

# Metagenomics Service

## Full Length 16S Bacterial Species Identification

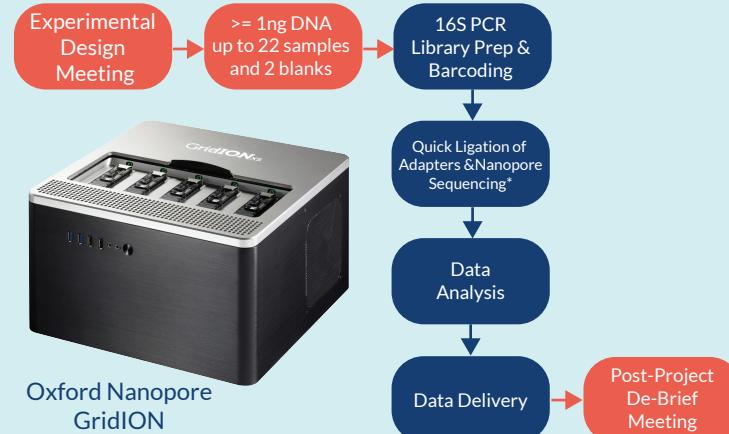
### with Oxford Nanopore Technology

16S based species identification in the past has typically relied upon PCR and subsequent sequencing of variable regions 3 and 4 in the 16S gene only. Advances in sequencing technology means that we can offer species identification using full length 16S amplicons using Oxford Nanopore long read sequencing technology. We have a GridION in house allowing us to run up to 120 samples simultaneously.

We can sequence with as little as 1ng of input DNA. To ensure the most robust analysis we recommend both an extraction and PCR reagent blank.

You will receive your raw sequencing data, which will be approximately 160,000 reads per sample based on 12 samples. We also perform basic bioinformatics analysis, including demultiplexing, correction, and a composition report of your sample.

## Our Nanopore Workflow



\*The only PCR reaction in this protocol is the 16S amplification. Adaptors are ligated directly to these products eliminating a potential confounding step usually present in illumina library preparation

## Nanopore Training

Want to learn how to get started with Nanopore?  
Want to understand how the sequencing, sample prep and analysis works?

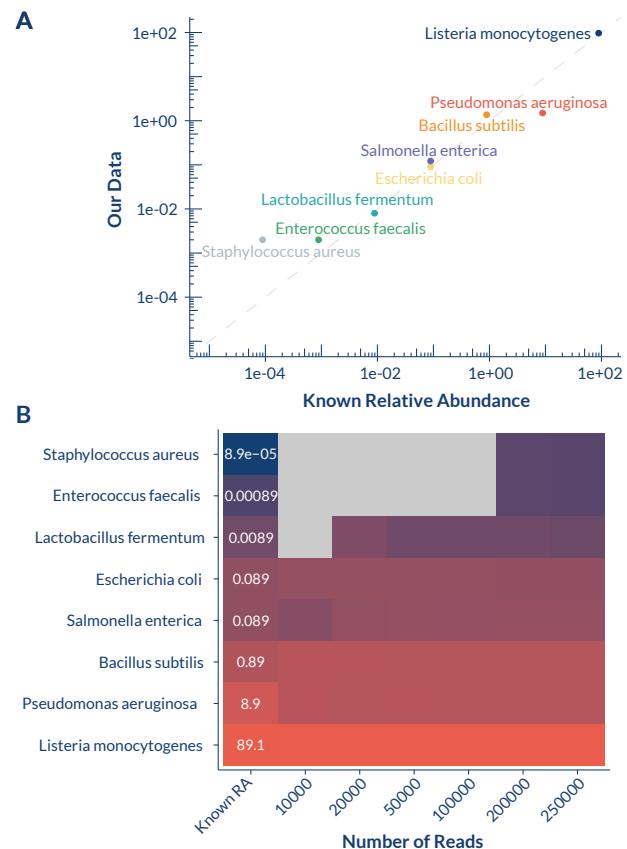
### Sign-Up to one of our training courses!

Hands on lab work and bioinformatics in one day. We have four MinIONs to use during training so in your groups you can load and sequence a standard or your own 16S sample



## Our Performance<sup>†</sup>

ZymoBIOMICS™ Microbial Community DNA Standard II (Log Distribution) is a mixture of genomic DNA of eight bacterial and two fungal strains. The DNA from each strain was quantified before pooling. After mixing, the microbial composition was confirmed using Illumina sequencing. In Figure 1A we show that we can recapitulate the true contribution of each bacterial species with 5ng of input DNA with 200,000 reads. To determine limits of detection we downsampled the number of reads (Figure 1B).



**Figure 1.** The predicted bacterial composition of the Zymo microbial community log standard using Nanopore sequencing compared to known relative abundance. **A.** Relative abundance calculated using 200,000 reads from 5ng of input material. **B.** Reads were downsampled to assess the performance at 10,20,50,100, 200 and 250 thousand reads. Grey indicates undetectable. I.e. at 10,000 reads 0.089% is the limit of detection

## £80 Per Sample\*

Including basic bioinformatic analysis.

We guarantee 2 million reads per flow cell.

Roughly 160,000 reads per sample based on 12 per flow cell with a 16S PCR blank/reagent background. With 24 samples this equates to approximately 80,000 reads per sample. Number of reads per sample varies based on input amount and quality.

\* based on barcoding 24 samples and excluding VAT (med school researchers pay no VAT)

Email us for more information  
[bioinformatics-core@sheffield.ac.uk](mailto:bioinformatics-core@sheffield.ac.uk)

<sup>†</sup> For this experiment 1 MinION flow cell was utilised. 1ng, 5ng and 10ng of the Zymo mock community log standard were prepared. These samples were subject to 16S PCR and library preparation separately using barcodes 1-3. Samples were pooled at as close to equimolar quantities as possible. From a roughly 24hr run 4.5million reads were generated. To determine how washing and reloading of the flow cell performed we subsequently re-prepared 1,5 and 10ng of the Zymo standard and again subject them to separate PCR and library prep with barcodes 4-6. After another 24 hours the flow cell generated a further 4.2 million reads. Reads were demultiplexed with porechop, using the settings '--barcode\_threshold 90 --discard\_middle\_middle\_threshold 75' to ensure cross-over is prevented and chimeric reads removed. Reads were mapped with minimap2 '-ax map-on' to the bacterial reference sequences present in the standard, provided by the manufacturer, re-capitulating the analysis performed by Zymo. Reads with a quality <=30 were excluded. Figure displays data from barcode 4 - 1ng input.