DTU

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23260 Applied methods in metagenomics

Resistance genes abundances in different pig feces samples from Danish farms

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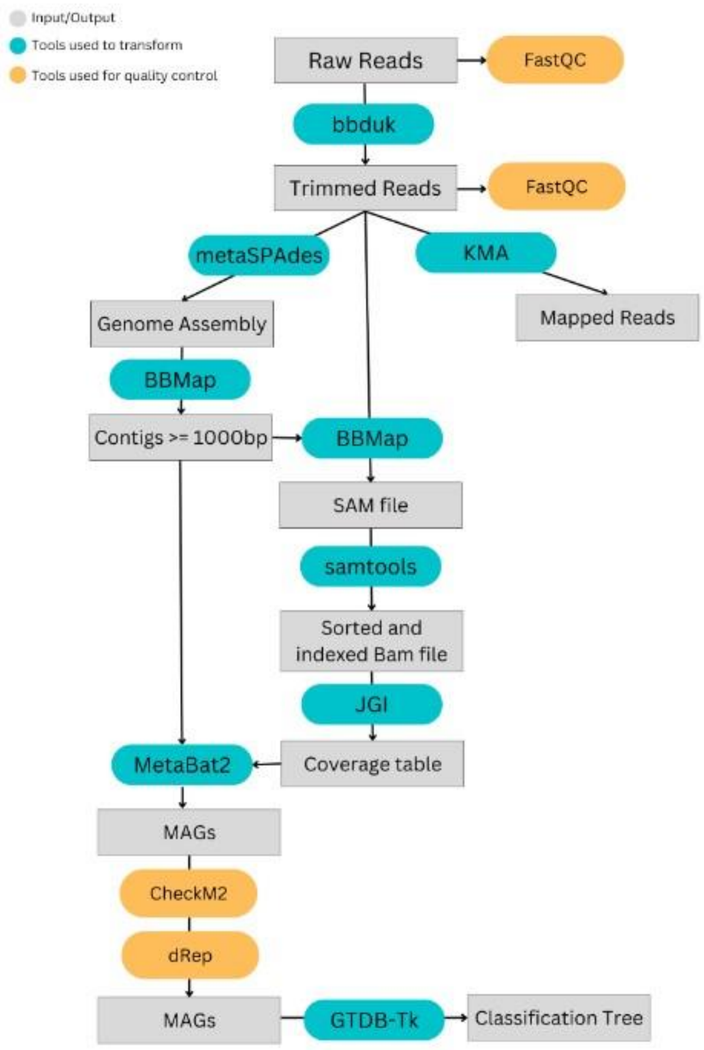
INTRO

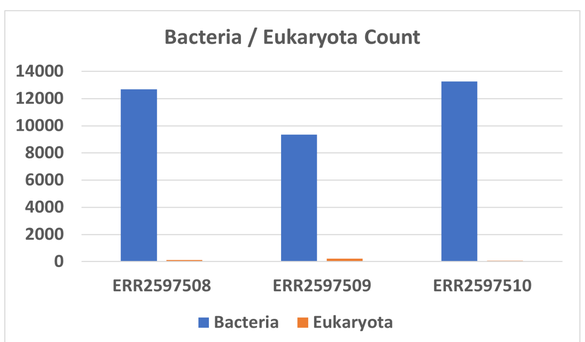
Metagenomics allows the study of a microbial community directly from its environment. In this project some metagenomic samples obtained from pig feces from different Danish farms have been analyzed. Each sample derives from 30 pigs' feces belonging to the same farm. Shotgun DNA sequencing has been performed and paired-ends reads per each sample have been obtained. Starting from these, de novo assembly and binning processes brought to identify different metagenomes assembled genomes (MAGs). In parallel, the different farms have been monitored for antimicrobial resistance (AMR). As a matter of fact, thanks to whole genome sequencing analysis tools it is possible to analyze genotypic basis of resistance directly, without the need of experimental phenotypical assays. In this work the phenotypical resistance has been inferred and analyzed using the ResFinder database, a collection of annotated resistance genes[1].

FLOWCHART



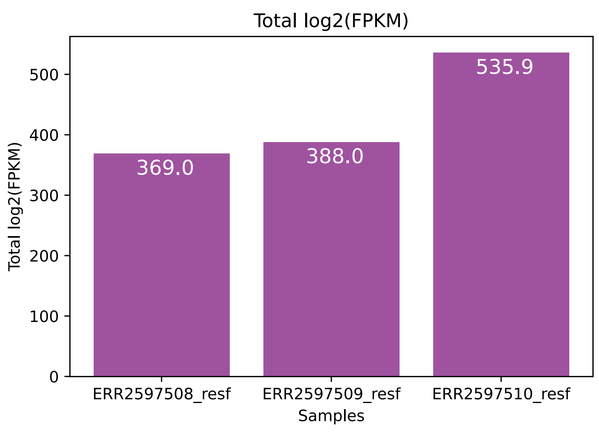
MAPPING WITH KMA and COMPOSITIONAL ANALISYS

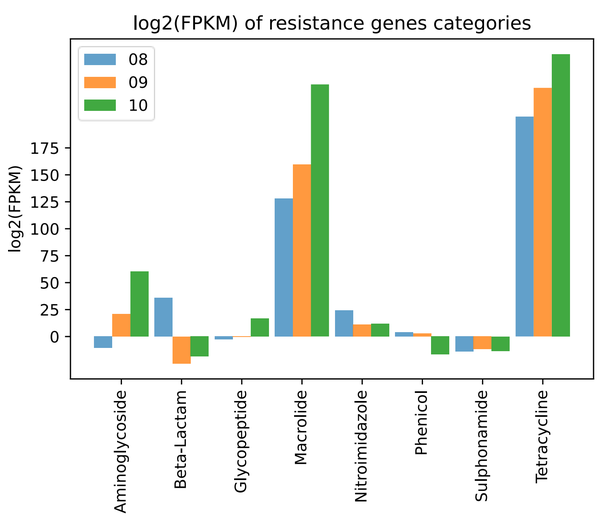
SILVA database contains 16S and 18S references from procaryotes, archaea and eukaryotes [4]. By mapping the trimmed sequences against it, the distribution of bacteria and eukaryote were obtained for each of the three samples, as shown in the following graph.



The *fragmentCountAln* values obtained from SILVA and ResFinder mapping were used to obtain values for calculation of Fragments per Kb of transcript, per M fragments.

$$\log FPKM = log\frac{[n°\ of\ fragments\ mapped\ to\ gene]\cdot 10^3\cdot 10^6}{[total\ n°\ of\ mapped\ bacteria][gene\ length\ in\ bp]}$$





TRIMMING and FASTQC

Trimming of raw sequences is performed with bbduk applying both K-trim and Q-trim. Both raw reads and trimmed reads are analyzed with FastQC to investigate the actual improvement in quality of the reads (Q-trim) and check that the adapters have been removed (K-trim).

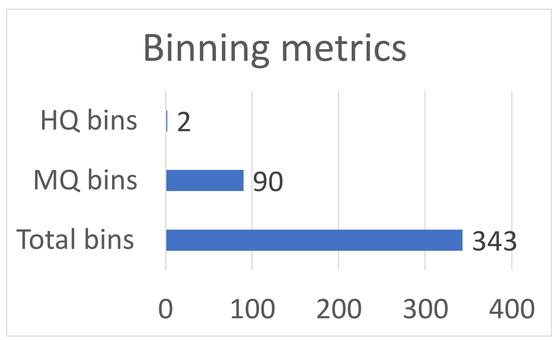
Examples of per-base sequence quality plots of the reads pre- and post-trimming.

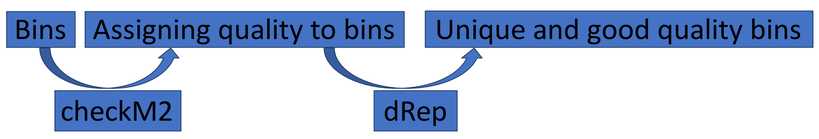
ASSEMBLY AND BINNING

De novo assembly by metaSPAdes applies De Bruijn graphs to transform the reads into assembly graphs, with the goal in the end to have long genomic fragments(contigs). In the table below the metrics describing the assembly is reported. The N50 value indicates that at least 50% of the assembly is composed by similar or longer contigs.

# sample	n° contigs	Longest contig (Kb)	N50 (bp)
8	1325029	121,657	800
9	1166561	71,952	739
10	1465684	189,185	832

Trimmed reads are mapped back to the contigs by BBMap for coverage information. MetaBat2 bin contigs together that are believed to come from one organism (draft genomes), using contigs >= 1000bp long and coverage table as input.





The 22 found dereplicated MAGs from medium and high quality bins (completeness higher than 75% and contamination lower than 25% as dRep default settings) are mapped to the GTDB database, giving the following abundances for phylum and for top 4 genus (see table below).These findings are in line with literature as MAGs from pigs' gut microbiome are known to mainly map to Firmicutes and Bacteroidota [2] [3].

Phylum	Counts
Bacteroidota	11
Firmicutes	9
Proteobacteria	1
Spirochaetota	1
Genus	
Prevotella	2
Limimorph	2
Cryptobacteroides	6
Lactobacillus	2

CONCLUSIONS

A higher relative abundance of resistance genes was found in sample 10, while sample 8 and 9 have a similar relative abundance. Two classes of resistance genes are being far most abundant in all three samples; resistance against Macrolide and Tetracycline antibiotics.

References:
[1] Patrick Munk, Vibe Dalhoff Andersen, Leonardo de Knegt, Marie Stengaard Jensen, Berith Elkær Knudsen, Oksana Lukjancenko, Hanne Mordhorst, Julie Clasen, Yvonne Agersø, Anders Folkesson, Sünje Johanna Pamp, Håkan Vigre, Frank Møller Aarestrup, A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herds, Journal of Antimicrobial Chemotherapy, Volume 72, Issue 2, February 2017, Pages 385–392, https://doi.org/10.1093/jac/dkw415
[2] Xiao, L., Estellé, J., Kiilerich, P. *et al.* A reference gene catalogue of the pig gut microbiome. *Nat Microbiol* **1**, 16161 (2016). https://doi.org/10.1038/nmicrobiol.2016.161
[3] Holman DB, Kommadath A, Tingley JP, Abbott DW. Novel Insights into the Pig Gut Microbiome Using Metagenome-Assembled Genomes. Microbiol Spectr. 2022 Aug 31;10(4):e0238022. doi: 10.1128/spectrum.02380-22. Epub 2022 Jul 26. PMID: 35880887; PMCID: PMC9431278.
[4] Christian Quast, Elmar Pruesse, Pelin Yilmaz, Jan Gerken, Timmy Schweer, Pablo Yarza, Jörg Peplies, Frank Oliver Glöckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Research, Volume 41, Issue D1, 1 January 2013, Pages D590–D596, https://doi.org/10.1093/nar/gks1219