

# Synthesizing and Printing High-Quality Oligonucleotide Arrays

## PolyPlex<sup>®</sup> Synthesizer & OmniGrid<sup>®</sup> Microarrays, Slides, and Reagents

### ▶ Abstract

This Application Note describes in detail how to produce high-quality oligonucleotide arrays at a fraction of commercial costs. Oligonucleotides are fast becoming the probe molecule of choice in microarray research. Digilab is uniquely positioned to support both the synthesis and fabrication of oligonucleotide arrays. Traditionally, self-spotted microarrays have utilized cDNA for expression features. The availability of low-cost oligos and high-quality genomic sequence is driving a transition to oligonucleotide arrays. The Digilab PolyPlex oligo synthesizer and OmniGrid microarrayers combine to deliver straightforward, high-quality, and cost-effective oligonucleotide array production. These two products put the power and flexibility of oligonucleotide arrays easily within reach of production facilities.

As microarray researchers increase the desired number of genes to monitor in a single expression experiment, the cost associated with producing the required oligos has increased dramatically. Although synthesis costs have fallen substantially for commercial oligos, the cost to purchase array-ready commercial oligos remains high. The PolyPlex synthesizer and OmniGrid microarrayer together deliver high-quality oligo arrays at a fraction of commercial costs by utilizing crude 5'-amino-modified oligos on OmniGrid microarray slides. As a result, tedious and expensive oligo purification steps are eliminated. Synthesis in 96-well format provides error-free handling in the transition between synthesizer and microarrayer. During the processing of the printed slide, the truncated oligos that did not receive the amino linker are washed off during the pre-hybridization steps. This complete synthesis and microarray package offers a flexible in-house alternative to costly commercial oligo arrays.

### ▶ Materials and Methods

Crude 50-mer oligos were synthesized at 30 nmol scale on the PolyPlex synthesizer using 1000Å standard controlled pore glass (CPG). All microarrays were printed on OmniGrid Aldehyde slides using either the OmniGrid or OmniGrid Accent microarrayer fitted with SMP3B (TeleChem International, Inc., Sunnyvale, CA) microspotting pins.

All oligo synthesis reagents, CE-phosphoramidites, and 5'-Amino Modifier C6-TFA were purchased from Glen Research (Sterling, VA). A 96-well filter plate was filled with 30nmol of standard CPG and a Digilab tested protocol was loaded into the PolyPlex software.

Three different 50mers were synthesized (Table 1). A full 96-well plate of oligos at this length takes approximately 6.7 hours to create on the PolyPlex synthesizer. After synthesis, 300µL of ammonium hydroxide were added to each well to cleave the oligos from synthesis plate and into a deep-well plate. The oligos in the deep-well plate

on the PolyPlex synthesizer using 1000Å

**Table 1. Oligos from the PolyPlex Synthesizer**

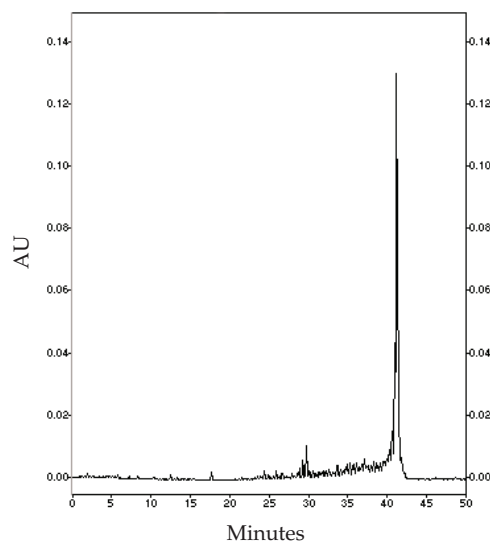
Tubulin BC006481_Oligo1 (TUB_1) caagcgtgcc ttgttctact ggtacgtggg tgaggggatg gaggaaggcg Length: 50 base pairs 60% GC content Molecular Weight: 15638
Tubulin BC006481_Oligo2 (TUB_2) gaattccaga ccaacctggt gccctacccc cgcctccact tcctctggc Length: 50 base pairs 62% GC content Molecular Weight: 15077
GAPDH_Oligo1 (GADPH_1) ctcaaggcca tctctgggcta cactgagcac caggtggtct cctctgact Length: 50 base pairs 58% GC content Molecular Weight: 15302

were deprotected at 55°C in a water bath overnight. After deprotection, the deep-well plate was dried down in a lyophilizer.

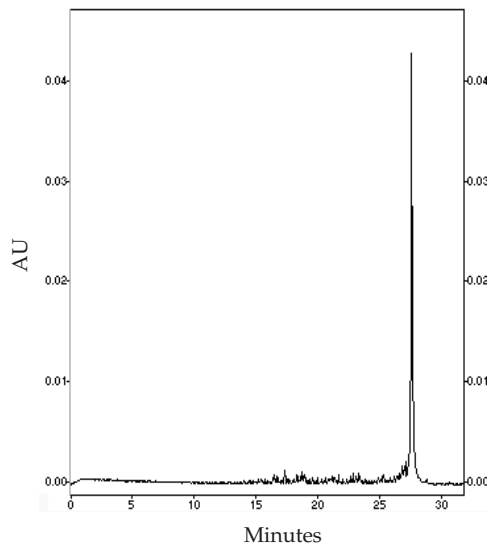
## Capillary Gel Electrophoresis for Quality Control and Analysis

The purity of crude oligonucleotides was analyzed by capillary electrophoresis separation. Before drying the deprotected oligonucleotides, a 10 µL aliquot of the 300 µL ammonium hydroxide oligo solutions was dried down, and then suspended with 10 µL deionized water. The resuspended oligo sample was then separated on a P/ACET<sup>TM</sup> MDQ Capillary Electrophoresis instrument (Beckman Coulter Inc., Brea, CA).

**Figure 1. Capillary Electropherogram of Tubulin BC006481\_Oligo 2 with Amino Linker. Coupling Efficiency = 98.5%**



**Figure 2. Capillary Electropherogram of Commercial, Purified 50mer with Amino Linker**



Separating voltage was set to 16.00 kV. Average coupling efficiencies of all 96 PolyPlex oligos were 98.4%.

## Microarraying

Microarray features were made using C6-amino-modified oligos (Table 1). Of the three oligos synthesized on the PolyPlex synthesizer, Tubulin BC006481\_Oligo1 (**TUB\_1**) and GAPDH\_Oligo1 (**GAPDH\_1**) were also ordered from a commercial source. Oligos **TUB\_1** and Tubulin BC006481\_Oligo2 (**TUB\_2**) are specific for the human  $\alpha$ -tubulin, and GAPDH\_1 oligos are specific for the GAPDH ORFs. The **TUB\_2** oligo was specific for a different region of the  $\alpha$ -tubulin ORF. These sets of oligos were spotted onto OmniGrid Aldehyde slides (Pt.# OGC-AL) along with a buffer blank using the OmniGrid microarrayer (Figure 3 and Figure 4). The features were spotted at a concentration of 250 ng/µL in 3XSSC using SMP3B microspotting pins. Replicates of 10 features were arranged in rows at 250 micron center to center spacing. The modified oligos were allowed to react with the slide surface overnight at room temperature. In some cases, the blocking of unreacted aldehyde groups was accomplished by incubating slides with a succinic anhydride/borate solution. All slides were rinsed for 2 minutes in 95°C water and dried by centrifugation in a plate spinner at 1500 rpm for 2 minutes.

## Hybridization Method

The hybridization was carried out in a Digilab HybChamber<sup>TM</sup> (Pt.# HYB-03, HYB-04) dual-slide hybridization chamber. 100 mL of water was applied to the slide's opposite end of the microarray to maintain humidity and feature integrity. The HybChamber containing the microarray was then incubated in a 42°C water bath and protected from ambient light for 5 hours. After this incubation, the

arrays were washed twice with 2X SSC, 0.2% SDS for 5 minutes and once in 2X SSC for 5 minutes and dried in a plate spinner at 1500 rpm of 2 minutes.

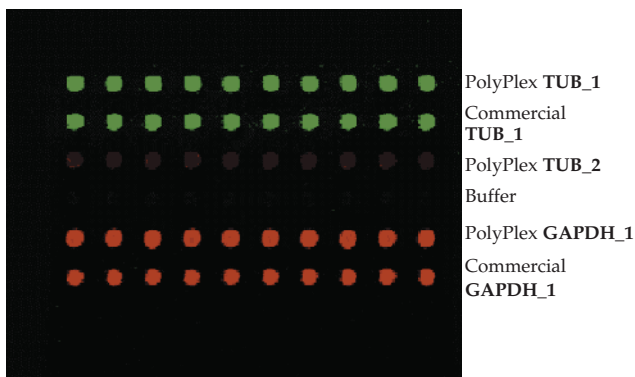
### Preparation of Complementary Oligos

In order to test the specificity and quality of the crude PolyPlex oligos, we compared their behavior to another commercially purchased set of purified oligos (Figure 3). The experiment involved designing two complementary oligonucleotides, one to TUB\_1 oligo and the other to GAPDH\_1 oligo. The complementary TUB\_1 oligo was modified with Cy3, and the complementary GAPDH\_1 oligo was modified with Cy5. These oligos were dissolved at a final concentration of 2  $\mu$ M in 5X SSC, 0.2% SDS, 0.2 mg/mL BSA. This solution, 15  $\mu$ L was applied to the microarray under a microscope cover slip and the microarray was hybridized using the Hybridization Method above.

### Preparation of cDNA

In order to better simulate actual microarray conditions used to monitor differential gene expression, Cy-labeled cDNA was prepared from human tissue culture cells (Figure 4). Total RNA was isolated from HeLa spinner cells using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). RNA, 20  $\mu$ g, was directly labeled with Cy3-labeled CTP and another 20  $\mu$ g with Cy5-labeled dCTP according to manufacturers instructions (Amersham Biosciences Corp, Piscataway, NJ), using anchored oligo-dT as a primer. One half of each reaction was used to hybridize the microarrays using the Hybridization Method above.

**Figure 3. Fluorescent Oligo Hybridization to Oligo Array**



OmniGrid Aldehyde slide spotted with amino-modified oligonucleotide 50mers. Cy3 labeled complementary 20mer for Tub\_1 ( $\alpha$ -tubulin) and Cy5 labeled complementary 50mer GAPDH\_1 (GAPDH ORFs) were hybridized at 42°C for 5 hours.

**Figure 4. cDNA Hybridization of Oligo Array**



OmniGrid Aldehyde slide spotted with amino-modified oligonucleotide 50mers. Total RNA HeLa cells were directly labeled with Cy3 and Cy5-dCTP and hybridized at 42°C for 5 hours.

## Results & Discussions

Figure 3 shows two sets of TUB\_1 features as green emissions and the GAPDH\_1 with red emissions, with little observed cross-hybridization (see TUB\_2 which lacked a complementary oligo probe). This result demonstrates that the oligos generated on the PolyPlex synthesizer serve as specific features, comparable to purified oligos purchased from commercial vendors.

Examination of Figure 4 shows that during actual gene expression experiments, crude PolyPlex oligos also perform similarly to purified commercial oligos. No hybridization could be detected where buffer alone was spotted to the slide.

Each output well from a PolyPlex run generates sufficient oligonucleotide material to spot well over 100,000 features. If the majority of the oligonucleotides are printed, taking into account loss due to handling (30%), the cost of the oligonucleotides for a 27,000-feature array is under \$2. Table 2 displays the cost breakdown for the typical yield from the PolyPlex for amino-linked 50mers spotted at

**Table 2. Cost of Synthesis and Array**

Cost amino-modified 50mer Synthesis costs (\$0.10/base) plus amino linker (\$0.12/oligo) 30nmol scale, 99% coupling efficiency	\$5.12
Maximum printable features Per oligo, printing concentration of 20 $\mu$ M, 600 nL print pin uptake volume, 100 slides per pin dip, 70% usable volume.	>100,000
Oligo costs per 27,000 feature slide	<\$2.00

27,000 features per slide. Realistically, the number of slides printed for any given batch of oligonucleotides will not approach this maximum throughput. While the oligonucleotides will remain usable, most of the material will be archived. Table 3 displays the oligonucleotide cost of these same microarrays if not all of the oligonucleotide is used, for several printing run sizes. At a throughput of 5,000 slides, the cost of the oligonucleotides rises to \$30 per slide, with a virtually unlimited repository of material to retrieve if necessary. Even at a modest printing run of 1000 slides, the oligonucleotides for the microarrays only cost \$150 per array.

**Table 3. Oligo Costs Per Slide (27,000 features)**

Number of features printed per oligo	Oligo cost
1000	<\$150
5000	<\$30
10,000	<\$15
100,000	<\$2

## ► Conclusions

The Digilab PolyPlex synthesizer and OmniGrid microarray products combine to form a powerful tool in producing oligonucleotide microarrays. Oligonucleotide arrays have quickly proven their value in a wide range of microarray research. By utilizing 5'-amino modifications in combination with the OmniGrid Aldehyde slide, this Application Note has demonstrated that high-quality microarrays can be produced from crude, unpurified oligonucleotides. The quality of these arrays is comparable to arrays produced with commercially purified oligonucleotides, but at a fraction of the cost.

When using a PolyPlex synthesizer, the oligonucleotide synthesis cost associated with array production can be extremely low. For a 27,000-feature array, minimum costs for synthesis approach \$2.00 in the highest production volumes (100,000 arrays). Even more modest production volumes (1000 arrays, 27,000 features) can be produced for just over \$150 per array.

Microarray producers will find that the PolyPlex synthesizer and OmniGrid microarrayers and slides offer an extremely cost-effective and high-quality alternative for oligonucleotide array production. The performance of the PolyPlex synthesizer and OmniGrid products put oligonucleotide arrays within reach of most production facilities.

### Author

Damien Luk, Anh Tuyet-Doan, Vivian Le, Keith Gulyas, Ph.D., and Robert Guettler, Ph.D.

## Worldwide Headquarters

Digilab, Inc.  
84 October Hill Road  
Holliston, MA 01746  
USA

Phone: (508) 893-3130  
Toll Free: (800) 935-8007  
Fax: (508) 893-8011  
E-Mail: [info@digilabglobal.com](mailto:info@digilabglobal.com)



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