## **Tutorial 1: Applying MED to sequences from one OTU**

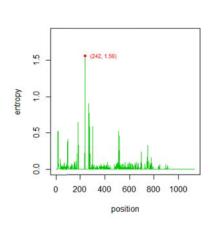
```
library(otu2ot)
#help(package = otu2ot)
```

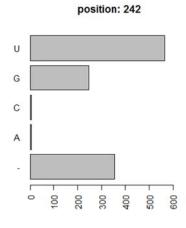
1) option a) The file is found in the working directory as a FASTA file containing aligned sequences.

File="HGB\_0013\_GXJPMPL01A30QX.fasta"

OT. seq. concat1 <- MED(File, minseq=21, entropymin=0. 6, Plot=TRUE)

```
#Position: 242
# - A C G U
#Nber 355.00 4 4 247.00 565.0
#Prop 0.31 0 0 0.22 0.5
```





The plots are only for the first entropy profile when all sequences are considered.

head(OT. seq. concat1)

system. time( MED(File, minseq=21, entropymin=0.6, Plot=FALSE))

#user system elapsed

#10.81 0.03 10.92

Option b) Importing first the sequences, and then applying MED, via the MEDMat function

```
Aln.list<- ImportFastaAlignment(File)
Names <- Aln.list[[1]]
Sequences <- toupper(Aln.list[[2]])
OT.seq.concat <- MEDMat(AlignedSequences=Sequences, minseq=21, entropymin=0.6, Plot=TRUE)
```

2) Retrieve the sample information from the FASTA headers directly. To see if the length of the substring in the FASTA headers is correct, use:

#### GetEnvironmentDatafromFileR(File, Start=2, Stop=9, test=TRUE)

[1] "HGB\_0010"

Then apply it to all headers:

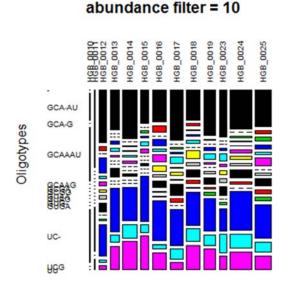
# ENV <GetEnvironmentDatafromFileR(File, Start=2, Stop=9, test=FALSE)</pre>

3) build the Sample-by-OT table and filter by abundance too if needed.

```
Table0 <- SampleXOT_Table(
    OT. seq. concat=OT. seq. concat,
    ENV=ENV,
    mosai cPlot=TRUE,
    filterByMinAbund= 0
)
```

#### unfiltered 9819 0014 0015 0016 0017 90019 0023 HGB\_0018 0024 0025 HGB ( HGB HGB BCA-AC GCA-AU Oligotypes 86AAGAC GCAAAU UC-HE9

```
Table10 <- SampleXOT_Table(
     OT. seq. concat=OT. seq. concat,
     ENV=ENV,
     mosai cPl ot=TRUE,
     fil terByMi nAbund= 10
)</pre>
```



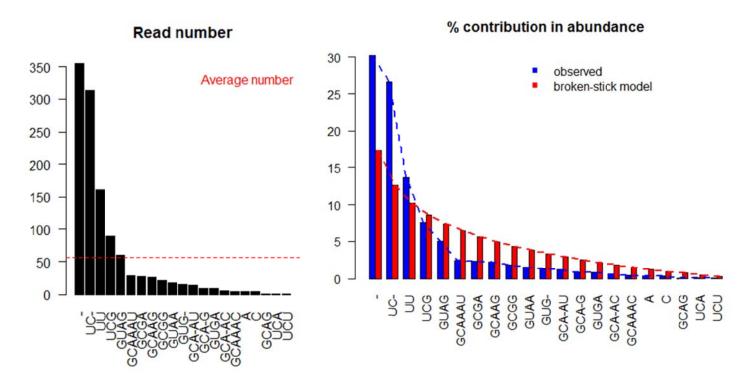
#### str(Tabl e0)

```
List of 1
$ SamplexOT. table: int [1:13, 1:21] 0 7 25 22 43 18 28 41 26 35 ...
..- attr(*, "dimnames")=List of 2
....$ Samples : chr [1:13] "HGB_0010" "HGB_0011" "HGB_0012" "HGB_0013" ...
....$ Oligotypes: chr [1:21] "-" "A" "C" "GCA-AC" ...
```

# Which OT in the raw table have abundances higher than predicted by using the Broken-Stick model approach?

```
OTAbund <- col Sums(TableO[[1]])
#overall abundance for each OT
```

```
OTAbund_BSM <- Count. BrokenStick(OTAbund, Plot = TRUE)
OTAbund_BSM
$Table
                          UC-
                                    UU
                                             UCG
                                                      GUAG
                                                             GCAAAU
                                                                         GCGA
          30. 21277 26. 63830 13. 70213 7. 574468 17. 35885 12. 59695 10. 21599 8. 628692
                                                          2. 468085 2. 382979
6. 485835 5. 692184
                                                 5. 106383 2. 468085
Observed
FromModel
                                                 7. 438216
                                  GUAA
              GCAAG
                         GCGG
                                            GUG-
                                                   GCA-AU
                                                               GCA-G
          2. 297872 1. 872340 1. 531915 1. 361702 1. 276596 0. 8510638 0. 8510638
Observed
FromModel
          5. 011912 4. 416674 3. 887574 3. 411383 2. 978483 2. 5816573 2. 2153569
              GCA-AC
                        GCAAAC
                                                            GCAG
          Observed
FromModel
          1.8752208 1.5577605 1.2601415 0.9800294 0.71547917 0.46485261
                  UCU
Observed
          0.08510638
FromModel 0.22675737
$Hi gherThanBSM
[1] "-" "UC-" "UU"
[1]
```



**Conclusions**: here only three OT have relative abundances higher than predicted by a random partition of sequence abundance.

# Table0\_BSM <- Table0[[1]][,OTAbund\_BSM\$HigherThanBSM] Table0\_BSM

Tableu_DSWI			
	Olig	gotyp	oes
Samples		UC-	UU
HGB_0010		2	0
HGB_0011	1 7	4	1
HGB_0012	2 25	15	2
HGB_0013	3 22	24	13
HGB_0014	1 43	30	25

```
HGB_0015 18 24 18

HGB_0016 28 35 15

HGB_0017 41 29 6

HGB_0018 26 29 21

HGB_0019 35 25 16

HGB_0023 21 10 6

HGB_0024 52 52 24

HGB_0025 37 34 14
```

## **BSM**

