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RNA extraction optimization for both microbiome and virome analysis

Alice Michel, Marie Leoz, Nicolas Nesi, Meriadeg Le Gouil, Hortense Petat,
Chervin Hassel, Jean-Christophe Plantier

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A. Michel¹, M. Leoz¹, N. Nesi², M. Le Gouil³, H. Petat⁴, C. Hassel¹, J.C. Plantier⁵

¹Univ Rouen Normandie, Univ de Caen Normandie, INSERM, DYNAMICURE UMR 1311 F-76000 Rouen, France ; ²Univ de Caen Normandie, Univ de Rouen Normandie, INSERM, DYNAMICURE UMR 1311 F-14000 Caen, France ; ³Univ de Caen Normandie, Univ Rouen Normandie, INSERM, DYNAMICURE UMR 1311, CHU Caen, Department of Virology, F-14000 Caen, France ; ⁴Univ Rouen Normandie, Univ de Caen Normandie, INSERM, DYNAMICURE UMR 1311, CHU Rouen, Department of Pediatrics, F-76000 Rouen, France ; ⁵Univ Rouen Normandie, Univ de Caen Normandie, INSERM, DYNAMICURE UMR 1311, CHU Rouen, Department of Virology, F-76000 Rouen, France

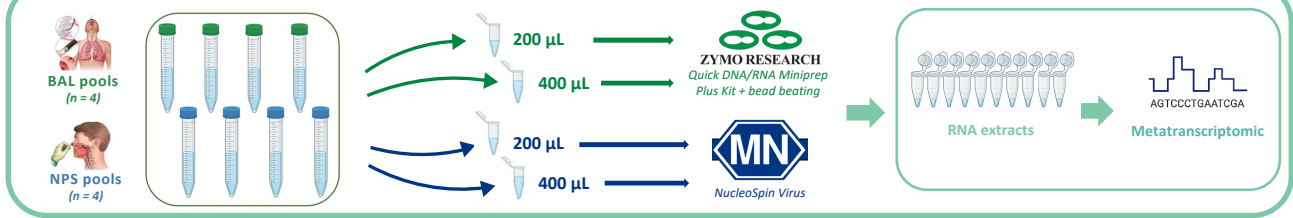
Introduction

The **respiratory microbiome** and its involvement in respiratory diseases have been increasingly studied since its discovery in the 2010's. The low biomass in respiratory samples makes it difficult to study by **next generation sequencing** (NGS).

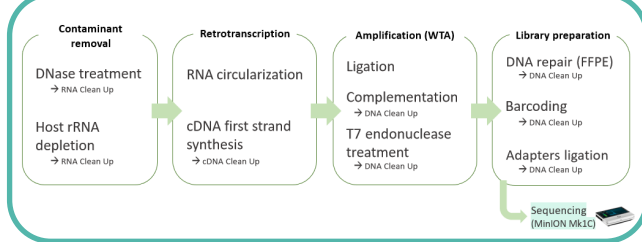
In this study, we assess two types of micro-organism **RNA extraction** from nasopharyngeal swabs (NPS) and bronchoalveolar lavages (BAL) pools to optimize **metatranscriptomic analysis** associated with **long-read sequencing** using **Nanopore Oxford Sequencing**.

Methods

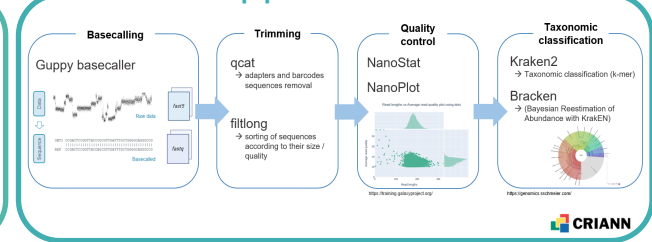
RNA extraction from respiratory samples



NGS



Bioinformatic pipeline



Results

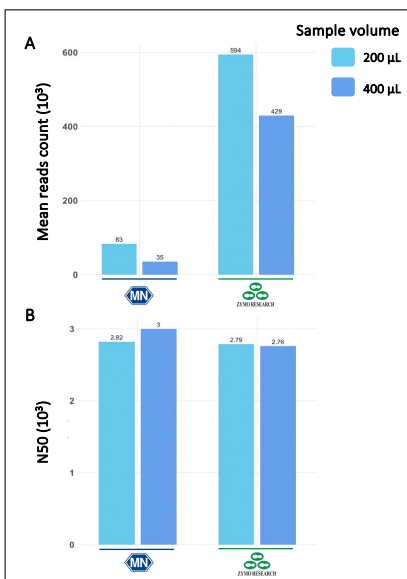


Figure 1. Mean reads count (A) and N50 values (B) depending on the RNA extraction kit (Zymo Reasearch ; Macherey-Nagel) and sample volume considered for input.

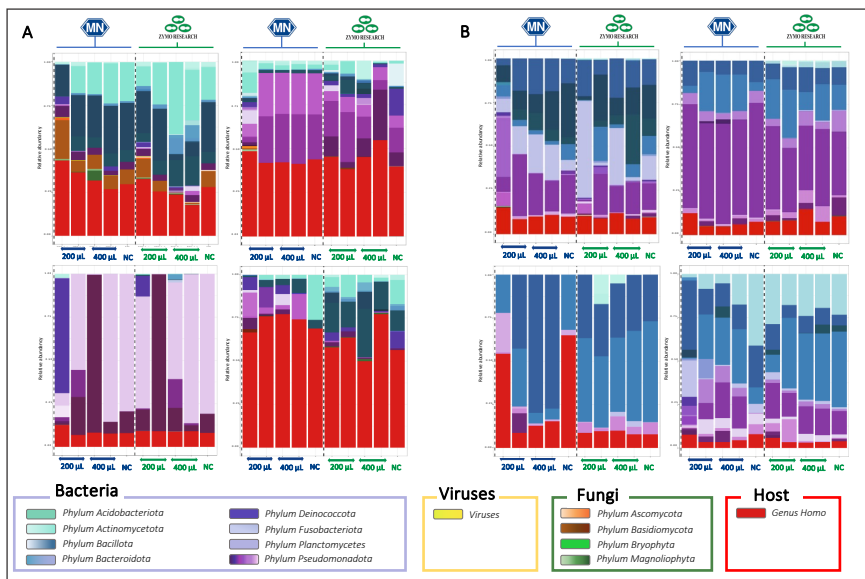


Figure 2. Taxonomic classification results at genus level, using a combination of Bracken and Kraken2 softwares for the four **BAL pools (A)** and **NPS pools (B)**, depending on the RNA extraction kit (Zymo Reasearch ; Macherey-Nagel) and sample volume considered for input. NaCl buffer and storage buffer, for BAL and NPS pools respectively, were considered as negative-controls (NC).

Outcomes

These results show that an **RNA extraction protocol** including bead beating provides a **large amount** of **bacterial taxonomic information**, without compromising its quality. Furthermore, this protocol does not appear to have a deleterious effect on the amount of **viral taxonomic information**, making it compatible with **microbiome analyses**, including **virome specific studies**.