Shell wrappers for EMBOSS utilities

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General Instructions

- It is assumed that EMBOSS utilities are already installed and available in the `$path` variable.
- Moreover, the newer version of `pcregrep` is installed which uses the switch `--buffer-size` to change the size of internal buffers. For extracting longer contigs, we have to increase the size from default `20K` to a higher value. We will set the alias `FASTAgrep` that will help us in extracting sequences from FASTA file based on a supplied pattern (You can also add this alias to `~/.bashrc`):

```
$ alias FASTAgrep="awk '{gsub(\"_\",\"\\\_\",\$0);\$0=\"(?s)^>\"\$0\".*?(?=\\\n(\\\z|>))\"
```

- The shell wrappers use the same name as the corresponding EMBOSS utility except that they have `.sh` extension and are primarly designed to work with metagenomic contigs.
- The shell wrappers accept input stream of FASTA sequences from `STDIN` using `less <&0` and unless otherwise specified,take ONLY one argument of space-delimited parameters list in inverted commas that are subsequently passed to the corresponding EMBOSS utility. Accepting sequences from `STDIN` makes it easier to incorporate `FASTAgrep` and also helps in integrating GNU's `parallel` which reduces the execution time. For example, here is the execution time for both sequential and parallel mode for `compseq.sh` on a given `contigs.fa` file for contigs > 1000bp:

```
$ time echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./
real    0m30.686s
user    0m22.476s
sys     0m9.250s
$ time echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | pa
real    0m6.420s
user    0m25.586s
sys     0m12.203s
```

  Here `parallel -kN300 --recstart '>' --pipe` splits the input FASTA stream into 300 records (`-N`) per processor using ">" (`--recstart '>'`) as a delimiter while maintaining the same order (`-k`) as input stream.
- The shell wrappers output records in a tab-delimited list with first column specifying the contig name. We have chosen this format as it is easier to collate the results (in some cases) later as well as plotting them using gplot. For example, if the data stream is of the form `[Contig]\t[Feature]\t[Value]`, then you can pipe the stream to `GENERATEtable.sh`:

```
$ cat test.tsv
contig1 F1      12.2
contig1 F2      34.2
contig1 F3      45.2
contig2 F2      56.3
contig2 F3      56.2
contig3 F1      45.4
contig3 F2      56.3
contig4 F1      23.5
```

```
contig5 F1      24.5
$ cat GENERATEtable.sh
#!/bin/bash
less <&0| \
        perl -ane '$r{$F[0].":".$F[1]}=$F[2];
                unless($F[0]~~@s){
                        push @s,$F[0];}
                unless($F[1]~~@m){
                        push @m,$F[1];}
        END{
        print "Contigs\t".join("\t",@s)."\n";
        for($i=0;$i<@m;$i++){
                print $m[$i];
                for($j=0;$j<@s;$j++){
                        (not defined $r{$s[$j].":".$m[$i]})?print "\t".0:print"\t".$r{$s[$
                print "\n";}}'
$ cat test.tsv | ./GENERATEtable.sh
Contigs contig1 contig2 contig3 contig4 contig5
F1      12.2    0       45.4    23.5    24.5
F2      34.2    56.3    56.3    0       0
F3      45.2    56.2    0       0       0
```

- We are using `perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1'` to linearise the sequences. If the input stream is always linearised, then this piece of code can be removed from the shell scripts to speed them up.
- Using `-s` switch, we can pass arguments to the perl one-liner, for example, the string `-- -o=$1` followed by the one-liner passes parameters to the original program in `$r=qx/`*`PROGNAME`* `-sequence=asis:$a[1] $o -stdout -auto /;`. Since we have written the parsers for a particular output (in most cases standard) format, the wrappers will work only with a subset of parameters given in *`PROGNAME`* `-help -verbose` and are listed next to each wrapper.

compseq.sh: calculate the composition of unique words in sequences

Content of [compseq.sh]:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/compseq -sequence=asis:$a[1] $o -stdout -auto /;
                $r=~s/#.*\n//g;
                $r=~s/\s+\n//g;
                $r=~s/^Word size\s+\d\nTotal count\s+\d+//g;
                $r=~s/Other.*?\n//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\
```

Parameters [from `compseq -help -verbose`]:

```
   -word               integer    [2] This is the size of word (n-mer) to
                                  count.
                                  Thus if you want to count codon frequencies
                                  for a nucleotide sequence, you should enter
                                  3 here. (Integer 1 or more)
   -frame              integer    [0] The normal behaviour of 'compseq' is to
                                  count the frequencies of all words that
                                  occur by moving a window of length 'word' up
                                  by one each time.
                                  This option allows you to move the window up
                                  by the length of the word each time,
                                  skipping over the intervening words.
                                  You can count only those words that occur in
                                  a single frame of the word by setting this
```

```
                                value to a number other than zero.
                                If you set it to 1 it will only count the
                                words in frame 1, 2 will only count the
                                words in frame 2 and so on. (Integer 0 or
                                more)
   -[no]ignorebz        boolean [Y] The amino acid code B represents
                                Asparagine or Aspartic acid and the code Z
                                represents Glutamine or Glutamic acid.
                                These are not commonly used codes and you
                                may wish not to count words containing them,
                                just noting them in the count of 'Other'
                                words.
   -reverse             boolean [N] Set this to be true if you also wish to
                                also count words in the reverse complement
                                of a nucleic sequence.
   -calcfreq            boolean [N] If this is set true then the expected
                                frequencies of words are calculated from the
                                observed frequency of single bases or
                                residues in the sequences.
                                If you are reporting a word size of 1
                                (single bases or residues) then there is no
                                point in using this option because the
                                calculated expected frequency will be equal
                                to the observed frequency.
                                Calculating the expected frequencies like
                                this will give an approximation of the
                                expected frequencies that you might get by
                                using an input file of frequencies produced
                                by a previous run of this program. If an
                                input file of expected word frequencies has
                                been specified then the values from that
                                file will be used instead of this
                                calculation of expected frequency from the
                                sequence, even if 'calcfreq' is set to be
                                true.
   -[no]zerocount       boolean [Y] You can make the output results file
                                much smaller if you do not display the words
                                with a zero count.
   -sbegin1             integer Start of each sequence to be used
   -send1               integer End of each sequence to be used
   -sreverse1           boolean Reverse (if DNA)
   -snucleotide1        boolean Sequence is nucleotide
   -sprotein1           boolean Sequence is protein
   -slower1             boolean Make lower case
   -supper1             boolean Make upper case
   -scircular1          boolean Sequence is circular
```

Output format:

```
[Contig]\t[Word]\t[Obs Count]\t[Obs Frequency]\t[Exp Frequency]\t[Obs/Exp Frequency]
```

Manual

Example usage:

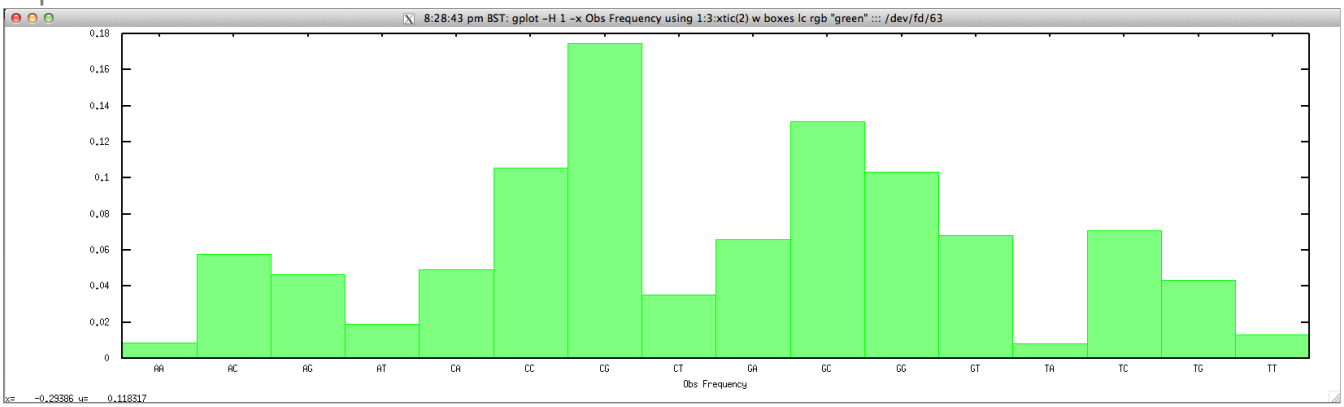
```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./comps
NODE_3_length_3390_cov_20.385250        AA      30      0.0086881       0.0625000       0.1
NODE_3_length_3390_cov_20.385250        AC      199     0.0576310       0.0625000       0.9
NODE_3_length_3390_cov_20.385250        AG      161     0.0466261       0.0625000       0.7
NODE_3_length_3390_cov_20.385250        AT      65      0.0188242       0.0625000       0.3
NODE_3_length_3390_cov_20.385250        CA      170     0.0492326       0.0625000       0.7
NODE_3_length_3390_cov_20.385250        CC      365     0.1057052       0.0625000       1.6
NODE_3_length_3390_cov_20.385250        CG      603     0.1746308       0.0625000       2.7
NODE_3_length_3390_cov_20.385250        CT      122     0.0353316       0.0625000       0.5
NODE_3_length_3390_cov_20.385250        GA      227     0.0657399       0.0625000       1.0
NODE_3_length_3390_cov_20.385250        GC      453     0.1311903       0.0625000       2.0
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./comps

NODE_3_length_3390_cov_20.385250        AAA     0       0.0000000       0.0156250       0.0
NODE_3_length_3390_cov_20.385250        AAC     18      0.0052144       0.0156250       0.3
NODE_3_length_3390_cov_20.385250        AAG     10      0.0028969       0.0156250       0.1
```

```
NODE_3_length_3390_cov_20.385250        AAT     2       0.0005794       0.0156250       0.1
NODE_3_length_3390_cov_20.385250        ACA     20      0.0057937       0.0156250       0.1
NODE_3_length_3390_cov_20.385250        ACC     76      0.0220162       0.0156250       1.4
NODE_3_length_3390_cov_20.385250        ACG     93      0.0269409       0.0156250       1.7
NODE_3_length_3390_cov_20.385250        ACT     10      0.0028969       0.0156250       0.1
NODE_3_length_3390_cov_20.385250        AGA     15      0.0043453       0.0156250       0.2
NODE_3_length_3390_cov_20.385250        AGC     82      0.0237543       0.0156250       1.5
```

For drawing the `Obs Frequency` for a given contig, we can simply use:

```
$ gplot -H 1 -x "Obs Frequency" 'using 1:3:xtic(2) w boxes lc rgb "green"' ::: <(echo "NODE
```

to produce:



We can also use GENERATEtable.sh script to compare 3-MERs `Obs Frequencey` for selected contigs:

```
$ echo "NODE_14372\d_length_\d+_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./comps
Contigs NODE_143720_length_167_cov_3.089820     NODE_143722_length_151_cov_2.304636     NO
AAA     0.1004367       0.0140845       0.0213675       0.0181818       0.0044444
AAC     0.0218341       0.0093897       0.0299145       0.0045455       0.0088889
AAG     0.0174672       0.0140845       0.0170940       0.0136364       0.0088889
AAT     0.0262009       0.0000000       0.0042735       0.0318182       0.0266667
ACA     0.0218341       0.0093897       0.0213675       0.0000000       0.0133333
ACC     0.0087336       0.0140845       0.0170940       0.0090909       0.0222222
ACG     0.0043668       0.0140845       0.0042735       0.0045455       0.0044444
ACT     0.0087336       0.0046948       0.0683761       0.0045455       0.0044444
AGA     0.0218341       0.0093897       0.0042735       0.0090909       0.0177778
AGC     0.0000000       0.0281690       0.0213675       0.0272727       0.0133333
AGG     0.0087336       0.0140845       0.0128205       0.0136364       0.0133333
AGT     0.0393013       0.0093897       0.0170940       0.0090909       0.0222222
ATA     0.0218341       0.0046948       0.0128205       0.0090909       0.0133333
ATC     0.0087336       0.0328638       0.0042735       0.0181818       0.0088889
ATG     0.0131004       0.0187793       0.0085470       0.0136364       0.0266667
ATT     0.0087336       0.0000000       0.0042735       0.0227273       0.0177778
CAA     0.0218341       0.0140845       0.0170940       0.0181818       0.0266667
CAC     0.0000000       0.0187793       0.0256410       0.0000000       0.0133333
CAG     0.0305677       0.0281690       0.0085470       0.0136364       0.0444444
CAT     0.0131004       0.0093897       0.0042735       0.0045455       0.0266667
CCA     0.0131004       0.0281690       0.0170940       0.0000000       0.0355556
CCC     0.0087336       0.0328638       0.0128205       0.0136364       0.0088889
CCG     0.0000000       0.0234742       0.0042735       0.0181818       0.0133333
CCT     0.0349345       0.0281690       0.0213675       0.0181818       0.0133333
CGA     0.0043668       0.0187793       0.0085470       0.0318182       0.0088889
CGC     0.0000000       0.0375587       0.0085470       0.0136364       0.0222222
CGG     0.0000000       0.0234742       0.0000000       0.0318182       0.0133333
CGT     0.0000000       0.0093897       0.0000000       0.0136364       0.0133333
CTA     0.0000000       0.0093897       0.0470085       0.0045455       0.0000000
CTC     0.0087336       0.0234742       0.0427350       0.0045455       0.0222222
CTG     0.0218341       0.0375587       0.0256410       0.0227273       0.0177778
CTT     0.0305677       0.0093897       0.0470085       0.0136364       0.0088889
GAA     0.0218341       0.0093897       0.0256410       0.0227273       0.0088889
GAC     0.0131004       0.0093897       0.0256410       0.0136364       0.0133333
GAG     0.0174672       0.0187793       0.0042735       0.0181818       0.0088889
GAT     0.0043668       0.0375587       0.0128205       0.0181818       0.0133333
GCA     0.0131004       0.0046948       0.0042735       0.0181818       0.0311111
GCC     0.0043668       0.0422535       0.0128205       0.0272727       0.0222222
GCG     0.0000000       0.0328638       0.0000000       0.0500000       0.0222222
GCT     0.0043668       0.0422535       0.0213675       0.0045455       0.0222222
```

```
GGA     0.0087336       0.0140845       0.0256410       0.0181818       0.0088889
GGC     0.0000000       0.0328638       0.0085470       0.0136364       0.0266667
GGG     0.0043668       0.0046948       0.0000000       0.0000000       0.0000000
GGT     0.0174672       0.0046948       0.0000000       0.0181818       0.0044444
GTA     0.0131004       0.0000000       0.0085470       0.0090909       0.0088889
GTC     0.0218341       0.0093897       0.0042735       0.0181818       0.0266667
GTG     0.0218341       0.0093897       0.0000000       0.0318182       0.0133333
GTT     0.0218341       0.0046948       0.0042735       0.0136364       0.0133333
TAA     0.0218341       0.0000000       0.0128205       0.0090909       0.0088889
TAC     0.0043668       0.0046948       0.0299145       0.0000000       0.0044444
TAG     0.0087336       0.0000000       0.0256410       0.0136364       0.0044444
TAT     0.0087336       0.0093897       0.0085470       0.0090909       0.0000000
TCA     0.0174672       0.0281690       0.0085470       0.0181818       0.0311111
TCC     0.0349345       0.0234742       0.0128205       0.0045455       0.0177778
TCG     0.0000000       0.0187793       0.0085470       0.0181818       0.0177778
TCT     0.0131004       0.0046948       0.0512821       0.0181818       0.0088889
TGA     0.0218341       0.0328638       0.0299145       0.0136364       0.0088889
TGC     0.0218341       0.0234742       0.0000000       0.0409091       0.0355556
TGG     0.0174672       0.0140845       0.0213675       0.0045455       0.0133333
TGT     0.0218341       0.0000000       0.0000000       0.0318182       0.0222222
TTA     0.0087336       0.0000000       0.0085470       0.0090909       0.0000000
TTC     0.0262009       0.0093897       0.0299145       0.0181818       0.0177778
TTG     0.0262009       0.0046948       0.0170940       0.0227273       0.0222222
TTT     0.0393013       0.0000000       0.0170940       0.0409091       0.0266667
```

where `perl -alne 'print join("\t",@F[0,1,3])'` extracts data in the right format for `GENERATEtable.sh`.

dan.sh: calculate nucleic acid melting temperature

Content of `dan.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/dan -sequence=asis:$a[1]  $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s+\n//g;
                $r=~s/\s+Start.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\n
```

Parameters [from `dan -help -verbose`]:

```
   -windowsize          integer    [20] The values of melting point and other
                                   thermodynamic properties of the sequence are
                                   determined by taking a short length of
                                   sequence known as a window and determining
                                   the properties of the sequence in that
                                   window. The window is incrementally moved
                                   along the sequence with the properties being
                                   calculated at each new position. (Integer
                                   from 1 to 100)
   -shiftincrement      integer    [1] This is the amount by which the window
                                   is moved at each increment in order to find
                                   the melting point and other properties along
                                   the sequence. (Integer 1 or more)
   -dnaconc             float      [50.] Enter DNA concentration (nM) (Number
                                   from 1.000 to 100000.000)
   -saltconc            float      [50.] Enter salt concentration (mM) (Number
                                   from 1.000 to 1000.000)
   -mintemp             float      [55.] Enter a minimum value for the
```

```
                                temperature scale (y-axis) of the plot.
                                (Number from 0.000 to 150.000)
   -product             toggle  This prompts for percent formamide, percent
                                of mismatches allowed and product length.
   -formamide           float   [0.] This specifies the percent formamide to
                                be used in calculations (it is ignored
                                unless -product is used). (Number from 0.000
                                to 100.000)
   -mismatch            float   [0.] This specifies the percent mismatch to
                                be used in calculations (it is ignored
                                unless -product is used). (Number from 0.000
                                to 100.000)
   -prodlen             integer [Window size (20)] This specifies the
                                product length to be used in calculations
                                (it is ignored unless -product is used).
                                (Any integer value)
   -thermo              toggle  Output the DeltaG, DeltaH and DeltaS values
                                of the sequence windows to the output data
                                file.
   -temperature         float   [25.] If -thermo has been specified then
                                this specifies the temperature at which to
                                calculate the DeltaG, DeltaH and DeltaS
                                values. (Number from 0.000 to 100.000)
   -rna                 boolean This specifies that the sequence is an RNA
                                sequence and not a DNA sequence.
   -sbegin1             integer Start of each sequence to be used
   -send1               integer End of each sequence to be used
   -sreverse1           boolean Reverse (if DNA)
   -snucleotide1        boolean Sequence is nucleotide
   -sprotein1           boolean Sequence is protein
   -slower1             boolean Make lower case
   -supper1             boolean Make upper case
   -scircular1          boolean Sequence is circular
```

Output format:

```
[Contig]\t[Start]\t[End]\t[Strand]\t[Tm]\t[GC]\t[DeltaG]\t[DeltaH]\t[DeltaS]\t[TmProd]\t[S
```

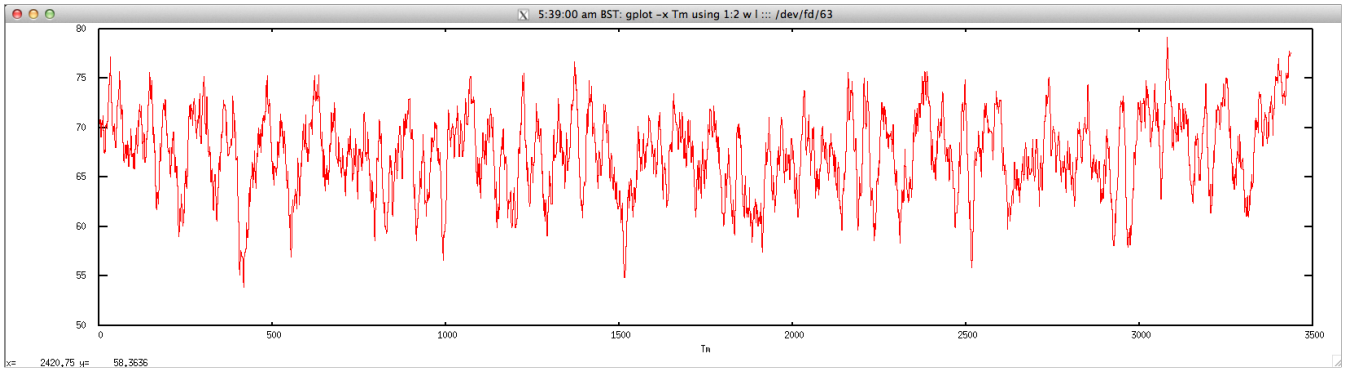
Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./dan.s

NODE_3_length_3390_cov_20.385250        1       20      +       70.8    80.0    -39.486 -1
NODE_3_length_3390_cov_20.385250        2       21      +       70.5    80.0    -39.451 -1
NODE_3_length_3390_cov_20.385250        3       22      +       70.3    80.0    -39.028 -1
NODE_3_length_3390_cov_20.385250        4       23      +       70.8    80.0    -39.486 -1
NODE_3_length_3390_cov_20.385250        5       24      +       70.8    80.0    -39.486 -1
NODE_3_length_3390_cov_20.385250        6       25      +       69.1    75.0    -38.500 -1
NODE_3_length_3390_cov_20.385250        7       26      +       69.1    75.0    -38.542 -1
NODE_3_length_3390_cov_20.385250        8       27      +       70.5    80.0    -39.451 -1
NODE_3_length_3390_cov_20.385250        9       28      +       70.3    80.0    -39.028 -1
NODE_3_length_3390_cov_20.385250        10      29      +       70.3    75.0    -39.028 -1
```

The graph for `Tm` is given below:

```
$ gplot -x "Tm" 'using 1:2 w l' ::: <(echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep
```



density.sh: calculate nucleic acid density

Content of density.sh:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/density -seqall=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s+\n//g;
                $r=~s/\s+Start.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\
```

Parameters [from `density -help -verbose`]:

```
   -window              integer    [100] Window length (Integer 1 or more)
   -sbegin1             integer    Start of each sequence to be used
   -send1               integer    End of each sequence to be used
   -sreverse1           boolean    Reverse (if DNA)
   -sprotein1           boolean    Sequence is protein
   -slower1             boolean    Make lower case
   -supper1             boolean    Make upper case
   -scircular1          boolean    Sequence is circular
```

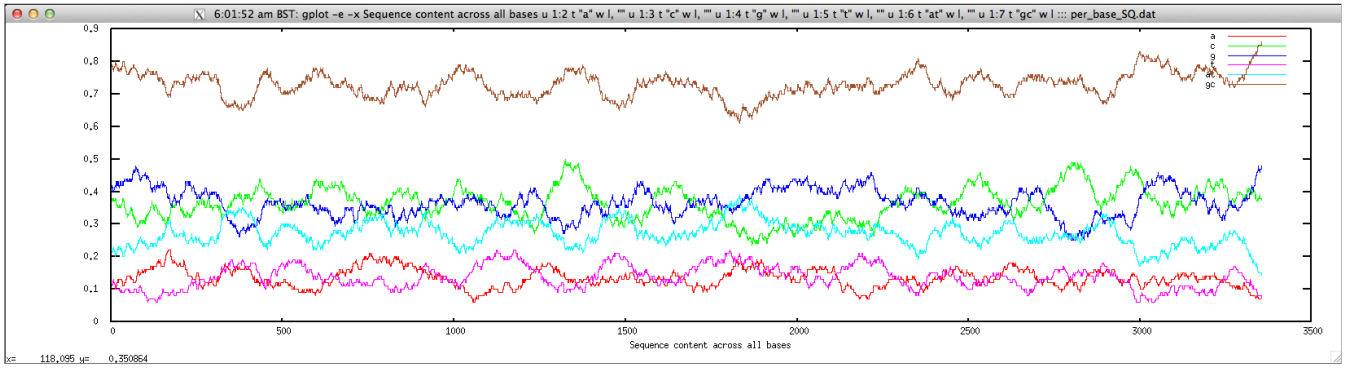
Output format:
`[Contig]\t[Start]\t[End]\t[Strand]\t[Score]\t[a]\t[c]\t[g]\t[t]\t[at]\t[gc]` Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./dens

NODE_3_length_3390_cov_20.385250        1       1       +       0.000   0.100   0.370   0.4
NODE_3_length_3390_cov_20.385250        2       2       +       0.000   0.100   0.380   0.4
NODE_3_length_3390_cov_20.385250        3       3       +       0.000   0.100   0.370   0.4
NODE_3_length_3390_cov_20.385250        4       4       +       0.000   0.100   0.370   0.4
NODE_3_length_3390_cov_20.385250        5       5       +       0.000   0.100   0.380   0.4
NODE_3_length_3390_cov_20.385250        6       6       +       0.000   0.100   0.380   0.4
NODE_3_length_3390_cov_20.385250        7       7       +       0.000   0.100   0.370   0.4
NODE_3_length_3390_cov_20.385250        8       8       +       0.000   0.100   0.380   0.4
NODE_3_length_3390_cov_20.385250        9       9       +       0.000   0.100   0.370   0.4
NODE_3_length_3390_cov_20.385250        10      10      +       0.000   0.100   0.370   0.4
```

We can plot them for a given contig as follows:

```
$ echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep --buffer-size=100000000 contigs.fa |
perl -alne 'print join("\t",@F[1,5,6,7,8,9,10])' > per_base_SQ.dat; \
gplot -e -x "Sequence content across all bases" u 1:2 t \"a\" w l, \"\" u 1:3 t \"c\" w l,
```



`cpgreport.sh`: identify and report CpG-rich regions in nucleotide sequence

Content of `cpgreport.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/cpgreport -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s*\n\s*/\n/g;
                $r=~s/CPGREPORT.*?\n//g;
                $r=~s/\nSequence.*?\n//g;
                $r=~s/asis\s+//g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\n
```

Parameters [from `cpgreport -help -verbose`]:

```
   -score              integer    [17] This sets the score for each CG
                                  sequence found. A value of 17 is more
                                  sensitive, but 28 has also been used with
                                  some success. (Integer from 1 to 200)
   -sbegin1            integer    Start of each sequence to be used
   -send1              integer    End of each sequence to be used
   -sreverse1          boolean    Reverse (if DNA)
   -snucleotide1       boolean    Sequence is nucleotide
   -sprotein1          boolean    Sequence is protein
   -slower1            boolean    Make lower case
   -supper1            boolean    Make upper case
   -scircular1         boolean    Sequence is circular
```

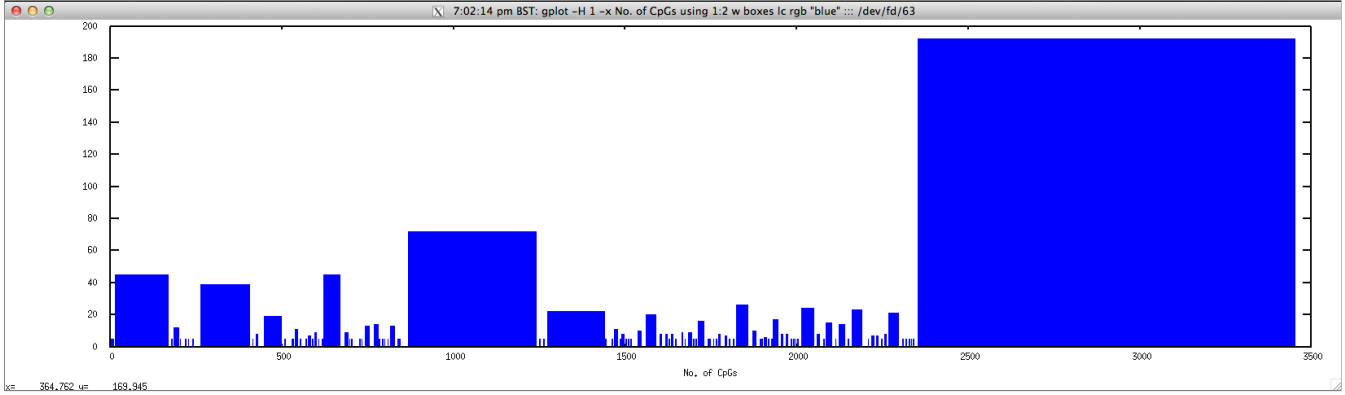
Output format:
`[Contig]\t[Begin]\t[End]\t[Score]\t[CpG]\t[%CG]\t[CG/GC]` Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./cpgr

NODE_3_length_3390_cov_20.385250        2       3452    7404    603     73.3    1.34
NODE_6_length_5675_cov_18.628546        1       5739    11380   951     73.2    1.20
NODE_11_length_1365_cov_14.082784       6       1424    3424    269     76.9    1.27
NODE_12_length_1944_cov_7.141975        5       1995    4292    349     71.9    1.54
NODE_13_length_1418_cov_18.503527       1       1480    2355    213     71.2    1.13
NODE_17_length_5129_cov_24.638330       5       5190    10835   890     73.9    1.21
NODE_18_length_2905_cov_18.701204       14      2966    5670    479     71.4    1.24
NODE_20_length_6239_cov_17.427153       2       6302    14022   1129    75.3    1.27
NODE_22_length_4091_cov_19.720362       3       4155    8250    689     73.3    1.21
NODE_24_length_4513_cov_14.317084       6       4575    7041    645     68.9    1.21
```

To graphically display No. of CpGs in the given range, use

```
$ gplot -H 1 -x "No. of CpGs" 'using 1:2 w boxes lc rgb "blue"' ::: <(echo "NODE_3_length_
./cpgreport.sh -score="5" | \
perl -alne 'for($i=$F[1];$i<=$F[2];$i++){print $i."\t".$F[3]}')
```



newcpgreport.sh: identify CpG islands in nucleotide sequence

Content of newcpgreport.sh:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/newcpgreport -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/^(.*?\n)+?FT/FT/g;
                $r=~s/FT\s+CpG island\s+(\d+)\.\.(\d+)\n/\n\1\t\2\t/g;
                $r=~s/FT\s+\/size=(\d+)\n/\1\t/g;
                $r=~s/FT\s+\/Sum C\+G=(\d+)\n/\1\t/g;
                $r=~s/FT\s+\/Percent CG=(\d+\.\d+)\n/\1\t/g;
                $r=~s/FT\s+\/ObsExp=(\d+\.\d+)\n/\1/g;
                $r=~s/FT\s+numislands\s+\d+\s*\n\/\///g;
                $r=~s/^\n//g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\n
```

Parameters [from `newcpgreport -help -verbose`]:

```
   -window              integer    [100] Window size (Integer 1 or more)
   -shift               integer    [1] Shift increment (Integer 1 or more)
   -minlen              integer    [200] Minimum Length (Integer 1 or more)
   -minoe               float      [0.6] Minimum observed/expected (Number from
                                   0.000 to 10.000)
   -minpc               float      [50.] Minimum percentage (Number from 0.000
                                   to 100.000)
   -sbegin1             integer    Start of each sequence to be used
   -send1               integer    End of each sequence to be used
   -sreverse1           boolean    Reverse (if DNA)
   -snucleotide1        boolean    Sequence is nucleotide
   -sprotein1           boolean    Sequence is protein
   -slower1             boolean    Make lower case
   -supper1             boolean    Make upper case
   -scircular1          boolean    Sequence is circular
```

Output format:
`[Contig]\t[Begin]\t[End]\t[Size]\t[Sum C+G]\t[Percent CG]\t[ObsExp]` Manual

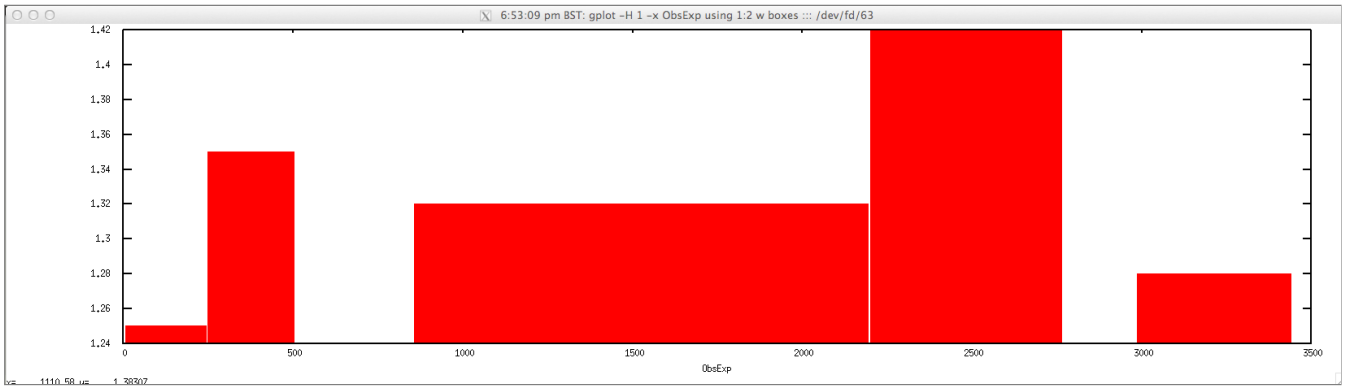
Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./newcp

NODE_3_length_3390_cov_20.385250        47      3400    3354    2444    72.87   1.30
NODE_6_length_5675_cov_18.628546        50      5684    5635    4122    73.15   1.24
NODE_11_length_1365_cov_14.082784       48      1374    1327    1025    77.24   1.27
NODE_12_length_1944_cov_7.141975        47      1952    1906    1370    71.88   1.36
NODE_13_length_1418_cov_18.503527       48      1426    1379    985     71.43   1.13
NODE_17_length_5129_cov_24.638330       47      5139    5093    3762    73.87   1.26
NODE_18_length_2905_cov_18.701204       47      2913    2867    2041    71.19   1.27
```

```
NODE_20_length_6239_cov_17.427153       47      6249    6203    4666    75.22   1.26
NODE_22_length_4091_cov_19.720362       47      4101    4055    2959    72.97   1.24
NODE_24_length_4513_cov_14.317084       47      4521    4475    3081    68.85   1.18
```

For plotting `ObsExp` for a selected contig:

```
$ gplot -H 1 -x "ObsExp" 'using 1:2 w boxes' ::: <(echo "NODE_3_length_3390_cov_20.385250"
FASTAgrep --buffer-size=100000000 contigs.fa | ./newcpgreport.sh "-window=20" | \
perl -alne 'for($i=$F[1];$i<=$F[2];$i++){print $i."\t".$F[6]}')
```



`fuzznuc.sh`: search for patterns in nucleotide sequences

Content of [`fuzznuc.sh`]:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/fuzznuc -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s+\n//g;
                $r=~s/\s+Start.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\
```

Parameters [from `fuzznuc -help -verbose`]:

```
   -pattern            pattern    The standard IUPAC one-letter codes for the
                                  nucleotides are used.
                                  The symbol 'n' is used for a position where
                                  any nucleotide is accepted.
                                  Ambiguities are indicated by listing the
                                  acceptable nucleotides for a given position,
                                  between square parentheses '[ ]'. For
                                  example: [ACG] stands for A or C or G.
                                  Ambiguities are also indicated by listing
                                  between a pair of curly brackets '{ }' the
                                  nucleotides that are not accepted at a given
                                  position. For example: {AG} stands for any
                                  nucleotides except A and G.
                                  Each element in a pattern is separated from
                                  its neighbor by a '-'. (Optional in
                                  fuzznuc).
                                  Repetition of an element of the pattern can
                                  be indicated by following that element with
                                  a numerical value or a numerical range
                                  between parenthesis. Examples: N(3)
                                  corresponds to N-N-N, N(2,4) corresponds to
                                  N-N or N-N-N or N-N-N-N.
                                  When a pattern is restricted to either the
```

```
                                5' or 3' end of a sequence, that pattern
                                either starts with a '<' symbol or
                                respectively ends with a '>' symbol.
                                A period ends the pattern. (Optional in
                                fuzznuc).
                                For example, [CG](5)TG{A}N(1,5)C
   -complement          boolean    [N] Search complementary strand
   -sbegin1             integer    Start of each sequence to be used
   -send1               integer    End of each sequence to be used
   -sreverse1           boolean    Reverse (if DNA)
   -snucleotide1        boolean    Sequence is nucleotide
   -sprotein1           boolean    Sequence is protein
   -slower1             boolean    Make lower case
   -supper1             boolean    Make upper case
   -scircular1          boolean    Sequence is circular
   -pmismatch           integer    Pattern mismatch
   -pname               string     Pattern base name
```

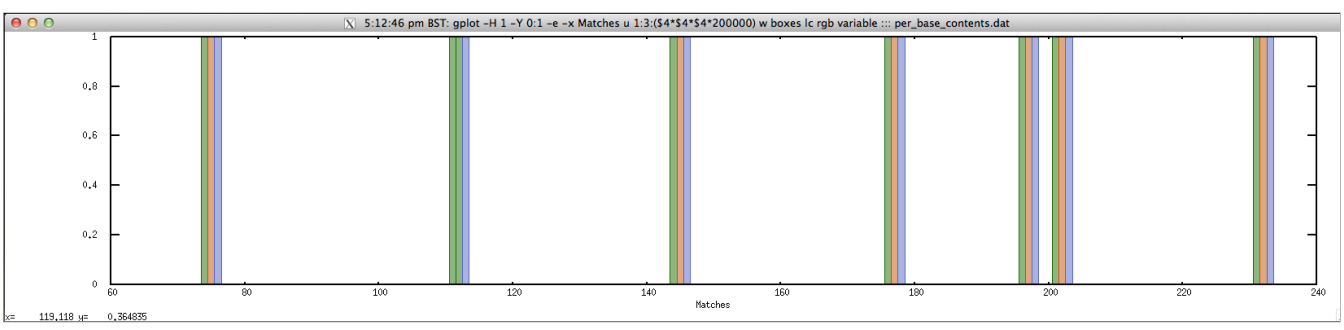
Output format:
`[Contig]\t[Start]\t[End]\t[Strand]\t[Pattern]\t[Mismatch]\t[Sequence]` Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./fuzzn

NODE_3_length_3390_cov_20.385250        3424    3433    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        3424    3436    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        3349    3361    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        3001    3010    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        3001    3011    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        3001    3013    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        2914    2923    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        2914    2924    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        2914    2926    +       pattern:[CG](5)TG{A}N(1,5)C
NODE_3_length_3390_cov_20.385250        2568    2580    +       pattern:[CG](5)TG{A}N(1,5)C
```

For shorter contigs, we can view the matches on the length of the contig:

```
$ echo "NODE_143725_length_172_cov_2.023256" | FASTAgrep --buffer-size=100000000 contigs.fa
./fuzznuc.sh "-pattern=C{A}G" | \
perl -ane '$j=0;@v=split("",$F[6]);for($i=$F[1];$i<=$F[2];$i++){$r{$i}=$v[$j++]}}{foreach m
$ cat per_base_content.dat | head -10
74      C       1       2
75      T       1       4
76      G       1       3
111     C       1       2
112     C       1       2
113     G       1       3
144     C       1       2
145     T       1       4
146     G       1       3
176     C       1       2
$ gplot -H 1 -Y 0:1 -e -x "Matches" 'u 1:3:($4*$4*$4*200000) w boxes lc rgb variable' ::: p
```



`fuzztran.sh`: search for patterns in protein sequences (translated)

Content of fuzztran.sh:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/fuzztran -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s*\n\s*/\n/g;
                $r=~s/\nStart.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\n
```

Parameters [from `fuzztran -help -verbose`]:

```
   -pattern            pattern    The standard IUPAC one-letter codes for the
                                  amino acids are used.
                                  The symbol 'x' is used for a position where
                                  any amino acid is accepted.
                                  Ambiguities are indicated by listing the
                                  acceptable amino acids for a given position,
                                  between square parentheses '[ ]'. For
                                  example: [ALT] stands for Ala or Leu or Thr.
                                  Ambiguities are also indicated by listing
                                  between a pair of curly brackets '{ }' the
                                  amino acids that are not accepted at a gven
                                  position. For example: {AM} stands for any
                                  amino acid except Ala and Met.
                                  Each element in a pattern is separated from
                                  its neighbor by a '-'. (Optional in
                                  fuzztran)
                                  Repetition of an element of the pattern can
                                  be indicated by following that element with
                                  a numerical value or a numerical range
                                  between parenthesis. Examples: x(3)
                                  corresponds to x-x-x, x(2,4) corresponds to
                                  x-x or x-x-x or x-x-x-x.
                                  When a pattern is restricted to either the
                                  N- or C-terminal of a sequence, that pattern
                                  either starts with a '<' symbol or
                                  respectively ends with a '>' symbol.
                                  A period ends the pattern. (Optional in
                                  fuzztran).
   -frame              menu       [1] Frame(s) to translate (Values: 1 (1); 2
                                  (2); 3 (3); F (Forward three frames); -1
                                  (-1); -2 (-2); -3 (-3); R (Reverse three
                                  frames); 6 (All six frames))
   -table              menu       [0] Code to use (Values: 0 (Standard); 1
                                  (Standard (with alternative initiation
                                  codons)); 2 (Vertebrate Mitochondrial); 3
                                  (Yeast Mitochondrial); 4 (Mold, Protozoan,
                                  Coelenterate Mitochondrial and
                                  Mycoplasma/Spiroplasma); 5 (Invertebrate
                                  Mitochondrial); 6 (Ciliate Macronuclear and
                                  Dasycladacean); 9 (Echinoderm
                                  Mitochondrial); 10 (Euplotid Nuclear); 11
                                  (Bacterial); 12 (Alternative Yeast Nuclear);
                                  13 (Ascidian Mitochondrial); 14 (Flatworm
                                  Mitochondrial); 15 (Blepharisma
                                  Macronuclear); 16 (Chlorophycean
                                  Mitochondrial); 21 (Trematode
                                  Mitochondrial); 22 (Scenedesmus obliquus);
                                  23 (Thraustochytrium Mitochondrial))
   -sbegin1            integer    Start of each sequence to be used
```

```
   -send1             integer    End of each sequence to be used
   -sreverse1         boolean    Reverse (if DNA)
   -snucleotide1      boolean    Sequence is nucleotide
   -sprotein1         boolean    Sequence is protein
   -slower1           boolean    Make lower case
   -supper1           boolean    Make upper case
   -scircular1        boolean    Sequence is circular
   -pmismatch         integer    Pattern mismatch
   -pname             string     Pattern base name
```

Output format:
`[Contig]\t[Start]\t[End]\t[Strand]\t[Score]\t[Pattern]\t[Mismatch]\t[Frame]\t[PStart]\t[PE`
Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./fuzz

NODE_31_length_15435_cov_19.081308      13504   13521   +       6       pattern:V{V}VVVL
NODE_729_length_5451_cov_18.101265      4561    4578    +       6       pattern:V{V}VVVL
NODE_934_length_8261_cov_16.588669      5125    5142    +       6       pattern:V{V}VVVL
NODE_1805_length_3627_cov_17.087124     1396    1413    +       6       pattern:V{V}VVVL
NODE_2046_length_9730_cov_19.413464     9202    9219    +       6       pattern:V{V}VVVL
NODE_2514_length_8896_cov_14.840940     7588    7605    +       6       pattern:V{V}VVVL
NODE_5074_length_2208_cov_10.750453     619     636     +       6       pattern:V{V}VVVL
NODE_8554_length_1025_cov_11.535610     847     864     +       6       pattern:V{V}VVVL
NODE_8554_length_1025_cov_11.535610     922     939     +       6       pattern:V{V}VVVL
NODE_10591_length_4696_cov_18.995316    3610    3627    +       6       pattern:V{V}VVVL
```

`freak.sh`: generate residue/base frequency table

Content of `freak.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/freak -seqall=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/\s+\n/\n/g;
                $r=~s/FREAK.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\
```

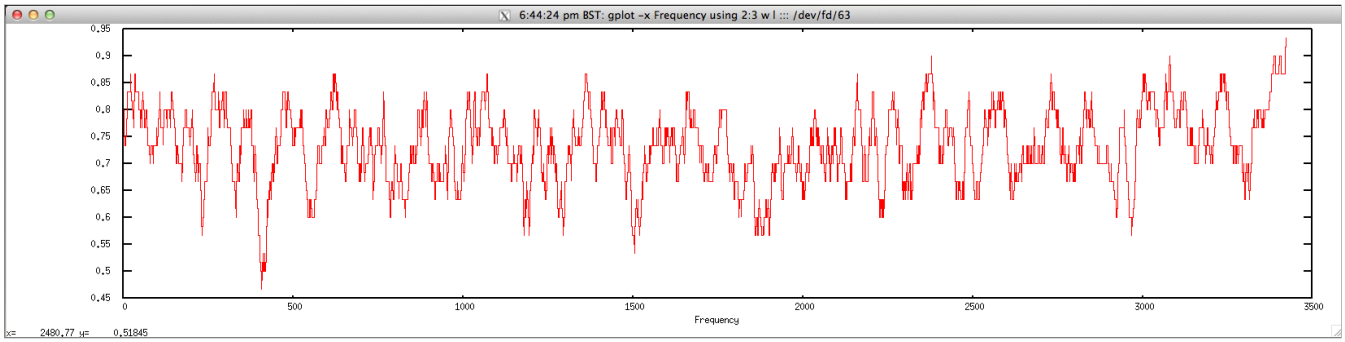
Parameters [from `freak -help -verbose`]:

```
   -letters           string     [gc] Residue letters (Any string)
   -step              integer    [1] Stepping value (Any integer value)
   -window            integer    [30] Averaging window (Any integer value)
   -sbegin1           integer    Start of each sequence to be used
   -send1             integer    End of each sequence to be used
   -sreverse1         boolean    Reverse (if DNA)
   -snucleotide1      boolean    Sequence is nucleotide
   -sprotein1         boolean    Sequence is protein
   -slower1           boolean    Make lower case
   -supper1           boolean    Make upper case
   -scircular1        boolean    Sequence is circular
```

Output format:
`[Contig]\t[Start]\t[Frequency]` Manual

Example usage:

```
$ echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep --buffer-size=100000000 contigs.fa |

NODE_3_length_3390_cov_20.385250        1       0.800000
NODE_3_length_3390_cov_20.385250        2       0.766667
NODE_3_length_3390_cov_20.385250        3       0.733333
NODE_3_length_3390_cov_20.385250        4       0.733333
NODE_3_length_3390_cov_20.385250        5       0.733333
NODE_3_length_3390_cov_20.385250        6       0.733333
NODE_3_length_3390_cov_20.385250        7       0.733333
NODE_3_length_3390_cov_20.385250        8       0.766667
NODE_3_length_3390_cov_20.385250        9       0.766667
NODE_3_length_3390_cov_20.385250        10      0.766667
$ gplot -x "Frequency" 'using 2:3 w l' ::: <(echo "NODE_3_length_3390_cov_20.385250" | FAST
```



`etandem.sh`: find tandem repeats in a nucleotide sequence

Content of `etandem.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/etandem -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s+Start.*?\n//g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\
```

Parameters [from `etandem -help -verbose`]:

```
   -minrepeat          integer    [10] Minimum repeat size (Integer, 2 or
                                  higher)
   -maxrepeat          integer    [Same as -minrepeat] Maximum repeat size
                                  (Integer, same as -minrepeat or higher)
   -threshold          integer    [20] Threshold score (Any integer value)
   -mismatch           boolean    Allow N as a mismatch
   -uniform            boolean    Allow uniform consensus
   -sbegin1            integer    Start of the sequence to be used
   -send1              integer    End of the sequence to be used
   -sreverse1          boolean    Reverse (if DNA)
   -snucleotide1       boolean    Sequence is nucleotide
   -sprotein1          boolean    Sequence is protein
   -slower1            boolean    Make lower case
   -supper1            boolean    Make upper case
   -scircular1         boolean    Sequence is circular
```

Output format:
`[Contig]\t[Start]\t[End]\t[Strand]\t[Score]\t[Size]\t[Count]\t[Identity]\t[Consensus]`
Manual

Example usage:

```
$ echo "NODE_\d+_length_(\d){4,}_" | FASTAgrep --buffer-size=100000000 contigs.fa | ./etand

NODE_554_length_5056_cov_16.977057      4517    4576    +       21      5       12      71
NODE_644_length_7589_cov_17.300699      5464    5578    +       20      5       23      60
NODE_1822_length_12545_cov_19.939497    6789    6903    +       38      5       23      68
NODE_1824_length_12270_cov_17.698696    8624    8698    +       20      5       15      66
NODE_1825_length_3666_cov_17.308783     570     624     +       20      5       11      72
NODE_1860_length_13418_cov_18.133999    616     730     +       20      5       23      60
NODE_2108_length_12563_cov_19.594921    1376    1440    +       22      5       13      70
NODE_2175_length_7718_cov_23.064524     1406    1480    +       24      5       15      69
NODE_2472_length_2810_cov_16.679716     484     578     +       20      5       19      63
NODE_2982_length_3833_cov_14.351161     547     726     +       43      5       36      63
```

`tcode.sh`: identify protein-coding regions using Fickett TESTCODE statistic

Content of `tcode.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/tcode -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r=~s/#.*\n//g;
                $r=~s/\s+Start.*?\n//g;
                $r=~s/No opinion/No_opinion/g;
                $r=~s/^\s+//g;
                $r=~s/\n\s+/\n/g;
                $r=~s/\h+/\t/g;
                if (defined $r and length $r){
                        print substr($a[0],1)."\t".join("\n".substr($a[0],1)."\t",split("\n
```

Parameters [from `tcode -help -verbose`]:

```
   -window              integer    [200] This is the number of nucleotide bases
                                   over which the TESTCODE statistic will be
                                   performed each time. The window will then
                                   slide along the sequence, covering the same
                                   number of bases each time. (Integer 200 or
                                   more)
   -step                integer    [3] The selected window will, by default,
                                   slide along the nucleotide sequence by three
                                   bases at a time, retaining the frame
                                   (although the algorithm is not frame
                                   sensitive). This may be altered to increase
                                   or decrease the increment of the slide.
                                   (Integer 1 or more)
   -sbegin1             integer    Start of each sequence to be used
   -send1               integer    End of each sequence to be used
   -sreverse1           boolean    Reverse (if DNA)
   -snucleotide1        boolean    Sequence is nucleotide
   -sprotein1           boolean    Sequence is protein
   -slower1             boolean    Make lower case
   -supper1             boolean    Make upper case
   -scircular1          boolean    Sequence is circular
```

Output format:
`[Contig]\t[Start]\t[End]\t[Strand]\t[Score]\t[Estimation]` Manual

Example usage:

```
$ echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep --buffer-size=100000000 contigs.fa |
NODE_3_length_3390_cov_20.385250        1       200     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        4       203     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        7       206     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        10      209     +       1.250   Coding
```

```
NODE_3_length_3390_cov_20.385250        13      212     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        16      215     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        19      218     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        22      221     +       1.250   Coding
NODE_3_length_3390_cov_20.385250        25      224     +       1.232   Coding
NODE_3_length_3390_cov_20.385250        28      227     +       1.232   Coding
$ echo "NODE_143725_length_172_cov_2.023256" | FASTAgrep --buffer-size=100000000 contigs.fa
NODE_143725_length_172_cov_2.023256     1       200     +       0.480   Non-coding
NODE_143725_length_172_cov_2.023256     4       203     +       0.469   Non-coding
NODE_143725_length_172_cov_2.023256     7       206     +       0.556   Non-coding
NODE_143725_length_172_cov_2.023256     10      209     +       0.624   Non-coding
NODE_143725_length_172_cov_2.023256     13      212     +       0.590   Non-coding
NODE_143725_length_172_cov_2.023256     16      215     +       0.590   Non-coding
NODE_143725_length_172_cov_2.023256     19      218     +       0.658   Non-coding
NODE_143725_length_172_cov_2.023256     22      221     +       0.527   Non-coding
NODE_143725_length_172_cov_2.023256     25      224     +       0.576   Non-coding
NODE_143725_length_172_cov_2.023256     28      227     +       0.548   Non-coding
```

`getorf.sh`: find and extract open reading frames (ORFs)

Content of `getorf.sh`:

```
#!/bin/bash
less <&0| \
        perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
        perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
        if ($. % 2 == 0){
                chomp $a[0];
                chomp $a[1];
                $r=qx/getorf -sequence=asis:$a[1] $o -stdout -auto 2>\/dev\/null/;
                $r =~ s/>asis/$a[0]/g;
                print $r}' -- -o=$2 | \
                perl -pe '/^>/?s/^>/\n>/:s/\s*$// if$.>1' | \
                perl -nse 'push @a, $_; @a = @a[@a-2..$#a];
                if ($. % 2 == 0){
                        chomp $a[0];
                        $a[0]=~/>(.*?) \[(\d+) - (\d+)\]\s*(.*)/g;
                        $s=(($4=~y===c)=="0")?"+":"-";
                        if($f eq "f"){
                                print ">".$1."_".$2."_".$3."_".$s."\n".$a[1]}
                        elsif($f eq "t"){
                                print $1."\t".$2."\t".$3."\t".$4."\t".$s."\t".$a[1]}}' --
```

Parameters [from `getorf -help -verbose`]:

```
   -table              menu       [0] Code to use (Values: 0 (Standard); 1
                                  (Standard (with alternative initiation
                                  codons)); 2 (Vertebrate Mitochondrial); 3
                                  (Yeast Mitochondrial); 4 (Mold, Protozoan,
                                  Coelenterate Mitochondrial and
                                  Mycoplasma/Spiroplasma); 5 (Invertebrate
                                  Mitochondrial); 6 (Ciliate Macronuclear and
                                  Dasycladacean); 9 (Echinoderm
                                  Mitochondrial); 10 (Euplotid Nuclear); 11
                                  (Bacterial); 12 (Alternative Yeast Nuclear);
                                  13 (Ascidian Mitochondrial); 14 (Flatworm
                                  Mitochondrial); 15 (Blepharisma
                                  Macronuclear); 16 (Chlorophycean
                                  Mitochondrial); 21 (Trematode
                                  Mitochondrial); 22 (Scenedesmus obliquus);
                                  23 (Thraustochytrium Mitochondrial))
   -minsize            integer    [30] Minimum nucleotide size of ORF to
                                  report (Any integer value)
   -maxsize            integer    [1000000] Maximum nucleotide size of ORF to
                                  report (Any integer value)
   -find               menu       [0] This is a small menu of possible output
                                  options. The first four options are to
                                  select either the protein translation or the
```

		original nucleic acid sequence of the open reading frame. There are two possible definitions of an open reading frame: it can either be a region that is free of STOP codons or a region that begins with a START codon and ends with a STOP codon. The last three options are probably only of interest to people who wish to investigate the statistical properties of the regions around potential START or STOP codons. The last option assumes that ORF lengths are calculated between two STOP codons. (Values: 0 (Translation of regions between STOP codons); 1 (Translation of regions between START and STOP codons); 2 (Nucleic sequences between STOP codons); 3 (Nucleic sequences between START and STOP codons); 4 (Nucleotides flanking START codons); 5 (Nucleotides flanking initial STOP codons); 6 (Nucleotides flanking ending STOP codons))
-[no]methionine	boolean	[Y] START codons at the beginning of protein products will usually code for Methionine, despite what the codon will code for when it is internal to a protein. This qualifier sets all such START codons to code for Methionine by default.
-circular	boolean	[N] Is the sequence circular
-[no]reverse	boolean	[Y] Set this to be false if you do not wish to find ORFs in the reverse complement of the sequence.
-flanking	integer	[100] If you have chosen one of the options of the type of sequence to find that gives the flanking sequence around a STOP or START codon, this allows you to set the number of nucleotides either side of that codon to output. If the region of flanking nucleotides crosses the start or end of the sequence, no output is given for this codon. (Any integer value)
-sbegin1	integer	Start of each sequence to be used
-send1	integer	End of each sequence to be used
-sreverse1	boolean	Reverse (if DNA)
-snucleotide1	boolean	Sequence is nucleotide
-sprotein1	boolean	Sequence is protein
-slower1	boolean	Make lower case
-supper1	boolean	Make upper case
-scircular1	boolean	Sequence is circular

Output format
"f":>[Contig]_[ORF.No]_[Start]_[End]_[Strand]\n[Sequence] Manual
"t":[Contig]_[ORF.No]\t[Start]\t[End]\t[Strand]\t[Sequence]

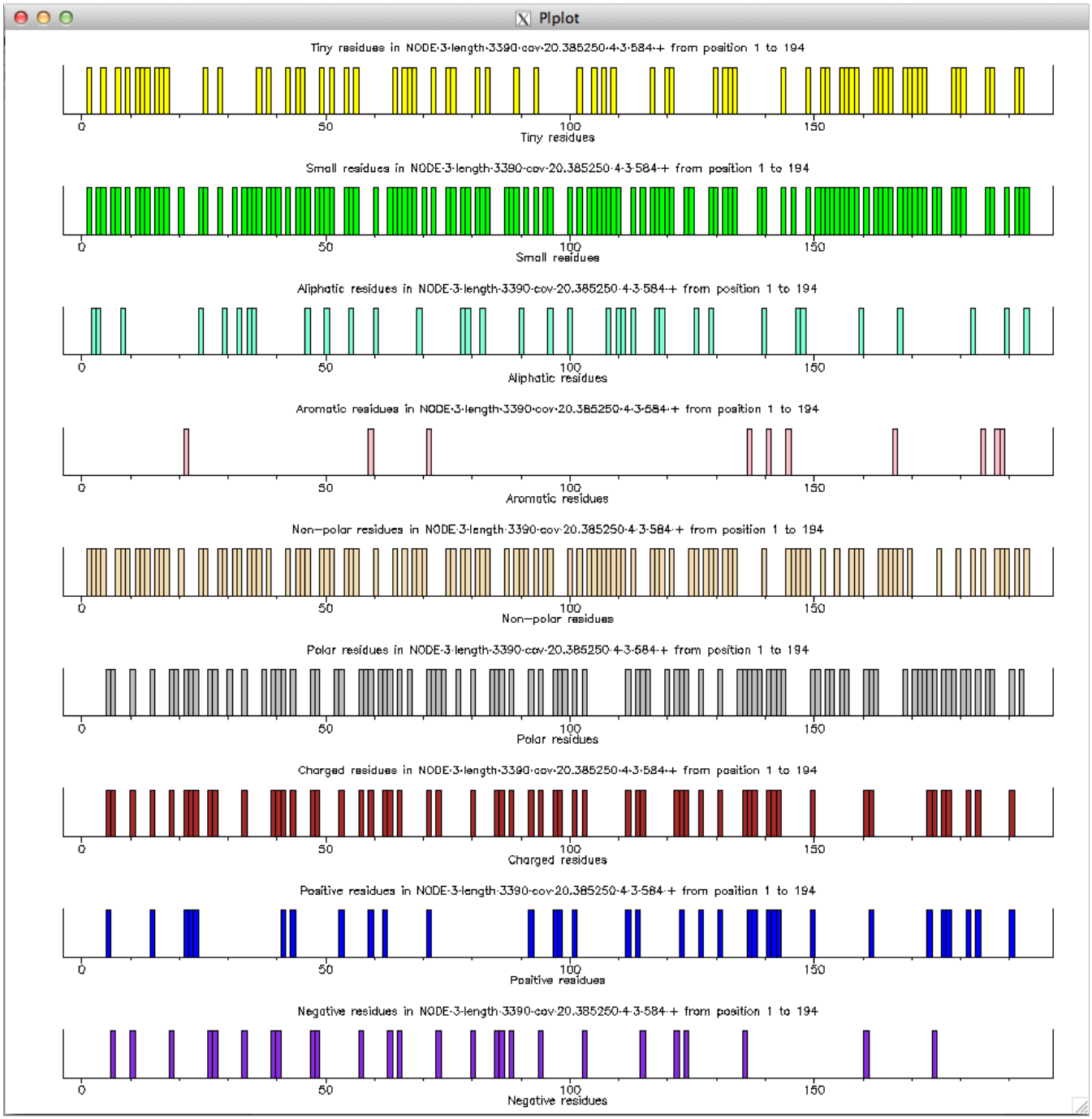
Example usage:

```
$ echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep --buffer-size=100000000 contigs.fa |

>NODE_3_length_3390_cov_20.385250_1_1_234_+
AGSLPATASVKPPPGPVVSSRTGASPRRRCSRSTSSGRQTTAVAPESRTAWRSGASGNSTFSGTPTPPACHTPSRPGR
>NODE_3_length_3390_cov_20.385250_2_56_343_+
AAAPARRRGGVAAARRRRAGRRPRSRRSRGRRGAAGRRGTARSAGRRRHRPATHRAGRAGSRGCWAGRTRRAPPVRARAPTGARRTGPRCI
>NODE_3_length_3390_cov_20.385250_3_238_504_+
SRVLGRKNPTRSPGASPCADRCAANRAPVSAYCAYVTRVSSRPSATRSAWSDALRRKSIAMFIDDPSLCSAGTPRTRRPRPWTAARRPG
>NODE_3_length_3390_cov_20.385250_4_3_584_+
GLVARDGLGEAAARAGGEQPHRRVAEEALQPLDVVGQADDRGRAGVEDGVAQRGVGEQHVQRDADATGLPHTEQAGQVVEGVGQEEPDALI
>NODE_3_length_3390_cov_20.385250_5_588_761_+
SGIAASSWAFATAVASAASASGSTTFAMPSSSARSAPISGASSISSRARCIPTIRGSR
$ echo "NODE_3_length_3390_cov_20.385250" | FASTAgrep --buffer-size=100000000 contigs.fa |

NODE_3_length_3390_cov_20.385250_1      1       234             +       AGSLPATASVKPPPGPVVS
```

```
NODE_3_length_3390_cov_20.385250_2      56      343             +       AAAPARRRGGVAAARRRRA
NODE_3_length_3390_cov_20.385250_3      238     504             +       SRVLGRKNPTRSPGASPCA
NODE_3_length_3390_cov_20.385250_4      3       584             +       GLVARDGLGEAAARAGGEG
NODE_3_length_3390_cov_20.385250_5      588     761             +       SGIAASSWAFATAVASAAS
NODE_3_length_3390_cov_20.385250_6      508     795             +       RPPAPGTPAGRRRGCGSPA
NODE_3_length_3390_cov_20.385250_7      799     831             +       RTPRRCGRRRT
NODE_3_length_3390_cov_20.385250_8      765     875             +       APATSGTMPRLTNTSQMRA
NODE_3_length_3390_cov_20.385250_9      879     944             +       AMPDTAATTGSRDSQIST
NODE_3_length_3390_cov_20.385250_10     865     1047            +       SRRRRRCPTPPRPRAAATP
```

You can then use pepinfo to draw amino-acid properties for `NODE_3_length_3390_cov_20.385250_4_3_584_+` by saving the sequence in a file `test.faa` and calling it as follows:

```
$ pepinfo test.faa -auto
```



Last Updated by Dr Umer Zeeshan Ijaz on 20/04/2014.