

PCR for Porphyromonas gingivalis and fimA genotypes

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Abstract

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Protocol

Set up the following reaction on ice. PCR amplification consists of 4.5ul 10*PCR buffer, 0.25 mM of each dNTP, 10 uM of each primers, 5 ul of template DNA, and 1.5 units of Taq DNA polymerase, and sterile Tris-distilled water, to a total volume of 25 ul.

Step 1.

PCR amplification was carried out in a Tetrad Thermal Cycle. Each sample was amplified for 5 min at 94°C and 30 cycles, with each cycle consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 1 min, and final extension for 10 min.

Step 2.

The amplified products were then electrophoresed on 1.5% agarose gel in Tris-acetate buffer (40 mM Tris acetate, 1 mM EDTA, pH8.0). The products were visualized with ethidium bromide by UV transillumination.

Step 3.