BLAST as a microcosm of all that is wrong with computational biology

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(NCBI) BLAST

- You've all used it?
- Very popular! Fast, sensitive way to find sequence similarity => putative homology.
- Primary sequence comparison tool used by biologists, computational biologists.

(NCBI) BLAST

- One or more query sequences…
- against a “subject” database.
- Finds core strong match, extends outwards.

```
SIRGGGVDHGISDDESQHSGDAGIS
S+RGGG++ G+S++       D+G
SVRGGGIEIGLSEE------DSGAE
```

Consideration #1

- BLAST *only* cares about sequence similarity.

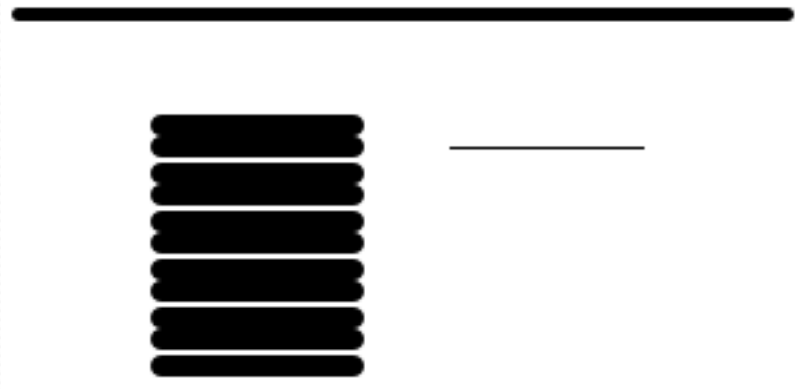
- No positional information taken into account, for e.g. protein domains.


Query: 1628 AHLLVNSQKC-KQTSSECIDTTDNAASVISARAS----TGTLEAEFPINTVASTTNPTPP 1682
N+Q C K C + S++ +A+ + TL F I++ ST T
Sbjct: 1537 MTFHANTQMCVKLDLQSCPTNVASVKSILGEKAAEFSTSSTLSRVFRIDSEGSTQTGT-- 1710
Query: 1683 PQDYTYXXXXXXXXXXXXXXXXXTHRKRKRETSTLWAPEGFNVTKKQRREPIGQDDLNG 1742
Y +KRKRE LW PEGF + KK+R+E ++LN
Sbjct: 1711 TNYLVYIIAGGGIMVLIIVIAGVIVSQKRKRENGNLWVPEGFQLFKKRRKE----NELNL 1878
Query: 1743 LNGSIHPGELTQLDT-AGTPFLNRWENTSLPQKSNHYHVQYTPENITFLPNNGTVPXXXX 1801
N L++ D A TPFL + + Q S + +L
Sbjct: 1879 NN-------LSKADMNAQTPFL---PHATEAQASKYSASSSDTPETDYL---------- 1995
Query: 1802 XXXXXXXXXXXXXEPTDNRKWTPQHLEAADLSRAGSACTPVTDLTPPPHIDVDEDDVNAR 1861
D R+WTP HLEAA+ S C + TPP + DD+NAR
Sbjct: 1996 --------HGSCASKEDKRQWTPHHLEAANNSNVN--CQIMN--TPPQSECPESDDINAR 2139
Query: 1862 GPDGVTPLMVASIRGGGVDHGISDDESQHSGDAGISGEGSDSMIXXXXXXXXXXXXTDR 1921
GPDG TPLM+AS+RGGG++ G+S++ D+G GEGSD+MI TDR
Sbjct: 2140 GPDGYTPLMIASVRGGGIEIGLSEE------DSGAEGEGSDNMIAGLILQGASLSATTDR 2301
Query: 1922 SGETXXXXXXXXXXXXXXXXXXXXXNMKDHSGRTPLHSAVAADAQGVFQILLRNRAT 1981
+GET NMKD +GRTPLH++VAADAQGVFQILLRNRAT
Sbjct: 2302 TGETALHLAARYARADAAKRLLDAGADANMKDQTGRTPLHNSVAADAQGVFQILLRNRAT 2481
Query: 1982 DLDARTNDGTTPMILASRLAVEGMVEELISANADVNAVDDHGKSALHWAAAVNNVDAVST 2041
DLDA+TNDGTTP+ILASRLAVEGMVE+LI+A+ADVNAVD+HGKS+LHWAAAVNN DA+
Sbjct: 2482 DLDAKTNDGTTPLILASRLAVEGMVEDLITAHADVNAVDNHGKSSLHWAAAVNNNDAIRA 2661

```
Query: 862  TCVCTPGFQGPTCANDINECMSPPCKNGGKCRNREPGYFCECLDGYSGVNCEENVDDCAS 921
            TC      QG T AN         C   G C N    + C C +G++G  CE  ++ C
Sbjct: 34   TCEVQAASQGTTVAN--------VCNGQGTCINSGNSHTCTCAEGFTGSYCETIINHCDP 189

Query: 922  DPCMNGGTCLDDVNSYKCLCKRGFDGNQCQNDVNECENEPCKNGATCTDYVNSYACTCPP 981
            +PC+N   C   +N Y+C C+ GF G+QCQ D++EC + PC NG TC + +N + C+CP
Sbjct: 190  NPCINAVKCTSGINGYECDCEAGFQGSQCQLDIDECTSNPCMNGGTCFNAINGFQCSCPR 369

Query: 982  GFRGTTCMENIDECNIGSCLNGGTCVDGINSYSCNCMAGFTGANCERDIDECVSSPC--K 1039
            G  G  C      C+   C N G C  GI S++C C  G+ G  C  DI+EC S+PC  +
Sbjct: 370  GTLGVLCEVVSSLCDPNPCQNNGHCTSGIGSFTCQCKPGYGGYLCNGDINECASNPCSTE 549

Query: 1040 NGAPCIHGINTFTCQCLTGYTGPTCAQMVDLCQNNPCRNGGQCSQTGTTSK---CLCTSS 1096
               C+ GIN F+C C  GY G TC+     C NNPC NG  C+          C CT+
Sbjct: 550  GSLDCVQGINEFSCLCKDGYYGDTCSNQASSCSNNPCLNGATCTDNSLEPLRYFCSCTND 729

Query: 1097 YSGVYCDVPRLSCSAAATWQGVEETSLCQHGGQCINSGSTHYCSCRAGYVGSYCETD--- 1153
            Y G  C++   +C +           +C + G+C++ GS  YC C  GY G+ C ++
Sbjct: 730  YRGKNCEMEFSTCPSLDM--------ICYNDGKCVD-GSAPYCKCPFGYTGTQCMSNTNT 882

Query: 1154 EDDCASY 1160
            E  C+SY
Sbjct: 883  EKQCSSY 903
```

Consideration #2

- BLAST is a *local* alignment algorithm.
- Strong matches are reported first; multiple matches may be out of order between query, subject.



Consideration #3

- BLAST creates gapped alignments.

```
SIRGGGVDHGISDDESQHSGDAGIS
S+RGGG++ G+S++       D+G
SVRGGGIEIGLSEE------DSGAE
```

- This means it's totally inappropriate for (for example) primer matching, unless you change the parameters.

- (Who here has actually changed BLAST parameters?)

Consideration #4

- BLAST e-values are database-size dependent.

- BLAST bit scores are not.

Score = 87.0 bits (214), Expect = 5e-16

- You can't technically compare e-values from BLASTs against different databases!

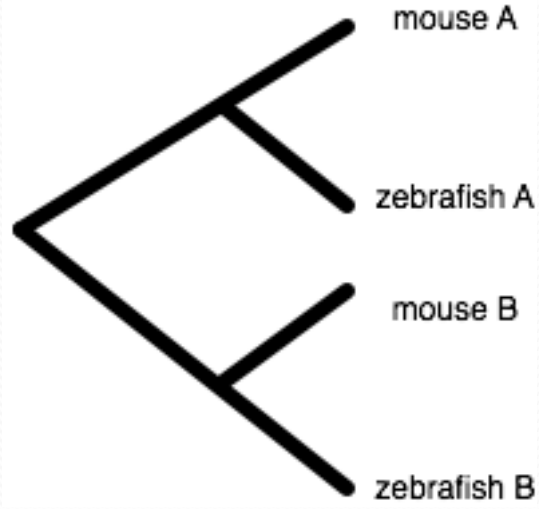
Consideration #5

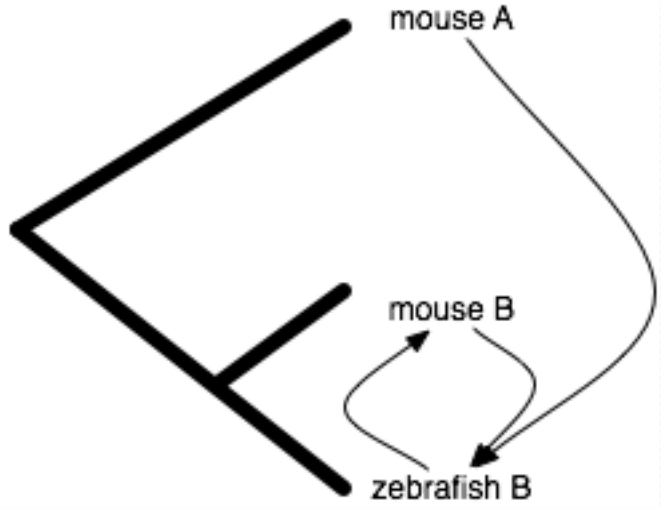
- BLAST uses an heuristic to speed things up: *requires* an **exact** match between 11 bases (DNA) or 3 amino acids in order to start an alignment.

```
Query: 862  TCVCTPGFQGPTCANDINECMSPPCKNGGKCRNREPGYFCECLDGYSGVNCEENVDDCAS 921
            TC      QG T AN         C   G C N    + C C +G++G  CE  ++ C
Sbjct: 34   TCEVQAASQGTTVAN--------VCNGQGTCINSGNSHTCTCAEGFTGSYCETIINHCDP 189

Query: 922  DPCMNGGTCLDDVNSYKCLCKRGFDGNQCQNDVNECENEPCKNGATCTDYVNSYACTCPP 981
            +PC+N   C   +N Y+C C+ GF G+QCQ D++EC + PC NG TC + +N + C+CP
Sbjct: 190  NPCINAVKCTSGINGYECDCEAGFQGSQCQLDIDECTSNPCMNGGTCFNAINGFQCSCPR 369
```

Consideration #6

- Reciprocal BLAST is a *horrible* (but frequently used) heuristic for "orthology". Intended for:





...but local alignments cause trouble here!

Considerations #7+

- BLAST implementation is (was?) impenetrable: completely inextensible, very optimized, built on a huge library.
  - Does it have bugs? Nobody knows…
  - V. difficult to embed => difficult to reuse

- BLAST text output format changes frequently and is designed for humans only to read; very hard for computers to parse.

BLAST is also kind of inconvenient

- No good Web interface for uploading your own databases (that I know of).

So, nobody uses BLAST, right?

- Absolutely wrong!
- Biologists love it: it's fast, sensitive, and has a nice Web interface at NCBI.
- Bioinformaticians love/hate it:
  - Biologists => programmers use it by default, and then spend a lot of time correcting for its problems.
  - Computer scientists => biologists often can't escape:
    - Lots of biology behind BLAST; tough to write your own.
    - Biologists *believe* in BLAST, and not your own dinky algorithm.

Digression: it's not BLAST's fault, really.

- Most of the "considerations" I presented are completely obvious and stated clearly all over the place.
- Everybody uses BLAST because it's there, it (mostly) works, and it's trusted by (almost) everyone.
- BLAST use may be starting to break down, though:
  - Doesn't scale to volume of data
  - Default gapping model is inappropriate for short-read mapping
  - Has significant false positive rate on very divergent proteins (metagenomics, "evolutionarily interesting" organisms)

This course & BLAST

- We'll be (mis)using BLAST just like everyone else.

- We'll show you how to run BLAST at the command line:
  - Run long jobs on some other computer
  - Make your own BLAST databases

- We'll show you BLAST output "parsing"
  - Make your *own* spreadsheet of matches
  - Your very own reciprocal BLAST script…

The UNIX command line

- Many computer folk, and most bioinformaticians, work with a text interface to their computers: "the command line".
- Sort of the grandaddy of all interfaces… think back to teletypes.
- Why?
  - Writing *new* programs is much easier if you write them for the command line (text, no graphics)
  - Simple & flexible (not nec. *good*) user interface design: **none**
  - Simple "pipelining" ability
- Almost all bioinformatics programs work at the command line, or via a Web interface.

The UNIX command line, part 2

- This software can be installed on your computer (Windows) or already exists (Mac OS X).
- …but we really, really, really don't want you to use your own computer to do analyses!
  - Laptops are slooooow
  - Data files are big
  - *Your* computer is here at KBS, and we don't want you transferring 50 gb+ of data here!
  - You'd much rather use your laptop as an interface!

- **Dilemma.** But we have a solution…

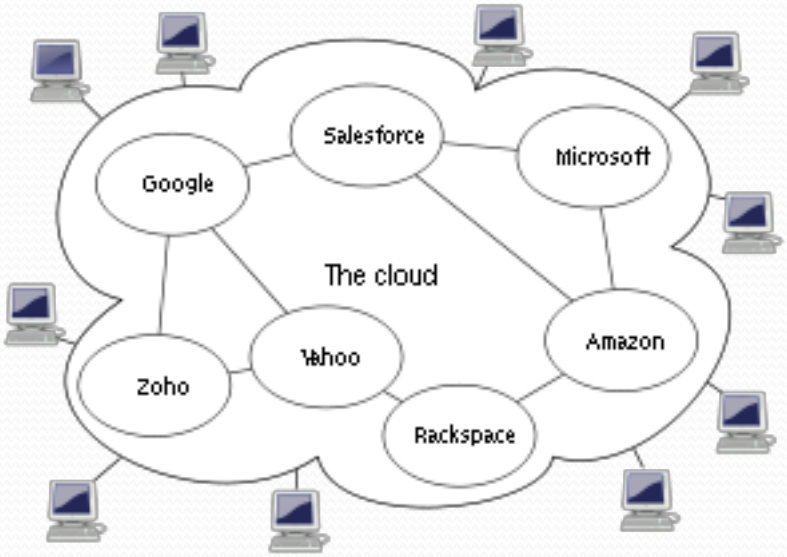
Part II: Cloud computing

What is cloud computing?

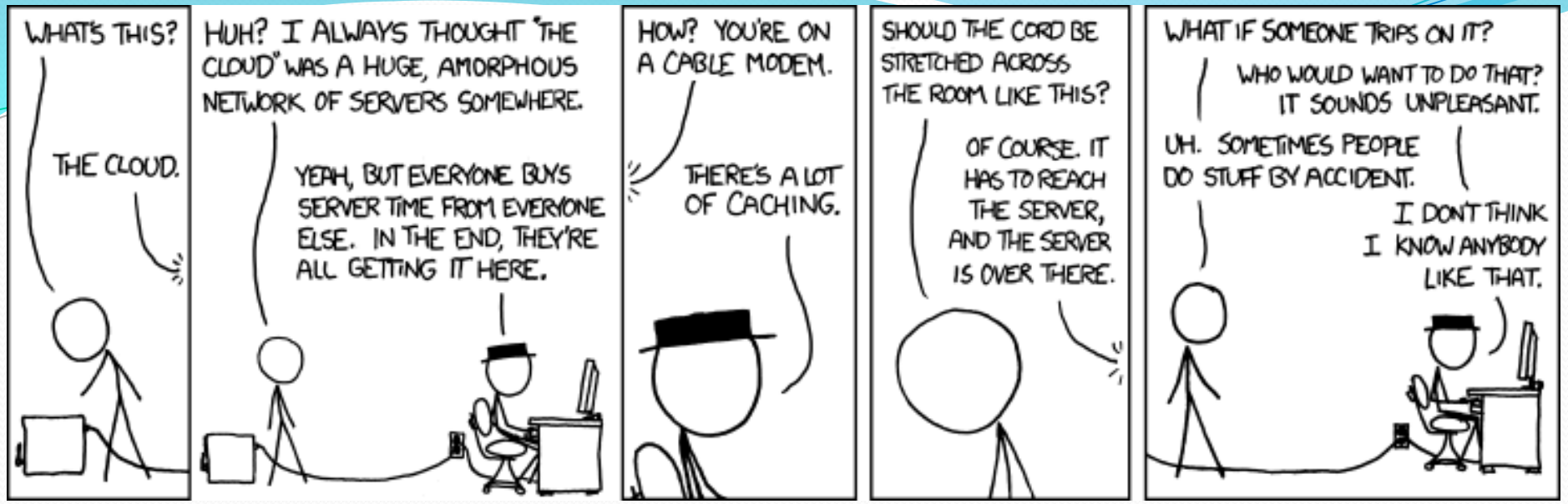
(for scientists)

- You can “rent” access to computers and disk space from a commercial provider of same.
- This provides you with a way to scale your computation for “burst” periods, without investing in hardware.
- Or you can just use a bigger, faster computer.
- (I will demonstrate.)

Why “cloud”?!



…because the diagram that CS people use to represent abstract compute resources looks like a cloud.


WHAT'S THIS?
THE CLOUD.
HUH? I ALWAYS THOUGHT "THE CLOUD" WAS A HUGE, AMORPHOUS NETWORK OF SERVERS SOMEWHERE.
YEAH, BUT EVERYONE BUYS SERVER TIME FROM EVERYONE ELSE. IN THE END, THEY'RE ALL GETTING IT HERE.
HOW? YOU'RE ON A CABLE MODEM.
THERE'S A LOT OF CACHING.
SHOULD THE CORD BE STRETCHED ACROSS THE ROOM LIKE THIS?
OF COURSE. IT HAS TO REACH THE SERVER, AND THE SERVER IS OVER THERE.
WHAT IF SOMEONE TRIPS ON IT?
WHO WOULD WANT TO DO THAT? IT SOUNDS UNPLEASANT.
UH. SOMETIMES PEOPLE DO STUFF BY ACCIDENT.
I DON'T THINK I KNOW ANYBODY LIKE THAT.

xkcd.com

Editor's note: Mr. Munroe has been missing for several days. We have received no submissions from him for some time, but we found this single panel on his desk in a folder labeled 'MY BEST IDEA EVER'. It is clearly part of a work in progress, but we have decided to post it in lieu of a complete comic.

Amazon is a major cloud computing provider

- Did you know they rent computers!?
- Rumors are that it's more lucrative than their book selling division…

Terms

EC2 – Elastic Cloud Computing, computer rental from Amazon.

EBS – Elastic Block Storage, virtual hard drive rental from Amazon.

Some quick calculations:

1 small machine, / yr:

1.7gb of RAM, a ~1.0 GHz single-core CPU, 160gb of local disk.

$.085 / hr

8760 hrs / year

=> ~$750 / year.

Not an effective server replacement.

1 high-memory quadruple extra-large instance / yr:

68.4 gb of RAM, 8 core @ ~3.2 GHz, 1.7tb of local disk.

$2.40 / hr
8760 hrs / year
=> $21,000 / year

20 high-CPU extra large machines, for a day:

7gb of RAM, 8 x 2.5 GHz CPUs, 1.7tb of local disk.

$.68 / hr
24 hrs / day
20 machines
=> ~$330/ day.

Why is EC2 so expensive??

- They cover *all* hardware, power, air conditioning and network costs.
- That's actually way more expensive than you think. (Talk to your sysadmin or HPC person…)
- They do not operate at 100% capacity,
- They want to make $$.

What are **we** using it for?

- Teaching workshops and classes.
- Running our own analyses/data sets in a timely manner.
- Sharing data within the lab via EBS snapshots.
- Providing data to other people via S3.
- Automated testing on clean machines with known software install.

Today's tutorials

1. Create (rent) a new machine from Amazon.
2. Install NCBI BLAST
3. Download & format some databases
4. Run BLAST
5. Produce an excel spreadsheet of best hits

…

1. Run 2-way BLAST (mouse x zfin, and vice versa)
2. Calculate reciprocal best hits
3. Produce an excel spreadsheet

PLEASE DO NOT

- Try to transfer 100mb+ of files via Dropbox ☺

- Transfer data here.