**Running Title: cONTsensus: A comprehensive pipeline to 16S subunits bacterial identification with Oxford Nanopore sequencing**

José M. Lázaro-Guevara1,2,3, Stefano M. Marino\*, and Adrian Odriozola3\*

1 Department of Botany and Biodiversity Research Centre, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

2 Facultad de ingeniería, Universidad de San Carlos de Guatemala, Guatemala,10012.

3 Univesidad del Pais Vasco

**Introduction**

The study of microbial communities through nucleotide sequencing has become an essential asset in environmental science, and is rapidly gaining popularity in clinical applications like high performance sports medicine; one technology holds great promises for its application on these scenarios due to its rapid and cost-effective processing times is the Oxford Nanopore Technologies (ONT) MinION sequencer is a portable and affordable device, that produces long reads, with a remarkable sequencing output (in terms of bases/hour). One of the most common approaches in microbiological investigations through sequencing is the analysis of the 16S rRNA gene, known as 16S metabarcoding. Only recently the application of MinION has extended to 16S metabarcoding; to date, a limitation is still represented by the available computational protocols: due to the intrinsic, unique features of the technology ONT long reads cannot be adequately analyzed with tools developed for previous technologies (e.g. for Illumina), and the existent protocols (e.g. EPI2ME) do not allow customization as needed for specific scientific endeavors, where the optimal parameters to obtain the desired results are a blurry line between an educated guess and a hit of luck. In this work a comprehensive and totally customizable computational pipeline, specifically tailored to the usage of ONT reads in 16S metabarcoding, is developed, assessed, and discussed. This study is particularly addressed to obtain the best possible outcome of multiple genomic (16S subunit) databases, up to date ONT workflow and permutation techniques that aims to obtain consensus results, equivalent to multiple individual experiments imbibed in one functional pipeline, were efficient usage of resources are particularly important.

**Key Words**: ONT; 16S Subunit, cONTsensus, oxford nanopore.