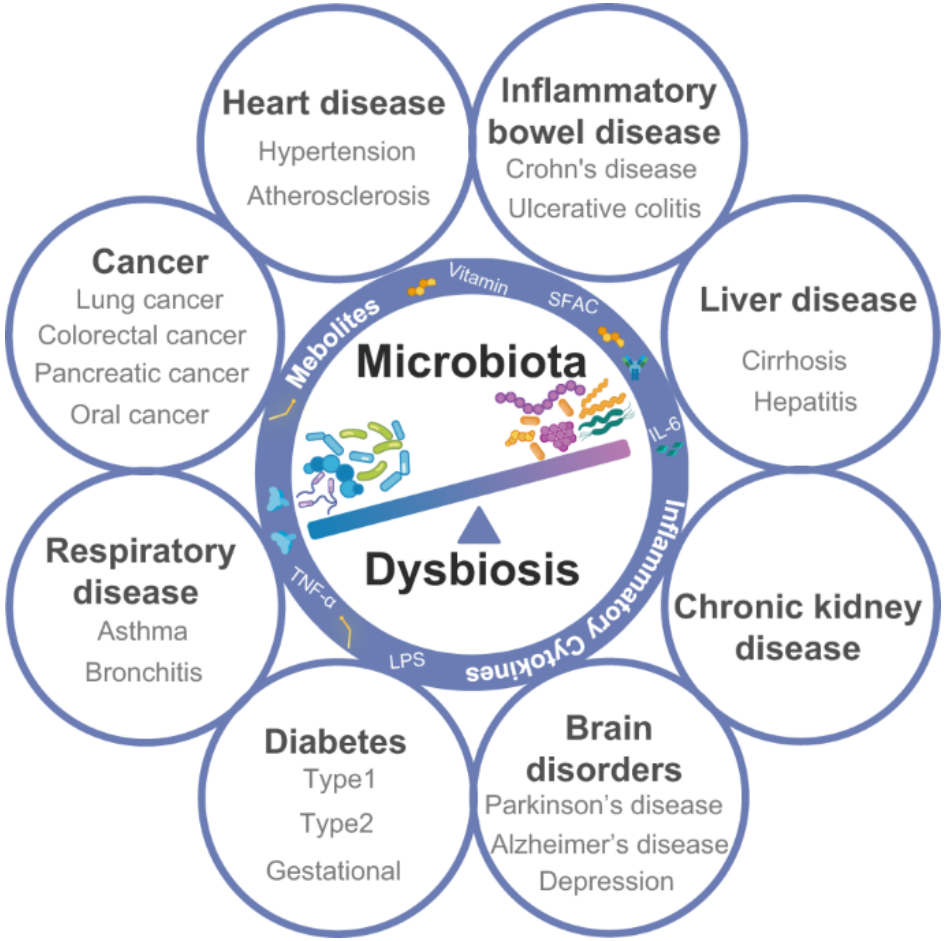
High-throughput cultivation of the faecal microbiome using FACS

ACS Presentation Allison McInnes

Centre for Microbiome Research

Introduction



Hou et al (2022)

- 1.3 bacteria cells per human cell (Sender et al 2016)
- Unified Human Gastrointestinal Genome collection estimates 4,644 prokaryote species (Almeida et al 2020)
- 100x the number of genes (Gilbert et al 2018

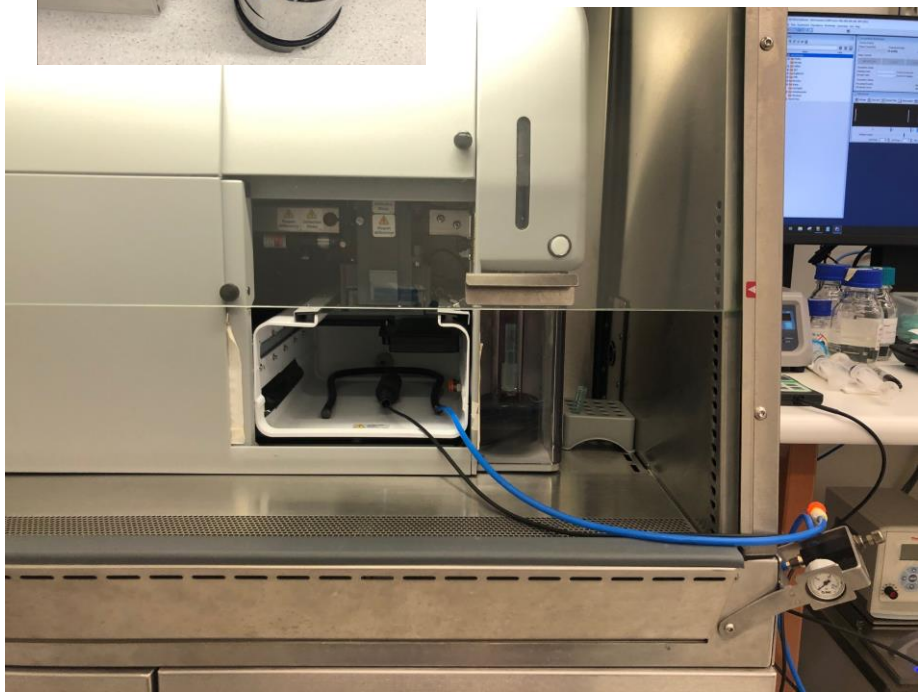
- Microbiome plays a role in host health:

Introduction

- Metagenomics – gives a full picture of who is there
  - Not quantitative
  - Function is inferred from genome
- Function must be confirmed through **isolation**.
  - Isolation also allows for manipulation and targeted use of microbes of interest
- Techniques for isolation are still slow, low throughput
  - Majority of human microbiome remains uncultured (~70% Almeida et al 2020)
  - 3,500 species still to be cultured

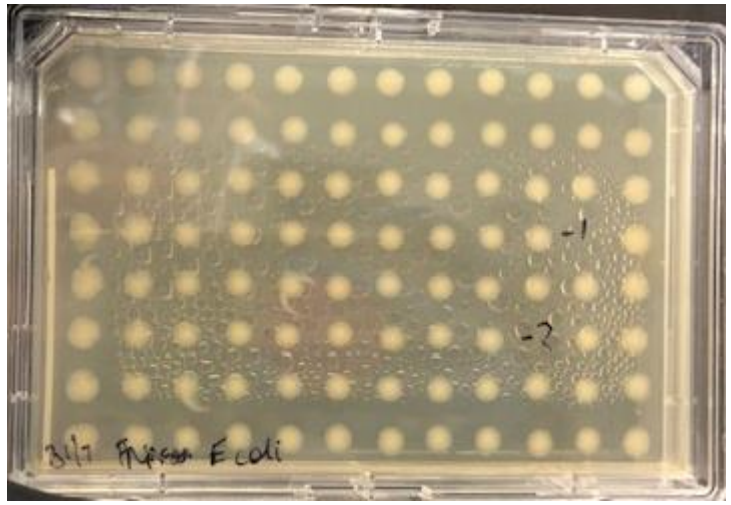
Anaerobic FACS

- Commercially available Fusion with SPO
- Plumbed in house nitrogen in addition to air
  - Pressurise system and run it off nitrogen for anaerobic sorts
  - Also allows us to sparge the sheath tank with nitrogen prior to sort
- Flood the sort chamber with Nitrogen for anaerobic sorting
  - Monitor with oxygen meter
- Transport sample and plate in an out of the anaerobic chamber using airtight boxes



Results – sorting viability

E. coli



Culture	% grown
Ecoli	96/96
B longum	58/96
M faecis	76/96
Fprau	86/96

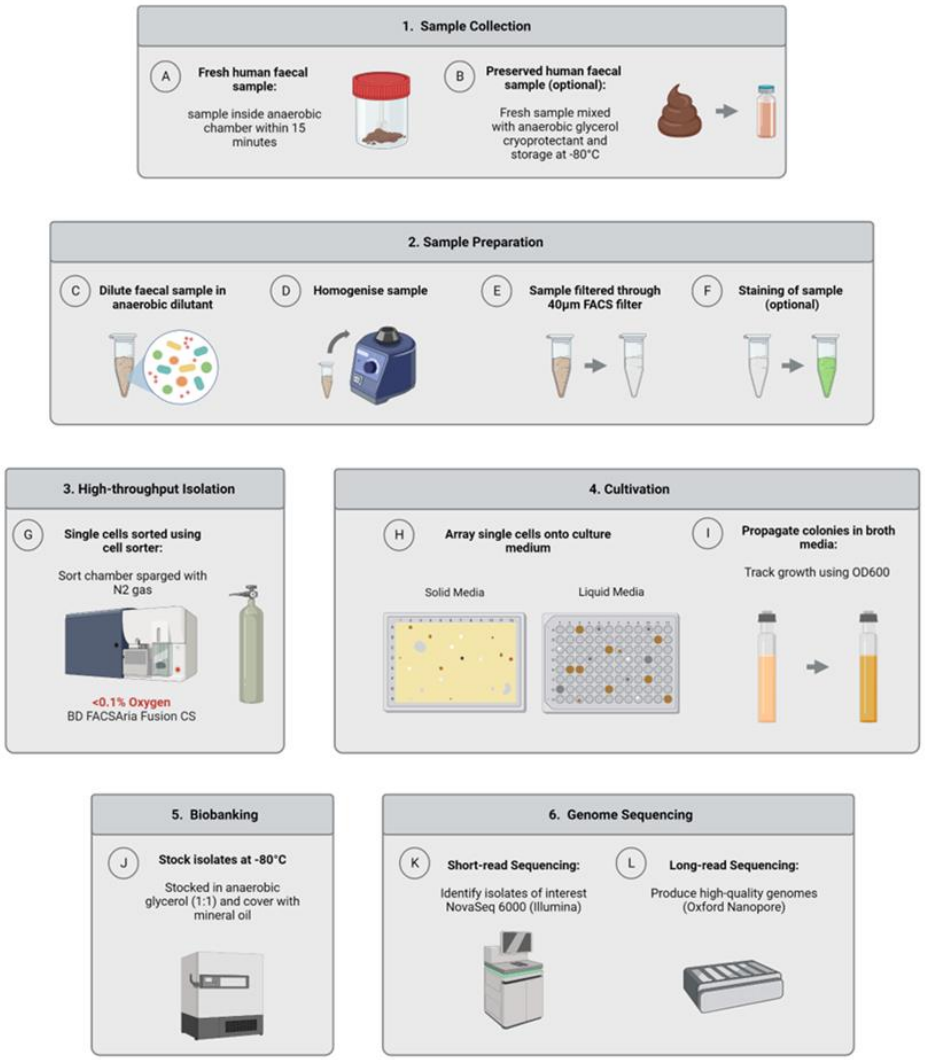
Faecal

Results – CMR biobank

- SUCCESS!
- 12,000 isolates
- >613 species
- 300 new to cultivation

	Isolated taxa	Uncultured taxa	Uncultured taxa %
Domain	2	0	0
Phylum	12	0	0
Class	14	0	0
Order	30	2	6.67
Family	60	9	15
Genus	234	41	17.52
Species	>613	300	48.93

A.



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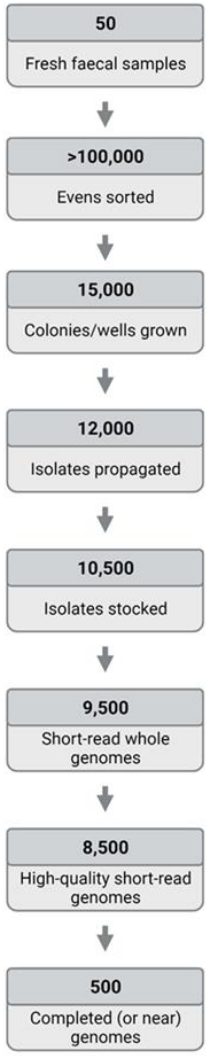
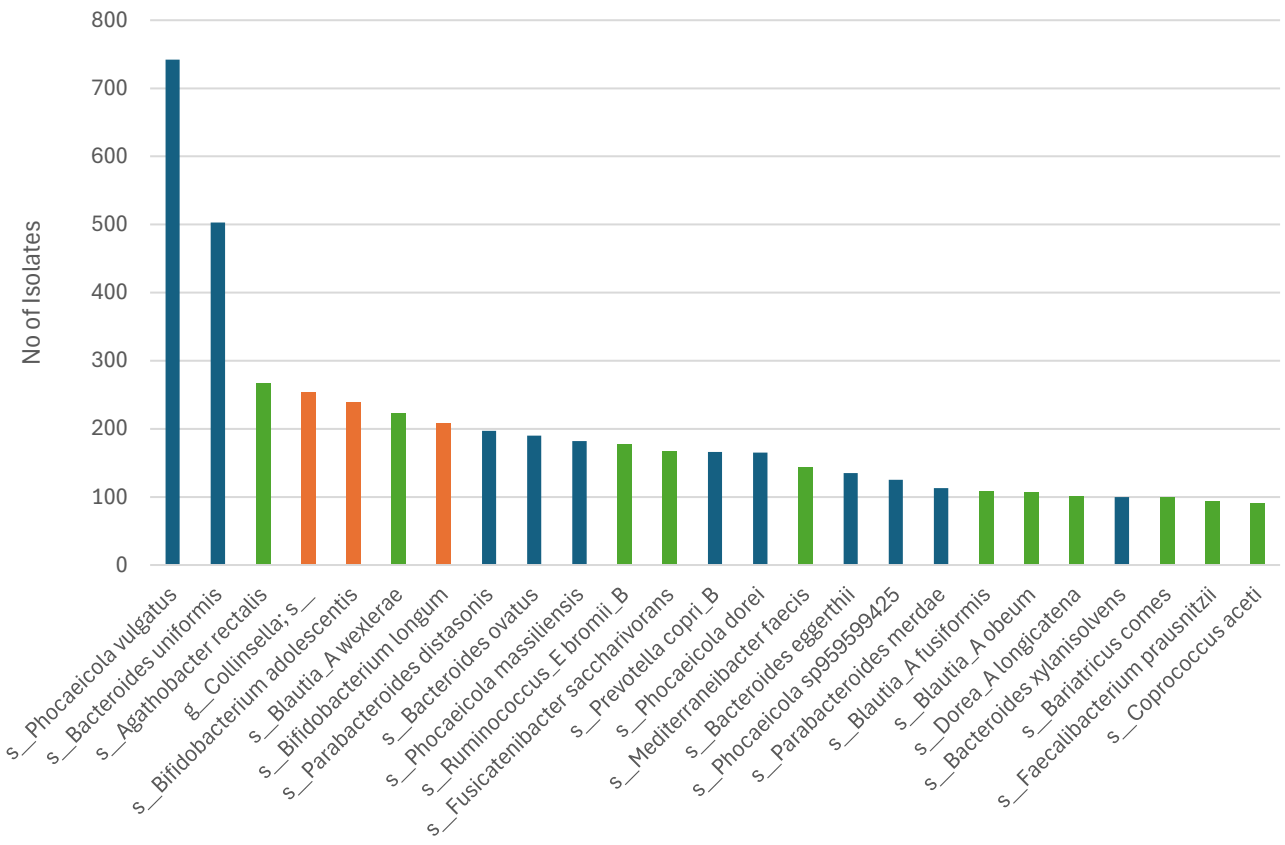


CMR biobank

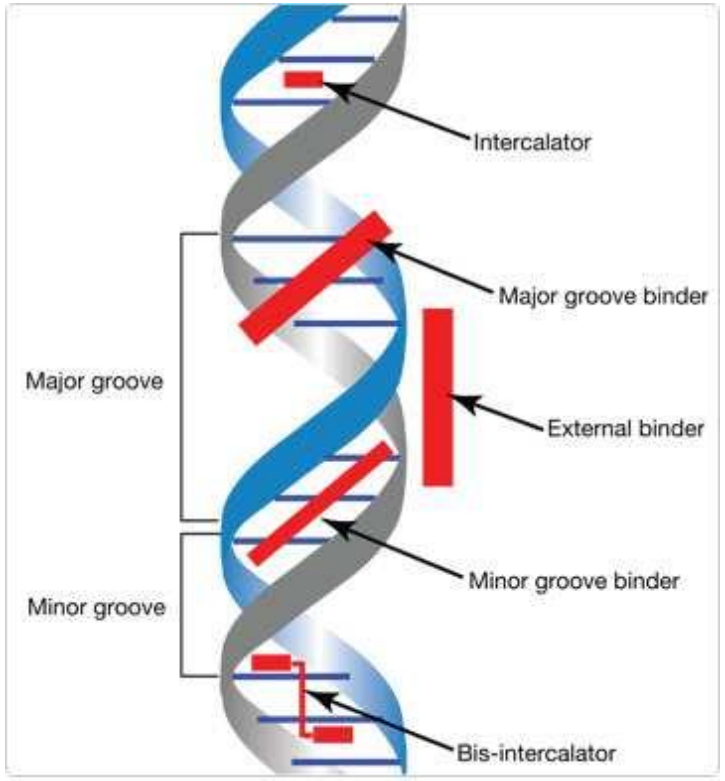


- Top 25 most common isolates represent 52.1% (4894/9388)
- We have almost 742 P. vulgatus isolates, which represent 7.9% of the biobank

  = Over representation of p_Bacteroidota
- 97.2% prevalence in donor profiles but only average relative abundance of 0.71%

  10-fold great in isolates

Targeting populations – DNA

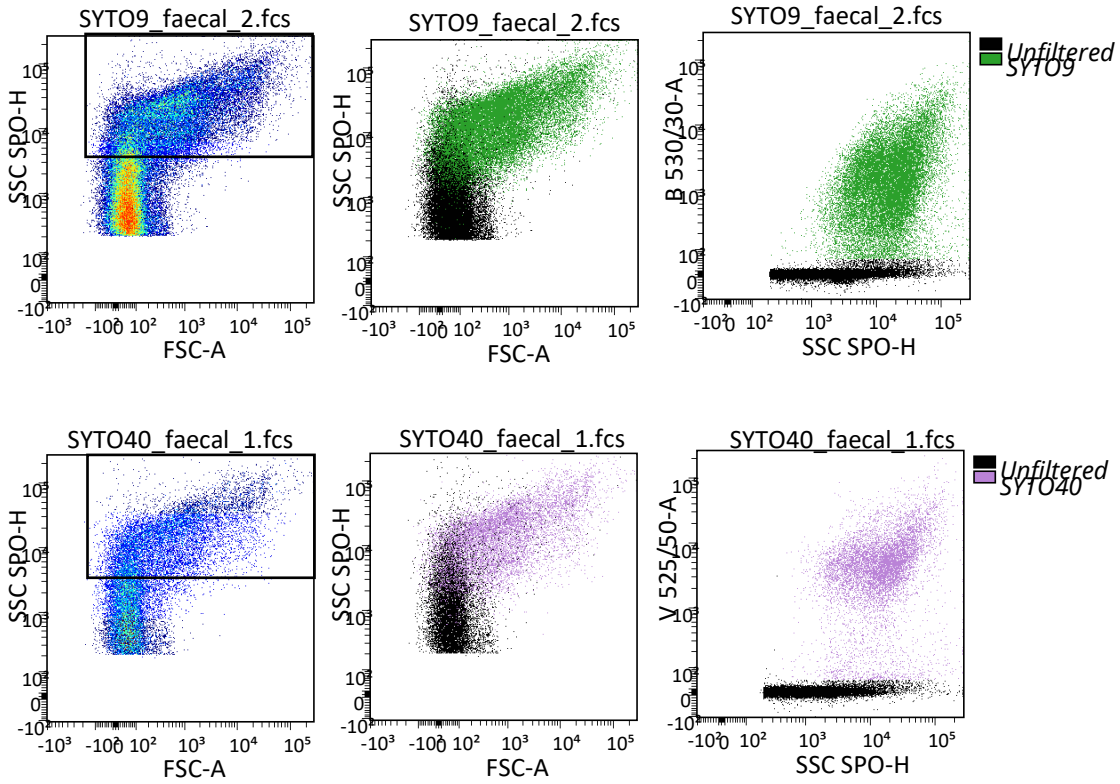


Molecular Probes Handbook - ThermoFisher

- Different DNA stains target different portions of the DNA.
- Some stains preferentially bind to AT rich regions and others GC

- Can we harness the differential staining to target components of the mixed assemblage?

Targeting populations – DNA results



Do different stains stain different portions of the microbial community?

Exp – stain 1 faecal sample with all CMR dyes (6) and sort positive populations.

(MDA amplification) (2 plates for each dye 5-12-23)

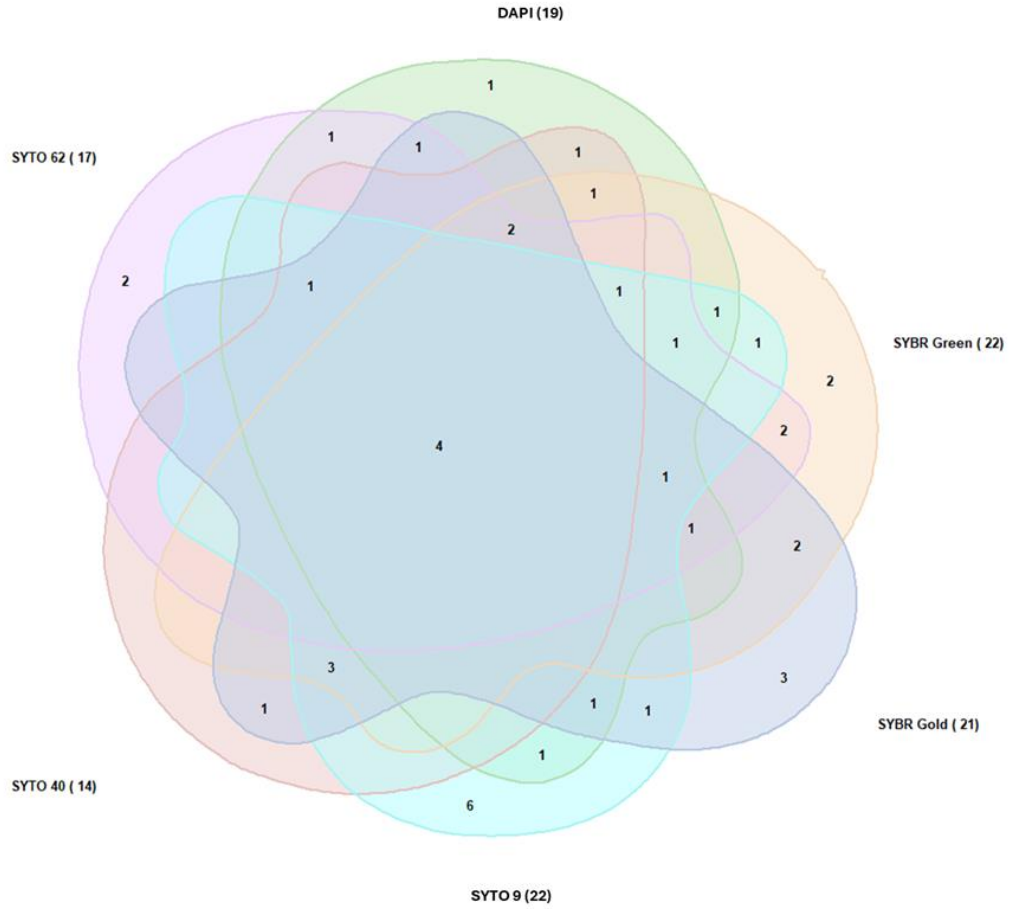
1. unstained
2. DAPI 72%
3. SYBRgreen 77%
4. SYBRgold 62%
5. SYTO9 83%
6. SYTO40 37%
7. SYTO62 79%

- Goal compare what is in one but not the other and can use various combinations to “target” sort for mini-metagenomes

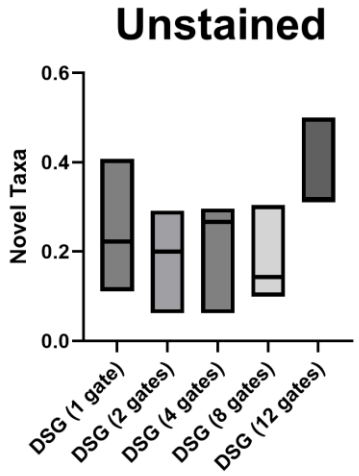
Targeting populations – DNA results

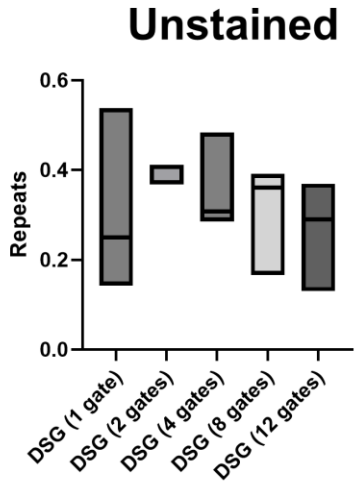
- We collected one fresh faecal sample and split it 7 ways: unstained and all dyes sorted 3 plates for each dye.

- Of the species cultivated using different dyes only 4 were isolated using all of them. The remaining 115 were only cultivated using one or more of the dyes tested.
- P vulgatus was isolated using unstained, DAPI, SYBR and SYTO40
- Not with SYBR gold, SYTO9, or SYTO62



Targeting populations – DSG





- Novel = species sorted only on that plate/total species
- Repeats = sum species sorted more than 1x/total isolates

- Increasing number of novel species with increasing number of gates
- Decreasing repeats with increasing number of gates

- DSG1 for repeats is skewed by 1 sample with very high diversity/evenness

Summary

- Proof of principle anaerobic cultivation with minimal modification to a commercially available instrument
- Created a biobank of 12,000+ clonal isolates over 613 species, half are new to cultivation

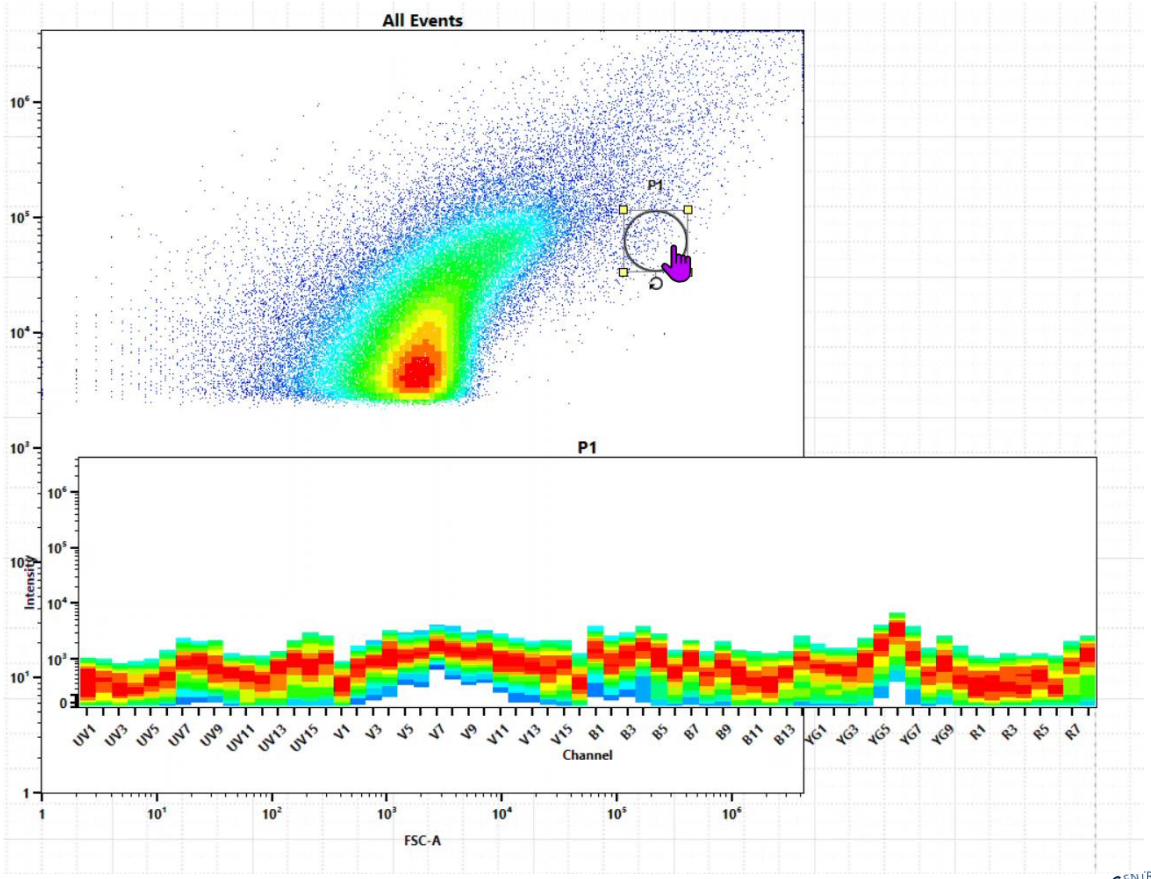
- Deep sort gates help reduce the number of the same species being isolated from the same sample
- Might be possible to target portions of the community using DNA dyes or use as an exclusion
  - *P. vulgatus* (the most problematic species) was only isolated unstained, DAPI, SYBR green, and SYTO40 but not with the remainder
  - Needs to be replicated

Future directions - AHMB

- Based on the existing work and preliminary spectral data the Australian Human Microbiome Biobank was funded 2023
- 5L Aurora CS in an anaerobic chamber
- Using enhanced sensitivity and spectral signatures paired with AI to differentiate components of the human microbiome for targeted sorting

Microbial Spectral Cytometry

- Currently working to optimise settings for maximum resolution of dim auto fluorescent populations and resolve microbes form the scatter
- Sorting in the chamber is viable
- More to come next year ☺



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