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.

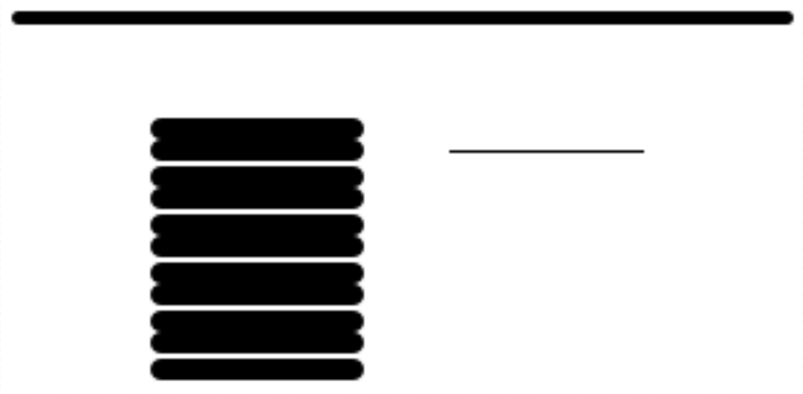
- No positional information taken into account, for e.g. protein domains.

```
Query: 1628 AHLLVNSQKC-KQTSSECIDTTDNAASVISARAS----TGTLEAEFPINTVASTTNPTPP 1682
                 N+Q C K     C     +  S++  +A+    + TL   F I++  ST   T
Sbjct: 1537 MTFHANTQMCVKLDLQSCPTNVASVKSILGEKAAEFSTSSTLSRVFRIDSEGSTQTGT-- 1710

Query: 1683 PQDYTYXXXXXXXXXXXXXXXXXXTHRKRKRETSTLWAPEGFNVTKKQRREPIGQDDLNG 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 GPDGVTPLMVASIRGGGVDHGISDDESQHSGDAGISGEGSDSMIXXXXXXXXXXXXXTDR 1921
            GPDG TPLM+AS+RGGG++ G+S++      D+G  GEGSD+MI             TDR
Sbjct: 2140 GPDGYTPLMIASVRGGGIEIGLSEE------DSGAEGEGSDNMIAGLILQGASLSATTDR 2301

Query: 1922 SGETXXXXXXXXXXXXXXXXXXXXXXXXNMKDHSGRTPLHSAVAADAQGVFQILLRNRAT 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!

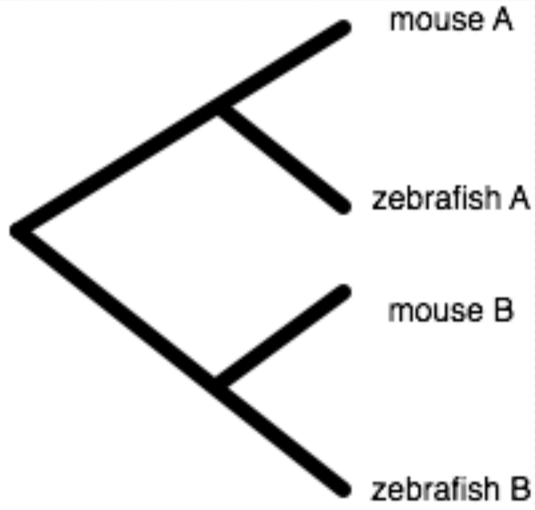
Consideration #5

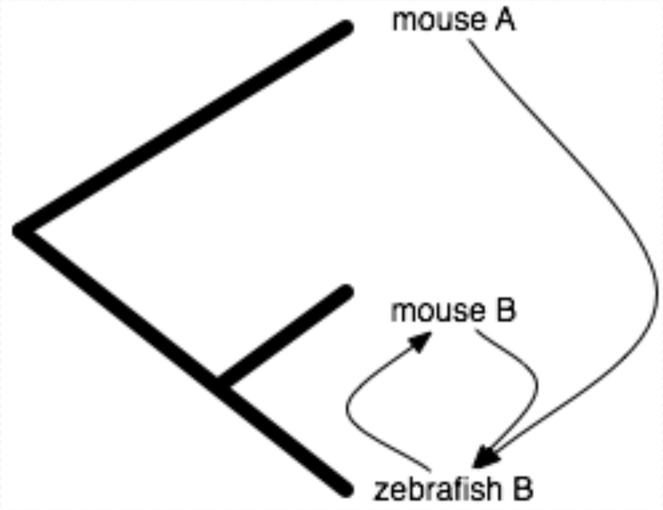
- BLAST uses an heuristic to speed things up: *requires* an **exact** match between 11 bases (DNA) or 3 amino acids in order to start an alignment.

```
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 workshop & 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.** => cloud computing…