

ULTRAmers™

Go the Distance



ABSTRACT

ULTRAMERS™ - THE LONGEST OLIGONUCLEOTIDES AVAILABLE WITH MASS SPECTROMETRY

Shawn D. Allen¹, Tina M. Luebke¹ and Scott D. Rose Ph.D.²

¹Synthetic Biology Department

²Molecular Genetics and Biophysics Department

Integrated DNA Technologies (IDT) has pioneered methods to synthesize and QC oligonucleotides of extreme length. Our R&D efforts resulted in improvements in both synthesis and QC methods that, taken together, permit commercial sale of oligonucleotides up to 200 bases in length. We call this new class of ultra high-quality long oligonucleotides "Ultramers". Ultramers have been used extensively by IDT's Synthetic Biology group for the de-novo manufacture of mini-genes.

The Synthetic Biology group routinely uses standard desalt Ultramers for site-directed mutagenesis. Ultramers are ideal candidates for use as templates for the synthesis of RNA (in-vitro transcription), and in a variety of other molecular biology applications that require high purity oligonucleotides ranging in length from 60-200 bases. The Ultramer product line contains the option of multiple 5' modifications as well as a number of internal modifications. For those needing an even higher quality oligonucleotide, IDT offers PAGE-purified Ultramers.

Ultramers™ - The Longest Oligonucleotides Available with Mass Spectrometry QC

Shawn D. Allen¹, Tina M. Luebke¹ and Scott D. Rose Ph.D²

¹Synthetic Biology Department

²Molecular Genetics and Biophysics Department

While enzymatic synthesis of nucleic acids has an extremely low error rate in the range of 10^{-6} – 10^{-7} (the native error rate of proof-reading eukaryotic DNA polymerases), chemical synthesis of DNA usually proceeds with a fidelity of around 99%, or 10^{-2} (the average efficiency of each base coupling step). This is fully ten thousand to a hundred thousand-fold less efficient and precise than biological systems. Thus, while cells can replicate chromosomes that exceed megabase size, chemically synthesized nucleic acids (oligonucleotides) are usually limited to a size range of 5-120 bases length. Although attempts have been made to synthesize longer oligonucleotides, successful commercial implementation has been hampered by the absence of any good QC tools to guarantee the quality of the final product.

Integrated DNA Technologies (IDT) has pioneered methods to synthesize and QC oligonucleotides of extreme length. Our R&D efforts resulted in improvements in both synthesis and QC methods that, taken together, permit commercial sale of oligonucleotides up to 200 bases in length and synthesis in an R&D setting of 300mers. We call this new class of ultra high-quality long oligonucleotides "Ultramers".

How do we synthesize "Ultramers" and what innovations were necessary to permit both synthesis and QC of a product like this? IDT employs proprietary DNA synthesis equipment (of our own design and manufacture) which permits rapid, high quality synthesis of nucleic acids. This platform can be adapted to a lower throughput mode with an "extra rich" synthesis cycle. Our R&D team has developed synthesis chemistry and cycles which improve coupling efficiency to average above 99.5% through an entire 200 base synthesis reaction. Along with improvements in the synthesis cycle, we developed a unique solid support which is specifically optimized for synthesis of low-yield, high-quality oligos of 200 bases or more in length.

Coupling Efficiency vs. Full Length Product

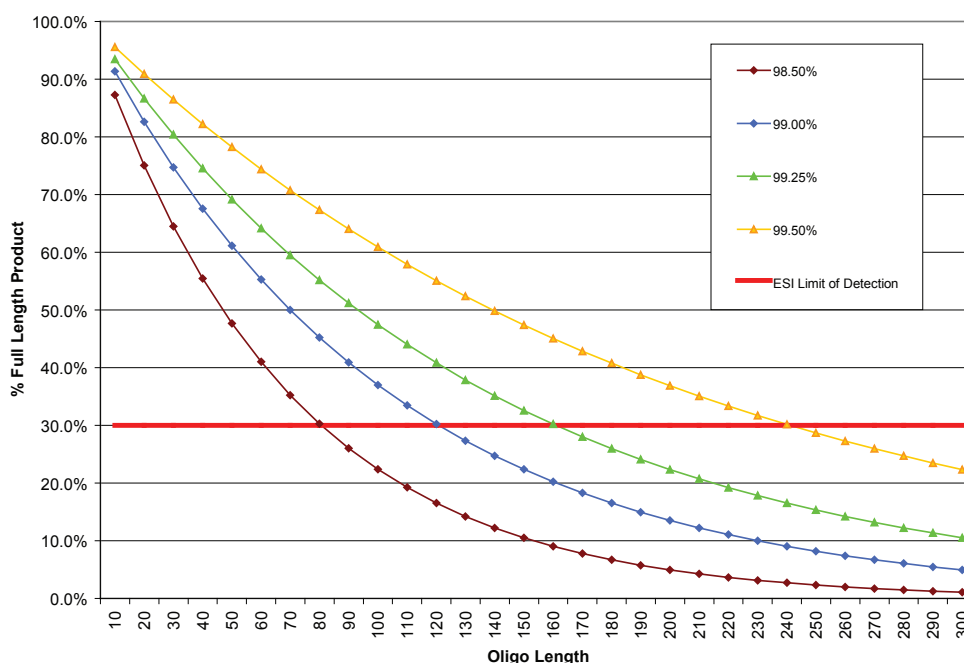


Figure 1

Each cycle of chemical synthesis proceeds with a finite coupling efficiency. The first base is attached to the solid support, thus a 20mer requires 19 coupling reactions and a 200mer requires 199 coupling reactions. Overall synthesis yield of full-length product is a function of both coupling efficiency and length according to the relationship:

$$\text{Full length product} = (\text{eff})^{(n-1)}$$

where eff = coupling efficiency (for example, 99.5% = 0.995) and (n-1) is the number of coupling reactions needed to make an oligo of length n. The relationship between yield (which directly relates to overall quality of a crude or unpurified synthesis product) and coupling efficiency is plotted in Fig. 1.

As demonstrated by the above graph (Figure 1), the only way to synthesize a 200-mer oligonucleotide with an appreciable yield of full length material (>30%) is to have a step wise coupling efficiency >99.25%.

It is not reasonable to sell a product commercially unless there is some method available to QC the final product. What methods are available to QC an oligonucleotide synthesis, especially in this size range? Typically, mass spectrometry is considered the gold standard for oligonucleotide synthesis QC. MALDI mass spectrometry is effective for products in the 10-50 base range. Above 50 bases, ESI (electrospray ionization) mass spectrometry can be employed and has an accuracy of better than 0.02% relative mass. Even with the sensitivity and accuracy of ESI-MS, performing QC on long oligonucleotides is complex. If a synthesis preparation is too impure, the various failure products will obscure the mass spectra, making it difficult to deconvolute and identify the desired species. In this case the old adage “garbage in, garbage out” is very true. Further, natural isotopic variation begins to make peaks broader or more imprecise for each sequence, so a functional limitation exists which is not dependent upon synthesis quality. Using our proprietary mass spectrometry methods, we can confidently synthesize and provide ESI-LC-MS quality documentation for oligonucleotides up to 200 bases in length. Although we have successfully made Ultramers in the 250-300 base range, we are unable to confidently provide QC and therefore do not offer these products commercially. ESI mass spectra for two Ultramers are shown in Figures 2 and 3 below.

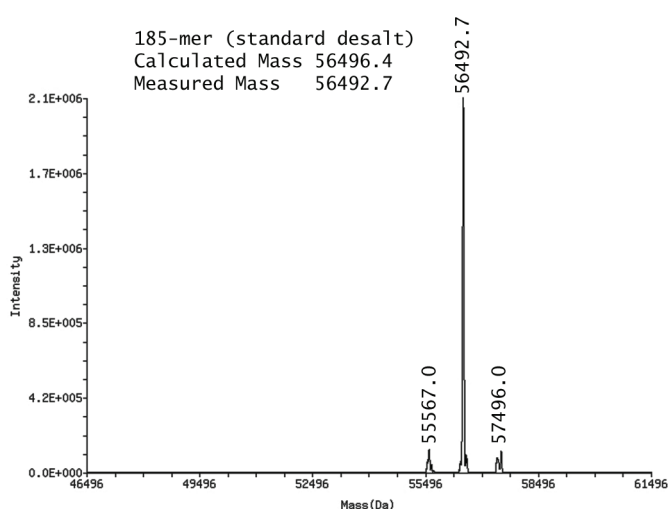


Figure 2

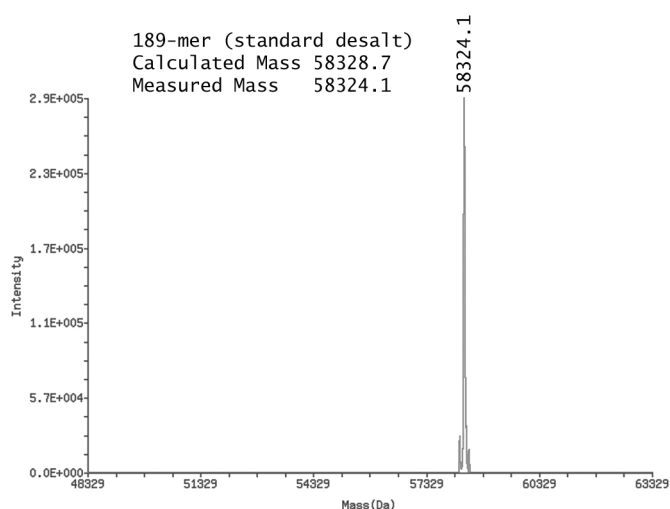


Figure 3

IDT's Custom Gene Synthesis group has pioneered the use of Ultramers for de novo gene construction. Many of the published procedures for constructing specific DNA sequences from oligonucleotides employ relatively short sequences (60 mers or less) with overlapping stretches of common sequence that permit annealing. The desired sequences are usually assembled using a PCR-based strategy^{1,2}. While these procedures work well for sequences of high complexity, cloning sequences containing regions of low complexity, such as direct or inverted repeats and homopolymeric runs, can be very difficult or impossible. Unless regions of unique sequence conveniently exist that permit overlaps to be placed at the necessary positions, the desired sequences can be very difficult to clone without deletions or insertions introduced by the PCR reaction. Ultramers can be designed to span long regions of low complexity in a single oligonucleotide and still leave room for unique terminal sequences

for assembly. Ultramers are also ideal for use in DNA-Directed RNAi (ddRNAi), where a duplex containing sequences capable of forming hairpins is cloned into an expression vector, such as Promega's siSTRIKE™ U6 Hairpin Cloning System—hMGFP.

Ultramers can be used to efficiently and quickly generate large insertions, deletions or change stretches of sequence identity by using site-directed mutagenesis. IDT has routinely inserted >100 bases into the middle of existing plasmid clones in a single round of mutagenesis using non-purified desalted Ultramers.

Absolute Quantitative PCR (qPCR) utilizes copy number standards made from either cloned amplicons or PCR products to create standard curves. Ultramers can serve as DNA standards in qPCR reactions or can be used as templates for in-vitro RNA synthesis.

IDT currently offers Ultramers with the following modifications as either standard desalted (yield 3 nmoles) or PAGE purified (for yield please inquire):

5' Phosphate	Inosine
5' Amino C6 and Amino C12*	Uridine
5' Biotin*	N-degenerate base

siSTRIKE™ is a trademark of the Promega Corporation

*Available only on standard desalted Ultramers

¹ Ai-Sheng Xiong, Quan-Hong Yao, Ri-He Peng, Xian Li, Hui-Qin Fan, Zong-Ming Cheng and Yi Li, A simple, rapid, high-fidelity and cost-effective PCR-based two-step DNA synthesis method for long gene sequences. *Nucleic Acids Research*, 2004, Vol. 32, No. 12 e98

² Ai-Sheng Xiong, Quan-Hong Yao, Ri-He Peng, Hui Duan, Xian Li, Hui-Qin Fan, Zong-Ming Cheng & Yi Li, PCR-based accurate synthesis of long DNA sequences. *Nature Protocols*, 2006, Vol.1, No. 2 pp.791-797

COMPANY ABOUT IDT

Since 1987, Integrated DNA Technologies (IDT) has been a major force in advancing biotechnology research both as a leading supplier of custom oligonucleotides and a developer of innovative new biotechnologies. In addition to the manufacturing headquarters in Coralville, Iowa USA, IDT has facilities in San Diego, California and Leuven, Belgium.



IDT World Headquarters, Coralville, IA USA