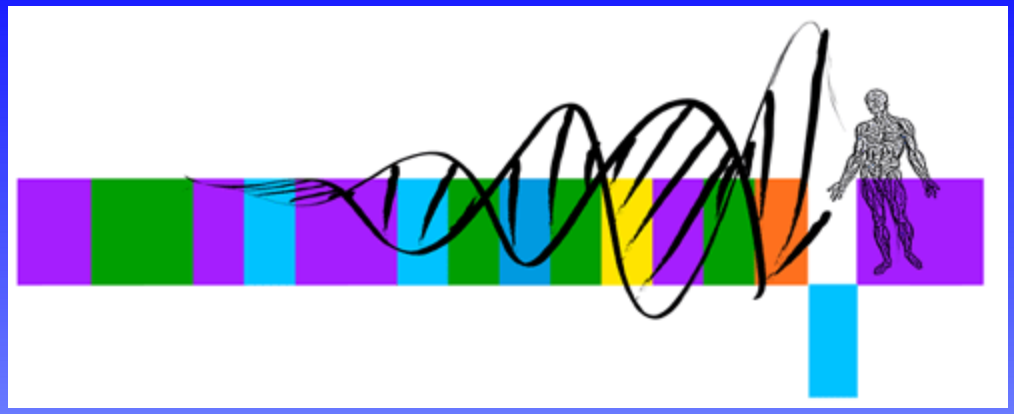

Unix, Perl and BioPerl

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
`[0-9]*`	>= 0 numbers
`\s+`	>= 1 space chars
`[^A]`	anything but 'A'
`\d{3}`	3 digit numbers

`\bword\b`	word anchor
`ATG/i`	ATG or atg
`ATG/g`	all ATG's

escaped characters: `\*` `\.` `\+` `\|` `\\` `\/` `\#` `\"`

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

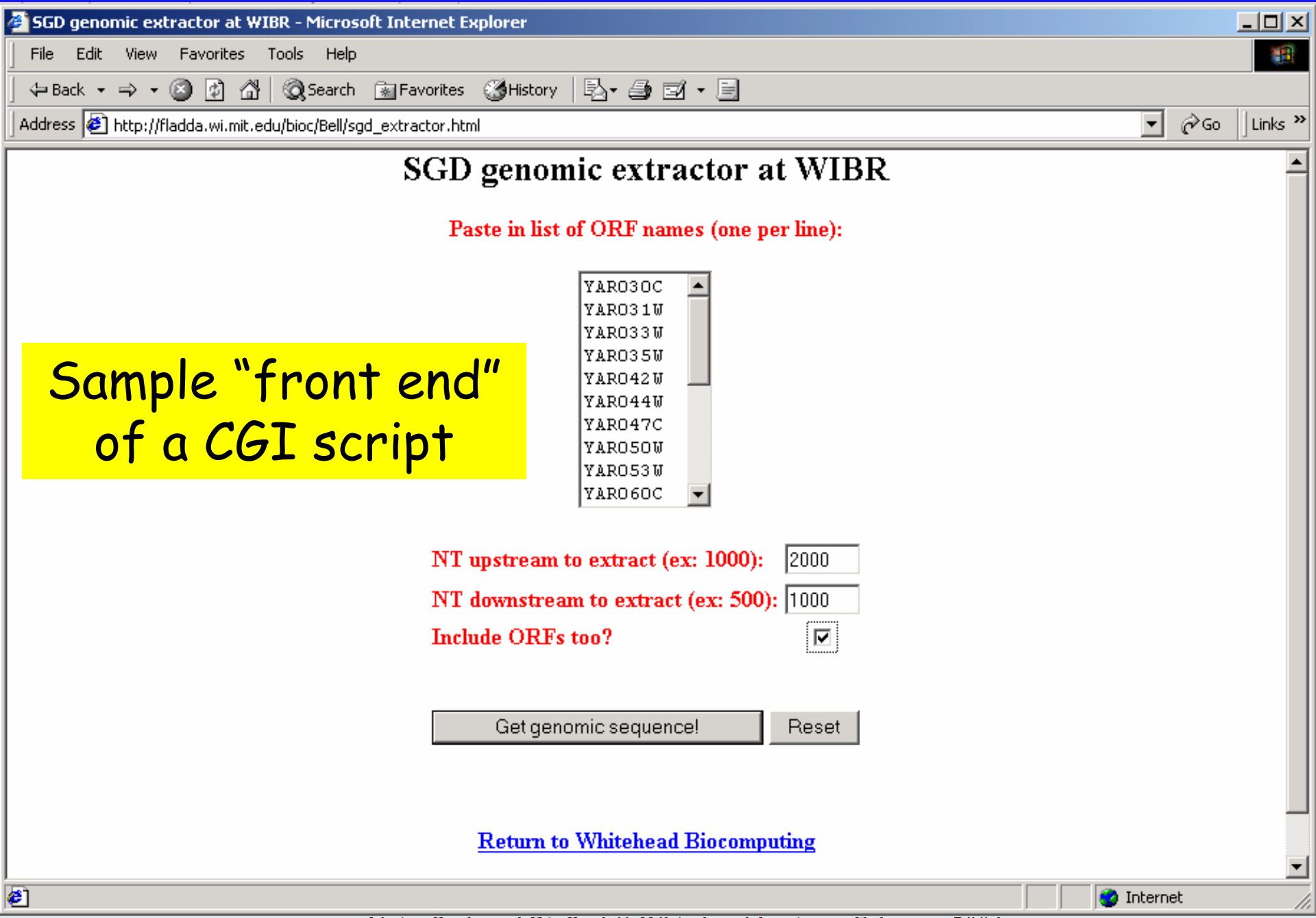
- objects are module-specific references to data
- a module can describe multiple objects
  - Bio::SeqIO::fasta
  - Bio::SeqIO::GenBank
- **->** send information about the data
- example of creating an object and performing methods on it:

```
$seqs = Bio::SeqIO->new(-file => "$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"; }
```

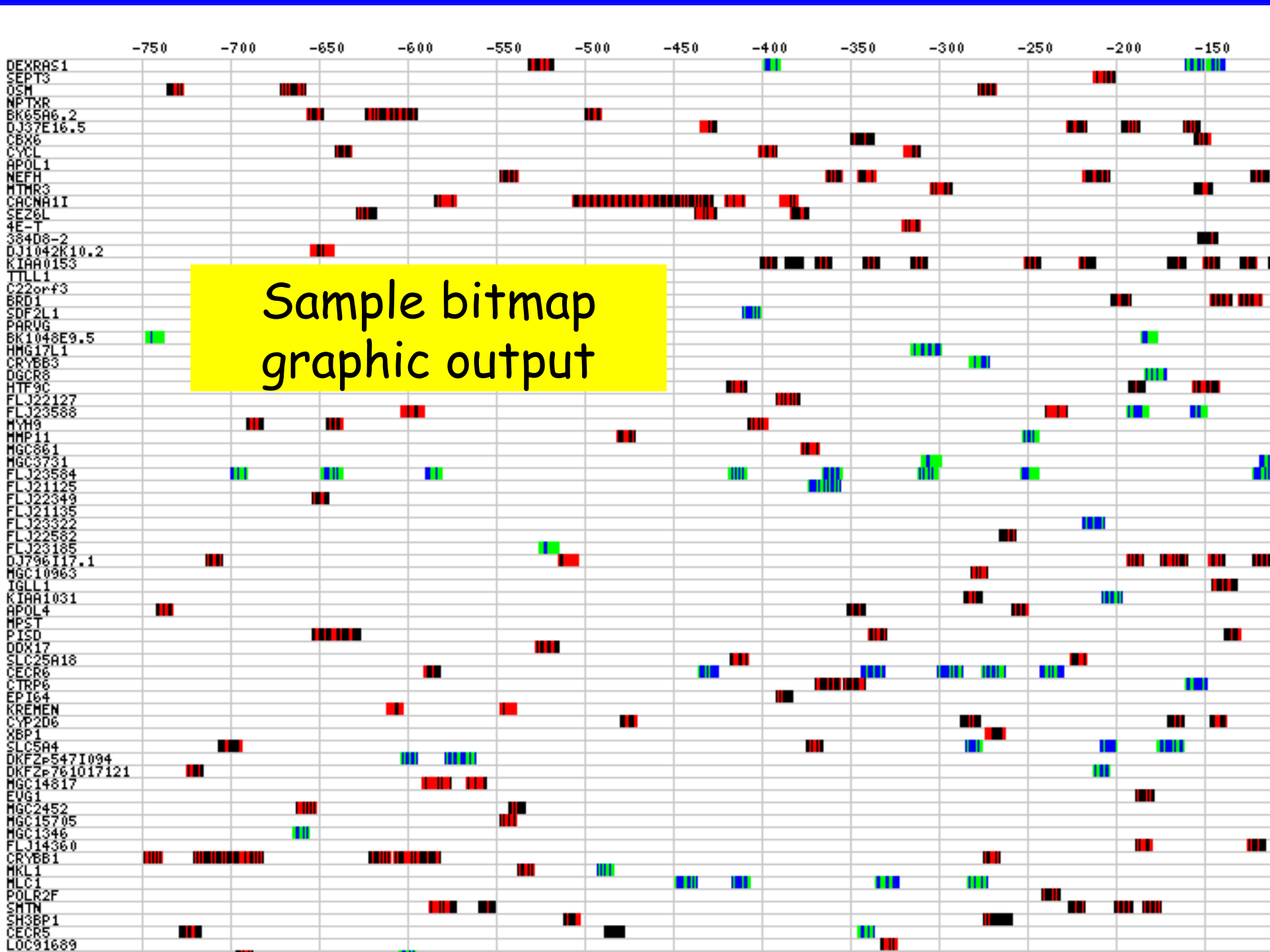


Sample "front end" of a CGI script

CGI: run scripts from the WWW

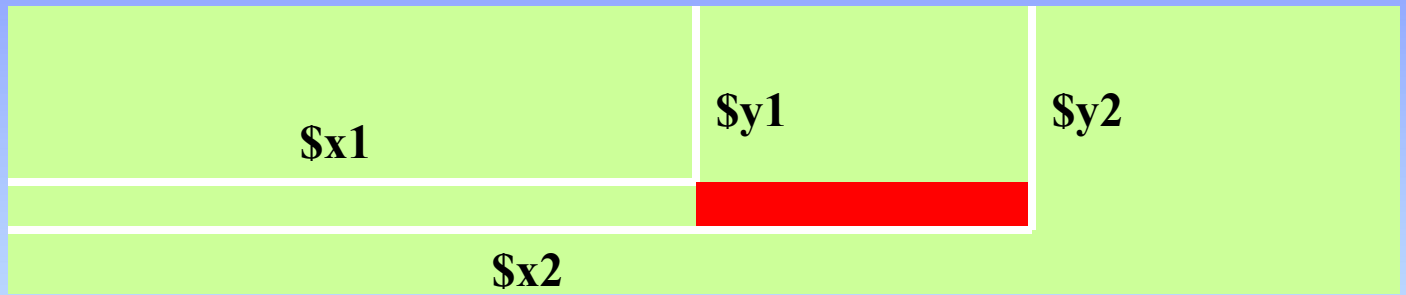
- gets input from HTML forms
- stdout writes document in browser
- execution controlled by server configuration
- example of usage:

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


-750
-700
-650
-600
-550
-500
-450
-400
-350
-300
-250
-200
-150
DEXRAS1
SEPT3
OSM
NPTXR
BK65A6.2
DJ37E16.5
CBX6
CYCL
APOL1
NEFH
MTMR3
CACNA1I
SE26L
4E-T
384D8-2
DJ1042K10.2
KIAA0153
TTLL1
C22orf3
BRD1
SDF2L1
PARVG
BK1048E9.5
HMG17L1
CRYBB3
DGCR8
HTF9C
FLJ22127
FLJ23588
MYH9
MMP11
MGC861
MGC3731
FLJ23584
FLJ21125
FLJ22349
FLJ21135
FLJ23322
FLJ22582
FLJ23185
DJ796I17.1
MGC10963
IGLL1
KIAA1031
APOL4
MPST
PISD
DDX17
SLC25A18
CECR6
CTRP6
EPI64
KREMEN
CYP2D6
XBP1
SLC5A4
DKFZp547I094
DKFZp761017121
MGC14817
EVG1
MGC2452
MGC15705
MGC1346
FLJ14360
CRYBB1
MKL1
MLC1
POLR2F
SMTN
SH3BP1
CECR5
LOC91689
Sample bitmap
graphic output

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);
```

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

Exercises

- 1: Parsing a file of multiple BLAST reports
- 2: Manipulating a GenBank file with BioPerl and creating a PNG image
- 3: Setting up a web-based search script