Bioinformatics

Computational Methods II:
Sequence Analysis with Perl

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Sequence Analysis with Perl

- Introduction
- Input/output
- Variables
- Functions
- Control structures
- Arrays
- Regular expressions

Objectives for this week

- write, modify, and run simple Perl scripts
- design customized and streamlined sequence manipulation and analysis pipelines with Perl scripts

Why Perl?

- Good for text processing (sequences and data)
- Easy to learn and quick to write
- Built from good parts of lots of languages/tools
- Lots of bioinformatics tools available
- Open source: free for Unix, PC, and Mac
- TMTOWTDI

First Perl program

- Create this program and call it hey.pl

```
#!/usr/local/bin/perl -w
# The Perl "Hey" program
print "What is your name? ";
chomp ($name = <STDIN>);
print "Hey, $name, welcome to the
  Bioinformatics course.\n";
```

- To run: `perl hey.pl` *or*
- To run: `chmod +x hey.pl`
  `hey.pl`

Perl Input/Output

- Types of input:
  - keyboard (STDIN)
  - files
- Types of output:
  - screen (STDOUT)
  - files
- Unix redirection can be very helpful
  ex: hey.pl > hey_output.txt

Variables

- Scalar variables start with $

```
$numSeq = 5;          # number; no parentheses
$seqName = "GAL4";    # "string"; use parentheses
$level = -3.75;       # numbers can be decimals too
print "The level of $seqName is $level\n";   # "\n" = new line
$_                    default input variable
```

- Arrays (lists of scalar variables) start with @:

```
@genes = ("BMP2", "GATA-2", "Fez1");
@orfs = (395, 475, 431);
print "The ORF of $genes[0] is $orfs[0] nt.";
# The ORF of BMP2 is 395 nt.
```

Perl functions – a sample

```
print    tr///    closedir open     m//

chomp    mkdir    split    close    die

length   chdir    join     chmod    rename

substr   opendir  pop      uc       use

s///     readdir  push     lc       sort
```

Control Structures 1

```
if (condition)      # note that 0, "", and (undefined) are false
{
    do this; then this;. . .
}
else        # optional; 'if' can be used alone; elsif also possible
{
    do this instead;
}
```

```
if ($exp >= 2)        # gene is up-regulated
{
  print "The gene $seq is up-regulated ($exp)";
}
```

Control Structures 2

```
while (condition)
{
    do this;
    then this;. . .
}
```

```
while ($orfLength > 100)
{                          # Add to table
  print "$seq\t";          # "\t" = tab
  print "$orfLength\n";
}
```

Control Structures 3

```
for (initialize; test; increment )
{
    do this;. . .
}
```

```
for ($i = 0; $i <= $#seqs; $i++)
# $#seqs = index of the last element in @seqs
{  # Add elements of @seqs and @orf to table
  print "$seq[$i]\t";
  print "$orf[$i]\n";
}
```

Arithmetic & numeric comparisons

- Arithmetic operators: `+ -   /  *  %`
- Notation: `$i = $i + 1;   $i += 1;   $i++;`
- Comparisons: > , < , <= , >= , = = , !=

```
if ($num1 != $num2)
{
  print "$num1 and $num2 are different";
}
```

- Note that = = is very different from =

= =	used as a test:	if ($num = = 50)
=	used to assign a variable:	$num = 50

String comparisons

- Choices: eq , ne

```
if ($gene1 ne $gene2)
{
  print "$gene1 and $gene2 are different";
 }
 else    # $gene1 eq $gene2
 {
   print "$gene1 and $gene2 are the same";
 }
```

Multiple comparisons

- AND &&
- OR ||

```
if ($exp > 2 || ($exp > 1.5 && $numExp > 10)
{
  print "Gene $gene is up-regulated";
}
```

Filehandles

To read from or write to a file in Perl, it first needs to be opened.
In general, `open(file handle, filename);`

Filehandles can serve at least three purposes:

```
open(IN, $file);        # Open for input
open(OUT, ">$file");    # Open for output
open(OUT, ">>$file");   # Open for appending
```

Then, get data all at once `@lines = <IN>;`
or one line at a time

```
  while <IN>    {
     $line = $_; do stuff with this line;
     print OUT "This line: $line"; }
```

Embedding shell commands

- use backquotes (`) around shell command
- example using EMBOSS to reverse complement:
  `` `revseq mySeq.tfa mySeq_rc.tfa`; ``
- Capture stdout from shell command if desired
- EMBOSS qualifier "-filter" prints to stdout

```
$date = `date`;
$rev_comp = `revseq mySeq.tfa -filter`;
print "$date";
print "Reverse complement:\n$rev_comp\n";
```

Programming issues

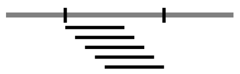
- What should it do and when is it "finished"?
- Who will be using/updating your software?
  - Reusability
  - Commenting
  - Error checking
- Development vs. execution time?
- Debugging tools: printing and commenting
- OBOB ("off-by-one bug"): for at least 3 reasons

Example: patscan_batch.pl

```
#!/usr/local/bin/perl -w
# Run patscan on all seqs in a folder
$myDir = "/usr/people/elvis/seqs";
$patFile = "/usr/people/elvis/patterns/polyA.pat";
chdir($myDir);                    # Go to $myDir
opendir(DIR, $myDir);             # Open $myDir

foreach $seqFile (sort readdir(DIR))
{
 if ($seqFile =~ /\.tfa$/)        # if file ends in .tfa
 {
  print "Processing $seqFile\n";
  $outFile = $seqFile;            # Create $outFile name
  $outFile =~ s/\.tfa/\.polyA\.out/;    # s/old/new/;
  ############  Run PATSCAN  ###############
  `scan_for_matches $patFile < $seqFile > patscan/$outFile`;
 }
}
```

Example: oligo analysis



sample fasta sequence:

```
>gi|16493450|gb|BB659629.1|BB659629
GCCTGCTTGAGTTTTGAAGTCTTGGAGCCACAGAA
AGCACTGGCCAGAGGAGAGGTAATCACTTCTAATG
CCAGGCCTGCTGTGCAGTGCGCATGTGTGATCTCA
GTCTGCTTCTGCCCTAGCTAATGAAGGCATGGACA
ATGGAATAGCCACATGGCAGCACCGGAAAACAAGC
TTACTTCTGCAGTACACAGCCTGCTTTGCCTGATT
TCTGTCCACTGG
```

Basic steps for oligos.pl

Open fasta sequence
Get raw sequence
Extract oligos
Analyze oligos
Print out results
(Modify script to analyze multiple seqs)

oligos.pl: part 1

```
#!/usr/local/bin/perl -w
# Extract oligos from a sequence and analyze %GC
$seq = "mySeq.tfa";     # input sequence
$mer = 35;              # length of oligo to make
$start = 5;             # nt to start oligos
$end = 11;              # nt to stop oligos

# Get continuous sequence from sequence file
open (SEQ, $seq) || die "cannot open $seq: $!";
@seq = <SEQ>;                  # make array of lines

$defline = $seq[0];            # get defline
$seq[0] = "";                  # delete defline
$seq = join ("", @seq);        # join($glue, @list)
$seq =~ s/\s*//g;              # delete whitespace
```

oligos.pl: part 2

```
$seqLength = length ($seq);
print "Oligos ($mer mers) for $defline
  ($seqLength nt) and % GC content\n";

# Beware of OBOB
for ($i = $start - 1; $i < $end - 1; $i++)
{
  # $oligo = substr(seq, start, length);
  $oligo = substr($seq, $i, $mer);
  $nt = $i + 1;
  $numGC = $oligo =~ tr/GC/GC/; # count GCs
  $pcGC = 100 * $numGC / $mer;  # find %
  print "$nt\t$oligo\t$pcGC\n";
}
```

Demonstration

- hey.pl
- input and output options
- patscan_batch.pl
- rev_comp.pl
- oligos.pl
- parse_genbank.pl

Next week

Computational Methods III:
Sequence analysis
with Perl and BioPerl

including

- regular expressions and hashes
- using LWP, GD, CGI, BioPerl, and other modules