

GOING ALL IN: BIG SILICA DEVICES, BIG RETURNS FROM BONE

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Because teeth and bones persist long after soft tissues have succumbed to decay, a few pieces of a disarticulated skeleton are often all that are ever recovered. The structure and unique mineral composition of bones/teeth contribute to DNA preservation, protecting it to some degree from microorganisms as well as the oxidative and hydrolytic damage that otherwise would rapidly occur. Collectively, these properties make bone-derived DNA a natural focal point in the field of missing persons identification. A primary area of interest has been improvement of the DNA isolation protocol, which should be streamlined and provide a maximum yield of DNA free of inhibitory compounds that can affect downstream applications.

In an effort to maximize recovery of DNA from bone, there has been a shift toward using larger volumes of digestion buffer to more fully demineralize pulverized bone samples. Many practitioners have employed ultrafiltration devices for volume reduction in order to reconcile larger volumes of crude extract with the necessarily small final volume containing the purified DNA. Unfortunately, ultrafiltration devices alone are insufficient for purification of crude bone extract and so must be paired with another method. Ultrafiltration is either preceded by one or more organic extraction steps or followed by purification with a small-scale silica device. Organic extraction is ineffective when dealing with common water-soluble inhibitors found in bone, such as humic acid. There are also inherent risks involved with the use of organic solvents. The number of handling steps is increased regardless of the method paired with ultrafiltration; and with this comes an increased risk of contamination, a greater demand on the time of the technician, and increased cost and hazard.

Silica based columns, slurries, and resins have long been available for DNA isolation, but the current methodology is geared toward extraction of DNA from small volumes. Limited volume has proven to be the greatest disadvantage of applying silica-based extraction methods to bone-derived samples. This study describes the preliminary design of a silica-based device that is large enough to process the entire volume of crude extract in a single step which greatly reduces time and manipulation necessary to perform purification. Additionally, the new design maximizes DNA recovery and minimizes the presence of compounds inhibitory to downstream enzymatic processes.