

# 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

# 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

# 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
```

**Once on O2, open an interactive shell:**

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

# 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/5.24.0
```

- 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 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

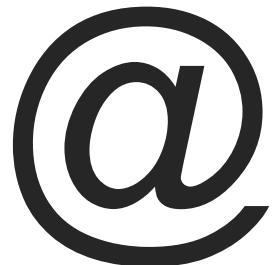
```
my @numbers = (1, 2, 3, 4);
```

```
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



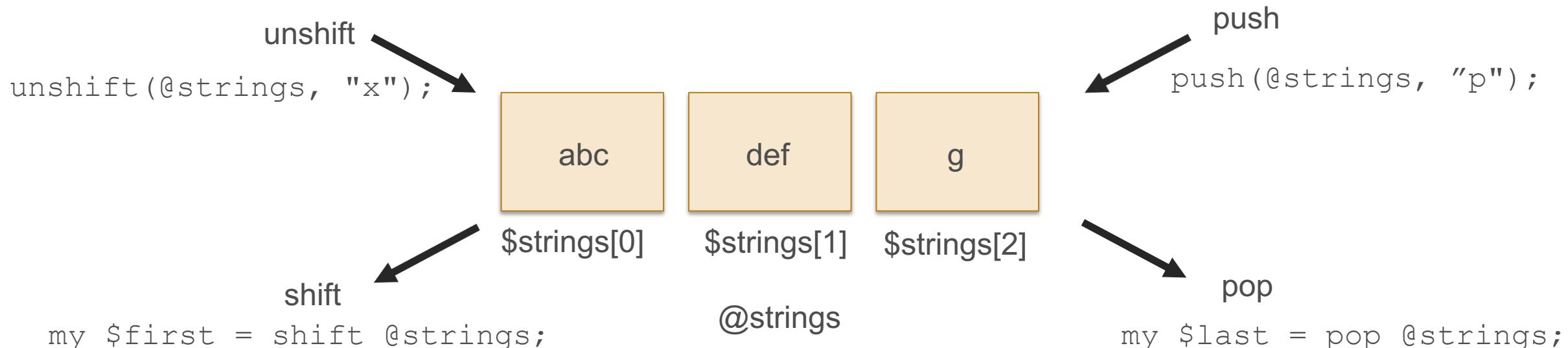
# Arrays – what do they contain?

- 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");
```

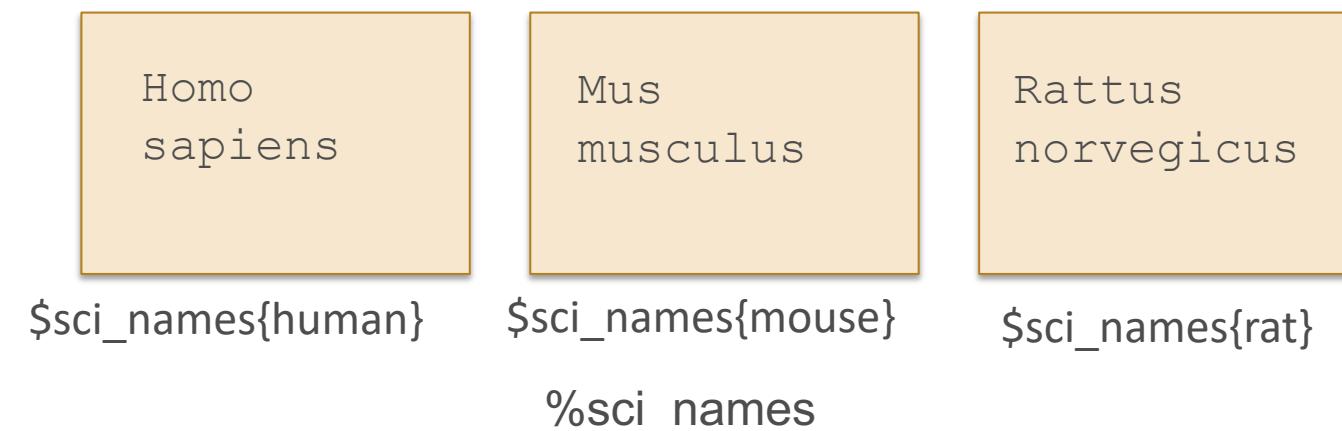
- Method 2 – with => operator

```
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
<code>==</code>	Equal to	<code>if (\$a == \$b)</code>
<code>!=</code>	Not equal to	<code>if (\$a != \$b)</code>
<code>&gt;</code>	Greater than	<code>if (\$a &gt; \$b)</code>
<code>&lt;</code>	Less than	<code>if (\$a &lt; \$b)</code>
<code>&gt;=</code>	Greater than or equal to	<code>if (\$a &gt;= \$b)</code>
<code>&lt;=</code>	Less than or equal to	<code>if (\$a &lt;= \$b)</code>

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 ( ($sequence eq "GAATTCT") || ($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, which is considered to be a **new language**, and **not a replacement** for Perl 5.
- If you'd like to see the Perl 5 to 6 changes, check out this guide:  
<https://docs.perl6.org/language/5to6-nutshell>

# 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 <http://www.cpan.org/> or <https://metacpan.org/> (better searching)
- 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 `~/perl5-02/` 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 `perl5-02/` 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](http://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](http://bioperl.org), extensive documentation available

# Contact information:

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