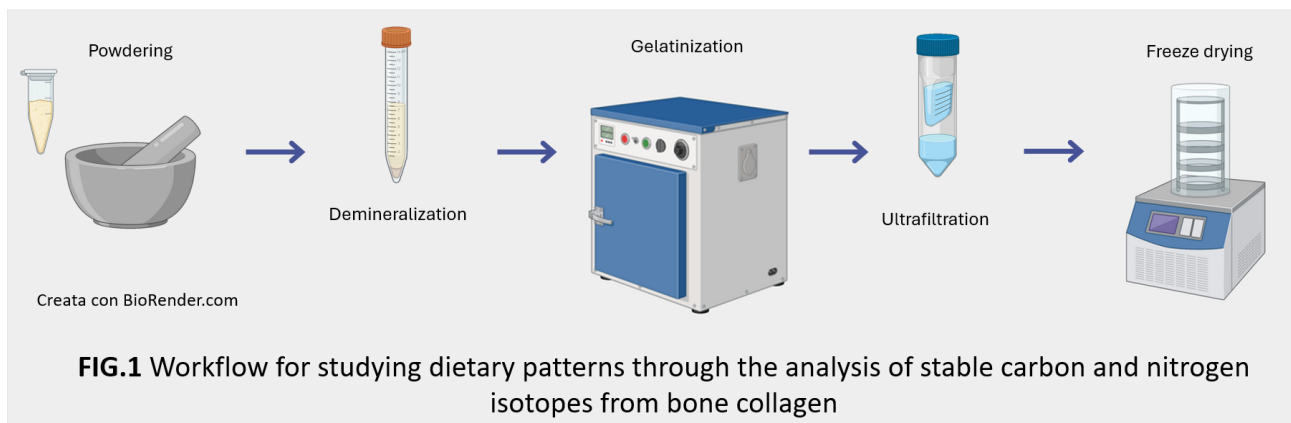


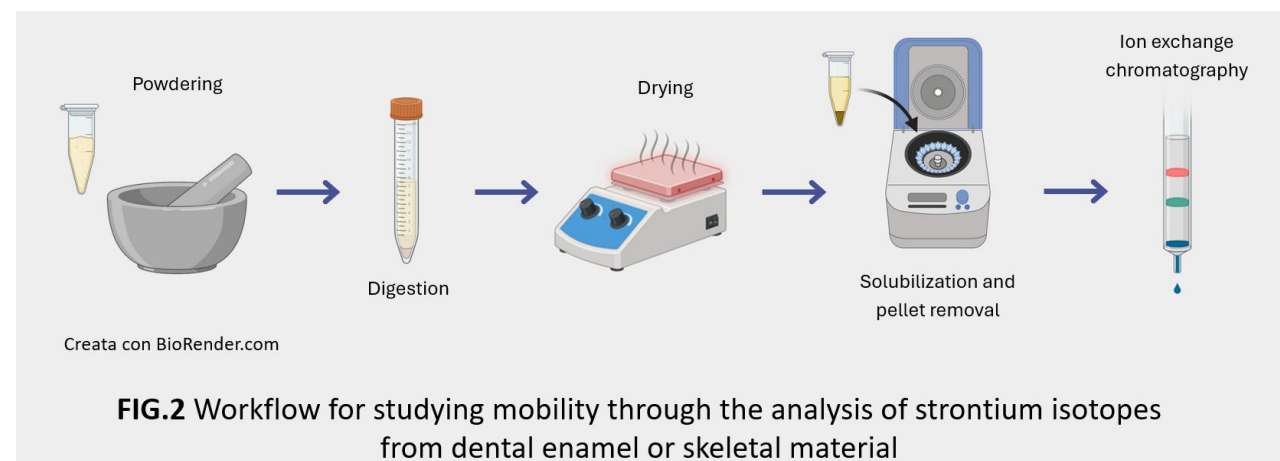
BIOMOLECULAR ANALYSES

Isotopic analyses were performed on 28 of the 38 individuals unearthed during the archaeological excavations at Piazza d'Armi and Cortaccione. The reconstruction of dietary patterns is possible through the study of carbon and nitrogen isotopes in collagen, one of the main proteins forming bone tissue. Specifically, $\delta^{13}\text{C}$ allows distinguishing whether the resources consumed derived from terrestrial or marine ecosystems and, in the former case, whether they came from plants adapted to temperate (C_3 photosynthesis) or hot and arid climates (C_4 photosynthesis). $\delta^{15}\text{N}$, on the other hand, provides information about the trophic level occupied within the food chain.

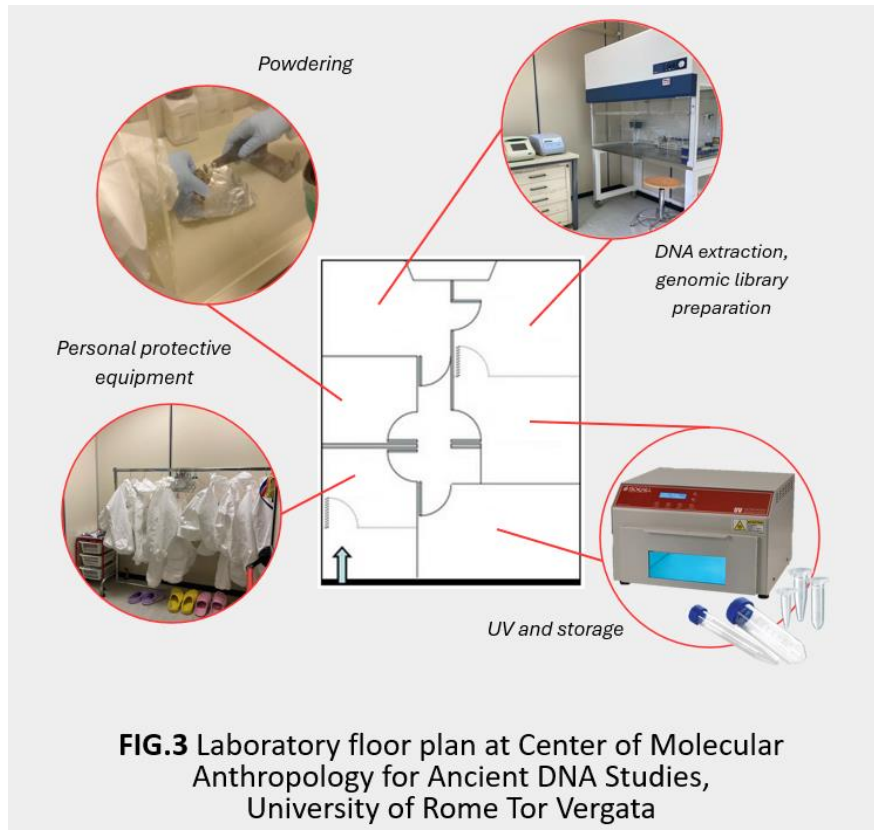
For collagen extraction, skeletal tissue was first cleaned, powdered, demineralized with hydrochloric acid, and gelatinized at high temperature, then ultrafiltered to remove low-molecular-weight organic acids. The resulting collagen was finally freeze-dried and analysed by mass spectrometry (Fig. 1). The results indicate that the Umbrians followed an omnivorous diet based on terrestrial resources such as wheat, barley, and oats, consistent with species common in central Italy during the Iron Age, supplemented with animal proteins. For some non-adult individuals, the data also suggest consumption of maternal milk at the time of death.



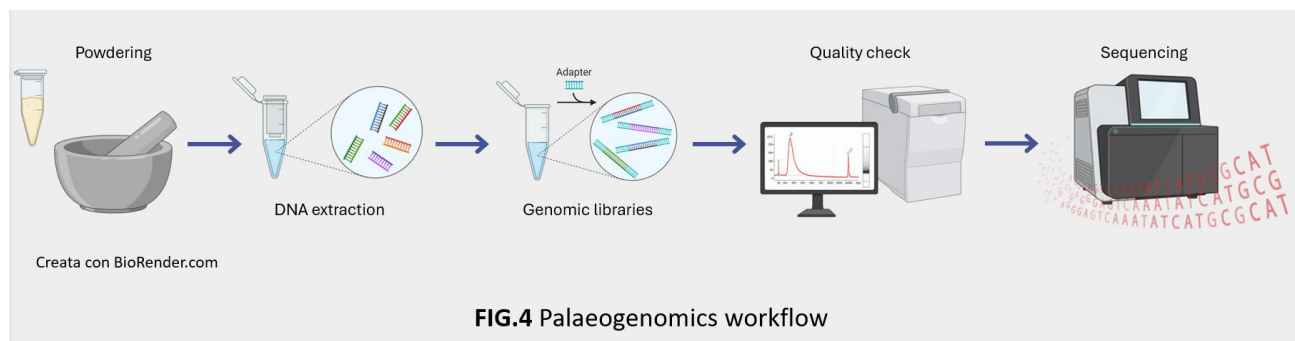
Strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) were used to study geographic mobility. This element, mainly released through rock erosion, enters the local ecosystem and, through the diet, is deposited into mineralized tissues such as tooth enamel. Since enamel forms during childhood, its isotopic signature reflects the area where the individual was born and raised. Through comparison of this signature with the local baseline measured in sediments, it is possible to detect any movement. For strontium analysis by mass spectrometry, samples were treated with various chemicals and subjected to multiple purification steps to isolate this element from the matrix using ion-exchange chromatography (Fig. 2). The study confirmed, consistent with some non-local grave goods, that several individuals had grown elsewhere.



Paleogenomic analyses were conducted on 29 inhumed individuals, selected based on the preservation of teeth and the temporal bone, tissues ideal for isolating ancient DNA (aDNA). To prevent contamination from modern material, aDNA extraction and the preparation of genomic libraries were carried out in a dedicated facility (Fig. 3) at the Center of Molecular Anthropology for Ancient DNA Studies of the University of Rome Tor Vergata (<http://bio.uniroma2.it/dna-antico/>).



The quality of libraries was assessed prior to sequencing (Fig. 4). About half of the samples were sequenced at higher depth to achieve the minimum genome coverage required for population-level analyses.



The data made it possible to characterize the genetic heritage of this Umbrian community, investigate kinship relationships hypothesized from the archaeological context, and confirm the biological sex of the individuals. Comparison with contemporary populations from the same area, such as the Picenes and Etruscans, helped to place the Umbrians in a broader context, providing new insights into social dynamics and the history of central Italian populations during the Iron Age.