July 24, 2014

Streamlined Purification of Assay-ready Oligonucleotides by Automated HPLC

Application Note PHA0114

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Introduction

Oligonucleotides used for standard molecular biology techniques such as qPCR, Next Generation Sequencing, and microarray analysis demand certain purity levels after large scale synthesis. Full-length products need to be separated from truncated sequences, inhibiting salts, and base protecting groups. Efficient purification of oligonucleotides of different lengths and at different production scales presents a manufacturing challenge. To overcome these hurdles, a single liquid handling platform configured with automated preparative and analytical HPLC capabilities was used to isolate, analyze, and desalt reagent-grade oligonucleotides in one continuous run. This liquid handling platform, Gilson GX-271 Purification System, was configured with both analytical and preparative injection ports, selection valves to accommodate different size chromatography columns, and with the ability to vary the sample load volume (up to 1.5L). Crude synthetic oligonucleotides (8-40mers) were first injected onto a preparative reverse phase or ion exchange HPLC column and peak-collected by Abs_{260} slope settings. Small samples were then removed from the fraction tubes and injected directly onto a reverse phase or anion exchange analytical column to confirm oligonucleotide size and purity. Using a size-exclusion desalting column in the final purification step >90% oligonucleotide purity was achieved. Effective salt removal was confirmed by in-line UV and conductivity monitoring. Modular and flexible programming of the TRILUTION LC® software facilitated seamless exchange of preparative and analytic methods without manual intervention. On-board fraction analysis during runs enabled the loading of sample lists for subsequent runs resulting in no down-time on the instrument. Additionally, built-in error handling conditions at various stages of the process prevented sample loss and allowed for fully automated, unattended operation. In the event of an error, the run can be stopped entirely or another method can be executed to enable sample recovery. The versatility of the Gilson Purification System permitted continuous sample injection, collection, reinjection, fraction pooling, and desalting on an automated platform providing bulk purified oligonucleotides as the end product for use in molecular biology applications.

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Materials & Methods

Gilson Automated Oligonucleotide Purification System

Figure 1. Fully Integrated and Flexible Oligonucleotide Purification System. A Gilson GX-271 Prep Liquid Handler was equipped with two GX Direct Injection valves and integrated with 322 Binary Mobile Phase Pumps, several ValveMates,152 UV/Vis Variable Wavelength Detectors [0.2 mm Flow Cell(Prep and Desalting); 5 mm Flow Cell (Analytical)], Hitachi Elite LaChrom L-2350 Column Oven, and Pharmacia Biotech Conductivity Meter. The complete system was controlled with Gilson TRILUTION® LC Software.

Luminex Oligonucleotide Products

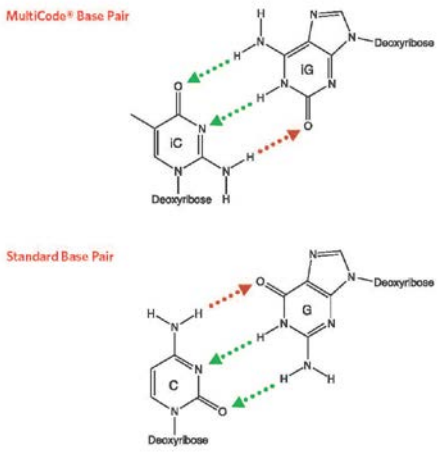

Figure 2. Luminex MultiCode® Technology. The isoC (iC) and isoG (iG) MultiCode bases form the building blocks for Luminex's next generation MultiCode assays for nucleic acid-based testing. MultiCode-based PCR assays are used for the early detection of infectious diseases and genetic-based conditions.
http://www.luminexcorp.com/TechnologiesScience/Real-Time-PCR/

Step 1: Isolation of Oligonucleotide by Ion Exchange HPLC (Preparative)

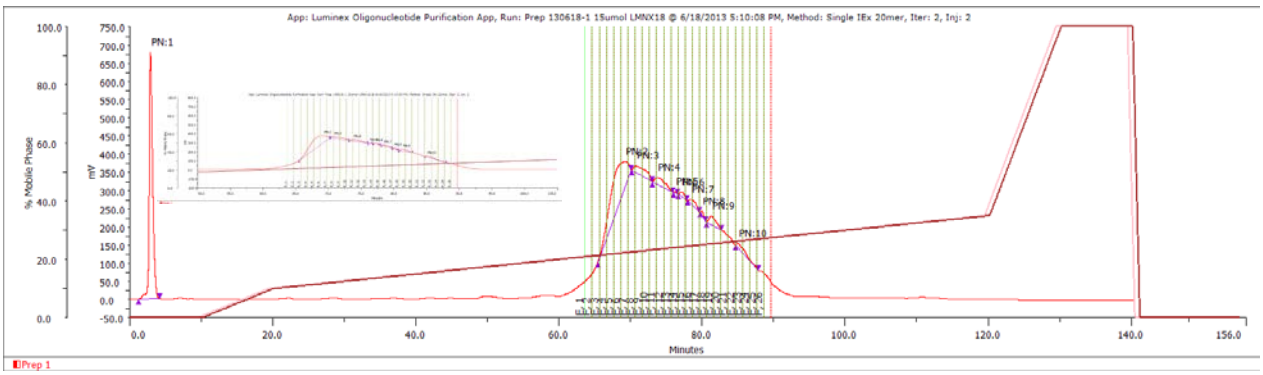

Figure 3. Pilot Scale Purification of 20-mer Oligonucleotide. Crude synthetic oligonucleotide (200 OD units) was injected onto a preparative ion exchange HPLC column (Tosoh SuperQ Column, 10x200 mm) and peak-collected by Abs_{260} slope settings. TRILUTION LC software allows the user to select the appropriate detector, column, solvents, and pumps for preparative HPLC. Variables also allow assignment of the sample location, injection location, peak collection settings, and fraction location suitable to the sample. Mobile Phase A = 20%ACN, $NaPO_4$, pH8.5; Mobile Phase B= A + 1M NaBr. See inset for expanded view of collected fractions.

Step 2: Verification of Oligonucleotide in Prep HPLC Fractions (Analytical)

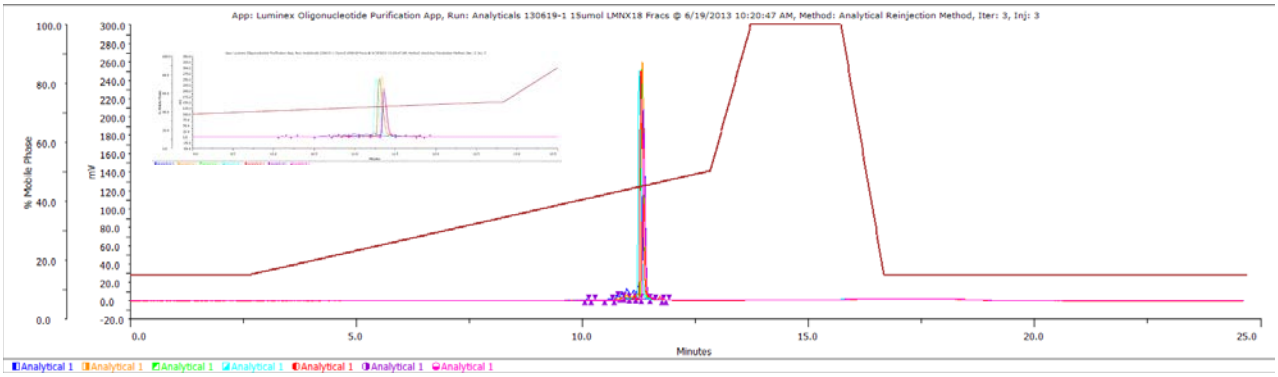

Figure 4. Analytic Verification of 20-mer Oligonucleotide. Selected early and late preparative HPLC column fractions (20 µL) were then re-injected onto a Dionex DNAPac PA-200 column using the analytical components of the GX-271 Purification System to verify 20-mer identity. Mobile Phase A = 20%ACN, NaPO4, pH11; Mobile Phase B= A + 1M NaBr. See inset for expanded view of collected fractions.

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Step 3: Removal of Salts and Other Impurities in Final Oligonucleotide Product

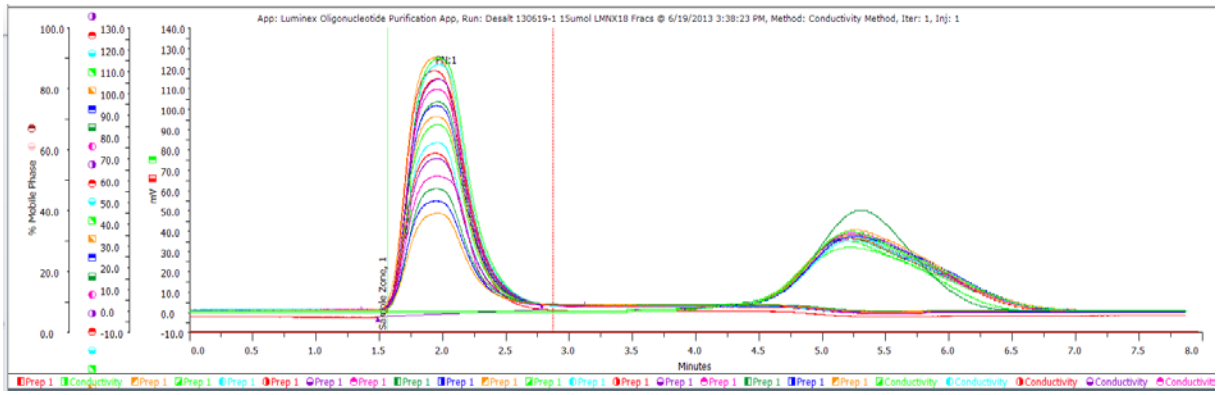

Figure 5. Desalting of 20-mer Oligonucleotide. Preparative HPLC fractions with established oligonucleotide identity were injected onto a GE HiPrep 26/10 size-exclusion desalting column and buffer exchanged with Milli-Q water. Greater than 90% oligonucleotide purity was achieved using a size-exclusion desalting column. Effective salt removal was confirmed by in-line UV and conductivity monitoring.

Summary

- The challenge of purifying a range of oligonucleotides (8-40mers) in various injection volumes (up to 1.5L) on different chromatography columns is overcome by the modularity of the Gilson instrumentation and the flexibility of the TRILUTION LC software.
- The versatility of the GX-271 Purification System and TRILUTION LC software permit easy switching between hardware components and columns accommodating sequential preparative HPLC, analytical HPLC, and large-scale desalting functionalities on one system.
- On-board fraction analysis during runs enables on-the-fly sample addition resulting in no down-time on the instrument.
- Built-in error handling conditions at various stages of the process prevent sample loss and assure full walk-away capabilities.
- Continuous sample injection, collection, reinjection, fraction pooling, and desalting on a single automated platform improves manufacturing efficiencies of oligonucleotides used in molecular biology applications.

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