Supplementary material for 'Drawing and editing the secondary structure(s) of RNA': Command-lines and code listings

Yann Ponty*
CNRS, LIX – UMR 7161 & INRIA AMIB. Ecole Polytechnique, Palaiseau, France
Fabrice Leclerc
CNRS, IGM – UMR 8621, Université Paris Sud, Orsay, France

Contents

*To whom correspondence should be addressed (yann.ponty@lix.polytechnique.fr)

1 Running VARNA (Linear layout → SVG output)

```
# Running VARNA (Linear layout/SVG output)
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd
   -i XXXX.yyy -o out.svg -algorithm line
```

2 Running R-chie (Linear layout → PDF output)

```
# Running R-chie (Linear layout/PDF output)
Rscript rchie.R --format1 "vienna" --pdf --output=out.pdf XXX.yyy
```

3 Running VARNA (Circular layout → PostScript output)

```
# Running VARNA (Circular layout/PostScript output)
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd
   -i XXXX.yyy -o out.eps -algorithm circular
```

4 Running RNAView to extract a 2D structure from a 3D model (PDB → RNAML)

```
# Running RNAView on RNA 3D model (PDB format)
rnaview -p XXXX.pdb
```

5 Running VARNA on the extended secondary structure (RNAML → Vector graphics)

```
# Running VARNA on the ext. sec. str. (RNAML -> Vector graphics)
java -cp VARNAvX-Y.jar fr.orsay.lri.varna.applications.VARNAcmd
   -i XXXX.pdb.xml -o YYYY.svg
```

6 Running VARNA (RNAML → VARNA session file)

```
# Running \Software{VARNA} (RNAML -> VARNA session file)
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd
   -i XXXX.pdb.xml -o YYYY.varna
```

7 Running VARNA (NAView algorithm → PostScript)

```
# Running VARNA (NAView algorithm -> PostScript output)
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd
   -i XXXX.pdb.xml -o YYYY.eps -algorithm naview
```

8 Running VARNA to draw the shadow of a secondary structure

```
# Running VARNA (NAView algorithm -> PostScript output)
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd -i XXXX -o YYYY
    -flat true -highlightRegion "1-ZZZ:fill=#A0A0A0,outline=#A0A0A0,radius=10"
    -drawBases false -backbone "#A0A0A0" -fillBases "#A0A0A0" -bpStyle simple
    -numPeriod 50
```

9 Running RNAplot (NAView algorithm → PostScript)

```
# Running RNAplot (NAView algorithm)
RNAplot < xxxx.yyy
```

10 Running RNAplot (Radial algorithm)

```
# Running RNAplot (Radial algorithm)
RNAplot  --layout-type=0 < xxxx.yyy
```

11 Running RNAplot with additional Postscript annotations

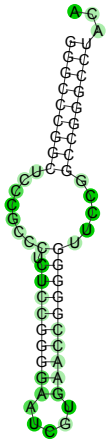
```
# Running RNAplot from a DBN file 'XXX.txt'

# Marking nucleotide 10
RNAplot --post "10 cmark" < XXX.txt
# Drawing backbone for region (10,15) using red color (r=1,g=b=0) and line thickness 2
RNAplot --pre "10 15 2 1. 0. 0. omark" < XXX.txt
# Defining a custom PS macro and using it to fill base number 10 in blue (r=g=0, b=1)
RNAplot --pre "/cfmark {setrgbcolor newpath 1 sub coor exch get aload pop fsize 2 div 0 360 arc fill} bind
    def 10 0. 0. 1. cfmark" < XXX.txt
```

12 Using RNAplot to display probing values as a color map

Listing 1: Using RNAplot to display probing values as a color map.

```
import os,sys
def getColor(val, minval, maxval,col1=(1.,1.,1.),col2=(0.0,0.8,0.0)):
    (r1,g1,b1),(r2,g2,b2) = col1,col2
    span = float(maxval)-float(minval)
    k = (float(val)-float(minval))/(span if span!=0 else 1.0)
    l = 1.-k
    return (r1*l+r2*k,g1*l+g2*k,b1*l+b2*k)
def formatValuesAsPS(values):
    minval,maxval = min(values),max(values)
    valtab = [" %s %s cfmark"%(i+1,"%.2f %.2f %.2f"%getColor(v,minval,maxval)) for i,v
        in enumerate(values)]
    return  "".join(valtab)
# Invokes RNAplot to display a color map
#   Arg1: path to DBN file
#   Arg2: list of comma-separated values (eg "1,2,3")
if __name__ == "__main__":
    inputFile = sys.argv[1]
    values = sys.argv[2].split(",")
    extraMacro = "/cfmark {setrgbcolor newpath 1 sub coor exch get aload pop fsize 2
        div 0 360 arc fill} bind def"
    os.system("RNAplot --pre \" %s %s\" < %s"%(extraMacro,formatValuesAsPS(values),
        inputFile))
```



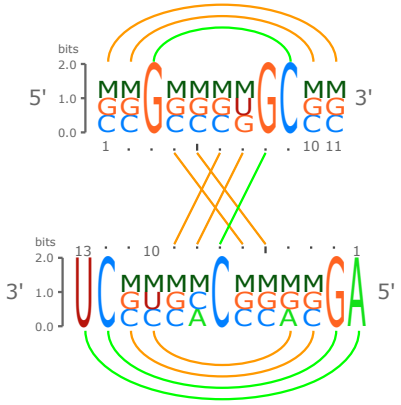
13 Script for formatting accessibility values as a color map using RNAplot

Listing 2: Fasta-like input file XXXX.txt expected by RILogo

```
>organism1
GCGGGGUGCGC&AGGACCCACUCCU
>organism2
CGGCCCGGCCG&AGCGGGCCGCGCU
>structure
(((AABBB)))&((((aabbb))))
```

Listing 3: Invoking RILogo

```
rilogo XXXX.txt > output.svg
```



14 Script for preprocessing a Stockholm file as input accepted by R2R

Listing 4: Transforming an Stockholm-formatted RFAM multiple sequence alignment into an unblocked alignment accepted by R2R as input.

```
import os,sys,string
def unblockStockholm(inpath,outpath):
    outfile = open(outpath,"w")
    (seqIDs,seqContents) = ([],{})
    for l in open(inpath):
                if len(l)>12 and ((not l.startswith("#")) or (l[:12] in ["#=GC
                    SS_cons","#=GC RF      "])):
                        (id,content) = (l[0:37].strip(),l[37:-1].strip())
                        if id not in seqContents:
                                seqIDs.append(id)
                                seqContents[id] = ""
                        seqContents[id] += content
                elif len(l)>1:
                        for id in seqIDs:
                                outfile.write(string.ljust(id, 37)+seqContents[id
                                    ]+"\n")
                        seqIDs,seqContents = [],{}
                        outfile.write(l)
    outfile.close()
# Transforms a 'blocked' STOCKHOLM file (eg RFAM alignment) into a linear one
#   Arg1: path to 'blocked' STOCKHOLM file
#   Arg2: path to 'linearized' STOCKHOLM output file
if __name__=="__main__":
    (inStoc,outFile) = (sys.argv[1],sys.argv[2])
    unblockStockholm(inStoc,outFile)
```

15 Running R2R to precompute conservation levels

```
# Compute conservation levels
```

```
r2r --GSC-weighted-consensus XXXX.sto XXXX.cons.sto 3 0.97 0.9 0.75 4 0.97 0.9
    0.75 0.5 0.1
```

16 Simple input meta file for R2R

```
XXXX.user.sto
XXXX.user.sto oneseq Pyrococcus_furiosus
XXXX.user.sto skeleton-with-pairbonds
```

17 Running R2R (Stockhlom/meta → SVG/PDF)

```
r2r XXXX.meta XXXX.pdf
r2r XXXX.meta XXXX.svg
```

18 Running RChie (Fasta multiple sequence alignment+DBN → PDF)

```
Rscript rchie.R --msafile XXXX.txt --colour1 "#4DAF4A" --msacol "#00A651,#0072BC
    ,#00B9F2,#F15A22,#231F20,#AAAAAA,#DA6FAB" --pdf --output="out.pdf" --format1
     "vienna" YYYY.txt
```

19 Script to generate a tree representation using GraphViz (DBN → DOT)

```
import sys
# Get custom styles by changing these lines (cf GraphViz manual)
STYLE_DEFAULT = "shape=\"rectangle\",style=filled,margin=\"0,0\",fontsize=20,color=grey40,fontcolor=grey20,
    fillcolor=grey90,fontname=\"Helvetica\""
STYLE_UNPAIRED = "shape=\"circle\",color=blue,fillcolor=aliceblue"
STYLE_PAIRED = "shape=\"hexagon\""
STYLE_EDGES = "color=grey50"
# Converts an RNA sequence/secondary structure (dot-parenthesis notation) into a DOT-formatted GraphViz input
    , written into a previously opened file f
def drawAsDOT (seq,secstr,f):
    print >> f, "digraph rna{\n  node [%s];\n  edge [%s];\n  -1 [label=\"Root\"];"%(STYLE_DEFAULT,STYLE_EDGES
        )
    stack = [-1]
    for i in range(len(secstr)):
        k = stack[-1]
        if secstr[i]=="(":
            stack.append(i)
            print >> f, "  %s -> %s;"%(k,i)
        elif secstr[i]==")":
            stack.pop()
            print >> f, "  %s [label=\"%s\",%s];"%(k,seq[k]+seq[i],STYLE_PAIRED)
        else:
            print >> f, "  %s -> %s;"%(k,i)
            print >> f, "  %s [label=\"%s\",%s];"%(i,seq[i],STYLE_UNPAIRED)
    print >> f, "}"

#Typical usage of this script (RNAToDOT.py):
#   python RNAToDOT.py (((...))) GGGAUACCC > rnaTree.dot
#   dot -Tpdf -o rnaTree.pdf rnaTree.dot
if __name__=="__main__":
  struct,seq = sys.argv[1],sys.argv[2]
  drawAsDOT (seq,struct,sys.stdout)
```

20 Another simple input meta file for R2R

```
RF00065_guide_target.cons.sto
RF00065_guide_target.sto    oneseq  Pa21-S892
RF00065_guide_target-hybrid.cons.sto
```

21 Advanced input meta file for R2R

```
RF00065_seed.cons.sto
RF00065_seed.sto     oneseq  Pyrococcus_furiosus
RF00065_seed.sto     oneseq  Pyrococcus_abyssi_GE
RF00065_seed.sto     oneseq  Pyrococcus_horikoshi
RF00065_seed.sto     oneseq  Thermococcus_kodakar
RF00065_seed.cons.sto    skeleton-with-pairbonds
RF00065_seed-ILOOP75.cons.sto
RF00065_seed-ILOOP85.cons.sto
```

22 Sample Stockholm file for interacting RNAs, usable as an input for R2R

```
# STOCKHOLM 1.0

Pa21-S892                    GGGCCCGGC.....UCCCGCCCUCUCCGGG...GA.............AUC.................GUGA.ACCG.
     GG.GGUUCCG..G.CCGGGCCU..ACA.UUUUUUUUUUUUUUAGGAAUUGGCGGGGGNNNNN
Ph21-S880                    GGGCCCGGU.....UCCCGCCCUCUCCGGG...GA.............AUC.................GUGA.ACCG.
     GG.GGUUCCG..A.CCGGGCCG..ACA.UUUUUUUUUUUUUUAGGAAUUGGCGGGGGNNNNN
Pf1-S880                     GGGCCCGGU.....UCCCGCCCUCUCCGGG...GA.............AUC.................GUGA.ACCG.
     GG.GGUUCCG..A.CCGGGCCC..ACA.UUUUUUUUUUUUUUAGGAAUUGGCGGGGGNNNNN
#=GC SS_cons                 <<<<<<<<<.....<<<...<<<<...<<.....________.....-..>>.>>>>-
     >>->.......->->>>>>>>>.....................................
#=GC SS_cons_1               ..............<<<<<<.....................................................................
     ...<<<<<.....................................>>>>>..>>>>>>......
#=GC R2R_LABEL               q...........1LL1LLL...e.f...........4.p.................p.........5KHH.HHiH.
     HHBHLLLLLj78H9HHHhHHHr....a.CCCCC20CCCCCCC.GGGGGmonGGGGGg.....
#=GC SUBFAM_hybrid_R2R_LABEL -------------AAaAAAbf-----------------------------------------------------------
     g.chHHHH----------------------------------DDDDDmonEEEEi------
#=GC R2R_XLABEL_KLOOP        ..............................KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK.......
     ..........................................................
#=GC R2R_XLABEL_KLOOPL       ..................................................k.............................
     ..........................................................
#=GC R2R_XLABEL_ILOOP        ..............PQXXXX..W.....................................................
     ...YYYYYRSU.V.............................YYYYY..PPPPPP......
#=GC R2R_XLABEL_ILOOPL       ................x....w.....................................................
     ......y..su.v.....................................................
#=GC R2R_XLABEL_ANA          ..........................................................................
     ......................CCC.....................................

#=GF R2R ignore_ss_except_for_pairs _1 outline_only_bp
#=GF R2R place_explicit 2 2-- -45 1 0 0 0 -90
#=GF R2R place_explicit n n-- -45 1 0 0 0 -90
#=GF R2R place_explicit m m-- -45 1 0 0 0 -90
#=GF R2R delcols 0
#=GF R2R tick_label l guide
#=GF R2R tick_label K K-Loop
#=GF R2R tick_label a ANA box
#=GF R2R tick_label g target
#=GF R2R keep q r
#=GF R2R var_backbone_range 4 5
#=GF R2R outline_nuc L
#=GF R2R outline_nuc l
#=GF R2R outline_nuc G
#=GF R2R outline_nuc n
#=GF R2R outline_nuc j
#=GF R2R circle_nuc 2 rgb:0,0,0
#=GF R2R circle_nuc C rgb:0,0,0
#=GF R2R nuc_color 2 rgb:0,0,0
#=GF R2R nuc_color C rgb:0,0,0
#=GF R2R var_backbone_range_size_fake_nucs 3 e f
#=GF R2R shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R shade_along_backbone ANA:C rgb:200,200,200
#=GF R2R outline_along_backbone ILOOP:Y rgb:255,228,196
#=GF R2R outline_along_backbone ILOOP:P rgb:193,255,193
#=GF R2R outline_along_backbone ILOOP:Q rgb:193,255,193
#=GF R2R outline_along_backbone ILOOP:X rgb:193,255,193
#=GF R2R shade_along_backbone ILOOP:Y rgb:255,228,196
#=GF R2R shade_along_backbone ILOOP:P rgb:193,255,193
#=GF R2R shade_along_backbone ILOOP:Q rgb:193,255,193
#=GF R2R shade_along_backbone ILOOP:X rgb:193,255,193

#=GF SUBFAM_PERL_PRED hybrid return 1;
#=GF SUBFAM_hybrid_R2R subst_ss _1 primary outline_only_bp
```

```
#=GF SUBFAM_hybrid_R2R box_nuc b rgb:200,0,0
#=GF SUBFAM_hybrid_R2R box_nuc f rgb:200,0,0
#=GF SUBFAM_hybrid_R2R box_nuc g rgb:200,0,0
#=GF SUBFAM_hybrid_R2R box_nuc c rgb:200,0,0
#=GF SUBFAM_hybrid_R2R outline_nuc E
#=GF SUBFAM_hybrid_R2R outline_nuc D
#=GF SUBFAM_hybrid_R2R outline_nuc m
#=GF SUBFAM_hybrid_R2R outline_nuc n
#=GF SUBFAM_hybrid_R2R outline_nuc i
#=GF SUBFAM_hybrid_R2R set_dir pos0 -90
#=GF SUBFAM_hybrid_R2R place_explicit b b-- +45 3 0 0 0 0
#=GF SUBFAM_hybrid_R2R place_explicit g g-- +90 1.5 0 0 0 90
#=GF SUBFAM_hybrid_R2R place_explicit c c-- 90 1 0 0 0 0
#=GF SUBFAM_hybrid_R2R place_explicit h h-- +45 3 0 0 0 90
#=GF SUBFAM_hybrid_R2R place_explicit m m-- 0 1 0 0 0 -90
#=GF SUBFAM_hybrid_R2R place_explicit o o-- 0 2.5 0 0 0 90
#=GF SUBFAM_hybrid_R2R tick_label i target
#=GF SUBFAM_hybrid_R2R tick_label a guide
#=GF SUBFAM_hybrid_R2R shade_along_backbone A rgb:193,255,193
#=GF SUBFAM_hybrid_R2R shade_along_backbone a rgb:193,255,193
#=GF SUBFAM_hybrid_R2R shade_along_backbone E rgb:193,255,193
#=GF SUBFAM_hybrid_R2R shade_along_backbone n rgb:193,255,193
#=GF SUBFAM_hybrid_R2R shade_along_backbone D rgb:255,228,196
#=GF SUBFAM_hybrid_R2R shade_along_backbone d rgb:255,228,196
#=GF SUBFAM_hybrid_R2R shade_along_backbone h rgb:255,228,196
#=GF SUBFAM_hybrid_R2R shade_along_backbone H rgb:255,228,196

#=GF R2R_oneseq Pa21-S892 shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq Pa21-S892 shade_along_backbone ILOOP:P rgb:193,255,193
#=GF R2R_oneseq Pa21-S892 shade_along_backbone ILOOP:Q rgb:193,255,193
#=GF R2R_oneseq Pa21-S892 shade_along_backbone ILOOP:X rgb:193,255,193
#=GF R2R_oneseq Pa21-S892 shade_along_backbone ILOOP:Y rgb:255,228,196
#=GF R2R_oneseq Pa21-S892 shade_along_backbone ANA:C rgb:200,200,200

//
```

23 Sample Stockholm file for a single RNA, usable as an input for R2R

```
# STOCKHOLM 1.0
#=GF ID    snoR9
#=GF AC    RF00065
#=GF DE    Small nucleolar RNA snoR9
#=GF AU    Bateman A, Daub J
#=GF GA    50.0
#=GF NC    49.8
#=GF TC    68.7
#=GF SE    Bateman A
#=GF SS    Published; PMID:12032319
#=GF TP    Gene; snRNA; snoRNA; CD-box;
#=GF BM    cmbuild  -F CM SEED; cmcalibrate --mpi -s 1 CM
#=GF BM    cmsearch  -Z 274931 -E 1000000  --toponly  CM SEQDB
#=GF DR    SO:0000593 SO:C_D_box_snoRNA
#=GF DR    GO:0006396 GO:RNA processing
#=GF DR    GO:0005730 GO:nucleolus
#=GF RN    [1]
#=GF RM    12032319
#=GF RT    Noncoding RNA genes identified in AT-rich hyperthermophiles.
#=GF RA    Klein RJ, Misulovin Z, Eddy SR;
#=GF RL    Proc Natl Acad Sci U S A 2002;99:7542-7547.
#=GF CC    snoRNA R9 is a member of the C/D class of snoRNA which contain
#=GF CC    the C (UGAUGA) and D (CUGA) box motifs.  R9 was identified in a
#=GF CC    computational screen in AT-rich hyperthermophiles [1]. R9 was
#=GF CC    found to overlap with the smaller snoRNA R19 which is currently a
#=GF CC    member of Pyrococcus C/D box snoRNA family Rfam:RF00095.
#=GF WK    http://en.wikipedia.org/wiki/Small_nucleolar_RNA_snoR9
#=GF SQ    5

#=GS Pyrococcus_furiosus    AC    AE009950.1/163991-163864
#=GS Pyrococcus_abyssi_GE   AC    AJ248283.1/230575-230449
#=GS Pyrococcus_horikoshi   AC    BA000001.2/215834-215709
#=GS P.furiosus             AC    AF468960.1/1-128
#=GS Thermococcus_kodakar   AC    AP006878.1/47908-47779

Pyrococcus_furiosus                GGGCCCGGUU.CCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGACCGGGCCCACA..
     AUGGGAUGAUGACCUUUUGCUUUACUGAACACAUGAUGACCACGCCCUUCGCUGAC.CUAAAUAUUUGAC
Pyrococcus_abyssi_GE               GGGCCCGGCU.CCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA..G..
     UUAUGAUGAACUUUUGCUUUGCUGAUGUGGUGAUGAGCACGCCCUUCGCUGAUACUCUCUCGUCCAU
Pyrococcus_horikoshi               CGGCCCGGUU.CCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGACCGGGCCGACA..GG.
     GGAUGAAGAGCUUUUGCUUUGCUGAGCAGAUGAUGACCACGCCCUUCGCUGAC.CU.GCUAUUUGAC
P.furiosus                         GGGCCCGGUU.CCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGACCGGGCCCACA..
     AUGGGAUGAUGACCUUUUGCUUUACUGAACACAUGAUGACCACGCCCUUCGCUGAC.CUAAAUAUUUGAC
```

```
Thermococcus_kodakar
     GGGCCUGGCGUCCCGCCCUCCCCGGGGAAACGUGAACCGGGGCUUCCUGCCAGGCCUACACCGGGGGAUGAAGAGCUUUUGCUUUGCUGAC..
     UGUGAUGAGCACGCCCUUCACUGACCCCGUAUCAGCUCU
#=GC SS_cons                      <<<<<<<<<.........<.<<<<<<<<<.....>>>>>>>>>>.....>>>>>>>>>.................
     ..........................................................
#=GC RF                           gGGCCCGGcu.CCCgCCCUCUCCGGGGAAUCGUGAACCGGGGGuUCCggCCGGGCCcACA..
     auguuAUGAUGAaCUUUUGCUUUaCUGAagagaUGAUGAgCACGCCCUUCGCUGAu.CUaaaUauUugAu
#=GR Pyrococcus_furiosus DEL_COLS  ..........................................................------------
     ----------------------------------------------------------
#=GR Pyrococcus_abyssi_GE DEL_COLS ..........................................................------------
     ----------------------------------------------------------
#=GR Pyrococcus_horikoshi DEL_COLS ..........................................................------------
     ----------------------------------------------------------
#=GR P.furiosus DEL_COLS           ..........................................................------------
     ----------------------------------------------------------
#=GR Thermococcus_kodakar DEL_COLS ..........................................................--------------
     ----------------------------------------------------------
#=GC R2R_LABEL                    p........1......2...........3..4...QQQQqQQQ5...6RRRRrRRRs...7.............
     ..........................................................8
#=GC R2R_XLABEL_KLOOP             ........................KKKKKKKKK.....................................
     ..........................................................
#=GC R2R_XLABEL_KLOOPL            ................................k.....................................
     ..........................................................
#=GC R2R_XLABEL_ILOOP             .........IIIIIIII.........................JJJJJ.........................
     ..........................................................
#=GC R2R_XLABEL_ILOOPL            .............i.................................j.........................
     ..........................................................
#=GC R2R_XLABEL_ANA               ..........................................................CCC.............
     ..........................................................
#=GC R2R_XLABEL_ANAL              ..............................................................c.............
     ..........................................................
#=GC SUBFAM_LABEL_TERM            ..........x.................................................................
     ..........................................................
#=GC SUBFAM_ILOOP75_R2R_LABEL     -------<..........B>---------------------<.KLMNO.>-------------------------
     ----------------------------------------------------------
#=GC SUBFAM_ILOOP85_R2R_LABEL     -------<..........B>---------------------<.KLMNO.>-------------------------
     ----------------------------------------------------------

#=GF R2R keep p s
#=GF R2R var_backbone_range 1 2
#=GF R2R var_backbone_range 3 4
#=GF R2R var_backbone_range 5 6
#=GF R2R var_backbone_range 7 8
#=GF R2R shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R shade_along_backbone ANA:C rgb:200,200,200
#=GF R2R tick_label ANAL:c ANA box
#=GF R2R tick_label KLOOPL:k K-Loop

#=GF SUBFAM_REGEX_PRED ILOOP75 TERM [.]
#=GF SUBFAM_REGEX_PRED ILOOP85 TERM [A-Z]

#=GF SUBFAM_ILOOP75_R2R no5
#=GF SUBFAM_ILOOP75_R2R shade_along_backbone ILOOP:I rgb:0,255,0
#=GF SUBFAM_ILOOP75_R2R tick_label ILOOPL:i 7 nt
#=GF SUBFAM_ILOOP75_R2R outline_nuc ILOOP:I
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 M M 5 nt
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 B B
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 K K
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 L L
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 N N
#=GF SUBFAM_ILOOP75_R2R var_backbone_range_size_fake_nucs 1 O O

#=GF SUBFAM_ILOOP85_R2R no5
#=GF SUBFAM_ILOOP85_R2R shade_along_backbone ILOOP:I rgb:0,255,0
#=GF SUBFAM_ILOOP85_R2R tick_label ILOOPL:i 8 nt
#=GF SUBFAM_ILOOP85_R2R outline_nuc ILOOP:I
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 M M 5 nt
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 B B
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 K K
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 L L
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 N N
#=GF SUBFAM_ILOOP85_R2R var_backbone_range_size_fake_nucs 1 O O

#=GF R2R_oneseq Pyrococcus_furiosus shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_furiosus tick_label ILOOPL:i 5' guide sequence
#=GF R2R_oneseq Pyrococcus_furiosus shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_furiosus tick_label ILOOPL:j 3' guide sequence
#=GF R2R_oneseq Pyrococcus_furiosus shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq Pyrococcus_furiosus var_backbone_range_size_fake_nucs 10 7 8
#=GF R2R_oneseq Pyrococcus_furiosus inline_nuc ILOOP:I
#=GF R2R_oneseq Pyrococcus_furiosus inline_nuc ILOOP:J
```

```
#=GF R2R_oneseq Pyrococcus_furiosus inline_nuc KLOOP:K
#=GF R2R_oneseq Pyrococcus_furiosus outline_nuc Q
#=GF R2R_oneseq Pyrococcus_furiosus outline_nuc q
#=GF R2R_oneseq Pyrococcus_furiosus outline_nuc R
#=GF R2R_oneseq Pyrococcus_furiosus outline_nuc r
#=GF R2R_oneseq Pyrococcus_furiosus outline_nuc s
#=GF R2R_oneseq Pyrococcus_furiosus tick_label q 8 bp
#=GF R2R_oneseq Pyrococcus_furiosus tick_label r 9 bp

#=GF R2R_oneseq Pyrococcus_abyssi_GE shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_abyssi_GE tick_label ILOOPL:i 5' guide sequence
#=GF R2R_oneseq Pyrococcus_abyssi_GE shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_abyssi_GE tick_label ILOOPL:j 3' guide sequence
#=GF R2R_oneseq Pyrococcus_abyssi_GE shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq Pyrococcus_abyssi_GE var_backbone_range_size_fake_nucs 10 7 8
#=GF R2R_oneseq Pyrococcus_abyssi_GE inline_nuc ILOOP:I
#=GF R2R_oneseq Pyrococcus_abyssi_GE inline_nuc ILOOP:J
#=GF R2R_oneseq Pyrococcus_abyssi_GE inline_nuc KLOOP:K
#=GF R2R_oneseq Pyrococcus_abyssi_GE outline_nuc Q
#=GF R2R_oneseq Pyrococcus_abyssi_GE outline_nuc q
#=GF R2R_oneseq Pyrococcus_abyssi_GE outline_nuc R
#=GF R2R_oneseq Pyrococcus_abyssi_GE outline_nuc r
#=GF R2R_oneseq Pyrococcus_abyssi_GE outline_nuc s
#=GF R2R_oneseq Pyrococcus_abyssi_GE tick_label q 8 bp
#=GF R2R_oneseq Pyrococcus_abyssi_GE tick_label r 9 bp

#=GF R2R_oneseq Pyrococcus_horikoshi shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_horikoshi tick_label ILOOPL:i 5' guide sequence
#=GF R2R_oneseq Pyrococcus_horikoshi shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R_oneseq Pyrococcus_horikoshi tick_label ILOOPL:j 3' guide sequence
#=GF R2R_oneseq Pyrococcus_horikoshi shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq Pyrococcus_horikoshi var_backbone_range_size_fake_nucs 10 7 8
#=GF R2R_oneseq Pyrococcus_horikoshi inline_nuc ILOOP:I
#=GF R2R_oneseq Pyrococcus_horikoshi inline_nuc ILOOP:J
#=GF R2R_oneseq Pyrococcus_horikoshi inline_nuc KLOOP:K
#=GF R2R_oneseq Pyrococcus_horikoshi outline_nuc Q
#=GF R2R_oneseq Pyrococcus_horikoshi outline_nuc q
#=GF R2R_oneseq Pyrococcus_horikoshi outline_nuc R
#=GF R2R_oneseq Pyrococcus_horikoshi outline_nuc r
#=GF R2R_oneseq Pyrococcus_horikoshi outline_nuc s
#=GF R2R_oneseq Pyrococcus_horikoshi tick_label q 8 bp
#=GF R2R_oneseq Pyrococcus_horikoshi tick_label r 9 bp

#=GF R2R_oneseq P.furiosus shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R_oneseq P.furiosus tick_label ILOOPL:i 5' guide sequence
#=GF R2R_oneseq P.furiosus shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R_oneseq P.furiosus tick_label ILOOPL:j 3' guide sequence
#=GF R2R_oneseq P.furiosus shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq P.furiosus var_backbone_range_size_fake_nucs 10 7 8
#=GF R2R_oneseq P.furiosus inline_nuc ILOOP:I
#=GF R2R_oneseq P.furiosus inline_nuc ILOOP:J
#=GF R2R_oneseq P.furiosus inline_nuc KLOOP:K
#=GF R2R_oneseq P.furiosus outline_nuc Q
#=GF R2R_oneseq P.furiosus outline_nuc q
#=GF R2R_oneseq P.furiosus outline_nuc R
#=GF R2R_oneseq P.furiosus outline_nuc r
#=GF R2R_oneseq P.furiosus outline_nuc s
#=GF R2R_oneseq P.furiosus tick_label q 8 bp
#=GF R2R_oneseq P.furiosus tick_label r 9 bp

#=GF R2R_oneseq Thermococcus_kodakar shade_along_backbone ILOOP:I rgb:0,255,0
#=GF R2R_oneseq Thermococcus_kodakar tick_label ILOOPL:i 5' guide sequence
#=GF R2R_oneseq Thermococcus_kodakar shade_along_backbone ILOOP:J rgb:0,255,0
#=GF R2R_oneseq Thermococcus_kodakar tick_label ILOOPL:j 3' guide sequence
#=GF R2R_oneseq Thermococcus_kodakar shade_along_backbone KLOOP:K rgb:0,129,255
#=GF R2R_oneseq Thermococcus_kodakar var_backbone_range_size_fake_nucs 10 7 8
#=GF R2R_oneseq Thermococcus_kodakar inline_nuc ILOOP:I
#=GF R2R_oneseq Thermococcus_kodakar inline_nuc ILOOP:J
#=GF R2R_oneseq Thermococcus_kodakar inline_nuc KLOOP:K
#=GF R2R_oneseq Thermococcus_kodakar outline_nuc Q
#=GF R2R_oneseq Thermococcus_kodakar outline_nuc q
#=GF R2R_oneseq Thermococcus_kodakar outline_nuc R
#=GF R2R_oneseq Thermococcus_kodakar outline_nuc r
#=GF R2R_oneseq Thermococcus_kodakar outline_nuc s
#=GF R2R_oneseq Thermococcus_kodakar tick_label q 8 bp
#=GF R2R_oneseq Thermococcus_kodakar tick_label r 9 bp

#=GF Makefile skeleton-with-pairbonds
//
```

24 Shell script demonstrating advanced usage of R2R

```
#!/bin/tcsh -f
#

set R2RPATH = ""
if ( $R2RPATH == "" ) then
echo "Usage: please set the R2RPATH variable"
exit
endif

set r2r = "$R2RPATH/src/r2r"
set subfam = "perl $R2RPATH/src/SelectSubFamilyFromStockholm.pl"
set GSCparams = '3 0.97 0.9 0.75 4 0.97 0.9 0.75 0.5 0.1'

$r2r --GSC-weighted-consensus RF00065_seed.sto RF00065_seed.cons.sto $GSCparams

$subfam RF00065_seed.cons.sto ILOOP75 > RF00065_seed-ILOOP75.sto
$r2r --GSC-weighted-consensus RF00065_seed-ILOOP75.sto RF00065_seed-ILOOP75.cons.sto $GSCparams
$subfam RF00065_seed.cons.sto ILOOP85 > RF00065_seed-ILOOP85.sto
$r2r --GSC-weighted-consensus RF00065_seed-ILOOP85.sto RF00065_seed-ILOOP85.cons.sto $GSCparams

setenv R2R_DUMP_FILE RF00065_seed.txt
$r2r RF00065_seed.meta RF00065_seed.pdf
$r2r RF00065_seed.meta RF00065_seed.svg


$r2r --GSC-weighted-consensus RF00065_guide_target.sto RF00065_guide_target.cons.sto $GSCparams

$subfam RF00065_guide_target.cons.sto hybrid > RF00065_guide_target-hybrid.sto
$r2r --GSC-weighted-consensus RF00065_guide_target-hybrid.sto RF00065_guide_target-hybrid.cons.sto $GSCparams

setenv R2R_DUMP_FILE RF00065_guide_target.txt
$r2r RF00065_guide_target.meta RF00065_guide_target.pdf
$r2r RF00065_guide_target.meta RF00065_guide_target.svg
```

25 Shell script for the generation of simplified representations using VARNA

```
# Top
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-highlightRegion "10-16:radius=10,fill=#acf269,outline=#acf269;43-47:radius=10,fill=#acf269,outline=#acf269"
     \
-o RF00065_testarc1.svg -algorithm line -drawBases False -spaceBetweenBases 0.6 -bpStyle line

# Bottom
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-chemProb "10-11:glyph=pin,dir=out,intensity=3,color=#f90000;17-18:glyph=pin,dir=out,intensity=1,color=#
     f90000;"\
-highlightRegion "26-27:radius=10,fill=#d2ccf4,outline=#d2ccf4;31-37:radius=10,fill=#d2ccf4,outline=#d2ccf4"
     \
-o RF00065_testarc2.svg -algorithm line -drawBases False -spaceBetweenBases 0.6 -bpStyle line
```

26 Shell script for using color maps and algorithms using VARNA

```
# panel A
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-highlightRegion "10-16:radius=10,fill=#acf269,outline=#acf269;43-47:radius=10,fill=#acf269,outline=#acf269"
     \
-o RF00065_panelA.svg -algorithm radiate -flat true -drawBases False -spaceBetweenBases 0.6 \
-title "RF00065: Pyrococcus abyssi GE" -titleSize 12

# panel B
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-chemProb "9-10:glyph=pin,dir=out,intensity=1,color=#3e844b;10-11:glyph=pin,dir=out,intensity=1,color=#3e844b
     ;"\
```

```
-o RF00065_panelB.svg -algorithm radiate -flat true -drawBases False -spaceBetweenBases 0.6

# panel C
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-colorMap "0;0;0;0;0;0;0;0;1;1;2;3;2;1;1;1;1;3;3;1;1;0;0;0;2;2;2;3;3;1;3;2;\
1;1;1;1;0;0;0;0;0;1;3;3;3;1;0;0;0;0;0;0;0;0;0;1;1;3" \
-colorMapMax 3 -colorMapMin 0 -colorMapStyle green \
-o RF00065_panelC.svg -algorithm radiate -flat true -drawBases True -spaceBetweenBases 0.6

# panels D, E, F
java -cp VARNAvx-y.jar fr.orsay.lri.varna.applications.VARNAcmd \
-sequenceDBN "GGGCCCGGCUCCCGCCCUCUCCGGGGAAUCGUGAACCGGGGGUUCCGGCCGGGCCUACA" \
-structureDBN "(((((((((.......(.(((((((((.....)))))))))).....)))))))))..." \
-chemProb "9-10:glyph=pin,dir=out,intensity=1,color=#3e844b;10-11:glyph=pin,dir=out,intensity=1,color=#3e844b
    ;"\
-highlightRegion "26-27:radius=10,fill=#d2ccf4,outline=#d2ccf4;31-37:radius=10,fill=#d2ccf4,outline=#d2ccf4"
    \
-o RF00065_panelD.svg -algorithm radiate -flat true -drawBases False -spaceBetweenBases 0.6
```