Introduction to Perl Programming for Bioinformatics

```
use Bio::Seq;
my $io = Bio::SeqIO->new( -file => "input.fasta",
                          -format => "fasta" );

my $firstSeq = $io->next_seq();

print "The sequence id is: " . $firstSeq->display_id . "\n";
print "The sequence is: " . $firstSeq->seq . "\n";
print "The sequence length is: " . $firstSeq->length . "\n";
print "The first 10 bases of the sequence is: " .
      $firstSeq->subseq(1,10) . "\n";
my $revComplement = $firstSeq->revcom();
print "The reverse complement of the sequence is: " .
      $revComplement->seq . "\n";
```

IDeA Network of Biomedical Research Excellence
INBRE II
Hawaii State Research and Education Partnership

Pacific Center for Emerging Infectious Diseases Research

RMATRIX
RCMI Multidisciplinary And Translational Research Infrastructure eXpansion

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Introduction to Perl Programming for Bioinformatics

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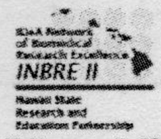
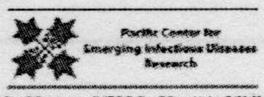




Day 1: Outline

- Introduction to Perl
- Working with scalars
- Conditional blocks and loops
- Lists and arrays
- Input, output and hashes

Day 2: Outline

- Subroutines
- Regular expressions
- Perl references
- Introduction to Bioperl
- Practical exercises and Open Questions

Quick Introduction to Perl

Meet Perl

- Perl stands for **P**ractical **E**xtraction and **R**eport **L**anguage
- Designed by Larry Wall and first released in 1987
- Borrows from many languages, such as C, awk, Lisp and many others
- Originally developed for text manipulation (System admin tasks)
- Now used for a wide range of tasks including web and network programming, astronomy, bioinformatics, etc.

What is Perl Good For

- Text processing
- Rapid prototyping
- Task automation
- Make portable code: write once, run anywhere
- Making large libraries of useful modules, such as in bioinformatics
- "Making easy things easy and complicated things possible"
  - There is more than one way to do it
  - From baby Perl to advanced perlish constructs

CPAN

What is Perl Bad For?

- Tasks where complex data-structures are needed
  - Very complex relationship between the data elements.
- Performance-critical applications
  - Complex, long-lived software projects with more than one author
- Doing important things in more than one way
  - Should there be one – and preferably only one – obvious way to do it?

Remember

- "Perl is an empirical Science. You learn about it but trying things out." Larry Wall
- You have to be diligent in testing what you are unsure of.
  - Or else you might introduce some nasty bugs

Hello World in Perl

```
#!/usr/bin/perl
# This is a sample program

use strict;
use warnings;

Print "Hello world!\n";
```

- Let's execute the program

```
mandi@mandi-laptop:~/Desktop$ perl helloWorld.pl
Hello World!
```

- A Perl "interpreter" is used to interpret the script.
- Scripts are interpreted from top to bottom
  - You can also run the script by executing it.
- Perl is **case sensitive.**
  - Use Warning is different from use warnings

Anatomy of a Perl Script

```
1: #!/usr/bin/perl
2: # This is a sample program

3: use strict;
4: use warnings;

5: Print "Hello world!\n";
```

```
Hello world
```

- **Line 1** is a directive to the shell.
  - Tells the shell which interpreter to use.
- **Line 2** is a comment.
  - Except for ***#!*** (hashbang), anything that starts with # is a comment.
- **Lines 3** and **4** are **pragmas**.
  - Pragmas are instructions to the interpreter
- **Line 5** Is a statement.
  - Calls the `print` function on a *string literal* and ends with ';' character. "\n" instructs Perl to print a new line.

Running Perl: Demonstration

- Download the workshop archive from:
  - http://dl.dropbox.com/u/28270/perl_workshop.zip
- Running a program from the command line.
- Using the IDE
  - Open the Komodo and create a new project
  - Add the helloWorld.pl file to the project
  - Run the file
  - Run with error "Missing end string delimiter"

Scalars

The Elementary Variable Type in Perl

How Is Information Handled?

- In Perl information is classified into three categories:
  - One dimensional
    - Ex. The string *"ATGTGA"*
  - Multi-dimensional
    - Ex. The x,y coordinates *(10, 12)*
  - Dictionary like
    - Ex. The side chain charge of different a.a
    - Alanine: Neutral, Arginine: positive, Asparagine: neutral, etc.
- In programming, variables are used to store information
  - Able to refer to information using meaningful names
  - Allows the modification of stored information
  - Ex. Having a variable called MolType, with a value of DNA or RNA, allows you to use it anywhere in the program.

Variables in Perl

- Variable names have a specific requirements
  - Start with alphabetical character
  - Contain any character [Aa-Zz] or [1-9] or _
  - Variable names are case sensitive
  - Should not contain any spaces
- Perl has three major variable types: **scalars**, **arrays** and **hashes**.
  - They can be distinguished with the "funny characters" prefixing the variable name.
  - Scalars have **$**, array have **@** and hashes have **%**
- A variable in Perl needs to be declared using the keyword "**my**" when it is first introduced.

one value

> one value

dictionary

Scalars

- Scalars are the simplest data-types that Perl manipulates
  - Store one dimensional information
  - Start with the "$" funny character
- To assign a value to a scalar, the "=" sign is used
- Scalar can hold a number or a string literal

```
my $length = 12;
my $evalue_threshold = -12e-16;
my $randomString = "ACGAGTGA";
my $stop_RNA = 'UAG';
```

- You can use single or double quotes to define a string.

Basic Scalar Operations

- Many operations available out of the box in Perl
  - They go in the middle of two operands
  - Can be applied to literals or variables
- For example, Perl provides basic arithmetic and string operations

```
2 + 3                    # addition
$lengths - $buffer       # subtraction
$length * 2              # multiplication
10 / 3                   # division
"2". "3"                 # concatenation of 2 and 3 -> "23"
$value++                 # auto increment. Eq. to $value = $value + 1
$value--                 # auto decrement. Eq. to $value = $value - 1
```

- If you do not assign a value to a variable, the variable is said to be undefined or **undef**
  - When using strict, Perl will complain if you try to do anything with an undef variable

Interpolation

important for later debugging

- In certain contexts, Perl replaces a variable with its content. It is called **interpolation**
- Interpolation takes place inside double quotes but not inside single quotes

```
my $x = "ACTG";
Print "1- The four letters coding for DNA are $x";
Print '2- The four letters coding for DNA are $x';
```

```
1- The four letters coding for DNA are ACGT
2- The four letters coding for DNA are $x
```

- We can use a "\" for **escaping**
  - literally printing a special character
- This works with all the special characters, including "\"

```
 my $myDNA = "ACTG";
 my $statement = "The value of \$myDNA is $myDNA";
print $statement;
```

```
The value of $myDNA is ACGT
```

Functions

- Functions in Perl are similar to Mathematical functions
  - Ex. f(x) = 1/(2x+10)
  - f(x) takes a value of x and computes (returns) 1/(2x+10)
- Functions in Perl do "almost" the same thing
  - The input, called argument, is placed between parentheses
    - Ex. f(3)
  - They execute some logic and may or may not return a value
- `print` is an example of a Perl function
  - Takes as input one or more arguments and prints it/them to the screen
  - Returns 1 (True) if the print went successfully
  - The parentheses are not always mandatory

Perl Functions to Quote Strings

- We use double quotes to delimit strings
- The **qq()** acts as a double quotes substitute (**q**uote **q**uote)

```
print "This is a polyA "AAAAAA" "          # Wrong bare-word AAAAAA
print qq(This is a polyA "AAAAAA")          # correct
```

- Similarly the q( ) function is a single quote function
  - Works the same way as qq( )

```
$x = 2;
print q($x)."\n";
Print qq($x)."\n";
---
$x
2
```

Mahdi doesn't use this

word not Known by Perl language

can also do this by escaping double quotes

Exercise

- Open the file **correctMe.pl**
- This program contains 3 errors. Find and correct them.
- Without running the program, what does it print?
- Run the program in Komodo and compare the results to your answer

```
#!/usr/bin/perl
use strict;
use warnings;

my $string = "ACG"

print q(my string is $string \n);

print "ACGT" + "$string\n";
```

Working with Strings

This is what perl is Known for

String Manipulation Overview

- Perl is ideal for working with strings
- A plethora of functions and perlish constructs to execute complicated operations
- We will examine the following operations
  - String length
  - Deleting the end of line character
  - Getting and replacing substrings
  - Changing the case of strings
- We will see other operations in later sections

String Length - 1

- The function `length()` returns the length of a string passed in as an argument
  - Counts all the characters (chars), including the end of line delimiter
  - Could be a source of bugs, be careful!
- Escaped chars, such as "`\$`" count as one char.
- To grab user input, we use the standard input handle `<STDIN>`

```
#!/usr/bin/perl
use strict;
use warnings;

print "Please enter your sequence:\n";

#We grab user input using <STDIN>
my $sequence = <STDIN>;
print "Sequence length before chomps is: " . Length($sequence) . "\n";
```

```
Please enter your sequence:
ACGTACGT
Sequence length before chomps is: 9
```

String Length - 2

- `chomp(STRING)` is another function from the Perl standard library
- Takes a string as input
- Deletes the end of line delimiter "\n"
  - Chomp won't do anything if there is not end of line char
  - Be careful not to use `chop(STRING)`
    - Removes the last char of the `STRING`

```
print "Please enter your sequence: ";
my $sequence = <STDIN>;
chomp($sequence);
print "\$sequence is $sequence and its length is: " .
        length($sequence) . "\n";
my $char = chop($sequence);
print "print \$sequence is $sequence and \$char is $char \n";
```

```
Please enter your sequence: ACGTACGT
$sequence is ACGTACGT and its length is: 8
print $sequence is ACGTACG and $char is T
```

chomp only removes \n

↓

use this whenever a user inputs its sequence

variables within qq get interpolated

String Concatenation

- We have seen concatenation with "."
- We can also use qq(STRING) to concatenate strings

```
my $x = "UAG";
my $y = "means";
my $z = "STOP";
my $s = " ";
print $x . $s . $y . $s . $z."\n";

$phrase = qq($x$s$y$s$z);
print $phrase . "\n";

$phrase = qq($x $y $z);
print $phrase . "\n";
```

```
UAG means STOP
UAG means STOP
UAG means STOP
```

- Can we use q(STRING)?

String Lists

- A list is an ordered group of scalars enclosed in parenthesis and separated by commas.
  - Ex (1,2,3)
- Instead of declaring each variable alone, we can use a list to assign values to more that one at a time.

```
my ($x, $y, $z, $s) = ("UAG", "means" "STOP", " ")
```

join concatenation function

Concatenation Function

- Given a list of strings, we can use join(EXP,LIST) to glue them together
  - EXP: Expression used to join the strings
  - LIST: List of strings to be joined

```
# We are assigning a list of literals into a list of variables
my ($x, $y, $z, $s) = ("UAG", "mean", "STOP", " ");
my $phrase = join($s, $x, $y, $z);
print $phrase . "\n";

my ($x, $y, $z, $s) = ("Perl", "is", "cool", ":");
my $phrase = join($s, $x, $y, $z);
print $phrase . "\n";
```

```
Perl is cool
Perl:is:cool
```

useful for creating a (eg. comma separated file)

Split, The Dual of Join

- Split is used to create a list by splitting a string according to a specific delimiter
- `split(/DELIMITER/, STRING)`
- `DELIMITER`: Can be a char, a string or a regular expression
- Return a list of literals

```
# Splits the string along colon
my ($x, $y, $z) = split(/:/, "AUG:means:STOP")

# Splits the string along colon
my ($x, $y, $z) = split(/ /, "AUG means STOP")
```

- Be careful when using / /
  - Splits across one single space. Use " " or regex when in doubt of the number of spaces used.

```
split(/ /, "Perl is   cool");
#returns ("Perl", "is", "", "", "", "cool")

split(" ", "Perl is   cool");
#returns ("Perl", "is", "cool")
```

- "\t" splits across tabs (key for splitting tsv files)

tab based delimiter function

Working With Substrings - 1

- In Perl, each character in a string has a position
  - The first char is at position (pos) 0, second at pos 1, etc.
  - Indices can also be negative if we count from right to left

```
         AUG is a start codon
Index +  01234567891111111111
                   0123456789
         AUG is a start codon
         21111111111987654321 - Index
         09876543210
```

- The `substr()` function allows us to extract/modify a substring
  - Flexible function that can take 2, 3, 4 arguments
- Basic usage is `substr(STRING,START)`

useful for parsing the end of a large string

Working with Substrings - 2

- `substr(STRING,START)`
  - Returns the substring starting at position `START`

```
my $phrase = "ATG is a start codon";
substr($phrase, 1);          # TG is a start codon
substr($phrase, 10);         # tart codon
substr($phrase, -8);         # rt codon
```

- `substr(STRING,START,LEN)`
  - Returns the substring of length `LEN` starting at position `START`

```
my $phrase = "ATG is a start codon";
substr($phrase, 1, 4);       # TG i
substr($phrase, 10, 10);     # tart codon
substr($phrase, -8, 1);      # t
# different than when a positive length is specified
substr($phrase, 2, -2);      # G is a start cod
```

- Useful to test one position

Substitution of a Substring

- `substr()` can also be used for replacing a substring
  - Used as an l-value in an expression

```
my $phrase = "Perl is cool";
substr($phrase, 8, 4) = "confusing sometimes";
print $phrase;
```

```
Perl is confusing sometimes
```

  - The right value does not have to be the same length as the substring

- The right side of the expression can be replaced by a fourth argument in the `substr()` expression

```
my $phrase = "Perl is cool";
substr($phrase, 8, 4, "confusing sometimes");
print $phrase;
```

```
Perl is confusing sometimes
```

will be used on a regular basis

Other Useful Methods

- `reverse(STRING)` – Returns a reversed a string
- `lc(STRING)` – Returns a string converted to lower case
- `uc(STRING)` – Returns a string converted to upper case
- `ucfirst(STRING)` – Returns string with the first char converted to upper case
- `lcfirst(STRING)` – Returns string with the first char converted to lower case
- `index(STRING,SUBSTR)` – Returns the index of the first occurrence of SUBSTR in STRING or -1 if SUBSTR is not present in STRING

```
my $dnaSeq = "ACGTacgt";
print reverse($dnaSeq);                    # tgcaTGCA
print lc($dnaSeq);                         # acgtacgt
print uc($dnaSeq);                         # ACGTACGT
print lcfirst($dnaSeq);                    # aCGTacgt

my $myStatement = "COG0004 is Metabolism";
print index($myStatement, "is");           # 8
print index($myStatement, "Signaling");    # -1
```

lower & upper case nucleotides can be weighed differently in BLAST e-value calculation

Exercise 1

- Open the program substrings.pl in your editor
- Complete the program by writing the code that
  - Gets the sequence name
  - Gets the sequence length
  - Chops off the polyA tail (last 6 bases)

Exercise 2

- The program "Blast" has an option to filter any region that is in lower case.
  - Mask Lower Case –U
- You want to take advantage of that option to filter the adapter in your sequences.
- Your adapter has the sequence: "**ATTGATTG**".
- You need to find it in the sequence and lower case it as well as anything before it.
- For example:
  - ATTGATTGTTATTAAAT becomes attgattgTTATTAAAT
  - TATGATTGATTGAATGTA becomes tatgattgattgAATGTA
  - Etc.
- A program structure is provided in maskAdaptor.pl

Conditional Blocks and Loops

Making Decisions - 1

- There are a number of useful ways to make decisions in Perl
- `if(CONDITION)` do something
  - Is a statement that can chose (or not) to do something.
- If `CONDITION` evaluates to `TRUE`, then the block is executed.
- You can group a set of statements between { }
  - Similar to a paragraph
- An `else{}` statement can be added to execute an alternative block if the condition is `FALSE`.

To test spacing, place -B-$seq-E-
want print

Example

```
if ($sequence eq "ATG") {
        print "This is a DNA codon";
        print "This is a methionine";
} else {
        print "This is not a methioine";
}
```

- The `if(CONDITION)` evaluates to TRUE whenever $sequence is equal to "**ATG**"
- When the CONDITION is FALSE, the `else` block is executed.
- If the outcome of `if` is one line, you can use the shortened construct (Perlish construct)

```
print "Codon is a methionine" if ($sequence  eq "ATG");
```

Booleans

- Conditional expressions can be any expressions that produce a **boolean** value.
  - They evaluate to True or False (1 or 0).
  - Ex. 'eq' and '==' are boolean expressions

all ← numbers only

- These expression can be combined using boolean operators
  - The "&&" operator refers to AND
  - The "||" operator refers to OR
  - The "!" operator refers to NOT

==

eq <=

ne >=

!=

Making Decisions - 2

- `unless(CONDITION)` statement is used to negate a condition
- `unless(CONDITION)` is equivalent to `if(NOT CONDITION)`

```
print "Codon is a methionine" if ($sequence  eq "ATG");
print "Codon is not methionine" unless ($sequence  eq "ATG");
```

- Ex.

```
if ( ($codon eq "ATG") ||
     ($codon eq "TAG") ||
     ($codon eq "TAA") ||
     ($codon eq "TGA"))
{
   print "This is a stop codon";
}
if ( (len($dnaSeq) > 8) && (index($sequence, "ATTGATTG") != -1 ) )
{
   print "This sequence does not contain the adapter and";
   print "the sequence is at least 10 bases long \n";
}
```

← return value if sequence found

if/not can be used instead of unless

Truth in Perl

- What makes a CONDITION TRUE or FALSE in Perl
  - undef, "" (empty string), 0, "0" are all False
  - All else is True

```
my $seq = "";
if($seq){
   print "sequence contains something";
}
if(len($seq)){
   print "sequence has length > 0";
}
# To avoid confusion, explicitly state the test your are trying to accomplish
if("" != $seq)
# and
if(len($seq) > 0  )
```

While Loops

- `while(CONDITION){}` allows you to keep on executing a block of code, for as long as a CONDITION is TRUE
- `while(CONDITION)` is a "sort of" iterated `if`

```
while( my $input = <STDIN>){
   print "Enter your text: ";
   print "You have entered: $input";
}
```

- The previous program creates an infinite loop

While Loop

- The following program creates an infinite loop
  - An **infinite loop** runs indefinitely (Unless some condition terminates it)

```
while(1){
   print "I am stuck here\n";
}
```

- You can break out of any loop using `last`
- You can also skip an iteration using `next`

```
my $i = 0;
while(1){
   $nbIterations = $nbIterations + 1;
   next if (3 == $nbIterations);
   print " I am in iteration $nbIterations\n"
   last if (5 == $nbIterations);
}
```

For Loop

- A Perlish way of implementing loops.
- Uses a **range**
  - (1..5) generates a list of all the integers between 1 and 5

```
For my $i (1..5){
   print "I've counted to: $i\n";
}
```

```
> perl for_loopv2.pl
I've counted to: 1
I've counted to: 2
I've counted to: 3
I've counted to: 4
I've counted to: 5
```

- `last` and `next` can be used with the for loop as well

Nested blocks

- **Loops, if statements** and **blocks** can be nested
- You can put a loop within a loop
  - A loop is not repeated until all the code within it has completed running

my $i

```
for (1..3){
   for my $j (4..6){
        print "i is: $i \t j is: $j \n";
   }
}
```

- For every value of $i, the internal loop is executed 3 times (for the value 4, 5 and 6)
- In total, the print statement will be executed 9 times.

Interactive Example

- We will write a program that reverse complements a sequence
- The complete program is in reverseComplement.pl
- We read a line from the user (standard input)
- We reverse the sequence
- We loop along the sequence
- For every base, we print its reverse complement.
- Using if, **elsif**, else (elsif used to groups if statement having the same outcome)
- Using die()

example of removing 3 different primers from tons of sequences

Using random()

- `rand(MAX)` returns a random fractional number greater than or equal to 0 and less than the value of MAX
- Perl has a function called `int()`
  - This function takes a fractional number and returns an integer
  - The function `int` is used for converting between fractional (`floats`) and integers (`ints`).
- We can use `int` and `rand` conjointly to generate random integers

```
my $fractionalRand = rand(10);
my $integerRandom = int($fractionalRand);
# or just
my $integerRandom = int(rand(10));
```

Exercise

- Print a list of random DNA fasta sequences
- Each DNA sequence is of size 10.
- Ex.
  >1
  GCTGATCGTA
  >2
  GCTGATCGTA
  >3
  GCTGATCGTA
  ...
- A program structure is given in randomFasta.pl

Lists and Arrays

Lists

- Remember that a list is a group of ordered elements
- Lists are elements enclosed in parenthesis
  - Ex.: (A, B, C), (1, 2, 3), (John, 23, Honolulu), etc.
- A list is a fixed collection literal
  - They are not tied to a variable
  - We can access each element separately, but we cannot modify it.
- Lists can be generated by other functions. Ex.
  - `qw(ATT ATG) → ("ATT", "ATG")`
  - `split(//, "ACGT") → ('A', 'T', 'G', 'T')`
  - `range (1..5) → (1, 2, 3, 4, 5)`
- We use an index to access a specific position **[index]**
  - (1,2,3,4)**[0]** → returns 1
  - (1,2,3,4)**[1]** → returns 2
  - etc.

Arrays

- Arrays are similar to a lists
- The array is a variable collection of ordered elements. The variable name starts with the "@" funny character
- Practically, you can think of an array as a list but with a name that can be used to access it.
- An array is initialized with a list

```
my @cellularProcesses = ("Translation", "Transcription", "Replication");
```

- Or using any function that returns a list

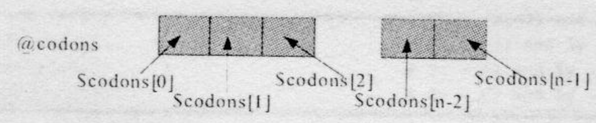
```
my @alphabet = (a..z);
my @tokens = split(//, "fructokinase    128 a.a.    Metabolism")
```

← Single or double-quoted (error)

↑ splitting on lack of space

Array Indexing

- Every element in the array is indexed by a position
- First element of the array is located at position 0
- If the array contains n elements, the last element is then located at position n-1
  - Or position -1 if using the negative index
- **Important**: Every element of the array is a scalar.



Accessing Array Elements

- Array elements can be accessed for display or modification, using their index

```
my @digits = (A..E);

print $digits[1];              # prints B
print $digits[4];              # prints E
print $digits[15];             # generates an error

$digits[20] = 'T';             # does not generate an error
print $digits[20];             # does not generate an error
print $digits[19];             # does generate an error
```

- The example `$digits[20]` does not generate an error.
  - Arrays grow and shrink dynamically
  - Arrays automatically add undef to compensate for gaps
  - Trying to access and undef element will generate an error
- Always, use the scalar funny character to access a single element of the array.

Array Operations - 1

- The `for()` allows us to iterate through the elements of an array, using user defined steps
- The perlish way to loop through an array is through a `foreach`.

```
my @digits = (1..10);
for my $i (0..9){
   print $digits[$i] . "\n";
}
```

```
my @digits = (1..10);
foreach my $element (@digits) {
   print $element . "\n";
}
```

- The first approach generates indexes
  - Requires to specify the size of the array
- The perlish approach iterates through the elements

Index of Last Element

- To find the position of the last element, prefix the array name with `@#`.
- We can use the index of the last element to generate a range and use it to loop through the array.

```
my @digits = (1..10);

# @#digits returns the index of the last element of the array
foreach my $i (0 .. @#digits){
   print $digits[$i];
}
```

Array Operations - 2

- We can use the size of the array to loop through all the elements

```
my @digits = (1..10);
foreach my $i (0 .. @#digits){
   print $digits[$i];
}
```

```
1
2
3
...
```

Adding to an Array

- push() is the most used way to add to an array
  - It can add a single element, a list or even another array

```
my @x = ();
push(@x, 1);              # @x now contains 1
push(@x, 2);              # @x now contains 1, 2
push(@x, 3, 4, 5);        # @x now contains 1, 2, 3, 4, 5
push(@x, qq(6 7) );       # @x now contains 1, 2, 3, 4, 5, 6, 7
my @y = (8, 9, 10);
push(@x, @y);             # @x now contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
```

Deleting From an Array

- To delete the last element, use pop()
- To delete the first element, use shift()
  - Both pop() and shift() automatically resize the array and return the element deleted

```
my @x = (1, 2, 3);
my $y = pop(@x);                    # @x = (1,2) and $y = 3
$y = pop(@x);                       # @x = (1) and $y = 2
$y = pop(@x);                       # @x = () and $y = 1
$y = pop(@x);                       # @x = () and $y = undef

@x = (1, 2, 3);
$y = shift(@x);                     # @x = (2, 3) and $y = 1
$y = shift(@x);                     # @x = (3) and $y = 2
$y = shift(@x);                     # @x = () and $y = 3
$y = shift(@x);                     # @x = () and $y = undef
```

Arrays and Context

- Array operation have different meaning in different contexts

```
my @x = ("A", "B", "C", "D");
print "@x\n";                    # prints A B C D
print @x;                         # prints ABCD
print @x . "\n";                  # prints 4
```

```
my @y = @x;
print "@y\n";                     # prints 1 2 3 4
my $value = @x;
print "$value \n";                # prints 4
($value) = @x;
print "$value \n";                # prints 1
print @x - 1;                     # prints 3
```

- Assignments can also be non-intuitive.
- In case 1, an array is being assigned to another array
  - @y will be an exact and independent copy of @x
- In case 2, an array is being assigned to a scalar
  - `$myVar` contains the number of elements in the array

Know arrays well, easy to introduce bugs here

Array Length

- If we put an array in a scalar context, we get the length of the array

```
@x - 1;                           #returns length - 1
```

- Array length can be used as a for loop argument
  - It is not equal to `$#ARRAY`
  - In a `for` loop, `@x-1` is easier to read that `$#x`

```
my @x = (1..5);

foreach my $i (0 .. (@x - 1) ){
   print "element $i is $x[$i] \n";
}
```

Reversing an Array

- The `reverse(LIST)` function returns the reversed list of the passed argument.
  - The returned list is reversed but the argument is **NOT**!
  - If you need to reverse the argument, assign it back to itself

```
my @x = (1, 2, 3);
my @y = reverse(@x);              # @y = (3, 2, 1) and @x = (1, 2, 3)
@x = reverse(@x);                 # @x = (3, 2, 1)
```

Sorting an Array

- The `sort()` function returns a list sorted in ASCII ascending order
  - By default, even digits are sorted using the ASCII order
  - Use `reverse()` if you need descending order
- To sort numerically redefine `sort()`
  - `sort ({$a <=>$b}, LIST)`
  - Compares the predefined variables $b and $a numerically
  - If you change the order of $a and $b, it changes the sorting order.

```
my @x = sort("small", "medium", "large");
print "@x\n";                          # prints large medium small
my @y = (1, 2, 4, 8, 16, 32, 64);
@y = sort(@y);
print "@y\n";                          # prints 1 16 2 32 4 64 8
@y = sort({$a <=> $b} , @y);
print "@y\n";                          # prints 1 2 4 8 16 32 64
```

tells perl to sort numerically

Exercises

- We will write a program that shuffles sequences for a boot strap
- Shuffled sequence contains the same letter composition, but a different order
- Ex:

```
my $dnaSeq = "ACGACGT";
my $shuffledSeq = "TACGCTAG";
```

- The algorithm will be explained first
- A program structure is given in shuffle.pl

Input Output

Input From a File

- Reading from a file is done in 3 steps
  1. Open the file for reading
  2. Grabbing input, we use the <FILEHANDLE> operator to get a line of input from the file
     - Similar to how we were reading from <STDIN>
  3. Closing the file

```
# open a file and create a file handle
open(INPUT, "/tmp/randSeqs.fasta") || die ("could not open the input file");
# fetch the next lie in the file, using the <> operator.
while (my $line = <INPUT>) {
   # do some work …
}
close(INPUT);
```

- File handles are capitalized by convention
- The <> operator returns TRUE while there are more lines to reads
  - Returns FALSE if we reach the end of a file!

Interactive Example

- Open the file or throw an exception if you cannot find it
  - If you are unsure what the relative path is, use the file info and the full file path to avoid any confusion
- Always the same structure
- Grab one line of input at a time
  - Remember that the line contains "\n" at the end
  - Do something useful with it
- Do not forget to close the file
  - Especially if you are opening the file for writing
- readFromFile.pl

Output to a File

- Writing to a file is similar to reading
  - Also done in 3 steps
- Open the file for writing.
  - Overwrite or create: `open(FILEHANDLE,">FILEPATH");`
  - Append or create: `open(FILEHANDLE,">>FILEPATH");`

```
# open a file and create a file handle
open(OUTPUT, "/tmp/someFile.txt") || die ("could not open the file $!");

# print to output file
print OUTPUT "Print this line to the file";

# close the file handle
close(OUTPUT);
```

- Not properly closing the files can cause the buffers not to be flushed
  - The text is not output to the file!

Interactive Example

- We modify the random fasta program to write the fasta sequences to a file
- We get the file name to write to from the user
- And add three modifications
  1. Open the file
  2. Write to the file, instead of the screen
  3. Close the file
- writeRandomFasta.pl

Hashes

What are Hashes?

- A hash is a dictionary-like structure
  - It holds a set of key/value elements
- Every element is indexed by a unique key
  - Position no longer relevant since we index by key
- A Hash variable is identified by the "%" *funny character*
- Every element of the hash is like a list of two elements
  - (key, value)

%codons	ATT	CTT	GTT	GGT	...	CGT	TAA
	ILE	LEU	VAL	GLY		ARG	STOP

There is no predetermined order for the elements of a hash structure

Store amino acid elements in a hash

($somval @anearray) = (1, 2, 3, 4, 5)

Initializing Hashes

- A hash is initialized using a list of elements
- Every element contains a key and a value, separated by the "=>" symbol
  - The key is not quoted but the value is

```
my %codons = (ATT => "ILE", CTT => "LEU", GTT => "VAL");
($codons{CGT}, $codons{TAA}) = ("ARG", "STOP");
```

- To access a hash value, we use $HASH{KEY}
- To get a list of the hash keys, we use keys()

```
my %codons = (ATT => "ILE", CTT => "LEU", GTT => "VAL", => "STOP");
foreach my $k ( keys(%codons) ){
   print "$k codes for $codons{$k} \n";
}
```

```
ATT codes for ILE
CTT codes for LEU
GTT codes for VAL
TAA codes for STOP
```

copy this one

FASTA comparison, any thing that would have a unique identifier (ie. taxonomy of a sequence)

Initializing Hashes

- Every element of the hash is a scalar
  - We use the scalar prefix $ to access an element

```
my %codons = (ATT => "ILE", CTT => "LEU", GTT => "VAL");
print $codons{GTT};                    # prints VAL
print $codons{ATT};                    # prints ILE
```

  - We can add a new value by specifying a key and a value

```
$codons{CGT} = "ARG";
$codons{TAA} = "STOP";
```

- We can use the **keys()** in the same way we have used lists
  - Print, loop, size, get the size, etc...

```
my %codons = (ATT => "ILE", CTT => "LEU", GTT => "VAL", TAA => "STOP");
# we return all the keys in codons
my @keys = keys(%codons);
# the keys are not necessarily in the order they were added
print "@keys \n";
```

Truth and Deleting From a Hash

- To check if a key exists in your hash, use exists(KEY)
- Same notion of "truth" as for scalars
- if(exists(HASH{KEY}))
  - TRUE if KEY exists (value may be undefined)
- The delete(KEY) function is used to remove entries from a hash
  - Deletes the KEY and VALUE from the hash.
  - Remember to use the scalar prefix ($)

```
my %codons = (ATT => "ILE", CTT => "LEU", GTT => "VAL", TAA => "STOP");
delete ($codons{TAA});                 # removes the key/value pair
```

COG functions table

Interactive Example

- Lets test the randomness of `rand()`
  - We generate a 1,000, 3bp sequences

```
int(rand(@array));
```

  - For every 3bp sequence created, we keep track, using a hash, of the number of times this triplet was obtained
  - Using a horizontal count plot, we illustrate the obtained statistics.

```
AAA  ***************
AAC  **************
AAG  ****************
AAT  ********************
ACA  *************
...
...
TTT  *******************
```

- See randomKmers.pl

Exercise

- Putting together annotations using the three following annotation files

```
1 - GeneCOG.txt association file
GeneID     COG
637671327 COG0001
637671102 COG0001
637671101 COG0002
...

2 - COGFunctions.txt association file
[J] COG0001
[A] COG0002
[K] COG0003
[K] COG0004
[L] COG0005
...

3 - COGcodes.txt file
J Translation, ribosomal structure and biogenesis
A RNA processing and modification
```

Exercise (cont'd)

- We need to assign the annotation (cogFunctions.txt) to each geneID.
  - Each geneID is assigned to a COG number, and each COG # has an assigned function
  - For the first example, we would print the following line:

```
637671327 COG0001  J        Translation, ribosomal structure and biogenesis
```

- Program structure is given in buildAnnotations.pl

```
1 - GeneCOG.txt association file
GeneID     COG
637671327 COG0001
...

2 - COGFunctions.txt association file
[J] COG0001
...

3 - COGcodes.txt file
J Translation, ribosomal structure and biogenesis
```

Subroutines in Perl

What Are Subroutines?

- A **subroutine** is a reusable block of code that performs a specific task
  - Similar to the functions we have used so far
  - `length()`, `keys()`, `rand()`
- Subroutines are a good programming practice
  - Add modularity to a script
  - Easier to read, debug and maintain
- A subroutine is defined by its name
  - Should be informative about the functionality

Using Subroutines

- A subroutine is defined with **sub** keyword, followed by the name
- A block of code, delimited by "{" and "}" defines the execution logic of the subroutine
- A return statement is used when the subroutine returns a result.

```
# definition of the subroutine
sub getGcRate{
    my $seq = $_[0];
    my $gcRate = 0;
    # do the necessary work to calculate the GC rate
    return $gcRate;
}

#call the subroutine
my $result = getGcRate("ACTGTAGCTGATCGTGATCGTGTAGCTGTAGCT");
```

recalls this, position zero

saved as @_

my $num = @_;
assigns length of array

Subroutine Arguments

- A subroutine accepts one or more arguments
  - ex. `length(STR)` takes one argument, the string to compute the length of, and returns the length.
- Inside the sub., an automatic array, "`@_`", is created
  - `@_` is an array where each element is a passed argument
  - Remember to use the scalar character prefix (`$`) when accessing one element of the array

```
# subroutine name: getBaseCount
# arguments:      $seq: a sequence
#                 $bt: the base type to count (ex. G)
# return value: the base count in the sequence
sub getBaseCount {
   my $ct = 0;
   ($seq, $bt) = @_;
   #do work
}
my $countType = getBaseCount($sequence, $baseType);
```

Example

- What does the following code print?
  - Hint: it contains a possible bug

```
# some subroutine … that does something!
sub my_square {
   my $num = @_;
   return $num**2;
}

print my_square(5);
```

Argument Validation

- It is always a good practice to verify the validity of the passed arguments
  - Depends on the function logic
- As a good practice, your function should assign default values or raise errors when necessary

```
sub my_square {
   my $num = @_;
   return $num**2;
}

print my_square();          # oops, use of uninitialized value!!!
```

```
# BETTER! … but who wants 0**2 ?
sub my_square {
   die("No arguments passed") if(!defined($_[0]))
   my $num = $_[0];
   return $num**2;
}
```

Return Values

- Your function can return any three of the variable types we have seen so far

```
($scalar, @array, %hash)
```

- Be careful when returning more than one array!
- Be careful when passing non scalars, as arguments are **flattened**
- Create a copy whenever it is possible and not too costly

Exercise – 1 of 2

- Write a subroutine that returns a k-mer
- The size is passed as an argument to the subroutine
- A program structure is provided in returnKmer.pl

Exercise – 2 of 2

- Write a function that parses a fasta file and returns an array of all the ids in the file
- The sequence filename is passed as an argument.
- A program structure is given in parseFastaSub.pl

Regular Expressions

A Brief Definition

- "A regular expression, abbreviated as **regex** or **regexp**, is a string that describes or matches a set of strings, according to certain syntax rules." *Wikipedia*
- A regex is a pattern used to give a concise description of a set, without listing all the elements of that set.
  - A regular expression is case sensitive
- It is used for "*matching*" (specifying and recognizing) strings of text, such as particular characters, words, etc.
- The simplest regular expression would contain the exact searched string
  - A binding site TRYA can be expressed as; TACA or TATA or TGCA or TGTA.

Matching Character

- When "matching", the regular expression is used as a parameter of a match expression `m/REGEX/`
  - The **`m`** is often dropped and only the **`/REGEX/`** `part` is kept
- To request a match, the "=~" operator is used to bind the regular expression to the target sequence
- If the absence of pattern is tested, the "!~" is used

```
$sentence = "A sequence of amino acids";
$sentence =~ m/sequence/;          # returns TRUE
$sentence =~ m/the/;               # returns FALSE
$sentence !~ m/the/;               # returns TRUE
```

Regular Expressions

- We use a character class to represent a set of possible characters
  - Character classes are denoted by "[]" with the possible matches inside
  - It means that at least one of the characters inside "[]" should be matched
- For example, TRYA, can be expressed as T[AG][CT]A

```
# ( ($sequence =~ /TACA/) || ($sequence =~ /TACA/ || … )
$sequence =~ /T[AG][CT]A/;                # matches 'TACA', 'TATA', …
```

Character Classes Abbreviations

- The special character "-" acts as a range indicator, within character classes.
  - When in the beginning or the end, it is considered a normal character

```
$sequence =~ /item[0-9]/;          # matches item0, item1, …, item[9]
```

- Several abbreviations for char classes are used. The most common are:

\d	matches a digit ([0-9])
\s	matches a whitespace
\w	matches a word character (alphanumeric or _)
\D	matches any non digit character. It is a negation of \d;
\S	matches any non-whitespace character. It is a negation of \s
\W	matches any non-word character (such as $, %, &, etc...). It is a negation of \w.

Some Useful Meta-characters

- The "^" and "$" meta-characters are used to specify the beginning and the end of a string, respectively
- To specify alternate choices, the special char "|" can be used.
- Ex.: To match AAA or TTT use the AAA|TTT regular expression
  - At every position in the sequence, AAA is tested first and if it doesn't match, the second alternate, TTT, is tested
  - The first alternative allowing a hit is chosen
  - By default, the match stops right after we get a hit

. means ~~any~~ wildcard

Matching Repetition

a?	match 'a' 0 or 1 times
a*	match 'a' 0 or more times
a+	match 'a' at least once
a{n,m}	match 'a' at least n times, but not more than m times.
a{n,}	match 'a' at least n or more times
a{n}	match 'a' exactly n times

```
/[a-z]+\s+\d*/;          # match a lower-case word of minimum size 1,
                         # at least on 1 or more spaces, and any
                         # number of digits

$year =~ /\d{2,4}/;      # make sure year is at least 2 but not more
                         # than 4 digits
```

- What is the problem with the year validation in the example above?
  - What would be a better regular expression?

Examples

```
$sequence =~ /A[ACGTN]A/;      # matches A followed by any other base
                               # Followed by A such as ATA, AGA, etc.
$sequence =~ /^gi\|\d*$/;      # matches the refseq id format
                               # >gi|46110415

"AAACCCTTT" =~ /AAA|TTT/;      # matches AAA
"AAACCCTTT" =~ /TTT|AAA/;      # matches TTT
"ACGT" =~ /A|AC|ACG|ACGT/;     # matches A
"ACGT" =~ /ACGT|ACG|AC|A/;     # matches ACGT

$sequence =! /CDS\s*\d*\.\.\d*/  # matches the coding sequence format of
                                 # the NCBI CDS 150..980
```

escaped dots

Grouping and Extracting Matches

- Grouping allows the creation of a sub-regex
- Implemented by enclosing the desired "unit" between parenthesis. "()"

```
#matches VERSION refseq of VERSION uniport
$domain =~ /VERSION (refseq|uniprot)/;
```

- The parenthesis are also used to capture the "hit"
  - The variables $1, $2, $3, etc. contain the hits of a regular expression, enclosed by parenthesis

```
my $version = "VERSION: refseq";
#matches VERSION refseq of VERSION uniport
$version =~ /VERSION: (refseq|uniprot)/;
print "The version is: $1";
```

```
The version is: refseq
```

master this for pulling out specific fields of a fasta file

Global Matching - 1

- We have seen that, by default, a regular expression finds and reports the first hit it finds
- Global matching is the option to find and report all matches obtained by a regular expression

```
my $sequence =~ m/REGEX/g;
```

  – We can assign the hits directly to an array

```
my $sequence = "A AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT";
my @hits = ($sequence =~ /A.A/g);
for my $hit (@hits){
   print "-$hit \n";
}
```

```
-A A
-ACA
-AGA
```

Global Matching - 2

- In a scalar context, the global search would return TRUE or FALSE, based on the fact that a hit was found or not
  – We can use this fact to loop through the found hits

```
my $sequence = "AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT";
while($sequence =~ /(A.A)/g) {
   print "$1 \n";
}
```

- What do you think is going to happen if we remove the global (g) modifier

/g need to make a regular expression greedy to find all motifs within a longer sequence

Hit Position

- To determine the position of a hit, use the "pos()" function on the searched sequence.
  – pos() returns the position of the first mismatch occurring after the last match
    - The result is 0 indexed

```
my $sequence = "AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT";
while($sequence =~ /(A.A)/g) {
   print "hit found $1 at position " . (pos($sequence) -3) . "\n";
}
```

```
hit found AAA at position 0
hit found ACA at position 16
hit found AGA at position 32
```

$1 $2 $3

(COG0001 T Transcription

/(^COG\d+)\s+(.)\s+(.+)$/

$1 $2 $3

String Substitution

- When the position of the desired text is known, we can use `substr()` to substitute a substring.
- An easier way to do that is through the substitution expression **s/REGEX/REPLACEMENT/**
  - Bound to a string using the =~ operator
  - **REPLACEMENT**: Exact text used to replace a substring
  - Substitution can be **global** or **minimal**
  - The operation returns the number of substitutions made

Example

```
my $sequence = "AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT";

$sequence =~ s/(A\SA)/NNN/;
print "The sequence after using the substitution " .
      "in a minimal context is: \n$sequence\n\n";

$sequence =~ s/(A\SA)/NNN/g;
print "The sequence after using the substitution " .
      "in a global context is: \n$sequence\n";
```

```
The sequence after using the substitution in a minimal context is:
NNN AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT

The sequence after using the substitution in a minimal context is:
NNN AAC AAG AAT NNN ACC ACG ACT NNN AGC AGG AGT
```

quick search and replace

String Translation

- To substitute a string of characters for another, the "`tr///`" operator is used.
- The expression allows character for character translation "`tr/TN/UX/`"
  - Replaces T by U and N for X
  - Returns the number of substitutions made
- Can sometimes be used to count the number of occurrences in a string.

```
my $count = ($sequence =~ tr/A/A/);
```

  - Assigns to `$count` the number of occurrences of A in `$sequence`.
- If used with the **c** modifier, reverses the search list. (Anything but the search list)

Exercise

- Rewrite the script reverseComplement.pl
- You script should replace all the non-standard DNA letter entered by the character **N**
- A program structure is given in betterReverseComplement.pl

compliment /c

eg. $seq=~ tr/ACGT/N/c;

Running a Shell Script From Perl

- Perl offers different ways to run a program from within the code.
  - Programmatically running programs is ideal for automation and building pipelines
- The easiest way is to simply enclose the command to run by back-ticks
  - Variables are interpolated within back-ticks (similar to a double quote context)

```
my $inputFile = "/tmp/input.fasta";
my $outputFile = "/tmp/output.fasta";

my $database = "/tmp/database.fasta";

#run blast
`blastall -p blastn -i $inputFile -o $outputFile -d $database -F F -U -e 1e-05`
```

Returning Output From a Shell Command

- If the command returns an output, you can assign the output to a variable.

```
my $directory = "/tmp/";

# get the files in a directory
my @filesInDir = `ls $directory`;

chomp($filesInDir[0]);
print $filesInDir[0] . "\n";
chomp($filesInDir[1]);
print $filesInDir[1] . "\n"
```

```
myRead.fasta
myRead.fastq
```

- runCommand.pl

Very Brief Intro to Objects

- BioPerl use the concept of Objects, another paradigm for code design
  - Procedural programming: Code arranged in subroutines and programs flows like a lab protocol (or a recipe)
  - Object oriented programming: Encapsulates the logic within entities called objects
- Each class represents an entity and all the code within one class has a common purpose (self contained)
- Choosing one approach or another depends on many factors.
  - The size of the project
  - The efficiency
  - The desired level of code reuse.
  - Etc.

Object Attributes and Methods

- Object oriented programming models the program using objects, classes.
- A class is a set of attributes and actions
  - Attributes are the fields that describe the object
  - Actions (Methods) are the tasks that can be applied to the object
    Methods are the way the user manipulate the objects
- For example, we can think of a Fasta Sequence as an Object, instead of a string
  - Attributes can be: Type(DNA|RNA|Protein), Length, Name,
  - Methods can be:
    - `complement()`
    - `rev_complement()`
    - `substrting()`,
    - etc.

A Brief Introduction to Bioperl

What is Bioperl?

- Bioperl is a collection of modules (libraries of code) that facilitates the development of bioinformatic applications
- Maintained by a dedicated group of volunteer developers.
- Latest developments can be found on www.bioperl.org website
- It gives access to a variety of functionality, such as plotting phylogenic trees, modeling sequences, alignments, communicating with databases, parsing popular sequence files, doing alignments,....
- The purpose is to interactively see some of the available functionality by looking at some simple examples

Objects in BioPerl

- Most concepts in BioPerl are objects
  - Sequence Objects(Seq, RichSeq, LargeSeq, SeqWithQuality, etc...),
  - Alignment objects (SimpleAlign, Stockholm)
  - Trees(Tree)
  - Sequence variations (SNP, DNAMutation)
  - Etc.
- Offers ways to create those objects and methods to operate on them

Parsing Bioinformatic Files

- Most objects in BioPerl have their parsers
  - SeqIO(Fasta, Fastq, genbank, Interpro, ace, etc...),
  - TreeIO(Newick, Nexus, Phyloxml, ...),
  - SearchIO(Blast, hmmer, fasta, etc..),
  - FeatureIO(Bed, GFF, GTF)
- Parsers open the file and build iterators
  - Iterators iterate through the objects in the file
  - You can iterate through a Fasta file by getting each sequence independently
- Most of the time, they offer ways to convert between formats
  - Convert fasta to fastq, newick to Nexus, etc.

can change trees formats instantly

Parsing a Fasta File

```
use Bio::SeqIO;

my $io = Bio::SeqIO->new( -file => "/tmp/myReads.fasta", -format => "fasta");
my $firstSeq = $io->next_seq();

print "The sequence id is: " . $firstSeq->display_id . "\n";
print "The sequence is: " . $firstSeq->seq . "\n";
print "The sequence length is: " . $firstSeq->length . "\n";
print "The first 10 bases of the sequence is: " . $firstSeq->subseq(1,10) .
    "\n";
my $revComplement = $firstSeq->revcom();
print "The reverse complement of the sequence is: ".$revComplement->seq .
    "\n";
print "The translated sequence is: ".$firstSeq->translate()->seq."\t";
```

```
The sequence id is: 1
The sequence is: AGATGATGGATAGAGTTACGGCATGATC
The sequence length is: 28
The first 10 bases of the sequence is: AGATGATGGA
The reverse complement of the sequence: GATCATGCCGTAACTCTATCCATCATCT
The translated sequence is: R*WIELRHD
```

firstBioPerl.pl

Parsing a Fasta File

```
my $io = Bio::SeqIO->new( -file => "/tmp/myReads.fasta",
                          -format =>"fasta");
```

- The `$io` object is the iterator
  - When asked for the next_seq, the iterator returns the next seq in line.
  - The sequence returned is a BioPerl Object
  - Returns False, when there no more sequences to return
- The id, seq and length are all attributes of the $firstSeq class.
- `subseq()` and `revcom()` are methods of the object
- Both methods and attributes are called by post fixing the objects name with the "->" characters
- Sequences have many other attributes and methods

Interactive Example

- Modifying the previous script to only print those sequences that have lengths above a user given threshold.
- We can use the same construct to write output files

```
my $seqout = Bio::SeqIO->new( -file => ">output.fa", -format =>"fasta");
```

- we can directly write BioPerl Seq objects to output files using the method write_seq()
- We use these methods to convert fasta-sanger to fasta-illumina
  - convertSangerToIllumina.pl
- We can read convert between many other formats
  - http://search.cpan.org/~cjfields/BioPerl-1.6.901/

Parsing Blast Results

- BioPerl offers the same parsing functionality as that used for parsing sequences
  - http://search.cpan.org/~cjfields/BioPerl-1.6.901/Bio/SearchIO/blast.pm
  - You can generate a parser using
    - `Bio::SearchIO->new()`
  - For get every result using
    - `$searchio->next_result()`
  - Then the hits and the hsps.
- All of these are objects: They have attributes and methods

Example: Parsing Blast Results

parseBlast.pl

```
use Bio::SearchIO

my $fileParser = new Bio::SearchIO( -file => '/tmp/metagenome.blast.out'
                                    -format => 'blast');

while(my $result = $fileParser->next_result) {
  print "----------------START----------------";
  while(my $hit = $result->next_hit){
    while(my $hsp = $hit->next_hsp){
      if($hit->significance < 1e-02){
        print $result->query_name . "\tvs\t" . $hit->name . " evalue: " .
              $hit->significance() . "\n";
        print "\tLength\t" . $hsp->length() .
              " Percent ID\t" .$hsp->percent_identity . "\n";
      }
    }
  }
  print "--------------- END ----------------";
}
```

query, hit, HSB

nested loops need to cover all elements within all elements within all elements

Reading And writing Trees

TreeOperations.pl

```
if ($tree->get_lca(\@nodes) == $tree->get_root_node()) {
    print "The lowest common ancestor is the root\n";
} else {
    print "The lowest common ancestor is NOT the root\n";
}

@nodes = $tree->find_node(-id => 'A');
push(@nodes, $tree->find_node(-id => 'E'));

if ($tree->get_lca(\@nodes) == $tree->get_root_node()) {
    print "The lowest common ancestor is the root\n";
} else {
    print "The lowest common ancestor is NOT the root\n";
}
```

Questions

perldoc