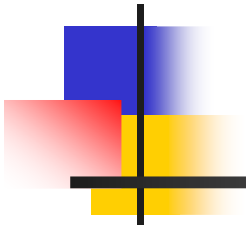
Fields Institute, Feb. 26, 2007



Modern Homology Search

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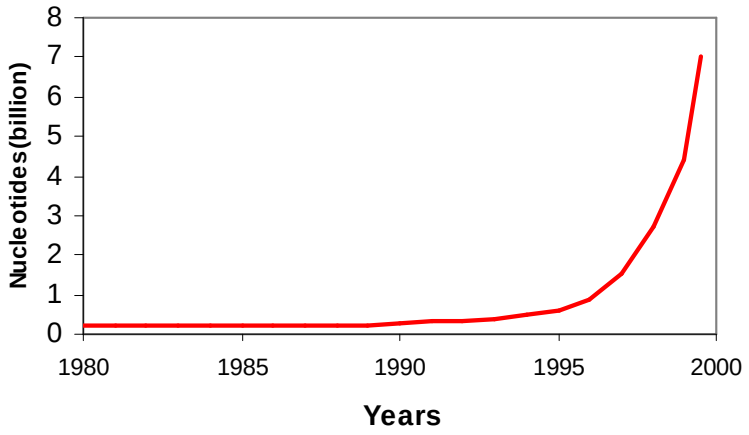
Joint work with Bin Ma, John Tromp
Joint work: X.F. Cui, T. Vinar, D. Shasha

I will present three simple ideas

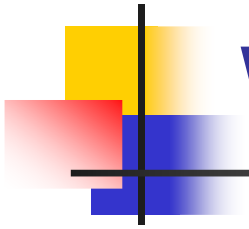
A gigantic gold mine

- The trend of genetic data growth



30 billion
in year 2005

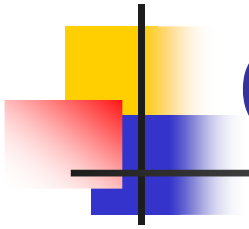
- 400 Eukaryote genome projects underway
- GenBank doubles every 18 months
- Comparative genomics 乇 all-against-all search



What is homology search

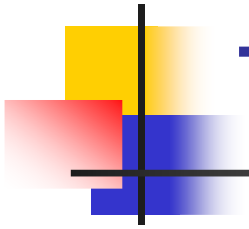
- Given two DNA sequences, find all local similar regions, using "edit distance" (match=1, mismatch=-1, gapopen=-5, gapext=-1).
- Example. Input:
  - E. coli genome: 5 million base pairs
  - H. influenza genome: 1.8 million base pairs

  Output: all local alignments.



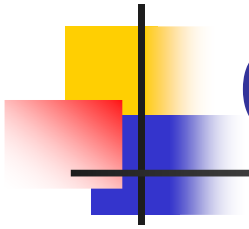
Comparing to internet search

- Internet search
  - Size limit: 5 billion people x homepage size
  - Supercomputing power used: ½ million CPU-hours/day
  - Query frequency: Google --- 112 million/day
  - Query type: exact keyword search --- easy to do
- Homology search
  - Size limit: 5 billion people x 3 billion basepairs + millions of species x billion bases
  - 10% (?) of world's supercomputing power
  - Query frequency: NCBI BLAST -- 150,000/day, 15% increase/month
  - Query type: approximate search --- topics today



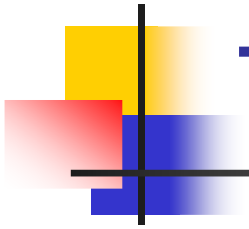
Tremendous Cost

- Bioinformatics Companies living on BLAST:
  - Paracel (Celera)
  - TimeLogic
  - TurboGenomics (TurboWorx)
- NSF, NIH, pharmaceuticals *proudly* support many supercomputing centers for homology search
- However: hardware become obsolete in 2-3 years. Software solution is indispensable.



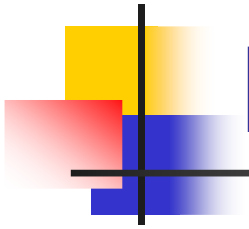
Old Homology Search

- Too slow (dynamic programming)
- Too lossy (BLAST) and in fact still too slow
- No specific. Hundreds of unrelated answers.



Time Flies

- Dynamic programming (1970-1980)
  - Human vs mouse genomes: 10^4 CPU-years
- BLAST, FASTA heuristics (1980-1990)
  - Human vs mouse genomes: 19 CPU-years
  - BLAST paper was referenced 100000 times
- PatternHunter
  - Human vs mouse genomes: 20 CPU-days
- PatternHunter II: dynamic programming sensitivity, BLAST speed.



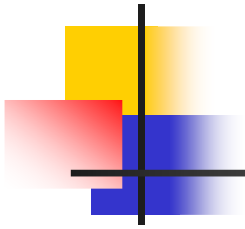
Model Homology Search

- ~100% sensitivity, approaching to dynamic programming.
- ~100% specificity: return only the correct match, not hundreds of junk alignments
- Still at higher (than BLAST) speed.

BLAST Algorithm & Example

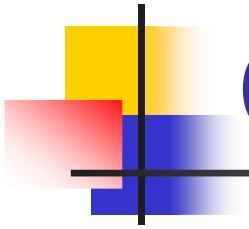
- Find seeded matches of 11 base pairs
- Extend each match to right and left, until the scores drop too much, to form an alignment
- Report all local alignments

Example:



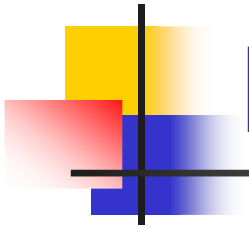
BLAST Dilemma:

- If you want to speed up, have to use a longer seed. However, we now face a dilemma:
  - increasing seed size speeds up, but loses sensitivity;
  - decreasing seed size gains sensitivity, but loses speed.
- How do we increase sensitivity & speed simultaneously? For 20 years, many tried: suffix tree, better programming ..



Outline

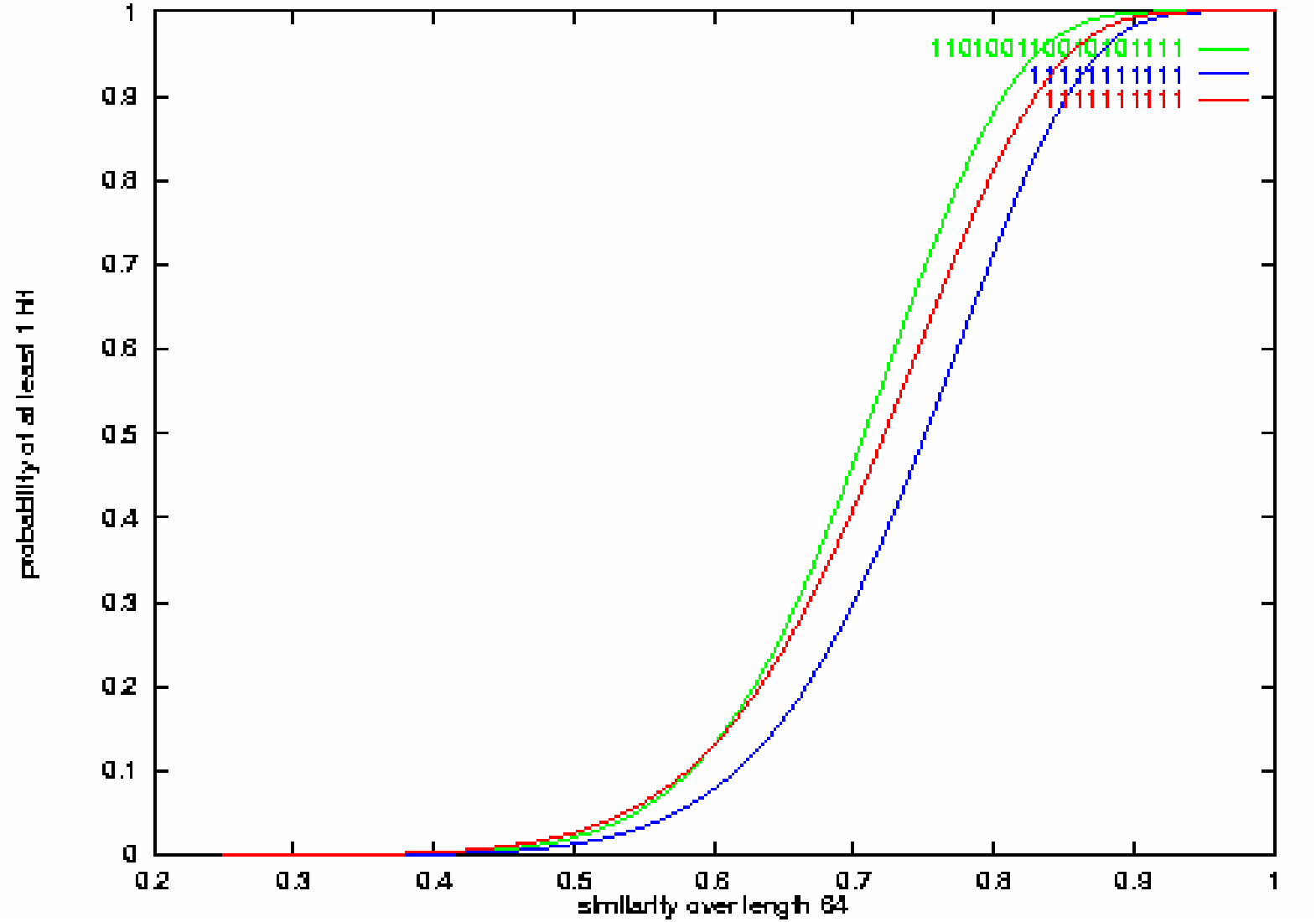
- **A simple (but profound) idea**
- A simpler (but working) idea
- Another simple idea

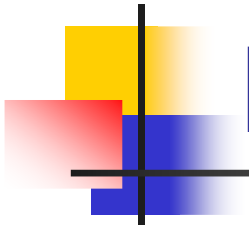


New Idea: Spaced Seed

- Spaced Seed: nonconsecutive matches and optimize match positions.
- Represent BLAST seed by 11111111111
- Spaced seed: 111*1**1*1**11*111
  - 1 means a required match
  - * means “don’t care” position
- This seemingly simple change makes a huge difference: significantly increases hit to homologous region while reducing bad hits.

Sensitivity: PH weight 11 seed vs BLAST 11 & 10





Formalize

- Given i.i.d. sequence (homology region) with Pr(1)=p and Pr(0)=1-p for each bit:

1100111011101101011101101011111011101
111*1**1*1**11*111

- Which seed is more likely to hit this region:
  - BLAST seed: 11111111111
  - Spaced seed: 111*1**1*1**11*111

Expect Less, Get More

- Lemma: The expected number of hits of a weight W length M seed model within a length L region with homology level p is

$$(L-M+1)p^W$$

Proof. $E(\#hits) = \sum_{i=1 \ldots L-M+1} p^W$ ■

- Example: In a region of length 64 with p=0.7
  - Pr(BLAST seed hits)=0.3
    E(# of hits by BLAST seed)=1.07
  - Pr(optimal spaced seed hits)=0.466, 50% more
    E(# of hits by spaced seed)=0.93, 14% less

Why Is Spaced Seed Better?

A wrong, but intuitive, proof: seed s, interval I, similarity p

E(#hits) = Pr(s hits) E(#hits | s hits)

Thus:

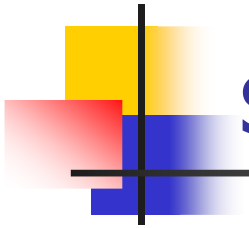
Pr(s hits) = Lp^w / E(#hits | s hits)

For optimized spaced seed, E(#hits | s hits)

111*1**1*1**11*111	Non overlap	Prob
111*1**1*1**11*111	6	p^6
111*1**1*1**11*111	6	p^6
111*1**1*1**11*111	6	p^6
111*1**1*1**11*111	7	p^7

.....

- For spaced seed: the divisor is $1+p^6+p^6+p^6+p^7+$ ...
- For BLAST seed: the divisor is bigger: $1+ p + p^2 + p^3 +$ ...



Complexity of finding the optimal spaced seed (Li, Ma, Zhang, SODA)

Theorem 1. Given a seed and it is NP-hard to find its sensitivity, even in a uniform region.

Theorem 2. The sensitivity of a given seed can be efficiently approximated with arbitrary accuracy, with high probability.

Computing Spaced Seeds

(Keich, Li, Ma, Tromp, *Discrete Appl. Math*)

Let $f(i,b)$ be the probability that seed s hits the length i prefix of R that ends with b.

Thus, if s matches b, then

$$f(i,b) = 1,$$

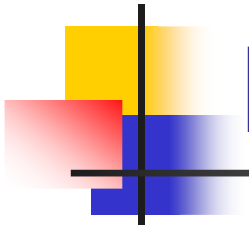
otherwise we have the recursive relationship:

$$f(i,b) = (1-p)f(i-1,0b') + pf(i-1,1b')$$

where b' is b deleting the last bit.

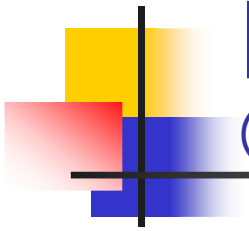
Then the probability of s hitting R is

$$\Sigma_{|b|=M} Prob(b)\, f(L-M,b)$$



Related Literature

- Random or multiple spaced q-grams were used in the following work:
  - FLASH by Califano & Rigoutsos
  - Multiple filtration by Pevzner & Waterman
  - LSH of Buhler
  - Praparata et al on probe design
- Optimizing & further work
  - Buhler-Keich-Sun
  - Brejova-Bronw-Vinar
  - Choi-Zhang
  - Tsur, Farach-Colton, Landau , Sahinalp
  - Over 100 research papers.



PatternHunter

(Ma, Tromp, Li: *Bioinformatics*, 18:3, 2002, 440-445)

- PH used optimal spaced seeds, novel usage of data structures: red-black tree, queues, stacks, hashtables, new gapped alignment algorithm.
- Written in Java.
- Used in Mouse Genome Consortium (*Nature*, Dec. 5, 2002), as well as in hundreds of institutions and industry.

Comparison with BLAST

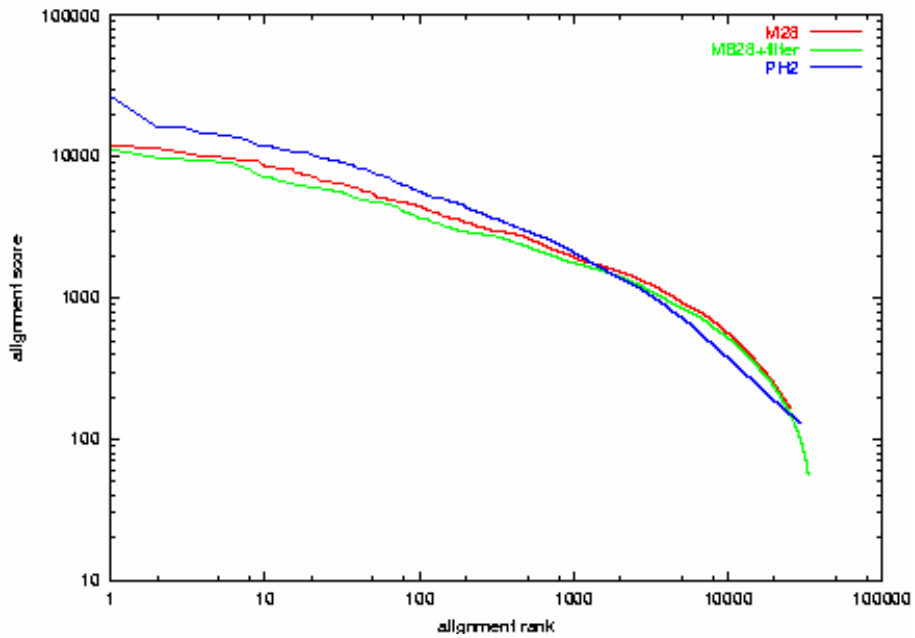
- On Pentium III 700MH, 1GB

	BLAST	PatternHunter
E.coli vs H.inf	*716s*	*14s/68M*
Arabidopsis 2 vs 4	*--*	*498s/280M*
Human 21 vs 22	*--*	*5250s/417M*
Human(3G) vs Mouse(x3=9G)*	*19 years*	*20 days*

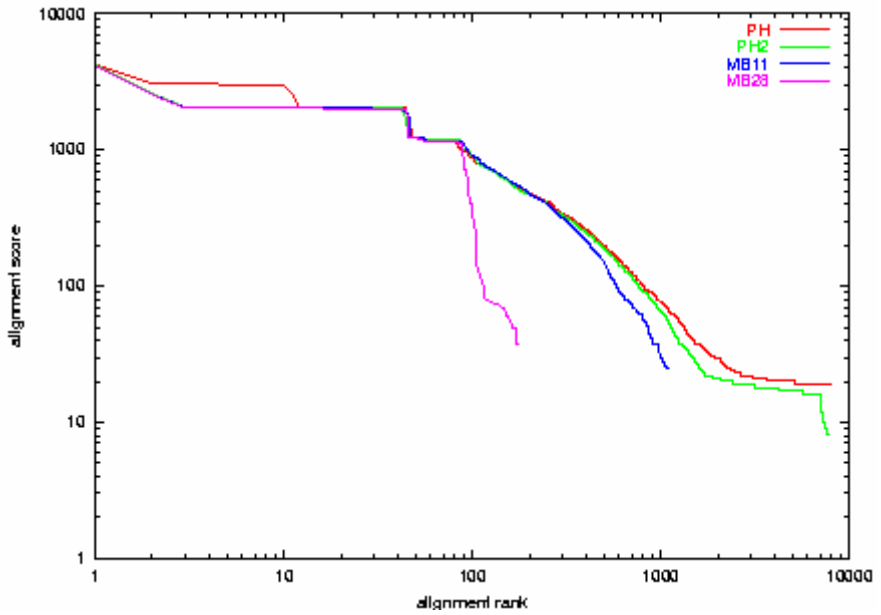
- All with filter off and identical parameters
- 16M reads of Mouse genome against Human genome for MIT Whitehead. Best BLAST program takes 19 years at the same sensitivity

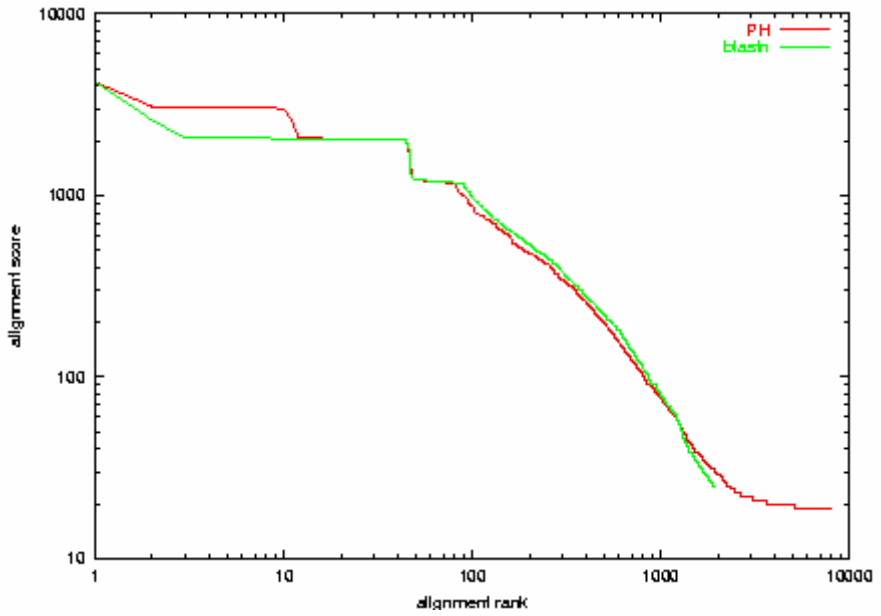
Quality Comparison:

x-axis: alignment rank
y-axis: alignment score
both axes in logarithmic scale

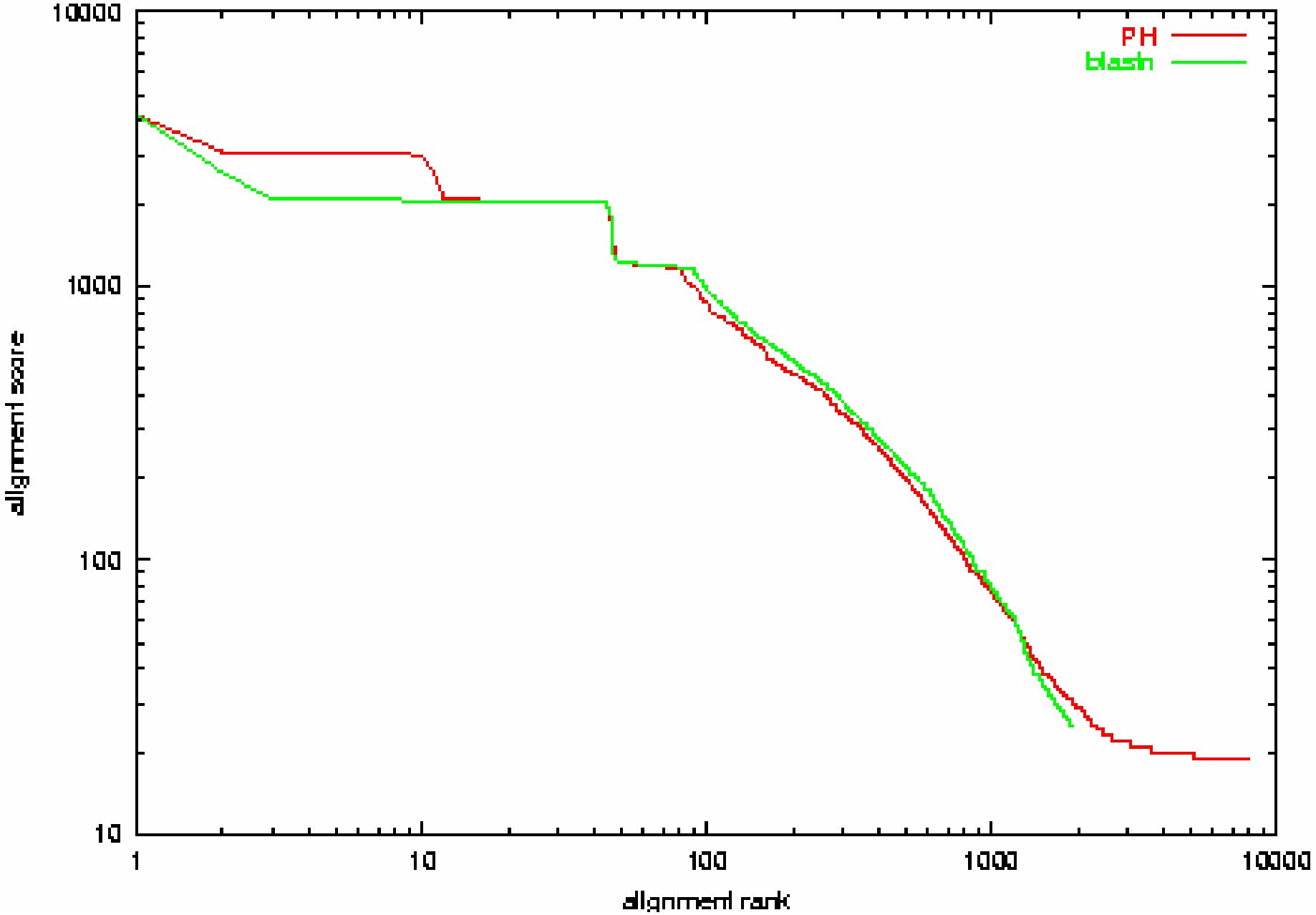


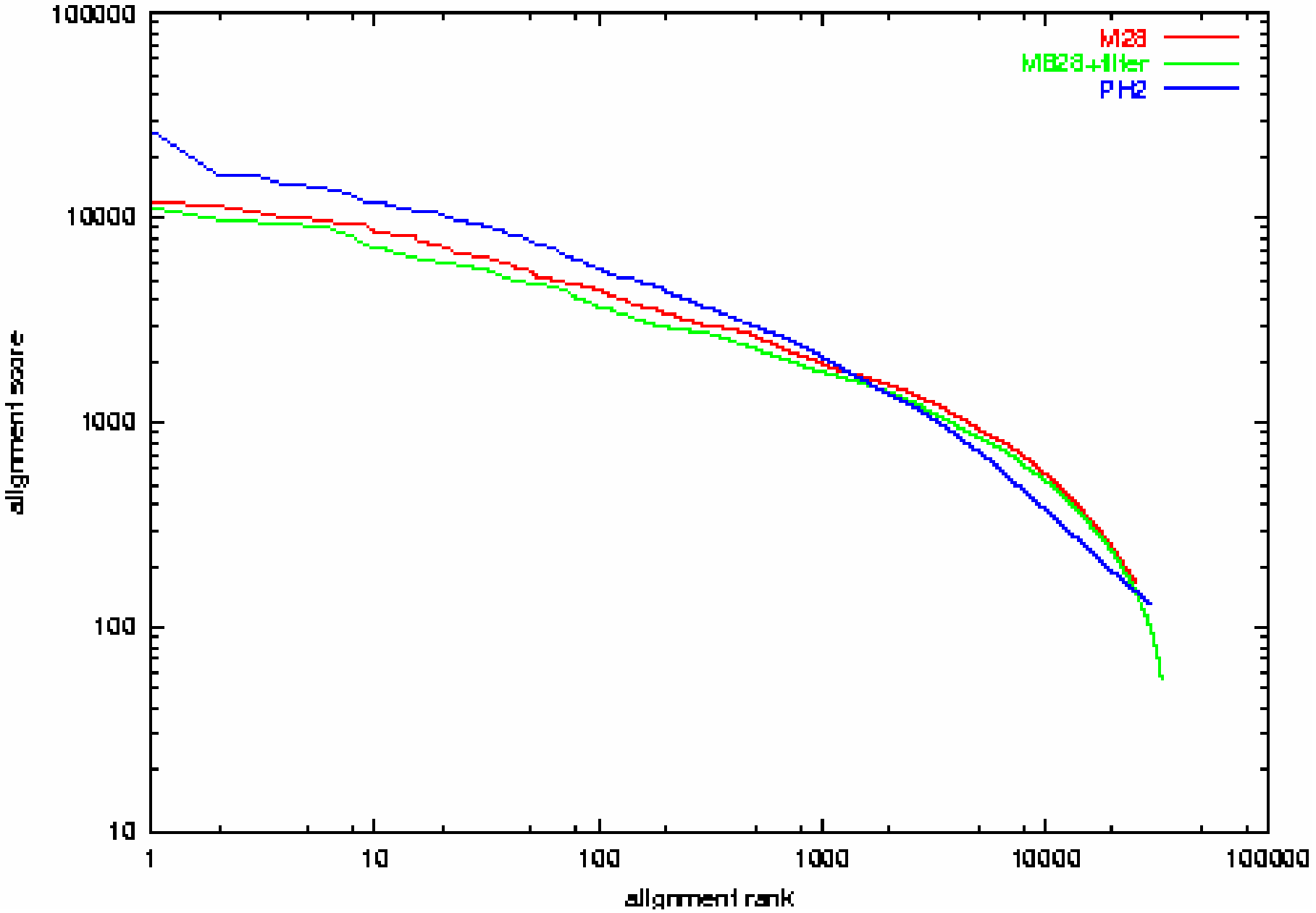
A. thaliana chr 2 vs 4



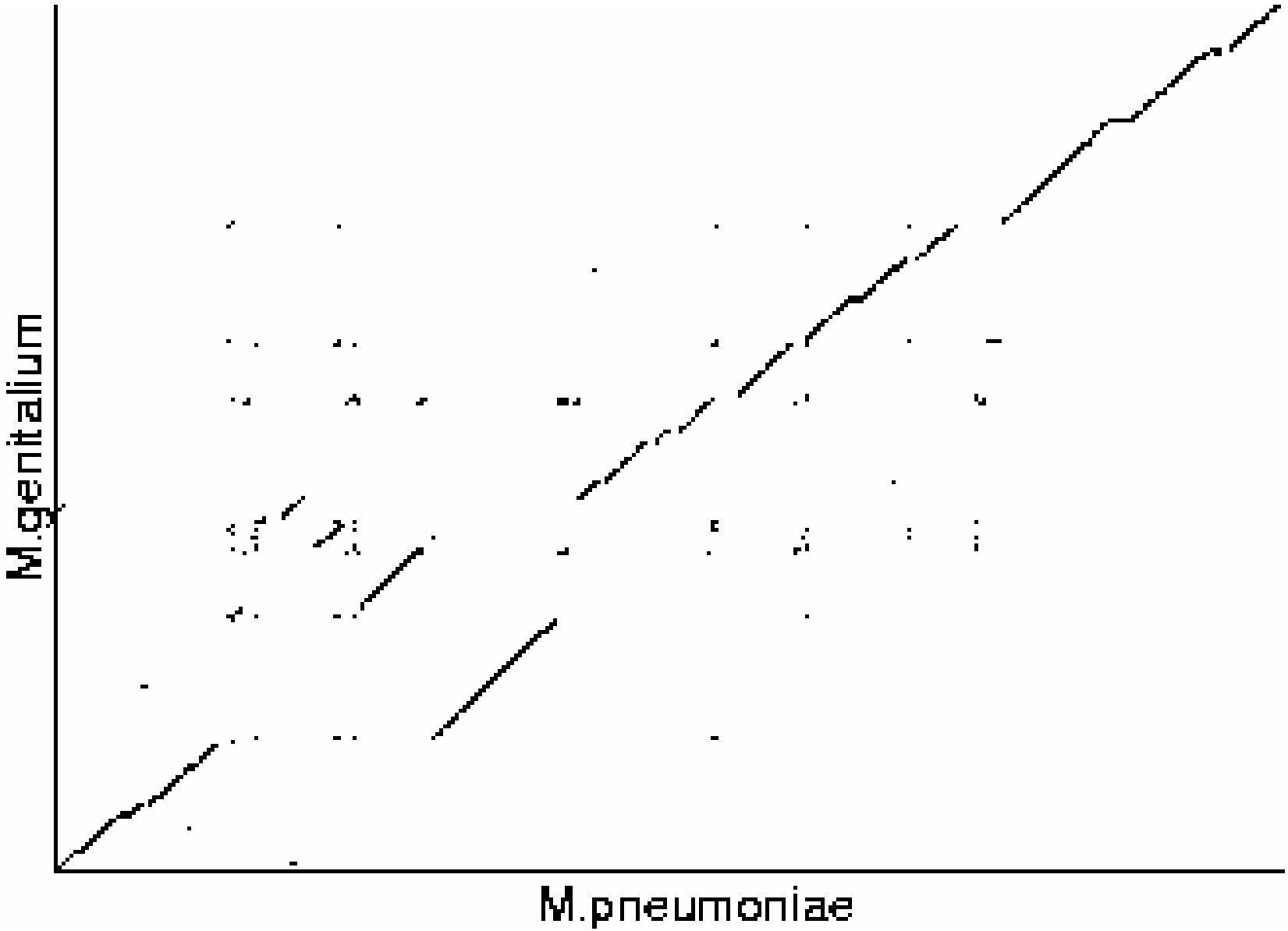


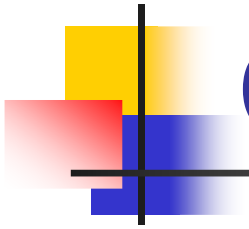
E. Coli vs *H. influenza*


PH
blastn
alignment score
alignment rank
10000
1000
100
10
1
10
100
1000
10000


M2B
M2B+filter
PH2
100000
10000
1000
100
10
1
10
100
1000
10000
100000
alignment score
alignment rank

Genome Alignment by PatternHunter (4 seconds)





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PattternHunter II:

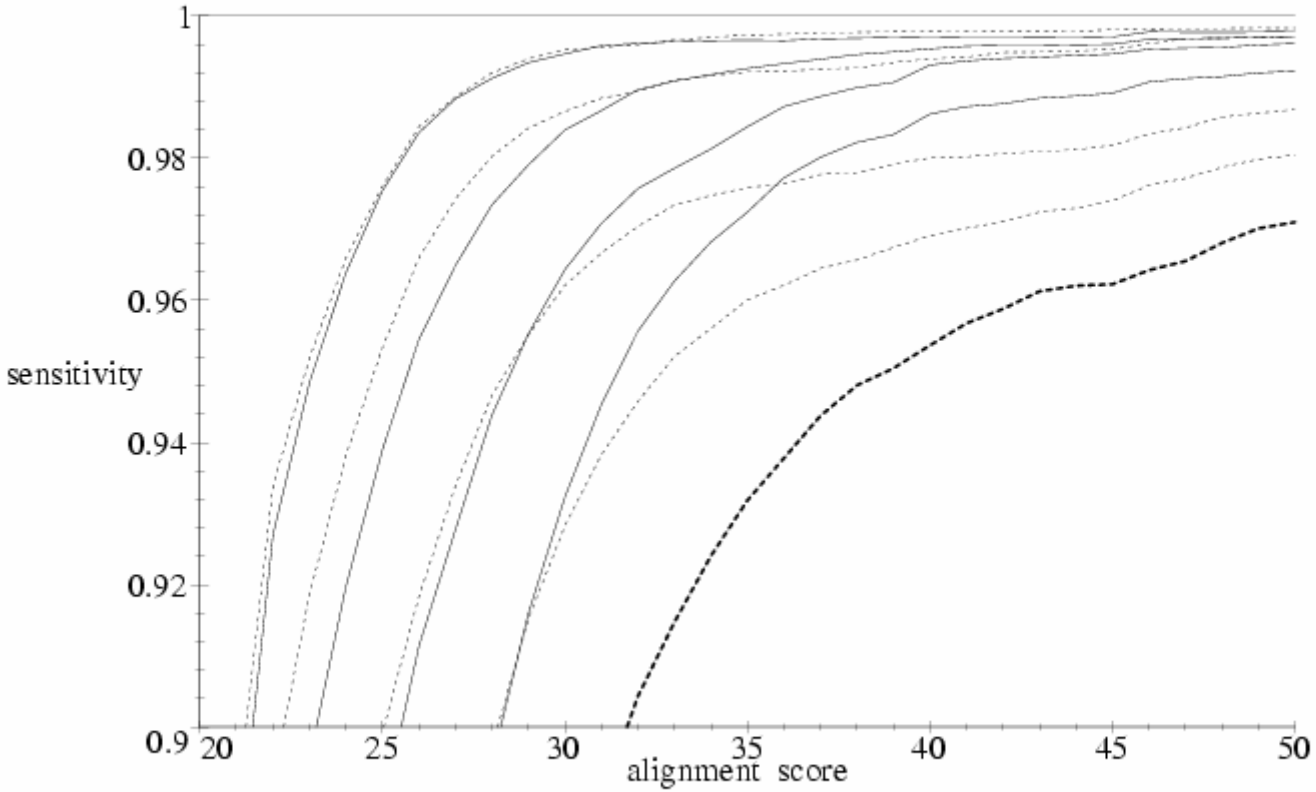
-- Smith-Waterman Sensitivity, BLAST Speed

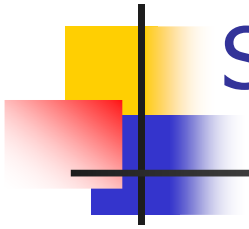
(Li, Ma, Kisman, Tromp, *J. Bioinfo Comput. Biol*. 2004)

- The biggest problem for BLAST was low sensitivity (and low speed). Massive parallel machines are built to do S-W exhaustive dynamic programming.
- Spaced seeds give PH a *unique* opportunity of using several optimal seeds to achieve optimal sensitivity, this was not possible by BLAST technology.
- We have designed PH II, with multiple optimal seeds.
- PH II approaches Smith-Waterman sensitivity, and 3000 times faster.
- Experiment: 29715 mouse EST, 4407 human EST.

Sensitivity Comparison with Smith-Waterman (at 100%)

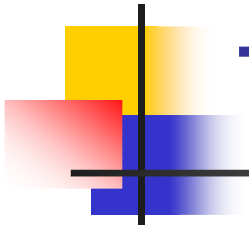
The thick dashed curve is the sensitivity of BLAST, seed weight 11.
From low to high, the solid curves are the sensitivity of PH II using
1, 2, 4, 8 weight 11 coding region seeds, and the thin dashed curves
are the sensitivity 1, 2, 4, 8 weight 11 general purpose seeds, respectively





Speed Comparison with Smith-Waterman

- Smith-Waterman (SSearch): 20 CPU-days.
- PatternHunter II with 4 seeds: 475 CPU-seconds. 3638 times faster than Smith-Waterman dynamic programming at the same sensitivity.



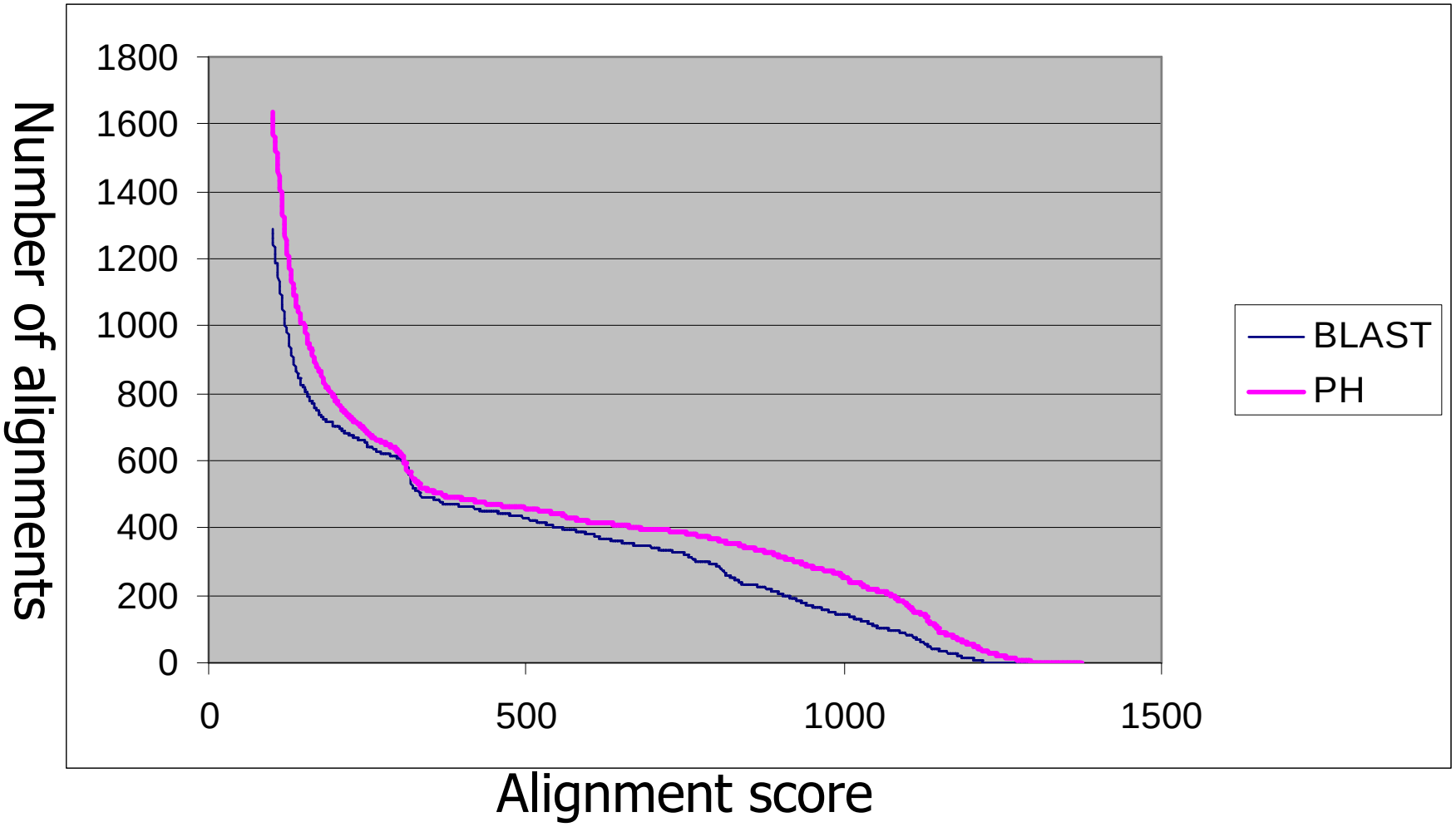
Translated PatternHunter

- Has all the functionalities of
  - Blastp
  - tBlastx – with gapped alignments
  - tBlastn, Blastx – with gapped alignments
- More sensitive and faster – new algorithm replacing 6-frame translation

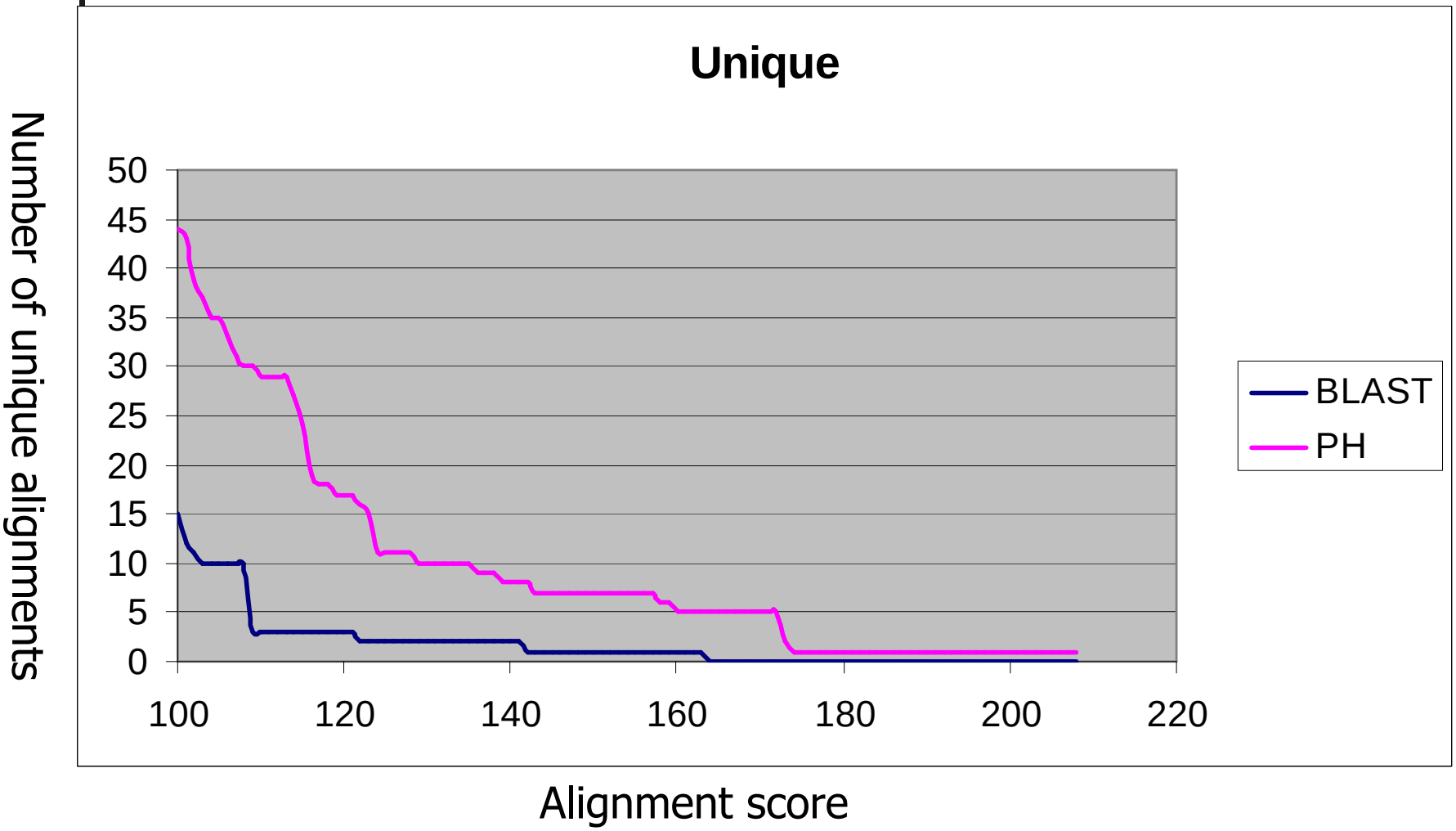
Alignment comparison: tBLASTx vs tPH

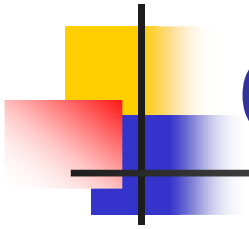
tPH: 253 seconds

tBLASTx: 807 seconds



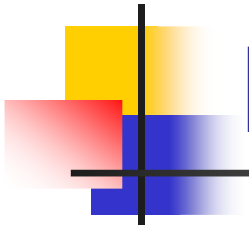
Unique Alignments: tBLASTx vs tPH





Old field, new trend

- Research trend
  - Over 30 papers on spaced seeds have appeared since our original paper, in 2 years.
  - Many more have used PH in their work.
  - Most modern alignment programs (including BLAST) have now adopted spaced seeds
  - Spaced seeds are serving thousands of users/day
- PatternHunter direct users
  - Pharmaceutical/biotech firms.
  - Mouse/Rat Genome Consortiums, *Nature, Dec. 5, 2002.*
  - Hundreds of academic institutions.

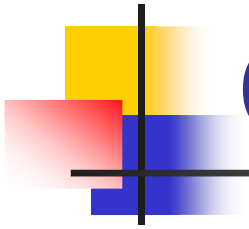


Running PH

Available at: www.BioinformaticsSolutions.com

Java –Xmx512m –jar ph.jar –i query.fna –j subject.fna –o out.txt

-Xmx512m --- for large files
-j missing: query.fna self-comparison
-db: multiple sequence input, 0,1,2,3 (no, query, subject, both)
-W: seed weight
-G: open gap penalty (default 5)
-E: gap extension (default 1)
-q: mismatch penalty (default 1)
-r: reward for match (default 1)
-model: specify model in binary
-H: hits before extension
-P: show progress
-multi 4: use 4 seeds



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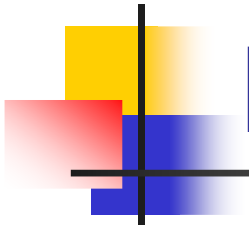
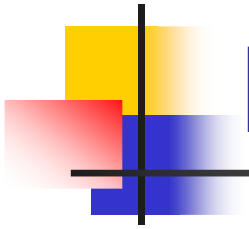


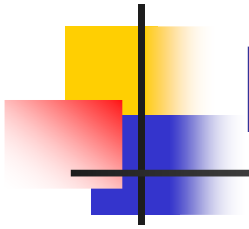
Meaningful Match?

- Given a gene sequence, BLAST or PH simply returns a bunch of meaningless alignments.
- Can we return a complete gene match?
- Idea: Combine PH with ExonHunter (Brejova, Brown, Li, Vinar, ISMB'2005): take the ab initio gene-finder (HMM) trained for the database genome, further train/bias it with the query gene model (its splice sites etc). Use PH to find possible hot regions and use this HMM to do extension, deciding on introns/exons.



Example:

- Given a human gene [GI:35560], want a homologous gene in mouse genome [GI:293767]

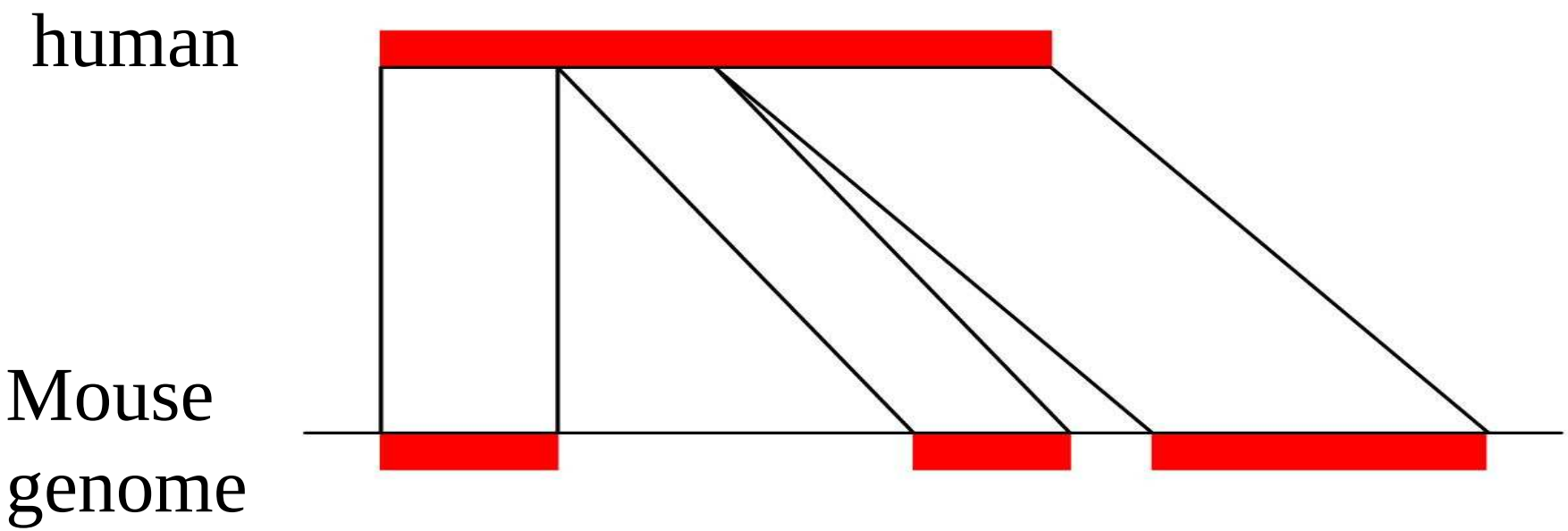


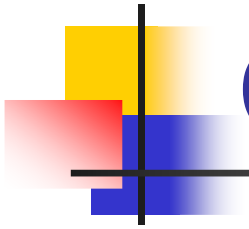
BLAST Result

- 249 alignments are returned
- Only 3 alignments are relevant
- Exons / Splice sites are not detected

New gPH results

- Fully correct homologous gene-match is returned. Just one alignment!





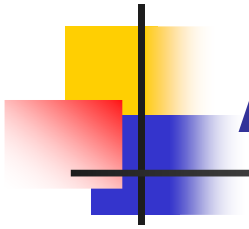
Conclusion

Best ideas are simple ones.

The most difficult theoretical studies are those that actually work.

Open questions:

- Polynomial time probabilistic algorithm for finding (near) optimal seed, multiple seeds.
- gPH for distant species, via 3D modeling?



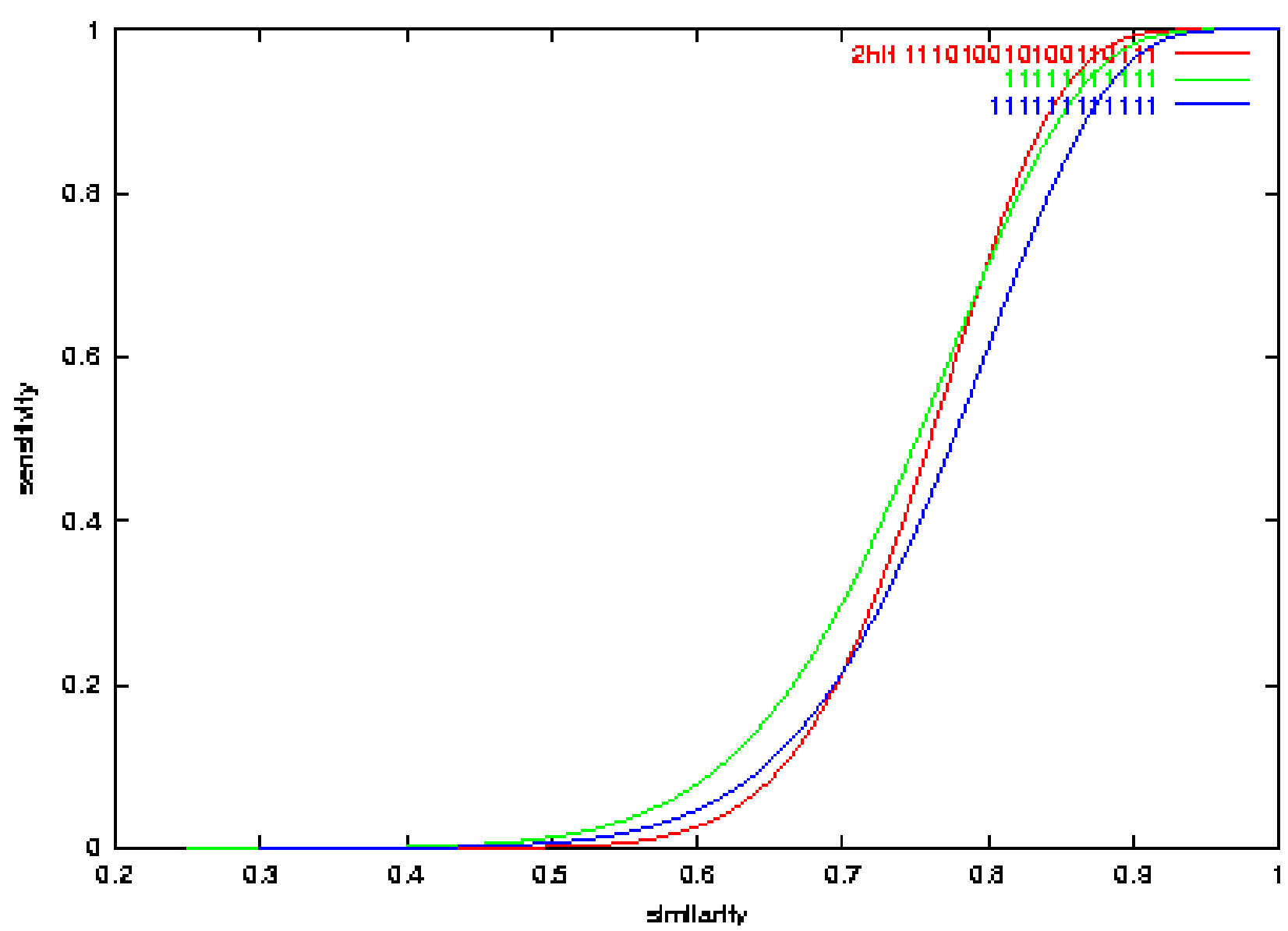
Acknowledgement

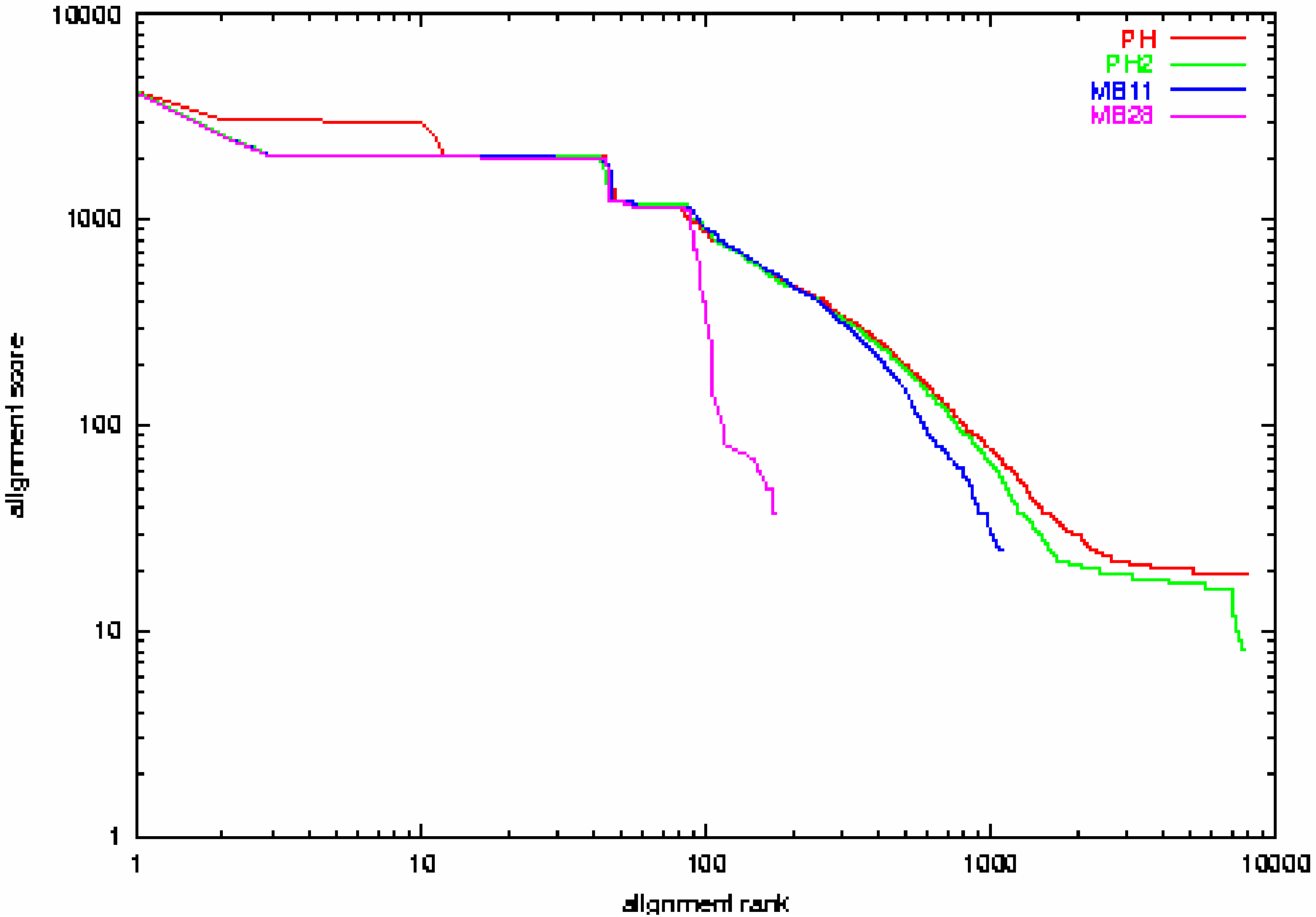
- PH is joint work with Bin Ma and John Tromp
- PH II is joint work with Ma, Kisman, and Tromp
- Some joint theoretical work with Ma, Keich, Tromp, Xu, and Brown.
- gPH is joint work with X.F. Cui, D. Shasha, T. Vinar.
- Financial support: Bioinformatics Solutions Inc, NSERC, Killam Fellowship, Steacie Fellowship, CRC chair program.

Conclusion – continued

- Another simple idea applied to data mining: from irreversibly computing 1 bit requires 1kT energy (von Neumann, Landauer), we derived shared information d(x,y) between x,y, to classify
  - Species & genomes, Li *et al*, in *Bioinformatics*, 2001
  - Chain letters, Bennett, Li, Ma, *Scientific American*, 2003
  - Languages, Benedeto,Caglioti,Loreto, *Phy. Rev. Let.*'02
  - Music, Cilibrasi, Vitanyi, de Wolf, *New Scientist*, 2003
  - Time series/anomaly detection, Keogh, Lonardi, Ratanamahatana, KDD'04. They compared d(x,y) with 51 methods/measures from SIGKDD, SIGMOD, ICDM, ICDE, SSDB, VLDB, PKDD, PAKDD and concluded our method the simplest & best --- Keogh tutorial ICDM'04.

PH 2-hit sensitivity vs BLAST 11, 12 1-hit

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