Perl in a Day

Peeking Inside the Oyster

Biology-Flavored Perl Overview

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Class Overview

- Introduction – Why learn Perl?
- Scripting – Reproducible Science
- Variables – Making Programs Reusable
- Control Structures – Doing Things Lots of Times (Or Not)
- Data Munging – Perl for Bioinformatics
- Arrays and Hashes – Groups of Things
- Subroutines & Modules – Making Programs *Really* Reusable
- Objects – Complex Data, Complex Workflow
- BioPerl – Doing (More) Bio With Perl

Research Computing Commercial

- Knowledge + experience in science + computers
    - We worry about computers so you can do science
    - Backup, installation, security, scripting…
- FAQ and more: http://rc.fas.harvard.edu
- Tickets
    - Research questions to rchelp@fas.harvard.edu
    - Other questions to help@fas.harvard.edu
    - The more detail, the better
- Talk to us **before** you do lots of work
    - Save time
    - Do better science

The Bad News

- You can't learn programming in such a short time
  - Too much syntax
  - Too many functions
  - Too many concepts
  - Too many special cases (especially in Perl)

The Good News

- You can do a lot knowing just a little Perl
- Perl is good at scientific computing
- Perl is fun!

Objectives

- Understand the basics of Perl
  - Focus on what kinds of things Perl can do
  - Don't worry too much about syntax
- Learn to read, modify, and run Perl scripts
- Learn some mistakes to avoid
- Answer your questions (maybe after class)
- Special focus on *data munging*
  - Data what?

Data Munging

- Doing stuff with data
  - Getting data from many sources
    - Keyboard, local files, databases, ftp, web, …
  - Reading (and understanding) data
    - Binary, Text, HTML, XML, zip, Graphics, …
    - BIG files, many files
  - Combining data
  - Analyzing data (e.g., mathematically)
  - Filtering data
  - Outputting data
- Lots of scientific computing is just data munging
- Perl is very (very) good at data munging

Why Perl?

- Easy to learn and quick to write
  - Rapid prototyping
  - But scalable to large programs
- Kitchen sink language
  - Combines parts of many other tools (C, sed, awk, sh, …)
  - Call other programs
- Cross-Platform: Windows, Mac, UNIX
- Open Source – lots of code already available
- TMTOWTDI - “There’s more than one way to do it”
- Very popular in Bioinformatics

What Makes Perl Different?

- More like English, less like Math
  - (Pluses or minuses…)
  - More messy (writing vs. reading)
  - Less orthogonal (TMTOWTDI vs. inelegance)
  - Huge set of libraries available (which is best?)
  - Regular expressions (power vs. complexity)
  - Interpreted, not compiled (fast writing vs. running)
  - DWIM – "Do what I mean" (convenience vs. confusion)

Why Not Perl? (A Biased View)

- Perl is not the fastest-running language
  - Not good for doing huge amounts of very complex math
  - But you often save time by developing code quickly
- Perl allows you to write messy code
  - "Write-only language"
  - But messy is fine in certain contexts
  - Perl can help you write clean code
- Not originally designed for huge programs
  - Older versions of Perl made it hard
  - But plenty of huge programs have been written in Perl
  - This class isn't for people writing huge programs

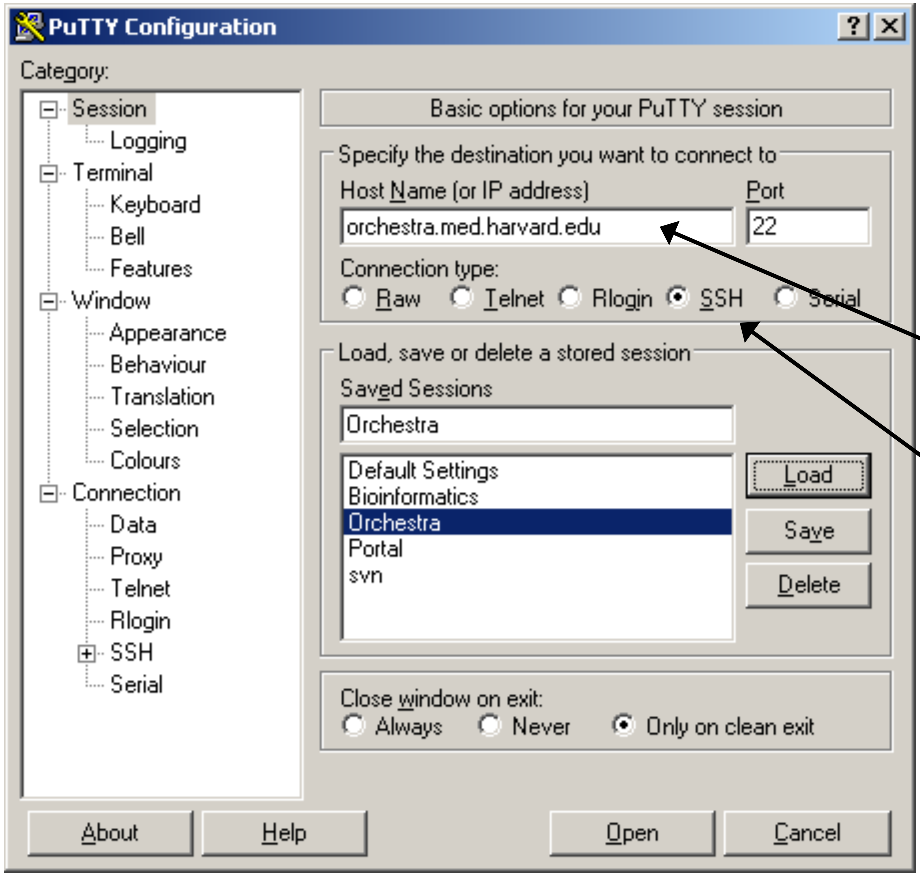
What Can Perl Do for Me?

- Automate other programs
  - Run 1,000 BLASTs
  - High-throughput downloading and analysis of biological databases
- Analyze, filter, merge, reformat data
  - Munge results of other programs
  - Write one-liners to explore your data
- Interact with SQL databases (MySQL, Oracle, etc.)
  - Store, read, change structured data
- Create interactive CGI web pages
  - UCSC, BLAST, a simple login form
- Other bioinformatics topics
  - Population Genetics, Ontologies, Alignments, Graphing, …

Getting Started

- Where is Perl?
  - On any UNIX (Linux, Mac) computer
  - On the HMS cluster (orchestra.med.harvard.edu)
  - On the FAS cluster (odyssey.fas.harvard.edu)
  - Windows: download from http://www.activestate.com/Products/ActivePerl
- Don’t run on your own laptop!
  - Unless you have BLAST+ installed

Logging In (If Necessary)



Host Name (or IP address): orchestra.med.harvard.edu

Port: 22

Connection type: ● SSH

Saved Sessions: Orchestra

Close window on exit: ● Only on clean exit

Terminal program: putty.exe (Google Putty and SSH)

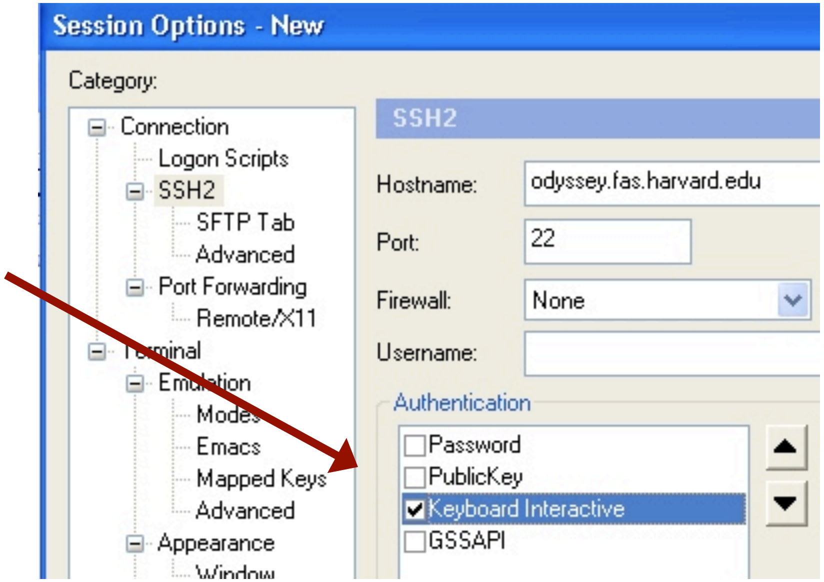
HMS cluster "head" node:

orchestra.med.harvard.edu

SSH, a secure telnet. (Port will change to 22)

Logging in to Odyssey

- Run Xming on your desktop (for editing files with gedit)
- Connect to Odyssey with SecureCRT
  - Use Keyboard Interactive **only**
- Start an interactive shell
  - Lets you run long programs from command line
- `module load hpc/perl5mods`
  - For Bioperl, fancy Perl stuff



Hostname: odyssey.fas.harvard.edu
Port: 22
Firewall: None
Username:

Authentication
☐ Password
☐ PublicKey
☑ Keyboard Interactive
☐ GSSAPI

Getting the Sample Code (Odyssey)

- Get the code
  `cp -r /n/nobackup2/workshop_perl .`
- Change to the sample directory
  - **`cd workshop_perl`**
  - Class demos are in `class_demos_UNIX`, etc.
- List of programs in class order (in demo directory)
  - **`more MANIFEST`**

Getting the Sample Code (UNIX/Mac)

- Get the zipped code
  `http://software.rc.fas.harvard.edu/training/perl/unixcode.zip`
- (Mac: open a Terminal window)
- Unzip code
  - **`unzip unixcode.zip`**
- Change to the sample directory
  - **`cd unixcode`**
  - Class demos are in `class_demos_UNIX`, etc.
- List of programs in class order (in demo directory)
  - **`more MANIFEST`**

Getting the Sample Code (Windows)

- Open a Cygwin window
  - Start->All Programs->Cygwin->Cygwin Bash Shell
- Get the zipped code
  - `ftp://ftp.cgr.harvard.edu/data/akarger/perlclass/doscode.zip`
- Unzip code
  - **`unzip doscode.zip`**
  - OR double-click **`doscode.zip`** and copy **`code`** folder to Desktop
- Change to the code directory
  - **`cd Desktop`** (if necessary)
  - **`cd code`**

Getting Ready to Run on orchestra (if applicable)

- Use LSF (Do this just once, each time you log in)
    - `bsub -Is -q shared_int_2h bash`

Before you start using Perl…

- Make sure Perl exists, find out what version it is
  - `perl -v`
- How do I get help?
  - `perldoc perl (general info, TOC)`
  - `perldoc perlop (operators like +, *)`
  - `perldoc perlfunc (functions like chomp: > 200!)`
  - `perldoc perlretut (regular expressions: /ABC/)`
  - `perldoc perlreref (regular expression reference)`
  - `perldoc -f chomp (what does chomp function do?)`
  - `perldoc File::IO (find out about a Perl module)`
- Type q to quit when viewing help pages,
- Space bar for next page

Editing your files graphically

- Use an editor to write your programs
  - pico, nano, emacs, vi (or vim) are non-graphical options
- Odyssey: `gedit &`
  - `&` lets you type in SecureCRT window while gedit is open
  - Running Xming lets gedit appear on your desktop
- Mac: use TextEdit, save as PLAIN text (Prefs)
- Windows: http://winscp.net edits remove files
  - Notepad or Wordpad to edit local files

Editing your files with pico

- Use an editor to write your programs
  - pico, nano, emacs, vi (or vim) are some UNIX options
  - Just type `pico` or `pico blah.pl`
- Type your program
  - "Enter" to start a new line
  - Arrow keys, not mouse, to move around
- Common commands at bottom of screen
  - Control-O Save (Don't type Control-S!!!)
  - Control-X Quit

(Odyssey) Exercise – "Hello, World!"

```
print "Hello, World!\n"; # A comment
```

- Create a new file in gedit
- Type in the above program (just one line)
- Save it as `hello.pl`
- Run it from the command line (`perl hello.pl`)

```
[iliadaccess02:workshop_perl] perl hello.pl
Hello, World!
```

You have written your first Perl program!

(Mac) Exercise – "Hello, World!"

```
print "Hello, World!\n"; # A comment
```

- Create a file called hello.pl in TextEdit
- Type in the above program (just one line)
- Save it as PLAIN text (not rich text)
- Run it from the Terminal (`perl hello.pl`)

```
% perl hello.pl
Hello, World!
```

You have written your first Perl program!

(UNIX) Exercise – "Hello, World!"

```
print "Hello, World!\n"; # A comment
```

- Create a file called hello.pl (`pico hello.pl`)
- Type in the above program (just one line)
- Save it (Control-O) and exit `pico` (Control-X)
- Run it from the command line (`perl hello.pl`)

```
% perl hello.pl
Hello, World!
```

You have written your first Perl program!

(Windows) Exercise – "Hello, World!"

```
print "Hello, World!\n"; # A comment
```

- Create a file called hello.pl with Notepad
- Type in the above program (just one line)
- Save it as hello.pl (Save as a “Text file”)
- Run it (`perl hello.pl` in the Cygwin window)

```
% perl hello.pl
Hello, World!
```

You have written your first Perl program!

First Perl Program

Comment - any text after # sign - doesn’t do anything

```
# Hack into government computers...
```

\n makes a new line (inside "double quotes")

```
print "Hello, World!\n";
```

Many Perl scripts start with a #! line

- For now, ignore this
- The -w is like typing "use warnings"

```
#!perl -w     (or maybe #!/usr/bin/perl -w)
```

First Perl Program II

```
print "Hello, World!\n"; # A comment
```

- " ; " is used at the end of each command
  - A command is *usually* one line
  - But multi-line commands, multi-command lines OK
  - Semicolons are (sometimes) optional
- Warning: Perl is case sensitive!
  - print is not the same as Print
  - $bio is not the same as $Bio

First Perl Program III

- `print` is a *function* which prints to the screen
  - `print("Hi")` is (usually) the same as `print "Hi"`
  - Inside "double quotes", \n starts new line, \t prints tab
  - A function is *called* with zero or more *arguments*
    - Arguments are separated by commas
    - `print` takes as many arguments as you give it

```
print ""; # legal, prints nothing, not even \n
print("Hi", "There"); # prints HiThere
print(Hi); # illegal (calls the function Hi)
print(1+1, 2+2, "\n"); # prints 24 and a newline
```

Scripting

Reproducible Science

via

Scripting command-line calls

3-Minute Introduction to Biology

- BLAST program
  - Finds DNA or protein sequences similar to your **query** sequence(s)
  - Better results have lower **E-value** (e.g., 1e-5 is better than .03)
  - Our results will be in tabular format

```
Query_id123   Subject_id456   92.20   510   86   1  602   1101   29   560   1e-20  459
```

  - A **hit** means part or all of the **query** sequence is similar to a **subject** sequence in the big search database
- FASTA file format
  - Standard format for storing DNA or protein sequences
  - Identifier, (optional) description, sequence

```
>blah|12345    Cytochrome c oxidase
ACTGGTCGAAGTTGGCGA
ACGGTTGGTACGCA
```

- Examples are biology-specific, but the Perl ideas aren’t

Embedding Shell Commands

Use shell commands in Perl programs:

```
system("ls"); # list files in current directory
```

Run a BLAST with tabular output:

```
system("blastn -task blastn -db fungi -query one_seq.fasta
     -outfmt 6 -evalue 1e-4 > one_seq.blast");
```

Search for text string “Cgla” in BLAST output file:

(UNIX, Mac, Cygwin in Windows. No Cygwin? Use “find”)

```
system("grep 'Cgla'" one_seq.blast);
```

Embedding shell commands II

Multiple commands in sequence → script

```
# Blast a yeast sequence against many fungi
system("blastn ... > one_seq.blast");

# Find Candida glabrata hits
system("grep 'Cgla' one_seq.blast");
```

Benefits over running from command line:

- Easy to repeat (reproducible science)
- Easy to rerun with slightly different parameters

Easier if parameters are at the top of the program

- … or program asked us for them

Exercise – Automate BLAST and grep

1. Run the script to BLAST and grep
   - `perl EX_Scripting_1.pl`
2. Now edit `EX_Scripting_1.pl` and change the way you're BLASTing and greping.
   a) How many Sklu hits are there?
   b) How many Kwal hits?
   c) BLAST with 1e-50 instead of 1e-4
   How many Cgla hits do you get now?

- Exercises are in exercises_UNIX/ or Windows/
- Solutions are in solutions_UNIX/ or Windows/
Look for "# CHANGED" lines

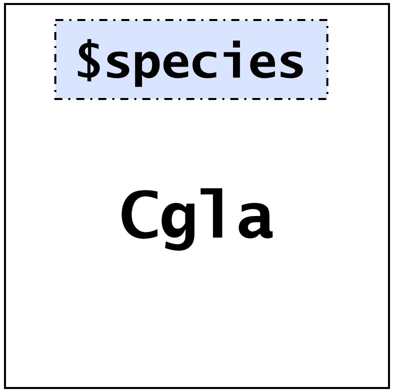
Variables

Making Programs Reusable
by
Storing and Manipulating Data

Scalar Variables

- A box containing a single “thing” (value)
  - `$e_value = 1e-4;`
  - `$string = "has spaces and $vars and \n";`
  - `$species = "";`
  - References, objects
- Has a name (label) starting with $
- Value can change during a program
  - `$species = "Cgla";`
  - `$species = "Ylip"; # later…`
- Variables encourage reusability
- See **`variables.pl`**

$



`$species`

`Cgla`

Scalar Variables II – Declaring Variables

- Declare variables with `my`
  - Tell the program there's a variable with that name
  - `my $e_value = 1e-4;`
  - Use `my` the first time you use a variable
  - Don't have to give a value (default is "", but –w may warn)
- Avoid typos
  - `use strict;`
  - Put this at the top of (almost) any program
  - Now Perl will complain if you use an undeclared variable
  - `$evalue = 1e-10; # "Global symbol…"`
- Better to get parameters from the user…

Reading Variables – From the Keyboard

- See `variables_ask.pl`
- Use `<>` to read in a line of input from the keyboard
  - `$species = <>;`
  - Result gets placed in variable `$species`
  - Typing Cgla and Enter yields same results as this code:

```
$species = "Cgla\n";
```

- `chomp()` removes the newline (\n) from the input
  - `$species` is now `Cgla`
  - `chomp()` only removes a newline
  - `chomp()` only removes newline at the end of a string

Reading Variables – From an Input File

- `<>` can also read from input files
  - Specify input file(s) on the command line
  - `perl variables_ask.pl variables_ask.in`
  - Use `<>` for multiple files of the same type
    - E.g., Multiple BLAST outputs, or multiple FASTA files
  - `<>` reads data from files as if you typed it on the keyboard
- Saving input files → Reproducible Science!
- But this is a lot of work, for one or two options…

Reading Variables – From the Command Line

- Getopt::Long
  - A module (library of functionality someone else wrote)
  - Allows you to input simple options on the command line
  - `perldoc Getopt::Long` for (much) more information
- Using Getopt::Long
  - `use Getopt::Long; # use the module`
  - `my $species = "Cgla"; # default value for variable`
  - `GetOptions("spec=s" => \$species);`
  - `spec` means you can type -spec, -sp, -s on command line
  - `=s` means text **s**tring (`=i` for **i**nteger, `=f` for “**f**loat” decimal)
  - `=>` is a fancy comma
  - `\$species` is a "reference" (pointer) to `$species` variable

Reading Variables – From the Command Line II

- See `get_s_opt.pl`
  - Run it like this: `perl get_s_opt.pl -s "Klac"`
  - **Not** like this: `perl -s "Klac" get_s_opt.pl`
  - If also giving files: `perl get_s_opt.pl -s "Klac" file1`
- You can input multiple parameters
  - Call `GetOptions` only *once* near beginning of program
  - Tell `GetOptions` about all possible options
  - `GetOptions(`
    - `"spec=s" => \$species,`
    - `"blast=s" => \$run_blast`
  - `);`
  - `GetOptions` will set `$species` and `$run_blast` (if user inputs -blast and -spec)

Getting output from shell commands

- Use backquotes (``) around shell command
- Runs the command (like `system()`)
- Gets the results in a variable
  - You get the standard output, i.e., what would have been printed to the screen
    - (But standard error will still print to the screen)
  - You can embed $variables in the command

```
$date = `date`; # UNIX command: guess what it does?
print "The date is $date";
# Note: returns a LONG string with \n's in it!
$blast = `blastn -task blastn -evalue $e_value …`
```

Exercise – Variables and Inputting Options

1. Input the E-value to use for BLAST from the user
   - Change `EX_Variables_1.pl`
   - Input E-value from the keyboard (*before* BLASTing!)
   - Using same program, input from a file (with two lines)
   - Input from two separate, one-line files. (Type file names in the right order!)
2. Use Getopt::Long
   - Start with `EX_Variables_2.pl`
   - Add –evalue parameter
   - E-value is a "float" (decimal number); use **`=f`**, not **`=s`**

Control Structures

Doing Things Lots of Times (Or Not)
using
Loops and Conditions

Loops and Conditions – Why?

- So far we have seen only linear programs
- Flowcharts are more interesting (and realistic)
  - Loops - do something more than once
  - Conditions - do something sometimes, but not other times

- Combining loops and conditions correctly is a major part of programming

Conditions

- Let's stop running BLAST every time
- Basic if statement:
  - `if (condition)` is true…
  - Run `{BLOCK}` of code

```
if (condition) {
    do some stuff;
    and more stuff;
}
```

```
if ($run_blast eq "y") {
    my $db = "other_fungi";
    print "Blasting $db\n"; #works
    system("blastn -db $db …");
}
print $db; # ERROR. Unknown var
```

- No semicolon after beginning and end braces
- Blocks are often indented for ease of reading
- One or more commands inside BLOCK, separated by ;
- `my` variable inside a BLOCK will lose its value at end

Conditions II – else

- Let's warn user when we're not running BLAST
  - `else` (if the condition wasn't true…)
  - Run the code inside the else `{BLOCK}`

```
if (condition) {
    do some stuff;
}
else {     # optional
   do other stuff;
}
```

```
if ($run_blast eq "y") {
    system("blastn …");
}
else {
    print "Not running blast";
}
```

- else blocks are optional

Conditions III – else if

- See `if_run_blast.pl`
- Only allow "y" or "n" as inputs –
- Otherwise `die` (exit with an error)
- You can have one or more `elsif`'s after an `if`
  - just if, if else, if elsif, if elsif else, if elsif elsif elsif …

```
if (condition) {
    do some stuff;
}
elsif (other cond.) {        # optional
   do other stuff;
}
else {                              # optional
    do this instead;
    blocks can have >1 cmd
}
```

```
if ($run_blast eq "y") {
    system("blastn …");
}
elsif ($run_blast eq "n") {
    print "Use saved BLAST\n";
}
else {
    die "Illegal -b option\n";
}
```

Comparisons for Conditions

- String (text) comparisons: `eq ne gt lt ge le`
  - Made of letters so you know we're comparing text

```
# Compare gene names
if ($gene1 ne $gene2) {
  print "$gene1 and $gene2 are different";
}

# Careful! "y" ne "Y"
if ($run_blast eq "y") { print "Yay!\n"; }
```

- When comparing strings, "0.1" ne ".1"
  - How do we test for numerical equality?

Comparisons for Conditions II

- Numeric Comparisons: `==  !=  >   <   >=   <=`

```
if ( $num1 >= 0 ) {
    print "$num1 is positive or zero\n";
}
if (0.1 == .1) {
    print ”Oh, good. It’s a numerical comparison\n”;
}
```

- Careful!
  - `=` used to assign a variable: `$num = 37;`
  - `==` used as a test: `if ($num == 37) {…}`

- Careful!
  - Text strings have numeric value 0, so "ACTG" == "GCTA"

Multiple Comparisons

- `&&` means a logical AND (all pieces must be true)
- `||` means a logical OR (at least one piece is true)
- Group comparisons with parentheses

```
if (($run_blast eq "y") || ($run_blast eq "Y")) {
  print "Running BLAST\n";
  system("blastn …");
}
```

- `!` negates a condition

```
if (!(some complicated expression)) {
    print "It wasn't true";
}
```

Loops - foreach

- A `foreach` loop loops over a `(list)`
  - Sets a `$variable` to first value in `(list)`
  - Runs a `{BLOCK}` using that value for the `$variable`
  - Repeats loop for every value in the `(list)`
- See `foreach.pl`

foreach my $variable (list) {
do some stuff;
do more stuff; # …
}

".." is great for making lists

```
# Find hits from several species
foreach my $species ("Cgla", "Klac") {
  print "Hits for $species\n";
  system("grep '$species' $blast_out");
}

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

Loops II - while

- A `while` loop keeps running while a `(condition)` is true
- It checks the `(condition)`
- Runs code in the `{BLOCK}` if it was true
- Then checks again…
- It's sort of like foreach + if

```
while (condition) {
   do some stuff;
    then do other stuff;
}
```

```
# Print numbers from 5 to 15 by fives
my $i = 5;
while ( $i < 20 ) {
    print "$i ";
    $i = $i + 5;
}
# Here, $i=20 BUT code never prints 20
# If we tested $i <= 20, we'd print 20
```

Loops III – Jumping Around

- `last` jumps out of a loop
- `next` skips to the `{BLOCK}` bottom, but then keeps looping
- Note: `if` is NOT a loop - `last` / `next` ignore `if` blocks

```
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;
}
```

Exercise – Loops and Conditions

1. Write a program to BLAST/grep four files
   - Use `"YAL001C.fasta", "YAL002W.fasta”, …`
   - Hint: Add a loop to `EX_Loops_1.pl`
2. Tell user what’ s happening
   - Start with solution to `EX_Loops_1.pl`
   - If file is `YAL002W.fasta`, print “It’s my favorite sequence!”
3. Input checking
   - If the user inputs an e-value other than 1e-4, then using a stored BLAST output would be bad.
   - Make the program `die` if the user inputs -e not equal to 1e-4 and also inputs -b n
   - Hint: what compound condition do you need to test?
   - Start with `EX_Loops_3.pl`

Data Munging

Perl for Bioinformatics
or
Reading, Filtering, Merging, Changing, and Writing Data

Math

- Arithmetic operators: + - / * %

```
$a = 10 + 5; # $a is now 15
$a = $a + 20; # add 20 to the value of $a
$a += 20; # short cut, similarly -= /= *=
$a++; # shorter cut, same as $a+=1
$a = "hi" + 5; # $a=5. A text string counts as zero
```

- % is "modulus", or the remainder after division: 11 % 3 = 2, 12 % 3 = 0

Math II - Functions

- A function takes one or more arguments
  - Math functions: sqrt, exp, log, int, abs, …
- A function returns a value
  - Set a variable equal to the return value
  - Or print it
- Parentheses are optional (sometimes)
  - Better to use them unless it's really obvious

```
$b = int(3.2); # Remove after the decimal. $b = 3
print int(-3.2); # (Or print int -3.2) prints -3
print int -3.2; # Same
```

Math III – Precedence

- Parentheses are **not** optional (sometimes)

```
$a = 4*3 + 2; # $a=14
$a = 4 * 3+2; # oops! Spaces can be dangerous
$a = 4 * (3+2); # correct. $a = 20
# quadratic equation
$x = (-$b + sqrt($b*$b - 4*$a*$c)) / (2*$a)
```

Text Functions – A Brief Overview

- `"abc" . "def"` → `"abcdef"`
- `join(":", "a", "b", "c")` → `"a:b:c"`
- `split(/:/, "a:b:c")` → `"a", "b", "c"`
- `substr("abcdefghi", 2, 5)` → `"cdefg"`
- `reverse("ACTG")` → `"GTCA" # NOT complement!`
- `"ACCTTG" =~ s/T/U/g` → `"ACCUUG" # DNA->RNA`
- `"ACCTTG" =~ tr/ACGT/UGCA/` → `"UGGAAC" # complement!`
- `length("abc")` → `3`
- `lc("ACTG")` → `"actg" # uc does the opposite`
- `index("ACT", "TTTACTGAA")` → `3 # -1 if not found`
- Wow! (**`perldoc -f split`**, etc.)

Regular Expressions

- Patterns for searching a text string
- Does the string FOO appear in variable $x?
  - `if ($x =~ /FOO/) { print "Found FOO!" }`
  - True for $x="FOO", "aFOO", "FOOFOOFOO", "FOOLISH"
  - False for $x="", "FO", "OOF", "foo", "F O O"
- Search for variables
  - `if ($line =~ /$species/) { print ”Got $species!" }`
- Search for wildcards (see below)
- One of Perl's greatest strengths (powerful)
- One of Perl's greatest weaknesses (confusing)
- **`perldoc perlretut, perlreref, perlre`**

Regular Expressions II

- `^` matches beginning of string, `$` matches end
- Many special characters must be \quoted
  - `^ $ ( ) { } [ ] ? . @ + * / \`
  - I.e., `\$` matches a literal dollar sign, not end of string
  - `\t` tab `\n` newline `\s` (space,`\t`,`\n`) `\S` non-space `\d` digit
- `/stuff/i` - the 'i' option ignores case
- See **`match.pl`**

```
$x =~ /ACTTGG/ # Finds subsequence ACTTGG in $x
$x =~ /^M/ # Finds seq starting with methionine
$x =~ /\*$/ # Sequence ends with stop codon
$x =~ /AACC/i # Find upper- or lower-case bases
```

Regular Expressions III

- `|` means or (sort of like `||`)
- `.` matches any character except `\n`
- `[ACT]` means any one of A, C, or T. `[A-Z]` any upper case
- `()` save (part of) a match in magic variables `$1`, `$2`, etc.
  - Can also be used to group together - see next slide

```
/ACAG|ACCG/ # Matches a profile
/A.C/ # matches ABC, A1C, A C, A~C, but not AC, A\nC
if (/AC([AC])G/) { # Note: ACACG will NOT match
  print "Wobbly base was $1\n";
}
```

Regular Expressions IV

- `+` matches 1 or more copies of the previous thing
- `*` matches 0 or more copies of the previous thing
- `?` matches if something appears or if it doesn't

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

Note: /ab*/ matches ac!
/^ab*$/ doesn’t match ac

	/a(bc)?d/	/a(bc)*d/	/a(bc)+d/
ad	✓	✓	X
abcd	✓	✓	✓
abccd	X	X	X
abcbcd	X	✓	✓

```
/CG?CA/ # Finds sequence with or without deletion
if (/^>(\S+)/) {$id=$1} # FASTA ID (\S = non-space)
```

Substitutions

- Replace first occurrence of FOO in variable $x with BAR
  - `$x =~ s/FOO/BAR/;`
  - "aaaFOObbbFOO" → "aaaBARbbbFOO"
- Replace all occurrences
  - `$x =~ s/FOO/BAR/g; # g stands for "global"`
  - "aaaFOObbbFOO" → "aaaBARbbbBAR"
- The thing to substitute can be a regular expression
  - `$x =~ s/a+/x/;`
  - "aaaFOObbbFOO" → "xFOObbbFOO"
- Matches are “greedy”
  - `$x =~ s/a.*F/x/;`
  - "aaaFOObbbFOO" → "aOO"
- If it can't find FOO, s/// does nothing
  - `$x =~ s/FOO/BAR/;`
  - "aaabbb" → "aaabbb"

Exercise – Regular Expressions

1. Edit `EX_Regexp_1.pl` to die unless the user inputs a valid species
   - One upper-case letter followed by three lower-case letters
2. Promise me you'll learn about regexps someday
   - **`perldoc perlretut, perlreref, perlre`**
   - "Mastering Regular Expressions" (O'Reilly)
   - Or just start using them (carefully)

I/O Overview

- Filehandle
  - A way to "hang on" to (name, refer to) a file
  - Not the same as a file name
  - Usually a name in all capital letters
- Open a filehandle to read from/write to a file
- `<FILEHANDLE>` reads a line from a file
- `print FILEHANDLE` … writes to a file
- Multiple read/write filehandles open at once
- Close filehandle when done reading/writing

Opening and Closing Files

- `open(FILEHANDLE, "filename")`
  - Must be done **before** reading/writing a file
  - Associates the file name with a filehandle
  - `"filename"` is the same as `"<filename"` - read from file
  - `">filename"` - write to file
    Note: `>` DELETES ANY PRIOR DATA IN THE FILE!
  - `">>filename"` - add to end of file. Doesn't delete anything.
  - `open(…) or die "Error: $!\n"` helps diagnose problems
- `close(FILEHANDLE)`
  - Finish writing/reading

Reading From Files

- `$x = <FILEHANDLE>;`
  - Reads from a filehandle
  - Gets one line at a time (by default)
- `<STDIN>` (abbreviated `<>`)
  - Reads from the keyboard
  - OR from files given as arguments to the script

```
perl blah.pl file1 file2
```

  - Automatically opened/closed

I/O: Reading from a file

- Let's replace UNIX grep with Perl regexps

```
open(BLAST, "<$blast_out")
    or die "Can't open $blast_out: $!\n";
$line = <BLAST>;
if ($line =~ /\t$species/) { # species name after a tab
    print $line;
}
close(BLAST);
```

- Great, but we're only reading one line
  - Can we read multiple lines (without Repeating Code)?
  - How do we know when the file is done?

I/O: Reading from a file II

- Using a `while` loop with `<FILEHANDLE>`
  - If there are no lines left, `<FILEHANDLE>` will return `undef`,
  - `undef` is default value for variables (`my $var;`), not `""`
  - `defined($line)` is true EXCEPT if `$line` is `undef`
  - See **read_file.pl**

```
open(BLAST, "<$blast_out")
    or die "Can't open $blast_out: $!\n";
while (defined(my $line = <BLAST>)) {
    if ($line =~ /\t$species/) { # species name after tab
        print $line;
    }
}
close(BLAST);
```

Writing To Files

- `print FILEHANDLE "string", $var, …`
  - Prints one or more things to a filehandle
  - Remember to explicitly write `"\n"`'s
  - Note: **no comma** between FILEHANDLE and stuff to print
- `STDOUT`
  - `print STDOUT …` is the same as a regular `print …`
  - Prints to screen even if one or more filehandles are open
- See **`write_file.pl`**
- Advanced: filehandles can be variables
  - `open(my $fh, ">", "file")`
  - `print $fh "something”`
  - `while (<$input_fh>) {…}`

Parsing BLAST Output with Regexps

- lcl|Scer--YAL036C Spar--ORFN:355 92.20 1103 86 0 1 1103 1 1103 0.0 1459
- `$line =~ /^\S+\t($species\S*)\t/ or die "Bad line $line";`
- `my $id = $1;` pull out just the hit ID
- The regular expression we're searching with is:
  - `\S+` Multiple non-space chars
  - `\t` a tab
  - `($species\S*)` species name, followed possibly by non-space characters (AND parentheses save this string in `$1`)
  - `\t` tab after the ID
- `or die "…"` exit informatively if we have unexpected format
- See **`get_hit_ids.pl`**

Exercises – Input/Output and Munging

1. Write Cgla results to `Cgla_hits.txt` and Sklu results to `Sklu_hits.txt`
   - Change `EX_Munging_1.pl`
   - The easy way: read BLAST results twice
   - Slightly harder: read BLAST results only once
     - (Hint: you can have multiple input or output files open at the same time, as long as they have different filehandles)
     - Solutions are `SOL_Munging_1a.pl` and `SOL_Munging_1b.pl`
2. Edit `EX_Munging_2.pl` to also get the percent identity (next column after ID)

The Scriptome

Advanced Data Munging for Beginners
or
Perl for Wimps Busy Biologists

The Default Variable $_

- Many functions act on `$_` by default
  - `print` prints `$_`
  - `chomp()` removes \n from end of `$_`
  - `while(<HANDLE>)` reads lines into `$_`
    - Same as `while(defined($_=<HANDLE>))`
    - `<>` only reads into `$_` inside a `while()`!
  - `/a/` matches against `$_`(no =~ necessary)
  - `s/A/B/` substitutes B for A in `$_`
- If you can't find a variable, assume it's `$_`
- Give variables descriptive names

One-Liners

- Perl has shortcuts for data munging
- (You won't be tested on this!)
- fancy grep with full Perl functionality: get FASTA IDs

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

- sed+awk with Perl functionality

```
perl -wpe 's/^\S+\t(Cgla--\S+).*/$1/' blast.out > IDs
```

- Add line numbers to a file

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

- Count times each value is in col 3 (tab-separated)

```
perl -wlanF"\t" -e '$h{$F[2]}++; END { foreach (keys
%h) {print "$_\t$h{$_}"}}' blah.tab > count.tab
```

One-Liners II: Serious Data Munging

- With practice, you can *explore* your data quickly
  - Much faster than opening up a graphing program
  - Also good for "sanity checking" your results
- Choose best BLAST hit for each query sequence

```
perl -e '$name_col=0;$score_col=1; while(<>) {s/\r?\n//;
@F=split /\t/, $_; ($n, $s) = @F[$name_col, $score_col]; if (!
exists($max{$n})) {push @names, $n}; if (! exists($max{$n}) || $s
> $max{$n}) {$max{$n} = $s; $best{$n} = ()}; if ($s == $max{$n})
{$best{$n} .= "$_\n"};} for $n (@names) {print $best{$n}}' infile

> outfile
```

Scriptome Motivation

- "You can't possibly learn Perl in a day "
- "But I need to get work done!"
- "If only someone would do all the work for me…"

The Scriptome In One Slide

- Scriptome: cookbook of Perl one-liners
  - No programming needed
  - No install needed (if you have Perl)
  - No memorization needed
- sysbio.harvard.edu/csb/resources/computational/scriptome
- Read the instructions
- Find BLAST results with > 80% identity (3rd col.=2)
- Expand code to see how it's done
- Build a protocol

Sample Scriptome Manipulations

- Manipulate FASTAs
- Filter large BLAST result sets
- Merge gene lists from different experiments
- Translate IDs between different databases
- Calculate 9000 orthologs between two species of *Drosophila*

- Please (please!) contact me about using Scriptome

(Odyssey) Scriptome on the Command Line

- Odyssey has shortcuts for running Scriptome Tools
  - More powerful; a bit more work
- `module load bio/hpc_data_tools`
- List all “change” tools on the Scriptome website

```
Scriptome -t change
```

- Run a tool

```
Scriptome -t change_fasta_to_tab allY.fasta > Y.tab
```

- Program will ask you for parameters, if needed

```
Scriptome -t choose_cols Y.tab > ids.txt
```

  - Voilà! Easy way to get FASTA IDs
- Or set parameters on command line: scriptable

```
Scriptome -t choose_cols -p '@cols=(0, 1, -2, 2)'
one_seq.blast > short.tab
```

- ScriptPack: runs on your laptop
  - Available on website Resources page

Exercises – Scriptome

1. Print BLAST hits from one_seq.blast with 80 – 85% identity (see `EX_Scriptome_1.txt`)
2. Use the Scriptome to *change* `allY.fasta`, which contains four sequences, to tabular format. (see `EX_Scriptome_2.txt`)

Arrays and Hashes

Groups of Things
for
High Throughput Munging

Why Arrays?

- What if we want to store the hit IDs?
  - Further analysis
  - Different kinds of filtering
  - Printing out
- We don't want to read the file multiple times!
- Store the IDs in an array

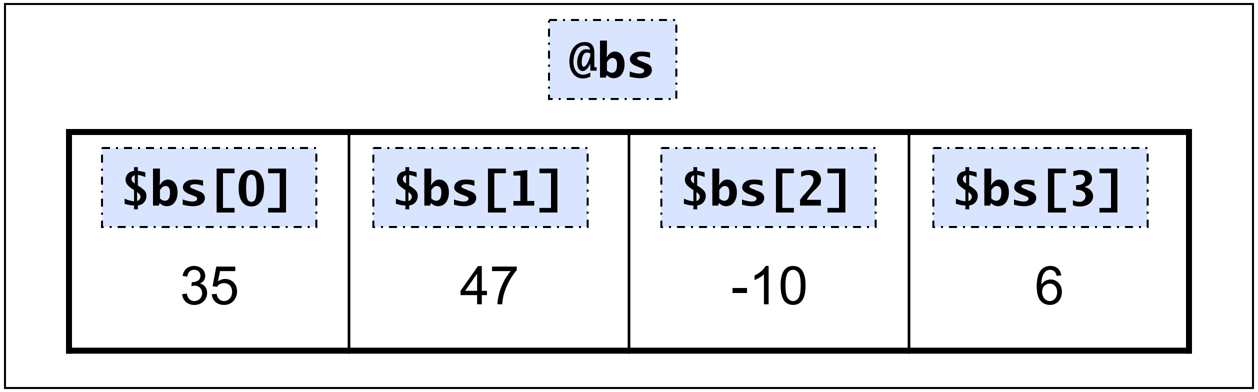
Arrays

- A box containing a **set** of “things”
    - `@bs = ( 35, 47, -10, 6 );`
    - `@strings = ("a", "b", "cde");`
    - `@scalars = ($a, $b, $c);`
- Array names start with @
- Best for many of the same kind of data
    - A set of sequences, a set of fold change values
    - Do the same thing to each array member
    - Filter to find certain useful members

@

Arrays II – Accessing the Insides

- Each thing in an array is like a scalar variable
  - So each scalar has a name that starts with $
  - It also has an index (number) to identify it
  - Indexes start from ZERO
  - `@bs = ( 35, 47, -10, 6 );`
  - `print $bs[2] # -10. Note the $`
  - `print @bs # 3547-106. Note the @`



@bs

`$bs[0]`	`$bs[1]`	`$bs[2]`	`$bs[3]`
35	47	-10	6

Arrays III – Manipulating

A single value in the array can change.

- `@letters = ( "a", "b", "c", "d" );`
- `$letters[2] = "x";`
- `print @letters; # abxd`

An array's size can change (unlike FORTRAN, C)

- `@nums = ( 9,8,7 );`
- `$nums[3] = 6;`
- `print @nums; # 9876`
- `push @nums, 5; # push onto end - 98765`
- `pop @nums; # pop off of the end - 9876`
- `print scalar (@nums); # Array size = 4`

Playing with Arrays

- `split()` splits a string into pieces
- Let's split our BLAST hits into columns
- `my @cols = split /\t/, $line;`
- Now easily access percent identity, target ID, etc.
- lcl|Scer--YAL036C Spar--ORFN:355 92.20 1103 86 0 1 1103 1 1103 0.0 1459
  - `my $percent_identity = $cols[2]; # count from 0!`
  - `print "Score: $cols[-1]\n"; # -1 is last thing in array`

  -
    ```
    # Set multiple scalars from a "slice" of an array
    my ($subj_id, $pct_ident, $align_len) = @cols[1..3];
    ```
- See **`get_hit_cols.pl`**

The Magical Array @ARGV

- `@ARGV` holds any arguments you gave your Perl script
- `perl script.pl 73 abc "Amir Karger" myfile.txt`
- `my $num = $ARGV[0]; # 73`
- `my $str = $ARGV[1]; # "abc"`
- `my $name = $ARGV[2]; # "Amir Karger"`
- `my $file = $ARGV[3]; # "myfile.txt"`
- OR `my ($num, $str, $name, $file) = @ARGV;`
- TMTOWTDI: parse `@ARGV` instead of using Getopt::Long
  - Getopt::Long will only remove –options. Files will still be in `@ARGV`
- `shift(@ARGV)` removes `$ARGV[0]`
  - `shift()` with no argument acts on `@ARGV`
  - BUT in a subroutine, `shift()` acts on `@_`

Why Hashes?

- Searching an array for a given value is slow
- Array indexes must be numbers – IDs are strings
- "A gene" has many associated pieces of data
  - Name
  - Alternate name(s)
  - Disease association(s)
  - English description
  - Coded protein(s)
- Storing diverse types of data in one array is messy
- Why can't we have arrays with string indexes?

Hashes

- A box containing a set of key/value pairs
  - Only one value per key (simple case)
- Give it a key, it returns a value
  - What NCBI ID represents "BRCA1"?
  - What amino acid does "ATG" code for?
  - What is the "DE" part of this Uniprot record? http://us.expasy.org/uniprot/Q92560
- Hash names start with %

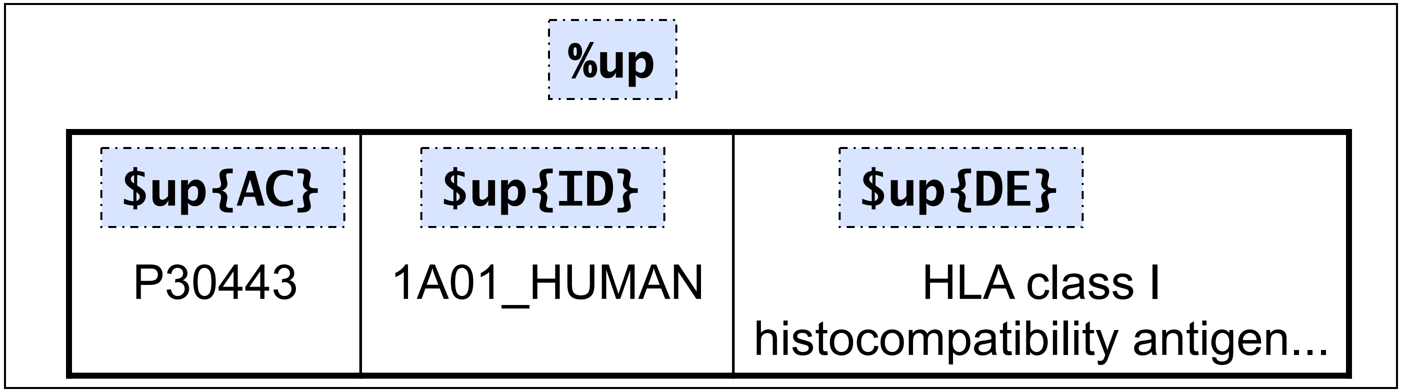
%

Hashes II - Declaration

%hash = (key1=>val1, key2=>val2, ...)

```
%up = (
  "AC" => "P30443",
  "ID" => "1A01_HUMAN",
  "DE" => "HLA class I…",
);
```

```
%translate = (
  "ATG" => "M", "GGT" => "G",
  "CAT" => "H", "TAG" => "*",
); # etc. . .
print "ATG encodes $translate{'ATG'}";
# ATG encodes M
```



Hashes III - Usage

- Accessing hashes
  - When looking at a whole hash, `%hash`
    `keys(%hash)` gets all keys in the hash
  - When accessing one value, `$hash{key}`
  - Setting one value: `$hash{key} = value;`
- Hashes vs. arrays
  - Hashes are NOT in any order
  - BUT you can get to a value instantly instead of searching through an array
  - Keys are usually text strings, not numbers
- See `unique_hits.pl`

Hashes IV – Common Hash Uses

- Translation table (codons, sequence IDs, etc.)
- Storing complicated records
  - Uniprot: store and manipulate ID, AC, DE separately
  - BLAST hits: manipulate ID, % identity, etc. separately
  - `my %hit = ( "ID" => $cols[1], "pct_id" => $cols[2], …);`
- See if we know about a particular thing
  - `if (! exists $known_ID{$ID}}) { do stuff…}`
- Make things unique (only one value per key)
  - Read lines into `%hash`, look at `keys(%hash)`

Exercises – Arrays and Hashes

1. Edit `EX_Array_1.pl` to print hits of any species with percent identity (third column) between 80 and 85
2. `EX_Array_2.pl` puts data from various columns (see "Hashes IV" above) into a `%hit` hash. Change the program to use that hash in the `if` and `print` statements in the `while` loop.

Class Overview

- Introduction – Why learn Perl?
- Scripting – Reproducible Science
- Variables – Making Programs Reusable
- Control Structures – Doing Things Lots of Times (Or Not)
- Data Munging – Perl for Bioinformatics
- Arrays and Hashes – Groups of Things
- The Scriptome –Data Munging for Perl Beginners
- Subroutines & Modules – Making Programs *Really* Reusable
- Objects – Complex Data, Complex Workflow
- BioPerl – Doing (More) Bio With Perl

Subroutines and Modules

Making Programs Really Reusable
by
Creating New Functions

Subroutines – Why?

```
my $dna1 = "CCGGCCGGAGTTCTTAGGCGTAGCCGGCCGG"; # UTR+CDS
# (Shortest possible exon: +? is a "non-greedy" +)
$dna1 =~ /(ATG(...)+?)TAG/; # start codon, 3N bp, stop
my $len = length($1)/3; # length of translated protein

# Later…
my $dna2 = <FASTA>; # Read in DNA from FASTA file

# Do the same thing to the new sequence
$dna2 =~ /(ATG(...)+?)TAG/;
$len = length($1)/3;
```

- Harder to read larger program
- What if there's a bug (TAG only)? Update every copy

Subroutines – Example

```
my $dna1 = "CCGGCCGGAGTTCTTAGGCGTAGCCGGCCGG";
my $len = &get_translated_length($dna1); # call sub
print "DNA with UTR: $dna1. Protein length: $len\n";

my $dna2 = <FASTA>;
# Call the subroutine again, with a different argument
$len = &get_translated_length($dna2);  print $len;

sub get_translated_length {
    my ($dna) = @_; # changing $dna won't change $dna1
    $dna =~ /(AGT(...)+?)TAG/; # Remove stop codon,3' UTR
    my $plen = length($1)/3; # resulting protein length
    return $plen;
}
```

- Only one copy of the code
- Main program becomes shorter and simpler

Subroutines – View from the Outside

- Subroutines: write your own Perl functions
- *main* program *calls* subroutine

  `&get_translated_length`
  - Ampersand is optional
- It *passes* zero or more *arguments* (`$dna1`)
  - Parentheses are (sometimes) optional
- Code in the subroutine gets executed
- Subroutine *returns* results to *caller*
  - Perl subroutines can return multiple values
  - Some subroutines return no values

Subroutines – View from the Inside

```
# Comments describe the subroutine
sub some_name {                            - starts a subroutine
  # Local copies of the arguments
  my ($thing, $other) = @_;                - gets the arguments
  # Put fancy code here…                   - calculates, prints,
  # More code…                               does other stuff
  # More                                     calls other subroutines?
  return ($first, $second);                - returns stuff to caller
}                                          - ends subroutine
```

- Some people use @_ or $_[0]… in subs - careful!
- See **`sub.pl`**

Subroutines – Extra credit/FYI

- Alternate way to get the arguments inside the subroutine
  - `my $thing = shift;`
  - `shift` is like `pop`, but pulls out `$array[0]`
  - Inside a subroutine, `shift()` does `shift(@_)`
  - I.e., put the first argument to the subroutine into `$thing`

- Passing 1 array/ hash to a sub: easy. Make it the *last* arg
  - `call_sub($a, $b, @c);` Pass array to sub
  - `my ($arg_a, $arg_b, @arg_c) = @_;` Get args inside sub
- Passing 2 arrays/hashes: harder. `perldoc perlreftut`
  - `call_sub(\@arr1, \@arr2);` References “pack” arrays into scalars
  - `my ($ref1, $ref2) = @_;` Get (scalar) args inside sub
  - `@in_array1 = @$ref1;` Unpack references - scalar back into array

Subroutines – Organizing Code By Function

- Code reuse
  - Call same subroutine from different parts of your program
  - More general: `$len = &get_protein_length($dna, $remove);`
- Organization
  - E.g., separate messy math from main program flow
  - Each subroutine can only mess up its own variables
- Easier testing
  - Test subroutine code separately
- Increased efficiency
  - Write code just once, optimize just one sub
- Coder's Creed: Never write the same code twice

Modules

- A set of related subroutines
  - Placed in a separate file
  - Included in the original file with the `use` command
- We've been using modules all day
  - `use Getopt::Long;`
  - Reads in the file **`/usr/…/perl5/…/Getopt/Long.pm`**
  - Now `&GetOptions()` acts like a regular Perl function
  - **`perldoc Getopt::Long`** gets module documentation
    - Documentation is stored inside the module
    - POD, a very simple HTML-ish language
  - `strict` is a special module called a "pragma"

Modules II

- Getting new modules
  - **Thousands** of modules available at www.cpan.org
  - search.cpan.org (E.g., search for "transcription factor")
  - Usually simple to install
  - Basically, installation places .pm file(s) in `/usr/…`
  - Or a different directory Perl knows to look in
- Benefits (like subroutine benefits, but more so)
  - Organization: separate a set of functionality
  - Code reuse: don't have to re-write code for every program
    - "Good composers borrow; great composers steal." -Stravinsky?
  - Modules also give you access to new classes…

Exercise – Subroutines

- Move BLAST (and deciding whether to run) to a subroutine
- `&maybe_run_blast($run_blast, $fasta_in, $e_value, $blast_out);`
- Now our main program is *much* easier to read:

```
GetOptions(…);

&maybe_run_blast($run_blast, $fasta_in, $e_value, $blast_out);

foreach $species ("Cgla", "Sklu") {
    &analyze_blast($species, $blast_out, $unique_hits);
}
exit;
```

Objects and Classes

Complex Data, Complex Workflow
or
How to Write Big Perl Programs Without Going Crazy

Objects

- Scalar variables storing multiple pieces of data
  - `$uniprot_seq` stores a whole Uniprot record
  - Easier than keeping track of complicated hashes
  - Store many Uniprot records in a hash/array
- Variables that can do things (by calling *methods*)
  - `$uniprot_seq->id` gets the ID
  - Like `&id($uniprot_seq)`, but better (see below)
  - `$rev = $uniprot_seq->revcom` reverse complements

Objects II – Bio objects

- Bioperl objects store biological information
- Bioperl objects do biological things

```
use Bio::Seq;

# $seq is a Bio::Seq object, which represents a sequence
# along with associated data...
print "Raw sequence: ", $seq->seq(); # Just a regular string
print "Species is ", $seq->species();

# Object's sub-pieces can be objects too!
@features = $seq->get_SeqFeatures(); # Coding sequences, SNPs, …
foreach $feat ( @features ) {
  print $feat->primary_tag, " starts at ",$feat->start\n";
}
```

Classes

- Really just a fancy module
- Every object belongs to one or more classes
- What kind of object is it?
  - Sequence, Feature, Annotation, Tree...
- What fields will this object have?
  - species, start/end, text, subtrees
- What can I DO with this object?
  - I.e., what methods can I call?
  - id, get_SeqFeatures, set_root_node

Classes II – Bio Classes

- Bioperl classes have Bioperl objects in them, which
  - Store biological information
  - Do biological things

```
# Bio::Seq object $seq can DO things, not just hold information
use Bio::Seq;
print "Sequence from 1 to 100: ", $seq->subseq(1,100);

# You can chain -> method calls.
# revcom returns Bio::Seq object. revcom->seq returns raw sequence
$rev_comp = $seq->revcom->seq();
print "Reverse comp. from 1 to 100:", $seq->revcom->subseq(1, 100);
```

Object Oriented Programming – Who Cares?

```
# User has pulled in sequences from different
 databases
my @seqs = ($uniprot_seq, $EMBL_seq, $GenBank_seq);

foreach my $seq (@seqs) {
    print $seq->id;
    print $seq->description;
}
```

- Different classes can have totally different ways to implement the `id` method
- User doesn't have to care!
  - Crucial for large programs
- Each object "automagically" does the right thing
  - Because each object knows which class it belongs to
- Congratulations: you're now an OOP expert!

Bioperl

Doing (More) Bio with Perl
by
~~Stealing~~ Using Collected Wisdom

BioPerl Overview

- Modules useful for doing bioinformatics in Perl
- Many specialized modules (Annotation, Parsing, Running BLAST, Phylogenetic Trees, …)
- Many scripts
  - `ls /odyssey/apps/perl5mods/bin/bp*.pl` on Odyssey
  - `` perldoc -F `which bp_seq_length.pl` ``
- Can be a bit overwhelming
  - Huge (> 800,000 lines of code, 2010)
  - Mostly uses objects
  - Documentation not always easy

BioPerl Tutorial TOC

- . Using bioperl
  - .1 Accessing sequence data from local and remote databases
    - .1.1 Accessing remote databases (Bio::DB::GenBank, etc)
    - .1.2 Indexing and accessing local databases (Bio::Index::*, bp_index.pl, bp_fetch.pl)
  - .2 Transforming formats of database/ file records
    - .2.1 Transforming sequence files (SeqIO)
    - .2.2 Transforming alignment files (AlignIO)
  - .3 Manipulating sequences
    - .3.1 Manipulating sequence data with Seq methods (Seq)
    - .3.2 Obtaining basic sequence statistics (SeqStats,SeqWord)
    - .3.3 Identifying restriction enzyme sites (Bio::Restriction)
    - .3.4 Identifying amino acid cleavage sites (Sigcleave)
    - .3.5 Miscellaneous sequence utilities: OddCodes, SeqPattern
    - .3.6 Converting coordinate systems (Coordinate::Pair, RelSegment)
  - .4 Searching for similar sequences
    - .4.1 Running BLAST remotely (using RemoteBlast.pm)
    - .4.2 Parsing BLAST and FASTA reports with Search and SearchIO
    - .4.3 Parsing BLAST reports with BPlite, BPpsilite, and BPbl2seq
    - .4.4 Parsing HMM reports (HMMER::Results, SearchIO)
    - .4.5 Running BLAST locally (StandAloneBlast)
  - .5 Manipulating sequence alignments (SimpleAlign)

BioPerl Tutorial TOC II

.6 Searching for genes and other structures on genomic DNA (Genscan, Sim4, ESTScan, MZEF, Grail, Genemark, EPCR)
  .7 Developing machine readable sequence annotations
    .7.1 Representing sequence annotations (SeqFeature,RichSeq,Location)
    .7.2 Representing sequence annotations (Annotation::Collection)
    .7.3 Representing large sequences (LargeSeq)
    .7.4 Representing changing sequences (LiveSeq)
    .7.5 Representing related sequences - mutations, polymorphisms (Allele, SeqDiff)
    .7.6 Incorporating quality data in sequence annotation (SeqWithQuality)
    .7.7 Sequence XML representations - generation and parsing (SeqIO::game)
    .7.8 Representing Sequence Features using GFF (Bio:Tools:GFF)
  .8 Manipulating clusters of sequences (Cluster, ClusterIO)
  .9 Representing non-sequence data in Bioperl: structures, trees, maps, graphics and bibliographic text
    .9.1 Using 3D structure objects and reading PDB files (StructureI, Structure::IO)
    .9.2 Tree objects and phylogenetic trees (Tree::Tree, TreeIO, PAML.pm)
    .9.3 Map objects for manipulating genetic maps (Map::MapI, MapIO)
    .9.4 Bibliographic objects for querying bibliographic databases (Biblio)
    .9.5 Graphics objects for representing sequence objects as images (Graphics)
  .10 Bioperl alphabets
    .10.1 Extended DNA / RNA alphabet
    .10.2 Amino Acid alphabet

Bio::Perl - Easy Bioperl

- Bio::Perl provides simple access functions.
  - Much easier than the rest of Bioperl
  - Much less functionality

• get_sequence	get a sequence from Internet databases
• read_sequence	read a sequence from a file
• read_all_sequences	read all sequences from a file
• new_sequence	make a Bio::Seq object from a string
• write_sequence	write one or more sequences to a file
• translate	translate a sequence. Return an <u>object</u>
• translate_as_string	translate a sequence. Return a <u>string</u>
• blast_sequence	BLAST a sequence *using NCBI computers*
• write_blast	write a BLAST report out to a file

Bio::Perl II - Getting Sequences

Retrieve EMBL sequence, write it out in FASTA format

```
use Bio::Perl;

# only works if you have an internet connection
$seq_object = get_sequence("embl","AI129902");

write_sequence(">cdna.fasta","fasta",$seq_object);
```

What could you do with `while()`? (Careful!)

Bio::Perl III - Automated BLAST

BLAST sequence at NCBI using default “nr” database

```
use Bio::Perl;

$seq_object = get_sequence("embl","AI129902");

# uses the default database - nr in this case
$blast_result = blast_sequence($seq);

# write results to a file
write_blast(">cdna.blast",$blast_result);
```

BioPerl - Objects

- Bio::Seq: main sequence object
  - Available when sequence file is read by Bio::SeqIO
  - It has many methods - `perldoc Bio::Seq`

```
# Make a new Bio::SeqIO object $myseqs
# by opening a file for reading
#(This command doesn't actually read any sequences)
$myseqs = Bio::SeqIO->new(
  '-file' => "<inputFileName", '-format' => 'Fasta'
);

# Get next (i.e., first) seq in Bio::SeqIO object
# $seqobj is a Bio::Seq object
$seqobj = $myseqs->next_seq();
```

BioPerl - SeqIO and Seq

- Bio::SeqIO: Sequence input/output
  - Formats: Fasta, EMBL, GenBank, uniprot, PIR, GCG, …
  - Parse GenBank sequence features: CDS, SNPs, Region
  - Uses Bio::Seq objects instead of storing only sequence bp in scalar text strings
- Bio::Seq: sequence manipulation
  - subsequence
  - translation
  - reverse complement, and much more
- **`See gb2fastas.pl`**

BioPerl - SeqIO and Seq II

```
#Using SeqIO and Seq
use Bio::SeqIO;
use Bio::Seq;
$in = Bio::SeqIO->new(-file=>"<$fin", "-format"=>"Fasta");
$out =
  Bio::SeqIO->new(-file => ">$fout", "-format" => "EMBL");
while ($seq = $in->next_seq()) {
  $out->write_seq($seq); # print sequence to $out
  print "Raw sequence:", $seq->seq();
  print "Sequence from 1 to 100: ", $seq->subseq(1,100);
  print "Type of sequence: ", $seq->moltype, "\n";
  if ($type eq "dna") {
    print "Reverse comp: ", $seq->revcom->seq(), "\n";
    print "Revcom 1-100:",$seq->revcom->subseq(1, 100);
  }
}
```

BioPerl - BPlite

- BPlite: Blast Parser "lite“
  - BLAST -outfmt 6 doesn't actually give us alignments
  - But BLAST output is Hard! (see `one_seq.long_blast`)
  - One of several BLAST parsers available
  - Each matching sequence can have multiple matching regions ("hsp", high scoring pair)

```
use Bio::Tools::BPlite;
$report = new Bio::Tools::BPlite(-file=>"$inFile");
while(my $sbjct = $report->nextSbjct) {
  while (my $hsp = $sbjct->nextHSP) {
    print $hsp->subject->seqname;
  }
}
```

Bioperl - Codon Tables

- Bioperl::Tools::CodonTable
  - Translate/reverse translate codons & amino acids
  - Handles alternate codon tables
  - See `codon_table.pl`
  - Also includes `is_start_codon`, `is_ter_codon`
  - Use these codon tables to translate Bio::Seqs

What’s missing

- More Bioperl, regexps, functions, OOP, ...
- Testing, debugging and proactive error checking
- Context and other shortcuts
  - $line = <FILE> reads just one line
  - @foo = <FILE> reads an entire file into an array
- Databases and web programming
- Graphics
- Perl Golf and Obfuscated Perl
  - `perl -le '$_*=$`%9e9,//for+1=~/0*$/..pop;print$`%10' 10`
- Etc.

Resources for After the Class

- **akarger@cgr.harvard.edu**
- perldoc perl (see "Tutorials" section)
  - perlintro, perltut, perlfunc, perlretut, perlboot
- http://bip.weizmann.ac.il/course/prog/
  - HUNDREDS of slides - many bio-related examples
  - Also look at "assignments" for practice
- http://www.oreilly.com/catalog/begperlbio/ and .../learnperl4
  - Beginning Perl for Bioinformatics is designed for biologists. (It has a sequel, too.)
  - *Learning Perl* is more general, but gets rave reviews

Resources for After the Class II

- search.cpan.org
  - 9000 modules and counting
- http://www.bioperl.org
  - Especially look at (and do) bptutorial
  - "Howtos" describe Sequence Analysis, Phylogenetics, etc. w/ Bioperl with lots of stealable sample code
  - bioperl-l@bioperl.org - ask questions to experts.
- http://www.pasteur.fr/recherche/unites/sis/formation/bioperl/
  - Big Bioperl course, with lots of examples and exercises
- The Scriptome
  - http://sysbio.harvard.edu/csb/resources/computational/scriptome