Assessment of Contaminants Associated with Gold-standard Ancient DNA Protocols

Ancient DNA (aDNA) techniques have rapidly evolved in recent years, especially the application of single-stranded DNA library construction protocol and the automation of lab work using liquid handling robots that has greatly improved the efficiency of ancient DNA research. These techniques are widely used in the study of ancient DNA. However, laboratory background contaminants introduced when using different protocols are still unclear, which has been one of the great challenges in the field of ancient DNA.

This study evaluates the microbial DNA introduced in different ancient DNA experimental protocols to achieve a better understanding of background contaminant DNA in ultra-clean laboratories and how
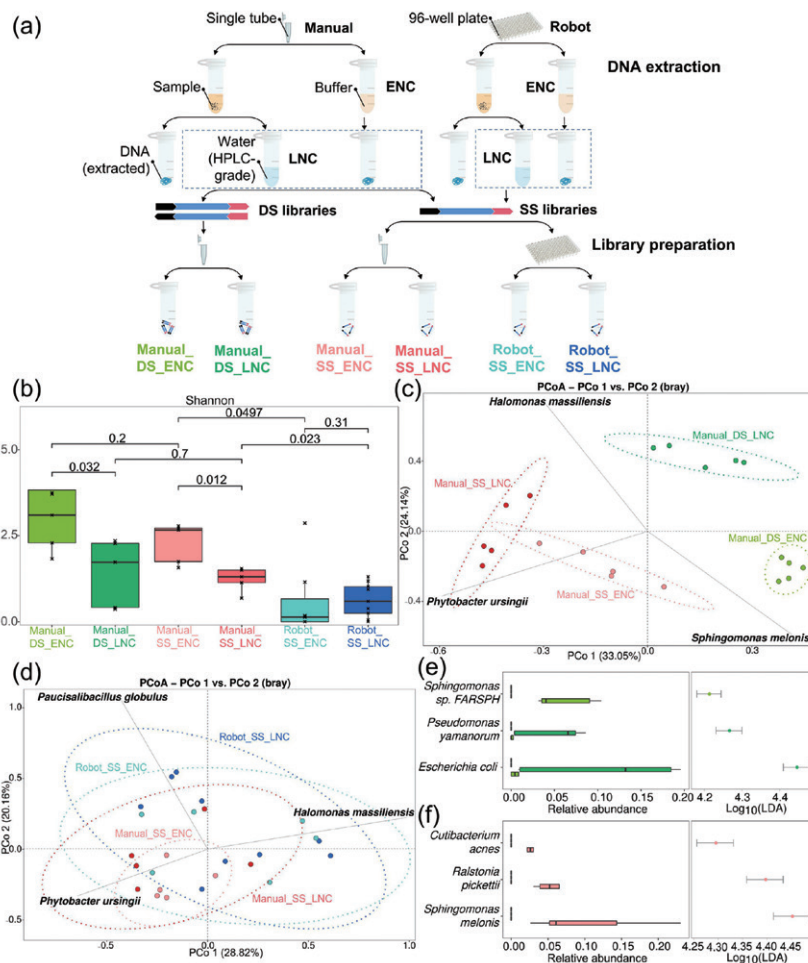

IMAGE: (a) A schematic diagram clarifying the experimental design. (b) The comparison of the Shannon index of different groups. The PCoA of (c) manually processed groups and (d) robot-processed groups. The LEfSe analysis of (e) manually processed samples and (f) robot-processed samples.

cutting-edge techniques can be applied to minimize contamination.

The metagenomes of 40 negative controls processed with different protocols in the ultra-clean laboratory are profiled and analyzed in detail. These samples contain extraction negative controls samples generated manually using double-stranded DNA library protocol (Manual_DS_ENC, 5 samples), library negative controls samples generated manually using double-stranded DNA library protocol (Manual_DS_LNC, 5 samples), extraction negative controls samples generated manually using single-stranded DNA library protocol (Manual_SS_ENC, 5 samples), library negative controls samples generated manually using single-stranded DNA library protocol (Manual_SS_LNC, 5 samples), extraction negative controls samples generated by robots using single-stranded DNA library protocol (Robot_SS_ENC, 9 samples), and library negative controls samples generated by robots using single-stranded DNA library protocol (Robot_SS_LNC, 11 samples). The single-stranded DNA library preparation protocol performs better in retrieving highly damaged DNA, whereas the protocol is lengthy and thus robot serves as a preferable choice.

The environmental microbial DNA like those from water, soil, air, and human skin are all potential sources of laboratory background contaminants and no obvious ancient DNA damage patterns were observed. Manually processed samples showed higher richness (237 species) and diversity (Shannon Index) compared to samples processed by robots (37 species). Finally, the combination of single-stranded DNA library construction protocol and the automation of lab work using liquid handling robots can significantly reduce the diversity of background microorganisms and reduce the possible background contaminants in the experiments.

In summary, this study provided new insights into the potential impacts of using different protocols and equipment on the introduction of microbial contaminant DNA in the ultra-clean laboratory environment.

(Source: IVPP)