



## Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes

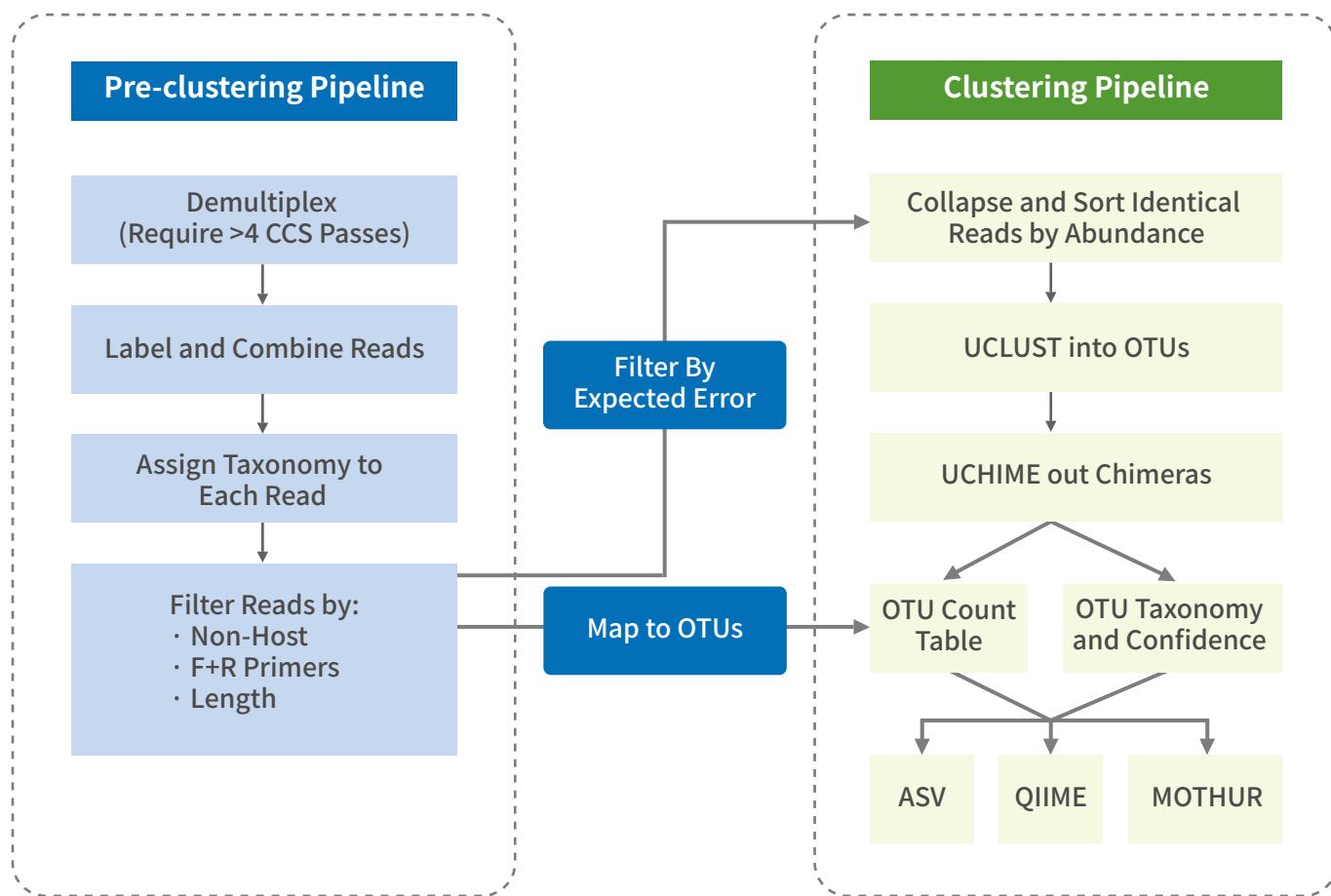


Fig. 1 Overview of the MCSMRT pipeline in Full-length 16S Amplicon analysis represented as a flowchart.

Research Pipeline	
 	<b>Samples</b> <ul style="list-style-type: none"> <li>Total DNA was isolated from sinonasal samples of patients undergoing sinonasal surgery for non-inflammatory and non-infectious indications (predominately pituitary tumors or other skull base neoplastic process) who had not received antibiotics in the preceding 8 weeks.</li> </ul>
 	<b>Library Preparation</b> <ul style="list-style-type: none"> <li>2kb SMRTbell library</li> </ul>
 	<b>Sequencing</b> <ul style="list-style-type: none"> <li>PacBio Sequel platform</li> </ul>
 	<b>Bioinformatics Analysis</b> <ul style="list-style-type: none"> <li>Circular Consensus Sequence (CCS) polishing</li> <li>ASV/OTU clustering</li> <li>Taxonomic classification</li> <li>Ecological analysis</li> </ul>

## Results

- 1.** Analysis of a mock community with 20 bacterial species demonstrated 100% specificity and sensitivity with regard to taxonomic classification.
- 2.** Examination of a 250-plus species mock community demonstrated correct species-level classification of > 90% of taxa, and relative abundances were accurately captured.
- 3.** We found greater variation among subjects than among sites within a subject, although significant within-individual differences were also observed. *Propiniobacterium acnes* (recently renamed *Cutibacterium acnes*) was the predominant species throughout, but was found at distinct relative abundances by site.

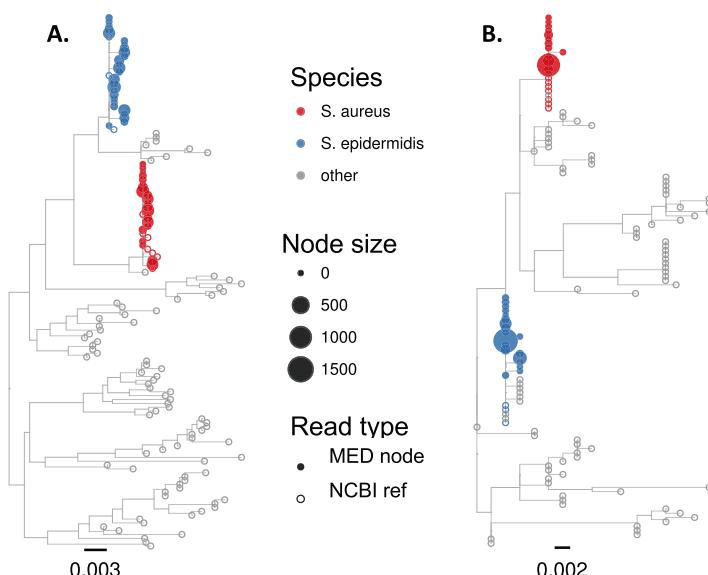


Fig.2 Approximate maximum likelihood phylogenetic tree reconstruction of staphylococcal 16S sequences representing the ASV nodes identified by MED, along with staphylococcal NCBI database entries (midpoint rooting). Each filled tip symbol represents a single MED node, and its size represents the number of reads belonging to that node. Unfilled symbols indicate NCBI database entries. Color indicates the taxonomic assignment for the two expected species with others indicated with gray. a Using FL16S (48 MED node representatives). b Using truncated V3-V5 16S (33 MED node representatives)

## Conclusions

- 01** Our microbial composition analysis pipeline for single-molecule real-time 16S rRNA gene sequencing overcomes deficits of standard marker gene-based microbiome analyses by using CCS of entire 16S rRNA genes to provide increased taxonomic and phylogenetic resolution (shown in Fig.2 above).
- 02** Extensions of this approach to other marker genes could help refine taxonomic assignments of microbial species and improve reference databases, as well as strengthen the specificity of associations between microbial communities and dysbiotic states.

## Reference

Earl J.P, Adappa N.D, Krol J et al. Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *Microbiome*, 2018, 6:190.

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