

## Efficient semi-preparative and analytical chromatography for the high throughput production of oligonucleotides

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Besides being the major provider for genotyping and DNA sequencing services, Eurofins Genomics Europe Pharma and Diagnostic Products & Services Synthesis GmbH is one of the world-leading oligonucleotide manufacturers for the industrial and research sector. Several different classes of standard and highly modified oligonucleotides are produced via solid phase oligonucleotide synthesis using the phosphoramidite approach [1] in a high throughput manner with short turnaround times (TAT). The portfolio is ranging from fluorescent probes for qPCR applications over Unique Dual Index (UDI) primer sets for state-of-the-art Next Generation Sequencing (NGS) methods to custom DNAs and RNAs.[2]

Since 2021, Eurofins Genomics can capitalise of next generation high throughput synthesizers, the so-called Continuous Flow Synthesizer (CFS), which allow a simultaneous production of 6 x 384 different oligonucleotides with elevated speed reaching an excellent coupling efficiency of up to 99.6%. This immensely reduces the need of purification, however the requirements for several products move towards high purity and in-depth analytics. Especially the exact impurity profiling of active pharmaceutical ingredients (API) is mandatory while going through clinical phases. Thus, a fast and accurate procedure to purify and analyse oligonucleotides is inevitable to fulfil the needs of the vastly growing business.

Here, we present an elaborated upstream and downstream process for the high throughput production of standard and modified oligonucleotides of length up to 200 nucleotides. The focus is set on the sample purification and analysis via RP-U/HPLC exploiting the Thermo Scientific™ Vanquish™. Commonly, the device is used in combination with customary C18 columns to obtain highly resolved analytical chromatograms for an unambiguous sample composition determination, however a semi-preparative application for the purification of dual-labeled oligonucleotides could successfully be demonstrated.[3] In addition, we show the crucial role of LC-ESI-MS investigation on a modified DNA longmer leading to an optimized production strategy and hence resulting in a significantly improved impurity profile which massively simplifies the subsequent chromatographic purification.

Literature:

[1] M.H. Caruthers et al. Chemical synthesis of deoxyoligonucleotides by the phosphoramidite method, *Methods in Enzymology*, 1987, 154, 287-313

[2] Oligonucleotide Synthesis - Portfolio overview, *Eurofins Genomics*, <https://eurofinsgenomics.eu/en/dna-rna-oligonucleotides/portfolio-overview/>, Accessed 11 March 2023

[3] Thermo Scientific Customer Application Note 001641, Semi-preparative reversed-phase liquid chromatographic purification of oligonucleotides, <http://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/cn-001641-hplc-vanquish-fraction-collection-purification-oligos-eurofins-cn001641-na-en.pdf>

## Biography

With more than seven years of experience in the field of oligonucleotides, Dr. Johannes Kremser has a proven track record of successful project management and producing quality outcomes through professional expertise and team motivation. He truly enjoys working with his clients to determine requirements and provide excellent service. His advanced education began in Innsbruck at the Leopold-Franzens University where his bachelor thesis in Organic Chemistry was supervised by Univ. Prof. Ronald Micura. Upon completion of his bachelor's degree in 2014, he continued there under the direction of Assoc. Prof. Christoph Kreutz where he defended his masters in Organic Chemistry in 2016. His passion for oligonucleotide chemistry in combination with NMR spectroscopy took him abroad to London as a visiting doctoral student at the University College London where in the Department of Structural and Molecular Biology guided by Dr. D. Flemming Hansen, his project focused on RNA entitled 'Elucidation of transiently formed excited state RNA structures by NMR spectroscopy'. With his successes in the UK, he returned in 2019 to his alma mater of Leopold-Franzens University to produce and defend his doctoral thesis "'Seeing the Invisible' - Investigation of RNA Excited States via NMR Spectroscopy" under supervision of Assoc. Prof. Christoph Kreutz.

Johannes transitioned from his academic career into his professional debut as the group leader in R&D for oligonucleotides in Switzerland at Bachem AG where he was responsible for a team which ranged from equipment qualification, customer care, and the creation of GMP-conform documents to the pilot scale production with a focus on oligonucleotide downstream and process development for therapeutic oligonucleotide GMP production. His subsequent and present employment with Eurofins Genomics in Ebersberg commenced in 2022 as Senior Scientist R&D for Oligonucleotides and has the dedication to manage the laboratory and process planning for RNA high throughput and mid-scale production and ensure ISO-conform oligonucleotide production. Furthermore, he develops and executes pilot projects for priority customers and as a business liaison on external events and representative here with the HPLC symposium: innovation transfer and acquisition of collaboration partners as scientific conference attendee.