# Project analysis code and data reproducibility Project analysis code available

# For SNP calling

Software: bowtie2, bcftools

Parameter:

bowtie2 -p 12 -x /lp082\_ref/lp --no-mixed --very-sensitive --n-ceil 0,0.01 -1

ISP\_single\_genome/hm/H1\_1.fq -2 /ISP\_single\_genome/hm/H1\_2.fq | samtools sort -O bam -@ 24 -o - > h1ref.bam

bcftools mpileup -C 50 -Ou -m 3 -F 0.0002 -f /ISP\_single\_genome/lp082\_ref/ref.fasta z1ref.bam > z1ref.bcf

bcftools call -c --variants-only -Ob --ploidy 1 z1ref.bcf > z1.bcf bcftools view -Ov z1.bcf | vcfutils.pl varFilter -d 100 > z1.vcf

# Merge all single \*.vcf to the SNP profile:

/ISP single genome/callsnp/tabix-0.2.6/bgzip H1.vcf

bcftools index H1.vcf.gz

bcftools merge --merge all -l listvcf -O v > snp.vcf

#### #For isolate single genome assembling

Software: Spades

Parameter:

spades.py -1 H1\_1.fq -2 H1\_2.fq -o spades\_error\_corrected\_reads -t 24 -m 250 --only-error-correction

spades.py -1 H1 1.00.0 0.cor.fastq.gz -2 H1 2.00.0 0.cor.fastq.gz -o

spades kmers set careful assembly -t 24 -k 21,33,55,77,89 --careful --only-assembler

#### **#Strain mobile element annotation**

sed 's/k141/H1\_node/g' /ISP\_single\_genome/assemble\_genome/H1/final.contigs.fa > /ISP\_single\_genome/assemble\_genome/assembled/H1.fasta

cat \*.fasta > whole\_assembled.fasta

blastall -a 12 -p blastn -i whole\_assembled.fasta -d ./ref/ref.fasta -o b1 -W 11 -e 0.00001 -b 1 -m 8

python extracnt blast.py -i whole assembled.fasta -b b1 -t 90 -o unreass.fasta

cd-hit-est -i unreass.fasta -o cdhitass.fasta -c 0.9 -M 100000 -aS 0.9 -B 1

awk -v min="500" 'BEGIN {RS = ">"; ORS = ""} length(\$2) >= min {print ">"\$0}'

cdhitass.fasta > unreass.fasta

bowtie2-build unreass.fasta unress

bowtie2 -p 24 -x ./unreass/unreass --no-mixed --very-sensitive -1

/ISP\_single\_genome/hm/H1\_1.fq -2 /ISP\_single\_genome/hm/H1\_2.fq -S h1ass.sam soap.coverage -cvg -sam -i h1ass.sam -refsingle ./unreass/unreass500.fasta -o h1ass.txt -depthsingle h1ass.coverage -plot hiassplot.txt 0 1000

#### **#For ANI calculation**

average\_nucleotide\_identity.py -i /ISP\_single\_genome/aug10/ani/ -o /ISP\_single\_genome/aug10/aniout -m ANIm -g

#### #For phylogenic tree construction

Software: MEGA-X, iTOL

### #Shotgun metagenomic sequencing data assembling

Software: megahit

Parameter:

megahit -1 /ISP\_metagenome/cleandata/MH1\_1.fq -2

/ISP\_metagenome/cleandata/MH1\_2.fq -m 100000000000 -t 12 -o

/ISP metagenome/assemble/MH1 --min-contig-len 500

#### **#For intestinal microbiota evolution analysis**

Software: MDIS Parameter:

run midas.py snps /path/to/outdir --species id Bacteroides vulgatus 57955 -1

/path/to/reads 1.fq.gz -2 /path/to/reads 2.fq.gz

#### #For metagenomic data taxonomy annotation

Software: Bracken

Parameter:

kraken2-build --db \${KRAKEN\_DB} --threads \${THREADS}

bracken-build -d \${KRAKEN\_DB} -t \${THREADS} -k \${KMER\_LEN} -I \${READ\_LEN}

kraken2 --db \${KRAKEN\_DB} --threads \${THREADS} --report \${SAMPLE}.kreport

\${SAMPLE}.fq > \${SAMPLE}.kraken

bracken -d \${KRAKEN\_DB} -i \${SAMPLE}.kreport -o \${SAMPLE}.bracken -r \${READ\_LEN}

-I \${LEVEL} -t \${THRESHOLD}

#### #For metagenomic data functional annotation

Software: HUMAnN2

Parameter:

humann2 --threads 12 --input examples/demo.fasta --output \$OUTPUT\_DIR

## #For metagenomic data phage, antibiotic resistant genes and mobile elements annotation

Software: Bowtie2, R

Parameter:

bowtie2 -p 24 -x /ISP\_metagenome/mge/mge\_ref/mge -D 20 -R 3 -N 1 -L 20 -i S,1,0.50 -1

/ISP metagenome/cleandata/MH1 1.fg -2 /ISP metagenome/cleandata/MH1 2.fg |

samtools view -bS | samtools sort -T PREFIX -@ 24 -o - >

/ISP\_metagenome/mge/MH1.outputPrefix.bam

samtools index MH1.outputPrefix.bam

samtools idxstats MH1.outputPrefix.bam | tee -a MH1.txt

bowtie2 -p 24 -x /ISP metagenome/card/card ref/card -D 20 -R 3 -N 1 -L 20 -i S,1,0.50 -1

/ISP metagenome/cleandata/MH1 1.fg -2 /ISP metagenome/cleandata/MH1 2.fg |

samtools view -bS | samtools sort -T PREFIX -@ 24 -o - >

/ISP metagenome/card/MH1.outputPrefix.bam

samtools index MH1.outputPrefix.bam

samtools idxstats MH1.outputPrefix.bam | tee -a MH1.txt

```
bowtie2 -p 12 -x /ISP_metagenome/phage/ref_phage/phage -1
/ISP_metagenome/cleandata/MH1_1.fq -2 /ISP_metagenome/cleandata/MH1_2.fq |
samtools view -bS | samtools sort -T PREFIX -@ 24 -o - >
/ISP_metagenome/phage/MH1.outputPrefix.bam
samtools index MH1.outputPrefix.bam
samtools idxstats MH1.outputPrefix.bam | tee -a MH1.txt
R
rm(list=ls())
files <- list.files(pattern = "txt$")
name <- c('gene_name','gene_length')</pre>
final data <- read.table(files[1], header=F, sep="\t")[,1:2]
for (filename in files)
{
 data <- read.table(filename, header=F, sep="\t")[,3]
 name <- c(name, filename)
 final_data <- cbind(final_data, data)</pre>
name <- gsub(".bowtie2.txt","",name)</pre>
colnames(final_data) <- name
write.table(file='card bowtie2 unique',final data, sep="\t", quote=F, row.names=F)
```

#### Data and figures repeatable

#### #Figure 1

- #(A) Experiment design of the present research.
- #(B) The phylogenetic tree was constructed based on the SNPs profile with the iTOL online version, the SNPs sequences were listed in "SNPS22\_tree.fasta" on

Github: https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 1B

#(C) Top panel; The curve was drawn by R program, the original code and the raw data were deposited on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure\_1C

Bottom panel: The figure was constructed by GraphPad Prism 8, the original code and the raw data were deposited on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 1C

#(D) The genomic cycle figure with SNPs and GC abundance was constructed with R, the original code and the raw data were deposited on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure\_1D

#(E)The simplified phylogenetic tree was constructed based on SNP sequences with the iTOL online version.

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure\_3

#(F) The evolutionary dynamics figures were drawn by Python and R program with the code and dataset "FIG 3CD.txt" on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 3

#### #Figure 2

#The FIG\_2A and 2B were constructed based on Table S2 of "Sup\_Tabs.xlsx" deposited in <a href="https://github.com/zhjch321123/Intestinal\_Selective\_Pressure\_project">https://github.com/zhjch321123/Intestinal\_Selective\_Pressure\_project</a>. The genes were annotated with the PROKKA annotation pipeline (<a href="http://kbase.us/">http://kbase.us/</a>).

# The FIG\_2C was drawn by R program, the original code and the raw data were deposited on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 2C

#### #Figure 3

#(A) The distance boxplot was constructed by the R program, the original code and the raw data were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 4A

#(B) The 2 curves were constructed by the R program, the original code and the raw data were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 4B

#(C) The 2 network figures were constructed by Cytoscape 3.7.1. The correlation strength was calculated by SpiecEasi using R program, the code and raw network file were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 4C

#### #Figure 4

#(A) The distance boxplot was constructed by the R program, the original code and the raw data were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 5A

- #(B) The boxplot was constructed by the R program, the raw data was listed in Table S8.
- #(C) The 4 figures were constructed by GraphPad Prism 8 with the dataset

"META\_SNP\_pro\_pla.pzfx" on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Figure 5B

#(D) The Heatmap was constructed by the R program, the raw data was listed in Table S8.

#### **#Supplementary figures**

**#Fig\_S1** A-B, The 2 figures were constructed by R, the original code and the raw data were deposited on

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Supp\_Figures

C-G, The phylogenetic tree was constructed based on SNP sequences with the iTOL online version. The raw data were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Supp\_Figures

**# Fig\_S2** The SNPs heatmap was constructed with the "FIG\_S2A.txt" by R program, the code and raw network file were deposited on Github:

https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Supp Figures

- # Fig\_S3 The indigenous microbiome responded to the probiotic invasion. The figures was constructed based on Table S6-S7. The raw data were deposited on Github:
- https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree/master/Supp\_Figures
- # Fig\_S4 The number of SNPs of host intestinal microbiota responded to probiotic consumption in the present study and other published probiotic related study based on the data in "FIG\_S5A.txt" and the correlation between the SNPs and the species alpha diversity was deposited in "FIG\_S5B.txt", The code and the raw data were deposited on Github:

 $\underline{https://github.com/zhjch321123/Host-dependent-co-evolution-of-supplemented-probiotic/tree} \\ \underline{/master/Supp\_Figures}$