Introduction to Perl

HMS Research Computing

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Overview

- Learn how to write and run Perl scripts on O2
- Become familiar with Perl syntax
- Understand the different data types in Perl
- Learn how to import and output files
- Example: parsing BLAST tabular output

- Slides available at github.com/hmsrc/user-training
  - IntroToPerl.pdf

Why Perl?

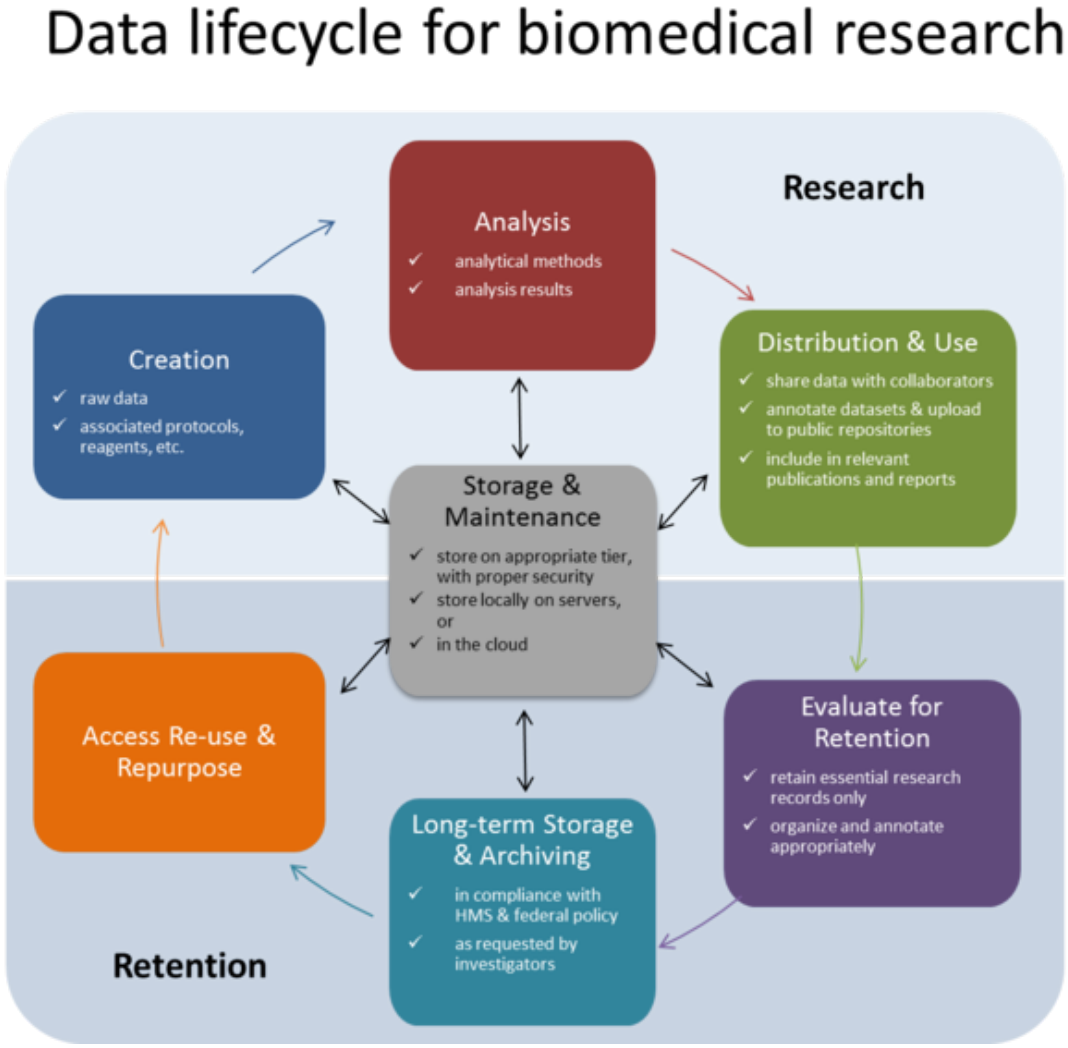
- Open source, and still under development
- Available for most operating systems: Windows, Mac, Unix
- Easy to learn, and scripts are quick to write
- Kitchen sink language
- TMTOWTDI – there's more than one way to do it
- Many bioinformatics modules and scripts available

What can you do with Perl?

- Data munging
  - Making your data set less “messy”
  - includes: combining, analyzing, filtering, reformatting data
- Automate analyses
  - Download many files from biological databases
- Interface with SQL databases
- Use other people’s code

Data Management

As you run more jobs, you'll probably end up creating a whole bunch of files. In the same way it's important to plan bench projects beforehand, it’s good to think early about how you should manage and organize all those files you'll be making. *Note: be sure to ask your PI and your department about standard practices in your field!*



Harvard Biomedical Data Management Website: https://datamanagement.hms.harvard.edu

Resources & Information: https://datamanagement.hms.harvard.edu/overview

Top Data Management Best Practices

- **Planning**: Document the activities for the entire lifecycle. Create a Data Management Plan including sponsorship requirements, realistic budget, assigned responsibilities, all the data to be collected, *and each of these topics*! https://datamanagement.hms.harvard.edu/planning-overview

- **Organization**: Define how the data will be organized, including what is your folder hierarchy and how did you get from raw data to the final product? Consider versioning control for changes for both software and data products. https://datamanagement.hms.harvard.edu/versioning-1

- **Documentation**: Explain how the data will be documented such as naming conventions, acronyms, data fields and units. Determine whether there is a community-based metadata standard that can be adopted. Create a README file to record the metadata that will be associated with data. https://datamanagement.hms.harvard.edu/readme-files

Top Data Management Best Practices

- **Storage**: Your storage plan is integral to data management. Consider how the data will be stored and protected over the duration of the project. Identify short-term and long-term storage options. Remember to link accompanying metadata and related code and algorithms. https://datamanagement.hms.harvard.edu/storage-overview

- **Sharing**: Describe what data will be disseminated, to who, when, and where. Identify sharing tools to work with collaborators during the project and publish data in an open repository. Be sure to use standard, nonproprietary approaches and provide accompanying metadata & associated code. https://datamanagement.hms.harvard.edu/data-sharing

- **Retention**: Think about your preservation strategy from the start & adhere to your lab's standard practices. Research records should generally be retained no fewer than seven (7) years after the end of a research project or activity. https://datamanagement.hms.harvard.edu/data-retention

Notation

- `[user123@login ~]$`

  O2 bash (your terminal)

- **1** `#!/usr/bin/env perl`

  2 `print "Hello world!\n";`

Perl script (you'd save this without line numbers and run)

- `my $scalar = 10;`

  Example Perl code snippet

Connecting to O2

Mac and Linux: Open a terminal, and ssh to O2:

```
$ ssh user123@o2.hms.harvard.edu
```

Windows: Use MobaXterm to connect to O2:

```
$ ssh user123@o2.hms.harvard.edu
```

If you do not have an O2 account, use a training account:

Details will be distributed in class.

Once on O2...

Start an interactive job:

```
[user123@login ~]$ srun --pty -p interactive -t 0-2
-c 1 bash
```

Copy the class materials:

```
[user123@compute ~]$ cp /n/groups/rc-
training/perl/perl_code.zip .
```

```
[user123@compute ~]$ unzip perl_code.zip
```

```
[user123@compute ~]$ cd perl_code
```

Where is Perl on O2?

- See available Perl modules

```
[user123@compute ~]$ module spider perl
```

- Load Perl module

```
[user123@compute ~]$ module load gcc/6.2.0
perl/5.30.0
```

- Check which Perl will be used:

```
[user123@compute ~]$ which perl
```

```
[user123@compute ~]$ perl -v
```

General workflow

- Write Perl script in text editor (e.g. nano, vim, emacs)
  - Script should conventionally end in `.pl`
- Save your script, exit text editor
  - **Nano -** `CTRL + O` then `ENTER` to save, `CTRL + X` to quit
- Run script on command line

```
[user123@compute ~]$ perl myscript.pl
```

- Run script non-interactively (with an sbatch job)
  - reference the Using Slurm Basic wiki page for an example sbatch script.

Syntax

- Comments start with #
  - Can be a full line, or at the end of a set of commands
- Commands end in ;
- Use = to assign variables
- Whitespace doesn't matter to Perl, but should be used for clarity
- For printing – double quotes ("") interpolate variables, ('') do not
- Characters may need to be "escaped" for printing or regular expressions

First script

```
1 # This is a comment!
2 print “Hello world!\n”;
```

Open up a text editor and type the lines above.

(Do not add the line numbers in your script!)

Save as `hello.pl`. Run by:

```
[user123@compute ~]$ perl hello.pl
```

What is happening in the script?

```
1 # This is a comment!
2 print “Hello world!\n”;
```

Comments start with `#`. Perl ignores these.

Each command needs to end in `;`

The `print` function sends text to the terminal, and `\n` is the newline character.

Functions take zero or more arguments

Shebang line

- The first line of your script describes what type of program it is, and how to execute it. This is called an interpreter directive.

```
#!/usr/bin/perl
```

- Or to make your program portable between Perl versions use:

```
#!/usr/bin/env perl
```

Variables

Scalars, Arrays, Hashes

Variable type: Scalars

- Hold one thing, such as a number, or text

```
$sequence = "GTCAGATTC";
$e_value = 1e-10;
```

- Name starts with $
- Can change the value of a scalar in a script

```
$gene_symbol = "BRCA2";
$gene_symbol = "INS";
```

Good practices:

- Declare variables with `my` the first time you use them, and don't need to give a value when it's declared.

```
my $sequence = "GTCAGATTC";
```

- Prevent misspellings:

```
use strict;
```

- Have Perl tell you when you're doing something wrong:

```
use warnings;
```

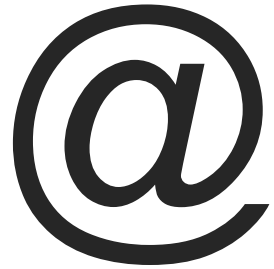
- `use` statements should be put at the top of your scripts.

Variable type: Arrays

- Hold a set of things, called elements

```
my @numbers = (1, 2, 3, 4);
my @scalars = ($a, $b, $c);
my @strings = (“abc”, “def”, “g”);
my @colors = qw(blue red green black);
```

- Name starts with @
- Common uses:
  - Saving contents of an input file
  - Performing an operation on all elements



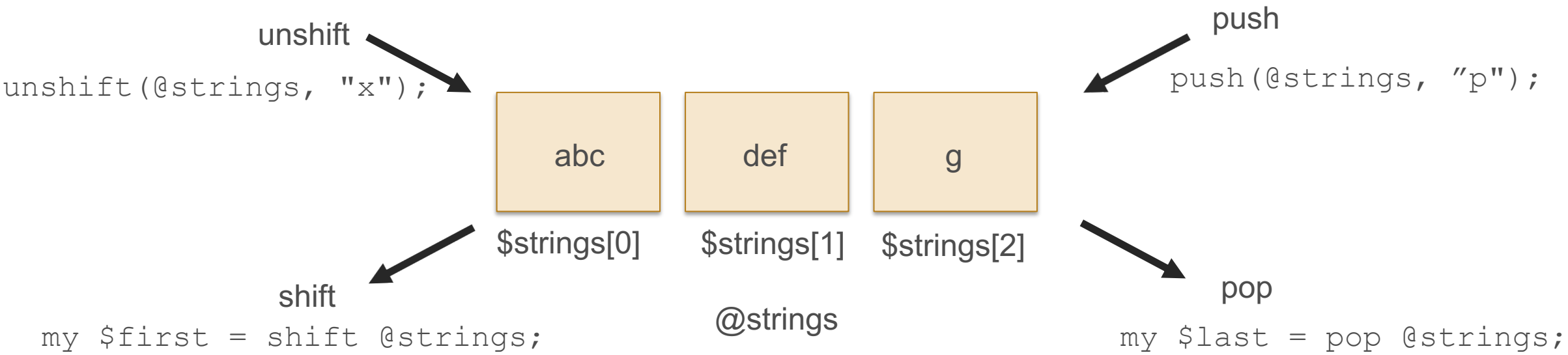
Arrays – what do they contain?

- Arrays have indexed elements
  - Each element is a scalar variable, so use $ not @
  - Indexes describe the element's position in the array, start from 0

```
my @strings;
@strings = (“abc”, “def”, “g”);
print $strings[2];
print @strings;
print “$strings[0]\t$strings[1]\t$strings[2]\n”;
print “Array size: ”, scalar(@strings),”\n”;
```

Array manipulation

- Arrays can change in size:



- Can also change a single value:

```
$strings[2] = "q";
```

Strings to Arrays

- Use the `split()` function to convert a string to an array
  - Need to split on a delimiter, such as tabs or spaces
  - Split a sequence on Cs:

```
my $sequence = "ATGCACGAA";
my @seq = split /C/, $sequence;
#("ATG","A","GAA")
```

  - Split a line on tabs:

```
my @columns = split /\t/, $line;
```

Special array @ARGV

- So far we have been using hard-coded variables. How do we accept user input? Use `@ARGV`!
- Anything you provide after the script name goes into `@ARGV`

```
[user123@compute ~]$ perl argv.pl "Abe Lincoln" 1809 blue

my $name = $ARGV[0]; # "Abe Lincoln"

my $year = $ARGV[1]; # 1809

my $color = $ARGV[2]; # blue
```

- Can alternatively use module `Getopt::Long` for command line options

Getopt::Long for command line parameters

- Getopt::Long is a module (library of functionality that someone else wrote) that allows you to input options on the command line

```
use Getopt::Long; # use the module
use strict;
my $bedfile = “file.bed”; #declaring default value
GetOptions('bed=s' => \$bedfile);
```

- =s is for text string, can also use =i (integer), or =f (float)
- now can specify a file with the –bed option on command line
  - Value will be assigned to $bedfile

Variable type: Hashes

%

- Hold a set of unordered key/value pairs
  - One value for key (unless using complex data structure)
  - Keys are unique – will overwrite if you give multiple pairs with the same key
- Name starts with %
  - When accessing part of hash $ is used
- General uses:
  - Holding varied data
  - Quickly searching through a dataset
  - Making a unique set

Declaring Hashes

- Method 1 – without assignment operator

```
my %sci_names = (“human”, “Homo sapiens”, “mouse”, “Mus
musculus”, “rat”, “Rattus norvegicus”);
```

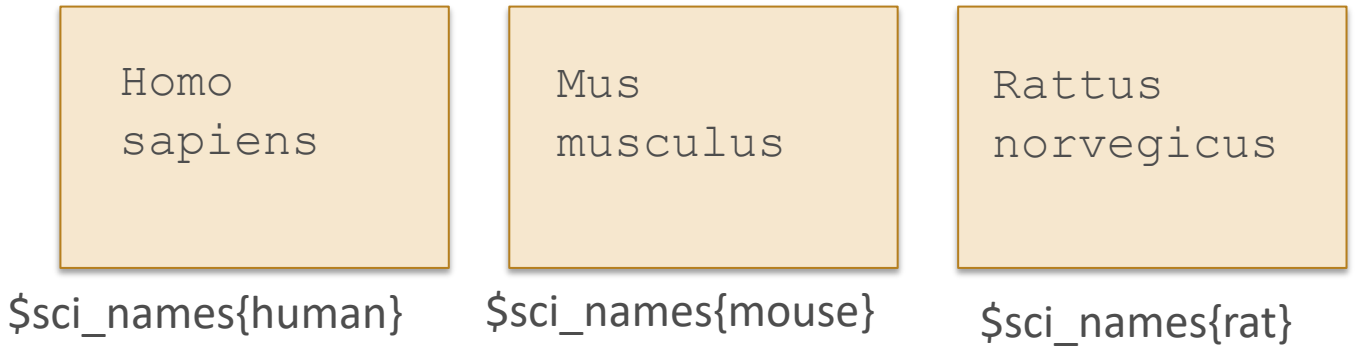
- Method 2 – with => operator

```
my %translate = (
   “ATG” => “M”, “GGT” => “G”,
   “CAT” => “H”, ”TAG” => “*”,
   …
)
```

- Adding one value: `$hash{$key} = $value;`

Accessing hash contents

- `my %sci_names = (“human”, “Homo sapiens”, “mouse”, “Mus musculus”, “rat”, “Rattus norvegicus”);`



- One value: `my $value = $hash{$key}`
- All keys: `my @keys = keys(%hash)`
- See if something is in a hash: `if(exists $hash{$key}){ … }`

Control Structures

Conditions and Loops

Conditional statements - if

- Basic format:

```
if (condition) {
  run some code here
}
```

- Example:

```
if (length($sequence) > 10 ) {
  print "Seq is longer than 10\n";
}
```

Conditional statements - else

- Basic format:

```
if (condition) {
   run some code here
}else {
   run other code here
}
```

- Example:

```
if (length($sequence) > 10 ) {
     print "Seq is longer than 10\n";
}else{
     print "Seq is not longer than 10\n";
}
```

Conditional statements - elsif

- Basic format:

```
if (condition1) {
   run some code here
}elsif(condition2){
   run something else here
}else {
   run other code here
}
```

- Example:

```
if (length($sequence)  >10 ) {
   print "Seq length is longer than 10\n";
}elsif(length($sequence) == 10){
   print "Seq length is 10\n";
}else{
   print "Seq length is less than 10\n";
}
```

Numerical comparisons

Operator	Meaning	Example
==	Equal to	`if ($a == $b)`
!=	Not equal to	`if ($a != $b)`
>	Greater than	`if ($a > $b)`
<	Less than	`if ($a < $b)`
>=	Greater than or equal to	`if ($a >= $b)`
<=	Less than or equal to	`if ($a <= $b)`

Table modified only slightly from “Perl and Unix to the Rescue”, page 126

String/text comparisons

Operator	Meaning	Example
eq	Equal to	`if ($a eq $b)`
ne	Not equal to	`if ($a ne $b)`
gt	Greater than	`if ($a gt $b)`
lt	Less than	`if ($a lt $b)`
ge	Greater than or equal to	`if ($a ge $b)`
le	Less than or equal to	`if ($a le $b)`

Multiple comparisons

- Put multiple comparisons together in (), separated by:
  - && - and (everything must be true)
  - || - or (at least one must be true)

```
if (($sequence eq “GAATTC”) || ($sequence eq “CTTAAG”)){
    print “Sequence is EcoRI site\n”;
}
```

Loops - while

- Basic format:

```
while (condition){
  do stuff while condition is true
}
```

- Example:

```
my $num = 12;
while ($num <=20){
  print "$num\n"; $num +=2;
}
```

Loops - foreach

- Iterate through a list

```
my @proteins = qw(histone, ubiquitin, actin);
foreach my $protein (@proteins){
  print “$protein\n”;
}

foreach my $i (1 .. length($DNA)) {
    print "Letter $i of the seq is ";
    print substr($DNA, $i-1, 1),"\n";
}
```

Loop control

- `next` – restarts loop
- `last` – terminates loop

```
my $count = 1;
while ($count <= 10) { # repeat for up to ten species
   print "Input species $count abbreviation, or Q to end: ";
   my $species = <>;
   chomp $species;
      if ($species eq "Q") { last; }
      elsif ($species eq "") {
             print "No species entered.\n";
             next; # no grep, counter doesn't change. Ask again.
      }
      system("grep '$species' $blast_out");
      $count = $count + 1;
}
```

Input and Output

Input and Output – File operator

- `<>` - file operator
  - Reads lines of text
  - Remembers how many lines it has seen (`$.`)

- To read in only one line from a file:

```
# if you already specified a file to open, such as
through @ARGV:
# if you didn't specify a file, it will read in a
line of input from the keyboard, equal to <STDIN>
my $line = <>;
```

Input and Output - Filehandles

- Filehandles are special variables used to read or write to files
  - not the same as the file name
  - can open multiple filehandles at once
  - three modes: < read, > write (will overwrite a file if it exists!), >> append
  - must close filehandles once you done operating on them
  - classically written in capital letters

```
open(FH, "<", "filename.txt");
close(FH);
```

Input and Output - Filehandles

- Newer, preferred way to specify filehandles, using scalar variables

```
open(my $input, "<", "in_file.txt") or die "Can't open in_file.txt:
$!\n";

open(my $output, ">", "out_file.txt") or die "Can't open out_file.txt:
$!\n";

while(my $line =<$input>){

      chomp $line;

      my $uc = uc($line);

      print $output "$uc\n";

}

close $input; close $output;
```

Math and functions

Math – operators

**+ - * / % **

```
$y = 6 + 7;    # $y is now 13
$y = $y * 2;  # $y is now 26
$y = $y + 4;   # $y is now 30
$y += 4;       # shortcut for adding, similar operators /=, *=, -=
$y = $y / 10;  # divide by 10
```

Numerical functions

- Functions take arguments, and return a value.
  - Save the returned value to a variable
- abs, int, log, rand, sin, exp
- If worried about operator precedence, use parentheses!

```
$abs = abs(-6); # returns 6

$integer = int(9.7); # returns 9

$y = (4+6) * 7; # force addition before
multiplication
```

Text functions

- Concatenation: `my $cat =“AGG” . “TAC”; # “AGGTAC”`
- Repetition: `print “la” x 40; # prints la 40 times`
- Length: `my $length = length(“xyz”); # yields 3`
- substr(): `my $fox = substr(“quick brown fox”, 12, 3);`
- uc(): `my $upper = uc(”gtcat”);#GTCAT, lc() for lower case`
- join(): `my $joined = join(':', "g", "a", "c"); # g:a:c`
- reverse(): `my $rev = reverse("ACTG"); # GTCA`
- index(): `my $index = index("grandma", "ma"); # 5, -1 if not found`

Regular expressions

Regular expressions

- Fancy pattern searching
- Useful for when you don't know the exact text you want to match
- Requires the =~ Binding operator
  - Matching:

```
print "Found Waldo!\n" if $x =~m/Waldo/; # note that
m can be omitted
```

  - Substitution:

```
$x =~ s/FOO/BAR/g; # global search and replace of
FOO to BAR
```

Regular expression metacharacters

- Anchors: `^` beginning, `$` end
- Characters to escape (\): `^ $ { } ( ) [ ] ? . @ + * / \`
  - `\^` will match a caret, not beginning of string
- Space matching: `\t` tab `\n` newline `\s` space
- Character matching: `\S` non-space `\d` digit `\D` non-digit `\w` word
- `|` or
- `.` Any character but `\n`
- `[^ACT]` any character but A,C, T `[A-Z]` one upper case letter
- `()` save part of the match in special variables - `$1`, `$2`, etc.

Regular expressions – number of matches

- ? – match if it appears or not (0 or 1 times)
- * - match 0 or more times
- + - match 1 or more times
- {n} match n times, {m, n} match minimum m times, maximum n times

	/ab?c/	/ab*c/	/ab+c/
ac	✓	✓	✗
abc	✓	✓	✓
abbc	✗	✓	✓

Regular expression examples

```
if ($x =~ /^M/){ print “Start codon!\n”; }


if($seq =~m/G{2}[UCAG]/i){ # i is case insensitive
  print “Found a glycine!\n”;
}


my $seq_name = $1 if $line =~m/^>(.*)/;
```

Subroutines

- Create your own Perl functions!
- Reuse code: don't write the same code over and over
- Great for organization, and avoiding bugs
- Call subroutine (pass zero or more arguments), code within subroutine will be executed:

```
&analyze_blast(); # & is optional
```

Subroutine example

```
1   #!/usr/bin/env perl
2   use strict; use warnings;
3
4   my $seq = "CCGGCCGGATGTCTTAGGCGTAGCCGGCCGG";
5   my $gc =  &get_gc_content($seq);
6   print "GC content of sequence: $gc \n";
7
8   sub get_gc_content{
9           my ($seq) = @_;
10          $seq = uc($seq);
11          my $num_g_and_c = $seq =~ tr/CG/CG/;
12          my $length = length($seq);
13          my $gc_content = ($num_g_and_c / $length) * 100;
14          return ($gc_content);
15  }
```

One-liners

- Single line of Perl code executed on command line
- Great for data munging and file manipulation
- Add line numbers to a file

```
perl -wpe 's/^/$.\t/' blah.txt > blah_lines.txt
```

- Global search and replace, retaining original files in file*.bak

```
perl -pi.bak -e 's/FOO/BAR/g' file1.txt file2.txt
```

- Get FASTA ids:

```
perl -wlne 'if (/^>(\S+)/) {print $1}' a.fasta > IDs
```

Example – parsing BLAST tabular output

- Context:
  - BLAST is an alignment tool that can be used with nucleotide or protein query sequence(s).
  - Input sequence(s): *Saccharomyces cerevisiae* sequence
  - Database contains: 12 sequences, including *S. cerevisiae*
- We'll parse the tab-delimited BLAST output in two different ways:
  - `blast_parse_array.pl` - keep lines in an array, parse for hits over a specified percent identity
  - `blast_parse_regex.pl` – parse lines using regex, parse for hits from a certain species

BioPerl

- Set of modules for doing bioinformatics in Perl, supported features:
  - Sequence input and output
  - Creating and analyzing alignments
  - Retrieving data from remote databases
  - Converting file formats
- Uses object oriented programming (we don't have time to discuss this today)
- BioPerl is available through module perl/5.24.0 on O2

BioPerl example – renaming seqs in assembly

```
use Bio::SeqIO;
my $in = Bio::SeqIO->new(-file  => $file,  -format => 'fasta');
my $out = Bio::SeqIO->new(-file  => '>redefined.fasta', -format => 'fasta');
my $ct = 1; # Starting numbering scaffolds from 1
while ( my $seqobj = $in->next_seq() ) {
    my $id = $seqobj->display_id; # gets ID from input file. Will move to
description.
    my $desc = $seqobj->description; # Get description field. Should be empty.
    print "Description field is not empty in this sequence:$id\n" if $desc;
    $seqobj->description("Old_ID:$id"); # Description field now has old ID
    my $newid = sprintf(”MYGENOME_scaffold_%06d", $ct); # Modify ID
    my $seq = $seqobj->seq;

    $seqobj->display_id($newid);
    $seqobj->seq($seq);
    $out->write_seq($seqobj);
    $ct++;
}
```

Note on Perl versions

- We've used Perl 5 for this class.
- There is another, newer Perl: Perl 6 or Raku, which is considered to be a **new language**, and **not a replacement** for Perl 5.
- If you'd like to see more information on Raku, please check out:
  - https://www.raku.org/

Perl Modules

- Other people's code you can reuse!
- Each module is in its own file – ending in `.pm`
  - `.pm` stands for Perl module
- Can access a module with the `use` command (goes at beginning of your script)
  - `use Getopt::Long;`

Where to find available modules?

- CPAN – Comprehensive Perl Archive Network
- Search for modules at https://metacpan.org/
- Install packages with `cpan` or `cpanm`
  - `cpan` – standard tool to install modules, comes with Perl distributions by default
  - `cpanm` – created after `cpan`, less verbose, more user friendly

```
cpan App::cpanminus
cpanm Module::Name
```

Installing Perl modules on O2

- Can install packages yourself using `local::lib`
- Will put packages in the `~/perl5-O2/` directory
- If you installed Perl packages on Orchestra, you will need to create a new personal Perl library for O2.
- Setup instructions can be found on our Personal Perl Packages page.

Example of installing a module on O2

- Search CPAN to find package
- Assuming `local::lib` is already set up:

```
$ cpanm Text::Fuzzy
```

Module will be installed in `perl5-O2/` in your home directory.

- To use this module, add this at the beginning of your script:

```
use Text::Fuzzy;
```

Documentation

- Use `perldoc` command or reference http://perldoc.perl.org/

`$ perldoc perlsyn`	Syntax
`$ perldoc perldata`	Data types
`$ perldoc perlfunc`	Functions
`$ perldoc -f chomp`	Specific function: chomp
`$ perldoc perlvar`	Predefined variables
`$ perldoc -v @ARGV`	Specific variable: @ARGV
`$ perldoc perlretut`	Regular expression tutorial
`$ perldoc Getopt::Long`	Specific Module

- Use q to exit `perldoc` help pages, arrow keys to maneuver

Resources for after class

- Unix and Perl to the Rescue! by Keith Bradnam and Ian Korf
  - rescuedbycode.com
- Beginning Perl for Bioinformatics by James D. Tisdall
- Learning Perl by Randal L. Schwartz, brian d foy, Tom Phoenix
- Perl One-Liners by Peteris Krumins
  - https://github.com/pkrumins/perl1line.txt/blob/master/perl1line.txt
- Perl introduction: http://perldoc.perl.org/perlintro.html
- bioperl.org, extensive documentation available

Contact information:

HMS Research Computing

https://wiki.rc.hms.harvard.edu/display/O2/O2

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https://rc.hms.harvard.edu/office-hours/