 100 000 000 000 000

User can't evaluate every character individually.

Complex beings have also larger genomes.

But this operations are easy compared with folding prediction software: a) CPU-time, b) Algo.

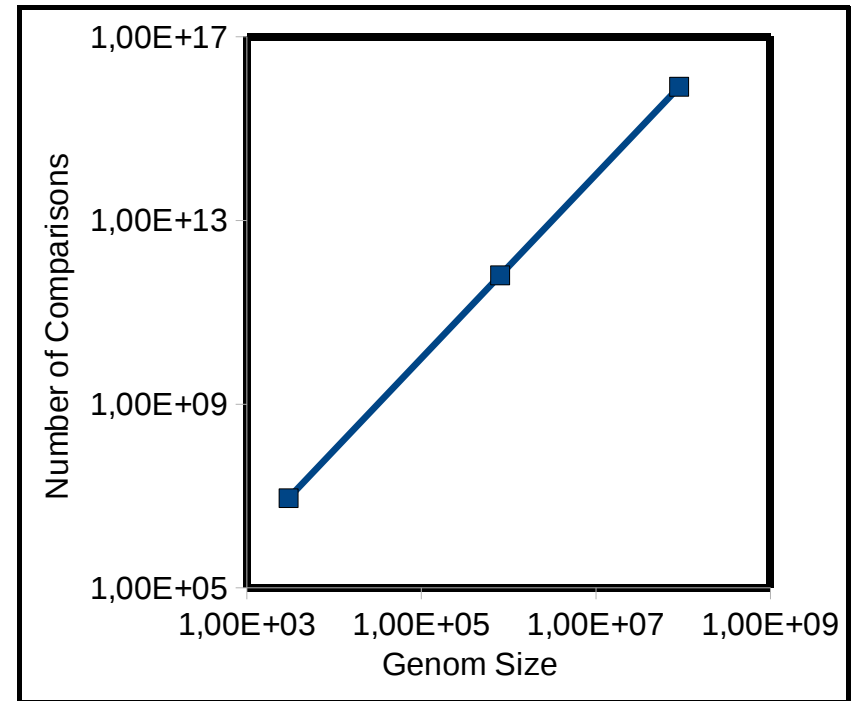
Plausibility check: Viruscode is so small, that it disappears within the human or even within the bacteria genome.

No fingerprint coded for proteins

The higher the organism the more useless code within the fingerprint.

Dotplot/ Principle/ PC Limit

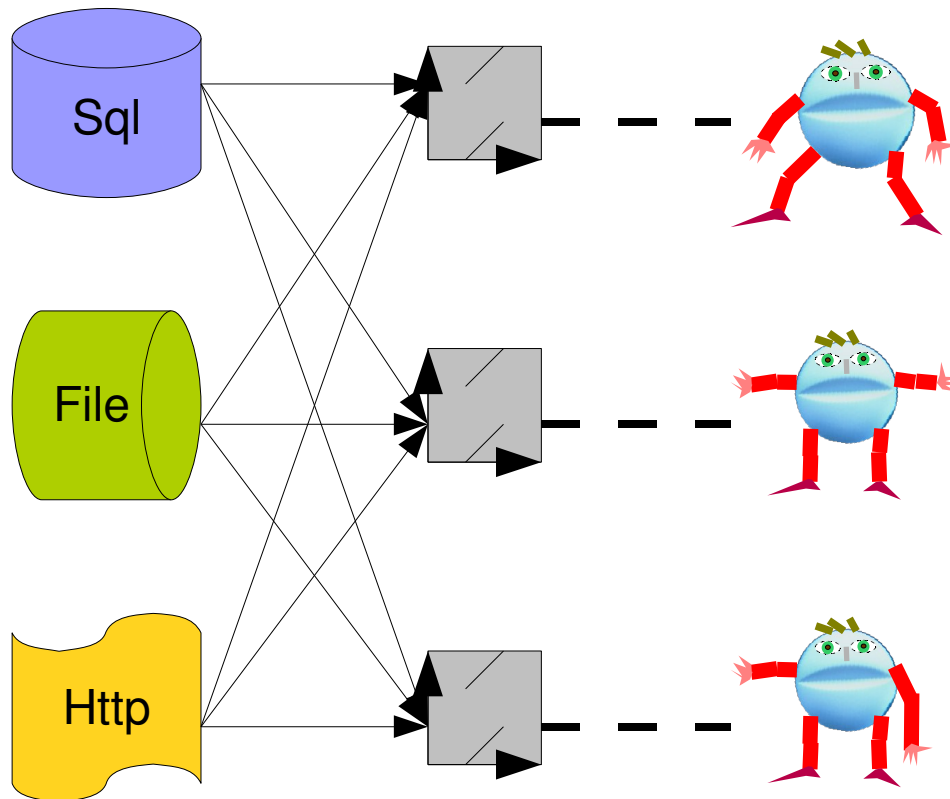
- Squarewise growth of the CPU-time if both genomes grow linear at the same time.
- Both genomes fit into the RAM of a normal PC.
- Program can compare viruses within online time, other organisms lay in the batch time range.
- OS calls are problematic when setting Pixels for comparison hits.



Dotplot/ Principle/ Why

- ★ What is similar between two genomes?
- ★ Which regions have moved?
- ★ Ignore the noise.
- ★ See the whole thing.
- ★ Understand large amounts of data.
- ★ Avoid subjective comparison parameters.
- ★ Low cost.

Dotplot/ Principle/ Use Cases



Zoom between two pixels

Align some amino acids

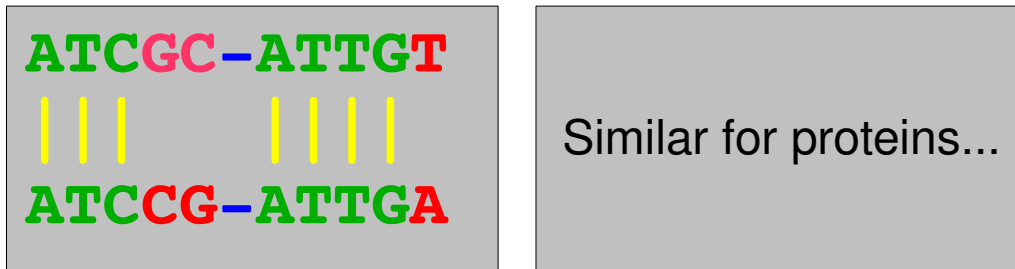
Search a protein in an organism

Find relationships

Nucleotide or
Proteine input

Deal with the
equalizer criterium

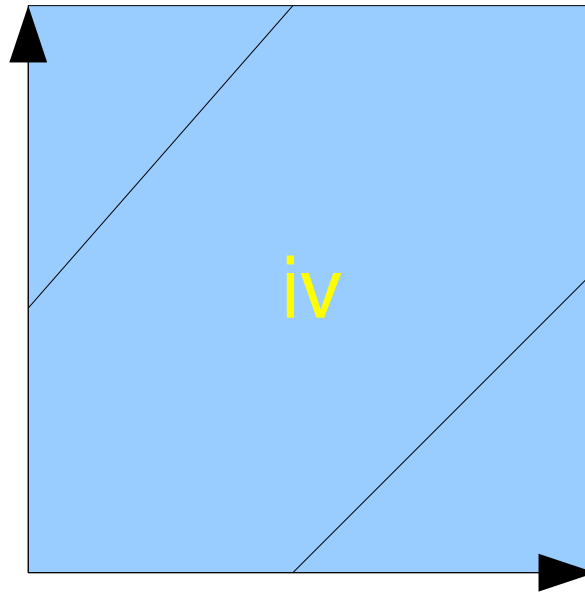
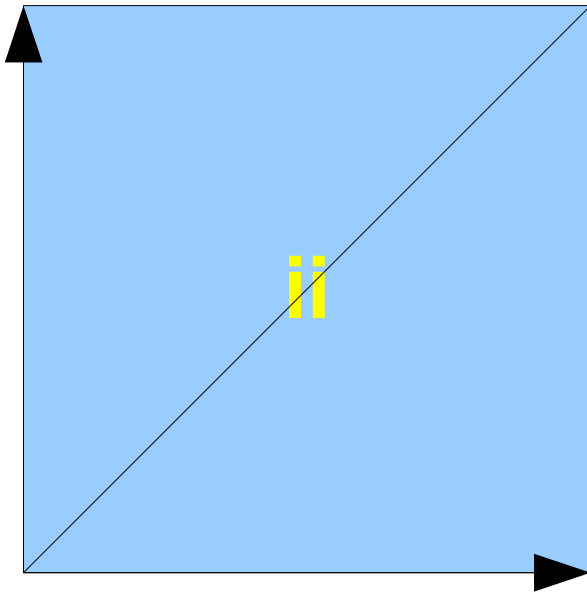
Dotplot/ Principle/ Alignment



The **alignment** is a colorful view of two or more parallel pieces of characters of the comparison operants. It is either the result of a BLAST search request or it can be obtained by a lookup operation within one of the DOTPLOT programs. Therefore the user can chose one point at the diagram area with the cursor. This information contains both startpoints within the sequences. Color-highlighting: Equal characters are green, different characters are red.

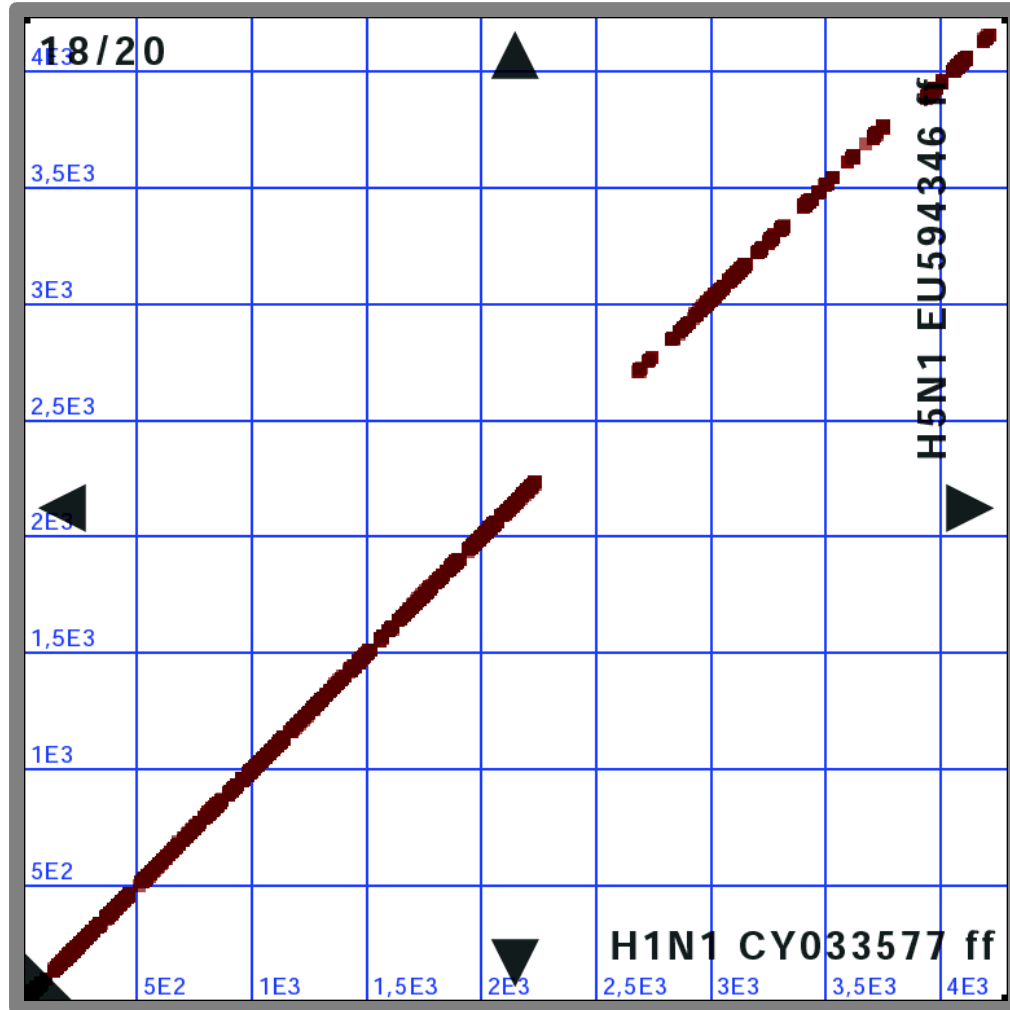
Dotplot/ In Use/ Exercise

- i. Create 2 files with an identical proteine sequence! You can use http://www.ncbi.nlm.nih.gov/protein/219903732?ordinalpos=1&itool=EntrezSystem2.PEntrez.Sequence.Sequence_ResultsPanel.Sequence_RVDocSum - copy the text block at the end of the page.
- ii. Compare both as dotplot in tidy-mode (7 hits per 20 characters). Why this result?
- iii. Take the second file. Cut the 1st half and append it after the end of the remaining file content.
- iv. Compare again. Point at the 1st half and the 2nd half (based on the original file) in the result!



Dotplot/ In Use / Flu

Influenza A virus
(A/duck/Hubei/Hangmei01/2006(H5N1))



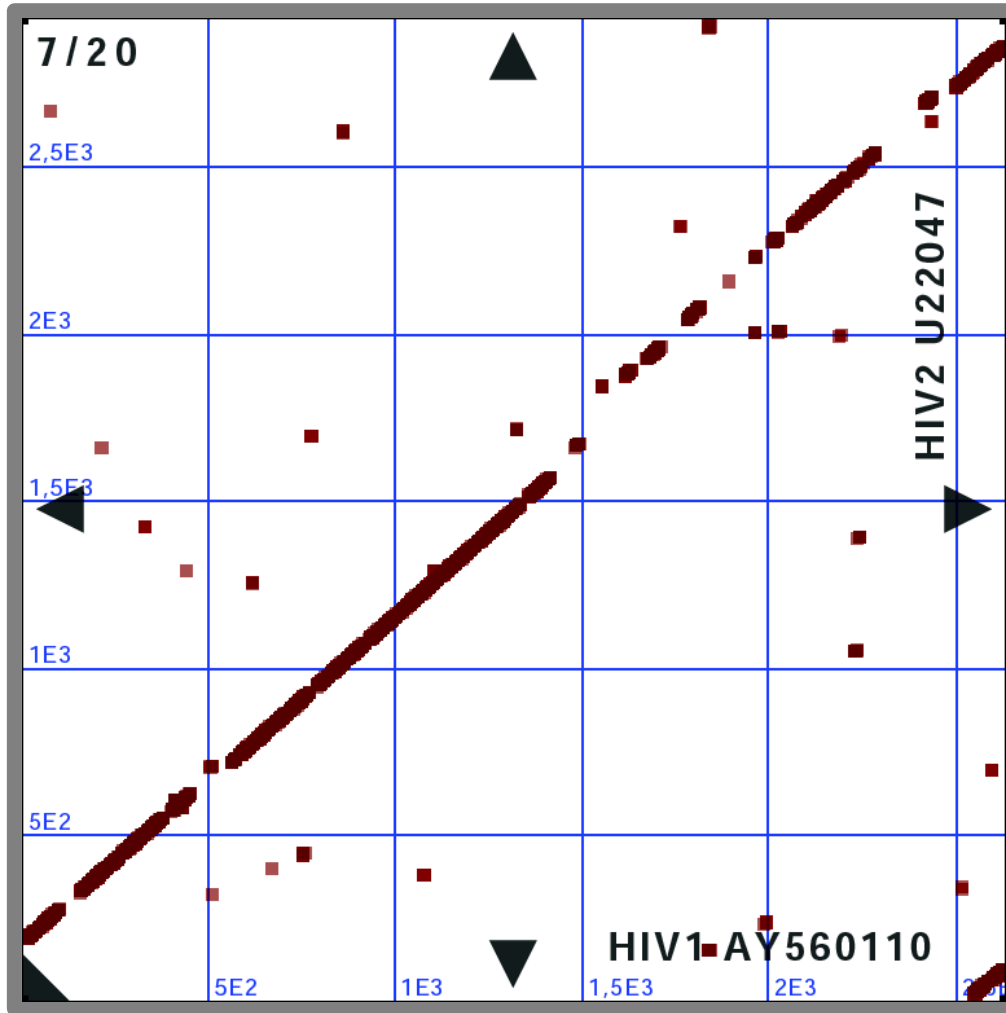
Influenza A virus
(A/Puerto Rico/8/34(H1N1))

- Swine flu ↔ Bird Flu.
- Proteine comparison. & positions.
- Single stranded RNA virus.
- Close similar in *core* (left-bottom).
- Distantly similar in *env* (right-top).
- Different env proteins on important regions claimed and no cross-immunization assumed.
- Why no immunization by MHC-I?

H1N1: ENTREZ Nucleotide Database; CY033577 ff.
H5N1: ENTREZ Nucleotide Database; EU594346 ff.

Dotplot/ In Use/ HIV1-HIV2

Human immunodeficiency virus type 2



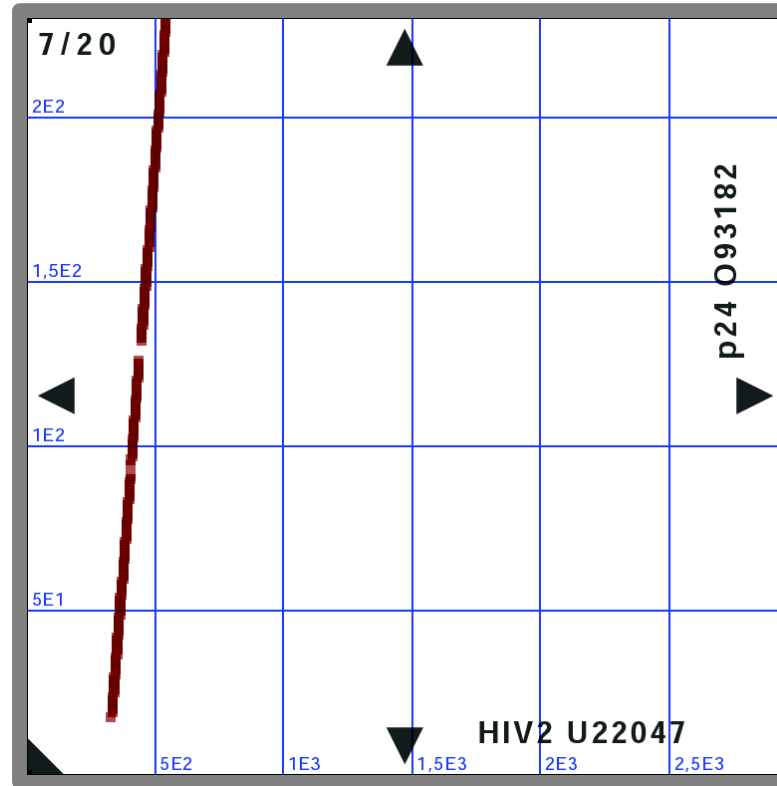
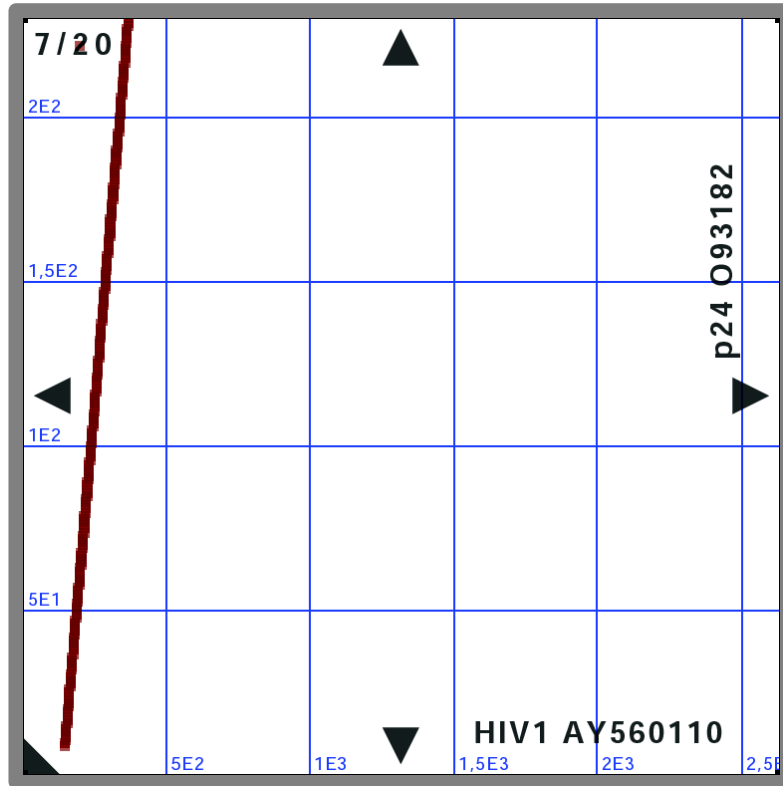
HIV-1 isolate 98USHVTN941c1 from USA

- AIDS 1 ↔ AIDS 2.
- Proteine comparison and positions.
- Similar in core.
- Some hits in env.
- More mutations than on HxN1.
- Length similar than HxN1.

HIV1: ENTREZ Nucleotide Database; AY560110.
HIV2: ENTREZ Nucleotide Database; U22047.

**HIV1 shifted
backwards. Reality or
sequencing defect?**

Dotplot/ In Use/ p24



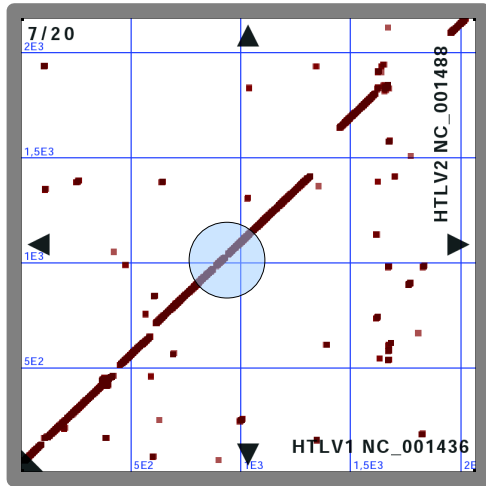
HIV1: ENTREZ Nucleotide Database; AY560110.

HIV2: ENTREZ Nucleotide Database; U22047.

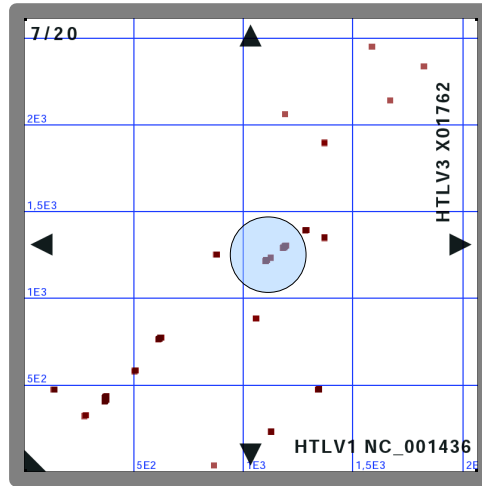
p24: The Uniprot Consortium; O93182[133-363], Gag polyprotein, Human immunodeficiency virus type 1 (isolate 90CF056 group M subtype H) (HIV-1).

Search of the proteine p24 from HIV1 (datasource is Uniprot, proteine format) in HIV1 (datasource is GenBank, parsed nucleotide format) and HIV2 (datasource also GenBank, parsed nucleotide format). Proteine found in the cores of both. Attention: The naming „p24“ does only specify the weight in kilo Dalton (kDa). Another organism may also have a p24 with the same weight but completely differently constructed!

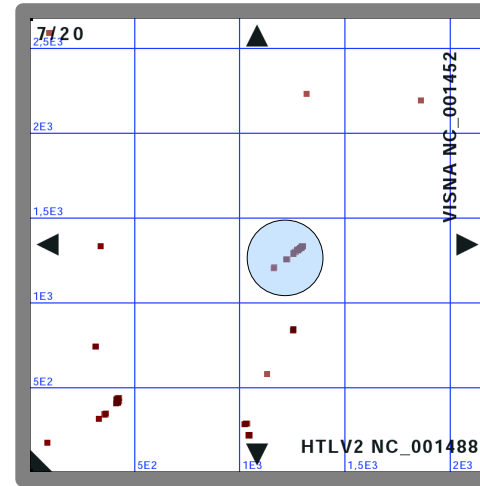
Dotplot/ In Use/ Lentivirus



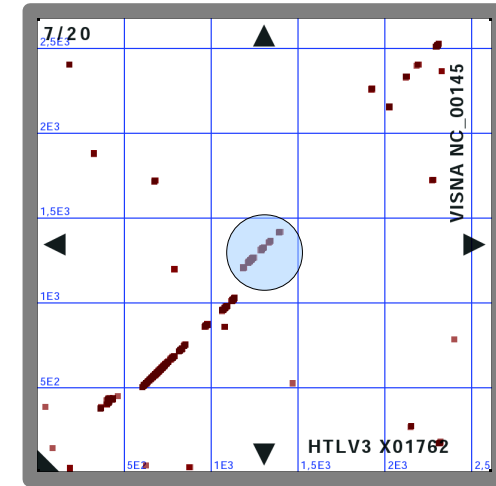
HTLV1-HTLV2



HTLV1-HTLV3



HTLV2-VISNA



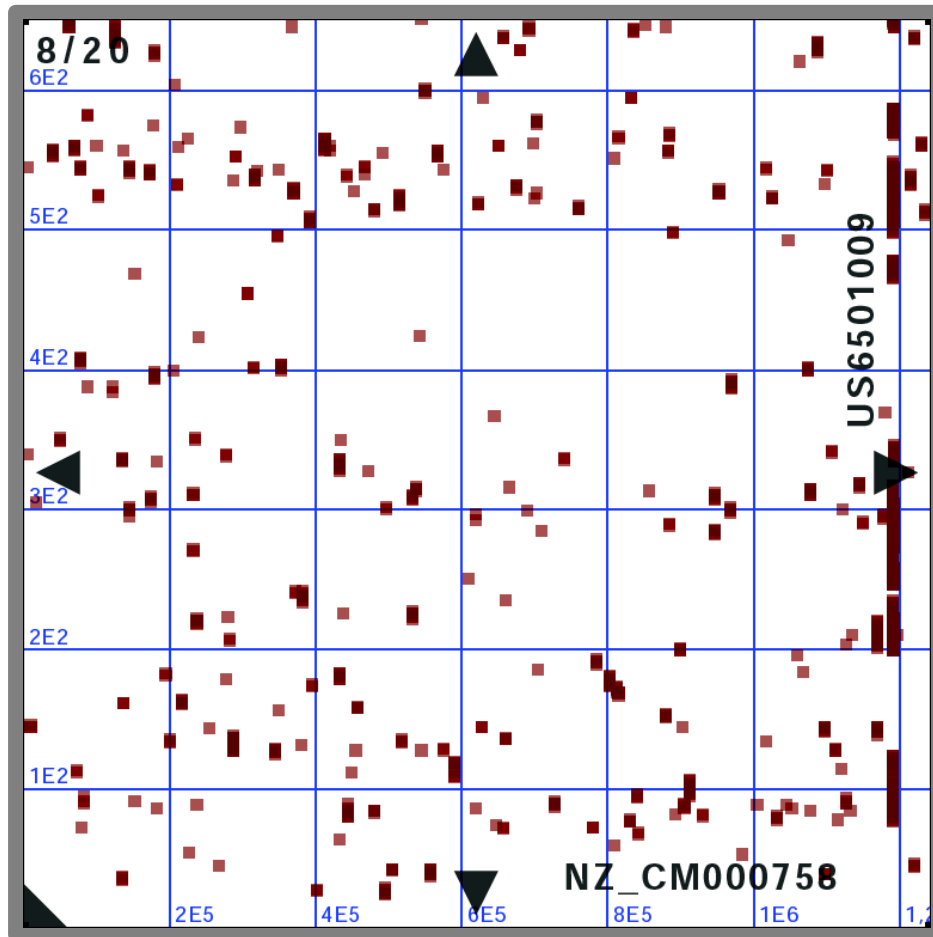
HTLV3-VISNA

ENTREZ Nucleotide Database; **HTLV1**-NC_001436/
HTLV2-NC_001488, **HTLV3**-X01762, **VISNA**-NC_001452.

- HTLV1 and HTLV2 are similar but HTLV3 is different from them. HTLV3 is the former name of HIV1 but the old naming convention had suggested an untrue relatedness between the human t lymphotropic viruses and the lentiviridae.
- VISNA is a lentivirus which resides in sheep and HTLV3 is a lentivirus which resides in human. Both have similar regions within their genome.
- The human t lymphotropic viruses are different from the lentiviridae except some specific regions.

Dotplot/ In Use/ MON863

MON863 Transgenic Maize,
US6,501,009 Toxic protein cryIIIB.



bacillus thuringiensis, producer of
insecticidal CRY toxins.

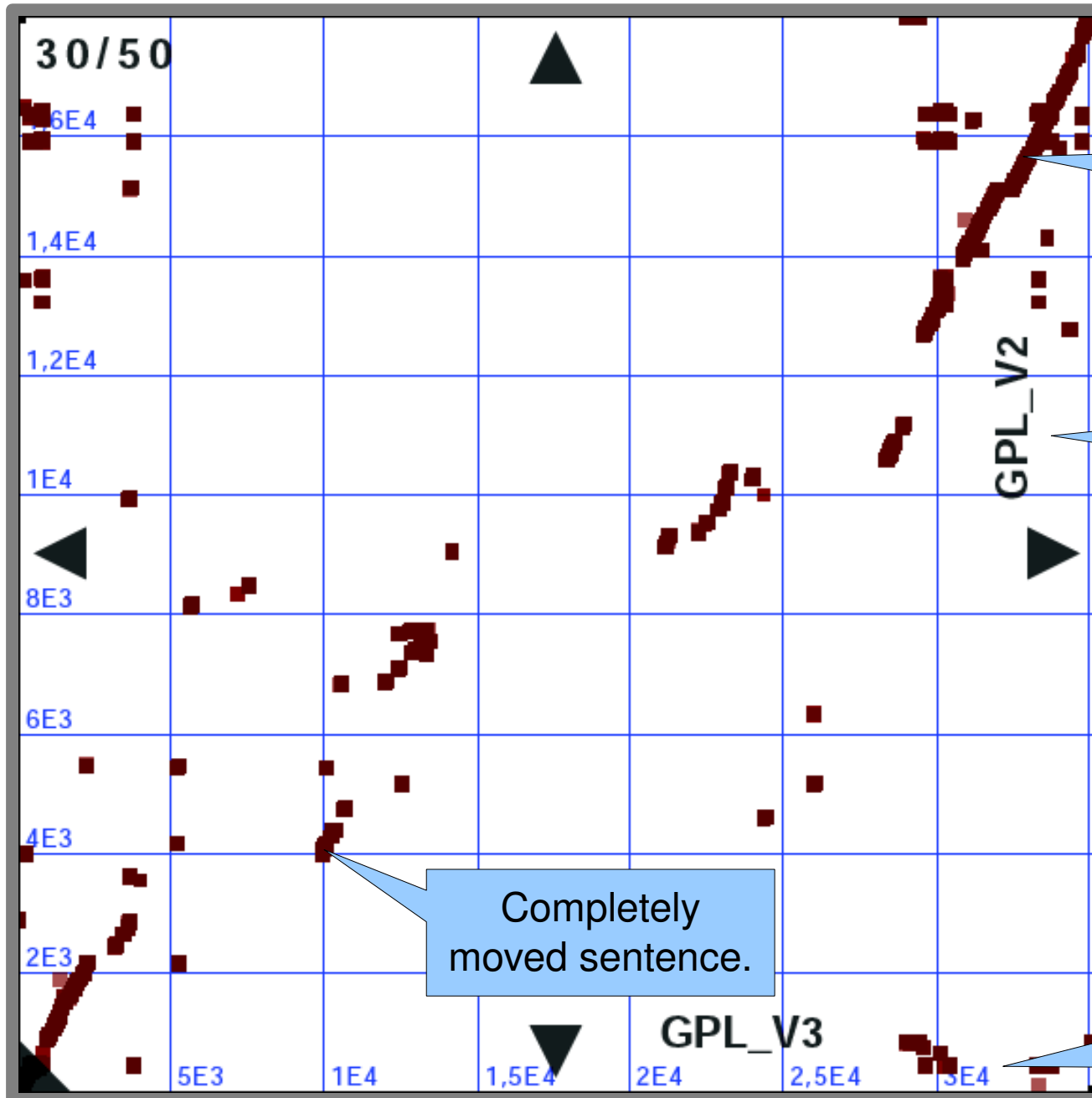
Bacterium Thuringiensis: ENTREZ Nucleotide Database; NZ_CM000758.
US Patent 6,501,009: ENTREZ Nucleotide Database; US6501009_1.

The MONSANTO company claims the insertion of insecticidal CRY toxins into the genome of the MAIZE plant within its PATENT. A comparison shows that the claimed gene sequence (y-axis) occurs within the bacterium *bacillus thuringiensis* (x-axis) like explained in the patent. The produced substance is toxic against BEEs and RATs.

- BEEs die.
- MAIZE only as MON863.
- Minister gets secret money.
- People not heard.
- Food makes \$-PROFIT.

Source of CRY toxine is copied from nature. Not created bottom up.

Dotplot/ In Use/ Text Compare



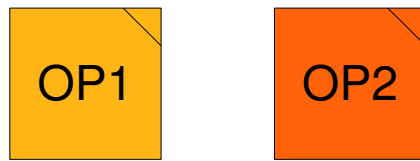
Same begin & end.
Modified in the middle.

GPL 2 and 3.

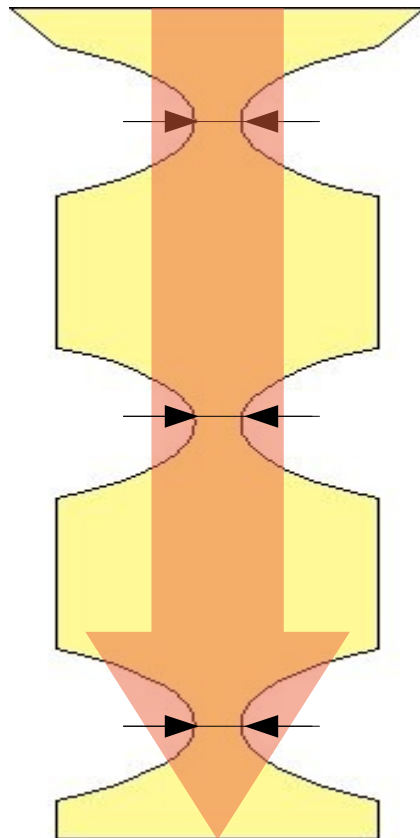
Completely
moved sentence.

GPL 3 two times big
than GPL 2.

Dotplot/ Implementation/ Bottleneck



Operands *size* = $2 * n$



CPU comparison flag $t \sim n * n$

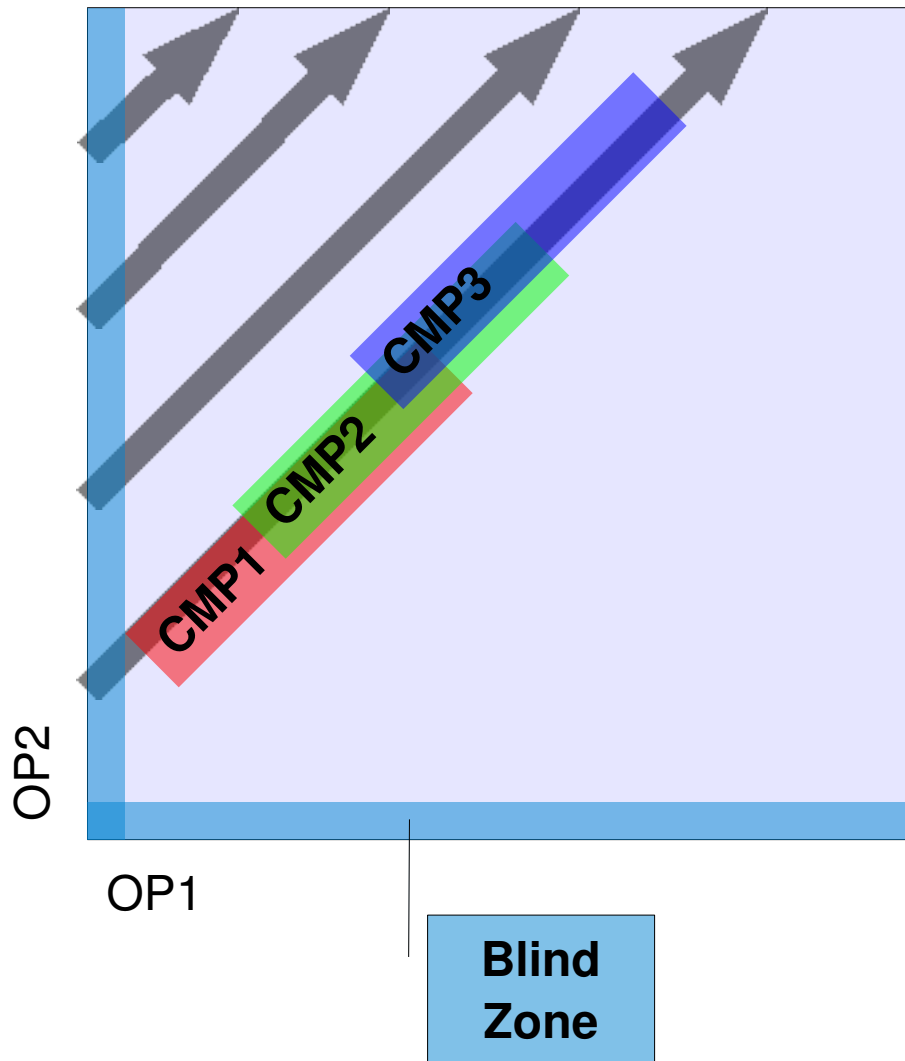
Set result pixel $t \sim \% \text{ hits}$

Result size *RAM* = $\text{width} * \text{height}$



Result *size* = $\text{width} * \text{height}$

Dotplot/ Implementation/ Sliding Window

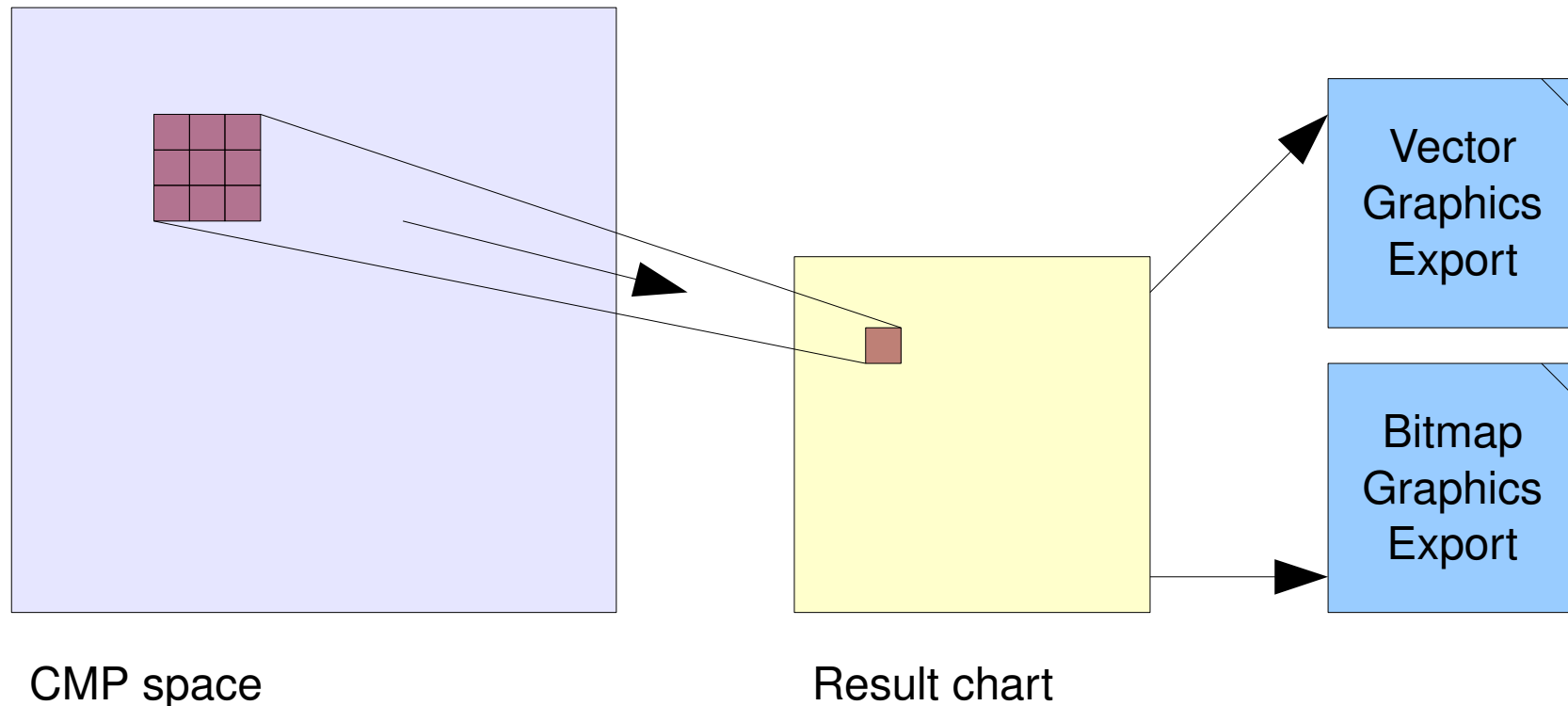


CMP(i, j):

```
WHILE i < len1 AND j < len2
  IF op1[i-20] = op2[j-20] THEN
    dec(sum)      # Old cmp.
  END IF
  IF op1[i] = op2[j] THEN
    inc(sum)      # Curr cmp.
  END IF
  inc(i)
  inc(j)
LOOP
```

All combinations of i and j start indexes for every diagonal. Algo is independent from the length of the sliding window (assumed as numerical 20) but grows square wise with diagonal length.

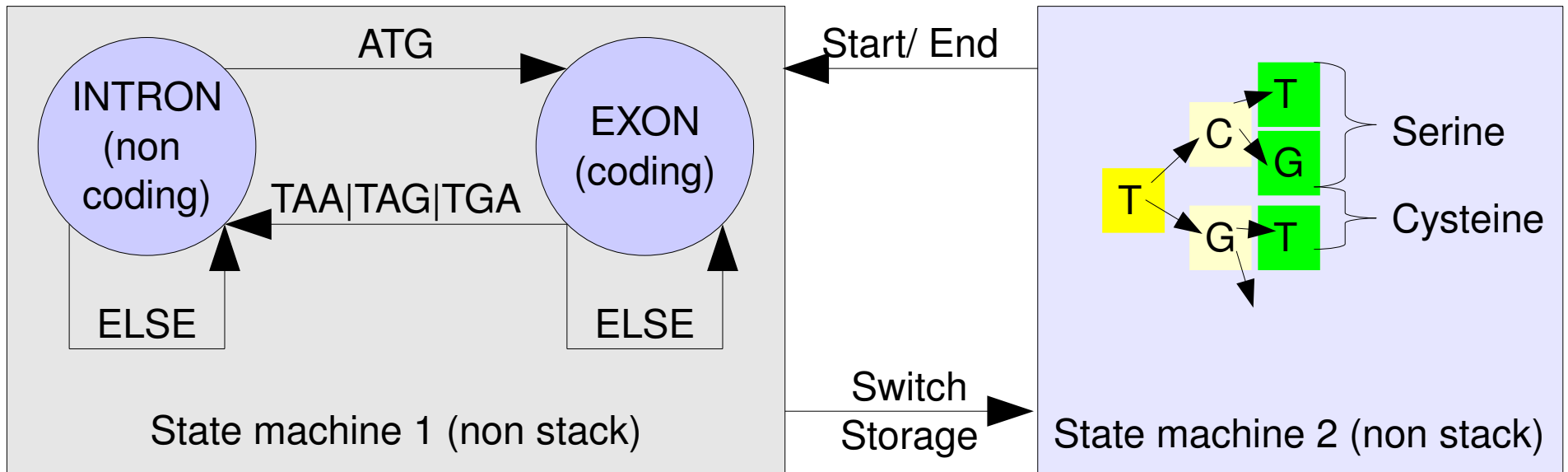
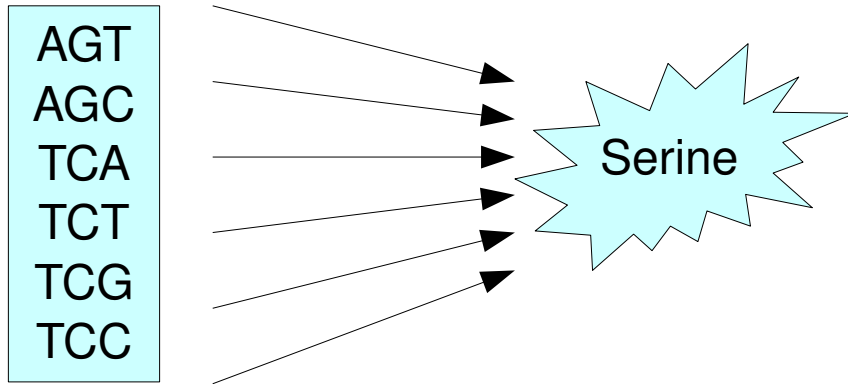
Dotplot/ Implementation/ Result Projection



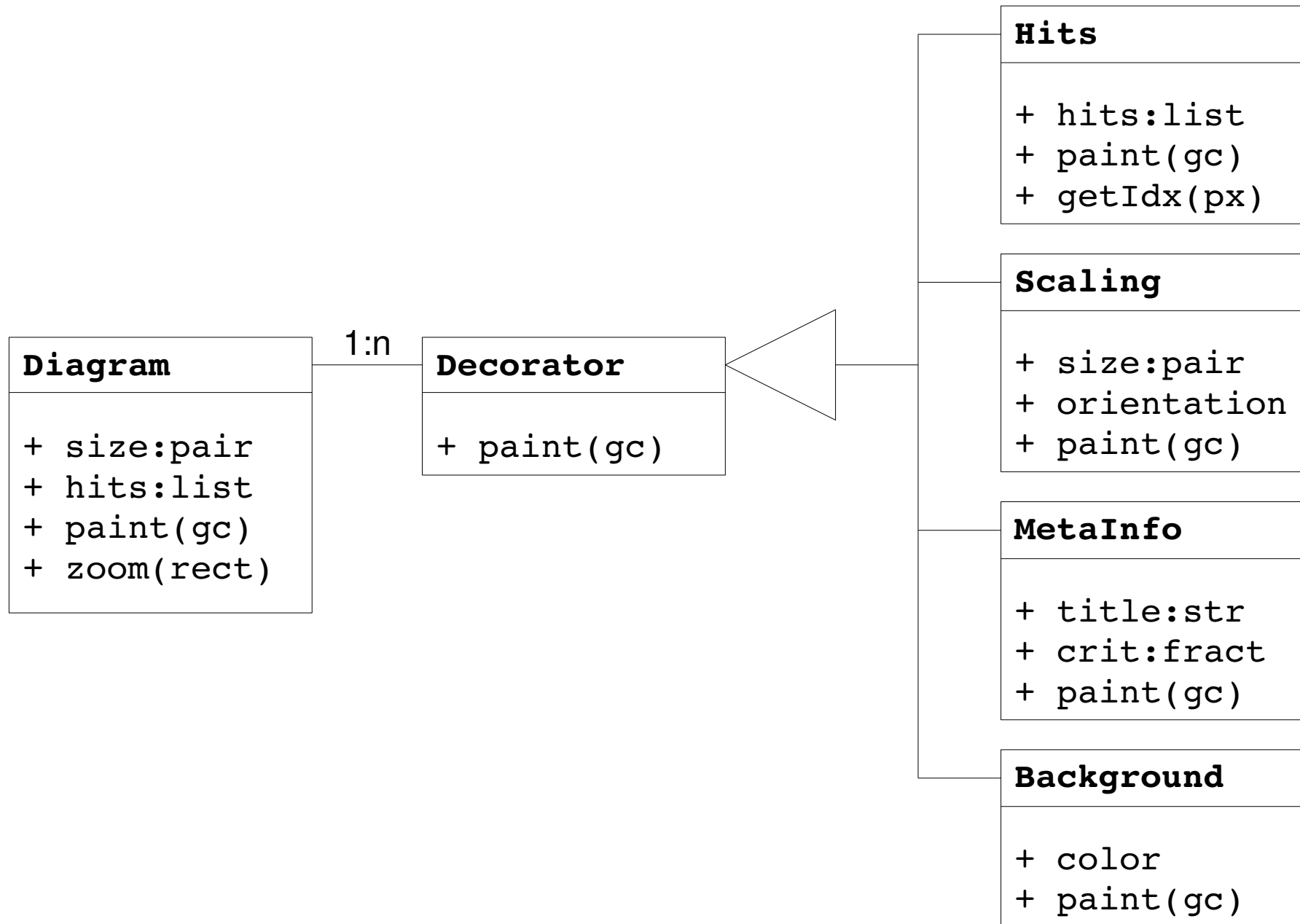
Result chart is often smaller than number actually performed comparisons. This is a surjective projection. A pixel in the result is set when at least one of the related comparisons has matched. The number of hits should be limited when one pixel set operation is done per OS-call. Otherwise the OS may block and it will be hard for the user to interrupt the dotplot process.

Dotplot/ Implementation/ Nucleotide Parser

Start codon = {ATG}
End codons = {TAA, TAG, TGA}



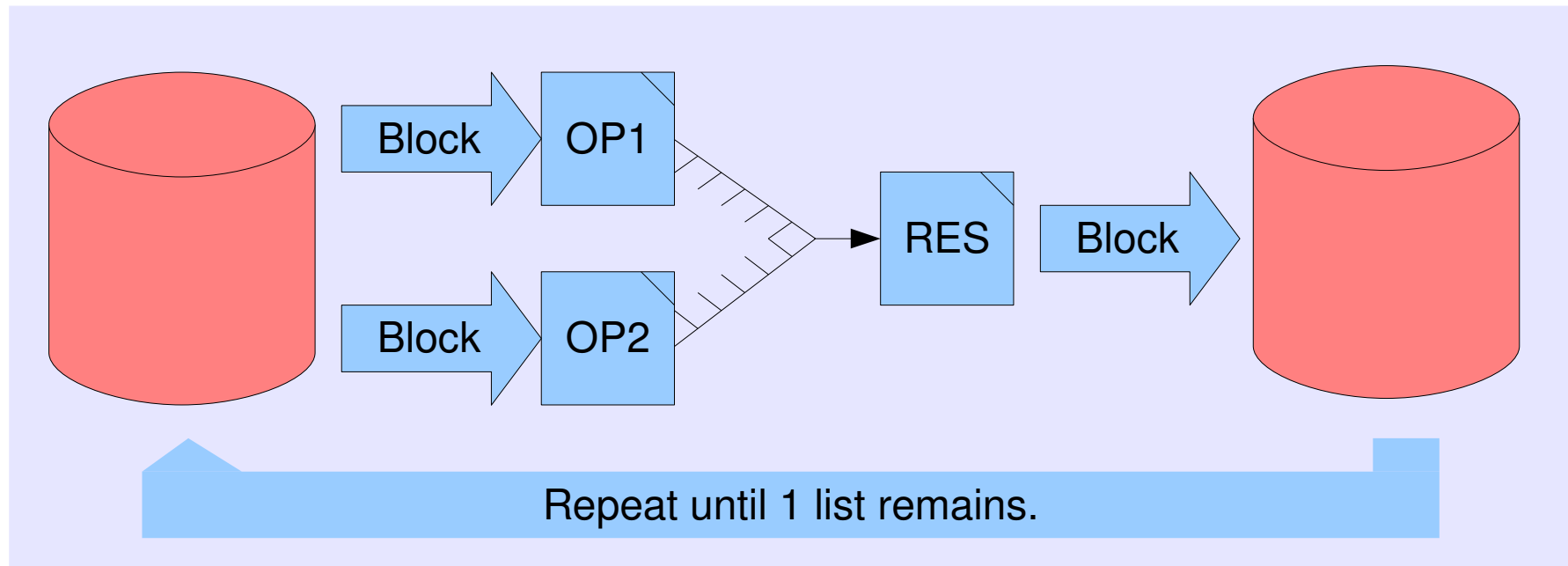
Dotplot/ Implementation/ Diagram API



Dotplot/ Implementation/ Disc Merge Sort ($t = n * \log(n)$)

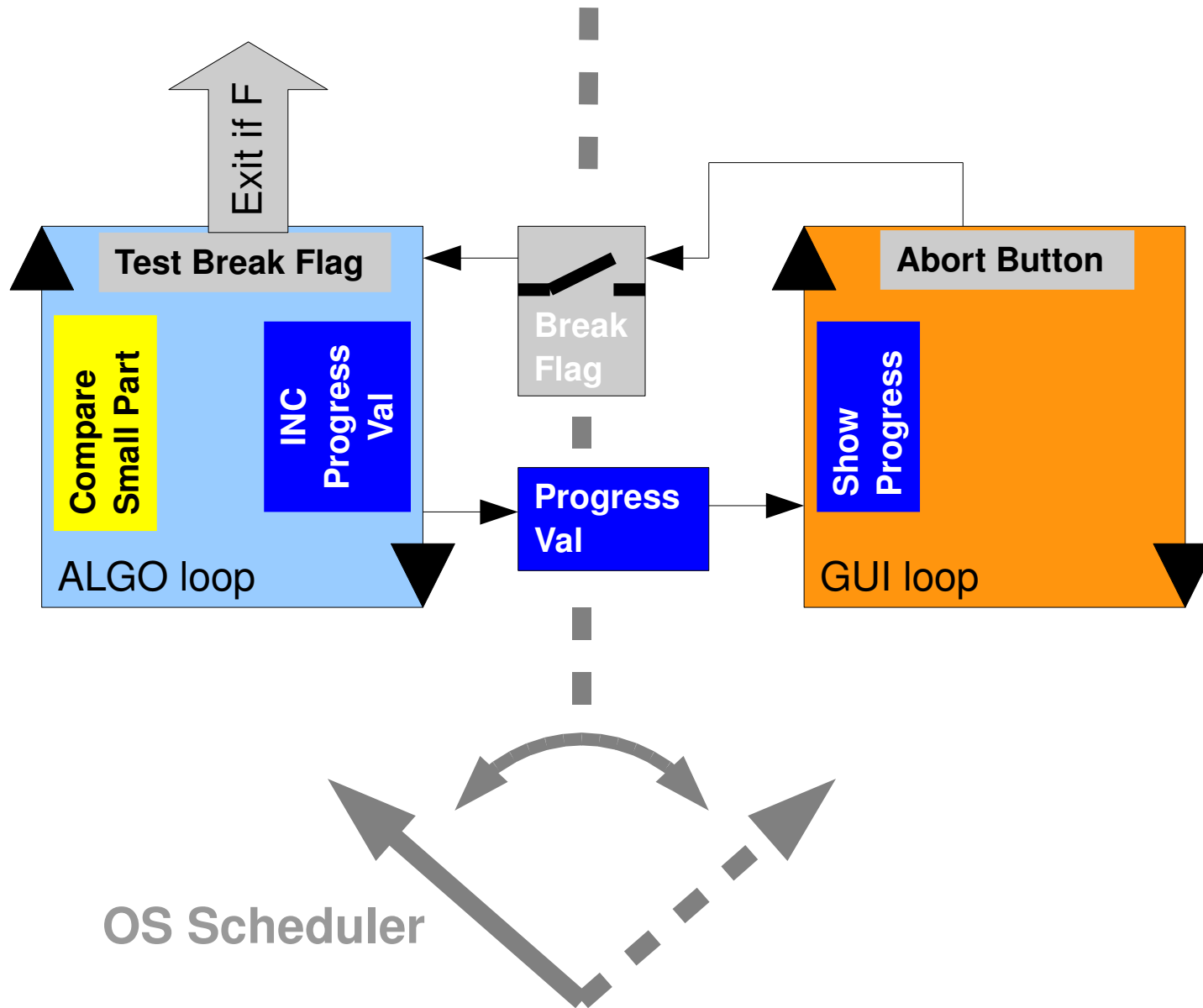
Record	[ch1][ch2][ch3] [POS]
--------	---------------------------------

SORT

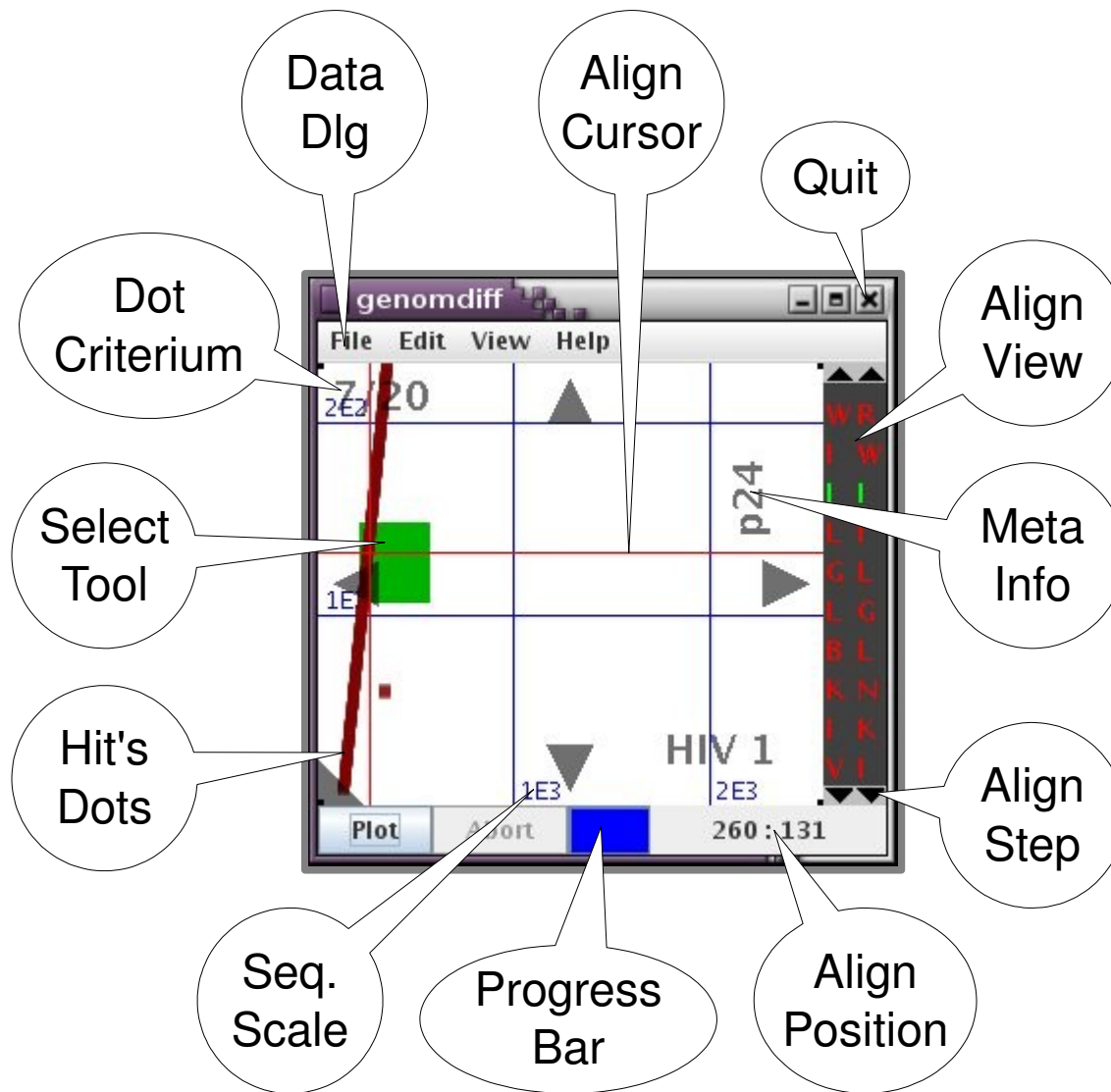


- 1) Split input into a number of one-entry-lists!
- 2) Make a merge sort pair-wise with all lists!
- 3) Until there is more than one result-list: Repeat step 2)!

Dotplot/ Implementation/ Progress Bar



Dotplot/ Software/ Genomediff



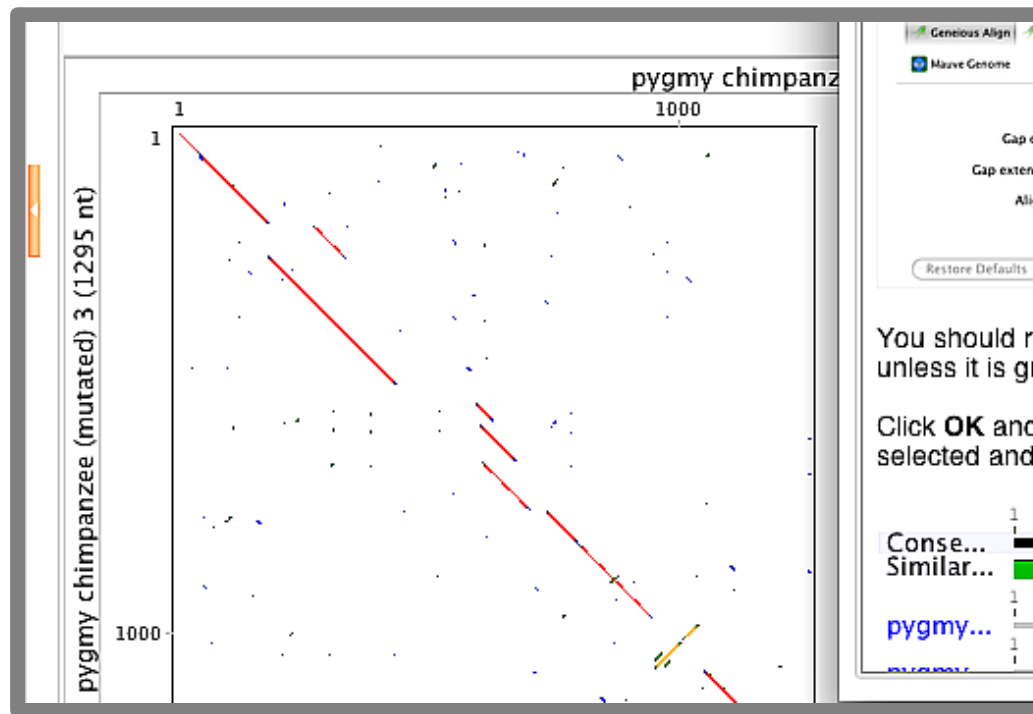
- Author's own free tool.
- Only forward compare.
- Only virus-size datasets.
- Java based desktop program.
- Simple Pdf export feature.
- Common DNA parser.
- <http://sourceforge.net/projects/genomdiff>



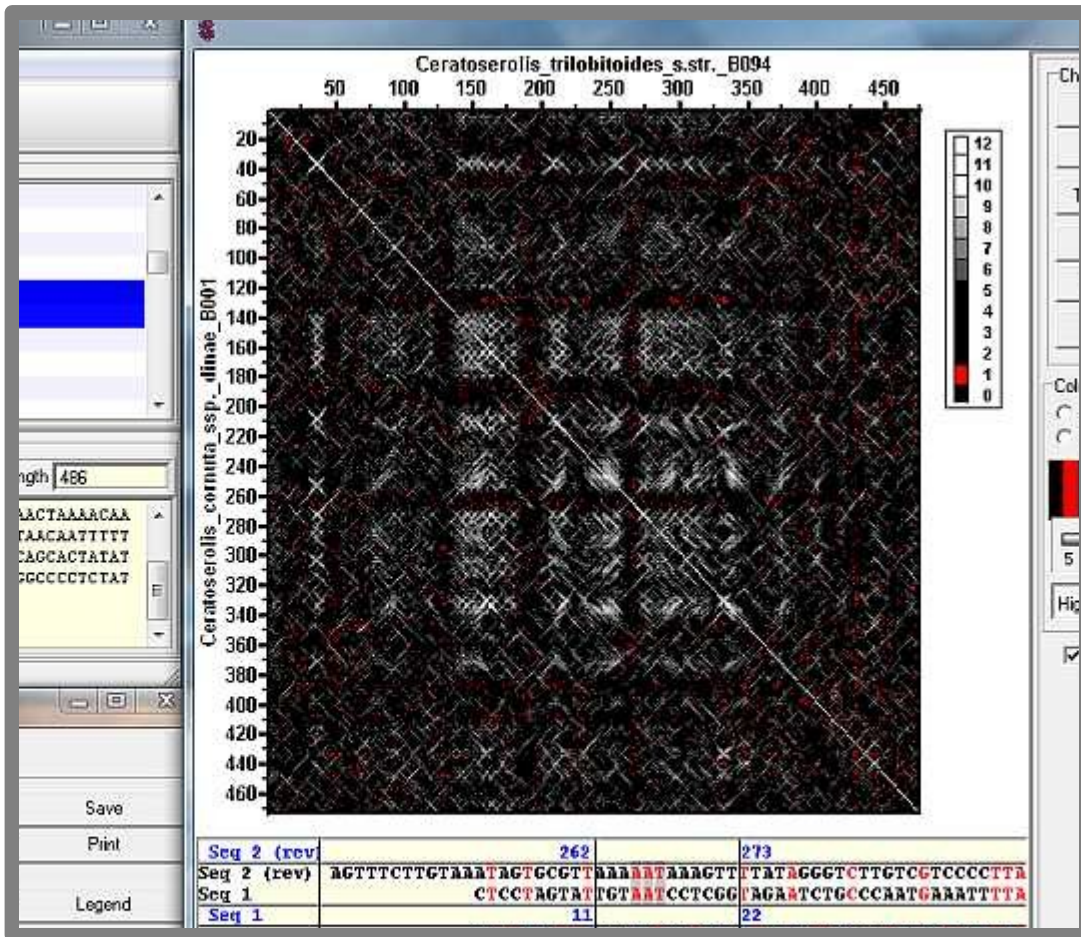
Dotplot/ Software/ Geneious



- Student Price = (250.00\$ - 1.00 \$).
- Provider: BIOMATTERS, New Zealand.
- Free BASIC version is hidden.
- MAC, SOLARIS, LINUX, WIN.
- Desktop-SW, more features than Dotplot.
- User forum & tutorials & support & ncbi search.
- <http://www.geneious.com/>



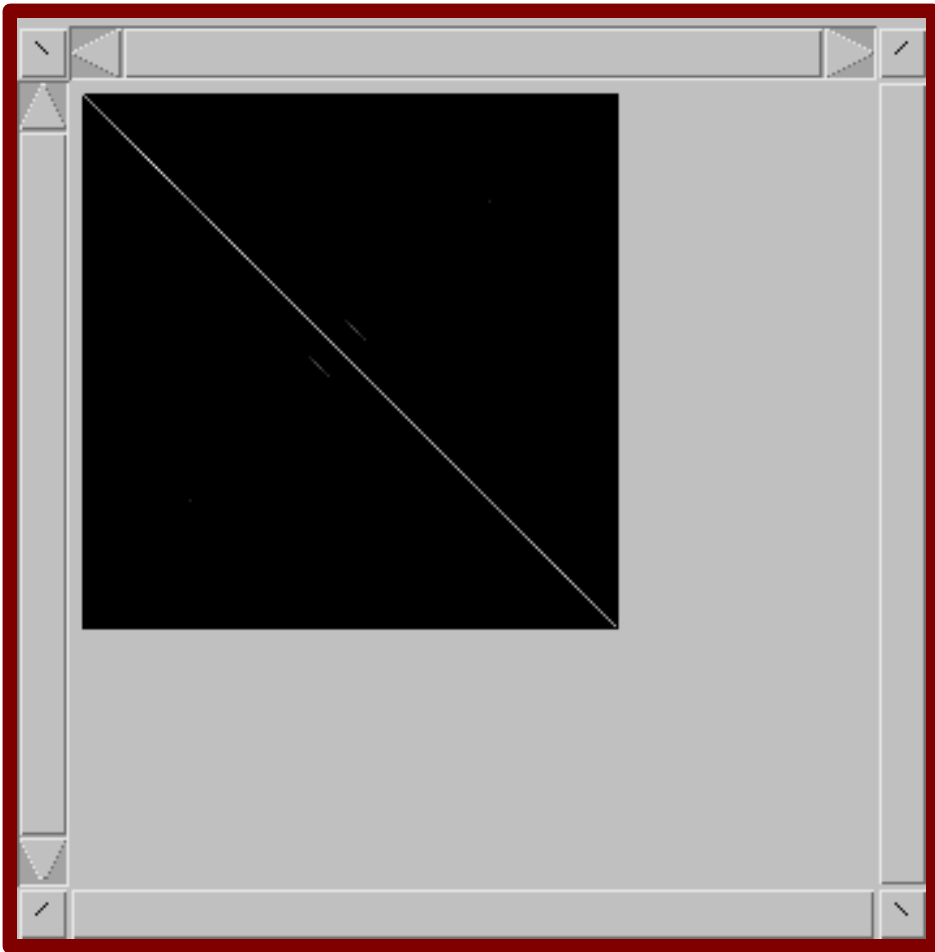
Dotplot/ Software/ Serolis



- Donationware, ~10.00€.
- Written by: Dr. Jan Schulz.
- 100% WINDOWS domain.
- DNA Parser.
- Desktop-SW.
- Tutorial offered.
- http://www.code10.info/index.php?option=com_content&view=category&id=50&Itemid=75



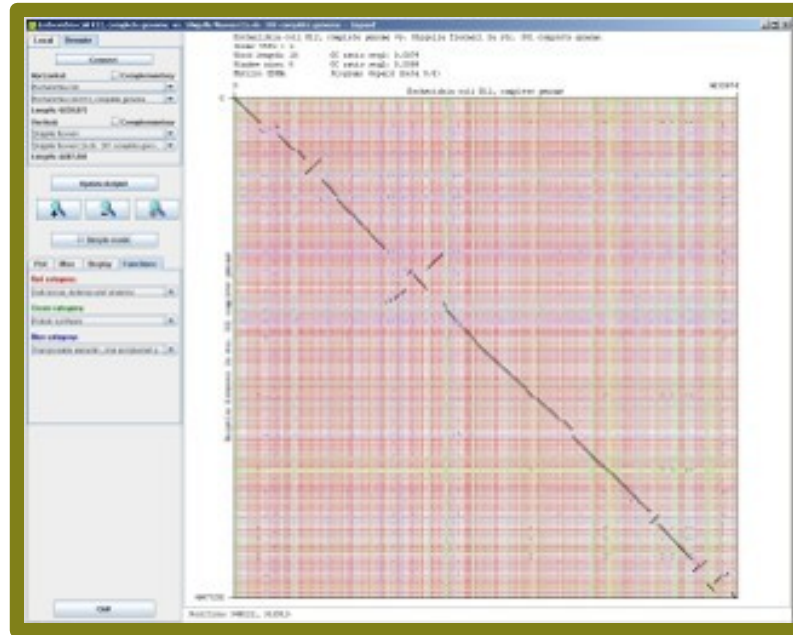
Dotplot/ Software/ Dotlet



- Java applet without fee.
- Thomas Junier & Marco Pagni.
- Dna parser.
- Pdf print service.
- Hit statistics.
- List of sequences held.
- <http://myhits.isb-sib.ch/cgi-bin/dotlet>



Dotplot/ Software/ Gepard



- Java-Web-Start application + Webservice.
- Author: Jan Krumsiek.
- Optimized for large genomes: $time = len \times \ln(len)$, uses sorted index.
- Offered by governmental „HELMHOLTZ“-center, but no license specified.
- Bitmap output.
- Zoom by entering exact range figures.
- <http://mips.helmholtz-muenchen.de/services/analysis/gepard>



Dotplot/ Software/ DotMatcher



- Http Form Webservice.
- Institut PASTEUR.
- Input as textfield, file or from internet DB similar GenBank.
- User's Email requested.
- Similarity matrixes.
- Pixelgraphic sent back.
- <http://mobyte.pasteur.fr/cgi-bin/portal.py?form=dotmatcher>



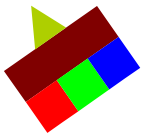
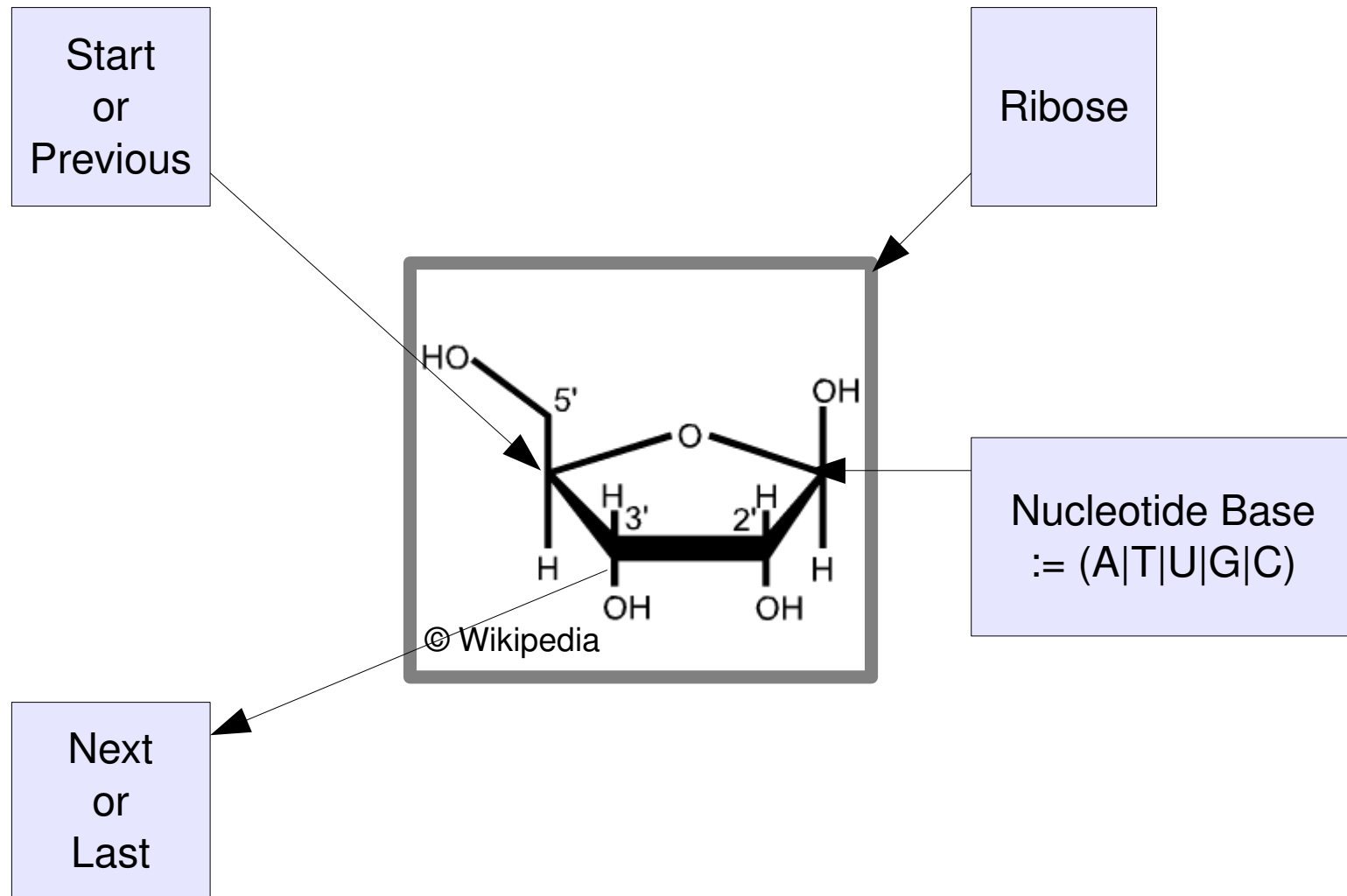
Dotplot/ Software/ SWdiff

Product	Price	Platform	Data-Size/ Bp *)	Reverse
GenomeDiff	0.00€	Java	~10 000	FALSE
Genious	>=249.00\$	Java	<= 2 500	TRUE
Serolis	~10.00€	Win	~12 000	TRUE
Dotlet	0.00€	Applet	~10 000	FALSE
Gepard	0.00€	Java WS	>150 000 000	TRUE
DotMatcher	0.00€	WebApp	>150 000 000	TRUE

*) As translated proteine, Virus ~ 10kBp, Bacterium ~ 5MBp, Animal/ Plant ~ 20 x 100MBp.



Dotplot/ Proteine Synthesis/ 5'-3' End of Nucleotide



Dotplot/ Proteine Synthesis/ Fasta File Format

Begin
Char

Meta Info

>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]

;Comment Line

LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
LLILILLLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFPLPIAGX
IENY

... Next Record

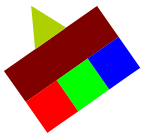
Header

Comment

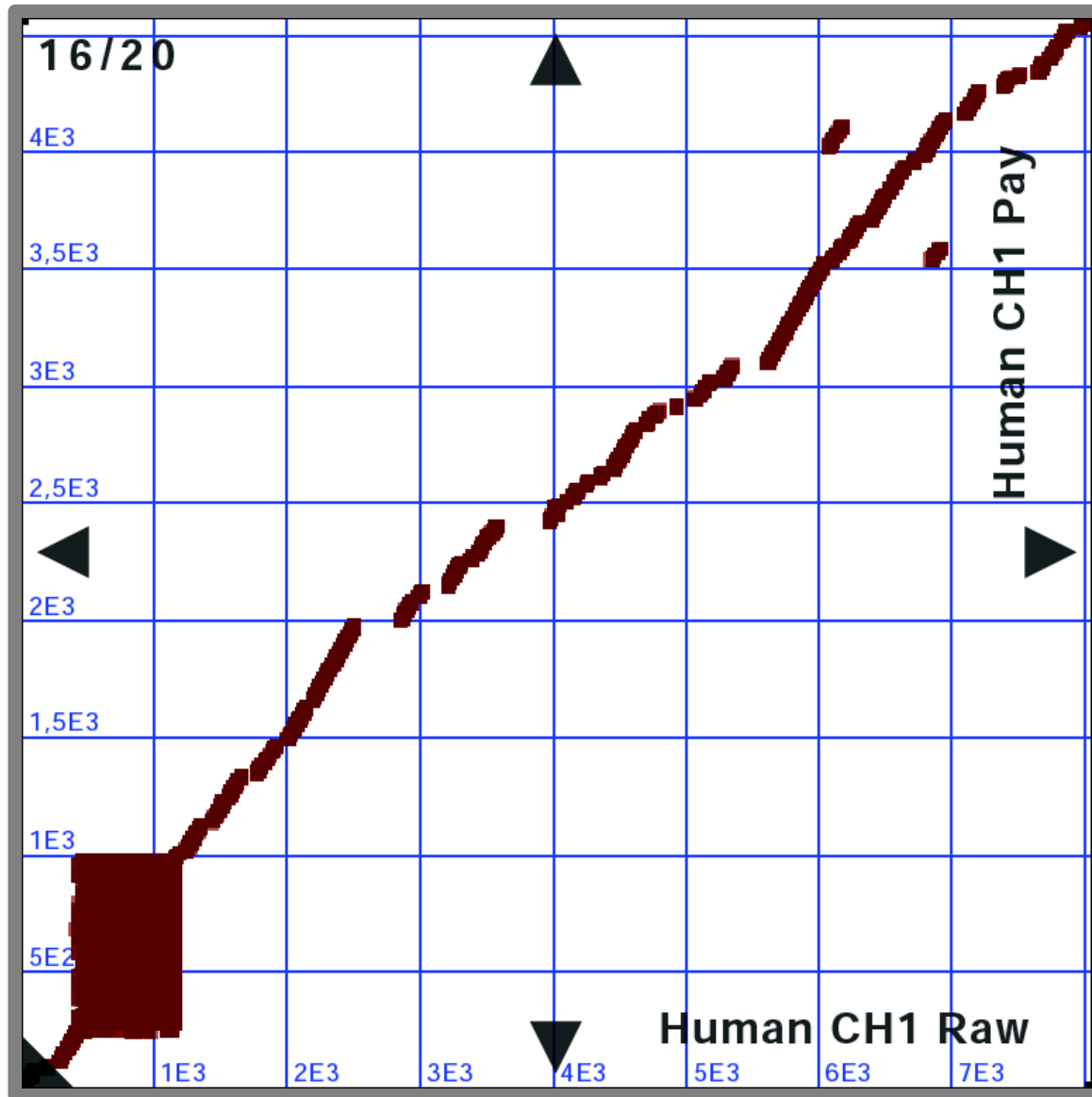
Sequence

80 columns

- Nucleotide Sequence: (A|C|G|T|U)
- Proteine Sequence: (A|B|C|D|E|F|G|H|I|J|K|L|M|N|P|Q|R|S|T|U|V|W|Y|Z|*)

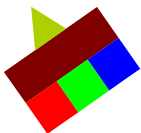


Dotplot/ Proteine Synthesis/ Fingerprint

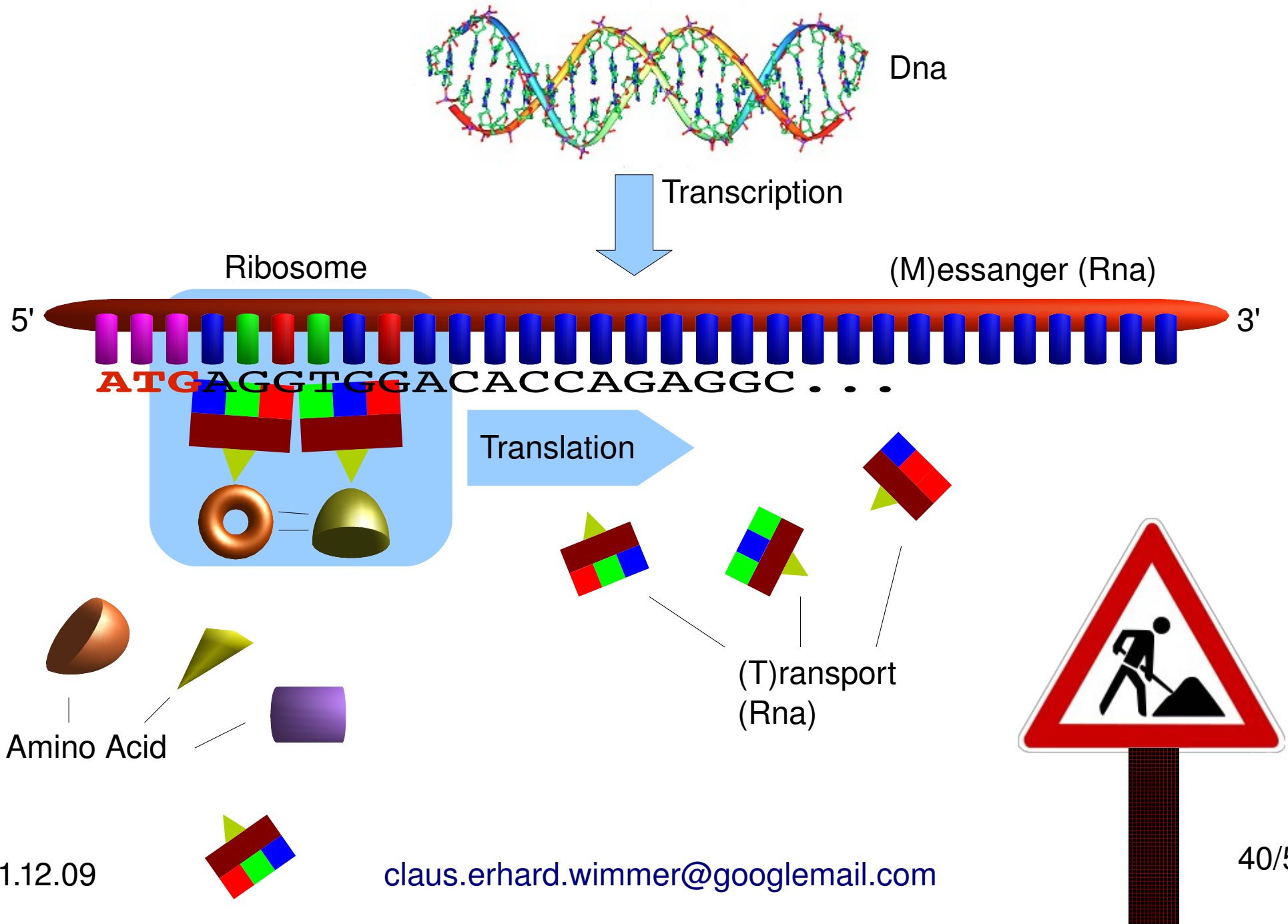


Comparison between: Piece of the human genome, chromosome 1 with its modified copy where all introns have been removed. Fingerprint sequences are visible at the RAW axis. A self-identical section is also present between positions 500 and 1000 (1E3).

Source: <http://www.gutenberg.org/etext/2201>



Dotplot/ Proteine Synthesis/ mRNA → tRna

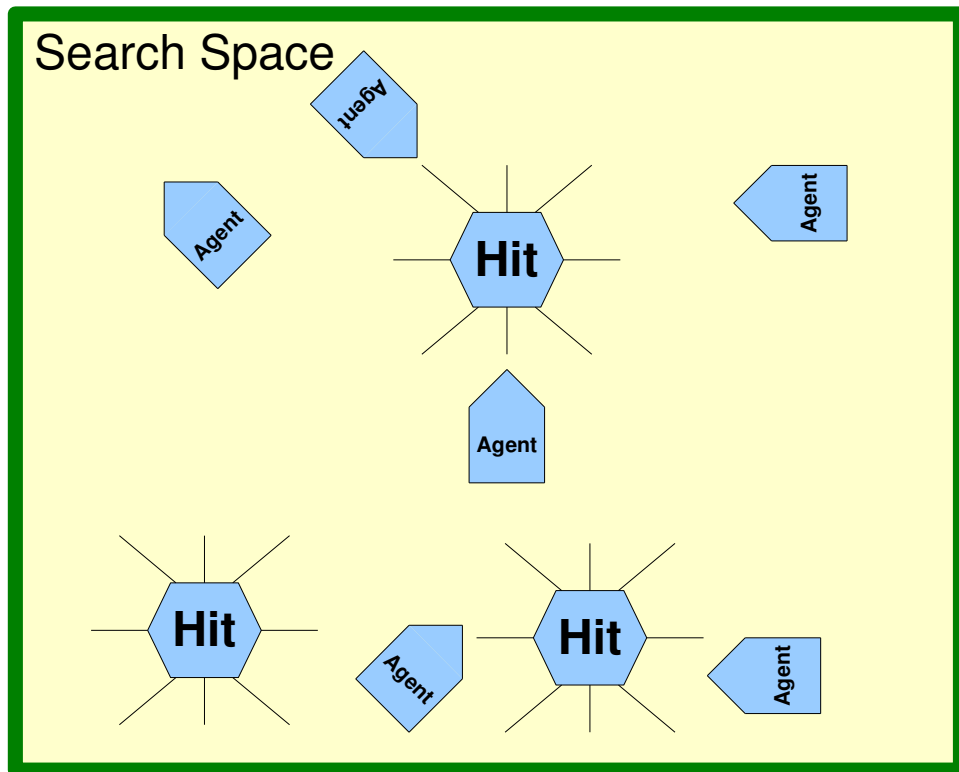


Dotplot/ Proteine Synthesis/ BLOSUM

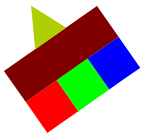
[illegible]

- **BLOck SUbstitution Matrix.**
- Different matrixes for several purposes.
- Similaritiy of Amino Acids in Proteins.
- The more similar the higher value.

Dotplot/ Proteine Synthesis/ GenAlgo as Search



Genomes (AGENT) drift to the fitness maxima (HIT). Hits send fitness gradients into the search space. Agents find this gradients and follow their +growth direction. Sometimes an agent has to decide between two different directions. Search space may be very large – agents are not able to explore it completely.



Dotplot/ Proteine Synthesis/ Commercial Services

GenAxxon/ Ulm GERMANY

- Proteine sequencing ~1000,-€
- Peptide synthesis
- <http://www.genaxxon.de>

Fraunhofer/ Munich GERMANY

- Dna sequencing
- Peptide synthesis
- <http://www.ime.fraunhofer.de>

EUROFINS/ 85560 Ebersberg GERMANY

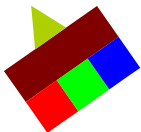
- Dna sequencing
- Dna/ Rna synthesis
- <http://www.eurofinsdna.com>

BioNexus/ Oakland CA USA

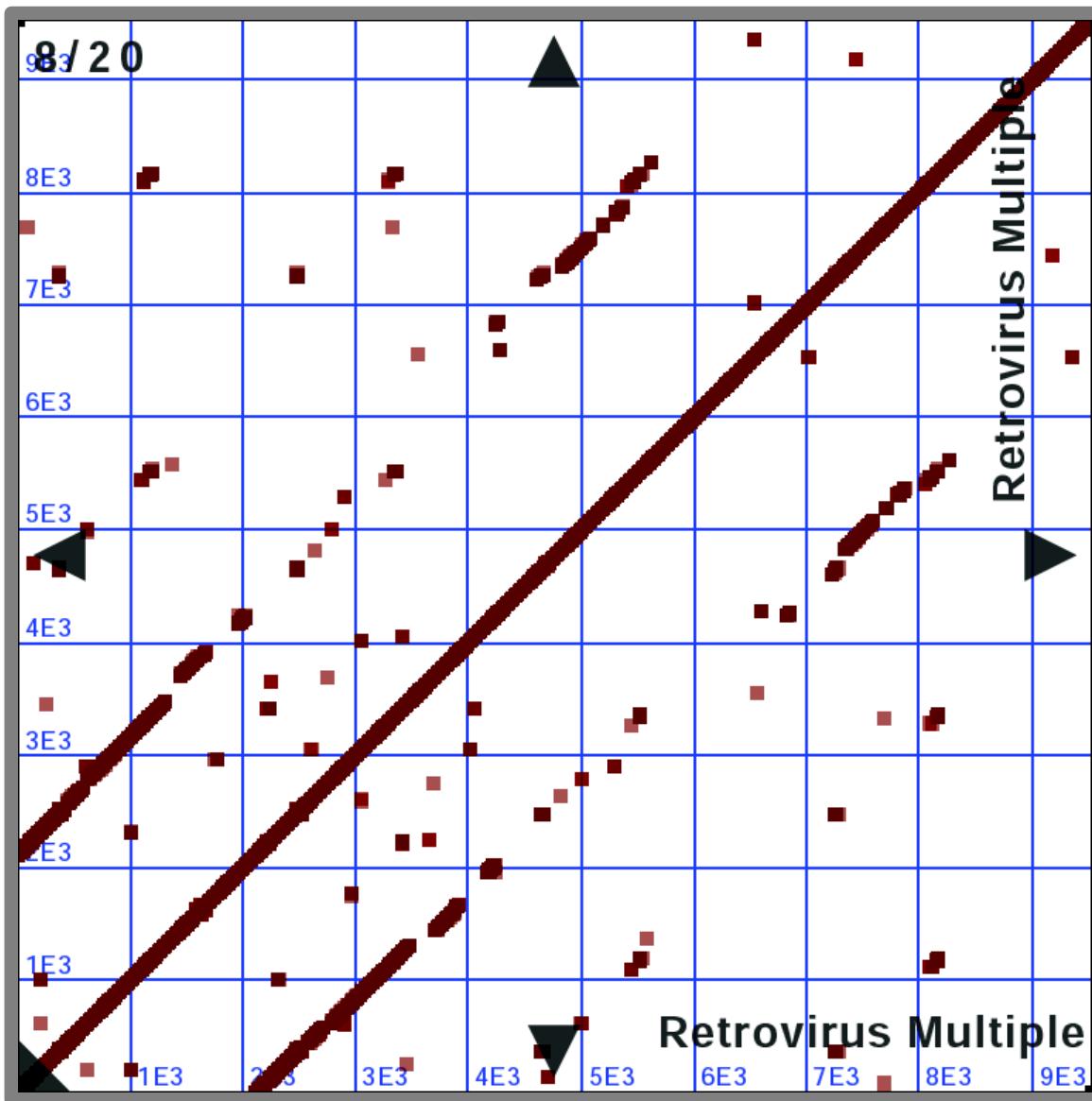
- Dna sequencing
- Gene synthesis
- Peptide synthesis
- <http://www.bionexus.net>

Proteome Factory/ Berlin GERMANY

- Proteine sequencing ~200,-€
- <http://www.proteomefactory.de>



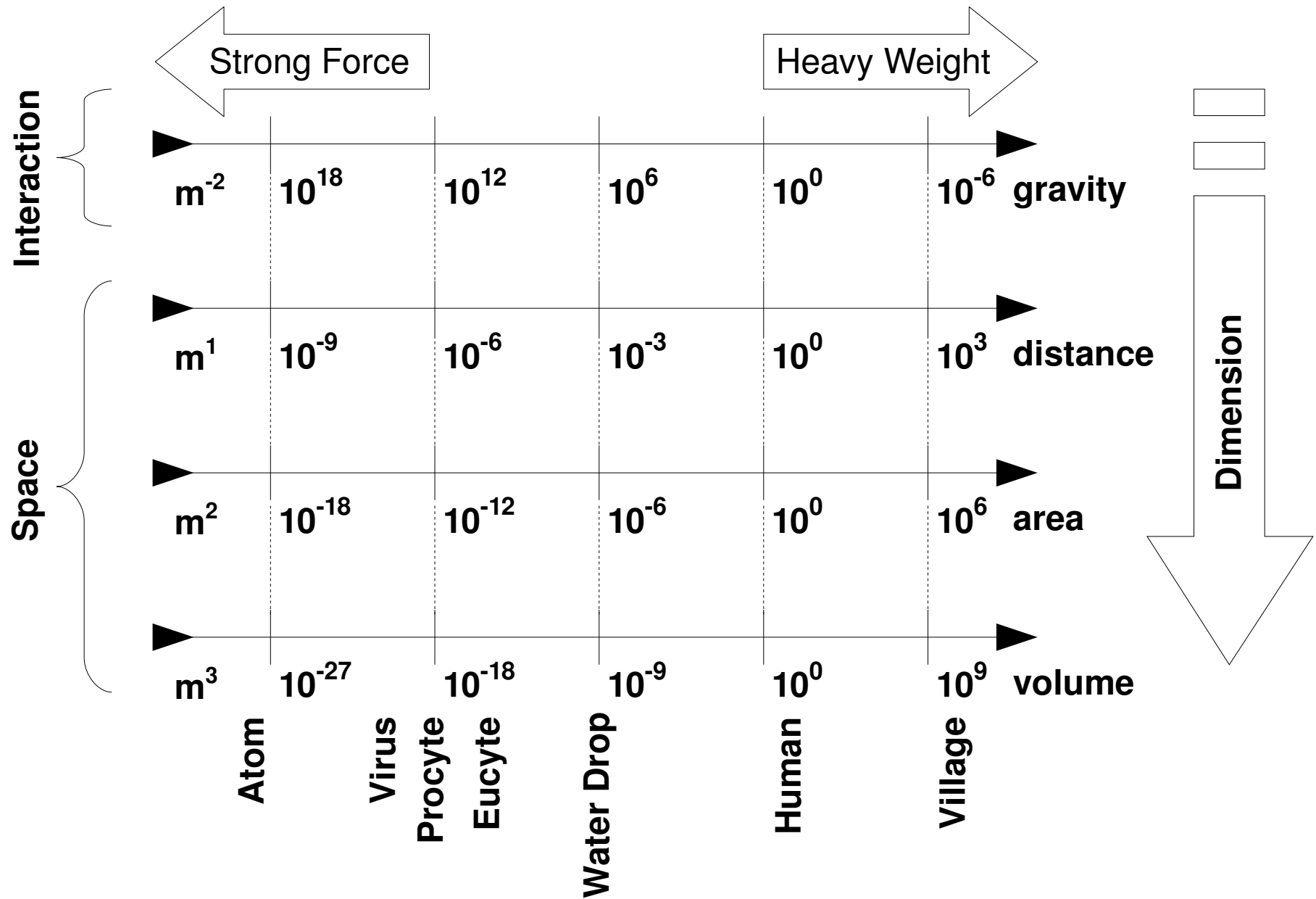
Dotplot/ Misc/ Multiple Plot



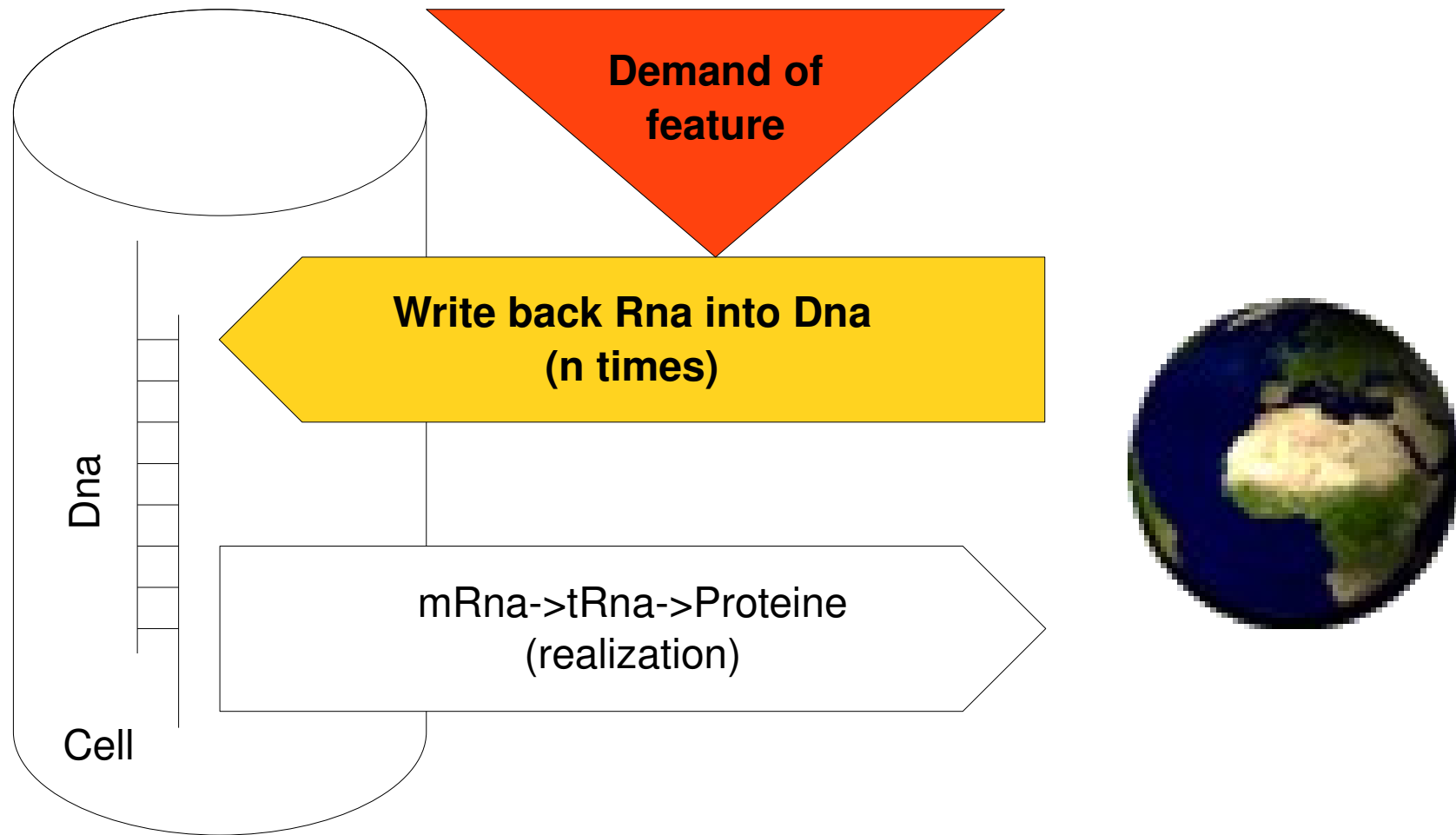
- Both axes: HTLV1-HTLV2-HIV-VISNA (NC_001436, NC_001488, X01762, NC_001452).
- Concatenated input file.
- Multiple-self-comparison.
- Protein based.
- NxN/2 combinations at one chart but also more CPU time.

Source: ENTREZ Nucleotide Database.

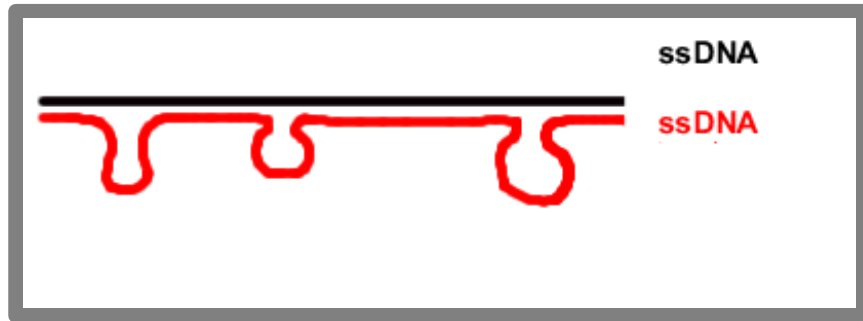
Dotplot/ Misc/ Dimensions



Dotplot/ Misc/ Gene Amplification



Dotplot/ Misc/ Heteroduplex Hybridisation



- ▶ DNA double strand.
- ▶ Two different organisms.
- ▶ Attractions between similar regions.
- ▶ Adjustable sensitivity/ tolerance.
- ▶ No hidden program logic.
- ▶ Done in laboratory.

Dotplot/ Misc/ Ressources

Author	Title	RefId
Dr. Jan Schulz	Dotplots (EN)	http://www.code10.info/index.php?view=category&id=52%3Acat_coding_algorithms_dot-plots&option=com_content&Itemid=56
Linder	Biologie (D)	Isbn: 3-507-10930-1
USA	NCBI(En-DB)	http://www.ncbi.nlm.nih.gov/
Japan	DDBJ (En-DB)	http://www.ddbj.nig.ac.jp/
Europe	EML (En-DB)	http://www.ebi.ac.uk/embl/
Europe	Uniprot (En-DB)	http://www.uniprot.org/

Dotplot/ Misc/ Quiz

- A) What does „maximum mean“ mean in geneology-tree-programs?**
- B) How fast does CPU-time grow with sequence length?**
- C) Which plant does the patent for MON863 claim?**
- D) Name the angle of the sliding window!**
- E) Which programs can compare one of the human chromosomes?**
- F) On which end does the FASTA file format start?**
- G) Explain the heteroduplex-hybridisation!**

Dotplot/ Misc/ License

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