

To Obtain Abundant Whole Genome DNA from a Single Hair Follicle

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A new technology termed Multiple Displacement Amplification (MDA) was recently developed to generate unlimited quantities of whole genomic DNA (gDNA) at 300C directly from blood specimen/cultured cells or use with as few as 1-10 copies of genomic DNA. This method utilizes the unique/high processivity of Φ 29 DNA polymerase, capable of replicating 70,000 nucleotides in one binding event, and initiates the displacing of the complementary strand as template for subsequent replication at the rate of 25-50 nucleotides/sec.

The reagent kit REPLI-g for MDA (QIAGEN Co., Hilden, Germany) has been validated exhaustively for a variety of down stream processing application including SNP & STR genotyping, RFLP and comparative genome hybridization etc. We report the first application of REPLI-g Kit to obtain abundant gDNA from a single hair follicle for STR typing. Method: The REPLI-g kit protocol was slightly modified to process a single hair follicle. A single hair follicle was separated from its shaft from 10 individuals. Each hair follicle was placed into a separate well of a 96-well plate containing 20 μ l TE. 20 μ l of cell lysis solution was added to each well. The plate was then placed on ice for 10 minutes. 20 μ l of stop buffer was added to each well. 5 μ l of the lysed solution was transferred to a new 96-well PCR plate. 20 μ l of MDA reaction master mix was added to each well. The tray was transferred to a thermocycler with a three step program. 300C -16 hours, 650C -10 minutes, 40C -hold. The MDA-gDNA can be used immediately or stored at -200C. The quantity/quality of MDA-gDNAs was assessed by OD readings at 260, 280 & 320 nm. The MDA-gDNA was electrophoresed onto a 0.7% gel along with uncut Lambda and Hind III λ digest as molecular weight marker.

Genomic DNA was extracted from blood specimens of the same 10 individuals using the QIAamp columns and tested in parallel with the MDA-gDNAs for STR typing using ABI AmpF/STR Identifiler Kit on the ABI Prism 3100 Sequencer. Result: The DNA yields of the 10 single hair follicle samples ranged from 39-57 μ g, which was close to the expected amount of 40 μ g from the manufacturer's insert. The OD of 260/280 was ≥ 1.65 . The size of the amplified gDNA via MDA was approximately 23Kb and the expected size is ≥ 10 Kb. Concordant STR typing results were obtained between DNAs obtained from blood or gDNAs via MDA. Our STR typing of 16 genetic markers located on 15 different chromosomes from a single hair follicle via MDA method demonstrates that high fidelity whole genome amplification using the REPLI-g kit generates abundant DNA across the entire genome with minimum sequence biases.

Conclusion: This new technique opens the door to facilitate typing for genetic markers on virtually any limited clinical or forensic sample sources: needle biopsy, single hair follicle or from less than 10 nucleated cells from trace of blood, saliva or body fluid left at crime scene. It also enables the generation of unlimited quantities of gDNA from valuable specimen for archiving or serve as QC samples. The MDA method is superior to

all previously described whole genome amplification methods because there is no amplification bias and purification of template is not required.