A76 Sex Determination Assay for Degraded or Low Quality DNA

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After attending this presentation, attendees will learn about a highly sensitive, pyrosequencing based sex determination assay for degraded or low quality DNA.

This presentation will impact the forensic science community by detailing a more sensitive technique for sexing challenging samples, such as aged skeletal remains or tissue with little or poor quality DNA, than is currently available. The pyrosequencing based method will better allow sexing of samples when the standard amelogenin method is not effective.

Current molecular based human sexing techniques target a region of the single copy amelogenin gene, which exists on both the X and Y chromosomes, with the X chromosome having a six base-pair deletion. When this region is amplified and sized, female samples result in a single allele (peak), while males produce two products. However, this method relies on a sufficient quantity of deoxyribonucleic acid (DNA) being present to produce a strong enough signal for detection, usually 0.5 – 1 ng. Unfortunately, many forensic samples, such as hair shafts, aged bone, or handled objects, have much lower quantities and/or quality of DNA, and traditional molecular sexing methods are unsuccessful.

In order to overcome shortcomings with targeting amelogenin in degraded or low copy DNA, multicopy loci can be assayed which increases the probability of amplification. For instance, X chromosome specific DXZ4 repetitive satellite sequences have successfully been used to help sex skeletal remains. Other sexing techniques utilize an autosomal multicopy Alu sequence and Y-specific multicopy DYZ5 sequence, with sensitivity as low as 4 pg. Different regions of the Y chromosome with even more copies have the potential to be utilized in this manner, including DYZ1, a family of repeats similar to but more prevalent than DYZ5, made up of a 3.4 kb repeat with 2000 to 4000 copies per cell.

In this study, a PCR multiplex for DYZ1 and Alu was created which was assayed using pyrosequencing technology. Pyrosequencing was particularly beneficial in these experiments because it is extremely effective for assaying small DNA fragments, consisting of as little as two primers flanking one or more internal bases. This technique differs from standard Sanger (dideoxy) sequencing as it is based on detection of pyrophosphate release upon nucleotide incorporation, which is converted to adenosine triphosphate (ATP) by ATP sulfurylase. A luciferase, in the presences of ATP, gives off light, while any unincorporated dNTPs are degraded by apyrase. Sequences can be generated beginning at the base directly beyond the primer, which are not possible using standard sequencing methods.

Small DYZ1 and Alu regions (<150 bp) were targeted and PCR primers were optimized and tested for specificity on male and female DNAs. Internal pyrosequencing primers were then developed for both regions which further increased the specificity of the reactions. Once the method was optimized on high molecular weight DNA, the sensitivity and accuracy were compared to traditional amelogenin amplification by analyzing artificially degraded DNA as well as low quality DNA extracted from forensic samples such as hair shafts, fingernails, aged skeletal remains, and touch DNA.

Modifications of standard pyrosequencing methods were also examined with a goal of increasing ease and throughput. Pyrosequencing generally utilizes biotinylated primers for post-PCR clean up, immobilizing single stranded products by strong noncovalent affinity to streptavidin. However, this process is time-consuming and requires a vacuum workstation and multiple costly reagents. Given this, the biotinylated primer technique was compared to an enzymatic purification utilizing the hydrolytic enzymes exonuclease I and shrimp alkaline phosphatase, thereby allowing for direct pyrosequencing of double stranded PCR products.

Ultimately, the DYZ1/Alu assay is far more sensitive and potentially less ambiguous sexing technique than the standard amelogenin method on low quality/quantity DNA, proving successful even with less than 1 pg of DNA. Pyrosequencing of multicopy sex-specific loci, along with autosomal controls, is a straightforward and fast molecular technique that could be highly useful to the forensic community.

DNA, Sex Determination, Pyrosequencing

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