

## Analyzing the oral metagenome

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The microflora of the oral cavity is one of the most diverse in the human body with greater than 800 bacterial species estimated to exist there. The oral cavity consists of distinct ecological niches (such as the surface of the tongue or the dental plaque) that harbour different bacterial species. Approximately half of oral species can not be cultured and, therefore, molecular approaches will be used to investigate this community.

Bacteria adhere to both oral surfaces and to each other to form biofilms. Bacteria will generally exist in equilibrium with the human host; however, some organisms are opportunistic and may cause disease. An understanding of adhesion mechanisms will provide insight into how such bacteria attach to human tissues and cause oral disease. A comprehensive metagenomic study of the oral microflora is being used to investigate the diversity and adhesion mechanisms of this unique bacterial community.

Previous studies of the oral metagenome have reported that up to 60% of clones in a BAC library contain human DNA. Therefore preferential isolation of prokaryotic DNA was desirable for the construction of an oral metagenomic library. High quality DNA is required for the production of metagenomic libraries and, therefore, a range of DNA extraction methods have been investigated. Differential centrifugation and selective lysis of human cells have been investigated as methods of removing human DNA from samples prior to library production. Field inversion gel electrophoresis and real-time PCR have been used to assess the molecular weight of DNA and quantity of human DNA respectively. The final sampling method, a combination of the two methods, was chosen accordingly. Whole genome amplification will be used to produce enough DNA for the production of metagenomic libraries.

Novel adhesins of the oral microbial community will be identified using phage display libraries. Phage display libraries are constructed by inserting DNA fragments into a bacteriophage host, which allows the bacterial peptide to be expressed on the surface of the phage particle. The library can be screened for peptides and protein domains that adhere to known ligands, which can be directly linked to the DNA sequence. Optimisation of methods for the production of fully representative phage display libraries have been, and will be further, investigated. Fosmid libraries will be used to complement phage display to obtain complete gene sequences and determine the species responsible for adhesion mechanisms identified.