Programming in bioinformatics:
BioPerl

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Programming and biology
Basic algorithm structures
Programming for biology

- Cultural divide between biologists and computer science
  - use programs, don't write them
  - write programs when there's nothing to use
  - programming takes time
- Focus on interesting, unsolved, problems
- Open Source tools comes as part of the rescue
Reasons for programming

- Scientific
  - Quantity of existing data
  - Dealing with new data
  - Automating the automation
  - Evaluating many targets

- Economic

... programmers going into biology often have the harder time of it ... biology is subtle, and it can take lots of work to begin to get a handle on the variety of living organisms. Programmers new to the field sometimes write a perfectly good program for what turns out to be the wrong problem! -- James Tisdall
Biology

- Science in different mediums
  - in vitro – in glass
  - in vivo – in life
  - in silico – in computer algorithms

- Huge amount of experimental data
  - collected, shared, analyzed
  - biologists forced to relay on computers
Basic programming

- Simple basic building blocks which enable us to describe desired behavior (algorithm) to computer

- Sequence

- Condition

- Loop

\[
i = 1 \quad i < 10 \quad i++ \quad i < 10
\]
Why perl?

- well suited to text manipulation tasks
- easy to learn
- CPAN modules, including BioPerl
- rapid prototyping
  - duct tape of Internet
- available on multiple platforms
  - Unix, Linux, Windows, VMS...
- TIMTOWTDI
  - There Is More Than One Way To Do It
### Java Code
```java
import java.io.*;
public class rot13 {
    public static void main(String args[]) {
        int abyte = 0;
        try {
            while((abyte = System.in.read())>=0) {
                int cap = abyte & 32;
                byte &= ~cap;
                byte = ((byte >= 'A') && (byte <= 'Z') ?
                        ((byte - 'A' + 13) % 26 + 'A') : byte) | cap;
                System.out.print(String.valueOf((char)abyte));
            }
        } catch (IOException e) { }
        System.out.flush();
    }
}
```

### Perl Code
```perl
#!/usr/bin/perl -p
y/A-Za-z/N-ZA-Mn-za-m/;
```
Art of programming

Different approaches
- take a class
- read a tutorial book
- get programming manual and plunge in
- be tutored by a programmer
- identify a program you need
- try all of above until you've managed to write the program
Programming process

- identify inputs
  - data from file or user input
- make overall design
  - algorithm by which program generate output
- decide how to output results
  - files, graphic
- refine design by specifying details
- write perl code
IUB/IUPAC codes

<table>
<thead>
<tr>
<th>Code</th>
<th>Nucleic Acid(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
</tr>
<tr>
<td>T</td>
<td>Thymine</td>
</tr>
<tr>
<td>U</td>
<td>Uracil</td>
</tr>
<tr>
<td>M</td>
<td>A or C (amino)</td>
</tr>
<tr>
<td>R</td>
<td>A or G (purine)</td>
</tr>
<tr>
<td>W</td>
<td>A or T (weak)</td>
</tr>
<tr>
<td>S</td>
<td>C or G (strong)</td>
</tr>
<tr>
<td>Y</td>
<td>C or T (pyrimidine)</td>
</tr>
<tr>
<td>K</td>
<td>G or T (keto)</td>
</tr>
<tr>
<td>V</td>
<td>A or C or G</td>
</tr>
<tr>
<td>H</td>
<td>A or C or T</td>
</tr>
<tr>
<td>D</td>
<td>A or G or T</td>
</tr>
<tr>
<td>B</td>
<td>C or G or T</td>
</tr>
<tr>
<td>N</td>
<td>A or G or C or T (any)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Code</th>
<th>Amino acid</th>
<th>TLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Alanine</td>
<td>Ala</td>
</tr>
<tr>
<td>B</td>
<td>Aspartic acid or Asparagine</td>
<td>Asx</td>
</tr>
<tr>
<td>C</td>
<td>Cysteine</td>
<td>Cys</td>
</tr>
<tr>
<td>D</td>
<td>Aspartic acid</td>
<td>Asp</td>
</tr>
<tr>
<td>E</td>
<td>Glutamic acid</td>
<td>Glu</td>
</tr>
<tr>
<td>F</td>
<td>Phenylalanine</td>
<td>Phe</td>
</tr>
<tr>
<td>G</td>
<td>Glycine</td>
<td>Gly</td>
</tr>
<tr>
<td>H</td>
<td>Histidine</td>
<td>His</td>
</tr>
<tr>
<td>I</td>
<td>Isoleucine</td>
<td>Ile</td>
</tr>
<tr>
<td>K</td>
<td>Lysine</td>
<td>Lys</td>
</tr>
<tr>
<td>L</td>
<td>Leucine</td>
<td>Leu</td>
</tr>
<tr>
<td>M</td>
<td>Methionine</td>
<td>Met</td>
</tr>
<tr>
<td>N</td>
<td>Asparagine</td>
<td>Asn</td>
</tr>
<tr>
<td>P</td>
<td>Proline</td>
<td>Pro</td>
</tr>
<tr>
<td>Q</td>
<td>Glutamine</td>
<td>Gln</td>
</tr>
<tr>
<td>R</td>
<td>Arginine</td>
<td>Arg</td>
</tr>
<tr>
<td>S</td>
<td>Serine</td>
<td>Ser</td>
</tr>
<tr>
<td>T</td>
<td>Threonine</td>
<td>Thr</td>
</tr>
<tr>
<td>V</td>
<td>Valine</td>
<td>Val</td>
</tr>
<tr>
<td>W</td>
<td>Tryptophan</td>
<td>Trp</td>
</tr>
<tr>
<td>X</td>
<td>Unknown</td>
<td>Xxx</td>
</tr>
<tr>
<td>Y</td>
<td>Tyrosine</td>
<td>Tyr</td>
</tr>
<tr>
<td>Z</td>
<td>Glutamic acid or Glutamine</td>
<td>Glx</td>
</tr>
</tbody>
</table>
Variables to store data

• Scalars
  – denoted by $sigil
  – store sequence of chars
  – join, substr, translate, reverse

• characters used
  – A, C, G, T – DNA nucleic acid
  – A, C, G, U – RNA
  – N – unknown

• $DNA= 'ATAGTGCCGAGTGATGTAGTA' ;
#!/usr/bin/perl -w
# Transcribing DNA into RNA

# The DNA
$DNA = 'ACGGGAGGACGGGAAAATTACTACGGCATTAGC';

# Print the DNA onto the screen
print "Here is the starting DNA:

";
print "$DNA

";

# Transcribe the DNA to RNA by substituting all T's with U's.
$RNA = $DNA;

$RNA =~ s/T/U/g;

# Print the RNA onto the screen
print "Here is the result of transcribing the DNA to RNA:

";
print "$RNA
";

# Exit the program.
exit;
String substitution

Here is the starting DNA:
ACGGGAGGACGGGAAAATTACTACGGCATTAGC

Here is the result of transcribing the DNA to RNA:
ACGGGAGGACGGGAAAAUUACUACGGCAUUAGC

$RNA \ =~ s/T/U/g;
#!/usr/bin/perl -w
# Calculating the reverse complement of a strand of DNA

# The DNA
$DNA = 'ACGGGAGGACGGGAAAATTACTACGGCATTAGC';

# Print the DNA onto the screen
print "Here is the starting DNA:

$DNA

";

print "$DNA

";

# Make a new (reverse) copy of the DNA
$revcom = reverse $DNA;

print "Reverse copy of DNA:

$revcom

";

# Translate A->T, C->G, G->C, T->A, s/// won't work!
$revcom =~ tr/ACGT/TGCA/;

# Print the reverse complement DNA onto the screen
print "Here is the reverse complement DNA:

$revcom

";

exit;
#!/usr/bin/perl -w
# Calculating the reverse complement of a strand of DNA

# read lines from file or STDIN
while ( $DNA = <> ) {
    # remove line ending
    chomp( $DNA );

    # Make a new (reverse) copy of the DNA
    $revcom = reverse $DNA;

    # Translate A->T, C->G, G->C, T->A
    $revcom =~ tr/ACGT/TGCA/;

    # Print the reverse complement DNA onto the screen
    print "$revcom\n";
}

$ cat dna.txt
ACGGGAGGACGGGAAAATTACTACGGCATTAGC
$ ./03-compleme nt-file.pl dna.txt
GCTAATGCCGTAGTAATTTTCCCGTCCTCCCGT
Introducing @array

- list of ordered elements
  - direct access to element by offset
    $first_element = $array[0];
  - can be created from scalars using split
    @array = split( //, 'ABCD' );
    @array = ( 'A', 'B', 'C', 'D' );
  - can be iterated, extended and consumed at both ends
    $first = shift @array;  # ( 'B', 'C', 'D' )
    $last = pop @array;    # ( 'B', 'C' )
    unshift @array, 'X';   # ( 'X', 'B', 'C' )
    push @array, 'Y';     # ( 'X', 'B', 'C', 'Y' )
How about mutations?

- perl provides random number generator
- we want to mutate 10% of nucleotides
  - length of DNA divided by 10
- store mutated DNA in array
- for each mutation
  - find $mutation_position
  - select new $random_nucleotide
  - modify @mutated_DNA
- print out @mutated_DNA as string
#!/usr/bin/perl -w
use strict;
# randomize 10% of nucleotides

my @nucleotides = ( 'A', 'C', 'G', 'T' );

while ( my $DNA = <> ) {
    chomp( $DNA );
    my $DNA_length = length( $DNA );
    warn "DNA has $DNA_length nucleotides\n";
    my $mutations = int( $DNA_length / 10 );
    warn "We will perform $mutations mutations\n";
    my @mutated_DNA = split( //, $DNA );
    for ( 1 .. $mutations ) {
        my $mutation_position = int( rand( $DNA_length ) );
        my $random_position = int( rand( $#nucleotides ) );
        my $random_nucleotide = $nucleotides[ $random_position ];
        $mutated_DNA[ $mutation_position ] = $random_nucleotide;
        warn "mutation on $mutation_position to $random_nucleotide\n";
    }
    warn "$DNA\n";
    print join( '', @mutated_DNA),"\n";
}
Evolution at work...

$ ./05-ran dom.pl dna 2.txt | tee dna 3.txt

DNA has 33 nucleotides
We will perform 3 mutations
mutation on 16 to A
mutation on 21 to A
mutation on 8 to A
ACGGGAGGACGGGAAAATTACTACGGCATTAGC
ACGGGAGGACGGGAAAATTACAACGGCATTAGC
DNA has 33 nucleotides
We will perform 3 mutations
mutation on 9 to G
mutation on 24 to A
mutation on 12 to A
GCTAATGCCGTAGTAATTTTCCCGTCCTCCCGT
GCTAATGCCGTAAATAATTTTCCCGACCTCCCGT
Introducing %hash

- unordered list of pair elements
  - stores key => value pairs
    ```perl
    %hash = ( foo => 42, bar => 'baz' );
    ```
  - can fetch all key values or pairs
    ```perl
    @all_keys = keys %hash;
    while (( $key, $value ) = each %hash) {
        print "$key=$value\n";
    }
    ```
- Examples
  - counters
  - lookup tables (mappings)
Let's count nucleotides!

- read input file for DNA line by line
- split DNA into @nucleotides array
- for each $nucleotide increment %count
  - key will be nucleotide code
  - value will be number of nucleotides
  - we don't care about order :-)
- iterate through %count and print number of occurrences for each nucleotide
- same as counting letters in string
#!/usr/bin/perl -w
use strict;
# Count nucleotides in input file

my %count;

while ( my $DNA = <> ) {
    chomp( $DNA );
    # $DNA = "ACGGGAGGACGGGAAAATTACTACGGCATTAGC"
    
    my @nucleotides = split( //, $DNA );
    # ("A","C","G","G","G","A","G","G","A","C","G","
    
    foreach my $nucleotide ( @nucleotides ) {
        $count{$nucleotide}++;
        # increment by one
    }
}

# %count = ( A => 11, C => 6, G => 11, T => 5 )
while ( my ($nucleotide,$total_number) = each %count ) {
    print "$nucleotide = $total_number\n";
}
Unix file handling

$ cat dna.txt
ACGGGAGGACGGGAAAATTACTACGGCATTAGC
# make new copy
$ cp dna.txt dna2.txt
# append complement of DNA from dna.txt to dna2.txt
$ ./03-complement-file.pl dna.txt >>> dna2.txt
# examine current content of file dna2.txt
$ cat dna2.txt
ACGGGAGGACGGGAAAATTACTACGGCATTAGC
GCTAATGCCGTAGTAATTTTCCCGTCCTCCCGT
# count nucleotides in dna.txt
$ ./04-count.pl dna.txt
A = 11
T = 5
C = 6
G = 11
# and again in dna2.txt - do numbers look OK?
$ ./04-count.pl dna2.txt
A = 16
T = 16
C = 17
G = 17
Translating Codons to Amino Acids

my %genetic_code = (
    'TCA'=> 'S', 'TCC'=> 'S', 'TCG'=> 'S', 'TCT'=> 'S',
    'TTC'=> 'F', 'TTT'=> 'F', 'TTA'=> 'L', 'TTG'=> 'L',
    'TAC'=> 'Y', 'TAT'=> 'Y', 'TAA'=> '_', 'TAG'=> '_',
    'TGC'=> 'C', 'TGT'=> 'C', 'TGA'=> 'I', 'TGG'=> 'W',
    'CTA'=> 'L', 'CTC'=> 'L', 'CTG'=> 'L', 'CTT'=> 'L',
    'CCA'=> 'P', 'CCC'=> 'P', 'CCG'=> 'P', 'CCT'=> 'P',
    'CAC'=> 'H', 'CAT'=> 'H', 'CAA'=> 'Q', 'CAG'=> 'Q',
    'CGA'=> 'R', 'CGC'=> 'R', 'CGG'=> 'R', 'CGT'=> 'R',
    'ATA'=> 'I', 'ATC'=> 'I', 'ATT'=> 'I', 'ATG'=> 'M',
    'ACA'=> 'T', 'ACC'=> 'T', 'ACG'=> 'T', 'ACT'=> 'T',
    'AAC'=> 'N', 'AAT'=> 'N', 'AAA'=> 'K', 'AAG'=> 'K',
    'AGC'=> 'S', 'AGT'=> 'S', 'AGA'=> 'R', 'AGG'=> 'R',
    'GTA'=> 'V', 'GTC'=> 'V', 'GTG'=> 'V', 'GTT'=> 'V',
    'GCA'=> 'A', 'GCC'=> 'A', 'GCG'=> 'A', 'GCT'=> 'A',
    'GAC'=> 'D', 'GAT'=> 'D', 'GAA'=> 'E', 'GAG'=> 'E',
    'GGA'=> 'G', 'GGC'=> 'G', 'GGG'=> 'G', 'GGT'=> 'G',
);

# Picture is based on RNA so uracil appears instead of thymine
# we are going directly from DNA to amino acids, So codons use
# thymine instead of uracil
# define subroutine (in separate file together with %genetic_code)
# and store it in module GeneticCode.pm to be reusable

sub codon2aa {
    my ( $codon ) = @_;

    # check does mapping for codon exists
    if ( exists $genetic_code{ $codon } ) {
        # if it does, return amino acid
        return $genetic_code{ $codon };
    } else {
        # if it doesn't exit with error
        die "bad codon: $codon";
    }
}

# now we can use module directly from command line;
$ perl -M GeneticCode -e "print codon2aa('ACG')"
#!/usr/bin/perl -w
use strict;

# load module (*.pm)
use GeneticCode;

while ( my $DNA = <> ) {
    chomp($DNA);

    my $protein = ''; 

    # start at beginning and move by three places through DNA for ( my $i = 0; $i <= (length($DNA) - 2); $i += 3 ) {
        # extract single codon starting at position $i
        my $codon = substr( $DNA, $i, 3 );
        # call subroutine from GeneticCode module
        $protein .= codon2aa( $codon );
    }

    print "$protein\n";
}
Decoding DNA proteins

$ cat dna2.txt dna3.txt
ACGGGAGGACGGGAAAATTACTACGGCATTAGC
GCTAATGCCGTAGTAATTTCCTCCGTCCTCCCGT
ACGGGAGGACGGGAAAATTACAACGGCATTAGC
GCTAATGCCTTAGTAATTTTCCCGACCTCCCGT

$ ./06-dna2protein.pl dna2.txt dna3.txt
TGGRENYYGIS
ANAVVIFPSSR
TGGRENYNGIS
ANAVVIFPSSR

# let's improve our GeneticCode.pm by extending it to DNA2protein.pm

sub DNA2protein {
    my ( $DNA, $offset ) = @_;  
    my $protein = '';  

    # start at $offset and move by three places through DNA  
    for ( my $i=$offset; $i<=(length($DNA)-2-$offset); $i+=3 ) {  
        # extract single codon starting at position $i  
        my $codon = substr( $DNA, $i, 3 );  
        # decode codon to amino acid  
        $protein .= codon2aa( $codon );  
    }  
    # return created protein  
    return $protein;  
}

sub revcom {
    my ( $DNA ) = @_;  
    my $revcom = reverse $DNA;  
    $revcom =~ tr/ACGT/TGCA/;  
    return $revcom;  
}
Decoding all reading frames

#!/usr/bin/perl -w
use strict;

# use module DNA2protein to implement reading frames
use DNA2protein;

while ( my $DNA = <> ) {
    chomp($DNA);

    foreach my $offset ( 0 .. 2 ) {
        print DNA2protein( $DNA, $offset ), "\n";
        print DNA2protein( revcom($DNA), $offset ), "\n";
    }
}

$ ./07-reading-frames.pl dna.txt
TGGRENYYGIS
ANAVVIFPSSR
REDGKITTAL
LMP__FSRPP
GRTGKLLRH__CRSNFPVLP
Review

- Why to pursue biology programming?
- Algorithmic way of thinking
- $Scalars, @arrays and %hashes
- Modules as reusable components made of subroutines
- Combination of small tools with pipes (the Unix way)
Find out more...

- James Tisdall: "Beginning Perl for Bioinformatics", O'Reilly, 2001
Questions?

3*7*2