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Improving ancient DNA extraction from waterlogged grape pips

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INTRODUCTION

- In VINICULTURE, we aim at mapping the genomic makeup of ancient grape cultivars in France during the last 4,000 years in order to better understand the history of wine-making in the region, including the selective and admixture processes underlying the origins of important cultivars that are key to the modern industry.
- The project builds on an extensive collection of archeological grape pips spanning the whole country from the Bronze Age to the Modern Era (Figure 1). In addition to DNA, samples are investigated for both their morphological variation using GMM techniques.
- This pilot study was aimed to assess the performance of five DNA extraction protocols (A-E) using NGS technologies so as to ensure that maximal endogenous DNA yields could be obtained for the collection.
- We found that protocol E, which is based on DNA purification through High Pure Viral Nucleic Acid Roche® columns (HPVNAc), is generally associated with increased endogenous DNA content and molecular complexity, while minimizes hands-on time.

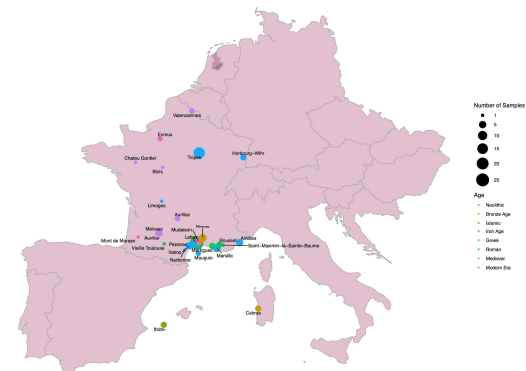
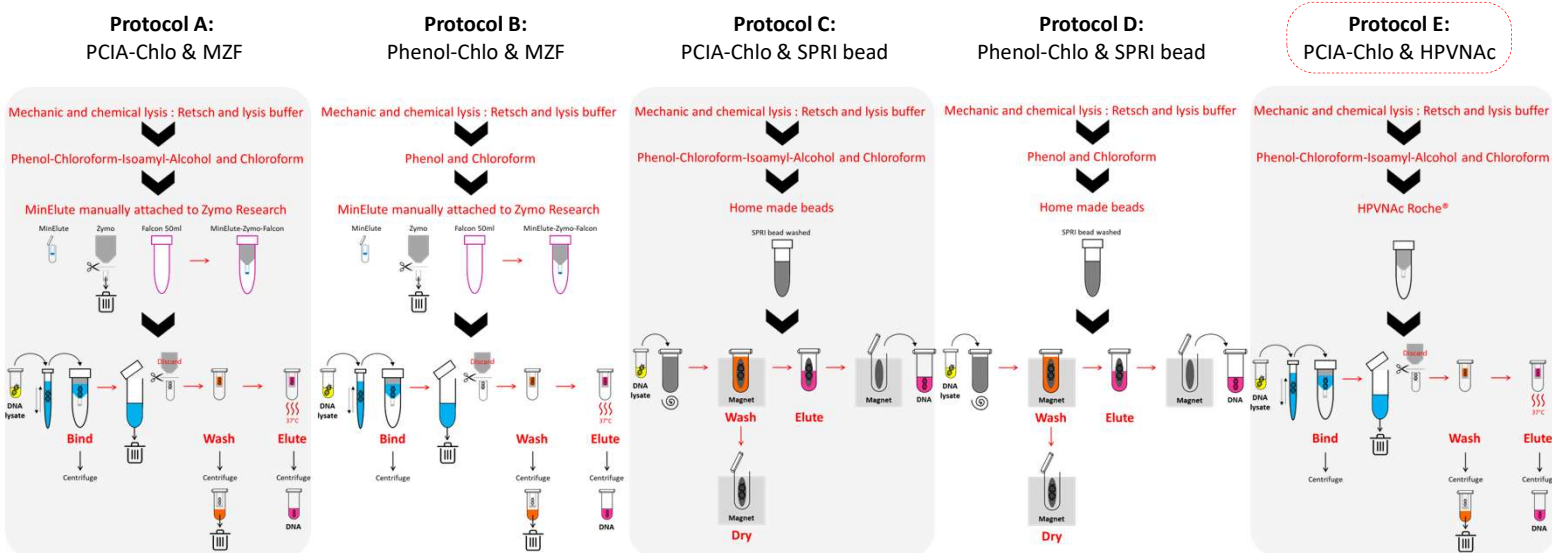


Figure 1. DNA libraries were prepared from a total of 154 DNA extracts. DNA amplification provided sufficient material for shotgun sequencing on the MiniSeq instrument for 121 DNA libraries (Figure 2).

METHOD



RESULTS

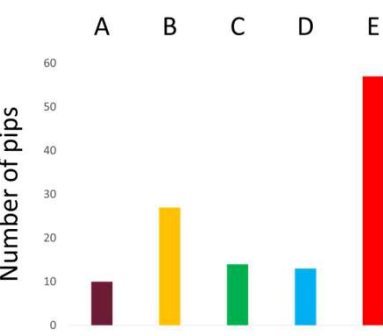


Figure 2. Number of archeological pips treated per conditions.

The size of individual pips is too small to provide sufficient material for multiple extractions. We thus randomized samples across space and time before proceeding with DNA extraction, library construction and shotgun sequencing.

Statistical comparisons are based on 45 random selections of 9 samples per condition (Figure 3).

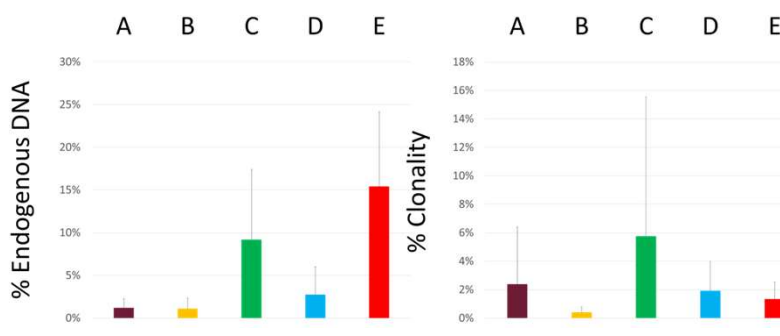


Figure 3. Endogenous DNA content and clonality estimates following shotgun sequencing of 0.1.xM to 3.xM read pairs on the MiniSeq instrument. Sequence processing, including trimming, collapsed and mapping was carried out using PALEOMIX (Schubert et al. 2014).



This study is part of the VINICULTURE project funded by the French National Research Agency (Grapes and wines in France from the Neolithic to the Middle Ages, an integrated approach in archeosciences).

CONCLUSION

We described a DNA extraction protocol that increases the recovery of endogenous DNA and the complexity from ancient pips while minimizing hands-on time compared to previous methods. The application of method E using Phenol-Chloroform-Isoamyl-Alcohol and Chloroform & HPVNAc facilitated the screening and whole-genome sequencing of 49 ancient grape samples as part of the ANR-funded Viniculture program.

REFERENCES

- Characterization of ancient and modern genomes by SNP detection and phylogenomic and metagenomic analysis using PALEOMIX, Schubert et al. 2014.
- Optimization of DNA Recovery and Amplification from Non-Carbonized Archaeobotanical Remains, Wales et al. 2014.