Some useful bits&pieces that every Perl programmer should know

Strictness

- Perl breaks a number of the ‘‘golden rules’’ of the traditional programming language
  - allows variables to be used before they are declared
  - Subroutines can be invoked before they are defined
- All variables are global by default
  - the use of my variables turns a global variable into a lexical.
  - By default, the use of my variables is optional
    - However, it is possible to have perl insist on the use of my variables, making their use mandatory.

Strictness

- This insistence is referred to as strictness, and is switched on by adding the following line to the top of a program:

  use strict;

- This is a directive that
  - tells perl to insist on all variables being declared before they are used,
  - all subroutines be declared (or defined) before they are invoked.

Strictness

- As programs get bigger, they become harder to maintain.
  - The use of use strict helps keep things organised and reduces the risk of errors being introduced into programs.
    - Anything that helps reduce errors is a good thing, even if it is sometimes inflexible.
- Thinking about the scope of variables, and using my and our to control the visibility of variables, becomes important as a program grows in size.

Strictness

- When strictness is enabled, perl checks the declaration of each of a program's variables before execution occurs.
- Consider the following program:

```
#! /usr/bin/perl -w
# bestrict - demonstrating the effect of strictness.
use strict;
$message = "This is the message.\n";
print $message;
```

- In the code, $message scalar is not declared as a lexical (my) or global (our) variable.

Results from bestrict

- When an attempt is made to execute the bestrict program, perl complains that the strictness rules have been broken:

```
>perl -w strict.pl
    Global symbol "$message" requires explicit package name at bestrict line 7.
    Global symbol "$message" requires explicit package name at bestrict line 9.
    Execution of bestrict aborted due to compilation errors.
>
```

- These ‘‘compilation errors’’ are fixed by declaring the $message scalar as a my variable

```
my $message = "This is the message.\n";
```

Results from bestrict

```
# bestrict - demonstrating the effect of strictness.
use strict;
my $message = "This is the message.\n";
print $message;
```

```
>perl -w mystrict.pl
This is the message.
>Exit code: 0
```

use subs

- Perl provides the use subs directive that ...
  - can be used in combination with use strict
    - to declare a list of subroutines at the top of the program

  For example:

```
use strict;
use sub qw( drawline biod2mysql);
```

- The use subs directive declares a list of subroutine names that are later defined somewhere in the program's disk-file.

Unless you have a really good reason not to, always switch on strictness at the top of your program

Perl One-Liners

- Perl usually starts with the following line:

  >#! /usr/bin/perl –w

  w: warning

  – instructs perl to warn the programmer when it notices any dubious programming practices

- -e switch checks whether a module installed correctly or not:

  >perl -e 'use ExampleModule'

  e: execute

  – instructs perl to execute the program statements included within the single quotes

Perl One-Liners

- Other examples :

  >perl -e 'print "Hello from a Perl one-liner.\n";‘

  – a single line of Perl code is provided to perl to execute immediately from the command-line

  >perl -e 'printf "%0.2f\n", 30000 * .12;'

  – turns perl into a simple command-line calculator

  - The printf subroutine is a variant of the more common print, and prints to a specified format.

- The ability to use the –e switch on the command-line in this way creates what is known in the perl world as...

  a one-liner.

Perl One-Liners: Equivalents

- Another useful switch is –n, which, when used with –e, treats the one-liner as if it is enclosed with a loop.
- Consider this one-liner:

  >perl -ne 'print if /ctgaatagcc/;' embl.data

  which is equivalent to the following program statement:

```
while ( <> )
{
        print if /ctgaatagcc/;
}
```

Perl One-Liners: Equivalents

- When the one-liner is executed, the following output is generated:

  attgtaatat ctgaatagcc actgattttg taggcacctt tcagtccatc tagtgactaa

- Same function can also be implemented using grep:

  >grep 'ctgaatagcc' embl.data

- When the -n switch is combined with -p, the loop has a print statement added to the end.

Perl One-Liners: More Options

- Here is a one-liner that prints only those lines from the embl.data disk-file that do not end in four digits:

```
>perl -npe 'last if /\d{4}$/;' embl.data
```

- The above one-liner is equivalent to this program:

```
while ( <> )
{
        last if /\d{4}$/;
}
continue {
        print $_;
}
```

- This one-liner is a little harder to do with grep.

```
> grep -v '[0123456789][0123456789][0123456789][0123456789]$' embl.data
```

Perl One-Liners: More Options

- When executed, the following output is produced:

```
gccacagatt acaggaagtc atatttttag acctaaatca ctatcctcta tctttcagca      60
agaaaagaac atctacttgg tttcgttccc tatccaagat tcagatggtg aaacgagtga     120
tcatgcacct gatgaacgtg caaaaccaca gtcaagccat gacaaccccg atctacagtt     180
tgatgttgaa actgccgatt ggtacgccta cagtgaaaac tatggcacaa gtgaagaaaa     240
acgctttgtt aagtttgttg caactcaaat tgacgagctt aaatcacgct acaagggtgc     300
agagatttac ctgatacgga atgaactcga ttattggttg tttagcccta aagatggtcg     360
tagattcagc cctgactaca tgctgatcat taatgatgct gaaaatagtg aaatgtacta     420
tcaatgctta attgagccta aaggtggtca tttgcttgaa aaggatactt ggaaagagga     480
agtattgatt agtttggatg atgaaagcca aattgttttt gatgcagatc aagatgattc     540
acaaaactat gttgagttct taaatgaagt taaagagcat ggttataagg aagttaaatg     600
tttaggcttc aaattctaca ataccgaacc acgatctgaa tcagattttg ctattgattt     660
tcacaatagg atgccgagtt aatctaggtt tctcactgta acctgctgat tattatcttt     720
ttgtgaagtt gctacataat attgttttta agatcattga ataaaaaagc cagctctata     780
ctggcttttt tattgcttaa aattatattc cgatgcttgg tcaaaactgc aagtatgcag     840
tcttgaccag gcatctaggg gtcgtctcag aattcggaaa ataaagcacg ctaaggcgta     900
gtcaccccgt gactcccccg cgccgatgca gcgagcttcg ttccgtcttg cagtgacgca     960
```

Running Other Programs From Perl

- There are two main ways to do this:
  - By invoking the program in such a way that after execution, the calling program can determine whether the called program successfully executed.
    - Perl's in-built system subroutine behaves in this way
  - By invoking the program in such as way that after execution, any results from the called program are returned to the calling program.
    - Perl's *backticks* and qx// operator behaves in this way
- Following example program demonstrates each of these mechanisms by invoking the DOS utility program, dir, that lists disk-files in the current directory

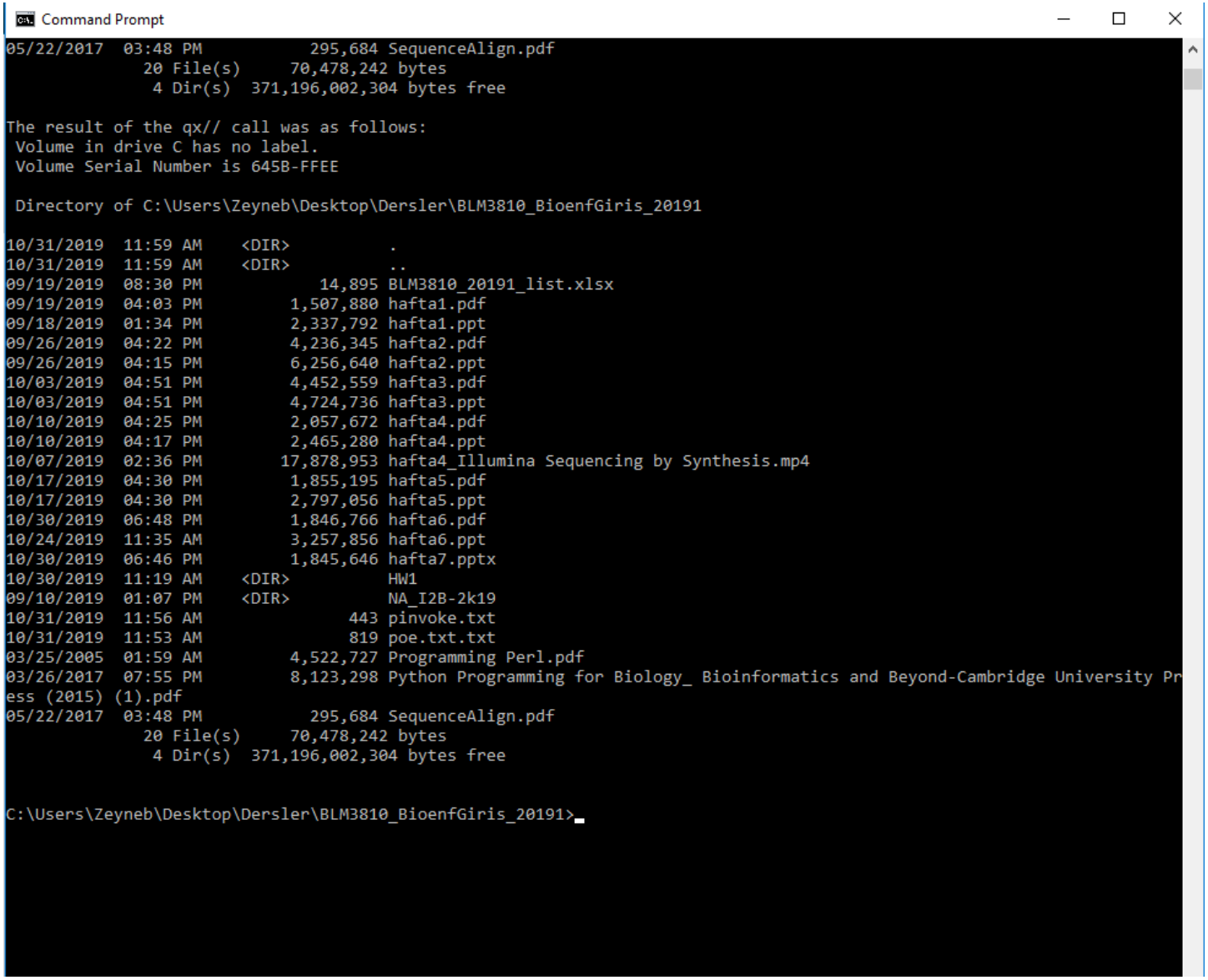
Running Other Programs From Perl

```
#! /usr/bin/perl –w
# pinvoke - demonstrating the invocation of other programs
# from Perl.
use strict;

my $result = system("dir *.*" );
print "The result of the system call was as follows:\n$result\n";

$result = `dir *.*`;  # warning: $result = 'dir *.*'; will not work
print "The result of the backticks call was as follows:\n$result\n";

$result = qx/dir *.*/;
print "The result of the qx// call was as follows:\n$result\n";
```

Running Other Programs From Perl

- The invocation of system results in the dir program executing. Any output from dir is displayed on screen (STDOUT) as normal.
  - As dir executed successfully, a value of zero is returned to pinvoke and assigned to the $result scalar.
    - The $result scalar is then printed to STDOUT as part of an appropriately worded message.
  - If the dır program fails, the $result scalar is set to −1.
- Perl's backticks (` and `) also execute external programs from within Perl.
  - The results from the program are captured and returned to the program.
    - In the pinvoke program, the results are assigned to the $result scalar, and then printed to STDOUT as part of an appropriately worded message.
- The qx// operator is another way to invoke the backticks behaviour:
  - it works exactly the same way as backticks

Results from pinvoke



Recovering from Errors

- It is not always appropriate to die whenever an error occurs.
  - Sometimes it makes more sense to spot, and then recover from, an error.
- This is referred to as exception handling.
- Consider the following code:

```
my $first_filename = "itdoesnotexist.txt";

open FIRSTFILE, "$first_filename"
        or die "Could not open $first_filename. Aborting.\n";
```

Recovering from Errors

- Executing the code

  >perl -w errorrec.pl

  Name "main::FIRSTFILE" used only once: possible typo at errorrec.pl line 4.

  Could not open itdoesnotexist.txt. Aborting.

  >Exit code: 2

- This assumes that the itdoesnotexist.txt disk-file does not exist.
  - The program terminates as a result of the invocation of die.
- It is possible to protect this code by enclosing it within an eval block.

Recovering from Errors

- The in-built eval subroutine takes a block of code and executes (or evaluates it).
- When perl invokes eval, anything that happens within the eval block that would usually result in a program terminating is caught by perl and does not terminate the program.
- Here's exception handling by an eval block:

```
eval {
    my $first_filename = "itdoesnotexist.txt";
    open FIRSTFILE, "$first_filename"
            or die "Could not open $first_filename.
Aborting.\n";
    };
```

Recovering from Errors

- If die is invoked within an eval block, the block immediately terminates and perl sets the internal $@ variable to the message generated by die.
- After the eval block, it is a simple matter to check the status of $@ and act appropriately.
- Adding the following if statement after the above eval block:

```
if ( $@ )
{
    print "Calling eval produced this message: $@";
}
```

prints the following message to STDOUT when the itdoesnotexist.txt disk-file does not exist:

```
Calling eval produced this message: Could not open itdoesnotexist.txt. Aborting.
```

Use eval to protect potentially erroneous code

Sorting

- Perl provides powerful in-built support for sorting.
  - sort and reverse,
    - can be used to sort lists of strings or numbers into ascending order, descending order or any other customized order.
- Following examples demonstrate usage of sort and reverse.
  - In the following program a list of four short DNA sequences is assigned to an array called @sequences, which is then printed to STDOUT

Sorting

```
#! /usr/bin/perl -w
# sortexamples - how Perl's in-built sort subroutine works.

use strict;

my @sequences = qw( gctacataat attgttttta aattatattc cgatgcttgg );
print "Before sorting:\n\t-> @sequences\n";

my @sorted = sort @sequences;
my @reversed = sort { $b cmp $a } @sequences;
my @also_reversed = reverse sort @sequences;
print "Sorted order (default):\n\t-> @sorted\n";
print "Reversed order (using sort { \$b cmp \$a }):\n\t-> @reversed\n";
print "Reversed order (using reverse sort):\n\t-> @also_reversed\n";
```

Results from sort examples ...

```
>perl -w sort1.pl
   Before sorting:
      -> gctacataat attgttttta aattatattc cgatgcttgg
   Sorted order (default):
      -> aattatattc attgttttta cgatgcttgg gctacataat
   Reversed order (using sort { $b cmp $a }):
      -> gctacataat cgatgcttgg attgttttta aattatattc
   Reversed order (using reverse sort):
      -> gctacataat cgatgcttgg attgttttta aattatattc
>Exit code: 0
```

Sorting

- my @sorted = sort @sequences;
  - created by invoking the in-built sort subroutine
  - sorts the array alphabetically in ascending order (from ‘‘a’’ through to ‘‘z’’).
- my @reversed = sort { $b cmp $a } @sequences;
  - also created by invoking the in-built sort subroutine
  - sorts the array alphabetically in descending order (from ‘‘z’’ through to ‘‘a’’).
- my @also_reversed = reverse sort @sequences;
  - created by first sorting the array, then reversing the sorted list by invoking the in-built reverse subroutine.
    - Note that the reverse subroutine reverses the order of elements in a list; it does not sort in reverse order.

Another Sorting Example

- It is also possible to sort in numerical order using sort
  - The following program defines a list of chromosome pair numbers and assigns them to another array, called @chromosomes, and the array is then printed to STDOUT:

```
my @chromosomes = qw( 17 5 13 21 1 2 22 15 );
print "Before sorting:\n\t-> @chromosomes\n";

@sorted = sort { $a <=> $b } @chromosomes;
@reversed = sort { $b <=> $a } @chromosomes;
print "Sorted order (using sort { \$a <=> \$b }):\n\t-> @sorted\n";
print "Reversed order (using sort { \$b <=> \$a }):\n\t->
@reversed\n";
```

And its results ...

```
>perl -w sort2.pl
  Before sorting:
  -> 17 5 13 21 1 2 22 15
  Sorted order (using sort { $a <=> $b }):
  -> 1 2 5 13 15 17 21 22
  Reversed order (using sort { $b <=> $a }):
  -> 22 21 17 15 13 5 2 1
>Exit code: 0
```

- To learn more, use the following command-line to read the on-line documentation for sort that comes with Perl:

```
>perldoc -f sort
>man sort
```

The sortfile Program

- The following program takes any disk-file and sorts the lines in the disk-file in ascending order

```
#! /usr/bin/perl -w
# sortfile - sort the lines in any file.
use strict;
my @the_file;
while ( <> )
    {
        chomp;
        push @the_file, $_;
    }
my @sorted_file = sort @the_file;
foreach my $line ( @sorted_file )
    {
        print "$line\n";
    }
```

The sortfile Program

- Before running sortfile

•We loved with a love that was more than love.

•All that we see or seem is but a dream within a dream.

•Those who dream by day are cognizant of many things which escape those who dream only by night.

•I became insane, with long intervals of horrible sanity.

•Deep into that darkness peering, long I stood there, wondering, fearing, doubting, dreaming dreams no mortal ever dared to dream before.

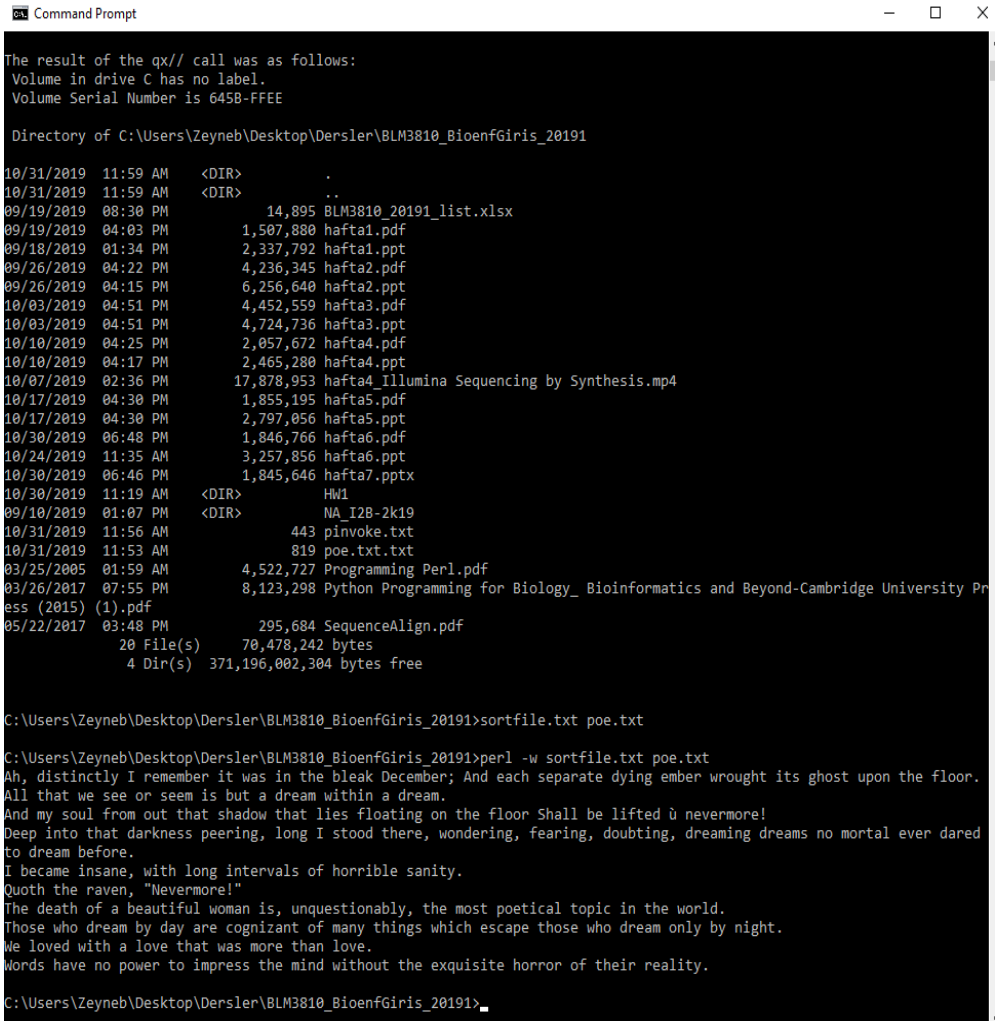
•Quoth the raven, "Nevermore!”

•And my soul from out that shadow that lies floating on the floor Shall be lifted ó nevermore!

•The death of a beautiful woman is, unquestionably, the most poetical topic in the world.

•Words have no power to impress the mind without the exquisite horror of their reality.

•Ah, distinctly I remember it was in the bleak December; And each separate dying ember wrought its ghost upon the floor.



- Same thing could also be done by using Linux sort utility in command-line:
  sort sort.data

Take the time to become familiar with the utilities included in the operating system

HERE Documents

- Consider the requirement to display the following text on screen in exactly the format shown from within a program:

```
Shotgun Sequencing

This is a relatively simple method of reading
a genome sequence.
It is "simple" because
it does away with the need to locate
individual DNA fragments on a map before
they are sequenced.

The Shotgun Sequencing method relies on
powerful computers to assemble the finished
sequence.
```

Without HERE Documents

- Could be done by using a sequence of print statements as follows:

```
print "Shotgun Sequencing\n\n";
print "This is a relatively simple method of reading\n";
print "a genome sequence. It is "simple" because\n";
print "it does away with the need to locate\n";
print "individual DNA fragments on a map before\n";
print "they are sequenced.\n\n";
print "The Shotgun Sequencing method relies on\n";
print "powerful computers to assemble the finished\n";
print "sequence.\n";
```

Output

```
>perl -w shotgun1.pl
      Shotgun Sequencing

      This is a relatively simple method of reading
      a genome sequence. It is "simple" because
      it does away with the need to locate
      individual DNA fragments on a map before
      they are sequenced.

      The Shotgun Sequencing method relies on
      powerful computers to assemble the finished
      sequence.
>Exit code: 0
```

With HERE Documents

- A better way to do this is to use Perl's HERE document mechanism

```
my $shotgun_message = <<ENDSHOTMSG;
Shotgun Sequencing

This is a relatively simple method of reading
a genome sequence. It is "simple" because
it does away with the need to locate
individual DNA fragments on a map before
they are sequenced.

The Shotgun Sequencing method relies on
powerful computers to assemble the finished
sequence.
ENDSHOTMSG

print $shotgun_message;
```

Output

```
>perl -w shotgun2.pl
      Shotgun Sequencing

      This is a relatively simple method of reading
      a genome sequence. It is "simple" because
      it does away with the need to locate
      individual DNA fragments on a map before
      they are sequenced.

      The Shotgun Sequencing method relies on
      powerful computers to assemble the finished
      sequence.
>Exit code: 0
```

Even Better HERE Documents

- It is possible to improve previous program by removing the need for the $shotgun message scalar and printing the HERE document directly, as follows:

```
print <<ENDSHOTMSG;
Shotgun Sequencing

This is a relatively simple method of reading
a genome sequence. It is "simple" because
it does away with the need to locate
individual DNA fragments on a map before
they are sequenced.

The Shotgun Sequencing method relies on
powerful computers to assemble the finished
sequence.
ENDSHOTMSG
```

Output

```
>perl -w shotgun3.pl
      Shotgun Sequencing

      This is a relatively simple method of reading
      a genome sequence. It is "simple" because
      it does away with the need to locate
      individual DNA fragments on a map before
      they are sequenced.

      The Shotgun Sequencing method relies on
      powerful computers to assemble the finished
      sequence.
>Exit code: 0
```

HERE Documents

- HERE documents are useful,
  - especially when it comes to dynamically producing HTML documents.
    - This use of HERE documents will be discussed later

Downloading Datasets

- Downloading from the Web
  - a highly interactive mechanism
  - useful for downloading individual data-files
  - cumbersome for downloading large number of data-files
- Some technologies allow the easy integration of data sources across the Internet.
- However, it is often convenient to download frequently used datasets and store them locally.

Downloading Datasets

- The advantages of downloading and storing datasets locally :
  - Ease of access
    - accessing data-files on a local hard disk easier than writing an interface routine to download them as needed from a – possibly congested – location on the Internet.
  - Speed
    - Local hard-disk access, even over a shared file system, is usually faster than operating through external networks to Internet locations.
      - When the processing is performed locally, it may be possible to allocate extra computational resources to the analysis.

Downloading Datasets

- Reliability
  - Accessing local hard-disk copies of data-files is more reliable than network connections and WWW servers.
- Stability
  - If the data changes frequently, it is often helpful to ‘‘freeze’’ it by downloading a copy and using it locally until all analyses are completed.
- Flexibility
  - Often the search facilities that exist on the WWW lack certain required functionality.
- Security
  - Data or results are often sensitive, and sending them to a remote, third-party Internet site may be unacceptable.

Downloading Datasets

- The disadvantages of downloading and storing datasets locally :
  - Stale data
    - The local copy is a one-time ‘‘snapshot’’ of the dataset at a particular point in time.
      - At some stage, it will need to be updated or replaced by newer data.
  - Storage
    - The dataset has to be stored somewhere, and some datasets can be large.
      - The Protein Databank (PDB) is close to four gigabytes, and the PDB is one of the smaller databases!
        - Consequently, storing multiple copies of the PDB is often impractical.
  - Performance
    - The centralised specialist services accessible from the WWW are often configured with dedicated parallelised systems, designed to service requests as quickly as possible.
      - If the stored dataset is designed with such systems in mind, it is unlikely that a local system will be able to match this advanced processing capability.
        - Consequently, some analyses may be slower locally when compared to those performed on the WWW.

Downloading Datasets

- can be accomplished in a number of ways:
  - by using established sequence analysis programs, such as EMBOSS
    - http://emboss.sourceforge.net/
    - have specific methods for performing downloads.
      - Typically, datasets are accessed via a standard network connection to remote Internet sites.
      - Frequently, downloads are automated to occur at regular intervals.
  - The wget program, included with most Linux systems, can be used to do just this.
    - wget is an excellent example of GNU software as distributed by the Free Software Foundation.
      - https://www.gnu.org/software/wget/
      - http://gnuwin32.sourceforge.net/packages/wget.htm

Using wget to download PDB data-files

- To download a single data-file via anonymous FTP, simply provide the URL of the data-file required after the wget command.
  - For example, to download the two Protein Data Bank (PDB) structures, use these commands:

```
mkdir structures
cd structures
wget ftp://ftp.rcsb.org/pub/pdb/data/structures/all/pdb1m7t.ent.Z
wget ftp://ftp.rcsb.org/pub/pdb/data/structures/all/pdb1lqt.ent.Z
```

Mirroring a dataset

- wget can be used to mirror datasets.
  - to download the entire PDB, which is four gigabytes of data, stored in over 18000 data-files:

    wget --mirror ftp://ftp.rcsb.org/pub/pdb/data/structures/all/pdb
- Such a command should be invoked only when there is a real need to mirror the PDB.
  - a download of this size takes a considerable amount of time and disk space.
    - If such a need exists, once complete, another invocation of the same command downloads only additions or updates to the PDB since the last mirror.

Smarter mirroring

- The wget command in the previous slide results in a deep directory tree.
  - The actual data-files are found in

    structures/ftp.rcsb.org/pub/pdb/data/structures/all/pdb
- Such a deep directory structure can be very inconvenient and frustrating to navigate.
- Following wget invocation can help with this problem:

```
wget --output-file=log --mirror --http-user=anonymous          \
     --http-passwd=email@where.ever.net                        \
     --directory-prefix=structures/mmCIF                       \
     --no-host-directories                                     \
     --cut-dirs=6 ftp://ftp.rcsb.org/pub/pdb/data/structures/all/pdb
```

Smarter mirroring

- The wget command sets a number of options:
  - --output-file
    - a disk-file into which any message produced by wget is placed.
  - --mirror
    - turns on mirroring.
  - --http-user
    - sets the web username to use (if needed).
  - --http-passwd
    - sets the web password to use (if needed).
  - --directory-prefix
    - the place to put the downloaded data-files.
  - --no-host-directories
    - the instruction not to use the hostname when creating a mirrored directory structure, which is the ‘‘ftp.rcsb.org’’ part.
  - --cut-dirs
    - instructs wget to ignore the indicated number of directory levels.
      - In the previous example, six directory levels are to be ignored, that is, the ‘‘pub/pdb/data/structures/all/pdb’’ part.

Downloading a subset of a dataset

- On many occasions, the entire contents of an FTP site might not be required
- wget can fetch a specific data-file, placing it in the current directory.
  - Use a command similar to this:

    wget ftp://beta.rcsb.org/pub/pdb/uniformity/data/mmCIF/all/1ger.cif.Z
- While multiple URLs to data-files can be supplied on the command-line (separated by spaces), it is often more convenient to place the URLs in a data-file and use the ‘‘--input file=’’ switch.

Downloading a subset of a dataset

- The pdbselect program takes the PDB-Select list produced in the Non-Redundant Datasets (discusssed later), builds a list of URLs, removes the duplicates and then downloads them:

```
#! /usr/bin/perl
# pdbselect <list of PDB IDs> - a program that takes a list of PDB ID
# codes; build a list of URLs for them;
# and automates the downloading of them
# using 'wget'.
use strict;
my $Base_URL = "ftp://ftp.rcsb.org/pub/pdb/data/structures/all/pdb";
my $Output_Dir = "structures";
open URL_LIST, ">pdb_select_url.lst"
```

Downloading a subset of a dataset

```
or die "Cannot write to file: ’pdb_select_url.lst’\n";
while ( <> )
{
if ( /Failed/ )
{
next;
}
s/ //g;
my ( $Structure, $Length ) = split ( ":", $_ );
my ( $ID, $Chain ) = split ( ",", $Structure );
$ID =~ tr /[A-Z]/[a-z]/;
print URL_LIST "$Base_URL/pdb$ID.ent.Z\n";
}
```

Downloading a subset of a dataset

```
close URL_LIST;
if ( !-e $Output_Dir )
{
system "mkdir $Output_Dir";
}
if ( !-w $Output_Dir or !-d $Output_Dir )
{
die "ERROR: Cannot access directory: '$Output_Dir'. Exiting\n";
}
system "sort -u pdb_select_url.lst > unique_urls.lst";
system "rm $Output_Dir/* > /dev/null";
system "wget --output-file=log --http-user=anonymous                \
                --http-passwd=email\@some.where.net                 \
                --directory-prefix=$Output_Dir -i unique_urls.lst";
```

Downloading a subset of a dataset

- This program takes a list of PDB ID codes from STDIN and downloads them from the URL specified in the scalar variable $Base_URL6.
  - Those structures marked as Failed are skipped, otherwise a URL is built and written to the pdb_select_url.lst file.
  - Duplicate structures are filtered out using the sort -u operating system utility.
  - Error-checking is performed to see if the output directory exists (otherwise it is created) and that the directory can be accessed.
    - All previous files in it are then deleted using the rm system call.
  - Finally, wget is invoked with the list of URLs.

Download a dataset only when absolutely necessary.

Consider the implications of doing so first.

The Protein Databank

- The similarity between the amino acid sequence of a ‘‘new’’ protein and one previously characterized can give an indication of the function of the new protein.
  - Sequence search algorithms assume some groups of amino acids have similar functional roles and consequently, occur in both sequences.
  - It is also assumed that these amino acids have similar local structures (the amino acids arrangement in space).
- It is these structures that determine the function of a protein.
  - Although these assumptions are useful as a working model.

The Protein Databank

- Determining the detailed structure of a protein is more difficult than finding a DNA or amino acid sequence.
- The aim of some structural studies
  - to know how the protein (or other biomolecule) ‘‘does what it does’’
  - to alter its function.
    - for example, to design a small molecule that binds to the protein, more commonly known as a ‘‘drug’’.

Determining Biomolecule Structures

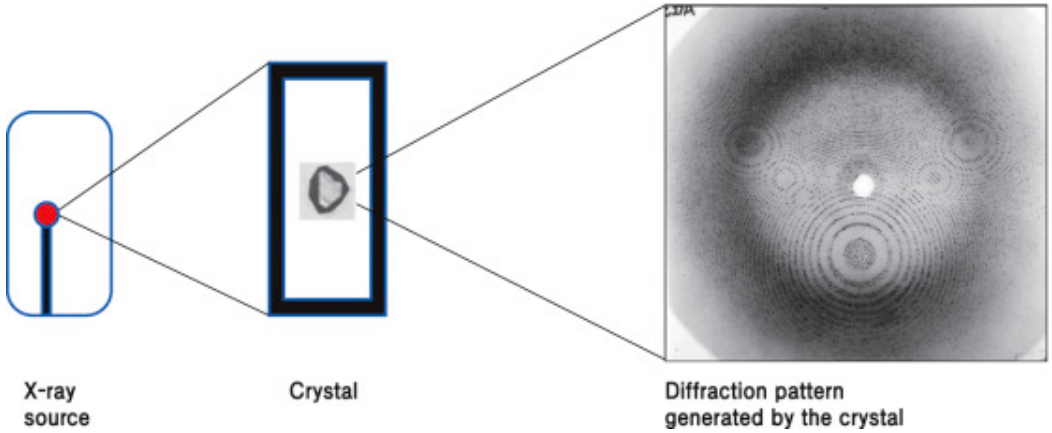
- There are many methods used for gaining information about the structure of a biomolecule
- The two major methods by which the location of atoms can be determined to a useful accuracy
  - X-Ray Crystallography
  - Nuclear Magnetic Resonance (NMR).

X-Ray Crystallography

- a technique for determining the three-dimensional structure of molecules,
  - including complex biological macromolecules such as proteins and nucleic acids.
- a powerful tool in the elucidation of the three-dimensional structure of a molecule at atomic resolution.
- Data is collected by diffracting X-rays from a single crystal, which has an ordered, regularly repeating arrangement of atoms.
- Based on the diffraction pattern obtained from X-ray scattering off the periodic assembly of molecules or atoms in the crystal, the electron density can be reconstructed.

X-Ray Crystallography

- a tool used for determining the atomic and molecular structure of a crystal.
- The underlying principle is that the crystalline atoms cause a beam of X-rays to diffract into many specific directions.

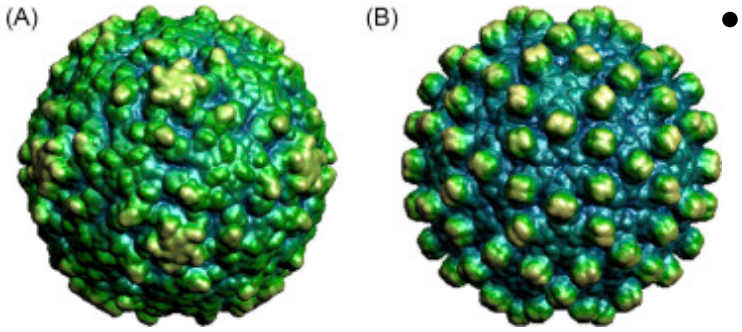


- By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a 3D picture of the density of electrons within the crystal.

X-Ray Crystallography

- From this electron density image, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder, and various other information.
  - The method revealed the structure and function of many biological molecules, including vitamins, drugs, proteins, and nucleic acids, such as DNA.
    - Note that the double helix structure of DNA discovered by James Watson and Francis Crick was revealed by X-ray crystallography.
- Recent advances in image reconstruction technology have made X-ray crystallography amenable to the structural analysis of much larger complexes, such as virus particles.

X-Ray Crystallography



- Viral capsid structure obtained by X-ray crystallography.
  - (A) Poliovirus capsid with T=3 symmetry.
  - (B) Hepatitis B virus capsid with T=4

- The major shortcomings of X-ray crystallography
  - it is difficult to obtain a crystal of virus particles, which is a prerequisite for X-ray crystallography.
  - X-ray crystallography generally requires placing the samples in nonphysiological environments, which can occasionally lead to functionally irrelevant conformational changes.

Nuclear magnetic resonance

- In NMR, no crystals are used in the process, and the protein remains in solution throughout the entire experiment.
- An intense and very linear magnetic field aligns the atomic nuclei of the protein into one of two spin states.
- A series of radio frequency pulses is used to perturb these by ‘‘flipping’’ some of the nuclei from one spin state to the other.
- As the total amount of energy absorbed is low, the protein remains undamaged and functions as normal.

Nuclear magnetic resonance

- Eventually, the ‘‘flipped’’ spin state of the nuclei realigns to the normal state, emitting a radio frequency pulse as it does so.
- The timing of this re-emission of energy is determined by the electronic environment in which the nucleus is embedded.
- A feature of this environment is the lectrostatic shielding effects of the surrounding nuclei.
- The nuclei, in addition to the bonds linking them, can be identified by their spin decay properties.

Nuclear magnetic resonance

- A problem with NMR methods is the size of the proteins that can be studied.
  - Using current techniques, this equates to a maximum of 200 amino acids.
    - This is low compared to the many hundreds of amino acids that can be studied using X-Ray Crystallography.
- The X-Ray Crystallography and NMR systems are complementary in many respects, as both determine, to a high accuracy, the coordinates of the atoms in protein structures.
  - If protein structures determined by X-Ray Crystallography and NMR are compared, they are generally consistent with each other and moreover are biologically plausible.
- This should give the researcher confidence when using them.

The Protein Databank

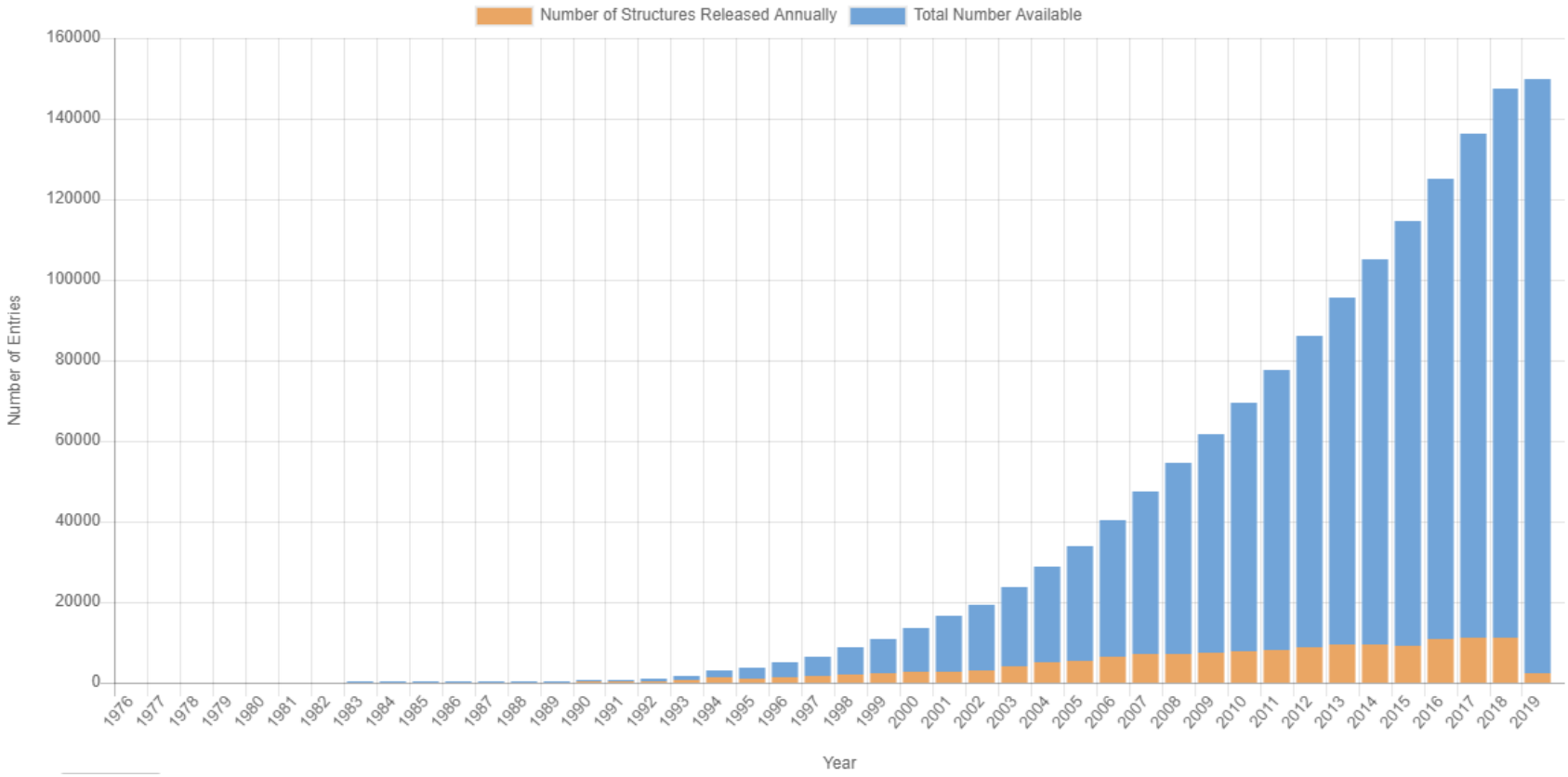
- contains a large collection of previously determined biological structures.
  - For inclusion in the PDB, the spatial locations of the atoms have to be determined with sufficient accuracy to usefully describe protein structures.
- also includes experimental details of how the structure was determined, what publications and other databases to consult for more information on the structure, some ‘‘derived data’’ and details of any ill-defined regions.
  - While this information is meant to be included in the PDB, some of it may be missing, incomplete or incorrect for some database entries.

The Protein Databank

- one of the oldest bioscience data stores, dating back to 1971.
- It originally stored the 3D coordinates of protein structures as determined by the Xray Crystallography method.
- Prior to the PDB, structures were typically published in journals, and many researchers re-entered the information manually into their computers so as to facilitate further manipulation of them.
- The original PDB data-file format adopted was a ‘‘flat’’ textual disk-file that was 80 columns wide.
- Today, the structures in the PDB are determined by either X-Ray Crystallography or NMR.
  - Often, many years of effort go into determining an individual structure.
- This is reflected in the growth of the number of entries in the PDB over some 40 years.

The Protein Databank

- PDB Statistics:
  - Overall Growth of Released Structures Per Year

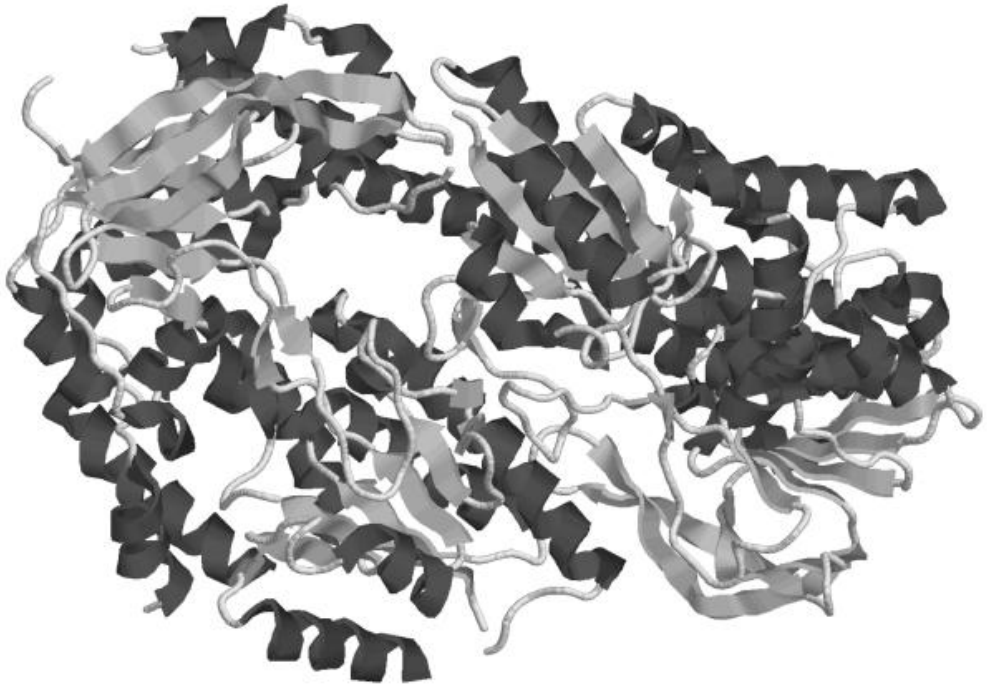


The PDB Data-file Formats

- available in one of two formats.
  - These formats are inter-convertible
- PDB flat file
  - The original, generic and highly unstructured PDB data-file format that is still widely used by researchers.
    - When biologists talk of ‘‘PDB files’’ or ‘‘PDB format’’, they are referring to this data-file format.
    - The current standard format is the 2.3 version.
- mmCIF
  - The new PDB data-file format that is designed to offer a highly structured, modern replacement to the original PDB Flat File format.
    - The mmCIF format is often informally referred to as the ‘‘new PDB format’’.

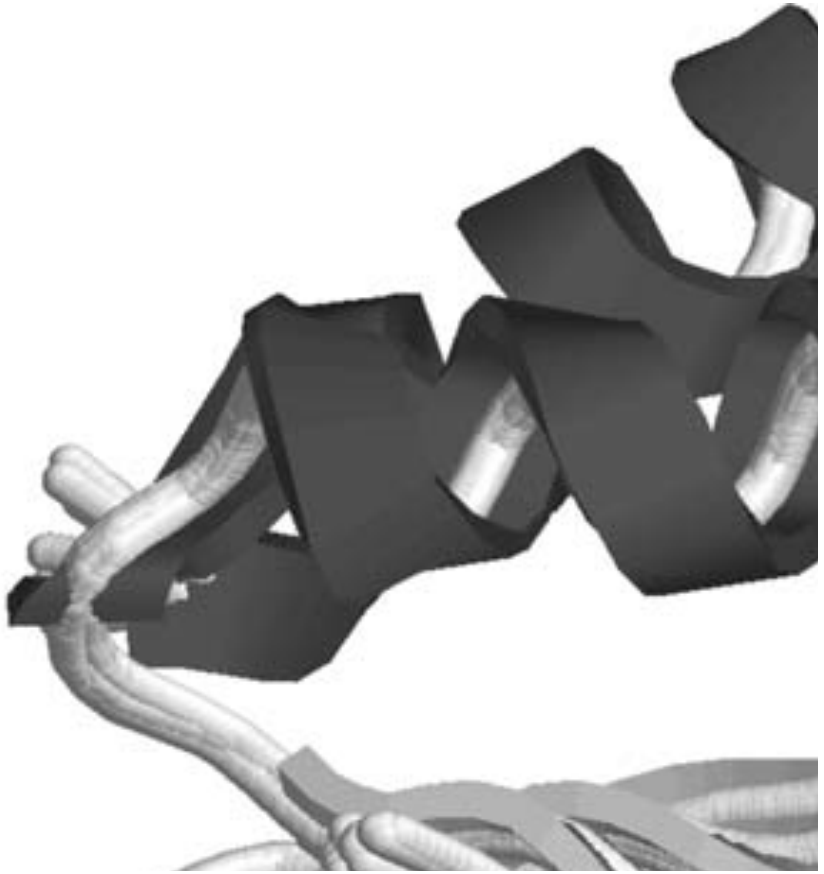
Example structures

- 1LQT
  - A modern, high-resolution ‘‘Oxidoreductase’’ enzyme structure produced using X-Ray Crystallographic techniques.



Example structures

- 1M7T
  - A modern protein structure of ‘‘Thioredoxin’’ produced using NMR.



Downloading PDB data-files

- PDB structure data-files can be downloaded from many web-site locations on the Internet.
- The RCSB web-site is always a good place to start:
  - http://www.rcsb.org/pdb/
- Alternatively, the EBI hosts a European mirror. Follow the links from:
  - http://www.ebi.ac.uk/services/

  to access the PDB from the EBI.

Accessing Data in PDB Entries

- There are some common sections to all PDB entries:
  - those concerned with
    - indexing,
    - bibliographic data,
    - notable features
    - 3D coordinates.
- Other sections are radically different from each other, as they depend on the experimental technique (X-Ray Crystallography or NMR) used to determine the structure.

Accessing Data in PDB Entries

- In a PDB data-file there is a left-right split (per line) and a top-bottom split (per data-file):
- Left-right
  - The left-most characters (a maximum of nine) on each line indicate what information is present on the right-hand side.
- Top-bottom
  - There is an upper HEADER section that contains the annotation about the structure (top) and a lower coordinates section that contains the 3D spatial locations of the atoms in the structure (bottom).

A short description of the most important fields in the PDB data-file

- HEADER
  - Contains a brief description of the structure, the date and the PDB ID code.
- TITLE
  - The title of the structure.
- COMPND
  - Brief details of the structure.
- SOURCE
  - Identifies which organism the structure came from.
- KEYWDS
  - Lists a set of useful words/phrases that describe the structure.
- AUTHOR
  - The scientists depositing the structure.
- REVDAT
  - The date of the last revision.

A short description of the most important fields in the PDB data-file

- JRNL
  - One or more literature references that describe the structure.
- REMARK 1 through REMARK 999
  - Details of the experimental methods used to determine the structure are contained in this subsection (see the example in the next section).
- DBREF
  - Cross links to other databases.
- SEQRES
  - The official amino acid sequence (protein, RNA or DNA) of the structure.
- HELIX/SHEET
  - Details of the regions of secondary structure found in the protein.
- ATOM/HETATM
  - The 3D spatial coordinates of particular atoms in the protein structure or other molecules such as water or co-factors.

Accessing PDB Annotation Data

- There are many examples of parsing data from the HEADER section of PDB data-files, all of which involve pattern matching.
  - Perl is exceptionally good at this.
- Two representative examples exploring
  - the relationship between the resolution of a structure and its Free R value, both of which are measures of the quality of the X-Ray Crystallographic structures.
    - the Free R value measures the agreement between the model and the observed x-ray reflection data.
    - The lower the Free R Value, the better the fit between the model and the observed data.
  - the database cross-referencing section used to link to other databases.

Free R and resolution

- The REMARK tag, type 2 subsection stores resolution, whereas the Free R value is quoted in REMARK tag, type 3.
  - Here's a small extract from the 1LQT entry:

    REMARK 2
    REMARK 2 RESOLUTION. 1.05 ANGSTROMS.
- In NMR structures, REMARK tag, type 2 and type 3 are present, but the data in them is ''NOT APPLICABLE'' for REMARK tag, type 2 and ''NULL'' or free text for REMARK tag, type 3.
  - Extract from the 1M7T structure's HEADER:

    REMARK 215 NMR STUDY
    REMARK 215 THE COORDINATES IN THIS ENTRY WERE GENERATED FROM SOLUTION
    REMARK 215 NMR DATA. PROTEIN DATA BANK CONVENTIONS REQUIRE THAT
    REMARK 215 CRYST1 AND SCALE RECORDS BE INCLUDED, BUT THE VALUES ON
    REMARK 215 THESE RECORDS ARE MEANINGLESS.

Free R and resolution

- Structural Refinement is the process of iteratively fitting the model structure into the electron density map, and details of this refinement are stored in REMARK tag, type 3.
- Here is an extract :

```
.
.
.
REMARK 3 FIT TO DATA USED IN REFINEMENT.
REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
REMARK 3 R VALUE (WORKING + TEST SET) : 0.134
REMARK 3 R VALUE (WORKING SET) : 0.134
REMARK 3 FREE R VALUE : 0.153
REMARK 3 FREE R VALUE TEST SET SIZE (%) : NULL
REMARK 3 FREE R VALUE TEST SET COUNT : 2200
```

A Perl program extracts the resolution and Free R Value from any PDB data-files

```
#! /usr/bin/perl -w
# free_res - Designed to extract the 'Free R Value' and 'Resolution'
# quantities from 'PDB data-files' containing structures
# produced by 'Diffraction'.
use strict;
my $PDB_Path = shift;
opendir ( INPUT_DIR, "$PDB_Path" )
        or die "Error: Cannot read from mmCIF directory: '$PDB_Path'\n";
my @PDB_dir = readdir INPUT_DIR;
close INPUT_DIR;
my @PDB_Files = grep /\.pdb/, @PDB_dir;
foreach my $Current_PDB_File ( @PDB_Files )
{
        my $Free_R;
        my $Resolution;
        open ( PDB_FILE, "$PDB_Path/$Current_PDB_File" )
                or die "Cannot open PDB File: '$Current_PDB_File'\n";
```

A Perl program extracts the resolution and Free R Value from any PDB data-files

```
while ( <PDB_FILE> )
{
        if ( /^EXPDTA / and !/DIFFRACTION/ )
        {
                last;
        }
        if ( /^REMARK 2 RESOLUTION/ )
        {
                ( undef, undef, undef, $Resolution ) = split ( " ", $_ );
        }
                if ( /^REMARK 3 FREE R VALUE / )
        {
                $Free_R = substr ( $_, 47, 6 );
                $Free_R =~ s/ //g;
```

A Perl program extracts the resolution and Free R Value from any PDB data-files

```
                    if ( $Free_R =~ /NULL/ or $Resolution eq "" )
                {
                    last;
                }
                else
                {
                    printf ( "%7s %4.2f %7.3f \n", $Current_PDB_File,
                        $Resolution, $Free_R );
                    last;
                }
            }
        }
        close ( PDB_FILE );
}
```

Database cross references

- The DBREF subsection gives a list of cross references to other Bioinformatics databases.
  - This makes it easier for researchers to integrate biological datasets.
- The second value on the DBREF line is the PDB identifier.
  - By examining this value, researchers and automatic parsing programs can tell to which structure the entry belongs.
- Example DBREF lines :

```
DBREF 1LQT A 1 456 GB 13882996 AAK47528 1 456
DBREF 1LQT B 1 456 GB 13882996 AAK47528 1 456

DBREF 1AFI 1 72 SWS P04129 MERP_SHIFL 20 91

DBREF 1M7T A 1 66 SWS P10599 THIO_HUMAN 0 65
DBREF 1M7T A 67 106 SWS P00274 THIO_ECOLI 68 107
```

Database cross references

- The DBREF lines identify the following fields, working from left to right:
  - PDB ID code.
  - Chain identifier (if needed).
  - The start of the sequence.
  - Insertion code.
  - End of the sequence.
  - The external database to which the cross reference refers.
  - The external database accession code.
  - The database external accession name.
  - The start, insertion and end of the sequence in the external database.

Database cross references

- The PDB publishes a table of database names and their associated, abbreviated codes.

Database Name	Database
BioMagResBank	BMRB
BLOCKS	BLOCKS
European Molecular Biology Laboratory	EMBL
GenBank	GB
Genome Data Base	GD
Nucleic Acid Database	NDB
PROSITE	PROSIT
Protein Data Bank	PDB
Protein Identification Resource	PIR
SWISS-PROT	SWS

Coordinates section

- The coordinate data for the locations of atoms in the macromolecular structure is straightforward, especially when compared to the annotation contained in the HEADER section of the PDB data-file.
  - The coordinates are presented as points in space, the atoms they represent are actually in motion.
  - In crystallographic structures, isotropic B-factors, commonly referred to as ‘‘Temperature Factors’’, give us an idea of the vibration of the molecule.
    - For very high-resolution structures, Anisotropic Temperature Factors may be included in the ANISOU lines.
      - These provide an idea of the vibration of the molecule in the directions of the coordinate axes.
  - In NMR structures, the variation in position of a particular atom between different models in the ensemble can be used as a similar measure of motion or as an indication of the error between the minimization models.
- Here is an example from 1M7T:

```
REMARK 210
REMARK 210 BEST REPRESENTATIVE CONFORMER IN THIS ENSEMBLE : 21
REMARK 210
```

Data section

- Referring to the 1LQT x-ray structure, an extract of lines from the coordinate section looks like this:

```
ATOM      1  N   ARG A   2      26.318  -8.010  39.090  1.00 20.71
ANISOU    1  N   ARG A   2    2040   3071   2755    114   -339   -39
ATOM      2  CA  ARG A   2      25.150  -8.702  38.505  1.00 18.85
ANISOU    2  CA  ARG A   2    2029   2677   2455     67   -321   -20
ATOM      3  C   ARG A   2      24.846  -8.176  37.123  1.00 17.23
ANISOU    3  C   ARG A   2    1689   2429   2429    143   -282   -25
ATOM      4  O   ARG A   2      25.151  -7.048  36.775  1.00 18.14
     .
     .
     .
TER    7215      GLY A 456
ATOM   7216  N   ARG B   2     -19.423  25.709   6.980  1.00 21.57
ANISOU 7216  N   ARG B   2    2476   3012   2707   -165   -370     9
ATOM   7217  CA  ARG B   2     -18.718  26.510   8.024  1.00 19.01
ANISOU 7217  CA  ARG B   2    2127   2672   2424    -63   -285     9
ATOM   7218  C   ARG B   2     -17.250  26.207   8.002  1.00 17.22
```

Data section

```
     .
     .
     .
TER   14289      GLY B 456
HETATM14290  C   ACT  1866     -13.075   1.733  10.218  1.00 27.25
ANISOU14290  C   ACT  1866     3493   3560   3299    -39    -36    -4
     .
     .
     .
CONECT1429014291142921 4293
CONECT1429114290
CONECT1429214290
TER
     .
     .
     .
CONECT1469014663
```

Data section

- For the 1M7T NMR structure, an extract of lines from the coordinate section looks like this:

```
MODEL        1
ATOM      1  N   MET A   1       3.110  -4.682  -3.025  1.00  0.00
ATOM      2  CA  MET A   1       2.546  -3.712  -2.053  1.00  0.00
ATOM      3  C   MET A   1       1.134  -3.295  -2.450  1.00  0.00
ATOM      4  O   MET A   1       0.882  -2.130  -2.758  1.00  0.00
ATOM      5  CB  MET A   1       3.466  -2.491  -2.002  1.00  0.00
ATOM      6  CG  MET A   1       3.781  -1.903  -3.370  1.00  0.00
ATOM      7  SD  MET A   1       4.256  -0.166  -3.285  1.00  0.00
ATOM      8  CE  MET A   1       6.004  -0.307  -2.920  1.00  0.00
ATOM      9 1H   MET A   1       2.906  -4.327  -3.980  1.00  0.00
ATOM     10 2H   MET A   1       2.650  -5.601  -2.859  1.00  0.00
ATOM     11 3H   MET A   1       4.134  -4.738  -2.858  1.00  0.00
ATOM     12  HA  MET A   1       2.517  -4.178  -1.079  1.00  0.00
ATOM     13 1HB  MET A   1       2.996  -1.724  -1.405  1.00  0.00
ATOM     14 2HB  MET A   1       4.397  -2.778  -1.536  1.00  0.00
ATOM     15 1HG  MET A   1       4.596  -2.461  -3.807  1.00  0.00
ATOM     16 2HG  MET A   1       2.907  -1.993  -3.998  1.00  0.00
ATOM     17 1HE  MET A   1       6.344  -1.302  -3.167  1.00  0.00
ATOM     18 2HE  MET A   1       6.160  -0.120  -1.860  1.00  0.00
```

Data section

```
      .
      .
      .
TER    1659      VAL A 107
ENDMDL
MODEL        2
ATOM      1  N   MET A   1       2.750  -6.779  -1.627  1.00  0.00
ATOM      2  CA  MET A   1       2.487  -5.475  -2.290  1.00  0.00
      .
      .
      .
TER    1660      VAL A 107
```

Data section

- In each ATOM line, the fields are as follows:

COLUMNS	DATA TYPE	FIELD	DEFINITION
1 - 6	Record name	"ATOM(s)	
7 - 11	Integer	serial	Atom serial number.
13 - 16	Atom	name	Atom name.
	Character	altLoc	Alternate location indicat
18 - 20	Residue name	resName	Residue name.
22	Character	chainID	Chain identifier.
23 - 26	Integer	resSeq	Residue sequence number.
27	AChar	iCode	Code for insertion of resi
31 - 38	Real(8.3)	x	Orthogonal coordinates for

Extracting 3D co-ordinate data

- The technique involves extracting the three substrings from each line that contains the X, Y and Z coordinates.
- Assuming the data is in $_, three invocations of Perl's substr subroutine do the trick:

```
my ( $X, $Y, $Z ) = ( substr( $_, 30, 8 ),

                      substr( $_, 38, 8 ),

                      substr( $_, 46, 8 ) );
```

The simple_coord_extract program

```
#! /usr/bin/perl -w

# simple_coord_extract <PDB File> - Demonstrates the extraction of
# C-Alpha co-ordinates from a PDB
# data-file.

use strict;

while ( <> )
{
    if ( /^ATOM/ && substr( $_, 13, 4 ) eq "CA " )
    {
        my ( $X, $Y, $Z ) = ( substr( $_, 30, 8 ),
        substr( $_, 38, 8 ),
        substr( $_, 46, 8 ) );

        $X =~ s/ //g;
        $Y =~ s/ //g;
        $Z =~ s/ //g;

        print "X, Y & Z: $X, $Y, $Z\n";
    }
}
```

Results from simple_coord_extract ...

```
X, Y & Z: 25.150, -8.702, 38.505
X, Y & Z: 23.675, -8.497, 35.069
X, Y & Z: 20.747, -6.252, 34.332
X, Y & Z: 17.545, -8.297, 34.292
X, Y & Z: 15.182, -7.484, 31.454
X, Y & Z: 11.736, -8.952, 30.942
X, Y & Z: 10.261, -9.014, 27.451
X, Y & Z:  6.507, -9.548, 27.173
```

Introducing Databases

- Many modern computer systems store vast amounts of structured data.
- Typically, this data is held in a database system.
- Database
  - a collection of one or more related tables.
- Table
  - a collection of one or more rows of data.
    - The rows of data are arranged in columns, with each intersection of a row and column containing a data item.
- Row
  - a collection of one or more data items, arranged in columns.
    - Within a row, the columns conform to a structure.

Introducing Databases

- For example,
  - if the first column in a row holds a date, then every first column in every row must also hold a date.
  - if the second column holds a name, then every second column must also hold a name, and so on.
- The following data corresponds to the structure, in that there are two columns, the first holding a date, the second holding a name:

```
1960-12-21          P. Barry
1954-6-14           M. Moorhouse
```

Structured data

- Each column can be given a descriptive name.

```
--------------   ---------
Discovery_Date   Scientist
--------------   ---------
1960-12-21       P. Barry
1954-6-14        M. Moorhouse
1970-3-4         J. Blow
2001-12-27       J. Doe
```

- In addition, the structure requires that each data item held in a column be of a specific type.

```
-----------          ----------------
Column name    Type restriction
-----------          ----------------
Discovery_Date       a valid Date
Scientist            a String no longer than 64 characters
```

- This type information generally goes by one of two names: metadata or schema.

Relating tables

- Extending the Discoveries table to include details of the discovery, an additional column is needed to hold the data

Discovery_Date	Scientist	Discovery
1960-12-21	P. Barry	Flying car
1954-6-14	M. Moorhouse	Telepathic sunglasses
1970-3-4	J. Blow	Self cleaning child
2001-12-27	J. Doe	Time travel

- The inclusion of this new column requires an update to the structure of the table

Column name	Type restriction
Discovery_Date	a valid Date
Scientist	a String no longer than 64 characters
Discovery	a String no longer than 128 characters

Relating tables, cont.

Column name	Type restriction
Discovery_Date	a valid Date
Scientist	a String no longer than 64 characters
Discovery	a String no longer than 128 characters
Date_of_birth	a valid Date
Telephone_number	a String no longer than 16 characters

Discovery_Date	Scientist	Discovery	Date_of_birth	Telephone_number
1960-12-21	P. Barry	Flying car	1966-11-18	353-503-555-91910
1954-6-14	M. Moorhouse	Telepathic sunglasses	1970-3-24	00-44-81-555-3232
1970-3-4	J. Blow	Self cleaning child	1955-8-17	555-2837
2001-12-27	J. Doe	Time travel	1962-12-1	-
1974-3-17	M. Moorhouse	Memory swapping toupee	1970-3-24	00-44-81-555-3232
1999-12-31	M. Moorhouse	Twenty six hour clock	1958-7-12	416-555-2000

The problem with single-table databases

- Although the above table structure solves the problem of uniquely identifying each scientist, it introduces some other problems:
  - If a scientist is responsible for a large number of discoveries, their identification information has to be entered into every row of data that refers to them.
    - This is time-consuming and wasteful.
  - Every time identification information is added to a row for a particular scientist, it has to be entered in exactly the same way as the identification information added already.
    - Despite the best of efforts, this level of accuracy is often difficult to achieve.
  - If a scientist changes any identification information, every row in the table that refers to the scientist's discoveries has to be changed.
    - This is drudgery.

Solving the one table problem

- The problems described in the previous section are solved by breaking the all-in-one Discoveries table into two tables.
- Here is a new structure for Discoveries:

Column name	Type restriction
Discovery_Date	a valid Date
Scientist_ID	a String no longer than 8 characters
Discovery	a String no longer than 128 characters

Column name	Type restriction
Scientist_ID	a String no longer than 8 characters
Scientist	a String no longer than 64 characters
Date_of_birth	a valid Date
Address	a String no longer than 256 characters
Telephone_number	a String no longer than 16 characters

Solving the one table problem, cont.

Discovery_Date	Scientist_ID	Discovery
1954-6-14	MM	Telepathic sunglasses
1960-12-21	PB	Flying car
1969-8-1	PB	A cure for bad jokes
1970-3-4	JB	Self cleaning child
1974-3-17	MM	Memory swapping toupee
1999-12-31	MM2	Twenty six hour clock
2001-12-27	JD	Time travel

Scientist_ID	Scientist	Date_of_birth	Address	Telephone_number
JB	J. Blow	1955-8-17	Belfast, NI	555-2837
JD	J. Doe	1962-12-1	Syndey, AUS	-
MM	M. Moorhouse	1970-3-24	England, UK	00-44-81-555-3232
MM2	M. Moorhouse	1958-7-12	Toronto, CA	416-555-2000
PB	P. Barry	1966-11-18	Carlow, IRL	353-503-555-91910

Relational Databases

- Relating data in one table to that in another forms the basis of modern database theory.
  - It also explains why so many modern database technologies are referred to as Relational Database Management Systems (RDBMS).
- When a collection of tables is designed to relate to each other they are collectively referred to as a database.
- It is usually a requirement to give the database a descriptive name.

Database system: a definition

- A database system is a computer program (or group of programs)
  - that provides a mechanism to define and manipulate
    - one or more databases
- A database system
  - allows databases, tables and columns to be created and named, and structures to be defined.
  - provides mechanisms to add, remove, update and interact with the data in the database.
- Data stored in tables can be searched, sorted, sliced, diced and cross-referenced.
- Reports can be generated, and calculations can be performed.

Available Database Systems

- Personal database systems:
  - Designed to run on PCs
    - Access, Paradox, FileMaker, dBase
- Enterprise database systems:
  - Designed to support efficient storage and retrieval of vast amount of data
    - Interbase, Ingres, SQL Server, Informix, DB2, Oracle
- Open source database systems:
  - Free!!! (Linux!!!)
    - PostgreSQL, MySQL

Chosing Database System

- Which type of database system is chosen depends on a number of factors, including (but not limited to):
  - The amount of data to be stored in the database.
  - Whether the data supports a small personal project or a large collaborative one.
  - How much funds (if any) are available towards the purchase of a database system.

SQL: The Language of Databases

- Defining data with SQL (structured query language)

- SQL provides two facilities:

  - A database definition Language (DDL)
    - provides a mechanism whereby databases can be created

  - A Data Manipulation Language (DML)
    - provides a mechanism to work with data in tables

Installing a database system

- MySQL is a modern, capable and SQL-enabled database system.
- It is Open Source and freely available for download from the MySQL web-site:

  http://www.mysql.com

- It comes as a standard, installable component of most Linux distributions
- The following commands switch on MySQL on RedHat and RedHat-like Linux distributions

  ```
  chkconfig --add mysqld
  chkconfig mysqld on
  ```

- If the first chkconfig command produces an error messages like this:

  ```
  error reading information on service mysqld: No such file or directory
  ```

  this means that MySQL is not installed and the second command will also fail.

Installing a database system

- Once MySQL is installed, it needs to be configured.
- The first requirement is to assign a password to the MySQL superuser, known as ‘‘root’’.
- The mysqladmin program does this, as follows:

```
mysqladmin -u root password 'passwordhere‘
```

- It is now possible to securely access the MySQL Monitor command-line utility with the following command, providing the correct password when prompted:

```
mysql -u root -p
```

A Database Case Study: MER

- A small collection of SWISS-PROT and EMBL entries are taken from the Mer Operon, a bacterial gene cluster that is found in many bacteria for the detoxification of Mercury Hg2+ ions.
- These provide the raw data to a database, which is called MER.
- The MER database contains four tables:
  - proteins – A table of protein structure details, extracted from a collection of SWISS-PROT entries.
  - dnas – A table of DNA sequence details, extracted from a collection of EMBL entries.
  - crossrefs – A table that links the extracted protein structures to the extracted DNA sequences.
  - citations – A table of literature citations extracted from both the SWISS-PROT and EMBL DNA entries.

A Database Case Study: MER

- Once the raw data is in the database, SQL can be used to answer questions about the data.
- For instance:
  - How many protein structures in the database are longer than 200 amino acids in length?
  - How many DNA sequences in the database are longer than 4000 bases in length?
  - What's the largest DNA sequence in the database?
  - Which protein structures are cross-referenced with which DNA sequences?
  - Which literature citations reference the results from the previous question?

Creating the MER database

- SQL queries can be entered directly at the MySQL Monitor prompt.

```
mysql> create database MER;
    Query OK, 1 row affected (0.36 sec)
mysql> show databases;
    +------------+
    | Databases  |
    +------------+
    | MER        |
    | test       |
    | mysql      |
    +------------+
    3 rows in set (0.00 sec)
```

- A list of databases is returned by MySQL.
- There are three identified databases:
  - MER – The just-created database that will store details on the extracted protein structures, DNA sequences, cross references and literature citations.
  - test – A small test database that is used by MySQL and other technologies to test the integrity of the MySQL installation.
  - mysql – The database that stores the internal ‘‘system information’’ used by the MySQL database system.

Creating the MER database

- It is possible to use the MySQL superuser to create tables within the MER database.
- However, it is better practice to create a user within the database system to have authority over the database, and then perform all operations on the MER database as this user.
- The queries to do this are entered at the MySQL Monitor prompt.
- Here are the queries and the messages returned:

```
mysql> use mysql;
        Database changed
mysql> grant all on MER.* to bbp identified by 'passwordhere';
        Query OK. 0 rows affected (0.00 sec)
mysql> quit
        Bye
```

- The first query tells MySQL that any subsequent queries are to be applied to the named database, which in this case is the mysql database.
- The second query does three things:
  - creates a new MySQL user called ‘‘bbp’’.
  - assigns a password with the value of ‘‘passwordhere’’ to user ‘‘bbp’’.
  - grants every available privilege relating to the MER database to ‘‘bbp’’.

Adding tables to the MER database

```
create table proteins
(
    accession_number    varchar (6)     not null,
    code                varchar (4)     not null,
    species             varchar (5)     not null,
    last_date           date            not null,
    description         text            not null,
    sequence_header     varchar (75)    not null,
    sequence_length     int             not null,
    sequence_data       text            not null
)
```

```
$ mysql -u bbp -p MER < create_proteins.sql
```

Understand the data before designing the tables

Databases and Perl

- Why Program Databases?
  - Customised output handling
    - Programs can be written to post-process the results of any SQL query and display them in any number of preferred formats.
  - Customised input handling
    - Users of customised input handling programs do not need to know anything about SQL – all they need to know and understand is their data.
  - Extending SQL
    - Some tasks that are difficult or impossible to do with SQL can be programmed more easily.
  - Integrating MySQL into custom applications
    - Having the power of MySQL as a component of an application can be very powerful.

Perl Database Technologies

- A number of third-party CPAN modules provide access to MySQL from within a Perl program.
  - One such module is Net::MySQL by Hiroyuki Oyama, which provides a stable programming interface to MySQL functionality.
- In fact, nearly every database system provides a specific technology for programmers to use when programming their particular database.
  - This technology is referred to as an API, an application programming interface.
- Unfortunately, the effort expended in learning how to use Net::MySQL is of little use when a program has to be written to interface with Oracle or Sybase

Perl Database Technologies

- The DBI module provides a database independent interface for Perl.
  - By providing a generalised API, programmers can program at a ‘‘higher level’’ than the API provided by the database system, in effect insulating programs from changes to the database system.
- To connect the high-level DBI technology to a particular database system, a special driver converts the general DBI API into the database system-specific API.
  - These drivers are implemented as CPAN modules.

Preparing Perl

DBI and DBD::mysql modules need to be installed

$ man DBI

$ man DBD::mysql

$ find `perl -Te 'print "@INC"' ` -name '*.pm' -print | grep 'DBI.pm'

$ find `perl -Te 'print "@INC"' ` -name '*.pm' -print | grep 'mysql.pm'

$ locate DBI.pm

$ locate mysql.pm

DBI (previously called DBperl) is a database independent interface module for Perl.

DBD: Data Base Description

Checking the DBI installation

```
#! /usr/bin/perl -w

# check_drivers - check which drivers are installed with DBI.

use strict;

use DBI;

my @drivers = DBI->available_drivers;

foreach my $driver ( @drivers )
{
   print "Driver: $driver installed.\n";
}
```

Programming Databases With DBI

```
#! /usr/bin/perl -w

# show_tables - list the tables within the MER database.
# Uses "DBI::dump_results" to display results.

use strict;
use DBI qw( :utils );
use constant DATABASE => "DBI:mysql:MER";
use constant DB_USER => "bbp";
use constant DB_PASS => "passwordhere";
my $dbh = DBI->connect( DATABASE, DB_USER, DB_PASS )
    or die "Connect failed: ", $DBI::errstr, ".\n";

my $sql = "show tables";
my $sth = $dbh->prepare( $sql );
$sth->execute;
print dump_results( $sth ), "\n";
$sth->finish;
$dbh->disconnect;
```

- DATABASE
  - Identifies the data source to use
- DB_USER
  - Identifies the username to use when connecting to the data source
- DB_PASS
  - Identifies the password to use when authenticating to the data source

The Sequence Retrieval System

The Sequence Retrieval System

- Sequence Retrieval System (SRS) is a web-based database integration system that allows for the querying of data contained in a maltitude of databases, all through a single user interface.
- This makes the individual databases appear as if they are really one big relational database, organised with different subsections:
  - one called SWISS-PROT,
  - one called EMBL,
  - one called PDB,
  - …

The Sequence Retrieval System

- SRS makes it very easy to query the entire data set, using common search terms that work across all the different databases, regardless of what they are.
- Everything contained within the SRS is ‘‘tied together’’ by the web-based interface.
- Figure in the next slide is the database selection page from the EBI’s SRS web-site, which can be navigated to from the following Internet address:
  - http://srs.ebi.ac.uk
    - SRS is a trademark and the intellectual property of Lion Bioscience

EBI's SRS Database Selection Page

3/29/2018

Pages

SRS

Created by Andrew Cowley, last modified on Jul 26, 2016

Service Retirement

The EMBL-EBI SRS service was decommissioned on **Thursday 19th December 2013**. The tables below detail alternative s

For services provided by EMBL-EBI, please see EMBL-EBI Services. User with a programmatic/systematic usage requireme

Instem, the developers of SRS, maintain a list of public SRS servers containing details of the databanks and tools provided b

If you have any queries about the retirement of the EMBL-EBI SRS service, please contact us via EMBL-EBI Support.

Databanks

Alternative sites which provide access to data which appeared in the SRS databanks, or provide equivalent data:

Library Group	Databank Name	Alternative Site
Active protein sequence databases	BCIPEP	http://www.imtech.res.in/raghava/b
	EPO_PRT	*see PATENT_PRT*
	IPI	ftp://ftp.ebi.ac.uk/pub/databases/IP
	IPIHISTORY	ftp://ftp.ebi.ac.uk/pub/databases/IP
	JPO_PRT	*see PATENT_PRT*
	KIPO_PRT	*see PATENT_PRT*

The Sequence Retrieval System

- SRS is important for two reasons:
  - It is a useful and convenient service that every Bioinformatician should know about.
  - It is an excellent example of what can be created when the World Wide Web, databases and programming languages are combined.

- Warning:
  - Don't create a new data format unless absolutely necessary.
  - Use an existing format whenever possible

Creating Content For The WWW with Perl

The Web Development Components

- Client-side programming
  - a technology used to program the web client, providing a way to enhance the user's interactive experience.
    - Java applets, JavaScript, Macromedia Flash, …
- Server-side programming
  - a technology used to program the web server, providing a mechanism to extend the services provided by the web server.
    - Java Servlets, JSP, Python, ASP, PHP, Perl, …
- Backend database technology
  - a place to store the data to be published, which is accessed by the server-side programming technology.
    - MySQL, …

Additional components

- The acronym LAMP is used to describe the favoured WWW development infrastructure of many programmers.
  - The letters that form the acronym are taken from the words Linux, Apache, MySQL and Perl/Python/PHP.
    - O'Reilly & Associates provides an excellent LAMP web-site, available on-line at
      - http://www.onlamp.com.
- The additional components turn the standard web development infrastructure into a dynamic and powerful application development environment.
- One of the reasons the WWW is so popular is the fact that creating content is so straightforward
  - Adding a programming language into the mix allows even more to be accomplished

Creating Content For The WWW

- There are a number of techniques employed to create HTML (Hyper Text Markup Language):
  - Creating content manually
    - Any text editor can be used to create HTML, since HTML is mostly text.
    - Special tags within the text guide the web browser when it comes to displaying the web page on screen.
    - The tags are also textual and any text editor can produce them
    - Advantages and disadvantages:
      - provides the maximum amount of flexibility as the creator has complete control over the process.
      - It can also be advantageous to know what's going on behind the scenes, so learning HTML is highly recommended
      - can be time-consuming and tedious, as the creator of the page has to write the content as well as decide which tags to use and where

Creating Content For The WWW

- Creating content visually
  - Special-purpose editors can create HTML pages visually, displaying the web page as it will appear in the web browser as it is edited.
    - Netscape Composer, Microsoft FrontPage and Macromedia Dreamweaver, ...
  - Advantages and disadvantages:
    - no need to know anything about HTML.
    - The editor adds the required tags to the text that's entered by the user
    - unnecessary tags added,
    - HTML pages are larger

Creating Content For The WWW

- Creating content dynamically
  - Since HTML is text, it is also possible to create HTML from a program.
  - Advantages and disadvantages:
    - HTML pages produced in this way can sometimes be useful when combined with a web server that allows for server-side programming of a backend database
  - needs a web page creator to write a program to produce even the simplest of pages

- A useful web-site:
  - http://www.htmlprimer.com

Producing HTML

- Producing HTML with a Perl program using a HERE document:

```
#! /usr/bin/perl -w

# produce_simple - produces the "simple.html" web page using
# a HERE document.

use strict;

print <<WEBPAGE;
<HTML>
<HEAD>
<TITLE>A Simple HTML Page</TITLE>
</HEAD>
<BODY>
This is as simple a web page as there is.
</BODY>
</HTML>
WEBPAGE
```

Producing HTML

- HTML file produced by the program:

```
<HTML>
<HEAD>
<TITLE>A Simple HTML Page</TITLE>
</HEAD>
<BODY>
This is as simple a web page as there is.
</BODY>
</HTML>
```

Producing HTML, cont.

- Another version of HTML generation
  - written to use Perl's standard CGI module

```
#! /usr/bin/perl -w

# produce_simpleCGI - produces the "simple.html" web page using
# Perl's standard CGI module.

use strict;

use CGI qw( :standard );

print       start_html( 'A Simple HTML Page' ),
            "This is as simple a web page as there is.",
            end_html;
```

- The CGI module is designed to make the production of HTML as convenient as possible.
- start_html subroutine produces the tags that appear at the start of the web page.
- end_html subroutine produces the following HTML, representing tags that conclude a web page:

  </body></html>

Results from produce_simpleCGI

- HTML file produced by the program:

```
<!DOCTYPE html
    PUBLIC "-//W3C//DTD XHTML 1.0 Transitional//EN"
     "http://www.w3.org/TR/xhtml1/DTD/xhtml1-transitional.dtd">
<html xmlns="http://www.w3.org/1999/xhtml" lang="en-US" xml:lang="en-US">
<head>
<title>A Simple HTML Page</title>
<meta http-equiv="Content-Type" content="text/html; charset=iso-8859-1" />
</head>
<body>
This is as simple a web page as there is.
</body>
</html>
```

Extra stuff at the start is optional. Extra tags tell the web browser exactly which version of HTML the web page conforms to. The CGI module includes these tags for web browser to optimise its behaviour to the version of HTML identified

Static creation of WWW content

- simple.html web page is static
- If the web page is put on a web server it always appear in exactly the same way every time it is accessed.
  - It is static, and remains unchanged until someone takes the time to change it.
- It rarely makes sense to create such a web page with a program unless you have a special requirement.
  - Create static web pages either manually or visually

The dynamic creation of WWW content

- When the web page includes content that is not static, it is referred to as dynamic web page.
  - For example a page including current date and time
- It is not possible to create a web page either manually or visually that includes dynamic content, and
  - this is where server side programming technologies come into their own.

The dynamic creation of WWW content

```
#! /usr/bin/perl -wT

# whattimeisit - create a dynamic web page that includes the
# current date/time.

use strict;

use CGI qw( :standard );

print start_html( 'What Date and Time Is It?' ),
    "The current date/time is: ", scalar localtime,
        end_html;
```

Results from whattimeisit ...

```
>perl -wT whattime.pl

<?xml version="1.0" encoding="iso-8859-1"?>
<!DOCTYPE html PUBLIC "-//W3C//DTD XHTML 1.0 Transitional//EN"
    "http://www.w3.org/TR/xhtml1/DTD/xhtml1-transitional.dtd">
<html xmlns="http://www.w3.org/1999/xhtml" lang="en-US" xml:lang="en-
    US"><head><title>What Date and Time Is It?</title>
</head><body>The current date/time is: Wed Oct 30 18:56:17 2019</
    body></html>>Exit code: 0
```

- This web page, if served up by a web server, changes with each serving, as it is dynamic.

And some time later ...

```
>perl -wT whattime.pl

<?xml version="1.0" encoding="iso-8859-1"?>
<!DOCTYPE html PUBLIC "-//W3C//DTD XHTML 1.0 Transitional//EN"
    "http://www.w3.org/TR/xhtml1/DTD/xhtml1-transitional.dtd">
<html xmlns="http://www.w3.org/1999/xhtml" lang="en-US"
    xml:lang="en-US"><head><title>What Date and Time Is It?</title>
</head><body>The current date/time is: Wed Oct 30 18:59:59 2019</
    body></html>>Exit code: 0
```

- Note that use of the "T" command-line option at the start of the program.
  - This switches on Perl's taint mode,
    - which enables a set of special security checks on the behaviour of the program.
- If a server-side program does something that could potentially be exploited and, as a consequence, pose a security treat, Perl refuses to execute the program when taint mode is enabled.
- Always enable ''taint mode'' for server-side programs
- Test your web-site on localhost prior to deployment on the Internet

Sending Data To A Web Server

- Switch on taint mode on the Perl command line
- Use CGI module, importing (at least) the standart set of subroutines
- Ensure the first print statement within the program is “print header”;
- Envelope any output sent to STDOUT with calls to the start_html and end_html subroutines
- Create a static web page to invoke the server-side program, providing input as necessary

Sending Data To A Web Server

```
#! /usr/bin/perl -wT

# The 'match_emblCGI' program - check a sequence against the EMBL
#                                                    database entry stored in the
#                                                    embl.data.out data-file on the
#                                                    web server.

use strict;

use CGI qw/:standard/;

print header;

open EMBLENTRY, "embl.data.out"
   or die "No data-file: have you executed prepare_embl?\n";

my $sequence = <EMBLENTRY>;

close EMBLENTRY;
```

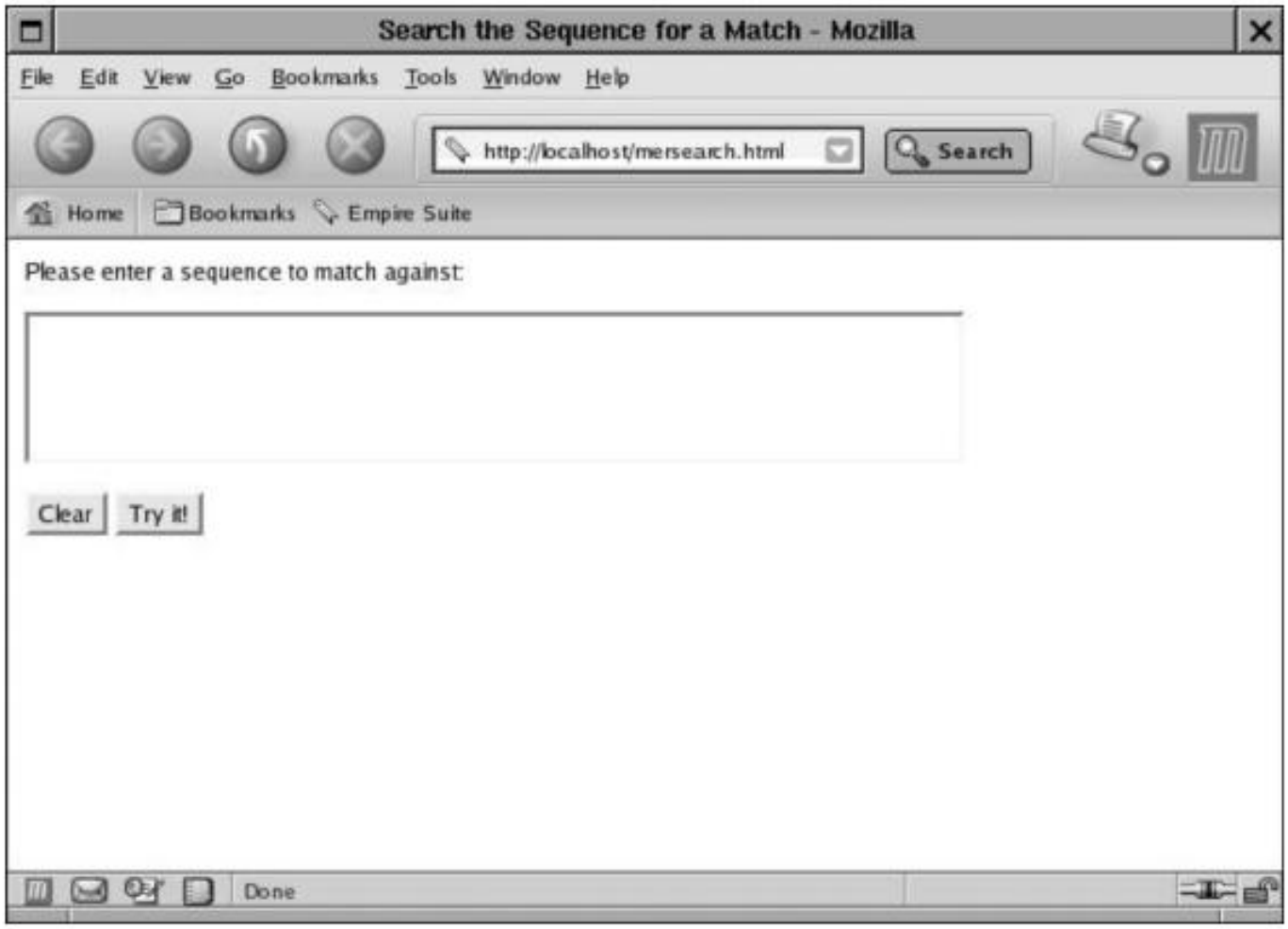
match_emblCGI, cont.

```
print start_html( "The results of your search are in!" );
print "Length of sequence is: <b>", length $sequence,
                    "</b> characters.<p>";
print h3( "Here is the result of your search:" );

my $to_check = param( "shortsequence" );

$to_check = lc $to_check;

if ( $sequence =~ /$to_check/ )
{
   print "Found. The EMBL data extract contains: <b>$to_check</b>.";
}
else
{
   print "Sorry. No match found for: <b>$to_check</b>.";
}
print p, hr, p;
print "Press <b>Back</b> on your browser to try another search.";
print end_html;
```

A Search HTML Page

```
<HTML>
<HEAD>
<TITLE>Search the Sequence for a Match</TITLE>
</HEAD>
<BODY>
Please enter a sequence to match against:<p>
<FORM ACTION="/cgi-bin/match_emblCGI">
<p>
<textarea name="shortsequence" rows="4" cols="60"></textarea>
</p>
<p>
<input type="reset" value="Clear">
<input type="submit" value="Try it!">
</p>
</FORM>
</BODY>
</HTML>
```

The ``Search the Sequence for a Match'' web page



The ``Results of your search are in!'' web page

The ``Sorry! Not Found'' web page

Installing CGIs on a Web Server

```
$ su

$ cp mersearch.html /var/www/html

$ cp match_emblCGI /var/www/cgi-bin

$ chmod +x /var/www/cgi-bin/match_embl

$ cp embl.data.out /var/www/cgi-bin

$ <Ctrl-D>
```

Using a HERE document

```
print <<MERFORM;

Please enter another sequence to match against:<p>
<FORM ACTION="/cgi-bin/match_emblCGIbetter">
<p>
<textarea name="shortsequence" rows="4" cols="60"></textarea>
</p>
<p>
<input type="reset" value="Clear">
<input type="submit" value="Try it!">
</p>
</FORM>
MERFORM
```

Better version: ``Results of your search are in!'' web page

Web Automation

Using Perl to automate web surfing

Automate repetitive WWW interactions whenever possible

Why Automate Surfing?

- Imagine you have 100 sequences to check.
- If it takes average 1 minutes to enter the sequence into text area, entering 100 sequences requires 100 minutes
- Why not automate it to save time

Perl module WWW::Mechanize allows programmer to automate interactions with any web-site

Strategy to follow when automating interactions with any web page

- Load the web page of interest into a graphical browser
- Wiev the HTML used to display the web page by selecting the Page Source option from browser's View menu
- Read the HTML, make a note of the names of the interface elements and form buttons that are of interest
- Write a Perl program that use WWW::Mechanize to interact with the web page
- Use an appropriate regular expression to extract the interesting bits from the results returned from the web server

The automatch program

```
#! /usr/bin/perl -w

# The 'automatch' program - check a collection of sequences against
# the 'mersearchmulti.html' web page.

use strict;

use constant URL => "http://pblinux.itcarlow.ie/mersearchmulti.html";

use WWW::Mechanize;

my $browser = WWW::Mechanize->new;

while ( my $seq = <> )
{
    chomp( $seq );

    print "Now processing: '$seq'.\n";
```

The automatch program, cont.

```
    $browser->get( URL );
    $browser->form( 1 );
    $browser->field( "shortsequence", $seq );
    $browser->submit;
    if ( $browser->success )
    {
        my $content = $browser->content;
        while ( $content =~
                m[<tr align="CENTER"                                    /><td>(\w+?)</
      td><td>yes</td>]g )
        {
            print "\tAccession code: $1 matched '$seq'.\n";
        }
    }
    else
    {
        print "Something went wrong: HTTP status code: ",
                $browser->status, "\n";
    }
}
```

Running the automatch program

```
$ chmod +x automatch

$ ./automatch sequences.txt
```

Results from automatch

```
Now processing: 'attccgattagggcgta'.
Now processing: 'aattc'.
   Accession code: AF213017 matched 'aattc'.
   Accession code: J01730 matched 'aattc'.
   Accession code: M24940 matched 'aattc'.
Now processing: 'aatgggc'.
Now processing: 'aaattt'.
```

Results from automatch ...

Accession code: AF213017 matched 'aaattt'.
    Accession code: J01730 matched 'aaattt'.
    Accession code: M24940 matched 'aaattt'.
Now processing: 'acgatccgcaagtagcaacc'.
    Accession code: M15049 matched 'acgatccgcaagtagcaacc'.
Now processing: 'gggcccaaa'.
Now processing: 'atcgatcg'.
Now processing: 'tcatgcacctgatgaacgtgcaaaaccacag'.
    Accession code: AF213017 matched 'tcatgcacctgatgaacgtgcaaaaccacag'.
    .
    .
Now processing: 'ccaaat'.
    Accession code: AF213017 matched 'ccaaat'.
    Accession code: J01730 matched 'ccaaat'.
    Accession code: M24940 matched 'ccaaat'.

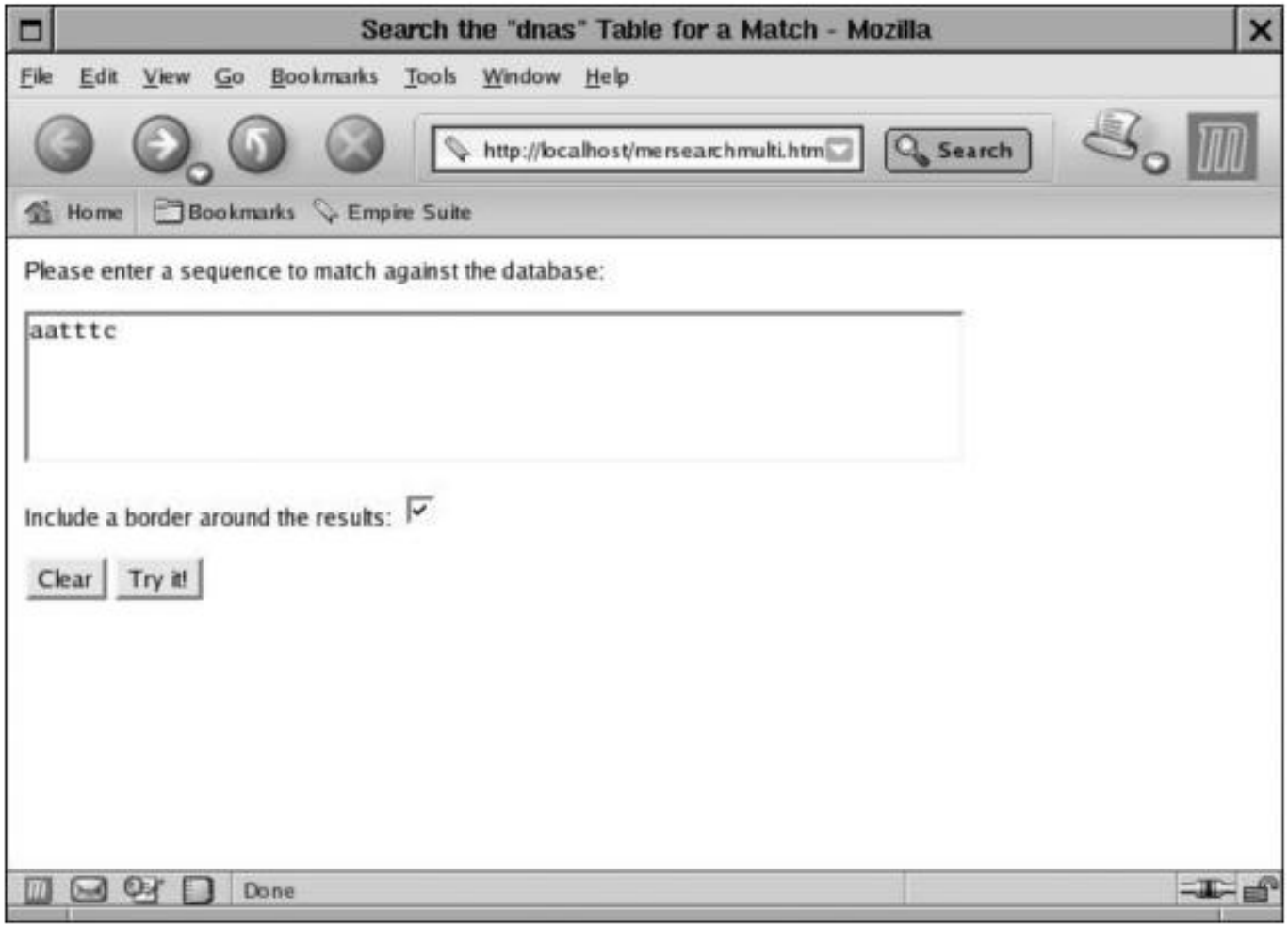
Viewing the source of the mersearchmulti.html web page

Source of: http://localhost/mersearchmulti.html - Mozilla

File Edit View Help

```
<html>
<head>
    <title>Search the "dnas" Table for a Match</title>
</head>
<body>

Please enter a sequence to match against the database:<p>
<form ACTION="/cgi-bin/db_match_emblCGI">
    <p>
        <textarea name="shortsequence" rows="4" cols="60"></textarea>
    </p>
    <p>
        Include a border around the results: <input type="checkbox"
name="printborder" value="on" checked="checked" />
    </p>
    <p>
        <input type="reset"  value="Clear">
        <input type="submit" value="Try it!">
    </p>
</form>
</body>
</html>
```

Searching all the entries in the dnas table



The ``results'' of the multiple search on the dnas table