PROGRAM NAME: maskedfastacoords.pl

LICENSE: GNU General Public License (GNU-GPL)

LAST UPDATE: September 26, 2001

DESCRIPTION: Retrieving positions for masked regions of masked sequences in

fasta format. Program can also output those coords in GFF format,

where you can define several fields from command-line.

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1 Introduction

1.1 Program description

Retrieving positions for masked regions of masked sequences in fasta format. Program can also output those coords in GFF format, where you can define several fields from command-line.

1.2 Input

The following example illustrates both, "classical" (using 'N') and soft (using lowercase letters), masking of fasta sequences as they can be obtained from a program such REPEATMASKER¹. Sequence is splitted into 50 chararacter-length lines.

The sequence 'Masked_fasta_sequence' is 325 nucleotides long. Masking is distributed in the following coords:

51	100	N-masking
128	188	soft-masking
189	200	N-masking
212	245	soft-masking
269	284	soft-masking
274	278	n-masking (soft)

1.3 Output

The program can produce four different outputs returning the coords of the sequence masked segments: only "classical" masking ('N' or 'n'), only soft-masking ('[a-z]'), both or merge contiguous masked segments as if there were only one class. From the previous example we must obtain the following results:

Default	-maski	ing	All-masking			Merged-masking		
51	100	N-masking	51	100	N-masking			N-masking
189	200	N-masking	128	188	soft-masking	128	200	merged-masking
274	278	n-masking	189	200	N-masking	212	245	soft-masking
Soft-masking			212	245	soft-masking	269	284	merged-masking
128	188	soft-masking	269	273	soft-masking			
212	245	soft-masking	274	278	n-masking (soft)			
269	284	soft-masking	279	284	soft-masking			

1.4 To Do

- We can define mask checking as three different subroutines that will be called by reference, so that a variable name (called for instance \$test) will be set depending on command-line options.[Section 3.2, page 7]

¹http://www.genome.washington.edu/UWGC/analysistools/repeatmask.htm

2 Implementation

2.1 Program outline

TO DO

• This is a first draft of the maskedfastacoords.pl.

```
\langle maskedfastacoords 2a \rangle \equiv
2a
          (PERL shebang 10a)
          #
          # MODULES
          #
          ⟨Use Modules 2f⟩
          # VARIABLES
          ⟨Global Vars 2g⟩
          # MAIN LOOP
          ⟨Main Loop 3a⟩
          # FUNCTIONS
          ⟨Functions 3c⟩
2b
       \langle Program Info 2b \rangle \equiv
          my $PROGRAM = 'maskedfastacoords.pl';
          my $VERSION = '0.1_alpha';
2c
       \langle Prog\ USAGE\ 2c \rangle \equiv
          #$PROGRAM [options] < fasta_file > coords_file
2d
       \langle Prog\ DESC\ 2d\rangle \equiv
          #Retrieving masked regions coords from fasta files.
2e
       \langle Program \ Description \ 2e \rangle \equiv
               ⟨Prog USAGE 2c⟩
          #
          #
          #
               \langle Prog\ DESC\ 2d \rangle
          #
2f
       \langle Use\ Modules\ 2f \rangle \equiv
          ⟨Use Modules - Benchmark 10c⟩
          ⟨Use Modules - Getopt 4a⟩
          ⟨Use Modules - Bio::Seq 3d⟩
        \langle Global\ Vars\ 2g\rangle \equiv
2g
          ⟨Boolean 10b⟩
          (Stderr subs vars 12a)
          my (\$id,\$seq) = (",");
          my %CmdLineVar = (
                                     ⟨cmdline defaults - masking 6b⟩
                                     ⟨cmdline defaults - fasta 6e⟩
                                     ⟨cmdline defaults - GFF 6h⟩
                                     );
```

```
\langle Main Loop 3a \rangle \equiv
3a
        &parse_cmdline(); # PROG-START
        &main();
        &report('PROG-FINISH',&timing($T));
        exit(0);
3b
      \langle messages: program running 3b \rangle \equiv
         'PROG-START'
                         => "$line$s\n$s Running $PROGRAM\n$s".
                              "$s HOST: $host".
                              "$s USER: $USER".
                              "$s DATE: $DATE\n$s\n$line$s" ,
         'PROG-FINISH' => "$s\n$line$s\n$s $PROGRAM FINISHED\n$s".
                              "$s TOTAL TIME: \%s\n$line" ,
      \langle Functions 3c \rangle \equiv
3c
        ⟨Parsing command line options 4c⟩
        ⟨Main program function 7b⟩
        ⟨Common PERL subs - Benchmark 10e⟩
        ⟨Common PERL subs - STDERR 11f⟩
```

2.2 BIOPERL modules

Bio::Seq² is the BIOPERL³ main sequence object while Bio::SeqIO⁴ is the BIOPERL support for sequence input/output into files. BIOPERL is also available from CPAN⁵.

For sequences larger than 50 Kbp it is recommended to use the largefasta file format, defined in Bio::Seq::LargeSeq⁶ module, if you run off memory. It slows down the program because it is using temporary files to access the sequence. The module must be accessed through Bio::SeqIO.

²http://bioperl.org/Core/POD/Bio/Seq.html

³http://bioperl.org/

⁴http://bioperl.org/Core/POD/Bio/SeqIO.html

⁵http://search.cpan.org/search?dist=bioperl

⁶http://bioperl.org/Core/POD/Bio/Seq/LargeSeq.html

3 Program functions

3.1 Processing command-line options

```
4a
      \langle Use\ Modules - Getopt\ 4a \rangle \equiv
        use Getopt::Long;
        Getopt::Long::Configure qw/ bundling /;
      \langle perl \ requires - Getopt \ 4b \rangle \equiv
4b
        "Getopt::Long" - processing command-line options.
          See 'man Getopt::Long' for further info about this package.
      \langle Parsing\ command\ line\ options\ 4c \rangle \equiv
4c
        sub parse cmdline() {
             $SIG{__WARN__} = sub { &warn('UNKNOWN_CL_OPTION',$T,$_[0]) };
             GetOptions(
                           (command-line options - masking 6a)
                           ⟨command-line options - fasta 6d⟩
                           ⟨command-line options - GFF 6g⟩
                           ⟨command-line options with exit 4e⟩
                           ) | (&warn('CMD_LINE_ERROR',$T), exit(1));
             $SIG{__WARN__} = 'DEFAULT';
             &report("PROG-START");
        } # parse cmdline
4d
      \langle warnings: command-line 4d \rangle \equiv
        'UNKNOWN_CL_OPTION' =>
           $Warn. "Error trapped while processing command-line: \n".(" "x16)."\%s\n",
        'CMD_LINE_ERROR' =>
          $spl.$spw." Please, check your command-line options!!!\n".$Error."\n".
           spw."".("."x12)." Type \"maskedfastacoords.pl -h\" for help.\n".$spl,
```

3.1.1 Defining help and auxiliary code chunks

The following command-line checkings look for those options exiting the program: 'help' and 'version'. Both need to output to screen without any other message/warning being displayed at the same time.

```
\langle command\mbox{-line options with exit 4e} \rangle \equiv
4e
         "version"
                          => \&prt version,
         "h|help|?" => \&prt_help,
4f
       \langle command-line\ help\ -\ help\ 4f \rangle \equiv
         -h, -help
                                       Shows this help.
         -version
                                       Shows current version and exits.
4g
       \langle Parsing\ command\ line\ options\ 4c \rangle + \equiv
         sub prt_version() {
               &report('SHOW_VERSION', $PROGRAM, $VERSION);
               exit(1);
         } # prt_version
4h
       \langle messages: parsing command-line 4h \rangle \equiv
         'SHOW_VERSION' => $sp."### \%s - Version: \%s\n".$sp,
```

Printing command-line help to STDERR: 5a $\langle Parsing\ command\ line\ options\ 4c \rangle + \equiv$ sub prt_help() { print STDERR «"+++EndOfHelp+++"; PROGRAM: \$PROGRAM - \$VERSION ⟨Prog DESC 2d⟩ **USAGE:** ⟨Prog USAGE 2c⟩ DESCRIPTION: Retrieving positions for masked regions of masked sequences in fasta format. Program can also output those coords in GFF format, where you can define several fields from command-line. **REQUIRES:** (perl requires help 5b) COMMAND-LINE OPTIONS: *(command-line help 5c)* BUGS: Report any problem to 'jabril\@imim.es'. AUTHOR: \$PROGRAM is under GNU-GPL (C) 2000 - Josep F. Abril +++EndOfHelp+++ exit(1); } # prt_help $\langle perl \ requires \ help \ 5b \rangle \equiv$ \$PROGRAM needs the following Perl modules installed in your system, we used those available from the standard Perl distribution. Those that are not in the standard distribution are marked with an '(*)', in such cases make sure that you already have downloaded them from CPAN (http://www.perl.com/CPAN) and installed. ⟨perl requires - BioPerl 3e⟩ (perl requires - Getopt 4b) (perl requires - Benchmark 10f)

```
⟨command-line help 5c⟩≡
A double dash on itself "-" signals end of the options
and start of file names (if present). After double dash,
you can use a single dash "-" as STDIN placeholder.
Available options and a short description are listed here:
+ General options:
```

⟨command-line help - help 4f⟩

5c

ба

6b

6c

6d

6e

6f

6g

6h

 $\langle cmdline\ defaults - GFF\ 6h \rangle \equiv$

=> 0, sequence => 'noname', source => 'masked', feature => 'masked', strand => '.',

=> ",

GFF

group

```
⟨command-line help - masking 6c⟩
    ⟨command-line help - fasta 6f⟩
  + GFF output:
    ⟨command-line help - GFF 7a⟩
3.1.2 Defining command-line options
\langle command\mbox{-line options} - masking 6a \rangle \equiv
  "a|all-masked"
                    => sub { $CmdLineVar{MASKING} = 2 },
  "s|soft-masked" => sub { $CmdLineVar{MASKING} = 4 },
  "m|merge-masked" => sub { $CmdLineVar{MASKING} = 8 },
\langle cmdline\ defaults - masking\ 6b \rangle \equiv
 MASKING => 0,
\langle command\text{-line help - masking 6c} \rangle \equiv
                            Default is looking for "N" masked sequence
  -a, -all-masked
                             segments. This option distinguish between
                             upper/lower-case and assumes lower-case to
                             define masked regions too (so called soft-masking).
  -s, -soft-masked
                            Only outputs soft-masked sequence segments.
  -m, -merge-masked
                            Previous options differentiate classical-masking
                             (with "N"s) from soft-masking. This option merges
                             both as if they were the same masking type
                             (it also enables soft-masking search so previous
                             options are not required when passing this one).
\langle command-line\ options\ -\ fasta\ 6d \rangle \equiv
  "l|large-fasta" => \$CmdLineVar{LARGE},
\langle cmdline\ defaults\ -\ fasta\ 6e \rangle \equiv
 LARGE
            => 0,
\langle command-line\ help\ -\ fasta\ 6f \rangle \equiv
  -1, -large-fasta
                            For large genomic sequence you can enable
                             Bio::SeqIO to work with temporary files
                             avoiding memory overload (though it makes
                             the script to run slowly).
\langle command\text{-line options} - GFF 6g \rangle \equiv
  "g|gff"
                   => \$CmdLineVar{GFF},
                   => \$CmdLineVar{sequence},
  "seq-name=s"
                   => \$CmdLineVar{source},
  "source=s"
  "f|feature=s" => \$CmdLineVar{feature},
                  => \$CmdLineVar{strand},
  "strand=s"
                   => \$CmdLineVar{group},
  "group=s"
```

```
7a ⟨command-line help - GFF 7a⟩≡
-g, -gff Output in GFF format (default coords).

-seq-name <string> Sets sequence GFF field (default "noname").
-source <string> Sets source GFF field (default "masked").
-f, -feature <string> Sets feature GFF field (default "masked").
-strand <string> Sets strand GFF field (default ".").
-group <string> Sets group GFF field (default none).
```

3.2 Main program function

```
\langle Main\ program\ function\ 7b \rangle \equiv
7b
         sub main() {
              (Opening sequence object 3f)
              while ($sequence = $seqin->next_seq()) {
                   my ($sid,$len,$seq,@nuc,@coords,$masked_flg,$match,$msk_num);
                   @coords = ();
                   (Setting sequence variables from fasta record 7d)
                   (Finding masked regions coords 7e)
                   (Writing masked regions coords in GFF 8)
              }; # while
         } # main
      \langle Global\ Vars\ 2g\rangle + \equiv
7c
7d
       \langle Setting \ sequence \ variables \ from \ fasta \ record \ 7d \rangle \equiv
        print STDERR "### READING FASTA....\n";
         $sid = $sequence->display_id();
         $len = $sequence->length();
         $seq = $sequence->seq();
```

TO DO

- We can define mask checking as three different subroutines that will be called by reference, so that a variable name (called for instance \$test) will be set depending on command-line options.
- It will be also necessary to define the parsing functions for largeseq in a different way from default fasta sequences.

```
\langle Finding\ masked\ regions\ coords\ 7e \rangle \equiv
7e
       print STDERR "###
                                   PARSING SEQUENCE: $sid ($len bp)\n";
       @nuc = split //og, $seq;
       (\$masked\_flg,\$match) = (\$F,\$F) ;
       for (my n = 0; n <= \#nuc; n++) {
           match = ( nuc[n] = /[NnXx]/o ) ? T : F;
           ( !$masked_flg && $match ) && do {
                $masked_flg = $T ;
                # $n contains the last non-masked nucleotide
               push @coords, ($n + 1);
               next;
           $masked_flg && do {
                $match && (next);
                $masked_flg = $F ;
                # $n contains the last masked nucleotide now
               push @coords, $n;
           };
       }; # for nuc in $seq
       # if last nucleotide is masked, previous loop not includes its coord.
       $masked_flg &&( push @coords, $len);
```

A empty appendix section

A.1 empty appendix subsection

B Common code blocks

B.1 PERL scripts

```
10a
       \langle PERL \ shebang \ 10a \rangle \equiv
         #!/usr/bin/perl -w
         # This is perl, version 5.005_03 built for i386-linux
         ⟨Program Description 2e⟩
         ⟨GNU License 12f⟩
         (Version Control Id Tag 12e)
         use strict;
         (Program Info 2b)
         my $DATE = localtime;
         my $USER = defined($ENV{USER}) ? $ENV{USER} : 'Child Process';
         my $host = 'hostname';
         chomp($host);
10b
       \langle Boolean \ 10b \rangle \equiv
         my (\$T,\$F) = (1,0); \# for 'T'rue and 'F'alse
```

B.1.1 Timing our scripts

The 'Benchmark' module encapsulates a number of routines to help to figure out how long it takes to execute a piece of code and the whole script.

```
10c ⟨Use Modules - Benchmark 10c⟩≡
use Benchmark;
⟨Timer ON 10d⟩
```

See 'man Benchmark' for further info about this package. We set an array to keep record of timing for each section.

```
\langle Timer\ ON\ 10d \rangle \equiv
10d
        my @Timer = (new Benchmark);
       ⟨Common PERL subs - Benchmark 10e⟩≡
10e
         sub timing() {
             push @Timer, (new Benchmark);
             # partial time
              $_[0] ||
                   (return timestr(timediff($Timer[$#Timer],$Timer[($#Timer - 1)])));
              # total time
             return timestr(timediff($Timer[$#Timer],$Timer[0]));
         } # timing
10f
       \langle perl \ requires - Benchmark \ 10f \rangle \equiv
         "Benchmark" - checking and comparing running times of code.
```

B.1.2 Printing complex Data Structures

With 'Data:: Dumper' we are able to pretty print complex data structures for debugging them.

```
10g ⟨Use Modules - Dumper 10g⟩≡
use Data::Dumper;
local $Data::Dumper::Purity = 0;
local $Data::Dumper::Deepcopy = 1;
```

B.1.3 Common functions

```
\langle Skip \ comments \ and \ empty \ records \ 11a \rangle \equiv
11a
         next if /^{\#/0};
         next if /^\s*$/o;
         chomp;
11b
       ⟨Common PERL subs - Min Max 11b⟩≡
         sub max() {
              my $z = shift @_;
              foreach my 1 (@) \{ z = 1 \text{ if } + 2 \};
              return $z;
         } # max
         sub min() {
              my $z = shift @_;
              foreach my 1 (@_) \{ z = 1 \text{ if } 1 < z \};
              return $z;
         } # min
11c
       \langle Common\ PERL\ subs - Text\ fill\ 11c \rangle \equiv
         sub fill_right() \{ \ \[0].(\[2] \ x \ (\[1] - length(\[0]))) \}
         sub fill_left() \{ (\$_[2] \times (\$_[1] - length(\$_[0]))).\$_[0] \}
         sub fill_mid()
              my $1 = length($[0]);
              my $k = int(($_[1] - $1)/2);
              (\$_[2] \times \$k).\$_[0].(\$_[2] \times (\$_[1] - (\$1+\$k)));
         } # fill mid
           These functions are used to report to STDERR a single char for each record processed (useful for reporting
       parsed records).
11d
       \langle Common\ PERL\ subs - Counter\ 11d \rangle \equiv
         sub counter { # $_[0]~current_pos++ $_[1]~char
              print STDERR "$ [1]";
              ((\$_{0}) \$ 50) == 0) \& (print STDERR "[".&fill_left(\$_{0}, 6, 0")."]\n");
         } # counter
```

B.1.4 Common functions for reporting program processes

sub counter_end { # \$_[0]~current_pos

} # counter_end

 $\langle Global\ Vars - Counter\ 11e \rangle \equiv$

11e

Function 'report' requires that a hash variable '%Messages' has been set, such hash contains the strings for each report message we will need. The first parameter for 'report' is a key for that hash, in order to retrieve the message string, the other parameters passed are processed by the sprintf function on that string.

\$_[1]~char

 $((\$_[0] \% 50) != 0) \& (print STDERR "[".&fill_left(\$_[0],6,"0")."]\n");$

```
11f ⟨Common PERL subs - STDERR 11f⟩≡
sub report() { print STDERR sprintf($Messages{ shift @_ },@_) }
```

my (\$n,\$c); # counter and char (for &counter function)

The same happens to 'warn' function which also uses the hash variable '%Messages' containing the error messages.

```
11g ⟨Common PERL subs - STDERR 11f⟩+≡
sub warn() { print STDERR sprintf($Messages{ shift @_ }, @_) }
```

Those are accessory variables for the messages strings:

```
12b ⟨Stderr subs vars 12a⟩+≡

my %Messages = (

# ERROR MESSAGES
⟨warnings: command-line 4d⟩

# WORKING MESSAGES
⟨messages: program running 3b⟩
⟨messages: parsing command-line 4h⟩

); # %Messages
```

B.2 BASH scripts

```
\langle BASH \text{ shebang } 12c \rangle \equiv
12c
         #!/usr/bin/bash
         # GNU bash, version 2.03.6(1)-release (i386-redhat-linux-gnu)
         (Version Control Id Tag 12e)
        SECONDS=0 # Reset Timing
         # Which script are we running...
        L="###############"
         { echo "$L$L$L$L";
           echo "### RUNNING [$0]";
           echo "### Current date: 'date' ";
           echo "###"; } 1>&2;
12d
       \langle BASH \ script \ end \ 12d \rangle \equiv
         { echo "###"; echo "### Execution time for [$0]: $SECONDS secs";
           echo "$L$L$L$L";
           echo ""; } 1>&2;
         exit 0
```

B.3 Version control tags

This document is under Revision Control System (RCS). The version you are currently reading is the following:

```
12e ⟨Version Control Id Tag 12e⟩≡
# $Id: maskedfastacoords.nw,v 1.3 2001/09/25 17:24:54 jabril Exp $
```

B.4 GNU General Public License

C Extracting code blocks from this document

From this file we can obtain both the code and the documentation. The following instructions are needed:

C.1 Extracts Script code chunks from the NOWEB file

Remember when tangling that '-L' option allows you to include program line-numbering relative to original NOWEB file. Then the first line of the executable files is a comment, not a shebang, and must be removed to make scripts runnable.

C.2 Extracting different Config Files

```
14c ⟨tangling 14a⟩+≡
notangle -R"root" $WORK/$nwfile.nw | \
cpif $DATA/root_config ;
```

C.3 Extracting documentation and LATEX'ing it

```
14d
      \langle tangling 14a \rangle + \equiv
        notangle -Rweaving $WORK/$nwfile.nw | cpif $WORK/nw2tex ;
        notangle -RLaTeXing $WORK/$nwfile.nw | cpif $WORK/ltx ;
        chmod a+x $WORK/nw2tex $WORK/ltx;
      \langle tangling\ complementary\ LaTeX\ files\ 14e \rangle \equiv
14e
        notangle -R"HIDE: LaTeX new definitions" $WORK/$nwfile.nw | cpif $DOCS/defs.tex;
        notangle -R"HIDE: TODO" $WORK/$nwfile.nw | cpif $DOCS/todo.tex ;
14f
      \langle weaving 14f \rangle \equiv
        (BASH shebang 12c)
        # weaving and LaTeXing
        ⟨BASH Environment Variables 15b⟩
        ⟨tangling complementary LaTeX files 14e⟩
        noweave -v -t4 -delay -x -filter 'elide "HIDE: *"' \
                  $WORK/$nwfile.nw | cpif $DOCS/$nwfile.tex ;
        # noweave -t4 -delay -index $WORK/$nwfile.nw > $DOCS/$nwfile.tex
        pushd $DOCS/ ;
        latex $nwfile.tex ;
        dvips $nwfile.dvi -o $nwfile.ps -t a4;
        popd;
        ⟨BASH script end 12d⟩
```

```
15a
      ⟨LaTeXing 15a⟩≡
        ⟨BASH shebang 12c⟩
        # only LaTeXing
        (BASH Environment Variables 15b)
       pushd $DOCS/ ;
       echo "### RUNNING LaTeX on $nwfile.tex" 1>&2;
        latex $nwfile.tex ;
       latex $nwfile.tex;
       latex $nwfile.tex ;
       dvips $nwfile.dvi -o $nwfile.ps -t a4;
        # pdflatex $nwfile.tex ;
       echo "### CONVERTING PS to PDF: $nwfile" 1>&2;
       ps2pdf $nwfile.ps $nwfile.pdf ;
       popd ;
        ⟨BASH script end 12d⟩
```

C.4 Defining working shell variables for the current project

```
⟨BASH Environment Variables 15b⟩≡
15b
        #
        # Setting Global Variables
        WORK="/home/ug/jabril/development/softjabril/maskedfastacoords" ;
        BIN="$WORK/bin";
        PARAM="$BIN/param" ;
        DOCS="$WORK/docs";
        DATA="$WORK/data" ;
        nwfile="maskedfastacoords" ;
        export WORK BIN PARAM DOCS DATA nwfile;
15c
      \langle tangling 14a \rangle + \equiv
        # BASH Environment Variables
        notangle -R'BASH Environment Variables' $WORK/$nwfile.nw | \
                  cpif $WORK/.bash_VARS ;
        source $WORK/.bash_VARS ;
```