



# Clinical Metagenomics in Post-Operative Endophthalmitis

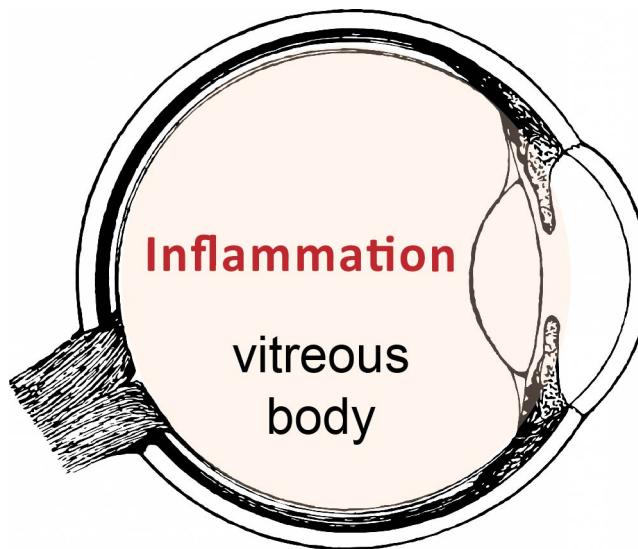
Sünje Johanna Pamp

---

Technical University of Denmark

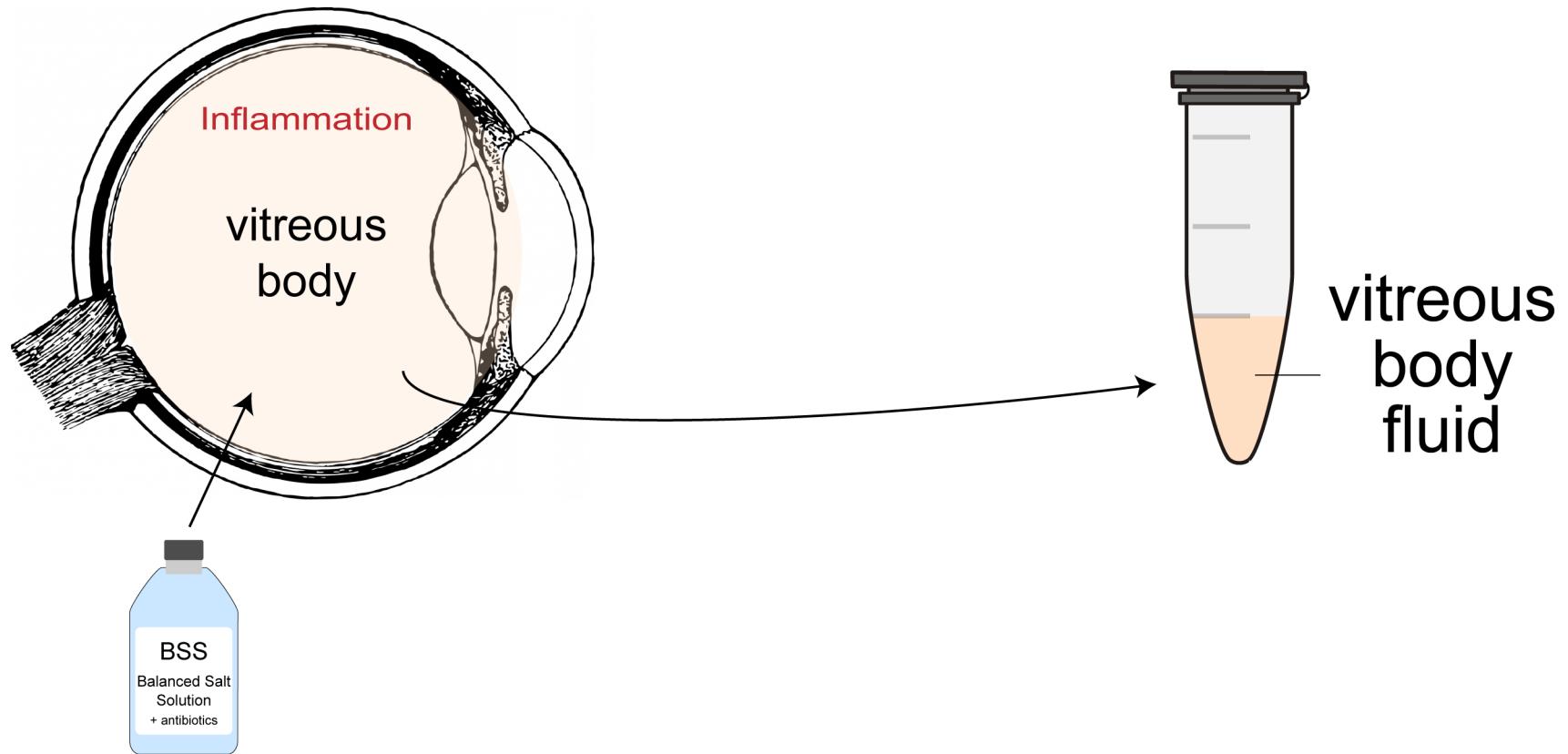
 sjpamp@food.dtu.dk  
 @SJPamp

# Post-Operative Endophthalmitis



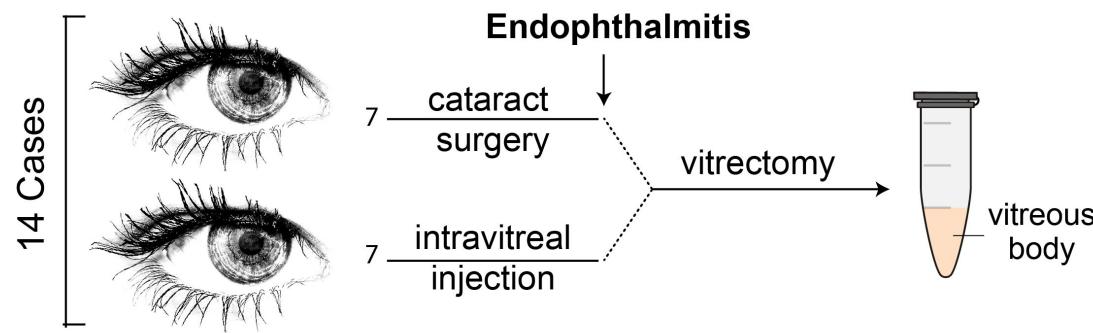
- Acute **intraocular inflammation**
- Develops in response to **microorganisms** (e.g. bacteria, fungi)
- Following **cataract surgery** or **intravitreal injection**
- Can lead to rapid permanent **loss of vision**

# Vitrectomy Surgery

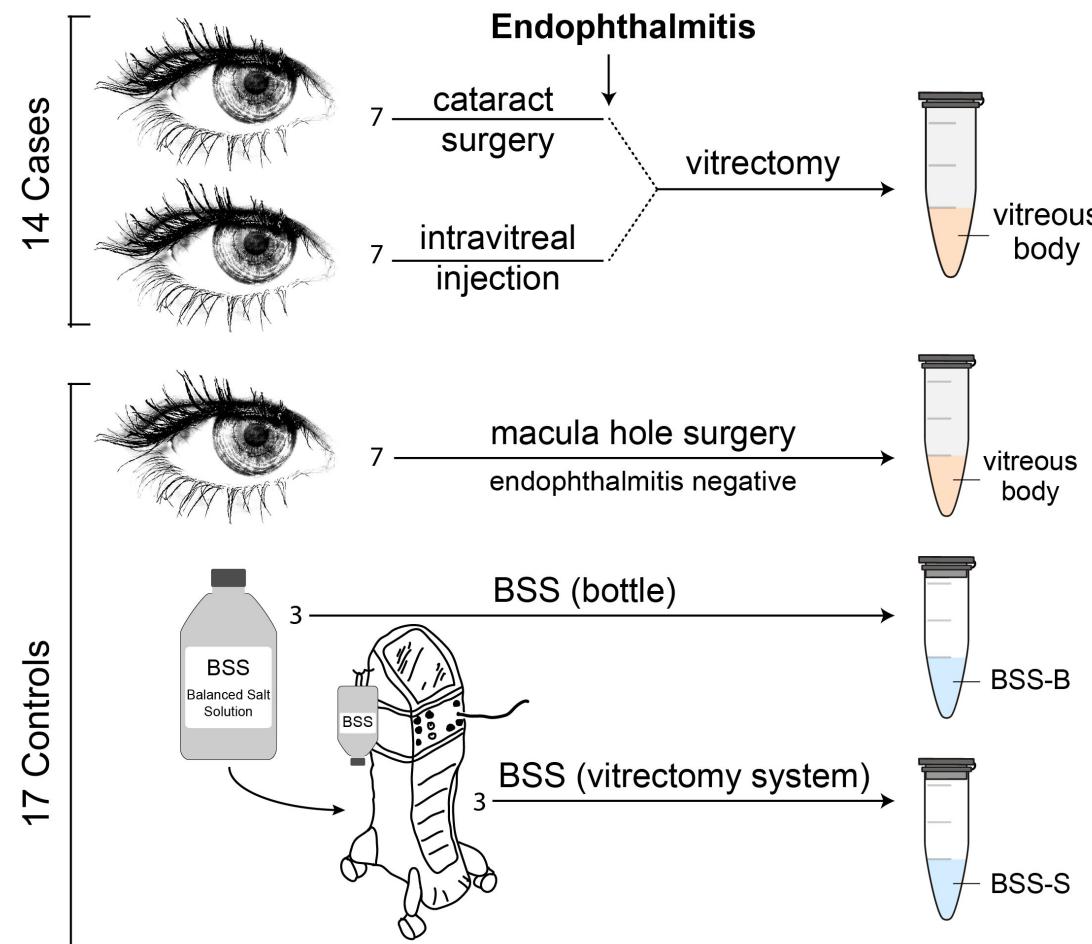


- Removal of the **vitreous body**
- Replace with **balanced salt solution + antibiotics**

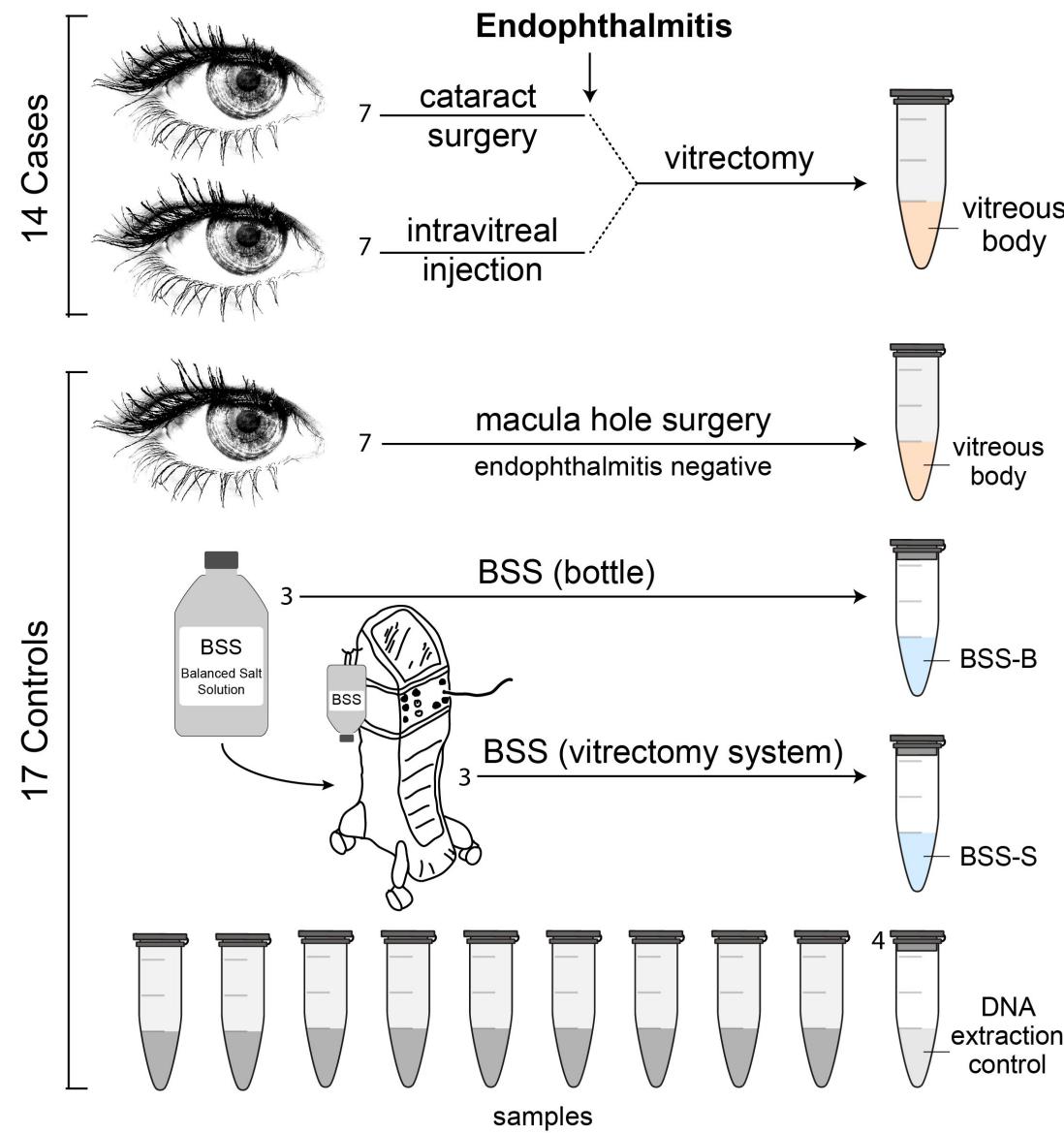
# Study Outline



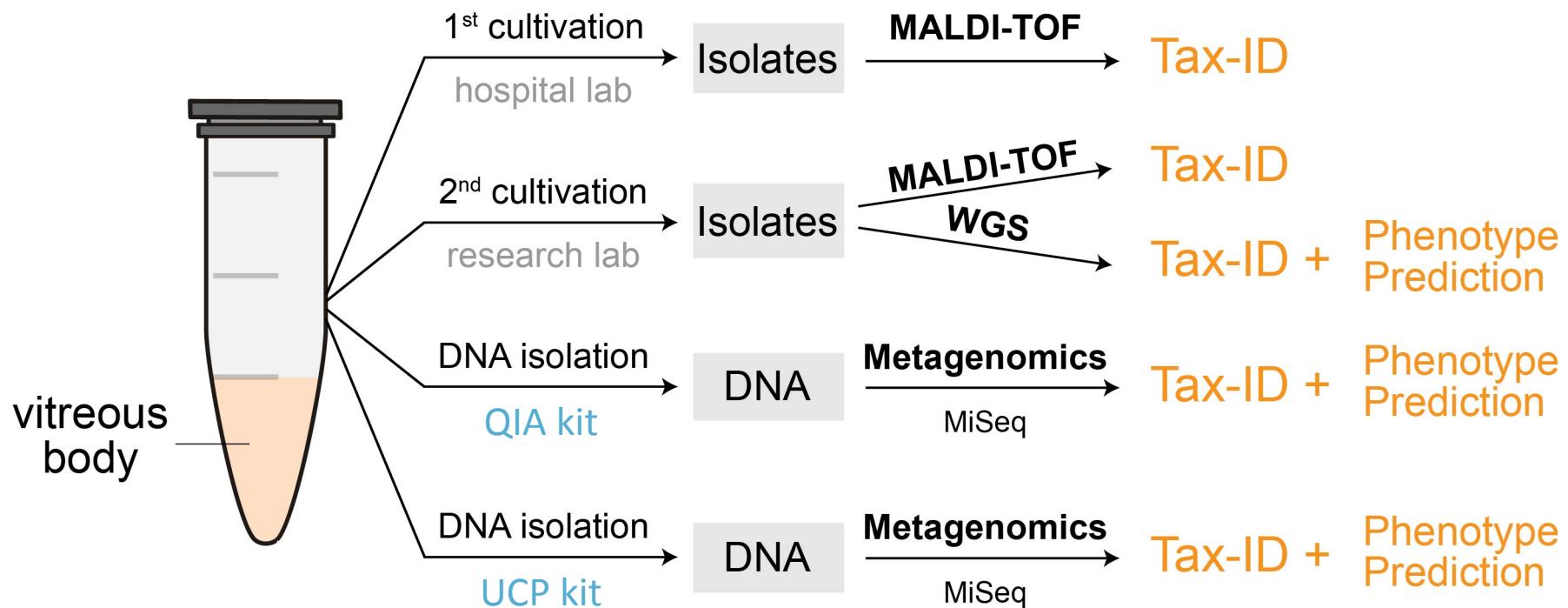
# Study Outline



# Study Outline



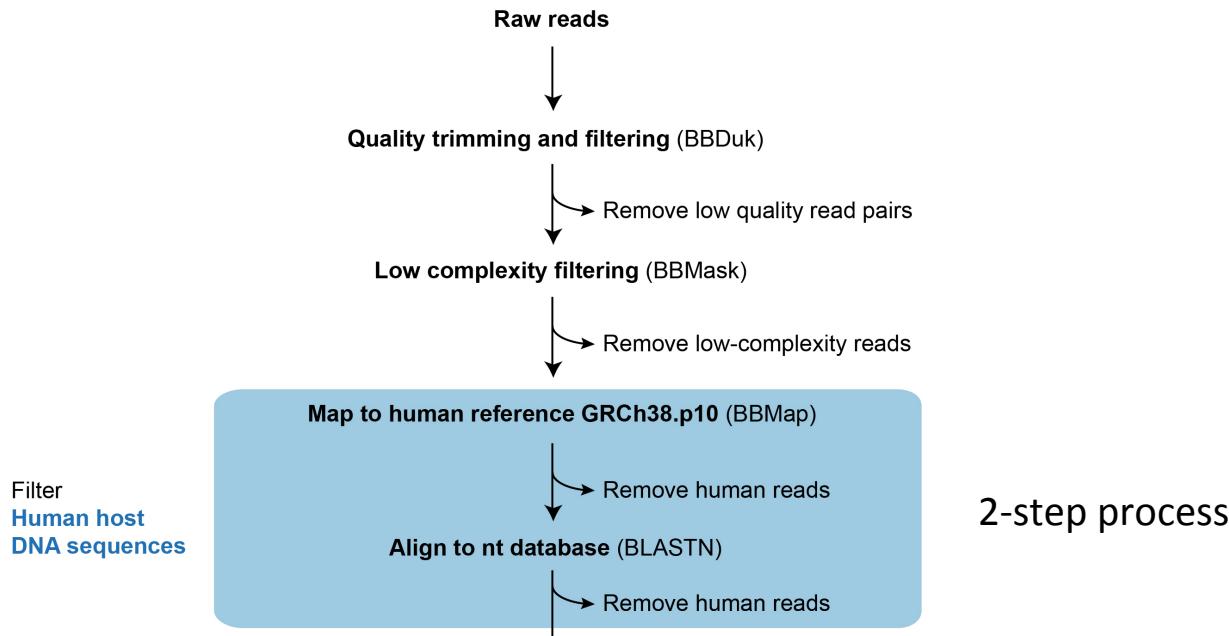
# Sample processing



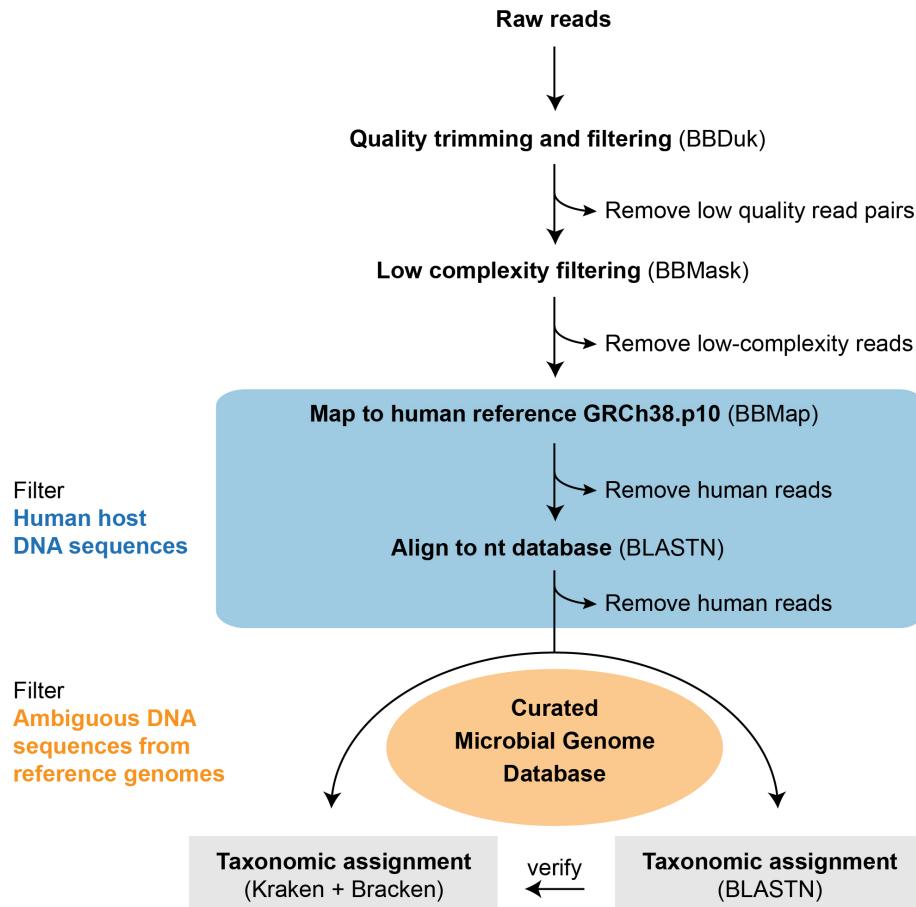
QIAamp DNA Mini Kit (QIA)

QIAamp UCP Pathogen Mini Kit (UCP)

# Metagenomics Analysis



# Metagenomics Analysis



**Curated  
Microbial Genome  
Database**

**5755**  
**Reference/Representative Genomes**

**251**  
**Archaea**

**5166**  
**Bacteria**

**225**  
**Fungi**

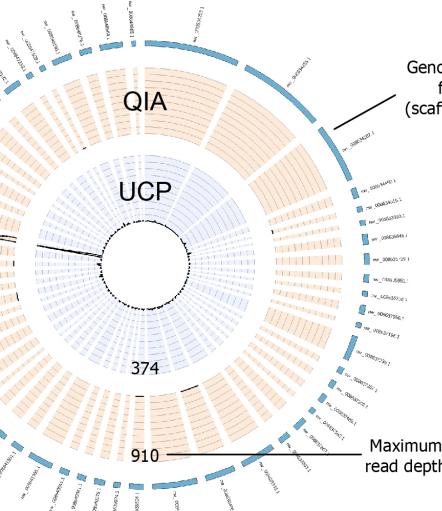
**73**  
**Protozoa**

**39**  
**Viruses**

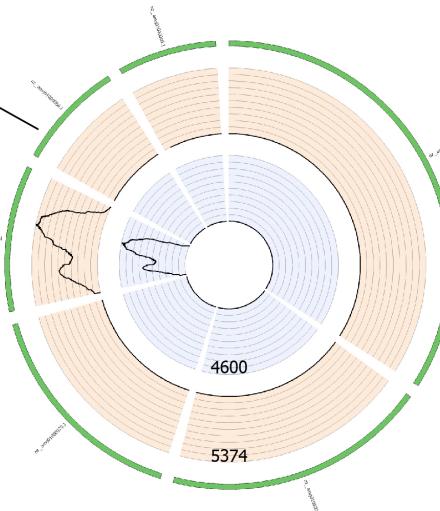
**1**  
**Human**

# Remove Ambiguous DNA Sequences from Reference Genomes

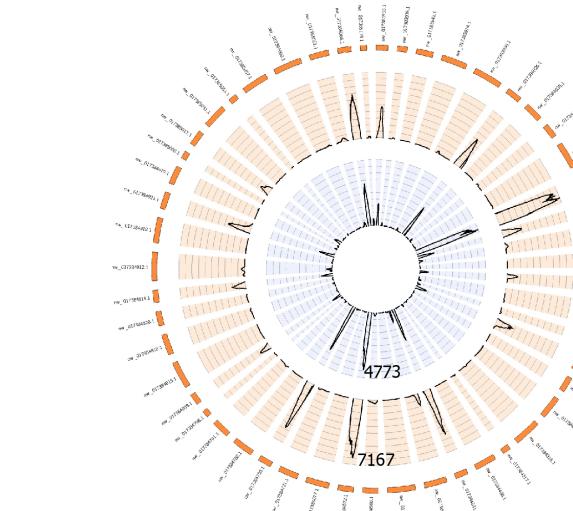
*Hammondia hammondi*  
strain H.H.34  
(parasite)



*Alcanivorax hongdengensis*  
Strain A-11-3  
(seawater bacterium)



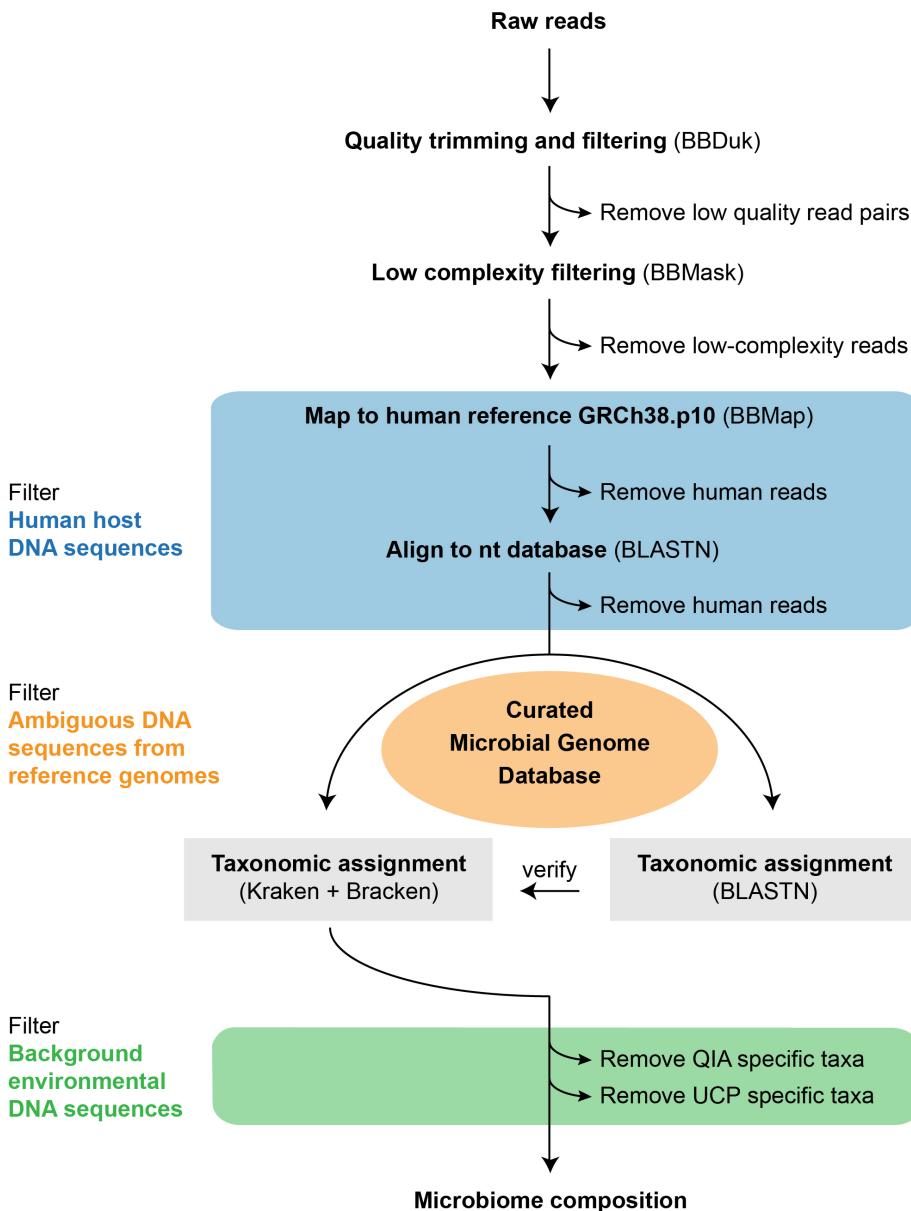
*Toxoplasma gondii*  
ME49  
(parasite)



|            | Total       |                       | Ambiguous sequences* |                    |             |
|------------|-------------|-----------------------|----------------------|--------------------|-------------|
|            | Genomes     | Bases                 | Genomes              | Bases              | Bases (%)   |
| Archaea    | 251         | 673,145,451           | 65                   | 1,813,095          | 0.27        |
| Bacteria   | 5166        | 20,854,687,300        | 2251                 | 75,888,994         | 0.36        |
| Fungi      | 225         | 6,486,874,847         | 126                  | 6,642,500          | 0.10        |
| Protozoa   | 73          | 2,930,167,033         | 53                   | 26,447,579         | 0.84        |
| <b>Sum</b> | <b>5714</b> | <b>30,944,874,631</b> | <b>2922</b>          | <b>110,858,157</b> | <b>0.35</b> |

\*Genomic sequence regions  $\leq 10$  kb (incl. contigs and scaffolds) who had a match (e-value  $\leq 1e-6$ ; query coverage  $\geq 70\%$ ) belonging to a different genus than their stated genus definition when aligned against the non-redundant nucleotide collection (nt) database from NCBI.

# Metagenomics Analysis



**Curated  
Microbial Genome  
Database**

**5755**  
**Reference/Representative Genomes**

**251**  
**Archaea**

**5166**  
**Bacteria**

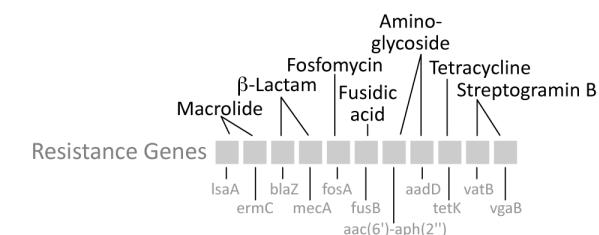
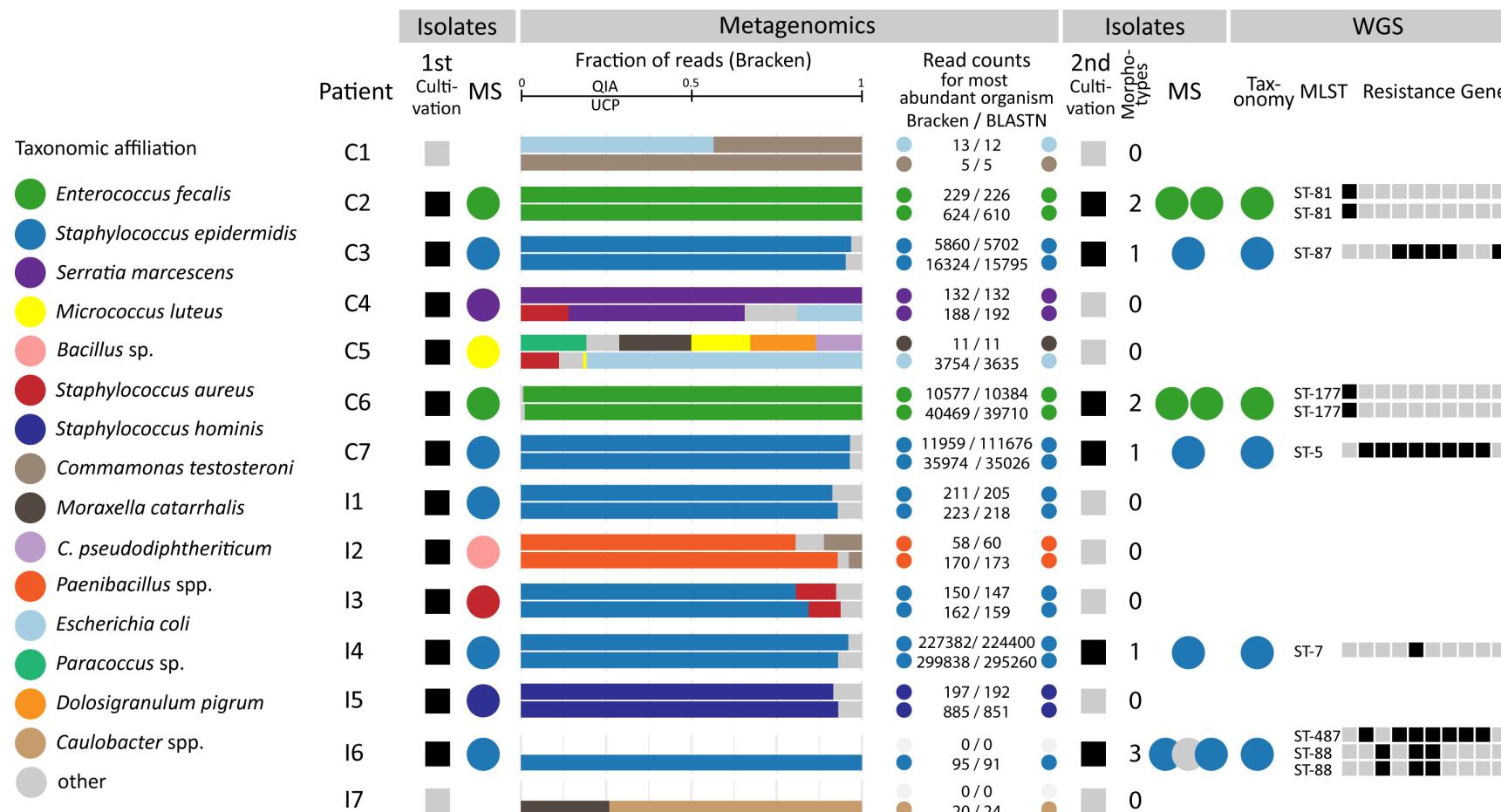
**225**  
**Fungi**

**73**  
**Protozoa**

**39**  
**Viruses**

**1**  
**Human**

# Summary of Results



## Take-home messages

- Ultra-clean reagents/supplies for nucleic acid extraction
- Range of negative controls
- Bioinformatics workflow with filtering steps:
  - i) human DNA, ii) ambiguous/contaminant seq in reference genomes, iii) environmental sequences (kit/lab)
- Curated reference databases

<https://figshare.com/s/2a0709b1f0c5e18754df>

5. July 2017



### Guidelines for Metagenomics Sequencing Projects

This list provides an overview of **considerations** and **controls** relevant for metagenomic sequencing projects. Project design, sample collection, and sample storage are discussed elsewhere. It is assumed that all samples of a particular project are being collected and stored in the same way.

# Acknowledgement

**Philipp Kirstahler** (DTU)

Søren Solborg Bjerrum (Glostrup hospital)

Alice Friis-Møller (Hvidovre hospital)

Morten la Cour (Glostrup hospital)

Frank M. Aarestrup (DTU)

Henrik Westh (Hvidovre hospital)



**bioRxiv** <https://doi.org/10.1101/176529>

**Figshare** See links in manuscript

**DDBJ/ENA/GenBank** PRJEB21503 (WGS assemblies, WGS reads, Metagenomics reads)

**Github** <https://github.com/phildTU/endoPublication>



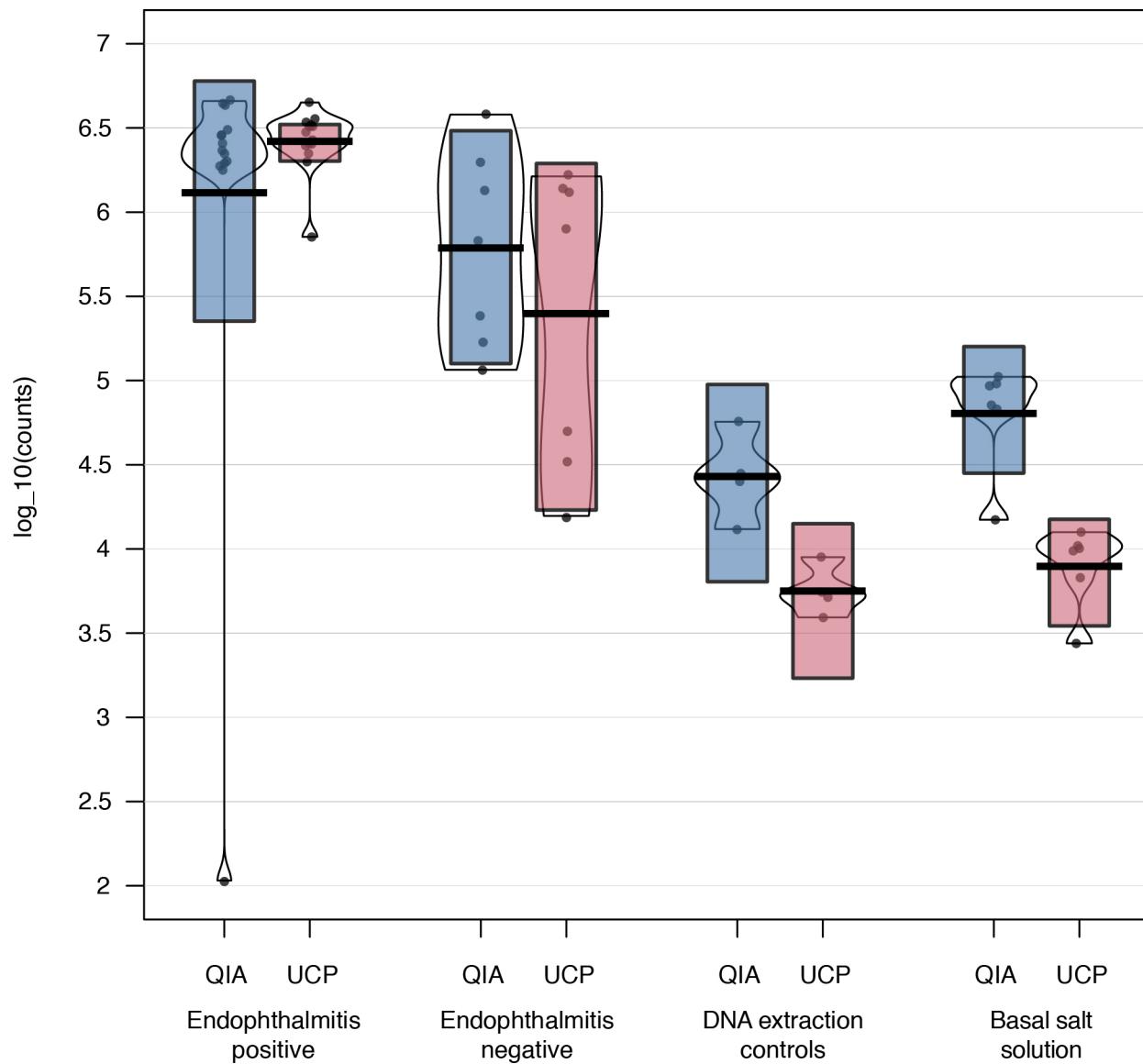
Thank You!

**Sünje Johanna Pamp**  
Technical University of Denmark

 sjpamp@food.dtu.dk  
 @SJPamp



# Lower number of reads for UCP-extracted control samples



# Kit-specific “fingerprints” + lab/staff-associated microorganisms

