Supplementary material 2

**The code used for sequence analysis and bioinformatics**

# 16S

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MinION\_data/16S\_mobile\_1\_3\_5/fast5\_pass

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MinION\_data/16S\_mobile\_2\_4/fast5\_pass

nohup /opt/software/MetONTIIME/MetONTIIME.sh /home/User/MetONTIIME/16S\_mobile /home/User/MetONTIIME/sample-metadata.tsv /home/User/MetONTIIME/DB/Silva132/16S/silva\_132\_99\_16S\_sequence.qza /home/User/MetONTIIME/DB/Silva132/16S/silva\_132\_99\_16S\_taxonomy.qza 30 Blast 1 0.8 0.85 &

# export:

qiime tools export \

--input-path /home/User/MetONTIIME/16S\_mobile/table.qza \

--output-path /home/User/MetONTIIME/16S\_mobile/export

biom convert -i /home/User/MetONTIIME/16S\_mobile/export/feature-table.biom -o /home/User/MetONTIIME/16S\_mobile/export/otu\_table.txt --to-tsv

qiime tools export \

--input-path /home/User/MetONTIIME/16S\_mobile/taxonomy.qza \

--output-path /home/User/MetONTIIME/16S\_mobile/export

# 18S

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MinION\_data/18S\_mobile\_1\_3\_5/fast5\_pass

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MinION\_data/18s\_mobile\_2\_4/fast5\_pass

nohup /opt/software/MetONTIIME/MetONTIIME.sh /home/User/MetONTIIME/18S\_mobile /home/User/MetONTIIME/sample-metadata.tsv /home/User/MetONTIIME/DB/Silva132/18S/silva\_132\_99\_18S\_sequence.qza /home/User/MetONTIIME/DB/Silva132/18S/silva\_132\_99\_18S\_taxonomy.qza 30 Blast 1 0.8 0.85 &

# export:

qiime tools export \

--input-path /home/User/MetONTIIME/18S\_mobile/table.qza \

--output-path /home/User/MetONTIIME/18S\_mobile/export

biom convert -i /home/User/MetONTIIME/18S\_mobile/export/feature-table.biom -o /home/User/MetONTIIME/18S\_mobile/export/otu\_table.txt --to-tsv

qiime tools export \

--input-path /home/User/MetONTIIME/18S\_mobile/taxonomy.qza \

--output-path /home/User/MetONTIIME/18S\_mobile/export

# ITS

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MetONTIIME/ITS\_mobile\_BC01-12/fast5\_pass

/opt/software/MetONTIIME/Launch\_MinION\_mobile\_lab.sh /home/User/MetONTIIME/ITS\_mobile\_BC13-19/fast5\_pass

nohup /opt/software/MetONTIIME/MetONTIIME.sh /home/User/MetONTIIME/ITS\_mobile /home/User/MetONTIIME/sample-metadata.tsv /home/User/UNITE/unite-ver8-dynamic-sequences-04.02.2020-dev.qza /home/User/UNITE/unite-ver8-dynamic-taxonomy-04.02.2020-dev.qza 30 Blast 1 0.7 0.7 &

# export:

qiime tools export \

--input-path /home/User/MetONTIIME/ITS\_mobile/table.qza \

--output-path /home/User/MetONTIIME/ITS\_mobile/export

biom convert -i /home/User/MetONTIIME/ITS\_mobile/export/feature-table.biom -o /home/User/MetONTIIME/ITS\_mobile/export/otu\_table.txt --to-tsv

qiime tools export \

--input-path /home/User/MetONTIIME/ITS\_mobile/taxonomy.qza \

--output-path /home/User/MetONTIIME/ITS\_mobile/export

# import:

library("phyloseq")

otu <- read.table(file = "otu\_table.txt", header = TRUE)

tax <- read.table(file = "taxonomy.tsv", sep = '\t', header = TRUE)

combined\_table <- merge(otu, tax, by.x = c("OTUID"), by.y = c("OTUID"))

write.table(combined\_table, file = "combined\_table", sep = '\t', col.names = TRUE, row.names = FALSE)

# Split combined\_table.txt file into two files: otu\_matrix.csv, taxonomy.csv

otu\_table <- read.csv("otu\_matrix.csv", sep=",", row.names=1)

otu\_table <- as.matrix(otu\_table)

taxonomy = read.csv("taxonomy.csv", sep=",", row.names=1)

taxonomy<-as.matrix(taxonomy)

metadata=read.csv("metadata.csv",sep=",",row.names=1)

OTU = otu\_table(otu\_table, taxa\_are\_rows = TRUE)

TAX = tax\_table(taxonomy)

META = sample\_data(metadata)

physeq = phyloseq(OTU,TAX,META)