Combining Novel Methods to Achieve Reliable Results in Extremely Low Yield Human/Animal Mixes

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When presented with a case where there are only minute and degraded quantities of DNA present and even the small quantity that is present is further diluted due to a mixture of human and animal DNA, approaches to analyzing the case become extremely tedious. Combining a number of novel or uncommon approaches to amplification can oftentimes yield not only desired results, but could be commonly incorporated in the laboratory to be used on a routine basis to enable cases that otherwise would not yield consistent and court-worthy results. One such example is the use of nested PCR for mitochondrial analysis. Though extreme caution must be used in order to prevent contamination, when using the proper protective equipment and techniques, it is easy to take a number of small sequence fragments generated by nested PCR, sequence them, and then combine them in order to achieve the entire mitotype. When seeking either human and/or animal DNA in a mixed sample, we chose to use universal mammalian primers amplify the DNA and then a yield gel to separate the amplified product into various different sized bands according to species. The bands or the regions where the bands should appear were then excised and purified using the Qiagen™ Gel Purification system. The amplified DNA was then reamplified using primers that generated fragment lengths of between 150 and 200 base pairs. Even when bands were barely visible and sometimes when no visible band appeared on the yield gel, reamplification yielded products in most cases that were viable for sequencing and had consistent reproducible controls as well as blank controls.