INTEGRATED DNA TECHNOLOGIES

Purification of Oligonucleotides
Quick Look

This is a modified, quick look version of the full Technical Report *Chemical Synthesis of Oligonucleotides.* Please see the full version for a more comprehensive explanation.

Methods of Purification

Purification of an oligonucleotide is application-dependent. For longer oligonucleotides, such as those that are typically used for cloning and hybridization applications, purification is important because the non-full-length mass might interfere with the applications. Similarly, oligonucleotides modified with non-standard bases, fluorescent dyes, linkers, etc., often benefit from additional purifications.

IDT uses two methods for post-production purification:

1. Polyacrylamide gel electrophoresis (PAGE)
2. High performance liquid chromatography (HPLC)

PAGE

High percentage acrylamide gels are used to separate the full-length product from all shorter species with great efficiency. PAGE is the most effective means of purification for oligonucleotides that are **unmodified** and only need to have the capped products removed. PAGE purification does result in an unavoidable loss of mass because it is physically impossible to recover every bit of full-length product from a gel slice. Thus, a PAGE-purified 50-mer will yield a substantially lower mass relative to the starting synthesis scale. However, the loss of mass is typically an acceptable trade for the increase in purity. PAGE purification is strongly recommended for oligos over 60 bases in length. For unmodified oligos, purity of >90% is routinely achieved.

HPLC

HPLC utilizes the concept that a 25-mer oligonucleotide will have a specific charge and a specific affinity, or lack of affinity for a hydrophobic matrix in the presence of mixed organic solvents. This method is for oligonucleotides that have been modified through the addition of a linker or spacer, a non-standard base or bases, or hydrophobic molecules. HPLC is a form of column chromatography that utilizes a column to hold a stationary phase while the sample is applied within a mobile phase. The analyte's motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it passes through the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition and, thus, retention time is unique for each analyte.

HPLC comes in two basic forms.

1) **Reverse-phase HPLC** - separation is on the basis of the difference in matrix affinity between modified and unmodified oligonucleotide.
2) **Ion-exchange HPLC** - separation is on the basis of the differences in length as detected by the net charge between modified and unmodified oligonucleotide.

The type of HPLC used is dependant on the particular sample. Again, there will be an unavoidable loss of mass due to purification but this will be offset by the gain in purity.

Purification Quality Control

Synthesizing and purifying an oligonucleotide does not provide information about the final quality of the oligonucleotide. The oligonucleotide can be tested for quality prior to its use in an experiment through two methods:

1) Mass spectrometry
2) Capillary electrophoresis (CE)

Mass spectrometry

Two types of Mass spectrometry may be employed:

1) MALDI-TOF: Matrix Assisted Laser Desorption Ionization – Time Of Flight
2) ESI: Electrospray Ionization

MALDI-TOF can resolve oligonucleotides up to 50 bases in length (~15,000 Daltons) and is based on the principle that the speed at which an ion moves is inversely proportional to its mass. Thus, in the ion chamber of a MALDI instrument, materials are ionized and given the same potential energy, eV, where V is the potential and e refers to the number of charges on the ion. As the ions emerge from the ion source, the potential is converted to kinetic energy in the moving ion. Ions in motion obey the rule $E = 1/2mv^2$, or $m = 2E/v^2$, where m is the mass of the ion, v is velocity, and E is energy. Since the amount of energy is a constant in the MALDI, the mass of the ion can be determined by velocity alone. Velocity is simply time over distance such that the time

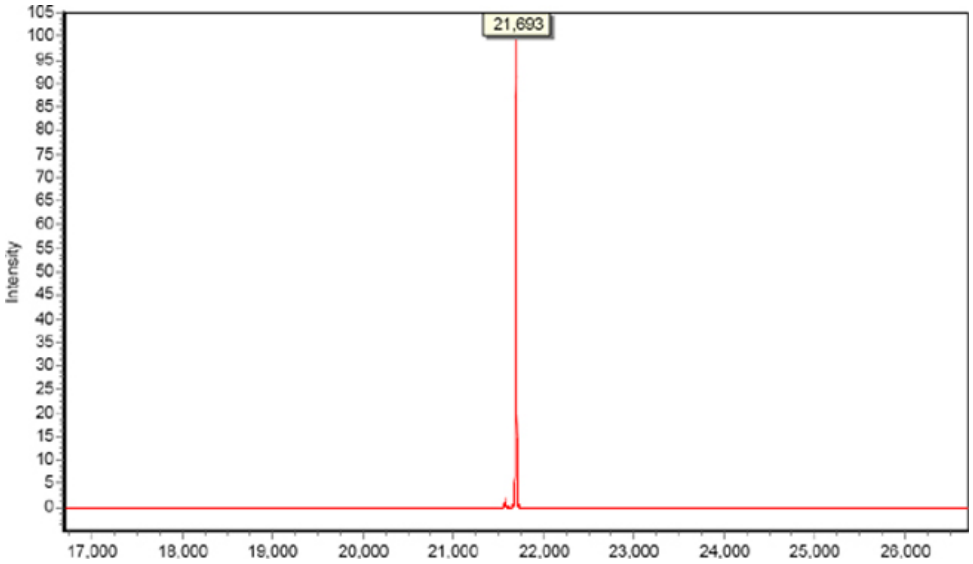

Figure 1. Example of a Mass spectrometry trace. The main peak is the main product at the indicated mass in Daltons.

of arrival of the ion in the detector of the MALDI (time of flight) is directly converted to velocity because the distance is a constant as well. In oligonucleotide synthesis QC, MALDI-TOF has the added advantage that the mass of an oligonucleotide can be estimated with precision since the masses of the individual nucleotides are fixed (Figure 1). For any given sequence, the expected arrival time will be given by the expected mass and any deviation from that time of arrival will indicate a deviation from ideal size and/or purity.

Electrospray Ionization (ESI) mass spectrometry can resolve longer oligonucleotides (up to ~200 bases in length). The material to be analyzed by ESI begins in liquid form and is dispersed into a fine aerosol by electrospray. The aerosol enters a heated capillary where any remaining solvent is evaporated from the charged droplets. Smaller droplets will evaporate more quickly than larger droplets. Once the solvent is evaporated, only the ions remain in the gas phase. The ions will have a variety of charges and so will hit the detector at different times based on the charge. Based on the peak from when an ion hits the detector, the charge state is determined and then, from this, the mass.

Capillary electrophoresis

Capillary electrophoresis uses a small amount of the final synthesis product that is subjected to a constant electrical field in a hair-thin capillary. As the product migrates into the capillary it is separated into component sizes in a manner exactly like gel electrophoresis. The fragments will migrate past an optical window and an ultraviolet beam detector will assess the density of the fragments. The "trace" that is produced is composed of a series of peaks corresponding to material densities flowing past the detector (Figure 2). This density profile is made quantitative by establishing a base line and integrating the area under the individual peaks. The purity of the product is the ratio of the main peak to the total area under all peaks. In practice, the main peak should be the last peak since no species should be longer than the full-length product. Peaks that appear to the right of the main peak indicate residual impurities and other potential contaminants.

Figure 2. A capillary electrophoresis of an oligonucleotide synthesis. The axes are uV absorbance units (AU) versus time on the column in minutes. The main peak is the dominant peak and the "shoulder" to the left is the (n-1)-mer peak. The ratio of the main peak to the total indicates purity greater than 95%.