

## VERIFILER™ DIRECT PCR AMPLIFICATION KIT FOR PATERNITY TESTING

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VeriFiler™ Direct is a PCR based amplification assay specifically developed to assist in difficult paternity testing such as in deficiency cases, and cases involving mutational events. The kit amplifies 9 autosomal STR loci (D10S1248, D1S1656, D2S1338, D22S1045, D19S433, TH01, D2S441, D6S1043, and D12S391) and the sex determining marker, Amelogenin. The kit uses the same 5-dye chemistry found in the AmpF $\lambda$ STR® Identifiler® PCR Amplification Kit and the loci encompass a size range between 72 to 287 nucleotides.

VeriFiler™ Direct was developed to be used in conjunction with an Identifiler® series STR kit to provide a total of 21 unique STR loci for higher discriminatory power. Four loci in the Verifiler™ Direct Kit overlap with the Identifiler® kit (D2S1338, D19S433, TH01 and Amelogenin) as an internal check to reduce the possibility of mixing up samples. The Probability of Paternity Exclusion (Pe) in Caucasians for VeriFiler™ Direct + Identifiler® is 0.999999999 versus 0.999999200 with Identifiler® alone.

VeriFiler™ Direct is a direct PCR amplification kit and we have demonstrated that it amplifies multiple sample types including buccal swab lysates and samples on FTA paper substrates without the need for DNA extraction/purification. The kit is validated for direct amplification of 2ul of buccal swab lysates isolated with the Prep-n-Go™ lysis buffer and 1.2mm punches of Blood and Buccal samples on FTA paper substrates in a 25ul PCR reaction. In order to successfully amplify Buccal on FTA samples directly, the kit requires the addition of 2ul of Prep-n-Go™ lysis buffer to the FTA punch prior to the PCR.

The buffers, primer mix and PCR thermal cycling conditions have been optimized to deliver optimal peak heights on a 3130xl Capillary Electrophoresis instrument when amplified with the Veriti® 96-well Thermal Cycler or GeneAmp® PCR System 9700 with 96-well silver or gold-plated silver block. Kit specific panels and bins have been developed for the allelic ladder and sample genotyping can be performed using the GMID-X software.

Data will be presented demonstrating the performance of this assay. Experiments performed for the developmental validation study include: a) reproducibility within samples, b) assay sensitivity, and c) first pass success rate in achieving full profiles. ☘