Perl for Bioinformatics

Perl and BioPerl I

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Outline

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Why Perl for data processing & bioinformatics

- Fast text processing
- Regular expressions
- Extensive module libraries for pre-written tools
- Large number of users in community of bioinformatics
- Scripts are often faster to write than full compiled programs

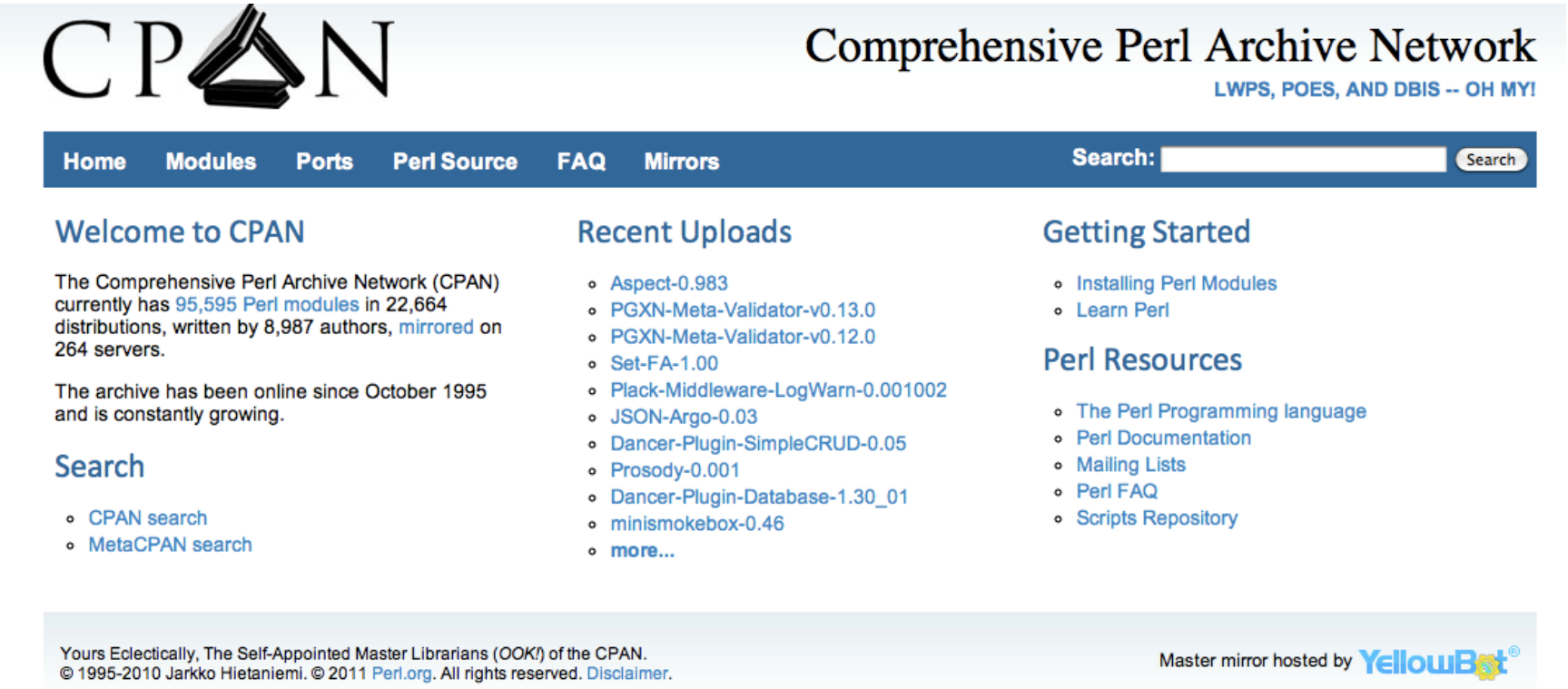
Cons Syntax sometimes confusing to new users; 'There's more than one way to do it' can also be confusing. (TMTOWTDI)

Cons Not a true object-oriented language so some abstraction is clunky and hacky

Scripting languages (Perl, Python, Ruby) generally easier to write simply than compiled ones (C, C++, Java) as they are often not strongly typed and less memory management control.

Perl packages

- CPAN - Comprehensive Perl Archive



`http://www.cpan.org`

Help!

- Perldoc online `http://perldoc.perl.org/`
- Or on your computer - type 'perldoc'
- For functions use -f 'perldoc -f sprintf'
- For modules just the name 'perldoc List::Util'

Hello world

```
#!/usr/bin/perl -w
use strict;
print "hello world\n";

>> perl hello.pl
>> hello world
```

Variables & Syntax

```
$scalar - single value, can be string,number
@array - list of values
%hash - paired values: key and value

# comments look like this
my $variable; # a var declared with my for this scope
my ($n1,$n2) = (10,20); # declared and initialized
```

Strings

```
my $number = "12";
my $msg = "This could be a message";
my $composite = "There are $number apples";
print $composite, "\n";

>> There are 12 apples
```

Quotable

```
my $str = 'literally';
my $str2 = "interpreted as $str";
my $executed = `/usr/bin/clustalw seqs.fa`;

# special characters
my $tab = "\t";
my $newline = "\t";
my $singlequote = "'";
my $dblquote = "\""; # OR '" ';
```

Numerics

```
my $n = 16;
my $g = 3**2; # 3^2
my $d = 12.34;
my $ir = 1/3;
my $h = 1e-3;
print $ir, "\n";
print $h, "\n";
printf "%e\n", $h; # print in scientific notation

>> 0.333333333333333
>> 0.001
>> 1.000000e-03
```

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Print me something

```
#!/usr/bin/perl -w
use strict;
 print "a message\n";
 my $v1 = 'CFTR';
 my $v2 = '20.34';
 printf "a LOD score for marker %s is %d\n", $v1, $v2;

>> a LOD score for the marker CFTR is 20
```

Logic

```
if ( $A < $B) { } # if this
elsif ( $B < $C ) { } # otherwise if this
else {} # do this as last resort

unless( $bool_not ) { } # do something if bool_not is false

while( $bool ) {} # loop if  bool is true
until ( $bool_not ) {} # loop if bool_not is false
for( INIT; TEST; INCREMENT ) { }
for( $i = 0; $i < 5; $i++ ) { print $i, "\n"; }
```

```
>> 1
>> 2
>> 3
...
```

Logic and Truth

```
if( $a == $b ) # a is numerically equivalent to b
if( $a eq $b ) # a is lexically equivalent to b
if( $a < $b ) # a is less than b
if( $a != $b ) # a is not equal to b (numerically)
if( $a ne $b ) # a is not lexically equal (string comparison)
if( @list ) # true if @list is not empty
if( $a ) # true if $a is not 0 and not undefined
```

Arrays and Lists

```
my @fruit = ('apple', 'pear', 'peach');
my @tropical = qw(kiwi passion star); # qw for quote words
my @all = (@fruit,@tropical); # lists are flattened
my @mixed = (1, 'pineapple', 18.3); # can be mixed types
my @sorted = sort @mixed; #alpha numeric sort
my @ordered = sort { $a<=>$b} (10,3,17,200,9);
print $ordered[0], "\n"; # print the 1st item
print $ordered[-1], "\n"; # print the last item
```

```
>> 3
>> 200
```

Lists and strings start counting at '0' not '1'

Arrays

To add or remove entries from lists: can operate as linked-lists and stacks

- `pop` to remove from end
- `push` to add to end
- `shift` remove from front `unshift` add to front
- `splice` to arbitrarily remove from any position

```
my @tools = qw(rake shovel);
push @tools, 'hammer'; # now there will be 3 items
my $first = shift @tools; # will be 'rake'
splice(@tools, 1,0,'saw'); # insert 'saw' after 'hammer'
print join(",",@tools),"\n";
```

```
>> rake,saw,shovel,hammer
```

Split and Join

```
my @lst = qw(In the locust wind comes a rattle and hum);
print join(",", @lst), "\n"; # combine list to a string
my @newlist = split(/\s+/, "GENE1 GENE2 GENE3 VALUE1");
print $newlist[2], "\n"; # split string, get 3rd item
```

```
>> In,the,locust,wind,comes,a,rattle,and,hum
>> GENE3
```

Hashes

```
my %fruit = ('apple' => 'red'); # initialize with value
$fruit{'banana'} = 'yellow';    # add a value
$fruit{'apple'} = 'green';      # update a value
my @keys = keys %fruit;         # get keys of hash
my @vals = values %fruit;       # get the values
print join(",", @keys), "\n";
print join(",", @vals), "\n";

>> banana,apple
>> yellow,green
```

Manipulate strings

- substr - get a substring and also manipulate in place
- length - get length of a string
- . - concatenate two strings

```
my $left = 'ABC';
my $right = 'XYZ';
my $concat = $left .$right;
print length($concat), " is length of string $concat\n";
print "pos 3-4 is ", substr($concat,2,2), "\n";
my $lastchar = substr($concat, -1,1);
substr($concat,1,2,''); # replace 2nd and 3rd characters
print "concat is $concat\n";
```

```
>> 6 is the length of string ABCXYZ
>> pos 2-4 is CX
>> concat is AXYZ
```

Manipulate strings 2

How about walking through each base in a sequence?
Could use split and turn it into an array and use a for loop OR
Can use substr to request each character in the string, one at a time. Turns out this is faster.

```
my $sequence = 'ACGGTAGCATA';
for( my $i = 0; $i < length $sequence; $i++ ) {
 my $base = substr($sequence, $i, 1); # get the i-th base
  print $base, "\n";
}
```

```
>> A
>> C
>> G
>> G
>> T
...
```

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References – pointers in Perl

```
my %info = ( 'gene123' => 1.02);
my $ref = \%info; # backslash to make reference
print $ref->{'gene123'}, "\n"; # arrow to dereference
my $ref2 = { 'gene1' => 2 }; # {} for anonymous hash reference
print $ref2->{'gene1'}, "\n";
print join(",", keys %{$ref2}), "\n"; # %{ } to deref a hash
my $refarray = ['gene1','gene2']; # [] for anonymous array referen
print join(";", @$refarray), "\n"; # @{} to dereference array

>> 1.02
>> gene1
>> gene1;gene2
```

References used for Hashes of Arrays (HoA)

If you wanted to store more than 1 thing per key in a hash

```
my %kitchen = ( 'ingredients' => [ qw(flour eggs milk) ] );
print join(",", @{$kitchen{'ingredients'}}), "\n";
$kitchen{'chefs'} = [qw( Anthony Emeril)];
push @{$kitchen{'chefs'}}, 'Julia';
for my $info ( keys %kitchen ) {
  print "$info: ",join("\t", @{$kitchen{$info}}),"\n";
}
```

```
>> flour,eggs,milk
>> chefs:        Anthony Emeril  Julia
>> ingredients:  flour   eggs    milk
```

Array of Hashes (HoH)

```
my @library = (  { 'title' => 'To Kill a Mockingbird',
                   'author_last'=> 'Lee',
                   'author_first'=> 'Harper'},
                 { 'title' => 'Moby Dick',
                   'author_last' => 'Mellville',
                   'author_first' => 'Herman'},
                 { 'title' => 'Old Man and the sea',
                   'author_last' => 'Hemmingway',
                   'author_first'=> 'Ernest' } );
for my $book ( sort { $a->{author_last} cmp $b->{author_last} }
                @library ) {
  printf "%s,%s %s\n", $book->{author_last},
         $book->{author_first},$book->title;
}

>> Hemmingway,Ernest Old Man and the sea
>> Lee,Harper To Kill a Mockingbird
>> Mellville,Herman Moby Dick
```

Array or Arrays (AoA) - great for a matrix

```
my @matrix = ();
$matrix[0]->[0] = 1;
$matrix[0]->[1] = 2;
$matrix[0]->[2] = 3;
$matrix[1] = [ 4,5,6];
$matrix[2] = [ 7,8,9];
for my $row ( @matrix ) {
  print join(" ",@$row),"\n";
}
```

```
>> 1 2 3
>> 4 5 6
>> 7 8 9
```

Hashes of Hashes and more complicated things

```
my %geneset;
$geneset{'YFG111'} = { 'name' => 'YFG111',
  'aliases' => [qw(IFU1 GEO887)],
  'chrom'   => 'chrom11',
  'start'   => '1002131',
  'end'     => '1003075',
  'strand'  => '+' };
my $ref = $geneset{'YFG11'};
$ref->{length} = $ref->{end} - $ref->{start} + 1;
```

Hash of Hashes (HoH)

```
# DATA FILE 1
# GENE    SCORE
# YFG123  0.1
# DATA FILE 2
# GENE    LENGTH
# YFG123  200
my %data;
while(<$fh1>) { # read file 1
 next if /^\#/;
 my ($gene,$score) = split;
 $data{$gene}->{score} = $score;
}

while(<$fh2>) { # read file 1
 next if /^\#/;
 my ($gene,$length) = split;
 $data{$gene}->{length} = $length;
}
for my $gene ( keys %data ) {
 print join("\t", $gene, $data{$gene}->{score},
 $data{$gene}->{length}), "\n";
}
```

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Subroutines

Subroutines are reusable set of code that can be called as a unit

```
sub add_up {
  my @arguments = @_;
   print "argument #1 is $arguments[0]\n";
 return $arguments[0] + $arguments[1];
}
print "sum of 10 and 23 is ", add_up(10,23), "\n";

>> argument1 is 10
>> sum of 10 and 23 is 33
```

Arguments to subroutines

Subroutines take list as arguments. If you want to pass in an array and NOT have to flattened, you have to pass in as a reference.

```
my ($A,@B) = (20,40,50);
my @C = (65,21);
doThis($A, \@B, @C); # sometimes you'll see &doThis(...)

sub doThis {
 my ($in_A, $in_B, @in_C) = @_;
  print "A= $in_A\n";
  print "B= ",join(",",@$in_B),"\n";
  print "C= ",join(",",@in_C),"\n";
}
```

```
>> A= 20
>> B= 40,50
>> C= 65,21
```

What is scope (why do we use my again?)

Scope defines the context where a variable is valid for.
Re-declaring a variable will cause a warning. Undeclared variables will cause compile time error.

```
use strict;
use warnings
my $score = 5;
my $score = 10; # Last declaration wins
if( $score == 10 ) {
  my $score = 20;
   print "In if score=$score\n";
}
print "At end score=$score\n";
```

```
"my" variable $score masks earlier declaration in same
scope at test_scope.pl line 4.
>> In if score=20
>> At end score=10
```

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Filehandles for reading

```
open(IN, "results.dat") || die $!; # open for reading
open(IN2,"<results2.dat") || die $!; # equivalent
open(my $fh => "<results3.dat") || die $!; # equivalent
while(<IN>) {
 my $line = $_;
}
```

Filehandles for writing

```
open(OUT, ">output.out") || die $!; # open for writing
open($fh => ">output2.out) || die $!;

print OUT join "\t", qw(onion 2.03),"\n";
print $fh join "\t", qw(garlic 0.78),"\n";
```

Implicit variable $_

```
while(<FH>) {
 # $_ is updated with each line
 my ($col1,$col2) = split; # split works on $_
}
```

Parsing a tab delimited file OR Stop using Excel for everything!

Tab (or comman, or space, or any other) delimited columns are easy to parse in Perl.
Here is a simple script to parse a BLAST tabular output (outfmt 7 for BLAST+ or -mformat 8 or 9 from blastall)

```
open($fh => $filename) || die $!;
while(<$fh1>) { # read file 1
 next if /^\#/;
 my @row = split;
 my ($query,$subject,$percent_id, $aln_len, $mismatches,$gaps,
     $qstart,$qend, $hitstart,$hitend,$evalue,$bitscore) = @row;
}
```

When things go wrong...

How to read the error messages and debug your code?

- Note the error line
- Try using 'print' to print out the variable's value
- You can see if you program will just compile with 'perl -w'
- always 'use strict'
- You can use the perl debugger (perl -d)
- Did you miss a ';'

Filehandle extras - running programs

```
open(my $fh => "zcat seqs.fa.gz |") || die $!;
open(my $fh2 => "zgrep '^>' seqs.fa.gz |") || die $!; # get FASTA
while(<$fh>) {
 my $id = $_;
 print $id, "\n";
}
```

Also can write to a dynamic filehandle

```
open(my $fh => "| gzip -c > output.gz") || die $!;
print $fh "Data1\n"; # etc
print $fh "Data2\n"; # etc
```

This will create a file called output.gz of compressed version of the output data.

Run a multiple alignment without writing a file

```
open(my $fh => "| muscle -in - -out $outfile.aln") || die $!;
print $fh ">seq1\n", "ACTAA\n";
print $fh ">seq2\n", "ACATCA\n";
```

Can you read and write to the same file handle?

Only if you use IPC::Run3 module which gives you access to STDIN, STDOUT, and STDERR for an external program.

Command line processing

Special variable @ARGV are the command line options

```
my ($arg1, $arg2) = @ARGV;
# OR (TMTOWTDI)
# my $arg1 = shift @ARGV;
# my $arg2 = shift @ARGV;
print "start wearing $arg1!\n";

>> perl cmdline.pl purple
>> start wearing purple!
```

Command line processing modules

Modules make it easier to process command line arguments with options

```
use Getopt::Long; # I really like this module,
# also see Getopt::Std
my ($name,$rank,$serialnum,active);
GetOptions(
            'n|name:s' => \$name,
            'r|rank:s' => \$rank,
            's|serial:s' => \$serialnum,
            'a|active!'  => \$active);
my @other_args = @ARGV; # all of these options are consumed
```

```
>> perl cmdline2.pl -n "James Davis" -r Private -s 867100 --active
>> perl cmdline2.pl -n "Leonard Lawrence" -r Private -s 811220 --noactive
```

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Regular expressions

- Very powerful feature of Perl built-in
- expressions go in / / (though this can be overriden)

  - match any of the symbols in there: [A-Z] all capital letters
- shortcuts: \d - all digits, \w - all alphanumeric characters + more, \s - white space,
- \D - not digits, \W - not a word character, \S - not whitespace

```
my $var = 'caterpillar';
if( $var =~ /cat/ ) { # would be true }
if( $var !~ /cat/ ) { # would be false (the string contains 'cat'
```

See the PerlRE page: `http://perldoc.perl.org/perlre.html`

Regular expressions to capture matches

```
my $var = 'GENE1:230-250';
if( $var =~ /(\w+):(\d+)-(\d+)/ ) {
 my ($gene,$start,$end) = ($1,$2,$3);
  print "gene=$gene start=$start end=$end\n";
}
```

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BioPerl

- Collection of Perl modules for life sciences data and analysis
- Modules are interfaces to data types: Sequences, Alignments, Features, Locations, Databases
- Example: Parser of sequence files, Alignment (BLAST) or Multiple alignment formats
- `http://github.com/bioperl` and `http://bioperl.org`
- `http://www.bioperl.org/` - Website has lots of information
- `http://www.bioperl.org/wiki/HOWTOs` - How To guides

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SeqIO: Read in a Fasta sequence file, count number of **sequences**

```
#!/usr/bin/perl -w
use strict;
use Bio::SeqIO;
my $seqfile = "sequences.fa";
my $in = Bio::SeqIO->new(-format=>'fasta',
                         -file=> $seqfile);
my $count = 0;
while( my $seq = $in->next_seq ) {
  $count++;
}
print "There are $count sequences\n";
```

SeqIO: Read in a Fasta sequence file, count number of **bases**

```
#!/usr/bin/perl -w
use strict;
use Bio::SeqIO;
my $seqfile = "sequences.fa";
my $in = Bio::SeqIO->new(-format=>'fasta',
                         -file=> $seqfile);
my $count = 0;
while( my $seq = $in->next_seq ) {
  $count += $seq->length;
}
print "There are $count bases\n";
```

SeqIO: Convert format & Write out sequences

```
use Bio::SeqIO;
my $seqfile = "sequences.gbk";
my $in = Bio::SeqIO->new(-format=>'genbank',
                         -file=> $seqfile);
my $out = Bio::SeqIO->new(-format=>'fasta',
                          -file=> ">outputfile.fa"));
while( my $seq = $in->next_seq ) {
  $out->write_seq($seq);
}
```

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GFF flavors

```
# GFF3
chr1    Curator gene    200     300     .       +       .       ID=GENE001;Name=YFG
chr1    Curator mRNA    200     300     .       +       .       ID=mRNA001;Parent=GENE001;Name=YFG.T0
chr1    Curator CDS     200     300     .       +       .       ID=CDS001;Parent=mRNA001
chr1    RMasker repeat  400     480     .       +       .       ID=Repeat1;Name=LINE1
chr1    RMasker repeat  600     750     .       +       .       ID=Repeat2;Name=hAT

#GFF2
chr1    RMasker repeat  600     750     .       +       .       ID Repeat2 ; Name hAT
#GTF
Chr_5 CC3_FINAL start_codon 871198 871200 . - 0 gene_id "CC1G_00004"; transcript_id "CC1G_00004T0";
```

Sequence file formats: Fasta

```
>gi|45552454|ref|NM_206028.1| Drosophila melanogaster Adh transcription factor 1 (Adf1), transcript varian
TAATTGGCAGAGACGCGACTGAGCTGGGACGTACCGTTACCGTTGGCAGAGACGCGACTGAGAAATAAAA
TTAAAACGTCGACGTTCCTTCCTCGTAGAAGAAACCAATCAAAATAAAAACAAACAGAGCGTGCGTTCGC
```

Sequence file formats: GenBank

```
LOCUS       NM_206028               1678 bp    mRNA    linear   INV 01-FEB-2011
DEFINITION  Drosophila melanogaster Adh transcription factor 1 (Adf1),
            transcript variant C, mRNA.
ACCESSION   NM_206028
VERSION     NM_206028.1  GI:45552454
KEYWORDS    .
SOURCE      Drosophila melanogaster (fruit fly)
  ORGANISM  Drosophila melanogaster
            Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota;
            Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha;
            Ephydroidea; Drosophilidae; Drosophila; Sophophora.
REFERENCE   1  (bases 1 to 1678)
  AUTHORS   Hoskins,R.A., Carlson,J.W., Kennedy,C., Acevedo,D., Evans-Holm,M.,
            Frise,E., Wan,K.H., Park,S., Mendez-Lago,M., Rossi,F.,
            Villasante,A., Dimitri,P., Karpen,G.H. and Celniker,S.E.
  TITLE     Sequence finishing and mapping of Drosophila melanogaster
            heterochromatin
  JOURNAL   Science 316 (5831), 1625-1628 (2007)
   PUBMED   17569867
FEATURES             Location/Qualifiers
     source          1..1678
                     /organism="Drosophila melanogaster"
                     /mol_type="mRNA"
                     /db_xref="taxon:7227"
                     /chromosome="2R"
                     /genotype="y[1]; cn[1] bw[1] sp[1]; Rh6[1]"
     gene            1..1678
                     /gene="Adf1"
                     /locus_tag="Dmel_CG15845"
                     /gene_synonym="Adf 1; adf-1; Adf-1; adf1; CG15845;
                     Dmel\CG15845; l(2)01349; l(2)04065; nal"
                     /note="Adh transcription factor 1"
                     /map="42C3-42C3"
                     /db_xref="FLYBASE:FBgn0000054"
                     /db_xref="GeneID:47082"
```

Bio::DB::Fasta - Fast random access to Fasta seq databases

```
     CDS             180..968
                     /gene="Adf1"
                     /locus_tag="Dmel_CG15845"
                     /gene_synonym="Adf 1; adf-1; Adf-1; adf1; CG15845;
                     Dmel\CG15845; l(2)01349; l(2)04065; nal"
                     /note="CG15845 gene product from transcript CG15845-RC;
                     CG15845-PC; Adf1-PC; nalyot; naylot"
                     /codon_start=1
                     /product="Adh transcription factor 1, isoform C"
                     /protein_id="NP_995750.1"
                     /translation="MHTLTAAIEMDKLDANLEQQFDLNLIEAVKLNPVIYDRSHYNYK
                     HFVRKAQTWKQIAETLGVPEQKCTKRWKSLRDKFAREMKLCQESRWRYFKQMQFLVDS
                     IRQYRESLLGKCANGSQSANQVADPSQQQQAQQQTVVDIFAQPFNGSATTSAQALTHP
                     HEITVTSDAQLATAVGKDQKPYFYEPPLKRERSEEEHSDNMLNTIKIFQNNVSQAVSA
                     EDQSFGMVVTDMLNTLGVRQKAEAKVHIIKYLTDMQLLAQHNKY"
ORIGIN
        1 taattggcag agacgcgact gagctgggac gtaccgttac cgttggcaga gacgcgactg
       61 agaaataaaa ttaaaacgtc gacgttcctt cctcgtagaa gaaaccaatc aaaataaaaa
      121 caaacagagc gtgcgttcgc gccaaatact taacaacaat tagcaaacgt aagaagcaaa
      181 tgcataccct cacggcggcc attgagatgg acaagctgga tgccaatctt gagcagcagt
      241 ttgatctcaa tctcatcgag gctgtcaagc tgaacccagt gatatacgac aggtcgcact
      301 acaattacaa gcactttgtg cgcaaggccc agacctggaa acaaatcgcc gaaacgctcg
      361 gtgtgcctga acaaaaatgt acgaagcgct ggaagagtct gcgcgacaag ttcgcccgcg
```

Sequence objects

- `Bio::SeqIO` to read and write, creates and uses `Bio::Seq` objects
- Methods for getting info from a sequence object
- `seq()` - sequence as a string
- `length()` - how long is the sequence
- `id()` - what is the ID for the sequence
- `description()` - what is the ID for the sequence

Sequence Features

GenBank and other rich formats have features. Features are elements that are located on a Sequence

- `get_SeqFeatures()` will return the sequence features
- `Bio::SeqFeature::Generic` objects
- `start,stop,strand,length` are all feature object methods for position info
- `primary_tag, source_tag` - the tags for the (source, type)

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Bio::DB::Fasta - Fast random access to Fasta seq databases

```
use Bio::DB::Fasta
# $dir can be a directory of *.(fa|fasta) files or
# a single dir or file to index
my $dir = shift @ARGV;
my $dbh = Bio::DB::Fasta->new($dir);
my $seq  = $dbh->get_Seq_by_acc("SEQ123");
# extract a sub-string from db
my $seqstr = $dbh->seq("chr1", 10012, 13454);
```

Bio::DB::Genbank - Query Genbank

```
use Bio::DB::GenBank;
use Bio::SeqIO;
my $db = Bio::DB::GenBank->new;
my $seq = $db->get_Seq_by_acc("NM_206028.1");
my $out = Bio::SeqIO->new(-format => 'fasta');
$out->write_seq($seq);
```

Bio::DB::Genbank - Query Genbank, output genbank

```
use Bio::DB::GenBank;
use Bio::SeqIO;
my $db = Bio::DB::GenBank->new;
my $seq = $db->get_Seq_by_acc("NM_206028.1");
my $out = Bio::SeqIO->new(-format => 'genbank');
$out->write_seq($seq);
```

Query Genbank with complex query

```
use Bio::DB::GenBank;
use Bio::DB::Query::GenBank;
use Bio::SeqIO;
my $db = Bio::DB::GenBank->new;
my $query = Bio::DB::Query::GenBank->new(-db => 'nucleotide',
 -query => 'Zea mays[Organism] and mRNA',
 -mindate=> 2010,
 -maxdate=> 2010);
print "there are ", $query->count," records\n";
my $stream = $db->get_Stream_by_query($query);
my $out = Bio::SeqIO->new(-format => 'genbank');
while (my $seq = $stream->next_seq) {
 $out->write_seq($seq);
 last;
}
```

Bio::DB::SeqFeature - Databases of features

```
use Bio::DB::SeqFeature::Store;
my $db = Bio::DB::SeqFeature::Store->new(-dir => 'demo',
                                         -adaptor=>'berkeleydb');
my @genes = $db->features(-type => 'gene');
print "querying genes\n";
for my $g ( @genes ) {
  print $g->name, " ", $g->location->to_FTstring, "\n";
}
print "querying repeats\n";
my $iterator = $db->get_seq_stream(-type => 'repeat');
while( my $feature = $iterator->next_seq ) {
  print $feature->name, " ", $feature->length, "\n";
}
```

Input and Output from feature db query

```
#input
chr1    Curator gene    200     300     .       +       .       ID=GENE001;Name=YFG
chr1    Curator mRNA    200     300     .       +       .       ID=mRNA001;Parent=GENE001;Name=YFG.T0
chr1    Curator CDS     200     300     .       +       .       ID=CDS001;Parent=mRNA001
chr1    RMasker repeat  400     480     .       +       .       ID=Repeat1;Name=LINE1
chr1    RMasker repeat  600     750     .       +       .       ID=Repeat2;Name=hAT
#output
>> querying genes
>> YFG chr1:200..300
>> querying repeats
>> LINE1 81
>> hAT 151
```

Outline

Getting rich feature data

```
my $seqfile = "sequences.gbk";
my $in = Bio::SeqIO->new(-format=>'genbank',
                         -file=> $seqfile);
my $out = Bio::SeqIO->new(-format => 'fasta',
                          -file => '>pepseqs.fa');
while( my $seq = $in->next_seq ) {
  for my $feat ( $seq->get_SeqFeatures ) {
     if( $feat->primary_tag eq 'CDS' ) {
      # get protein_id name
      warn("all tags are ", join(",",$feat->get_all_tags),"\n");
      if ( $feat->has_tag('protein_id') ) {
        my ($protein_id) = $feat->get_tag_values('protein_id');
        my ($pseq) = $feat->get_tag_values('translation');
        my $pepseq = Bio::Seq->new(-id => $protein_id,
                   -description => $seq->accession_number,
                                -seq => $pseq);
        $out->write_seq($pepseq);
      }
    }
  }
}
```

Sequence input file

```
LOCUS       BT069887                973 bp    mRNA    linear   PLN 25-FEB-2009
DEFINITION  Zea mays full-length cDNA clone ZM_BFb0307L22 mRNA, complete cds.
ACCESSION   BT069887
VERSION     BT069887.1  GI:224035416
KEYWORDS    FLI_CDNA.
SOURCE      Zea mays
  ORGANISM  Zea mays
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; PACCAD
            clade; Panicoideae; Andropogoneae; Zea.
FEATURES             Location/Qualifiers
     source          1..973
                     /mol_type="mRNA"
                     /db_xref="taxon:4577"
                     /strain="B73"
                     /clone="ZM_BFb0307L22"
                     /organism="Zea mays"
     CDS             98..613
                     /db_xref="GI:224035417"
                     /codon_start=1
                     /protein_id="ACN36784.1"
                     /translation="MGIISKLVPILTEGNFVERCLEILRNLSDMEEAVARITRTDRCL
                     ASVAEYLDTGSPTERQHAVVILLAVCSCSAEDCLLVMKEGVIPALVDLSVNGTEEAKG
                     CSTKLLHLLRDMRRSDQFTNSCSQEVAATGMVVEDAPKNSVHKQPASKSSRFFQRKLN
                     IFSKPRSLTLF"
                     /product="unknown"
ORIGIN
        1 aatactggca tctgaagata ccgaaggcct cgaattgtct ctgaagatca tctgcgagct
       61 ttcatccgac gccgatataa gatcttcgtt agtttcaatg ggaataatct cgaagcttgt
      121 tcccatttta accgaaggaa acttcgtcga gcgctgtttg gagatcctgc ggaacttaag
```

Sequence output

```
>ACN36784.1 BT069887
MGIISKLVPILTEGNFVERCLEILRNLSDMEEAVARITRTDRCLASVAEYLDTGSPTERQ
HAVVILLAVCSCSAEDCLLVMKEGVIPALVDLSVNGTEEAKGCSTKLLHLLRDMRRSDQF
TNSCSQEVAATGMVVEDAPKNSVHKQPASKSSRFFQRKLNIFSKPRSLTLF
>ACN36708.1 BT069811
MGNMMDNLLVRSLTSKSKGRVDDIAPPSPVKAPDDDETDKAEGEESPMMETVRSKCITQL
LLLGAIDSIQKRYWSRLKATQQIAIMDILLSLLEFASSYNSPSNFRTRMHHIPLERPPLN
LLRQELVGTTIYLDILHKSTVEQDKIDSIEETNGLNVESGDQEKIKYLAEGKLVSFCGQI
LKEASVLQPSTGEAASADIHRVLDLRAPVIVKVLKGMCIMDAQIFRRHLKEFYPLITKLI
CCDQMDVRGALGDLFSKQLTPLMP
```

Outline

Tree objects

- `Bio::TreeIO` Tree parser/writer can read/write trees in New Hampshire/Newick, nexml, phyloxml, and Nexus formats
- Build up `Bio::Tree::Tree` objects with `Bio::Tree::Node` objects in memory (on dev branch, code to represent in SQLite db to reduce memory)

Convert from nexus to newick

```
use Bio::TreeIO;
my $in = Bio::TreeIO->new(-format => 'nexus',
                          -file => shift @ARGV);
my $out = Bio::TreeIO->new(-format => 'newick');
while( my $tree = $in->next_tree ) {
 $out->write_tree($tree);
}
```

Tree querying and manipulations

```
use Bio::TreeIO;
my $in = Bio::TreeIO->new(-format => 'newick',
                          -file => shift @ARGV);
while( my $tree = $in->next_tree ) {
 my @nodes = $tree->get_nodes;
 my (@tips) = grep { $_->is_Leaf() } @nodes;
 my @tips; # TMTOWTDI
 for my $n ( @nodes ) {
  if( $n->is_Leaf() ) {
     push @tips2, $n;
  }
 my ($cat) = grep { $_->id eq 'cat' } @tips;
 my ($dog) = grep { $_->id eq 'dog' } @tips;
 my $lca = $tree->get_lca($cat,$dog); #get least common ancestor
}
```

Outline

Multiple Alignment objects

```
use Bio::AlignIO;
my $in = Bio::AlignIO->new(-format => 'clustalw',
                           -file   => shift @ARGV);

my $out = Bio::AlignIO->new(-format => 'phylip',
                           -file   => shift @ARGV);
while(my $aln = $in->next_aln ) {
 $out->write_aln($aln);
}
```

Multiple Alignment objects

- Formats supported: Clustalw, FastA, PHYLIP, pfam, stockholm, selex, psi-blast,xmfa,mega
- Alignment objects can be queried for conserved residues, consensus sequence, gapped positions

Outline

Seq Database Search objects

- Parsing results from BLAST, FastA, HMMER, BLAT, etc
- `Bio::SearchIO` is the main framework for this
- `Bio::Search::Result` - result objects
- `Bio::Search::Hit` - Hit/Subject objects
- `Bio::Search::HSP` - Alignments (High scoring Segment Pairs)

See the HowTo
`http://www.bioperl.org/wiki/HOWTO:SearchIO`

Seq Database Search objects

```
my $in = Bio::SearchIO->new(-format => 'blast',
                            -file   => shift @ARGV);
while( my $r = $in->next_result ){
  print $r->query_name, "\n";
 while( my $h = $r->next_hit ) {
  print "\t", $h->name, " ", $h->significance\n";
  while( my $hsp = $h->next_hsp ) {
   print "\t\t", $hsp->query->start, "..",$hsp->query->end, "\n";
   print "\t\t", $hsp->hit->start, "..",$hsp->hit->end, "\n";
   print "\t\t", $hsp->evalue, " ",$hsp->frac_identical, " ",
$hsp->frac_conserved, "\n";
   print "\t\t", $hsp->query_string, "\n";
  }
 }
}
```

Outline

- `List::Util` for several list utilities
- `Getopt::Long` for command line argument processing
- `Statistics::Descriptive` can calculate median, mean for data distribution