Application Note SI-01998

Synthesis of ampR1 Primer using StratoSpheres™ DNA

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Introduction

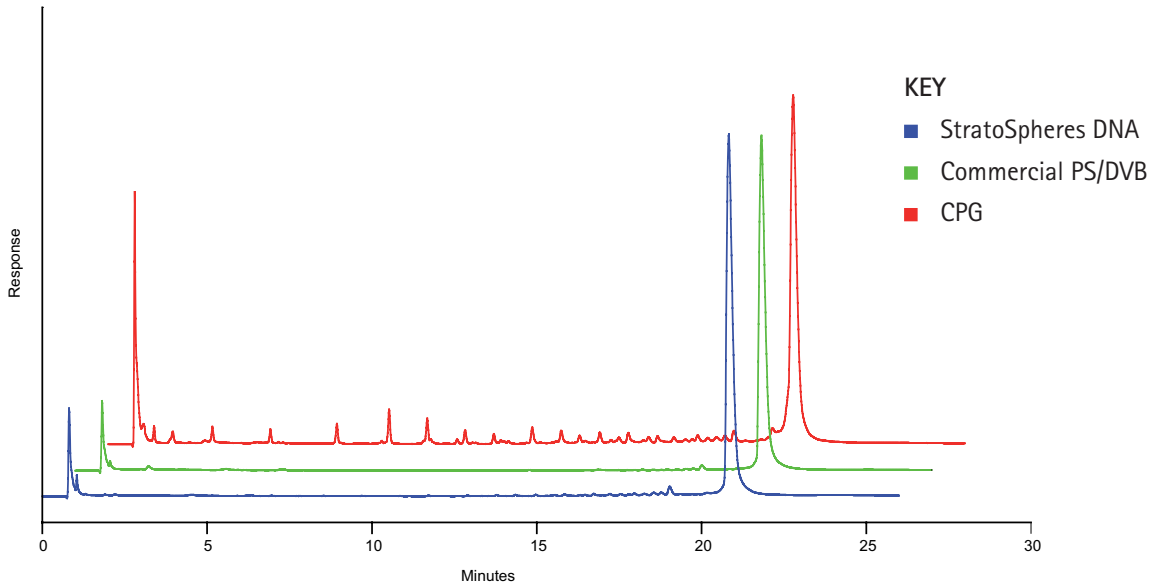
The synthesis of primer sequences (for PCR, cloning and sequencing applications) is perhaps the most commonly used application for oligonucleotide synthesis. The ampR1 (or SR1) reverse primer sequence 5' CTT TCT GCT ATG GAG GTC AGG TAT G 3' is recommended for use in sequencing and amplification with the pSMART cloning vector. The StratoSpheres DNA dG support matches the performance of the leading commercial macroporous PS/DVB in the synthesis of this primer 20-mer sequence, and exceeds the capability of a controlled pore glass support (CPG).

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



E. J. Summer *et al.* (2004) *Burkholderia cenocepacia* phage BcepMu and a family of Mu-like phages encoding potential pathogenesis factors. *J. Mol. Biol.*, **340**, 49-65.

These data represent typical results.
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