Comparative genomics tools for biological discovery

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Outline

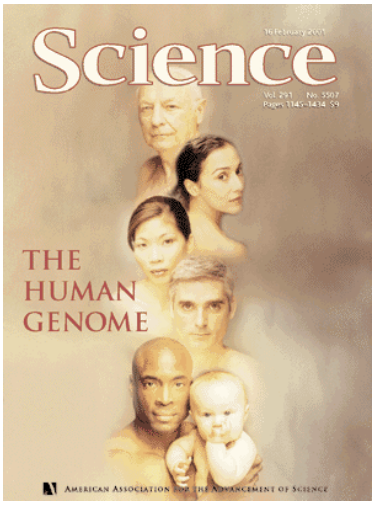
What is comparative genomics?

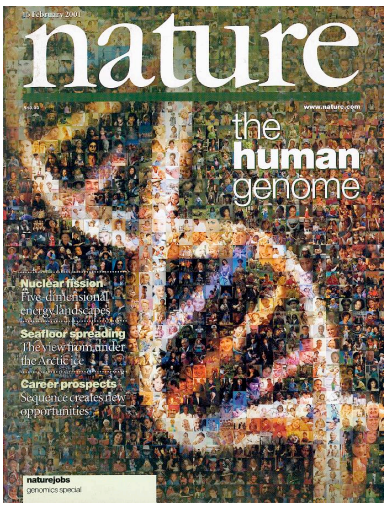
VISTA tools developed for comparative genomics.

Related biological stories

Large scale VISTA applications including automatic computational system for comparing the human and mouse genomes

The Human genome





From the Nature paper:

The next steps:

Developing the IGI (integrated gene index) and IPI (integrated protein index)

Large-scale identification of regulatory regions

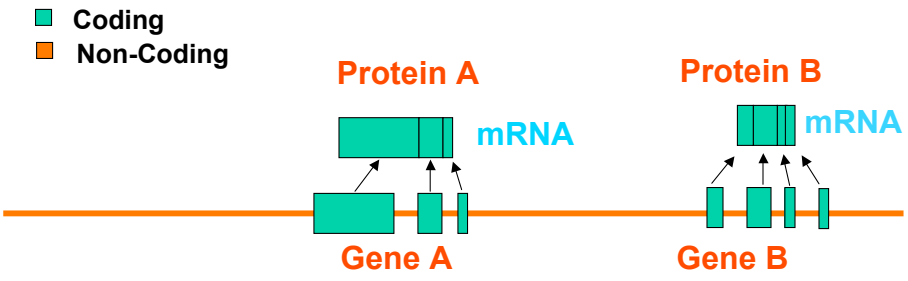
Sequencing of additional large genomes

Completing the catalogue of human variation

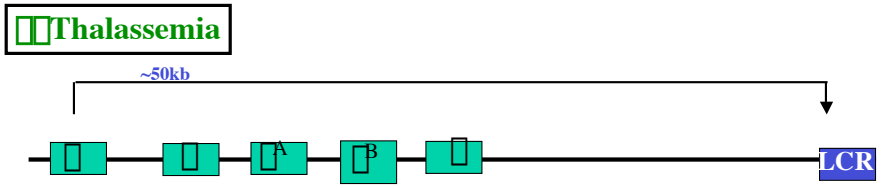
From sequence to function

"

1-2% Coding



Distant Non-Coding Sequences Causing Disease

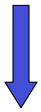


Disease	Gene	Distance
Campomelic displasia	SOX9	850kb
Aniridia	PAX6	125kb
X-Linked Deafness	POU3F4	900kb
Saethre-Chotzen syndrome	TWIST	250kb
Rieger syndrome	PITX2	90kb
Split hand/split foot malformation	SHFM1	450kb

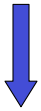
Background

Evolution can help!

In general, functionally important sequences are conserved



Conserved sequences are functionally important



Raw sequence can help in finding biological function

Comparison of 1196 orthologous genes
(Makalowski et al., 1996)

- Sequence identity:
  - exons: 84.6%
  - protein: 85.4%
  - introns: 35%
  - 5' UTRs: 67%
  - 3' UTRs: 69%
- 27 proteins were 100% identical

Integrating data into more powerful gene prediction models than with human genomic sequence alone

Comparing sequences of different organisms

- Helps in gene predictions
- Helps in understanding evolution
- Conserved between species non-coding sequences are reliable guides to regulatory elements
- Differences between evolutionary closely related sequences help to discover gene functions

Challenges

- Sequence at different stages of completion, difficult to compare
-
- Whole genome shotgun → Partial Assemblies
- Finished BACs
- Fast and accurate analysis
- Scaling up to the size of whole genomes

http://www-gsd.lbl.gov/vista

Processed ~ 11000 queries on-line, distributed > 560 copies of the program in 34 countries

Modules of VISTA:

- Program for global alignment of DNA fragments of any length
- Visualization of alignment and various sequence features for any number of species
- Evaluation and retrieval of all regions with predefined levels of conservation

Sequence comparisons. How?

Three variations:

Find the best OVERALL alignment.

Global alignment

Find ALL regions of similarity.

Local alignment

Find the BEST region of similarity.

Optimal local alignment

Aligning large genomic regions

- Long sequences lead to memory problems
- Speed becomes an issue
- Long alignments are very sensitive to parameters
- Draft sequences present a nontrivial problem
- Accuracy is difficult to measure and to achieve

References for some existing programs:

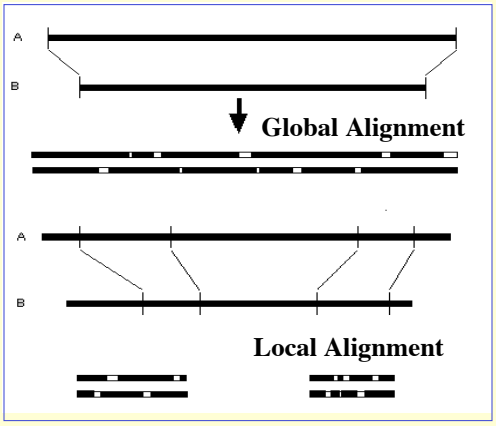
Glass:
Domino Tiling, Gene Recognition, and Mice.
Pachter, L. *Ph.D. Thesis, MIT* (1999)
Human and Mouse Gene Structure: Comparative Analysis and Application to Exon Prediction.
Batzoglou, S., Pachter, L., Mesirov, J., Berger, B., Lander, E. *Genome Research* (2000).
MUMmer
Delcher, A.L., Kasif S., Fleischmann, R.D., Peterson J., White, O. and Salzberg, S.L.
Alignment of whole genomes. *Nucleic Acids Research* (1999)
PipMaker
PipMaker: A Web Server for Aligning Two Genomic DNA Sequences.
Scott Schwartz, Zheng Zhang, Kelly A. Frazer, Arian Smit, Cathy Riemer, John Bouck, Richard Gibbs, Ross Hardison, and Webb Miller. *Genome Research* (2000)
Scan2
Dbscan/Scan2: Fast alignment of mega-sequences.
Seledtsov I.A., Solovyev V.V. To Appear. Web site http://softberry.com/

Local alignment algorithms are designed to search for highly similar regions in two sequences that may not be highly similar in their entirety. The algorithm works by first finding very short common segments between the input sequence and database sequences, and then expanding out the matching regions as far as possible.

For cross-species comparison one needs to accurately align two complete sequences. It is insufficient to find common similar regions in the two sequences, rather, what is needed is a global map specifying how the two sequences fit together, much like understanding how the pieces in a puzzle connect up with each other.

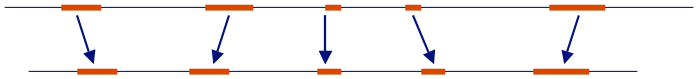
This problem is called **global alignment**

Local vs global alignment



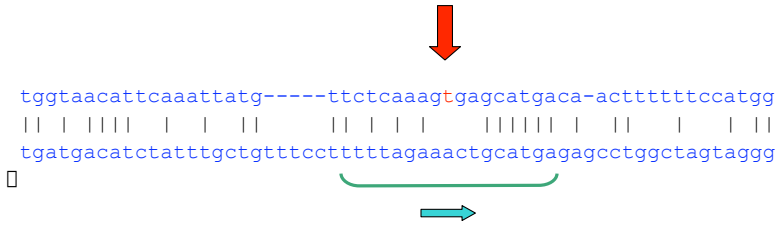
AVID- the alignment engine behind VISTA

- Very fast global alignment of megabases of sequence.
- Provides details about ordered and oriented contigs, and accurate placement in the finished sequence.
- Full integration with repeat masking



- ORDER and ORIENT
- FIND all common k-long words (k-mers)
- ALIGN k-mers scoring by local homology
- FIX k-mers with good local homology
- RECURSE with smaller k (shorter words)

Visualization



Window of length L is centered at a particular nucleotide in the base sequence

Percent of identical nucleotides in L positions of the alignment is calculated and plotted

Move to the next nucleotide

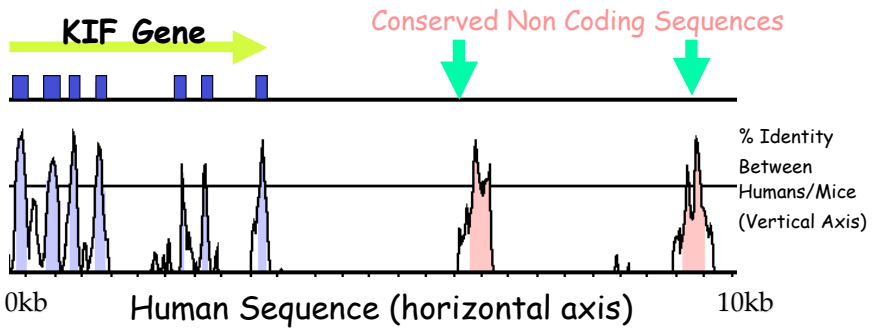
Finding conserved regions with percentage and length cutoffs

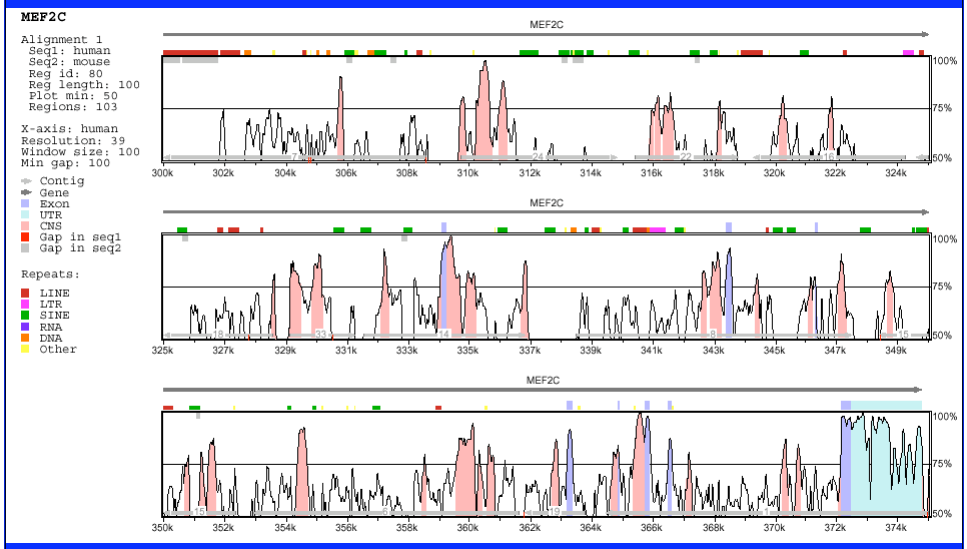
Conserved segments with percent identity X and length Y - regions in which every contiguous subsegment of length Y was at least X% identical to its paired sequence. These segments are merged to define the conserved regions.

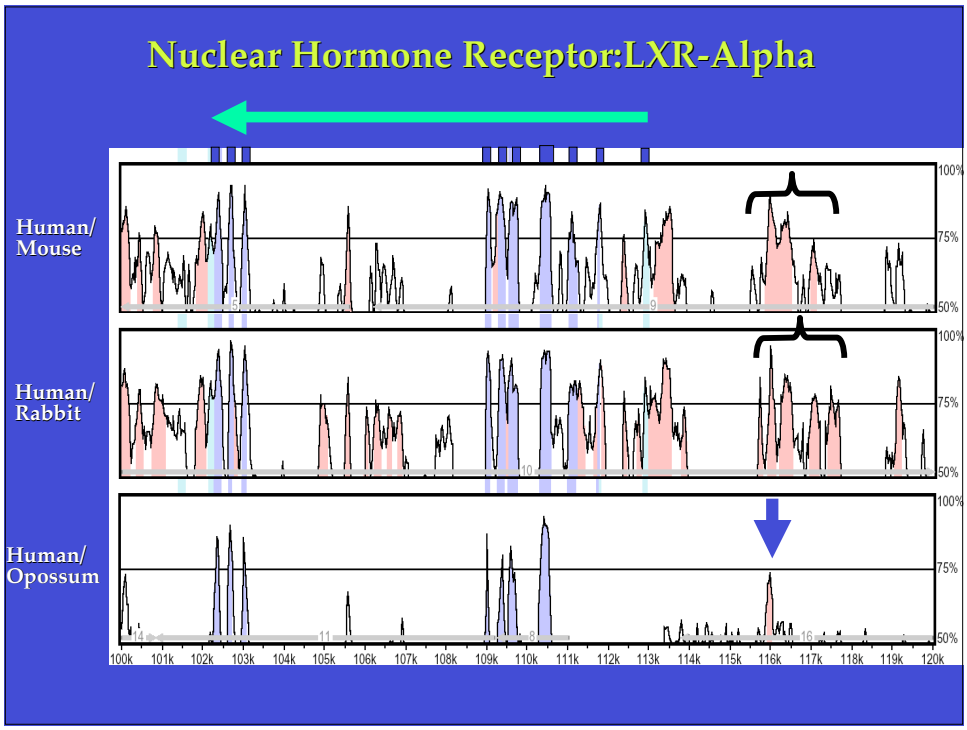
Output:

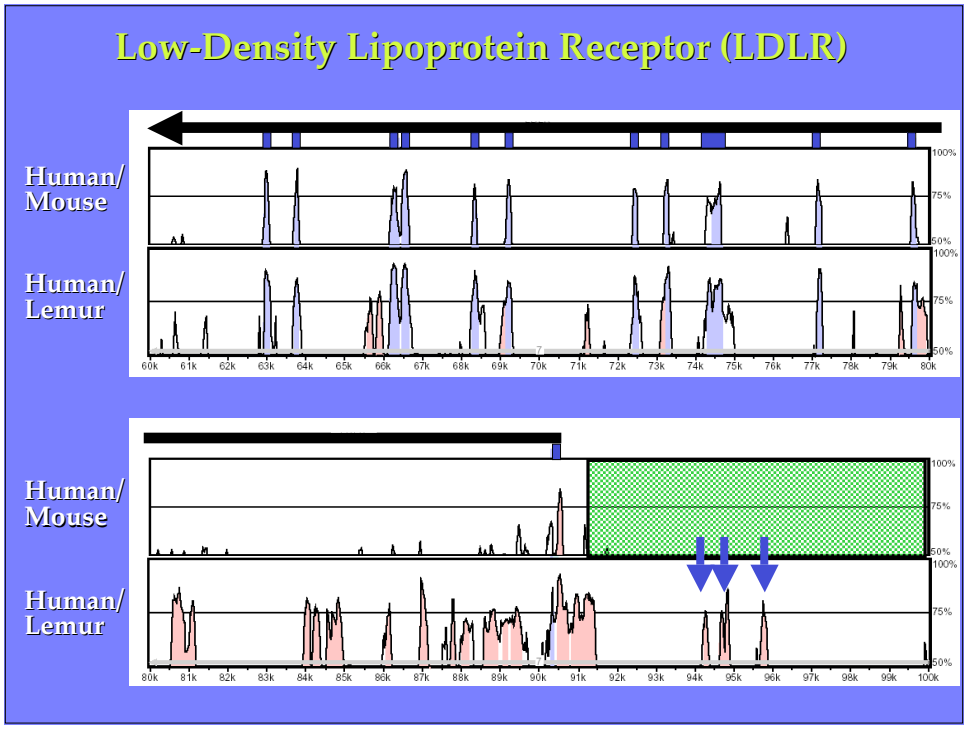
11054 - 11156 = 103bp at 77.670% NONCODING
13241 - 13453 = 213bp at 87.793% EXON
14698 - 14822 = 125bp at 84.800% EXON

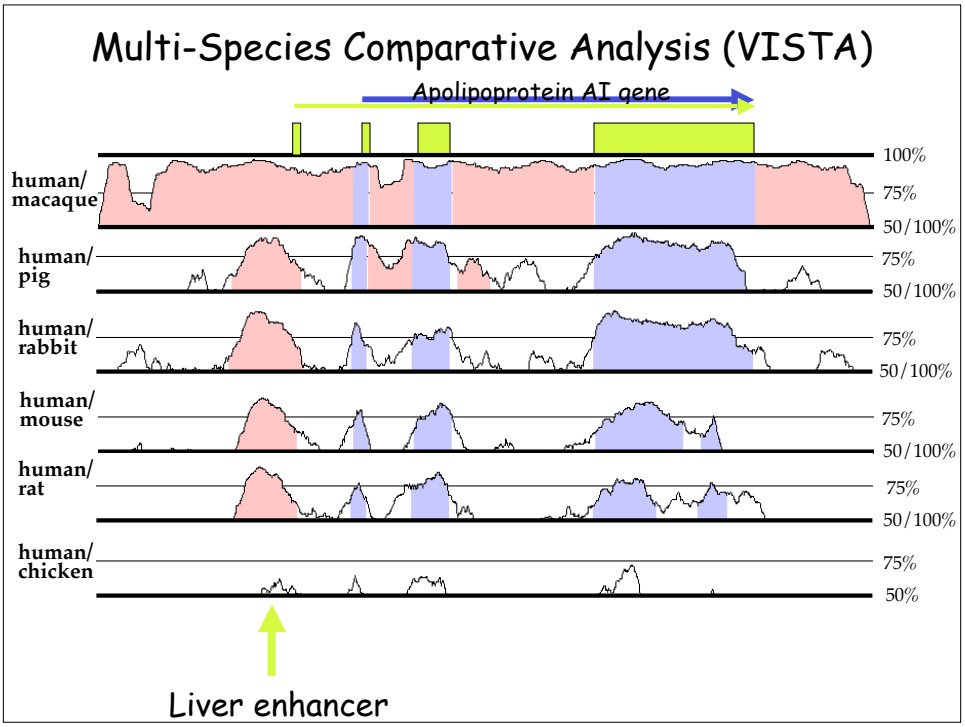
VISTA plot




MEF2C
Alignment 1
Seq1: human
Seq2: mouse
Reg id: 80
Reg length: 100
Plot min: 50
Regions: 103
X-axis: human
Resolution: 39
Window size: 100
Min gap: 100
Contig
Gene
Exon
UTR
CNS
Gap in seq1
Gap in seq2
Repeats:
LINE
LTR
SINE
RNA
DNA
Other
MEF2C
100%
75%
50%
300k 302k 304k 306k 308k 310k 312k 314k 316k 318k 320k 322k 324k
MEF2C
100%
75%
50%
325k 327k 329k 331k 333k 335k 337k 339k 341k 343k 345k 347k 349k
MEF2C
100%
75%
50%
350k 352k 354k 356k 358k 360k 362k 364k 366k 368k 370k 372k 374k


Nuclear Hormone Receptor:LXR-Alpha
Human/
Mouse
Human/
Rabbit
Human/
Opossum
100%
75%
50%
100k 101k 102k 103k 104k 105k 106k 107k 108k 109k 110k 111k 112k 113k 114k 115k 116k 117k 118k 119k 120k


Low-Density Lipoprotein Receptor (LDLR)
Human/ Mouse
Human/ Lemur
Human/ Mouse
Human/ Lemur


Multi-Species Comparative Analysis (VISTA)
Apolipoprotein AI gene
human/ macaque
human/ pig
human/ rabbit
human/ mouse
human/ rat
human/ chicken
100%
75%
50/100%
50%
Liver enhancer

VISTA input files

- Sequences of two or more organisms
- Annotation file for a base sequence if available

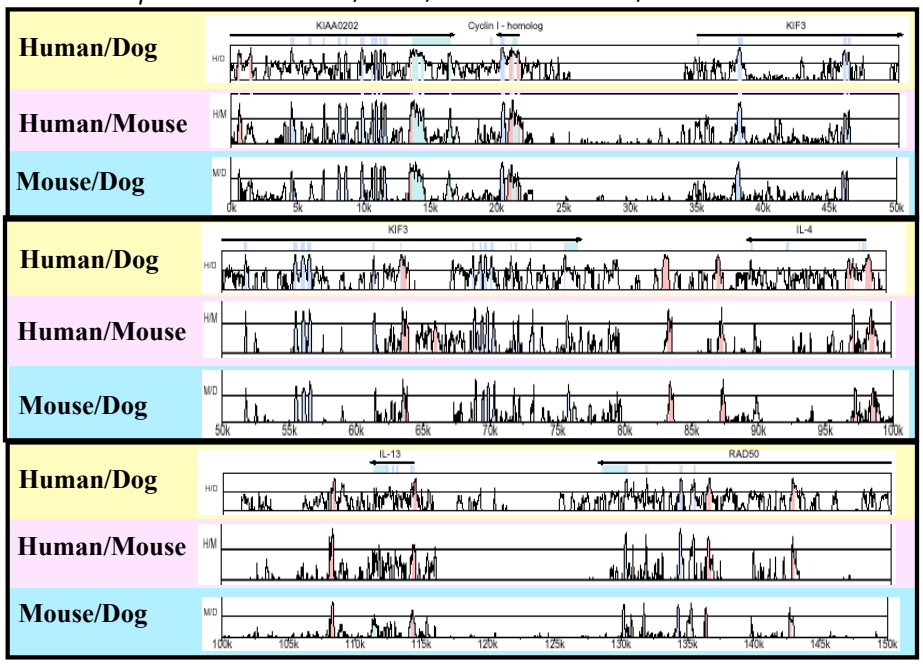
VISTA output files

- All pair wise global alignments of the sequences
- VISTA plot
- The list of conserved regions at predefined by the user length and conservation cutoffs

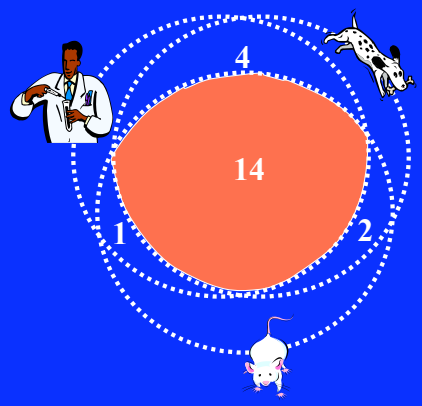
VISTA flavors

- VISTA – comparing DNA of multiple organisms
- for 3 species - analyzing cutoffs to define actively conserved non-coding sequences
- cVISTA - comparing two closely related species
- rVISTA – regulatory VISTA

Example: Dubchak et al., 2000, Genome Research, 10: 1304-1306.



Active conservation of noncoding sequences – present in more than two mammals



% Cutoff

sum of three pair wise Intersection/Union values is maximal

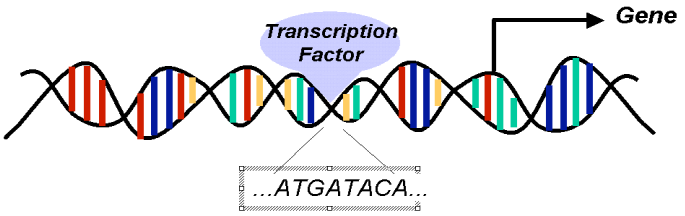
Over 120 basepairs:

H/D > 92%

H/M > 80%

D/M > 77%

Identifying non-coding sequences (CNSs) involved in transcriptional regulation



rVISTA - prediction of transcription factor binding sites

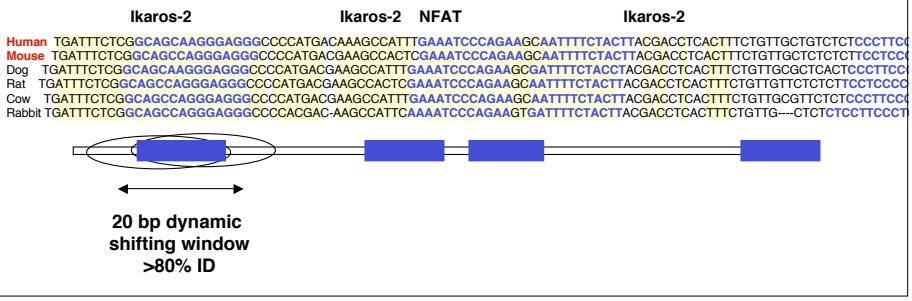
- Simultaneous searches of the major transcription factor binding site database (Transfac) and the use of global sequence alignment to sieve through the data

- Combination of database searches with comparative sequence analysis reduces the number of predicted transcription factor binding sites by several orders of magnitude

Regulatory VISTA (rVISTA)

1. Identify potential transcription factor binding sites for each sequence using library of matrices (TRANSFAC)
2. Identify aligned sites using VISTA
3. Identify conserved sites using dynamic shifting window

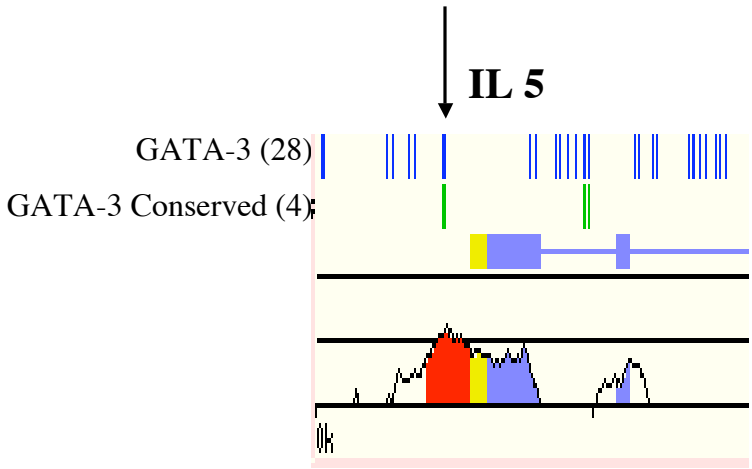
Percentage of conserved sites of the total 3-5%

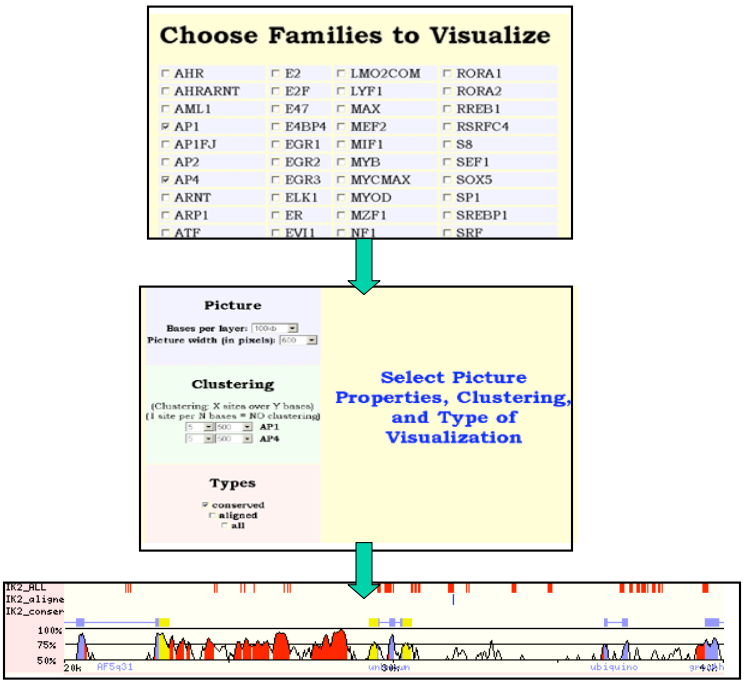


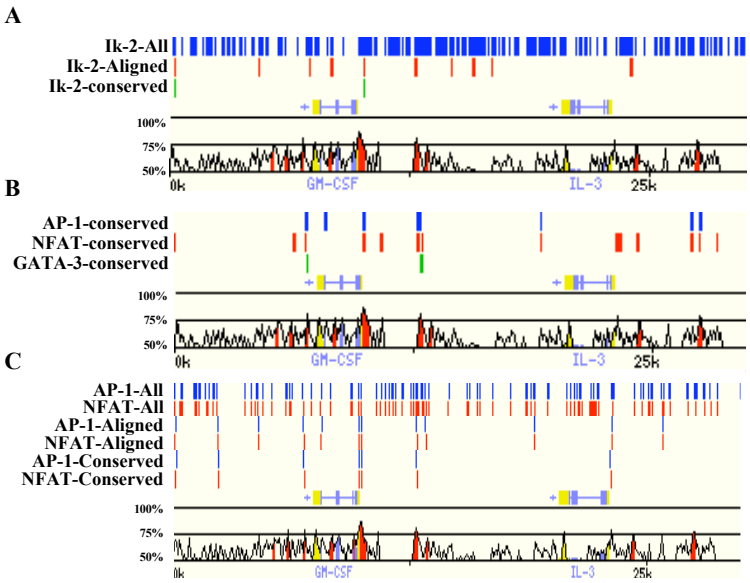
~1 Meg region, 5q31

	Coding	Noncoding
Human interval Transfac predictions for GATA sites	839	20654
Aligned with the same predicted site in the mouse seq.	450	2618
Alligned sites conserved at 80% / 24 bp dynamic window	303	731
Random DNA sequence of the same length		29280

2 Exp. Verified GATA-3 Sites







Sequence motif recognition

+

multiple sequence alignment of syntenic regions,

↓

a high throughput strategy for filtering and prioritizing putative DNA binding sites

↓

genomically informed starting place for globally investigating detailed regulation

Main features of VISTA

- Clear , configurable output
- Ability to visualize several global alignments on the same scale
- Alignments up to several megabases
- Working with finished and draft sequences
- Available source code and WEB site

Related publications

- **O N E**
- Loots GG, Locksley RM, Blankespoor CM, Wang ZE, Miller W, Rubin EM, Frazer KA. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons.
- Science. 2000 Apr 7;288(5463):136-40.

- **T W O**
- Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing.
- Science. 2001 Oct 5;294(5540):169-73.

What if you don't have a sequence of other species for the region of your interest?

Are there publicly available comparative genomics data?

Large scale VISTA applications:

Cardiovascular comparative genomics database
http://pga.lbl.gov

Godzilla – comparing the human and mouse genome
http://pipeline.lbl.gov

Godzilla - automatic computational system for comparative analysis of genomes

http://pipeline.lbl.gov http://www-gsd.lbl.gov/vista

DATA

Base Human Genome – Golden Path Assembly

Mouse assemblies:

Arachne	October 2001
Phusion	November 2001
MGSC v3	April 2002

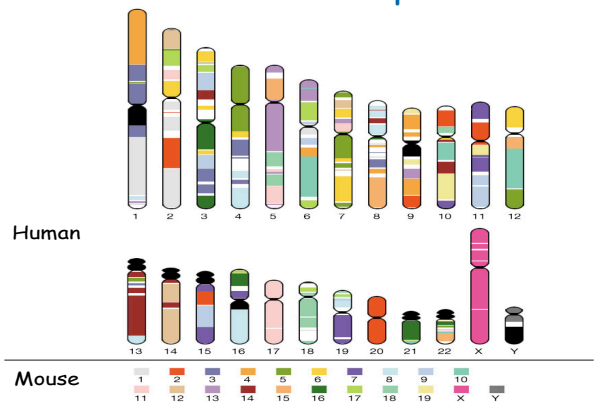
Main modules of the system

Mapping and alignment of mouse contigs against the human genome

Visualization

Analysis of conservation

Chromosome Comparison

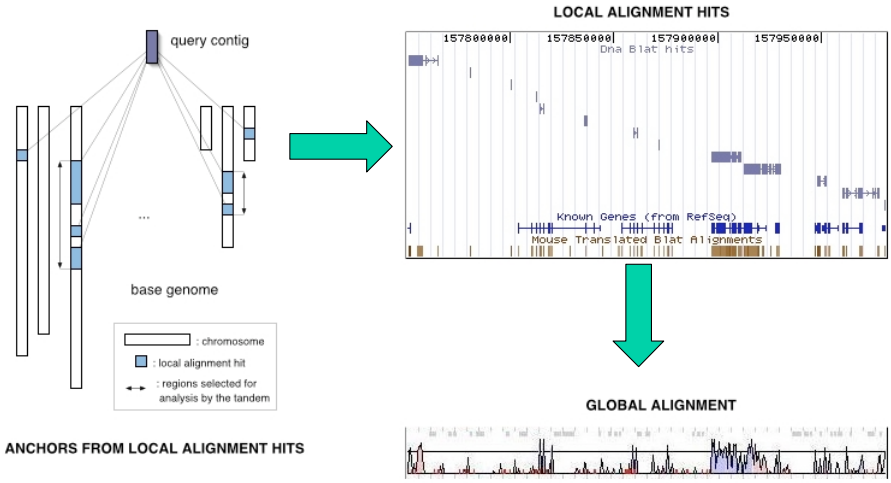


Base pair alignment

```
247 GGTGAGGTCGAGGACCCTGCA  CGGAGCTGTATGGAGGGCA   AGAGC
    |:   ||  |||||:  |||| --:||  ||| |::|   |||---||||
368 GAGTCGGGGGAGGGGGCTGCTGTTGGCTCTGGACAGCTTGCATTGAGAGG
```

Tandem Local/Global Alignment Approach

Sequence fragment anchoring (DNA and/or translated BLAT)
Multi-step verification of potential regions using global alignment (AVID)

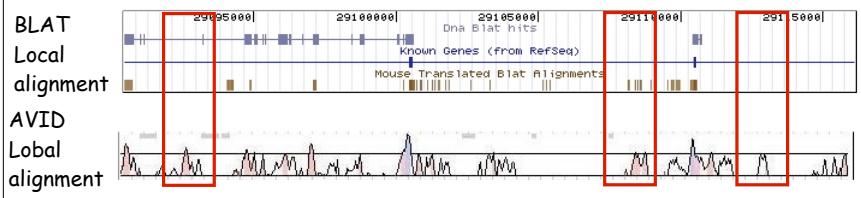


Advantage of the tandem approach:

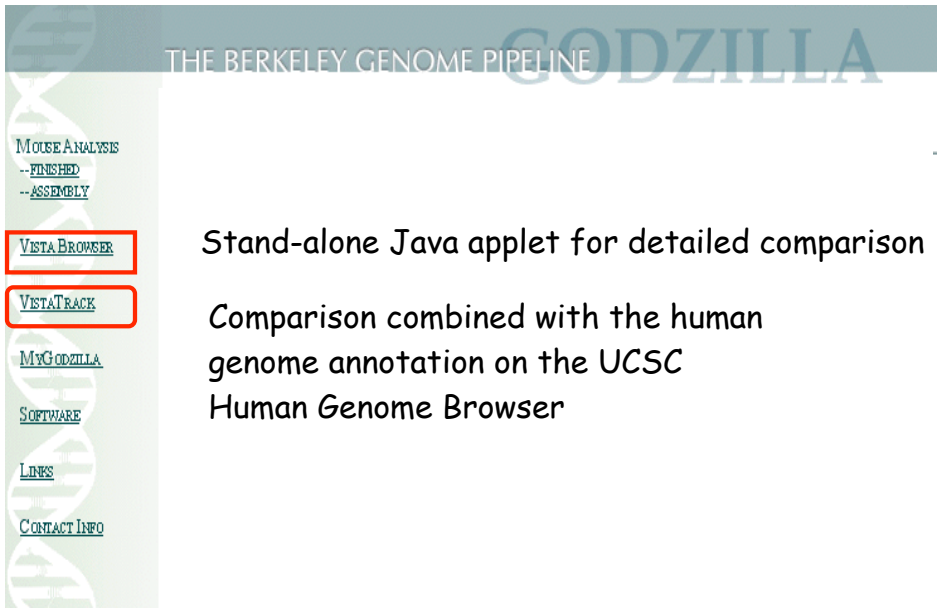
better sensitivity/specificity trade-off

fill-in effect
scoring longer alignments

NT_002606 at Chr.17:2909457-29116113



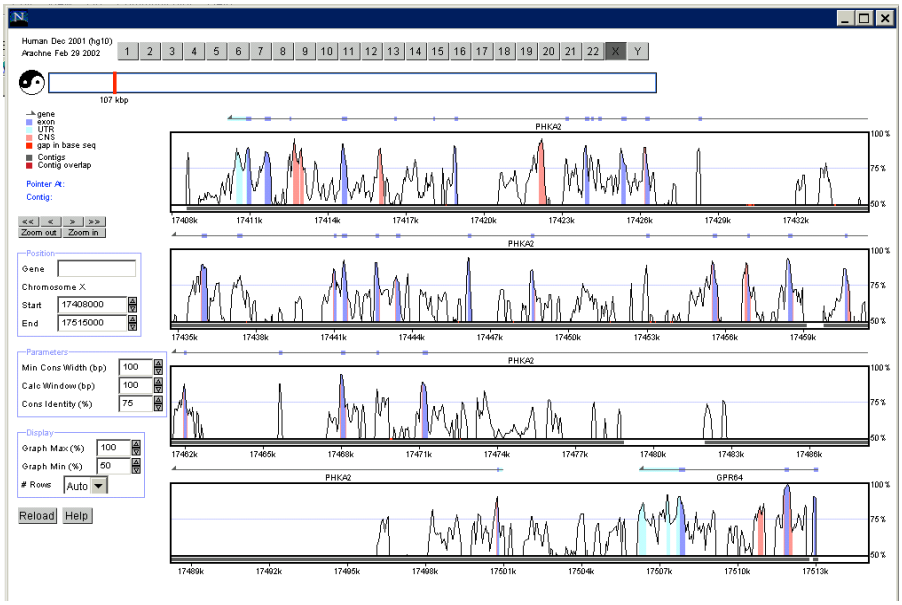
Visualization – VistaBrowser & VistaTrack



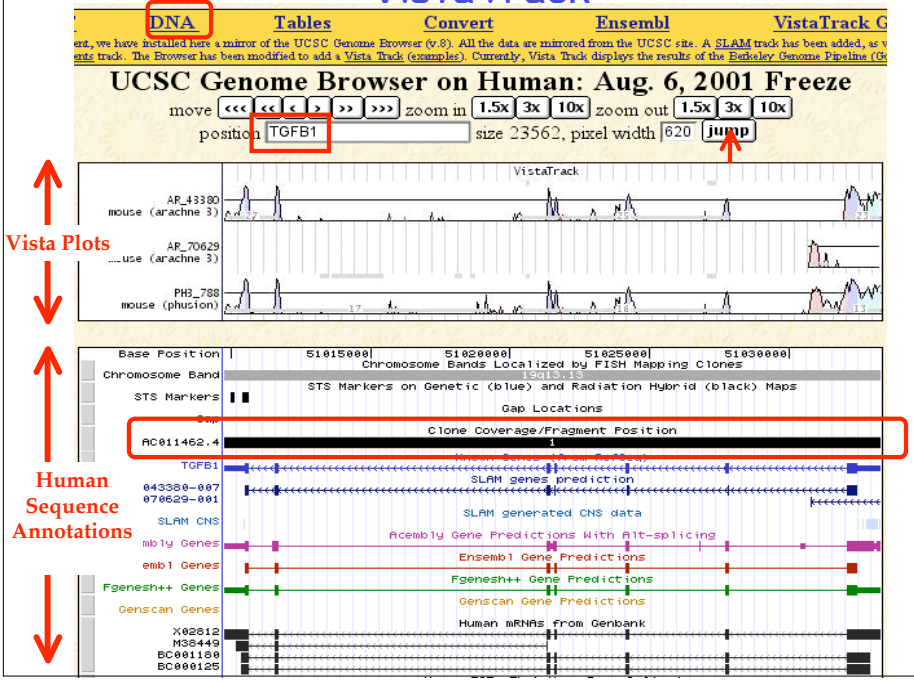
Stand-alone Java applet for detailed comparison

Comparison combined with the human genome annotation on the UCSC Human Genome Browser

VistaBrowser



VistaTrack



http://pipeline.lbl.gov/

MyGodzilla - is an interactive web tool for comparing your favorite sequence against the human genome

MyGodzilla Tool

Submit a DNA sequence of ANY organism...

... or submit a whole chromosome and analyze another Genome

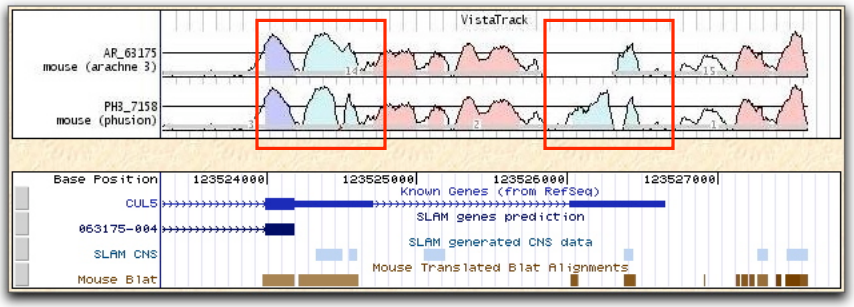
Your request is submitted and the results will be ready at this link: http://pipeline.lbl.gov/cache24h/22599550.8711

to receive an automatic notification type your email here: email me

Examples of Results

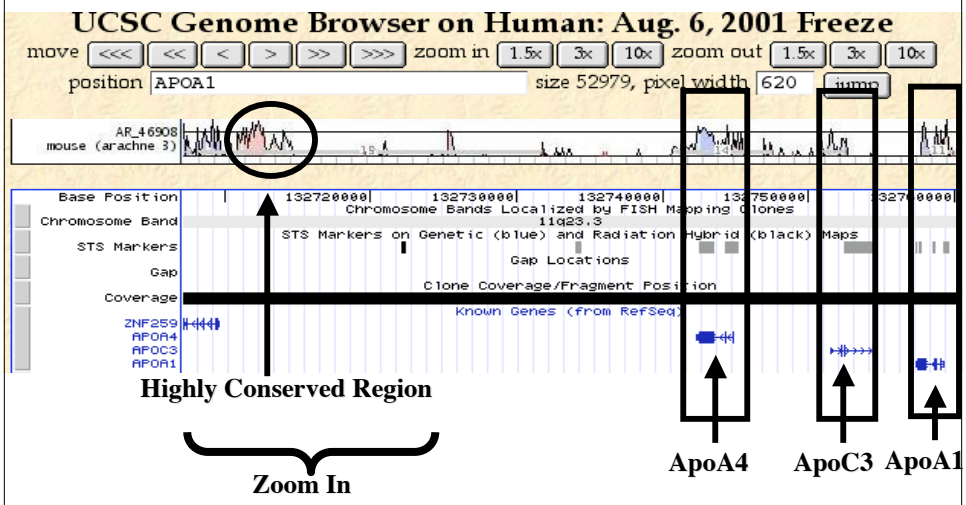
- Understanding the structure of conservation
- Identification of putative functional sites
- Discovery of new genes
- Detection of contamination and misassemblies

Two assemblies are better than one



Identification of a New Apo Gene on Human 11q23

Godzilla



Identification of a New Apo Gene on Human 11q23

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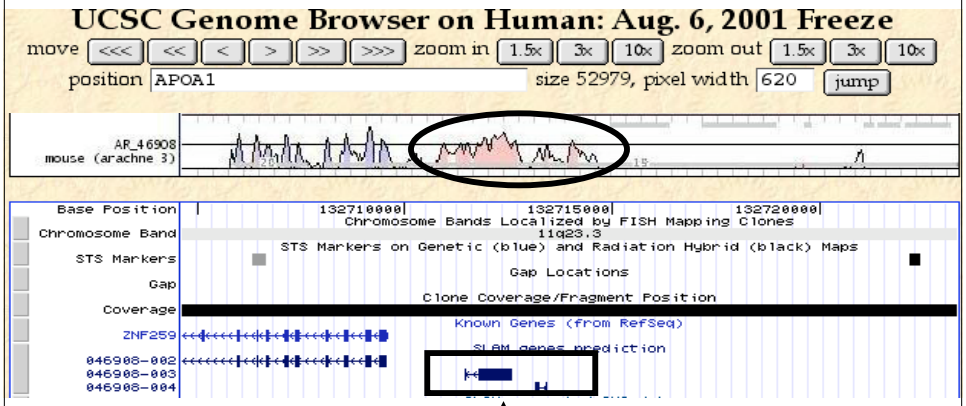
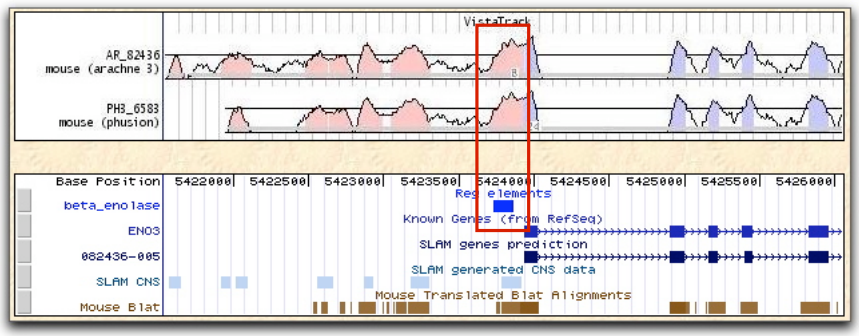


New Gene (ApoA5)
Pennacchio LA et al.
Science. 2001. 294:169-73.

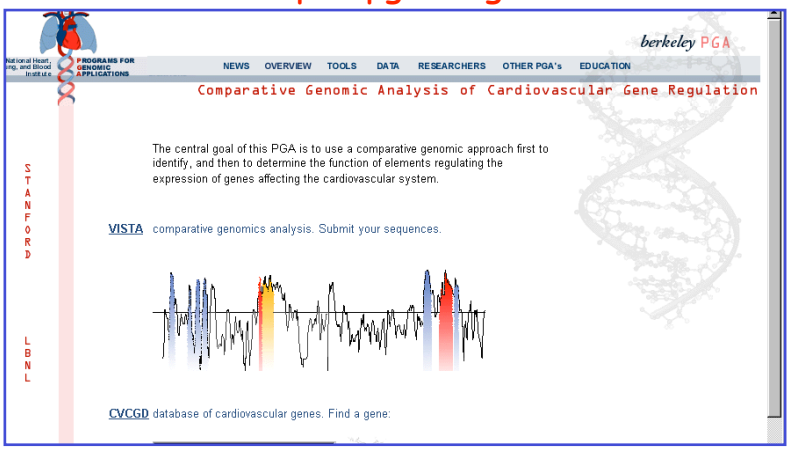
Finding regulatory regions

Muscle Specific Regulatory Region: human beta enolase intronic enhancer

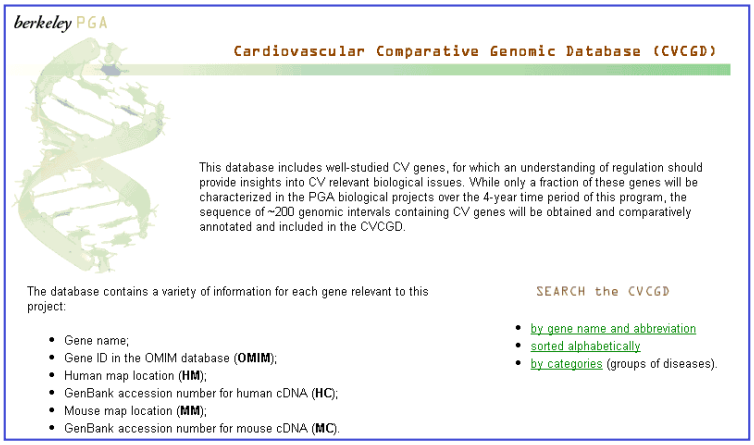


Comparative analysis of genomic intervals containing important cardiovascular genes

http://pga.lbl.gov



http://pga.lbl.gov/cvcgd.html



Example of CVCGD entry

Solute carrier family 22, organic cation transporter member 4 (SLC22A4, OCTN1) - Netscape

File Edit View Go Communicator Help

CVCGD search results | Reload | Home | Search | Netscape | Print | Security | Shop | Stop

Bookmarks | Location: http://pga.lbl.gov/cgi-bin/get_gene?id=234

Bioinformatics,

Solute carrier family 22, organic cation transporter member 4 (SLC22A4, OCTN1)

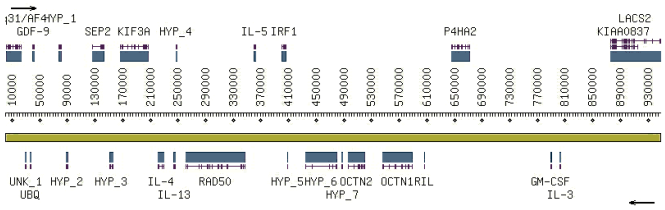
- Category: Atherosclerosis
- Gene ID in the OMIM database: 604190
- Human map location: 5q31
- GenBank accession number for human cDNA: NM_003059
- Mouse map location: 11
- GenBank accession number for mouse cDNA: NM_019687
- Annotation of the human sequence
- Human_mouse alignment: Whole sequence | 1-100000 | 100001-200000 | 200001-300000 | 300001-400000 | 400001-500000 | 500001-600000 | 600001-700000 | 700001-800000 | 800001-900000 | 900001-967696 *(see important note below)* | Printable version (PDF)
- List of conserved regions

Note: *If your browser hangs or crashes on the alignment page you can try this link instead.*

Short annotation of the region

Annotation of the VA5q31 region *

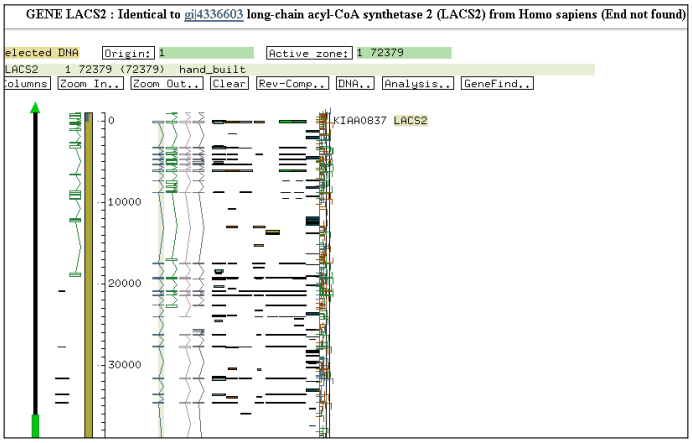
948869 bp



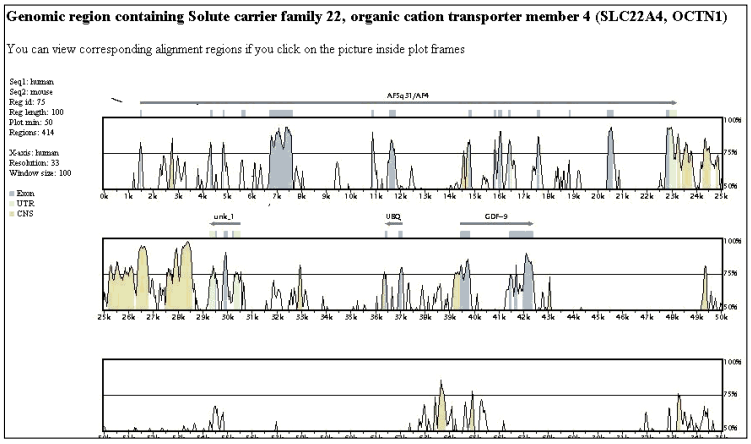
*Assembly contains a deletion of 18822 bp after the first exon of the RIL gene

Gene Name	Identity/Similarity
AF5q31/AF4	Identical to gi|6601437 Homo sapiens AF5q31 protein (AF5q31) (Start not found)
GDF-9	Identical to gi|488526 growth differentiation factor 9 from Homo sapiens
HYP_1	hypothetical
SEP2	Identical to gi|1503987 KIAA0202/Septin 2 gene from Homo sapiens

Detailed annotation in AceDB format



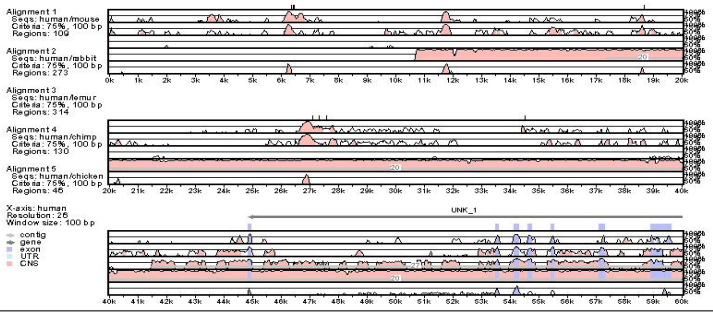
VISTA plot of the region



multiVISTA plot of the region

Genomic region containing Apolipoprotein A-IV (APOA4)

This plot is not clickable. In order to view alignment regions please go back to the gene page and click o
alignment you are interested in.



Alignment

Genomic region containing Solute carrier family 22, organic cation transporter membe

```
seq1 = human
seq2 = mouse

                6990      7000      7010      7020      7030      7040
seq1      CAGAGTGACAGCACAACACAGAGAAGAACTGTAGGCAAAAAACAACCCAAAAAGGCTGAG
          ||||| ||||| || || ||||| |||||||| |||||||||||||||||||  |||||
seq2      CAGAGCGACAGTACCACTCAGAGGAGAACTGTCGGCAAAAAACAACCCAAAAAACCTGAG
             8130      8140      8150      8160      8170      8180

                7050      7060      7070      7080      7090      7100
seq1      AAGGCAGCTGCTGAAGAGCCTCGTGGAGGCCTGAAGATAGAAAGTGAAACCCCTGTAGAC
          ||| ||||||||||||||||||||||||||||||||||||||||||| |||||||| |||
seq2      AAGTCAGCTGCTGAAGAGCCTCGTGGAGGCCTGAAGATAGAAAGTGAGACCCCTGTGGAC
             8190      8200      8210      8220      8230      8240

                7110      7120      7130      7140      7150      7160
seq1      TTGGCTAGCAGCATGCCCTCCAGCAGACACAAAGCAGCCACCAAAGGCTCAAGGAAACCC
           |||||  |||||||||||||||||| |||||||||||||||| ||||| |||||||||
seq2      ATGGCTGCCAGCATGCCCTCCAGCAGGCACAAAGCAGCCACCAAGGGCTCGAGGAAACCC
             8250      8260      8270      8280      8290      8300
```

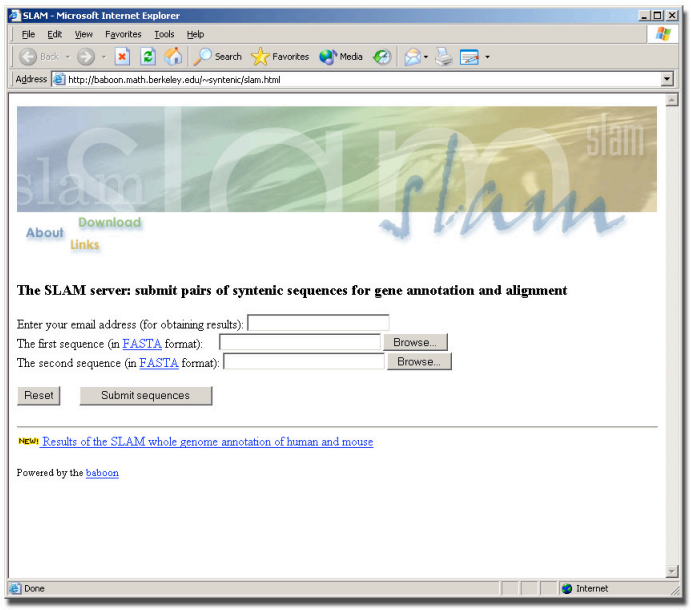
Conserved regions

Genomic region containing Solute carrier family 22, organic cation transporter member 4 (SLC22A4, OCTN1)

```
Criteria: 75% identity over 100 bp

*************** Conserved Regions - human (mouse) ***************

    1469      (580)  to     1515      (626)  =       47bp  at  85.1%  exon
    2668     (2043)  to     2817     (2191)  =      153bp  at  80.4%  noncoding
    4316     (4531)  to     4370     (4585)  =       55bp  at 100.0%  exon
    4816     (6136)  to     4853     (6173)  =       38bp  at  97.4%  exon
    6717     (7860)  to     7634     (8777)  =      918bp  at  87.8%  exon
   10839    (10749)  to    10927    (10837)  =       89bp  at  91.0%  exon
   11553    (12627)  to    11793    (12873)  =      247bp  at  81.8%  exon
   14508    (15706)  to    14622    (15823)  =      119bp  at  76.5%  noncoding
   14671    (15886)  to    14783    (16003)  =      118bp  at  74.6%  noncoding
   14784    (16004)  to    14878    (16098)  =       95bp  at  89.5%  exon
   15797    (17526)  to    15860    (17589)  =       64bp  at  93.8%  exon
   15975    (17703)  to    16111    (17839)  =      137bp  at  90.5%  exon
   16365    (18045)  to    16436    (18116)  =       72bp  at  91.7%  exon
   16437    (18117)  to    16535    (18217)  =      101bp  at  75.2%  noncoding
   17554    (18914)  to    17647    (19007)  =       94bp  at  87.2%  exon
```



http://bio.math.berkeley.edu/slam/

SLAM components

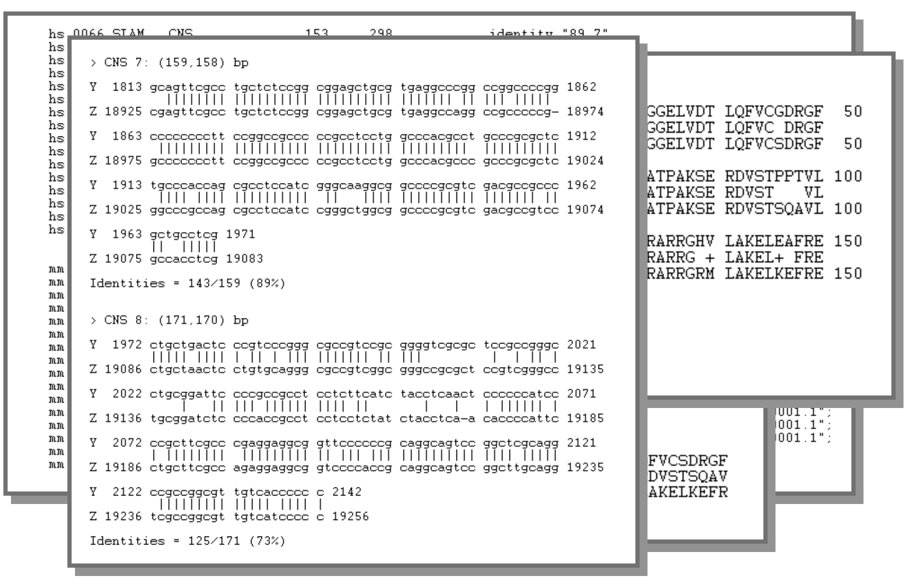
- Splice site detector
  - VLMM
- Intron and intergenic regions
  - 2nd order Markov chain
  - independent geometric lengths
- Coding sequence
  - PHMM on protein level
  - generalized length distribution
- Conserved non-coding sequence
  - PHMM on DNA level

SLAM input and output

- Input:
  - Pair of syntenic sequences (FASTA).
- Output:
  - CDS and CNS predictions in both sequences.
  - Protein predictions.
  - Protein and CNS alignment.

Input:

Output:



Publications on our tools:

- I. Dubchak, M. Brudno, L.S. Pachter, G.G. Loots, C. Mayor, E. M. Rubin, K. A. Frazer. (2000) Active conservation of noncoding sequences revealed by 3-way species comparisons. *Genome Research*, 10: 1304-1306.
- C. Mayor, M. Brudno, J. R. Schwartz, A. Poliakov, E. M. Rubin, K. A. Frazer, Lior S. Pachter, I. Dubchak. (2000) VISTA: Visualizing global DNA sequence alignments of arbitrary length. *Bioinformatics*, 16: 1046-1047.
- G. G. Loots, I. Ovcharenko, L. Pachter, I. Dubchak and E. M. Rubin. (2002) Comparative sequence-based approach to high-throughput discovery of functional regulatory elements. *Genome Res.*, 12:832-839
- I. Dubchak, L. Pachter. (2002) The computational challenges of applying comparative-based computational methods to whole genomes. *Briefings in Bioinformatics*, 3, 18.

Related sites

- The Human Genome Browser & BLAT program http://genome.ucsc.edu/
- ENSEMBLE Project (Sanger Center) http://www.ensembl.org/
- AVID alignment program

  http://baboon.math.berkeley.edu/~syntenic/avid.html
- SLAM comparative gene prediction program http://bio.math.berkeley.edu/slam/mouse/
- PSU group's MHC Human-Mouse comparison results http://bio.cse.psu.edu/mousegroup/MHC/
- PSU Pipmaker program http://bio.cse.psu.edu/pipmaker/

Summary

Suite of comparative genomics tools VISTA
http://www-gsd.lbl.gov

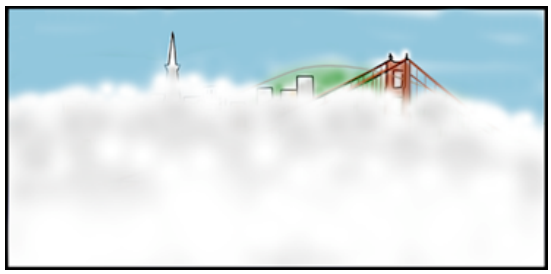
Godzilla comparing the human and mouse genome
http://pipeline.lbl.gov

Cardiovascular comparative genomics database
http://pga.lbl.gov

Questions? Write to vista@lbl.gov

Towards Better VISTAs

Information from a Single Sequence Alone



Multi-Organism High Quality Sequences

Thanks

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	Chris Mayor
	Ivan Ovcharenko
	Alexander Poliakov
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