

Exploring the genetic identities of the Medieval Spanish Royal House of Aragon through mitochondrial lineages and nuclear SNPs detected by a novel enrichment approach

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The Medieval Royal House of Aragon was one of the most powerful and influential dynasties of its time. Their reign over the kingdom united the territories of Aragon and Catalonia, leading to the return of the rich domains of the northeast Iberian Peninsula (Ebro Valley and current region of Valencia, among others). Through collaborative efforts of archaeologists/anthropologists, historians, analytical chemists, and forensic geneticists, the putative identities of the members of the royal dynasty were able to be attributed to the skeletal remains discovered within specified Spanish pantheons. Previous efforts investigated the use of radiocarbon dating and stable isotope testing as well as historical accounts of the time period in an attempt to identify the remains believed to belong to the Medieval Royal House of Aragon. Currently, genetic analyses are underway exploring both mitochondrial and nuclear DNA contained within these ancient bones (estimated to be approximately 850-950 years old). Mitochondrial DNA (mtDNA) testing often is performed on skeletal remains due to its relatively high copy number and capacity to resist degradation. Genetic testing of the mtDNA control regions (HVI and HVII; 16,024-576) was performed using an early version of the ForenSeq™ mtDNA Control Region Solution kit (Verogen; San Diego, CA). The nuclear genome of ancient remains typically is highly degraded and damaged, and thus genetic information often cannot be obtained. Current forensic methodologies are incapable of typing such low quality samples. A novel one-step PCR technology, Reverse Complement PCR (RC-PCR), designed to target 27 human identity single nucleotide polymorphisms (SNPs) was explored. The EasySeq™ NGS Reverse Complement-PCR kit (NimaGen B.V.; Nijmegen, Netherlands) was used to amplify these target SNPs requiring DNA fragments to be only approximately 50 basepairs in length. The RC-PCR assay is highly sensitive (tested down to 60 pg of input DNA) and has the added feature of being a single, closed system which reduces chances of contamination and improves workflow. Genetic profiles from both mtDNA sequencing and RC-PCR were obtained from the highly degraded DNA of the Royal House of Aragon skeletal remains. The impact of these assays on forensic and historical investigations, to be able to obtain genetic profiles from compromised samples and potential identification of the remains of the Medieval Royal House of Aragon, will be discussed.