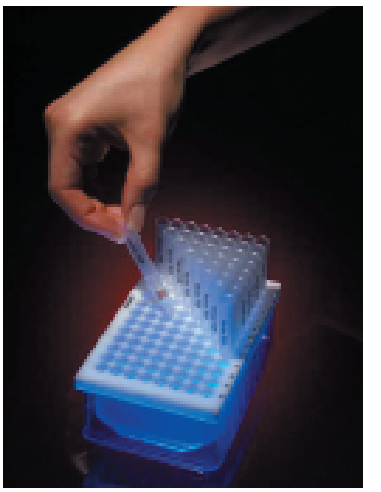
High-Throughput Purification System for Oligonucleotides Improves Synthetic Gene Production



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Synthetic genes have become fundamental tools for biological research and development in laboratories around the world. They are becoming increasingly important in the development of clinical diagnostics as well. Such genes are produced by assembling oligonucleotides into larger pieces of DNA by ligation. For a synthesized gene to be useful in molecular biology, its sequence must be 100% accurate. Assembly errors will introduce mutations into the final product that are costly to correct. The most direct solution for reducing mutations in the final gene is to use purified, error-free oligonucleotides containing no n-1 failure sequences. However, traditional oligo purification techniques — such as high-performance liquid chromatography (HPLC) and polyacrylamide gel electrophoresis (PAGE) — provide only limited throughput and require high capital equipment expenditure and/or labor costs.

Blue Heron Biotechnology (http://blueheronbio.com) needed a high-throughput tool to purify large quantities of oligonucleotides. The company uses its proprietary, fully automated GeneMaker technology to produce synthetic full-length genes and fragments on a fee-for-service basis to biotechnology and pharmaceutical customers. To ensure 100% sequence fidelity for its customers, Blue Heron partnered with Varian, Inc. (www.varianinc.-com), to develop a high-throughput, simple, and cost-effective solution for oligo purification. The Trityl-on Oligonucleotide Purification (TOP) product leverages Varian's sorbent chemistry and 96-well plate technology with Blue Heron's synthetic oligo and gene synthesis expertise. TOP was developed to balance speed, cost, yield, and purity by incorporating a unique 96-well plate with removable tubes, a streamlined gravity flow procedure, and a high-affinity polymeric resin.

High-Throughput Format

In manual oligo purification, TOP coupled with commercially available multichannel pipettes increases productivity by reducing the number of pipetting steps. Up to 96 oligos can be purified simultaneously in 45 minutes using the full-plate option. When used with a liquid handling system, TOP can process multiple plates unattended. It can purify thousands of high-quality oligos per day, increasing accuracy and precision by reducing the incidence of operator error.

Unlike standard fixed 96-well designs, TOP was engineered with a modular format that consists of a reusable base plate and removable tubes. The ability to customize the plate and purchase individual tubes reduces the overall cost per oligo by eliminating wasted-well or cross-contamination issues associated with fixed-well designs.

Gravity Flow Procedure

TOP's gravity flow procedure facilitates sample handling and improves robotic system compatibility. Processing multiple samples on conventional cartridge purification devices requires constant stopcock and vacuum control manipulation to avoid accidentally drying the resin and adversely affecting results. That is especially important when using a 96-well plate system on an automated workstation where monitoring and manipulating individual wells is difficult. TOP's gravity flow capability provides high yields, improved well-to-well reproducibility, and a simple, walk-away process for purification.

The streamlined procedure balances high performance with increased throughput. It uses one-third the solvent of similar products, significantly reducing disposal and reagent costs and maximizing space on robotic platforms — enabling more plates to be processed without manual intervention. Throughput increases also are realized during the postpurification evaporation stage. The collection solvent requires less total water at the outset, which can cut lyophilization times by up to eight hours.

High-Affinity Polymeric Resin

TOP's binding efficiency is based on a highly pH-stable polymeric resin that permits direct loading of the deprotection solution. Obviating the traditional evaporation step for the basic ammonia sample before purification saves up to four hours. This also ensures more reproducible yields by reducing the risk of accidentally removing the trityl group. The chemistry of the resin eliminates the need to reprocess samples for multiple passes to increase yield, an all too common makeshift technique used with other cartridge based systems. Single loading makes this a more robot-compatible product,

reduces hands-on time, minimizes the cost of collection tubes and pipette tips, and increases yields by reducing the number of transfer steps.

Varian's innovative TOP system provided Blue Heron with a simple, economical, and high-throughput solution for oligonucleotide purification. By incorporating a unique 96-well plate with removable tubes, streamlined gravity-flow procedure, and high-affinity polymeric resin, TOP efficiently and cost-effectively improved productivity in an oligo production setting while providing high, reproducible yields and purity.

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