2019.04.05版

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*

日本乳酸菌学会誌の連載第13回

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Abstract To unravel the biological function of the widely used probiotic bacterium Lactobacillus rhamnosus GG, we compared its 3.0-Mbp genome sequence with the similarly sized genome of L. rhamnosus LC705, an adjunct starter culture exhibiting reduced binding to mucus. Both genomes demonstrated high sequence identity and synteny. However, for both strains, genomic islands, 5 in GG and 4 in LC705, punctuated the colinearity. A significant number of strain-specific genes were predicted in these islands (80 in GG and 72 in LC705). The GG- specific islands included genes coding for bacteriophage components, sugar metabolism and	Similar a Characte pili [App] Mucosal a the [App] Genomic	articles rization o Environ I adhesior Environ I characte	of th Mici 1 pro Mici eriza
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日本乳酸菌学会誌の連載第13回

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

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①Table 1で、②*L. rhamnosus* GGの基本情報が見られる。

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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.

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Acknowledgments

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	Abstract Lactobacillus rhamnosus is a facultatively heterofermentative lactic acid bacterium and is frequently isolated from human gastrointestinal mucosa of healthy individuals. L. rhamnosus ATCC 53103, isolated from a healthy human intestinal flora, is one of the most widely used and well-documented probiotics. Here, we report the finished and annotated genome sequence of this organism.			
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Complete genome sequence of til X Complete Genome Sequence of t × + https://jb.asm.org/content/191/24/7630.long Θ С \cap The complete genome sequence of Lactobacillus rhamnosus ATCC 53103 was determined by a wholegenome 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 t 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

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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.



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The sequence data for the L. rhamnosus ATCC 53103 genome are available in DDBJ/GenBank/EMBL

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ACKNOWLEDGMENTS

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

ACKNOWLEDGMENTS

①AP011548のページにアクセス。

W6: AP011548.fasta

S Lactoba	tillus rhamnosus ATCC 58 × +		-		×
\leftrightarrow \rightarrow c	ttps://www.ncbi.nlm.nih.gov/nuccore/AP011548	☆	x	Θ	:
S NCBI	Resources 🗹 How To 🖸				^
Nucleotic	e Nucleotide Advanced				
① The Nu	eleotide database will include EST and GSS sequences in early 2019. <u>Read more</u> .				
GenBank - Lactob GenBank: /	acillus rhamnosus ATCC 53103 DNA, complete	gei	nom	Send to	
FASTA G	aphics				_
Go to: () LOCUS DEF INITION ACCESSION VERSION DBLINK KEYWORDS SOURCE ORGANISM	APO11548 3005051 bp DNA circular BCT 07-OCT-2016 Lactobacillus rhamnosus ATCC 53103 DNA, complete genome. APO11548 APO11548.1 BioProject: <u>PRJDA40637</u> BioSample: <u>SAMD00060369</u> Lactobacillus rhamnosus GG <u>Lactobacillus rhamnosus GG</u> Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus.				*

W6: AP011548.fasta

Cactobacillus rhamnosus	ATCC 58 × +		-		×
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GenBank Format Summary GenBank GenBank (full) FASTA	nosus ATCC 53103 DNA, comple	ete ge	nom	Send to	
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www.ncbi.nlm.nih.gov を待機して	いより				•

W6: AP011548.fasta

全選択して、中身をAP011548.fasta というファイル名で保存。

× S Lactobacillus rhamnosus ATCC 53 × + Θ С https://www.ncbi.nlm.nih.gov/nuccore/AP011548.1?report=fast... ☆ J. i \cap actobacillus rhamnosus ATCC 53103 <u>DNA, complete *g*enome</u> AGGA GOTT TGGGOT TA COTGAA TGAT AA AT TOOGTGAA GA GT TGACCOCCA GT OGGT GGAT TCAA ACAGOCAA GCOGGT CA AA TT GAOCCA AGAT AA ACTOGA AA TOGA AGTA OOT 'GCA CAAGGCCTAC TGGGAGAAAAAT CTGGTTACCAAAGTGGTGGAAGGCGTCTATGAGTTTGC XAA TTA GAA GTOGAT OCTGTCAT CA TGACCA AA GA OGAGTT GCAGOCOGCT TOCA OGCA TCAGOA TCAA O TGACGA TGAT GACCAA CA ACTCACCTTT AA GGCA AA AA CGCA TT TA AA TCOGAA AT AT A FGACCGCTT OGTGAT TGGT AA AGGT AA OCAA AT GGCT CA OGOGGCGGCT TT AGCA GT AGCT GA GGOGOC TACAACCCGCTGTTTATTTACGGCGGCGTTGGCTTGGGGAAGACGCATTTGAT DTGGT CT TGGAAAAT AA TOODGOTGODAA CA TT AAAT AT GT CA ODAGOGAGAA TT TT GODAA A AT TOCA TOCA AA CA AA AC AGCA AGAA CA AT TTOGTO AA GA GTACOGAA AT GGTT GATGAT AT CCAA TT CT TC GGTGACAA AGAA GCCA CGCA AGAA GA GT TC TT CCAT ACAT TT A GT ACGA AA AT AT GA AGCA AA TT GT OCTA ACCA GT GA TOGT OT GOOT AA TGAGAT TOOCAA GT TGGTAT CCCGCTTCAA TA AA GGACTA TCCGTT GA CGTCACCCCGCCG GCCAT OT TA OGCA AT AA AGOC AA TGCA GA AGAT OT CA GT AT TO OGGA TGAC AC GOT I GGGCAA AT TGAA AGTA ACGT CCGT GA TCTT GA AGGGGCGCTGGT TCGGGT TCAGG. GAA OGAGGA CA TOACAA OCAGTT TA GOGGOA GA TGOA OT AA AA GOOOTT AA ACTOGA TGAT OG A GC GGACAA CT CA CCAT T C OG CA GA TT CT OG AT GC GG T C GC CA AA TA TT TT CA AG T C ACOG TT CA GGA FAAAA GGCAAAAAA GCGGGT TAAACAAA TT GT CA TT OCOCGGCAAA TC GCCA TG TA CT TA GCACGCGAGA GACOGACAA TA GOTTACOGAA AA TOGGOOAGGA AA TTGGOGGA AA AGAT CA CA OGACTGTOAT TOACGO AA AT CA TGGCTGCCAT GA CCACCA ACGA AGAT TT AA AA GCACAT GT GGTCGA T COTGAA AA A T CGTGGT TAAT COACTT GT GGACAA OT GOCA GAAA CA GAACTT TT TGTOOCTA TO GGT GCAT AA CT TCAT GATT TGCT GT TCCGCA AA GT TA TCCA CA GT TT GA ACACGCO. CTAAAAGCTTTATATATATATATATAAAACGACGTACAGGAGGCTCTTATGAAATTTACGAT XAT TOTT GAAAACOT TGAA TGAT GT TT OCOGOGOT AT TT CAACCAAAACCA OGAT OCOGAT GGGTT AA AA AT OGTGOT CAAT GA TA OT GGAOTA GT GOTGAOOGGT AG TGAT GOTGAOAT TT 1A CCAT TA ACGCTGCT GA TGAA AG TA ACGA TT TA CAAA TCGGCA GCACTGGT GA AA TT GT GT TT CA GOGA AA TOGT TA AA OGGOTGOO AGAA AGCA COAT GA CA GT AGAA GT CAGACGGTCATTACTTCAGGA GCTTCCGA GTTTACGATTCGTGGA GAAGATGCGAACAATTAT ACOGGA AA TTACOGCA GA TGAA GOOTTA TOTGTTOOGGOTGA TGTOTTACGOCA ACTGATTA A CCGT TA TOGOOG TA TOCAAT CA GGAA AGTOGGOO AA TT OT GA COGGGO TGOA TT TA ACGA TI XAA CT OGTT GOOGTT GOGA OO GA TT OGCA TO GGTT AT OGCA AC GT ACTO TGA CAAGTAA TA TOGA TTACGA CA TCA TCA TTOCOGGTAA AA GTCTGA CA GA ACTA AG TCGCA TG TA GGTG. GATGTGACTAAAAT CGAAAT TOGCAT TGCCGAGAAT CAGGTATTAT TTGTAT TTGGACAA GCTT GAAGGCAATT AT CCAGACACAA GT CGACTT AT CCCAACGT CCAGCAAT ACGCAAG T OGAT GOGOOGGCACTGOT GGOT TOCATT CA GOGGGOGA GOTT GOTGAGTOACGA AA GOAGCA GT GOGT TT AGTOOT CA ACAT TGGOGA GOAA AGAGCA ACCA TO TA TGGT AA TT OGCOAGAT GTCGA AGAA GTCTTA AGCTTTAA CA AGCTGA CCGGTCAGGA TCTGGA AA TCTCCTTCAA CCCTG ATT ACAT GAAGGAOGOGOT GOAA GGOT TT GGOCAA ACAGOGAT TGAA GT TGACTT TA OGGOACOGOT GOG 'CACCT TGGT TCCA ACCGAA GA TA AA GA GA AGTT TA TT CA GT TGAT TA CGCC GGT GCGGA CG'

①Gepardプログラムの場所に移動

W7: Gepard

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S Gepard: a rapid and sensitive too × +	- 🗆 ×
← → C ↑ https://www.ncbi.nlm.nih.gov/pubmed/17309896	☆ 📕 \varTheta :
S NCBI Resources 🖂 How To 🖂	▲
Public gov US National Library of Medicine National Institutes of Health Advanced	Sear
Format: Abstract - Send to	•
Bioinformatics. 2007 Apr 15;23(8):1026-8. Epub 2007 Feb 19. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. Krumsiek J ¹ , Arnold R, Rattei T. ⊕ Author information	Full text links OXFORD Academic Save items
Abstract Gepard provides a user-friendly, interactive application for the quick creation of dotplots. It utilizes suffix arrays to reduce the time complexity of dotplot calculation to Theta(m*log n). A client-server mode, which is a novel feature for dotplot creation software, allows the user to calculate dotplots and color them by functional annotation without any prior downloading of sequence or annotation data. AVAILABILITY: Both source codes and executable binaries are available at http://mips.gsf.de/services/analysis/gepard PMID: 17309896 DOI: 10.1093/bioinformatics/btr [Indexed for MEDLINE]	Similar articles JDotter: a Java interface to multiple do [Bioinformatics.] PhyloGenaa user-friendly system for [Bioinformatics.] Idiographica: a general-purp web applic [Bioinformatics.] Review VEGA, the genome browser w [Brief Bioinform.]

Krumsiek et al., Bioinformatics, 23: 1026-1028, 2007

①URLがちょっと変わっていますが、気にしない。

W7: Gepard

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Genome Pair Rapid Dotter (gepa 🗙 🕂	_
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CENOME DAID DATTED (CEDADD)	
GENOME PAIR RAPID DOTTER (GEPARD) Gepard (German: "cheetah", Backronym for "GEnome PAir - Rapid Dotter") allows the calculation of dotplots even for large sequences like chromosomes or bacterial genomes. Reference: Krumsiek J, Arnold R, Rattei T. Gepard: A rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics 2007; 23(8): 1026-8. PMID: 17309896 USE CASES	CUBE NEWS >> Happy new year 2019 01.01.19 >> New paper in Nature Biotechnology 21.12.18 >> Hiring: Open group leader position at CUBE 05.12.18

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

W7: Gepard

①論文自体は若干古いですが、②バクテリアゲノム 程度ならこれで十分です。③ちょっと下に移動。

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GENOME PAIR RAPID DOTTER (GEPARD) Gepard (German: "cheetah", Backronym for "GEnome PAir - Rapid Dotter") allows the calculation of dotplots even for large sequences like chromosomes or bacterial genomes. Reference: Krumsiek J, Arnold R, Rattei T. Gepard: A rapid and sensitive for creating dotplots on genome scale. Bioinformatics 2007; A. (3): 1026-8. PMID: 17309896 USE CASES	CUBE NEWS >> Happy new year 2019 01.01.19 >> New paper in Nature Biotechnology 21.12.18 >> Hiring: Open group leader position at CUBE 05.12.18
Local comparison two of nucleotide or amino acid sequences from user- specified files. Batch dotplot functionality provided by command line access to Gepard.	LATEST PUBLICATIONS Minimum Information about an Uncultivated Virus Genome

W7:Gepard

②このあたりまで移動。GepardはJavaプログラムな ので、③Javaをインストールしておきましょう。



TUTORIAL

An offline version of the tutorial is included in the download package

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閉じる(C)

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更新設定の詳細

×

W7:Gepard

🚺 Genome Pair Rapid Dotter (gepa 🛛 🗙

→ C 合 ③ 保護されていない通信 | cube.univie.ac.at/gepard

+

data remotely and does not contain any form of malware.

SYSTEM REQUIREMENTS

Gepard requires the Java Runtime Environment Version 5.0 or later (http://www.java.com/download/). It has been tested on the following operating systems:

- Microsoft Windows XP and later
- Linux/Un*x systems
- MacOS 10.x

DOWNLOAD



Please download the **jar file** if you want to run gepard on your computer (Java needs to be installed). On MacOS, nagivate to your Downloads folder and open the jar file via right-click. Confirm the execution of this program.

SOURCE CODE

The source code is available in our GitHub repository gepard.

TUTORIAL

An offline version of the tutorial is included in the download package

①jar fileをクリックしてダウンロードしましょう。これが Gepardプログラムの実体です。Javaプログラムの場 合は、Javaさえ予めインストールしておけば、「ダウ ンロード完了 = インストール完了」なのがよいところ

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	galeobdolon.	Lannu	m		
,	The Genetic Trans	sformat	ion of		
	Chlamydia pneun	noniae.			
1	DALTA OT				

CONTACT

X

- +43 1 4277 76681
- +43 1 4277 876681
- 🔯 contact.cube@univie.ac.at

W7:Gepard

1

基本どこでもよいと思いますが、ここでは①デスクトップ 上に、②Gepard-1.40.jarファイルを、③保存しました。



W8:Gepard実行



W8:Gepard実行	<mark>)ファイル(FM17</mark> P011548.fasta)	9322.fastaと をそれぞれ指定。
A Gepard	 – 🗆 X	
Sequences		
Sequence 1 (horizontal):		
Select me		
Sequence 2 (vertical):		
Complementary Select file		
Create dotplot		
>> Advanced mode		
Quit		

①予め作成しておいた比較したい2つ
🛃 Gepard \times _ Sequences Sequence 1 (horizontal): uments\2019\執筆\乳酸菌第13回\FM179322.fasta Complementary Select file Sequence 2 (vertical): uments\2019\執筆\乳酸菌第13回\AP011548.fasta Complementary Select file Create dotplot >> Advanced mode Quit



🐣 Gepard		-	Х
Sequences			
Sequence 1 (horizontal):			
uments\2019\\$\\$\\$\\$\\$\$\$ \$\\$\$\$\$ \$\\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$ \$\\$\$\$\$\$\$			
Sequence 2 (vertical): uments\2019)執筆以酸菌第13回\AP011548.fasta			
Complementary Select file	Working ×		
Create dotplot	Calculating dot matrix Abort		
Quit			

遅くとも、20秒ほどで描画されると思います。

W8:Gepard実行

🎇 FM179322.1 Lactobacillus rhamnosus GG wł	ole gen vs. AP011548.1 Lactobacillus rhamnosus ATCC 53103 D Gepard	<u> 32</u>		×
FM179322.1 Lactobacillus rhamnosus GG wh Sequences Sequence 1 (horizontal): uments\2019\執筆以配菌第13回\FM179322.fasta Complementary Select file Sequence 2 (vertical): uments\2019\執筆以配菌第13回\AP011548.fasta	nole gen vs. AP011548.1 Lactobacillus rhamnosus ATCC 53103 D Gepard FM179322.1 Lactobacillus vs. AP011548.1 Lactobacillus Zoom: 8234 : 1 Word length: 10 GC ratio seq1: 0.4669 Window size: 0 GC ratio seq2: 0.4669 Matrix: DNA Program: Gepard (1.40 final) 0 FM179322.1 Lactobacillus rhamnosus GG whole 3010110 0 FM179322.1 Lactobacillus rhamnosus GG whole			×
Update dotplot	P011548.1 Lactobacillus rhannosus AICC 53103 D		Hor : - Vert: -	
Quit	Position: 3010110, 798698			

W8:Gepard実行

①のあたりをクリックしたら、②その座標付近のアラ インメントが下のほうに表示されることが分かります。



W8:Gepard実行

①クリックする場所を変えると、②その座標付近のア ラインメントが下のほうに表示されます。このような青 線が出ないようにしたい場合は、③を押すとよい。



Advanced mode.

W9: Advanced mode







①Plotタブで、任意の描 画領域を指定可能です。



①ここでは2つの配列ともに、領域 [610000, 640000 bp]としました。② Update dotplot。

💫 FM179322.1 Lactobacillus rhamnosus GG whole gen... vs. AP011548.1 Lactobacillus rhamnosus ATCC 53103 D... - Gepard X FM179322.1 Lactobacillus ... vs. AP011548.1 Lactobacillus ... Sequences Zoom: 8234 : 1 Word length: 10 GC ratio seq1: 0.4669 Sequence 1 (horizontal): GC ratio seq2: 0.4669 Window size: 0 uments\2019\執筆\乳酸菌第13回\FM179322.fa Matrix: DNA Program: Gepard (1.40 final) 3010110 Complementary Select file FM179322.1 Lactobacillus rhamnosus GG whole... 0 Sequence 2 (vertical): ö uments\2019\執筆\乳酸菌第13回\AP011548.fa 111 DNA, Complementary Select file 53103 ATCC 2 Update dotplot 5HH 4 inter-# 12 | 41 12'L TRAAT BORRE IN M. I. BR. rhamnosus -**₽** AP011548.1 Lactobacillus << Simple mode -Plot Misc Display Coordinates Start 1: 610000 Stop 1: 640000 titt at the state of a late state 5 10 H H H Start 2: 610000 3005050 Stop 2: 640000 Position: 0, 3005050 ✓ Auto zoom Small plots Zoom: Auto params Word length: Window size: Auto matrix -

①確かに逆位になっている 領域がありました。



①Export image、②Fig1b.png として保存。③OK

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

①FM179322中に存在する赤矢印の長さに相 当する部分の塩基配列が…





①赤枠内のコマンドを、Rでコピペ実行

W11:Fig. 2a

配列k同士のドットプロットです	
<pre>seq1 <- "ACTCGTAGTCTATCATACGA" seq2 <- "ACTCGTAGTCTATCATACGA" out_f <- "Fig2a.png" param_fig <- c(300, 300)</pre>	#1つめの塩基配列を指定(配列k) #2つめの塩基配列を指定(配列k) #出力ファイル名を指定してout_fに格納 #ファイル出力時の横幅と縦幅を指定(単位はビ
#必要なパッケージをロード library(seqinr)	#バ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=par par(mar=c(0, 0, 0, 0)) dotPlot(s2c(seq1), s2c(seq2), xlab dev.off()	am_fig[1], height=param_fig[2])#出力ファイル #下、左、上、右の順で余白(行)を指定 ="", ylab="")#ブロット #おまじない

こんな感じ。①作業ディレクトリ上に、 ②Fig2a.pngができているはずです。 W11:Fig. 2a seginrでドットプロット seginr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 #1つめの塩基配列を指定(配列k) seq1 <- "ACTCGTAGTCTATCATACGA"</pre> seg2 <- "ACTCGTAGTCTAT_ATACGA"</pre> #2つめの塩基配列を指定/ 起加し out_f <- "Fig2a.png" (2) #出力ファイル名を指定し RGui (64-bit) X #ファイル出力時の横幅な param fig <- c(300, 30 ファイル 編集 閲覧 その他 パッケージ ウインドウ ヘルプ #必要なバッケージをロード 🖆 🖆 🖬 🖻 🔂 😐 🎒 #バッケージの読み込み library(seqinr) R Console #ファイルに保存(pngファイル) png(out f, pointsize=13, width=param_fig[1], height=param_f > getwd() par(mar=c(0, 0, 0, 0)) #下、左、上、右の順でタ [1] "C:/Users/kadota/Desktop" dotPlot(s2c(seq1), s2c(seq2), xlab="", ylab="")#プロット > seq1 <- "ACTCGTAGTCTATCATAC</pre> #1つめの塩基\$ #おまじない dev.off() #2つめの塩基\$ > seq2 <- "ACTCGTAGTCTATCATACGA</p> > out f <- "Fig2a.png" #出力ファイル\$ 4 #ファイル出力\$ > param fig <- c(300, 300) > #必要なパッケージをロード #パッケージの\$ > library(seginr) > #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(seql), s2c(seq2), xlab="", ylab="")#プロ\$ > dev.off() #おまじない null device 1 >

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> ...

W11:Fig. 2a

実物です。同一配列同士のドットプロットなので 、左下端から右上端にかけて、①キレイに直線 状のドットが描かれていることがわかります。



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。



56

W12:Fig. 2b



このコードも実行して、①Fig2b.pngも作成。

赤枠内が反転させた領域のドットプロット。

W12:Fig. 2b



#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



4

確かに反転させることで、赤枠の領域内のドットが「左下端から右上端」ではなく「左上端から 右下端」に切り替わっていることがわかる。

W12:Fig. 2b

W12 : Fig2b
 配列kとlのドットプロットです

seq1 <- "ACTCGTAGTCTATCATACGA"
seq2 <- "ACTCGACTATCTGATTACGA"
out_f <- "Fig2b.png"
param fig <- c(300, 300)</pre>

#必要なバッケージをロード library(seqinr)

#バッケージの読み込み

#1つめの塩基配列を指定(配列k) #2つめの塩基配列を指定(配列1)

#出力ファイル名を指定してout flc格納

#ファイル出力時の横幅と縦幅を指定(単位はビクセル

#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="")#ブロット dev.off() #おまじない



W13: Ensembl Bacteria rhamnosus GGのページ。③と④から 得られるファイルと同じものが…

el Lactobacillus rhamnosus GG - En 🗙 🕂	X
	rhamnosus_gg/Info/Index 🖈 🗷 🛛 🔂 :
	Login/Register ▲ Documentation More ▼ Lactobacillus rhamnosus Q
actobacillus rhamnosus GG	
Lactobacillus rhamnosus GG Lactobacillus rhamnosus GG Provider <u>European Nucleotide Archive</u> P Taxonomy ID <u>568703</u>	
Search Lactobacillus rhamnosus GG Go	
e.g. is1 or FM179322:17235-18251 or synthetase	
Cenome assembly: <u>ASM2650v1</u> C	Gene annotation
 More information and statistics Download DNA sequence (FASTA) Display your data in Ensembl Bacteria 	 What can rink? Protein-cooling and non-cooling genes, spice variants, cDNA and protein sequences, non-coding RNAs. More about this genebuild Download genes, cDNAs, ncRNA, proteins - FASTA - GFF3
Comparative genomics What can I find? Gene families based on HAMAP and PANTHER classification.	Update your old Ensembl IDs Example transcript
 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

Ensembl Bacteria release 42 - Dec 2018 © EMBL-EBI

(1)Ensembl Bacteriaの、(2)L.

①Ensembl Bacteriaの、②L. W13: Ensembl Bacteria W13: Ensembl Bacteria のページ。③と④から 得られるファイルと同じものが⑤です

🛃 Lactobacillus rhamnosus GG - En 🗙 🕂		– 🗆 X	
C ① 保護されていない通信 bacteria.ensembl.org/Lactobacillus_rh	namnosus_gg/Info/Index	☆ 📕 😝 :	
	Documentation More ▼ 🔐 - Lactobacillus rhamnosus	Login/Register	
actobacillus rhamnosus GG			
Lactobacillus rhamnosus GG			
Search Lactobacillus rhamnosus GG • W13: Ensembl Bac	teria : Cunningham et al Nucleic Acid	ls Res 2019	
e.g. is1 or FM179322:17235-18251 or synthetase o Lactobacillus	rhamnosus GG	<u>13 ((63, 2015</u>	
About Lactobacillus rhamnosus GG オリジナルはつ Information and statistics orelease42のグ orelease42のア	<u>7ァイル名が長いので短くしています。念</u> [●] ノム配列ファイル: <u>ASM2650v1.fa</u> (約 7ノテーションファイル: <u>ASM2650v1.gf</u>	<u>のためここでも</u> 3MB) <u>f3</u> (約2MB)	ダウンロードできるようにしています。
Genome assembly: <u>ASM2650v1</u> ₽			
More information and statistics	What can I find? Protein-coding and non-coding genes, splice variants, cDNA and protein sequences, non-coding RNAs.	InisA ^{npt} lacZ accD	
Display your data in Ensembl Bacteria	Download genes, cDNAs, ncRNA, proteins - FASTA - GFF3		
Comparative genomics	Copdate your old Ensembli IDs		
What can I find? Gene families based on HAMAP and PANTHER classification.		Example transcript	
More about comparative analyses	Variation		
Phylogenetic overview of gene families	This species currently has no variation database. However you can variants using the Variant Effect Predictor:	n process your own	
	Variant Effect Predictor		
Ensembl Bacteria release 42 - Dec 2018 © FMBI -FBI			~

W14:SRA

公共DBの1つである①SRA上で、②「 Lactobacillus RNA-seq」で、③キーワード検索。

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AACGCC TTGCATT TAA	Sequence Read Archive (SRA) makes be community to enhance reproducibility an The SRA stores raw sequencing data an sequencing platforms, including Roche 4 Applied Biosystems SOLiD System®, He	iological sequence data available to th d allow for new discoveries by compar d alignment information from high-thro 154 GS System®, Illumina Genome An elicos Heliscope®, Complete Genomic	e resea ing data ughput alyzer® s®, and	rch i sets. I Pacific	
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SRA Fact Sheet (.pdf)

W14:SRA

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日本乳酸菌学会誌	の連載第13回				

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①原著論文がある場合は、ここに表示 されるようだ。②ページ下部に移動。

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W14:SRA

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Accession	PRJNA419802; GEO: GSE107337	Navigate Across	Taxonomy
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Project Data:	,		See more

①このGSE107337というIDを頼りにして W15:L. rhamnosus G 、のちに原著論文の確認を行いました。

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W15:L. rhamnosus G(^{プルで、発現変動遺伝子検出まで行う} にとができるデータセットであることを知

①この部分をみて、実験デザインがシン り、詳細に調べていく決断をしました。

NCBI Resources How To Sign in to NCBI BioProject BioProject Advanced Browse by Project attributes Search Display Settings: + Send to: + Related information BioSample To understand transcriptional regulation of probiotic bacteria under acidic condition, RNAseq analysis was carried out over different growth conditions by See Genome Genome Overall design: Comparison of acidic (pH4) and neutral (pH7) conditions by See Genome GEO DataSets SRA Accession PRJNA419802; GEO: GSE 107337 Tasconomy Tasconomy Recent activity Recent activity Data Type Transcriptome or Gene expression Tum Off Clear RNA-seq analysis of Lactobacillus rhamnosus GG RNA-seq analysis of Lactobacillus rhamnosus GG SRA Links for BioProject (Select 475032) (36) SRA Scope Muttisolate Tum Off Clear Rescent activity Tasconomy Organism Bacteria; Fimicutes; Bacilli; Lactobacillus rhamnosus; Lactobacillus rhamnosus GG SRA Links for BioProject (Select 475032) (36) SRA Submission Registration date: 25-Nov-2017 Computational and Synthetic Biology Laboratory, Department of Biotechnology, Korea University Recobacillus RNA-seq) AND bioproject_srafifitter] No BioProject_srafifiter] No BioProject_srafifite	$\leftrightarrow \rightarrow G$	https://www.ncbi.nlm.nih.gov/bioproject/419802 ■		☆ 📕 \varTheta
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日本乳酸菌学会誌の連載第13回

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Project Data:

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Sequence data		
SRA Experiments		9
Other datasets		
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GEO DataSets		1
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Data volume, Supplementary Mbytes		1
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A https://www.pcbi.plm.pib.gov/sra?lipkpa

赤枠内の、①と②の情報から、トータル9サンプル からなるこのデータセットは、全部で3状態の比較 (3群間比較)を行っており、各群につき3反復のデ ータを取得しているのだろうと判断できる。また、 ③Illumina MiSeqが用いられていることもわかる。

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- 1 ILLUMINA (Illumina MiSeg) run: 1.4M spots, 690.4M bases, 428.8Mb downloads Accession: SRX3422364
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- 1 ILLUMINA (Illumina MiSeg) run: 1.8M spots, 883.8M bases, 562.8Mb downloads Accession: SRX3422363
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- 8. 1 ILLUMINA (Illumina MiSeg) run: 1.5M spots, 738.2M bases, 467.2Mb downloads Accession: SRX3422362
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全9サンプル分を表示。ここでも大まかに全体像 を理解することができる。これは①通常のpH環 境と、酸性ストレス環境下(pH = 4)の比較を行っ ている。②酸性ストレス条件で1h経過後と、③ 24h経過後のデータを取得していることがわかる



3.

①GSE107337の原著論文はここでは見られないが 、②Registrationから1年以上経過しているため、もし かしたらPubMedで見つかるかもという視点で探す。

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To understa condition, F Overall des differentially	and transcriptional regulation of probiotic bacteria under acidic RNAseq analysis was carried out over different growth conditions ign: Comparison of acidic (pH4) and neutral (pH7) conditions by expressed genes	See Genome Information for Lactobacillus rhamnosus	Genome GEO DataSets SRA
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Organism	Lactobacillus rhamnosus GG [Taxonomy ID: 568703] Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus rhamnosus; Lactobacillus rhamnosus	5 years 10 years Custom range Species	Front Microbiol. 2018 Aug 23; 2018. PMID: 30210466 Free PMC Similar articles	9:1978. doi: 10.3389/fmicb.2018.01978. eColle C Article	ection Search details RNA-seq [All Fields] AND ("analysis" [Subheading] OR "enalysis" [All Fields]) AND
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論文著者情報の手がかりを得るため、① ページ下部に移動して、②GEO DataSets のリンク先にいく。このやり方でなくても、 GSE107337で単純にググるのでもよい。





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①GEOの、②GSE107337のページ。こ こでもまだ③原著論文情報はない。

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	Submission data	Nov 25, 2017				
	Last undate date	lan 24, 2018				
	Contact name	kucchl submitter				
	E-mail	kucsbi submittei				
	Organization name	Kacabi.group@gmail.com				
	Dopartmont	Department of Biotechnology				
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①と②の情報を用いて PubMed検索することで…

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Con	ntributor(s)	Choi I, Oh S (2)				
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Con	ntact name	kucsbl submitter				
E-m	nail	kucsbl.group@gmail.com				
Org	anization name	e Korea University				
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それっぽい論文を発見!

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<u>J Microbiol Biotechnol.</u> 2018 Oct 28;28(10):1604-1613. doi: 10.4014/jmb.1807.07033. Transcriptional Response and Enhanced Intestinal Adhesion Ability of	Full text links	xt schnol			
Lactobacillus rhamnosus GG after Acid Stress.	Save items				
Bang M ¹ , Yong CC ¹ , Ko HJ ^{2.3} , Choi IG ² , Oh S ¹ .					
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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	Similar articles Lactobacillus rhamnosus GG SpaC pilin subunit binds [Anim Sci J. 2016]				
and glycolysis-related genes were upregulated, whereas genes involved in gluconeogenesis, amino acid	Shear-Enhance	ed Dynamic s rha [] angi	: Adhesio	on 181	
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 from the probiotic [ACS Nano. 201					
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 fat ζ [Colloids Surf B Biointer					
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 dis [Minerva Gastroenterol Dieterva Gastroent					
first report showing that acid stress enhanced the intestine-binding ability of LGG through the induction See reviews of pili-related genes.					

	GSE107337中の記述内容と同じ。
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<u>J Microbiol Biotechnol.</u> 2018 Oct 28;28(10):1604-1613. doi: 10.4014/jmb.1807.07033.	Full text links
Transcriptional Response and Enhanced Intestinal Adhesion Ability of Lactobacillus rhamposus GG after Acid Stress.	
Bang M^1 , Yong CC^1 , Ko HJ ^{2,3} , Choi IG ² , Oh S ¹ .	Save items
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Abstract	
Lactobacillus rhamnosus GG (LGG) is a probiotic commonly used in fermented dairy products. In this	Similar articles
data revealed that the exposure of LGG to acid at pH 4.5 (resembling the final pH of fermented dairy	Lactobacillus rhamnosus GG SpaC pilin subunit binds [Anim Sci. J. 2016]
products) for 1 h or 24 h provoked a stringent-type transcriptomic response wherein stress response-	Chart Enhanced Dunamia Adhesian
and glycolysis-related genes were upregulated, whereas genes involved in gluconeogenesis, amino acid	of Lactobacillus rha [Langmuir. 2018]
spaC, and spaF were significantly upregulated upon exposure to acid-stress. The transcriptomic results	Adhesion and nanomechanics of pili
were further confirmed via quantitative polymerase chain reaction analysis. Moreover, acid-stressed	from the probiotic [ACS Nano. 2013]
bound to a mucin layer in a 96-well culture plate as compared to the control. The enhanced intestinal	Adhesive interactions between milk fat (Colloids Surf B Biointerfaces 1
binding ability of acid-stressed LGG was confirmed in an animal study, wherein significantly more viable	Poview Probletics in directive
LGG cells (≥ 2 log CFU/g) were observed in the ileum, caecum, and colon of acid-stressed LGG-treated	dis [Minerva Gastroenterol Dietol]
first report showing that acid stress enhanced the intestine-binding ability of LGG through the induction	See reviews
of pili-related genes.	
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①ENA上で、②GSE107337を、③検索

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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)	FAST files (Gala	Q axy)	
	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		

日本乳酸菌学会誌の連載第13回

①ここから個別にダウンロード

• W22: <u>SRAdb</u>: <u>Zhu et al., BMC Bioinformatics, 2013</u> 原著論文(Bang et al., J Microbiol Biotechnol., 2018)の記述からGSE107337を頼りに、 SRP125028(こ)こ

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。 「<u>(Rで)塩基配列解析</u>」の 「イントロ | NGS | 配列取得 | FASTQ or SRA | <u>SRAdb(Zhu_2013)</u>」の 例題3以降と基本的に同じです。

W22:失敗した場合の対応策2

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"SR/ 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パッケージを利用して

、①SRP125628を与えてgzip

圧縮FASTQファイルをダウ

ンロードすることができます



①DRASearch画面上で、②Accession の場所でGSE107337で、③Search

W23:DRA

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<u>Sa</u> Ru	<u>mple</u> n	4947100 6227253							
		Organism			Study Type			Center Name	
#	Orga	nism Name	Study	#	Study Type	Study	#	Center Name	Study
1	<u>Homo sa</u>	<u>ipiens</u>	16356	1	<u>Other</u>	69729	1	<u>BioProject</u>	108088
2	<u>Mus mus</u>	sculus	13445	2	Whole Genome Sequencing	56641	2	GEO	29441
3	<u>soil meta</u>	<u>agenome</u>	5530	3	<u>Metagenomics</u>	26156	3	DOE - JOINT GENOME INSTITUTE	2590
4	<u>Zea may</u>	<u>'S</u>	3281	4	Transcriptome Analysis	24085	4	UMIGS	2557
5	Populus	trichocarpa	3064	5	Population Genomics	802	5	JGI	2365

W23:DRA

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Accession : GSE107337		
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Show 20 • records Sort by Study • Search	Clear	
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Website policy © DNA Data Bank of Japan		

W24:DRA

①Keywordの場所でGSE107337で、②Searchすると、③3つヒットします。④SRP125628をクリック

	🖹 Result List - DR/	A Search X	+							_		×
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	SRA633931.su 1 xml version=</td 8"?> <submiss< td=""> GSE107337" b</submiss<>	<u>Ibmission.xml</u> "1.0" encoding="UTF- ION alias="GEO: roker	<u>SRA633931</u>	SRP125628	RNA-seq analysis of Lactobacillus at acidic stress	<u>Transcriptome</u> <u>Analysis</u>	<u>Lactobacillus</u> <u>rhamnosus</u> <u>GG</u>	0		<u>GE0</u>		
	SRA633931.stu .org/2001/XMLS 2 <study center<br="">alias="GSE107. accession="SRP <identifiers></identifiers></study>	udy.xml Schema-instance"> _name="GEO" 337" *125628"> > <primary< td=""><td><u>SRP125628</u></td><td>SRP125628</td><td>RNA-seq analysis of Lactobacillus at acidic stress</td><td><u>Transcriptome</u> <u>Analysis</u></td><td><u>Lactobacillus</u> rhamnosus GG</td><td>0</td><td></td><td><u>GEO</u></td><td></td><td></td></primary<>	<u>SRP125628</u>	SRP125628	RNA-seq analysis of Lactobacillus at acidic stress	<u>Transcriptome</u> <u>Analysis</u>	<u>Lactobacillus</u> rhamnosus GG	0		<u>GEO</u>		
			<u>SRX3422361</u> SRX3422362									

①こんな感じでSRP125628のページに飛びます。

W24:DRA

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SRP125628	1	
Study Detail		Navigation
Title	RNA-seq analysis of Lactobacillus at acidic stress	Submission <u>SRA633931</u>
Study Type	Transcriptome Analysis	🔮 Experiment <u>SRX3422361</u> 😫 FASTQ 😫 SRA
	To understand transcriptional regulation of probiotic bacteria under acidic	SRX3422362 SRA
	condition, RNAseq analysis was carried out over different growth conditions	SRX3422363 🗳 FASTQ 🗳 SRA
Abstract	Overall design: Comparison of acidic (pH4) and neutral (pH7) conditions by	SRX3422364 🐸 FASTQ 🐸 SRA
	differentially expressed genes	SRX3422365 🖺 FASTQ 🖺 SRA
Description		SRX3422366 SRA
Center Name	GEO	SRX3422367 SRX3422367
		SRX3422368 SRA
		SRX3422369 🖺 FASTQ 🖺 SRA
		🔮 Sample <u>SRS2714081</u>
		<u>SRS2714082</u>
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		<u>SRS2/14084</u>
		<u>SRS2714085</u> SPS2714086
		SRS2714087
		SRS2714088
		SRS2714089

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①Accessionの場所でSRP125628で、②Search。

W25:DRA

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#	Organism Name	Study	#	Study Type	S	tudy	#	Center Name	Study	
1	<u>Homo sapiens</u>	16356	1	<u>Other</u>	6	9729	1	BioProject	108088	\$
2	<u>Mus musculus</u>	13445	2	Whole Genome Seque	encing 5	6641	2	GEO	29441	
3	<u>soil metagenome</u>	5530	3	Metagenomics	2	6156	3	DOE - JOINT GENOME INSTITUTE	2590	
4	<u>Zea mays</u>	3281	4	Transcriptome Analysi	<u>is</u> 24	4085	4	UMIGS	2557	
5	Populus trichocarpa	3064	5	Population Genomics	8	02	5	JGI	2365	-

W25:DRA

①こんな感じでSRP125628のページに飛びます。 ②FASTQおよびSRAファイルともにまだ提供され ていないことが分かります。

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SRP125628		
Study Detail		Navigation
Title	RNA-seq analysis of Lactobacillus at acidic stress	Submission <u>SRA633931</u>
Study Type	Transcriptome Analysis	🕑 Experiment <u>SRX3422361</u> 😫 FASTQ 😫 SRA
	To understand transcriptional regulation of probiotic bacteria under acidic	SRX3422362
	condition, RNAseg analysis was carried out over different growth conditions	SRX3422363 BASTQ SRA
Abstract	Overall design: Comparison of acidic (pH4) and neutral (pH7) conditions by	SRX3422364 BASTQ SRA
	differentially expressed genes	SRX3422365 🗳 FASTQ 🗳 SRA
Description		SRX3422366
Center Name	GEO	SRX3422367 BASTQ SRA
		SRX3422368 BFASTQ SRA
		SRX3422369 BASTQ SRA
		Sample <u>SRS2714081</u>
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		<u>SRS2714088</u>
		<u>SRS2714089</u>

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①GEOの、②GSE107337のページ。③下部に移動 W26:実験デザイン Х GEO Accession viewer × https://www.ncbi.nlm.nih.gov/geo/guery/acc.cgi?acc=GSE107337 \cap ন্দ্র NCBI Gene Expression Om MIAME HOME | SEARCH | SITE MAR **GEO Publications** FAQ Email GEO NCBI > GEO > Accession Display 🕑 Not logged in | Login 🛽 Scope: Self Format: HTML Amount: Quick V GEO accession: GSE107337 • GO Series GSE107337 Query DataSets for GSE107337 Public on Nov 29, 2017 Status RNA-seg analysis of Lactobacillus at acidic stress Title Organism Lactobacillus rhamnosus GG Experiment type Expression profiling by high throughput sequencing To understand transcriptional regulation of probiotic bacteria under acidic Summary 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 Has this study been published? Please login to update or notify GEO. Citation missing Submission date Nov 25, 2017 Jan 24, 2018 Last update date kucsbl submitter Contact name
W26:実験デザイン

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(2)	GSM2864943 pH4_1h rep3						
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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	
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4		pH4.5_1h rep2	SRR6322563	1,470,602	265,838,592	296,910,848	3
5		pH4.5_1h rep3	SRR6322564	1,760,461	319,807,488	354,557,952	2
6		pH4.5_24h rep1	SRR6322565	1,375,368	241,553,408	268,341,248	3
7		pH4.5_24h rep2	SRR6322566	3,869,088	682,504,192	759,697,408	3
8		pH4.5_24h rep3	SRR6322567	1,795,874	315,613,184	353,099,776	5
9		pH7_CCG rep1	SRR6322568	3,095,834	551,383,040	615,870,464	ŀ
10		pH7_CCG rep2	SRR6322569	2,570,876	451,117,056	510,308,352	2
11		pH7_CCG rep3	SRR6322570	846,623	148,500,480	167,350,272	2
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W28: GSM2864941

W26で見ているものと同じ。 ①GSM2864941をクリック

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Relations BioProject SRA	PRJNA419802 SRP125628							
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W28: GSM2864941

①GSM2864941のページに移動しました。
 ②少しページ下部に移動。

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Title	pH4_1h rep1		•				
Sample type	SRA						
Source name	whole transcriptome						
Organism	Lactobacillus rhamnosus GG						
Characteristics	ph: 4						
Extracted molecu	e total RNA						
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	to sequencing using standard i		00013				
Library strategy	RNA-Seq						
Library source	transcriptomic						
Library selection	Library selection cDNA						
Instrument mode	Illumina MiSeq					-	
日本乳酸菌学会誌の	連載第13回						

W28:GSM2864941

①このあたりまで移動すると、②Data processing 情報が見つかります。リファレンスゲノムとして、③ NC_013198.1が利用されていることがわかります。

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	Source name Organism Characteristics Extracted molecule Extraction protocol	whole transcriptome Lactobacillus rhamnosus GG ph: 4 total RNA TruSeq RNA Sample Preparation Kit (Illumina) was used. RNA libraries were prepared for sequencing using standard Illumina protocols					•	
	Library strategy Library source Library selection Instrument model	RNA-Seq transcriptomic cDNA 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.		2				
	Submission date Last update date Contact name E-mail Organization name Department	Nov 25, 2017 Nov 29, 2017 kucsbl submitter kucsbl.group@gmail.com Korea University Department of Biotechnology					•	

W29:BowtieとRPKM

 \times

GEO Accession viewer

①mapping (alignment)にはBowtieが使われており、②RPKMの作成にはedgeRが用いられていると書かれています。ここではDESeqも書かれていますが、別のところではedgeRのみしか書かれていなかったのでDESeqは無視しています

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Source name Organism Characteristics Extracted molecule Extraction protocol	whole transcriptome Lactobacillus rhamnosus GG ph: 4 e total RNA TruSeq RNA Sample Preparation Kit (Illumina) was used. RNA libraries were prepared for sequencing using standard Illumina protocols			
Library strategy Library source Library selection Instrument model	RNA-Seq transcriptomic cDNA 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 Last update date Contact name E-mail Organization name Department	Nov 25, 2017 Nov 29, 2017 kucsbl submitter kucsbl.group@gmail.com e Korea University Department of Biotechnology			
平孔酸困子会誌の	里戰弗13回			

W26と同じく、①GSE107337のページ。 ②RPKMデータファイルと、③カウントデ ータファイルをダウンロードしておく。

W30:CountとRPKM

S GEO Accession view	wer × +			—		×
\leftrightarrow \rightarrow C \triangle	https://www.ncbi.nlm.nih.g	jov/geo/query/acc.cgi?	acc=GSE107337	☆ 🏃	Θ	:
Platforms (1)	GPL24302 Illumina MiSeq (La	ctobacillus rhamnosus (3G)			
Samples (9) ∃ Less	GSM2864941 pH4_1h rep1 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 SRA	PRJNA419802 SRP125628					
Download family	/		Format			
SOFT formatted fa	amily file(s)		SOFT 🕐			
MINiML formatted Series Matrix File(family file(s) s)		MINIML 🛿 TXT 🔋			
Sup	plementary file	Size 2 vnload	File type/resource			
GSE107337_RPKM	1.csv.gz	21.1 Kb (ftp)(http)	CSV			
GSE107337_RawC	Counts.csv.gz	20.8 Kb (ftp)(http)	CSV			
Raw data are avai	lable in SRA	3				
Processed data is	available on Series record					-

1		/31	1 : カウント 回 ᠀· ᠬ・ জ	データ E107337_RawCounts.csv 1	①raw countデータフ averageと書かれてし うに、3状態間比較で 」 値になっている。だか	マイルの いることか いんしました いら3列ら	Dほう。② からもわかるよ データの平均 うしかない。
77	ッイル	<u></u> ₩−Δ	挿入 ページレイアウト 数	式 データ 校閲 表示 /	∿レプ ♀ 操作アシスト ♂ 共	有 □	
E2	.7	Ŧ	$\therefore \checkmark \checkmark f_x$			*	
		A				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		
	()	G	SE107337_RawCounts	+ : •		•	
Scr	ollLock	¢				+ 100%	

2	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 				
ן דר	自動保存 マイル :	• • 7 7 ѫ–ь) 日 り・C・・ GSI 挿入 ページレイアウト 数5	E107337_RawCounts.csv···· く 式 データ 校閲 表示	フント、(4)4列目かpH/0 のカウント、であることを マレン マ またアンヘー ビ キ)コントロ 覚えてる	コールの平均 おく。		
E2	7	•	$\therefore \checkmark f_x$			٧]		
					(4)	E	•		
1	Gene	-	pH4_1h_average_rawcount	pH4_24h_average_rawcount	pH7_CCG_average_rawcount				
2	LGG_0	00001	286	676	545				
3	LGG_0	00002	776	1601	1450				
4	LGG_0	00003	71	114	91				
5	LGG_0	00004	201	470	368				
6	LGG_0	00005	368	1160	1447				
7	LGG_0	00006	1041	2742	2880				
8	LGG_0	00007	7	10	2				
9	LGG_0	80000	73	76	499				
10	LGG_0	00009	16	45	42				
11	LGG_0	00010	20	65	57				
12	LGG_0	00011	2281	2596	2150				
13	LGG_0	00012	2510	3631	3097				
14	LGG_0	00013	971	1301	1424				
15	LGG_0	00014	17	78	16				
16	LGG_(00015	SE107227 DowCountr	255	33	_			
	•	G	SET07337_KawCounts	(+)					
Scr	ollLock					+ 100%			

W	32	:記載内	容と-	「 「 の の の の の	著論文のp1608の G_02240(GroES 発現が、③コント)左上の _{gene})の ロールに	ほうで ②24E 比べ	⁵ は、① 時間ストレス て、2.53倍変
		명 월· 연· ፣ G	SE107337_Rav	vCounts.csv <mark>化比</mark>	したと記載されて	いる。		
ファイル	<u>₩</u> −₽	挿入 ページレイアウト 数	対式 データ	校閲 表示 へ	ルプ 🛛 操作アシスト	132 共有		
A2158	-	$\therefore \checkmark f_x$ L	GG_02240				*	
	А	В		С	D		E 🔺	
2151 LGG	02232		33	95		70		
2152 LGG	02233	22	27	331		539		
2153 LGG	02234	40	06	791		1093		
2154 LGG	02235	5	32	999		1289		
2155 LGG	02236	2'	77	467		647		
2156 LGG	02238	70	58	674		577		
2157 LGG	02239	514	46	18308		5690		
2158 LGG	02240	110	06	2444	2	422	3	
2159 LGG_	02241		37	107		130		
2160 LGG_	02242	3'	77	340		540		
2161 LGG_	02244		36	116		44		
2162 LGG_	02245		19	101		29		
2163 LGG	02246		23	63		8		
2164 LGG_	02247	3	34	531		556		
2165 LGG	02248	24	45	405		318		
2166 LGG_	02249	12	25	188		227	-	
${}^{\bullet} \rightarrow$	GS	E107337_RawCounts	(+)	E 4				
ScrollLock						+ 1	00%	

N 81 1			原著論文の	p1608の左上のほう	では、①	
₩32・記載内容と				LGG_02240 (GroES gene)の224時間ストレス		
				3)コントロールに比べ	、て、2.53倍変	
) 🗄 垳 🤆 न 🖓 🖬 GSE	107337_RawCounts	.csv 化したと記載	或されている。 倍率変	北なので、4	
	ぼう ページョンフロト 粉ゴ	・ ニーム 広明	<mark>5.79倍変化Ⅰ</mark>	したと書くべきだとは	思われるが、	
ノア176 ホーム	挿入 ハーンレイアリト 数式	テータ 牧開	🏝 <mark>⑤log2変換l</mark>	した結果と確かに一致	<mark>改する。</mark> 論文の	
A2158 -	$\times \sqrt{f_x}$ LGC	t 02240	Table S2の	脚注部分で、確かに	og2変換したも	
			のをFold ch	angeと表現している・	…まあ、妥当	
A	В	С				
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	1106		2444	422 3		
2159 LGG_02241	87		107	130		
2160 LGG 02242	377		340	540		
2161 LGG 02244	36		116	R Console		
2162 LGG 02245	19		101	> 2444/422	^	
2163 LGG 02246	23		63	2444/422		
2164 LGG 02247	384		531	[1] 5.79146	9 (4)	
2165 LGG 02248	245		405	$(5) > \log^2(2444)$	/422)	
2166 LGG 02249	125		188	[1] 2.53392	9	
GS	E107337_RawCounts	(+)		>		
Scrolll ock	_	0			~	
SCIOILOCK				¢	> ,	