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<td><strong>Author(s)</strong></td>
<td>Chia, Aletheia Zhi Hui</td>
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<td><strong>Citation</strong></td>
<td>Chia, A. Z. H. (2016, March). Culturing the Unculturable: A DIY, high-throughput system for the culturing, isolation and characterisation of bacterial microbiomes. Presented at Discover URECA @ NTU poster exhibition and competition, Nanyang Technological University, Singapore.</td>
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<td><strong>Date</strong></td>
<td>2016</td>
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<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10220/41625">http://hdl.handle.net/10220/41625</a></td>
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Culturing the Unculturable: A DIY, high-throughput system for the culturing, isolation and characterisation of bacterial microbiomes

INTRODUCTION
Recent advances in genomic analysis have enabled an explosion in microbiomics, leading to new insights into the millions of bacteria we carry around and their impact on human health and disease. However, current culture-independent methods (16s rRNA, metagenomics) are limited, and a true understanding of these bacterial ecosystems would require phenotypic studies; i.e. culturing. E.g.: • Genome – Phenome correlation to understand pathogenesis • Proteomics & Metabolomics for drug/antibiotic discovery • Constituting a minimal microbiome for Faecal transplantation But: 99.9% of bacteria have not been cultured; labor & time intensive

Approach to culturing ‘unculturables’
Nutrients and conditions • Commercial agars: limited number, designed for known bacteria • Reviews of strategies (Steward 2012, Vartoukian et al 2010), include filtration, simulated environments, long incubation times and co-cultures Sources • Microbiome studies have largely been of the human gut Growth kinetics • Culture selects for fast-growing bacteria, overgrowing other strains • Existing strategies: dilution down to single cells + long incubations

AIM A low-cost, automated system that enables the culture of previously uncultured bacteria and their subsequent isolation and characterisation

APPROACH

DIY AUTOMATION
Pipetting system: serial dilution, pipette onto plate • Magnetic beads + shaker: spreading of bacteria

PRELIMINARY RESULTS
3mm stainless steel beads: • Used as automated alternative to L-spreaders • Shaking plate with beads for 2 minutes gives evenly-spread colonies, with numbers comparable to manual spreading

Figures 2-4: Scanned images of a mixed specimen grown on TSA plates at 24, 30, 36 hrs

Fig. 1: Schematic of Scanner-incubator interface

Fig. 5: Photo of preliminary system and remotely-accessed image

Fig. 6: Raw image (left), outlines of colonies highlighted in red (right)

CellProfiler: an open-source software for the visual analysis of cell assays; adapting for colony identification and characterization

FUTURE PLANS
System development: full automation • Integration with labmates’ DIY machines (e.g. automated pipette) • Validation: vs standard methods Discovery of previously unculturable bacteria • Characterisation of the oral / faecal microbiome, compare with genotyping • Build up library of phenotypes and growth dynamics of known bacteria • Further exploration of methods to elicit previously unculturable bacteria (e.g innovative agar, co-cultures)