# Comparison of Biotage and Competitor Oligonucleotide Synthesis Methodologies

Synthesizing oligonucleotides often leads to the production of impurities that can be detrimental to downstream applications. These impurities are typically shorter, truncated versions of the full-length oligonucleotide. To ensure high-quality results, the solid phase synthesis and chromatographic purification processes must be carefully optimized and precisely controlled to minimize the presence of truncated side-products, leaving the oligonucleotide in a highly pure form. Biotage recognizes the need for continuous optimization of our synthesis and purification processes to ensure that our oligonucleotides are of the highest quality. This application note compares a Biotage oligonucleotide to the same sequence supplied by another vendor.

## The Problem with Impurities

When Synthesizing an oligonucleotide each iteration of the synthesis cycle can result in a small amount of incomplete coupling which leads to the formation of shorter failure sequences as by-products, i.e., oligonucleotide missing one or more nucleotides, so N -1 nucleotide, -2 nucleotides, - 3 nucleotides and so on. If these failure sequences are not removed their presence can have negative impacts on downstream applications such as PCR or enzymatic reactions, interfering with the target molecule's performance and distorting results. Furthermore, these impurities possess intrinsically similar chemical and physical properties to the desired full-length sequence and hence achieving chromatographic separation on a preparative scale is highly challenging. As such, in order to obtain high purity oligonucleotides a two-fold strategy is required: firstly, it is essential that all parameters associated with oligonucleotide synthesis are carefully controlled to ensure maximum coupling efficiency and minimal formation of side-products; secondly the purification process must be carefully optimized for each individual oligonucleotide sequence in order to ensure maximum resolution of any impurities which do form.

## High Quality Oligonucleotide Preparation Techniques

At Biotage, we use our specialist chemical knowledge to ensure maximum efficiency during each step of the oligonucleotide synthesis cycle, with minimal side-product formation. In addition, we have developed a range of proprietary purification techniques designed specifically for removing failure sequences and other common impurities from synthesized oligos and delivering the highest levels of purity possible. Our expertize in purification ensures that our services consistently deliver extremely high-quality molecules at an affordable price point for our customers. Furthermore, investigations into materials from competitor companies have shown that our techniques are far superior when it comes to producing pure molecules free from unwanted side-products or contaminants.



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Figures 1 and 2 show comparative capillary electrophoresis (CE) traces of the same oligonucleotide sequence – a 74-mer oligonucleotide containing standard phosphorothioate and 5'-phosphate modifications – synthesized by Biotage and also by a competitor: the presence of a series of deletion sequences is evident in the electropherogram of the competition sample. Final purities of the two samples as delivered to the customer were ›95% for the Biotage material and ›65% for the competitor.

## Conclusion

In conclusion, utilizing proper purification techniques is essential for ensuring high-quality synthesized oligonucleotides free from any unwanted by-products or contaminants.
At Biotage, we leverage our expertize in purification technologies to provide highly pure molecules that you can confidently apply to your research and development projects without worry about potential problems caused by unwanted impurities in your sample. Investigate our range of products today and see how we can help you achieve success with your next project.

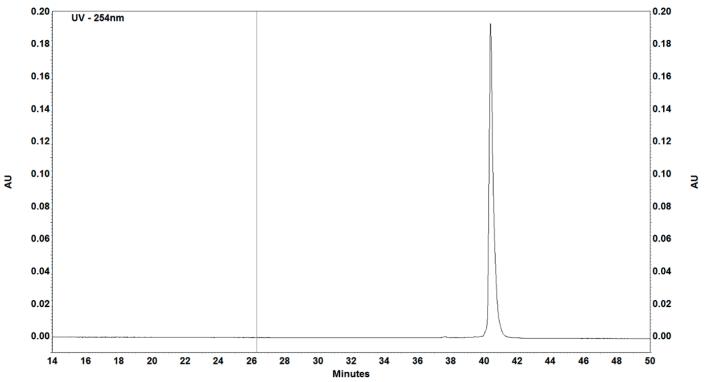


**Figure 1.** CE trace of an oligonucleotide synthesized by Biotage at >95% purity. The gel used for the CE analysis was ssDNA 100r kit and the length of the capillary was 30.2 cm. The voltage of separation that was be 9.0 kV over 60 min.

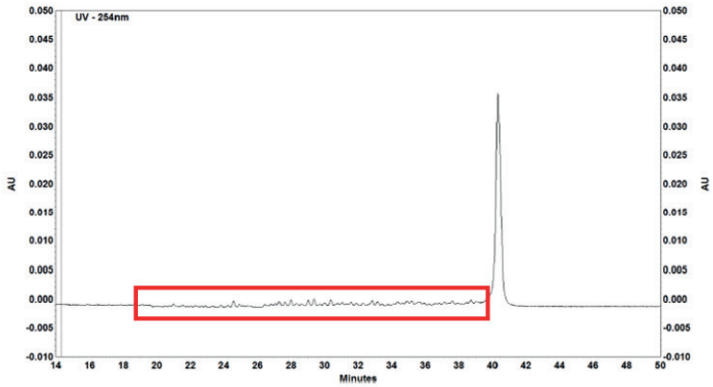


**Figure 2.** CE trace of the same oligonucleotide synthesized by a competitor at >65% purity. The gel used for the CE analysis was ssDNA 100r kit and the length of the capillary was 30.2 cm. The voltage of separation that was be 9.0 kV over 60 min.

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