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Agilent Technologies

Application Note SI-02001

Synthesis of a primer test sequence using StratoSpheres™ DNA

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Introduction

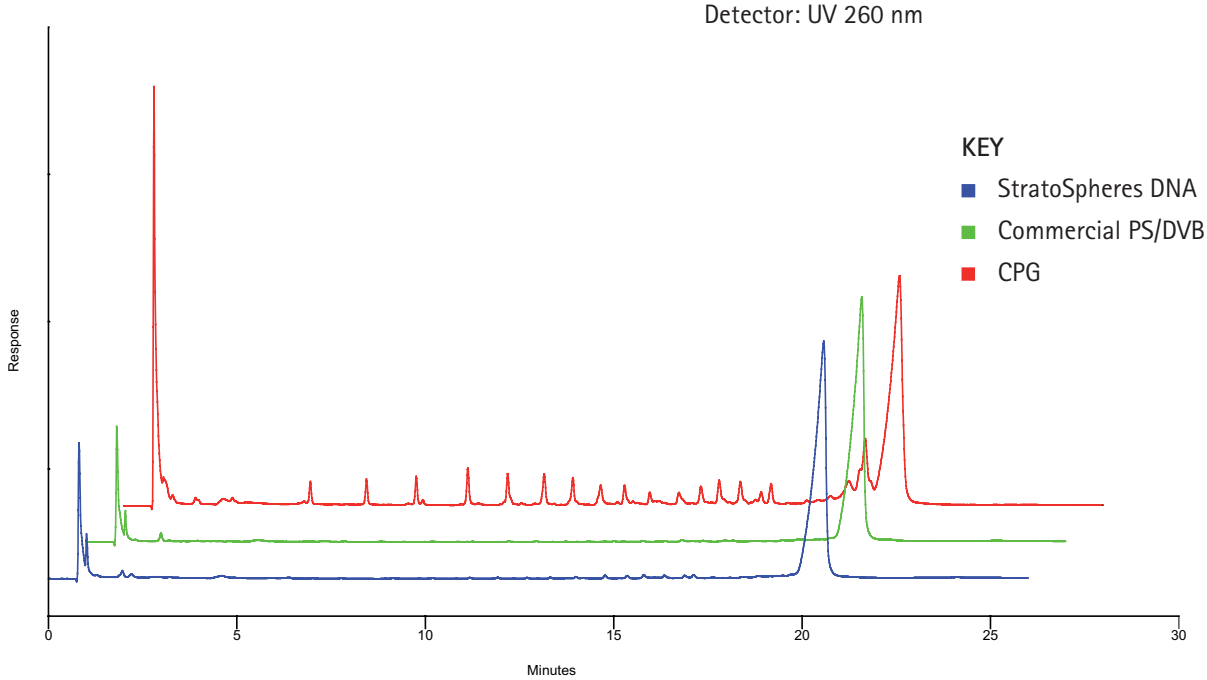
The synthesis of primer sequences for PCR is perhaps the most commonly used application for oligonucleotide synthesis. 5' ATA CCG ATT AAG CGA AGT TT 3' is a commonly used 20-mer test sequence for evaluating synthesis supports or automated instrumentation. The StratoSpheres DNA dT support matches the performance of the leading commercial macroporous PS/DVB.

Oligonucleotide Synthesis

The oligonucleotides were prepared on 0.2 µM scale using an Applied Biosystems 392 DNA/RNA Synthesizer and standard chemistry. Following synthesis, the oligonucleotides were cleaved using ammonium hydroxide. Deprotection of the side chain protecting groups (A, C and G) was accomplished by heating to 55 °C overnight as required. The "DMT on" oligonucleotides were diluted with water in order to give an on-scale response during HPLC analysis.

HPLC Analysis

Column: Pellicular SAX, 4 x 250 mm
Eluent: A = 25 mM Tris-HCl, 0.5% ACN, pH 8.0; B = 25 mM Tris-HCl, 0.8 M Ammonium Chloride, 0.5% ACN, pH 8.0
Gradient: 0 -100% B in 26 min
Flow Rate: 1.5 mL/min
Temp: 60 °C
Detector: UV 260 nm



J. Shanagar (2005) Purification of a synthetic oligonucleotide by anion exchange chromatography: method optimisation and scale-up. *J. Biochem. Biophys. Methods*, **64**, 216-225.

These data represent typical results.
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