A46 Application of Circular Ligase to Provide Template for Rolling Circle Amplification of Low Amounts of Fragmented DNA

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After attending this presentation, attendees will be familiarized with the use of a ligase enzyme that circularizes DNA, which in turn may serve as a template for Rolling Circle Amplification and the product of that amplification can be subjected to DNA analysis.

This presentation will impact the forensic science community and/or DNA community by providing a new protocol for analysis of degraded/low copy number (LCN) DNA.

Degraded/LCN template is a frequently encountered obstacle when performing DNA analysis. The described methodology attempts to recover useful information from such templates using a robust form of Whole Genome Amplification (WGA), termed Rolling Circle Amplification (RCA), in which a circular DNA template is amplified by degenerate primers and a highly processive polymerase. Because human genomic DNA exists in linear form, a novel commercially-available circular ligase (CL), which circularizes linear ssDNA, was investigated towards the goal of producing templates which are suitable for RCA. Also described is a polyacrylamide gel electrophoresis (PAGE) method for the detection and analysis of template circularization.

Initial studies on CL involved optimization of ssDNA template circularization utilizing synthetic DNA oligonucleotides. These studies involved varying reagent concentration, incubation temperature, and reaction time. Circularization was observed to increase with increased enzyme amounts, however a maximum activity level was observed. High concentrations of manganese chloride were found to adversely affect the circularization of longer DNA templates via non-specific degradation.

The application of CL for circularizing dsDNA, the native form of human DNA, was also investigated. dsDNA was first heat denatured and snap-cooled, to generate ssDNA templates for circularization. However, while these were immediately subjected to CL, the results revealed that complementary ssDNA strands readily re-annealed to form dsDNA just prior to circularization. CL-treated dsDNA templates nonetheless formed exonuclease III-resistant products suggesting that CL is directly active on dsDNA templates. Furthermore, restriction digestion analysis confirmed that the dsDNA was circularized. In contrast, control experiments using T4 DNA ligase resulted in the formation of linear concatemers of dsDNA. CL optimization studies on dsDNA were conducted; however, no variation in template circularization was observed when incubation time, temperature, and ATP concentration were altered.

Additional experiments revealed that specific 5' and 3' terminal nucleotides of the linear CL template can affect the efficiency of circularization. To this end, an adaptor sequence was developed to contain nucleotide ends which are optimal for ligation. Ligation of such adapters to both ends of a linear template which contained suboptimal terminal nucleotides yielded successful circularization.

The results described form the foundation for further development of a method to analyze degraded/LCN samples via the circularization of human DNA fragments and subsequent RCA. The technique shows promise for obtaining partial or perhaps complete nuclear DNA and/or mtDNA profiles from compromised samples.

Degraded/LCN DNA, Template Circularization, Rolling Circle Amplification

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