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Quantitative analaysis of polymerase chain reaction products with high performance liquid chromatography and fluorometric detection

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Polymerase chain reaction (PCR) has been widely applied in basic and clinical research, as well as in the routine diagnosis of genetic, malignant and infectious diseases. PCR analysis is sensitive but generally qualitative. We have developed an automated method which can quantify PCR products, using High Performance Liquid Chromatography (HPLC). The assay can be completed in 15 min. Sample (20 µL) was injected into a weak anion exchange DEAE column, running at room temperature in the following stepwise linear gradient mode (buffer mobile phase pH 9 maintained throughout the HPLC run): 0-2 min 0.50-0.55 mol/L NaCl, 0.25 mmol/L tris buffer; 2-4 min 0.55-0.58 mol/L NaCl, 0.25 mmol/L tris buffer; 4-6 min 0.58-0.60 mol/L NaCl, 0.25 mmol/L tris buffer; 6-6.5 min 0.6-1 mol/L NaCl, 0.25 mmol/L tris buffer; 6.5-9 min 1 mol/L NaCl, 0.25 mmol/L tris buffer; 9-9.2 min 1.0-0.5 mol/L NaCl, 0.25 mmol/L tris buffer and 9.2-15 min 0.5 mol/L NaCl, 0.25 mmol/L tris buffer. The column was washed with 0.2 mol/L NaOH every 20 runs and separation efficiency was checked against a linearized pUC19 plasmid control. Within-run and day-to-day imprecision (CV%) for a 160 ng DNA standard were 0.7 and 2.4 respectively. The recovery of added DNA standard ranging from 8-160 ng was 100 ± 2%. Linearity was in the range of 2-4000 ng DNA standard sample load/injection. The detection limit of the PCR products was 1 ng when UV detection was used. When one primer was labelled at the 5'-end with fluorescein, the imprecision and recovery were similar to UV detection while the detection limit of the PCR products was 25-50 pg, using fluorometric detection. We conclude that quantitation of PCR products can be easily achieved by HPLC analysis and that fluorometric detection is 20-40 times more sensitive than UV spectrophotometric detection.