



Unix, Perl and BioPerl

II: Sequence Analysis with Perl

George Bell, Ph.D.

WIBR Bioinformatics and Research Computing

Sequence Analysis with Perl

- Introduction
- Input/output
- Variables
- Functions
- Control structures
- Comparisons
- Sample scripts

Objectives

- 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

A 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 quotes
$seqName = "GAL4";    # "string"; use quotes
$level = -3.75;       # numbers can be decimals too
print "The level of $seqName is $level\n";
$_                  # 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

<code>print</code>	<code>tr///</code>	<code>closedir</code>	<code>open</code>	<code>m//</code>
<code>chomp</code>	<code>mkdir</code>	<code>split</code>	<code>close</code>	<code>die</code>
<code>length</code>	<code>chdir</code>	<code>join</code>	<code>chmod</code>	<code>rename</code>
<code>substr</code>	<code>opendir</code>	<code>pop</code>	<code>uc</code>	<code>use</code>
<code>s///</code>	<code>readdir</code>	<code>push</code>	<code>lc</code>	<code>sort</code>

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"; # "\n" = newline
```

```
}
```

Control Structures 3

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

```
for ($i = 0; $i <= $#seqs; $i++)  
# $#seqs = index of the last element in @seqs  
{ # Add elements of @seqs and @orf to table  
    print "$seqs[$i]\t";  
    print "$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 $gene2 are different";
}
else
{
    print "$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(filehandle, 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.fa mySeq_rc.fa`;`

- Capture stdout from shell command if desired

- EMBOSS qualifier “-filter” prints to stdout

```
$date = `date`;
```

```
$rev_comp = `revseq mySeq.fa -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
- Beware of OBOB ("off-by-one bug")

Example: patscan_batch.pl

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

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

Example: oligo analysis



sample fasta sequence:

>BB659629

```
GCCTGCTTGAGTTTTGAAGTCTTGGAGCCACAGAA
AGCACTGGCCAGAGGAGAGGTAATCACTTCTAATG
CCAGGCCTGCTGTGCAGTGCGCATGTGTGATCTCA
GTCTGCTTCTGCCCTAGCTAATGAAGGCATGGACA
ATGGAATAGCCACATGGCAGCACCGGAAAACAAGC
TACTTCTGCAGTACACAGCCTGCTTTGCCTGATT
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
$seqFile = "mySeq.fa";      # 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, $seqFile ) || die "cannot open $seqFile: $!";
@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 $define
      ($seqLength nt) and % GC content\n";

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

Summary

- Input/output
- Variables
- Functions (scalars and arrays)
- Control structures
- Comparisons
- Sample scripts:
 - patscan_batch.pl
 - oligos.pl

For more information, books:

- Learning Perl (Schwartz et al.) - O'Reilly
- The Perl CD Bookshelf - O'Reilly
- Beginning Perl for Bioinformatics – Tisdall
- ‘Using Perl to Facilitate Biological Analysis’ (Stein) in *Bioinformatics* (Baxevanis & Ouellette)
- ‘Bioinformatics Programming using Perl and Perl Modules’ in *Bioinformatics: Sequence and Genome Analysis, 2nd ed.* (Mount)

AND several good web sites (see course page)

Demo scripts on the web site

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

Exercises

- Retrieving and aligning a list of human-mouse orthologs
- Retrieving a set of genes encoding growth factors, extracting their proximal promoters, and analyzing them.