Barcode of life: simple laboratory and analysis workflows for 16S and CO1 analysis

Genus- and species-level identification by 16S or CO1 analysis made easy using a rapid laboratory protocol for adapter attachment and new data-analysis workflow

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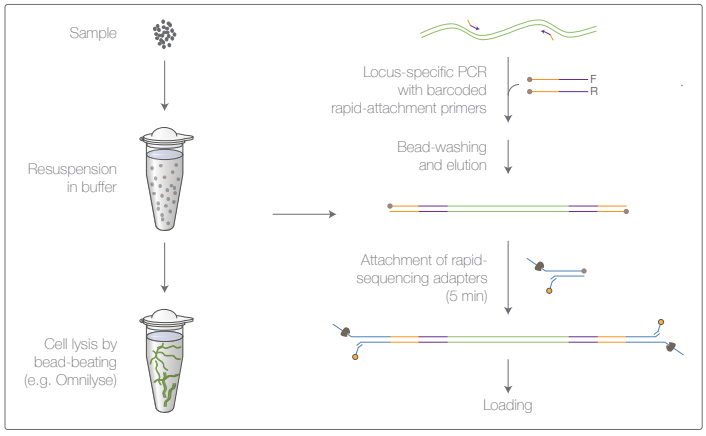

Fig. 1 Laboratory workflow for barcoded 16S and CO1 sequencing

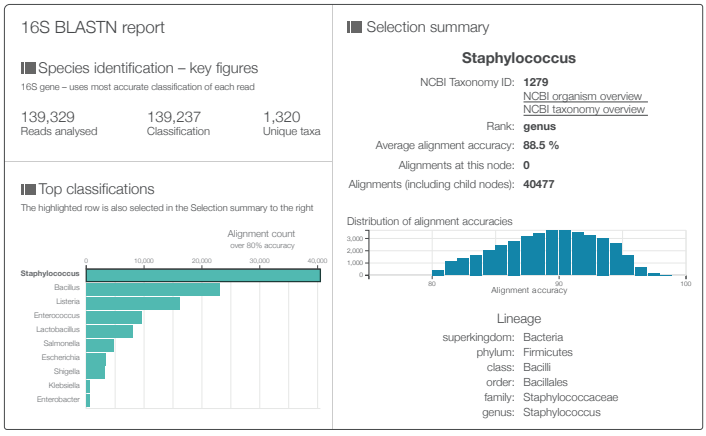

Fig. 2 Analysis report for species identification, shown here for Staphylococcus 16S

Locus-specific PCR coupled with rapid adapter attachment for 16S and CO1

It is often desirable to be able to identify the species present in a complex mixture. This can be achieved by amplifying the bacterial 16S or mammalian cytochrome oxidase (CO1) loci, and comparing the results with a reference database. PCR amplification of specific loci can allow enrichment of the target region in the presence of a large background of other organisms. By modifying the 5' ends of standard PCR primers used for amplification of these loci we have developed a protocol that attaches our sequencing adapters to the amplicons in approximately 5 minutes, enabling more rapid species identification (Fig. 1).

Analysis workflow and report simplifies amplicon-based species identification

In the 16S analysis workflow, reads are compared to the NCBI 16S bacterial database using the Basic Local Alignment Search Tool (BLAST), immediately after each read has been basecalled. To validate the workflow, we prepared 1D 16S libraries by PCR amplification of the ZymoBIOMICS Microbial Community DNA Standard, sequenced the libraries on MinION™ flowcells and passed the basecalled results through the analysis workflow (Fig. 2). We were able to classify all eight bacteria in the mock community to genus level. We calculated the precision at genus level to be 99% for 1D data.

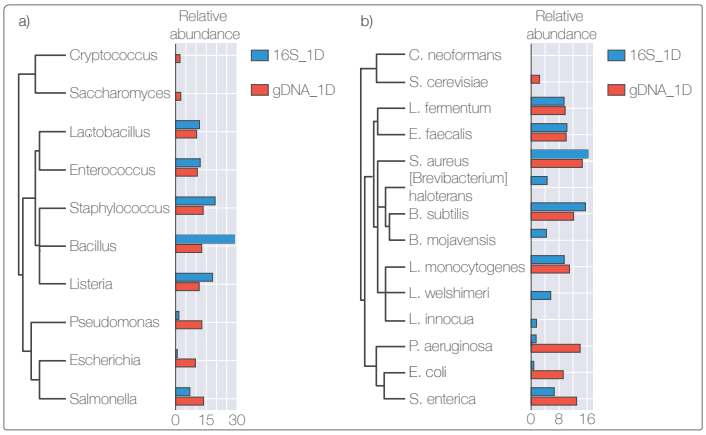

Fig. 3 Comparison of whole-genome and 16S identification at a) genus and b) species levels

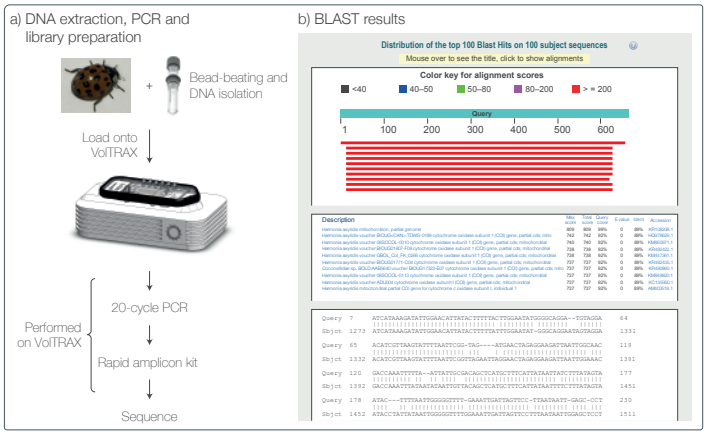

Fig. 4 DNA extraction, followed by CO1 PCR and library prep on VolTRAX

16S compared to whole genome identification at genus and species levels

We generated whole genome and 16S data from the ZymoBIOMICS Microbial Community DNA Standard and compared the number of calls from the WIMP and 16S workflows at the genus (Fig. 3a) and species (Fig. 3b) levels. As expected for a quantitative workflow, the gDNA WIMP calls agree well with the theoretical levels. The identity calls from the 16S data correlate closely with those from the gDNA data, particularly at genus level, but the correct abundance of genera and species is not reflected in the 16S data, possibly due to PCR bias. This is most noticeable for Pseudomonas, to which our 16S primers had mismatches. At species level, the 16S data reveals some false positive calls, reflecting the similarity of the 16S sequences of some genera.

Sample to result: identification of insect species by CO1 sequencing using VolTRAX

In some situations it is an advantage to be able to identify species outside of a laboratory environment. VolTRAX is a portable device which is designed to perform the necessary steps to convert a raw biological sample to a form ready for analysis on a nanopore sensing device, without the need for human intervention. We extracted DNA from the invasive ladybird species Harmonia axyridis by bead-beating, and loaded the crude extract onto VolTRAX. We performed PCR of a 650 bp region of the cytochrome oxidase gene followed by addition of sequencing adapters using our rapid-attachment chemistry, on VolTRAX, and sequenced the resulting library for 1 hour (Fig. 4a). BLAST analysis of the reads confirmed the identity of the sample (Fig. 4b).