

## CALCULATING THE TOTAL MICROBIAL DIVERSITY IN AN INDIVIDUAL HUMAN GUT

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**Introduction:** Sequencing of 16S rRNA genes is a well established method for quantifying the diversity of a microbial community. Diversity is measured as the number of clusters or operational taxonomic units (OTUs) at a given level of sequence dissimilarity. 3% OTUs are often taken as approximating species. The advent of next generation sequencing technologies, principally 454 pyrosequencing, has allowed much larger sample sizes than were possible using clone based methods. It is now feasible to completely characterise the gut microbiome of an individual, and determine the total diversity. However, with these very large samples it is critically important to remove both sequencing and PCR noise that will inflate diversity estimates (Quince et al. 2009). We applied novel noise removal algorithms to two individual data sets, each with over 500,000 pyrosequenced reads, determined OTU number, and extrapolated to the true diversity.

**Materials and Methods:** Microbial DNA was extracted from the faecal material of two individuals, and the V2 region of the 16S rRNA gene amplified and pyrosequenced. We also sequenced mixtures of known organisms or 'mock communities'. We used these mock communities to validate the noise removal algorithms and total diversity estimators.

**Results and Discussion:** We were able to accurately reconstruct the known diversity in the mock communities. For the real communities we were able to show that the majority of diversity was captured and that total OTU numbers are actually relatively low – just hundreds of OTUs – an order of magnitude smaller than was previously estimated from smaller noisy samples.

Quince, C., Lanzen, A., Curtis, T.P., Davenport, R., Hall, N., Head, I.M., Read, L.F. and Sloan, W.T. (2009). Accurate determination of microbial diversity from 454 pyrosequencing data. *Nature Methods* **6** (9), 639-641.