

DPC 2026 - Freiburg

Distangling contaminants from true intratumoural microbial signals in pancreatic ductal adenocarcinoma – A benchmarking approach

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2026-03-07

Background

- PDAC harbors its own bacteria community,
- New biomarker for prognoses and therapy.

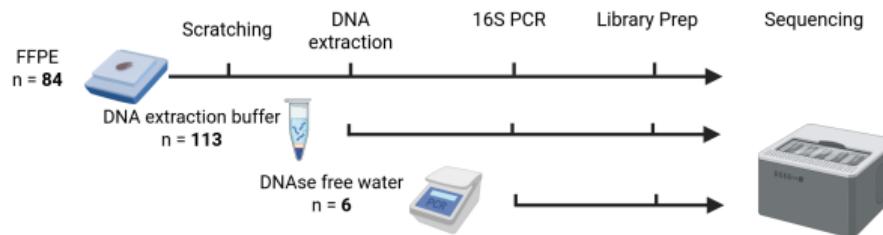
Cubersome

- Low bio-mass samples, prone to contamination

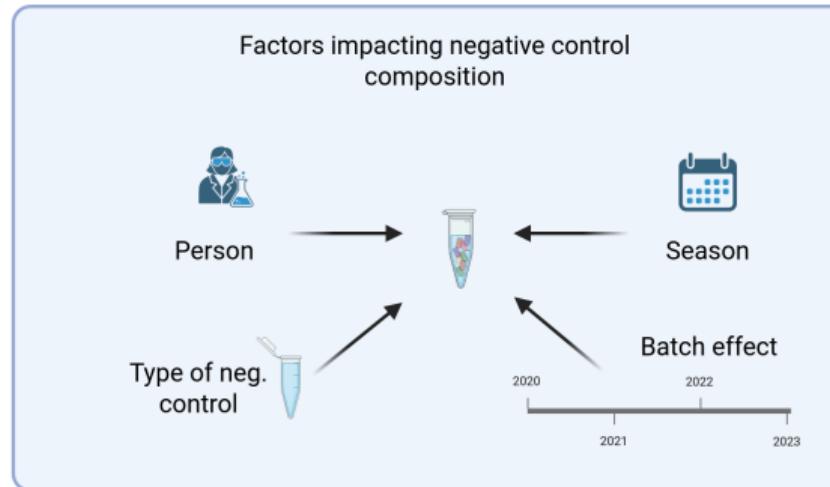
Section 1

Negative Control Survey in the Lab

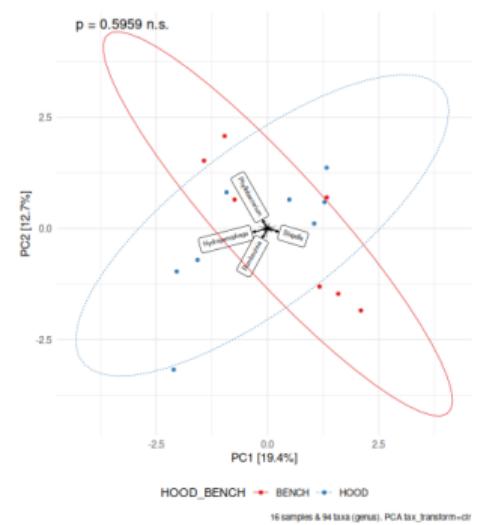
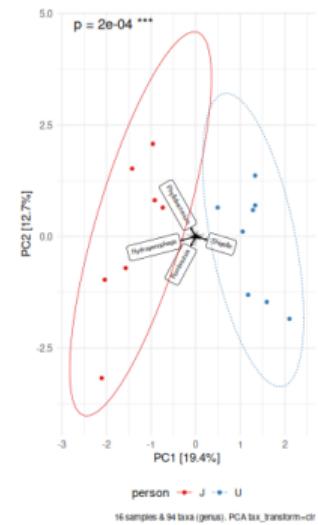
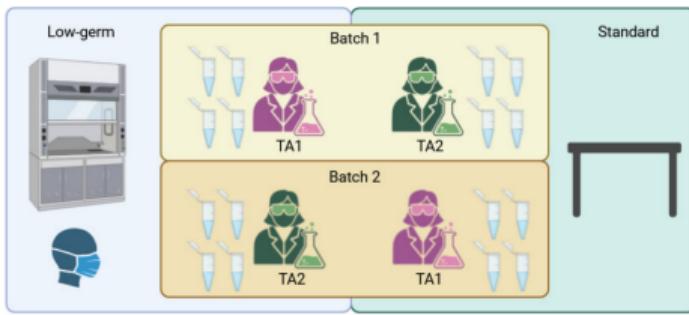
Negative Sample Collection



- In total 203 negative control samples over 4 years
 - 113 buffer controls,
 - 84 paraffin controls,
 - 6 PCR control.



Example

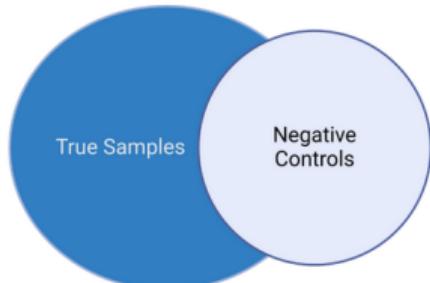


Section 2

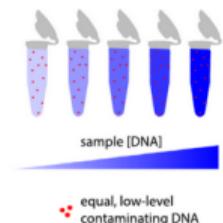
Intratumor Microbiome in PDAC and Assessment of

Decontamination Strategies

Restrictive

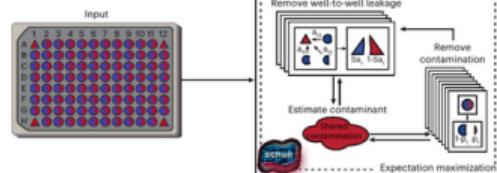


Decontam



contaminant DNA correlates inversely with total DNA

SCRuB

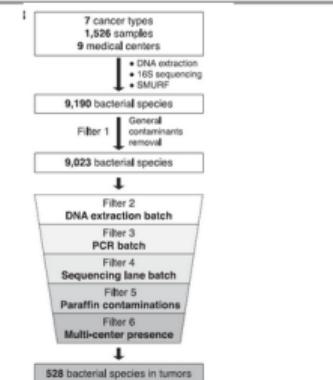


Austin et al. 2023 Nature Biotechnology

Davis et al. 2018 Microbiome



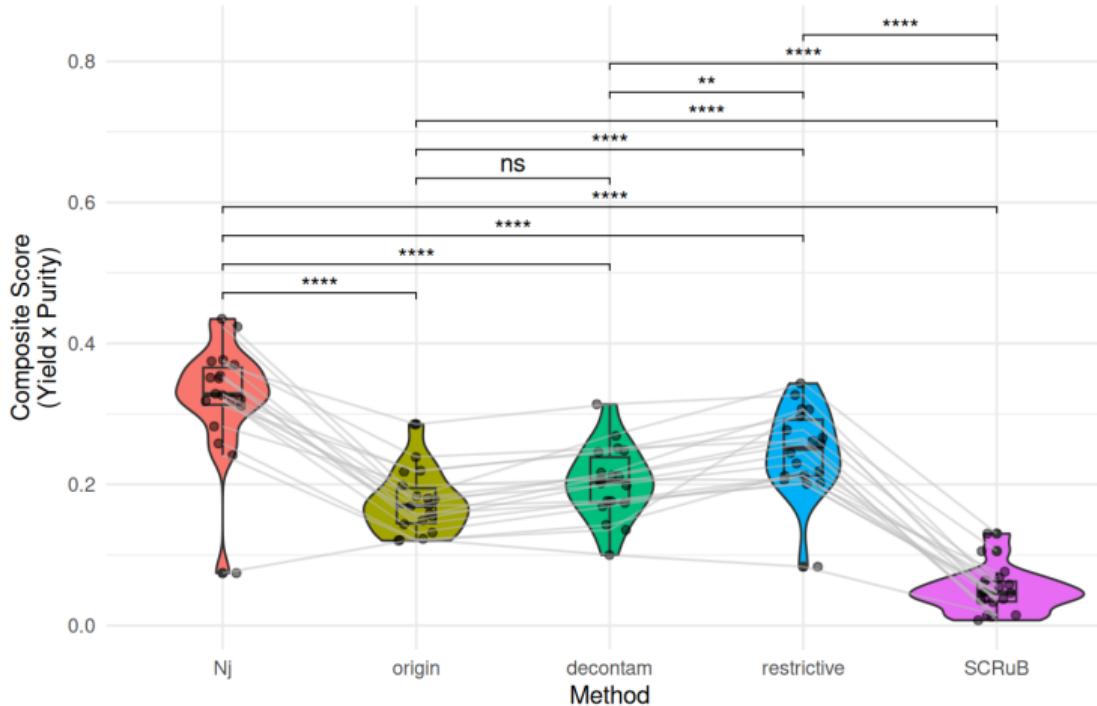
Nejman et al. 2020 Science



Assessment of Decontamination Approach

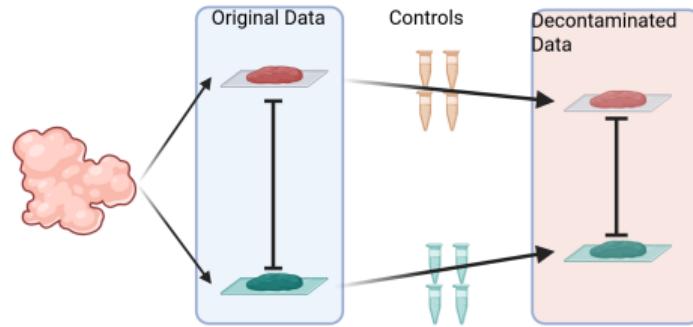
Comparison of Decontamination Methods - LMM

Metric: Penalized Clean Yield (Higher is Better)



Integrate Replica

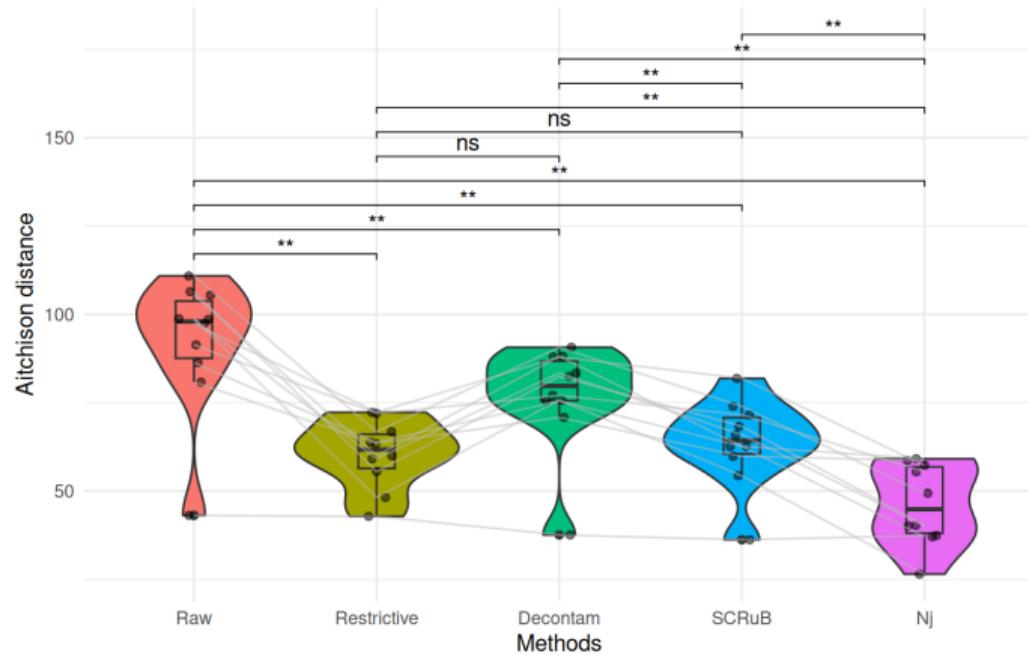
Utilize Replica for Assessment



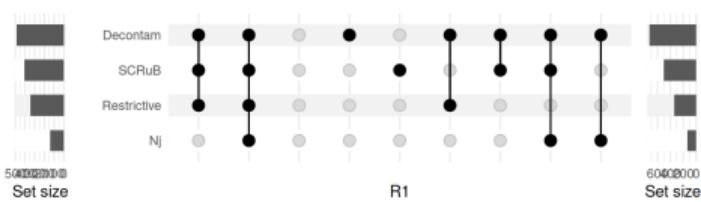
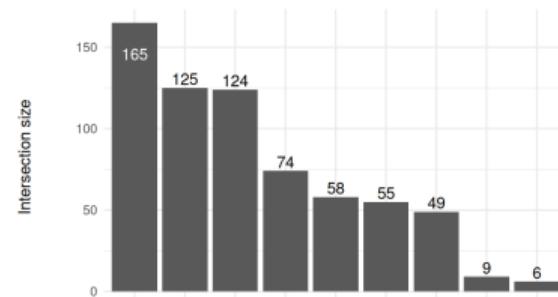
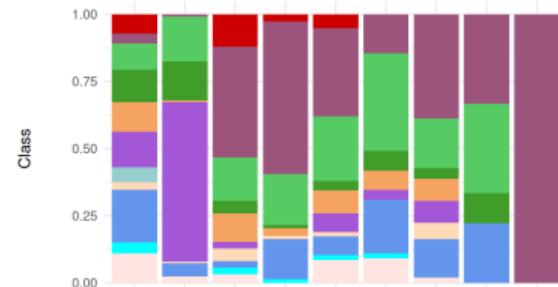
- 10 PDAC sample, each with 2 replica
- Aitchison distance with paired Wilcox test.

Paired Wilcox test

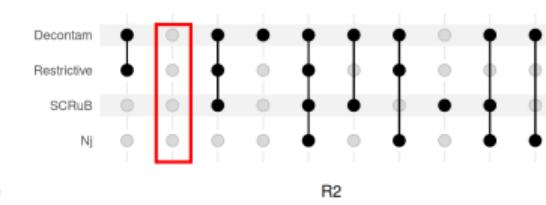
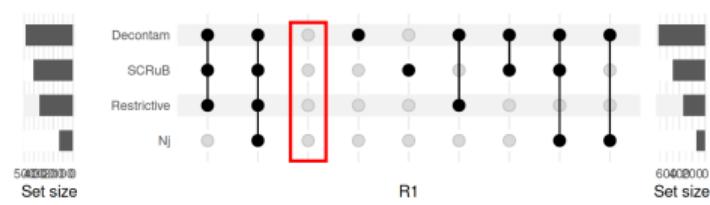
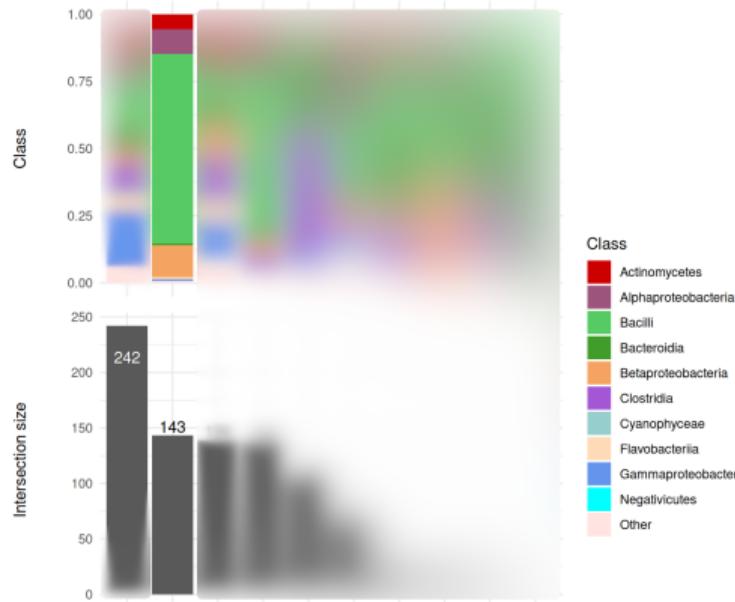
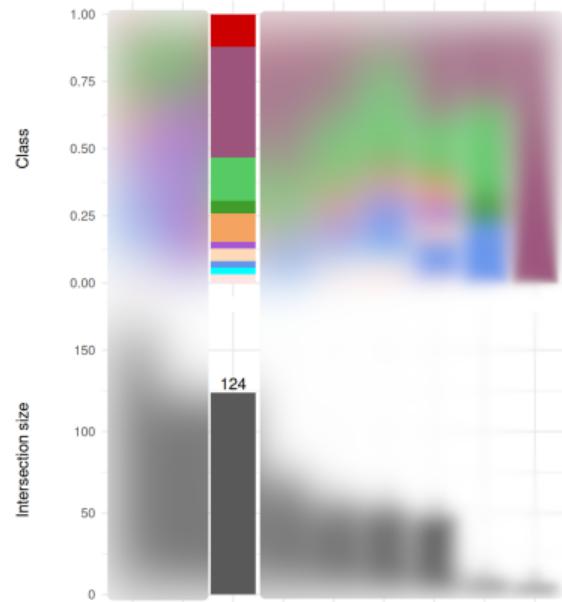
The lower the better



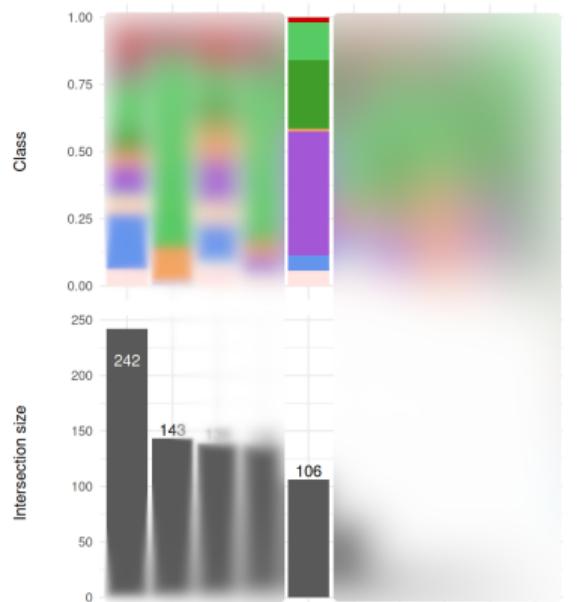
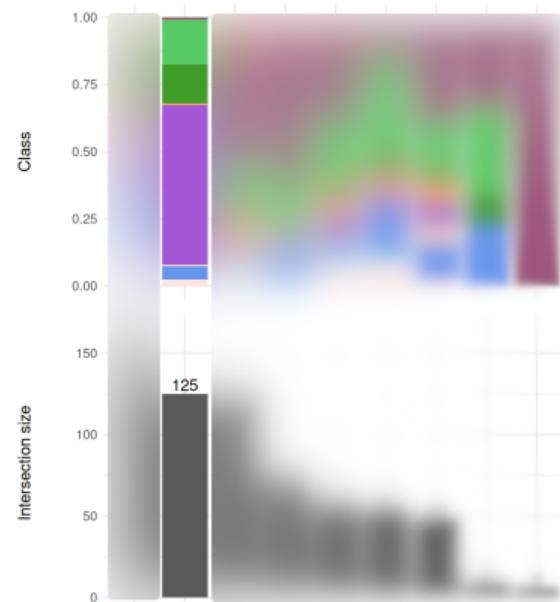
Overlap - Microbiome profile of PDAC in mice



Overlap - Microbiome profile of PDAC in mice

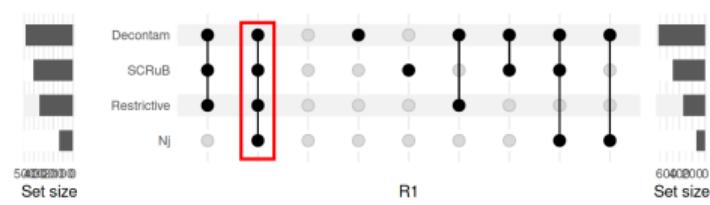


Overlap - Microbiome profile of PDAC in mice



Class

- Actinomycetes
- Alphaproteobacteria
- Bacilli
- Bacteroidia
- Betaproteobacteria
- Clostridia
- Cyanophyceae
- Flavobacteriia
- Gammaproteobacteria
- Negativicutes
- Other



Conclusions

Decontamination Methods

- Negative control samples are essential to avoid spurious taxa.
- Each batch has its own contaminant profile.
- In our study, thoroughly remove contaminant such as Nejman et al. procedure outperforms others.

Intratumor microbiome in PDAC

- Re-sequencing and various decontamination methods with proper negative controls could yield a reliable set of intratumor microbiota.
- Almost identified taxa are well-known gut microbiota.

Acknowledgements

AG Neesse

Christoph Ammer-Herrmenau

Jacob Hamm

Jutta Blumberg

Ulrike Wegener

Sören Buchholz

Catharina Lingens

Cathleen Heuer

Areej Shahbaz

Richard Meier

Rahel Scholz

Louisa Eskelson

Helena Lescow

Charlotte Vogel

Linh Dang

Isabel Toussaint

Johannes Richter

Annemarie Dresbach

Leoni Florschütz



P-MAPS Consortium

UNIVERSITÄTSMEDIZIN
GÖTTINGEN **UMG**

UMG

AG Beissbarth (Bioinformatik)

AG Ströbel (Pathologie)

AG Schneider (Chirurgie)

AG Papantonis (Pathologie)

AG Sax (Medizinische Informatik)

Externe Kooperationspartner

Johannes Zimmermann, Jena

Gabriel Nussbaum, Jerusalem

Bernd Heinrich, Hannover

Magdalena Huber, Marburg

Matthias Gaida, Mainz

Paco Real, CNIO Madrid

Nuria Malats, CNIO Madrid

Evgenij Shumilov, Münster

Ulrike Bacher, Bern

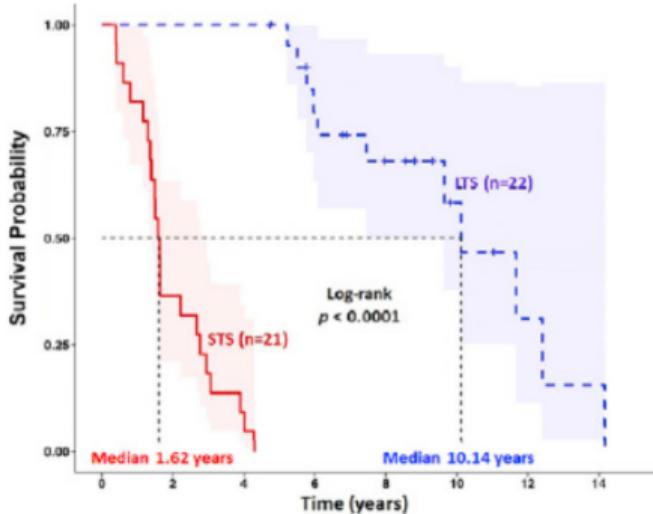
Section 3

Backup Slides

Contamination in Microbiome Study

Cell

Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes



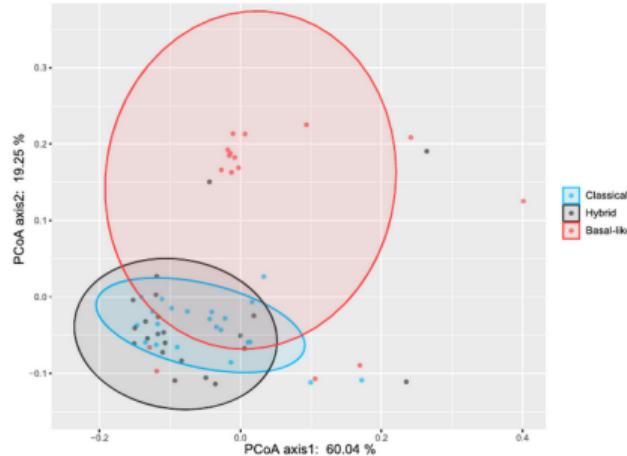
Riquelme et al. Cell 2019

Key taxa: Streptomyces, Pseudoxanthomonas, Saccharopolyspora

Article | Open access | Published: 31 August 2021

Tumor microbiome contributes to an aggressive phenotype in the basal-like subtype of pancreatic cancer

Wei Guo, Yuchao Zhang, Shiwei Guo, Zi Mei, Huiping Liao, Hang Dong, Kai Wu, Haocheng Ye, Yuhang Zhang, Yufei Zhu, Jingyu Lang, Landian Hu, Gang Jin & Xiangyin Kong



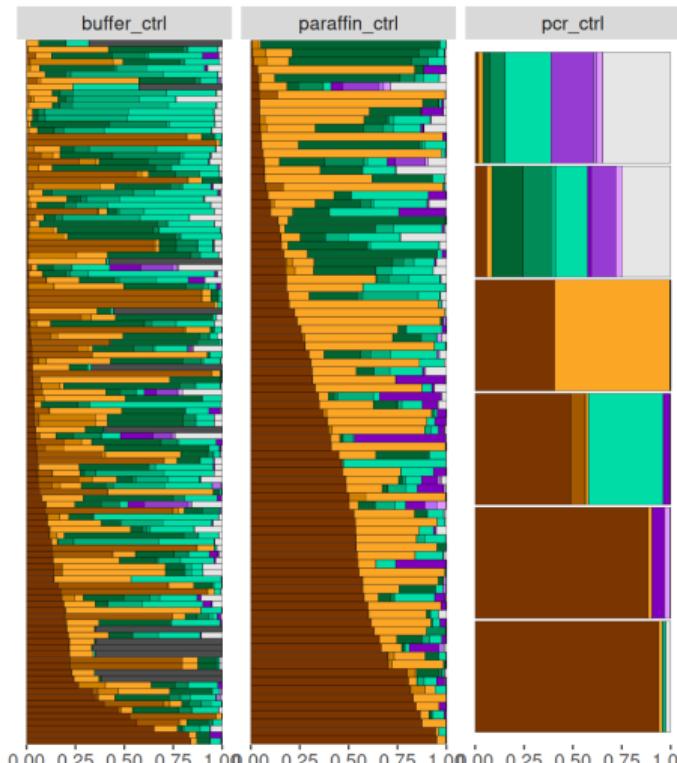
Guo Communication biology 2021

Key taxa: Acinetobacter, Pseudomonas, Sphingopyxis

Bacterial Composition

Composition of NCT Samples

Order by sum

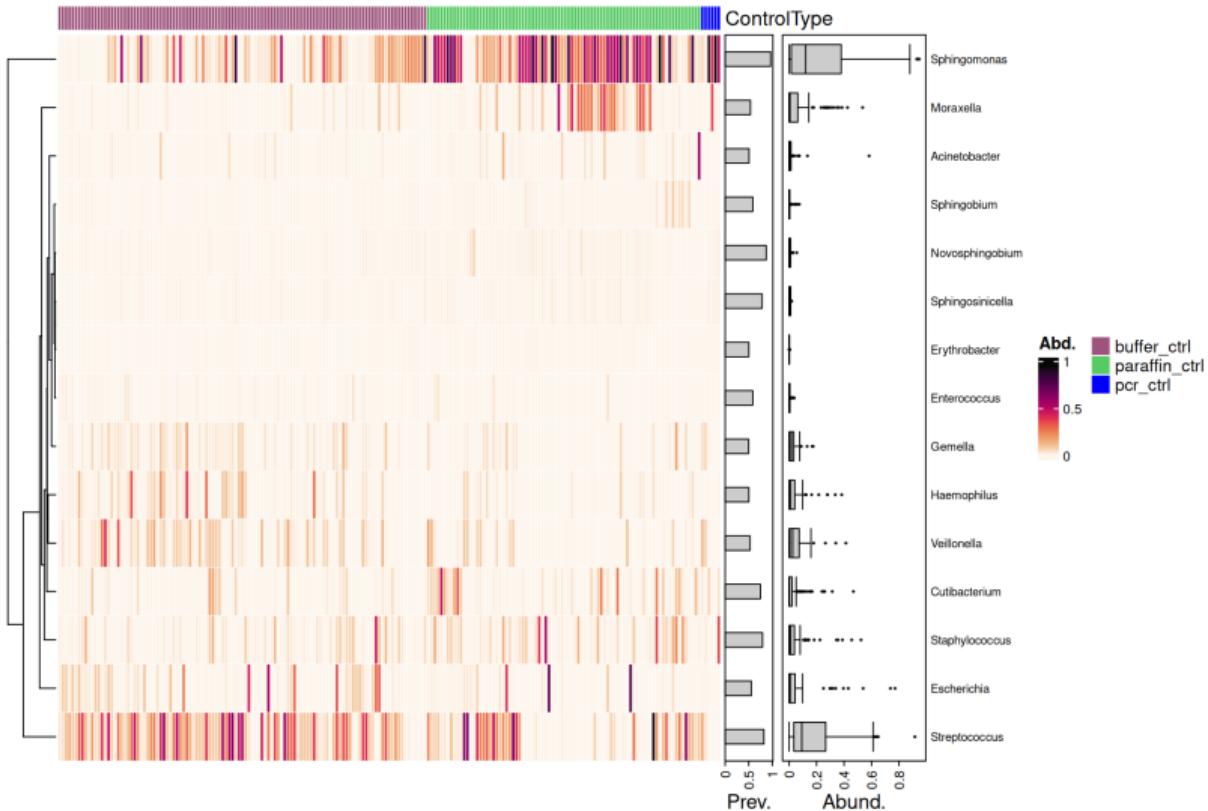


phylum: family

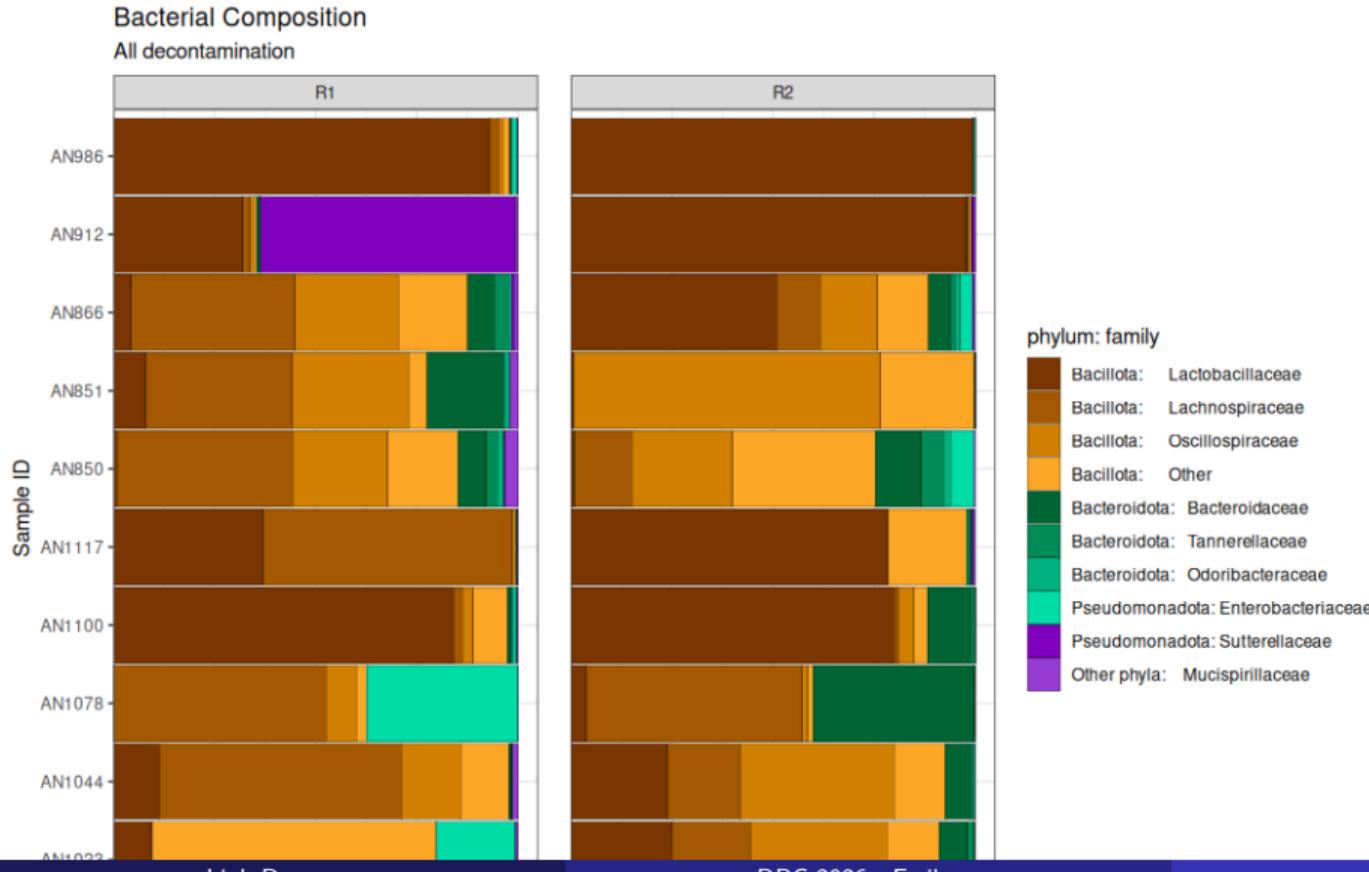
- Pseudomonadota: Sphingomonadaceae
- Pseudomonadota: Burkholderiaceae
- Pseudomonadota: Pasteurellaceae
- Pseudomonadota: Other
- Bacillota: Streptococcaceae
- Bacillota: Veillonellaceae
- Bacillota: Peptoniphilaceae
- Bacillota: Other
- Actinomycetota: Propionibacteriaceae
- Actinomycetota: Actinomycetaceae
- Actinomycetota: Micrococcaceae
- Actinomycetota: Other
- Other phyla: Bacteria_unclassified_superkingdom
- Other phyla: Candidatus Babeliaceae
- Other phyla: Deltaproteobacteria_unclassified_class
- Other phyla: Other

Heatmap from Negative Controls

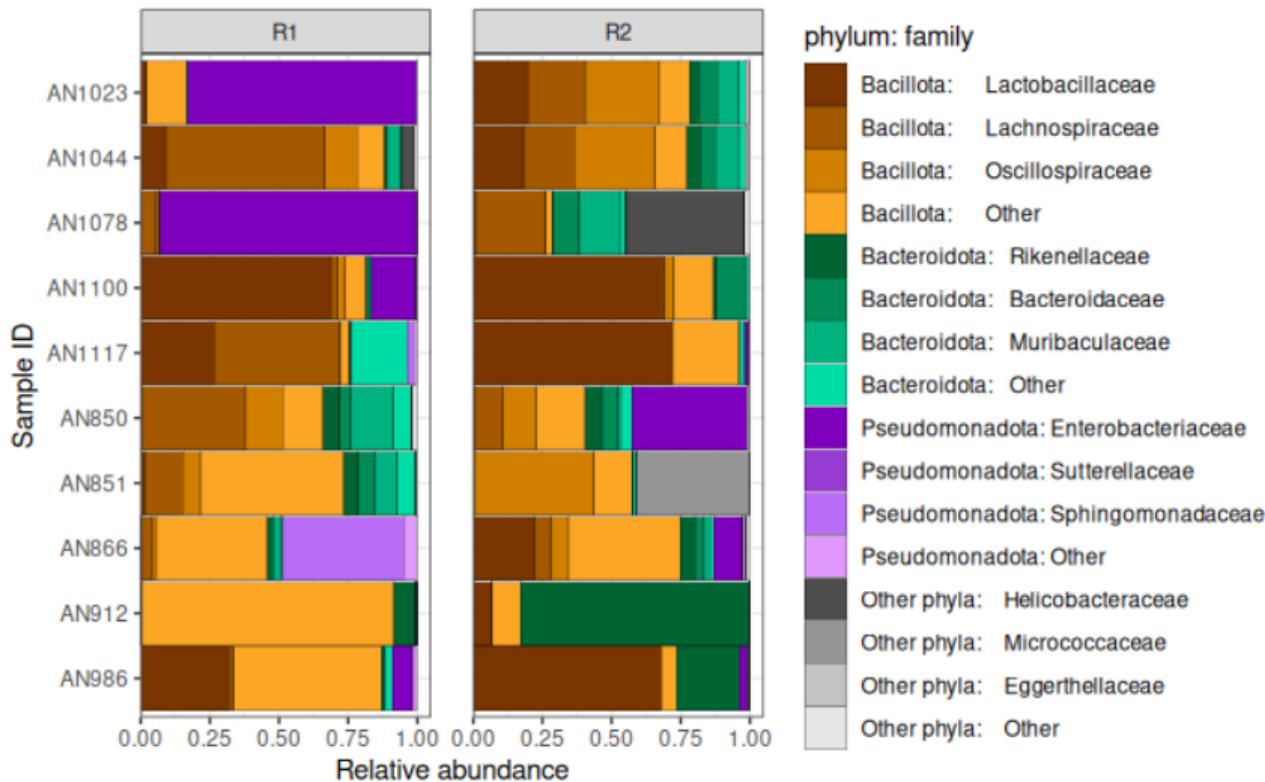
Top 15 genera by prev



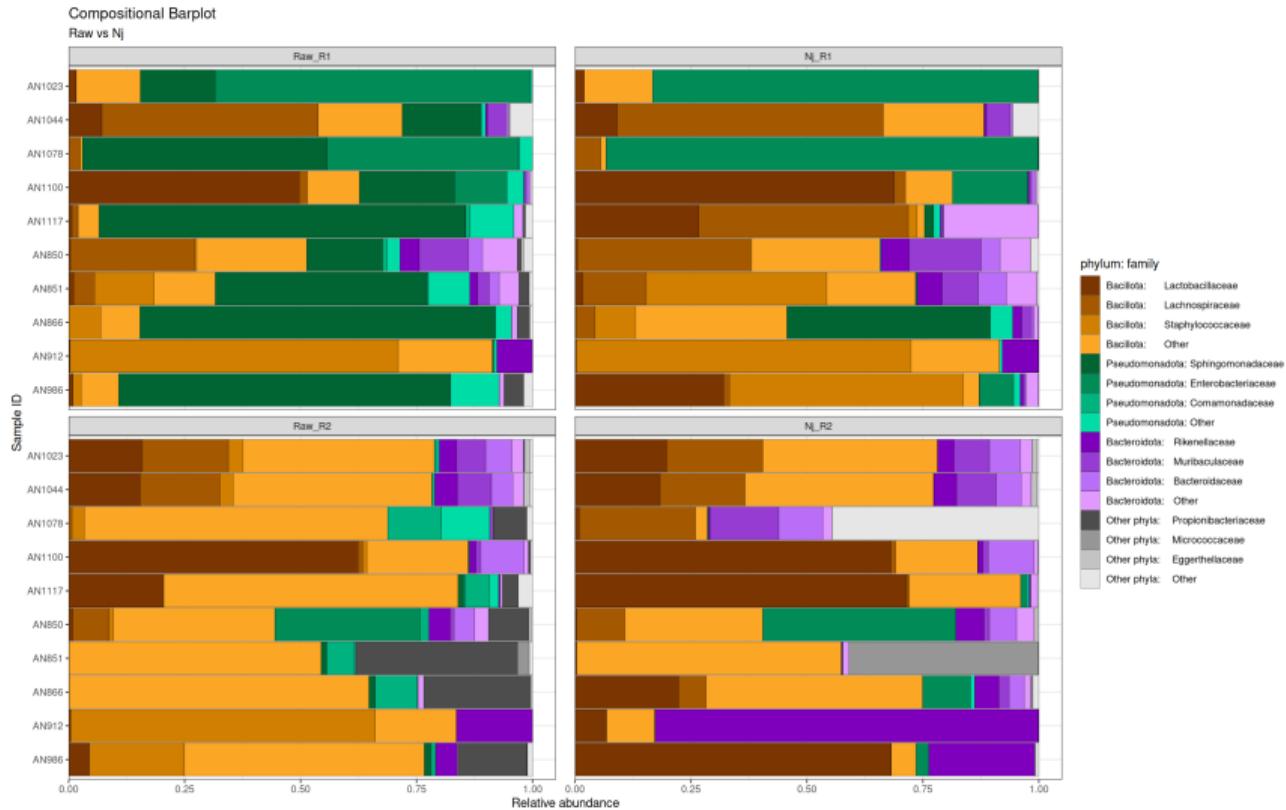
Core microbiome - All decontamination method - phylum: family



Core microbiome after Nj approach



Core microbiome after Nj approach



Assessment of Decontamination Approach

- **Yield:**

$$\frac{\# \text{ putative true taxa} \notin \text{NCT}}{\# \text{ observed species}}$$
 - **Purity:**

$$\frac{\# \text{ putative true taxa} \notin \text{NCT}}{\# \text{ putative contaminants}}$$
 - **Composite Score =**

$$\text{Yield} \times \text{Purity}$$

