

Ultramer[®] Oligonucleotides

Long Oligos with Mass Spectrometry QC

Integrated DNA Technologies (IDT) has pioneered methods to synthesize long oligonucleotides and assess them individually by mass spectrometry for quality control (QC). Our research and development efforts have enabled improvements in synthesis and QC methods that permit manufacture of Ultramer[®] oligonucleotides—high quality, long oligos up to 200 nucleotides (nt) in length. Ultramer Oligonucleotides form the basis of synthetic biology products from IDT, including gBlocks[®] Gene Fragments, Genes, and MiniGene[™] Synthetic Genes.

Standard desalted Ultramer Oligonucleotides are perfect for site-directed mutagenesis, for use as templates for in vitro transcription, and in a variety of other molecular biology applications that require high purity oligonucleotides of length 45–200 nt. For applications in which higher purity is required, IDT offers PAGE purification.

Enzymatic synthesis of nucleic acids has an extremely low error rate in the range of 10^{-6} – 10^{-7} (the native error rate of proofreading eukaryotic DNA polymerases); however, chemical synthesis of DNA usually proceeds with a fidelity of only approximately 99%, or 10^{-2} (the average coupling efficiency of each nucleoside phosphoramidite), making it 10,000- to 100,000-fold less efficient and precise than biological systems. Thus, while cells can replicate chromosomes of megabase lengths, chemically synthesized nucleic acids (oligonucleotides) are usually limited to lengths of 5–120 nt.

IDT has pioneered synthesis and QC methods that permit commercial production and mass spectrometry QC of oligonucleotides up to 200 nt in length. These long oligos, called Ultramer[®] Oligonucleotides, are synthesized using proprietary oligonucleotide synthesis equipment, designed and manufactured by IDT, that permit rapid, high quality synthesis of nucleic acids. IDT synthesis chemistry and cycles have an average coupling efficiency >99.5% through an entire 200-base synthesis reaction. A unique solid support, also developed at IDT, is specifically optimized for synthesis of low-yield, high quality oligos of lengths in excess of 200 nt.

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 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 Figure 1. The graph demonstrates that an appreciable yield of full-length material (>30%) for a 200mer oligonucleotide can only be achieved if the stepwise coupling efficiency is >99.25%.

To facilitate commercialization of these Ultramer Oligonucleotides, IDT developed a proprietary QC method that allows us to guarantee the product. Typically, mass spectrometry is considered the gold standard for QC of

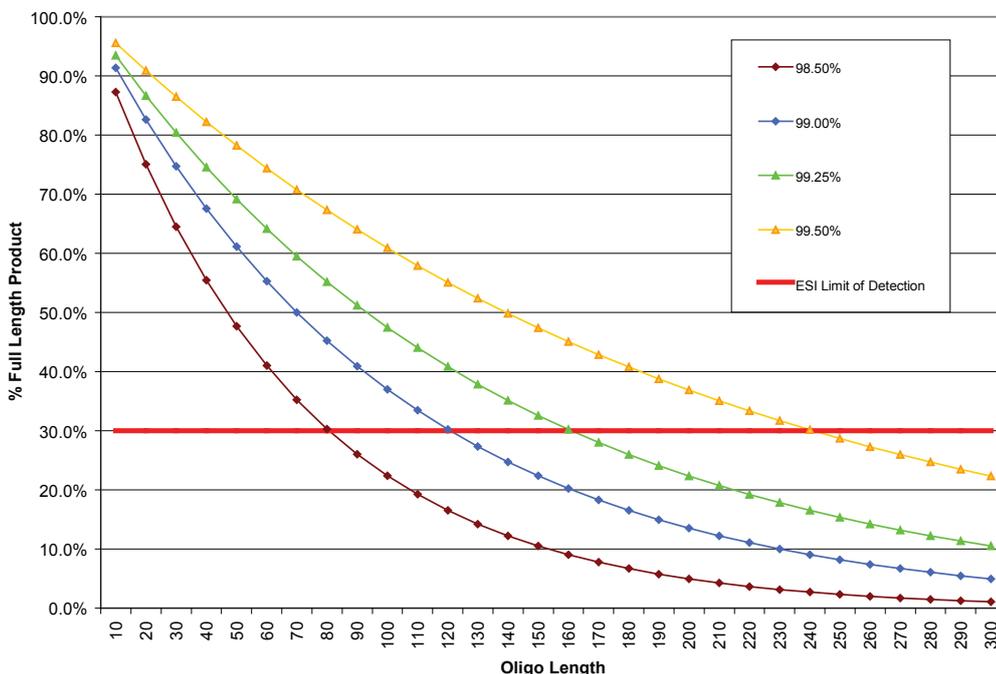


Figure 1. Oligonucleotide Yield is Determined by Coupling Efficiency.

oligonucleotide synthesis. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is effective for products of length 10–50 nt. Electrospray ionization (ESI) mass spectrometry, which has an accuracy better than 0.02% relative mass, can be used for oligos ≥50 nt. However, even with the sensitivity of ESI mass spectrometry, performing QC on long oligonucleotides is complex. When a synthesis preparation contains high proportions of truncated prod-

uct, the various failure products obscure the mass spectra, complicating identification of the desired species. Additionally, natural isotopic variations make peaks broader or more imprecise for each sequence, introducing a functional limitation that is independent of synthesis quality. IDT proprietary mass spectrometry methods enable provision of ESI documentation for oligos up to 200 nt. ESI mass spectra for 2 Ultramer Oligonucleotides are shown in Figure 2.

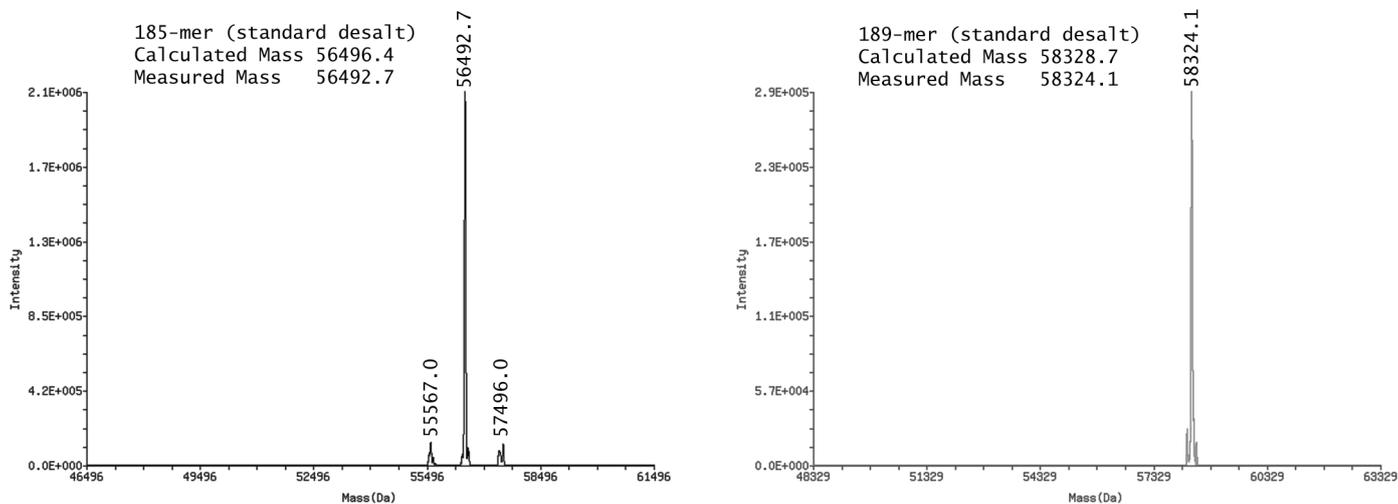


Figure 2. ESI Mass Spectra Show High Purity of Two Ultramer® Oligonucleotides. (A) 185mer; (B) 189mer.

Ultramer® Oligonucleotides—Long Oligos with Mass Spectrometry QC

The Custom Gene Synthesis group at IDT pioneered the use of Ultramer Oligonucleotides for *de novo* gene construction. Many of the published procedures for constructing specific DNA sequences from oligos use relatively short sequences (≤ 60 mer) with overlapping stretches of common sequence that permit annealing, followed by assembly using PCR-based strategies [1,2]. While these procedures work well for oligos of high sequence variability, they often fail for sequences containing regions of low complexity, such as direct or inverted repeats and homopolymeric runs. It is difficult to avoid deletions and insertions introduced by the PCR-based methods if there exist no conveniently located regions of unique sequence that allow overlaps to be placed at the necessary positions. Ultramer Oligonucleotides, however, can be designed to span long regions of low complexity in a single oligonucleotide while leaving room for unique terminal sequences for assembly.

Ultramer Oligonucleotides are also suited for a wide variety of other molecular biology applications. They are useful in DNA-directed RNAi (ddRNAi), where a duplex containing sequences capable of forming hairpins is cloned into an expression vector, such as GeneClip™ Hairpin Cloning

System—hMGRP (Promega) and BLOCK-iT™ shRNA entry vectors (Life Technologies). Ultramer oligos can also be used to efficiently and quickly generate large insertions or deletions, and for site-directed mutagenesis. Scientists at IDT routinely insert >100 nucleotides into existing plasmid clones in a single round of mutagenesis using unpurified, desalted Ultramer Oligonucleotides. Additionally, Ultramer oligos can replace cloned amplicons and PCR products as copy number standards when creating standard curves for qPCR, and can be used as templates for *in vitro* transcription during RNA synthesis.

Ultramer Oligonucleotides are offered as either standard desalted (4 or 20 nmole yield) or PAGE-purified (yields are reduced upon purification), with the following modifications:

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

* Available only for standard desalted Ultramer® Oligonucleotides

References

1. Xiong AS, Yao QH, et al. (2004) A simple, rapid, high-fidelity and cost-effective PCR-based two-step DNA synthesis method for long gene sequences. *Nucleic Acids Res*, 32(12):e98.
2. Xiong AS, Yao QH, et al (2006) PCR-based accurate synthesis of long DNA sequences. *Nat Protoc*, 1(2):791–797.