

Oligonucleotide Yield, Resuspension, and Storage Quick Look

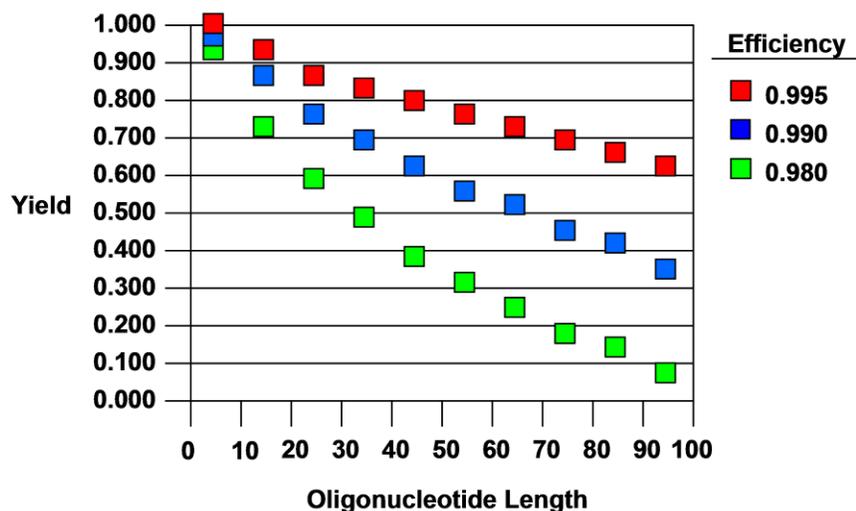
This is a modified, quick-look version of the full Technical Report *Oligonucleotide Yield, Resuspension, and Storage*. Please see the full version for a more comprehensive explanation.

Introduction

Scale refers to the amount of starting material which is composed solely of the most 3' nucleotide of a sequence attached to a solid support and housed within the column used to make the oligonucleotide.

Yield refers to the amount of final product recovered after all the synthesis and purification steps associated with the oligonucleotide have been completed.

Based on the relationship between expected yield and actual scale of synthesis, IDT routinely offers yield guarantees based upon a combination of oligonucleotide length and synthesis scale. Because of chemical and physical restraints, coupling efficiency is less than 100% at each step in the synthesis. In addition, coupling efficiency varies for each base both by type and position in the growing oligonucleotide [1, 2]. IDT's experience gained from the synthesis of millions of oligonucleotides shows that some sequences will result in better yields than others. **Monitoring efforts at IDT confirm that our average coupling efficiency for oligonucleotides exceeds 99%.** Thus, theoretical yield for a 24mer will be 89.1% full-length product (FLP) at 99.5% average coupling efficiency and 79.4% FLP at 99.0% average coupling efficiency.



Synthesis of very long oligonucleotides (those greater than 60 bases) is problematic. Thus, IDT does not offer any yield guarantees for oligonucleotides greater than 60 bases at our highest synthesis scale and greater than 100 bases at any scale. In addition, as a general rule, IDT recommends that any oligonucleotide longer than 40 bases should receive further purification. In addition, for demanding applications such as site-directed mutagenesis, cloning, and gel-shift protein-binding assays, additional purification is recommended even for oligonucleotides shorter than 40 bases. Finally, in specific instances, IDT requires additional purification of an oligonucleotide synthesis. Please note that additional purification will result in a decrease in final oligonucleotide yield.

Resuspension

Oligonucleotides are shipped in dry (lyophilized) form unless otherwise requested. Lyophilized DNA is usually very easy to resuspend in an aqueous solution. However, not all oligonucleotides dry identically and some can take more time to completely go into solution than others. In some cases it is possible for the dried oligonucleotide to become dislodged from the tube during shipping. Thus, it is very important to spin every oligonucleotide prior to opening the tube for resuspension.

What to use:

1) TE buffer. We recommend using TE buffer because the buffer will maintain a constant pH. The oligo should not be exposed to conditions that are either too acidic or too basic. IDT supplies a nuclease-free IDTE buffer (10 mM Tris pH 8.0; 0.1 mM EDTA; pH 8.0). See the IDT Catalog for ordering information.

2) Water. If using water for resuspension, be sure to use only nuclease-free water, pH 7.0 (HPLC-grade is preferable). DEPC water will harm oligonucleotides and water from a Millipore, or other types of deionizing, system can be acidic with pH as low as 5.0.

How to resuspend oligos:

1) Spin down the tube before opening. The oligonucleotide pellet may become lodged in the lid; spinning is important to insure it does not fall out when the lid is opened.

2) Decide on the final concentration. We recommend a 100 μ M concentration. This is an easy concentration to make and is an easy concentration to dilute. This will be the stock concentration which will be diluted for the working solution for each experiment.

All yield information is contained on the “spec sheet” for each oligonucleotide. This contains the actual yield of the oligonucleotide synthesis in three forms: optical density (OD) units; mass in milligrams (mg); and copy number in nanomoles (nm).

To make a 100 μ M concentration: Take the number of nmoles of oligo in the tube and multiply that by 10. This number will be the number of μ L of buffer to add to get a 100 μ M

solution. For example, if you have 9 nmoles of oligo, add 90 μL of buffer to make a 100 μM solution.

Alternatively, dilution using mass units involves the OD units and weight of the oligo. A 20mer oligonucleotide primer with random base composition will have a molecular weight of ~ 6100 and a molar extinction coefficient of 196,900 L/mole-cm. 1 OD_{260} unit of this oligonucleotide will therefore correspond to 31 μg , or 5 nmoles, of DNA. Dissolving 500 μg of DNA (16 OD units) in 500 μl of TE will yield a stock primer concentration of 1 $\mu\text{g}/\mu\text{L}$, or about a 160 μM solution. This converts to 160 picomoles of oligonucleotide per μL .

An oligonucleotide can be stored at any concentration. However, concentrations lower than 1 μM may change over time as some of the oligo may be absorbed into the plastic of the tube. In addition, a 5–10 mM solution is generally the highest concentration at which an oligo will go into solution.

3) For hard to suspend oligos, heat at 55° C for 1–5 minutes, then thoroughly vortex. If this does not work, the tube might have Trityl (flakey appearance) or CPG (a pellet at bottom of tube). Neither of these should affect the performance of the oligo, and both can be removed with a Sephadex G50 column, or by removing the supernatant.

Storage

Store resuspended oligonucleotides as several small aliquots at -20°C .

References

1. Temsamani J, Kubert M, and Agrawal S. (1995) Sequence identity of the n-1 product of a synthetic oligonucleotide. *Nucleic Acids Res*, 23(11): 1841–1844.
2. Hecker KH and Rill RL. (1998) Error analysis of chemically synthesized polynucleotides. *Biotechniques*, 24(2): 256–260.