RESOLUTION OF MIXTURES BY CLONING OF THE MITOCHONDRIAL DNA CONTROL REGION

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The purpose of the research is to develop a standard method to resolve mixtures by cloning of the mtDNA control region. Evidence containing DNA mixtures from two individuals can be resolved using appropriate statistical analysis. If the evidence involves mixtures of multiple individuals or the mixture involves low copy number samples, then standard statistical methods cannot resolve the mixtures. We have developed a method to resolve mixtures from multiple individuals or from low copy number samples by cloning the hypervariable region of the mtDNA genome.

The mtDNA control region is cloned into the PCR-Blunt EndII cloning vector of the *Zero Blunt TOPO PCR Cloning Kit* developed by Invitrogen Co. Theoretically, each individual colony corresponds to the control region of mtDNA from one individual. The process of cloning the mtDNA was developed using the following strategy:

- Determine the level of detection using mtDNA cloning.
- Develop a method to clone one, two, and five individuals using 50 pg of genomic DNA at equal ratios.
- Develop a method to clone two individuals at varying ratios of input DNA
- Develop method to clone mtDNA from one, two, and five individuals from handled objects
- Perform blind studies to determine if “unknown” individuals can be identified from handled objects.
- Study the effect that high fidelity DNA polymerases will have on cloning efficiency and on the frequency of amplification errors.
- Determine the amplification error rate and intrinsic rate of heteroplasmy through dual amplification.

The results indicated that mixtures involving one, two, and five individuals could be resolved by cloning the mtDNA region. Amplifying the extracted DNA in duplicate, followed by cloning and sequencing the DNA permitted the identification of individuals contributing to the mixture, the identification of clones associated with heteroplasmy, and the identification of errant clones arising from amplification errors. Results indicated that the rate of errant clones due to amplification error was 3×10^{-5}, while the observed rate of heteroplasmy was 3×10^{-4}.

Studies were performed to evaluate the effect that high fidelity DNA polymerase would have on reducing the amplification error rate. Using *Herculase* High Fidelity DNA polymerase the amplification error rate was lowered to 8×10^{-6}. The amplified mtDNA resulting from the use of high fidelity DNA polymerase could be successfully cloned into appropriate vectors and sequenced to identify the individuals contributing to a five person mixture.

Amplifying the extracted DNA in duplicate, followed by cloning and sequencing the DNA insert permitted the identification of multiple individuals contributing to the mixture, the identification of errant clones arising from amplification errors, and the identification of clones arising due to heteroplasmic events. Although this technique can be used to identify multiple individuals

contributing to a mixture, the following limitations need to be considered before using this technique on casework.

Cloning of the mtDNA control region can be used to identify individuals; however amplification errors and heteroplasmic events will occur and result in a background of clones that differ in their sequences from the original DNA sequences associated with the individuals.

Different individuals may have polymorphisms at primer binding sites, this would affect the cloning efficiency of that individual. Different individuals shed cells at different rates, therefore unequal ratios of cloned mtDNA profiles will be observed. Maternal relatives cannot be differentiated from each other using mtDNA. The power of exclusion of the mtDNA is approximately 99%; therefore the technology should be used as an investigative tool or a tool to exclude the falsely accused.