次世代シーケンサーデータの解析手法
第13回RNA-seq解析（その1）
ウェブ資料

寺田朋子、坂本光央、清水謙多郎、門田 幸二*

W1 : *L. rhamnosus* GG

Kankainen et al.,PNAS, **106**: 193–198, 2009

W1：*L. rhamnosus* GG

Comparative genomic analysis of ×　Comparative genomic analysis of ×　＋

https://www.pnas.org/content/106/40/17193.long

①

Table 1.
General genomic features of *L. rhamnosus* GG and LC705 and selected *Lactobacillus* spp.

Organism	*L. rhamnosus* GG	*L. rhamnosus* LC705	*L. casei* ATCC334	*L. plantarum* WCFS1
Genome size, Mbp	3.01	3.03	2.92	3.35
No. genes	2,944	2,992	2,771	3,057
Plasmids	0	1	1	3
rRNA operons	5	5	5	5
tRNA genes	57	61	59	70
GC content, %	47	47	47	44
Coding efficiency, %	85	85	82	84
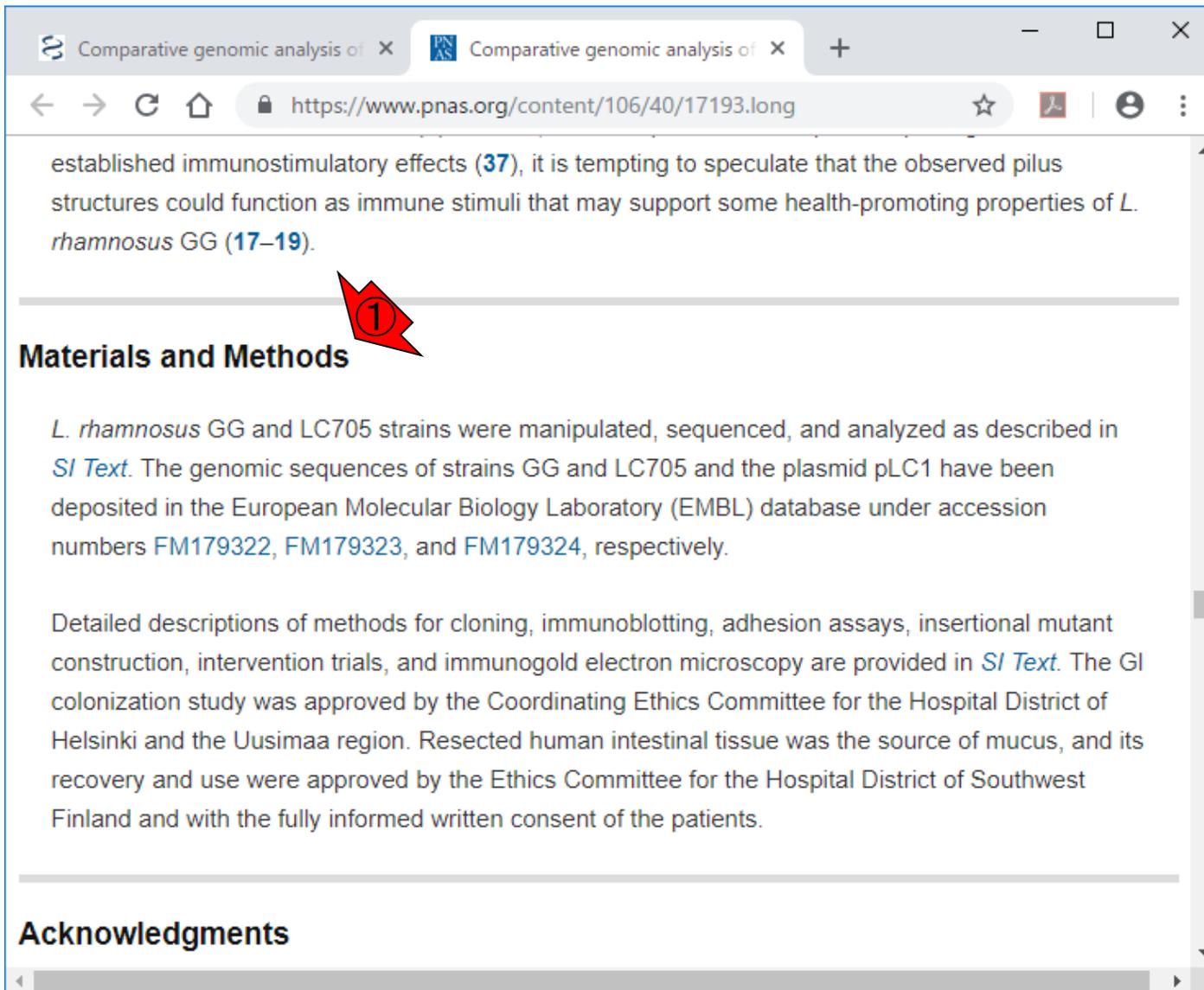
②

W1：*L. rhamnosus* GG

Comparative genomic analysis o × | Comparative genomic analysis o × | +

https://www.pnas.org/content/106/40/17193.long

established immunostimulatory effects (**37**), it is tempting to speculate that the observed pilus structures could function as immune stimuli that may support some health-promoting properties of *L. rhamnosus* GG (**17–19**).

Materials and Methods

L. rhamnosus GG and LC705 strains were manipulated, sequenced, and analyzed as described in *SI Text*. The genomic sequences of strains GG and LC705 and the plasmid pLC1 have been deposited in the European Molecular Biology Laboratory (EMBL) database under accession numbers FM179322, FM179323, and FM179324, respectively.

Detailed descriptions of methods for cloning, immunoblotting, adhesion assays, insertional mutant construction, intervention trials, and immunogold electron microscopy are provided in *SI Text*. The GI colonization study was approved by the Coordinating Ethics Committee for the Hospital District of Helsinki and the Uusimaa region. Resected human intestinal tissue was the source of mucus, and its recovery and use were approved by the Ethics Committee for the Hospital District of Southwest Finland and with the fully informed written consent of the patients.

Acknowledgments

W1 : *L. rhamnosus* GG

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

W2：FM179322

①Send to、②File、③がFASTA形式になっていることを確認して、④Create File。こんな感じになります。⑤デフォルトはsequence.fastaなので、⑥FM179322.fastaというファイル名で、⑦保存。

W3：FM179322

W3：FM179322

W4：注意点

W4：注意点

W4：注意点

Kankainen et al.,PNAS, **106**: 193-198, 2009

W4：注意点

Comments and References: ③

genome sequence

Determination of the DNA genome sequence of this strain has been or is being determined either in whole or in part.

④

Morita H et al. (2009)

Morita, H., Toh, H., Oshima, K., Murakami, M., Taylor, T.D., Igimi, S., and Hattori, M. "Complete genome sequence of probiotic Lactobacillus rhamnosus ATCC 53103." J. Bacteriol. (2009) 191:7630-7631.

External Information Resources (NCBI LinkOut)

①

LinkOut	Subject	LinkOut Provider
Lactobacillus rhamnosus	meta-databases	BacDive
Lactobacillus rhamnosusGG	organism-specific	BioCyc
GOLD: Go0001197	organism-specific	Genomes On Line Database
Show Biotic Interactions	taxonomy/phylogenetic	Global Biotic Interactions
2 records from this provider	organism-specific	Integrated Microbial Genomes
OMA	taxonomy/phylogenetic	OMA Browser: Orthologous MAtrix

②

W5：別の論文

Morita et al., J Bacteriol., **191**: 7630–7631, 2009

W5：別の論文

W5：別の論文

The complete genome sequence of *Lactobacillus rhamnosus* ATCC 53103 was determined by a whole-genome shotgun strategy with the Sanger method. Genomic libraries containing 2-kb inserts were constructed and sequenced, and 39,936 sequences were generated, giving 8.6-fold coverage from both ends of the genomic clones. Sequence reads were assembled with the Phred-Phrap-Consed program (2). Remaining gaps between contigs were closed by direct sequencing of clones. Prediction and annotation of protein-coding genes were performed as described previously (5).

The genome of *L. rhamnosus* ATCC 53103 consists of a circular 3,005,051-bp chromosome containing 2,834 predicted protein-coding genes and has no plasmid. Of all predicted protein-coding genes, we could assign 1,939 (68%) to known functions, 610 (22%) as conserved hypothetical genes, and 285 (10%) as novel hypothetical genes. This strain has a relatively high number of proteins involved in carbohydrate and amino acid metabolism and transport and defense mechanisms, compared with other sequenced intestinal lactobacilli. The genome encodes 28 complete phosphoenolpyruvate-carbohydrate phosphotransferase-type transporter systems (PTSs) and 25 putative glycosyl hydrolases, which classified into 12 different carbohydrate-active enzyme families (http://www.cazy.org/). Of the 12 f alpha-L-fucosidase (GH29) and alpha-mannosidase (GH38) are not found in other sequenced intestinal lactobacilli. Of the 28 PTSs, 12 are encoded by genes adjacent to glycosyl hydrolase genes and transcriptional regulator genes, allowing localized transcriptional control. This organism carries 22 multidrug ABC transporters, eight antimicrobial peptide ABC transporters, and seven beta-lactamases, suggesting its broad range of antibiotic resistance. The genome contains 17 complete two-component

W5：別の論文

contain imperfect repeats consisting of serine and alanine. The genes for both proteins could encode mucin-like cell surface adhesives, because both genes are located adjacent to glycosyltransferase genes (7). The presence of genes encoding proteins for a diverse number of fermentable sugars, a variety of cell surface adherence proteins, bacteriocin biosynthetic proteins (LRHM_2289 to LRHM_2312), and bile salt hydrolase (LRHM_0484) is likely to contribute to the organisms' gastric survival and promote interactions with the intestinal mucosa and microbiota.

During the preparation of this article, the genome sequence (3,010,111 bp) of *L. rhamnosus* GG, the original strain of *L. rhamnosus* ATCC 53103, was deposited in a public database (GenBank accession no. FM179322) (3). The genome of *L. rhamnosus* ATCC 53103 is 5 kb shorter than that of *L. rhamnosus* GG. Furthermore, an alignment analysis of both genome sequences shows that the 8.9-kb region (genome coordinates 618415 to 627294) of *L. rhamnosus* ATCC 53103 is inverted.

Nucleotide sequence accession number.

The sequence data for the *L. rhamnosus* ATCC 53103 genome are available in DDBJ/GenBank/EMBL under accession no. AP011548.

ACKNOWLEDGMENTS

W5：別の論文

W6：AP011548.fasta

W6：AP011548.fasta

W6：AP011548.fasta

W7：Gepard

W7：Gepard

Krumsiek et al., Bioinformatics, **23**: 1026–1028, 2007

W7：Gepard

W7：Gepard

W7：Gepard

W7：Gepard

W8：Gepard実行

Gepard-1.40.jar

①

Gepard

Sequences

Sequence 1 (horizontal):

☐ Complementary　　Select file

Sequence 2 (vertical):

☐ Complementary　　Select file

Create dotplot

>> Advanced mode

Quit

First start

Welcome to the Gepard dotplot software.

It is recommended to read the tutorial / short manual to get an overview over the functionality of the program. The tutorial is included with the download archive.

(This user message will only show up once)

②

Close

W8：Gepard実行

Gepard

Sequences

Sequence 1 (horizontal):

☐ Complementary　　Select file ①

Sequence 2 (vertical):

☐ Complementary　　Select file ①

Create dotplot

>> Advanced mode

Quit

W8：Gepard実行

W8：Gepard実行

W8：Gepard実行

W8：Gepard実行

W8：Gepard実行

W9：Advanced mode

W9：Advanced mode

W9：Advanced mode

W9：Advanced mode

W9：Advanced mode

W9：Advanced mode

W9：Advanced mode

W10：ずれの理由

①メインの直線が若干右上側にずれている理由は、FM179322にはあるがAP011548にはない領域が存在するためだと解釈すればよい。

W10：ずれの理由

W10:ずれの理由

W10：ずれの理由

W11：Fig. 2a

seqinrでドットプロット

seqinrパッケージ(Charif et al., Bioinformatics, 2005)中のdotPlot関数を用いてドットプロットを作成。

- W11：Fig2a
  配列k同士のドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"        #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGTAGTCTATCATACGA"        #2つめの塩基配列を指定(配列k)
out_f <- "Fig2a.png"                  #出力ファイル名を指定してout_fに格納
param_fig <- c(300, 300)              #ファイル出力時の横幅と縦幅を指定(単位はピクセ

#必要なパッケージをロード
library(seqinr)                       #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_fig[2])#出力ファイルの各
par(mar=c(0, 0, 0, 0))                #下、左、上、右の順で余白（行）を指定
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                             #おまじない
```

①

W11：Fig. 2a

seqinrでドットプロット

seqinrパッケージ(Charif et al., Bioinformatics, 2005)中のdotPlot関数を用いてドットプロットを作成。

- W11：Fig2a
配列k同士のドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"        #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGTAGTCTATCATACGA"        #2つめの塩基配列を指定(配列k)
out_f <- "Fig2a.png"        ②          #出力ファイル名を指定し
param_fig <- c(300, 300)              #ファイル出力時の横幅と

#必要なパッケージをロード
library(seqinr)                       #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_f
par(mar=c(0, 0, 0, 0))                #下、左、上、右の順で
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                             #おまじない
```

```
R RGui (64-bit)                                            □ ×
ファイル  編集  閲覧  その他  パッケージ  ウインドウ  ヘルプ

R R Console                                              □ □ ▣

> getwd()
[1] "C:/Users/kadota/Desktop"  ①
> seq1 <- "ACTCGTAGTCTATCATACGA          #1つめの塩基$
> seq2 <- "ACTCGTAGTCTATCATACGA"         #2つめの塩基$
> out_f <- "Fig2a.png"                   #出力ファイル$
> param_fig <- c(300, 300)               #ファイル出力$
>
> #必要なパッケージをロード
> library(seqinr)                        #パッケージの$
>
> #ファイルに保存(pngファイル)
> png(out_f, pointsize=13, width=param_fig[1], height=$
> par(mar=c(0, 0, 0, 0))                 #下、左、上、$
> dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロ$
> dev.off()                              #おまじない
null device
          1
> |
```

W11：Fig. 2a

seqinrでドットプロット

seqinrパッケージ(Charif et al., Bioinformatics, 2005)中のdotPlot関数を用いてドットプロットを作成。

- W11：Fig2a
  配列k同士のドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"          #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGTAGTCTATCATACGA"          #2つめの塩基配列を指定(配列k)
out_f <- "Fig2a.png"                    #出力ファイル名を指定してout_fに格納
param_fig <- c(300, 300)                #ファイル出力時の横幅と縦幅を指定(単位はピク

#必要なパッケージをロード
library(seqinr)                         #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_fig[2])#出力ファイルの
par(mar=c(0, 0, 0, 0))                  #下、左、上、右の順で余白（行）を指定
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                               #おまじない
```

①

W12：Fig. 2b

- W12：Fig2b

  配列kとlのドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"        #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGACTATCTC TTACGA"        #2つめの塩基配列を指定(配列1)
out_f <- "Fig2b.png" ①                 #出力ファイル名を指定してout_fに格納
param_fig <- c(300, 3                  #ファイル出力時の横幅と縦幅を指定(単位はピクセル

#必要なパッケージをロード
library(seqinr)                        #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_fig[2])#出力ファイルの各種
par(mar=c(0, 0, 0, 0))                 #下、左、上、右の順で余白（行）を指定
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                              #おまじない
```

①

W12：Fig. 2b

- W12：Fig2b

  配列kとlのドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"          #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGACTATCTGATTACGA"          #2つめの塩基配列を指定(配列1)
out_f <- "Fig2b.png"                    #出力ファイル名を指定してout_fに格納
param_fig <- c(300, 300)                #ファイル出力時の横幅と縦幅を指定(単位はピクセル

#必要なパッケージをロード
library(seqinr)                         #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_fig[2])#出力ファイルの各種
par(mar=c(0, 0, 0, 0))                  #下、左、上、右の順で余白(行)を指定
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                               #おまじない
```

W12：Fig. 2b

- W12：Fig2b

  配列kとlのドットプロットです

```
seq1 <- "ACTCGTAGTCTATCATACGA"          #1つめの塩基配列を指定(配列k)
seq2 <- "ACTCGACTATCTGATTACGA"          #2つめの塩基配列を指定(配列1)
out_f <- "Fig2b.png"                    #出力ファイル名を指定してout_fに格納
param_fig <- c(300, 300)                #ファイル出力時の横幅と縦幅を指定(単位はピクセル

#必要なパッケージをロード
library(seqinr)                         #パッケージの読み込み

#ファイルに保存(pngファイル)
png(out_f, pointsize=13, width=param_fig[1], height=param_fig[2])#出力ファイルの各種
par(mar=c(0, 0, 0, 0))                  #下、左、上、右の順で余白（行）を指定
dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット
dev.off()                               #おまじない
```

W13：Ensembl Bacteria

Lactobacillus rhamnosus GG - En... ✕ +

← → C ⌂ ⓘ 保護されていない通信 | bacteria.ensembl.org/Lactobacillus_rhamnosus_gg/Info/Index ☆ 📄 ❚ ❸ ⋮

Login/Register

e!EnsemblBacteria ① MMER | BLAST | Tools | Downloads | Documentation | More ▼ 🄱 ▾ Lactobacillus rhamnosus 🔍

Lactobacillus rhamnosus GG

Lactobacillus rhamnosus GG ②
Lactobacillus rhamnosus GG

Provider European Nucleotide Archive ⊠ | Taxonomy ID 568703 ⊠

[Search Lactobacillus rhamnosus GG...] Go

e.g. **is1** or **FM179322:17235-18251** or **synthetase**

About *Lactobacillus rhamnosus GG*

ⓘ Information and statistics

Genome assembly: ASM2650v1 ⊠

ⓘ More information and statistics

⬇ Download DNA sequence (FASTA) ③

🗂 Display your data in Ensembl Bacteria

Example region

Gene annotation

What can I find? Protein-coding and non-coding genes, splice variants, cDNA and protein sequences, non-coding RNAs.

ⓘ More about this genebuild

⬇ Download genes, cDNAs, ncRNA, proteins - FASTA - GFF3 ④

🔧 Update your old Ensembl IDs

phnI metC
hisA hpt
lacZ accD

mple gene

Example transcript

Comparative genomics
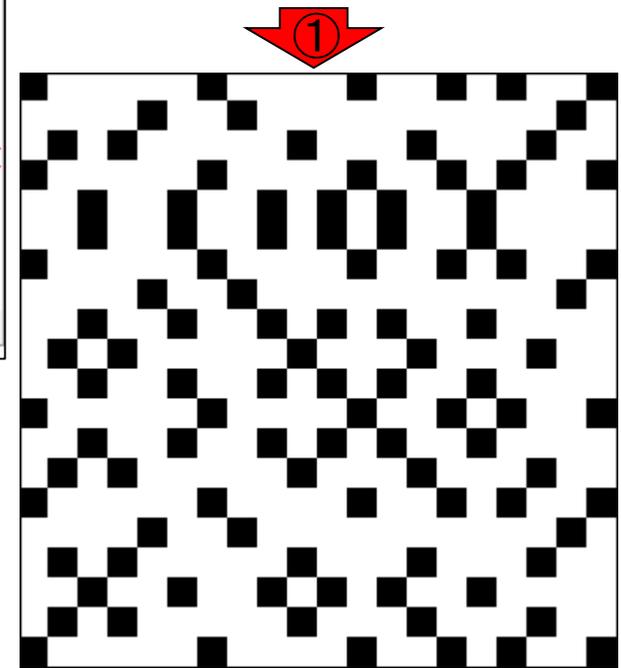
What can I find? Gene families based on HAMAP and PANTHER classification.

ⓘ More about comparative analyses

ⓘ Phylogenetic overview of gene families

Variation

This species currently has no variation database. However you can process your own variants using the Variant Effect Predictor:

Variant Effect Predictor **Ve!P**

Ensembl Bacteria release 42 - Dec 2018 © EMBL-EBI

W13：Ensembl Bacteria

- W13: Ensembl Bacteria : Cunningham et al., Nucleic Acids Res., 2019
  - Lactobacillus rhamnosus GG
  オリジナルはファイル名が長いので短くしています。念のためここでもダウンロードできるようにしています。
  - release42のゲノム配列ファイル：ASM2650v1.fa （約3MB）
  - release42のアノテーションファイル：ASM2650v1.gff3 （約2MB）

W14：SRA

W14：SRA

W14：SRA

W14：SRA

W14：SRA

日本乳酸菌学会誌の連載第13回 65

W15：*L. rhamnosus* GG

W15：*L. rhamnos*

5. **Bilophila wadsworthia worsens high-fat diet-induced metabolic impairments in inflammation dependent and independent manners [bacterial gene expression]**
Taxonomy: *uncultured bacterium*
Project data type: Transcriptome or Gene expression
Scope: Multiisolate
ILTOO Pharma
Accession: PRJNA445875 ID: 445875

6. **RNA-seq analysis of Lactobacillus at acidic stress** ③
Taxonomy: *Lactobacillus rhamnosus GG*
Project data type: Transcriptome or Gene expression
Scope: Multiisolate
Computational and Synthetic Biology Laboratory, Department of Biotechnology, Korea U
Accession: PRJNA419802 ID: 419802

7. **Lactobacillus plantarum**
Global transcriptional responses to oxidative stress conditions in **lactobacillus** plantarum
Taxonomy: *Lactobacillus plantarum*
Project data type: Transcriptome or Gene expression
Scope: Multiisolate
College of Food Science and Nutritional Engineering, China Agricultural University
Accession: PRJNA387288 ID: 387288

8. **Candida albicans**
Antifungal defense of probiotic **Lactobacillus** rhamnosus GG is mediated by blocking adhesion and nutrient depletion
Taxonomy: *Candida albicans*
Project data type: Transcriptome or Gene expression
Scope: Multiisolate
MBT, Fraunhofer IGB

W15：*L. rhamnosus GG*

W15：*L. rhamnosus* GG

日本乳酸菌学会誌の連載第13回

W15：*L. rhamnosus GG*

RNA-seq analysis of Lactobacillus × +

https://www.ncbi.nlm.nih.gov/bioproject/419802

NCBI   Resources ⊙ How To ⊙   Sign in to NCBI

BioProject   [BioProject ▼] [   ] [Search]
              Advanced   Browse by Project attributes   Help

Display Settings: ▼   Send to: ▼

RNA-seq analysis of Lactobacillus at acidic stress   Accession: PRJNA419802   ID: 419802

To understand transcriptional regulation of probiotic bacteria under acidic condition, RNAseq analysis was carried out over different growth conditions
Overall design: Comparison of acidic (pH4) and neutral (pH7) conditions by differentially expressed genes   ①

See Genome Information for Lactobacillus rhamnosus

NAVIGATE ACROSS

74 additional projects are related by organism.

Related information
BioSample
Genome
GEO DataSets
SRA
Taxonomy

Accession	PRJNA419802; GEO: GSE107337
Data Type	Transcriptome or Gene expression
Scope	Multiisolate
Organism	Lactobacillus rhamnosus GG [Taxonomy ID: 568703] Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus rhamnosus; Lactobacillus rhamnosus GG
Submission	Registration date: 25-Nov-2017 Computational and Synthetic Biology Laboratory, Department of Biotechnology, Korea University
Relevance	Industrial

Recent activity
                                                Turn Off   Clear

📄 RNA-seq analysis of Lactobacillus at acidic st BioProject
🔍 SRA Links for BioProject (Select 475032) (36)   SRA
📄 How Lactobacillus plantarum Shapes its Transcriptom BioProject
🔍 (Lactobacillus RNA-seq) AND bioproject_sra[filter] NO BioProject

                                                See more...

Project Data:

W16：GSE107337

W16：GSE107337

W16：GSE107337

W16：GSE107337

GSM2864949: pH7_CCG rep3; Lactobacillus rhamnosus GG; RNA-Seq
1. 1 ILLUMINA (Illumina MiSeq) run: 846,623 spots, 425M bases, 267.3Mb downloads
Accession: SRX3422369

GSM2864948: pH7_CCG rep2; Lactobacillus rhamnosus GG; RNA-Seq
2. 1 ILLUMINA (Illumina MiSeq) run: 2.6M spots, 1.3G bases, 810.6Mb downloads
Accession: SRX3422368

①

GSM2864947: pH7_CCG rep1; Lactobacillus rhamnosus GG; RNA-Seq
3. 1 ILLUMINA (Illumina MiSeq) run: 3.1M spots, 1.6G bases, 967.5Mb downloads
Accession: SRX3422367

GSM2864946: pH4_24h rep3; Lactobacillus rhamnosus GG; RNA-Seq
4. 1 ILLUMINA (Illumina MiSeq) run: 1.8M spots, 901.5M bases, 564Mb downloads
Accession: SRX3422366

GSM2864945: pH4_24h rep2; Lactobacillus rhamnosus GG; RNA-Seq
5. 1 ILLUMINA (Illumina MiSeq) run: 3.9M spots, 1.9G bases, 1.2Gb downloads
Accession: SRX3422365

②

GSM2864944: pH4_24h rep1; Lactobacillus rhamnosus GG; RNA-Seq
6. 1 ILLUMINA (Illumina MiSeq) run: 1.4M spots, 690.4M bases, 428.8Mb downloads
Accession: SRX3422364

GSM2864943: pH4_1h rep3; Lactobacillus rhamnosus GG; RNA-Seq
7. 1 ILLUMINA (Illumina MiSeq) run: 1.8M spots, 883.8M bases, 562.8Mb downloads
Accession: SRX3422363

GSM2864942: pH4_1h rep2; Lactobacillus rhamnosus GG; RNA-Seq
8. 1 ILLUMINA (Illumina MiSeq) run: 1.5M spots, 738.2M bases, 467.2Mb downloads
Accession: SRX3422362

③

GSM2864941: pH4_1h rep1; Lactobacillus rhamnosus GG; RNA-Seq
9. 1 ILLUMINA (Illumina MiSeq) run: 301,126 spots, 151.2M bases, 94.2Mb downloads
Accession: SRX3422361

W17：原著論文を探す

RNA-seq analysis of Lactobacillus

https://www.ncbi.nlm.nih.gov/bioproject/419802

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BioProject   [BioProject ▼] [   ] [Search]
                  Advanced   Browse by Project attributes   Help

Display Settings: ▾   Send to: ▾

RNA-seq analysis of Lactobacillus at acidic stress   Accession: PRJNA419802   ID: 419802

To understand transcriptional regulation of probiotic bacteria under acidic condition, RNAseq analysis was carried out over different growth conditions Overall design: Comparison of acidic (pH4) and neutral (pH7) conditions by differentially expressed genes

See Genome Information for Lactobacillus rhamnosus

Accession	PRJNA419802; GEO: GSE107337 ①
Data Type	Transcriptome or Gene expression
Scope	Multiisolate
Organism	Lactobacillus rhamnosus GG [Taxonomy ID: 568703] Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus rhamnosus; Lactobacillus rhamnosus GG
Submission	Registration date: 25-Nov-2017 ② Computational and Synthetic Biology Laboratory, Department of Biotechnology, Korea University
Relevance	Industrial

Navigate Across

74 additional projects are related by organism.

Related information
BioSample
Genome
GEO DataSets
SRA
Taxonomy

Recent activity
Turn Off   Clear
- RNA-seq analysis of Lactobacillus at acidic s BioProject
- SRA Links for BioProject (Select 475032) (36) SRA
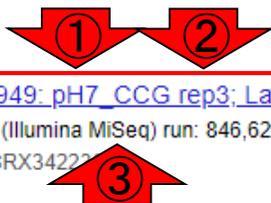- How Lactobacillus plantarum Shapes its Transcriptom BioProject
- (Lactobacillus RNA-seq) AND bioproject_sra[filter] NO BioProject

See more...

Project Data:

W17：原著論文を探す

W17：原著論文を探す

W17：原著論文を探す

RNA-seq analysis of Lactobacillus ✕ ＋

https://www.ncbi.nlm.nih.gov/bioproject/419802

Organism	Lactobacillus rhamnosus GG [Taxonomy ID: 568703] Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus rhamnosus; Lactobacillus rhamnosus GG
Submission	Registration date: 25-Nov-2017 Computational and Synthetic Biology Laboratory, Department of Biotechnology, Korea University
Relevance	Industrial

RNA-seq analysis of Lactobacillus at acidic s BioProject

SRA Links for BioProject (Select 475032) (36) SRA

How Lactobacillus plantarum Shapes its Transcriptom BioProject

(Lactobacillus RNA-seq) AND bioproject_sra[filter] NO BioProject

See more...

Project Data:

Resource Name	Number of Links
SEQUENCE DATA	
SRA Experiments	9
OTHER DATASETS	
BioSample	9
GEO DataSets	1 ②

▾ GEO Data Details

Parameter	Value
Data volume, Supplementary Mbytes	1

▾ SRA Data Details

Parameter	Value
Data volume, Gbases	9
Data volume, Mbytes	5634

①

W17：原著論文を探す

W17：原著論文を探す

W17：原著論文を探す

GEO Accession viewer

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107337

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Scope: Self ▼　Format: HTML ▼　Amount: Quick ▼　GEO accession: GSE107337　[GO]

Series GSE107337　　　　　　　　　　Query DataSets for GSE107337

Status	Public on Nov 29, 2017
Title	RNA-seq analysis of Lactobacill.. at acidic stress
Organism	Lactobacillus rhamnosus GG　①
Experiment type	Expression profiling by high throughput sequencing
Summary	To understand transcriptional regulation of probiotic bacteria under acidic condition, RNAseq analysis was carried out over different growth conditions
Overall design	Comparison of acidic (pH4) and neutral (pH7) conditions by differentially expressed genes
Contributor(s)	Choi I, Oh S　②
Citation missing	*Has this study been published? Please login to update or notify GEO.*
Submission date	Nov 25, 2017
Last update date	Jan 24, 2018
Contact name	kucsbl submitter
E-mail	kucsbl.group@gmail.com
Organization name	Korea University
Department	Department of Biotechnology
Lab	Computational and Synthetic Biology Laboratory

W17：原著論文を探す

W17：原著論文を探す

Transcriptional Response and Enh × +

https://www.ncbi.nlm.nih.gov/pubmed/?term=Lactobacillus+rhamnosus+GG+Choi+I%2...

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J Microbiol Biotechnol

J Microbiol Biotechnol. 2018 Oct 28;28(10):1604-1613. doi: 10.4014/jmb.1807.07033.

Transcriptional Response and Enhanced Intestinal Adhesion Ability of *Lactobacillus rhamnosus* GG after Acid Stress.

Bang M[1], Yong CC[1], Ko HJ[2,3], Choi IG[2], Oh S[1].

⊕ Author information

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Abstract
Lactobacillus rhamnosus GG (LGG) is a probiotic commonly used in fermented dairy products. In this study, RNA-sequencing was performed to unravel the effects of acid stress on LGG. The transcriptomic data revealed that the exposure of LGG to acid at pH 4.5 (resembling the final pH of fermented dairy products) for 1 h or 24 h provoked a stringent-type transcriptomic response wherein stress response- and glycolysis-related genes were upregulated, whereas genes involved in gluconeogenesis, amino acid metabolism, and nucleotide metabolism were suppressed. Notably, the pilus-specific adhesion genes, *spaC*, and *spaF* were significantly upregulated upon exposure to acid-stress. The transcriptomic results were further confirmed via quantitative polymerase chain reaction analysis. Moreover, acid-stressed LGG demonstrated an enhanced mucin-binding ability in vitro, with 1 log more LGG cells ($p < 0.05$) bound to a mucin layer in a 96-well culture plate as compared to the control. The enhanced intestinal binding ability of acid-stressed LGG was confirmed in an animal study, wherein significantly more viable LGG cells (≥ 2 log CFU/g) were observed in the ileum, caecum, and colon of acid-stressed LGG-treated mice as compared with a non-acid-stressed LGG-treated control group. To our knowledge, this is the first report showing that acid stress enhanced the intestine-binding ability of LGG through the induction of pili-related genes.

Similar articles

Lactobacillus rhamnosus GG SpaC pilin subunit binds [Anim Sci J. 2016]

Shear-Enhanced Dynamic Adhesion of Lactobacillus rha [Langmuir. 2018]

Adhesion and nanomechanics of pili from the probiotic [ACS Nano. 2013]

Adhesive interactions between milk fat ᴳ [Colloids Surf B Biointerfaces....]

Review Probiotics in digestive dis [Minerva Gastroenterol Dietol. ...]

See reviews...

See all...

W18：ENA

W18：ENA

W18：ENA

https://www.ebi.ac.uk/ena/data/v ×   +

← → C ⌂   🔒 European Bioinformatics Institute [GB] | https://www.ebi.ac.uk/ena/data/view/PRJNA419802   ☆ 👤 ⋮

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🟢 Bulk Download Files ⚠ (If the downloader app doesn't open, please try using Firefox to launch it.)

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Study accession	Sample accession	Secondary sample accession	Experiment accession	Run accession	Tax ID	Scientific name	Instrument model	Library layout	FASTQ files (FTP)	FASTQ files (Galaxy)
PRJNA419802	SAMN08098216	SRS2714081	SRX3422361	SRR6322562	568703	Lactobacillus rhamnosus GG	Illumina MiSeq	PAIRED	File 1 File 2	File 1 File 2
PRJNA419802	SAMN08098215	SRS2714083	SRX3422362	SRR6322563	568703	Lactobacillus rhamnosus GG	Illumina MiSeq	PAIRED	File 1 File 2	File 1 File 2
PRJNA419802	SAMN08098214	SRS2714082	SRX3422363	SRR6322564	568703	Lactobacillus rhamnosus GG	Illumina MiSeq	PAIRED	File 1 File 2	File 1 File 2
PRJNA419802	SAMN08098213	SRS2714084	SRX3422364	SRR6322565	568703	Lactobacillus rhamnosus GG	Illumina MiSeq	PAIRED	File 1 File 2	File 1 File 2

W19：ダウンロード

W19：ダウンロード

W19：ダウンロード

W19:ダウンロード

W19：ダウンロード

W19：ダウンロード

W19：ダウンロード

W19:ダウンロード

W20：成功例

W20：成功例

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Remote Files

FASTQ ✕ | Submitted | SRA

Downlo...	Accession	Name	Size	Progress	MD5 ...
✓	SRR6322566	SRR6322566_2.fastq.gz	724.30 MB		⊘
✓	SRR6322567	SRR6322567_1.fastq.gz	300.99 MB		⊘
✓	SRR6322567	SRR6322567_2.fastq.gz	336.74 MB		⊘
✓	SRR6322568	SRR6322568_1.fastq.gz	525.84 MB		⊘
✓	SRR6322568	SRR6322568_2.fastq.gz	587.34 MB		⊘
✓	SRR6322569	SRR6322569_1.fastq.gz	430.22 MB		⊘
✓	SRR6322569	SRR6322569_2.fastq.gz	486.67 MB		⊘
✓	SRR6322570	SRR6322570_1.fastq.gz	141.62 MB		⊘
✓	SRR6322570	SRR6322570_2.fastq.gz	159.59 MB		⊘

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W20：成功例

W21：失敗例

①こんな感じで、②失敗することもありますが、めげずに③を押してリトライ。この日は何度トライしても同じ失敗が繰り返されました。あまり失敗しまくるようだと、諦めて個別にダウンロードでもよいと思います。

W22：失敗した場合の対応策1

W22：失敗した場合の対応策2

- W22：SRAdb：Zhu et al., BMC Bioinformatics, 2013

原著論文(Bang et al., J Microbiol Biotechnol., 2018)の記述からGSE107337を頼りに、 SRP125628にたどり着いています。 したがって、ここで指定するのは"SRP125628"となります。 「(Rで)塩基配列解析」の「イントロ | NGS | 配列取得 | FASTQ or SRA | SRAdb(Zhu_2013)」の 例題3以降と基本的に同じです。

```
param <- "SRP125628"                    #取得したいSRA IDを指定

#必要なパッケージをロード
library(SRAdb)                          #パッケージの読み込み

#前処理
#sqlfile <- "SRAmetadb.sqlite"          #最新でなくてもよく、手元に予めダウンロードしてある"SRA
sqlfile <- getSRAdbFile()               #最新のSRAmetadb SQLiteファイルをダウンロードして解凍
sra_con <- dbConnect(SQLite(), sqlfile) #おまじない

#前処理(実験デザインの全体像を表示)
hoge <- sraConvert(param, sra_con=sra_con)#paramで指定したSRA IDに付随するstudy (SRP...), s
hoge                                    #hogeの中身を表示
apply(hoge, 2, unique)                  #hoge行列の列ごとにユニークな文字列を表示させている。
getFASTQinfo(in_acc=hoge$run, sra_con=sra_con)#hoge$runで指定したSRRから始まるIDのFASTQファ

#本番(FASTQファイルのダウンロード)
getFASTQfile(hoge$run, sra_con=sra_con, srcType='ftp')#hoge$runで指定したSRRから始まるIDのF
```

SRAdb：Zhu et al., *BMC Bioinformatics*, **14**: 19, 2013

W22：失敗した場合の対応策

- W22：SRAdb：Zhu et al., BMC Bioinformatics, 2013

原著論文(Bang et al., J Microbiol Biotechnol, 2018)の記述からGSE107327を頼りに、SRP125628にたどり着いています。 したがって、ここで指定
「イントロ | NGS | 配列取得 | FASTQ or SR

```
param <- "SRP125628"

#必要なパッケージをロード
library(SRAdb)

#前処理
#sqlfile <- "SRAmetadb.sqlite"
sqlfile <- getSRAdbFile()         ①
sra_con <- dbConnect(SQLite(), sqlfil

#前処理(実験デザインの全体像を表示)
hoge <- sraConvert(param, sra_con=sra
hoge
apply(hoge, 2, unique)
getFASTQinfo(in_acc=hoge$run, sra_con

#本番(FASTQファイルのダウンロード)
getFASTQfile(hoge$run, sra_con=sra_co
```

R RGui (64-bit)　　　　　　　　　　　　　— □ ×

ファイル　編集　閲覧　その他　パッケージ　ウインドウ　ヘルプ

R Console

```
       anyDuplicated, append, as.data.frame, basename, cbind, colMeans,
       colnames, colSums, dirname, do.call, duplicated, eval, evalq,
       Filter, Find, get, grep, grepl, intersect, is.unsorted, lapply,
       lengths, Map, mapply, match, mget, order, paste, pmax, pmax.int,
       pmin, pmin.int, Position, rank, rbind, Reduce, rowMeans,
       rownames, rowSums, sapply, setdiff, sort, table, tapply, union,
       unique, unsplit, which, which.max, which.min

 要求されたパッケージ RCurl をロード中です
 要求されたパッケージ bitops をロード中です
Setting options('download.file.method.GEOquery'='auto')
Setting options('GEOquery.inmemory.gpl'=FALSE)
>
> #前処理
> #sqlfile <- "SRAmetadb.sqlite"          #最新でなくてもよく、手元に予めダウ$
> sqlfile <- getSRAdbFile()               #最新のSRAmetadb SQLiteファイルをダ$
 URL 'https://s3.amazonaws.com/starbuckl/sradb/SRAmetadb.sqlite.gz' を試して$
Content type 'binary/octet-stream' length 2660684574 bytes (2537.4 MB)
downloaded 2537.4 MB


Unzipping...
```

W23：DRA

W23：DRA

W24：DRA

W24：DRA

W25：DRA

W25：DRA

W26：実験デザイン

W26：実験デザイン

W26：実験デザイン

Platforms (1)　　GPL24302　Illumina MiSeq (Lactobacillus rhamnosus GG)

Samples (9)
⊟ Less...

GSM2864941　pH4_1h rep1
GSM2864942　pH4_1h rep2
GSM2864943　pH4_1h rep3
GSM2864944　pH4_24h rep1
GSM2864945　pH4_24h rep2　①
GSM2864946　pH4_24h rep3
GSM2864947　pH7_CCG rep1
GSM2864948　pH7_CCG rep2
GSM2864949　pH7_CCG rep3

Relations
BioProject　　PRJNA419802
SRA　　SRP125628

Download family	Format
SOFT formatted family file(s)	SOFT ⍰
MINiML formatted family file(s)	MINiML ⍰
Series Matrix File(s)	TXT ⍰

Supplementary file	Size	Download	File type/resource
GSE107337_RPKM.csv.gz	21.1 Kb	(ftp)(http)	CSV
GSE107337_RawCounts.csv.gz	20.8 Kb	(ftp)(http)	CSV

Raw data are available in SRA

URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107337

W27：表1

	A	B	C	D	E	F
1						
2		サンプル名	SRR ID	リード数（片側のみ）	ファイルサイズ（forward側）	ファイルサイズ（reverse側）
3		pH4.5_1h rep1	SRR6322562	301,126	53,833,728	59,662,336
4		pH4.5_1h rep2	SRR6322563	1,470,602	265,838,592	296,910,848
5		pH4.5_1h rep3	SRR6322564	1,760,461	319,807,488	354,557,952
6		pH4.5_24h rep1	SRR6322565	1,375,368	241,553,408	268,341,248
7		pH4.5_24h rep2	SRR6322566	3,869,088	682,504,192	759,697,408
8		pH4.5_24h rep3	SRR6322567	1,795,874	315,613,184	353,099,776
9		pH7_CCG rep1	SRR6322568	3,095,834	551,383,040	615,870,464
10		pH7_CCG rep2	SRR6322569	2,570,876	451,117,056	510,308,352
11		pH7_CCG rep3	SRR6322570	846,623	148,500,480	167,350,272

W27：表1

	A	B	C	D	E	F
1						
2		サンプル名	SRR ID	リード数（片側のみ）	ファイルサイズ（forward側）	ファイルサイズ（reverse側）
3		pH4.5_1h rep1	SRR6322562	301,126 ②	53,833,728	59,662,336
4		pH4.5_1h rep2	SRR6322563	1,470,602	265,838,592	296,910,848
5		pH4.5_1h rep3	SRR6322564	1,760,461	319,807,488	354,557,952
6		pH4.5_24h rep1	SRR6322565	1,375,368	241,553,408	268,341,248
7		pH4.5_24h rep2	SRR6322566	3,869,088 ①	682,504,192	759,697,408
8		pH4.5_24h rep3	SRR6322567	1,795,874	315,613,184	353,099,776
9		pH7_CCG rep1	SRR6322568	3,095,834	551,383,040	615,870,464
10		pH7_CCG rep2	SRR6322569	2,570,876	451,117,056	510,308,352
11		pH7_CCG rep3	SRR6322570	846,623	148,500,480	167,350,272
12						

W28：GSM2864941

W28：GSM2864941

W28：GSM2864941

GEO Accession viewer

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM2864941

Source name	whole transcriptome
Organism	Lactobacillus rhamnosus GG
Characteristics	ph: 4
Extracted molecule	total RNA
Extraction protocol	TruSeq RNA Sample Preparation Kit (Illumina) was used. RNA libraries were prepared for sequencing using standard Illumina protocols
Library strategy	RNA-Seq
Library source	transcriptomic
Library selection	cDNA
Instrument model	Illumina MiSeq

Data processing   Read data were aligned on the reference genome sequence.
DESeq and edgeR package in the Bioconductor/R packages were used for making RPKM values.
Base-calling by the MiSeq platform
Alignment by Bowtie software
Using R packages for DEG analysis
Genome_build: Lactobacillus rhamnosus GG (ATCC 53103, RefSeq ID: NC_013198.1)
Supplementary_files_format_and_content: CSV file of normalized RPKM value from edgeR for every samples.

Submission date	Nov 25, 2017
Last update date	Nov 29, 2017
Contact name	kucsbl submitter
E-mail	kucsbl.group@gmail.com
Organization name	Korea University
Department	Department of Biotechnology

W29：BowtieとRPKM

GEO Accession viewer

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM2864941

Source name	whole transcriptome
Organism	Lactobacillus rhamnosus GG
Characteristics	ph: 4
Extracted molecule	total RNA
Extraction protocol	TruSeq RNA Sample Preparation Kit (Illumina) was used. RNA libraries were prepared for sequencing using standard Illumina protocols
Library strategy	RNA-Seq
Library source	transcriptomic
Library selection	cDNA
Instrument model	Illumina MiSeq
Data processing	Read data were aligned on the reference genome sequence. ② DESeq and edgeR package in the Bioconductor/R packages were used for making RPKM values. Base-calling by the MiSeq platform ① Alignment by Bowtie software Using R packages for DEG analysis Genome_build: Lactobacillus rhamnosus GG (ATCC 53103, RefSeq ID: NC_013198.1) Supplementary_files_format_and_content: CSV file of normalized RPKM value from edgeR for every samples.
Submission date	Nov 25, 2017
Last update date	Nov 29, 2017
Contact name	kucsbl submitter
E-mail	kucsbl.group@gmail.com
Organization name	Korea University
Department	Department of Biotechnology

W30：CountとRPKM

GEO Accession viewer

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107337 ①

Platforms (1)	GPL24302 Illumina MiSeq (Lactobacillus rhamnosus GG)
Samples (9)	GSM2864941 pH4_1h rep1
Less...	GSM2864942 pH4_1h rep2
	GSM2864943 pH4_1h rep3
	GSM2864944 pH4_24h rep1
	GSM2864945 pH4_24h rep2
	GSM2864946 pH4_24h rep3
	GSM2864947 pH7_CCG rep1
	GSM2864948 pH7_CCG rep2
	GSM2864949 pH7_CCG rep3

Relations
BioProject   PRJNA419802
SRA   SRP125628

Download family	Format
SOFT formatted family file(s)	SOFT ?
MINiML formatted family file(s)	MINiML ?
Series Matrix File(s)	TXT ?

Supplementary file	Size	② Download	File type/resource
GSE107337_RPKM.csv.gz	21.1 Kb	(ftp)(http)	CSV
GSE107337_RawCounts.csv.gz	20.8 Kb	(ftp)(http)	CSV

③

Raw data are available in SRA
Processed data is available on Series record

W31：カウントデータ

①raw countデータファイルのほう。②averageと書かれていることからもわかるように、3状態間比較で、反復データの平均値になっている。だから3列分しかない。

自動保存 ● オフ   🔒 🖫 ↻ ▾ ᴄ ▾ ▾   GSE107337_RawCounts.csv ①   サ

ファイル　ホーム　挿入　ページレイアウト　数式　データ　校閲　表示　ヘルプ　♡ 操作アシスト　ⒶⓍ 共有　💬

E27　　▾　⦂　✕　✓　fx

	A	②	②	②	E
1	Gene	pH4_1h_average_rawcount	pH4_24h_average_rawcount	pH7_CCG_average_rawcount	
2	LGG_00001	286	676	545	
3	LGG_00002	776	1601	1450	
4	LGG_00003	71	114	91	
5	LGG_00004	201	470	368	
6	LGG_00005	368	1160	1447	
7	LGG_00006	1041	2742	2880	
8	LGG_00007	7	10	2	
9	LGG_00008	73	76	499	
10	LGG_00009	16	45	42	
11	LGG_00010	20	65	57	
12	LGG_00011	2281	2596	2150	
13	LGG_00012	2510	3631	3097	
14	LGG_00013	971	1301	1424	
15	LGG_00014	17	78	16	
16	LGG_00015	82	255	33	

◀ ▶　　GSE107337_RawCounts　　⊕　　⦂　◀

ScrollLock

W32：記載内容と一致

①1列目が遺伝子名、②2列目がpH4.5の酸ストレスを1時間与えた平均のカウント、③3列目がpH4.5の酸ストレスを24時間与えた平均のカウント、④4列目がpH7のコントロールの平均のカウント、であることを覚えておく。

	① Gene	② pH4_1h_average_rawcount	③ pH4_24h_average_rawcount	④ pH7_CCG_average_rawcount	E
1	Gene	pH4_1h_average_rawcount	pH4_24h_average_rawcount	pH7_CCG_average_rawcount	
2	LGG_00001	286	676	545	
3	LGG_00002	776	1601	1450	
4	LGG_00003	71	114	91	
5	LGG_00004	201	470	368	
6	LGG_00005	368	1160	1447	
7	LGG_00006	1041	2742	2880	
8	LGG_00007	7	10	2	
9	LGG_00008	73	76	499	
10	LGG_00009	16	45	42	
11	LGG_00010	20	65	57	
12	LGG_00011	2281	2596	2150	
13	LGG_00012	2510	3631	3097	
14	LGG_00013	971	1301	1424	
15	LGG_00014	17	78	16	
16	LGG_00015	82	255	33	

GSE107337_RawCounts

ScrollLock

W32：記載内容と一致

GSE107337_RawCounts.csv・

	A	B	C	D	E
2151	LGG_02232	33	95	70	
2152	LGG_02233	227	331	539	
2153	LGG_02234	406	791	1093	
2154	LGG_02235	582	999	1289	
2155	LGG_02236	277	467	647	
2156	LGG_02238	768	674	577	
2157	LGG_02239	5146	18308	5690	
2158	LGG_02240 ①	1106	2444 ②	422 ③	
2159	LGG_02241	87	107	130	
2160	LGG_02242	377	340	540	
2161	LGG_02244	36	116	44	
2162	LGG_02245	19	101	29	
2163	LGG_02246	23	63	8	
2164	LGG_02247	384	531	556	
2165	LGG_02248	245	405	318	
2166	LGG_02249	125	188	227	

A2158　LGG_02240

GSE107337_RawCounts

ScrollLock

W32：記載内容と一致

原著論文のp1608の左上のほうでは、①LGG_02240（GroES gene）の②24時間ストレスの発現が、③コントロールに比べて、2.53倍変化したと記載されている。倍率変化なので、④5.79倍変化したと書くべきだとは思われるが、⑤log2変換した結果と確かに一致する。論文のTable S2の脚注部分で、確かにlog2変換したものをFold changeと表現している…まあ、妥当