



Genome Assembly and Differential Expression of *Enterococcus faecium* E745

Paper I – RNA-seq and Tn-seq reveal fitness
determinants of vancomycin-resistant *Enterococcus*
faecium during growth in human serum (Zhang *et al.* 2017)

Overview

- 01 Introduction**
Background and aim of study
- 02 Preprocessing**
Quality control, trimming
- 03 Assembly**
Different assemblers
- 04 Annotation**
Finding genes

- 05 Synteny**
Aligning different species
- 06 Transcriptomics**
Mapping, counting, comparing RNA
- 07 Results**

Enterococcus faecium

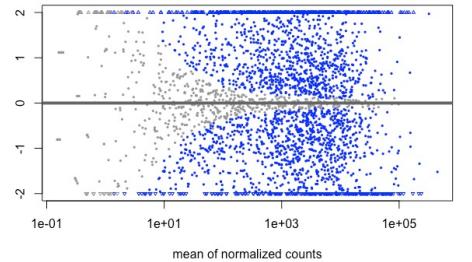
- Multi-resistant (Clade A)
- Hospital disease
- Urinary tract and bloodstream
- Strain: E745 from rectal swab (Clade A-1, NL)



Photo: <https://www.indiamart.com/proddetail/enterococcus-faecium-probiotics-8411604530.html>

Aim

Identify genes responsible for growth in human serum



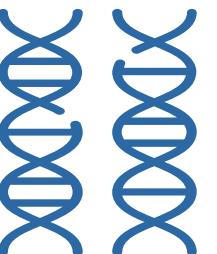
Data

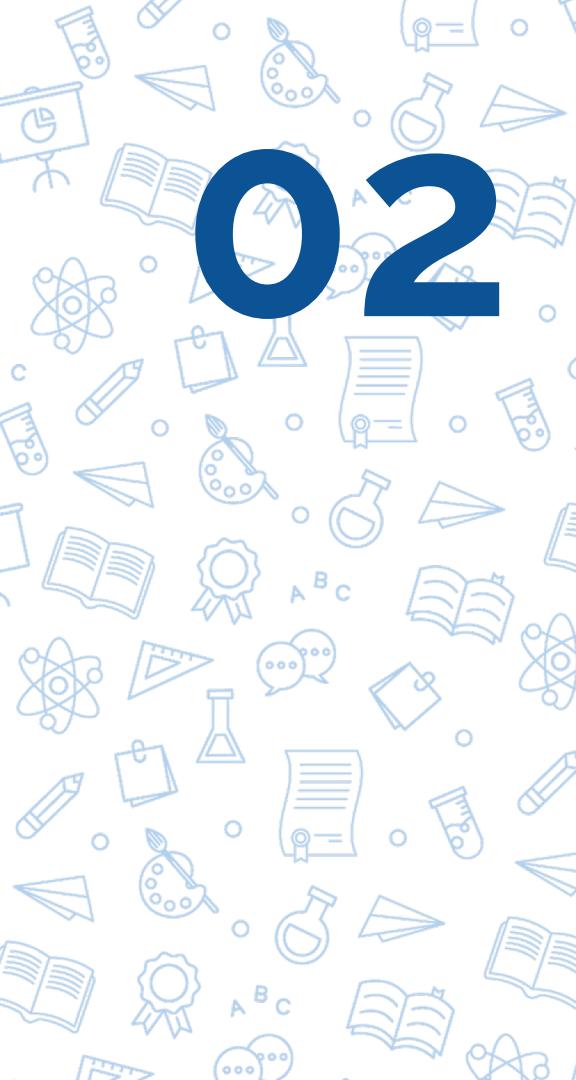
DNAseq from:

- PacBio RSII
- illumina HiSeq 2500
- NanoPore MinION 2D

RNAseq (and Tn):

- illumina HiSeq 2500





02

Preprocessing

Quality Control with
FastQC

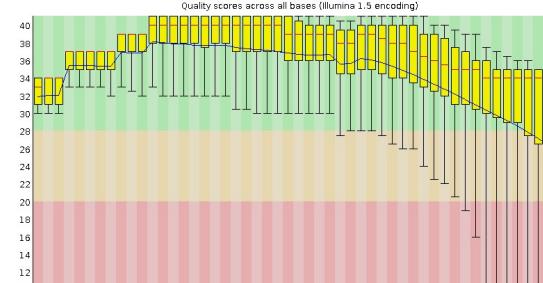
Trimming with
Trimmomatic

FastQC example

Illumina DNA read 1

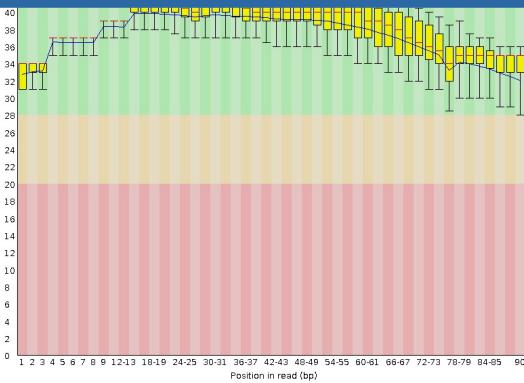


Illumina DNA read 2

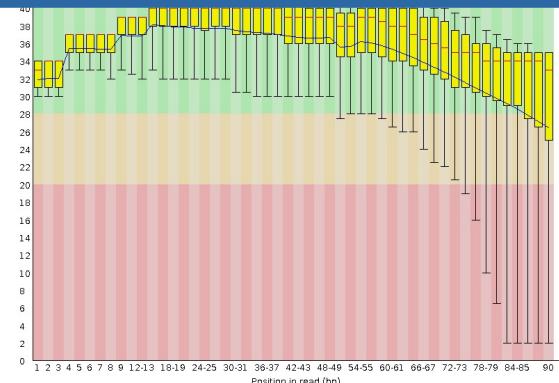


Before trimming

No significant difference – files already trimmed



After trimming





03

Assembly + evaluation

Assemblers

Canu

PacBio

Spades

illumina

illumina + NanoPore

illumina + PacBio

illumina + NanoPore + PacBio

Pilon

Canu_PacBio

+

Spades_*

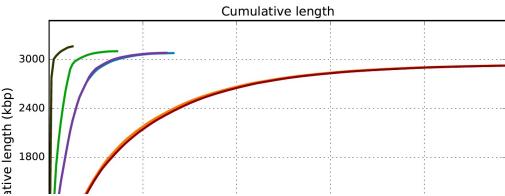
Evaluation

BCF

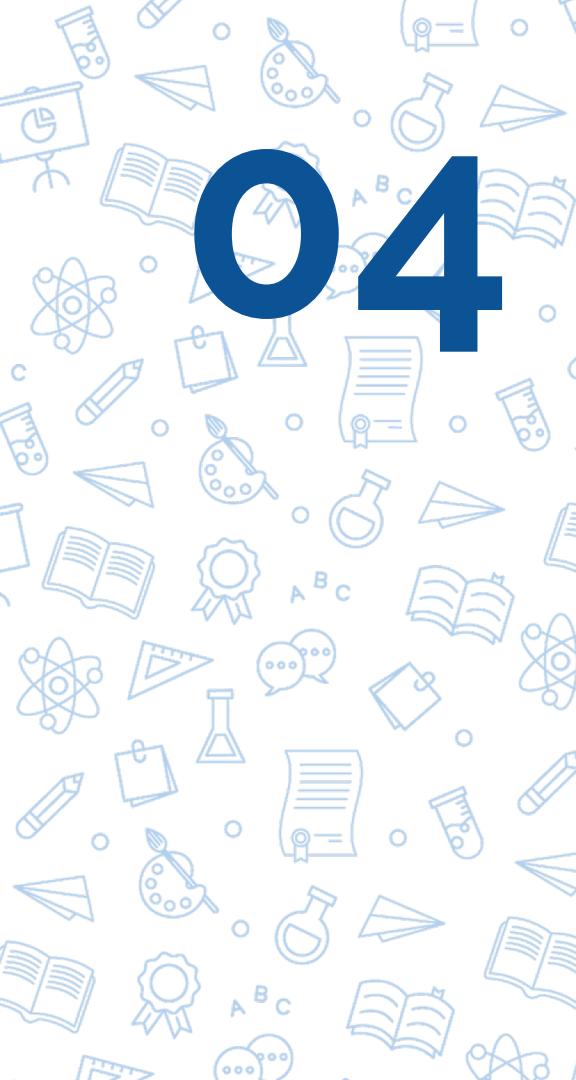
MUMmerPlot

QUAST

QUAST report



| Column1 | <input checked="" type="checkbox"/> illumina.contigs | <input type="checkbox"/> illumina_Nanopore.contigs | <input type="checkbox"/> PacBio.contigs | <input type="checkbox"/> Pilon_illumina_Nanopore_PacBio | <input type="checkbox"/> Pilon_illumina_PacBio |
|----------------------------|--|--|---|---|--|
| #contigs(>=0bp) | 488 | 175 | 10 | | 10 |
| #contigs(>=1000bp) | 163 | 46 | 10 | | 10 |
| #contigs(>=50000bp) | 17 | 16 | 2 | | 2 |
| Total_length(>=0bp) | 2975893 | 3101302 | 3162825 | 3162825 | 3162984 |
| Total_length(>=1000bp) | 2905560 | 3075336 | 3162825 | 3162825 | 3162984 |
| Total_length(>=50000bp) | 1402640 | 2724412 | 3006416 | 3006416 | 3006523 |
| #contigs | 195 | 53 | 10 | | 10 |
| Largest_contig | 114283 | 458911 | 2775639 | 2775639 | 2775725 |
| Total_length | 2926792 | 3080395 | 3162825 | 3162825 | 3162984 |
| GC(%) | 37.65 | 37.64 | 37.77 | 37.77 | 37.77 |
| N50 | 49767 | 162629 | 2775639 | 2775639 | 2775725 |
| N75 | 19664 | 102479 | 2775639 | 2775639 | 2775725 |
| L50 | 19 | 6 | 1 | | 1 |
| L75 | 43 | 11 | 1 | | 1 |
| Mismatches: #N's per 100k | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| L75 | 43 | 11 | 1 | 1 | 1 |
| Mismatches: #N's per 100kt | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |



04

Annotation Prokka

Findings:

- rRNA
- tRNA
- tmRNA
- CDS

Prokka annotation



(Excerpt from Artemis visualization)

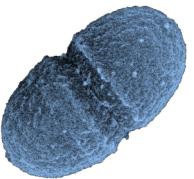


05

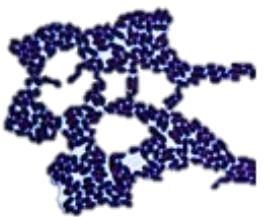
Synteny

Align, compare

Synteny – E. faecium



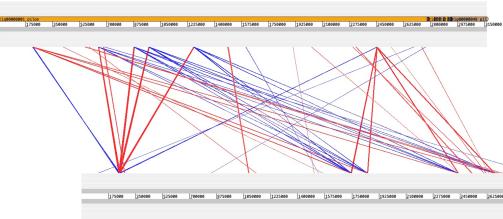
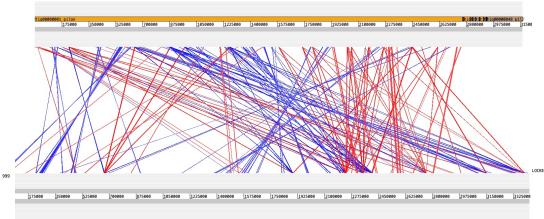
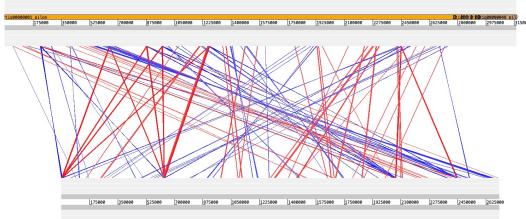
E. faecalis



E. casseliflavus



L. monocytogenes



blastn alignment of whole genome. Visualized with Artemis ACT

06

Transcriptomics

Mapping, counting reads, compare

RNA seq

BWA

Mapping RNA reads
onto genome
assembly

HTSeq count

Count number of reads
mapped to each gene

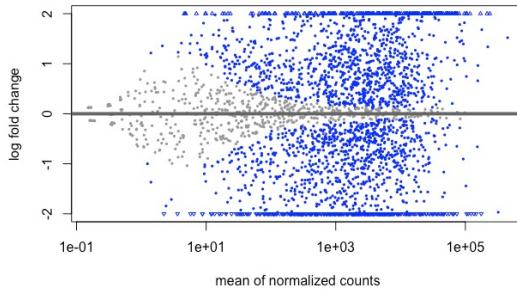
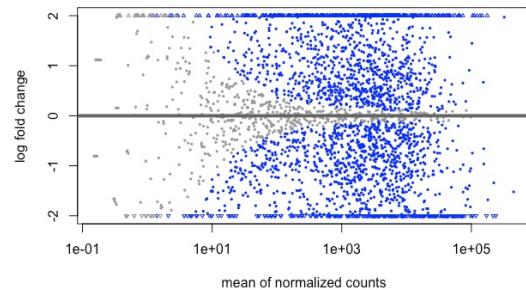
DeSeq2

Differential
expression -
visualized

07

Results

DeSeq2



(Excerpt from RStudio visualization. Log fold change vs LFC shrinkage)

Example of Transposon effect

- When these genes have transposon
- Showing number of mutant survivors after 24h in serum and medium
- Significant negative effect on viability in serum

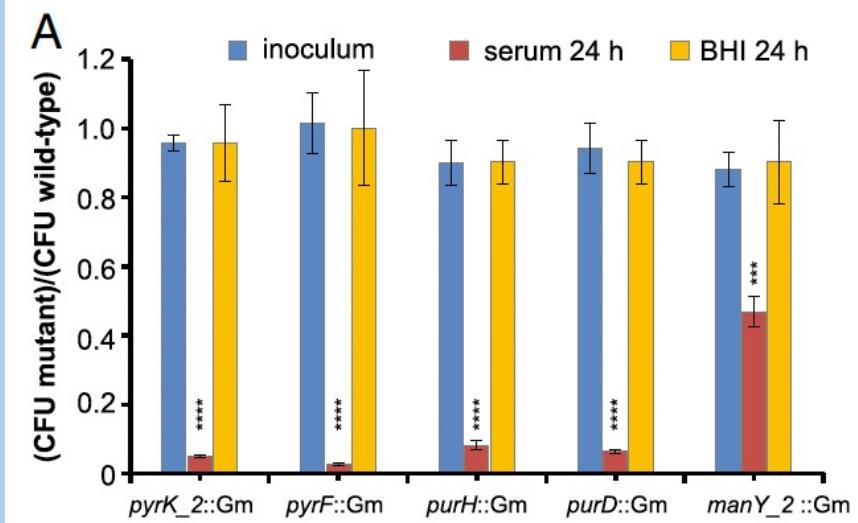


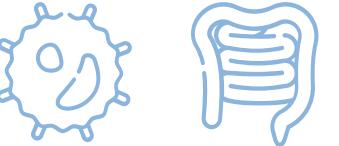
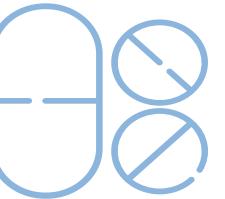
Diagram from Zhang et al. 2017

Conclusion

Good targets:

Genes involved in the nucleotide synthesis and carbohydrate metabolism pathways

(Zhang et al. 2017)



References

- Zhang X, de Maat V, Guzmán Prieto AM, Prajsnar TK, Bayjanov JR, de Been M, Rogers MRC, Bonten MJM, Mesnage S, Willems RJL, van Schaik W. 2017. RNA-seq and Tn-seq reveal fitness determinants of vancomycin-resistant Enterococcus faecium during growth in human serum. BMC Genomics 18: 893.
- <https://canu.readthedocs.io/en/latest/tutorial.html#canu-the-command>
- <https://cab.spbu.ru/files/release3.14.0/manual.html>
- <https://support.illumina.com/bulletins/2016/04/fastq-files-explained.html>
- <http://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#htseq-count-input>

THANK YOU