Lecture 3

Torgeir R. Hvidsten
Assistant professor in Bioinformatics
Umeå Plant Science Center (UPSC)
Computational Life Science Centre (CLiC)

This lecture

- Introduction to Perl 3
  - regular expressions
  - parallelization
  - running external programs and commands
- Object-oriented programming
- BioPerl
- Go through Lab 2

Examples of regular expression

```
if ($player =~ m/Kaká/) {
    print ”Traitor!\n”;
}

if ($player =~ /[K|k]aka/) {
  print “Still a traitor\n";
}

my @genes = split /\s+/, $line;
```

Regular expression elements

Symbol classes

\s,\S	Whitespace character
\w, \W	Word character
\d, \D	Digit
[...]	Character set
[^...]	Set complement
.	Wildcard; any character
\$, \\, \[, \., *, \+, etc.	Quoted literals, meta-characters

Quantifiers

*	Zero or more
+	One or more
?	Zero or one
{a,}	a or more
{,b}	b or less
{a,b}	a to b inclusive

Logic

|	Logical “or”
(...)	Grouping for quantifiers

Anchors

^	Beginning of string
$	End of string
\b	Word-boundary
\B	Non-word boundary

Perl regular expression

/a+/	# Match one or more a’s, ex: a,aa,aaa…
/[aeiou]/	# Match a vowel
/[^aeiou]/	# Match a non-vowel
/\s+/	# Match one or more whitespaces
/\S+/	# Match one or more non-whitespaces
/\d+/	# Match unsigned integer = /[0-9]+/
/\d+\.\d+/	# Match unsigned floats, ex: 3.1415
/\w*/	# Zero or more word characters = /[a-zA-Z_0-9]*/
/\W?/	# Zero or one non-word character = /[^a-zA-Z_0-9]?/
/ this | that /	# Match *this* or *that* = / th(is|at) /
/c.t/	# Match cat, cut, ctt, c@t, c t, tic tac, …
/ b.{2,4}t /	# Match boot, beat, blast, b- t, bastat, bttttt
/ b[^t]{2,4}t /	# Match *blast* and *beast* but not bttttt

Perl regular expression

/\bhunt/	# Match *hunt*, *hunter*, but not *shunt* or *_hunt*
/\bsearch\B/	# Match *searching*, *searches* but not *search*
/\[[^]]*\]/	# Match anything surrounded by []
/^[A-Z][a-z]*/	# Capitalized word at beginning of string
/\.$/	# Match a period at the end of the string
/\n/	# Match a new-line character
/http:\/\//	# You need to quote literal slashes with backslashes

Basic comparision

- Returns true if string $string contains substring "sought_text", false otherwise:
  `$string =~ m/sought_text/;`
- Returns true if string $string contains substring "sought_text" at the very beginning:
  `$string =~ m/^sought_text/;`
- Returns true if the sought text is the very last text in the string:
  `$string =~ m/sought_text$/;`
- Returns true only if $string contains the sought text and nothing but the sought text:
  `$string =~ m/^sought_text$/;`
- Case insensitive comparision:
  `$string =~ m/^sought_text$/i;`
- Note: `m` is optional
- `=~` return true if `$string` matches the pattern, file `!~` returns true if `$string` does not match the pattern

Back-references in Perl

- Besides for grouping (e.g. / th(is|at) /), parentheses save the part they match for use later in the Perl code ($1 form).
- The value matched by the first set of parentheses is accessed with $1, the value matching the second set in $2 and so on.

```
my $string = "Protein structure: 1awa (P < 0.0001)";
$string =~ m/^.*: (\w{4}) \(P < (\d\.\d+)\)$/;
print "$1 $2\n";
```

```
1awa 0.0001
```

Non-greedy versions of quantifiers

- By default, regular expression match: 1) the left-most valid substring, and 2) extend as far right as possible.
- In Perl, you can change the second behavior with *non-greedy* versions of quantifiers, e.g. +?, *?, {2,4}?

```
my $text = "milk and cookies";
$text =~ /(\w+)/;
print "$1\n";
milk

$text =~ /(m.*i)/;
print "$1\n";
milk and cooki
```

```
$text =~ /(m.*?i)/;
print "$1\n";
mi

my $buttons = "<top>  <bottom>";
$buttons =~ /(<.*>)/;
print "$1\n";
<top>  <bottom>

$buttons =~ /(<.*?>)/;
print "$1\n";
<top>
```

Regex Substitutions: s/// and s///g

- The *substitution operator* s/// is an incredibly powerful tool for text transformation.
- The pattern between the first two delimiters is replaced by the string between the last two.
- Use the g modified-form s///g to replace all matches in a line.

```
my $text = "milk and cookies";
$text =~ s/\s/_/;
print "$text\n";
milk_and cookies
$text =~ s/\s/_/g;
print "$text\n";
milk_and_cookies
```

Object-oriented (OO) programming

- The key idea of OO programming is that all data is stored and modified with special data structures called objects,
- and each kind of object can be accessed only by its defined subroutines called methods.
- The user of an OO class is typically spared the effort of directly manipulating data, and can use class methods for this instead.

Understanding *objects*

- Object = Collection of data that logically belongs together.
  - E.g., a "genome" object has parts ("attributes") such as…
    - Name of the species
    - Its DNA sequence
    - A list of genes, each associated with one or more transcripts
    - A list of start and end points for each exon
    - etc
- A type of object (e.g., genome object) is called a *class*
  - All objects derive from a class

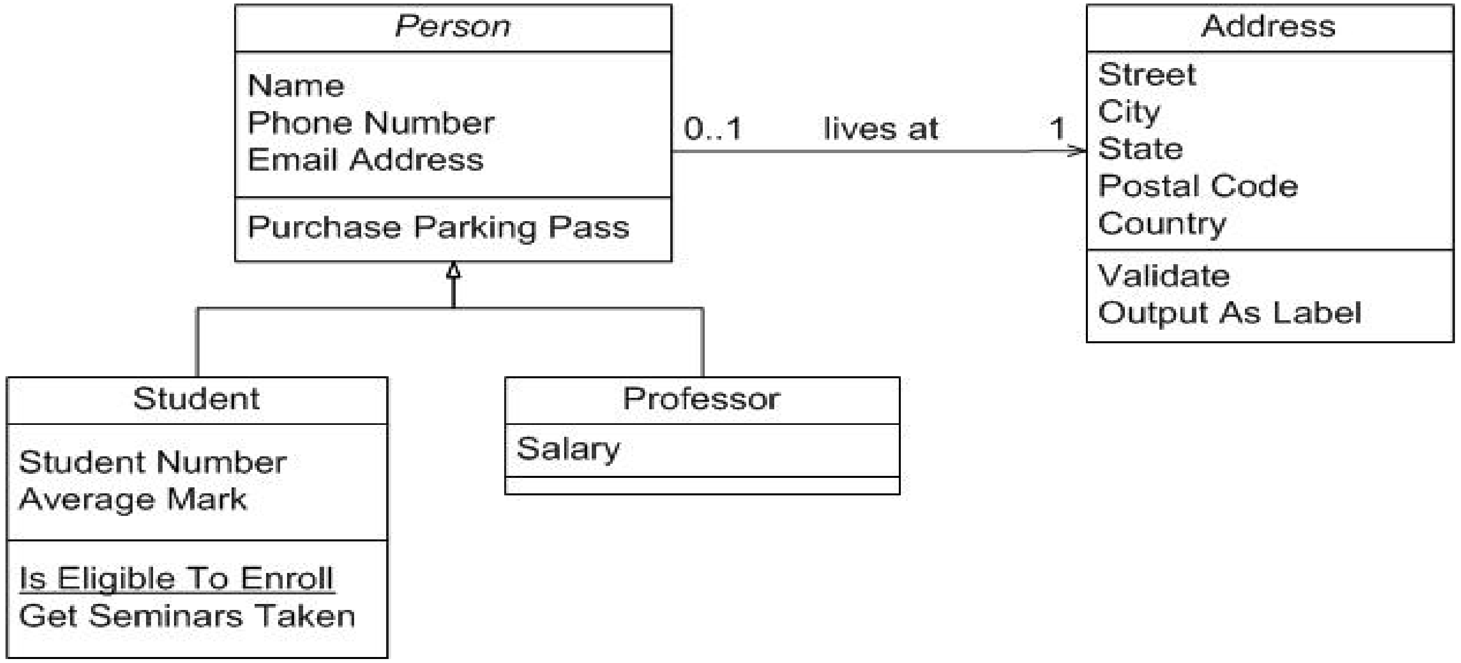
Understanding *methods*

- A *Method* is just like a subroutine but associated specifically with a class; they are not shared, except by "inheritance"
- Each type of object has one or more methods that it can call, and only those methods
  - The only way to access the data in an object is via the methods defined for that class.
- E.g., a genome object might have …
  - A *compare* method, for whole-genome comparisons
  - A *list-gene-families* method, for listing all gene families known to exist in a genome
  - A *GC-percent function*, for calculating %GC in specific areas of the genome, or all of it.

Understanding *classes*

- A Class is an object definition + a collection of methods.
- A specific object (e.g. a genome object for *H. sapiens*) is called an *instance* of a class.

Example of class definition and inheritance



OO in Perl

Class

```
package Person;
# The object constructor
sub new {
    my $self  = {};
    $self->{NAME}   = undef;
    $self->{AGE}    = undef;
    $self->{PEERS}  = [];
    bless($self);
    return $self;
}
# Methods to access object data
sub name {
    my $self = shift;
    if (@_) { $self->{NAME} = shift }
    return $self->{NAME};
}
sub age {
    my $self = shift;
    if (@_) { $self->{AGE} = shift }
    return $self->{AGE};
}
sub peers {
    my $self = shift;
    if (@_) { @{ $self->{PEERS} } = @_ }
    return @{ $self->{PEERS} };
}
1;
```

Program

```
use Person;

my $him = Person->new();
$him->name("Jason");
$him->age(23);
$him->peers( "Norbert", "Rhys", "Phineas" );

my @all_recs;
push @all_recs, $him;  # save object in array for later

printf "%s is %d years old.\n", $him->name, $him->age;
print "His peers are: ", join(", ", $him->peers), "\n";

printf "Last rec's name is %s\n", $all_recs[-1]->name;
```

```
Jason is 23 years old.
His peers are: Norbert, Rhys, Phineas
Last rec's name is Jason
```

BioPerl

BioPerl: >1,000 modules divided into several packages

- Free
- "Open Source" software

Bioperl Group	**Functions**
bioperl (the core)	Most of the main functionality of Bioperl.
bioperl-run	Wrappers to a lot of external programs.
bioperl-ext	Interaction with some alignment functions and the Staden package.
bioperl-db	Using bioperl with BioSQL and local relational databases.
bioperl-microarray	Microarray specific functions.
bioperl-gui	Some preliminary work on a graphical user interface to some Bioperl functions.

BioPerl

BioPerl provides object classes for various types of bioinformatics analysis

- external programs (e.g. BLAST, FASTA, clustalw and EMBOSS).
- various types of databases for storage and retrieval of data
- sequence analysis
- gene expression analysis
- etc

Bio::Perl module designed for beginners

- Bio::Perl is a module designed for beginners with easy access to a small number of Bioperl's functionality
- Bio::Perl is not object-oriented

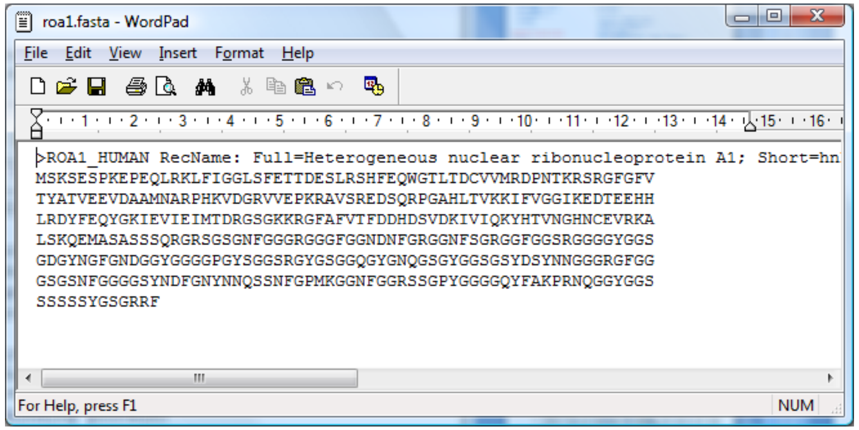
get_sequence	- gets a sequence from standard, internet accessible databases
read_sequence	- reads a sequence from a file
read_all_sequences	- reads all sequences from a file
new_sequence	- makes a Bioperl sequence just from a string
write_sequence	- writes a single or an array of sequence to a file
translate	- provides a translation of a sequence
translate_as_string	- provides a translation of a sequence, returning back just the sequence as a string
blast_sequence	- BLASTs a sequence against standard databases at NCBI
write_blast	- writes a blast report out to a file

Bio::Perl example

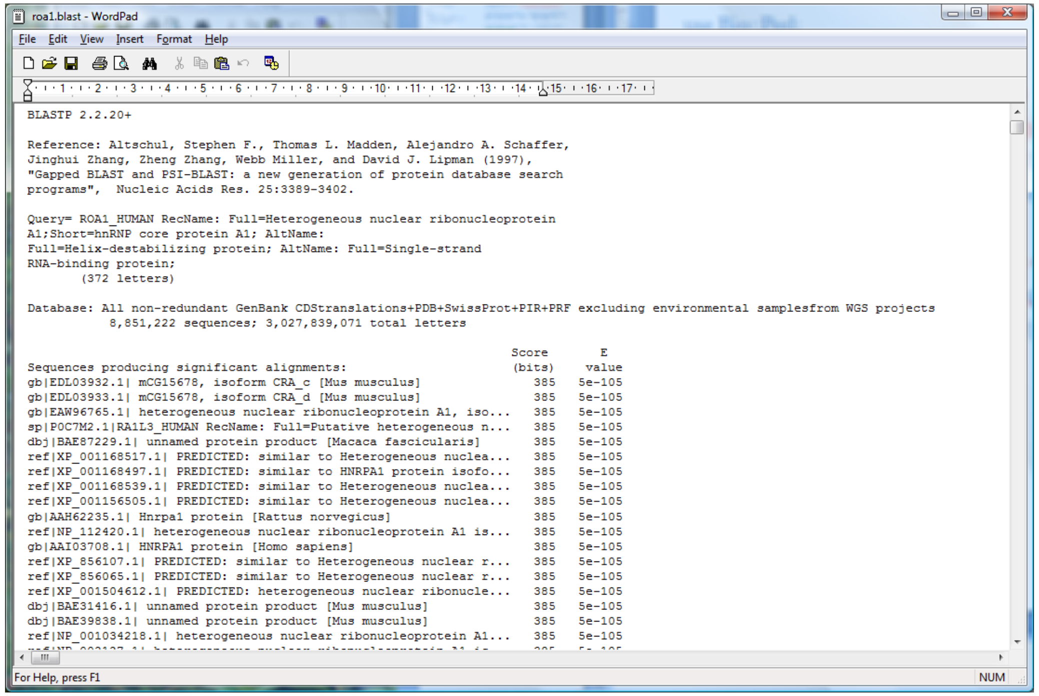
```
use Bio::Perl;

# the databases you can get sequences from
# are 'swiss', 'genbank', 'genpept', 'embl', and 'refseq'
my $seq = get_sequence('swiss',"ROA1_HUMAN");
write_sequence(">roa1.fasta",'fasta',$seq);

# uses the default database - nr in this case
my $blast_result = blast_sequence($seq);
write_blast(">roa1.blast",$blast_result);
```



```
>ROA1_HUMAN RecName: Full=Heterogeneous nuclear ribonucleoprotein A1; Short=hn
MSKSESPKEPEQLRKLFIGGLSFETTDESLRSHFEQWGTLTDCVVMRDPNTKRSRGFGFV
TYATVEEVDAAMNARPHKVDGRVVEPKRAVSREDSQRPGAHLTVKKIFVGGIKEDTEEHH
LRDYFEQYGKIEVIEIMTDRGSGKKRGFAFVTFDDHDSVDKIVIQKYHTVNGHNCEVRKA
LSKQEMASASSSQRGRSGSGNFGGGRGGGFGGNDNFGRGGNFSGRGGFGGSRGGGGYGGS
GDGYNGFGNDGGYGGGGPGYSGGSRGYGSGGQGYGNQGSGYGGSGSYDSYNNGGGRGFGG
GSGSNFGGGGSYNDFGNYNNQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGYGGS
SSSSSYGSGRRF
```



```
BLASTP 2.2.20+

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.

Query= ROA1_HUMAN RecName: Full=Heterogeneous nuclear ribonucleoprotein
A1;Short=hnRNP core protein A1; AltName:
Full=Helix-destabilizing protein; AltName: Full=Single-strand
RNA-binding protein;
       (372 letters)

Database: All non-redundant GenBank CDStranslations+PDB+SwissProt+PIR+PRF excluding environmental samplesfrom WGS projects
           8,851,222 sequences; 3,027,839,071 total letters


                                                                 Score     E
Sequences producing significant alignments:                      (bits)  value
gb|EDL03932.1| mCG15678, isoform CRA_c [Mus musculus]               385   5e-105
gb|EDL03933.1| mCG15678, isoform CRA_d [Mus musculus]               385   5e-105
gb|EAW96765.1| heterogeneous nuclear ribonucleoprotein A1, iso...   385   5e-105
sp|P0C7M2.1|RA1L3_HUMAN RecName: Full=Putative heterogeneous n...   385   5e-105
dbj|BAE87229.1| unnamed protein product [Macaca fascicularis]       385   5e-105
ref|XP_001168517.1| PREDICTED: similar to Heterogeneous nuclea...   385   5e-105
ref|XP_001168497.1| PREDICTED: similar to HNRPA1 protein isofo...   385   5e-105
ref|XP_001168539.1| PREDICTED: similar to Heterogeneous nuclea...   385   5e-105
ref|XP_001156505.1| PREDICTED: similar to Heterogeneous nuclea...   385   5e-105
gb|AAH62235.1| Hnrpa1 protein [Rattus norvegicus]                   385   5e-105
ref|NP_112420.1| heterogeneous nuclear ribonucleoprotein A1 is...   385   5e-105
gb|AAI03708.1| HNRPA1 protein [Homo sapiens]                        385   5e-105
ref|XP_856107.1| PREDICTED: similar to Heterogeneous nuclear r...   385   5e-105
ref|XP_856065.1| PREDICTED: similar to Heterogeneous nuclear r...   385   5e-105
ref|XP_001504612.1| PREDICTED: heterogeneous nuclear ribonucle...   385   5e-105
dbj|BAE31416.1| unnamed protein product [Mus musculus]              385   5e-105
dbj|BAE39838.1| unnamed protein product [Mus musculus]              385   5e-105
ref|NP_001034218.1| heterogeneous nuclear ribonucleoprotein A1...   385   5e-105
```

BioPerl: the *Sequence* object

```
use Bio::Seq;

my $seq_obj = Bio::Seq->new(-seq =>
                     "aaaatgggggggggggccccgtt",
                   -display_id => "#12345",
                   -desc => "example 1",
                   -alphabet => "dna" );

print $seq_obj->display_id(), ": ", $seq_obj->seq();
```

```
#12345: aaaatgggggggggggccccgtt
```

- use Bio::Seq; tells Perl to use a module on your machine called "Bio/Seq.pm".
- The variable $seq_obj is a *Sequence* object
- Arguments are passed to the method new() using ”hash syntax”
- display_id() and seq() are methods that returns the id and sequence as strings.

BioPerl: The *SeqIO* object

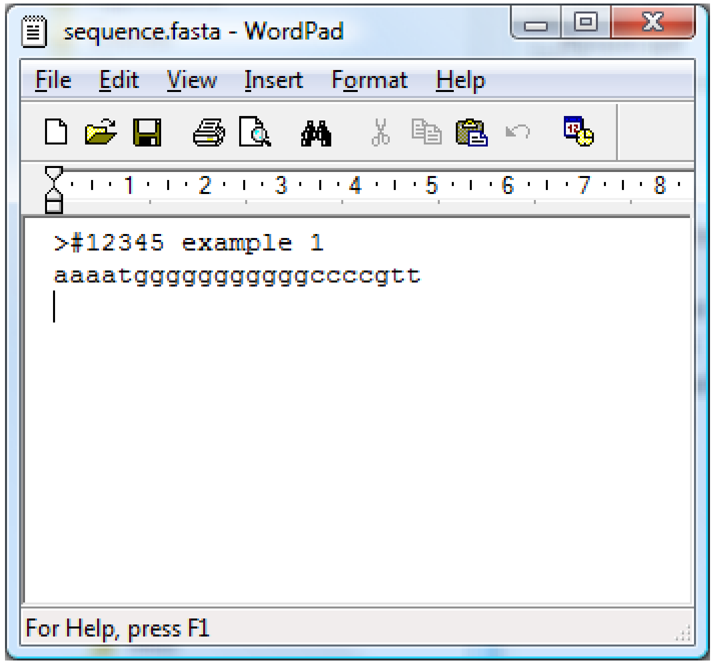
```
use Bio::Seq;
use Bio::SeqIO;

my $seq_obj = Bio::Seq->new(-seq =>
                    "aaaatgggggggggggccccgtt",
                  -display_id => "#12345",
                  -desc => "example 1",
                  -alphabet => "dna" );


my $seqio_obj = Bio::SeqIO->new(-file =>
    '>sequence.fasta', -format => 'fasta' );


$seqio_obj->write_seq($seq_obj);
```

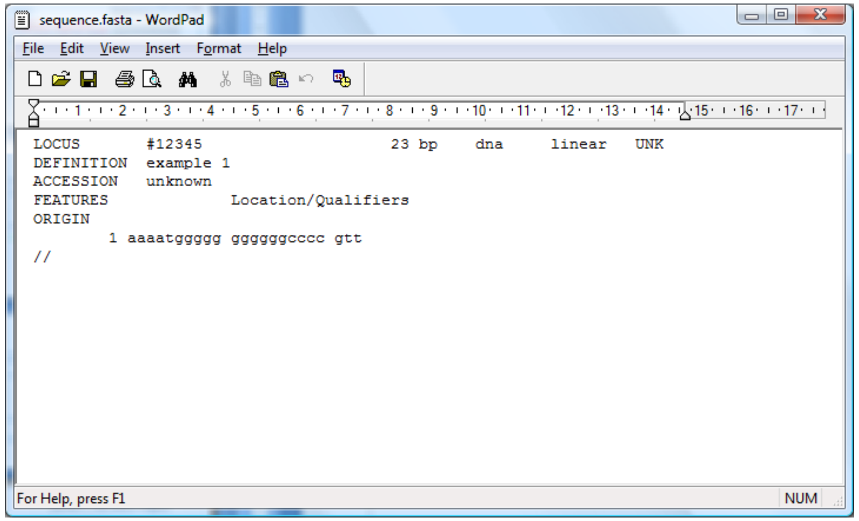
- The variable $seqio_obj is a *SeeqIO* object
- The “>” in the -file argument indicates that we're going to write to the file



```
>#12345 example 1
aaaatgggggggggggccccgtt
```

BioPerl: The *SeqIO* object

- Changing format from fasta to genbank in the previous program changes the output
- This illustrates some of the flexibility and power of using an IO object over open



```
LOCUS       #12345                  23 bp    dna     linear   UNK
DEFINITION  example 1
ACCESSION   unknown
FEATURES             Location/Qualifiers
ORIGIN
        1 aaaatggggg ggggggcccc gtt
//
```

BioPerl: The *SeqIO* object

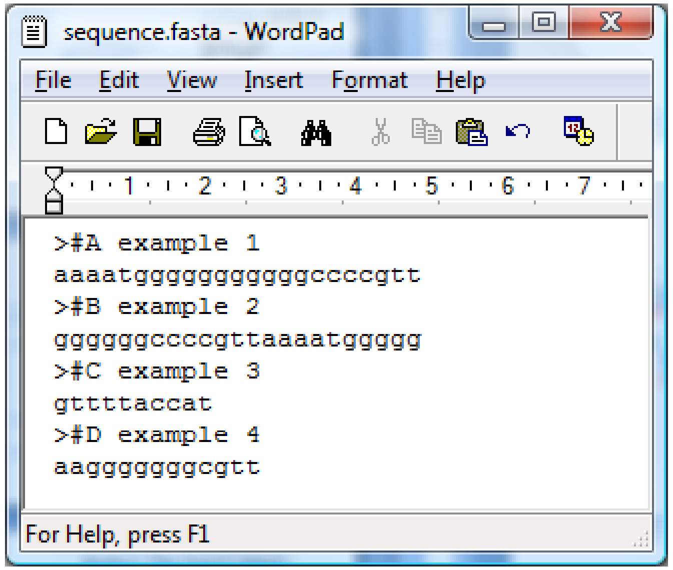
```
use Bio::SeqIO;

$seqio_obj = Bio::SeqIO->new(-file =>
    "sequence.fasta", -format => "fasta" );

while (my $seq_obj = $seqio_obj->next_seq()){
  # print the sequence
  print $seq_obj->seq(),"\n";
}
```

```
aaaatgggggggggggccccgtt
ggggggccccgttaaaatggggg
gttttaccat
aagggggggcgtt
```

- No “>” in the -file argument indicates that we're going to read from the file
- The method next_seq() is typical for BioPerl



```
>#A example 1
aaaatgggggggggggccccgtt
>#B example 2
ggggggccccgttaaaatggggg
>#C example 3
gttttaccat
>#D example 4
aagggggggcgtt
```

Retrieving a sequence from a database

```
use Bio::DB::SwissProt;

my $db_obj = Bio::DB::SwissProt->new;

my $seq_obj = $db_obj->get_Seq_by_acc("ROA1_HUMAN");

print $seq_obj->seq(),"\n";
```

```
MSKSESPKEPEQLRKLFIGGLSFETTDESLRSHFEQWGTLT
DCVVMRDPNTKRSRGFGFVTYATVEEVDAAMNARPHKV
DGRVVEPKRAVSREDSQRPGAHLTVKKIFVGGIKEDTEE
HHLRDYFEQYGKIEVIEIMTDRGSGKKRGFAFVTFDDHD
SVDKIVIQKYHTVNGHNCEVRKALSKQEMASASSSQRGR
SGSGNFGGGRGGGFGGNDNFGRGGNFSGRGGFGGSRG
GGGYGGSGDGYNGFGNDGGYGGGGPGYSGGSRGYGS
GGQGYGNQGSGYGGSGSYDSYNNGGGRGFGGGSGSNF
GGGGSYNDFGNYNNQSSNFGPMKGGNFGGRSSGPYGG
GGQYFAKPRNQGGYGGSSSSSSYGSGRRF
```

- Bio::DB retrieves sequences from online databases
- Other alternatives:
  - GenBank (Bio::DB::GenBank)
  - GenPept (Bio::DB::GenPept)
  - EMBL (Bio::DB::EMBL)
  - SeqHound (Bio::DB::SeqHound)
  - Entrez Gene (Bio::DB::EntrezGene)
  - RefSeq (Bio::DB::RefSeq)

Retrieving sequences from a database

```
use Bio::DB::GenBank;
use Bio::DB::Query::GenBank;

my $query = "Arabidopsis[ORGN] AND topoisomerase[TITL] AND 0:3000[SLEN]";
my $query_obj = Bio::DB::Query::GenBank->new(-db    => 'nucleotide',  -query => $query );

my $gb_obj = Bio::DB::GenBank->new;

my $stream_obj = $gb_obj->get_Stream_by_query($query_obj);

while (my $seq_obj = $stream_obj->next_seq) {
        print $seq_obj->display_id, "\t", $seq_obj->length, "\n";
}
```

- Use a stream object whenever you expect to retrieve a stream or series of sequence objects
- The stream object has a next_seq() method to retrieve one seqeunce at a time
- 0:3000[SLEN] limits hits to 3000 nucleotides

Running external programs/system commands

- Both Perl's exec() function and system() function execute a system shell command
- system() runs the command and returns when done.

```
system("mkdir TEST");
print "Finished!\n";
```

```
Finished!
```

- exec() runs the command and do not return.

```
exec("mkdir TEST");
print "Finished!\n";
```

Running external programs/system commands

- To capture the output of a system command, use the backtick operator:

```
my $result = `dir bioperl.pl`;
print "$result\n";
```

```
Volume in drive C is OS
Volume Serial Number is CA2C-F64B

 Directory of C:\Labs

2009-05-12  14:20                732 bioperl.pl
                 1 File(s)        732 bytes
                 0 Dir(s)         848 bytes free
```

Running BLAST from Perl

Standard approach:

```
system(”blastall -d C:\Blast\db\yeast.nt -i sequence.txt >hits.txt”);

open (H, "hits.txt");
# Parse output
...
close(H);
```

External programs in BioPerl

Bio::Tools::Run contains a large number of modules for running bioinformatics tools

```
use Bio::Seq;
use Bio::Tools::Run::StandAloneBlast;

my $blast_obj = Bio::Tools::Run::StandAloneBlast->new(program  => 'blastn', database => 'yeast.nt');

my $seq_obj = Bio::Seq->new(-id  =>"test_query", -seq =>"TTTAAATATATTTTGAAGTATAGATTATATGTT");

my $report_obj = $blast_obj->blastall($seq_obj);

while(my $result = $report_obj->next_result ) {
   while(my $hit = $result->next_hit ) {
     while(my $hsp = $hit->next_hsp ) {
       if ( $hsp->percent_identity > 75 ) {
         print "Hit: ", $hit->name, ”, Length: ", $hsp->length('total'), ”, Percent_id: ",
         $hsp->percent_identity, "\n";
       }
     }
   }
}
```

```
Hit: gi|6323989|ref|NC_001146.1|, Length: 15, Percent_id: 100
Hit: gi|6322623|ref|NC_001143.1|, Length: 17, Percent_id: 94.1176470588235
...
```

Parallelization

- Many bioinformatics problems can be divided into smaller ones, which are then solved concurrently ("in parallel").
  - E.g. finding paralogs in a huge genome. How?
- Parallelization has become more important in recent years do to the commonness of multicore processors

Parallelization in Perl

- A process is an instance of a computer program that is being sequentially executed
- fork(): Create a duplicate process (child) of the current process (parent)
- Each process is given a process ID by the operating system
- fork() returns the child process ID to the parent on success, 0 to the child on success and undef on failure to fork

Parallelization in Perl

```perl
my @genome = get_all_gene_sequences();

my $num = 10;
my @children;

for (my $i = 0; $i < $num; $i++) {
  my $pid = fork();
  if ($pid) { # parent
    push @children, $pid;
  } elsif ($pid == 0) { # child
    print "child $i\n";
    find_paralogs($i,  $num, \@genome);
    exit;
  } else {
    print "couldn't fork\n";
  }
}

foreach my $child (@children) {
  waitpid($child, 0);
}

collect_paralogs($num);
```

```perl
sub get_all_gene_sequences {
   # Read all gene sequences in the genome from file
   return  @genome;
}

sub find_paralogs {
  my $i = $_[0];
  my $num = $_[1];
  my @genome = @{$_[3]};

  my $n = @genome;
  my @slice = @genome[$i*$n/$num..$i*$n/$num+$n/$numb-1];

  # Blast @slice against @genome and write paralogs to file
}

sub collect_paralogs {
   my $num = $_[0];
   # Read pralogs from files and write them to a common file
}
```

Acknowledgements

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