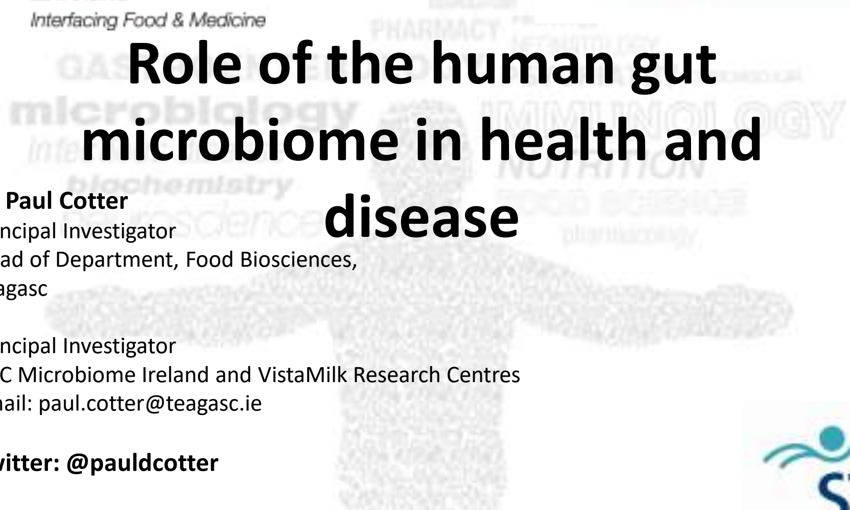


Role of the human gut microbiome in health and disease

Dr Paul Cotter
Principal Investigator
Head of Department, Food Biosciences,
Teagasc

Principal Investigator
APC Microbiome Ireland and VistaMilk Research Centres
Email: paul.cotter@teagasc.ie

Twitter: @pauldcotter

Microbiome research in Cork

http://apc.ucc.ie

Teagasc is the Agriculture and Food Development Authority
- Research & Innovation
- Farm advisory
- Education

SFI Centre Focussed on microbiome research
~300 researchers across Teagasc and UCC

Our microbes - Some impressive facts

http://apc.ucc.ie

Microbes are a part of human life, living on all the surfaces and cavities of the human body.

-Majority of these microbes are found in the gut
(but those located at other sites can also have significant roles)

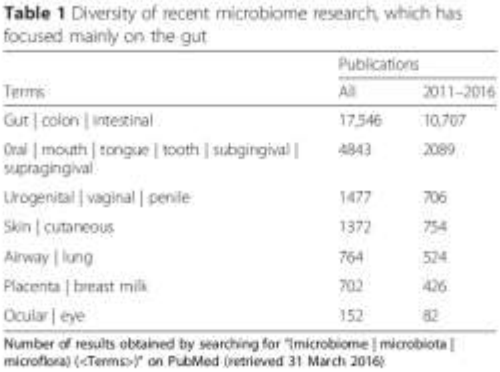


Table 1 Diversity of recent microbiome research, which has focused mainly on the gut

Terms	Publications All	 2011–2016
Gut | colon | intestinal	17,546	10,707
Oral | mouth | tongue | tooth | subgingival | supragingival	4843	2089
Urogenital | vaginal | penile	1477	706
Skin | cutaneous	1372	754
Airway | lung	764	524
Placenta | breast milk	702	426
Ocular | eye	152	82

Number of results obtained by searching for "(microbiome | microbiota | microflora) (<Terms>)" on PubMed (retrieved 31 March 2016)

Lloyd-Price et al 2016 Genome Med

Unlike our human genome, our microbial genome can change….contributing to health or disease

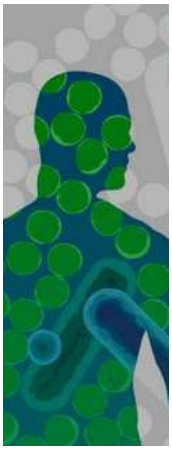
Modulation

http://apc.ucc.ie

One can use
Human studies
Animal trials
***In vitro/ex vivo* models**
to assess the ability of
Antimicrobials
Other drugs
Probiotics
Prebiotics
Faecal transplant
Diet
Exercise (?)
to positively influence the gut microbiota

Gut microbiota and health

http://apc.ucc.ie



Function

Vitamin synthesis

Digestion and absorption

Immune stimulation

Control pathogens

Metabolism of drugs

Influencing the efficacy of energy harvest from ingested food

Intestinal epithelial cell proliferation & differentiation, pH

Signaling from the periphery to the brain

Etc.

Why Culture independent approaches?

Traditional, culture-based, approaches to microbiology only reveal the 'tip of the iceberg'

The success of culture based approaches is dependent on having types of agar/culture media/growth conditions that allow all microbes to grow

Such an agar does not exist!!

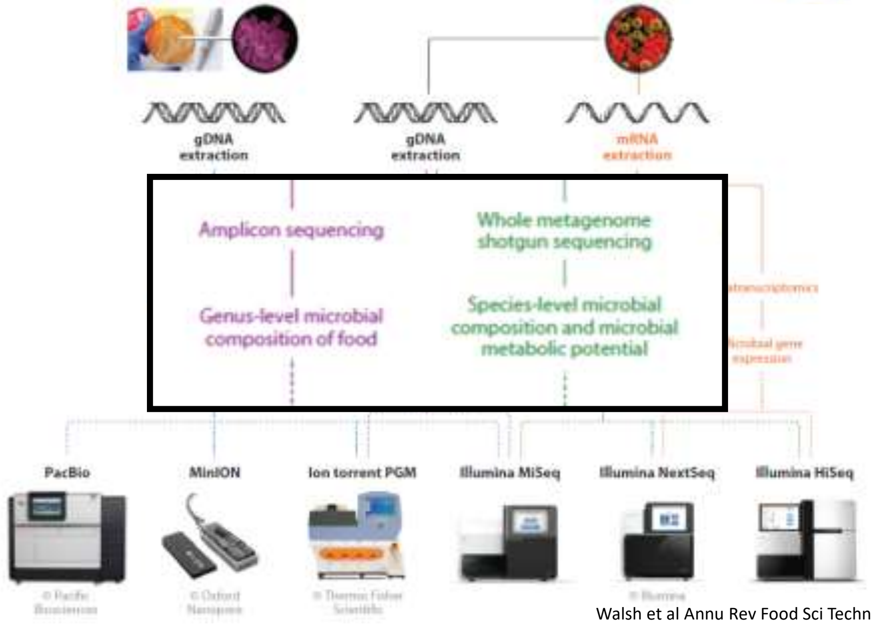
Indeed only a small % of microbes are easily cultured in the laboratory (see next slide)

DNA sequencing-based approaches can allow an analysis to the entire population regardless of whether it can be grown or not

All of the DNA from the microbes present in a particular environment = Metagenomic DNA

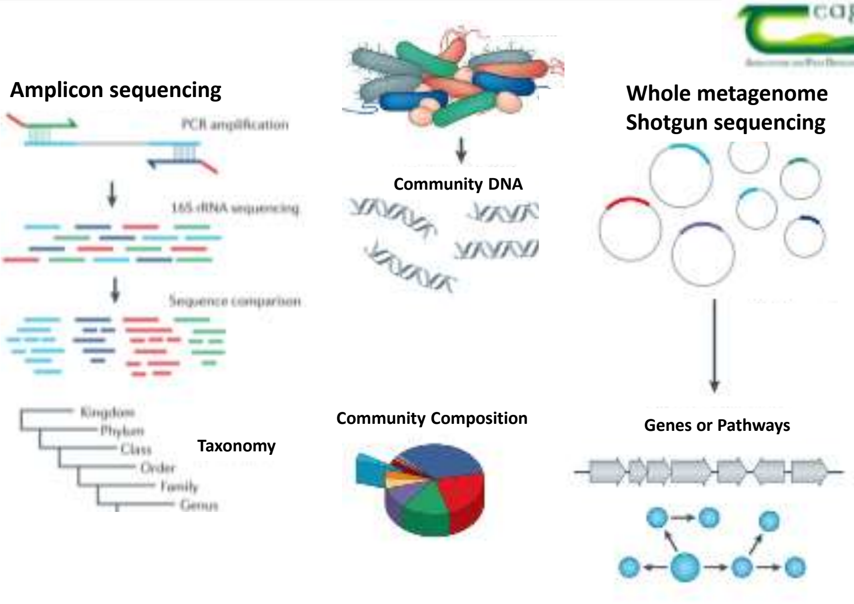
Sequencing based approaches to study microbes

http://apc.ucc.ie



Walsh et al Annu Rev Food Sci Technol 2017

DNA-based community analysis



Amplicon sequencing

Taxonomy

Community DNA

Community Composition

Whole metagenome Shotgun sequencing

Genes or Pathways

Evolution of Sequencing Platforms

http://apc.ucc.ie

1 M reads

Roche - GSFLX

- Whole-genome
- Composition

4 M

ION - PGM

- Whole-genome
- Composition
- Shotgun

15 M

Illumina - MiSeq

- Shotgun

30 M

ION - Proton

- Shotgun

130 – 400 M

Illumina - NextSeq

Oxford -MinION

Associated Infrastructure

http://apc.ucc.ie

Extracting DNA

Preparing DNA libraries

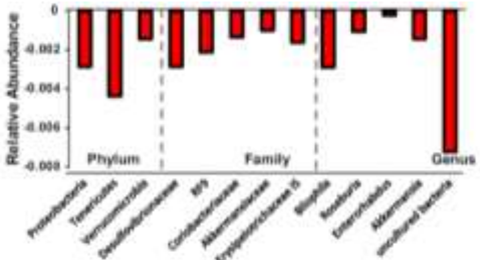
Quantifying DNA

Quality assurance

Data storage and analysis

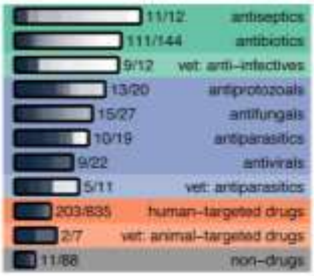
Undesirable impact of drugs on gut bacteria

http://apc.ucc.ie



Impact of rosuvastatin on the faecal microbiota

Nolan et al Am J Physiol Gastrointest Liver Physiol. 2017



A recent study screened a collection of >1000 clinically approved drugs, mostly designed for human targets, against 40 representative gut bacterial strains and demonstrated that many of them inhibited bacterial growth in vitro

Anticommensal activity of 203 (24%) human-targeted drugs

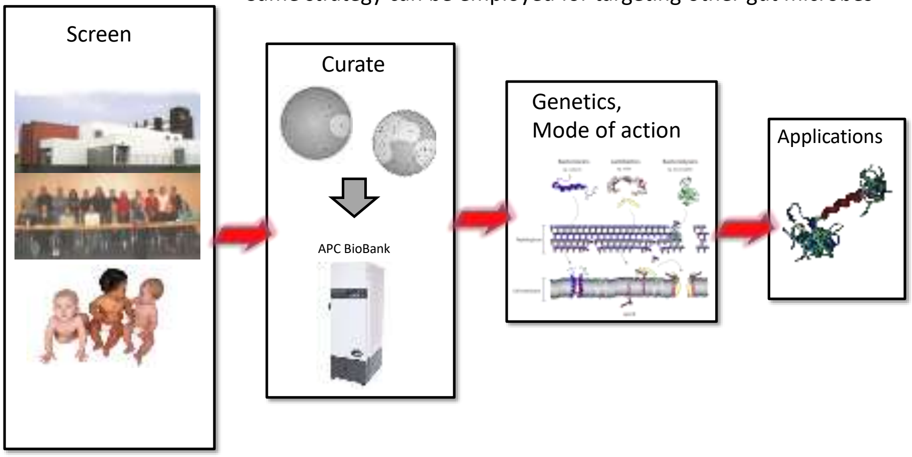
from Maier et al., 2018 Nature 2018

Narrow spectrum antimicrobials

http://apc.ucc.ie

Screening for anti *C. difficile* Antimicrobials

Same strategy can be employed for targeting other gut microbes



Screen

Curate

APC BioBank

Genetics,
Mode of action

Applications

Bacteriocins

http://apc.ucc.ie

Bacteriocins are bacterially produced, ribosomally synthesized, small, heat-stable antimicrobial peptides that are active against other bacteria and to which the producer has a specific immunity mechanism

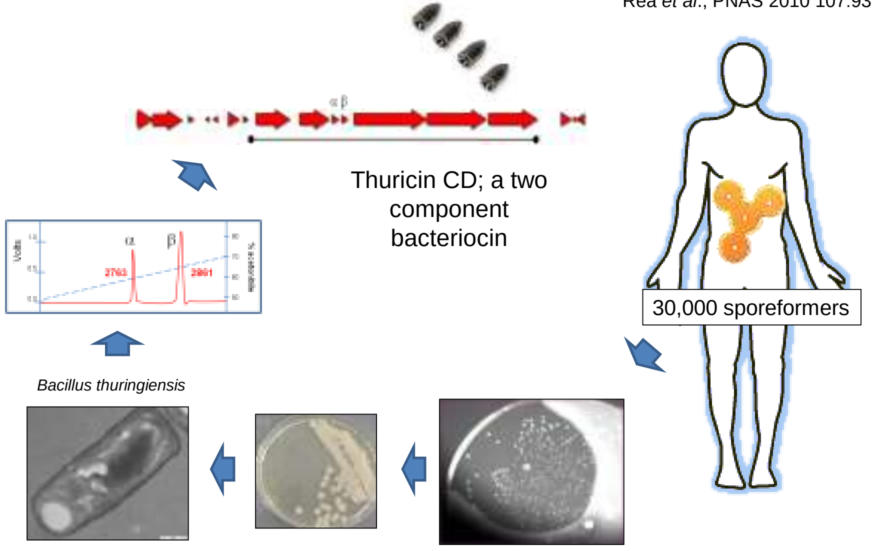
Bacteriocin production is widespread among bacteria, including gut commensals.
Can be applied in a purified form or produced in situ by probiotics

Cotter *et al*., Nature Rev Microbiol 2005 3:777

Narrow spectrum antimicrobials

http://apc.ucc.ie

Rea *et al*., PNAS 2010 107:9352



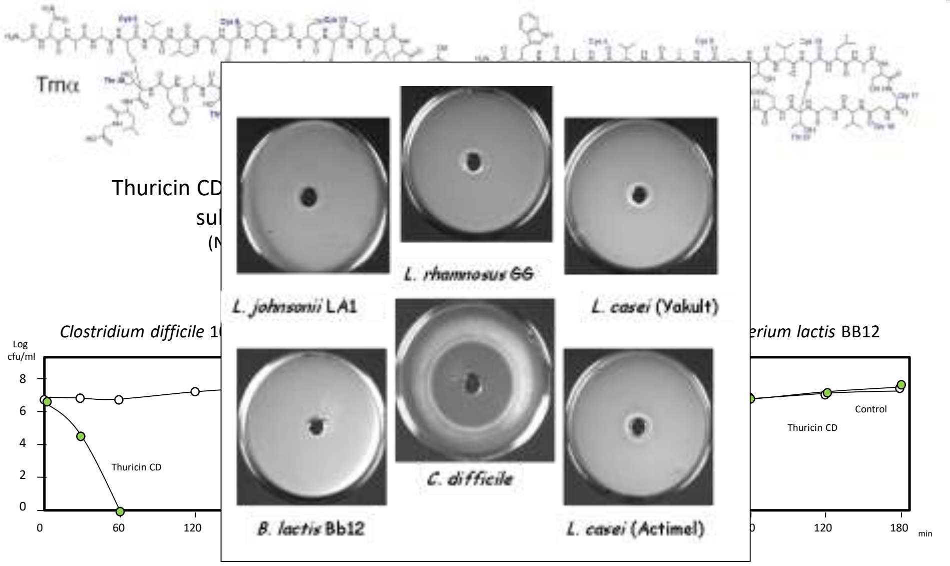
Thuricin CD; a two component bacteriocin

30,000 sporeformers

Bacillus thuringiensis

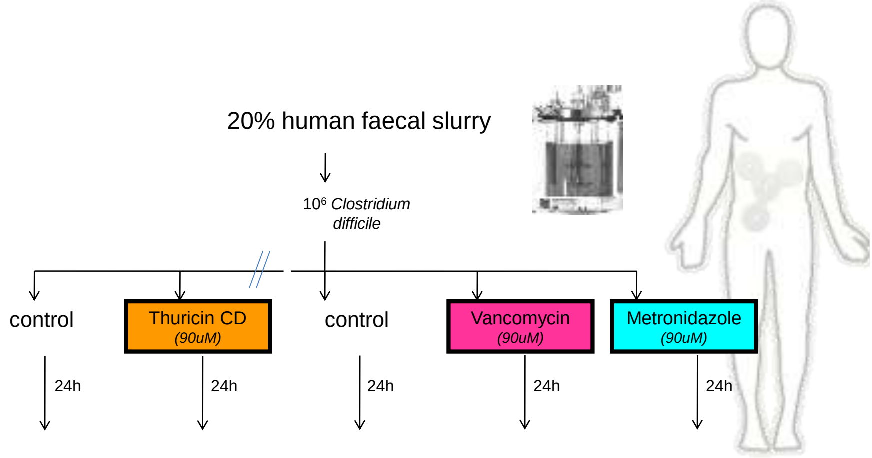
Overlaid with *Clostridium difficile*

Thuricin CD



In vitro distal colon model

http://apc.ucc.ie



20% human faecal slurry

10^6 *Clostridium difficile*

- control
- Thuricin CD *(90uM)*
- control
- Vancomycin *(90uM)*
- Metronidazole *(90uM)*

24h

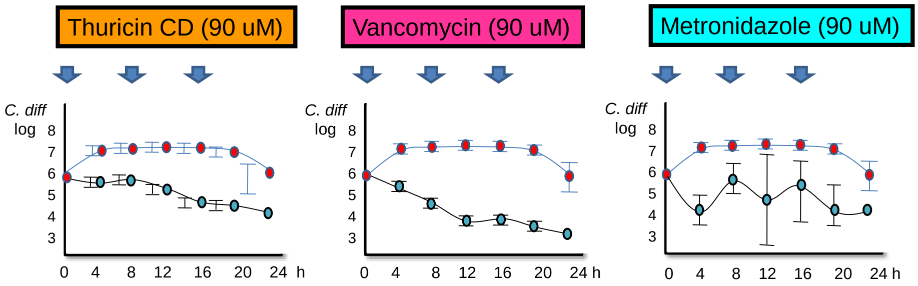
Total DNA purified, amplified V4 region of 16S rRNA sequencing, MEGAN

Rea et al. PNAS 2011 108 Suppl 1:4639

Narrow spectrum antimicrobials

http://apc.ucc.ie

Distal colon model

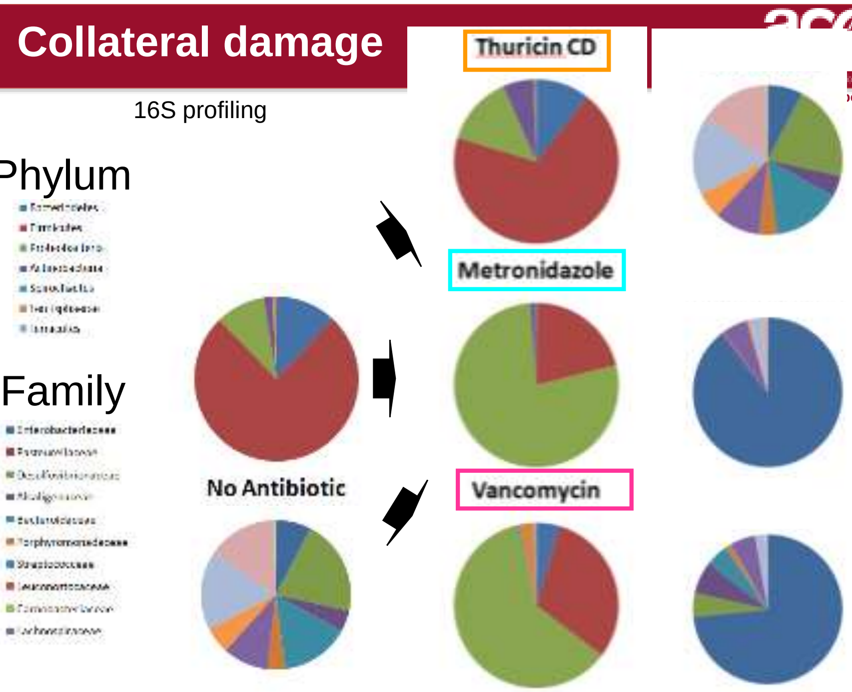


Collateral damage

16S profiling

Phylum

Family



Rea et al. PNAS 2011 108 Suppl 1:4639

Microbiota - terminology

http://apc.ucc.ie

Alpha diversity
How many types of sequences in a sample?

Beta diversity
How different types are distributed among samples?

Microbiota - diversity

http://apc.ucc.ie

Low total diversity within the gut microbiota is generally regarded as less desirable and has been observed in children that are more susceptible to allergies as well as sufferers of IBD, IBS, obesity and *C. difficile* infection (among others).

Some individuals appear to have unusually high gut microbiota diversity

Gut microbiota

ORIGINAL ARTICLE

Exercise and associated dietary extremes impact on gut microbial diversity

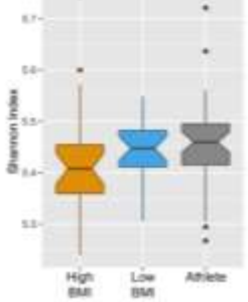
Siobhan F Clarke, Eileen F Murphy, Orla O'Sullivan, Alice J Lucey, Margaret Humphreys, Aileen Hogan, Paula Hayes, Maeve O'Reilly, Ian B Jeffery, Ruth Wood-Martin, David M Kerins, Eamonn Quigley, R Paul Ross, Paul W O'Toole, Michael G Molloy, Eanna Falvey, Fergus Shanahan, Paul D Cotter

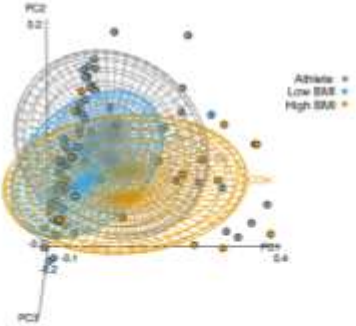
Clarke et al . Gut. 2014.
Barton et al. Gut. 2017.

Microbiota Diversity - Pathways

http://apc.ucc.ie

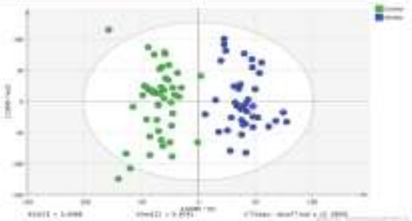
Barton et al. Gut. 2017.

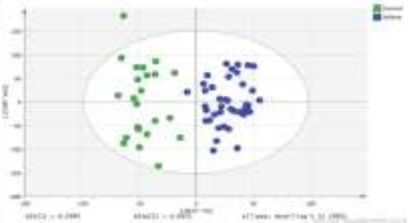




URINE NMR

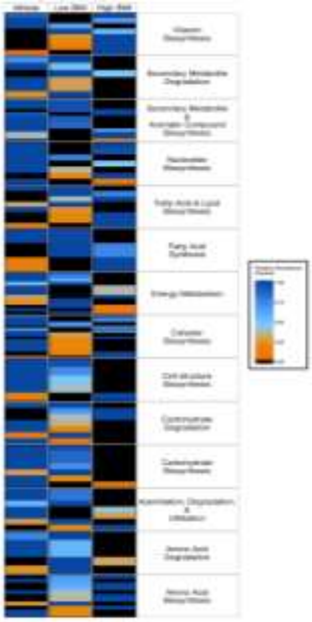
Faecal NMR





Functional Metagenomic Variation

http://apc.ucc.ie



Thousands of pathways to analyse!!!

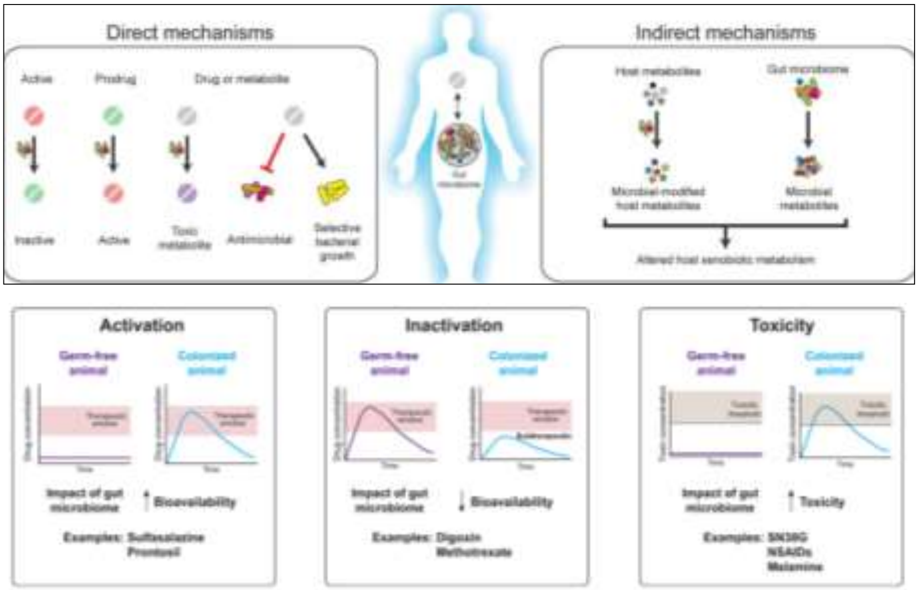
High BMI control group had the lowest average abundance scores across 31 metabolic pathway categories.

Athlete group had the highest mean abundance across 29 of the 34 metabolic categories

Barton et al. Gut. 2017.

Impacts of Gut Microbes on Drugs

http://apc.ucc.ie



From Spanogiannopoulos et al Nat Rev Microbiol 14, 273 (2016)

Drugs metabolised by Gut Microbes

http://apc.ucc.ie

Metabolism via Reduction	
5-Fluorouracil	Neoprontosil
Balsalazide	Nitrazepam
BILR 355	Nizatidine
Chloramphenicol	Olsalazine
Clonazepam	Omeprazole
Deleobuvir	Potassium [illegible]
Digoxin	Prontosil
Eltrombopag	Ranitidine
Glyceryl trinitrate	Risperidone
Indicine N-oxide	Sennosides
Levodopa	Sulfasalazine
Loperamide N-oxide	Sulfinpyrazone
Methamphetamine	Sulindac
Metronidazole	Zonisamide
Misonidazole	
Metabolism via Hydrolysis	
Azetirelin	Levamisole
Benzylpenicillin	Lovastatin
Calcitonin	Methotrexate
Chloramphenicol	Morphine 6-glucuronide
Diclofenac glucuronide	Phenacetin
Glycyrrhizin	Sennosides
Indomethacin glucuronide	SN-38G
Insulin	Sodium picosulfate
Isosorbide dinitrate	Sorivudine
Ketoprofen glucuronide	Succinyl sulfathiazole
Metabolism via Acylation	
5-Aminosalicylic acid	
Sulfapyridine	
Metabolism via Oxidation	
Flucytosine	

Oxford -MinION

From Spanogiannopoulos et al Nat Rev Microbiol 14, 273 (2016)

Summary

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- Our understanding of the composition and function of the gut microbiota has increased dramatically thanks to high throughput DNA sequencing
- In parallel, the role of the gut microbiota in health and disease has become clearer
- The gut microbiota can be negatively impacted on by a wide variety of drugs
- Gut microbes can also activate/inactivate different drugs
- Potential to sequence an individual's gut microbiota to assess its likely impact on a drug being considered for use
- Potential to enhance efficacy of a drug through co-administration with an antibiotic or biotherapeutic strain (probiotic)

Acknowledgements

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Food, Gut, Rumen, Exercise, Mycobiome, Probiotics, Bacteriocins

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Gwynneth Halley
Laura Coughlan
Ronan Whiston
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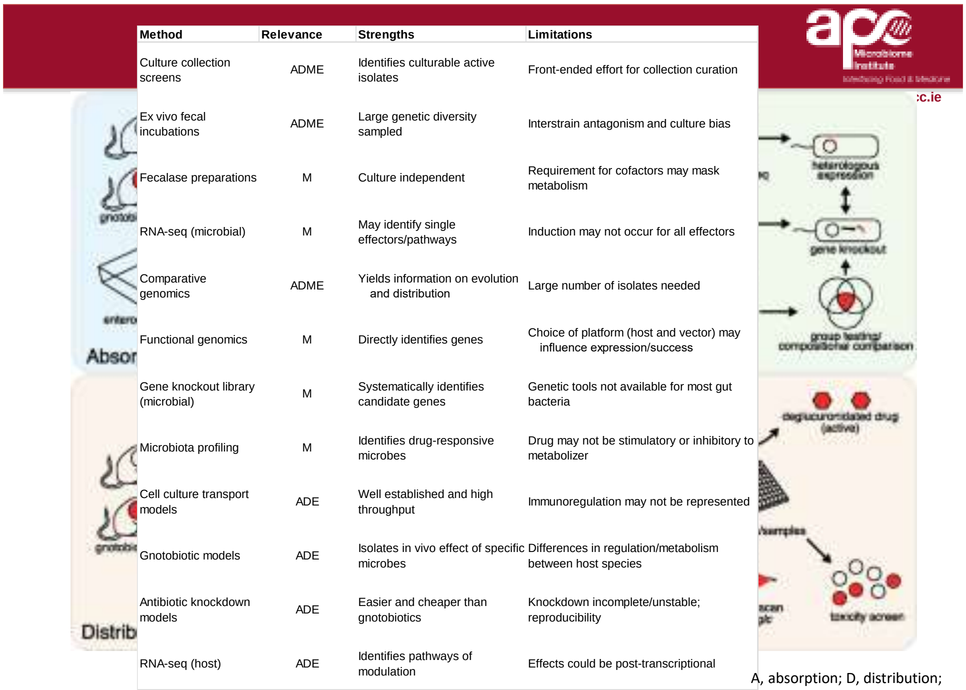
Ruben Miranda
Calum Walsh



Method	Relevance	Strengths	Limitations
Culture collection screens	ADME	Identifies culturable active isolates	Front-ended effort for collection curation
Ex vivo fecal incubations	ADME	Large genetic diversity sampled	Interstrain antagonism and culture bias
Fecalase preparations	M	Culture independent	Requirement for cofactors may mask metabolism
RNA-seq (microbial)	M	May identify single effectors/pathways	Induction may not occur for all effectors
Comparative genomics	ADME	Yields information on evolution and distribution	Large number of isolates needed
Functional genomics	M	Directly identifies genes	Choice of platform (host and vector) may influence expression/success
Gene knockout library (microbial)	M	Systematically identifies candidate genes	Genetic tools not available for most gut bacteria
Microbiota profiling	M	Identifies drug-responsive microbes	Drug may not be stimulatory or inhibitory to metabolizer
Cell culture transport models	ADE	Well established and high throughput	Immunoregulation may not be represented
Gnotobiotic models	ADE	Isolates in vivo effect of specific microbes	Differences in regulation/metabolism between host species
Antibiotic knockdown models	ADE	Easier and cheaper than gnotobiotics	Knockdown incomplete/unstable; reproducibility
RNA-seq (host)	ADE	Identifies pathways of modulation	Effects could be post-transcriptional

A, absorption; D, distribution; M, metabolism; E, excretion

Bisanz et al. Drug Metab Dispos. 2018 Nov;46(11):1588-1595

Things to consider!

http://apc.ucc.ie

- What biological material are you collecting? Faeces?
- How should it be stored?
- What DNA extraction protocols should be employed?
- What DNA sequencing platform should be used?
- What bioinformatic pipeline should be used?

16S rRNA analysis – Taxonomic Assignment

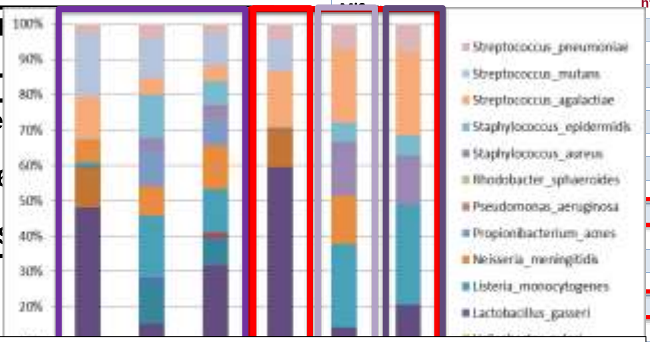
http://apc.ucc.ie

Defined microl
(defined ratios

Different e

Different 16

MiS

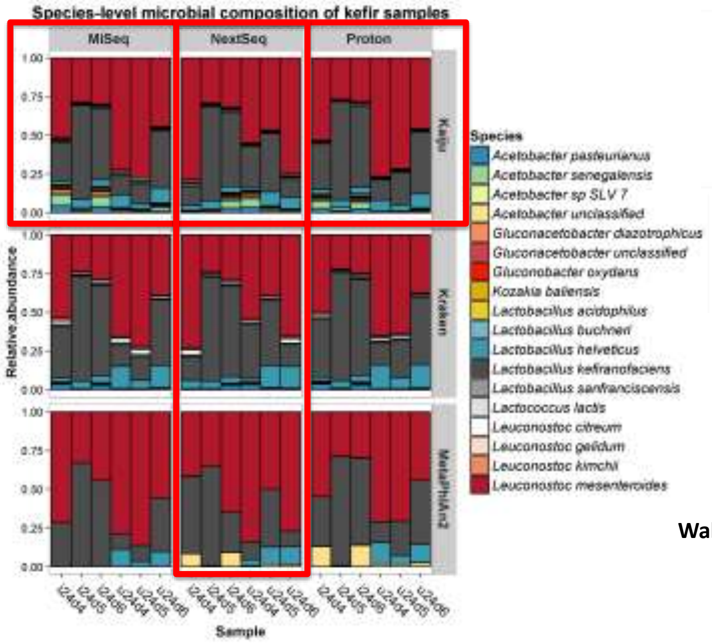


	Expected	No. detected
		16
		16
		15
		13
		14
		14
		16
		14
		11
		13
		11
		20
		20
		20
		20
		17
		17
		17
		17
		18
TdeglonQiagenGlycerol	21	19
TdeglonRBBGlycerol	21	20
TdeglonRBBPBS	21	18

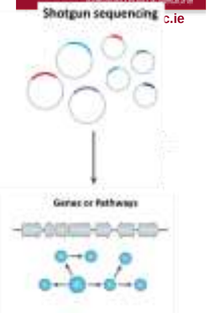
Bottom line:
Care needs to be taken when standardising protocols re. treatment of samples, extraction, polymerase, PCR primers, cycling conditions and platform
(and when comparing results from other labs)

Fouhy et al BMC Microbiology
Cooney et al PLoS One

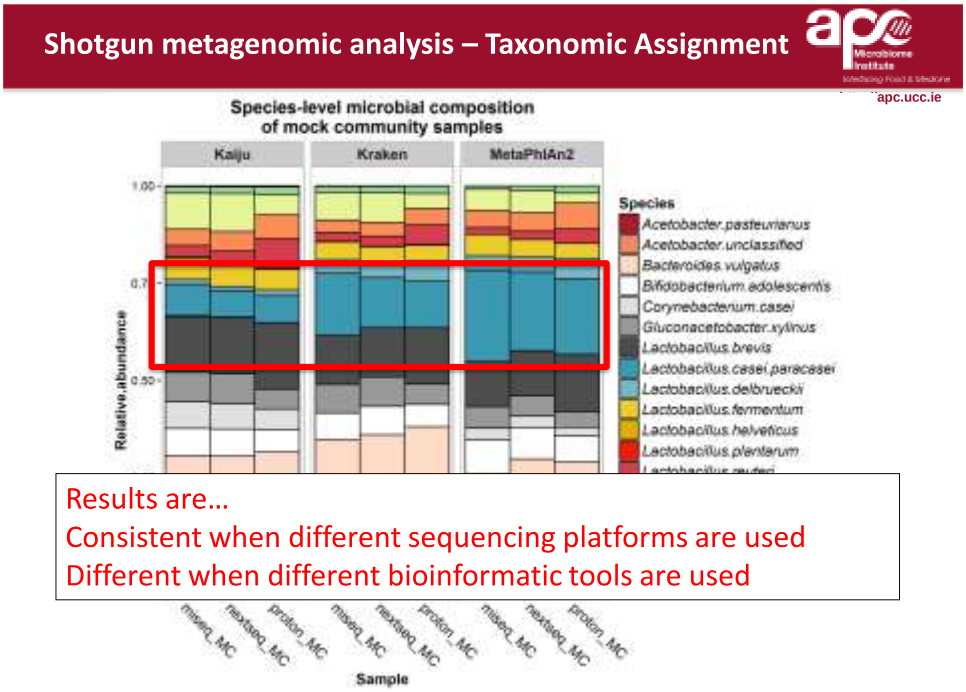
Shotgun metagenomic analysis – Taxonomic Assignment



c.ie



Walsh et al In prep.



Shotgun metagenomic analysis – Taxonomic Assignment

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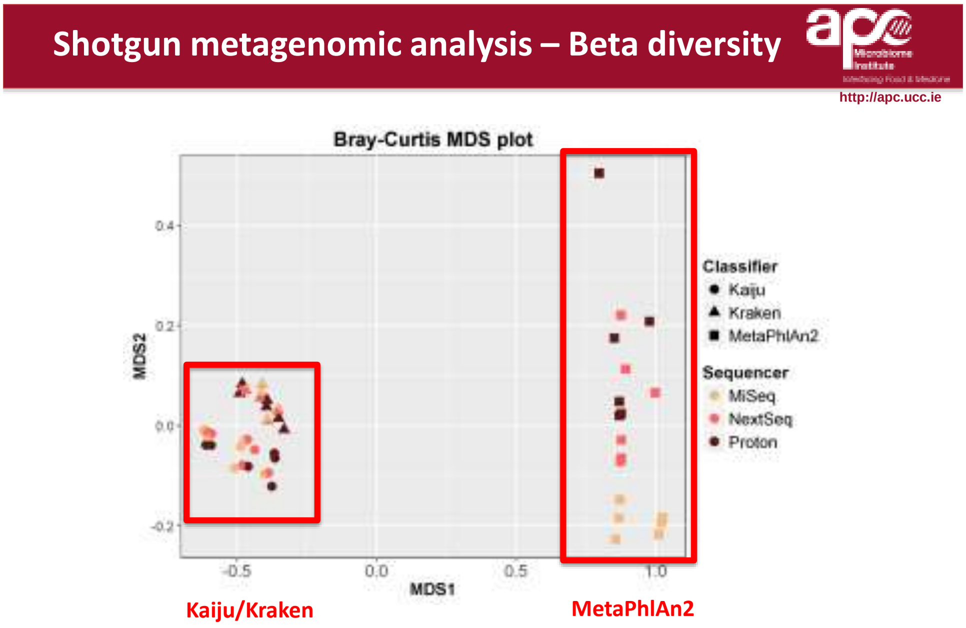
Results are…
Consistent when different sequencing platforms are used
Different when different bioinformatic tools are used



Shotgun metagenomic analysis – Beta diversity

http://apc.ucc.ie

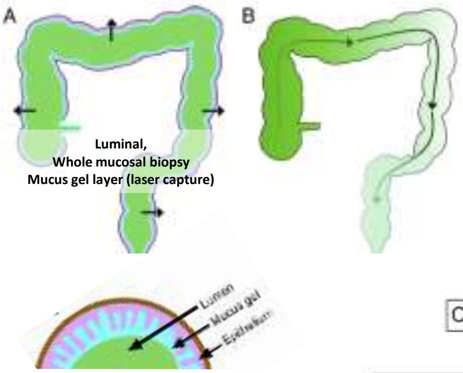
Kaiju/Kraken **MetaPhlAn2**

Sample site: Ulcerative colitis vs Controls

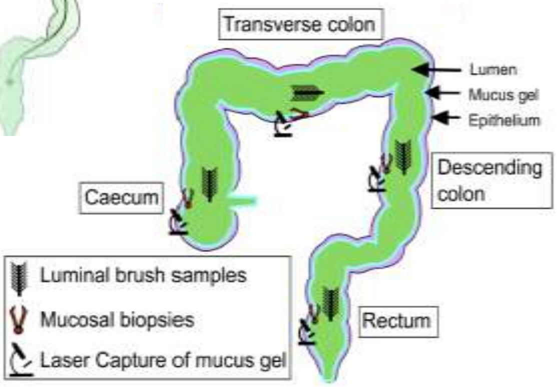
http://apc.ucc.ie

Cross sectional gradients

Longitudinal gradients



Luminal,
Whole mucosal biopsy
Mucus gel layer (laser capture)



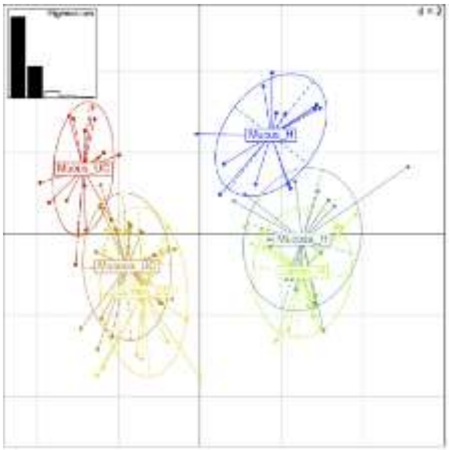
Sampling – 4 colorectal levels x 3 layers

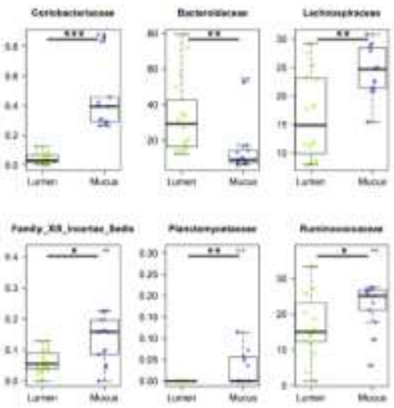
Lavelle et al Gut 2015

Differences in Microbiota depending on sample site

http://apc.ucc.ie

UC (surgical colectomy due to medically refractory UC) vs Controls (routine colonoscopy)
Sequencing revealed differences between mucus gel and luminal microbiota





A between-class analysis (BCA) based on a principal component analysis of Hellinger-transformed family-level taxon abundance, comparing the luminal, mucosal and mucus gel microbiota of controls and UC.

Summary

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- Characterisation of the gut microbiota can involve gut dependent and/or independent approaches
- DNA sequencing can contribute through genomics, metagenomics and metatranscriptomics
- Metagenomics typically involve amplicon or shotgun based approaches
- Outputs focus on assignment of taxonomy, functional potential or a determination of diversity
- The outputs from these analyses depend on a number of factors including bioinformatics pipelines used and sampling site

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Fergus Shanahan

Microbiome research in Cork

http://apc.ucc.ie

Teagasc is the Agriculture and Food Development Authority

- Research & Innovation
- Farm advisory
- Education

SFI Centre Focussed on microbiome research
~300 researchers across Teagasc and UCC

Directly funded projects

http://apc.ucc.ie

Extremes of life

Health vs Disease

Dietary & other interventions

Food products

Food chain

Animal Health

Environment

- **Athletes vs Non-athletes; Exercise** (Orla O'Sullivan SIRG, APC, IRFU, ISI)
- **Obese vs Lean** (SFI PI Obesibiotics; UCD; Brunel Univ London)
- **Mycobiome** (APC – innovation platform)
- **Mother-infant** (SFI – New APC Spoke - Microbe Mom)
- **Vaginal microbiome** (UCD – Pre-term birth)
- **Impact of specific diets** (DAFM – NIHAM Foods; RMIS – LIFE Microbiota; Whey)
- **Impact of fermented foods on the gut** (Univ Edmonton; John Cryan)
- **Microbiota of fermented foods** (Irish industry, APC; Fermented Healthy Beverages)
- **Dairy powders** (DAFM - SACCP, ThermodurOut, Glanbia, Dairygold and others)
- **Identification of new spoilage agents** (Pinking, numerous companies including in US)
- **Production and processing facilities** (Dairybiota)

Collaborations

http://apc.ucc.ie

Extremes of life

Health vs Disease

Dietary & other interventions

Food products

Food chain

Animal Health

Environment

- **Pigs** (Feed Conversion Efficiency; Peadar Lawlor, Orla O'Sullivan, Biomin [Austria])
- **Cow rumen** (Nitrogen utilisation; Super-shedding, Mycobacterium)
- **Mystery diseases in horses** (Irish Equine Centre)
- **Plant/soil microbiome** (IT Carlow, University of Limerick)
- **Poultry microbiome** (Spain)
- **Cystic fibrosis in children** (lung; Trinity, Tallaght)
- **Bladder disease** (Niall Hyland, UCC)
- **Impact of statins** (Cormac Gahan, UCC)
- **Short bowel syndrome** (Australia)
- **IBS, IBD, Appendicitis, Immune system knock outs** etc (APC, Trinity, Sweden, AgResearch)

Culturomics

http://apc.ucc.ie

Culture based approaches can be improved through use of:

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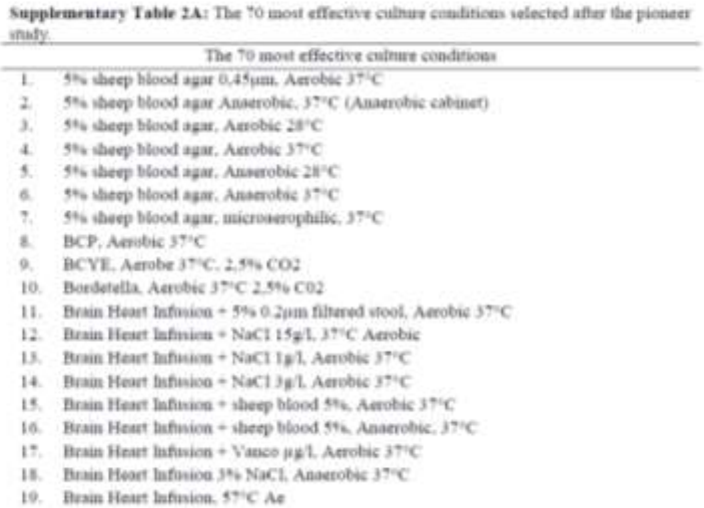


Supplementary Table 2A: The 70 most effective culture conditions selected after the pioneer study.

	The 70 most effective culture conditions
1.	5% sheep blood agar 0.45µm, Aerobic 37°C
2.	5% sheep blood agar Anaerobic, 37°C (Anaerobic cabinet)
3.	5% sheep blood agar, Aerobic 28°C
4.	5% sheep blood agar, Aerobic 37°C
5.	5% sheep blood agar, Anaerobic 28°C
6.	5% sheep blood agar, Anaerobic 37°C
7.	5% sheep blood agar, microaerophilic, 37°C
8.	BCP, Aerobic 37°C
9.	BCYE, Aerobe 37°C, 2,5% CO2
10.	Bordetella, Aerobic 37°C 2,5% C02
11.	Brain Heart Infusion + 5% 0.2µm filtered stool, Aerobic 37°C
12.	Brain Heart Infusion + NaCl 15g/l, 37°C Aerobic
13.	Brain Heart Infusion + NaCl 1g/l, Aerobic 37°C
14.	Brain Heart Infusion + NaCl 3g/l, Aerobic 37°C
15.	Brain Heart Infusion + sheep blood 5%, Aerobic 37°C
16.	Brain Heart Infusion + sheep blood 5%, Anaerobic, 37°C
17.	Brain Heart Infusion + Vanco µg/l, Aerobic 37°C
18.	Brain Heart Infusion 3% NaCl, Anaerobic 37°C
19.	Brain Heart Infusion, 57°C Ae

Targeting of components other than bacteria (e.g. fungi)

Lagier et al. Nature Microbiology 1: 16203 (2016); Huseyin et al. Front. Micro. 2017 and others

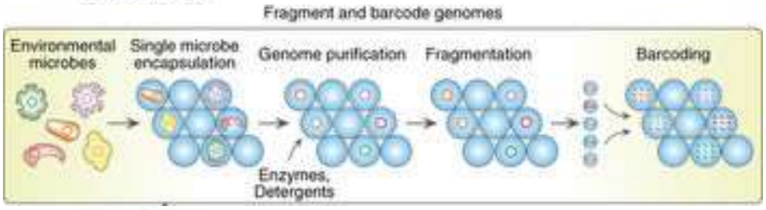
Characterisation

http://apc.ucc.ie

Once you do successfully culture microbes……

Culture independent approaches to study microbes

http://apc.ucc.ie



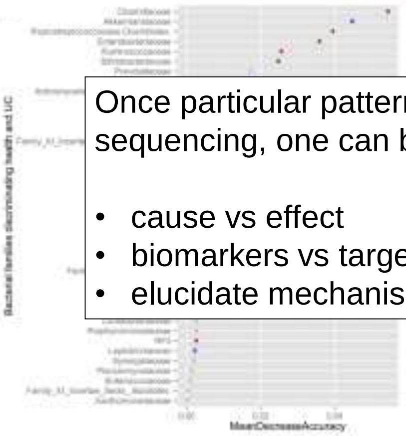
Lan et al Nature Biotechnology 2017

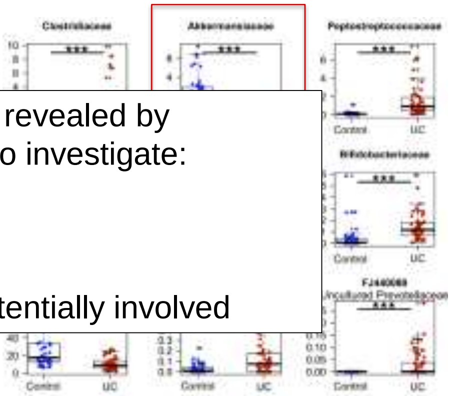
Walsh et al Annu Rev Food Sci Technol 2017

Ulcerative colitis vs Controls

http://apc.ucc.ie

Sequencing also revealed differences between UC and Controls





Once particular patterns are revealed by sequencing, one can begin to investigate:

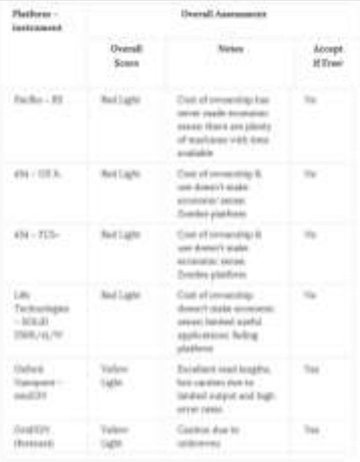
- cause vs effect
- biomarkers vs targets
- elucidate mechanisms potentially involved

Boxplots of the relative abundances of the nine bacterial families that were both discriminatory (with a mean decrease in accuracy of the classifier of greater than 0.01 when removed from the analysis) and significantly different in terms of abundance by the Wilcoxon rank test after a Bonferroni correction.

Results from the Random Forests classifier, demonstrating bacterial families that are most discriminatory between the two cohorts in descending order. Samples are coloured by whether they are significantly increased in controls (blue), UC (red) or not (grey) using the Wilcoxon rank test with a Bonferroni correction for multiple comparisons.

Lavelle et al Gut 2015

Sequencing Platforms



Longer reads; but not so many

Short reads; but very many of them

http://www.molecularecologist.com