MCB 5472

Blast, Psi BLAST,
Perl: Arrays, Loops

J. Peter Gogarten
Office: *BPB 404*
phone: *860 486-4061,*
Email: *gogarten@uconn.edu*

homology

Two sequences are homologous, if there existed an ancestral molecule in the past that is ancestral to both of the sequences

Types of Homology

Orthologs: “deepest” bifurcation in molecular tree reflects speciation.
These are the molecules people interested in the taxonomic classification of organisms want to study.

Paralogs: “deepest” bifurcation in molecular tree reflects gene duplication. The study of paralogs and their distribution in genomes provides clues on the way genomes evolved. Gen and genome duplication have emerged as the most important pathway to molecular innovation, including the evolution of developmental pathways.

Xenologs: gene was obtained by organism through horizontal transfer. The classic example for Xenologs are antibiotic resistance genes, but the history of many other molecules also fits into this category: inteins, selfsplicing introns, transposable elements, ion pumps, other transporters,

Synologs: genes ended up in one organism through fusion of lineages. The paradigm are genes that were transferred into the eukaryotic cell together with the endosymbionts that evolved into mitochondria and plastids
(the -logs are often spelled with "ue" like in orthologues)

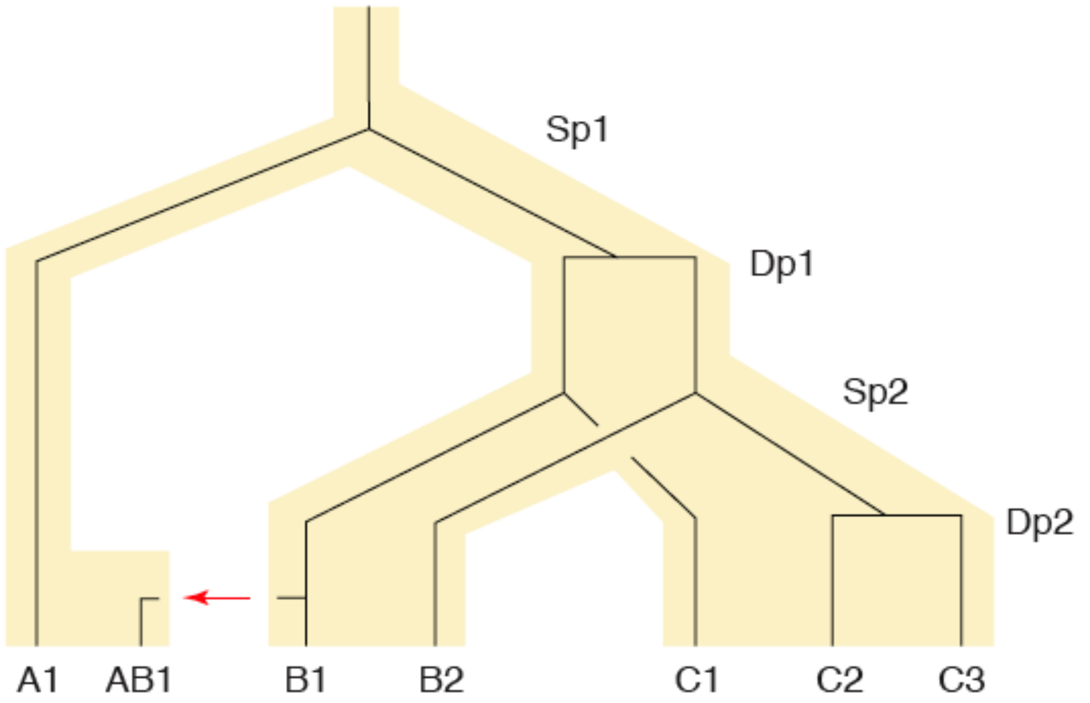
see Fitch's article in TIG 2000 for more discussion.

Homologs, orthologs, and paralogs

- Homologous structures or characters evolved from the same ancestral structure or character that *existed in some organism in the past*.
- Orthologous characters present in two organism (A and B) are homologs that are derived from a structure *that existed in the most recent common ancestor* (MRCA) of A and B (orthologs often have the same function, but this is NOT part of the definition; e.g. human arms, wings or birds and bats).
- Paralogous characters in the same or in two different organisms are homologs that are not derived from the same character in the MRCA, rather they are *related* (at their deepest node) *by a gene duplication event*.

Examples

FIGURE 1. Orthology, paralogy and xenology



trends in Genetics

B1 is an ortholog to C1 and to A1
C2 is a paralog to C3 and to B1;
BUT
A1 is an ortholog to both B1, B2,and to C1, C2, and C3

From: Walter Fitch (2000): *Homology: a personal view on some of the problems*, TIG 16 (5) 227-231

Uses of Blast in bioinformatics

The Blast web tool at NCBI is limited:

- custom and multiple databases are not available
- tBlastN (gene prediction) not available
- “time-out” before long searches are completed

What if researcher wants to use tBlastN to find all olfactory receptors in the mosquito? Or, if you want to check the presence of a (pseudo)gene in a preliminary genome assembly?
Answer: Use Blast from command-line

Also: The command-line allows the user to run commands repeatedly

by Bob Friedman

Types of Blast searching

- blastp compares an amino acid query sequence against a protein sequence database

- blastn compares a nucleotide query sequence against a nucleotide sequence database

- blastx compares the six-frame conceptual protein translation products of a nucleotide query sequence against a protein sequence database

- tblastn compares a protein query sequence against a nucleotide sequence database translated in six reading frames

- tblastx compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

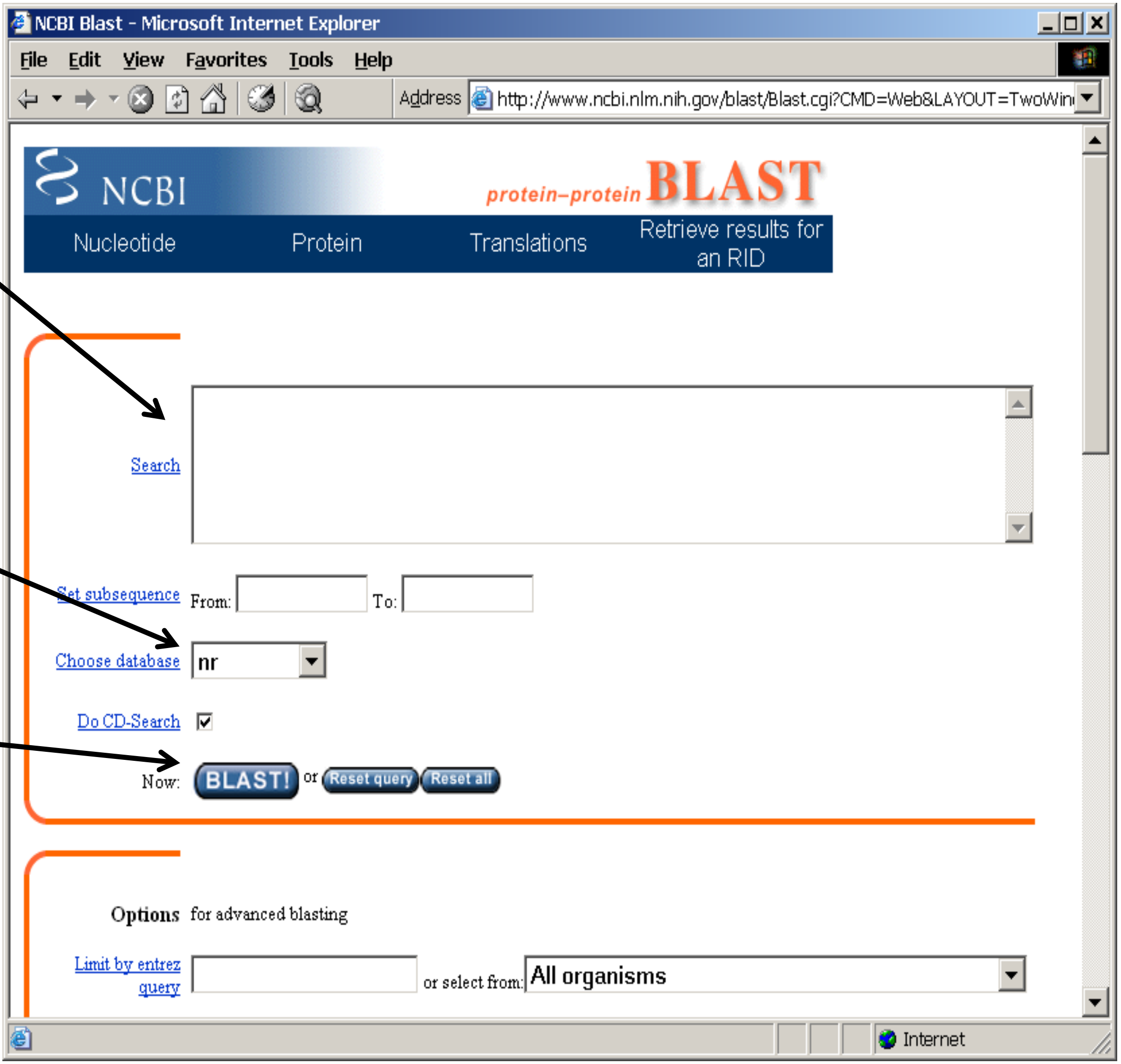
by Bob Friedman

Routine BlastP search

FASTA formatted text
or Genbank ID#

Protein database

Run


NCBI Blast - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Address http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&LAYOUT=TwoWin

NCBI protein–protein BLAST

Nucleotide | Protein | Translations | Retrieve results for an RID

Search

Set subsequence From: To:

Choose database: nr

Do CD-Search ☑

Now: BLAST! or Reset query Reset all

Options for advanced blasting

Limit by entrez query   or select from: All organisms

by Bob Friedman

BlastP parameters

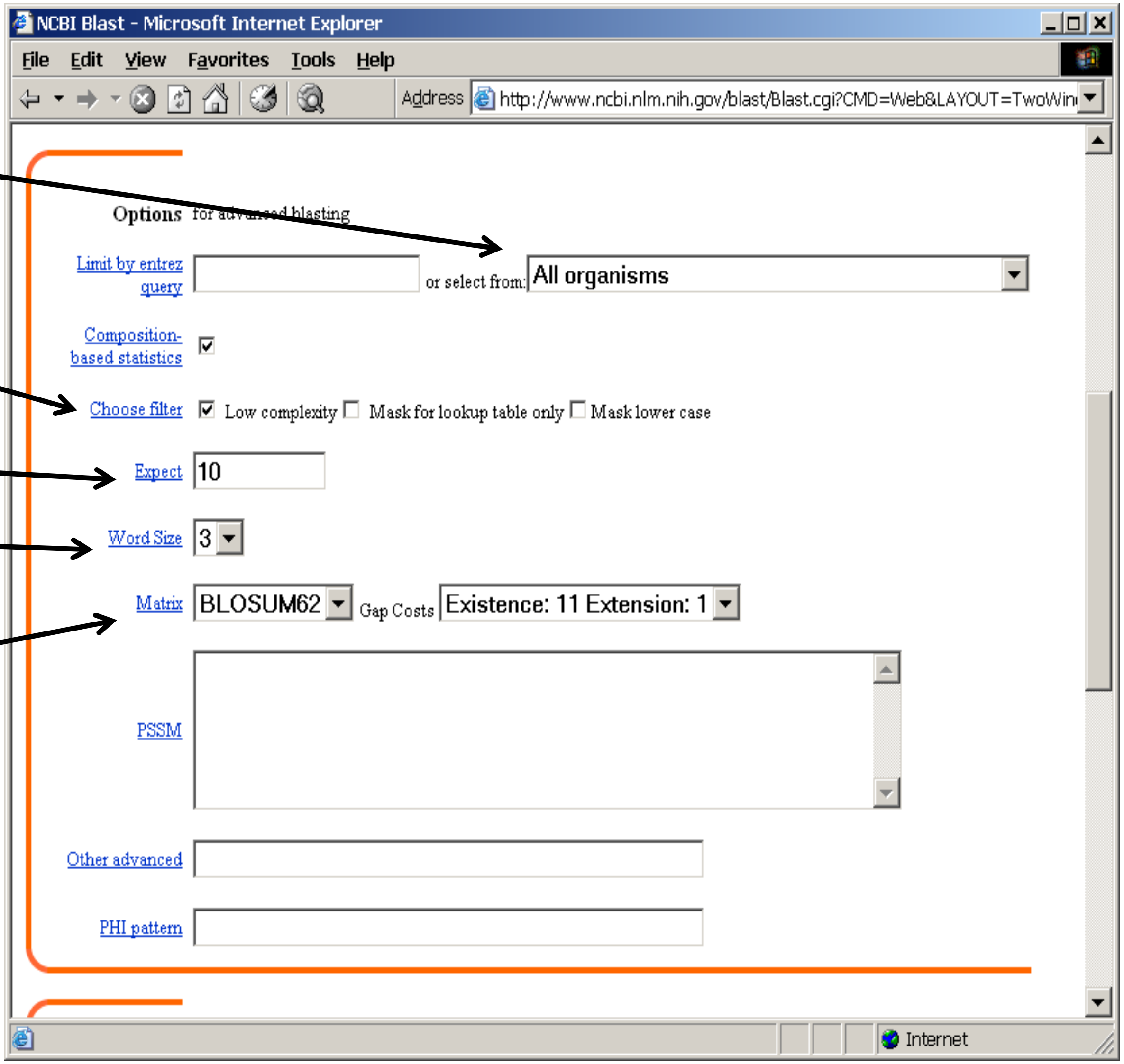
Restrict by taxonomic group

Filter repetitive regions

Statistical cut-off

Size of words in look-up table

Similarity matrix (cost of gaps)



http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&LAYOUT=TwoWin

Options for advanced blasting

Limit by entrez query or select from: All organisms

Composition-based statistics ☑

Choose filter ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Expect 10

Word Size 3

Matrix BLOSUM62 Gap Costs Existence: 11 Extension: 1

PSSM

Other advanced

PHI pattern

by Bob Friedman

Establishing a significant "hit"

Blast's E-value indicates statistical significance of a sequence match

Karlin S, Altschul SF (1990) Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. PNAS 87:2264-8

E-value is the Expected number of sequence (HSPs) matches in database of *n* number of sequences

- database size is arbitrary
- multiple testing problem
- E-value calculated from many assumptions
- so, E-value is not easily compared between searches of different databases

Examples:

E-value = 1 = expect the match to occur in the database by chance 1x

E-value = .05 = expect 5% chance of match occurring

E-value = 1×10^{-20} = strict match between protein domains

by Bob Friedman

When are two sequences significantly similar? PRSS

One way to quantify the similarity between two sequences is to

1. compare the actual sequences and calculate an alignment score

2. randomize (scramble) one (or both) of the sequences and calculate the alignment score for the randomized sequences.

3. repeat step 2 at least 100 times

4. describe distribution of randomized alignment scores

5. do a statistical test to determine if the score obtained for the real sequences is significantly better than the score for the randomized sequences

z-values give the distance between the actual alignment score and the mean of the scores for the randomized sequences expressed as multiples of the standard deviation calculated for the randomized scores.

For example: a z-value of 3 means that the actual alignment score is 3 standard deviations better than the average for the randomized sequences. z-values > 3 are usually considered as suggestive of homology, z-values > 5 are considered as sufficient demonstration.

E-values and significance

Usually E values larger than 0.0001 are not considered as demonstration of homology.

For small values the E value gives the probability to find a match of this quality in a search of a databank of the same size by chance alone.

E-values give the expected number of matches with an alignment score this good or better,
P-values give the probability of to find a match of this quality or better.
P values are [0,1], E-values are [0,infinity).
For small values E=P

Problem: If you do 1000 blast searches, you expect one match due to chance with a P-value of 0.0001

“One should” use a correction for multiple tests, like the Bonferroni correction.

Psi-Blast: Detecting structural homologs

Psi-Blast was designed to detect homology for highly divergent amino acid sequences

Psi = position-specific iterated

Psi-Blast is a good technique to find “potential candidate” genes

Example: Search for Olfactory Receptor genes in Mosquito genome

Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zwiebel LJ (2002) G protein-coupled receptors in Anopheles gambiae. Science 298:176-8

Psi-Blast Model

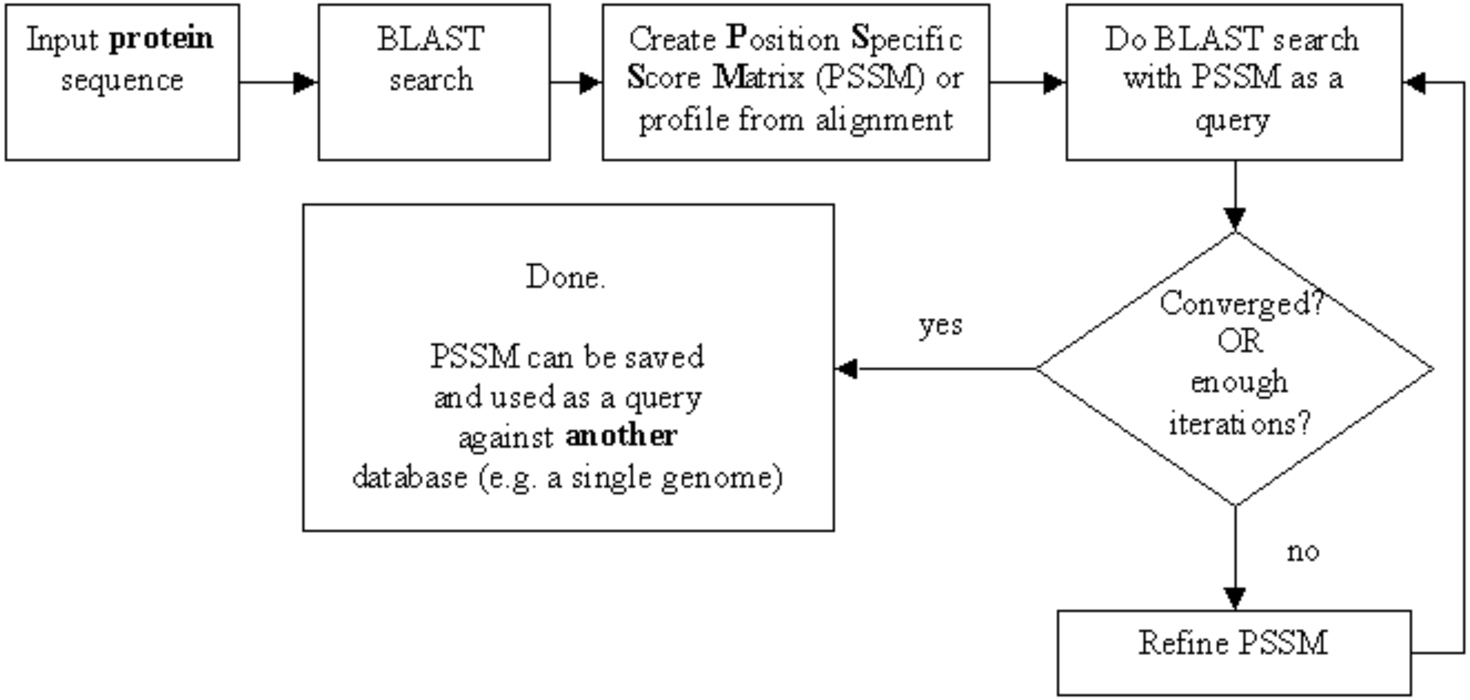
Model of Psi-Blast:
1. Use results of gapped BlastP query to construct a multiple sequence alignment
2. Construct a position-specific scoring matrix from the alignment
3. Search database with alignment instead of query sequence
4. Add matches to alignment and repeat

Similar to Blast, the E-value in Psi-Blast is important in establishing matches
E-value defaults to 0.001 & Blosom62

Psi-Blast can use existing multiple alignment - particularly powerful when the gene functions are known (prior knowledge) or use RPS-Blast database

by Bob Friedman

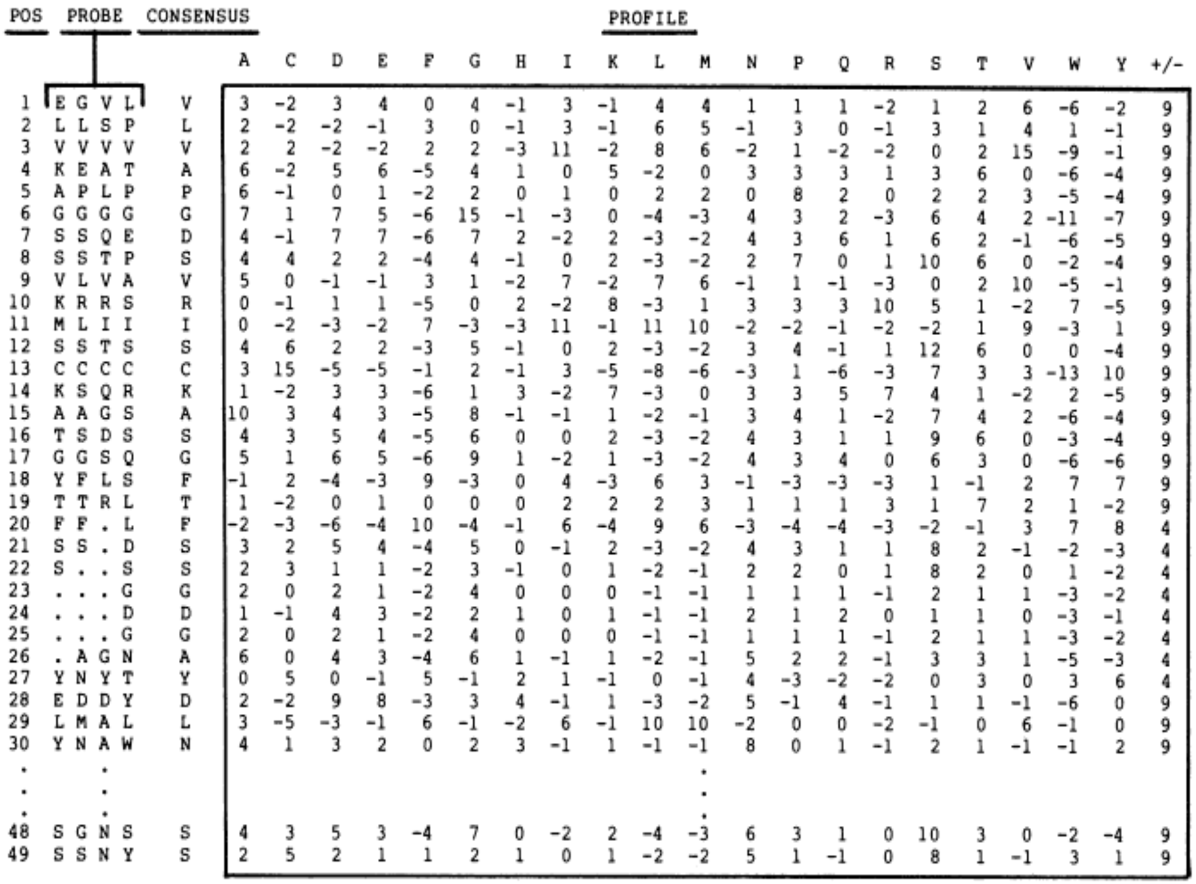
PSI BLAST scheme



Input **protein** sequence
BLAST search
Create **P**osition **S**pecific **S**core **M**atrix (PSSM) or profile from alignment
Do BLAST search with PSSM as a query
Converged? OR enough iterations?
yes
Done.
PSSM can be saved and used as a query against **another** database (e.g. a single genome)
no
Refine PSSM

© Olga Zhaxybayeva

Position-specific Matrix



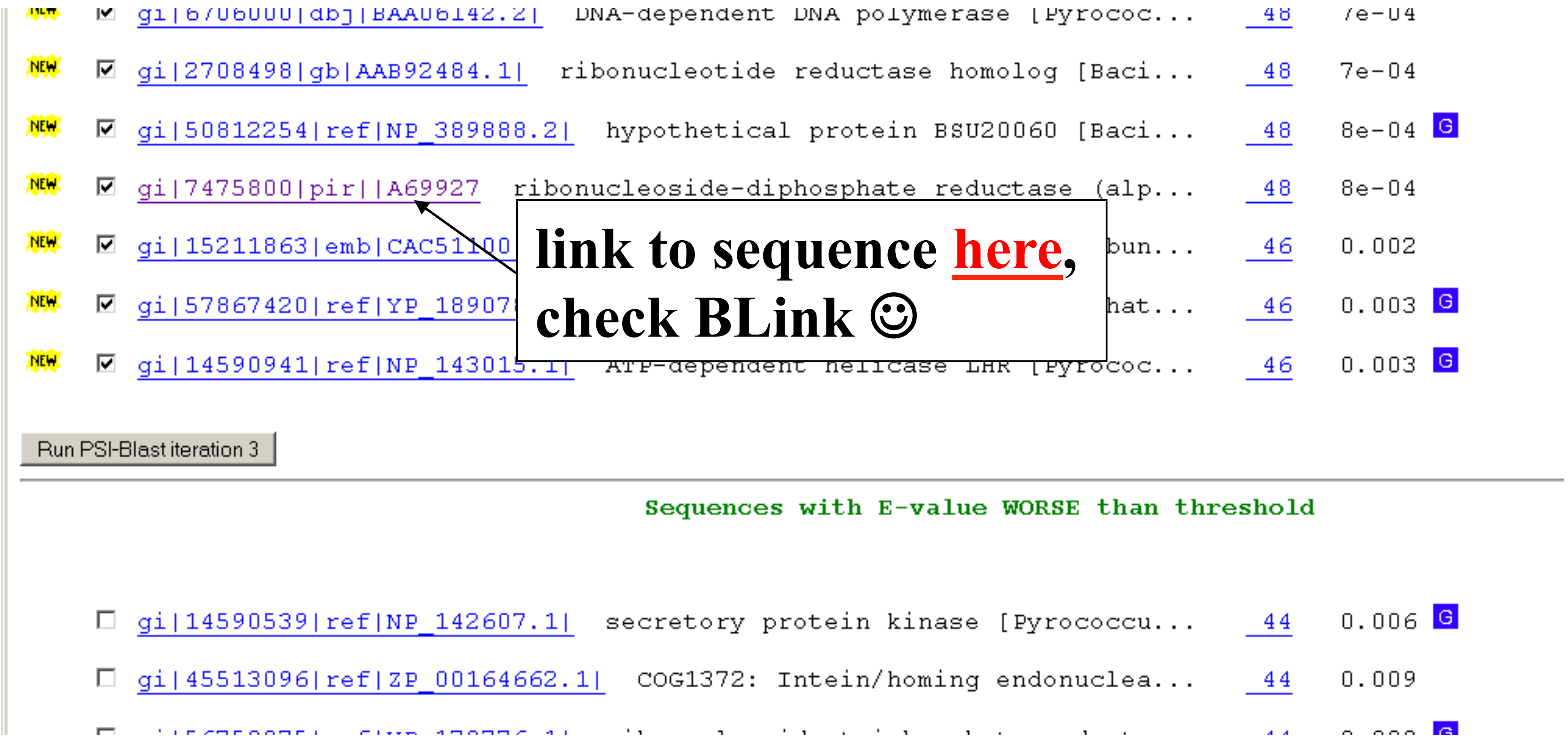
POS	PROBE	CONSENSUS	PROFILE																				
			A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	+/-
1	E G V L	V	3	−2	3	4	0	4	−1	3	−1	4	4	1	1	1	−2	1	2	6	−6	−2	9
2	L L S P	L	2	−2	−2	−1	3	0	−1	3	−1	6	5	−1	3	0	−1	3	1	4	1	−1	9
3	V V V V	V	2	2	−2	−2	2	2	−3	11	−2	8	6	−2	1	−2	−2	0	2	15	−9	−1	9
4	K E A T	A	6	−2	5	6	−5	4	1	0	5	−2	0	3	3	3	1	3	6	0	−6	−4	9
5	A P L P	P	6	−1	0	1	−2	2	0	1	0	2	2	0	8	2	0	2	2	3	−5	−4	9
6	G G G G	G	7	1	7	5	−6	15	−1	−3	0	−4	−3	4	3	2	−3	6	4	2	−11	−7	9
7	S S Q E	D	4	−1	7	7	−6	7	2	−2	2	−3	−2	4	3	6	1	6	2	−1	−6	−5	9
8	S S T P	S	4	4	2	2	−4	4	−1	0	2	−3	−2	2	7	0	1	10	6	0	−2	−4	9
9	V L V A	V	5	0	−1	−1	3	1	−2	7	−2	7	6	−1	1	−1	−3	0	2	10	−5	−1	9
10	K R R S	R	0	−1	1	1	−5	0	2	−2	8	−3	1	3	3	3	10	5	1	−2	7	−5	9
11	M L I I	I	0	−2	−3	−2	7	−3	−3	11	−1	11	10	−2	−2	−1	−2	−2	1	9	−3	1	9
12	S S T S	S	4	6	2	2	−3	5	−1	0	2	−3	−2	3	4	−1	1	12	6	0	0	−4	9
13	C C C C	C	3	15	−5	−5	−1	2	−1	3	−5	−8	−6	−3	1	−6	−3	7	3	3	−13	10	9
14	K S Q R	K	1	−2	3	3	−6	1	3	−2	7	−3	0	3	3	5	7	4	1	−2	2	−5	9
15	A A G S	A	10	3	4	3	−5	8	−1	−1	1	−2	−1	3	4	1	−2	7	4	2	−6	−4	9
16	T S D S	S	4	3	5	4	−5	6	0	0	2	−3	−2	4	3	1	1	9	6	0	−3	−4	9
17	G G S Q	G	5	1	6	5	−6	9	1	−2	1	−3	−2	4	3	4	0	6	3	0	−6	−6	9
18	Y F L S	F	−1	2	−4	−3	9	−3	0	4	−3	6	3	−1	−3	−3	−3	1	−1	2	7	7	9
19	T T R L	T	1	−2	0	1	0	0	0	2	2	2	3	1	1	1	3	1	7	2	1	−2	9
20	F F . L	F	−2	−3	−6	−4	10	−4	−1	6	−4	9	6	−3	−4	−4	−3	−2	−1	3	7	8	4
21	S S . D	S	3	2	5	4	−4	5	0	−1	2	−3	−2	4	3	1	1	8	2	−1	−2	−3	4
22	S . . S	S	2	3	1	1	−2	3	−1	0	1	−2	−1	2	2	0	1	8	2	0	1	−2	4
23	. . . G	G	2	0	2	1	−2	4	0	0	0	−1	−1	1	1	1	−1	2	1	1	−3	−2	4
24	. . . D	D	1	−1	4	3	−2	2	1	0	1	−1	−1	2	1	2	0	1	1	0	−3	−1	4
25	. . . G	G	2	0	2	1	−2	4	0	0	0	−1	−1	1	1	1	−1	2	1	1	−3	−2	4
26	. A G N	A	6	0	4	3	−4	6	1	−1	1	−2	−1	5	2	2	−1	3	3	1	−5	−3	4
27	Y N Y T	Y	0	5	0	−1	5	−1	2	1	−1	0	−1	4	−3	−2	−2	0	3	0	3	6	4
28	E D D Y	D	2	−2	9	8	−3	3	4	−1	1	−3	−2	5	−1	4	−1	1	1	−1	−6	0	9
29	L M A L	L	3	−5	−3	−1	6	−1	−2	6	−1	10	10	−2	0	0	−2	−1	0	6	−1	0	9
30	Y N A W	N	4	1	3	2	0	2	3	−1	1	−1	−1	8	0	1	−1	2	1	−1	−1	2	9
.	.												.										
.	.												.										
.	.												.										
48	S G N S	S	4	3	5	3	−4	7	0	−2	2	−4	−3	6	3	1	0	10	3	0	−2	−4	9
49	S S N Y	S	2	5	2	1	1	2	1	0	1	−2	−2	5	1	−1	0	8	1	−1	3	1	9

FIG. 1. The concept of a profile. (*a*) A flow diagram of profile analysis. (*b*) A 49-residue sample profile for the immunoglobulin variable-region domain, generated from the four-probe sequences shown at the left (see Fig. 2*b* for details). The profile is shown in the box. The rightmost column of the profile gives the penalty for insertion/deletion (+/−). Positions 31–47 of the profile are omitted from the figure for clarity. Notice that where gaps appear in some of the probe sequences, the insertion/deletion penalty is lower than elsewhere.

M Gribskov, A D McLachlan, and D Eisenberg (1987) Profile analysis: detection of distantly related proteins. PNAS 84:4355-8.

by Bob Friedman

Psi-Blast Results   Query: 55670331 (intein)



```
NEW  ☑  gi|6706000|dbj|BAA06142.2|   DNA-dependent DNA polymerase [Pyrococ...   48   7e-04
NEW  ☑  gi|2708498|gb|AAB92484.1|   ribonucleotide reductase homolog [Baci...   48   7e-04
NEW  ☑  gi|50812254|ref|NP_389888.2|   hypothetical protein BSU20060 [Baci...   48   8e-04  G
NEW  ☑  gi|7475800|pir||A69927   ribonucleoside-diphosphate reductase (alp...   48   8e-04
NEW  ☑  gi|15211863|emb|CAC51100                                    bun...   46   0.002
NEW  ☑  gi|57867420|ref|YP_18907                                    hat...   46   0.003  G
NEW  ☑  gi|14590941|ref|NP_143015.1|   ATP-dependent helicase LHR [Pyrococ...   46   0.003  G
```

```
                    Sequences with E-value WORSE than threshold

     □  gi|14590539|ref|NP_142607.1|   secretory protein kinase [Pyrococcu...   44   0.006  G
     □  gi|45513096|ref|ZP_00164662.1|   COG1372: Intein/homing endonuclea...   44   0.009
```

link to sequence here, check BLink ☺

PSI BLAST and E-values!

Psi-Blast is for finding matches among divergent sequences (position-specific information)
WARNING: For the nth iteration of a PSI BLAST search, the E-value gives the number of matches to the profile NOT to the initial query sequence! The danger is that the profile was corrupted in an earlier iteration.

PSI Blast from the command line

Often you want to run a PSIBLAST search with two different databanks - one to create the PSSM, the other to get sequences:
To create the PSSM:

```
blastpgp -d nr -i subI -j 5 -C subI.ckp -a 2 -o subI.out -h 0.00001 -F f

blastpgp -d swissprot -i gamma -j 5 -C gamma.ckp -a 2 -o gamma.out -h 0.00001 -F f
```

Runs a 4 iterations of a PSIblast
the -h option tells the program to use matches with E <10^-5 for the next iteration, (the default is 10^{-3})
-C creates a checkpoint (called subI.ckp),
-o writes the output to subI.out,
-i option specifies input as using subI as input (a fasta formated aa sequence).
The nr databank used is stored `in /common/data/`
-a 2 use two processors

(It might help to use the node with more memory (017)
(command is `ssh node017`)

To use the PSSM:

```
blastpgp -d /Users/jpgogarten/genomes/msb8.faa -i subI -a 2 -R
subI.ckp -o subI.out3 -F f

blastpgp -d /Users/jpgogarten/genomes/msb8.faa -i gamma -a 2 -R
gamma.ckp -o gamma.out3 -F f
```

Runs another iteration of the same blast search, but uses the databank `/Users/jpgogarten/genomes/msb8.faa`

-R tells the program where to resume
-d specifies a different databank
-i input file - same sequence as before
-o output_filename
-a 2 use two processors

PSI Blast and finding gene families within genomes

use PSSM to search genome:

A) Use protein sequences encoded in genome as target:

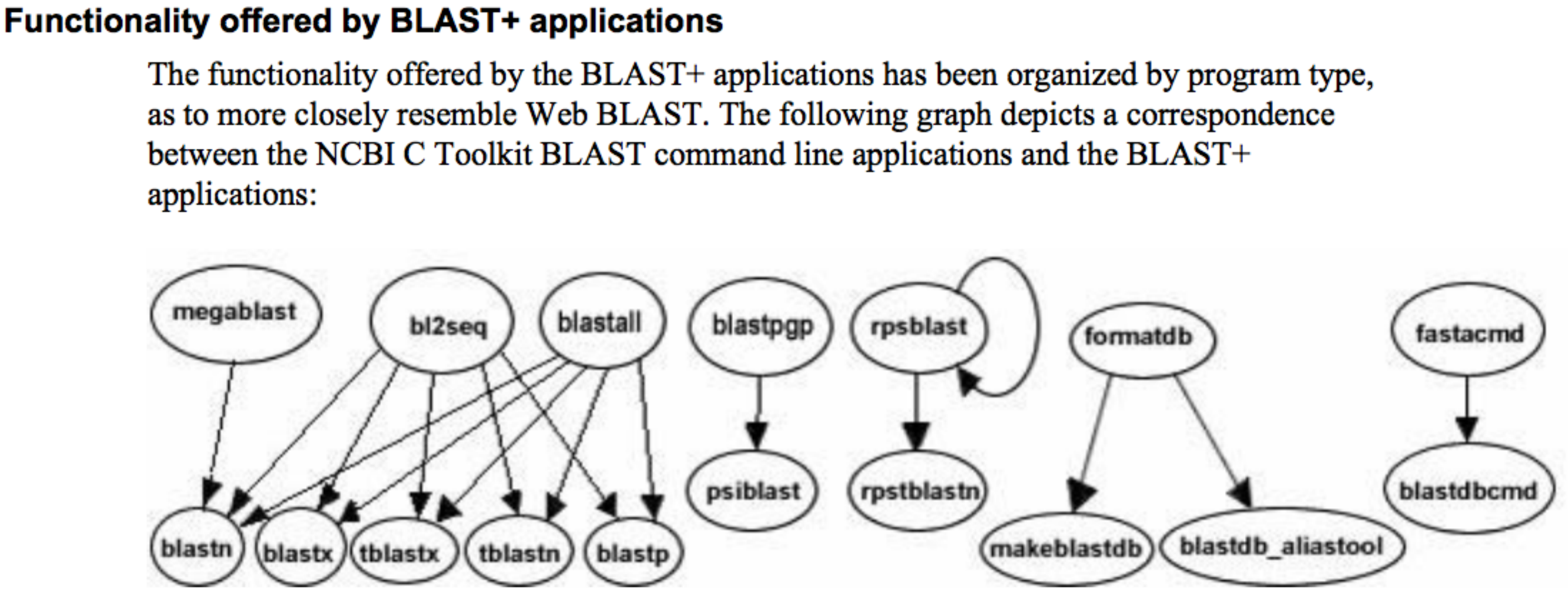
```
blastpgp -d target_genome.faa -i query.name -a 2 -R query.ckp -o
    query.out3 -F f
```

B) Use nucleotide sequence and tblastn. This is an advantage if you are also interested in pseudogenes, and/or if you don’t trust the genome annotation:

```
blastall -i query.name -d target_genome_nucl.ffn -p psitblastn -R
    query.ckp
```

The NCBI has released a new version of blast.   The command line version is blast+ . The new version is faster and allows for more flexibility, both versions should be running it on the cluster.

The new commands are equivalent to the blastall commmands:



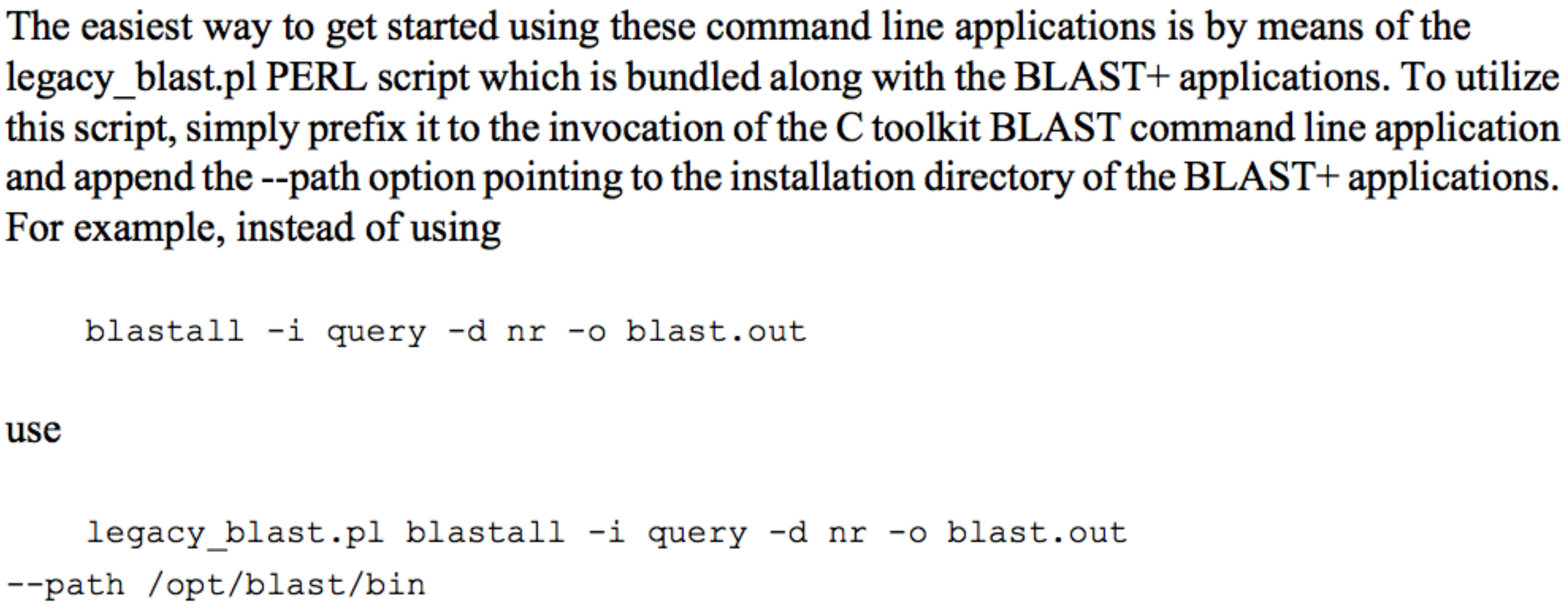
Functionality offered by BLAST+ applications

The functionality offered by the BLAST+ applications has been organized by program type, as to more closely resemble Web BLAST. The following graph depicts a correspondence between the NCBI C Toolkit BLAST command line applications and the BLAST+ applications:

The legacy_blast.pl script that is part of blast+ translates blastall commands into the blast+ syntax. E.g.:

```
$ ./legacy_blast.pl megablast -i query.fsa -d nt -o mb.out --print_only
/opt/ncbi/blast/bin/blastn -query query.fsa -db "nt" -out mb.out
$
```

From the blast+ manual:



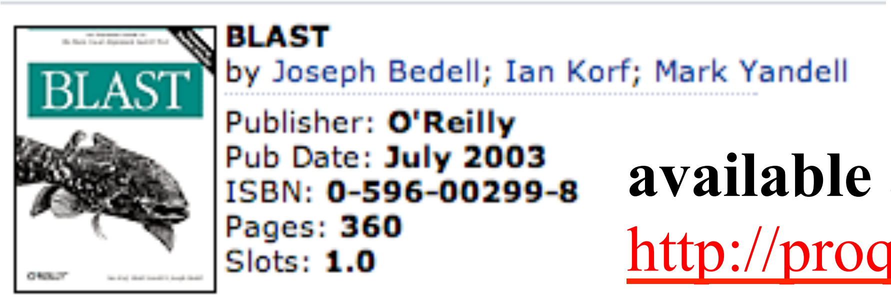
The easiest way to get started using these command line applications is by means of the legacy_blast.pl PERL script which is bundled along with the BLAST+ applications. To utilize this script, simply prefix it to the invocation of the C toolkit BLAST command line application and append the --path option pointing to the installation directory of the BLAST+ applications. For example, instead of using

```
    blastall -i query -d nr -o blast.out
```

use

```
    legacy_blast.pl blastall -i query -d nr -o blast.out
--path /opt/blast/bin
```

More on blastall:



BLAST
by Joseph Bedell; Ian Korf; Mark Yandell
Publisher: **O'Reilly**
Pub Date: **July 2003**
ISBN: **0-596-00299-8**
Pages: **360**
Slots: **1.0**

available at safari books online
http://proquestcombo.safaribooksonline.com/

Installation instructions and info on parameters at the NCBI:
http://www.ncbi.nlm.nih.gov/staff/tao/URLAPI/blastall/
ftp://ftp.ncbi.nlm.nih.gov/blast/documents/formatdb.html
ftp://ftp.ncbi.nlm.nih.gov/blast/documents/blast.html
ftp://ftp.ncbi.nlm.nih.gov/blast/documents/blastpgp.html
ftp://ftp.ncbi.nlm.nih.gov/blast/documents/fastacmd.html
ftp://ftp.ncbi.nlm.nih.gov/blast/documents/

http://en.wikipedia.org/wiki/BLAST

Old assignments:

1) What is the difference between a compiler and an interpreter?

A compiler takes program and translates it in low level executable language/ code.

An interpreter goes through a program line by line and executes commands.

The traditional distinction between compiled and interpreted languages is being blurred.

2) When is it useful to make a script executable, when not?

You save a little bit of typing when you make it executable, but else it is pretty equivalent. (If you start the program with `$ perl script_name.pl`, you don't **need** the shebang line. But the –w flag to use warnings is recognized.

Comments on `use strict`; and `use warnings,`.

Old assignments:

3) What is the value of $i after each of the following operations?

```
$i=1;
$i++;
$i *= $i;
$i .= $i;
$i = $i/11;
$i = $i . “score and” . $i+3;
```

First make a guess, then test your prediction using a script.

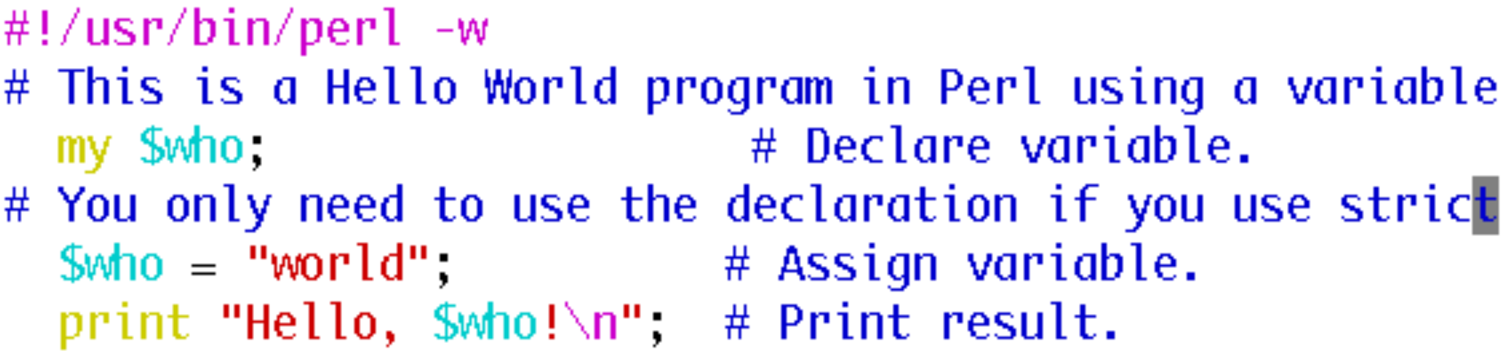
```
#!/usr/bin/perl #-w
my $i=";
print "\$i= $i\n";
$i = 1;
print "\$i= $i\n";
$i++;
print "\$i= $i\n";
$i *= $i;
print "\$i= $i\n";
$i .= $i;
print "\$i= $i\n";
$i = $i/11;
print "\$i= $i\n";
$i = $i . "score and" . $i+3 ;
print "\$i= $i\n";
$i = $i+3 . "score and" . $i;
print "\$i= $i\n";
```

```
$i=
$i= 1
$i= 2
$i= 4
$i= 44
$i= 4
$i= 7
$i= 10score and7
```

discuss and run test.pl with and without –w flag

Discuss and run the hello_world script with variable and input

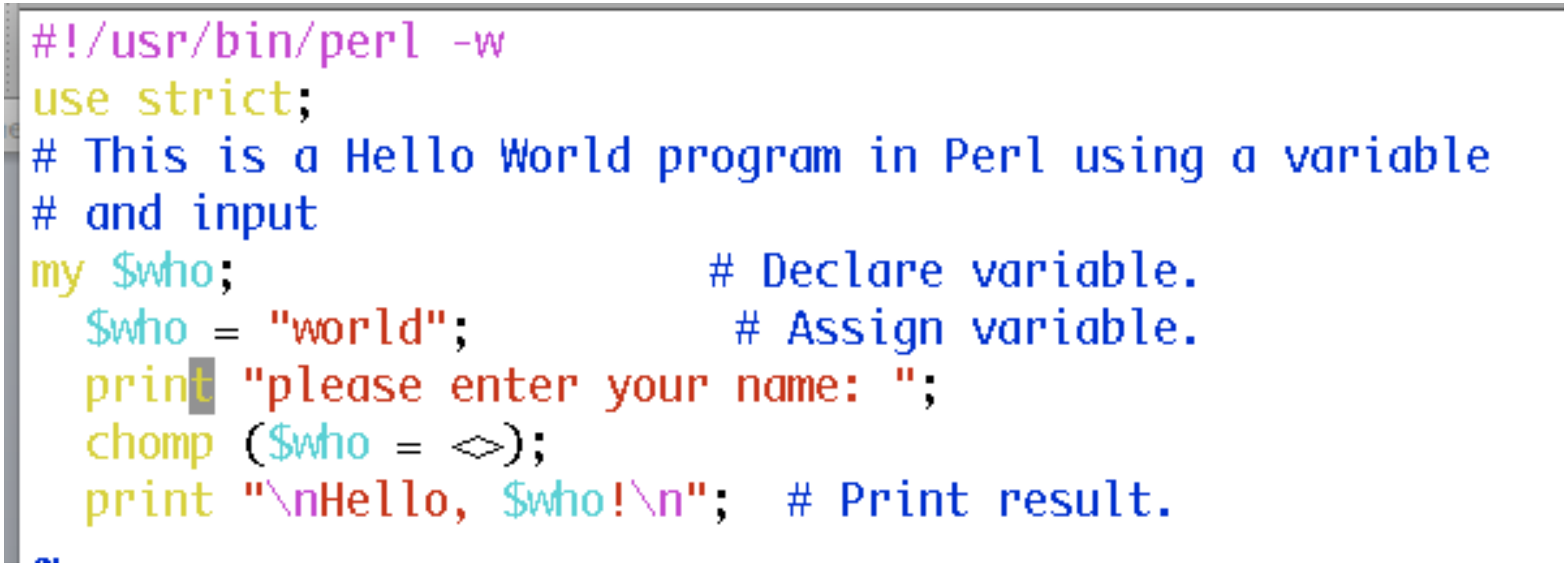
hello_world_variable.pl



```
#!/usr/bin/perl -w
# This is a Hello World program in Perl using a variable
  my $who;                  # Declare variable.
# You only need to use the declaration if you use strict
  $who = "world";          # Assign variable.
  print "Hello, $who!\n";  # Print result.
```

Discuss and run the hello_world script with variable and input

hello_world_variable_input.pl



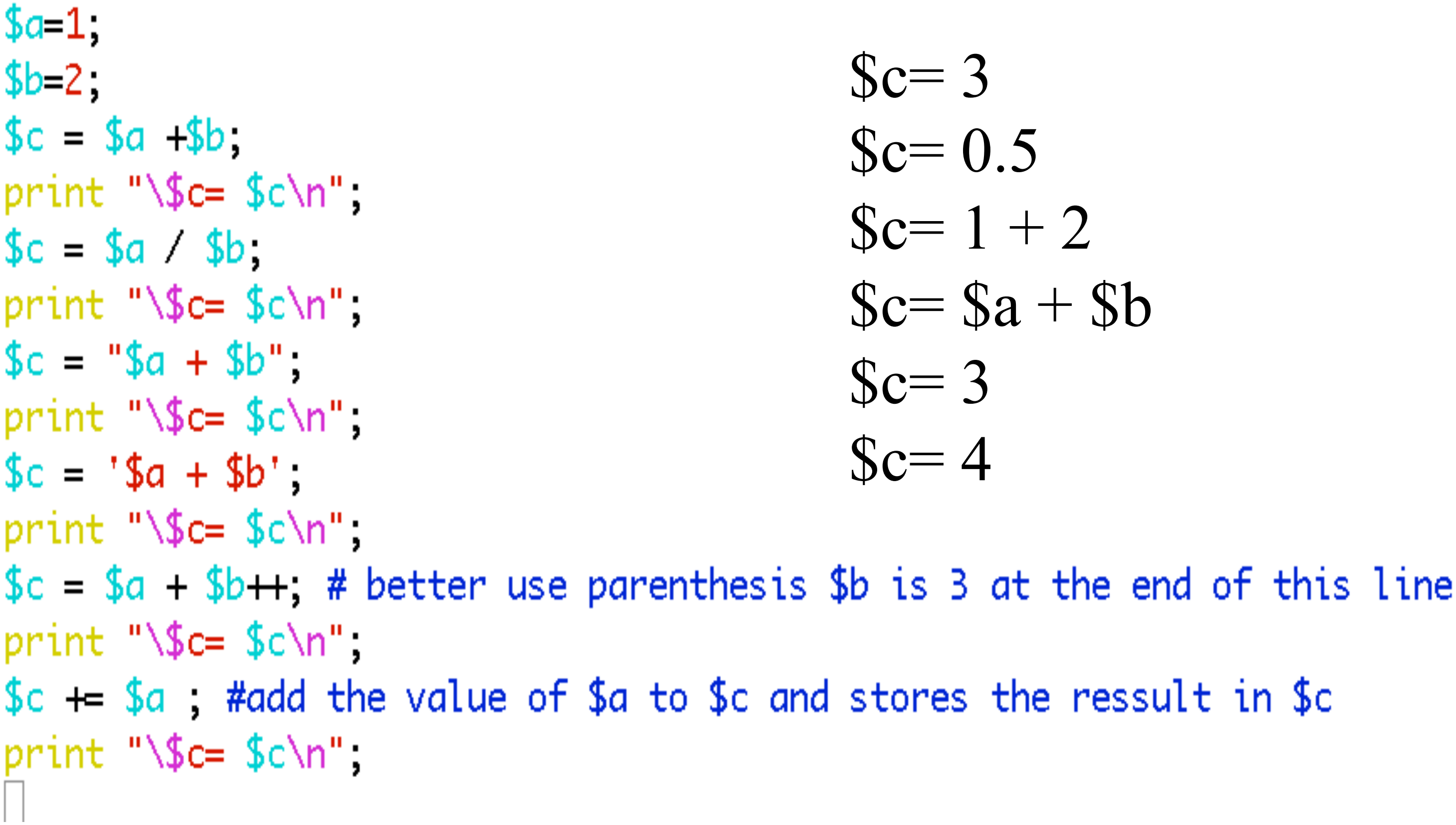
```
#!/usr/bin/perl -w
use strict;
# This is a Hello World program in Perl using a variable
# and input
my $who;                  # Declare variable.
  $who = "world";          # Assign variable.
  print "please enter your name: ";
  chomp ($who = <>);
  print "\nHello, $who!\n";  # Print result.
```

Old assignments:

4) If $a = 2 and $b=3, what is the type and values of the scalar stored in $c after each of the following statements:

```
$c = $a + $b;
$c = $a / $b;
$c = $a . $b;
$c = "$a + $b";
$c = '$a + $b';
```

First make a guess, then test your prediction using a script.



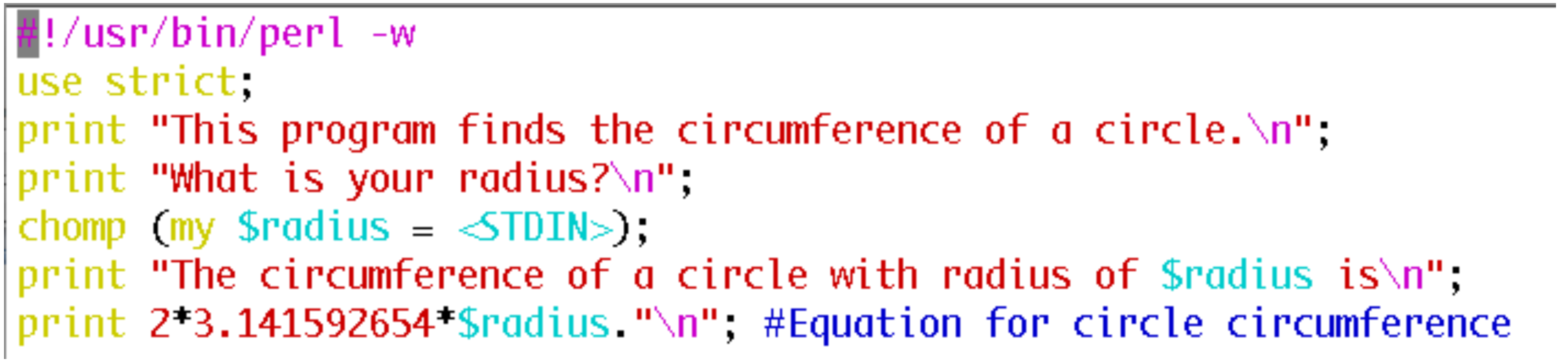
```
$a=1;
$b=2;
$c = $a +$b;
print "\$c= $c\n";
$c = $a / $b;
print "\$c= $c\n";
$c = "$a + $b";
print "\$c= $c\n";
$c = '$a + $b';
print "\$c= $c\n";
$c = $a + $b++; # better use parenthesis $b is 3 at the end of this line
print "\$c= $c\n";
$c += $a ; #add the value of $a to $c and stores the ressult in $c
print "\$c= $c\n";
```

```
$c= 3
$c= 0.5
$c= 1 + 2
$c= $a + $b
$c= 3
$c= 4
```

Run and discuss test2.pl

2) Why does the first of these get along without `chomp ($line);` (chomp is a built-in command in Perl to remove a trailing newline, if any, from a string).

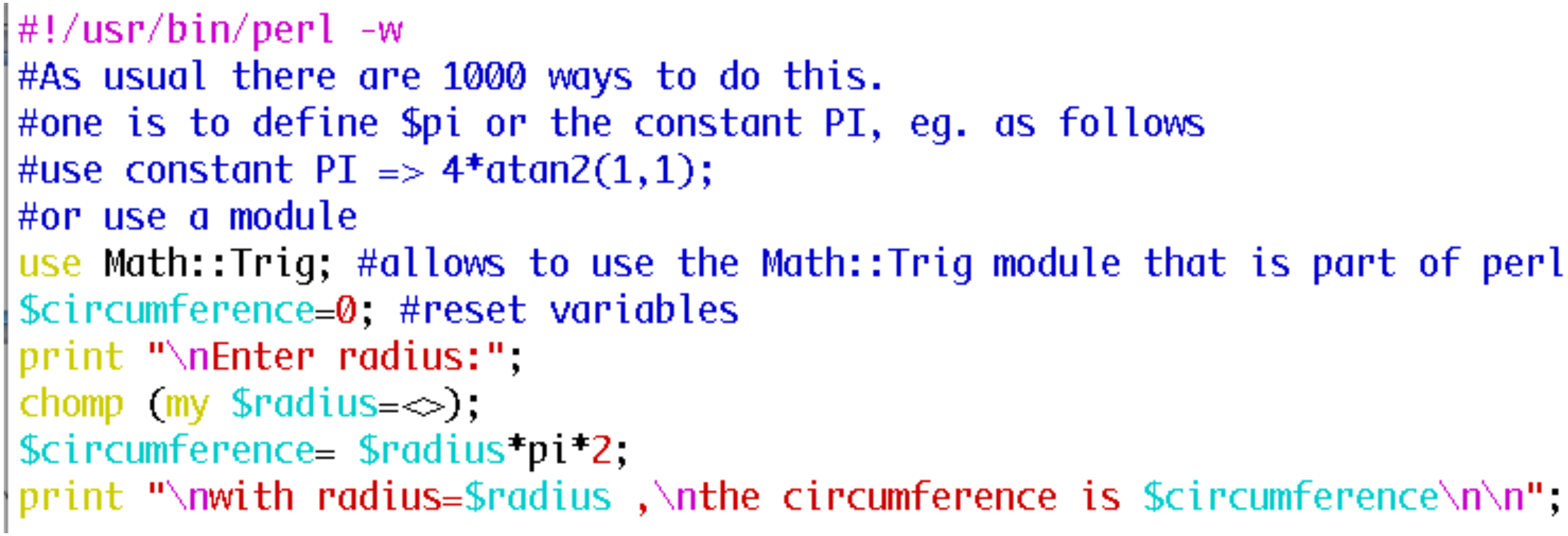
3) Write a short Perl script that calculates the circumference of a circle given a radius provided by the user.



```
#!/usr/bin/perl -w
use strict;
print "This program finds the circumference of a circle.\n";
print "What is your radius?\n";
chomp (my $radius = <STDIN>);
print "The circumference of a circle with radius of $radius is\n";
print 2*3.141592654*$radius."\n"; #Equation for circle circumference
```

2) Why does the first of these get along without `chomp ($line);` (chomp is a built-in command in Perl to remove a trailing newline, if any, from a string).

3) Write a short Perl script that calculates the circumference of a circle given a radius provided by the user.



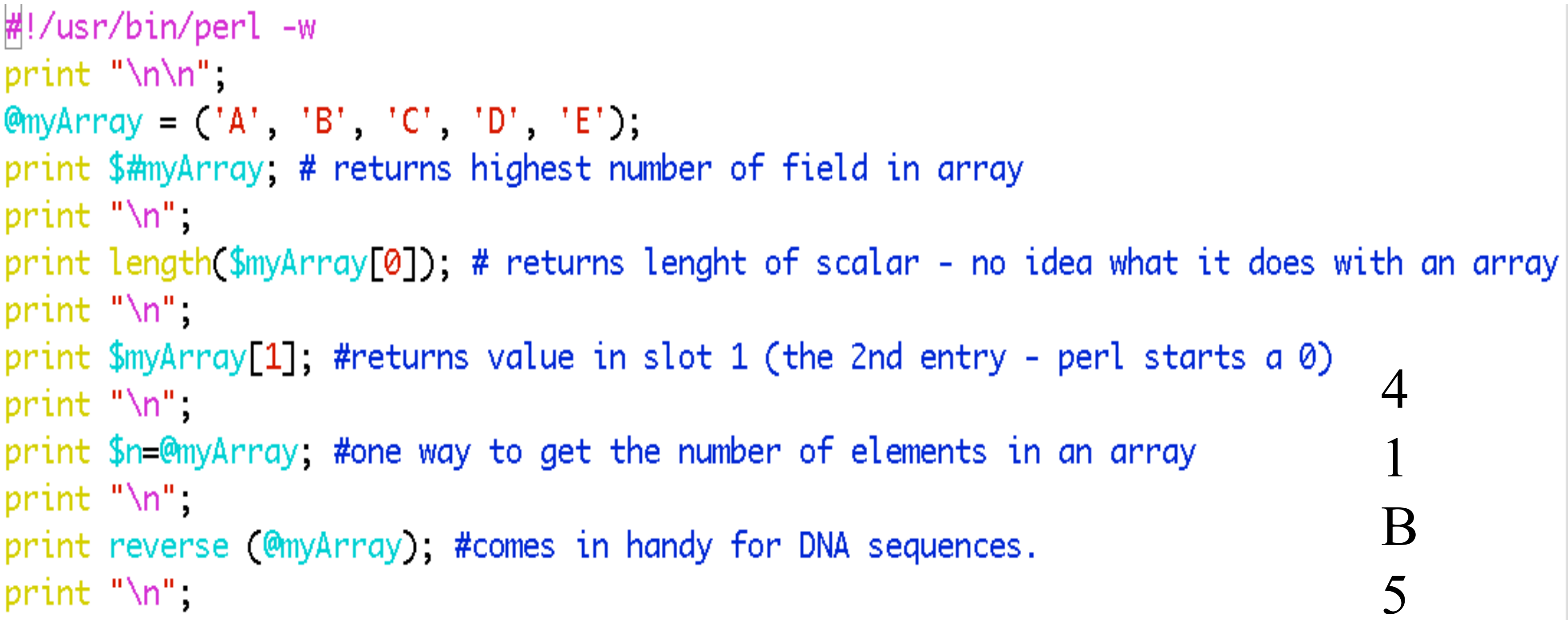
```
#!/usr/bin/perl -w
#As usual there are 1000 ways to do this.
#one is to define $pi or the constant PI, eg. as follows
#use constant PI => 4*atan2(1,1);
#or use a module
use Math::Trig; #allows to use the Math::Trig module that is part of perl
$circumference=0; #reset variables
print "\nEnter radius:";
chomp (my $radius=<>);
$circumference= $radius*pi*2;
print "\nwith radius=$radius ,\nthe circumference is $circumference\n\n";
```

The best way to find which module to use is google. You can search core modules at http://perldoc.perl.org/search.html?

From Wednesday:

For the following array declaration @myArray = ('A', 'B', 'C', 'D', 'E'); what is the value of the following expressions:

```
$#myArray
length(@myArray)
$myArray[1]
$n=@myArray
reverse (@myArray)
```



```
#!/usr/bin/perl -w
print "\n\n";
@myArray = ('A', 'B', 'C', 'D', 'E');
print $#myArray; # returns highest number of field in array
print "\n";
print length($myArray[0]); # returns lenght of scalar - no idea what it does with an array
print "\n";
print $myArray[1]; #returns value in slot 1 (the 2nd entry - perl starts a 0)
print "\n";
print $n=@myArray; #one way to get the number of elements in an array
print "\n";
print reverse (@myArray); #comes in handy for DNA sequences.
print "\n";
```

```
4
1
B
5
EDCBA
```

Run and discuss myArray.pl

Assignment for Monday (class 4)

1) Write a 2 sentence outline for your student project
2) Read chapter P5 and P12 conditional statements and on “for, foreach, and while” loops.
   http://korflab.ucdavis.edu/Unix_and_Perl/unix_and_perl_v2.3.3.pdf

Background:

```
@a=(0..50);
```

# This assigns numbers from 0 to 50 to an array,

# so that $a[0] =0; $a[1] =1; $a[50] =50

3) Write perl scripts that add all numbers from 1 to 50. Try to do this using at least two different control structures.
4) Create a program that reads in a sequence stored in a file handed to the program on the command line and determines GC content of a sequence. Use class3.pl as a starting point.

5)

For the following array declaration

@myArray = ('A', 'B', 'C', 'D', 'E');

what is the value of the following expressions:

```
$#myArray
length(@myArray)
$myArray[1]
$n=@myArray
reverse (@myArray)
```

6)

Create a program that reads in a sequence stored in a file handed to the program on the command line and determines GC content of a sequence.

Details in class3.pl. See the challenge!

Go through class3.pl script.

Coding sequences example:
http://www.ncbi.nlm.nih.gov/protein/AEE95833.1
Ctrl click open CDS in new window.

If time do
chomp_example.pl (also in scripts)