16S METAGENOMIC ANALYSIS OF DNA FROM HUMAN SKELETAL SAMPLES USING THE ROCHE 454 GS JUNIOR SEQUENCER

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DNA extracts from samples such as bone, carpet, clothing, food items and anal swabs typically contain microbial DNA along with human DNA. The presence of significant amounts of microbial DNA mixed with human DNA can lead to non-specific amplification in the STR results. Having performed STR DNA testing on thousands of skeletal remains, a number of different non-human amplicons have been produced that likely originate from the mis-priming of the STR primers on microbial DNA. In many cases, the non-specific amplicons are off ladder alleles with a signal strength that is much stronger than the STRs, although some have been identified that are within an allelic bin and of similar height to the rest of the STR alleles.

To take a closer look at the bacteria present in the DNA extracts from two human skeletal samples, 16S metagenomic analysis was performed. DNA extract from sample 31, a fourth left metatarsal, and sample 122, a left fibula from a different individual, both had additional non-specific amplicons when tested using the AmpFLSTR® Identifiler® PCR Amplification Kit. The human DNA, as determined by the Quantifiler kit, was approximately 0.25 ng/µl while the total DNA content for both samples, as determined by a Qubit® 2.0 Fluorometer, was more than 60 fold higher. Two regions of the 16S rRNA gene were amplified for both samples, and then sequenced on a Roche 454 GS junior sequencer using the titanium chemistry.

Samples 31 and 122 yielded ~75,000 and ~50,000 key pass sequence reads, respectively. The individual reads were cleaned to remove low quality bases, clustered, and matched to the Silva Gold Bacterial Database. A total of 329 different species of bacteria were detected in sample 31 and 178 were detected in sample 122. A total of 76 species were present in both samples although almost all were different percentages of the reads. Many of the species detected are what would be expected for a decaying body such as *Clostridium putrefaciens*, bacteria known to inhabit the soil such as *Rhodococcus yunnanensis*, airborne microbes such as *Intrasporangium calvum*, and microbes previously found in insects such as *Janibacter anophelis.* Most of the species detected were different between the samples, including 250 bacteria detected in sample 31 that were not found in sample 122 and 106 that were detected in sample 122 and not detected in sample 31.