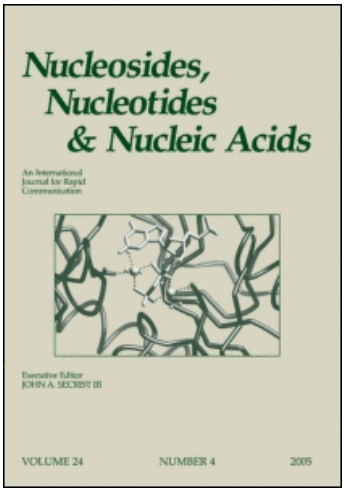


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# Significant Improvement of Quality for Long Oligonucleotides by Using Controlled Pore Glass with Large Pores

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# SIGNIFICANT IMPROVEMENT OF QUALITY FOR LONG OLIGONUCLEOTIDES BY USING CONTROLLED PORE GLASS WITH LARGE PORES

**Igor A. Kozlov, Minh Dang, Ken Sikes, Theofilos Kotseroglou, David L. Barker, and Chanfeng Zhao** ▫ *Illumina, Inc., San Diego, California, USA*

▫ *A key factor influencing the quality of long oligonucleotides is the choice of controlled pore glass (CPG) which is used as a solid support during oligonucleotide synthesis. We studied the influence of CPG pore size on the quality of 75-mer oligonucleotides. Using electrophoresis and HPLC, we demonstrated failure modes that can occur at certain oligo lengths with 1000A pore size, and compared yield and purity of 75-mer oligos using 1000A and larger pore size CPG. We showed that oligonucleotides with much better quality are obtained using CPG with pore sizes of 1400A and larger. We also identified the key characteristics for CPG selection that lead to the best CPG performance.*

**Keywords** Oligonucleotide Synthesis, Solid Support, Controlled Pore Glass



## INTRODUCTION

Availability of high quality long oligonucleotides is very important in large scale genomics research. One of the most common applications for long oligonucleotides is generation of spotted microarrays to monitor gene expression.[1,2] Due to the large-scale nature of this application, the oligonucleotides are most often used without purification. This creates a need for long oligonucleotides with maximal full-length products and minimal truncated by-products. We therefore investigated ways to improve our proprietary Oligator® DNA synthesis platform so that high-quality long oligonucleotides that can be manufactured and used without purification. We found that a key factor influencing the quality of long oligonucleotides is the choice of controlled pore glass (CPG) used as the solid support during phosphoramidite oligonucleotide synthesis. Most published reports suggest that 1000 Å pore size CPG is the best choice for synthesizing

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oligonucleotides longer than 30 nucleotides and that 2000 Å pore size CPG is best for oligonucleotides longer than 150 nucleotides.[3–5] There is also a report indicating that longer oligonucleotide sequences (50–150 bases) can be prepared on either 1000 Å or 2000 Å pore size CPG.[6] Since there is no clear indication which pore size of CPG is the best choice for the synthesis of oligonucleotides longer than 70-mers, we systematically studied the influence of CPG pore size on the quality of 75-mer oligonucleotides made using an ABI Expedite 8909 and Oligator DNA synthesizers.

## RESULTS AND DISCUSSION

Using 2000 Å CPG, we found that the coupling efficiency for monomers remains consistent during the synthesis of 75-mer oligonucleotides. We also found that the coupling efficiency decreased dramatically after 30 steps of synthesis when we used 500 Å CPG, and after 40–50 steps of synthesis using 1000 Å CPG. The syntheses of 75-mer oligonucleotides were performed at 1 μmol scale on an ABI Expedite 8909 synthesizer using different dT CPG. Typical trityl deprotection reports from the syntheses of 75-mer oligonucleotides using CPG with different pore sizes and separation profiles of the products obtained by electrophoresis in polyacrylamide gel are shown in Figure 1.

We calculated the yields of full length 75-mer oligonucleotides using final DMT deprotection data and absorbance of gel-purified oligonucleotide solution at 260 nm. The yields of oligonucleotides were calculated based on absorption of the

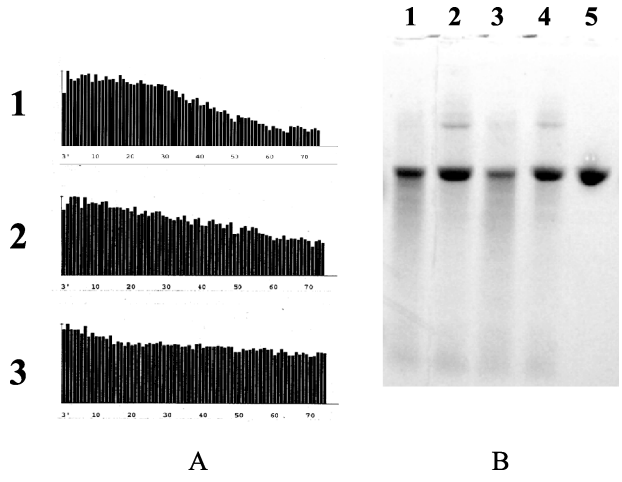
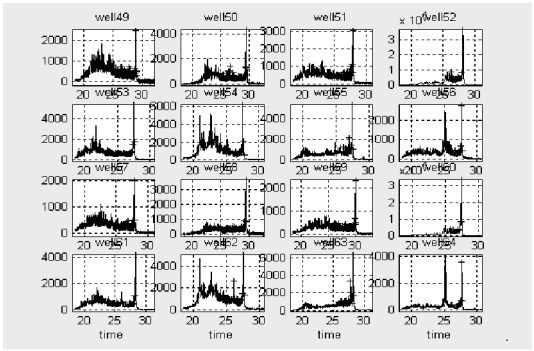



**FIGURE 1** (A) Trityl deprotection reports from the syntheses of 75-mer oligonucleotides at 1-mmol scale on ABI Expedite 8909 DNA synthesizer using dT CPG with different pore sizes. 1, 500 Å CPG; 2, 1000 Å CPG; 3, 2000 Å CPG; (B) 15% 7M urea PAGE analysis of 75-mer oligonucleotides at 1-μmol scale on ABI Expedite 8909 synthesizer using dT CPG with different pore sizes. Lanes 1 and 3, 1000 Å CPG; lanes 2 and 4, 2000 Å CPG; lane 5, gel-purified 75-mer oligonucleotide.

DMT group using the extinction coefficient ε = 70,000 at 500 nm. The typical yields obtained with 1000 Å CPG were in the range of 100–150 nmols while the yields obtained with 2000 Å CPG were 300–500 nmols. The yields for gel-purified oligonucleotides were calculated based on absorbance of the oligonucleotide solution at 260 nm. The typical yields for gel-purified oligonucleotides obtained with 1000 Å CPG were in the range of 30–40 nmols while the yields for oligonucleotides obtained with 2000 Å CPG were 70–80 nmols. Both methods indicate that 2000 Å CPG generates twice as much full length 75-mer oligonucleotide compared to 1000 Å CPG. We believe that the CPG with larger pore size allows more space within its pores for growing long oligonucleotides and enables better flow of the reagents during later steps of the synthesis.

We also compared the performance of CPG with different pore sizes using the Oligator Farm® oligonucleotide manufacturing facility at Illumina. Illumina's highly automated and fully integrated Oligator Farm is one of the largest oligonucleotide synthesis facilities in the world, with a current capacity of greater than



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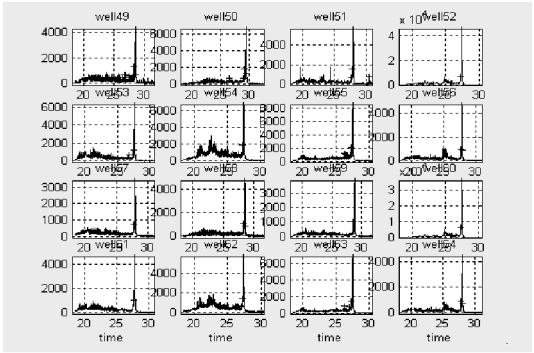


B

**FIGURE 2** Comparison of CE analysis profiles for the same 75-mer oligonucleotides synthesized using 1000 Å (A) and 1400 Å CPG (B). Corresponding diagrams on panels A and B represent the separations for 75-mer oligonucleotides with the same sequences.

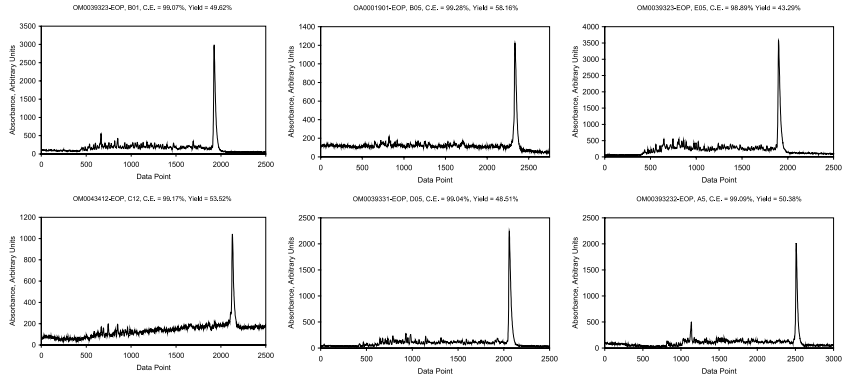


**FIGURE 3** Synthesis of 75-mer oligonucleotides using 1400 Å pore size CPG at Illumina's Oligator Farm® facilities. The typical CE profiles of different 75-mer oligonucleotides are shown. The profiles were provided by Illumina's QC/QA department.



17 million 20-mer oligonucleotides per year. Illumina has deployed two models of its proprietary DNA synthesizers that perform oligonucleotide synthesis in 96- or 384- well plates.[7,8] We collected substantial data indicating much better quality of long oligonucleotides synthesized using 2000 Å CPG comparing to traditionally used 1000 Å CPG. However, the 2000 Å CPG typically has low loading (typical units are μmol/g) of monomers on its surface. Most high throughput synthesis systems dispense a certain amount of CPG by volume before the synthesis starts. This means that CPG with low loading will generate less product. This is a significant drawback to the use of this type of CPG in an industrial oligonucleotide production environment. Through further investigation, we found that certain lots of 1400 Å CPG provide the ideal combination of reasonably high monomer loading and ability to yield oligonucleotides with quality comparable to that obtained using 2000 Å CPG. A comparison of capillary electrophoresis (CE) analysis profiles for the same 75-mer oligonucleotides synthesized using 1000 Å and 1400 Å CPG is shown in Figure 2.

The data show that optimized 1400 Å pore-size CPG generates long oligonucleotides without the truncation problems found with 1000 Å CPG. During mid-2004, Illumina produced 30,372 oligonucleotides of lengths between 70 and 80 nucleotides using 1400 Å CPG. Typical CE profiles of different 75-mer oligonucleotides are shown in Figure 3. These indicate high-quality oligonucleotides with extremely high monomer coupling yields.

## CONCLUSIONS

The use of 1400 Å CPG enables the synthesis of long oligonucleotides with higher yield than obtained with 2000 Å CPG and better quality than obtained using 1000 Å CPG. We also identified the key characteristics for CPG selection that lead to the best CPG performance. These finding combined with continuous

research and development efforts and unique proprietary Oligator DNA synthesis technology give Illumina the capability to produce oligonucleotides with the highest quality available today.

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