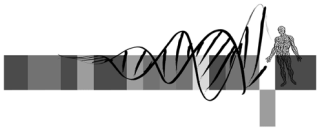

Bioinformatics for Biologists

Computational Methods III:
Sequence Analysis with Perl -
Modules and BioPerl

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Sequence analysis with Perl Modules and BioPerl

- Regular expressions
- Hashes
- Using modules
- Library for WWW access in Perl (LWP)
- Common Gateway Interface Class (CGI)
- GD and SVG graphics libraries
- BioPerl (SeqIO, SearchIO)

Objectives

- Start to take advantage of the power of Perl's regular expressions
- Start to use modules to extend the power of Perl's core functions
- Start to use BioPerl modules for sequence analysis

Regular expressions

- "a pattern to be matched against a string"
- found in Unix, Perl, and elsewhere
- used in Perl for matching and substitution
- Regexps use lots of special characters
- Perl example: extracting human fasta headers

```
@hdrs = grep (/^>.*(human|homo)/i, @lines);
```

`^`	beginning of word anchor
`.`	any character but newline
`*`	0 or more of preceding character
`|`	logical 'OR'
`i`	pattern is case insensitive

Some uses of regular expressions

- biological applications you've seen:
  - sequence motifs
  - transcription factor binding sites
- other biological applications:
  - parsing GenBank and BLAST reports
  - reformatting data from a file (ex: EMBOSS output)
  - extracting references from a manuscript

Writing a regular expression

- Describe the pattern in English
- What part of match do you want to extract?
- Translate into Perl (see below)

`[A-Z]`	any capital letter	`\bword\b`	word anchor
`[0-9]*`	>= 0 numbers	`ATG/i`	ATG or atg
`\s+`	>= 1 space chars	`ATG/g`	all ATG's
`[^A]`	anything but 'A'	escaped characters: `\* \. \+ \\| \\ \/ \# \"`	
`\d{3}`	3 digit numbers		

Regex examples for GenBank files

- ORGANISM Mus musculus

```
if (/(ORGANISM\s*)(.*)/)
    { $org = $2; }
```

- VERSION NM_007553.1 GI:6680793

```
if (/VERSION (.*) GI:(\d*)/)
    { $acc = $1;  $gi = $2;}
```

- CDS 357..1541

```
if (/(CDS\s*)(\d*)(\.\.)(\d*)/)
    {$start = $2; $end = $4; }
```


Hashes

- pairs of scalar data represented as a lookup table
- a hash can be created all at once:
  %hash = (key1, value1, key2, value2, etc.)
- examples: creating %translate and %gi

key	value
ATG ⇨	M
GGT ⇨	G
CAT ⇨	H
TAG ⇨	*

```
%translate = (
"ATG", "M",          "GGT", "G",
"CAT", "H",          "TAG", "*",
); # etc. . .

print "ATG is the codon for $translate{'ATG'}";
#      ATG is the codon for M
# In general, $hash{key} = value;
```


Hashes (cont.)

- a hash can also be created one key/value pair at a time:
  $hash{key} = value
- Example: given corresponding arrays of GI numbers (**@gi**) and sequence names (**@seqs**), create **%gi2seq**

```
for ($i = 0; $i <= $#seqs; $i++)
  {
      $gi2seq{$gi[$i]} = $seq[$i];
  }
print "GI:$gi[$i] represents $gi2seq{$gi[$i]}.";
# example:    GI:6680793 represents mouse BMP-2.
# To separate out keys and values:
@mykeys = keys(%gi2seq);
@myvalues = values(%gi2seq);
```


Introduction to modules

- "a unit of software reuse"
- adds a collection of commands related to a specific task
- core modules vs. other modules
- see http://www.cpan.org/ to find documents and downloads, etc.

Using modules

- Before using a module that you installed yourself,

```
use lib 'full/path/to/module';
```

- For all modules,

```
use module_name;
```

- Example:

```
# full path to directory with GD.pm

use lib '/home/elvis/modules';
use GD;      # The .pm is optional
```


Object-oriented Perl

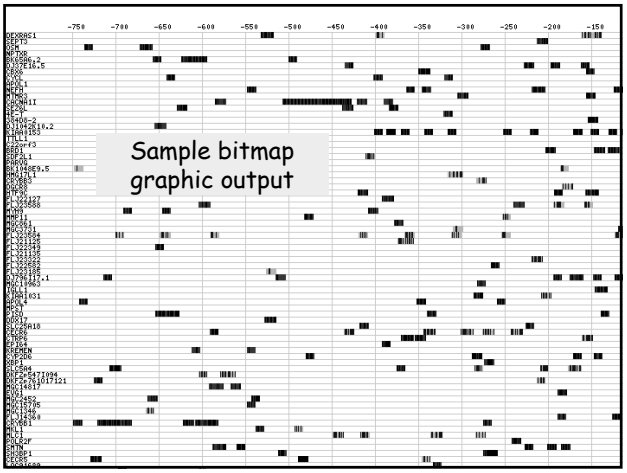
- objects are module-specific references to data
- a module can describe multiple objects
  - Bio::SeqIO::fasta
  - Bio::SeqIO::GenBank
- **->** send information about the data
- example of creating an object and performing methods on it:

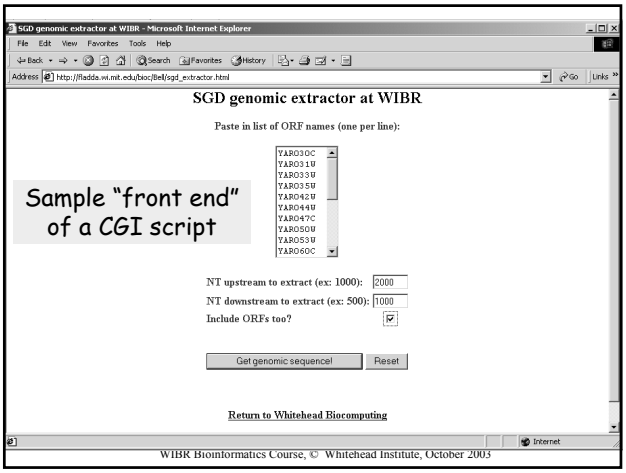
```
$seqs = Bio::SeqIO->new(-file => "$inFile",
    '-format' => 'Fasta');      # makes a SeqIO object

$seqobj = $seqs->next_seq(); # makes a Seq object
$rawseq = $seqobj->seq();

$rev_comp = $seqobj->revcom->seq();
```

LWP: fetch WWW documents

- To automate WWW access
- LWP::Simple - procedural interface to LWP
- Example of usage:

```
use LWP::Simple;
$url = "http://www.whatever.com/data.html";
$page = get($url);
if ($page)
   { # do something }
else    { print "Problems getting $url"; }
```

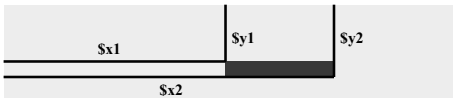





Sample "front end" of a CGI script

GD: generate bitmap graphics

- GD generates figures (png, gif(?)) from rectanges, polygons, circles, lines, and text
- For all methods, position is in pixels from top left corner of figure



- method examples:

```
$img->filledRectangle($x1, $y1, $x2, $y2, $red);

$img->string(gdSmallFont, $x, $y, $text, $green);
```


CGI: run scripts from the WWW

- gets input from HTML forms
- stdout writes document in browser
- execution controlled by server configuration
- example of usage:

```
use CGI qw(:standard);      # import :group shortcuts
$input = new CGI;
print $input->header('text/html');
# print content here
print $input->end_html;
```


SVG: generate vector graphics

- Vector graphics
  - images are made up of objects
  - magnification maintains resolution
  - figures can be edited in Illustrator
- based on XML (text)
- SVG images can be viewed in a web browser BUT require a free plug-in

(http://www.adobe.com/svg/)

BioPerl

- modules designed to simplify the writing of bioinformatics scripts
- uses objects (references to a specific data structure)
- Seq: main sequence object
  - available when a sequence file is read

```
$seqs = Bio::SeqIO->new('-file' =>
"inputFileName", '-format' => 'Fasta');
$seqobj = $seqs->next_seq();
```


BioPerl's SeqIO module

- sequence input/output
- formats: Fasta, EMBL, GenBank, swiss, SCF, PIR, GCG, raw
- parse GenBank sequence features
  - CDS, SNPs, Region, misc_feature, etc.
- sequence manipulation:
  - subsequence, translation, reverse complement

Using SeqIO

```
$in = Bio::SeqIO->new(-file => "$in", '-format' => 'Fasta');
$out = Bio::SeqIO->new(-file => ">$out", '-format' =>
   'Genbank');

while ($seqobj = $in->next_seq())
{
   $out->write_seq($seqobj);      # print sequence to $out
   print "Raw sequence:", $seqobj->seq();
   print "Sequence from 1 to 100: ", $seqobj->subseq(1,100);
   print "Type of sequence: ", $type = $seqobj->alphabet();
   if ($type eq "dna")
   {
       $rev_comp = $seqobj->revcom->seq();
       print "Reverse complement: $rev_comp";
       print "Reverse complement from 1 to 100";
              $seqobj->revcom->subseq(1, 100);
   }
}
```


Parsing BLAST reports with SearchIO

- best BioPerl blast parser

```
use Bio::SearchIO;
$report = new Bio::SearchIO(-file=>"$inFile",
      -format => "blast");
while($result = $report->next_result)
{
  while($hit = $result->next_hit)
  {
      while ($hsp = $hit->next_hsp)
      {
          print "Hit=", $hit->description, "\t",
            "PercentID=", $hsp->percent_identity, "\n";
} } }
```


Summary: Perl and BioPerl

- Regular expressions
- Hashes
- Using modules
- Library for WWW access in Perl (LWP)
- Common Gateway Interface Class (CGI)
- GD and SVG graphics libraries
- BioPerl (SeqIO, BPlite)

Summary: Bioinformatics tools

- individual applications (Blast, Genscan, etc.):
  - web
  - command line
- analysis packages: EMBOSS, etc.
- Unix tools
- Perl tools
  - core commands
  - core modules
  - BioPerl and other "add-on" modules

Demo scripts on the web site

get_web_data.pl	use LWP to automate web file access
draw_figure.pl	draw a PNG figure using the GD module
draw_figure_SVG.pl	draw a figure with vector graphics
fastaToGenbank.pl	sequence conversion
genbank_parse.pl	parse GenBank sequence features
manipulate_seq.pl	manipulate a sequence
blast_parse.pl	parse BLAST output files using BioPerl's SearchIO